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(54) Title: BIO-ENGINEERED HYPER-FUNCTIONAL "SUPER" HELICASES

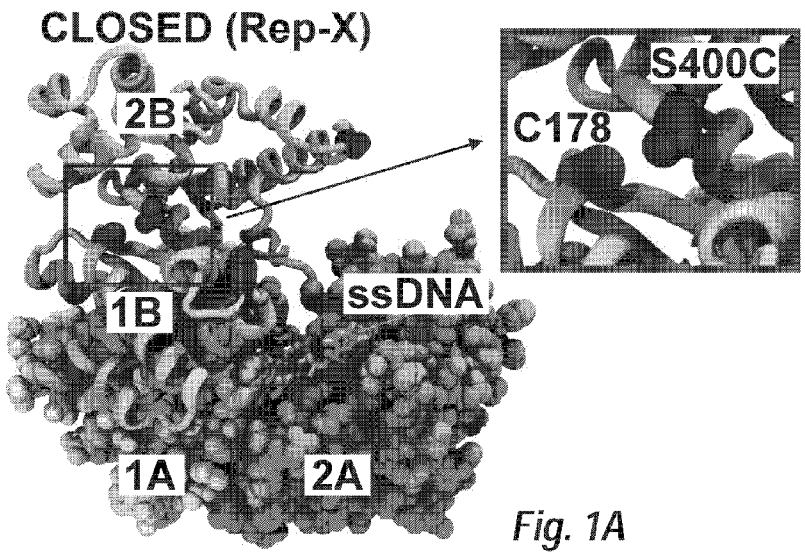

Fig. 1A

(57) Abstract: Conformationally- constrained helicases having improved activity and strength are provided. Methods of making conformationally-constrained helicases having improved activity and strength are provided. Methods of using confbrmationally-constrained helicases having improved activity and strength are provided. The present invention is based on the discovery of novel modified helicases that show dramatically enhanced helicase activity and increased strength as compared to unmodified helicases. As described further herein, it has been surprisingly discovered that, by controlling the conformation of certain subdomains such that the helicase remains in a closed form (e.g., by covalently crosslinking the 2B domain to the 1A domain or the IB domain in a Rep helicase), a highly active and strong form of the helicase is achieved

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BIO-ENGINEERED HYPER-FUNCTIONAL "SUPER" HELICASES

RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No. 62/079,183, filed November 13, 2014, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED **RESEARCH OR DEVELOPMENT**

[002] This invention was made with government support under GM065367 awarded by the National Institutes of Health. The United States Governm ent has certain rights in the invention.

SEQUENCE LISTING

[002.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.

FIELD OF **THE INVENTION**

[003] The present disclosure relates to compositions and methods for helicase-mediated DNA unwinding activity.

BACKGROUND

[004] A traditional definition of a helicase is an enzyme that catalyzes the reaction of separating/unzipping'unwinding the helical structure of nucleic acid duplexes (DNA, RNA or hybrids) into single-stranded components, using nucleoside triphosphate (NTP) hydrolysis as the energy source (such as ATP). However, it should be noted that not all helicases fit this definition anymore. A more general definition is that they are motor proteins that move along the single-stranded or double stranded nucleic acids (usually in a certain direction, 3' to 5' or 5 to 3, or both), i.e. translocases, that can or cannot unwind the duplexed nucleic acid encountered. In addition, some helicases simply bind and "melt" the duplexed nucleic acid structure without an apparent translocase activity.

[005] Helicases exist in all living organisms and function in all aspects of nucleic acid metabolism. Helicases are classified based on the amino acid sequences, directionality, oligomerization state and nucleic-acid type and structure preferences. The most common classification method was developed based on the presence of certain amino acid sequences, called motifs. According to this classification helicases are dividai into 6 super families: SFl, SF2, SF3, SF4, SF5 and SF6. SFl and SF2 helicasesdo not form a ring structure around the nucleic acid, whereas SF3 to SF6 do. Superfamily classification is not dependent on the classical taxonomy.

[006] DNA helicases are responsible for catalyzing the unwinding of double-stranded DNA (dsDNA) molecules to their respective single-stranded nucleic acid (ssDNA) forms. Although structural and biochemical studies have shown how various helicases can translocate on ssDNA directionally, consuming one ATP per nucleotide, the mechanism of nucleic acid unwinding and how the unwinding activity is regulated remains unclear and controversial (T.M. Lohman, **E.J.** Tomko, C.G. Wu, "Non-hexameric DNA helicases and translocases: mechanisms and regulation," *NatRevMol CellBiol* **9:391-**401 (2008)). Since helicases can potentially unwind all nucleic acids encountered, understanding how their unwinding activities are regulated can lead to harnessing helicase functions for biotechnology applications.

BRIEF **SUMMARY OF** THE **INVENTION**

[007] The present invention is based on the discover}' of novel modified helicases that show dramatically enhanced helicase activity and increased strength as compared to unmodified helicases. As described tùither herein, it has been surprisingly discovered that, by controlling the conformation of certain subdomains such that the helicase remains in a closed form (e.g., by covalently crosslinking the 2B domain to the 1A domain or the 1B domain in a Rep helicase), a highly active and strong form of the helicase is achieved.

[008] In one aspect, a composition for catalyzing an unwinding reaction on double-stranded DNA is provided that includes a conformationally-constrained helicase.

[009] In another aspect, a method of catalyzing an unwinding reaction of a double-stranded DNA is provided. The method includes the step of contacting the double-stranded DNA with a conformationally-constrained helicase in the presence of ATP.

[0010] In another aspect, an isolated nucleic acid that encodes a helicase polypeptide having the capability to be constrained in a confonnatioii by an intramolecular crosslinking agent is provided.

[0011] In another aspect, a modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid is provided. Said first amino acid is at least about 30 A from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 A from said second amino acid when the helicase is in an active conformation. A side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conform ati onal 1y-con strai ned helicase.

[0012] In certain exemplary embodiments, the modified helicase is a Super Family 1 (SF 1) helicase (e.g., an SF 1A or an SF 1B helicase) or a Super Family 2 (SF2) helicase.

[0013] In certain exemplary embodiments, the first amino acid is less than about 20 Å, about 19 A, about 18 A, about 17 A, about 16 A, about 15 A, about 10 A, about 9 A, about 8 A, about 7 A, about 5 A, or about 4 A from the second amino acid when the helicase is in an active conformation.

[0014] In certain exemplary embodiments, the first amino acid is at least about 30 A, about 40 A, about 50 A, about 55 A, about 60 A, about 65 A, about 70 A, about 75 A, about 80 A or about 85 A from the second amino acid when the helicase is in an inactive conformation.

[0015] In certain exemplary embodiments, the helicase is selected from the group consisting of a Rep helicase (e.g., from *E. coll.),* a LJvrD helicase (e.g., from *E. coli.)* and a PcrA helicase (e.g., from *B. stearothermophihis).*

[0016] In certain exemplary embodiments, the first amino acid is at any one of positions 84-116 or 178-196 of the modified helicase amino acid sequence, and the helicase is a Rep, PcrA or UvrD helicase, or homolog thereof.

[0017] In certain exemplary embodiments, the first amino acid is at any one of positions 92-116 or 178-196 of the modified helicase amino acid sequence, and the helicase is a Pcr A helicase, or homolog thereof

[0018] In certain exemplary embodiments, the first amino acid is at any one of positions 84-108 or 169-187 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof

[0019] In certain exemplary embodiments, the first amino acid is at any one of positions 90-114 or 175-193 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

[0020] In certain exemplary embodiments, the first amino acid at position 178 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

[0021] In certain exemplary embodiments, the first amino acid is at position 187 of the modified helicase amino acid sequence, and the helicase is a PerA helicase, or homolog thereof.

[0022] In certain exemplary embodiments, the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 13 or SEQ ID NO: 14, and the helicase is a Rep helicase, or homolog thereof.

[0023] In certain exemplary embodiments, the second amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 15 or SEQ ID NO: 16, and the helicase is a Rep helicase, or homolog thereof.

[0024] In certain exemplary embodiments, the second amino acid residue is at any one of positions 388-411, 422-444 and 518-540 of the modified helicase amino acid sequence, and the helicase is a Rep, PerA or UvrD helicase, or homolog thereof.

[0025] In certain exemplary embodiments, the second amino acid is at any one of positions 397-411, 431-444 or 526-540 of the modified helicase amino acid sequence, and the helicase is a PerA helicase, or homolog thereof.

[0026] In certain exemplary embodiments, the second amino acid is at any one of positions 388-402, 422-435 or 519-531 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

[0027] In certain exemplary embodiments, the second amino acid is at any one of positions 393-407, 427-440 or 523-540 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

[0028] In certain exemplary embodiments, the second amino acid is at position 400 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

[0029] In certain exemplary embodiments, the second amino acid is at position 409 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

[0030] In certain exemplary embodiments, the first amino acid is at any one of positions 60-82 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof. In certain exemplary embodiments, the first amino acid is at any one of positions 68-79 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

[0031] In certain exemplary embodiments, the first amino acid is at any one of positions 69-89 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof. In certain exemplary embodiments, the first amino acid is at any one of positions 77-87 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

[0032] In certain exemplary embodiments, the first amino acid is at any one of positions 67-87 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof. In certain exemplary embodiments, the first amino acid is at any one of positions 75-85 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

[0033] In certain exemplary embodiments, the second amino acid is at any one of positions 509-536 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof. In certain exemplary embodiments, the second amino acid is at any one of positions 519-525 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

[0034] In certain exemplary embodiments, the second amino acid is at any one of positions 516-534 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof. In certain exemplary embodiments, the second amino acid is at any one of positions 526-532 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

[0035] In certain exemplary embodiments, the second amino acid is at any one of positions 513-531 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase.

or homolog thereof. In certain exemplary embodiments, the second amino acid is at any one of positions 523-529 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

[0036] In certain exemplary embodiments, said first subdomain and said second subdomain comprise no more than a total of two cysteine residues.

[0037] In certain exemplary embodiments, the helicase comprises one cysteine residue and/or is from a bacterium selected from the group consisting of Deinococcus geothermalis, Meiothermus sp., Marinithermus hydrothermalis, Marinithermus hydrothermalis and Oceanithermiis profundus.

[0038] In certain exemplary embodiemnts, the helicase comprises one cysteine residue or no cysteine residues and/or is from a bacterium selected from the group consisting of Thermococcus sp. EXT9, Thermococcus sp. IRI48, Thermococcus sp. IRI33, Thermococcus sp. AMT7, Thermococcus nautili, Thermococcus onnurineus (strain NA1), Thermococcus kodakarensis (strain ATCC BAA-918 / JCM 12380 / KOD1) (Pyrococcus kodakaraensis (strain KOD1)), Thermococcus sibiricus (strain MM 739 / DSM 12597), Thermococcus paralvinellae. Thermus aquaticus Y51MC23, Thermus aquaticus Y51MC23, Thermus aquaticus Y51MC23, Thermus sp. RL, Thermus sp. RL, Thermus sp. 2.9, Salinisphaera hydrothermalis C41B8, Thermus filiformis, Meiothermus ruber, Thermus sp. NMX2.AI, Thermus thermophilus JL-18, Thermus scotoductus (strain ATCC 700910 / SA-01), Thermus scotoductus (strain ATCC 700910 / SA-01), Oceanithermiis profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermiis profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermiis profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermiis profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermiis profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Thermus oshimai JL-2, Thermus oshimai JL-2, Thermus oshimai JL-2, Thermomonospora curvata (strain ATCC 19995 / DSM 43183 / JCM 3096 / NCIMB 10081), Thermodesulfatator indicus (strain DSM 15286 / JCM 11887 / CIR29812), Geobacillus stearothermophilus (Bacillus stearothermophilus), Coprothermobacter proteolyticus (strain ATCC 35245 / DSM 5265 / BT), Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) (Thermus silvanus), Anaerolinea thermophila (strain DSM 14523 / JCM 11388 /

NBRC 100420 / UNI-1), Thermoanaerobacterium thermosaccharolyticum M0795, Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Deinococcus radiodurans (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051/ NBRC 15346 / NCIMB 9279 / R1 / VKM B-1422), Thermodesulfobium narugense DSM 14796, Thermus thermophilus (strain HB8 / ATCC 27634 / DSM 579), Dictyoglomus thermophilum (strain ATCC 35947 / DSM 3960 / H-6-12), Thermus thermophilus (strain SG0.5JP17-16), Thermus thermophilus (strain SG0.5JP17-16), Thermus thermophilus (strain SG0.5JP17-I6), Thermus sp. CCB_US3_UF1, Deinococcus geothermalis (strain DSM 11300), Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039), Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039), Marinithermus hydrothermalis (strain DSM 14884 / JCM 11576 / T1).

[0039] In certain exemplary embodiments, the first amino acid and the second amino acid are each independently an unnatural amino acid or a natural amino acid.

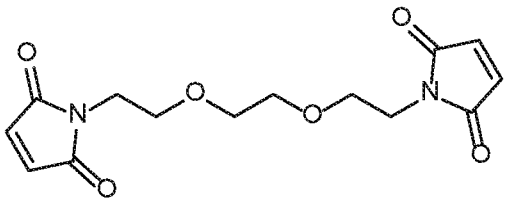
[0040] In certain exemplary embodiments, one or more of an amino acid of the helicase is substituted with an unnatural amino acid or a natural amino acid (e.g., a cysteine or a homocysteine).

[0041] In certain exemplary embodiments, said helicase comprises a sequence selected from SEQ ID NOs:4 and 12.

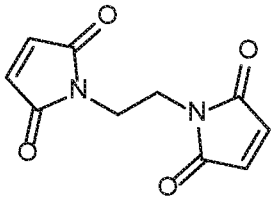
[0042] In certain exemplary embodiments, the first amino acid is covalently crosslinked to the second amino acid by a disulfide bond or by a chemical crosslinker (e.g., a chemical crosslinker having a length of from about 6Å to about 25A).

[0043] In certain exemplary embodiments, the chemical crosslinker is a bis-maleimide crosslinker.

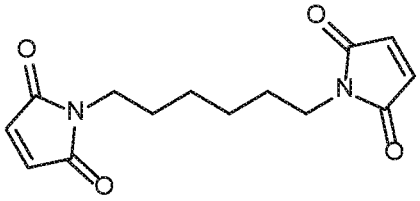
[0044] In certain exemplary embodiments, the chemical crosslinker is selected from the group consisting of



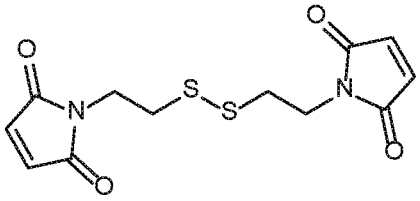
l~[2-[2~[2-(2,5-dioxopyrrol-l-yl)ethoxy]ethoxy]ethyl]pyrrole-2,5-dione,



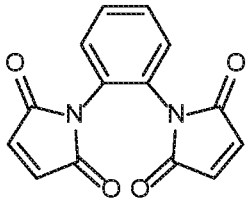
l-[2-(2,5-dioxopyrrol-l-yl)ethyl]pyrrole-2,5-dione,



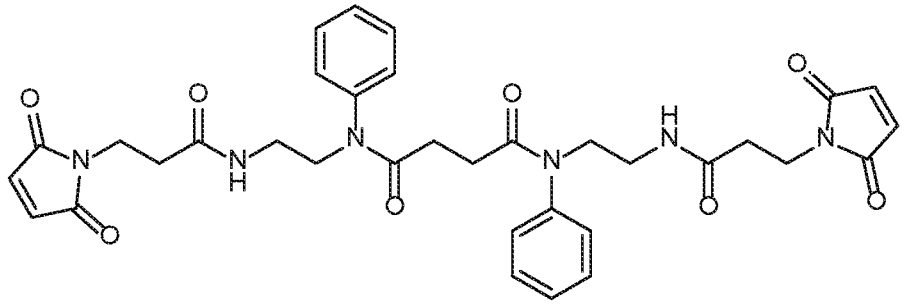
l~[6-(2,5-dioxopyrrol-l-yl)hexyl]pyrrole-2,5-dione,



1-[2-[2-(2,5-dioxopyrrol-1-yl)ethyldisulfanyl]ethyl]pyrrole-2,5-dione,

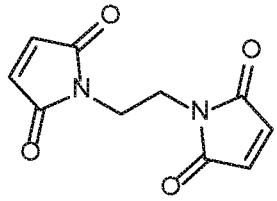


1-[2-(2,5-dioxopyrrol-l -yl)phenyl]pyrrole-2,5-dione, and



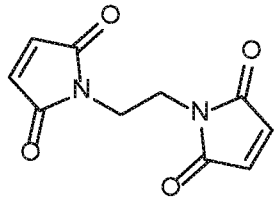
N,N'-bis[2-[3-(2,5-dioxopyrrol-l-yl)propanoy{amino]ethyi]-N,N'-diphenylbutanediamide.

[0045] In certain exemplary embodiments, the chemical crosslinker is



l-[2-(2,5-dioxopyrrol-l-yl)ethyl]pyrrole-2,5-dione.

[0046] In one aspect, a modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid, wherein said first amino acid is at least about 30 Å from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 Å from said second amino acid when the helicase is in an active conformation, and wherein a side chain of the first amino acid is chemically crosslinked to a side chain of the second amino acid using



1-[2-(2,5-dioxopyrrol- 1-yl)ethyl]pyrrole-2,5-dione to form an active, conformationally-constrained helicase is provided.

[0047] In another aspect, a modified Rep, PerA or UvrD helicase or homolog thereof, comprising a first subdomain having a first amino acid at any one of positions 84-1 16 and a second subdomain having a second amino acid at any one of positions 388-4 l 1, 422-444 and 518-540, wherein a side chain of the first amino acid is covalently crosslinked to a side chain

of the second amino acid with a linker to form an active, conformationally-constrained Rep, PerA or UvrD helicase, or homolog thereof is provided.

[0048] In another aspect, a modified Rep helicase or homolog thereof comprising an amino acid at position 178 covalentiy crosslinked to an amino acid at position 400 to form an active, conformationally-constrained Rep helicase or homolog thereof is provided.

[0049] In another aspect, a modified Rep helicase or homolog thereof comprising an amino acid at position 187 covalentiy crosslinked to an amino acid at position 409, to form an active, conformationally-constrained helicase is provided.

[0050] In another aspect, a modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid, wherein said first amino acid is at least about 30 A from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 Å from said second amino acid when the helicase is in an active conformation, and wherein a side chain of the first amino acid is covalentiy crosslinked to a side chain of the second amino acid with a chemical crosslinker to form an active, conformationally-constrained helicase, and wherein one or more of an amino acid of the helicase is substituted with an unnatural amino acid or a natural amino acid is provided.

[0051] In one aspect, a method of making an active, conformationally-constrained helicase is provided. The method includes the steps of selecting in a helicase a first amino acid in a first subdomain that is at least about 30 A from a second amino acid in a second subdomain when the helicase is in an inactive conformation, and the first amino acid is less than about 20 A from the second amino acid when the helicase is in an active conformation, and covalentiy crosslinking the first amino acid to the second amino acid when the helicase is in an active conformation to form an active, conformationally-constrained helicase.

[0052] In a certain exemplary embodiment, the method includes two steps. The first step includes expressing a helicase polypeptide having the capability to be constrained in a conformation by an intramolecular crosslinking agent from an isolated nucleic acid selected from a group consisting of SEQ ID NOs: 2, 3, 5 and 6. The second step includes reacting the helicase polypeptide with an intramolecular crosslinking agent to form the conformationally-constrained helicase.

[0053] In certain exemplary embodiments, the modified helicase is a Super Family 1 (SF1) helicase (e.g., SF1A or SFIB) or a Super Family 2 (SF2) helicase.

[0054] In certain exemplary embodiments, the first subdomain comprises a 1A subdomain or a IB subdomain and the second subdomain comprises a 2B subdomain.

[0055] In certain exemplary embodiments, the first amino acid is less than about 20 A, about 19 A, about 18 A, about 17 A, about 16 A, about 15 A, about 10 A, about 9 A, about 8 A, about 7 A, about 5 A, or about 4 A from the second amino acid when the helicase is in an active conformation.

[0056] In certain exemplary embodiments, the first amino acid is at least about 30 A, about 35 A, about 40 A, about 45 A, about 50 A, about 55 A, about 60 A, about 65 A, about 70 A, about 75 A, about 80 A or about 85 A from the second amino acid when the helicase is in an inactive conformation.

[0057] In certain exemplary embodiments, the helicase is selected from the group consisting of a Rep helicase, a UvrD helicase and a PcrA helicase.

[0058] In certain exemplary embodiments, the helicase comprises a sequence selected from SEQ ID NOs:4 and 12.

[0059] In certain exemplary embodiments, the first amino acid is covalently linked to the second amino acid by a disulfide bond or a chemical crosslinker.

[0060] In another aspect, a method of catalyzing an unwinding reaction of a double-stranded DNA, comprising contacting the double-stranded DNA with a modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid is provided. Said first amino acid is at least about 30 A from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 A from said second amino acid when the helicase is in an active conformation. A side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conformationally-constrained helicase.

[0061] In certain exemplary embodiments, the conformationally-constrained helicase comprises SEQ ID NO: 4 or SEQ ID NO: 12.

[0062] In certain exemplary embodiments, the conformationally-constrained helicase is chemically crosslinked.

[0063] In certain exemplary embodiments, the linker comprises an alkyl having a length in the range from C7 to C23 or from C8 to C13.

[0064] In another aspect, a method of performing isothermal DNA amplification, comprising combining a DNA template, the conformationaliy-constrained heiicase described above and amplification reagents under conditions compatible for performing isothermal DNA amplification.

[0065] In certain exemplary embodiments, the method includes two steps. The first step includes forming a mixture. The mixture includes a double-stranded DNA template having a first strand and a second strand; a conformationaliy-constrained heiicase; a DNA-dependent DNA polymerase; a first oligonucleotide primer complementary to a portion of the first strand; a second oligonucleotide primer complementary to a portion of the second strand; and an amplification buffer cocktail. The second step includes incubating the mixture at a temperature compatible for activating the conformationaliy-constrained heiicase and DNA-dependent DNA polymerase.

[0066] In certain exemplary embodiments, the conformationaliy-constrained heiicase comprises SEQ ID NO:4 or 12. In certain exemplary embodiments, the DNA-dependent DNA polymerase is selected from a group consisting of E. coli DNA Pol I, E. coli DNA Pol I Large Fragment, Bst 2.0 DNA Polymerase, Bst DNA Polymerase, Bst DNA Polymerase Large Fragment, Bsu DNA Polymerase I Large Fragment, T4 DNA Polymerase, T7 DNA polymerase, PyroPhage® 3173 DNA Polymerase and phi29 DNA Polymerase.

[0067] In certain exemplary embodiments, the conformationaliy-constrained heiicase is chemically crossl inked.

[0068] In certain exemplary embodiments, the chemical crosslinker comprises a length in the range from about 6Å to about 25A.

[0069] In certain exemplary embodiments, the chemical crosslinker comprises an alkyl having a length in the range from C7 to C23 or from C8 to C13.

[0070] In another aspect, a kit for performing heiicase dependent amplification is provided. The kit includes a conformationaliy-constrained heiicase and amplification reagents (e.g., an amplification buffer cocktail).

[0071] In certain exemplary embodiments, the conformationaliy-constrained heiicase is

selected from SEQ ID NOs: 4 and 12.

[0072] In certain exemplary embodiments, the kit further comprising a DNA-dependent DNA polymerase, e.g., selected from a group consisting of E. coli DMA Pol I, E. coli DNA Pol I Large Fragment, Bst 2.0 DNA Polymerase, Bst DNA Polymerase, Bst DNA Polymerase Large Fragment, Bsu DNA Polymerase I Large Fragment, T4 DNA Polymerase, T7 DNA polymerase, PyroPhage® 3173 DNA Polymerase and phi29 DNA Polymerase.

[0073] In one aspect, an isolated nucleic acid encoding a modified helicase described herein is provided.

[0074] In certain exemplary embodiments, the isolated nucleic acid is selected from the group consisting of SEQ ID NOs: 2, 3, 10 and 11.

[0075] In one aspect, a modified *E coli.* Rep helicase comprising a first subdomain having a first amino acid, a second subdomain having a second amino acid, and an axis vector defined by the alpha carbon of ILE371 from which the vector originates and the alpha carbon of SER280 or the alpha carbon of ALA603, wherein theta is an angle of rotation of said first amino acid and said second amino acid around the axis vector is provided. A first theta between said first amino acid and said second amino acid is between about 60 degrees and about 155 degrees when the helicase is in an inactive conformation, and a second theta between said first amino acid and said second amino acid is between about 355 degrees and about 25 degrees when the helicase is in an active conformation. A side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conformationally-constrained helicase.

[0076] In certain exemplary embodiments, the first theta is about 133 degrees and/or the second theta is about 0 degrees.

[0077] In certain exemplary embodiments, the axis vector is defined by the alpha carbon of ILE371 and the alpha carbon of SER280.

[0078] In certain exemplary embodiments, the first amino acid is at any one of positions 84-108 or 169-187 or at position 178 of the modified helicase amino acid sequence. In certain exemplary embodiments, the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 13 or SEQ ID NO: 14. In certain exemplary embodiments, the first amino acid is at any one of positions 60-82 of the modified

helicase amino acid sequence. In certain exemplary embodiments, the first amino acid is at any one of positions 68-79 of the modified helicase amino acid sequence.

[0079] In certain exemplary embodiments, the second amino acid is at any one of positions 388-402, 422-435 or 519-531 or at position 400 of the modified helicase amino acid sequence. In certain exemplary embodiments, the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 15 or SEQ ID NO: 16. In certain exemplary embodiments, the second amino acid is at any one of positions 509-536 of the modified helicase amino acid sequence. In certain exemplary embodiments, the second amino acid is at any one of positions 519-525 of the modified helicase amino acid sequence.

[0080] These and other features, objects and advantages of the present invention will become better understood from the description that follows. In the description, reference is made to the accompanying drawings, which form a part hereof and in which there is shown by way of illustration, not limitation, embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0081] The foregoing and other features and advantages of the present invention will be more fully understood from the following detailed description of illustrative embodiments taken in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessaiy fee.

[0082] FIG. 1A depicts the closed form Rep crystal structure (PDB entry 1UAA), wherein subdomains are colored and named accordingly and 3' end of the ssDNA (gray) is visible. Residues that were mutated to cysteine and crosslinked to lock the conformation are shown as pink, blue and red van der Waals spheres in both conformations as reference. Boxed area is magnified view showing the two residues that were crosslinked for engineering Rep-X.

[0083] FIG. 1B depicts the open form Rep crystal structure (PDB entry 1UAA), wherein subdomains are colored and named accordingly and 3' end of the ssDNA (gray) is visible. Residues that were mutated to cysteine and crosslinked to lock the conformation are shown as pink, blue and red van der Waals

spheres in both conformations as reference. Boxed area is magnified view showing the two residues that were crosslinked for engineering Rep-Y.

[0084] FIG. 1C depicts a schematic showing that helicase-catalyzed unwinding of a DNA labeled with a donor and an acceptor would convert high FRET efficiency (£FRET) to IOWEFRET. Shading level of the donor and acceptor color represents the relative intensity changes. Figure discloses "(dT)io" as SEQ ID NO: 33.

[0085] FIG. 1D depicts an ensemble unwinding kinetics of DNA from FIG. 1C by Rep and Rep-X shows the enhanced helicase activity of Rep-X over Rep as measured via ensemble E_{FRET}- Solid lines are fitted exponential decay curves as guides to the eye.

[0086] FIG. 1E depicts exemplary data of ensemble unwinding kinetics of the Rep-Y, Rep-X and non-crosslinked Rep using an assay containing 10 nM helicase, 5 nM 50-bp ensemble unwinding DNA with 3'-$(dT)_{30}$(SEQ ID NO: 17) overhang in buffer D and 1 mM ATP).

[0087] FIG. 2A depicts a schematic of unwinding stages of dual labeled DNA by a Rep-X monomer. Color liglitness of the donor (green) and acceptor (red) on the DNA represents the change in the emission intensities as the unwinding progresses.

[0088] FIG. 2B depicts representative single molecule time traces show the DNA binding, unwinding and dissociation for the acceptor strand for Rep-X, wherein the donor fluorescence signal is in green, acceptor in red and J¾¾ET in blue.

[0089] FIG. 2C depicts representative single molecule time traces showing the DNA binding, unwinding and dissociation for the donor strand for Rep-X, wherein the donor fluorescence signal is in green, acceptor in red and E_{FRET} in blue. Unwinding period is denoted by *At.*

[0090] FIG. 2D depicts representative single molecule time traces showing the DNA binding and dissociation behavior for the donor strand for Rep, wherein the donor fluorescence signal is in green, acceptor in red and E_{FRET} in blue.

[0091] FIG. 2E depicts representative single molecule time traces showing the DNA binding and dissociation behavior for the donor strand for Rep-Y, wherein the donor fluorescence signal is in green, acceptor in red and J¾¾ET in blue.

[0092] FIG. 2F depicts a representative distribution of Rep-X unwinding period *At* .

[0093] **FIG. 2G** depicts fractions of DNA binding events that led to unwinding (i.e. exhibited an E_{FRET} increase phase) in smFRET experiments for Rep, Rep-Y and Rep-X. Error bars represent 95% confidence bounds.

[0094] **FIG. 3**A depicts a schematic of the optical tweezers assay depicts a Rep-X molecule tethered to the bead surface that just loaded on the free ssDNA overhang and started to unwind the 6-kbp DNA=.

[0095] FIG. 3B depicts unwinding traces showing the extent of processive unwinding by Rep-X on the 6-kbp DNA (colored according to conditions of overhang length, SSB and force, and offset for clarity). Background is color coordinated with the inset to show the two laminar flows.

[0096] FIG. 3C depicts an exemplary distribution of Rep-X unwinding velocities (N=38). Mean velocity of unwinding and the standard deviation for each molecule were plotted above (colors as in B). Figure discloses "(dT)i$_0$," "(dT)$_{15}$" and "(dT)$_{75}$" as SEQ ID NOS 33-35, respectively.

[0097] FIG. 3D depicts exemplar}' data comparing the fraction of the complete DNA binding events for Rep, Rep-Y and Rep-X. Error bars represent the 95% confidence bounds.

[0098] FIG. 3E depicts unwinding by five representative Rep-X molecules in the fixed trap assay are plotted. Pulling force increases during unwinding as the Rep-X pulls the beads closer. Tether breaks appear as sudden force drops.

[0099] FIG. 3F depicts exemplar}' data showing the average of normalized unwinding velocities of 58 Rep-X molecules plotted against the pulling force that shows the high force tolerance of the engineered super-helicase Rep-X. Error bars represent standard error of the mean.

[00100] **FIG. 4A** illustrates a consensus sequence alignment of TxGx motif for 27 organisms within 10 out 11 families, wherein Cys is present at position 96. Leuconostocaceae family species have an alanine at this position. Figure discloses SEQ ID NOS 109-142, respectively, in order of appearance.

[00101] FIG. 4B illustrates a consensus sequence alignment of motif III for 27 organisms within 10 families, wherein Cys is present at position 247. Leuconostocaceae family species have an alanine at this position. Figure discloses SEQ ID NOS 143-176, respectively, in order of appearance.

[00102] FIG. 5A depicts exemplar}' ATPase activity of mutant PcrA before ("PcrA") and after crosslinking ("PcrA-X"). Error bars represent standard deviation over multiple preparations.

[00103] **FIG.** SB depicts exemplar}' data of an ensemble unwinding assay for PcrA-X and wild type PcrA. Solid lines are fitted exponential decay curves as visual guides.

[00104] FIG. 6A depicts representative single molecule time traces for DNA binding and unwinding by PcrA-X monomers.

[00105] **FIG. 6B** depicts representative single molecule time traces for DNA binding and unwinding by t PcrA monomers, which are incapable of DNA unwinding.

[00106] FIG. 6C depicts exemplary data of fractions of enzyme-DNA binding events that led to an unwinding phase for PcrA and PcrA-X in the smFRET assay. Error bars represent the 95% confidence bounds

[00107] **FIG. 6D** depicts exemplary data showing processive unwinding of 6-kbp DNA by four representative PcrA-X molecules in the optical tweezers assay. Figure discloses "(dT)i$_5$" and "(dT)$_{75}$" as SEQ ID NOS 34 and 35, respectively.

[00108] **FIG. 6E** depicts exemplary data for fractions of enzyme-DNA binding that led to the unwinding of 6-kbp DNA in the optical tweezers assay. Error bars represent the 95% confidence bounds

[00109] FIG. 6F depicts a schematic (in subpanel (/)) ofthe conformational effect of RepD, a stimulatory partner of Per A, on PcrA as measured in a smFRET assay and £FRET histograms (sub-panel (*i*/)) showing that the PcrA bound to RepD adduct is biased toward the closed form (high E_{FRET} population) compared to PcrA bound to the bare *ori-D* DNA.

[00110] FIG. 7A shows an exemplary SDS-PAGE analysis of Rep-Y intra-crosslinking, wherein the typical three-band pattern on SDS polyacrvi amide gels is evident. Rep-X intra-crosslinking pattern is shown for comparison, wherein the dominant middle band is slightly shifted for Rep-X compared with the corresponding band for Rep-Y. Lane designated as Rep is non-crosslinked Rep.

[00111] FIG. **7B** shows an exemplary SDS-PAGE analysis of Rep-Y intra-crosslinking in comparison to uncrosslinked Rep ("Rep"). Lane denoted as Rep-Y* depicts β-ME reduced Rep-Y (crosslinked with a di-sulfide crosslinker DTME).

[00112] FIG. 7C shows an exemplary size exclusion chromatography (SEC) elution profile for Rep (dotted line) and the Rep-Y sample (solid line).

[00113] FIG. 7D shows an exemplary SDS-PAGE analysis of Rep-Y fractions, F1-F7, collected from SEC (FIG. 5C) in comparison with Rep-Y.

[00114] FIG. 7E depicts exemplary data of ssDNA dependent ATPase levels of Rep-Y and Rep. Error bars represent standard deviation over multiple preparations.

[00115] FIG. **8** depicts a schematic of an isothermal DNA amplification process called helicase dependent amplification, wherein in step 1: DNA helicase (104) contacts a double-stranded DNA **(101)** to unwind the first and second single strands (102 and **103)** and first and second oligonucleotide primers (105 and **106)** hybridize to the first and second single strands (102 and **103),** respectively; in step 2: DNA-dependent DNA polymerases (107) bind to the 3'-tennini of the first and second oligonucleotide primers (105 and **106)** to initiate chain elongation of new strands **(108** and 109); and in step 3: continued DNA polymerization results in DNA amplification and formation of new double-stranded DNA **(110** and 111).

[00116] **FIG.** 9A shows target residues in Rep (SEQ ID NO: 32), PerA (SEQ ID NO: 177) and UvrD (SEQ ID NO: 178), for - X fonn crosslinking, calculated basal on the criteria and ciystal structures in open (inactive) and closed (active) conformations. One residue is chosen from LA or IB domain (shaded), and another from 2B (shaded).

[00117] FIG. 9B shows 56 representative Rep homologs/orthologs with 90% identity to and 80% overlap, and the corresponding region of domain 1A where crosslinking residues can be chosen. Figures 9B-9C disclose SEQ ID NOS 179-235, respectively, in order of appearance.

[00118] FIG. 9C shows 56 representative Rep homologs/orthologs with 90% identity to and 80% overlap, and the corresponding region of domain IB where crosslinking residues can be chosen.

[00119] FIG. 9D shows 56 representative Rep homologs/orthologs with 90% identity to and 80% overlap, and the corresponding region of domain 2B where crosslinking residues can be chosen. Figures 9D-9F disclose SEQ ID NOS 236-292, respectively, in order of appearance.

[00120] FIG. 9E shows 56 representative Rep homologs/orthologs with 90% identity to and 80% overlap, and the corresponding region of domain 2B where crosslinking residues can be chosen in addition to those shown in FIG. 9D.

[00121] **FIG. 9F** shows 56 representative Rep homologs/orthologs with 90% identity to and 80% overlap, and the corresponding region of domain 2B where crosslinking residues can be chosen in addition to those shown in FIG. 9E.

[00122] FIG. 9G shows target residues in drUvrD, Rep, PcrA and UvrD, for - X form crosslinking, calculated based on the criteria and crystal structures in open (inactive) and closed (active) conformations. One residue is chosen from 1A or 1B domain (shaded), and another from 2B (shaded). Figure discloses SEQ ID NOS 293-304, respectively, in order of appearance.

[00123] **FIG. 10** shows the reaction of maleimide-activated compounds to sulfhydryl-bearing compounds.

[00124] FIG. 11 shows a closed form crystal structure of/A *radiodnransO\xD* (drUvrD; Q9RTJ9) with target crosslinking regions of domains 1A, IB and 2B indicated by arrows.

[00125] FIG. 12 shows selected target residue pairs for crosslinking, and the specific distances between the pairs, in a ribbon diagram of a structure of RecD2.

[00126] FIG. 13 is a ribbon diagram of a CsRecQ/DNA crystal structure.

[00127] FIG. **14** shows a schematic diagram of RecQ DNA helicase, and an overlay of RecQ structures which highlight the mobility of the WH domain.

[00128] FIG. 15 shows alternate ribbon diagrams of a RecQl crystal structure.

[00129] **FIG.** 16 shows a stereo view of a ribbon diagram of a 5-3' SF1 superhelicase (T4 Dda).

[00130] FIG. 17 shows Rep helicase' s 2B domain structure in two different orientations that differ through a rotation around an axis coming out of the plane of the paper. 2B domain orientation can be described by the rotation angle Θ with respect to the closed form. θ =0 when the helicase is in the closed form, and θ is 133 degrees when the 2B rotates to the open form.

DETAILED DESCRIPTION

[00131] The present disclosure provides details of the discovery of robust enzymes of the superfamily 1 helicases. The helicase enzymes are engineered as crosslinked, conformationally-constrained monomelic configurations providing enhanced unwinding activity on dsDNA substrates. The "super" helicases display inherently strong physical properties having superior characteristics to all presently known natural helicases. The disclosed helicases have utility in isothermal PCR and helicase-dependent amplification processes, as well as in next generation sequencing applications, including nanopore sequencing methods and the like.

Terminology and Definitions

[00132] The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. With respect to the use of plural and/or singular terms herein, those having skill in the art can translate from the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for the sake of clarity.

[00133] Terras used herein are intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.).

[00134] Furthermore, in those instances where a convention analogous to "at least one of A, **B** and C, etc." is used, in general such a construction is intended in the sense of one having ordinary skill in the art would understand the convention (e.g., "a system having at least one of A, B and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C togetlier, and/or A, B, and C togetlier). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description or figures, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or 'B or "A and B."

[00135] All language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can subsequently be broken down into sub-ranges.

[00136] A range includes each individual member. Thus, for example, a group having 1-3 members refers to groups having 1, 2, or 3 members. Similarly, a group having 1-6 members refers to groups having 1, 2, 3, 4, or 6 members, and so forth.

[00137] The modal verb "may" refers to the preferred use or selection of one or more options or choices among the several described embodiments or features contained within the same. Where no options or choices are disclosed regarding a particular embodiment or feature contained in the same, the modal verb "may" refers to an affirmative act regarding how to make or use and aspect of a described embodiment or feature contained in the same, or a definitive decision to use a specific skill regarding a described embodiment or feature contained in the same. In this latter context, the modal verb "may" has the same meaning and connotation as the auxiliary verb "can."

[00138] The present invention provides modified helicases that have enhanced enzymatic activity. As used herein, a "helicase" refers to a class of enzymes that function as motor proteins which move directionally along a nucleic acid phosphodiester backbone, separating two annealed nucleic acid strands (i.e., DNA, UNA, or RNA-DNA hybrid) using energy derived from ATP hydrolysis. Helicases are often used to separate strands of a DNA double helix or a self-annealed RNA molecule using the energy from ATP hydrolysis, a process characterized by the breaking of hydrogen bonds between annealed nucleotide

bases. They also function to remove nucleic acid-associated proteins and catalyze homologous DNA recombination. Metabolic processes of RNA such as translation, transcription, ribosome biogenesis, RNA splicing, RNA transport, RNA editing, and RNA degradation are all facilitated by helicases. Helicases move incrementally along one nucleic acid strand of the duplex with a directionality and processivity specific to each particular enzyme.

[00139] Six super families ofhelicases are known in the art that are classified based on their shared sequence motifs. Helicases not forming a ring structure are classified in Super Families 1 **(SF1**) and 2 **(SF2).** Ring-forming helicases form Super Families 3 (SF3), 4 (SF4), 5 (SF5) and 6 (SF6).

[00140] SF1 is further subdivided into SF1A and SF1B helicases. In this group, helicases can have either **3'-5'** (SF1 A subfamily) or 5'-3'(SFlB subfamily) translocation polarity. SF1 A helicases include, but are not limited to are Rep and UvrD in gram-negative bacteria and PcrA heiicase from gram-positive bacteria. SF1B helicases include, but are not limited to RecD and Dda helicases.

[00141] SF2 is the largest family ofhelicases, which are involved in varied cellular processes. They are characterized by the presence of nine conserved motifs: Q, I, Ia, lb, and II through **VI.** This family primarily comprises DEAD-box RNA helicases ("DEAD" disclosed as SEQ ID NO: 18). Other helicases in SF2 family are the RecQ-like family and **Snf2-like** enzymes. Most of the SF2 helicases are type A, with a few exceptions such as the XPD family.

[00142] SF3 comprises helicases encoded mainly by small DNA viruses and some large nucleocytoplasmic DNA vimses. They have a 3'-5' translocation directionality (therefore they are all type A helicases). SF3 heiicase include viral helicases such as the papilloma vims E **1** heiicase.

[00143] SF4 helicases have a type B polarity (5'-3'), and function in bacterial or bacteriophage DNA replication. Gp4 from bacteriophage ***11*** is an SF4 heiicase.

[00144] SF5 helicases have a type B polarity (5'-3'), and include only the bacterial termination factors Rho.

[00145] SF6 helicases contain the core AAA+ that is not included in the SF3 classification. SF6 helicases include, but are not limited to. Mini Chromosome Maintenance (MCM), RuvB, RuvA, and RuvC.

[00146] Exemplary helicases according to the invention include, but are not limited to RecD, Upfl, PcrA, Rep, UvrD, Hel308, Mtr4, XPD, NS3, Mssl 16, Prp43, Recti, RecQ, TIR, RapA, Hef , RecB, Pifl , Dna2, Dda, U15, RecD2, Tral, Senlp, SETX, **IBP** 160, ZNFX1, Upflp, UPF1, Hcslp, IGHMBP2, **Dna2p, DNA2,** Mttl p, MO**VI**0, MO**VI**0L1, HELZ, PR285, ptMRDFLl and the like.

[00147] In certain embodiments of the invention, a helicase comprises subdomains. For example, SF1 helicases comprise subdomains 1A, IB, 2A and 2B. The 2B subdomain has been shown to rotate between an open conformation and a closed conformation.

[00148] As used herein, an "open conformation" refers to the inactive conformation of a helicase in which minimal or no helicase activity occurs. As used herein, a "closed conformation" refers to the active form of a helicase which has helicase activity. Crystal structures depicting the open and/or closed conformations of many helicases have been published in the art.

[00149] As described further herein, it has been discovered that, by stabilizing the active (i.e., closed) confomiation and destabilizing the inactive (i.e., open) conformation, a modified helicase can be obtained having greatly enhanced helicase activity and strength relative to the corresponding unmodified helicase. According to certain embodiments ofthe invention, a modified helicase that stabilizes the active (i.e., closed) conformation and destabilizes the inactive (i.e., open) conformation can be generated by covalently linking one or more amino acids in the 2B subdomain to one or more amino acids in the LA and/or the IB domain ofthe helicase. Such a modified helicase is refeired to herein as an "active, conformational ly constrained helicase" or a "helicase-χ polypeptide." Exemplary helicase-χ polypeptides include, but are not limited to, Rep-x, PcrA-χ and UvrD-χ. In certain embodiments, a helicase-x polypeptide forms a loop around a target nucleic acid sequence (e.g., a DNA sequence). In other embodiments, a helicase-χ polypeptide does not form a loop around a target nucleic acid sequence (e.g., a DNA sequence).

[00150] In other embodiments, a helicase is provided that is stabilized in its inactive (i.e., open) conformation and destabilized in its active (i.e., closed) conformation. Such a helicase is referred to as an "inactive, conformationally constrained helicase" or a "helicase-γ polypeptide." Heiicase-γ polypeptides exhibit little or no helicase activity.

[00151] In certain embodiments, a helicase-x polypeptide has an increased nucleic acid (e.g., DNA) unwinding activity relative to a corresponding unmodified helicase. In certain aspects, the number of base pairs that can be unwound by a helicase-x polypeptide is increased by about 1000%, about 10,000%, about 100,000% or more (or any ranges or points within the ranges) relative to a corresponding unmodified helicase.

[00152] In certain embodiments, a helicase-χ polypeptide can unwind at least about 500 base pairs, about 1000 base pairs, about 1500 base pairs, about 2000 base pairs, about 2500 base pairs, about 3000 base

pairs, about 3500 base pairs, about 4000 base pairs, about 4500 base pairs, about 5000 base pairs, about 5500 base pairs, about 6000 base pairs, about 6500 base pairs, about 7000 base pairs, about 7500 base pairs, about 8000 base pairs, about 8500 base pairs, about 9000 base pairs, about 9500 base pairs, about 10,000 base pairs or more (or any ranges or points within the ranges) without dissociating from the nucleic acid sequence (e.g., DNA).

[00153] In certain embodiments, a helicase-χ polypeptide is stronger that the corresponding unmodified helicase, withstanding opposing forces of at least about 10 pN, about 15 pN, about 20 pN, about 25 pN, about 30 pN, about 35 pN, about 40 pN, about 45 pN, about 50 pN, about 55 pN, about 60 pN, or more (or any ranges or points within the ranges).

[00154] In certain embodiments, a helicase-χ polypeptide comprises a first subdomain comprising a first amino acid and a second subdomain comprising a second amino acid, wherein the first amino acid is at least about 35 Å from the second amino acid when the helicase is in an inactive conformation, and wherein the first amino acid is less than about 25 Afrom the second amino acid when the helicase is in an active conformation. In certain embodiments, the first amino acid is at least about 40 A, about 45A, about 50 A, about 55 Å, about 60 A, about 65 Å, about 70 A, about 75 A, about 80 A, about 85 A, or more from the second amino acid (or any ranges or points within these ranges) when the helicase is in an inactive (i.e., open) conformation. In certain embodiments, the first amino acid is at most about 20 A, about 15A, about 10 A, about 9 A, about 8 A, about 7 Å, about 6 A, about 5 A, about 4 A, or less from the second amino acid (or any ranges or points within the ranges) when the helicase is in an active (i.e., closed) conformation. In certain embodiments, the linker in a heiicasex polypeptide has a length in the range from about 6Ato about 25A.

[00155] In certain embodiments, the first amino acid of a helicase-χ polypeptide is present in a 1A or a 1B subdomain and the second amino acid of a heiicasex polypeptide is present in a 2B subdomain.

[00156] In certain embodiments, the Rep-χ polypeptide forms a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of a Rep-χ polypeptide that forms a loop is at any one of positions 84-108 or 169-187, or at position 178 of the Rep amino acid sequence. In certain embodiments, the second amino acid of a Repx polypeptide that forms a loop is at any one of positions 388-402, 422-435 or 519-536, or at position 400 of the Rep amino acid sequence.

[00157] In certain embodiments, the PcrA-χ polypeptide forms a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of a PcrA-$_x$ polypeptide that forms a loop

is at any one of positions 92-116 or 178-196, or at position 187 of the PcrA amino acid sequence. In certain embodiments, the second amino acid of a PcrA-χ polypeptide that forms a loop is at any one of positions 397-411, 431-444 or 526-540, or at position 409 of the PcrA amino acid sequence.

[00158] In certain embodiments, the UvrD-χ polypeptide forms a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of a UvrD-χ polypeptide that forms a loop is at any one of positions 90-114 or 175-193 of the UvrD amino acid sequence. In certain embodiments, the second amino acid of a UvrD-χ polypeptide that forms a loop is at any one of positions 393-407, 427-440 or 523-540 of the UvrD amino acid sequence.

[00159] In certain embodiments, the Rep-χ polypeptide does not form a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of the Rep-χ polypeptide that does not form a loop is at any one of positions 60-82 (i.e., at any one of AREMKERVGQTLGRKEARGLMIS (SEQ ID NO: 19)), or at any one of positions 68-79 (i.e., at any one of GQTLGRKEARGL (SEQ ID NO: 20)) of the Rep amino acid sequence. In certain embodiments, the second amino acid of the Rep-χ polypeptide that does not form a loop is at any one of positions 509-536 (i.e., at any one of FSWMTEMLEGSELDEPMTLTQ VVTRFTL (SEQ ID NO: 21)), or at any one of positions 519-525 (i.e., at any one of SELDEPM (SEQ ID NO: 22)) of the Rep amino acid sequence.

[00160] In certain embodiments, the PcrA-χ polypeptide does not form a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of the PcrA-χ polypeptide that does not form a loop is at any one of positions 69-89 (i.e., at any one of **AREMRERVQSLLGGAAEDVWI** (SEQ ID NO: 23)), or at any one of positions 77-87 (i.e., at any one of QSLLGGAAEDV (SEQ ID NO: 24)) of the PcrA amino acid sequence. In certain embodiments, the second amino acid of the PcrA-χ polypeptide that does not form a loop is at any one of positions 516-534 (i.e., at any one of LSVTKHFENVSDDKSLIAF (SEQ ID NO: 25)), or at any one of positions 526-532 (i.e., at any one of SDDKSLI (SEQ ID NO: 26)) of the PcrA amino acid sequence.

[00161] In certain embodiments, the UvrD-χ polypeptide does not form a loop around the target nucleic acid (e.g., DNA) sequence. In certain embodiments, the first amino acid of the UvrD-χ polypeptide that does not form a loop is at any one of positions 67-87 (i.e., at any one of AAEMRHRIGQLMGTSQGGMWV (SEQ ID NO: 27)), or at any one of positions 75-85 (i.e., at any one of GQLMGTSQGGM (SEQ ID NO: 28)) of the UvrD amino acid sequence. In certain

embodiments, the second amino acid of the UvrD-χ polypeptide that does not form a loop is at any one of positions 513-531 (i.e., at any one of **VTATRQF**SYNEEDEDLMPL (SEQ ID NO: 29)), or at any one of positions 523-529 (i.e., at any one of **EEDEDLM** (SEQ ID NO: 30)) of the UvrD amino acid sequence.

[00162] In certain embodiments, the first amino acid and/or the second amino acid of a helicase-χ polypeptide is present in a particular amino acid sequence having at least about 15%, about 20 %, about 25 %, about 30 %, about 35 %, about 40 %, about 45 %, about 50%, about 55%, about 60%, about 65%, about 70%, about 75% about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% or more sequence identity to that of a reference sequence (e.g., a Rep helicase, A PerA helicase, a UvrD helicase, or a homolog or ortholog thereof).

[00163] In certain embodiments, the first amino acid is present in a Rep helicase at an amino acid sequence having at least about 15%, about 20 %, about 25 %, about 30 %, about 35 %, about 40 %, about 45 %, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80 %, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% or more amino acid sequence identity (or any ranges or points within the ranges) to FHTLGLDDKREYAALGMKANFSLF (SEQ ID NO: 13). In certain embodiments, the first amino acid is present in a Rep helicase at an amino acid sequence having at least about 15%, about 20 %, about 25 %, about 30 %, about 35 %, about 40 %, about 45 %, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% or more amino acid sequence identity (or any ranges or points within the ranges) to **GLYDAHLKACNVLDFDDLI (SEQ ID NO: 14).**

[00164] In certain embodiments, the second amino acid is present in a Rep helicase at an amino acid sequence having at least about 15%, about 20 %, about 25 %, about 30 %, about 35 %, about 40 %, about 45 %, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80 %, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% amino acid sequence identity (or any ranges or points within the ranges) to AYLRVLTNPDDDSAF (SEQ ID **NO: 15**). In certain embodiments, the second amino acid is present in a Rep helicase at an amino acid sequence having at least about 15%, about 20 %, about 25 %, about 30

%>, about 35 %, about 40 %, about 45 %, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% amino acid sequence identity (or any ranges or points within the ranges) to GEWAMTRNKSMFTA (SEQ ID NO:16).

[00165J Suitable amino acid positions for modifying to engineer helicase-χ polypeptides (and homologs and orthologs thereof) according to the invention can be identified by one of ordinary skill in the art using this disclosure and well-known local sequence alignment tools.

[00166] Techniques for determining nucleic acid and amino acid "sequence identity" are known in the art. Typically, such techniques include determining the nucleotide sequence of genomic DNA, mRNA or cDNA made from an mRNA for a gene and/or determining the amino acid sequence that it encodes, and comparing one or both of these sequences to a second nucleotide or amino acid sequence, as appropriate. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100.

[00167] An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Watemian, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppi. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and nonnalized by Gribskov (1986) Nucl. Acids Res. 14:6745. An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, Wis.) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, Wis.).

[00168] One method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John **F.** Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, Calif). **From** this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six).

From the data generated the "match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code= standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; **sort by** .dbd.HlGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+Swiss protein+Spupdate+PIR. Details of these programs can be found at the NCBFNLM web site.

[00169] In certain embodiments of the invention, a helicase is provided that is conformationally-constrained. The term "conformationally-constTained" refers to a conformation having **a** least one degree of freedom (that is, motion or range of motion) that is less than a reference conformation. In certain embodiments, a conformationally-constrained helicase has a least one degree of freedom that is less than a helicase that is not conformationally constrained.

[00170] In certain embodiments of the invention, **a** helicase is constrained via a covalent linkage between two or more amino acids of the helicase. **A** covalent linkage is a chemical linkage between **two** atoms or radicals formed by the sharing **of a** pair of electrons (i.e., a single bond), two pairs of electrons (i.e., a double bond) or three pairs of electrons (i.e., a triple bond). Covalent linkages are also known in the art as electron pair interactions or electron pair bonds.

[00171] In certain embodiments, a covalent linkage is formed via a crosslink between the side chains of two (or more) amino acids of a polypeptide (e.g., between two (or more) amino acids of a modified helicase).

[00172] As used herein the term "crosslink" refers to the joining of two or more molecules by a covalent bond. Crosslinking can occur via disulfide bonds, e.g., between cysteine residues. Crosslinking can occur via the use of crosslinking reagents (or chemical crosslinkers), which are molecules that contain two or more reactive ends capable of chemically attaching to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules.

[00173] The terms "intramolecular crosslinking agent" and "chemical crosslinking agent" refer to a compound that can form covalent bonds via specific functional groups (e.g., primary amines, sulfhydryls, etc.) on proteins or other molecules. An example of an intramolecular or chemical crosslinking agent includes a compound having two bifunctional groups in its structure.

[00174] Chemical crosslinkers are known in the art, and are commercially available (e.g., from Thermo Fisher Scientific, Waltham, **MA).** In certain embodiments, a crosslinker is cieavable (e.g., by reducing one or more of the functional groups of the crosslinker). In other embodiments, a crosslinker is not cieavable.

[00175] Examples of chemical crosslinkers include, but are not limited to, those having the following functional groups: maleimide, active esters, succinimide, azide, alkyne (such as dibenzocyclooctynol (DIBO or DBCO), difluoro cycloalkynes and linear alkynes), phosphine (such as those used in traceless and non- traceless Staudinger ligations), haloacetyl (such as iodoacetamide), phosgene type reagents, sulfonyl chloride reagents, isothiocyanates, acyl halides, hydrazines, disulphides, vinyl sulfones, aziridines and photoreactive reagents (such as aryl azides, diaziridines). Reactions between amino acids and functional groups may be spontaneous, such as cysteine/maleimide, or may require external reagents, such as Cu(I) for linking azide and linear alkynes.

[00176] Linkers can comprise any molecule that stretches across the distance required. Linkers can vary in length from one carbon (phosgene-type linkers) to many Angstroms. In certain embodiments, the linker includes an alky! having a length in the range from C_7 to C23. In some embodiments, the linker includes an alkyl having a length in the range from C_8 to C13.

[00177] Examples of linear molecules include but are not limited to, polyethyleneglycols (PEGs), polypeptides, polysaccharides, deoxyribonucleic acid (DNA), peptide nucleic acid (PNA), threose nucleic acid (TNA), glycerol nucleic acid (GNA), saturated and unsaturated hydrocarbons, and polyamides. These linkers may be inert or reactive, in particular they may be chemically cieavable at a defined position, or may be themselves modified with a ligand. In certain embodiments, the linker is resistant to dithiothreitol (DTT).

[00178] Examples of crosslinkers include, but are not limited to 2,5-dioxopyrrolidin-l-yl 3-(pyridin-2-yldisulfanyl)propanoate, 2,5-dioxopyrrolidin-l-yl 4-(pyridin-2-yldisulfanyl)butanoate and 2,5-dioxopyrrolidin-l-yl 8-(pwdin-2-yidisulfanyi)octananoate, di-maleimide PEG Ik, di-maleimide PEG 3.4k, di-maleimide **PEG** 5k, di-maleimide **PEG** 10k, bis(maleimido)etliane (BMOE), bis-maleimidohexane **(BMH),** 1,4-bis-maleimidobutane (BMB), 1,4 bis-maleimidyl-2,3- dihydroxybutane (BMDB), BM[PEO]2 (1,8-bis-maleimidodiethyieneglycol), BM[PEO]3 (1, 11-bis-maleimidotriethylene glycol), tris[2~maleimidoethyl]amine **(TMEA),** dithiobismaleimidoethane (DIME), bis-maleimide PEG3, bis-maleimide PEGU, DBCO-maleimide, DBCO-PEG4-maleimide,

DBCO-PEG4-NH2, DBCO-PEG4-NHS, DBCO-NHS, DBCO-PEG- DBCO 2.8kD& DBCO-PEG-DBCO 4.0kDa, DBCO- 15 atoms-DBCO, DBCO-26 atoms-DBCO, DBCO-35 atoms-DBCO, DBCO-PEG4-S-S-PEG3-biotin, DBCO-S-S-PEG3-biotin, DBCO-S-S- PEG! 1-biotin and (succinimidyl 3-(2-p\ridyldithio)propionate (SPDP).

[00179] In certain embodiments, a covalent linkage refers to the linkage between two or more amino acids. One or more of the linked amino acids may be naturally occurring or non-natural!y occurring. One or more of the linked amino acids may be chemically modified.

[00180] As used herein, a "natural amino acid" refers to the twenty genetically encoded alpha-amino acids. See, e.g., Biochemistry by L. Stryer, 3rd ed. 1988, Freeman and Company, New York, for structures of the twenty natural amino acids.

[00181] As used herein, an "unnatural amino acid," "modified amino acid" or "chemically modified amino acid" refers to any amino acid, modified amino acid, or amino acid analogue other than the twenty genetically encoded alpha-amino acids. Unnatural amino acids have side chain groups that distinguish them from the natural amino acids, although unnatural amino acids can be naturally occurring compounds other than the twenty proteinogenic alpha-amino acids. In addition to side chain groups that distinguish them from the natural amino acids, unnatural amino acids may have an extended backbone such asbeta-amino acids.

[00182] Non-limiting examples of unnatural amino acids include selenocysteine, pyrrolysine, homocysteine, an O-methyl-L-tyrosine, anL-3-(2-naphthyl)alanine, a 3-methyl-phenylalanine, an 0-4-allyl-L-tyrosine, a 4-propyl-L-tyrosine, a tri-0-acetyl-GlcNAc(5-serine, an L-Dopa, a fluorinated phenylalanine, an isopropyl-L-phenylalanine, a p-azido-L-phenylalanine, a p-acyl-L-phenylalanine, a p-benzoyl-L-phenylalanine, an L-phosphoserine, aphosphonoserine, aphosphonotyrosine, ap-iodo-phenylalanine, a p-bromophenylalanine, ap-amiiio-L-phenylalanine, an isopropyl-L-phenylalanine, an unnatural analogue of a tyrosine amino acid; an unnatural analogue of a glutamine amino acid; an unnatural analogue of a phenylalanine amino acid; an unnatural analogue of a serine amino acid; an unnatural analogue of a threonine amino acid; an alkyl, aryl, acyl, azido, cyano, halo, hydrazine, hydrazide, hydroxy!, alkenyl, alkynl, ether, thiol, sulfonyl, seleno, ester, thioacid, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, hydroxy!amine, keto, or amino substituted amino acid, or any combination thereof; an amino acid with a photoactivatable cross-linker; a spin-labeled amino acid; a fluorescent amino acid; an amino acid with a novel functional group; an amino

acid that covalently or noncovalently interacts with another molecule; a metal binding amino acid; a metal-containing amino acid; a radioactive amino acid; aphotocaged and/or photoisomerizable amino acid; a biotin or biotin-analogue containing amino acid; a glycosylated or carbohydrate modified amino acid; a keto containing amino acid; amino acids comprising polyethylene glycol or polyether; a heavy atom substituted amino acid; a chemically cleavable or photocleavable amino acid; an amino acid with an elongated side chain; an amino acid containing a toxic group; a sugar substituted amino acid, e.g., a sugar substituted serine or the like; a carbon-linked sugar-∞ ntaining amino acid; a redox-active amino acid; an cx-hydroxy containing acid; an amino thio acid containing amino acid; an α,α disubstituted amino acid; a β-amino acid; and a cyclic amino acid other than proline. In an embodiment of the helicases described herein, one or more amino acids of the helicase are substituted with one or more unnatural amino acids and/or one or more natural amino acids.

[00183] In certain embodiments, a helicase-χ is a closed form, conformationally-constrained helicase monomer generated from a helicase polypeptide that was reacted with an intramolecular crosslinMng agent. In certain embodiments, a helicase-γ is an open form, conformationally-constrained helicase monomer generated from a helicase polypeptide that was reacted with an intramolecular crosslinMng agent.

[00184] The chemical structures described herein are named according to IUPAC nomenclature rules and include art-accepted common names and abbreviations where appropriate. The IUPAC nomenclature can be derived with chemical structure drawing software programs, such as ChemDraw ® (PerkinElmer, Lnc.), ChemDoodle* (iChemLabs, LLC) and Marvin (ChemAxon Ltd.). The chemical structure controls in the disclosure to the extent that an IUPAC name is misnamed or otherwise conflicts with the chemical stmcture disclosed herein. *li coli* Rep mutants can be engineered that are intramolecularly crosslinked to constrain the 2B subdomain in open or closed conformations. Residues for the cysteine substitution mutagenesis and the length of the bis-maleimide crosslinkers were selected such that when crosslinked, the 2B subdomain cannot rotate appreciably, effectively locking the protein in one conformation (FIG. 1A, B). The *closed* form of a helicase that is crosslinked in a constrained conformation is denoted with the suffix "-X", and the *open* form of a helicase that is crosslinked in a constrained conformation is denoted with the suffix "-Y." For Rep, Rep-X and Rep-Y represent the confonnationally-coiistrained closed and open forms, respectively Enzymatic activities of Rep-X and Rep-Y monomers were studied

in single molecule and ensemble assays employing fluorescence resonance energy transfer (FRET), total internal reflection fluorescence (TIRF) microscopy, and optical tweezers force spectroscopy.

[00185] The Rep mutant sequences used to generate Rep-X and Rep-Y include those nucleotide and amino acid sequences identified in Table 1.

Table 1. Amino Acid and Nucleotide Sequences for exemplary Rep-X and Rep-Y proteins

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
Wild type Rep helicase (gene sequence) >gi|556503834:3960677-3962698 Escherichia coli str. K-12 substr. MG1655, complete genome (SEQ ID NO: 31)	ATGCGTCTAAACCCCGGCCAACAACAAGCTGTCGAATTCGTT ACCGGCCXXTGCCTGGTGCTGGCGGGCGCGGGTTCCGGTAAA ACTCGTGTTATCACCAATAAAATCGCCCATCTGATCCGCGGTT GCGGTTATCAGGCGCGGCACATTGCGGCGGTOACCTTTACTA ATAAAGCAGCGCGCGAGATGAAAGAGCGTGTAGGGCAGACG CTGGGGCGCAAAGAGGCGCGTGGGCTGATGATCTCCACTTTC CATACGTTGGGGCTGGATATCATCAAACGCGAGTATGCGGCG CTTGGGATGAAAGCGAACTTCTCGTTGTTTGACGATACCGATC AGCTTGCTTTGCTTAAAGAGTTGACCGAGGGGCTGATTGAAG ATGACAAAGTTCTCCTGCAACAACTGATTTCGACCATCTCTAA CTGGAAGAATGATCTCAAAACACCGTCCCAGGCGGCAGCAAG TGCGATTGGCGAGCGGGACCGTATTTTTGCCCATTGTTATGGG CTGTATGATGCACACCTGAAAGCCTGTAACGTTCTCGACTTCG ATGATCTGATTTTATTGCCGACGTTGCTGCTGCAACGCAATGA AGAAGTCCGCAAGCGCTGGCAGAACAAAATTCGCTATCTGCT GGTGGATGAGTATCAGGACACCAACACCAGCCAGTATGAGCT GGTGAAACTGCTGGTTGCCGCGCGCGCTTTACCGTGG GGGTGACGATGACCAGTCGATCTACTCCTGGCGCGGTGCACG TCCGCAAAACCTGGTGCTGCTGAGTCAGGATTTTCCGGCGCTG AAGGTGATTAAGCTTGAGCAGAACTATCGCTCTTCCGGGCGT ATTCTGAAAGCGGCGAACATCCTGATCGCCAATAACCCGCAC GTCTTTGAAAAGCGTCTGTTCTCCGAACTGGGTTATGGCGCGG

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	AGCTAAAAGTATTAAGCGCGAATAACGAAGAACATGAGGCTG AGCGCGTTACTGGCGAGCTGATCGCCCATCACTTCGTCAATAA AACGCAGTACAAAGATTACGCCATTCTTTATCGCGGTAACCAT CAGTCGCGGGl'GTTTGAAAAATTCCTCATGCAAAACCGCATC CCGTACAAAATATCTGGTGGTACGTCGTTTTTCTCTCGTCCTG AAATCAAGGACTTGCTGGCTTATCT^ GCGTGCTGACTAACCC GGACGATGACAGCGCATTTCTGCGTATCGTTAACACGCCGAA GCGAGAGATTGGCCCGGCTACGCTGAAAAAGCTGGGTGAGTG GGCGATGACGCGCAATAAAAGCATGTTTACCGCCAGCTTTGA TATGGGCCTGAGTCAGACGCTTAGCGGACGTGGTTATGAAGC ATTGACCCGCTTCACTCACTGGTTGGCAGAAATCCAGCGTCTG GCGGAGCGGGAGCCGATTGCCGCGGTGCGTGATCTGATCCAT GGCATGGATTATGAATCCTGGCTGTACGAAACATCGCCCAGC CCGAAAGCCGCCGAAATGCGCATGAAGAACGTCAACCAACTG TTTAGCTGGATGACGGAGATGCTGGAAGGCAGTGA ACTGGAT GAGCCGATGACGCTCACCCAGGTGGTGACGCGCTTTACTTTGC GCGACATGATGGAGCGTGGTGAGAGTGAAGAAGAGCTGGATC AGGTGCAACTGATGACTCTCCACGCGTCGAAAGGGCTGGAGT TTCCTTATGTCTACATGGTCGGTATGGAAGAAGGGTTTTTGCC GCACCAGAGCAGCATCGATGAAGAT 'AAT'ATCGATGAGGAGCG GCGGCTGGCCTATGTCGGCATTACCCGCGCCCAGAAGGAATT GACCTTTACGCTGTGTAAAGAACGCCGTCAGTACGGCGAACT GGTGCGCCCGGAGCCGAGCCGCTTTTTGCTGGAGCTGCCGCA CKMTGATCTGATTTGGG/VACAGGACGCAAAGTGGTCAGCGC CGAAGAACGGATGCAGAAAGGGCAAAGCCATCTGGCGAATCT GAAAGCGA 'TGATCXJCGGCAAAACGAGGGAAA TAA
Wild type Rep helicase (amino acid sequence)	MRLNPGQQQAVEFVTGPCLVLAGAGSGKTRVlŒ KIAHLIRGCG YOARHIAAVTFrNKAAREMKERVGQTLGRKEARGLMISTFHTLG

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) **N → C** (amino acid sequence)
>gi|48994965|gb|AAT48209.1 DNA helicase and single-stranded DNA-dependent ATPase [Escherichia coli str. K-12 substr. MG1655] (SEQIDNO: 32)	LDnKREYAALGMKANFSLFDDTDQ LALLKELTEGLIEDDKVLLQ QLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKAC **NVLDFDDLDJ-PTLIXQRNEEWKRWQISIKIRYJ^** VDEYQDTNTS QYE^TO.LVGSRARFTVVGDDDQSiYSWRGARPQNLVLLSQDFP **ALKV1KLEQNYRSSGRILKAANILIANNPHVFEKRLFSELGYGAEL** K\XSANNEEHEAERWGELIAHHFVNKTQYKDYA1LYRGNHQSR **VFEKFLMONR1PYKISGGTSFFSRPE1KDLLAYLRVLTNPDDDSAF** **LRIVNnTKREIGPATLKKLGEWAMITlNKSMFTASFD** MGLSQTLS **GRGYEAL'rRPlHWLAElQRLAEREPlAAVRDLIHGMDYESWLYE** TSP SPK AAEM RM KNY'NQLF SWMTEMI_EG SELD EPMTLTQV VTR **FT'LRDMMERGESEEELDQVQLMI'LHASKGLEFPYVYMVGMEEG** **FLPHQSSIDEDNIDEERRLAYVGITRAQKELTFTLCKERRQYGELV** IlPEPSlIFLLELPQDDLI\^QERKVVSAEERMQKGQSHLANLKy\M **MAAKRGK**
Rep-x polypeptide[1] (SEQIDNO:!)	**MRLNPGQQi)AVEFVTGPLLVLAGAGSGKTRVITNKIAHLIRGSG** YQARHIAAVTFTNKAAREMKERVGQTLGRKEARGT MTSTFI-ITLG **LD11KREYAALGMKANFSLFDDTDQLALLKELTEGL1EDDKVLLQ** QUSTISNWKNDLXTPSQAAASAIGERDRffAII\'TGLYDAIO<:AC **'NVLDFDDLILLPTLLLQRNEEVRKRVV'QNKIRYLLVDEYQDTNTS** **QYELVKLLVGSRARFTVVGDDDQS1YSWRGARPQNLVLLSODFP** ,ALKVIKLEQNYRSSGMLKy\ANlLIANNPHVFEKRLFSELGYGAEL K\l.SANNEEIiEAER\TGELLAHFIF\NKTQYKDYAILYRGNHQSR YTEKFLMQNRIPYKISGGTSFFSRPEIKDLLAYLRVLTNPDDDCAF LRI\'mPKREIGPATLKKLGEW^rTRNKS^IFTASFDMGLSQTLS GRGYE/\LTIlFTHWLAEIQRLAEREPIAy\Y¾DLIHG^lDYESWLYE **TSPSPKAAEMRMKN^'NQLJSWMTEMLEGSELDEPMTLTQVXTR** FIXRDM^mGESEEELDQVQLWLHASKGLEFPYA^MVGMEEG **FLPHQSS1DEDN1DEERRLAYVG1TRAQKELTFTLAKERRQYGELV**

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	RPEPSRFLI£ LPQDDLIWEQERK VV¾AEERMQKGQSII iLANLKAM MAAKRGK
Rep-xDNA [2] (SEQK)NO:2)	ATGCGTCTAAACCCCGGCCAACAACAAGCTGTCGAATTCGTT ACCGGCCCCTTGCTGGTGCTGGCGGGCGCGGGTTCCGGTAAA ACTCGTGTTATCACCAATAAAATCGCCCATCTGATCCGCGGTA GCGGGTACCAGGCGCGGCACATTGCGGCGGTGACCTTTACTA ATAAAGCAGCGCGCGAGATGAAAGAGCGTGTAGGGCAGACG CTGGGGCGCAAAGAGGCGCGTGGGCTGATGATCTCCACTTTC CATACGTTGGGGCTGGATATCATCAAACGCGAGTATGCGGCG CTTGGGATGAAAGCGAACTTCTCGTTGTTTGACGATACCGATC AGCTTGCTTTGCTTAAAGAGTTGACCGAGGGGCTGATTGAAG ATGACAAAGTTCTCCTGCAACAACTGATTTCGACCATCTCTAA CTGGAAGAATGATCTCAAAACACCGTCCCAGGCGGCAGCAAG TGCGATTGGCGAGCGGGACCGTATITTTGCCCATGTTTATGGG CTGTATGATGCACACCTGAAAGCCTGTAACGTTCTCGACTTCG ATGATCTGATTTTATTGCCGACGTTGCTGCTGCAACGCAATGA AGAAGTCCGCAAGCGCTGGCAGAACAAAATTCGCTATCTGCT GGTGGATGAGTATCAGGACACCAACACCAGCCAGTATGAGCT GGTGAAACTGCTGGTGGGCAGCCGCGCGCGCTTTACCGTGGT GGGTGACGATGACCAGTCGATCTACTCCTGGCGCGGTGCACG TCCGCAAAACCTGGTGCTGCTGAGTCAGGATTTTCCGGCGCTG AAGGTGATTAAGCTTGAGCAGAACTATCGCTCTTCCGGGCGT ATrCTGAAAGCGGCGAACATCCTGATCGCCAATAACCCGCAC GTCTTTGAAAAGCGTCTGTTCTCCGAACTGGGTTATGGCGCGG AGCTAAAAGTATrAAGCGCGAATAACGAAGAACATGAGGCTG AGCGCGTTACTGGCGAGCTGATCGCCCATCACTTCGTCAATAA AACGCAGTACAAAGATTACGCCATTCTTTATCGCGGTAACCAT CAGTCGCGGGTGTTTGAAAAATTCCTGATGCAAAACCGCATC

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) **N → C** (amino acid sequence)
	CCGTACAAAATATCTGGTGGTACGTCGTTTTTCTCTCGTCCTG AAATCAAGGACTTGCTGGCTTATCTGCGCGTGCTGACTAACCC GGACGATGACTGCGCATTTCTGCGTATCGTTAACACGCCGAA GCGAGAGATTGGCCCGGCTACGCTGAAAAy\GCrrGCK^GAGlXi GGCGATGACGCGCAATAAAAGCATGTTTACCGCCAGCTTTGA **TATGGGCCTGAGTCAGACGCTTAGCGGACGTGGTTATGAAGC** ATTGACCCGCTTCACTCACTGGTTGGCAGAAATCCAGCGTCTG **GCGGAGCGGGAGCCGATTGCCGCGGTGCGTGATCTGATCCAT** GGCATGGATTATGAATCCTGGCTGTACGAAACATCGCCCAGC CCGAAAGCCGCCGAAATGCGCATGAAGAACGTCAACCAACTG TTTAGCTGGATGACGGAGATGCTGGAAGGCAGTGAACTGGAT GAGCCGATGACGCTCACCCAGGTGGTGACGCGCTTTACTTTGC **(}{X}AC**ATGAT**GGAG(X}**TGGT**GA(}AGTGAA**(}AAGA**(}CTG(}**ATC AGGTGCAACTGATGACTCTCCACGCGTCGAAAGGGCTGGAGT **TTCCTTATGTCTACATGGTCGGTATGGAAGAAGGGTTTTTGCC** GCACCAGAGCAGCATCGATGAAGATAATATCGATGAGGAGCG **GCGGCTGGCCTATGTCGGCATTACCCGCGCCCAGAAGGAATT** GACCTTTACGCTGGCTAAAGAACGCCGTCAGTACGGCGAACT GGTGCGCCCGGAGCCGAGCCGCTTTTTGCTGGAGCTGCCGCA CXMTGATCTGATTTGGG/VACAGGACJC GCAAAGTGGTCAGCGC CGAAGAACGGATGCAGAAAGGGCAAAGCCATCTGGCGAATCT **GAAAGCGATGATGGCGGCAAAACGAGGGAAATAA**
Rep-x **RNA**[3] (SEQIDNO:3)	**AUGCGUCUAAACCCCGGCCAACAACAAGCUGUCGAAUUCGU** **UACCGGCCCCUUGCUGGUGCUGGCGGGCGCGGGUUCCGGUA** **AAACUCGlJGUUAlJC'ACC'AAUAAAAUCGCCCAUCljGAUCC)GC** **GGUAGCGGGUACCAGGCGCGGCACAUUGCGGCGGUGACCUU** **UACUAAUAAAGCAGCGCGCGAGAUGAAAGAGCGUGUAGGGC** AGACGCUGGGGCGCAAAGAGGCGCGUGGGCUGAUGAUCUCC

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	ACUUUCCAUACGUUGGGGCUGGAUAUCAUCAAACGCGAGUA
	UGCGGCGCUUGGGAUGAAAGCGAACUUCUCGUUGL1UUGACG
	AUACCGAUCAGCXJUGCUUUGCUUAAAGAGUUGACCGAGGGG
	CUGAUUGAAGAUGACAAAGUUCUCCUGCAACAACUGAUUUC
	GACCAUCUCUAACUGGAAGAAUGAUCUCAAAACACCGUCCC
	AGG(X}GC'AGCAA(}U;GCGAUUGGiCCiAGCGGiGAC(X}U;AUUUlJlJ
	GCCCAUGUUUAUGGGCUGUAUGAUGCACACCUGAAAGCCUG
	UAACGUUCUCGACUUCGAUGAUCUGAUUUUAUUGCCGACGU
	UGCUGCUGCAACGCAAUGAAGAAGUCCGCAAGCGCUGGCAG
	AACAAAAUUCGCUAUCUGCUGGUGGAUGAGUAUCAGGACAC
	CAACACCAGCCAGUAUGAGCUGGUGAAACUGCUGGUGGGCA
	GCCGCGCGCGCUUUACCGUGGUGGGUGACGAUGACCAGUCG
	AUCUACUCCUGG(X}CGGUJG(}ACGUCCGC'AAAACCUGGUGCU
	GCUGAGUCAGGAUUUUCCGGCGCUGAAGGUGAUUAAGCUUG
	AG(}AGAACUAUCGCUCUUCCGGGCGUAUUCUGAAAGCGGCG
	AACAUCCUGAUCGCCAAUAACCCGCACGUCUUUGAAAAGCG
	UCUGUUCUCCGAACUGGGUUAUGGCGCGGAGCUAAAAGUAU
	UAAGCGCGAAUAACGAAGAACAUGAGGCUGAGCGCGUUACU
	GGCGAGCUGAUCGCCCAUCACUUCGUCAAUAAAACGCAGUA
	CAAAGAUUACGCCAUUCUUUAUCGCGGUAACCAUCAGUCGC
	GGGUGUUUGAAAAAUUCCUGAUGCAAAACCGCAUCCCGUAC
	AAAAUAljC'UCXiUGGUAC}GUCGlJUUlJUCUCUC}GUCCUGAAAU
	CAAGGACUUGCUGGCUUAUCUGCGCGUGCUGACUAACCCGG
	ACGAUGACUGCGCAUUUCUGCGUAUCGUUAACACGCCGAAG
	CGAGAGAUUGGCCCGGCUACGCUGAAAAAGCUGGGUGAGUG
	GGCGAUGACGCGCAAUAAAAGCAUGUUUACCGCCAGCUUUG
	AUAUGGGCCUGAGUCAGACGCUUAGCGGACGUGGUUAUGAA
	GCAUUGACCCGCUUCACUCACUGGUUGGCAGAAAUCCAGCG

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	UCUGGCGGAGCGGGAGCCGAUUGCCGCGGUGCGUGAUCUGA UCCAUGGCAUGGAUUAUGAAUCCUGGCUGUACGAAACAUCG CCCAGCCCGAAAGCCGCCGAAAUGCGCAUGAAGAACGUCAA CCAACUGUUUAGCTJGGAIJGACGGAGAUGCTJGGAAGGCAGUG AACUGGAUGAGCCGAUGACGCUCACCCAGGUGGUGACGCGC UUUACUUUGCGCGACAUGAUGGAGCGUGGUGAGAGUGAAG AAGAGCUGGAUCAGGUGCAACUGAUGACUCUCCACGCGUCG AAAGGGCUGGAGUUUCCUUAUGUCUACAUGGUCGGUAUGG AAGAAGGGUUUUUGCCGCACCAGAGCAGCAUCGAUGAAGAU AAUAUCGAUGAGGAGCGGCGGCUGGCCUAUGUCGGCAUUAC CCGCGCCCAGAAGGAAUUGACCUUUACGCUGGCUAAAGAAC GCCGUCAGUACGGCGAACUGGUGCGCCCGGAGCCGAGCCGC UUIJIJUGCUGGAGCUGCCGC `AGGAIJGAUCUGAUUUGGGAACA GGAGCGCAAAGUGGUCAGCGCCGAAGAACGGAUGCAGAAAG GG(`AAAGCCAUCUGGCGAAUCUGAAAGCGAUGAUGGC `GGCA AAACGAGGGAAAUAA
Rep-x polypeptide[4] (SEQK)NO:4)	SEQ ID NO: 1and formula no 2 in Table 2 (l-[2-(2,5-dioxopyiTol-l-yl)ethyl]pyiTole-2,5-dione)
Rep-Y polypeptide[5] (SEQIDNO:5)	MRLWGQQQAVEFWGPLLVLAGA GSGKTRVITNKIAHLIRGSG YQARHIAAVTETmAAREMKERVGQTXGRKEARGL^nST^fflXG LDnKREYAALG^lKANFSLFDDTDQLAIJXELTEGIJEDDKA-lXQ QLISTISNWKNDLKTPSQAAASAIGERDRIFAHVYGLYDAHLKAC NVLDFODLIIJ_PTTXLQRNEEVRKRWQ]N^ RYLLVDEYQDTNTS QYELVKLLVGSRARFT\'VGDDDOSIYSWRGARPQNLX'LLSQDFP ALKViKl£ QNYRSSGRILKA ANILIANNPFIVTEKRIE SELGYGAEL KVLSANNEEHE/VERVTGELIAHHFVNKTQYKDYAILYRGNHQSR VFEKFLMONRIPYKISGGTSFFSRPEIKDLLAYLRVLTNPDDDC`AF

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) **N → C** (amino acid sequence)
	LR1VNTT KREKGPATLKKLGEW AN'ITRNKSMFTASFDMGLSQTLS **GRGYEAL'rRF' rHWLAEIORLAEREPIAAVRDLIHGMDYESVVLYE** T SPSPKA AE M RMKNV¾ QLF SWMTEMLEGSELDEPMTLTQVVTR FIXRDM^mGESEEELDQVQLjVrrLHASKGLEFPYA^MVGMEEG **FLPHOSSIDEDNIDEERRLAYVGITRAOKELTFTLAKERROYGELV** RPEPSRFLLELPQDDLIWEQERKWSAEERA^QKGQSHLANLKAM **MAAKRGK**
Rep-Y DNA [6] (SEQ IDNO :6)	ATGCGTCTAAACCCCGGCCAACAACAAGCTGTCGAATTCGTT ACCGGCCCCTTGCTGGTGCTGGCGGGCGCGGGTTCCGGTAAA ACTCGTGTTATCACCAATAAAATCGCCCATCTGATCCGCGGTA GCGGGTACCAGGCGCGGCACATTGCGGCGGTGACCTTTACTA **AT**AA AGCAGCGCGCGAGATGAAAGAGCGTGTAGGGCAGACG CTGGGGCGCAAAGAGGCGCGTGGGCTGATGATCTCCACTTTC **CA1ACG1TGGGG(TGGATATCATCAAACGCGAG1ATGCGGCG** CTTGGGATGAAAGCGAACTTCTCGTTGTTTGACGATACCGATC AGCTTGCTTTGCTTAAAGAGTTGACCGAGGGGCTGATTGAAG ATGACAAAGTTCTCCTGCA4CAACTGATTTCGACCATCTCTA4 CTGGAAGAATGATCTCAAAACACCGTCCCAGGCGGCAGCAAG TGCGATTGGCGAGCGGGACCGTATTTTTGCCCATGTTTATGGG CTGTATGATGCACACCTGAAAGCCTGTAACGTTCTCGACTTCG **ATGATCTGATTTTATTGCCGACGTTGCTGCTGCAACGCAATGA** AGAAGTCCGCAAGCGCTGGCAGAACAAAATTCGCTATCTGCT GGTGGATGAGTATCAGCiACACC/VACACCAGC'CAGTyVrGACiCrr GGTGAAACTGCTGGTGGGCAGCCGCGCGCGCTTTACCGTGGT **GGGTGACGATGACCAGTCGAT(TACT(X)TGGCGCGGTGCACG** TCCGCAAAACCTGGTGCTGCTGAGTCAGGATTTTCCGGCGCTG **AAGGTGATTAAGCTTCAGCAGAACTATCGCTCTTCCGGGCGT** ATTCTGAA4GCGGCGA4CATCCTGATCGCCA4TAACCCGCAC

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	GTCTTTGAAAAGCGTCTGTTCTCCGAACTGGGTTATGGCGCGG AGCTAAAAGTATTAAGCGCGAATAACGAAGAACATGAGGCTG AGCGCGTTACTGGCGAGCTGATCGCCCATCACTTCGTCAATAA AACGCAGTACAAAGATTACGCCATTCTTTATCGCGGTAACCAT CAGTCGCGGGTGTTTGAAAAATTCCTGATGCAAAACCGCATC CCGTACAAAATATCTGGTGGTACGTCGTTTTTCTCTCGTCCTG AAATCAAGGACTTGCTGGCTTATCTGCGCGTGCTGACTAACCC GGACGATGACTGCGCATTTCTGCGTATCGTTAACACGCCGAA GCGAGAGATTGGCCCGGCTACGCTGAAAAAGCTGGGTGAGTG GGCGATGACGCGCAATAAAAGCATGTTTACCGCCAGCTTTGA TATGGGCCTGAGTCAGACGCTTAGCGGACGTGGTTATGAAGC ATTGACCCGCTTCACTCACTGGTTGGCAGAAATCCAGCGTCTG GCGGAGCGGGAGCCGATTGCCGCGGTGCGTGATCTGATCCAT GGCATGGATTATGAATCCTGGCTGTACGAAACATCGCCCAGC CCGAAAGCCGCCGAAATGCGCATGAAGAACGTCAACCAACTG TTTAGCTGGATGACGGAGATGCTGGAAGGCAGTGAACTGGAT GAGCCGATGACGCTCACCCAGGTGGTGACGCGCTTTACTTTGC GCGACATGATGGAGCGTGGTGAGAGTGAAGAAGAGCTGGATC AGGTGCAACTGATGACTCTCCACGCGTCGAAAGGGCTGGAGT TTCCTTATGTCTACATGGTCGGTATGGAAGAAGGGTTTTTGCC GCACCAGAGCAGCATCGATGAAGATAATATCGATGAGGAGCG GCGGCTGGCCTATGTCGGCATTACCCGCGCCCAGAAGGAATr GACCTTTACGCTGGCTAAAGAACGCCGTCAGTACGGCGAACT GGTGCGCCCGGAGCCGAGCCGCTTnTGCTGGAGCTGCCGCA GGATGATCTGATTTGGGAACAGGAGCGCAAAGTGGTCAGCGC CGAAGAACGGATGCAGAAAGGGCAAAGCCATCTGGCGAATCT GAAAGCGATGATGGCGGCAAAACGAGGGAAATAA
Rep-yRNA'	AUGCGUCUAAACCCCGGCCAACAACAAGCUGUCGAAUUCGU

Polypeptide/DNA/RNA (SEQ ID NO: ___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
(SEQIDNO:7)	UACCG^JCCCCLJUGCUGGUGCUGGCGGGCGCGGGUUCCGGUA AAACUCGUGUUAUCACCAAUAAAAUCGCCCAUCUGAUCCGC GGUAGCGGGUACCAGGCGCGGCACAUUGCGGCGGUGACCUU UACUAAUAAAGCAGCGCGCGAGAUGAAAGAGCGUGUAGGGC AGACGCUGGGGCGCAAAGAGGCGCGUGGGCUGAUGAUCUCC ACUUUCCAUACGUUGGGGCUGGAUAUCAUCAAACGCGAGUA UGCGGCGCUUGGGAUGAAAGCGAACUUCUCGUUGUUUGACG AUACCGAUCAGCXJUGCUUUGCUUAAAGAGUUGACCGAGGGG CUGAUUGAAGAUGACAAAGUUCUCCUGCAACAACUGAUUUC GACCAUCUCUAACUGGAAGAAUGAUCUCAAAACACCGUCCC AGGCGGCAGCAAGUGCGAUUGGCGAGCGGGACCGUAUUUULT GCCCAUGUUUAUGGGCUGUAUGAUGCACACCUGAAAGCCUG UAACGUUCUCGACUUCGAUGAUCUGAUUUUAUUGCCGACGU UGCUGCUGCAACGCAAUGAAGAAGUCCGCAAGCGCUGGCAG AACAAAAl JUCGCUAUCUGCUGGUGGAUGAGUAUCAGGACAC CAACACCAGCCAGUAUGAGCUGGUGAAACUGCUGGUGGGCA GCCGCGCGCGCUUUACCGUGGUGGGUGACGAUGACCAGUCG AUCUACUCCUGGCGCGGUGCACGUCCGCAA.AACCUGGUGCU GCUGAGUCAGGAULJUUCCGGCGCUGAAGGUGALJUAAGCLJUG AG(AGAACUAUCGCUCUUCCGGGCGUAUUCUGAAAGCGGCG AACAUCCUGAUCGCCAAUAACCCGCACGUCXJUUGAAAAGCG UCUGUUCUCCGAACUGGGUUAUGGCGCGGAGCTJAAAAGUAU UA AGCGCG^A ATUTAA ACGA AGA ACA U GA GGC U CA CCGCGU U A CU GG(GAGCUGAUCGCCC AUCACUUCGUCAAUAAAACGCAGUA CAAAGAULLACGCCAUUCUULLAUCGCGGUAACCAUCAGUCGC GGGUGUIJUGAAAAAUUCCUGAUGCAAAACC GCAUCCCGUJAC AAAAUAUCUGGUGGUACGUCGULIUUUCUCUCGUCCUGAAAU CAAGGACUUGCUGGCUUAUCUGCGCGUGCUGACUAACCCGG

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	ACGAUGACUGCGCAUUUCUGCGUAUCGUUAACACGCCGAAG CGAGAGAUUGGCCCGGCUACGCUGAAAAAGCUGGGUGAGUG GGCGAUGACGCGCAAUAAAAGCAUGUUUACCGCCAGCUUUG AUAIJGGGCCIJGAGUCAGACGC)UUAGCGGACGUGGUUAUGAA GCAUUGACCCGCUUCACUCACUGGUUGGCAGAAAUCCAGCG UJCUGG(X}GAG(X}GGAG(X)CiAUlJGCCGCCiCiUGCGUCiAljClJCiA UCCAUGGCAUGGAUUAUGAAUCCUGGCUGUACGAAACAUCG CCCAGCCCGAAAGCCGCCGAAAUGCGCAUGAAGAACGUCAA CCAACUGUUUAGCUGGAUGACGGAGAUGCUGGAAGGCAGUG AACUGGAUGAGCCGAUGACGCUCACCCAGGUGGUGACGCGC UUUACUUUGCGCGACAUGAUGGAGCGUGGUGAGAGUGAAG AAGAGCUGGAUCAGGUGCAACUGAUGACUCUCCACGCGUCG AAAGGGCTJGGAGIJUIJCCUUAIJGUCUACAUGGUCGGUAUGG AAGAAGGGUUUUUGCCGCACCAGAGCAGCAUCGAUGAAGAU AAUAIJCGAUGAGGAGCGGCGGCUGGCCUAUGIJCGGCAUUAC CCGCGCCCAGAAGGAAUUGACCUUUACGCUGGCUAAAGAAC GCCGUCAGUACGGCGAACUGGUGCGCCCGGAGCCGAGCCGC UUUUUGCUGGAGCUGCCGCAGGAUGAUCUGAUUUGGGAACA GGAGCGCAAAGUGGUCAGCGCCGAAGAACGGAUGCAGAAAG GGCAAAGCCAUCUGGCGAAUCUGAAAGCGAUGAUGGCGGCA AAACGAGGGAAAUAA
Rep-Y polypeptide[8] (SEQ IDNO:8)	SEQ ID NO:5 and formula no 2 in Table 2 (l-[2-(2,5-dioxopyrrol-l-yl)ethyl]pyrrofe-2,5-dione).

'This Rep mutant encodes mutations removing natural cysteine residues found in the wild-type Rep and include further amino acid mutations to facilitate intramolecular aossl inking to an intramolecular crosslinMng agent to generate the Rep-χ polypeptide.

[2]The DNA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:l.

[3]The RNA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:l.

[4]The Rep-x polypeptide closed form monomer following reaction of Repx polypeptide (SEQ ID NO:1) with an intramolecular crosslinking agent.

[5]This Rep mutant encodes mutations that remove natural cysteine residues found in the wild-type Rep and include further amino acid mutations to facilitate intramolecular crosslinking to an intramolecular crosslinking agent to generate the Rep-γ polypeptide.

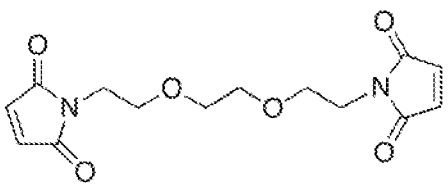
[6]The DNA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:5.

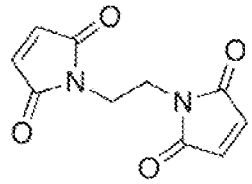
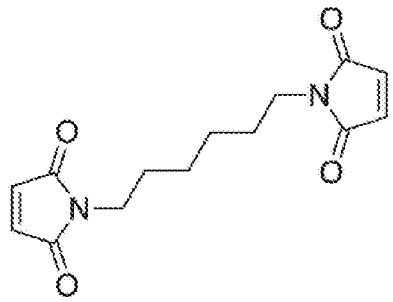
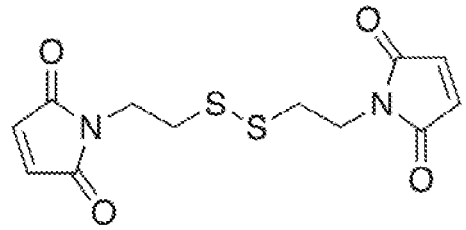
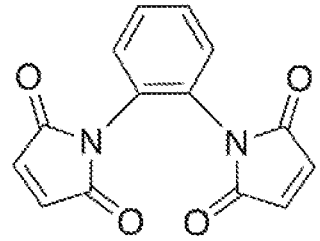
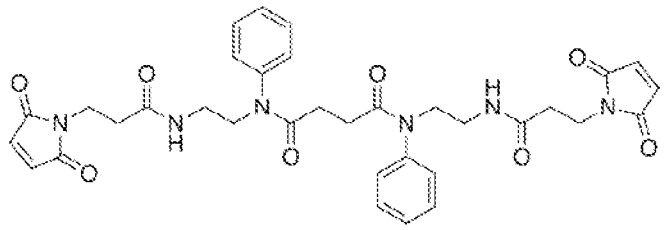
[7]The RNA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:5.

[8]The Rep-Y polypeptide open form monomer following reaction of Repy polypeptide (SEQ ID NO:5) with an intramolecular crosslinking agent.

[00186] The intramolecular crosslinking agents suitable for generating versions of Rep-χ and Rep-γ include those identified in **Tabic 2.**

Table 2. Exemplary intramolecular crosslinking agents for generating **Rep-χ** and **Rep-y**

Formula No.	Compound Structure (IUPAC Name)
1	1-[2-[2-[2-(2,5-dioxopyrrol-1-yl)ethoxy]ethoxy]ethyl]pyrrole-2,5-dione 
2	1-[2-(2,5-dioxopyrrol-1-yl)ethyl]pyrrole-2,5-dione

Formula No.	Compound **Structure** (IUPAC Name)
	
3	l-[6-(2,5<iioxopyrrol-l-yl)hexyl]pyrrole-2,5-dione 
4	1-[2-[2-(2,5-dioxopyrFol-1-yl)ethyldisulfanyl]ethyl]pyrrole-2,5-dione 
5	l-[2-(2,5-dioxopynOl-l-yl)phenyl]pynOle-2,5-dione 
6	N,N'-bis[2-[3-(2,5-dioxopyiTol-l-yl)propanoylamino]ethyl]-N, N'-diphenylbutanediamide 

[00187] These intramolecular crosslinking agents yield intramolecular crosslinked monomer stnictures when reacted with Rep-$_X$ and Rep-γ polypeptides. The linkers can have a length in the range from about 6Å to about 25Å. These types of linkers have an alkyl length in the range corresponding from about C_7 to about C20, wherein highly preferred linkers have a length in the range from about C10 to about C12. Methods and conditions for generating intramolecular crosslink formation in proteins are well known in the art for these types ofintramolecular crosslinking agents, and such methods and conditions are applicable to the helicases of this disclosure.

[00188] Rep-x would be inefficient in DNA unwinding even at high concentrations that make the wild type Rep active if the closed form is inactive for unwinding. In multiple turnover ensemble unwinding reactions using FRET-labeled DNA (see, for example, FIG. 1C), however, Rep-X unwound an 18-bp substrate with a 3'-(dT)$_{10}$ overhang (SEQ ID NO: 33) at a much faster rate and higher reaction amplitude than the wild type Rep (FIG. ID). In contrast, Rep-Y unwinding rates were similar to that of Rep (FIG. IE), indicating that the dramatic unwinding enhancement is specifically achieved in the *closed* conformation. Because the large enhancement in unwinding activity observed in bulk solution can result from the activation of a monomer or from enhanced oligomerization, single molecule FRET (smFRET) experiments were performed to test if a single Rep-X can unwind DNA.

[00189] Rep and Rep-X monomers were immobilized to a polymer-passivated quartz surface using antibodies against the N-terminal hexa-histidine-tag (SEQ ID NO: 36) on the protein (FIG. 2A) to ensure that the observed activity belonged to monomers (T. Ha *etal.* Initiation and re-initiation of DNA unwinding by the Escherichia coli Rep helicase. *Nature* 419, 638-641 (2002)). For the unwinding substrate, we used a 18-bp duplex DNA with a 3'-(dT)$_{20}$ overhang (SEQ ID NO: 37) labeled with a donor (Cy3) and an acceptor (Cy5) at two opposite ends of the DNA duplex, allowing us to identify unwinding reactions as increases in FRET efficiency (E_{FRET} FIG. 2A) (G. Lee, M. A. Bratkowski, F. Ding, A. Ke, T. Ha, Elastic Coupling Between RNA Degradation and Unwinding by an Exonbonuclease. *Science (New York, NY336,* 1726-1729 (2012)). When the DNA and ATP were added to the reaction chamber, we could observe the capture of a single DNA molecule by a single protein as the sudden appearance of fluorescence signal (FIG. **2B-E).** Subsequent DNA unwinding generated ssDNA strands that coil up due to high flexibility and E_{FRET} increased (M. C. Murphy, I. Rasnik, W. Cheng, T. M. Lohman, T. Ha, Probing single-stranded DNA confbrmational flexibility using fluorescence spectroscopy. *Biophysicaljournal* 86, 2530-2537 (2004)). Once the duplex was completely

unwound, the acceptor-labeled strand was released, which was marked by sudden disappearance of the acceptor signal and recovery of the donor signal. The donor-labeled strand then dissociated, resulting in complete loss of fluorescence. The mean duration of unwinding measured from the E_{FRET} increase to acceptor strand release was ~0.6 s, giving a lower limit on the unwinding speed of 30 bp/s for the 18-bp substrate (FIG. 2F). About 82% of the DNA molecules (661 out of 809) that initially bound to Rep-X monomers were unwound (FIG. 2G). In contrast, only 2% of the DNA molecules (13 out of 847) that bound to Rep (i.e. without crosslinking) showed unwinding, and the unwinding yield for Rep-Y was 16% (357 out of 2212) (FIG. 2G), showing that constraining Rep into the *closed* form selectively activates the unwinding activity of a monomer. The nonzero amplitude of unwinding for Rep and Rep-Y may be due to conformational constraints caused by surface tethering in a small fraction of molecules.

[00190] *In vitro* studies have shown that the unwinding processivity of Rep and related helicases is limited even in their oligomeric forms, ranging from 30-50 bp (A. Niedziela-Majka, M. A. Chesnik, E. J. Tomko, T. M. Lohman, Bacillus steaiOthemiophilus PcrA monomer is a single-stranded DNA translocase but not a processive helicase in vitro. *The Journal of biological chemistry* 282, 27076-27085 (2007);Ha *etal.* (2008) *supra,* J. A. Ali, T. M. Lohman, Kinetic measurement of the step size of DNA unwinding by Escherichia coli UvrD helicase. *Science (New York, N. Y.)* **275,** 377-380 (1997)). In order to investigate the processivity of Rep-X, we employed a dual optical tweezers assay (FIG. 3A; J. R. Moffitt *et al.,* Intersubunit coordination in a homomeric ring ATPase. *Nature* 457, 446-450 (2009)) that can monitor unwinding amplitudes and speeds over thousands of base pairs of DNA. The two traps held two streptavidin functionalized sub-micron sized polystyrene beads. The first was coated with 6-kbp dsDNA attached via a biotin on the blunt end and containing a 3' poly-dT ssDNA overhang on the other end ((dT)io (SEQ ID NO: 33), $(dT)_{35}$ (SEQ ID NO: 34), and $(dT)_{75}$ (SEQ ID NO: 35) see Example 7)). The other bead was coated with Rep-X molecules via biotinylated antibody against the hexa-histidine-tag (SEQ ID NO: 36). A laminar flow cell with two parallel streams of buffer was created for controlling the initiation of the unwinding reaction (inset of FIG. 3B; L. R. Brewer, P. R. Bianco, Laminar flow cells for single-molecule studies of DNA-protein interactions. *Nature methods* 5, 517-525 (2008)). When the two beads were brought in proximity in the first laminar stream (Buffer C with 100 μM ATP and 100 μM ATP-γS), a single Rep-X binding to the 3' overhang of the DNA formed a tether between the two beads without initiating unwinding. When the tethered beads were moved to the second laminar stream (Buffer C and 1 nM ATP), the DNA tether between the beads progressively shortened as the Rep-X monomer

unwound and pulled the DNA. Unless otherwise stated, SSB was added to the second laminar stream in order to prevent any subsequent interaction of unwound ssDNA with other Rep-X on the bead surface. The optical tweezers experiments that were performed without SSB yielded the same Rep-X behavior (Example 7). By operating the trap under force feedback control, the tension was maintained on the DNA at 10-22 pN, as indicated. Additional controls and considerations ascertained that the observed activity stemmed from a single Rep-X regardless of the 3'-tail length and inclusion/omission of SSB (Example 7). Remarkably, 95% (38 out of 40) of the Rep-X-DNA complexes tethered through a 3'-tail unwound the entire 6-kbp DNA in a processive manner (FIG. **3B, D)** and the average pause-free speed was 136 bp/s (FIG. 3C). In comparison, only 3% (2 out of 61 at 4 pN tension, none at higher forces) of wild type Rep and 7% (5 out of 70) of Rep-Y complexes displayed such processive unwinding events (FIG. **3D).** Rep-X may have even greater processivity than 6-kbp, currently only limited by the length of the DNA used. The processive activity of a crosslinked Rep-X monomer shows the innate potential of these helicases that can be harnessed via conformational control.

[00191] The amount of force Rep-χ can generate during unwinding was evaluated by performing measurements without the force feedback. Fixing trap positions led to a rapid build-up of force on the Rep-x in the opposite direction of unwinding until the measurement was terminated due to the breakage of connection between the two beads (FIG. **3E).** The highest loads achieved in this experiment were not enough to stall the helicase permanently. More detailed analysis showed that the pause free unwinding rate of Rep-χ was not impeded by increasing loads up to the limits of the linear regime of our trap (FIG. **3F),** approximately 60 pN. These results suggest that the engineered Rep-X is the strongest helicase known to date (T. T. Perkins, H. W. Li, R. V. Dalai, J. Gelles, S. M. Block, Forward and reverse motion of single RecBCD molecules on DNA. *Biophysicaljournal* **86,** 1640-1648 (2004); J. G. Yodh, M. Schlierf, T. Ha, Insight into helicase mechanism and function revealed through single-molecule approaches. *Quarterly reviews of biophysics* **43,** 185-217 (2010))

In order to test if generation of a super active helicase can be reproduced for other helicases, thereby providing additional evidence of the conformational control mechanism, a PcrA-X helicase was engineered from *Bacillus stearothermophilus* Per**A.** The Rep mutant sequences used to generate PciA-χ include those nucleotide and amino acid sequences identified in **Tabic** 3.

Table 3. Amino Acid and Nucleotide Sequences for exemplary PcrA-$_x$ proteins

Polypeptide/DNA/RNA (SEQ ID NO:___)	**5' → 3' (nucleotide sequence) N → C (amino acid sequence)**
Wild type Per**A** helicase (gene sequence) >gij696477066:c17795-15621 Geobacillus stearothermophilus ATCC 7953 GBScontig000QQ36_2, whole genome **shotgun** sequence **(SEQ ID NO:** 38)	**ATGAACTTTTTATCGGAACAGCTGCTCGCCCATTTAAACAAAG** **AGCAACAAGAAGCCGTCAGGACGACGGAAGGCCCGCTGCTCA** **TTATGGCGGGGGCGGGAAGCGGGAAAACGCGGGTGTTGACGC** **ACCGCATCGCCTATTTGATGGCGGAAAAGCATGTGGCGCCGT** **GGAACATTTTGGCCATTACGTTTACGAACAAGGCGGCGCGCG** **AAATGCGGGAACGTGTGCAGTCGCTCTTAGGTGGGGCGGCGG** **AAGACGTCTGGATTTCGACGTTCCACTCGATGTGCGTCCGCAT** TTTGCGCCGCGACATTGACCGCATCGGCATCAACCGCAATTTT **TCCATCCTTGATCCGACGGACCAGCTTTCAGTCATGAAAACGA** TTTTAAAAGAAAAAAACATAGACCCGAAAAAATTTGAGCCGC GGACGA TTTTAGGCACGATC AGCGCGGCG AAAAACGAGCTGT TGCCTCCGGAGCAATTCGCGAAGCGGGCCTCGACGTATTACG **AAAAAGTCGTCAGCGATGTGTATCAAGAATACCAACAGCGCC** **TGCTTCGCAATCATTCGCTCGATTTTGACGATTTGATCATGAC** GACGATCCAACTTTTTGACCGCGTGCTGCATTGCTTCACTAT **TACCAATATAAGTTTCAGTACATTCATATTGATGAGTACCAGG** ATACGAACCGCGCTCAATATACGCTGGTCAAAAAGCTGGCGG AACGCTTTCAAAACATTTGCGCCGTCGGCGACGCCGACCAAT CGATTTATCGGTGGCGCGGGGCGGACATCCAAAACATTTTGTC GTTCGAGCGCGACTATCCGAACGCAAAAGTCATTTTGCTTGAA CAAAACTACCGCTCGACGAAGCGCATTTTGCAAGCGGCGAAC GAAGTCATCGAGCATAACGTCAACCGGAAGCCGAAACGGCTT TGGACGGAAAACCCGGAAGGAAAGCCGATTCTTTATTATGAG GCGATGAACGAAGCGGACGAAGCGCAGTTTGTCGCTGGACGC ATCCGCGAGGCGGTGGAGCGCGGCGAACGCCGCTACCGTGAT TTTGCTGTCTTGTACCGCACGAATGCCTAGTCGCGTGTCATGG **AGGAAATGTTGCTGAAAGCGAACATTCCGTATCAAATTGTCG**

Polypeptide/DNA/RNA (SEQ ID NO: ___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	GCGGCTTAAAGTTCTATGACCGGAAAGAAATTAAAGACATTC TCGCCTATTTGCGCGTCATTGCCAATCCGGACGATGATTTAAG CTTGCTTCGC ATCATT AACGTGCCAAAACGCGGC ATTGGCGCC TCGACGATCGACAAy\CTCGTCCGCrrATGCAGCCGATCATGAG CTGTCCTTGTTTGAGGCGCTCGGCGAGCTAGAGATGATCGGGC T VGG€ G€ CAAAGCGG€ CGGGGCGCTCGCCGCGTTCCGGAGCC AGCTCGAGCAATGGACACAGCTGCAAGAATACGTCTCCGTCA CCGAACTCGTCGAAGAAGTGCTCGACAAATCGGGCTACCGCG AGATGCTCAAGGCGGAGCGGACGATTGAAGCACAAAGCCGG CTCGAGAACTTGGATGAGTTTTTGTCGGTGACGAAGCATTTTG AAAATGTGAGCGACGATAAATCGCTCATCGCCTTTTTAACCGA CTTGGCGCTCATTTCCGATTTGGACGAGCTGAACGGGACGGA ACAGGCCGCTGAAGGAGATGCCGTCATGTTGATGACGTTGCA TGCCGCCAAAGGGCTCGAGTTTCCGGTCGTCTTTTTGATCGGC ATGGiAAGAA(X}CA'n TTCC(X}CACAACCGCTCTXT(X}AGGA'r GACGATGAGATGGAAGAAGAACGGCGGCTGGCGTACGTCGG CATCACCCGCGCGGAGGAAGAACTTGTGCTGACGAGCGCGCA AATGCGGACGTTGTTTGGCAACATCCAAATGAACCCGCCGTC GCGCTTTTTGAATGAAATTCCGGCGCATTTGCTTGAGACAGCC TCGCGCCGCCAAGCGGGCGCCTCCCrc^ CGGCCGTTTCGCGCC CGCAGGCAAGCGGCGCCGTGGGATCGTGGAAAGTCGGCGATC (XJGCXIAA1X'ACXXXJAAATGGG(X'A1XX}(X'A(XX}TCGTCAGCG TCCGCGGCGGCGGCGACGACCAAGAGCTCGACATCGCCTTCC CGAGCCCGATCGGCATTAAACGGTTGCTTGCCAAATTTGCGCC GATTGAGAAAGTGTAG
Wild type PerA helica.se (amino acid sequence) >gii696477065|ref|WP_0	MNFLSEQLLAHLNKE(X)EAVRTTEGPLLIMAGAGSGKTRVLTHR LAYLMAEKHVAPWNILA1TFTNK,AAREMIERVQSLLGGA,AEDV W1STFHSMCVR1LRRD1DR1G1NRNFS1LDPTDQLSVMKTILKEKN1

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) **N → C** (amino acid sequence)
33016687.1| ATP-dependent DNA helicase PerA [Geobacillus steaiOthemiophilus] (SEQ ID NO: 39)	DPKKFEPRTILGTISAAKNEILPPEQFAKRASTCEKVVSDVYQE **YWRLLRNHSLDFDDLIMITIOLFDRVPDVLHYYOYKFQYIHIDE** YQDTNRAQra,\¾KLAERFONICAVGDADQSTYRWRGADIQNn. SFERDYPNAKVILLEQNYRSTKRILQAANEVIEHNVNRKPKRLWT **ENPEGKPILYYEAMNEADEAQFVAGRIREAVERGERRYRDFAVL** YRTNAQSRVMEEMLLKANIPYQIVGGLKFYDRKEIKDILAYLRVI ANPDDDLSLLRnmTKRGIGASTIDKLWYAADHELSLFEALGEL E^nGLGAKAAGALAAFRSOLEQWTQLQEY\¾\nTLWEVLDKS **GYREMLKAERTIEAOSRLENLDEFLSXTKHFENVSDDKSLIAFLT** DLALISDLDELNGTEQAAEGDA VM[*MTU* iAAKGLEFPVVFLIGM **EEGlFPHNRSLEDDDEMEEERRLAYVGriRAEEELVL 'ΓSAOMR**TL FGNIQMNPPSRFLNEPAHLLETASRRQAGASRPAVSRPQASGAV GSWKVGDRANHRKWGIGTVVSVRGGGDDQELDLAFPSPIGIKRL **LAKFAPIEKV**
PcrA-$_X$ polypeptide[1] (SEQIDNO:9)	**MNFLSEQLLAHILNKE(X)EAVRTTEGPLLIMAGAGSGKTRVLT1IR** IAYLMAEKiWAP WNILAITFTNKAAREMRERVQSLLGGAAEDV WISTFHSMAVRILRRDn RIGINRNFSILDPTDQLSVMKTILKEKNI DPKKFEPRTILGTISAAKNEIX PPEQFAKRASTYYEKW SDVY QE **YOORLLRC'HSLDFDDLIMITIOLFDRVPDVLHYYOYKFOYIHIDE YODTNRAOYTLVKKLAERFONIAAVGDAEX)SIYRWRGADIONIL** SFERDYPNAKMLLEQNYRSTKRlLQAy\NEVIEHNVNRKPKRLWT **ENPEGKPILY'YEAMNE.ADEAOFVAGRIREAVERGERRYRDFAVL** YRTOAQSRVMEEMLLK ANIPYQIVGGVKFYDRKEIKDILAYLRVI ANPDDDCSLLRIINVTKRGIGASTIDKLVRYAADHELSLFEALGEL EMIGLGAKAAGALAAFRSQLEQWTQLQEYVSVIELVEE\1.DKS **GYREMLKAERTIEAOSRLENLDEFLSVTKHFENVSDDKSLIAFLT** DLALISDLDELNGTEQAAEGDAVMLMTLHAAKGLEFPW FLIGM **EEGIFPHNRSLEDDDEMEEERRLAYVGrrRAEEELVLTSAOMRI 'L**

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) **N → C** (amino acid sequence)
	FGNIQMNPPSRFLNEIPAPnXETASRRQAGASRPA\ ʼSKPQASGAV **GSWKVGDRAN^RKWGlG ʽrVVSVRGGGDDQELDIAFPSPlGlKRL** LAKFAPIEKV
PcrA-xDNA2 (SEQ ID NO: 10)	ATGAACTTTTTATCGGAACAGCTGCTCGCCCATTTAAACAAAG **AGCAACAAGAAGCCGTCAGGACGACGGAAGGCCCGCTGCTCA** TTATGGCGGGGGCGGGAAGCGGGAAAACGCGGGTGTTGACGC ACCGCATCGCCTATTTGATGGCGGAAAAGCATGTGGCGCCGT GGAACATTTTGGCCATTACGTTTACGAACAAGGCGGCGCGCG AAATGCGGGAACGTGTGCAGTCGCTCTTAGGTGGGGCGGCGG AAGACGTCTGGATTTCGACGTTCCACTCGATGGCCGTCCGCAT TTTGCGCCGCGACATTGACCGCATCGGCATCAACCGCAATTTT **TCCATCCTTGATCCGACGGACCAGCTTTCAGTCATGAAAACGA** TTTTAAAAGAAAAAAACATAGACCCGAAAAAATTTGAGCCGC **GGACGATTTTAGGCACGATCAGCGCGGCGAAAAACGAGCTGT** TGCCTCCGGAGCAATTCGCGAAGCGGGCCTCGACGTATTACG AAAAAGTCGTCAGCGATGTGTATCAAGAATACCAACAGCGCC TGCTTCGCTGTCATTCGCTCGATTTTGACGATTTGATCATGACG ACGATCCAACTGTTrGACCGCGTGCCGGATGTGCTTCACTATT ACCAATATAAGTTTCAGTACATTCATATTGATGAGTACCAGGA TACGAACCGCGCTCAATATACGCTGGTCAAAAAGCTGGCGGA ACGCTTTCAAAACASTGCCGCCGTCGGCG^ \CGCCGACCAATC GATTTATCGGTGGCGCGGGGCGGACATCCAAAACATTTTGTC GTTCGAGCGCGACTATCCGAACGCAAy\AGTCATTTTGCTTGAy\ **CAAAACTACCGCTCGACGAAGCGCATTTTGCAAGCGGCGAAC** **GAAGTCATCGAGCATAACGTCAACCGGAAGCCGAAACGGCTT** TGGACGGAAAACCCGGAAGGAAAGCCGATTCTTTATTATGAG **GCGATGAACGAAGCGGACGAAGCGCAGTTTGTCGCTGGACGC** ATCCGCGAGGCGGTGGAGCGCGGCGAACGCCGCTACCGTGAT

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	TTTGCTGTCTTGTACCGGACGAACGCCCAGTCGCGTGTCATGG AGGAAATGTTGCTGAAAGCGAACATTCCGTATCAAATTGTCG GCGGCGTAAAGTTCTATGACCGGAAAGAAATTAAAGACATTC TCGCCTATTTGCGCGTCATTGCCAATCCGGACGATGATTGCAG CTTGCTTCGCATCATTAACGTGCCAAAACGCGGCATTGGCGCC TCGACGATCGACAAACTCGTCCGCTATGCAGCCGATCATGAG CTGTCCTTGTTTGAGGCGCTCGGCGAGCTAGAGATGATCGGGC TTGGCGCCAAAGCGGCCGGGGCGCTCGCCGCGTTCCGCAGCC AGCTCGAGCAATGGACACAGCTGCAAGA.ATACGTCTCCGTCA CCGAACTCGTCGAAGAAGTGCTCGACAAATCGGGCTACCGCG AGATGCTCAAGGCGGAGCGGACGATTGAAGCACAAAGCCGG CTCGAGAACTTGGATGAGTTTTTGTCGGTGACGAAGCATTTTG AAAATGTGAGCGACGATAAATCGCTCATCGCCTTnTAACCGA CTTGGCGCTCATTTCCGATTTGGACGAGCTGAACGGGACGGA ACAGGCCGCTGAAGGAGATGCCGTCATGTTGATGACGTTGCA TGCCGCCAAAGGGCTCGAGTTTCCGGTCGTCTTTTTGATCGGC ATGGAAGAAGGCATTTTCCCGCACAACCGCTCTCTCGAGGAT GACGATGAGATGGAAGAAGAACGGCGGCTGGCGTACGTCGG CATCACCCGCGCGGAGGAAGAACTTGTGCTGACGAGCGCGCA AATGCGGACGTTGTTTGGCAACATCCAAATGAACCCGCCGTC GCGCTTTTTGAATGAAATTCCGGCGCATTTGCTTGAGACAGCC TCGCGCCGCCAAGCGGGCGCCTCCCGCCCGGCCGTTTCGCGCC CGCAGGCAAGCGGCGCCGTGGGATCGTGGAAAGTCGGCGATC GCK}(X}AAICACC(}GAAATG(}GGCATC(jGCACCGl(X}TC`A(}(X} TCCGCGGCGGCGGCGACGACCAAGAGCTCGACATCGCCTTCC CGAGCCCGATCGGCATTAAACGGTTGCTTGCCAAATTTGCGCC GATTGAGAAAGTGTAG
PcrA-$_x$ RNA^3	AUGA.ACUUUL1UAUCGGAACAGCUGCUCGCCCAUUUAAACAA

Polypeptide/DNA/RNA (SEQ ID NO:___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
(SEQ ID NO:11)	AGAGCAACAAGAAGCCGUCAGGACGACGGAAGGCCCGCUGC UCAUUAUGGCGGGGGCGGGAAGCGGGAAAACGCGGGUGUU GACGCACCGCAUCGCCUAUUUGAUGGCGGAAAAGCAUGUGG CGCCGUGGAACAUljlJUGGCCAUUACGlJUUACGAACAAGGCG GCGCGCGAAAUGCGGGAACGUGUGCAGUCGCUCUUAGGUGG CKXCJGCGCI/VAGACGUCUGGAUIJIJCGACGIAJCCACUCGAUGG CCGUVVVVCA UUUU[illegible]CAUUGACCGCAUCUGGCAUC AACCGCAAUUUUUCCAUCCUUGAUCCGACGGACCAGCUUUC AGUCAUGAAAACGAUUUUAAAAGAAAAAAACAUAGACCCG AAAAAAUUUGAGCCGCGGACGAUUUUAGGCACGAUCAGCGC GGCGAAAAACGAGCUGUUGCCUCCGGAGCAAUUCGCGAAGC GGGCCUCGACGUAUUACGAAAAAGUCGUCAGCGAUGUGUAU CAAGAAUAC'CAACAGCGCCUGCUUCGCUGUCAUUCGCUCGA UUUUGACGAULIUGAUCAUGACGACGAUCCAACUGUUUGACC GCGUGCCGGAUGUGCIJUCACUAUUACCAAUAIJAAGUUUCAG UACAUUCAUAUUGAUGAGUACCAGGAUACGAACCGCGCUCA AUAUACGCUGGUCAAAAAGCUGGCGGAACGCUUUCAAAACA UUGCCGCCGUCGGCGACGCCGACCAAUCGAUUUAUCGGUGG CGCGGGGCGGACAUCCAAAACAUUUUGUCGUUCGAGCGCGA CTJAUCCGAACGC^\AAAGUCAlJLJUlJGClJLJGAACAAy\ACUACC GCUCGACGAAGCGCAUUUUGCAAGCGGCGAACGAAGUCAUC GAGCAUAACGUCAACCGGAAGCCGAAACGGCUUUGGACGGA AAACCCGGAAGGAAAGCCGAUUCUUUAUUAUGAGGCGAUGA ACGAAGCGGACGAAGCGCAGUIJUGUCGCUGGACGCAUCCGC GAGGCGGUGGAGCGCGGCGAACGCCGCUACCGUGAUUUUGC UGIJCUUGUACCGGACGAACGCCCAGUCGCGUGUCAUGGAGG AAAUGUUGCUGAAAGCGAACAUUCCGUAUCAAAUUGUCGGC GGCGUAAAGUUCUAUGACCGGAAAGAAAUUAAAGACAUUC

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Polypeptide/DNA/RNA (SEQ ID NO: ___)	5' → 3' (nucleotide sequence) N → C (amino acid sequence)
	UCGCCUAUUUGCGCGUCAUUGCCAAUCCGGACGAUGAUUGC AGCUUGCUUCGCAUCAUUAACGUGCC 'AAAACGCGGCAUUGG CGCCUCGACGAUCGACAAACUCGUCCGCUAUGCAGCCGAUC AUGAGCUGUCCUUGUUUGAGGCGCUCGGCGAGCUAGAGAUG AUCGGGCUUGGCGCCAAAGCGGCCGGGGCGCUCGCCGCGUU CCGC'AGCCAGCUC 'GAGCAAUGGACAC 'AGCUGCAAGAAUACG UCUCCGUCACCGAACUCGUCGAAGAAGUGCUCGACAAAUCG GGCUACCGCGAGAUGCUCAAGGCGGAGCGGACGAUUGAAGC ACAAAGCCGGCUCGAGAACUUGGAUGAGUUL1UUGUCGGUGA CGAAGCAUUUUGAAAAUGUGAGCGACGAUAAAUCGCUCAUC GCCULTJUUAACCGACUUGGCGCUCAUWCCGAUULTGGACGA GCUGAACGGGACGGAACAGGCCGCUGAAGGAGAUGCCGUCA UGUUGAUGAC 'GUUGCAUGCC 'GCCAAAGGGCUCGAGUUUCCG GUCGUCXJUUUUGAUCGGCAUGGAAGAAGGCAUUUUCCCGCA CAACCGCUCUCUCGAGGAUGACGAUGAGAUGGAAGAAGAAC GGCGGCUGGCGUACGUCGGCAUCACCCGCGCGGAGGAAGAA CUUGUGCUGACGAGCGCGCAAAUGCGGACGUUGUUUGGCAA CAUCCAAAUGAACCCGCCGUCGCGCUUUUUGAAUGAAAUUC CGGCGCAUUUGCUUGAGACAGCCUCGCGCCGCCAAGCGGGC GCCUCCCGCCCGGCCGUUUCGCGCCCGCAGGCAAGCGGCGCC GUGGGAUCGUGGAAAGUCGGCGAUCGGGCGAAUCACCGGAA AUGCiCX}CAUCG<iCACX'CiU(X}UCACiC'CiU(XXXX}GC:GC(XX}('G ACGACCAAGAGCUCGACAUCGCCUUCCCGAGCCCGAUCGGC AUUAAACGGUUGCUUGCCAAAUUUGCGCCGAUUGAGAAAGU GUAG
PcrA-x polypeptide [4] (SEQ ID NO: 12)	SEQ ID NO:9 and formula no 1 in Table 2 (l-[2-[2-[2-(2,5-dioxop>Trol-l-yl)ethox\']ethoxy]ethyl]pyrrole-2,5-dione).

[1]This PcrA mutant encodes mutations removing natural cysteine residues found in the wild-type PcrA and include further amino acid mutations to facilitate intramolecular crosslinking to an intramolecular crosslinking agent to generate the PσA -χ polypeptide.

[2]The D MA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:9.

[3]The RNA sequence corresponds to the open reading frame that encodes the polypeptide of SEQ ID NO:9.

[4]The PcrA-x polypeptide closed form monomer following reaction of PcrA-$_x$ polypeptide (SEQ ID NO:9) with an intramolecular crosslinking agent.

[00192] Exemplary intramolecular crosslinking agents suitable for generating versions of PcrA-$_X$ include those identified in Table 2. Methods and conditions for generating intramolecular crosslink formation in proteins are well known in the art for these types of intramolecular crosslinking agents, and such methods and conditions are applicable to the Pcr A helicases of this disclosure.

[00193] Mutations involved replacing two highly conserved Cys residues in this helicase (FIG. 4A, B) which reduced the apparent ssDNA-dependent ATPase activity from approximately 40 ATP/s (wild type) to 5 ATP/s. Upon crosslinking in the closed form, PcrA-χ retained the low ATPase activity (4.3 ATP/s), but exhibited an enhanced helicase activity in comparison to both the wild type and non-crosslinked mutant in ensemble reactions (FIG. **5A,** B). smFRET experiments showed that PcrA-X monomers can unwind 39 % (228 out of 578) of the 18-bp dsDNA they bind compared to only 4% (26 out of 617) for wild type PcrA (FIG. **6A**-C). In the optical tweezers assay, PcrA-X monomers, like Rep-X, were capable of processively unwinding of 1-6 kbp long DNA, albeit at a much lower rate (2-15 bp/s, FIG. 6D) whereas no PcrA molecule (0 out of 51) was able to do the same (FIG. 6E). Despite the impaired activity levels of the PcrA mutant, conversion to PcrA-X made its monomers into highly processive helicases, thus indicating a general mechanism of conformational control for this class of helicases.

[00194] Strong helicase activity of Rep-X and PcrA-X raises the possibility that the cellular partners of Rep or PcrA may switch on the powerful unwinding activity intrinsic to these enzymes by constraining them in the *closed* conformation. One such partner of PcrA is RepD, a plasmid replication initiator protein from ***Staphylococcus*** *aureus* that recognizes and forms a covalent adduct with the *oriD* sequence

of the plasmid, and then recruits PcrA for highly processive unwinding (A. F. Slatter, C. D. Thomas, M. R. Webb, PcrA helicase tightly couples ATP hydrolysis to unwinding double-stranded DMA, modulated by the initiator protein for plasmid replication, RepD. *Biochemistry* **48,** 6326-6334 (2009); W. Zhang *et a!.,* Directional loading and stimulation of PcrA helicase by the replication initiator protein RepD. *Journal of molecular biology* **371,** 336-348 **(2007);** C. Macton *etal,* RepD-mediated recruitment of PcrA helicase at the Staphylococcus aureus pC221 plasmid replication origin, oriD. *Nucleic acids research* **38,** 1874-1888 (20**10)).** Based on the similar results from PcrA-X and the homologous *E. coli* counterpart Rep-X, but not Rep-Y, we hypothesized that the RepD-induced PcrA activity enhancement is in fact the result of the conformational constraint of the helicase in the PcrA-X-like closed form. To test this prediction, we prepared an *oril)* DNA-RepD adduct, and measured the intramolecular conformation of PcrA bound to this adduct. We used a double cysteine mutant of PcrA, PcrA-DM 1, stochastically labeled with a mixture of donor and acceptor fluorophores that would be expected to generate high E_{FRET} in the *closed* fomi and low E_{FRET} in the *open* form (J. Park *et al,* PcrA helicase dismantles RecA filaments by reeling in DNA in uniform steps. *Cell* **142,** 544-555 (2010); **(FIG.** 6F). The E_{FRET} distributions of PcrA-DM 1 bound to the *oril)* DNA-RepD adduct and the *oril)* DNA alone are shown in **FIG. 6F.** Only the PcrA-DMl molecules with a fluorescence active Cy3-Cy5 pair were included in the analysis. The results revealed that the presence of RepD indeed biases PcrA toward the *closed* high E_{FRET} conformation. Without the invention being limited to any particular mechanism, the regulation mechanism of this class ofhelicases may involve *in vivo* partner proteins that constrain the conformation of 2B subdomain to the *closed* form to activate its function.

[00195] The basis for constraining Rep and PcrA into the *closed* form that converts an enzyme with undetectable unwinding activity to a super helicase is unknown. One possibility is that the intrinsic unwinding activity itself requires the *closed* form, for example via the torque-wrench mechanism proposed for UvrD (J. Y. Lee, W. Yang, UvrD helicase unwinds DNA one base pair at a time by a two part power stroke. (*Cell* 127, 1349-1360 (2006)). Another possibility is that the ***o/x'ti*** form inhibits helicase function and crosslinking to the *closed* form prevents this inhibitor}' mechanism. Without the invention being limited to any particular theory of operation, we prefer the latter for the following reasons. First, Rep-Y crosslinked in the *open* form does unwind DNA as well as the wild type when the protein is at high concentrations in excess of DNA (FIG. IE). Therefore, the *closed* form *per se* is not absolutely required for unwinding activity. Second, using ultra-high resolution optical tweezers combined with

smFRET capability, we found that UvrD assumes the *closed* conformation when it unwinds DNA but after it unwinds about **10** bp it switches to the *open* conformation and rewinds the DNA likely after strand switching. Therefore, we suggest that Rep-X becomes a highly processive super-helicase because crosslinMng prevents the *open* conformation required for strand-switching and rewinding that have been observed for UvrD (M. N. Dessinges, T. Lionnet, X. G. **Xi,** D. Bensimon, **V.** Croquette, Single-molecule assay reveals strand switching and enhanced processivity of UvrD. *Proc. Natl. Acad. Sci, U.S.A.* **101,** 6439-6444 (2004)) and BLM (**J.** G. Yodh, B. C. Stevens, **R** Kanagaraj, P. Janscak, T. Ha, BUM helicase measures DNA unwound before switching strands and hRPA promotes unwinding reinitiation. *The IiMBO journal* **28,** 405-41 6 (2009)). The enhancement of unwinding activity via the deletion of 2B domain in Rep (W. Cheng *et al.*, The 2B domain of the Escherichia coli Rep protein is not required for DNA helicase activity. *Proc. Natl. Acad Sci., USA.* **99, 16006-1601 1** (2002)) may also be due to inhibition of strand switching (M. J. Com stock, K.D. Whitley, H Jia, T.M. Lohman, T. Ha and Y. R. Chemla, "Direct observation of stnicture-liinction relationship in a nucleic acid processing enzyme," *Science* **348:** 352-354 (2015).

[00196] Most conforniational control of protein functions demonstrated so far first locks the naturally active protein to an artificially inhibited conformation so that additional controls imposed by researchers can be used to recover the original activity (B. Choi, G. Zocchi, Y. Wu, S. Chan, L. Jeanne Perry, Allosteric control through mechanical tension. *Phys Rev Lett* **95,** 078102 (2005); M. Tomishige, R. D. Vale, Controlling kinesinby reversible disulfide cross-linking. Identifying the motility-producing conformational change. *J Cell Biol* **151,** 1081-1092 (2000): D. M. Veine, K. Ohnishi, C. H. Williams, Jr., Thioredoxin reductase from Escherichia coli: evidence of restriction to a single conformation upon formation of a crosslink between engineered cysteines. *Protein science : a publication of the Protein Society* 7, 369-375 (1998); B. X. Huang, H. **Y.** Kim, Interdomain conformational changes in Akt activation revealed by chemical cross-linking and tandem mass spectrometry. *Mol Cell Proteomics* **5,** 1045-1053 (2006)). Our work is innovative and unique in that we found a conformational control that activates a naturally inhibited unwinding function, and the resulting enzyme is a super-helicase that has unprecedentedly high processivity for a single motor helicase. RecBCD, another **SF-1** helicase, has similarly high processivity but contains two motors and associated nucleases. Moreover it is known to backslide at opposing forces below 10 pN whereas Rep-X can be active against forces as high as 60 pN (Perkins *et al.* **(2004)** ***supra).*** This super helicase with high processivity and high tolerance against load

without nuclease activities may also be useful for biotechnological applications such as single molecule nanopore sequencing (D. Branton *etal.* The potential and challenges of nanopore sequencing. *Nature biotechnology?* **26,** 1146-1153 (2008); A.H. Laszfo ***etal.,*** Decoding long nanopore sequencing reads of natural DNA. *Nature biotechnology,* (2014)) and isotheniial DNA amplification **(M.** Vincent, Y. Xu, H. Kong, Helicase-dependent isothermal DNA amplification. *EMBO re/x>rts* **5,** 795-800 (2004).

[00197] In this regard, one type of isotheniial DNA amplification for which these super helicases have application include helicase dependent amplification. Referring to FIG. 8, the helicase dependent amplification can be characterized in three steps. In step 1, DNA helicase **(104)** contacts a double-stranded DNA **(101)** to unwind the first and second single strands **(102** and **103)** to provide the ability of first and second oligonucleotide primers **(105** and **106)** hybridize to the first and second single strands **(102** and **103),** respectively. In step 2: DNA-dependent DNA polymerases **(107)** bind to the 3'-termini of the first and second oligonucleotide primers **(105** and **106)** to initiate chain elongation of new strands **(108** and **109).** In step 3. continued DNA polymerization results in DNA amplification and formation of new double-stranded DNA **(110** and **111).**

Nucleic Acid **Amplification**

[00198] In certain exemplary embodiments, methods for amplifying nucleic acid sequences are provided. Exemplary methods for amplifying nucleic acids include the polymerase chain reaction (PGR) (see, e.g., Mullis et al. (1986) Cold Spring Harb. Symp. Quant. Biol. 51 Pt 1:263 and Cleary et al. (2004) Nature Methods 1:241; and U.S. Pat. Nos. 4,683,195 and 4,683,202), anchor PCR, RACE PGR, ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077-1080; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91:360-364), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. U.S.A. 87: 1874), transcriptional amplification system (Kwohet al. (1989) Proc. Natl. Acad. Sci. U.S.A. 86:1173), Q-Beta Replicase (Lizardi et al. (1988) BioTechnology 6:1197), recursive PCR (Jaffe et al. (2000) J. Biol. Chem. 275:26I9; and Williams et al. (2002) J. Biol. Chem. 277:7790), the amplification methods described in U.S. Pat. Nos. 6,391,544, 6,365,375, 6,294,323, 6,261,797, 6,124,090 and 5,612,199, isothermal amplification (e.g., rolling circle amplification (RCA), hyperbranched rolling circle amplification (HRCA), strand displacement amplification (SDA), helicase-dependent amplification (HDA), PWGA, or any other nucleic acid amplification method using techniques well known to those of skill in the art.

[00199] "Polymerase chain reaction," or "PGR," refers to a reaction for the in vitro amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of **DNA.** In other words, PGR is a reaction for making multiple copies or replicates of a target nucleic acid flanked by primer binding sites, such reaction comprising one or more repetitions of the following steps: (i) denaturing the target nucleic acid, (ii) annealing primers to the primer binding sites, and (iii) extending the primers by a nucleic acid polymerase in the presence of nucleoside triphosphates. Usually, the reaction is cycled through different temperatures optimized for each step in a thermal cycler instrument. Particular temperatures, durations at each step, and rates of change between steps depend on many factors well-known to those of ordinary skill in the art, e.g., exemplified by the references: McPherson et al., editors, PGR: A Practical Approach and PCR2 : A Practical Approach (IRL Press, Oxford, 1991 and 1995, respectively). For example, in a conventional PGR using Taq DNA polymerase, a double stranded target nucleic acid may be denatured at a temperature greater than 90 °C, primers annealed at a temperature in the range 50-75 °C, and primers extended at a temperature in the range 72-78 °C.

[00200] The term "PGR" encompasses derivative forms of the reaction, including but not limited to, RT-PCR, real-time PGR, nested PGR, quantitative PGR, multiplexed PGR, assembly PGR and the like. Reaction volumes range from a few hundred nanoliters, e.g., 200 nL, to a few hundred microliters, e.g., 200 microliters. "Reverse transcription PGR," or "RT-PCR," means a PGR that is preceded by a reverse transcription reaction that converts a target RNA to a complementary single stranded DNA, which is then amplified, e.g., Tecott et al., U.S. Pat. No. 5,168,038. "Real-time PGR" means a PGR for which the amount of reaction product, i.e., amplicon, is monitored as the reaction proceeds. There are many fomis of real-time PGR that differ mainly in the detection chemistries used for monitoring the reaction product, e.g., Gelfand et al., U.S. Pat. No. 5,210,015 ("Taqman"); Wittwer et al., U.S. Pat. Nos. 6,174,670 and 6,569,627 (intercalating dyes); Tyagi et al., U.S. Pat. No. 5,925,517 (molecular beacons). Detection chemistries for real-time PGR are reviewed in Mackay et al., Nucleic Acids Research, 30:1292-1305 (2002). "Nested PGR" means a two-stage PGR wherein the amplicon of a first PGR becomes the sample for a second PGR using a new set of primers, at least one of which binds to an interior location of the first amplicon. As used herein, "initial primers" in reference to a nested amplification reaction mean the primers used to generate a first amplicon, and "secondary primers" mean the one or more primers used to generate a second, or nested, amplicon. "Multiplexed PGR" means a PGR wherein multiple target sequences (or a single target sequence and one or more reference sequences) are simultaneously

carried out in the same reaction mixture, e.g. Bernard et al. (1999) Anal. Biochem., 273 :221-228 (two-color real-time PGR). Usually, distinct sets of primers are employed for each sequence being amplified. "Quantitative PCR" means a PCR designed to measure the abundance of one or more specific target sequences in a sample or specimen. Techniques for quantitative PCR are well-known to those of ordinary skill in the art, as exemplified in the following references: Freeman et al, Biotechniques, 26:1 12-126 (1999); Becker-Andre et al., Nucleic Acids Research, 17:9437-9447 (1989); Zimmerman et al., Biotechniques, 21 :268-279 (1996); Diviacco et al., Gene, 122:3013-3020 (1992); Becker-Andre et al., Nucleic Acids Research, 17:9437-9446 (1989); and the like.

[00201] In one aspect of the invention, a method of performing isothermal DNA amplification is provided. The method can includes two steps. The first step includes forming a mixture. The mixture includes a double-stranded DNA template having a first strand and a second strand; a confonnationally-constrained helicase; a DNA-dependent DNA polymerase; a first oligonucleotide primer complementary to a portion of the first strand; a second oligonucleotide primer complementary to a portion of the second strand; and an amplification buffer cocktail. The second step includes incubating the mixture at a temperature compatible for activating the conformationally-constrained helicase and DNA-dependent DNA polymerase. In some embodiments of this aspect the conformationally-constrained helicase is selected from SEQ ID NOs: 4 and 12.

Nucleic Acid Sequencing

[00202] In certain exemplary embodiments, methods of determining the sequence identities of nucleic acid sequences are provided. Determination of the sequence of a nucleic acid sequence of interest can be performed using variety of sequencing methods known in the art including, but not limited to, sequencing by hybridization (SBH), sequencing by ligation (SBL), quantitative incremental fluorescent nucleotide addition sequencing (QIFNAS), stepwise ligation and cleavage, fluorescence resonance energy transfer (FRET), molecular beacons, TaqMan reporter probe digestion, pyrosequencing, fluorescent in situ sequencing (FISSEQ), FISSEQ beads (U.S. Pat. No. 7,425,43 1), wobble sequencing (PCT/USQ5/27695), multiplex sequencing (U.S. 2008/0269068; Porreca et al (2007) Nat. Methods 4:931), polymerized colony (POLONY) sequencing (U.S. Pat. Nos. 6,432,360, 6,485,944 and 6,51 1,803, and PCT/US05/06425), nanogrid rolling circle sequencing (ROLONY) (U.S. 2009/0018024), nanopore sequencing (using platforms such as those from Agilent, Oxford, Sequenom, Noblegen, NABsys,

Genia), allele-specific oligo ligation assays (e.g., oligo ligation assay (OLA), single template molecule OLA using a ligated linear probe and a rolling circle amplification (RCA) readout, ligated padlock probes, and/or single template molecule OLA using a ligated circular padlock probe and a rolling circle amplification **(RCA)** readout) and the like. High-throughput sequencing methods, e.g., on cyclic array sequencing using platforms such as Roche 454, Illumina Solexa, ABI-SOLiD, ION Torrents, Complete Genomics, Pacific Bioscience, Helicos, Polonator platforms (Worldwide Web Site: Polonator.org), and the like, can also be utilized. High-throughput sequencing metliods are described in U.S. 2010/0273164. **A** variety of light-based sequencing technologies are known in the art (Landegren et al. (1998) Genome Res. 8:769-76; Kwok (2000) Pharmocogenomics **1:95-100;** and Shi (2001) Clin. Chem. **47:164-172).**

[00203] In certain exemplary embodiments, the DNA-dependent DNA polymerase is selected from **a** group consisting of *K coli* DNA Pol I, *K coli* DNA Pol I Large Fragment, *Bst* 2.0 DNA Polymerase, *Bst* DNA Polymerase, *Bst* DNA Polymerase Large Fragment *Bsu* DNA Polymerase I Large Fragment T4 DNA Polymerase, T7 **DNA** polymerase, PyroPhage® 3173 **DNA** Polymerase and phi29 **DNA** Polymerase. In some embodiments, the confomiationally-constrained helicase includes a helicase selected from supertamily 1, wherein the helicase has a first amino acid residue and a second amino acid reside, and wherein the first and second amino acid residues are in proximity. The confbniiationally-constrained helicase also includes a linker, wherein the linker comprises a first covalent bond with the first amino acid residue and a second covalent bond with the second amino acid residue. In some embodiments of this aspect, the conformationally-constrained helicase includes **a** crosslinked, closed form helicase monomer.

Expression of Helicase-^ Polypeptides

[00204] The nucleic acids encoding the Rep-$_X$ and PcrA-$_X$ polypeptides can be adapted to suitable expression systems for producing the helicasex polypeptides for helicase-χ production. For DNAs encoding helicasex genes, the representative genes can be operably-linked to suitable expression vectors for expressing the proteins in bacterial, fungal, insect or other suitable expression host. For RNAs encoding helicase-χ polypeptides, the representative RNAs can be engineered for enabling efficient expression *in vitro* of the polypeptides in extract lysates produced from bacterial, fungal, insect or other suitable expression host sources. Such systems are well known in the art. Following expression, the

helicase-χ polypeptides can be purified by methods known in the art, including affinity-tag chromatography, SDS-PAGE, and size-exclusion chromatography, among others.

[00205] In certain exemplary embodiments, vectors such as, for example, expression vectors, containing a nucleic acid encoding one or more helicase-χ polypeptides described herein are provided. As used herein, the term "vector" refers to a nucleic acid molecule capable of transpoiting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

|00206| In certain exemplar}' embodiments, the recombinant expression vectors comprise a nucleic acid sequence (e.g., a nucleic acid sequence encoding one or more helicase-χ polypeptides described herein) in a form suitable for expression of the nucleic acid sequence in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence encoding one or more helicase-χ polypeptides is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *i/ι vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 1**85,** Academic Press, San Diego,

Calif. (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors described herein can be introduced into host cells to thereby produce proteins or portions thereof, including fusion proteins or portions thereof, encoded by nucleic acids as described herein (e.g., one or more helicasex polypeptides).

[00207] Recombinant expression vectors of the invention can be designed for expression of one or more encoding one or more helicase-χ polypeptides in prokaryotic or eukaryotic cells. For example, one or more vectors encoding one or more helicase-χ polypeptides can be expressed in bacterial cells such as *li coli,* insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro,* for example using *11* promoter regulatory sequences and *11* polymerase.

[00208] Expression of proteins in prokaiyotes is most often carried out in *K coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) Gene 67:3 1-40); pMAL (New England Biolabs, Beverly, Mass.); and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[00209] In another embodiment, the expression vector encoding one or more helicase-χ polypeptides is a yeast expression vector. Examples of vectors for expression **in** yeast S. cerevisiae include pYepSec 1 (Baldari, et. al, (1987) EMBO J. 6:229-234); pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943); pJRY88 (Schultz et al., (1987) Gene 54:1 13-123); pYES2 (Invitrogen Corporation, San Diego, Calif); and picZ (Invitrogen Coiporation).

[00210] Alternatively, one or more helicase-χ polypeptides can be expressed in insect cells using baculovirus expression vectors. Baeulovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith et al. (1983) Mol. Cell. Biol. 3 2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:3 1-39).

[00211] In certain exemplar}' embodiments, a nucleic acid described herein is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and **pMT2PC** (Kaufman et **al.** (1987) EMBO J. 6:187-195). When used **in** mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see Green M., and Sambrook, J. Molecular Cloning: A Laboratory Manual. 4th, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012.

[00212] In certain exemplar}' embodiments, host cells into which a recombinant expression vector of the invention has been introduced are provided. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur **in** succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00213] **A** host cell can be any prokaryotic or eukaryotic cell. For example, one or more helicase-χ polypeptides can be expressed in bacterial cells such as *K coli,* viral cells such as retroviral cells, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[00214J Delivery of nucleic acids described herein (e.g., vector DNA) can be by any suitable method **in** the art. For example, deliver}' may be by injection, gene gun, by application of the nucleic acid in a gel,

oil, or cream, by electroporation, using lipid-based transtection reagents, or by any other suitable transtection method.

[00215] As used herein, the terms "transformation" and "transtection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transtection, lipofection (e.g., using commercially available reagents such as, for example, LIPOFEO TIN™ (Invitrogen Corp., San Diego, Calif), LIPOFECT AMINE™ (Invitrogen), FUGENE™ (Roche Applied Science, Basel, Switzerland), JETPEI™ (Polyplus-transfection Inc., New York, NY), EFFECTENE ™ (Qiagen, Valencia, Calif), DREAMFECT™ (OZ Biosciences, France) and the like), or electroporation (e.g., *in vivo* electroporation). Suitable methods for transforming or transfecting host cells can be found in Green and Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 4th, ed., Cold Spring Harbor Laboratoiy, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, 2012), and other laboratoiy manuals.

Kits

[00216] In another aspect, kits are contemplated in this disclosure. For example, a kit for performing helicase dependent amplification is provided. The kit can include a conformationally-constrained helicase and an optional amplification buffer cocktail. The conformationally-constrained helicase of the kit includes one or more helicasex polypeptides having a covalent linkage (e.g., reacted with a suitable intramolecular crosslinking agent) to form closed form helicase-χ monomers having super helicase activity of the type described for Rep-X and PcrA-X. In particular, the conformationally-constrained helicase can be generated form reacting SEQ ID NOs:4 and 9 with a suitable intramolecular crosslinking agent. Representative conformationally-constrained helicases include those of SEQ ID NOs:4 and 12.

[002 17] The kit can further include a DNA-dependent DNA polymerase. Exemplary DNA-dependent DNA polymerases for inclusion in kit include a polymerase selected from a group consisting of *E. coli* DNA Pol I, *K coli* DNA Pol I Large Fragment, *Est* 2.0 DNA Polymerase, *Est* DNA Polymerase, *Est* DNA Polymerase Large Fragment *Esu* DNA Polymerase I Large Fragment, T4 DNA Polymerase, T7 DNA polymerase, PyroPhage® 3173 DNA Polymerase, phi29 DNA Polymerase and the like.

EXAMPLES

Example **1.** *Mutagenesis and purification of protein*

[00218] Preparation of pET expression plasmids containing cysteine-less *rep* (CI 8L, C43S, C167V, C178A, C612A) and *pcrA* (C96AC247A) with N-terminal hexa-histidine-tags (SEQ ID NO: 36) were performed as described previously (Park *et al.* (2005) ***supra;*** I. Rasnik, S. Myong, W. Cheng, T. M. Lohman, T. Ha, DNA-binding orientation and domain conformation of the E. coli rep helicase monomer bound to a partial duplex junction: single-molecule studies of fluorescently labeled enzymes. *J. Mol. Biol.* 336, 395-408 (2004)). Site-directed mutations to introduce two Cys residues for crosslinking (Rep-X: A178C/S400C, Cys178 is a native cysteine in the wild type, Rep-Y: D127C/S494C, PcrA-X: N187C/L409C) were done using QuikChange Lightning kit (Life Technologies, Inc.) and mutagenic primer oligonucleotides (Integrated DNA Technologies Inc., Coralville, IA). Protein purifications were pafonned as described previously (Park *et al.* (2005) ***supra,*** Rasnik *et al.* (2004) *supra).* Catalytic activity levels of purified proteins as well as those of the crosslinked samples were determined in a ssDNA-dependent ATPase activity assay using the Invitrogen EnzChek phosphate assay kit (Life Technologies Inc.), the oligonucleotide (dT)4$ (SEQ ID NO: 305) and 1 niM ATP in buffer D (see ensemble FRET unwinding assay).

[00219] Wild type RepD from *Staplrylococcus aureus* was purified as described in (Slatter *et al.* ((2009) *supra;* Zhang *etal,* (2007) *supra)* with the following differences. A wt-RepD encoding pET 11m-RepD plasmid was constructed for expression in B834 (pLysS). The gene sequence contained silent mutations to introduce restriction sites for AgeI, PstI, SacI, and to modify the nick site (TCT'AAT to TCGAAT) to prevent premature cleavage by RepD during expression. An ammonium sulfate precipitated pellet (from 0.5 L culture) was resuspended and run through serially connected 5 ml Q-Sepharose (removed once the sample was through) and 5 ml heparin-Sepharose cartridges connected in series (GE Healthcare), and eluted on an ÄKTA purifier 10 FPLC system.

Example 2, **Intra-crosslinking** of Rep and PcrA

[00220] Dual-cysteine Rep mutants were incubated overnight at 4 °C with 2- to 100-fold excess ofbis-maleimide crosslinkers DTME (13 A) and BMOE (8 A) purchased from Thermo Fisher Scientific, Rockford, IL (Fig. 10). PcrA-X was crosslinked with DTME and BM(PEG)2 (14.7 A) from the same manufacturer. Excess crosslinkers were removed by Bio-Rad P-30 desalting column. Crosslinked

Rep-X, Rep-Y and PcrA-X samples were stored at -20°C or -80°C as described (Park *etal* (2005) ***supra,*** Rasnik ***et id.*** (2004) *supra).* Data presented in this manuscript used BMOE (8 A), but other crosslinkers of various lengths gave similar results. DIME is a di-sulfide containing crosslinker that we reduced with β-mercaptoethanol (β-ME) or tris(2-carboxyethyl) phosphine (TCEP) to revert the crosslinked helicase to the non-crosslinked form for control purposes.

[00221] Crosslinking of the double Cys mutants with thebis-maleimide linkers has the potential of producing covalently attached multimeric species, in addition to the intended internally crosslinked monomelic species. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) can distinguish these species from the non-crosslinked monomers (I L. Urbatsch *et a/.,* Cysteines 431 and 1074 are responsible for inhibitory disulfide cross-linking between the two nucleotide-binding sites in human P-glycoprotein. *J Biol Chem.* 276, 26980-26987 (2001)). Here we show a representative analysis of a crosslinked Rep-Y sample. Crosslinked Rep-X and Rep-Y produced three bands on a SDS polyacrylamide gel **(FIG.** 7A): a bottom band at -76 kDa that was the same as the non-crosslinked Rep, a slightly retarded dominant middle band at -100 kDa for Rep-Y and -90 kDa for Rep-X and a much more slowly migrated, very faint top band at -300 kDa. FIG. 7B shows three such bands of a Rep-Y sample (lane Rep-Y) crosslinked with a cleavable di-sulfide containing crosslinker (DTME). The dominant middle band and the fainter top band were the crosslinked species because they disappeared upon cleavage of the crosslinker using beta-mercaptoethanol (β-ME) (lane Rep-Y*). Relative shift between the middle bands of Rep-X and Rep-Y (FIG. 7A) was a strong indication of an internally crosslinked monomelic species, because the denatured Rep-X and Rep-Y would be likely to migrate at different rates due to the different size of peptide loops introduced by the internal crosslinker (denatured Rep-Y has a loop of 368 amino acids (aa) whereas Rep-X loop is 223 aa long). In order to ensure that the dominant middle band is not multimeric but is the intramoleailarly crosslinked monomelic species, a Rep-Y sample was fractionated according to molecular size on a Superdex 200 size exclusion chromatography (SEC) column controlled by an FPLC apparatus. Elution profiles of Rep-Y and non-crosslinked Rep are shown in the **FIG.** 7C. Eluted fractions were analyzed on an SDS polyacrylamide gel (FIG. 7D, lanes F1-F7). The multimeric species that was eluted in the early SEC fractions (11-13 ml) displayed only the top band whereas the dominant middle band was eluted together with the non-crosslinked Rep monomer in the SEC analysis, showing that the middle band represents the intramolecularly crosslinked species and the top band is multimeric. After establishing that the intra-

crosslinked protein shows up as a retarded band compared to the non-crosslinked form on the SDS polyacrylamide gels (such as the Rep-Y data presented here), we used this assay to check the efficiency of crosslinking reactions for Rep-X, Rep-Y and PcrA-X (86%, 73% and 58% respectively for the samples used in this manuscript). The Rep-Y form exhibited ATPase activity on par with non-crosslinked Rep (FIG. 7E).

Example 3. Size **exclusion** chromatography and SDS-PAGE analysis

[00222] Crosslinked Rep and PcrA samples were separated from mu!timeric byproducts using Superdex 200 grade 10/300GL or HiLoad 16/600 gel filtration columns on an ÄKTA purifier 10 FPLC system. The crosslinking efficiency was monitored by SDS-PAGE analysis on 7.5-10% Tris-glycine gels (Bio-Rad). As needed for gel analysis, reduction of samples crosslinked with DTME was achieved by adding 5% (v/v) β-ME during the SDS denaturation step.

Example 4, Ensemble FRET **unwinding** assay

[00223] Multiple turnover ensemble unwinding kinetics was used to gauge the effect of the mutations and conformational modifications to the helicase activity. We used an 18-bp FRET labeled DNA substrate with a 3'-$(dT)i_0$ overhang (SEQ ID NO: 33) (FIG. IC), constructed by annealing complementary oligonucleotides DNA7 (Cy5-GCC TCG CTG CCG TCG CCA (SEQ ID NO: 40)) and amino-dT labeled DNA8 (TGG CGA CGG CAG CGA GGC-(T-Cy3)-T_{10} (SEQ ID NO: 41)). Alternatively, another similarly labeled 50-bp DNA with a 3'-$(dT)_{30}$ overhang (SEQ ID NO: 17) was also used. This construct was made by annealing oligonucleotides **DN**A9 (Cy5-TCA ACT AGC AGT CAT AGG AGA AGT ATT AAC ATG CCT CGC TGC CGT CGC CA (SEQ ID NO: 42)) and amino-dT labeled DNA10 (TG GCG **ACG GCA** GCG AGG CAT GTT **AAT** ACT **TCT** CCT ATG ACT GCT AGT TGA (T-Cy3) T_{29} (SEQ ID NO: 43)). Unless otherwise stated, 5 nM ensemble FRET DNA was mixed with 50 nM helicase in buffer D (10 mM Tris-HCl [pH 8.0], 15 mMNaCl, lOmMMgC!$_2$, 10% (v/v) glycerol, 0.1 mg/ml BSA) and I mM ATP was added to start the unwinding reaction in a quartz cuvette. A Gary Eclipse fluorescence spectrophotometer was used to measure the donor *(Isssnm)* and the acceptor signals (I_{667nm}) under 545-nm excitation (5-nm slit, 2-10 Hz acquisition rate and 600-900V photomultiplier voltage). Unwinding of the substrate was monitored by the decrease in ensemble E_{FRET} value, defined as

$E_{FRET\text{-}ensemble} = I_{667nm}/(I_{555nm} - I_o + I_{667nm})$ where I_o was the baseline donor signal of unpaired Cy3 prior to addition of ATP.

Example 5. **smFRET** unwinding and **RepD-PerA** interaction assays

[00224] All smFRET experiments were conducted on a custom-built prism type TIRF microscopy stage with an Andor EMCCD camera as described in R. Roy, S. Holing, T. Ha, A practical guide to single-molecule FRET. *NatMethods* 5, 507-516 (2008) and C. Joo, T. Ha, in *Cold Spring Harh Protoc.* (2012), vol. 2012. Reaction chambers were formed by quartz slides and glass coverslips passivated with polyethyleneglycol (PEG) and 1%biotinylated PEG (mPEG-SC and bio-PEG-SC, LaysanBio, Arab, AL), followal by 5 min incubation with Neutravidin (Thermo Scientific, Newington, NIT) for immobilization of biotinylated molecules on the chamber surface as described below.

[00225] For the smFRET unwinding experiments, the reaction chamber was first incubated with biotinylated anti penta-histidine tag (SEQ **ID NO:** 44) antibody (Qiagen, Valencia, CA), followed by 10-30 min incubation of Ffise-tagged (SEQ ID NO: 36) helica.se sample (0.5-1 nM). The unwinding of the DNA was initiated by flowing 1 **n**M smFRET **DSN A** and 1 **m**M ATP in the reaction buffer **A** (10 **m**M Tris-HCl [pH 8.0], lOmMMgCb, 15 mMNaCi, 10% (v/v) glycerol, , 1% (v/v) gloxy and 0.2% (w/v) glucose, an oxygen scavenging system (Y. Harada, K. Sakurada, T. Aoki, D. D. Thomas, T. Yanagida, Mechanochemical coupling in actomyosin energy transduction studied by in vitro movement assay. *J. Mol. Biol.* 216, 49-68 (1990).) and 3-4 mM Trolox (T. Yanagida, M. Nakase, K. Nishiyama, F. Oosawa, Direct observation of motion of single F-actin filaments in the presence of myosin. *Nature* 307, 58-60 (1984); I. Rasnik, S. A. McKinney, T. Ha, Nonblinking and long-lasting single-molecule fluorescence imaging. *NatMethods* 3, 891-893 (2006)). The smFRET DNA substrate was constructed by annealing the oligonucleotides DNA3 (Cy5-GCC TCG CTG CCG TCG CCA (SEQ **ID** NO: 40)) and DNA4 (Cy3-TGG **CGA** CGG CAG CGA GGC-T_{20}(SEQ ID **NO:** 45)). The **PcrA-RepD** interaction assay involved preparation of the RepD-*oriZ)* DNA adduct as described in Slatter *et al.* (2009) *supra.* A biotinylated *oriD* DNA substrate was constructed by annealing oligonucleotides **DN**A1 (CTA ATA GCC GGT TAA GTG GTA ATT TTT TTA CCA CCC AAA GCC TGA AGA GCT AAT CGT TCG G (SEQ ID **NO:** 46)) and DNA2 (biotin-CCG AAC GAT TAG CTC TTC **AGG** CTT TGG GTG GTA AAA AAA TTA CCA $CTT)_{15}$ (SEQ ID NO: 47)). In one chamber, only *oriD* DNA (50-100 pM) was immobilized on the surface. In a second chamber the RepD-*oriZ)* **DNA** adduct was immobilized. 100-

500 pM dual labeled PcrA-DMl was injected into the chambers in buffer B (10 niM Tris [pH7.5], **10%** glycerol, 15 mMNaCl, 50 **niM** KC1, 5 **mM** $MgCl_2$, 3.4 **niM** Trolox, 1% **(v/v)** gloxy, 0.2% **(w/v)** glucose). Short movies of multiple chamber regions were recorded. Since the **two** Cys residues of PcrA-DMl were randomly labeled with Cy3-Cy5 mixture, each movie contained a brief initial 633-nm laser excitation period to determine the molecules with a fluorescent Cy5, followed by turning on the 532-nm laser for Cy3 excitation. Only the PcrA-DMl molecules with a colocalized donor-acceptor pair were factored in the E_{FRET} histograms.

[00226] smFRET signals were acquired by an Andor **EM**CCD camera operated with a custom software at 16-100-ms time resolution. E_{FRET} was calculated as described in **R**. Roy, S. Hohng, T. Ha, A practical guide to single-molecule FRET. *Nat Methods* **5,** 507-516 (2008). Unwinding periods were measured as described in the text. The fraction of unwinding events was calculated as the proportion of the all DNA binding events that displayed an E_{FRET} increase phase. Error bars were calculated according to Clopper-Pearson binomial proportion confidence interval method (C. J. Clopper, E. S. Pearson, The use of confidence or fiducial limits illustrated in the case of the binomial. *Biomettika* **26,** 404-413 (1934)).

Example 6. Optical tweezers assay

[00227] The optical trap handle was a 6098-bp long DNA, amplified from λ-phage DNA and flanked by a 5'-biotin and a 3'-$(dT)i_{0,i5,75}$ overhang **(SEQ** ID NOS 33-35, respectively) on the other end. First, a **5'**-tailed 6083-bp fragment was amplified by the auto-sticky PCR reaction **(J.** Gal, R. Schnell, S. Szekeres, M. Kalman, Directional cloning of native PCR products with preformed sticky ends (autosticky PCR). *MolGen Genet.* **260,** 569-573 (1999)) using primers P I (biotin-GGC **AGG GAT ATT CTGGCA** (SEQ ID NO: 48)) and P2 (GAT CAG TGG ACA GA-abasic-A AGC CTG AAG AGC TAA TCG TTC GG (SEQ ID NO: 49)). Subsequently the amplicon was annealed and ligated with oligonucleotide DNA5 (TTC **TGT** CCA CTG ATC-$(T)_{10,15,75}$ (SEQ ID **NOS** 50-52, respectively)) to create the **3'**-overhang for the initial helicase binding (10, 15 or **75-nt,** as specified in figures). DNA beads were prepared by adding biotinylated 6-kbp DNA to the streptavidin-coated polystyrene beads (0.79 μηι **in** diameter, Spherotech, Lake Forest, **IL),** and incubated at 25°C for 30 min. Protein samples were pre-incubated with biotinylated anti penta-histag (SEQ ID NO: 44) antibody (Qiagen, Valencia, CA) on ice for 1hour. One microliter of this mixture, 1 μï **of** streptavidin beads, and 8 μï buffer (100 mM **Tris-HCl** [pH 7.5], 100 mM NaCl, 10% glycerol **(v/v))** were mixed and incubated for 30 min on ice to make the

protein coated beads. Reactions were performed in laminar flow chambers that were designed and assembled as described in Z. Qi, R. A. Pugh, M. Spies, Y. R. Chenila, Sequence-dependent base pair stepping dynamics in XPD helicase unwinding. *FJife (Cambridge)* 2, e00334 (201 3). Reaction buffer C consisted of 100 niM Tris pH 8.0, 15 mMNaCI, 10% (v/v) glycerol, 10mMMgCl $_2$, and an oxygen scavenging system (100 μg/ml glucose oxidase, 20 μg/ml catalase, and 4 mg/ml glucose) to reduce photo damage to the sample (M. P. Landry, P. M. McCall, Z. Qi, Y. R Chemla, Characterization of photoactivated singlet oxygen damage in single-molecule optical trap experiments. *Biophysical journal* 97, 2 128-2136 (2009)). The reaction chamber contained two laminar streams of buffer C with different ATP, ATP-yS and SSB concentrations as described in the text. The dual-trap optical tweezers were set up and calibrated as described in (C. Bustamante, Y. R. Chemla, J. R. Moffitt, *High-resolution dual-trap optical tweezers with differential detection.* Single-molecule techniques: a laboratory manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2008); K. Berg-Sorensen, H. Flyvbjerg, Power spectrum analysis for optical tweezers. *Review of Scientific Instruments* 75, 594-612 (2004)). All measurements were recorded at 100 Hz with a custom Lab View software (8.2; National Instruments, Austin, TX) and smoothed with a 100 Hz boxcar filter. In the "force-feedback" mode, unwinding was allowed to occur against a constant force of 10-22 pN (as specified). The contour length of DNA was calculated from the measured force and end-to-end extension of the molecule and using the worm-like chain model (persistence length of 53 nm, stretch modulus of 1,200 pN and distance per base-pair of 0.34 ran). The velocity of DNA unwinding in the force feedback mode was determined from a linear fit of the contour length of DNA in a sliding window ofO.2 s (21 data points). Pauses longer than 0.2 s were removed and then the velocity was averaged in 1s bins. Error for the fraction of unwinding events per tether formation was calculated with the Clopper-Pearson binomial proportion confidence interval method (CloppertVi//. (1934) *s¾ora*)).

[00228] The force dependence of Rep-X unwinding activity was measured in the "fixed-trap" mode, by stopping the force feedback. The force data (100 Hz) was smoothed with a gaussian filter (by applying a 33-Hz moving average filter 10 times). Paused regions (velocity < 10 bp/s) were removed. The pause-free unwinding velocities were calculated and normalized by the velocity at 20 pN for each molecule, and binned against the dynamic force values up to 60 pN to create the V_{norm} vs. /7 plot (**FIG.** 3F). We previously found that the force response of our trap was linear against bead displacements up to 72 nm (determined in a separate experiment measuring where the force vs. extension curve of dsDNA started to

deviate from the theoretical worm like chain. At a trap stiffness of 0.167 pN/nm, the deviation occurred above 12 pN). Hence we calculated the maximum reliable force to be at least 59 pN at a trap stiffness of 0.82 pN/nm.

Example 7, Ensuring **monomelic** Rep-X **activity** in optical tweezers assay

[00229] We considered the possibility that the highly processive unwinding observed in our optical tweezers assay was caused by multiple Rep-X acting on the same DNA. If multimeric Rep-X had been required for highly processive unwinding, then the majority ofbinding events (i.e. formation of a tether) would not have displayed unwinding activity, because single Rep-X binding is the statistically the most probable event during the brief period of contact between the two beads. However, the majority of tethers formed displayed highly processive unwinding, suggesting that the processive unwinding is caused by a single Rep-X protein.

[00230] To further establish that the unwinding of the 6-kbp DNA was achieved by single Rep-X molecule, we repeated the experiment using beads incubated in lower concentrations of Rep-X, thus decreasing the number of Rep-X molecules per bead. Consequently, Rep-X binding (tether formation) took longer and required more trials ofbumping the two beads. As the Rep-X concentration was lowered (20 nM, 4 nM and 0.4 nM) during the pre-incubation with 20 nM biotinylated antibody, the efficiency of tether formation was also reduced (7 out of 11, 9 out of 27 and 2 out of 16 beads, respectively). However, the subsequent unwinding was still the prevalent behavior (7 out of 7, 8 out of 9 and 2 out of 2 tethers, respectively).

[00231] As another test to ensure that the highly processive unwinding was due to a single Rep-X molecule, not multiple molecules, we compared the unwinding reaction of DNA with 75nt vs. 10- and 15-nt3' overhangs. Since the footprint of Rep is reported to be 8-10 nt(S. Korolev, J. Hsieh, G. H. Gauss, T. M. Lohman, G. Waksman, Major domain swiveling revealed by the crystal structures of complexes of E. coli Rep helicase bound to single-stranded DNA and ADP. *Cell* 90, 635-647 (1997)), 10 or 15-nt overhang would increase the chance of single Rep-X binding. Rep-X exhibited the same highly processive behavior on the short overhang DNA molecules (17 out of 18 tethers formed with 10- and 15-nt overhang DNA vs. 21 out of 22 tethers formed with 75nt overhang DNA, FIG. 3B, C), further indicating that the high processivity of unwinding is the property of a Rep-X monomer.

[00232] To test the possibility that the unwound ssDNA interacted with additional Rep-X on the bead surface, possibly increasing the processivity of unwinding, we added 66 nM of *E. coli* ssDNA binding protein (SSB) in the unwinding reaction stream in order to render the unwound ssDNA inaccessible to other Rep-X molecules. Inclusion of SSB did not change the highly processive behavior of unwinding (17 out of 18 tethers formed in the absence of SSB vs. 21 out of 22 tethers formed in the presence of SSB, FIG. 3B), suggesting that DNA unwinding by Rep-X is highly processive whether the unwound ssDNA is sequestered by SSB or not. This observation is probably due to the design of the dual optical tweezers assay, in which the DNA is under tension only between the "front runner" Rep-X molecule and the streptavidin on the other bead. Binding of a second Rep-X to the already unwound ssDNA should not affect the measurements because the second Rep-X, which is also tethered to the bead, cannot interact with the front runner that is tethered elsewhere on the bead.

Example 8. Selection of crosslinking sites and crosslinker length

[00233] Open (inactive) and closed (active) form crystal structures of Rep and similar helicases were used as a visual guide. The target residue pair for crosslinking and the crosslinker were selected based on these criteria.

[00234] One target residue of the target residue pair should be located on the mobile 2B domain and the other target residue should be located on the immobile body of the helicase (for example on 1B or 1A domains). Preferably, target residue pair should not be part of functional helicase motifs known in the literature to prevent detrimental effects of amino acid engineering. Preferably the target residue pair should not be conserved residues. Preferably the target residue pair should be as far away as possible from the ssDNA binding sites. These measures reduce the potentially detrimental effects of the target residue mutations and crosslinking on the basic translocation function of the helicase.

[00235] The target residues should be as close as possible to each other in the closed (active) conformation of 2B domain, and at the same time should be as far as possible from each other in the open (inactive) conformation. For example, the distance between the target residue pair should be less than 15 Å in the closed form (measured from alpha carbon coordinates) and should increase by more than 30 Å during transition to open form, so that a short crosslinker can prohibit the transition to an inactive (open) form. Residues that satisfy such criteria can be determined for helicases with known crystal structures in closed or open forms.

[00236] By sequence alignment, the corresponding crosslinking target residues can be found in helicases with unknown structures to convert those to superhelicases, as well. Sequence homology models can also be employed.

[00237] Target residues should be preferably on the surface of the protein, and their side chains should be facing outward and more preferably facing toward each other.

[00238] The crosslinker should be as short as possible, preferably only long enough to efficiently link the target residue pair in the desired conformation. Crosslinker length should be considerably shorter than the distance between the target residues in the unwanted conformation.

[00239] A representative 56 Rep homologs/orthologs with 90% identity to and 80% overlap are shown in Table 4, which are also shown in Figs. **9**A-G. The target residues of Figs. 9A-G were selected from one residue from domain LA or domain IB, and one residue from domain 2B which satisfy the all these considerations. For PerA, or a homolog thereof, the target residues are selected from residues 92-1 16 of domain 1A or 178-196 of domain IB, and 397-41 1, 43 1-444 or 526-540 of domain 2B. For Rep, or a homolog thereof, the target residues are selected from 84-108 of domain 1A or 169-187 of domain IB, and 388-402, 422-435 or 519-536 of domain 2B. For UvrD, or a homolog thereof, the target residues are selected from residues 90-1 14 of domain 1A or 175-193 of domain 1B, and 393-407, 427-440 or 523-540 of domain 2B.

Table 4.

Rep homolog	Organism
REP_BUCAP	Buchnera aphidicola subsp. Schizaphis gram inum (strain Sg)
REP_BUCAJ	Buchnera aphidicola subsp. Acyrthosiphon pisum (strain APS) (Acyrthosiphon pisum symbiotic bacterium)
REP_ECOLI	Escherichia coli (strain K12)
REP_HAEIN	Haemophilus influenzae (strain ATCC 51907 / DSM 11121 / KW20 / Rd)
REP_SALTY	Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)
A0A077ZIR6 _TRITR	Trichuris trichiura (Whipworm) (Trichocephalus trichiurus)

S3IEG5_9ENTR	Cedecea davisae DSM 4568
J1R585_9ENTR	Kosakonia radicincitans DSM 16656
K8ABZ8_9ENTR	Cronobacter muytjensii 530
A0A060V.I91_KLEPN	Klebsiella pneumoniae
A()A090V5M6_ESCVU	Escherichia vulneris NBRC 102420
A0A083 YZC2_CITAM	Citrobacter amalonaticus
A0A0J6D7T8_SALDE	Salmonella derby
A OA085 ITL8_RAOPL	Raoiiltella planticola ATCC 3353 1
E7T4Q1_SHTO0	Shigella boydii ATCC 9905
A OA O85GMM2_9ENTR	Buttiauxella agrestis ATCC 33320
A OA085HAK 1_9ENTR	Leclercia adecarboxylata ATCC 232 16 = NBRC 102595
D4BE16_9ENTR	Citrobacter youngae ATCC 29220
A0A0H5PMJ7_SALSE	Salmonella senftenberg
A0A0J1JQT3__CITFR	Citrobacter freundii
A0A OJ8VI05_9ENTR	Cronobacter sp. DJ34
F5S3F4_9ENTR	Enterobacter hormaechei ATCC 49162
D2ZMA5_9ENTR	Enterobacter cancerogenus ATCC 353 16
A0A084ZTW9_9ENTR	Trabulsiella guamensis ATCC 49490
A OA03 8CLT3_RAOOR	Raoultella omithinolytica (Klebsiella ornithinolytica)
Q8Z385_SALTI	Salmonella typhi
0831X8_SHIFL	Shigella llexneri
A0A0D5WYP4_9ENTR	Klebsiella michiganensis
A0A0H3FM3 1_ENTAK	Enterobacter aerogenes (strain ATCC 13048 / DSM 30053 / JCM 1235 / KCTC 2190 / NBRC 13534 / NCIMB 10102 / NCTC 10006) (Aerobacter aerogenes)
A0A0H2WUK6_SALPA	Salmonella paratyphi A (strain ATCC 9150 / SARB42)
A0A0H3H1F3__KLEOK	Klebsiella oxytoca (strain ATCC 8724 / DSM 4798 / JCM 2005 1 / NBRC 3318 / NRRL B-199 / KCTC 1686)

X7H46_CITFR	Citrobacter freundii UCI 31
A0A0H3CTF5_ENTCC	Enterobacter cloacae subsp. cloacae (strain ATCC 13047 / DSM 30054 / NBRC 13535 / NCDC 279-56)
D2TH67_CITRI	Citrobacter rodentium (strain ICC168) (Citrobacter freundii biotype 4280)
Q329V6_SHIDS	Shigella dysenteriae serotype 1 (strain Sd197)
W6J7C4_9ENTR	Kosakonia sacchari SPl
I2BE87_SHTOC	Shimwellia blattae (strain ATCC 29907 / DSM 4481 / JCM 1650 /NBRC 105725 / CDC 9005-74) (Escherichia blattae)
B5EZ38_SALA4	Salmonella agona (strain SL483)
A0A0F5SGU2_CITAM	Citrobacter amalonaticus
G9YY11_9ENTR	Yokenella regensburgei ATCC 43003
A0A090UXU3_9ENTR	Citrobacter werkmanii NBRC 105721
A9MJ31_SALAR	Salmonella arizonae (strain ATCC BAA-731 / CDC346-86 / RSK2980)
Q3YVI6_SHISS	Shigella sonnei (strain Ss046)
D3RHB6_KLEVT	Klebsiella variicola (strain At-22)
Q57HT8_SALCH	Salmonella choleraesuis (strain SC-B67)
B5RFS5_SALG2	Salmonella gallinarum (strain 287/91 / NCTC 13346)
A0A089Q204_9ENTR	Cedecea neteri
A0A0H3BNR1_SALNS	Salmonella newport (strain SL254)
C9Y4T0_SICTZ	Siccibacter turicensis (strain DSM 18703 / LMG 23827 / z3032) (Cronobacter turicensis)
B7LU77_ESCF3	Escherichia fergusonii (strain ATCC 35469 / DSM 13698 / CDC 0568-73)
A0A0H3TAW8_SALEN	Salmonella enteritidis
G2S5J6JENTAL	Enterobacter asburiae (strain LF7a)
A0A0F7JC30_SALET	Salmonella enterica I
A7MQI4_CROS8	Cronobacter sakazakii (strain ATCC BAA-894) (Enterobacter sakazakii)
L0M8J0_ENTBF	Enterobacteriaceae bacterium (strain FGI 57)

A0A0KQHFU2_SALB< C	Salmonella bongori (strain ATCC 43975 / DSM 13772 / NCTC 12419)
A8ACT1 _CITK8	Citrobacter koseri (strain ATCC BAA-895 / CDC 4225-83 / SGSC4696)

[00240] Use of shorter crosslinkers increase the efficiency of crosslinking reaction by favoring the intramolecularly crosslinked species rather than intermolecularly crosslinked multimeric species. These rules also ensure that tlie 2B domain is restricted to the active (closed) conioniiation, and cannot attain an open (inactive) conformation. Thus conformational control is achieved, and the possibility of 2B domain to swinging open to access an inactive (open) conformation is virtually eliminated.

[00241] Without being bound by theory, one possible explanation for the super activation would be the decreased dissociation rate due to tlie crosslinked protein encircling the ssDNA strand (indicated by the crystal structure, so that tlie protein cannot dissociate from the ssDNA easily. However, it was found that despite both Rep-X and Rep-Y encircling tlie ssDNA (as indicated by the crystal structure), only Rep-X was super-active. Thus, in order to create the super active helicase, immobilization of the correct conformational state of the 2B domain is necessary.

Example 9. **Identifying** Suitable Crosslinking Sites in Homologous **Helicases**

[00242] Based on the crosslinking target site selection criteria established in Example 8, potential crosslinking target residues in helicases were determined using known crystal structures. By sequence alignment and structural homology modeling, tlie corresponding crosslinking target residues are identified in helicases with unknown stmctures. Subsequently these helicases can be converted to superhelicase forms. For example, based on the criteria that the distance between tlie target residue pairs should be less than 15 A in closed form and should increase by more than 30 A in open form, we identified the residues in Rep, PcrA and UvrD helicases as shown in Figs. 9A-G. Homologous helicases are identified, for example, by 50% sequence identity and 80% overlap to the helicase with the known stmcture. For example, we found 3147 such proteins homologous to E. coli Rep, 1747 proteins homologous to B. st PcrA, and 1209 proteins homologous to E. coli UvrD helicases were found (Tables 5-7, respectively). Then the corresponding crosslinking residues are identified in any of the homologs. For example, we chose an example of 56 Rep homologs (Table4), and found the regions where the crosslinking residues can be engineered (Figs. 9.A-G). Despite the fact that the three model superfamily 1

helicases, U_{VTD}, Rep and PcrA, have only 35-40% sequence identity, they exhibit >90% structural homology according to their crystal structures. Hence it is reasonable to expect a highly similar structural homology from the proteins with 50% identity to and 80% overlap to the helicase with the known crystal structure; these are suitable candidates for crosslinking in the superhelicase (-X) form.

[00243] E. coli UvrD (ecUvrD) has 33% sequence identity with E. coli Rep (ecRep) and 42% sequence identity with Bacillus stearothermophillus PcrA (bsPcrA). Highlighted regions in Fig. 9A and 9G show the crosslinking sites obtained from the open form and closed form crystal structures and the criteria established in Example 8. These regions align well in the sequence showing that a sequence alignment can be used in helicases with unknown structures to determine the crosslinking target sites in helicases with unknown structures. For example, the crosslinking regions (boxed sequences of Fig. 9G) in *D. radiodurans* UvrD (drUvrD) were found by aligning its sequence to bsPcrA, ecRep and ecUvrD, 1A/IB residues: 92-116, 182-200, 2B residues: 400-414, 434-447 and 528-544. drUvrD (Q9RTI9) has 33%, 36% and 41% sequence identity to bsPcrA, ecUvrD and ecRep, respectively. These four proteins have 21% sequence identity as a group. Only closed form crystal structures of drUvrD are known. Boxed regions shown in Fig. 9G are shown in the crystal structure of drUvrD (Fig. 11) to demonstrate the suitability of the regions for crosslinking.

[00244] *D. radiodurans* UvrD (drUvrD, Q9RTI9_DEIRA) has only 1 Cys residue, and a crystal structure is known. drUvrD has 31 entries in the 50% identity cluster of the Uniprot database, some of which are mildly thermophilic (40 °C - 68 °C; optimum growth at 60 °C), making them better candidates for helicase dependent nucleic acid amplifications. In certain exemplary embodiments, a suitable UvrD helicase is selected from following species: Deinococcus geothermalis, Meiothermus sp., Marinithermus hydrothermalis, Marinithermus hydrothermalis, Oceanithermus profundus. Selected thermophilic ortholog species of drUvrD are shown in Table 8.

[00245] In another embodiment, the helicase is selected from those shown in Tables 9 and Table 10.

Table 5. List of 3137 unique non-redundant helicases that Slave 50% sequence identity and 80% overlap with E. coli Rep. (**Uniref50_P09980** cluster, citable UniProtKB and UniParc accession numbers are shown).

P09980	UPI000518 77AD	UPI000509 97D4	A0A063KTD 1	W0QF97	A0A0C3I5L 6

A0A0G3HMG0	UPI0002CB7C3E	UPI00041BBC9F	A0A0F9UW26	A3MZ01	UPIOOOSEDEB6E
AOAO 69YUU2	UPI0 002CB6CB4	Q31J65	UPI0 0057A0DA3	A0A0A7MFM3	Q4F7B3
A0A0 69XK09	UPI0003EF7150	UPI0 004A751IF	UPI000427 35E 9	UPI000371 27A9	H2IB31
A0A0 90J554	A0A0B5IRM0	J3VUI0	AOAO B2JU34	W0QB54	UPI00039DFE7 6
J2LMP0	UPI0005F8A649	B3PF82	A0Y2 42	D9P8T6	UPI0 002D3CD90
A0A0E1CLG8	UPI0 0036A6E72	W1J4L6	G7F2 59	B0BT27	UPI00031FC355
W8VIF4	E7T4Q1	A0A0 68QMH5	F3BJI5	E0EII5	A0A034TPW5
AOAO J2JAV4	UPI0 002C94EDF	UP1000645DEF6	UPI000464 1B0 9	A0A011P8 92	UPI0003B1A99F
A0A0H4Z590	UPI0 004A19D90	A0A0J1C9T2	W1Z619	UPI00047C9D5E	A0A0 90P8C9
A0A0H4YMK4	AOAO 24KL85	A0A07 7P2A7	N6W1D7	W0QMN7	U3AJD4
A0A0H4ZZ54	A0A02 9K3Y0	AOAO 77PGN3	G7EU4 6	B3H0C1	A0A0H0Y6H0
W1MB14	A0A02 9LST3	A0A07 7Q5V6	UPI0 003F6330E	E0FKS8	D0WV97
A0A0H4ZHU7	A0A07 4IDU1	A0A0B6XFA1	UPI000417 5A32	E0E690	UPI0 0039CA46E
A0A0G3T2 52	A0A07 0UMT6	D3VHW6	G7EBR9	E8KIM6	K5UKZ2
A0A0H3GKB6	AOAOHONZ 89	N1NN52	UPI000231 7DDE	K0FXU5	A0A0 61Q0T1
AOAOKOGSG9	UPI0 004DACF34	A0A0A8NWA1	UP1000412 695D	A0A0D6UGN8	D0X594
A0A0G8G0X7	UPI0 0054 3FDCF	W1J8H9	A0A0 99L6T2	M9X512	UPI000681 4400
A0A0 60VJ91	A0A0H3MJI3	A0A07 7NN58	UPI0 002DA5F7A	D9P4Y2	UPI000 6AA085C
W1HUU8	UPI0 002A2C4E1	A0A0 68RA85	UP1000316 2143	UPI000255 6C8C	UPI0 0039C63E0
W1EG0 6	AOAOHOKMW	A0A0A8LWM	U1MBQ7	AOAOBOHCL	UPI0 005EF

	7	3		6	DC1A
W1HYL7	F4NQI1	A0A07 7PLN 5	U lJK11	UPI00041A 33FA	UPI00063C 4F58
W1EC12	ACA0G9FGL 3	AOAO 77PEC 1	U1IZP2	AOAO 81FYF 7	UPI0002B7 057 6
V0AP50	UPI000530 5422	D3V6L9	Q3IJF4	A0A0B3BWV 0	UPI000237 55B4
W1E3B2	I2X0N8	AOAO J5FTN 5	E6RJ61	A0A0B2DBM 6	UPI00069F BAOA
A0A0 80SZ3 3	UPI000 63C 108B	A0A07 7N2X 7	Z9K3Y4	UPI000337 D905	A7K6B6
A6TGG4	UPI0 0053A B8E4	UPI00037E A902	G7G5E8	D2TCP1	U4DZN2
W1BG54	UP1000390 34IB	AOAO 94NXP 4	UPI00034C 744C	UP1000196 0924	L8XB50
Q57HT8	UPI0 002C9 7E3 6	F9QBX8	A0A0F4PWX 2	V5Z3P5	UPI000597 5B9F
C0Q2V7	T8JFG3	UPT000364 DEC7	I3CJI7	UPI000 65F AA51	UPI00039C 5A41
A0A0G2NT5 8	A OA OJOIRL 7	A3V0 94	A1T091	UPI00054F 6EBB	UPI0005F0 AEC8
V2QUW5	J1GHC8	UPI0 002D3 C2F3	UPI000361 33E4	UPI000554 232A	A7N1B5
B5RFS5	UPI0005EF AD7E	F9SI11	A0A0A8UTG 2	UPI00044B 0A8 2	U4K9S4
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UPI 00032 9 8127	M9JK2 3	UPI 0004E1 B20E	UPI 0004E8 3861	W2V1F8	A0A0F3KHK 4
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A0A0D6TML 9	B7NF83	AOAO9OS7S 9	U4SES2	UPI 00037E 8B5D	UPI 000 6A9 A72 3
A0A0E8MKU 3	A0A0H2VE7 0	A0A0 90SBH 7	U4S4 59	M1FGB5	UP10004 11 12OA
V1L7R7	UPI 0002CA CCD9	G3IVH2	UPI 0002CB FAC9	UPI 000572 A7F6	UPI 000422 1DBE
AOAOJ8M09 4	N2RLH9	UPI00004DF 8545	UPI 0003AC 4DB6	UPI 000474 4F39	UPI 0004 85 D7F1
S4IGJ2	AOAOJl LI I	UPI 00047F	UPI 0004ED	AOAOFODKQ	UPI 00045E

	8	1C5C	825E	4	A9DE
S4JI21	UPI0003DE 3024	UPIOOOIEC FED8	U4RRC2	V9HKH3	B9YZ61
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S4T960	UPI000530 15A9	UPI000686 0AC7	AOAO 84EXM 6	S9ZLZ2	UPI0 002DE 6662
V7VTQ5	UPI000 6A5 F9C7	A0A0 80UY5 2	U4SEE3	N6YTH6	Q8D1K7
V1U62 3	UPI0 002C9 392A	L7Z9H4	UPT00049F FDBE	AOAO J8Q92 9	UPI0 00267 BACB
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S4JDD8	Q31UK7	U2L7 89	A0A0F9VQX 3	I3DJM6	Q66G21
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V2N1K8	R9VPF2	UPI000325 C91D	A0A0C1MVM 0	UPI00069E E2BB	A0A0H2YE3 5

S4K7P0	K8CWZ5	UPI000668 8668	U1LW91	UPI00069E 268B	A0A0E8MJ7 0
A0A0 J8LWR 5	UPI0 002C9 9AA5	AOAO J5CK0 9	A0A0F4QKC 2	UPI00069D 73₁8	AOAO 88KV6 3
AOAO J8HEC 7	UPI00038F B95B	S5EBF4	V4JJ09	UPI0003FD FDBC	A0A0B6FKI 9
AOAO J8JAK 5	UPI0 002C9 6518	AOAO 69CNT 5	A0A0F6A4M 8	A0A0 99KBQ 4	A0A0H5LYI 6
AOAO H5PMJ 7	W9BJ58	A0A0 87L2V η	UPT000 627 0414	UPI0003FB A996	UPI0 005DF OBEB
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X5MWS2	UPI000500 BFOE	UP1000667 70B0	UPI0004BB 1652	UPI00051D 9F62	A0A0H5JCZ 5
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S4LIK5	B5XYZ5	A0A0F6KPT 4	D0Z154	A0A0E9B35 7	UPI0 005E9 ED67
V2A692	D3RHB6	AOAO J8POE 6	A0A0D8MPB 6	A0A0E9BHY 8	UPI0 005DD 7BF8
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B3YF3 6	AOAO J4SW6 9	AOAO J5CHL 9	UPI000307 D228	A0A0A3YME 8	UPI0 005DB 455D
S5ILC5	UPI000616 F32D	UPT0004 68 70F9	UPI00040F 27C9	U2MK51	UP1000302 9766
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1	634B	5			3
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V2JQQ7	UPI000576 2BA1	UPI000487 A67F	Q1ZK16	UPI00034B F070	W8U8V7
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X2KI94	AOAOJ8VI0 5	C6C798	AOAOJ1GP2 7	UPI000508 FC34	UPI0005E0 0E96
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B5EZ38	C8TL39	UPT00040F FE96	A0A0J1H81 2	A0A0A6YFE 9	UPT0005DD B975
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V7RZ28	K8BE03	UPI000308 B214	A0A0B9GYU 2	J3D9Z5	A0A0H5MV8 9
M7S3Q8	UPI00044C EDIF	UPI000413 FA42	L8J7D2	UPI0002A6 B5C9	AOAOH5PMI 9
V1GIN0	K6KFS6	UPI00037F CD36	UPI000595 9C45	U3TSP9	A0A0B6LTD 8
S5HF97	UPI0006662A0A	A0A068Z3I 4	UPI0005099EA27	A0A0F3LWH 1	C4UBW2
V0GNY3	UPI000448 B406	E9CNV6	N8QMA4	UPI0005C5 2466	UPI0005E9 D7FF
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G4C8N2	A OA OJ8ZB7 6	UPI0002E7 F8E9	N9RHD1	UPI00050F 5FID	C4T54 0
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V5ZRF3	A0A0E0WT3 0	D3SDE5	A0A022TP2 3	UP1000535 8D4C	A7FD4 4
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B5Q5L3	UPI000 665 B1C4	UPI000381 348D	A0A0 09KTD 8	UPI000534 2DB0	UPI0005E8 8594
V7IN29	UPI000 664 F825	UPI0 0036A 352B	N8RGJ8	A0A0A3YQZ 0	C4S2J8
E8XIM3	X4BC54	UPI000363 8A7F	V2TJN5	J2UWK2	UPI0005E1 5072
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G5LVZ4	A0A07 0TA8 1	UPI000368 9D3E	N9KD21	D4HUI5	UPI0005E9 DE69
UPI000 67F CA22	F3VD48	UPI00035E 83C6	N9TJB8	E5B0I1	UPI0005E6 D02E
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G5P.MY1	K8ABZ8	UPI0 0036C B07E	N8XKN2	B2VG71	UPI0005E2 1E25
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G5QW51	AOAO J9AK2	UPI0 0037 3	N8VTA9	Q1YW5	UPI0 005E0

	8	4BC2			3B74
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A0A0 J0N6G 9	UPI0 003BC E0F3	UPI000365 3619	N8U6B7	UPI0 002F5 3240	M4R9M0
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VISFT8	A0A0K0HP6 1	UPI0 0035F 39C1	S7WVT1	B7VH95	AOAO 94IJP 1
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UPI0 005AB 2AE4	A0A0B8ZV6 3	UPI000368 288B	N9S JA9	UPI000 63A 1E73	C9R7F6
UPI000620 81F9	UP1000583 7FE4	UPI0 0036D 136F	N8W7 59	A0A0C1NZP 7	E6KV3 0
AOAO J5KVY 8	G9Y0Y6	UPI00037F 35BB	N8RKD0	UPI000 647 60CF	UPI000519 4C7 3
UPI00029C 3FF8	A0A0C5VQS 4	UPI00035E C95C	V5C2I3	A0A0F4NTM 0	U1S9B3
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D2ZMA5	AOAO 85GZB 9	UPI000423 C045	AOAO 61JWL 5	A0A0A6T9A 9	UPI000660 A603
UPI0 003A1 A991	UPI000622 53DC	UPI000373 D30C	UPI0 0036C BF04	UPI0005E2 F450	G4A8W6
D7XM77	UPI0003A5 9E18	UPI000362 43F4	W8QTS8	F9RNX1	G3Z8Y9
UPI0 00267 7FD2	C9P8Z5	UPI00037D 0EF9	A0A07 8LYT 9	A0A0F4NNJ 4	I1XRY3
D4BE16	F2JTM7	UPI000371 14DA	A0A0 99RR4 3	E8LUB9	UPI000683 1C68

UPI000579 4143	A4C7W3	B5FCU9	A0A0D9ATR 1	UPI0002E7 10F1	G4AZ10
UPI000260 B9F8	UPI00039F 0D67	Q5E1T0	14JQR3	UPI00031D 8F80	L8UHA2
UPI000283 07CF	UPI0 00255 8A91	B6EP51	I7A9J8	F7YTE8	UPI000 67C F184
UPI000 666 0AB8	UPI0005B8 6EE4	UPI000247 865A	K5XG8 3	A0A0 66UM2 6	UPI000681 5E34
A0A0E0VC5 5	AOAO 98GSR 1	A0A0 90IP0 2	A 0A 0A 1GK 0 7	F9S3 63	HOKG96
T8Z104	UPI000326 FBAO	T2L6T4	UPI0 0040F 147B	UP1000310 2D21	A 0A 0E 1YSI 3
S0YAD1	UPI00037E 68F8	L9UDC2	UPI000419 4356	A0A0 99LPD 8	X2JQA6
S0X3V2	UPI00034D 7EBF	UPI00037E 36DB	UPI0 003B3 9C7B	UPI000316 7DE1	UPI000 67F F093
SIDNS 3	UPI0 0056C F143	A0A0D7UZT 0	UPI0 005BA 4855	UPI0002E0 F676	C6AQX1
S0XCC9	A0A0B8V8Y 9	A0A0F9VK3 4	UPI0 005B7 EB79	A0A0C2P7 J 1	UPI000 6A7 1057
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T9IPB3	A0A0H4R6 J 4	G4F9U9	TJ1AEW3	UPI00031E 1DE0	L8UIY4
T6LBU7	A0A0B8USV 8	A0A0B1PVT 1	UPI000 617 AEB5	A0A0A5I59 0	Q7MYL0
S1GJ0 6	UPI000368 EE93	H0J1C6	UPI000618 2BB7	UPI000304 3227	A0A022PH4 2
D8ADY5	A0A0 95VW1 4	UPI000488 42D1	Q7NQR9	U3BS4 3	W3VA31
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UPI0002C9	R4YVB5	F7SNT3	UPT000380	UPI000699	AOAO J9EYL

B880			0078	D72A	2
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C8TYS8	C6XCN5	G9EBD3	UPI000369 273A	U0FTU6	UPI00058B F6A9
AOAO J2E1P 9	UPI00035F CCBC	A0A0F4RA3 5	W8KPW4	UPI000307 15AD	T0PH03
UPI0 005B2 C8D4	UPI00058D 9CB0	W1N5Q2	D5C0J9	UPI000 630 3856	AOAO 85JM9 5
UPI0002C9 5CB8	AOAOAOBTG 5	UPI0 004CE 4C17	A0A0A3AKX 9	E3BPB4	AOAO 95VZP 6
A4WG32	UPI0 002DE 5ECC	A0A0 81K8A 4	UPI000 6A9 FOFO	E8MCD7	UPI00046F 3C99
UP1000342 OEOF	F5T1S3	UPI000477 FE94	F7NR22	Q7MQG8	UPI0 004A3 375F
UPI00036F 08F6	A0A0F9NIL 3	A0A0 94JA2 8	A0A0 66T3V 5	A0A0 87IWU 5	A0A0F9VYU 9
UPI0005C4 EB5F	C0N7C2	A0A090KED 5	A0A0 80LJV 3	UPI000 6A9 8AC0	I1XM63
UPI0002C9 1779	F6DAI6	A6FE65	X2GZR9	UPI000 6A9 8232	12JF40
A0A07 8L9V 2	UPI0005C9 F562	UPI00030A 12FE	AOAO J5P3I 3	UPI000248 2DB8	F9ZY05
UPI000512 B6F1	W0DYM2	UPI0002DF 92C0	A0A0F2P6J 6	F9REI7	A0A0F5V8 3 6
UPI0 002CC 209C	UPI00022C 089B	UPI000464 C875	W7R0N4	UPI0 004F5 E7D9	S6GDK2
A0A0D7LJA 0	UPI000684 4205	UPI000427 CABO	UPI000475 2339	UPI000318 35E8	S6HCZ4
A0A073VC4 8	W7QF72	B9CXM2	UPI0 004E1 47DD	UPI00031E 4412	I8U5K5
X7I14 6	UPI00058D EE31	C5RZQ1	UPI000479 BC17	A0A0H2MLA 0	H3ZE8 4
X7HFY3	UPI000289 826B	UPI0 0035C ABIA	UPI0 0047B 5018	UPI000619 5856	J1YGP6

A0A0 8 9Q2 0 4	U7NY7 6	UPI 0003B4 8I E4	W9V34 1	UPI 000 67F 45 62	H2 IWS7
UPI 000 675 D9DD	A0A098RE9 9	UPI 00042 1 2DE4	UPI 0005C1 5FEC	A0A0 97QPF 1	UPI 0005D3 39A3
UPI 0004D8 E2 9C	E1VGA4	A0A0C4WTU 2	E0FET2	A0A0H0Y0 9 2	I0QQI6
B7UMN3	UPI 00030B 6E67	C1DJY5	UPI 00024 8 B5E1	UPI 0002F5 88B8	UPI 00038 0 60C2
H4I3H7	UP1000 614 8CBA	M9YDT7	J4TTN5	A0A0G9M02 6	H8NUJ7
H4 JUI 7	UPI 0005B7 89BB	UPI0004E1 F9C3	S9YCL8	UPI 0002DD 07EC	A0A0H3FLE 0
H4KPD2	S2KK42	W0E158	I2NC8 1	A0A0B4 IM6 5	UPI 000554 A92 9
H4L5 65	UP1 0003 67 4641	A0A0F7K0Q 6	UPI 000313 5SEC	A0A0A3EMP 0	A0A0 85G3A 6
H3KW73	UP1000343 B180	UPI 0004 8B F236	E0F2E9	K5V6E0	UPI 0004 1A BFF4
H4LJK6	UPI 00037 6 C869	UPI 000395 D43A	E0F8 J 5	UPI 000 66B 2D3D	UPI 000308 9400
H4 IZH1	S5T4K5	UPI 0004 6F 29D9	E0EW4 9	UPI 0005F9 A9ED	UPI 00047 1 947 9
H4 JFJ8	K0C8L7	A0A0F5ARC 9	E2P8X6	M7RIV0	UPI 00058F 6D4E
H4K9V6	A0A0F9YVG 2	Z5XTJ6	W0Q1J8	K5TSH9	C8NBT1
E3XW38	UPI 0004 05 7986	UPI 0002AA 68F1	UPI 000585 642 1	C9QC04	UPI 000 660 E94E
H4I IJ0	D2TWS6	A0A0F4S82 1	A0A0B5BWF 7	A8T649	

Table 6. List **of Bacillus** stearothermophiliis **PcrA** homologs **that have 50%** identity **to and 80%** overlap. 1747 members of **Uniref** 50% identity cluster is shown (citable UniProtKB and UniParc **accession numbers are** shown).

P56255	J7M5U5	T0TN09	A0A0I 6PI 8 8	R3VBE4	UPI 0005CD 7F53
S7T032	A0A0H2UUM 0	F8LQ0 3	A5LVX9	E0G4K8	UPI 0004 17 CODE

UPI000518 15BF	Q1JLF2	C2LSM3	UPI0005E4 1D7E	E6GJJ0	UPI0 005CD 905E
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U2YC97	UPI0 00254 D55F	UPI000 66E 20BD	UPI000 66C DBC6	C2H162	UPI000406 2509
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UPI0004D4 E3B7	G4NUA0	S8HPX5	UPI0005E0 6D6A	A0A0F5MJY 5	U5SAC6
A0A091BNX 2	A0A068LM6 5	UPI00046C 8FD9	UPI000318 BEDD	F3UWM7	A8U4Y8
E8JNI7	UPI00025F 1BD8	K9AHW3	A0A064BXT 9	UPI000660 3308	UPI000559 0D06
UPI00040D 1DBE	I3DZY5	UPI000554 B661	A0A098ZS0 0	F0INA4	UPI000550 B1B9
UPI00041D 9E3E	A0A0F4M49 1	C2E4W5	S2UT89	UPI000660 5CF8	UPI00054D 85C0
W7ZBP8	UPI000514 5490	UPI0002FE 2AC2	UPI0005DB EB5A	UPI0001FB B9D8	UPI000553 C539
A0A060M0P 3	A0A0F4LJ2 8	A0A087QEX 7	UPI0005E4 4ED7	A0A0F2CJE 0	UPI0005C4 A619
V6M8R1	A0A0F4LIP 7	UPI000045 9CDE	UPI00067A 8E24	UPI0003D2 BF73	UPI0003B3 6792
A0A075LMV	A0A089Y3J	A0A0A8B47	UPI0005EA	A3CM74	UPI0005CE

5	1	2	38C1		4434
U5L6Q7	A0A0F4LHL3	UPI00057E31OA	UPI0 005DFA93B	UPI00066124BC	I7LCW7
Q2BBV3	UPI000 66D84FA	F7SF58	UPI0005E43F3 8	UPI000204C9B3	UPI0004942515
J9YKR2	UPI00047168BE	UPI00069ED3EB	UPI0 005EA228D	E8JTX8	U1N3Z8
B9E831	UPI00031D0F4F	D0R5K3	UPI0 002D988F7	F3SII1	UPI0 004DFB32B
A0A0A5GC60	A0A0 33UAP9	Q74I48	UPI00016C30E9	F3UBI9	UPI000495DE5F
UPI00068F9B5A	UPI000 65B9E05	V5P42 9	A0A0E9GYK1	F0FE53	C4L2B1
D5XD67	UPI00030B5B12	F4AC33	UPI0005DF550 6	F9E172	AOAO 99DEL3
S4D0X9	R2S3I5	UP1000660D235	UPT000 66C8BEE	F9HGV1	UPI0 00554029F
UPI0 0040BD27E	UPI0 005BB07E7	UPT00066974FD	D2ER82	F3URE9	S3FYE4
W4QBS4	A0A0D1LT71	UPI000665A968	G6JAI2	AOAOAODGR7	UPI0004787EFF
UPI0001E98CB8	A0A0D1LUL0	UPI0003421E15	AOAO 18YKR7	UPI000 66B8271	A0A0 69EVD4
C8PBZ7	UPI0002191623	UPI0001A57BCC	A0A0 81R5K4	W4F98 5	UPI000426A771
UPI0001E2B095	D8MGK9	D1YI75	A0A0B7MAG0	UPI000 6A9F3B8	S2KHH9
UPI0001FDB18A	D5T1C2	UPI00050F2122	A0A0I9KK18	UPI00052475A3	Q1GBF8
UPI0002072E3B	F6DPJ5	UPI00050F4C6F	J1DH18	A0A0 87N1S5	UPI0006800765
A0A0E1XZC6	UPI0002E9BB91	AOAO J7K5W0	V8I62 9	A0A07 8MFK8	A0A0 61C4M6
E3BTD7	L0E9U7	AOAO J7HHM3	UPI0 005E3DDI 8	UPI00037E072F	E4SWU2
G3YXZ2	UPI000401406C	D3L7Q3	UPI000 66D82D8	UPI0 002F64287	D8FRH5
E1NV4 3	A0A0A6NWI1	Q04GN7	E1LLK4	UPI00040FF64 8	F0HX91

UPIOOOIFD 875B	UPI000471 66B2	UPI00050F 2BFE	AOAO I6S ON 2	UPI0 002FB C45B	Q71HW2
E1NLU9	A0A0A1MVS 7	UPI00050D E50 8	I0QC96	S2XYX9	UPI000051 0635
UPI0001E5 D8E8	UPI000595 B757	UPI00050F 1464	UPI0 005DC F59C	A0A0E4H7N 7	AOAO 61CFU 6
A0A061P6S 2	J3F9X4	A0A0E2VR2 9	AOAO D6J8D 1	UPI000475 8748	UPI000682 9E7 6
A0A0 61NMB 4	UP1000410 FF91	A0NL4 9	UPT000 66C DBD2	T0BRJ1	F0K0A9
UP1000364 DDD7	UPI0 002F4 C1DE	UPI000277 B8D1	UPI0 005E4 COOS	UPI0 004CE CB95	A0A0 61BVE 6
A0A0A3I33 0	UPI0 0046A 2EE8	UPI00050F B3CA	J5GJF0	UPI0 002DA 018 9	G6F4X4
A0A0A3 J75 7	F0NVP6	UP1000277 B786	UPI0 005E2 E74F	UPI0002E0 B711	UPI00032F 7094
UP1000310 7DC7	UPI0 002DD 6873	UPI00050D A477	AOAO E8AXP 8	UPI00037D 657F	A0A0 61CH3 5
UPI000465 B065	W5XDQ0	UPI000277 B3EF	F3VID1	UPI0005B6 51F3	AOAO 6IBSV 4
UPI0002B5 9EE5	UPI00046C 96B8	UPI00050E BFE8	E0TNB3	UPI00031B 78EB	AOAO 6ICOY a
UPI00031E D71B	C2KB80	C2EVD7	UPI000681 36F1	UPI0 002DA 8172	G6EVU1
UPI000422 5966	UPI0002E9 360C	UPI000495 3241	UPI0 005DD 3E82	UPI0 002FE 654 3	UPI00069A 32A5
UPI0002B5 5DD2	UPI0001B2 ADB 8	UPI000425 6505	AOAO I6F78 3	UPI0 002EB 9E2 8	UPI00034B 89F3
UPI000268 A7D1	D5H1T9	R9TUH2	UP1000680 414A	UPI000362 867B	V4QC95
UPI0002DD 3475	V5E0D8	AOAO J6H03 9	I0Q42 7	E3CGX4	UPI000287 FE90
UP1000314 0F32	U6FUY0	A0A0D7XCT 2	UPI0 005E1 E1D9	UPI000367 5904	UPT000493 F21F
UPI0 002DF 577A	U6FDW7	A0A0D7XAM 9	F9HM8 8	UPI0 002BE 1554	UPI0 002F6 4068
UPI0 002B5 2183	U4QEF3	UPT000470 EB15	R0NC4 7	UPI0 0036D 9746	G9ZMU8
UPI00030A	C2ELL7	A0A0H4X8R	UPI0005E3	UPI00031B	I7JG8 0

AF2 8		6	138C	D2D4	
UPI0002B5 4D20	F6CEU6	T5HV53	F9HDZ5	U2KML1	UPI000249 0095
UPI00030E D4DD	A0A0D5MK9 9	Q65MR3	UPI0 005DF 6040	UPI0002F0 FB 31	AOAO 69DTV 4
UPI000317 D564	U6F2P2	UPI0 003A9 2B3A	UPI000 66D F5D2	UPI0002DF 3A61	UPI000371 8539
UPI0002B5 AC18	A8YTZ2	M5P7T2	UPI0005E7 8EF0	UPI 000309 D239	A0A0A5GG7 6
UPI0 002B5 3160	UPI000468 D9FF	A0A0F5KXW 3	AOAO 17SM 9	UPI0002E4 AADA	UPI00040E 6B55
UPI0002B5 578F	UPI000698 FD57	AOAO J6ENX 9	UPI000399 E54 7	UPI0 002EF E60F	UPI0 005CD 2072
UPI0 002D5 DE55	F0TDD1	AOAO J6ES4 1	UPI0 005E5 D243	UPI 000371 4975	UPI000487 D20C
UPI0002FF CB1B	F2M355	UPI0 005A1 6A5C	UPI0 005DB EC58	UPI000377 3AA6	UPI000372 0301
UPI0 002B5 0165	R5ZGC9	H6NTK0	UPI0 005E3 FBD1	UPI 00037 5 ABE) 2	M3HNA0
UPI0002B5 E93C	E4SM94	F8FKW5	D4FT83	UPI0 002DE 032 0	UPI00020C BEE 6
UPI0002B5 60C6	C7XLR3	UPI0 003F5 0A8 6	G6CA7 0	UPI00035F C09C	A0A0A6UX7 7
UPI0002B5 SAB 2	J4BVM3	G9WIE0	A0A0B7LL6 0	G5JQP7	A0A0E2USS 7
A0A0E2ES 0 3	D0DI93	UPI00054F 4F52	R0MM0 9	UPI0002E2 9189	F1Z0K5
UPI00030C 4A19	K1MXE4	A0A0 90IVN 1	Q8DPIJ8	UPI00037C A55E	UPI00041B 294C
UPI000464 8E2C	U6FHU9	A0A0D0FW9 9	A0A0H2ZN8 8	UPI000379 270F	UPI000513 CEDF
UPI0 002B5 88C8	F3MR07	AOAODOFHN 9	AOAO I5SPG 7	K8Z8B4	GOVS 69
UPI000311 1877	W7YRH9	A0A0D0F8 3 3	E0PRK3	AOAO 87EPN 1	UPI000422 1C8D
UPI0 002DA 34OC	W7Z816	UPI 000219 5446	UPI000 66A C65D	A0A0 63B7B 8	UPI000485 8886
UPI0 002EE 7FD7	V6J250	J2ZKY4	12J7J6	UPI00054D F8E8	UPI000356 EF52

UPI0002D4 555C	C0WSC1	UPI000219 2DE8	F5W0L7	UPI000 6B2 51F7	R7MZQ6
UPI0 002B5 13D0	C2D3L0	AOAO J1HWV 3	S3BFG0	UPI000 6B2 5740	UPI000550 AF6D
UPI0002B5 E0D9	C0XHM8	R9BWL2	E8K074	UPI000 6B2 5166	AOAOBOIKT 7
UPI0 002D9 F995	F5L4 44	AOAO 73JY5 2	UPI000 66B 28A6	A0A0C2WKV 7	W4QVT6
UPI0002E8 D24F	A0A0G9MI1 8	UPI000482 AB97	J1S8C6	K0DDF5	UPI00054D 333 9
UPI0002B5 8C8 4	Q03Q12	A0A0E9GQ0 5	UPI000 66E 5C86	S0NID8	F9VEL8
UPI0002B5 3813	U2PJH7	UPI0002D3 2EA8	F9PWL8	UPI0 003F6 F988	V8ANU3
UPI0002B5 E4B1	M5AEL4	G6LUK0	UPI000 66E 06C6	UPI00047D 412F	UPI000622 6EE6
UPI0 002EF F9CD	A0A0H4QAV 4	UPI000 67A B63B	UPI000 66C F5FD	UP1000401 6A4F	UPI0002D6 6EB7
UPI0002B5 8C9A	UPI0005B6 4156	A0A0I8X5X 0	A0A0F3H5E 8	UPI00040A D781	K2PIY1
UPI0002B5 8360	UPI000488 2072	UPI000 66B 62FD	UPI000 66B 0571	G7SH52	A0A0 98CN3 4
A0A0E2EHS 3	A0A0C1PU4 7	UPI0001DD D26E	A0A0F2DYG 6	UPI0003FB 4C2E	UPI000266 D5EF
UPI00030C 12E8	UPI00041E 7D06	UPI0 005DB 8FEA	AOAO 95ZC6 3	UPI0 005CE 1998	UPI0 002FD 1A2 7
UPI0002B5 5BAG	J7LCK3	UPI000 66E E l37	AOAO 81SAK 2	UPI0 005CD 605D	UPI00031F 2D35
UPI0 002B4 E249	A0A0A7U0Q 8	lOSAQO	UPI000534 2342	UPI0 005CD F885	UPI00031F 5691
UPT0 002EC 6AC7	UPI000681 91BE	F9MKL8	A0A0 81Q0K 0	UPI0 0042 4 E658	UPI0 005AA 507F
UPI000466 520E	Q03YP2	F2QD7 4	UP1000535 8034	UPI0 005CC F850	UPI00040E 6C2A
UPI0002B5 A6DE	UPI0 005A1 E84F	A0A0F2DG5 0	UPI000 67A 78F3	UPI0003F5 12C0	UPI000472 C52 3
UPI000467 53B6	C2KL56	UPT00069C F310	UPI000 67B FD62	UPI0 005CC F8E5	UPT000373 D4CE
UPT0 0031B	T0VWY9	A0A0F2D2Q	H7QP48	UPI0003FB	UPI000672

4772		6		972A	387D
UPI000463 12FB	UPI000234 1443	UPI0005EA 00F8	J1V408	UPI000407 9443	E7RJR7
UPI0002D7 CB42	UPI0 005A6 A464	A0A0I8VWS 0	E1MA03	UPI000403 0821	A0A0A2UWH 4
UPI000466 DCC8	UPI00046C A6CE	A0A0I6VM1 5	UPI000411 F965	UPI00040E 7BC1	UPI000288 0C4F
UPI0002B5 50C2	UPI000681 422 8	A0A0I8S59 4	UPI000375 1A95	UPI0 005CD 07D9	A0A07 5TVF 2
UPI0 002B5 9082	A0A0H4N93 7	A0A0 81QA4 8	C8P6C2	UPI0 005CD B669	UPI00054E 004D
UPI0002B5 EB7C	A0A0 95AHY 9	A0A0I9J5Y 7	UPI000312 AA9F	UPI0 005CE AA59	UPI0 005A6 5B8B
UPI000463 3B7 4	UPI00037F 25A9	M5N7 94	E3CAD 3	UPI0 005CE 657 6	W9ANP4
UPI0 00264 EF92	B9DUG 5	UPI0 005E6 7219	UPI00021A 3A7 3	UPI000409 F746	M9LGB1
UPI0 002E7 D773	UPI000 620 28C1	G0I9B0	K0U2M0	UPI0005D1 6915	H3S9K5
UPI0 002D3 B5B3	UPI000 620 3F88	xA0A0E2P69 3	U1ES8 9	UPI0 005CE BAOO	UPI000378 CC96
UPI0002B5 77F4	A0A0F5I4 J 0	F9NZH5	UPI000660 DA3F	UPI00040F 513E	K2FNB3
UPI000466 2568	A0A0F5HRS 0	UPI0005E9 21D9	C8WTI7	UPI0 005CD 9361	UPI00067F 085F
UPI0 002FB B47E	Q5M4H1	A0A0H5LNP 6	B7DQB4	UPI0 00232 2E59	UPI0001E2 EBD3
UPI0002B5 379E	UPI0 002DE 5460	A0A0E0X8 J 2	F8IDV6	UPI0005CF 78F9	UPI0001FD B5AD
UPI0002B5 1579	V8LWU0	UPI0 005E6 4E83	UPI000559 9D20	R4NWS6	D4W7 31
UPI0 002B4 E9DA	E9DMH4	F9LXX0	UPI0 00509 968E	UPI0 005CE AB2F	UPI000490 D40D
UPI000464 DB1E	UPI000312 1E5D	X8K6Y7	UPI0 005CA BE96	UPI00040E 0B64	UPI0 00255 C522
UPI0 002B5 29E6	J7T7E4	I0T8M4	A0A0J5S2 9 0	UPI0 005CD 124C	A0A0F7D4N 3
UPI000467 IB60	UPI00031A FBDD	UPI0 005E2 2D3B	AOAO J5WFP 0	U5UIJ5	UPI00058E 169D

UPI000319EA31	E8KV65	E1LS08	A0A0J5YA29	UPI00042996E4	UPI000624F4AA
UPI0002FFFF03	UPI0002E8600B	A0A024DEK7	UPI0006A9C586	UPI00042A2929	A0A0A8JEM1
Q8DTY6	F8HD36	UPI0005E25BC5	C8NHG1	A4VUA8	UPI00047BFF10
UPI0002B59757	A0A0E2QHQ8	UPI0005E30B11	UPI0005874702	A0A0H3MVK6	I9B3V6
UPI00035CDC0C	F8LX97	E1M4S7	UPI00066C1DCE	UPI0005CD2519	A0A075K9S1
A0A084GLL3	A0A0F6BVJ6	UPI0005E14CCC	D4YVQ1	UPI000409C6E9	I9NQ12
A0A084H1D9	A0A0E2RHF6	F5VXC9	E6FS51	UPI0003FE3351	UPI0004883363
E6TWN0	UPI000264F340	A0A0E8T7V0	UPI0002EA5AD2	UPI00041E695A	S4NRZ4
K1LG40	UPI0000E563DC	E6KMR2	S4CP69	UPI0005CDA05A	J9W320
F2F7J1	UPI000660EC4F	UPI0005E76F14	UPI0003FECF16	UPI0004038E95	F4FSH6
J1GP52	UPI00066C13CA	UPI0005E0C70E	F2MQT5	UPI0005CE89C0	UPI000403AE07
F8HYK0	F8LIZ1	UPI0005E6F0D4	UPI0002A3D37C	UPI0004018E0C	A0A084HBI0
UPI00044D3C3A	G2GTJ2	S7YYN6	E0H8L5	UPI000404D8AB	D3FTF3
A0A0C6G2S0	X8J9A0	A0A0I8TLZ0	R1W0H5	UPI00041CFDD8	U6SL82
U2W3N6	UPI00066AA528	E9FJW6	S4FW64	UPI0005CDED2A	UPI00036426F2
A0A0E1ENC5	A0A074IU47	UPI0005E93C3F	R3UP49	UPI0005D236D1	UPI00047A28D8
Q99ZE1					

Table 7. List of E. **coli** PcrA **homologs** that **have** 50% **identity to and 80% overlap. 1029 members** of **Uniref** 50% **identity** duster is shown (citable UniProtKB and UniParc accession **numbers** are shown).

P03018	K8BG21	UPI0002C8F355	UPI0005A9630D	UPI0003EF5338	AOAOJ0DJ77
A0A0G3HMD3	A0A060VDV3	V1HN20	A0A0A3YR40	UPI0001F6648A	AOAOJOSUX3
U9ZBE3	A0A0E1CLV1	UPI0002C9B17D	UPI0005EB7A8B	UPI000678B341	AOAOJ0M6S9
A0A071CB77	W8V249	UPI0006811593	UPI0058E54A4	A0A0K0IDG2	AOAOFOXZS7
SIJ559	AOAOJ2G3Q6	AOAOJ4VXC9	H1C573	UPI002CC80BD	UPI0005D0A9E9
V2S4E7	A0A0H4Z3E1	UPI0025C7C5C	UPI005CD86D3	AOAOJ5K2Q0	A0A0C8UHF8
A0A073G662	V0AU35	UPI0005304A96	UPI0044E7286	A0A0H3MJV2	A0A0C9HTD3
I2SQY0	A0A0H4YPU3	UPI002CAB12A	UPT0037EE7F7	A0A0E0VDJ7	Q8Z3B0
B3X3W4	A0A0H5AHT5	UPT005CCA08F	AOAOJOGVC5	A0A0G2SID2	A0A0E7LC59
N2GY76	W1HG62	UPI00330B244	A0A0H0CXK2	UPI000542989F	W6J799
W1F3C2	A0A0H4ZLF1	UPI002CCBAB8	V3PV69	A0A070RYI3	UPI004DA823D
E1HNQ6	W9BQA0	F3WPX7	A0A0D1KFS4	H3MUW4	V8MJC9
A0A070SNS2	A0A0H3GGJ9	A0A0F6YD20	H5V6H2	A0A070H7E9	UPI00049F5927
H4URJ5	A0A0K0GRR7	K8DQF9	D7YBR7	UPI003BC8E89	N3EUQ7
M9G7C2	A6TGJ6	K8C9V5	UPI002C8B609	UPI0003910486	UPI0004693D87
N2IIQ0	A0A0G8G1B7	F5VR52	UPI00063CD924	A0A090UJD6	UPI0002CCC2BC
S1HRC3	UPI00058FD925	A0A0D1QDQ1	A0A0C2AR33	UPI0004977D3D	F5N8N5
D8E9M5	UPI00058F	A7MQJ8	M9I6S8	UPI0003EF	UPI0006A5

	49FC			3FD7	855E
A0A07 4HPP 5	W1HTQ0	V5U5I0	N3K33 0	UPI0 002CC 54C7	Q83IW7
L2VEY2	W0ZY91	UPI000518 7950	I6CD07	A0A0G3S4T 9	A0A0C7MG1 0
K5CJK9	F4T661	K8D2A7	E7SHD7	A0A0H3HA9 5	AOAO G3KPN 2
D7YG58	F4V8D3	UPI0 002CA 0405	B2TUW9	AOAOEOWSN 3	Q0SZ04
W1BJG9	F4TMH3	AOAO JOI5H 8	UP1000390 185B	AOAO 68H45 2	D2ABY6
N2QEY3	A0A02 9LAE 5	UPI000579 149A	UPI0 005EE DAF9	UPI0 004A0 FDEC	A0A0F6MJ8 5
A0A0 69YVJ 3	U9YHH0	UPT000 665 0689	B5RFP5	A0A0H0GX6 2	F5P1 J3
A0A07 0Y0G o	AOAO 80IB9 3	N2J8A4	UPI000473 3206	UPI0 002CB 804F	A0A0F6EK0 0
A0A073GWJ 7	AOAO 83YZ9 3	AOAO 63XKV 2	UP1000267 21AF	UPI0 002CB 6B71	I6BAB 7
V0RR87	UPI0 005C4 8DC6	UPI0005C6 3608	UPI0 003A8 0309	UPI0 004D7 856B	UPI00050B 7641
V0ACC7	UPI0 005A8 BFOl	E7T4T6	A8ACW1	AOAO 84ZTZ 9	UPI0 0050B 2FF7
N2RS67	UPI000371 0649	A0A0G2XIC 2	A0A0A5IRH 8	A0A0 62Y21 2	I6FW66
A0A0 69XHA a	A4WG04	I6DJM1	A0A0F1WNC 5	A0A0 64DKM 1	UPI000 67F 497D
A0A07 9H1K 8	AOAOJ8F6L 5	K0WUD4	V3DAP7	AOAO 8OEWZ 5	UPI000530 716C
A0A07 4IWT 6	UPI000 666 003A	E7TCS8	A0A0E2K1D 2	UPI000668 F9A7	A0A0B1RCP 6
F8XAY4	A0A0I1EMQ 9	Q31UH5	UPI0004D8 D514	V5AU63	B6T4F4
A0A07 4HJR 7	AOAO J5U9E 7	AOAO 85HAH 3	UPI0003EF 42B5	UPI0002CC 06C9	E9TMV0
V1BCC5	AOAO J6MG0 9	AOAO J5L0 8 5	UPI0 005A8 7CA8	A0A0B1FRQ 9	UPI0004D7 2F99
AOAO8OHWB 3	UPI000668 4F9F	T2BE57	UPI00016A 0FB4	S1FP2 7	UPI0 0025A BCDF

I2X3X5	UPI00058D 9C39	A0A0F1BI7 8	UPI000496 CFDD	S1L396	UPI000 627 F480
A0A07 0FA8 4	A0A0A5RML 6	AOAO JORXX 3	UPI000 6AB EED8	S1CI55	UPI000326 F8B9
L3K8 J5	A0A0 85ITJ 0	Y1GM95	UPI000464 613 0	L2VN93	A0A0F4HLT 5
A OA O8OGHX 3	A0A0 38CQJ 1	E8C7D9	B7MR33	A0A0 89U9W 2	T9FRL3
A0A073FPS 6	A 7ZU 18	V 2JXK2	A0A02 9TIQ 6	D2TV17	H5E8S0
S1GRU8	UPI0 002CA 1DFD	V1LV18	AOAO 29HFT 5	UPI000 667 BF5F	I4S2D3
H5J8D4	A0A0F3LUY 4	E8D343	AOAO J9KSZ 0	UPI000 620 7A91	V8FG33
D8ERJ1	UPI000248 1DE4	S4INC0	AOAO HORN6 7	12X2 71	I2RVR0
D6I369	A OA OJ8LYC 2	E7ZSL5	UPI0 002CB 9IAD	A0A0D6IZH 2	A0A07 0D8G 2
A0A071CFC 4	A OA OJ8MSQ 3	A0A038D0Z 4	C3SKC2	B7MH77	A0A02 6UZE 9
M8SKZ8	A OA OJ8HX7 3	E7YUD9	A0A0H8C2 8 7	A0A0E2KYP 4	A0A02 8CBA 2
S1EV3 8	A OA OJ8QFU 8	S4J0L5	V0VC55	UPI000512 AED4	V0U5F8
S1CHB8	AOAO J8KFX 8	E7YT71	V0SS57	UPI0 002C8 F6BF	H5A4E5
I6CYG1	AOAO J81WX 2	E8F002	T8ZCA9	G9YXY2	G2AN47
H5IRF1	AOAO J8NMQ 9	E8EDF3	N4MZW5	UPI0 002CC 829C	K3QI J3
I2WCK0	AOAO J8JFF 5	G5LW37	A0A07 0K8G 3	UPI0004B0 01CB	AOAO 7OSY6 9
A 0A 071DAV 1	AOAO J8M4K 3	E8FVB6	WIBBJO	UPI0 004E3 7056	I2UC63
A0A07 0DJ7 1	AOAO J8HJM 3	E7VG83	V0XWV9	UPI0 002C9 8364	M9EF0 5
A0A07 9D8 0 η	AOAO J8M7Y 3	E8AMN0	L2X7H9	UPI0 00267 F8CE	A0A02 7TGT 7
V0YB46	AOAO J8LY2	V1PEK5	T8JFJ4	UPI0 00267	A0A0E1SZY

	1			3104	6
D8AZQ6	A OA OJ8K6V 3	E7ZUE1	T5TRC8	UPI000 66D 844D	A0A0E2U3 9 a
L3IME4	A OA OJ8JD3 5	E8B3Y5	H4I3L1	UPI000508 3EE7	A0A02 7ZJG 3
14 J58 7	A OA OJ8KYA 0	G5PV87	AOAO J3V9C 5	UPI0 002CA BCFF	C STL 04
T9CEL0	AOAO J8M2A 9	T2Q2W7	N4NRN6	UPI0 002C9 4803	A0A02 8E3K 3
A0A07 GULP 7	AOAO J8KZU 2	E7VUY1	U9Z163	UPI000530 9A93	A0A02 6HN9 3
H4V8 76	J1GHE8	E8BI66	X7NZ16	AOAO J5MIB 1	A0A02 5G7T 3
F3VD13	UPI0 00472 CO58	S4TB83	S0YT63	UPI0 002CC 9136	K4VZX0
K3KG98	UPI0005F8 A7CD	E7XYR0	H4JTJM1	UPI000269 547E	K4XMA4
G0F7H1	UPI0 005ED 3E27	V7WD7 4	A0A07 3H2N 3	UPI0 0034 7 30CE	A0A0H3XBG 3
E6B0S3	S1I248	AOAO J6D7Q 1	AOAO 17131 2	UP1000391 0F4 9	H9UZ11
EOJ3Y2	UPI000512 EA8D	V1U5Z5	AOAO 8OECD 1	UPI00057C 0D33	C8UJJ5
A0A037Y8I 6	UPI0 002CB 816A	E8EQ65	L5GW49	A0A0G3PID 9	A0A0A8UGD 6
A0A0E2U8R 4	UPI000681 5C5F	E8GKX8	S1P4I2	AOAO J4WXG 0	UPI0005B3 4SAD
E8Y8R2	A0A0H7LQT 5	E7Y7G4	V2T0S1	V3D6C7	I6FW9 6
A0A0E0U5P 0	UPI0 002CA 127F	E7WDV6	A0A07 3UI6 6	AOAO 60UYE 6	UPI000471 3F51
B7L973	UPI0 003BB 4FC5	E8H1P2	V6FB5 6	M7P8V6	UPI0 002CC 83F4
E3PP00	UPI0002C9 2D2D	S4M012	J7RN2 4	W8XG71	A0A0F5SGW 9
A0A0E0Y7I 2	A0A0F0YW9 7	E8CHG5	S0XLH4	V3KJ7 9	UPI00069B E650
A0A0E3H4E 0	A0A0F6K2Y 9	S4JDIO	A0A0 64T2Q 3	W8XNG3	AOAO 69X2G 5

C8TYP3	UPI000251 5E81	V2N4 00	M9F52 8	A0A0 98GXV 9	A0A0 80FIP 4
A0A0E1M3W 0	UPI000699 EF6E	S4LF58	S1D3C3	UPI0004D5 4D12	A0A07 3T7U 4
A0A090L9E 8	V3IA60	E8DQ33	H4KPG7	UPI0 005ED DA4 8	AOAOFIAYX 8
A0A0A0F8P 2	A0A0I2HXR 0	V2P0K5	V8KDE4	UPI0005F0 8B1C	AOAO J1YCS 9
UPI0 005E6 9EA7	UPI00057 9 D3C9	A0A0H5PMN 6	A0A070P4C 7	UPI0 002CC 5FF6	AOAO JOHLB 6
W1G67 9	AOAO J9AH4 8	V2ISV7	U9Y365	R0D8R6	A0A0F0RX5 9
C0VZH1	T9ARP5	V1SA8 8	V0YN02	UPI0 002CB 96B1	UPI0 005CA F560
UPI000697 8729	U9ZZ52	T2PQM4	V4B7K3	A0A0H0HV0 4	A0A0D7LBX 3
UPI0 003EE 8CC5	B7NFB5	E8AAS2	E9YLR4	S3IGV1	H4JFN3
W1WHJ6	UPI000445 D59E	E8CTA8	M8LCT6	UPI00068E 1050	AOAO JIM 12 3
D8ASL4	UPI0003EF 87D0	E7WWF9	M9GJI0	AOAO J4LFX 4	X7HIN0
UPI000669 5A3 6	ACA0F4BA8 8	E8FMH0	L3Q9J9	A0A0H0CH2 9	A0A0 64CY9 1
UPI0002C9 5A2 3	V5KL37	E7WRB0	S0X3S7	AOAO J0K9Y 5	S0TTK4
W1XFI9	V2MBS7	E7X696	V0Y7G3	W7NZ36	UPI000 652 0C97
UPI0 0050A DC02	A0A0H3T6D 2	E8GBI9	A0A07 0PK7 4	B5EZS8	UPI0 002CC 3250
W1WI72	X5GT01	V1K5C8	H4L599	H7EDN3	UPI0 002CB 3E81
UPI000509 7CC3	UPI0 0056E BE4B	X0NNF5	S1EDJ9	UPI0 002E3 BEE 2	UPI0004DA 7107
Q8KI59	J1QP03	V2AKC0	T9TU72	UPI0004E2 422C	Q05311
UPI00044F BFBE	AOAO J2C8A 9	E7VT49	A0A07 9Y2R 2	AOAO J5MX4 3	A0A0D6TPI 8
UPT0 005CC	E1ITF3	AOAO J6JML	A0A0G3J26	A0A0 85HQF	A0A0E8MI4

FA15		4	3	9	2
Q9R2U0	A0A0D1CQK0	V1I8L5	H3KWA8	A0A07 8LAH7	A0A021WR03
UPIOOOSOA604C	V6FP78	E8E0U7	H4LJP0	A0A0H0R1B4	S5IH33
V0V67 4	V2ASN4	S4JVA6	T6GSY1	UPI0005575061	V2KFI5
A0A0A7A0U6	UPI000 627EB2 4	E7ZFI6	T6LNG9	UPI0004D8B75C	A0A0H3SHZ2
E2X518	UPI000237C903	V2H9R2	H4IZK5	UPI000452C3C5	E8XJD9
Q32 9Y9	AOAO J5L635	E8BMX2	T5NEX8	R8WLR8	A0A0H3NUG9
AOAOJlJGH9	A0A0 85GMJ6	G5NKF6	N3MX37	S0XDX3	V7QPA0
W1FYY2	UPI0005E94CCS	V2A9V9	A0A02 9P4R5	A0A0A1B3 85	V1H94 5
A0A0A1R5N6	U1VBA4	A0A0G2MMZ1	A0A02 7YRP2	UPI00016C8460	A0A0F6B9B3
A0A07 3VBC0	UPI000 66656EE	VlMAMl	L3PWK5	UP1000675DF8 5	A0A0F7 JES7
I6FY95	A0A0H3FP62	B3YFM1	S1GVU2	A0A0E1LGB9	UPI0 005F937F6
A0A0D7LIV8	UPI0005014921	A0A0H2WUN6	M9K6A8	D2ZMD4	L0MA8 9
X7I032	UPI000 63C446F	V1XNT7	T9TBM0	UPI0003ED146B	UPI0004B98CEC
D4BE43	UPI0 002CAC6D5	A0A0H3S2Qa	D7ZK11	A0A0E2A5Z6	E1I441
UPI0001C3403D	UPI00026947D6	X2KCL1	L3NT10	AOAO I2G829	D8ADU8
G5P1B6	UPI0002B6ODFC	A0A0H3IIW8	H4K9Z1	UPI0 002C8DCIE	UPI0001FB4B2C
G5LGM2	I6FIC1	C0Q3C2	S0VUG2	UPI000 66659C9	D7XDB2
A0A0H2VE91	AOAO 73W J1	V2NKZ3	M2P544	UPI0 003EF354 6	UPI00050B0CB8
Q1R4C1	UPI0003F93F50	A0A0H4VNJ1	E3XW04	UPI000370A2F0	AOAOIOYDW9

W8ZQE8	L4IV51	V7UEH9	S0V315	A0A0J0PQF7	UPI0003FF3A54
A0A024KJK2	A0A0J8YSU2	M4LQ08	N2JTA5	E6WHH6	UPI00067E3DB0
UPI00050B495A	UPI0002CAC228	Q57HQ6	H4IIM4	UPI00057BE5A7	UPI00050ABBE7
A0A090ND62	A0A0F0R0L1	A0A089GCQ8	A0A026RVL8	UPI0003BF7FA1	UPI00050BC0F6
A0A024L7U7	U2MK71	S5HQI6	E9XUJ2	T8XXA5	UPI0001FB4D65
I0VX51	UPI0005755034C	A0A0H3BQS9	A0A017JGC0	A0A0H0BBN2	A0A0I2EFX3
C9XT80	UPI000282E630	V1SQB1	D6JHC8	A0A0F3XJB2	V6E727
UPI0003027365	UPI0005307602	A0A0H3RDJ9	T5ZU25	A0A085PA08	V0VKJ6
K8BR96	UPI0006A629C6	V0GAX0	Q8X8P5	D6IG48	UPI000589632A
W0AUM0	UPI0006A6039B	A0A0F0IT73	B1LLY4	L4UZM9	UPI0002A4D3B7
UPI0002B9DE03	UPI0002CC68E2	A0A0D5WNL4	R6TVJ8	L3C1J2	UPI000628182D
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UPI0005196C1F	A0A0D5WY30	A0A0G2NZ21	D3QXA5	UPI0006800C6C	UPI00053A6F37
E3G3X3	A0A0K0HFZ6	V5ZRD0	D3H4V1	UPI0002CCBDA0	UPI0005CEF8A7
A0A0B5INH2	UPI00056ED442	V7IJT2	A0A023Z641	UPI0002CB0C5E	A0A0H7L7Z4
UPI0006969E0D	UPI0002CA6A43	V2D935	C6EG01	A0A0J1LKQ0	A0A0I1QVM4
A0A0J8ZBK9	F1ZPQ8	B5Q5I2	B7UND0	A0A0G2NT28	UPI0004643C70
UPI0002EE2722	E9Z1A2	V1RGT6	L9HYA4	V2PRV4	A0A0I2RPB4
A0A0J1RJH0	I2REU8	X4BR52	A0A0H3PUC7	UPI0002CBC0D7	UPI000281D683
A0A066P4B	UPI0005EA	UPI0004A8	A0A0F6GUU	B3HAV2	A0A0I0V6U

2-	4E43	DEFA	7		1
F5S3C5	UPI 0003BC DF55	G5P17 6	Q3YVF3	UPI OOOSOA 9E0 0	W1WLH4
A0A0E2M6M 6	UPI 0002C9 35D9	G9WCL2	AOAOF6FES 0	UPI 0004D4 FB82	UPI 000 69A 9A9D
AOAOJ0P9D 3	A0A0 90V7 I 4	G5MAR0	A0A0G3KBA 3	M8PMP0	A0A0H7RCS 5
AOAOJOVSA 8	A0A0 89Q42 8	UPI 000 67C 89D7	A0A0F6CBC 7	UPI 0004 83 DDB5	UPI 00050B 374 0
AOAOJoQVP 3	UPI 000398 07B5	UPI 000 67A C747	J2YWY3	A0A0D7ES I 8	WIAS VI
AOAOJOLCW 8	UPI 0004DA 8D8C	UPI 000 69F 6BC0	B7LU43	AOAOJ0DP9 2	W1DW14
A0A0A6EFN 1	UPI 000463 5F02	UPI0002A6 DF22	AOAO25C61 6	UPI 000352 C78C	J2X0N7
A0A0F1A8N 0	UPI 0004 63 708C	UPI 000 67D 0E8D	A0A0H4S4M 4	G4C8R9	G5LGM3
A0A0F1HG J 1	R9VNE2	UPI00028D E27E	A0A0H2Z4N 7	UPI 0005AA 8C72	UPI0002B9 DB1F
AOAO74TPI 3	UPI 0002CC 3EDA	UPI 0005F8 57AB	F0JWA1	UPI 0002CC 9A6F	X3YLW0
AOAOJ9AGF 8	UPI 0002 69 5288	UP100057 9 7D3A	UPI 0005EA F698	UPI 0005C6 74E6	UP1000438 1BCD
UPI 000 668 E496	UPI 00034D 611A	J5W6W9	D7ZU66	UPI 000 665 8EEE	UPI 0002AE B5B0
AOAOJISRM 4	M7RF8 0	A0A0F1L5B 3	UPI 000 696 EBE1	AOAOJ4TS2 4	G5QS93
AOAOHOABS 0	K8AAR4	UPI 000 667 5A7B	UPI 000 699 5D61	A0A0C7L0 9 9	G5MAU7
AOAOJ2H3P 7	A0A0I2D6J 9	ElJ5X4	UPI 00053B 46E6	Z5CP12	X3UNX0
A0A0E2R9B 6	M8KEA1	E6BNN4	UPI 000 681 EBB3	D3RH84	B3PGX1
UPI 0004B5 8C5B	UPI 000574 FBCF	D7XMB5	UPI 0002C9 25C5	B5XYK3	UPI 0002DB 7E8 1
AOAOJ2FBS 7	UPI 0002A1 343F	A0A07 9F6E 9	B1ERG0	UPI 0005CC 1957	UPI 0003B6 1D19
AOAOJ9AI3 3	UPI 000537 C7CA	A0A07 1AVK 4	UPI 0002CB 7FF2	UPI 000 666 ABBF	UPI 00037A E6F5

A0AOJ8Z8W7	AOAO J8Y5W3	A0A07 9FJR3	A0A02 9K3W3	R5WI8 8	UPI00040A8AC5
H3MDK3	UPI000472771C	UPI0 005AB IB13	A0A02 9LTL0	AOAO 89PHR7	A1SQW8
A0A0G3PTS1	UPI0 002B580C6	UPI0 002CB B03B	UPI000390 DC2A	A0A0H3CV27	G5QNH0
A0A0I1EU55	E8DBL4	UPI000 69C 7IE8	S5N2R7	A0A0H0C2 42	G5S3E4
UPT000669A104	E7XTB6	X SMS 66	G2S5G8	A0A0F0TB45	G5S J34
W0BDW4	UP10003915F4D	UPI000614 634C	A 0A 0F2AUK2	A0A071M1C1	X3XE62
G8LKV0	UPI00038F A10B	UPI0 005ED 8E6D	AOAO JOJZA2	UPI0 0035E 9F50	UPI000689139D
AOAO J0TK85	A0A0F6TXR6	V1GX81	UPI000 676 9073	F4W2 69	G5R9I0
AOAO JOGZG0	UPI00037F 6D42	A0A0G3QEA8	AOAO 90U681	UPI00038F A53E	G5P3F2
A0A0G4BNQ9	UPI000614 5584	UPI000 666 A5AA	A0A02 3V4X1	A0A0B7GI73	UPI0003D2 FA70
A0A0I0T9Z3	UPI0004DA E8E7	UPI000315 529E	AOAO I2BUS0	A0A0G2MHY8	AOAO 84CN62
R4Y7F0	AOAO J8XHN7	A9MJ02	R8WJE0	AOAOHODHS6	UPI00068E1512
C8T0H7	UPI0 003BE CD47	S1HNI3	AOAO JOIRI6	AOAO JOSS F2	UPI0005D0 93A3
F4VLD8	H3N5H9	UPI0 003BB 87D8	A0A0F3YGX7	AOAO JOB472	A0A0H4R3L7
F4SRL8	K6KT52	UPI000353 E7DB	UPI0 002CB 932D	AOAO JOENJ8	A0A0B8UZ32
F4NQE6	AOAO H3ENI0	A0A0F5B4P9	S0UJP4	AOAO H ODM28	A0A0B8V3X1
K8B2N0	UPI0004D4 C5A1	UPI000250 COIF	M8X9A7	AOAO JOPB20	U4TEK6
UPI0 003A8 00E6	UPI000598 DBB2	N4NWV1			

Table 8. *1), m diodurans* **UvrI)** and its **Orthologs** in Thermophilic Species

Accession #	Entry name	Protein names	Organism	Gene name
Q9RTI9	Q9RTI9_DEIRA	DNA helicase	Deinococcus radiodurans (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051 / NBRC 15346 / NCIMB 9279 / Rl / VKM B-1422)	DR_1775
F0RMJ1	F0RMJ1_DEIPM	DNA helicase	Deinococcus proteolyticus (strain ATCC 35074 / DSM 20540 / JCM 6276 / NBRC 101906 / NCIMB 13154 / VKM Ac-1939 / CCM 2703 / MRP)	Deipr_0885
H8GTP8	H8GTP8_DEIG!	DNA helicase	Deinococcus gobiensis (strain DSM 21396 / JCM 16679 / CGMCC 1.7299 / I-0)	**uvrD2,** DGo_CA1449
C1CVA3	C1CVA3_DEIDV	DNA helicase	Deinococcus deserti (strain VCD115 / DSM 17065 / LMG 22923)	**uvrD,** Deide_12100
A0A016QL30	A0A016QL30_9DEIO	DNA helicase	Deinococcus phoenicis	DE!PH__ctg079orf0093
Q1J014	Q1J014_DEIGD	DNA helicase	Deinococcus geothermalis (strain DSM 11300)	Dgeo_0868
D3PR99	D3PR99_MEIRD	DNA hehcase	Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber)	K649_05745
A0A0D0N7B7	A0A0D0N7B7_MEIRU	DNA helicase	Meiothermus ruber	SY28_04645
E8U932	E8U932_DEIML	DNA helicase	Deinococcus maricopensis (strain DSM 21211 / LMG 22137 / NRRL B-23946 / LB-34)	Deima_1926
D7BGJ6	D7BGJ6_MEISD	DNA helicase	Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) (Thermus silvanus)	Mesil_1937
A0A0A7KLI4	A0A0A7KLI4_9DEIO	DNA helicase	Deinococcus swuensis	QR90_10300
F2NK78	F2NK78_MARHT	DNA hehcase	Marinithermus hydrothermalis (strain DSM 14884 / JCM 11576 / Tl)	Marky_1312
A0A0F7JIM6	A0A0F7JIM6_9DEIO	DNA hehcase	'Deinococcus soli' Cha et al. 2014	SY84_01165
E4U8J8	E4U8J8_OCEP5	DNA helicase	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	Ocepr_1221

L0A7L7	L0A7L7_DEIPD	DNA heiicase	Deinococcus pera ridilitoris (strain DSM 19664 / LMG 22246 / CIP 109416 / KR-200)	Deipe_ 3ij22

Table 9. 36 **seed sequences** of UvrD-like **heiicase group** PF00580

ADDA_BACSU	EX5B_MYCTU	O53348_MYCTU	PCRA_GEOSE	Q9ZJE1_HELPJ	UVRD_ECOLI
ADDA_LACLM	HMI1_YEAST	O66983_AQUAE	PCRA_MYCTU	REP_BUCAP	UVRD_HAEIN
EX5B_BORBU	O24736_THETH	O83140_TREPA	PCRA_STAA8	REP_ECOLI	UVRD_MYCGE
EX5B_CHLTR	O25569_HELPY	O83991_TREPA	Q46538_DICNO	REP_HAEIN	UVRD_MYCPN
EX5B_ECOLI	O26611_METTH	O84614_CHLTR	Q9Z7D4_CHLPN	SRS2_SCHPO	UVRD_RICPR
EX5B_HAEIN	O51319_BORBU	P73465_SYNY3	Q9ZCJ7_RICPR	SRS2_YEAST	Y340_MYCPN

Table **10.** Selected Low-Cysteioe or No-Cysteme Wild-Type **PcrA Helicases**

PcrA with **no** cysteine from L. **citreuni** MK20	
/gene="pcrA" /locus_tag="LCK_00476" /EC_number="3.6.1.-" /note="COG0210L; TIGR01073" /codon_start=l /transl_table=ll /product="ATP-dependent DNA heiicase PcrA" /protein_id="ACA82309.1" /db_xref="GI:169803691" (SEQ ID NO: 53)	MSW.n,TNGMNNKQAEAVQTTE,GPLLIMAGAGSGKTR VL'rHRIA HLVQDLN VFPWRILAI'r F0 KA AIRE MRERIA A **LLSEDVARDIWVSTFHALAVRILRRDGEAIGLAKNFTIID** **TSAQRTXMKRVINDLNLDTNQYDPRTILGMISNAKNDM** **LRPRDYAKAADNAFOETVAEVYTAYOAELKRSOSVDF** **DDLIMI**.TroiJQSAPE\TARYQQQFEYLHVDEYQDTND **AOYTIVNLLAORSKNLAVVGDADQSIYGWRGANMNNl** LNFEKDYPNAI -m¾lLEQNYRSTQNLDAANAVINI-{NNE **RVPKKLWTENGKGDOITYYRAOTEHDEANFILSNKJOLR** **ETKHMAYSDFAVLYRTNAQSRNIEESLVKANMPYSMV** **GGHKFYERKEILDIMAYMSLITNPDDNAAFERVVNEPKR** GLGATSLTRLRELANRLN\¾YMKATESIELAPSITTKAAS **KFLTFAEMMHNLROQSEFLNVTELIELVM 'rOSGYROM** **LAEKNDPDSOARLENLEEFLSVTKEFDDKYQPEDPESIDP**

	VTDFLGTTALMSDLDDFEEGDGAWLMTLHAAKGLEFP VWLIGLEEGIFPLSRAMMDEDLLEEERRLAYVGriRAM KKLFLTNAFSRLLYGRTQANEPSRFLAEISPELLETAYSGL SRDKTQKK^PFDRKMQRATATTYQATPVTKrTNGWG GDQTSWSTGDKVSHKKWGVGTVISVSGRADDQELKVA FPSEGVKQLLAAFAPIQKVD
Selected Low **Cysteine count thermophilic PcrA helicases**	
>tr!B5Y6N2 |B5Y6N2___COPPD DNA helicase OS=Coprothermobacter proteolyiicus (strain ATCC 35245 / DSM 5265 / BT) GN=pcrA PE=4 SV=1 (SEQ ID NO: 54)	MALPOENLIPPSPSHNHLTLSLRSHIGGFFIYNE-DVDSVDL SKLNEAQKQAVTAPPKPLAIIAGPGSGKTRVLTYRALFA VKEWHLPPERILA1TPTNKAADELKERLGRL1PEGDRIFA ATM HSFAARMIJRYFAPYAGISQNFWDDDDSKGLIEDI LKQ^lNMDTKRFRPND\^NfflSAAK,ARMFDCNTFPEFIR QRYGSWGWFDTVTIQWMTYERI,KEQSQALDFDDLIM VLAQRMEDRPELREMIAGLFDLVMVDEFQDTNFAQYQ MLLYMTNPHYSGMNNVTIVO DPDQSIYGFRAAEYYNIK RFIDDYNPEVVFLDLNYRSMITIVDSASALINDSPS/VLFE RKLESIKGAGNKL1LRRPFDDADAAITAAFEV0RLHKMG IPYEEIAVlMRTRAlXARVEREFATRNIQYIfflGGVTFFAR REIKDILAYLRLSRNAMDRVSLKRILTMKKRGFGTASLE KLFNFAEENKLTLLEAMKAA\/ESIX FKKLSMNDYI E SL YTLIOTIOEIAEPSOAIYLVMEQENLLDHFRSISKSEEEYIE RTENVK0L1S1AEESADMDDFL0RSALGTRENNGGVEGV AISTVHG\TCGLEFQAV1LYYVFDGFFPHSLSVTTAEKEEE RRLLYVAMTRAKEHLIFYVPYKQPWGNGFEQMARPSPF LRSIPKELWDGKPNEIESLYAPYSPQQKWSE
>tr|E8MZN5 E8MZN5_ANATU DNA helicase OS=Anaerolinea therraophila (strain DSM 14523 / JCM 11388 / NBRC 100420 / UNI-1) GN=pcrAPE=4 SV=1	MDSLEI^NPQQllAAVrASAGPVLVLAGPGSGKm\l.TF RIGYLLSOLGVAPHHILAVTFTNKAAREMOSRVEKLLGH SLQGMWLGTFHAICARILRREQQYLPLDANFVIFDEDDQ OALIKRALRDLNLDEKLYRPTSVHAAISNAKNNLILPED YPTATYRDEWAR^XRYQELLVSSNAVDFDDLLLYA

(SEQIDNO: 55)	WKLLNEFSTVREQYARRFEHILVDEFODTNLAOYELVK IXASYIIRM-JVVGDEDQSTYRWRGADYRN^ RFEEDFP DRQKILLEONYRSTORVLDAAQAVINRNRNRTPKRLKST PEFLGEGEKL\A.YEA\TDDYGEAAFWDTIQQLVAGGKA RPGDFAIMYRTNAQSRLLEEAFLRAGVPYRLVGAMRFY GRREVKDMIAYLRLVQNPADEASLGRVINVPPRGIGDKS QLALQMEAQRTGRSAGLILMELGREGKDSPHWQALGR NASLLADFGSLLGEWHRLKDE1SLPSLFOR1LNDLAYREY lDDNTEEGQSRWENA^QELLRly\YEYEEKGLTAFLENLy\L VSF^DTLPENVEAPTLLTLHAAKGLEFPIVFITGLDFXJLIP HNRSLDDPEAkiAEERRLFWGLTRAKKRVTL\¾AAQR STYGSFQDSPSRFLKDPADLIQQDGRGRRMGRSWQSES RRSWDDNYAGTWGSRPERAKPSFIAPILQPRF¾PGMRVK HPSWGEGL^OSRIQDEDETVDIFFDSVGFKRVIASIANL EILS
>tr|E8PM35pPM35_THESS DNA helicase OS- Ī'herraus scotoductus (strain ATCC 700910 / SA-01) GN==pcrAl PE=4 SV=1 (SEQIDNO: 56)	MCXiPOSSHPGDELLRSLNEAQROAVLHFEGPALVVAGA GSGKmTVVHRVAYLL\KilGVFPSEILAVlTTNKAAEEM RERLKRMVKGGGELWVS ĪFHSAALRILRVYGERVGLKP GFV\TDEDDQTALIKEVLKELGLAARPGPLKALLDRAK NRGEAPESLLSELPDYYAGLSRGRLLDVLKRYEEALKA QGALDFGDIIX YALRl LEEDPEVLKRVRRRARFIHVDEY QDTNPVQYRFTKLLAGEE/VNL^IAVGDPDQGIYSFIIAAD IKMLEFTRDF^GAKVYmEEmTlSTEAILRFANALrvNN ALRLEKTLRPVKPGGEP\¾LYRARDAilDEARFVAEEILR LGPPFDRVAVLYRTNAOSRLLEOTLASRGVPARVVGGV GFFERAEVKDLLAYARLSLNPIJXiVSLKRVLNTPPRGlG PATVEKVEALAREKGLPLFEALRVAAEVLPRPAPLRHFL ALMEELQELAFGPAEGFFRFILLEATDYPAYLREAYPED YEDRLENVEELLRAAKEAEGLMEFLDKVALTARAEEPG EPAGKVALWLFMAKGIEFPV\'TVVCrWEGLLPFIRSSL

	STLEGLEEERRLFYVGVTRAQERLYLSYAEEREVYGRTE ATRPSRFI-EEVEGGLYEEYDPYRASAKVSPSPAPGEARA SKPGAYRGGEK\WRFGQGTWAAMGDEVTVFIFEGV GLKRLSLKYADLRPVG
>tr!E8PL08|E8PIO8JllESS DNA helicase OS=Thermus scotoductus (strain ATCC 700910 / SA-01) GN=pcrA2 PE=4 SV=1 (SEQIDNO: 57)	IVILl^EQEAVANHFTGPALVIAGPGSGKTRTVVHRIARLI RKGVDPETVTAVTFTKKAAGEMRERLVHLVGEETATK VFTATFHSLAYHVLKDTGTVRVLPAEOARKLIGEILEDL QAPKKLTAKVAQGAF SRVBCNSGGGRRELIAL YTDF SPYI ERAWDAYEAYKEEKRLLDFDDLLHOAVHELSTDIDLOA RWQFIRARFLIVDEYQDTNLVQFM LRLLLTPEENLMAV GDPNOAIYAWRGADFRLILEFKKHFPNATVYKLHTNYR SHNGIVTAAKKVITHNTQREDLDLKALRNGDLPTLVQA OSREDEALAVAEVVKRHLDQCiTPPEEIAILLRSLAYSRPl EATLRRYRIP\TIVGGLSFWNRKEVOLYLHLLOAASGNP ESTVEVLASLVPGMGPKKARKALETGKYPKEAEEALQL LODLRAYTGERGEHLASAVQNTLHRHRKTLWPYLLELA DGlEEAAWDRWANLEEyWSTLFAFAFIFflTEGDLDTYLA DILLQEEDPEDSGDCiVKlMTLHASKGLEFAVVLLPFLVE GAFPSWRSAQNPATIEEERRLFYVGLTRAKEHAYLSYH LVGERGATSPSRFARETPANLIHYNPTIGYQGKETDTLSK LAELF

Example 10. **Cysteine reactive crosslinkers and alternative crosslinkers**

[00246] Bis-maleimide crosslinkers with contour length varying from 6 to 25 Angstrom were used as exemplary crosslinkers (Table 2): BMPEG2, BMOE, BMH, OTME , (1,2-Phenylene-bis-maleimide), and (Succinyl Bis[(phenylimino)-2, 1-ethanediyl]bis(3-ma]eimidopropanamide)). Alternatively bis-maleimide crosslinkers such as BMPEG3, BMB, BMDB , (1,4-Phenylene-bis-maleimide), (Bis-maleimidomethyl), and (N,N-|pitliobis[(caroonylphenylimido)-2, l~ethanediyl]]bis(3~ maleimidopropanamide)) or homobifunctionai vinylsulfone crosslinker such as HBVS can be used. An alternative crosslinker can be of any crosslinker of desired length that fits the criteria set forth in Example

8 with suitable functional end groups. For crosslinking two cysteines, suitable end groups can be any of the maleimide, haloacetyi, kxloacetyl, pyridyl disulfide, vinylsulfone and other suitable moieties. Table 1l shows examples of bis-maleimide linkers with corresponding lengths.

Table **11.** Selected **Bismaleimide Crossliiikers**

Crosslinker	Spacer Arm Length (A)	**Spacer Ann** Composition (between **maleimide** groups)
BMOE	8.0	Alkane
BMDB	10.2	Cis-diol (periodate cleavable)
BMB	10.9	Alkane
BMH	13.0	Alkane
DIME	13.3	Disulfide (reducing agent cleavable)
BM(PEG)2	14.7	Polyethylene glycol (PEG)
BM(PEG)3	17.8	Polyethylene glycol (PEG)

Example **11.** Alternative crosslinking methods to cysteine crosslinking

[00247] As an alternative to cysteine crosslinking chemistry, one can introduce a pair of unnatural amino acids for crosslinking with linkers using different chemistries as defined herein. This may be advantageous over cysteine engineering, because it may eliminate the extra steps of site directed mutagenesis of potentially interfering native cysteines and potentially detrimental effects of such mutations in other related helicases. For example, it was shown herein that in the PcrA helicase, there are two native cysteines that are higlily conserved across diverse species (Figures 4A and 4B). The mutating out of these two cysteines in PcrA from ***Bacillus stearoihermophilus*** reduced the ATPase activity by more than 80%. However replacing all five native cysteines in Rep from E. coli had a very minimal effect.

[00248] Alternatively, a target residue pair can be introduced, one of which is an unnatural amino acid and the other is a cysteine. Alternatively, one can introduce two or more pairs of target residues, preferably each pair can be specifically targeted with specific crosslinkers that employ orthogonal

chemistries so that unwanted inter-pair crosslinking is avoided (for example, one pair of cysteines and one pair of unnatural amino acid residues) for enhanced conformational stability and activity.

Example 12. Unnatural amino acids as an alternative to cysteine **crosslinking**

[00249] There are nearly one hundred unnatural amino acids (Uaa) that have been genetically incorporated into recombinant or endogenous proteins. These Uaa provide a wide spectrum of side chains that can be covalently crosslinked using a homo or hetero bi-functional linker with suitable end groups. Additionally a multi-branched multi- or homo-functional crosslinkers can be used for secondary conjugation other chemicals, biomolecules such as a DNA polymerase enzyme, in addition to the main crosslinking reaction. Uaa can incorporate specific reactive groups to the specific sites on the proteins, such as aryl iodides, boronic acids, alkynes, azides, or others, or they can be post-transcriptionally or chemically modified to prepare for desired crosslinking chemistry. Examples of Uaa include, but are not limited to, homopropargylglycine, homoallylglycine, azido-phenylalanine, azidohomoalanine and others. Uaa modification and crosslinking reactions include, but are not limited to, azides and cyclooctynes in copper-free click chemistry, nitrones and cyclooctynes, oxime/hydrazone formation from aldehydes and ketones, tetrazine ligation, isonitrile based click reaction, quaricyclane ligations, copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition, copper acetylide to activate terminal alkynes toward reaction with azides, Staudinger ligation, cyclooctyne reactions, and Huisgen cycloaddition. Suitable end groups of these crosslinkers would include, but are not limited to, azide, alkyne, succinimide, phosphine, etc.

Example 13. Selected Super-Family 1B (SF1B) and Super-Family 2 (SF2) **Helieases**

[00250] Selected SF1B and SF2 helieases are described herein. In an embodiment, the helicase is RecD2. In an embodiment, the RecD2 helicase is from ***D. radioduram.*** Selected target residue pairs for crosslinking, and the specific distances between the pairs, in RecD2 are shown in Fig. 12 and Table 12.

Table 12. Selected Crosslinking **Pairs for** 5' **to** 3' **SF1B Superhelicase RecD2**

	Rec02	**Backbone C- C distance in Å**
ALA632	**LE170**	**18.0**

ALA632	ASN171	**17.0**
PHE635	GLY200	**18.0**

1B domain amino acid (RecD2; *D. radiodurans)*	**2B domain amino acid (RecD2; *D. radiodurans)***	**Backbone C- C distance in Å**
ARG 410 **(B-sheet)**	**ASN** 596 (loop)	12.91
PRO 413 (B-sheet)	PHE 603 (loop)	13.04
GLN 414 (B-sheet)	ASN 596 (loop)	11.13
GLY 415 (loop)	GLU 601 (loop)	8.38
PHE 416 (loop)	ARG 417 (loop)	6.36
ARG 417 (loop)	**ASN** 599 (loop)	12.43
GLY 418	TYR 598 (loop)	11.00
LEU 411 **(B-sheet)**	PHE 603 (loop)	13.62
ARG 417 (loop)	ARG 417 (loop)	10.14

[00251] RecQ helicase has a winged helix domain (denoted by WH, shown in green in Fig. 13 and Fig. 14) that rotates 90 degrees and makes contact with the duplex in the unwinding conformation (Mathei et al., "Structural mechanisms of DNA binding and unwinding in bacterial RecQ helicases" Proc Natl Acad Sci U S A. 2015 Apr 7;] 12(14).4292-7). In an embodiment, stabilization of the WH domain of RecQ leads to superhelicase activation. Stabilization of the closed form of the WH domain can be achieved by crosslinking it to the catalytic core using the residue pairs shown in Table 13.

Table 13. Selected **Crosslinking Pairs** for **Superhelicase RecQ**

Catalytic domain	WH domain	Backbone C- C distance in Å
PHE221	**VAL470**	**7.91**
GLU219	**ARG514**	**5.61**
LYS212	**GLU467**	**8.90**
PHE221	**GLU467**	**6.52**

[00252] RecQl helicase also has a **winged** helix domain (denoted by WH, shown in green in Fig. 15) that rotates 90 degrees and makes contact with the duplex in the unwinding conformation. In an embodiment, stabilization of the **WH** domain of RecQl leads to superhelicase activation. Stabilization of the closed form of the **WH** domain can be achieved by crosslinkmg it to the catalytic core using the residue pairs shown in Table **14.**

Table 14. Selected **Crosslinking Pairs for** Superhelicase **RecQl**

Zinc finger alpha helix domain amino acid	WH beta hairpin domain amino acid	Backbone C- C distance in Å
MET429	**TYR564**	**12.17**
VAL431	**THR566**	**8.31**
MET429	**ALA565**	**8.77**
MET429	**THR566**	**7.10**

[00253] 5-3' SF1 superhelicase T4 Dda (Fig. 16) is known to unwind dsDNA as a monomer, and has sequence similarity to *K coli* recD (exonuclease V). In an embodiment, stabilization of the tower/hook

and pin domains leads to superhelicase activation. Stabilization of the closed fomi of the tower/hook and pin domains can be achieved by crosslinking them using the residue pairs shown in Table 15. Wild-type T4 Dda has 439 amino acids, a 5-3' unwinding polarity, and 5 cysteines. It is a DNA helicase that stimulates DNA replication and recombination reactions in vitro, and has been suggested to play a role in the initiation of T4 DNA replication in vivo. It acts by dissociating and associating with the DNA molecule being unwound, interacting with UvsX and binding tightly to the gene 32 protein. Selected crosslinking pairs that parallel SF1A helicases are located in the tower/hook and the pin domains based on the crystal structure (Fig. 16) and are listed in Table 15.

Table 15. Selected Crosslinking Pairs for Superhelkase T4 Dda

1B domain (pin) amino acid	2B domain (tower/hook) amino acid	Backbone C-C distance in Å
THR91 (B-sheet)	TRP 374 (Alpha helix)	9.77
TYR 92 (B-sheet)	TYR 363 (Alpha helix)	11.78
TYR 92 (B-sheet)	TYR 363 (Alpha helix)	11.73
TYR 92 (B-sheet)	LYS 364 (Alpha helix)	10.42
GLU 93 (loop)	LYS 364 (Alpha helix)	6.83
GLU 93 (loop)	ALA 372 (loop)	9.25
GLU 93 (loop)	PRO 373 (loop)	10.45
GLU 93 (loop)	SER 375 (Alpha helix)	10.38
GLU 94 (loop)	TRP 374 (Alpha helix)	8.25
GLU 94 (loop)	ALA 372 (Alpha helix)	8.25

GLU 94 (loop)	SER 375 (Alpha helix)	10.73
GLU 94 (loop)	TRP 378 (Alpha helix)	8.58
VAL 96 (B-sheet)	LYS 381 (Alpha helix)	12.55
VAL 96 (B-sheet)	TRP 374 (Alpha helix)	12.36
VAL 96 (B-sheet)	TRP 378 (Alpha helix)	10.56

[00254] Structural data have been obtained for the SF1B RNA helicase Upfl (5'-3' SF1B **RNA/DNA** helicase) **in** complexes with phosphate, ADP and the non-hydrolysable **ATP** analogue, ADPNP (Cheng et al, 2006), although a structure with bound **RNA** remains lacking. These structures reveal a conformational change that accompanies binding of ATP and which is very similar to that which occurs during catalysis **in** SF1A helicases such as PerA.

Example 14. Identifying suitable crosslinking sites **for immobilizing 2B domain at a particular rotational conformation between the open and closed form**

[00255] It has been shown herein that the closed and open forms captured **in** the crystal structures are the active and the inactive states of the Rep helicase, respectively, which can be interconverted by a 133 degree rotation of the 2B domain around an axis. Therefore, the active conformation can be defined through definition of the range of a rotational angle, Θ (theta), relative to the closed form with $\Theta = 0$ (Fig. 17). For example, in an embodiment, Rep-X becomes a superhelicase if $\theta < 40$ degrees. In addition, arresting the helicase in an intermediate conformation, such as, e.g. Θ = 40 degrees, may allow a new function. While immobilizing the 2B domain at an angle $\theta = 40$ degrees, it was found that residue pairs distances increase more than 10 A when Θ changes from 40 degrees to 0 degrees (to closed form), and increase more than 20 A when Θ changes from 40 degrees to 130 degrees (to open form). Positions of residues at the desired Θ, can be interpolated from open and closed form crystal structures via rigid body rotation of the 2B domain around an axis. Having performed this calculation for θ = 40 degrees of Rep helicase, it was found that 2B residues that satisfy this criteria are residues 515 and 518-525, and the

residues on the rest of the protein structure satisfying the criteria are residues 543-547. For example, crosslinking residues 521 to residue 547 on with a crosslinker with a length of about 10 Å, restricts the 2B domain to a conformation of Θ= 40 degrees. Similar to restricting the 2B conformation to θ = 0 degrees (closed form), corresponding residues to restrict in helicases with unknown structures can be determined via sequence alignment.

[00256] Rigid body rotation of the 2B domain around a chosen axis can convert the closed form to the open form or vice versa. In the case of £ *coli* Rep, the chosen axis intersects the alpha carbons of residue ILE371 and residue SER280 or residue ALA603 . In an embodiment, the chosen axis intersects the alpha carbons of residue ILE371 and residue SER280. Theta is the angle of rotation around this chosen axis from the closed form toward the open form. According to this definition, theta is 0 degrees for the closed form. In the case of *K coli* Rep, theta increases to 133 degrees when it is rotated around the chosen axis to obtain the open form. Theta for the open form may vary between different helicases.

[00257] Thus, in an embodiment of a modified helicase described herein, the first amino acid and second amino acid, together with an axis vector defined by an alpha carbon of ILE371, from which the vector originates, and an alpha carbon of SER280 or an alpha carbon of ALA603 of *E. coli* Rep helicase, define an angle, theta, wherein theta is about 355 degrees to about 25 degrees in an active conformation. In an embodiment, theta is about 355 degrees, about 0 degrees, about 5 degrees, about 10 degrees, about 15 degrees, about 20 degrees or about 25 degrees, or any increment or point between about 355 degrees to about 25 degrees. In another embodiment, theta is about 0 degrees in an active confomiation. In an embodiment theta is about 60 degrees to about 155 degrees in an inactive conformation. In an embodiment, theta is about 60 degrees, about 65 degrees, about 70 degrees, about 75 degrees, about 80 degrees, about 85 degrees, about 90 degrees, about 95 degrees, about 100 degrees, about 105 degrees, about 110 degrees, about 115 degrees, about 120 degrees, about 125 degrees, about 130 degrees, about 133 degrees, about 135 degrees, about 140 degrees, about 145 degrees, about 150 degrees, or about 155 degrees, or any increment or point between about 60 degrees to about 155 degrees. In another embodiment, theta is about 133 degrees in an inactive conformation. In an embodiment, the axis vector is defined by an alpha carbon of ILE371 and an alpha carbon of SER280 of *K coli* Rep helicase. In another embodiment, the axis vector is defined by an alpha carbon oflLE 371 and an alpha carbon of SER280 of *E. coli* Rep helicase.

Example 15. **Examples of thermophilic** orthoSogs/homologs **of Uvrl), Rep and** PcrA

[00258] Based on the crosslinMng target site selection criteria established in Example 8, and analogous to identification of suitable crosslinking sites in hologous helicases as described in Example 9, by sequence alignment and structural homology modeling, the corresponding crosslinMng target residues are identified in helicases with unknown structures. Subsequent!y these helicases can be converted to superhelicase forms. Thus, in an embodiment. Rep-like thermophilic helicases featuring low or no cysteine content, and homologs or orthologs thereof are also suitable candidates for cross-linking to form a thermophilic superhelicase. Selected examples of thermophilic orthologs or homologs of LJvrD, Rep and PcrA are shown in Tables 16-18. In certain exemplary embodiments, a suitable UvrD, Rep or PcrA helicase is selected from the following species: Thermococcus sp. EXT9, Thermococcus sp. IRI48, Thermococcus sp. IRI33, Thermococcus sp. AMT7, Thermococcus nautili, Thennococcus onnurineus (strain NA1), Thermococcus kodakarensis (strain ATCC BAA-918 / JCM 12380 / KOD 1) (Pyrococcus kodakaraensis (strain KOD 1)), Thennococcus sibiricus (strain MM 739 / DSM 12597), Thermococcus paralvineliae, Thennus aquaticus Y51MC23, Thermus aquaticus Y51MC23, Thennus aquaticus Y51MC23, Thermus sp. RL, Thermus sp. RL, Thermus sp. 2.9, Salinisphaera hydrothermalis C41B8, Thermus filifoniiis, Meiothermus ruber, Thennus sp. NMX2.A1, Thermus thermophilus JL-18, Thermus scotoductus (strain ATCC 700910 / SA-01), Thermus scotoductus (strain ATCC 700910 / SA-01), Oceanithemius profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithemius profundus (strain DSM 14977/ NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Thermus oshimai JL-2, Thermus oshimai JL-2, Thermus oshimai JL-2, Theniiomonospora curvata (strain ATCC 19995 /DSM 43183 /JCM 3096/ NCIMB 10081), Thermodesulfatator indicus (strain DSM 15286 / JCM 11887 / CJR29812), Geobacillus stearothermopMlus (Bacillus stearotheniiophilus), Coprothermobacter proteolyticus (strain ATCC 35245 / DSM 5265 / BT), Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) (Thermus silvanus), Anaerolinea thermophila (strain DSM 14523 /JCM 11388 / NBRC 100420/ UNI-1), Thermoanaerobacterium thermosaccharolyticum M0795, Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Meiothermus tuber (strain ATCC 35948 /DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Deinococcus radiodurans (strain ATCC 13939 /DSM 20539/

JCM 16871 /LMG4051 /NBRC 15346/NCIMB 9279/Rl /VKM B-1422), Themiodesulfobium narugense DSM 14796, Thermus thermophiius (strain HB8 / ATCC 27634 / DSM 579), Dictyoglomus thermophi!um (strain ATCC 35947 / DSM 3960 / 1:1-6-12), Thennus thennophilus (strain SGQ.5JP17-16), Thennus thennophilus (strain SG0.5JP17-16), Thennus thennophilus (strain SG0.5JP17-16), Thennus sp. CCB_US3_UF1, Deinococcus geothermalis (strain DSM 11300), Thermus thennophilus (strain HB27 / ATCC BAA-163 / DSM 7039), Thermus thennophilus (strain HB27 / ATCC BAA-163 / DSM 7039), Marinithermus hydrotliemialis (strain DSM 14884 / JCM 11576 / Tl).

Table 16

Entry (3D)	Entry name	Protein names	Organism	Length	Gene names (primary)/ Gene encoded by
L0B9N8	L0B9N8_9EURY	UvrD Rep helicase SFI	Thermococcus sp. EXT9	591	Plasmid pEXT9a
L0B9J0	L0B9J0_9EURY	UvrD Rep helicase SFI	Thermococcus sp. IRI48	547	Plasmid pIRI48
L0BAD9	L0BAD9_9EURY	UvrD Rep helicase SFI	Thermococcus sp. IRI33	591	Plasmid pIRI33
L0BAT5	L0BAT5_9EURY	UvrD Rep helicase	Thermococcus sp. AMT7	591	Plasmid pAMT7
W8NUG2	W8NUG2_9EURY	Superfamily 1 DNA and RNA helicase and helicase subunits	Thermococcus nautili	665	
B6YXQ7	B6YXQ7_THEON	UvrD/REP helicase	Thermococcus onnurineus (strain NA1)	533	

Q5JFK3	Q5JFK3_THEKO	DNA helicase, UvrD/REP family	Thermococcus kodakarensis (strain ATCC BAA-918 / JCM 12380 / KOD1) (Pyrococcus kodakaraensis (strain KOD1))	661
C6A075	C6A075_THESM	DNA helicase, UvrD/REP family	Thermococcus sibiricus (strain M M 739 / DSM 12597)	716
W0I5I1	W0I5I1_9EURY	DNA helicase, UvrD/REP family protein	Thermococcus paralvinellae	659
B7AA42	B7AA42_THEAQ	DNA helicase (EC 3.6.4.12)	Thermus aquaticus Y51MC23	701
B7A5I6	B7A5I6_THEAQ	DNA helicase (EC 3.6.4.12)	Thermus aquaticus Y51MC23	868
B7A954	B7A954_THEAQ	DNA helicase (EC 3.6.4.12)	Thermus aquaticus Y51MC23	542
H7GEQ7	H7GEQ7_9DEIN	DNA helicase (EC 3.6.4.12)	Thermus sp. RL	1030
H7GH69	H7GH69_9DEIN	DNA helicase (EC 3.6.4.12)	Thermus sp. RL	693

A0A0B0SAG4	A0A0B0SAG4_9DEIN	DNA hehcase (EC 3.6.4.12)	Thermus sp. 2.9	692	
A0A084IL47	A0A084IL47_9GAMM	ATP-dependent DNA helicase Rep (EC 3.6.4. 12)	Salinisphaera hydrothermalis C41B8	670	rep
A0A0A2WMV1	A0A0A2WMV1_THEFI	DNA helicase (EC 3.6.4. 12)	Thermus filiformis	665	
A0A0D0N7B7	A0A0D0N7B7_MEIRU	DNA helicase (EC 3.6.4. 12)	Meiothermus ruber	706	
W2U4X3	W2U4X3_9DEIN	DNA helicase (EC 3.6.4. 12)	Thermus sp. NMX2.A1	710	
H9ZQB5	H9ZQB5_THETH	DNA helicase (EC 3.6.4.12)	Thermus thermophilus JL-18	693	
E8PM35	E8PM35_THESS	DNA helicase (EC 3.6.4.12)	Thermus scotoductus (strain ATCC 700910 / SA-01)	708	pcrAl
E8PL08	E8PL08_THESS	DNA helicase (EC 3.6.4.12)	Thermus scotoductus (strain ATCC 700910 / SA-01)	621	pcrA2

E4U8J8	E4U8J8_OCEP5	DNA helicase (EC 3.6.4.12)	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	719	
E4U4N5	E4U4N5_OCEP5	DNA helicase (EC 3.6.4.12)	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	917	
E4UAI1	E4UAI1_OCEP5	DNA helicase (EC 3.6.4.12)	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	889	Plasmid pOCEPROI
E4UAI8	E4UAI8_OCEP5	DNA helicase (EC 3.6.4.12)	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	638	Plasmid pOCEPROI

E4UAI4	E4UAI4_OCEP5	AAA ATPase	Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)	606	Plasmid pOCEPROI
K7QW32	K7QW32_THEOS	DNA helicase (EC 3.6.4.12)	Thermus oshimai JL-2	693	
K7QWX5	K7QWX5_THEOS	DNA helicase (EC 3.6.4.12)	Thermus oshimai JL-2	694	Plasmid pTHEOSOI
K7QTS9	K7QTS9_THEOS	DNA helicase (EC 3.6.4.12)	Thermus oshimai JL-2	854	
D1AF88	D1AF88_THECD	DNA helicase (EC 3.6.4.12)	Thermomonospora curvata (strain ATCC 19995 / DSM 43183 / JCM 3096 / NCIMB 10081)	799	
F8A884	F8A884_THEID	DNA helicase (EC 3.6.4.12)	Thermodesulfatator indicus (strain DSM 15286 / JCM 11887 / CIR29812)	503	
A0A087LEB0	A0A087LEB0_GEOSE	Uncharacterized protein	Geobacillus stearothermophilus (Bacillus stearothermophilus)	807	

B5Y6N2	B5Y6N2_COPPD	DNA helicase (EC 3.6.4.12)	Coprothermobacter proteolyticus (strain ATCC 35245 / DSM 5265 / BT)	696	pcrA
D7BJL0	D7BJL0_MEISD	DNA helicase (EC 3.6.4.12)	Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) (Thermus silvanus)	645	Plasmid pMESIL02
E8MZN5	E8MZN5.ANATU	DNA helicase (EC 3.6.4.12)	Anaeroiinea thermophila (strain DSM 14523 / JCM 11388 / NBRC 100420 / UNI-1)	737	pcrA
L0INW7	L0INW7_THETR	ATP-dependent exoDNAse (Exonuclease V), alpha subunit/helicase superfamily I member	Thermoanaerobacterium thermosaccharolyticum M0795	769	Plasmid pTHETHEOl

D3PPQ9	D3PR99_MEIRD	DNA helicase (EC 3.6.4.12)	Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber)	706
D3PLL2	D3PLL2_MEIRD	DNA helicase (EC 3.6.4.12)	Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber)	920
Q9RTI9 (X-ray crystallography (3))	Q9RTI9_DEIRA	DNA helicase (EC 3.6.4.12)	Deinococcus radiodurans (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051 / NBRC 15346 / NCIMB 9279 / RI / VKM B-1422)	745
M1E5C5	M1E5C5_9FIRM	DNA helicase (EC 3.6.4.12)	Thermodesulfobium narugense DSM 14796	610
Q5SIE7	Q5SIE7_THET8	DNA helicase (EC 3.6.4.12)	Thermus thermophilus (strain HB8 / ATCC 27634 / DSM 579)	692

B5YD55	B5YD55_DICT6	DNA helicase (EC 3.6.4.12)	Dictyoglomus thermophilum (strain ATCC 35947 / DSM 3960 / H-6-12)	656	
F6DJA4	F6DJA4_THETG	DNA helicase (EC 3.6.4.12)	Thermus thermophilus (strain SG0.5JP17-16)	722	Plasmid pTHTHE1601
F6DIL2	F6DIL2_THETG	DNA helicase (EC 3.6.4.12)	Thermus thermophilus (strain SG0.5JP17-16)	692	
F6DJ67	F6DJ67_THETG	DNA helicase (EC 3.6.4.12)	Thermus thermophilus (strain SG0.5JP17-16)	1014	Plasmid pTHTHE1601
G8N9P8	G8N9P8_9DEIN	DNA helicase (EC 3.6.4.12)	Thermus sp. CCB_US3_UF1	704	
Q1J014	Q1J014_DEIGD	DNA helicase (EC 3.6.4.12)	Deinococcus geothermalis (strain DSM 11300)	741	
Q745W4	Q745W4_THET2	DNA helicase (EC 3.6.4.12)	Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039)	551	Plasmid pTT27

Q72IS0	Q72IS0_THET2	DNA helicase (EC 5.6.4.12)	Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039)	692	uvrD
F2NK78	F2NK78_MARHT	DNA helicase (EC 3.6.4.12)	Marinithermus hydrothermalis (strain DSM 14884 / JCM 11576 / TI)	716	

Table 17

Entry	Sequence	SEQ ID NO:
L0B9N8	MSEALPVTSFEFSLPEESVIKIYGPPGTGKTTTLVRIIEHLIGFHDHTEFLESYGLSLLFGQYGAEDV IFMTFQTSALKEFEARTGIKVKDRQNKPGRYYSTVHGIAFRLLIDSGAIDGVITQNFGSLSPEDW FRLFCRQNGLRFESSEMGYSNVFNDGNRLWNALTWAYNVYYPTKGPKARHEALKRLAPKL WKYPPLWEEYKTEKGILDYNDMLVKAYEGLKSGEIDPRNLPGHKYSPKVLIVDEFQDLSPLQFE IFRLLANYMDLVIIAGDDDQTIFSYQGADPRLMNYVPGREIVLKRSYRLPIVVQAKAMTVISKTR HRKEKTVAPRTDLGDFKYKLFWFPDFLNDLVREAQEGHSIFILVRTNRQVLKLGKELILAGVHF RHLKVDYRSIWEAGSKEWGTFRDLVQALLKARRGEELEIADLVTILYYSELIDWHLGEKLPEKER YKKIAEQMEKTIEAIEKGLMPFDILKVKDDPFSVLDLEKIESLSPRHGKVAVELIREIMKEKSQVV SVPRDAEIYLDTLHASKGREADVVFLINDLPRKWSSILKTREELDAERRVWYVGLTRARKKVYLL NGKHPFPVL	58
L0B9J0	MRVKIYGPPGTGKTTTLQRTIDYTLGNSSEPPIPLPESFPTDLEPKNLAFVSFTNTAIDVIGKRTGI TTRSKEAPYMRTIHGLILSVLAEHFDPVAVDNLGKLADIQAEFSMRMGYYYSKDPFEFAEGN MKFNVITRALELYLPKTGDVEEALKLIDNREDRKFALAWYRYKRQKKIMDFDDILVIGYEHLEDF YVPVEVAFIDEGQDNGPLDYILLEKGFEGAKFVFLAGDPLQSIYGFKGADPRLFVRWKADKEIIL PRSYRLPKKVWLLSQSWALSLGIKGAVVRYAPSEKLGRVSRMKFIEALSYAVEQAKRGRSVLIL	59

	ARTNSLVKFVG NİLSI EFGVAYGHLKRASYWESHLLKFIEGLQM LKLWDGVTPI KVQDTKPITG L IRKLKDKHAREVLRRWRDSRQWSLEVQAVLQRIKKNPSEYFYITDFDRQALKAYFSKARLDLTE ELIIDTIHAAKGEEADWIFLDFIPTRSEERINPEELQEKLVAYVGFTRAREELIIVPTPAIKYHPMR DFMG VRQ! LG W N FH KHUJ KELVGGL	
L0BAD9	MSbALPV T SbbhSLPKbRi!KLYGAPG TGK 1T 1LVKIIbHLIGhQUH T bhLbNYGINLPFGQYbPGb VIFMTFQTSALKEFEARTGIKVKDRQNKPGRYYSTVHGIAFRLLIDSGAVDGUTQNFGSLSPED WFRNFCRQNGLRFESSEMGYSNVFNEGNQLWNALTWAYNVYYPTKGPKARYEALKRLAPK LWKFPPLWEEYKKGRGILDYNDMLVRAYEGLRSGEIDPRNLPGHKYSPKVUVDEFQDLSPLQ FEIFRLLANHMDLVIIAGDDDQTIFSYQGADPRLMNYVPGLEWLRKSHRLPIWQAKALTVISK TRHRKEKTVAPRTDLGDFKYKLFWFPDFLNDLVREAQEGHSIFILVRTNRQVLKLGKEULAGV HFEHLKVDYRSIWEAGSKEWGTFRDLVQALLKAKRGEELEVADLVTILYYSEUDWHLGEGISE KERYKKIAEQMEKTiEAIEKGLMPFDVLRVKENPFSVLDLEKIESLSPRHGKVAVELiKELMKEKS QWSIPKDARIYLDTLHASKGREADWFUNDLPRKWSSILKTREELDAERRVWYVGLTRARKK VYLLNGKHPFPVL	60
L0BAT5	MSEALSITSFDFTLPRERIIKIYGPPGTGKTTTLVRIiEHLiGFQDHTEFLENYGLSLPFGQYGAEDV IFMTFQTSALKEFEARTGIKVKDRQNKPGRYYSTVHGIAFRLUDSGAVDGUTQNFGSLSPED WFRHFCRQNGLRFESSEMGYSNIFNEGNQLWNALTWAYNVYYPTKGPKARYEALKRLAPKL WKFPPLWEEYKKEKGILDYNDMLIRAYEGLKSGEIDPRNLPGHKYSPKVLIVDEFQDLSPLQFEI FRLLANHMDLVIIAGDDDQTIFSYQGADPRLMNYVPGREIVLSKSYRLPIWQAKALTVISKTRH RKEKTVAPRTDLGDFKYKLFWFPDFLNDLVREAQEGHSIFILVRTNRQVLKLGKEULAGVHFEH LKVDYRSIWEAGSKEWGTFRDLVQALLKAKKGEELEVADLVTILYYSEUDWHLGERISEKERYK KIAEQMEKTiEAIEKGLMPFDILKVKENPFSVLDLEKiESLSPRHGKVAVEUKELMKEKSQWSIP KDAKIYLDTLHASKGREADWFL!NDLPRKWSNILKTREELDAERRVWYVGLTRARKKVYLLNG KHPFPIL	61
W8NUG2	MNENEKLSKFIAKLKVUEMERKAEIEAMRAEMRRLSGREREKVGRAVLGLNGKVIGEELGYFL VRYGREREIKTEISVGDLVVISKRDPLKSDLVGTWEKGKRFITVALETVPEWALKSVRIDLYAND ITFKRWLENLENLRESGRRALELYLGLREPEGGEEVEFTPFDKSLNASQRRAIAKALGSPDFFLIH G PFGTG KTRTLVELIRQEVARG N RVLATAESNVAVDNLVERLVDSG LKWRVG HPSRVSRG LH	62

	ETTLAYLMTQH ELYGELRELRVIG EN LKEKRDTFTKPAPKYRRG LTDRQJLRLAEKGIGTRG VPA RLIREMAQWLKINEQVQKTFDDARKLEERIAREIIREADWLTTNSSAGLEWDYGSYDVAIIDE ATQATI PSVU PIN RAG RFVLAG D H KQLPPTI LSEKAKELSKTLFEG LÍERYPG KSEM LTVQYRMN ERLMEFPSREFYDGRIEADESIRRITLADLGVKSPEDGDAWAEVLKPENVLVFIDTARREDRFER QRYGSESRENPLEARLVKEAVEGLLRLGVKAEWIGVITPYDDQRDLISSLLPEEIEVKTVDGYQG REKEVIVLSFVRSNRKGELGFLKDLRRLNVSLTRAKRKLILIGDSSTLSSHPTYRRLVEFVRERETV VDAKRLIGKVKIK	
B6YXQ7	MTAPIPTTYSILGVAGAGKTTQLIDLLNYLNFENSRNEKIWERHFEPVELNRIAFISFSNTAIQEIA N RTGI E KARKKSAPG RYFRTVTG LAEVLLYEN N LMTFEEVRSVSKLEGFRI KWAREHGMYYKP RDNDISYSGNEFFAEYSRLVNTYYHVKSLSEIIEMHSKSHLLLDYIREKEKLGIVDYEDILMRAYDY RNDIWDLEYMIIDEAQDNSLLDYATLLPIAKNNATELVLAGDDAQUYDFRGANYKLFHKUER SEIILNLTETRRFGSEIANLATAIIDDMNYIQKREVLSAATHSTKVAHIDLFQMMSILQNMATTD LTVYILARTNAVLNYVAKVLDEYKIQYKKNERITDFDRFLLSLNRLMRNEYTNDDIYTIYNYLRNK VAREEELKERLFQH KLHWTEKDVLGI LLLAYEQTTAKRI LTTAKNTNFKI KLSTI HSAKGSEADW FLINSVPHKTKMKILENYEGEKRVLYVAVTRARKFLFIVDQPVARRYEQLYYIRSYESRAQGSLV NRVAVPVA	63
Q5JFK3	MNEKEVLLSKFIAHLKELVEMERRAEIEAMRLEMRRLSGREREKVGRAVLGLNGKVIGEELGYF LVRYGRDREIKTEISVGDLWISKRDPLKSDLVGTWEKGKRFLTVAIETVPEWALKGVRIDLYAN DITFKRWMENLDNLRESGRKALELYLGLREPEESEPVEFQPFDKSLNASQRGAIAKALGSGDFF LVHGPFGTGKTRTLVEURQEVARGHKVLATAESNVAVDNIVERLADSGLKWRIGHPSRVSKA LHETTLAYLITQHDLYAELRELRVIGENLKEKRDTFTKPAPKYRRGLSDREILRLAEKGIGTRGVPA RLIREMAEWIRINQQVQKTFDDARKLEERIAREIIQEADWLTTNASAGLEWDYGEYDVAVID EATQATI PSVU PINRAKRFVLAGDHKQLPPTI LSEKAKELSKTLFEG U ERYPEKSEMLTVQYRM NERLMEFPSREFYDGKIKAHESVKNITLADLGVSEPEFGNFWDEALKPENVLVFIDTSKREDRF ERQRRGSDSRENPLEAKLVTETVEKLLEMGVKPDWIGVITPYDDQRDLISSMVGEDIEVKTVD GYQGREKEIIVLSFVRSNRRGELGFLTDLRRLNVSLTRAKRKLIAVGDSSTLSNHPTYRRFIEFVRE RGTFIEIDGKKH	64
C6A075	MTRVQIPAGAPKYGPVAQPGQSARLISGRSGVRSPAGPPKALLKERFRELFIHKNPVITMHVK	65

	NYIAKLVDLVELEREAEIEAMREEMRRLKGYEREKVGRAILNLNGKIIGEEFGFKLVKYGRKEAF KTEIGVGDLWISKGNPLASDLVGTWEKGSRFIWALETVPSWAFRNVRIDLYANDITFRRQLE NLKKL5ESGIRALKLILGKETPLKSSPEEFTPFDRNLNQSQKEAVSYALGSEDFFUHGPFGTGKTR TLVELIVQEVKRG NKILATAESNVAVDN LVERLWG KVKLVRLG HPSRVSVHLKESTLAFQVESH ERYRKVRELRNKAERLAVM RDQYKKPTPQM RRG LTN NQi LKLAYRGRGSRGVPAKDI KQ M A QWITLNEQIQKLYKFAEKIESEIIQEIIEDVDWLSTNSSAALEFIKDAEFDVAIIDEASQATIPSVUP lAKARRFVLAGDHKQLPPTiLSEEARALSETLFEKUELYPFKAKMLEiQYRMNQLLMEFPSREFY NGKIKADGSVKDITLADLKVREPFFGEPWDSILKREEPUFVDTSNRTDKWERQRKGSTSRENP LEALLVREIVERLLRMGIKKEWIGIITPYDDQVDSIRSIIQDDEIEIHTVDGYQGREKEIIILSLVRSN KKGELGFLMDLRRLNVSITRAKRKLWIGDSETLVNHETYKRUHFVKKYGRYIELGDTGIN	
W0I5I1	MNURYINHLKELVELEREAEIEAMREEMRKLTGYEREKVGRAVLGLNGKIIGEEFGYKLVKYGR KQEiKTEISVGDLWiSKGNPLASDLiGTVTEKGKRFLWALETVPSWALRNVRiDLYANDITFKR QIENLDKLSESGKRALRFILGLEKPKESIDIEFKPFDEQLNESQKKAVGLALGSEDFFLIHGPFGTG KTRTVAEVI LQEVKRG KKVLATAESNVAVDNLVERLWG KVKLVRLGHPSRVSKH LKESTLAYQ VEIHEKYKRVREFRNKAERLAMLRDQYTKPTPQWRRGLTDRQILRLAEKGIGARGIPARVIKS MAQWITFNEKVQRLYNEAKKIEEEIVKEIIRQADWLSTNSSAALEFIKDIKFDVAVIDEASQATI PSVL! PIAKAN KFILAG DHKQLPPTI LSEEAKELSETLFEKLi ELYPSKAKM LEI Q YRMIN ERLMEFPS KEFYNGKIKAYDGVKNITLLDLGVRVFSFGEPWDSILNLKEPLVFVDTSKHPEKWERQRKGSLS RENLLEAELVKEIVQKLLRMG IKPESIGVITPYDDQRDUSLLLENDEI EVKTVDGYQGREKEVI ILS FVRSNKKGELGFLTDLRRLNVSLTRAKRKUAIGDSETLSAHPTYKRFVEFVKEKGIFVQLNQYVS QTS	66
B7AA42	MGEAH PSEEALLSSLN EAQRQAVLHFEGPALWAGAGSG KTRTWH RVAYLIARRGVFPSEIL AVTFTN KAAEEM KARLKAM VRG A G ELWVSTFH AAALRI LRVYG ERVGLKPG FWYDEDDQT ALLKEVLKELGLAAKPGP!KSLLDRAKNQGVPPEHLLLELPEFYAGLSRGRLQDVLHRYQEALRA QGALDFGDILLYALKLLEEDGEVLKRVRKRARFIHVDEYQDTNPVQYRFTRLLAGEEANLMAV GDPDQGIYSFRAADIRNILDFTQDYPKARVYRLEDNYRSTEAILRFANAVIVKNALRLEKTLRPV KKGGEPVRLFRAESARDEARFVAEEIARLGPPFDRVAVLYRTNAQSRLLEQALASRGIPARWG GVGFFERAEVKDLLAYARLSLNPLDAVSLKRVLNTPPRGIGPATVEKVQAIARERGLPLFEALKV	67

	AALTLPRPEPLRAFLALMEELMDLAFGPAEAFFRHLLLATDYPAYLKEAYPEDAEDRLENVEELL RAAKEAESLMDFLDKVALTARAEEPAEAEG RVALMTLH NAKG LEFPWFLVG VEEG LLPH RSS LSTQEGLEEERRLFYVGVTRAQERLYLSYAQEREIYGRLEPVRPSRFLEEVDEGLYEVYDPYRQSS RKPTPPPHRALPGAFRGGEKWHPRFGPGTWAAAGDEVTVHFEGVGLKRLSLKYADLRPA	
B7A5I6	MRWU^AGTGKTHALVEELKGLiQSGVPLRRIAALTFTRKAAEELRGRAKRAVLAL5AEDPRLK EAEREVHGALFTT!HGFMAEALRHTAPLLSLDPDFALLDTFLAEALFLEEARSLLYRKGLDGGLA RALLHLYRKRTLAETLHPLPGAEGVFALYLEALEGYRRRLPAFLSPSDLEALALRILENPEALRRW ERFPHILLDEYQDTGPLQGRFFQGLKEAGARLVWGDPKQSIYLFRNARVEVFREALKQAEEVR YLSTTYRHAQAVAEFLN RFTALFGEEG VRVRPH RQEVG RVEVH WWG EGG LEEKRRAEAHLL LDRLMALREEGYAFSQMAVLVRSRSSLPPLEAAFRARGVPYALGRGRSFFARPEVRDLYHALR LSLLEG PPGPEERLALLAFLRG PWVG LDLSEVEEALKAQDPI PLLPEAVRAKLRALRALAG LPPLE ALKRLSRDEAFLRRLSPRARVNLDALLLLAAMERFPDLEALLEWLRLRAEDPEAAELPEGEEGV QVLTVHGAKGLEWPWALFDLSRGENPKEEDLLVGLGGEVALRGTPAYKEVRKALRKAQAEE ARRLLYVALSRARDVLIVTGSASGRPGPWVEALERLGLGPESQDPLVRRHPFKALPPLGDRPQ TPPPPPLPAPYAHLAFPERPLPFVYSPSAFTKAKEPVPLAEALEKEALPEFYRALGTLVHYAIARH LDPEDEGAMAGLLLQEVAFPFAEGEKRRLLEEVRDLLRRYRGMLGPSLPPLEAREEDHAELPLV LPLGGTVWYGILDRLYRVGGRWYLEDYKTOREVRPEAYRFQLAIYRRALLEAWGVEAEARLVY LRHGLVHPLDPEELERALKEGFPGMGPGEGGEKA	68
B7A954	M KG LTGSSRLRVYGPPGTGKTTWLKN EVERLLRSG VPG EEI AVCAFSRAAFREFASRLAGQVP EENLGTIHSLAYRAIGRPPLALTKDALSDWNRRVPDTWRVTPRVDGRGADLLDVMDPYEDE DSRPPGDKLYDRVAYLRNTLAPMAAWSEEERAFFQAWKSWMNAKGLVDFPGMLEAALAK PGG LG ARFLLVDEAQDLTPLQLLLVEKWAQGARLALVG DDDQAI YG FMG ADGASFLG VPVE DELVLGQSYRVPARVQRVAEAVIRRVQNRAPKRYAPRGDEGEVRLLWVPPEDPYHAWDAL ERVNRGESVLFLATAKYLLEELKRELLRVGEPYANPYAPHRHSFNLFPQGARSAWEKARSFLFP NRIAADVKAWTKHVSSKVFAVKGEEARRYIESFPDEEKVGDDHPIWNVFRPEHRPHAVGRD VSWLLDHLLG NAPKTM RQSLMVALKSPEAVLQG RARVWIGTIHSVKGG EADWVYVWPGY TRKAAREHPDQLHRLFYVAATRARKGLVLMDQGKAPHGYVWPRVDEFWGEVWV	69
H7GEQ7	M EAN LYVAGAGTG KTYTLAERYLGFLEEG LSPLQWAVTFTERAALELRH RVRQMVGERSLG	70

	HKERVLAELEAAPIGTLHALAARVCREFPEEAGVPADFQVMEDLEAALLLEAWLEEALLEALQ DPRYAPLVEAVGYEG LLDTLREVAKDPLAARELLEKG LG EVAKALRLEAWRXLRRRM EELFHG ERPEERYPGFPKGWRXEEPEWPDIIAWAGEVKFNKKPWLEYKXDPALXRLLKLLGGVKEGFS PGPADERLEEVWPLLRELAEGVLARLEERRFRARRLGYADLEVHALRALEXEEVRAYYRGRFRR LLVDEFQDTNPVQVRLLQALFPDLRAWTWGDPNQSiYSFRRADPKVMERFQXEAAKEGLRV RRLEKSHRYHQGLADFHNRFFPPLLPGYGAVSAERKPEGEGPWVFHFQGDLEAQARFIAQEV G RLLSEGFQVYDLG EKAYRPMSLRDVAVLG RTWRDLARVAEALRRLEVPAVEAGGG N LLETR AFKDAYLALRFLGDPXDEEALVGLLRSPFFALTDGEVRRLAEARGEGETLWEVLEREGDLSAEA ERARETLRGLLRRKALEAPSRLLQRLDGATGYTGVAARLPQGRRRVKDWEGTLDLVRKLEVGS EDPFLVARH LRLLLRSG LSVERPPLEAG EAVTLLTVHG AKG LEWPWFVLN VGG W N RLGSWK N N KTKPLFRPG LALVPPVLDEXG N PSALFHLAKRRVEEEEKQEEN RLLYVAATRASERLYLLLSP DLSPDKGDLDPQTUGAGSLEKGLEATEPERPWSGEEGEVEVLEERIQGLPLEALPVSLLPLAAR DPEAARRRLLGEPEXEGGEAWXPXXPQETEEEVPGGAGVGRMTHALLERFEAXEDLEREGRA FLEESFPGAEGEEVEEALRLARTFLTAEVFAPYRGNAVAKEVPVALELLGVRLEGRADRVGED WVLDYKTDRGVDAXAYLLQVGVYALALGKPRALVADLREGKLYEGASQQVEEKAEEVLRRLM GGEGQG RQPYPLAATD PG HGAPG	
H7GH69	MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVTFT N KAAEEMRERLRG LVPGAG EVWVSTFH AAALR! LRVYG ERVG LRPGFWYDEDDQTALLKEV LKELALSARPGPI KALLDRAKN RGVG LKALLG ELPEYYAGLSRGRLGDVLVRYQEALKAQG ALD FGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEEANLMAVGDPDQG lYSFRAADIKN İLDFTRDYPEARVYRLEENYRSTEAILRXANAVIVKNALRLEKALRPVKRGGEPV RLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLLEQALAGRGIPARWGGVGFFE RAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATWARVQLLAQEKGLPPWEALKEAART FXRAEPLRHFVALVEELQDLVFGPAEAFFRHLLEATDYPTYLREAYPEDAEDRLENVEELLRAAK EAEDLQDFLDRVALTAKAEEPAEAEGKVALMTLHNAKGLEFPWFLVGVEEGLLPHRNSLSTLE GLEEERRLFYVG!TRAQERLYLSHAEEREVYGRREPARPSRFLEEVEEGLYEVYDPYRXPKPXPPP HRPRPGAFRGGERWHPRFGPGTWAAQGDEVTVHFEGXGLKRLSLKYAELXPA	71
A0A0B0SAG4	M D EALLSSLN EAQRQAVLH FQG PALWAG AGSG KTRTWH RVAYLIA H RGVYPTEI LAVTFTN	72

	KAAEEMRERLKG MVRGAG EVWVSTFH AAALR! LRVYGERVG LKPG FWYDEDDQTALLKEV LKELG LSAKPG PIKALLDRAKN RG EPPEALLAELPEYYAGLSRRRLLDVFFRYQEALKAQGALDF GDILLYALRLLEEDQEVLARVRKRARF!HVDEYQDTNPVQYRFTKLLAGEEANLMAVGDPDQG IYSFRAADIKNILQFTADFPGAKVYRLEENYRSTEAILRFANAVIVKNALRLEKTLRPVKRGGEPV RLFRAKDAREEARFVAEEILRLGPPFDRIAVLYRTNAQSRLLEQALAGRGVGARWGGVGFFER AEVKDLLAYARLALNPLDSVSLKRILNTPPRGIGPATVEKVARLAQEKGLPLFEALKRAELLPRPE PVRHFVALMEELMDLAFGPAEAFFRHLLQATDYPAYLREAYPEDHEDRLENVEELLRAAKEAE SLLDFLDKVALTARAEEPAGAEGKVFLMTLHNAKGLEFPWFLVGVEEGLLPHRNSLNTLEALE EERRLFYVGVTRAQERLYLSYAEEREVYGRLEATRPSRFLEEVEEGLYQEYDPYRSPRPVPPSHR PKPGAFKGGEKWHPRFGPGTWAASGDEVWHFEGVGLKRLSLKYADLRPA	
A0A084IL47	M ALPKLNPQQDAAM RYLDGPLLVLAG AGSG KTGV!TRKI A HLİARGYDARRWAVTFTN KAA REMKQRASKUSADDARGLTVSTFHSLGLQMIREEHAALGYKPRFSIFDSEDADKVLADLVGR DGDHRKATKAAISNWKSAUDPETATAQATGSDIPLARAYGEYQRRLKAYNAVDFDDLLALPV HLLSTDH EARERWQSRFRYLLVDEYQDTNAAQYEMM RLLAGARAAFTWG DDDQSi YAWR GARPGNIADLSRDFPHLKVIKLEQNYRSVGNVLSAANQLIGASNQRAYEKTLWSAMGPGDRV RVIAAPDEAGEAERIASEISSHKLRLGTAYGDYAILYRGNFQSRAFEKALRERDIPYRVSGGRSFF ERSEIRDLVTYLKLMVNPDDDAAFLRIVNLPRREIGPATLEALGRYAGSRHISLFDAARGIGLAG GVGERSGRRLADFVDWLRNLTQDSEGMTPRELVSQUVD!DYRNWLRDTSANTKAARKRiEN LDDF!GWLDRLDNAEDGKPVTLEDWRRLSLMDFANQSEKDVENQVHLLTLHAAKGLEFDH VFU^GLEEGMLPHHACLEDDKIEEERRLLYVGiTRARKTLALTYARKRRRGGEESDSVPSRFLEEL PADELDWPSATGTRSKAANAEQGRDQVAALRAMLGASADS	73
A0A0A2WMV1	MPQVGFTDHFFKGLEALSREEQNRVREAVFAFMQDPKHPSFKLHRLEDIKTDRFWSARVSK DLRLi LYHHPEMGWi FAYVG H HDDAYRWAETHQAEVH PKLG LLQ İFRWEEVRVEPRKI KPLL PDYPDDYLLDLGVPPSYLKPLRLVETEDQLLGUEGLPQDVQERLLDLAAGRPVTLPPKLAPSEE WFKHPLSRQHIHFIQNLDELRQALSYPWERWMVFLHPAQREAVERVFQGPARVTGPAGTG KTWALHRAAALARRYPEEPLLLTTFNRFLASRLRSGLQRLLGEVPPNLTVENLHSLARRLHEQH VG PVKLVKEEDYG PWLLEAAQG LEYG KN FLLSEFAFADAWG LYTWEAYRG FPRTGRGVPLTA RERLKLFGAFQKVWGRMENEGALTFNGLLHRLRQRAEEGALPRFRAWVDEAQDLGPAELLL	74

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A0A0D0N7B7	MSDLLSSLN PSQREAVLH FEG PALWAG AGSGKTRTWH RIAYLLRERRVYPAE! LAVTFTNKA A G EM KERLEKM VG RSARDLWVTTFHAAAVRI LRTYG EYVGLKPG FVI YDEDDQNTLLKEVLK ELELEAKPGPFRSMIDRIKNRGAGLAEYMREAPDFIGGVPRDVAAEVYRRYQNSLRMQGALD FNDLLLLTIELFEQHPEVLHKVQQRARFIHVDEYQDTNPVQYRLTRLLAGERPNLMWGDPDQ SIYGFRNADI NNiLDFTKDYPG A RV!RLEENYRSSSSI LRVAN AVI EKNALRLEKVLRPTKPGGEPV RLYRAPNAREEAAFVAREIVKLGGYQQVAVLYRTNAQSRLLEEHLRRANVPVRLVGAVGFFER REIKDLLAYGRVAVNPDDSINLRRIVNTPPRGIGATTVARLVEHAQKTGITVFEAFRAAEQVISR PQQVQAFVRLLDELMEAAFESGPTAFFQRVLEQTGFREALKQEPDGEDRLQNVEELLRAAQD WEEEEGGSLADFLDSVALTAKAEEPQGDAPVEAVTLMTLHNAKGLEFPTVFLVGLEENLLPHR NSLHRLEDLEEERRLFYVGITRAQERLYLSYAEERETYGKREYTRPSRFLQDIPQDLLKEVGAFGD GETRVLSQARPEPKPRTQPAEFKGGEKVKHPKFGSGTWAAMGGEVTVMFPGVGLKRLAVK FAGLERLE	75
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B5Y6N2	MALPQENUPPSPSHNHLTLSLRSHIGGFFIYNEDVDSVDLSKLN EAQKQAVTAPPKPLAIIAG P GSGKTRVLTYRALFAVKEWHLPPERILAI TFTNKAADELKERLGRUPEGDRIFAATMHSFAAR	91

	MLRYFAPYAGISQNFVIYDDDDSKGUEDILKQMNMDTKRFRPNDVLNHISAAKARMFDCNT FPEFIRQRYGSWGYYFDTVHQVFMTYERLKEQSQALDFDDUMVLAQRMEDRPELREMIAGL FDLVMVDEFQDTNFAQYQMLLYMTNPHYSGMNNVTIVGDPDQSIYGFRAAEYYNIKRFIDD YNPEWFLDLNYRSN RT1VDSASALIN DSPSALFERKLESI KG A G N KLI LRRPFDDADAAITAAFE VQRLHKMGIPYEEIAVLMRTRALTARVEREFATRNIQYHIIGGVPFFARREIKDILAYLRLSRNA MDRVSLKRILTMKKRGFGTASLEKLFNFAEENKLTLLEAMKAAVESLLFKKLSMNDYLESLYTLI QTIQEIAEPSQAIYLVMEQENLLDHFRSISKSEEEYIERTENVKQUSIAEESADMDDFLQRSALG TRENNGGVEGVAISTVHGVKGLEFQAVILYYVTDGFFPHSLSVTTAEKEEERRLLYVAMTRAKE HLIFYVPYKQPWGNGFEQMARPSPFLRSIPKELWDGKPNEIESLYAPYSPQQKWSE	
D7BJL0	MNDPIRHKEG PALVFAGAGAGKTRTLTQRVKWLVEEG EDPYSITLVTFTNKAAG EM KERIAR LVEAPLAEAVWVGTFH RFCLQSLQVYG REIG LEKVAVLDSAAQRKLAER! IA G LFPAKPPRGFT PMAALGAVSRAANSGWDDIQLATMYADLTEKiVNFRWAYEEAKKGLGALDYDDLLLRGVRL LKLSEGAARMVRRRAAYLMVDEFQDTNGVQLELVRAIAPGTSPNLMWGDPDRSIYGWRG ANYRTI LEFRQHYPG AAVYG LYTNYRSQAGWEVANRI IAQN ATRKPEMQEAH LPQSEEPFLL VAKNRWEEAHFVAQAVEFYRGQGIALEEMAVLMRANFLSRDLEQALRLRGIPYQFTGGRSFF ERREIQLGMAVLKVLANPKDSLAVAALVEEMVEGAGPLGiQKVLEAAKAANLSPLEAFRNPA M VKG LRG KEVQAEAM RLAEVLQDQVARLAAEAPEYH ALLKETLDRLG FEAWLDRLG EESEQ VYSRKANLDRLLQGMQEWQEVNPGAPLQDLVGTLLLEAGDTPAEEGQGVHLMTVHASKG MEFRWFViGLNEGLFPLSKASSSFEGLEEERRLMYVAWRAKEVLHLSYAADGWSRFAQEAR VPVEEYDPRLGWSGRQNQQALKALLfIA	92
E8MZN5	M DSLEHLN PQQRAAVTASAG PVLVLAG PGSG KTRVLTFRIGYLLSQLGVAPHHI LAVTFTN KA AREMQSRVEKLLGHSLQGMWLGTFHAICAR!LRREQQYLPLDANFVIFDEDDQQAUKRALR DLNLDEKLYRPTSVHAAISNAKNNL!LPEDYPTATYRDEWARVYKRYQELLVSSNAVDFDDLLL YAWKLLNEFSTVREQYARRFEHILVDEFQDTNLAQYELVKLLASYHRNLPWGDEDQSIYRWR GADYRNVLRFEEDFPDRQKILLEQNYRSTQRVLDAAQAVINRNRNRTPKRLKSTPEHGEGEKL VLYEAVDDYGEAAFWDTIQQLVAGGKARPGDFAIMYRTNAQSRLLEEAFLRAGVPYRLVGA MRFYGRREVKDMIAYLRLVQNPADEASLGRVINVPPRGIGDKSQLALQMEAQRTGRSAGLIL MELGREGKDSPHWQALGRNASLLADFGSLLGEWHRLKDEISLPSLFQRILNDLAYREYIDDNT	93

	EEGQSRWENVQELLRIAYEYEEKGLTAFLENLALVSDQDTLPENVEAPTLLTLHAAKGLEFPIVFI TG LDDG LİPH NRSLDDPEAMAEERRLFYVG LTRAKKRVYLVRAAQRSTYGSFQDSI PSRFLKDi PADLIQQDGRGRRMGRSWQSESRRSWDDNYAGTWGSRPERAKPSHAPILQPRFKPGMRV KH PSWG EGLWDSRIQDEDETVDI FFDSVG FKRVIASIAN LEI LS	
L0 INW 7	MDINGQIIKLNRNKTQGTLKLTNGQKIKFKINSDSVKPIFLYEYYKFKGNMIEDTLIIDDIYGIAND IN!NDFTELFPSVAHDKINNICNRFNVLHVGNLIDLINDENFITWNDTIGEEKATIFLSNLQK!KD RQEYI DVWDII KKTNPTFDI NVP! KIVNALKYRASM NNITVSQLI KESPWI 1EQLDIFDSITERKKIA ENIATHYGLSNDSNKAVISYAIAMTNNYIQQGHSYIPYYTLVSRVSNSLKLDFNKVNDTLKFLPN DNKSGYURDNKYKDEIENEYNSDKKIGYSVYLPKIFHMEKYIADIISSILKKKSTINKIELQKNLKLY RSEN KLİ FSKEQEEAI FSISDNKITVITGGAGTG KTTVI KA İ DLVN KMGYTPWLAPTG IASQRVA PNVGSTIHKYARIFDTYDPVFDEIEENKENNSGKVIIVDEMSM!TVPVFAKLLSVTLDADSFIFVG DPNQLPPIGAGGVFEAUELGNKNINNINTWLNQSFRSKNSIVKNAQNILEDKPIYEDDNLNIIE AKSWNKIADEWNLIRKLLDNGVQYSDIMVLSSKRGEGKNGVSLLNERIRKEIFNNKGKYAVG DIVITTRNDYDNKSSYFRSKELKKYINSIRHEERPTIFNGTVGVIKDISDNEVilEYNTPMPVEAKY N M EELDWYI EYG FAITVHKAQGGQAKYI 1FASDEPRN!SREM LYTA!TRCKNG KVFLIGG EN ED WKI KKEHSFVLSKLKYRILDNIHQQEKESKI NSKIV U NQ	94
D3PR99	MSDLLSSLN PSQQEAVLHFEG PALWAGAGSG KTRTWH RIAYLLRERRVYPAEILAVTFTNKA A G EM KERLEKM V G RPARDLWVSTFH AAAVRi LRTYG EYVGLRPG FV İYDEDDQNTLLKEVLK ELELEAKPGPFRAMIDRIKNRGAGLAEYMREAPDFIGGVPKDAAAEVYRKYQSGLRMQGALD FNDLLLLTIELFEQHPEVLHKVQQRARFIHVDEYQDTNPVQYKLTRLLAGERPNLMWGDPDQ SIYGFRSADINNILDFTKDYPGARVIRLEENYRSSSSILRVANAV!EKNALRLEKVLRPTRPGGEPV RLYRAPNAREEAAFVAREIVKLGNFQQIAVLYRTNAQSRLLEEHLRRANVPVRLVGAVGFFER REIKDLLAYGRVAVNPADSINLRRIVNTPPRGIGATTVSRLVEHAQKTGTTVFEAFRVAEQVISR PQQVQAFVRLLDEL!EAAFESGPTAFFQRVLEQTGFREALKQEPDGEDRLQNVEELLRAAQD WEEEEGGSLSDFLDSVALTAKAEEPQGDAPAEAVTLMTLHNAKGLEFPTVFLVGLEENLLPHR NSLHRLEDLEEERRLFYVGITRAQERLYLSYAEERETYGKREYTRPSRFLEDIPQDLLKEVGAFGD SEVRVLPQARPEPKPRTQLAEFKGGEKVRHPKFGSGTWAAMGGEVTVMFPGVGLKRLAVK FAGLERLE	95

D3PLL2	MKVRVASAGTGKTASLVLRYLELIAKGTPLRRIAGVTFTRKAADELRVRVAAAIEEVLQTGRHLS FVASGGSRAAFQEAAREIAGATLST!HGFMAQCLRLAAPLLHLDPDFSMLGDWEAQA!FEEE WQTLRYLAQDAH HPLFG LVSDELTEPLLHLFSRRSQAEVFEPAAŒANQH LLQVYQTVYAAY EARLGANLLSPSELERKALELARNDRAMKRVLERVRVLLVDEYQDVNPVQGAFFAALEQARLP IEIVGDPKQSIYAFRNADVSVFRKALREGKSEPPLTHSYRHSRVLVRFLNGLTGYLAKEGLGFGLE EAPPVEGVRPEQG RLEVHWWG ELPLEELRKQEARVLAGRLAALRG Pi EYSQMAVLVRSYGS VRFLEEALAEAQIPYVLLQGRGYYERQEVRDLYHALRAALDPRGLSLAVFLRSPFGQHTEAGPL KPLELPQIEGVLRADDPLGRLAQHWPSVYERLRQIQAQVRLMAPLEVLKFURAPLMDGRPYH DFLEPRARENVDALLFYFAPRPPQNLEGLLERLELLSRQADAGDVPQSGEGVQILTVHQAKGL EWPLVAVFDLG RM NV HRPQPLYLGQGPNGG DGG RLRRWVALPETPQFEAFRQQVKLQEE EESYRLLYVAASRARDTLLLTASASHGQPEGWGKVLEAMNLGPASKPYHRPDFHLQTWPYQ PAPPVRVLSQPAPLQPSPWVDARFEPEPFPPLFSPSALKRLEAEPLPLPDPEEGEAVPGRARAI GTLVHYAIGQNWRPDNPQHLANLEAQEVMFPFGPDERRGIMAEVQALLEHYQELLGRALP WPRDEDYPEFAVALPLGSTVWQGVIDRLYRVGQQWYLEDYKTDQEMRPERYLVQLGIYLAA IRQAWQIEPEVRLVYLRFGWVERLDKAILEAALGEIMPKGEGLRR	96
Q9RTI9	MTSSAGPDLLQALNPTQAQMDHFTG PALVIAGAGSG KTRTLIYRIAH LIGHYGVH PG EI LAVT FTNKAAAEMRERAGHLVPGAGDLWMSTFHSAGVRILRTYGEHIGLRRGFVIYDDDDQLDIIK EVMGSIPGIGAETQPRVIRGIIDRAKSNLWTPDDLDRSREPFISGLPRDAAAEAYRRYEVRKKG QNAIDFGDUTETVRLFKEVPGVLDKVQNKAKFIHVDEYQDTNRAQYELTRLLASRDRNLLWG DPDQSIYKFRGADIQNILDFQKDYPDAKVYMLEHNYRSSARVLEAANKUENNTERLDKTLKPV KEAGQPVTFHRATDHRAEGDYVADWLTRLHG EG RAWSEMAI LYRTNAQSRVI EESLRRVQI PARIVGGVGFYDRREIRDILAYARLALNPADDVALRRIIGRPRRGIGDTALQKLMEWARTHHTS VLTACANAAEQNILDRGAHKATEFAGLMEAMSEAADNYEPAAFLRFVMETSGYLDLLRQEG QEGQVRLENLEELVSAAEEWSQDEANVGGSIADFLDDAALLSSVDDMRTKAENKGAPEDAV TLMTLHNAKGLEFPWFIVGVEQGLLPSKGAIAEGPSGIEEERRLFYVGITRAMERLLMTAAQN RMQFGKTNAAEDSAFLEDIEGLFDTVDPYGQPIEYRAKTWKQYRPTVPAATTAVKNTSPLTAE LAYRGGEQVKHPKFG EGQVLAVAG VG ERQEVTVH FASAGTKKLMVKFANLTKL	97
M1E5C5	MDLNLNEDQKRAVYSDSRALLIVAGAGTGKTRVLTTRAARUKENPDARYLLLTFTKKAAREM	98

	TTRVRELIEEDTKNRLYSGTFHSFCSNIIRRRSERVGLTNDFVIIDESDSLDLMKKVFSRIYSKEKID SLIFKPKDIL5LYSYARNNNQDFIEIVQRKYKYVNFEDIKKIISLYELNKKERNYLDFDDLLMYGLLA IKTLEKSPFDEVLVDEFQDTNQIQAEMLYYFYDLGSRISAVGDDAQSIYSFRGAYYENMFNFIKR LDAEKIILSSNYRSTQQILDIANSIIQSSYSSIKKELVANVRLKEIWKPKLVIVSDDWEEARYVARE MQKFGEKGLKVAALYRAAYIGRNLESQLNSMGIKYSFYGGQKLTESAHAKDFMSFLRVFVNP KDEIAURILKMFPGIGEKKAEKIKDAVISGDNLKKALSKEKNLfELNIFFDKLFKITDWHDLLELVF DFYKDIMNRLYPENYEEREEDUKFMDMSSNYDNLVEYLEAFTLDPVEKSEFDNNNVILSTIHS AKGLEFDWFLLSVi ESVYPHFRAQSTDEIEEERRLFYVAITRAKQRUFTFPRHSKKSRGYFAKNTI SPFLREKDNYLEVFIAR	
Q5SIE7	MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVTFT NKAAEEMRERLRG LVPGAG EVWVSTFHAAALRI LRVYGERVGLRPGFWYDEDDQTALLKEV LKELALSARPGPI KALLDRAKN RGVG LKALLG ELPEYYAGLSRGRLGDVLVRYQEALKAQG ALD FGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEEANLMAVGDPDQG lYSFRAADIKN 1LDFTRDYPEARVYRLEENYRSTEAILRFANAVIVKNALRLEKALRPVKRGGEPV RLYRAEDAREEARFVAEE!ARLGPPWDRYAVLYRTNAQSRLLEQALAGRGIPARWGGVGFFE RAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATWARVQLLAQEKGLPPWEALKEAART FSRPEPLRHFVALVEELQDLVFGPAEAFFRHLLEATDYPAYLREAYPEDAEDRLENVEELLRAAK EAEDLQDFLDRVALTAKAEEPAEAEGRVALMTLHNAKGLEFPWFLVGVEEGLLPHRNSVSTL EGLEEERRLFYVGITRAQERLYLSHAEEREVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPP HRPRPGAFRGGERWHPRFGPGTWAAQGDEVTVHFEGFGLKRLSLKYAELKPA	99
B5YD55	MNNQFDSEKKIFI!PSRKKKEFLERIEKDLNEEQRKWLEADGPSLVIAGPGSGKTRTIVYRVGYL VALGYSPKNIMLL ïFT NQAARHMINRTQALIRESIEEIWGGTFHHVGNRILRVYGKIIGINEQY NILDREDSLDUDEaEELFPEENLGKGILGELFSYKVNTGKNWDEVLKIKAPQIIDKIEIVQKVFER YEKRKRELNVLDYDDLLFFWYRLLLESEKTRKiLNDRFLYILVDEYQDTNWLQGEIIRLTREENKN 1LWG DDAQSI YSFRGAT1ENILSFPEI FPGTRI FYLVFNYRSTPEIINLANEII KRNTRQYFKEI KPVL KSGSKPKLVWVRDDEEEAQFWEVIKELHKEGVKYKDIGVLFRSNYHSMAVQMELTLQGIPYE VRGGLRFFEQAHIKDMISLLKILFNPQDEISAQRFFKLFPGIGRAYAKKLSQVLKESKDFDKIFQ MQFSGRTLEGLRILKNIWDKIKVIPVQNFSEILRVFFNEYYKDYLERNYPDFKDREKDVDQLILLS	100

	ERYDDLEKFLSELTLYTYAGEKLLEEEEEEKDFWLSTIHQAKGLEWHAVFILRLVQGDFPSYKS MDNIEEERRLFYVAVTRAKRELYVITYLTRKVKDMNVFTKPSIFLEELPYKELFEEWIVQREI	
F6DJA4	MLSPFGGEEETKAIPLEEE!LLAWRVFSAALPPNFLAPVSASLHTLVREAEGKEGAELEAYAWER LEELARTSWKDAiQSFLEVAAEKPEVLRAGLLWFRTWNRLSPEEREALYRKAERFKPTAELASK **ASFLQGPPPPPKPLSPSVQAARSSPPRFTPTPEQEEAVRAFLSREDMKLVAVAGSGK ILRL** MAQSAPKERLLWAFNRSVRDEAERTFPGNVEVLTLHGLAHRHWRGSGAYQRKLAARNGR VTPGDVLEALELPRERYALAYViRSTLEAFLRSASEVPTPAHIPPEYREVLQRRDKDPFSERYVLK **AVRLIWKLMQDPDDSFPL5FDGFVKIWAQAGAKIRGYDAVLVDEAQDLSPVFLQVLEAHRGE** LRRVYVGDPRQQIYGWRGAVNAMDKLDAPERKLTWSFRFGEDLARGVRRFLAHVGSPIELH GKAPWDTEVSLARPEPPYTALCRTNAGAVEAVTSFLLEEGREGARVFWGGVDE!AWLLRDA HLLKVGGEREKPHPELALVENWEELEELAKEVNHPQARMLVRLARRYDLLELARLLKHAQADE EGKADLWSTLHKAKGREWDRWLWGDFiPVWDEKVREFYRKQGALDELKEEENWYVALT **RARRFLGLDQLPDLHERFFQGEGLVKPPSVSPLSVGGAGVSADLLRELEVRVLAKLEDRLKEVA EVLAALLVEEASKAVAEAMREMGLLGEEG**	101
F6DIL2	**MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVTFT** NKAAEEMRERLRG LVPGAG EVWVSTFH AAALRi LRVYG ERVGLRPGFWYDEDDQTALLKEV LKELALSARPGPi **KALLDRAKN RGVG LKALLG ELPEYYAGLSRG RLG DVLVRYQEALKAQGALD** FGDiLLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEEANLMAVGDPDQG **IYSFRAADIKNILDFTRDYPEARVYRLEENYRSTEAILRFANAVIVKNALRLEKALRPVKRGGEPV** RLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLLEQALAGRGIPARWGGVGFFE RAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATWARVQLLAQEKGLPPWEALKEAART FPRAEPLRHFVALVEELQDLVFGPAEAFFRHLLEATDYPTYLREAYPEDAEDRLENVEELLRAAK **EAEDLQDFLDRVALTAKAEEPAEAEGKVALMTLHNAKGLEFPWFLVGVEEGLLPHRNSLSTLE** GLEEERRLFYVGITRAQERLYLSHAEEREVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPPH **RPRPGAFRGGERWHPRFGPGTWAAQGDEVTVHFEGVGLKRLSLKYAELKPA**	102
F6DJ67	**M** EAN **LYVAGAGTG KTYTLAERYLGFLEEG LSPLQWAVTFTERAALELRH RVRQMVGERSLG HKERVLAELEAAPIGTLHALAARVCREFPEEAGVPADFQVMEDLEAALLLEAWLEEALLEALQ** DPRYAPLVEAVGYEG LLDTLREVAKDPLAARELLEKG LG EVAKALRLEAWRALRRRM EELFHG	103

	ERPEERYPGFPKGWRTEEPEWPDLLAWAGEVKFNKKPWLEYKGDPALERLLKLLGGVKEGF SPGPADERLEEVWPLLRELAEGVLARLEERRFRARRLGYADLEVHALRALEREEVRAYYRGRFR RLLVDEFQDTNPVQVRLLC^IJPDLRAWTVVGDPNQSIYSFRRADPKVMERFQAEAAKEGL RVRRLEKSHRYHQGLADFHNRFFPPLLPGYGAVSAERKPEGEGPWVFHFQGDLEAQARFIAQ EVGRLLSEGFQVYDLGEKAYRPMSLRDVAVLGRTWRDLARVAEALRRLEVPAVEAGGGNLLE TRAFKDAYLALRFLGDPKDEEALVGLLRSPFFALTDGEVRRLAEARGEGETLWEVLEREGDLSA EAERARETLRGLLRRKALEAPSRLLQRLDGATGYTGVAARLPQGRRRVKDWEGTLDLVRKLEV GSEDPFLVARHLRLLLRSGL5VERPPLEAGEAVTLLTVHGAKGLEWPWFVLNVGGWNRLGS WKNNKTKPLFRPGLALVPPVLDEEGNPSALFHLAKRRVEEEEKQEENRLLYVAATRASERLYLL LSPDLSPDKGDLDPQTLiGAGSLEKGLEATEPERPWSGEEGEVEVLEERIQGLPLEALPVSLLPLA ARDPEAARRRLLGEPEPEGGEAWEPDGPQETEEEVPGGAGVGRMTHALLERFEAPEDLERE GRAFLEESFPGAEGEEVEEALRLARTFLTAEVFAPYRGNAVAKEVPVALELLGVRLEGRADRVG EDWVLDYKTDRGVDAKAYLLQVGVYALALGKPRALVADLREGKLYEGASQQVEEKAEEVLRR LMGGDRPEA	
G8N9P8	M DAFPSGKPLDEAWLSSLNEAQRQAVLHFEG PALWAGAGSG KTRTWH RVAYLMARRGV YPSEILA VTFiNKMEEMRERLKAMVKGAGELWVSTFHAAALRILRFYGERVGLKPGFVVYDE DDQTALLKEVLKELGVSAKPGPIKALLDRAKNRGEPPERLLADLPEYYAGLSRGRLLDVLHRYQ QALWAQGALDFGDILLLALKLLEEDPEVRKRVRKRARFIHVDEYQDTSPVQYRLTKLLAGEEAN LMAVGDPDQGIYSFRAADiKNiLQFTEDFPGAKVYRLEENYRSTERILRFANAVIVKNALRLEKT LRPVKSGGEPVRLFRARDAREEARFVAEEVLRLGPPYDRVAVLYRTNAQSRLLEQALASRGIGA RWGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATVEKVQAIAQEKGLPLY EALKVAAQVLPRPEPLRHFLALMEELMDLAFGPAEAFFRHLLEATDYPAYLKEAYPEDLEDRLE NVEELLRAAREAEGLMDFLDKVALTARAEEPGEAGGKVALMTLHNAKGLEFPWFLVGVEEG LLPHRSSVSTLEGLEEERRLFYVGVTRAQERLYLSYAEEREVYGRPEASRPSRFLEEVEEGLYEEY DPYRLPPPKPVPPPHRAKPGAFRGGEKWHPRFGLGTWAASGDEVTVHFDGVGLKRLSLKY ADLRPA	104
QU014	M PDLPASSLLAQLNPNQAQAAN HYTG PALVIAGAGSGKTRTLVYRIAHLIGHYGVDPGEI LAV TFiNKAAAEMRERARHLVEGADRLWMSTFHSAGVRILRAYGEHIGLKRGFVIYDDDDQLDIL	105

	KEIMGSiPGIGAETHPRVLRGILDRAKSNLLTPADLARHPEPFISGLPREVAAEAYRRYEARKKG QNAIDFGDLITETVRLFQEVPAVLERVQDRARFIHVDEYQDTNKAQYELTRLLASRDRNLLWG DPDQSIYRFRGADIQNILDFQKDYLDAKVYMLEQNYRSSARVLTIANKLIENNAERLEKTLRPVK EDGHPVLFHRATDQRAEGDFVAEWLTRLHAEGMRFSDMAVLYRTNAQSRVIEESLRRVQIP AKIVGGVGFYDRREIKDVLAYARLAINPDDDVALRRIIGRPKRGIGDTALERLMEWARVNGTSI LTACAHAQELNILERGAQKAVEFAGLMHAMSEAADNDEPGPFLRYVIETSGYLDLLRQEGQE GQVRLENLEELVSAAEEWSRENEGT!GDFLDDAALLSSVDDMRTKQENKDVPEDAVTLMTL HNAKGLEFPWFIVGTEEGLLPSKNALLEPGGIEEERRLFYVGITRAMERLFLTAAQNRMQYGK TLATEDSRFLEEIKGGFDTVDAYGQVIDDRPKSWKEYRPTESARPGAVKNTSPLTEGMAYRGG EKVRHPKFG EGQVLAVAG LGDRQEVTVHFPSAGTKKLLVKFAN LTRA	
Q745W4	MALRPTEEQLKAVEAYRSGQDLKWAVAGSGKTTTLRLMAEATPGKRGLYLAFNRSVQQEA ARKFPRNVRPYTLHALAFRMAVARDEGYRAKFQAGKGHLPAQAVAEALGLRNPLLLHAVLGT LEAFLRSEAASPDPGMIPLAYRTLRAGTKTWPEEEAFVLRGVEALWRRMTDPKDPFPLPHGA YVKLWALSEPDLSFAEALLVDEAQDLDPiFLKVLEAHRGRVQRVYVGDPRQQiYGWRGAINA MDRLEAPEARLTWSFRFAETLARFVRNLTALQDRPVEVRGKAPWATRVDAALPRPPFTVLCR TNAGWGAWVTHEVHRGRVHWGGVEELVHLLRDAALLKKGEKRTDPHPDLAMVETWEE LEALAEAGYAPAYGVLRLAQEHPDLEALAAYLERAVVTPVEVAAGVWSTAHKAKGREWDRV VLWDDFYPWWEEGAAARVNWGSDPAHLEEENLLYVAATRARKHLSLAQIRDLLEAVDRMG VYRVAEEATRAYLLLSAEVLRGVATDPRVPAEHRVRALKALGYLERGEEALDSPGKPGGQG	106
Q72IS0	MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVTFT NKAAEEMRERLRG LVPGAG EVWVSTFHAAALRi LRVYG ERVGLRPGFWYDEDDQTALLKEV LKELALSARPGPI KALLDRAKN RGVG LKALLG ELPEYYAGLSRGRLGDVLVRYQEALKAQGALD FGDiLLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEEANLMAVGDPDQG lYSFRAADIKNILDFTRDYPEARVYRLEENYRSTEAILRFANAVIVKNALRLEKALRPVKRGGEPV RLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLLEQALAGRGIPARWGGVGFFE RAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATWARVQLLAQEKGLPPWEALKEAART FPRPEPLRHFVALVEELQDLVFGPAEAFFRHLLEATDYPAYLREAYPEDAEDRLENVEELLRAAK EAEDLQDFLDRVALTAKAEEPAEAEGRVALMTLHNAKGLEFPWFLVGVEEGLLPHRNSVSTL	107

	EGLEEERRLFYVGiTRAQERLYLSHAEEREVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPP HRPRPGAFRGGERWHPRFGPGTWAAQGDEVWHFEGFGLKRLSLKYAELKPA	
F2NK78	M DLLRDLN PAQREAVQHYTG PALWAGAGSG KTRTWHRIAYU RH RGVYPTEI LAVTFTN KA AGEMKERLARMVGPAARELWVSTFHSAALRiLRVYGEYIGLKPGFWYDEDDQLALLKEVLG G LG LETRPQYARG V İ D R İ KN RMWSVDAFLREAED WVGG LPKEQMAAVYQAYEARM RALG AVDFNDLLLKVIGLFEAHPEVLHRVQQRARFIHVDEYQDTNPAQYRLTRLLAGAERNLMWG DPDQSIYGFRNADIHNILNFEKDYPDARVYRLEENYRSTEAILRVANAVIEKNALRLEKTLRPVRS GGDPVFLYRAPDHREEAAFVAREVQRLKGRGRRLDEIAVLYRTNAQSRVLEEAFRRQNLGVRI VGGVGFYERREVKDVLAYARAAVNPADDLAVKRVLNVPARGIGQTSLAKLSQLAETARVSFFE ALRRAG EVLARPQAQAVQRFVAU EGLAN AAYDTG PDAFLRLVLAETGYADM LRREPDG EAR LENLEELLRAAREWEEQHAGTIADFLDEVALTARAEEPEGEVPAEAVTLMTLHNAKGLEFPW FIVGVEEGLLPHRSSTARVEDLEEERRLFYVGITRAQERLYLTLSEERETYGRREAVRASRFLEDIP EAFLQPLSPFGEPLGAGREPVAVRPTRRSSAAGGFRGGEKVRHPRFGQGLWAASGEGDRQE VTVH FAGVG LKKLLVKYAG LERI E	108

Table 18

>tr | L0B9N8 |L0B9N8_9EURY UvrD Rep helicase SFT OS=Thermococcus sp. EXT9 GN=e9a-l PE=4 SV=1 (SEQ ID NO: 58)
MSEALPVTSFEFSLPEESVIKI YGPPGTGKTTTLVRI IEHLIGFHDHTEFLESYGLSLLF GQYGAEDVI FMT FQT S7\LKE FEARTG IKVKDRQNKPGRYYS TVHG IAFRLL ID SGAI DGV ITQNFGSLSPEDWFRLFCRQNGLRFESSEMGYSIWFNDGNRLWNALTWAYNVYYPTKGPK ARHEALKRLAPKLWKYPPLWEEYKTEKGILDYNDMLVKAYEGLKSGEIDPRNLPGHKYSP KVLIVDE FQDLS PLQFE IFRL LANYMDL V I TAGDDDQT I F SYQGADPRLMNYVPGRE IVL KRSYRLPIWQAKAMTVISKTRHRKEKTVAPRTDLGDFKYKLFWFPDFLNDLVREAQEGH SIF ILVRTNRQVLKLGKEL ILAGVH FRHLKVD YRS IWEA GSKEWGT FRDLVQALLKARRG EELEIADLVTILYYSELIDWHLGEKLPEKERYKKIAEQMEKTIEAIEKGLMPFDILKVKD DPF SVLDLEKI ESL SPRHGKVAVEL IRE IMKEKS QVV SVPRDAE IYLDTLHASKGREADV VFLINDLPRKWSS ILKTREELDAERRVW YVGLTRARKKVYLLNGKHPFPVL

>tr | L0B9 J O |L0B9 J0_9EURY UvrD Rep helicase SFT OS=Thermococcus sp. IRI48 GN=i48-l PE=4 SV=1 (SEQ ID NO: 59)
MRVKI YGPPGTGKTTTLQRTIDYTLGNSSEPPIPLPESFPTDLEPKNLAFVSFTNTAIDV IGKRTGITTRSKEAPYMRTIHGLILSVLAEHFDPVAVDNLGKLADIQAEFSMRMGYYYSK DPFEFAEGNMKFNVITRALELYLPKTGDVEEALKLIDNREDRKFALAWYRYKRQKKIMDF

DDILVIGYEHLEDFYVPVEVAFIDEGQDNGPLDYILLEKGFEGAKFVFLAGDPLQS IYGF
KGADPRLFVRWKADKEIILPRSYRLPKKVWLLSQSWALSLGIKGAWRYAPSEKLGRVSR
MKF IEAL SYAVE QAKRGRS VL ILARTNS LVKFVGN ILSIE FGVAYGHLKRAS YWE SHLLK
F IEGLQMLKLWDGVT P IKVQDTKP ITGLI RKLKDKHAREVLRRWRDSRQWS LEVQAVLQR
IKKNPSEYFYITDFDRQALKAYFSKARLDLTEELI IDTIHAAKGEEADWI FLDFIPTRS
EERINPEELQEKLVAYVGFTRAREELIIVPTPAIKYHPMRDFMGVRQILGWNFHKHLLI
RELVGGL

>tr jL 0BA D 9 |L0BAD9_9EURY UvrD Rep helicase SFT OS=Thermococcus
sp. IRI33 GN=i33-l PE=4 SV=1 (SEQ ID NO: 60)
MSEALPVTSFEFSLPRERI IKLYGAPGTGKTTTLVKI IEHLIGFQDHTEFLENYGINLPF
GQYEPGEVI FMTFQTS7\LRE FEARTGIKVKDRQNKPGRYYSTVHGIAFRLLIDSGAVDGL
ITQNFGSLSPEDWFRNFCRQNGLRFESSEMGYSIWFNEGNQLWNALTWAYNVYYPTKGPK
ARYEALKRLAPKLWKFPPLWEEYKKGRGILDYNDMLVRAYEGLRSGEIDPRNLPGHKYSP
KVLIVDE FQDLS PLQFE IFRL LANHMDL V ITAGDDDQT I FSYQGADPRLMNYVPGLEWL
RKSHRLPIWQAKALTVISKTRHRKEKTVAPRTDLGDFKYKLFWFPDFLNDLVREAQEGH
SIF ILVRTNRQVLKLGKEL ILAGVHFEHLKVDYRS IWEA GSKEWGT FRDLVQALLKAKRG
EELEVADLVTILYYSELIDWHLGEGISEKERYKKIAEQMEKTIEAIEKGLMPFDVLRVKE
NPFSVLDLEKIESLSPRHGKVAVELIKELMKEKSQWSIPKDARIYLDTLHASKGREADV
VFLINDLPRKWSS ILKTREELDAERRVW YVGLTRARKKVYLLNGKHPFPVL

>tr | L0BAT5 |L0BAT5_9EURY UvrD Rep helicase OS=Thermococcus sp .
AMT7 GN=a7-l PE=4 SV=1 (SEQ ID NO: 61)
MSEALS ITSFDFTLPRERI IKI YGPPGTGKTTTLVRI IEHLIGFQDHTEFLENYGLSLPF
GQYGAEDV I FM T FQT SALKE FEARTG IKVKDRQNKPGRYYS TVHG IAFRLL IDSGAVDGL
ITQNFGSLSPEDWFRHFCRQNGLRFESSEMGYSNI FNEGNQLWNALTWAYNVYYPTKGPK
ARYEALKRLAPKLWKFPPLWEE YKKEKG ILDYNDML IRAYEGLKS GE IDPRNLPGHKYS P
KVLIVDE FQDLS PLQFE IFRL LANHMDL V ITAGDDDQT IFSYQGADPRLMNYVPGRE IVL
SKSYRLPIWQAKALTVISKTRHRKEKTVAPRTDLGDFKYKLFi/VFPDFLNDLVREAQEGH
SIF ILVRTNRQVLKLGKEL ILAGVHFEHLKVDYRS IWEAGSKEWGT FRDLVQALLKAKRG
EELEVADLVTILYYSELIDWHLGERISERERYRRIAEQMERTIEAIERGLMPFDILRVRE
NPFSVLDLERIESLSPRHGRVAVELIRELMRERSQWS IPRDARI YLDTLHASRGREADV
VFL INDLPRRWSNI LRTREELDAERRVWYVGLTRARRRVYLLNGRHPFP IL

>tr | W8NUG2 !W8NUG2_9EURY Superfamily I DNA and RNA helicase and
helicase subunits OS=Thermococcus nautili GN=BD0 1 __ 1302 PE=4
SV=1 (SEQ ID NO: 62)
MNENE RLSR F IARLRVL IEME RKAE IEAMRAEMRRL SGRE RERVGRAVL GLNGRV IGEEL
GYFLVRYGRERE IKTE I SVGDLWI SRRDPLRSDLVGTVVERGRRFI TVALET VPEWALR
SVRI DLYAND ITFRRWLENLENLRE SGRRALEL YLGLRE PEGGEE VE F T P FDRS LNAS QR
RAJARALGSPDFFLIHGPFGTGRTRTLVELTRQEVARGNRVLATAESNVAVDNLVERLVD
SGLRWRVGHPSRVSRGLHETTLAYLMTQHELYGELRELRVIGENLRERRDTFTRPAPRY
RRGLTDRQILRLAEKGIGTRGVPARLIREMAQWLKINEQVQKTFDDARKLEERIAREIIR
EADWLTTNS SAGLEWDYGS YDVAI IDEATQAT IPSVL IP INRAGRFVLAGDHRQL PPT
ILSERARELSRTLFEGLIERYPGRSEMLTVQYRMNERLMEFPSREFYDGRIEADES IRRI
TLADLGVKSPEDGDAWAEVLKPENVLVFIDTARREDRFERQRYGSESRENPLEARLVKEA

VEGLLRLGVKAEWIGVITPYDDQRDLISSLLPEEIEVKTVDGYQGREKEVIVLSFVRSNR
KGE LG FLKDLRRLNVS LTRAKRKL IL I GDS STLS SH P T YRRLVE FVRE RE TWDAKRL I G
KVKIK

>tr jB6YXQ7 |B6YXQ7 _THEON UvrD/REP helicase OS=Thermococcus onnurineus (strain NA1) GN=TON_138 0 PE=4 SV=1 (SEQ ID NO: 63)
M TA P IP T T Y S ILGVAGAGKTTQLIDLLNYLNFENSRNEKIWERHFEPVELNRIAFISFSN
T A IQE I A N R T G IE IKARKKS APGRY FR T V TGLAE VLL YENNLMT FEE VRS V SKLE G FR IK
WAREHGMYYKPRDNDISYSGNEFFAEYSRLVNTYYHVKSLSEI IEMHSKSHLLLDYIREK
EKLGIVDYEDILMRAYDYRNDIWDLEYMI IDEAQDNSLLDYATLLPIAKNNATELVLAG
DDAQL IY D FRGANYKL FHKL IERSE 11LNLTE TRRFGSE IANLATAI IDDMNY IQKREVL
SAATHS TKVAH IDL FQMMS ILQNMAT TDLTVY ILARTNAVLNYVAKVLDE YKI QYKKNER
ITDFDRFLLSLNRLMRNEYTNDDIYTI YNYLRNKVAREEELKERLFQHKLHWTEKDVLGI
LLLAYEQTTAKRILTTAKNTNFKIKLSTIHSAKGSEADWFLINSVPHKTKMKILENYEG
EKRVLYVAVTRARKFLFIVDQPVARRYEQLYYIRSYESRAQGSLVNRVAVPVA

>tr I Q5JFK3 |Q5JFK3 _THEKO DNA helicase, UvrD/REP family OS=Thermococcus kodakarensis (strain ATCC BAA- 918 / JCM 12380 / KOD1) GN=TK017 8 PE=4 SV=1 (SEQ ID NO: 64)
MNEKEVLLSKFIAHLKELVEMERRAEIEAMRLEMRRLSGREREKVGRAVLGLNGKVIGEE
LGYFLVRYGRDRE IKTE I SVGDLWI SKRDPLKSDLVGTWEKGKRFLTVAIETVPEWAL
KGVR IDLYAND ITFKRWMENLDNLRE SGRKALE LYLGLRE PEE SE PVE FQPFDKS LNAS Q
RGAIAKALGSGDFFLVHGPFGTGKTRTLVELIRQEVARGHKVLATAESNVAVDNIVERLA
DSGLKWRIGHPSRVSKALHETTLAYLITQHDLYAELRELRVIGENLKEKRDTFTKPAPK
YRRGLSDREILRLAEKGIGTRGVPARLIREMAEWIRINQQVQKTFDDARKLEERIAREI I
QEADWLTTNASAGLEWDYGE YDVAVI DEA TQA T IPSVL I P INRAKRFVLAGDHKQLPP
T ILSEKAKE LSK TL FEGL I ERYPEKS EML T V QYRMNERLME FPSRE FYDGK IKAHE SVKN
ITLADLGVSEPEFGNFWDEALKPENVLVFIDTSKREDRFERQRRGSDSRENPLEAKLVTE
TVEKLLEMGVKPDW IGVIT PYDDQRDL ISSMVGED IEVKTVDGYQGREKE 11VLS FVRSN
RRGELGFLTDLRRLNVSLTRAKRKLIAVGDSSTLSNHPTYRRFIEFVRERGTFIEIDGKK
H

>tr | C6AG75 !C6A075_THESM DNA helicase, UvrD/REP family OS=Thermococcus sibiricus (strain MM 739 / DSM 12597) GN=TSIB_2 009 PE=4 SV=1 (SEQ ID NO: 65)
M TRVQ IPAGAP KYGPVAQ PGQSARL ISGRSG V RSPAG P PKAL LKERFRELFI HKN P V I T M
HVKNYIAKLVDLVELEREAEIEAMREEMRRLKGYEREKVGRAILNLNGKI IGEEFGFKLV
KYGRKEAFKTEIGVGDLWISKGNPLASDLVGTWEKGSRFIWALETVPSWAFRNVRID
LYAND ITFRRQLENLKKLSE SG IRALKL ILGKE TPLKS SPEE FTP FDRNLNQS QKEAVSY
ALGSEDFFLIHGPFGTGKTRTLVELIVQEVKRGNKILATAESNVAVDNLVERLWGKVKLV
RLGHPSRVSVHLKESTLAFQVESHERYRKVRELRNKAERLAVMRDQYKKPTPQMRRGLTN
N Q ILKLAYRGRG SRGVPAKD IKQMAQW ITLNE Q IQKL Y K FAE KIESEIIQEIIE DVD V V L
STNS SAALE F IKDAE FDVAI IDEAS QxAT IPSV L IP IAKARRFVLAGDHKQL PPT ILSEEA
RALSETLFEKLIELYPFKAKMLEIQYRMNQLLMEFPSRE FYNGKIKADGSVKDITLADLK
VRE P FFGE PWDS ILKREE PL IFVDT SNRT DKWE RQRKG STSRENPLEALLVRE IVERLLR
M G I KKE W IG 1 I TPYDDQVDS IRS11QDDE IE IHTVDG YQGREKE 11 I L SLVRSNKKGELG

FLMDLRRLNVS ITRAKRKLVVIGDSETLVNHETYKRLIHFVKKYGRYIELGDTGIN

>tr|W01511|W01511_9EURY DNA helicase, UvrD/REP family protein OS=Thermococcus paralvinellae GN=TES1_2 001 PE=4 SV=1 (SEQ ID NO: 66)
MNLIRYINHLKELVELEREAEIEAMREEMRKLTGYEREKVGRAVLGLNGKI IGEEFGYKL
VKYGRKQEIKTEISVGDLVVISKGNPLASDLIGTVTEKGKRFLVVALETVPSWALRNVRI
DLYAND ITFKRQIENLDKL SESGKRALRF ILGLEKPKE SIDIE FKP FDEQLNE SQKKAVG
LALGSED FFL IHGPFGTGKTRTVAEV ILQEVKRGKKVLAT AESNVAVDNL VERLWGKVKL
VRLGHPSRVSKHLKESTLAYQVEIHEKYKRVRE FRNKAERLAMLRDQYTKPTPQWRRGLT
DRQ ILRLAEKG IGARG IPARVI KSMAQWITFNEKVQRL YNEAKKI EEE IVKE 11RQADW
LSTNSSAALE FIKD IKFDVAVI DEAS QATIPSVLIP IAKANKF ILAGDHKQL PPTILSEE
AKELSETLFEKLIELYPSKAKMLEIQYRMNERLMEFPSKEFYNGKIKAYDGVKNITLLDL
GVRVFSFGEPWDSILNLKEPLVFVDTSKHPEKWERQRKGSLSRENLLEAELVKEIVQKLL
RMGIKPE SIGVITPYDDQRDL ISLLLENDE IEVKTVDGYQGREKEVT ILSFVRSNKKGEL
GFLTDLRRLNVSLTRAKRKLIAIGDSETLSAHPTYKRFVEFVKEKGI FVQLNQYVSQTS

>tr|B7AA42|B7AA42_THEAQ DNA helicase OS=Thermus aquaticus Y51MC23 GN=TaqDRAFT__3 809 PE=4 SV=1 (SEQ ID NO: 67)
MGEAHPSEEALLSSLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLIARRGVFPS
EILAVTFTNKAAEEMKARLKAMVRGAGELWVS TFHAAALRI LRVYGERVGLKPGFWYDE
DDQTALLKEVLKELGLAAKPGPIKSLLDRAKNQGVPPEHLLLELPEFYAGLSRGRLQDVL
HRYQEALRAQGALDFGDILLYALKLLEEDGEVLKRVRKRARFIHVDEYQDTNPVQYRFTR
LLAGEEANLMAVGDPDQGIYSFRAADIRNILDFTQDYPKARVYRLEDNYRSTEAILRFAN
AVIVKNALRLEKTLRPVKKGGEPVRLFRAESARDEARFVAEEIARLGPPFDRVAVLYRTN
AQSRLLEQALASRGIPARWGGVGFFERAEVKDLLAYARLSLNPLDAVSLKRVLNTPPRG
IGPATVEKVQAIARERGLPLFEALKVAALTLPRPEPLRAFLALMEELMDLAFGPAEAFFR
HLLLATDYPAYLKEAYPEDAEDRLENVEELLRAAKEAESLMDFLDKVALTARAEEPAEAE
GRVALMTLHNAKGLEFPWFLVGVEEGLLPHRSSLSTQEGLEEERRLFYVGVTRAQERLY
LSYAQEREI YGRLEPVRPSRFLEEVDEGLYEVYDPYRQSSRKPTPPPHRALPGAFRGGEK
WHPRFGPGTWAAAGDEVTVHFEGVGLKRLSLKYADLRPA

>tr|B7A51 6|B7A51 6_THEAQ DNA helicase OS=Thermus aquaticus Y51MC23 GN=TaqDRAFT_5 093 PE=4 SV=1 (SEQ ID NO: 68)
MRVYLASAGTGKTHALVEELKGLIQSGVPLRRIAALTFTRKAAEELRGRAKRAVLALSAE
DPRLKEAEREVHGALFTTIHGFMAEALRHTAPLLSLDPD FALLDTFLAEALFLEEARSLL
YRKGLDGGLARALLHLYRKRTLAETLHPLPGAEGVFALYLEALEGYRRRLPAFLSPSDLE
ALALRILENPEALRRWERFPHILLDEYQDTGPLQGRFFQGLKEAGARLVWGDPKQSIY
LFRNARVEVFREALKQAEEVRYLS TTYRHAQAVAE FLNRFTAL FGEEGVRVRPHRQE VGR
VEVHWVVGEGGLEEKRRAEAHLLLDRLMALREEGYAFSQMAVLVRSRSSLPPLEAAFRAR
GVPYALGRGRSFFARPEVRDLYHALRLSLLEGPPGPEERLALLAFLRGPWVGLDLSEVEE
ALKAQDP IPLLPEAVRAKLRALRALAGLPPLEALKRLSRDEAFLRRLS PRARVNLDALLL
LAAMERFPDLEALLEWLRLRAEDPEAAELPEGEEGVQVLTVHGAKGLEWPWALFDLSRG
ENPKEEDLLVGLGGEVALRGTPAYKEVRKALRKAQAEEARRLLYVALSRARDVLIVTGSA
SGRPGPWVEALERLGLGPESQDPLVRRHPFKALPPLGDRPQTPPPPPLPAPYAHLAFPER
PLPFVYSPSAFTKAKEPVPLAEALEKEALPEFYRALGTLVHYAIARHLDPEDEGAMAGLL

LQEVAFPFAEGEKRRLLEEVRDLLRRYRGMLGPSLPPLEAREEDHAELPLVLPLGGTVWY
GILDRLYRVGGRWYLEDYKTDREVRPEAYRFQLAI YRRALLEAWGVEAEARLVYLRHGLV
HPLDPEELERALKEGFPGMGPGEGGEKA

>t r IB7A954 | B7A954 THEAQ DNA helicase OS=Thermus aquaticus
Y51MC23 GN=Ta qDRAFT__4 7 64 PE=4 SV=1 (SEQ I D NO: 69)
MKGLTGSSRLRVYGPPGTGKTTWLKNEVERLLRSGVPGEEIAVCAFSRAAFREFASRLAG
QVPEENLGTIHSLAYRAIGRPPLALTKDALSDWNRRVPDTWRVTPRVDGRGADLLDVMDP
YEDEDSRPPGDKLYDRVAYLRNTLAPMAAWSEEERAFFQAWKSWMNAKGLVDFPGMLEAA
LAKPGGLGARFLLVDEAQDLTPLQLLLVEKWAQGARLALVGDDDQAI YGFMGADGASFLG
VPVEDELVLGQSYRVPARVQRVAEAVIRRVQNRAPKRYAPRGDEGEVRLLWVPPEDPYHA
WDALERVNRGESVLFLATAKYLLEELKRELLRVGEPYANPYAPHRHSFNLFPQGARSAW
EKARSFLFPNRIAADVKAWTKHVSSIWFAVKGEEARRYIESFPDEEKVGDDHPIWWFRP
EHRPHAVGRDVSWLLDHLLGNAPKTMRQS LMVALKSPEAVLQGRARVWI GT I HSVKGGEA
DWVYVWPGYTRKAAREHPDQLHRLFYVAATRARKGLVLMDQGKAPHGYVWPRVDEFWGEV
WV

>tr jH7GEQ7 | H7GEQ7 9DEIN DNA helicase OS=Thermus s p. RL
GN=RLTM_02 9 1 6 PE=4 SV=1 (SEQ I D NO: 70)
MEANLYVAGAGTGKTYTLAERYLGFLEEGLSPLQVVAVTFTERAALELRHRVRQMVGERS
L GHKE RVLAE LEAAP I GTL HALAARVCRE F PEEAGVPA DF QVME DLEAAL L LEA WLEEAL
LEALQDPRYAPLVEAVGYEGLLDTLREVAKDPLAARELLEKGLGEVAKALRLEAWRXLRR
RMEELFHGERPEERYPGFPKGWRXEEPEWPDLLAWAGEVKFNKKPWLEYKXDPALXRLL
KLLGGVKEGFSPGPADERLEEVWPLLRELAEGVLARLEERRFRARRLGYADLEVHALRAL
EXEEVRAYYRGRFRRLLVDE FQD TNPVQVRL L QAL FPDLRAWTWGDPNQS I YS FRRADP
KVMERFQXEAAKEGLRVRRLEKSHRYHQGLADFHNRFFPPLLPGYGAVSAERKPEGEGPW
VFHFQGDLEAQARFI AQEVGRLLSEGFQVYDLGEKAYRPMSLRDVAVLGRTWRDLARVAE
ALRRLEVPAVEAGGGNLLETRAFKDAYLALRFLGDPXDEEALVGLLRSPFFALTDGEVRR
LAEARGEGETLWEVLEREGDLSAEAERARETLRGLLRRKALEAPSRLLQRLDGATGYTGV
AARL P QGRRRVKDWE GTL DLVRKLEVGSE DP F L VARHLRL L L RS GL SVERP P LEAGEAVT
LLTVHGAKGLEWPWFVLNVGGWNRLGSWKNNKTKPLFRPGLALVPPVLDEXGNPSALFH
LAKRRVEEEEKQEENRLLYVAATRASERLYLLLSPDLSPDKGDLDPQTLIGAGSLEKGLE
ATEPERPWSGEEGEVEVLEERIQGLPLEALPVSLLPLAARDPEAARRRLLGEPEXEGGEA
WXPXXPQETEEEVPGGAGVGRMTHALLERFEAXEDLEREGRAFLEESFPGAEGEEVEEAL
RLART F L TAEVFAP YRGNAVAKEVPVALE LLGVRLE GRADRVGEDWVLDYKT DRGVDAXA
YLLQVGVYALALGKPRALVADLREGKLYEGASQQVEEKAEEVLRRLMGGEGQGRQPYPLA
ATDPGHGAPG

>tr | H7GH69 |H7GH69_9DEIN DNA helicase OS=Thermus s p . RL
GN=RLTM_07 977 PE=4 SV=1 (SEQ I D NO: 71)
MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVT
FTNKAAEEMRERLRGLVPGAGEVWVST FHAAALRI LRVYGERVGLRPGFWYDEDDQTAL
LKEVLKELALSARPGPIKALLDRAKNRGVGLKALLGELPEYYAGLSRGRLGDVLVRYQEA
LKAQGALDFGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEE
ANLMAVGDPDQGI YS FRAAD I KNI LDFTRDYPEARVYRLEENYRS TEA I LRXANAVI VKN
ALRLEKALRPVKRGGEPVRLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLL

EQALAGRGIPARWGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATW
ARVQLLAQEKGLPPWEALKEAARTFXRAEPLRHFVALVEELQDLVFGPAEAFFRHLLEAT
DYPTYLREAYPEDAEDRLENVEELLRAAKEAEDLQDFLDRVALTAKAEEPAEAEGKVALM
TLHNAKGLEFPWFLVGVEEGLLPHRNSLSTLEGLEEERRLFYVGITRAQERLYLSHAEE
REVYGRREPARPSRFLEEVEEGLYEVYDPYRXPKPXPPPHRPRPGAFRGGERWHPRFGP
GTVVAAQGDE VTVHFEGXGLKRL SLKYAE LXPA

>tr jA0A0B0SAG4 !A 0A 0B 0SA G4_ 9DE IN DNA helicase OS=Thermus sp .
2.9 GN=QT17_08170 PE=4 SV=1 (SEQ ID NO: 72)
MDEALLSSLNEAQRQAVLHFQGPALWAGAGSGKTRTWHRVAYLIAHRGVYPTEILAVT
FTNKAAEEMRERLKGMVRGAGEVWVS TFHAAALRI LRVYGERVGLKPGFWYDEDDQTAL
LKEVLKELGLSAKPGPIKALLDRAKNRGEPPEALLAELPEYYAGLSRRRLLDVFFRYQEA
LKAQGALDFGDILLYALRLLEEDQEVLARVRKRARFIHVDEYQDTNPVQYRFTKLLAGEE
ANLMAVG DPDQGIYS FRAAD IKNILQFTADFPGAKVYRL EENYRSTEAILRFANAV IVKN
ALRLEKTLRPVKRGGEPVRLFRAKDAREEARFVAEEILRLGPPFDRIAVLYRTNAQSRLL
EQALAGRGVGARW GGVGFFERAEVKDLLAYARLALNPLDSVSLKRILNTPPRGIGPATV
EKVARLAQEKGLPLFEALKRAELLPRPEPVRHFVALMEELMDLAFGPAEAFFRHLLQATD
YPAYLREAYPEDHEDRLENVEELLRAAKEAESLLDFLDKVALTARAEEPAGAEGKVFLMT
LHNAKGLEFPVVFLVGVEEGLLPHRNSLNTLEALEEERRLFYVGVTRAQERLYLSYAEER
EVYGRLEATRPSRFLEEVEEGLYQEYDPYRSPRPVPPSHRPKPGAFKGGEKWHPRFGPG
TWAAS GDEVTVH FEGVGLKRL SLKYADLRPA

>tr |A0A084IL47 |A0A084IL47_9GAMM ATP-dependent DNA helicase Rep
OS=Salinisphaera hydro thermal is C41B8 GN=rep PE=3 SV=1 (SEQ ID
NO: 73)
MALPKLNPQQDAAMRYLDGPLLVLAGAGSGKTGVITRKIAHLIARGYDARRVVAVTFTNK
AAREMKQRASKLISADDARGLTVSTFHSLGLQMIREEHAALGYKPRFS IFDSEDADKVLA
DLVGRDGDHRKATKAAI SNWKSAL IDPE TATAQATGS DIPLARAYGE YQRRLKAYNAVDF
DDLLALPVHLLSTDHEARERWQSRFRYLLVDEYQDTNAAQYEMMRLLAGARAAFTWGDD
DQS IYAWRGARPGNIADLSRDFPHLKVIKLEQNYRSVGNVLSAANQLIGASNQRAYEKTL
WSAMGPGDRVRV IAAPDEAGEAERIASE ISSHKLRLGTAYGDYAI LYRGN FQSRAFE KAL
RERDI PYRVSGGRS FFERSE IRDLVTYLKLMVNPDDDAAFLRI VNLPRRE IGPATLEALG
RYAGS RH ISLFDAARG IGLAGGVGERS GRRLAD FVDWLRNL TQDS EGM TPRE LVS QL IVD
IDYRNWLRDT SANTKAARKR IENLDDF IGWLDRLDNAE DGKPVT LEDWRRL SLMD FANQ
SEKDVENQVHLLTLHAAKGLEFDHVFLAGLEEGMLPHHACLEDDKIEEERRLLYVGITRA
RKTLALTYARKRRRGGEESDSVPSRFLEELPADELDWPSATGTRSKAANAEQGRDQVAAL
RAMLGASADS

>tr |A0A0A2WMV1 !A0A0A2WMV1__THEFI DNA helicase OS=Thermus
filiformis GN=THFILI_00990 PE=4 SV=1 (SEQ ID NO: 74)
MPQVGFTDHFFKGLEALSREEQNRVREAVFAFMQDPKHPSFKLHRLEDIKTDRFWSARVS
KDLRL ILYHHPEMGW IFAYVGHHDDAYRWAE THQAEVHPKLGLLQ IFRWEEVRVE PRKI
KPLLPDYPDDYLLDLGVPPSYLKPLRLVETEDQLLGLIEGLPQDVQERLLDLAAGRPVTL
PPKLAPSEEWFKHPLSRQHIHFIQNLDELRQALSYPWERWMVFLHPAQREAVERVFQGPA
RVTGPAGTGKTWALHRAAALARRYPEEPLLLTTFNRFLASRLRSGLQRLLGEVPPNLTV
ENLHSLARRLHEQHVGPVKLVKEEDYGPWLLEAAQGLEYGKNFLLSEFAFADAWGLYTWE

AYRGFPRTGRGVPLTARERLKLFGAFQKVWGRMENEGALTFNGLLHRLRQRAEEGALPRF
RAWVDEAQDLGPAELLLVRALAQEAPDSLFFALDPAQRI YKSPLSWQALGLEVRGRS I R
LKVNYRTTREIAKRAEAVLPKEVEGEMREVLSLLQGPEPEIRGFPTQEACQAELVRWLRW
LLEQGVRPEEVAVLARVRKLAEGLAEGLRRAGIPWLLSDQEDPGEGVRLGTVHSAKGLE
FRAVALFGANRGLFPLESLLREAPSEADREALLAQERNLLYVAMSRARERLWVGYWDEGS
PFLTP

>tr jA0AGD0N7B7 |A0A0D0N7B7_METRU DNA helicase OS=Meiothermus ruber GN=SY2 8_04 645 PE=4 SV=1 (SEQ I D NO: 7 5)
MSDLLSSLNPSQREAVLHFEGPALWAGAGSGKTRTWHRIAYLLRERRVYPAEILAVTF
TNKAAGEMKERLEKMVGRSARDLWVTTFHAAAVRILRTYGEYVGLKPGFVIYDEDDQNTL
LKEVLKELELEAKPGP FRSMI DRI KNRGAGLAEYMREAPDFI GGVPRDVAAEVYRRYQNS
LRMQGALDFNDLLLLTIELFEQHPEVLHKVQQRARFIHVDEYQDTNPVQYRLTRLLAGER
PNLMW GDPDQS I YGFRNADINNI LDFTKDYPGARVI RLEENYRS S S S I LRVANAVIEKN
ALRLEKVLRPTKPGGEPVRLYRAPNAREEAAFVAREIVKLGGYQQVAVLYRTNAQSRLLE
EHLRRANVPVRLVGAVGFFERREIKDLLAYGRVAVNPDDS INLRRIVNTPPRGIGATTVA
RLVEHAQKTGITVFEAFRAAEQVISRPQQVQAFVRLLDELMEAAFESGPTAFFQRVLEQT
GFREALKQEPDGEDRLQNVEELLRAAQDWEEEEGGSLADFLDSVALTAKAEEPQGDAPVE
AVTLMTLHNAKGLEFPTVFLVGLEENLLPHRNSLHRLEDLEEERRLFYVGITRAQERLYL
SYAEERETYGKREYTRPSRFLQDIPQDLLKEVGAFGDGETRVLSQARPEPKPRTQPAEFK
GGEKVKHPKFGSGTWAAMGGEVTVMFPGVGLKRLAVKFAGLERLE

>tr W2U4X3 | W2U4X3_9DEIN DNA helicase OS=Thermus sp. NMX2 .A1 GN=TNMX 07060 PE==4 SV=1 (SEQ I D NO: 7 6)
MQGPQSSHPGDELLRSLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLIAKRGVF
PSE I LAVTFTNKAALEMRERLKRMVKGAGELWVSTFHSAALRI LRVYGERVGLKPGFWY
DEDDQTALIKEVLKELGLAARPGPLKALLDRAKNRGEAPESLLSELPDYYAGLSRGRLLD
VLKRYEEALKAQGALDFGDILLYALRLLEEDPEVLKRVRRRARFIHVDEYQDTNPVQYRF
TKLLAGEEANLMAVGDPDQGI YS FRAAD I KNI LE FTRDFPGAKVYRLEENYRS TEAI LRF
ANALIVNNALRLEKTLRPVKPGGEPVRLYRARDARDEARFVAEEILRLGPPFDRVAVLYR
TNAQSRLLEQALASRGVPARWGGVGFFERAEVKDLLAYARLSLNPLDGVSLKRVLNTPP
RGIGPATVEKVEALAREKGLPLFEALRVAAEVLPRPAPLRHFLALMEELQELAFGPAEGF
FRHLLEATDYPAYLREAYPEDHEDRLENVEELLRAAKEAEGLMEFLDKVALTARAEEPGE
PAGKVALMTLHNAKGLEFPWFWGVEEGLLPHRSSLSTLEGLEEERRLFYVGVTRAQER
LYLSYAEEREVYGRTEATRPSRFLEEVEGGLYEEYDPYRASAKVSPSPAPSEARASKPKP
GAYRGGEKVIHPRFGQGTWAAMGDEVTVHFEGVGLKRLSLKYADLRPVG

>tr|H9ZQB5 |H9ZQB5_THETH DNA helicase OS=Thermus thermophilus JL-1 8 GN=Tt JL18_0 620 PE=4 SV=1 (SEQ I D NO: 7 7)
MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVT
FTNKAAEEMRERLRGLVPGAGEVWVSTFHAAALRILRVYGERVGLRPGFWYDEDDQTAL
LKEVLKELALSARPGPIKALLDRAKNRGVGLEALLGELPEYYAGLSRGRLADVLVRYQEA
LKAQGALDFGDILLYALRLLKEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEE
ANLMAVGDPDQGI YS FRAAD I KNI LDFTRDYPEARVYRLEENYRS TEAI LRLANAVI VKN
ALRLEKALRPVKRGGEPVRLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLL
EQALAGRGIPARVVGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATW

ARVQLLAQEKGLPPWEALKEAARTSSRVEPLRHFVALVEELQDLVFGPAEAFFRHLLEAT
DYPTYLREAYPEDAEDRLENVEELLRAAKEAEDLQDFLDKVALTAPCAEEPAEAEGKVALM
TLHNAKGLEFPWFLVGVEEGLLPHRNSLSTLEGLEEERRLFYVGITRAQERLYLSHAEE
REVYGRREPARPSRFLEEVEEGLYEVYDPYRVPKPAPPPHRPRPGAFRGGERWHPRFGP
GTWAAQGDE VTVHFEGFGLKRL SLKYAE LRPA

>tr|E8PM35|E8PM35_THESS DNA helicase OS=Thermus scotoductus (strain ATCC 700910 / SA-01) GN=pcrA1 PE=4 SV=1 (SEQ ID NO: 78)
MQGPQSSHPGDELLRSLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLIAKRGVF
PSE ILAVT FTNKAAEEMRERLKRMVKGGGELWVS TFHSAALRI LRVYGERVGLKPGFWY
DEDDQTALIKEVLKELGLAARPGPLKALLDRAKNRGEAPESLLSELPDYYAGLSRGRLLD
VLKRYEEALKAQGALDFGDILLYALRLLEEDPEVLKRVRRRARFIHVDEYQDTNPVQYRF
TKLLAGEEANLMAVG DPDQGIYSFRAAD IKNI LE FTRDFPGAKVYRLEENYRS TEAI LRF
ANALIVNNALRLEKTLRPVKPGGEPVRLYRARDARDEARFVAEEILRLGPPFDRVAVLYR
TNAQSRLLEQTLASRGVPARWGGVGFFERAEVKDLLAYARLSLNPLDGVSLKRVLNTPP
RGIGPATVEKVEALAREKGLPLFEALRVAAEVLPRPAPLRHFLALMEELQELAFGPAEGF
FRHLLEATDYPAYLREAYPEDYEDRLENVEELLRAAKEAEGLMEFLDKVALTARAEEPGE
PAGKVALMTLHNAKGLEFPWFWGVEEGLLPHRSSLSTLEGLEEERRLFYVGVTRAQER
LYLSYAEEREVYGRTEATRPSRFLEEVEGGLYEEYDPYRASAKVSPSPAPGEARASKPGA
YRGGEKVIHPRFGQGTWAAMGDEVTVHFEGVGLKRLSLKYADLRPVG

>tr|E8PL08|E8PL08_THESS DNA helicase OS=Thermus scotoductus (strain ATCC 700910 / SA-01) GN=pcrA2 PE=4 SV=1 (SEQ ID NO: 79)
MLNPEQEAVANHFTGPALVIAGPGSGKTRTWHRIARLIRKGVDPETVTAVTFTKKAAGE
MRERLVHLVGEETATKV FTATFHSLAYHVLKDTGTVRVLPAEQARKLIGEILEDLQAPKK
LTAKVAQGA FSRVKNSGGGRRELIALYTDFSPYIERAWDAYEAYKEEKRLLDFDDLLHQA
VHELSTDIDLQARWQHRARFLIVDEYQDTNLVQFNLLRLLLTPEENLMAVGDPNQAI YAW
RGADFRLILEFKKHFPNATVYKLHTNYRSHNGIVTAAKKVITHNTQREDLDLKALRNGDL
PTLVQAQ SREDEA LAVAE VVKRH LDQGTPPEEIAILLRS LAY SRPIEATLRRYRIPYTIV
GGLSFWNRKEVQLYLHLLQAASGNPESTVEVLASLVPGMGPKKARKALETGKYPKEAEEA
LQLLQDLRAYTGERGEHLASAVQNTLHRHRKTLWPYLLELADGIEEAAWDRWANLEEAVS
TLFA FAHHTPEGDLDT YLAD ILLQEEDPEDSG DGVKIMTLHAS KGLE FAWL LPFLVEGA
FPSWRSAQNPATLEEERRLFYVGLTRAKEHAYLSYHLVGERGATSPSRFARETPANLIHY
NPTIGYQGKETDTLSKLAELF

>tr|E4U8J8|E4U8J8_OCEP5 DNA helicase OS=Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506) GN=Ocepr_1221 PE=4 SV=1 (SEQ ID NO: 80)
MSARDLLSSLNEQQQAAVQHFLGPALVIAGAGSGKTRTWHRVAYLLAEREVYPAEVLAV
TFTNKAAGEMRERLSRMVGRAAGELWVS TFHSASLRI LRRYGERI GLKPGFWYDDDDQR
VLLKEVLGSLGLEARPTYVRAVLDRIKNRMWSVDEFLAHADDWVGGLTKQQMAEVYARYQ
QRLAENNAVDFNDLLLRTIELFERHPEALEAVRQRARFII-IVDEYQDTNPAQYRLTKLLAG
DEANLMWGDPDQS IYGFRNAD IQNILGFERD YRGAWYRLEANYRS TAAI LRVANAL IE
RNQQRLEKTLRPVKPAGEPVRLYRAPDHREEAAFVAREVARLAGERALDDFAVLYRTNAQ

SRVLEEAFRRLNL PAR I VGGVG FYERRE VKDVLAYARLAVNPADD V A L R R V INVPARGVG
AASVGKLAAWAQAQGVSLLEAAHRAGELLAARQAAAVAKFTDLLTTLREAAEGTGPEAFL
RLVLAETGYSEMLRREGDSEPRLENLEELLRAAAEWEEEHGGSVAEFLDEIALTARAEEP
NAAPEKSVTLMTLHNAKGLEFPWFWGVEEGLLPHRSSLGSDAEIEEERRLLYVGITRA
QERLYLTLSEERETWGQRERVRPSRFLEEIPEDFLKPVGPFGDAHEPAPAPLSSAPVNRA
AKGSASGFRGGEKVRHPRYGEGTVVATSGEGARQEVTVHFAEAGLKRLLVKYAGLERIE

>tr | E4U4N5 |E4U4N5_OCEP5 DNA helicase OS=Oceanithermus
profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)
GN=Ocepr_157 5 PE=4 SV=1 (SEQ ID NO: 81)
MKVRIASAGTGKTYALTSRFTAALAEHPPYRLAAVTFTRSAAAELKARLRERLLAIAAGR
FQPSGAEDVPPEAWRRAGALATEVLGATVTTIHGFFAELLRQNALALGLEPDFLRIDAS
E S Q Q I FAEEARAYVYLNEEDDALAE VLGRL FAKRS LAAE L R P Q G E A A E A L W A H F R A V L E R
YARRLGGEALGPADIELHAWRLLERAGREEALAARIRSRLARVFVDEYQDTSPLQGRVFA
ALEALGVEVEVVGDPKQS IYAFRNADVEVFREAMRRGEPLPPLVTSWRHDRALVRFLNRY
VDWVAEERPEAFARAEAPPVEARPDAGPGRVRLQLVQGEARQDALRPYEADQLARWLQER
HA E HA W R DM A V L V R SH S SV P L L V RA LA A H G L PH V A A 7 GGRGFYDLIEVRDLVI-IAARVALDP
RGRFSLAAFLRGPFAGLDLGRVERVLAAEDPLAELERAAPEVAERVDRLVRWVQTLRPLD
FFERMVRTPFLEGASYLERLEPPARANVDQLLFKLASRRYGRLEFLLRDLEDLRGSDEAG
VPEGGFDAVRI YTMHGSKGLEWPVVAVFDLNRGQPDGAEPFYVRPGSGEFAAEGDPDYPR
FAAEWKERERQEAYRLLYVALSRPRSRLLLSLSVQLKPDGEGLRPKFWRRTLGRTLIEEM
NLAAWDALEVERLDAARLPAPKAAAAAPRRAADVDERLRAPVEPLARPPVYSPSALKAER
PAPPELDDEGDVAVELEEPGVDPGLVARTVGILVHYAIGQDWGPERLQDLWNQEAVQRLT
EPERTRVKTEVAQRLETYWRLLGTELPALDERDEDYAEFPLLLPTRTARLDTVWEGVIDR
LYRVGDVWVLEDYKTDRELHPERYHFQLALYRRAVAAAWGIEPEARLVYLRFGEVVPLEA
QLLEEAFERGTREAEEV

>tr | E4UAI 1 |E4UAI l__OCEP5 DNxA helicase OS=Oceani thermus
profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506)
GN=Ocepr 2312 PE===4 SV=1 (SEQ ID NO: 82)
MKVIVASAGTGKTTRLTQRYLEHLEQHPPQRVAAVTFTNKAAAELRERI FEALGRGSFYD
FTPSPALAERLADYQVRVLEAPIGTIHSFFGYLLRLTAPMLGLDPHFEVIDPATARAWFL
E E V R N L A I I E G A E V D E T V T T A L V E L F K R R S I S E A F E G T G D A S R S L V A G F K K V Y A R W L T R L
G G R Y L D P S E I E R R A L A L I R H P E A L E R V R S R L G W L V D E Y Q D T A P I Q A R V F E A L E E A G V P I
E W G D P K Q S I Y A F R D A D V E G F R E A H R R A R E N G N V E T L T V S Y R H P P A L A D F L N x A F T S x A E A A
L G K A F T A E E A P E V K P G R E G D A R V E L I T V T P G D G K A T L D A L R N G E A R L L A R E L R R L H D E E G
Y D Y G Q M L V L F R R R H Q L P P L L R A L R G A G L P F A W G L R G L Y E E P E V R E L Y H A L R L A T G E A P R
D S L A V F L S G P F G G L T L G Q V R E V L A Q D A P E S Y L T L H H P E A A E R L L R L R A D A E K M R P A E A L T
R L I E A P T A K G P P F L D L L E L E M A D T V L Y V L G R I E H T R T Y P E A V A T L E S F R S G G E E E A S L A R
L G G D A V R V M S A H A A K G L Q A P V V V I F D A D R T F N G N S D E L V I E P R T G R V A L N G E D A Y E S I A Q
A L K A R K E G E D H R L I Y V A L S R S S E R L I V S A A V K E P R K G S W L H H L T E V L N L G S K F E H R N V T L
A E I A L E E P I E Q E A A T L P V D P E L A T P L P P A P P A V S S P T A L K A E R E L E V P D P E E A W P A D P E A
R L L G R I V G I L V H E G I Q R D W D P D D P E V L L A L E G E Q V L E E V P A D R R P A V I E E V A T L L R V Y R T
L L G S A I P S L E E R E V D L A E L P L V Y P L G A T A W E G V I D R L Y R V G D V W Y L E D Y K T D R E V H P E R Y
H S Q L A L Y R E A V R K H W G I E P E V R L V Y L R T G Q V V P L D A A A L K E G L A S Y T G G

>tr IE4UAI 8 | E4UAI 8_OCEP5 DNA helicase OS=Oceani thermus profundus (strain DSM 14977 / NBRC 1004 10 / VKM B-227 4 / 506) GN=Ocepr_231 9 PE=4 SV=1 (SEQ ID NO: 83)

MNEHERVIAHEVGPAAWAGAGSGKTRAATLRAARLARTGERVGLVTFTASAAEEMRQRV
LAE DVPAKHVWAGTFHS LAFQ I LRQ FPEAGGYE GFPE VL TPNDE LRL FRRL WAELLDQDL
DAELRRKLVKALGFFRKARAEEALEGWAARAGESLELDAEMLEALMIS FQLRKREAGLAS
FDDLIEGASRALGDKDVRKWADRRFPFLIVDEYQDTSRAQETFLAALMPGEAPNLMVIGD
PNQAI YGWRGAGSRTFERFQARYPQAVLYPLRKNYRSTRAVLRLAERAIARLYRSGQEAY
YRLE GVKEE GE P PVLLT P PNAAAEATDVAREVARAVASGVP PEE I AVLARS SMQLAGVE D
RLARLGVATRLLGGIRLSERREVKTLVQLLKAAWSLHERALVDFIEEAVPGLGERTLTRV
EHAARPYNLVDRIMNDGAFVRGFSTRVQQGLFMTRTLLQLARATFEGVTGEAFAERFREF
AQDLYGELLPGYLARIGKQGPNEEARRRHLERFVATVEAFAREEAEGGLDDLLARLAFLE
QQDGPAVTLGTVHAVKGLEFEWFWGMVEGAFPILADDSDPEEERRLFYVAATRAKRRL
YLSAPTYGPRGKILQPSRYLEEALDEGLVRLQKVRPAA

>tr | E4UAI4 | E4UAI4 OCEP5 AAA ATPase OS=Oceanithermus profundus (strain DSM 14977 / NBRC 1004 10 / VKM B-2274 / 506) GN=Ocepr_2315 PE=4 SV=1 (SEQ ID NO: 84)

MVSEGRWKIERWYLKDGFAWAVRNEAGERHTAVGEMPTPVEGTWVRMETEHTVHPRYG
PRLRWRFLGLAPPPSKELAKIEGYLKLGFSEEAASWLAARFGSRPERAFDKPQELLVPG
VPREVLRRVFPRLERLLGGL I DLLGEGHTAAPLFLLAERS GLGKEE I QELAREARKQRL I
VEEQGRYGLVQPYRTERS IADGLLFRLKPGRGLRLTPPAGHGLSDEQARI FKLVRENRW
VLTGGPGSGKTTTIATLLAAPELHRMRFGIAAPTGKAARRIAEVARLPAETIHRLLGLGE
ARRPLYHARNPLPYDLLVIDETSMLDAEIAAFLVDALAPSTSVIFVGDPDQLPPVGPGQF
LRDLMTRVATLRLTQI FRQAQDSPI VNGAYALREGRMPLADGERLRLLPFEEEAAQTTLR
TLLDELQRLEQIVGERPQVLVPGNRGPLGVRRLSPFLQQQLNPGGKPLGPIGWGMEAREG
DPAVWIHNDYELGIMNGEVGVLRGGGSLGLTFETPTDRFAIPGNKRSRLVLAYAMTVHRS
QGSEWPAVI TI LPKAHMALLSRELVYTALTRSKQYHTLLFHPEALYRARAVQASRRYTWL
DVLLRG

>tr IK7QW32 !K7QW32JTHEOS DNA helicase OS=Thermus oshimai JL-2 GN=Theos_17 87 PE=4 SV=1 (SEQ ID NO: 85)

MTAPGHPDALLAPLNPAQQEAVLHFQGPALWAGAGSGKTRTWHRVAYLMAHRGVYPGE
I LAVTFTNKAAEEMKGRLKALVPGAGELWVATFHSAALRI LRVYGEAI GLKPGFWYDEA
DQEALLKEVLKELGLSAKPGPLKALLDRAKNRGEAWEALEIPDYYAGLPKGKVLDVLRRY
QEALRAQGALDFGDILVYALRLLEENPEVLAKVRKRARFIHVDEYQDTSPVQYRFARLLA
GEEANLMAVGDPDQGIYSFRAADIRNILDFTRDFPGARVYRLEENYRSTEAILRFANAVI
QKNRLRLEKTLRPVKPGGEPVRVYAAPEAREEARFVAEEIFRLGPPYERFAVLYRTNAQS
RLLE QALAAKGL PYRVVGGVGF FERAEVKDLLAYARLSLNPEDGVS LKRVLNTP PRG I GP
ATLARLEALAQAEGVPLLGAIRLGAERFPKPEPLRAFLALLDELADLAFGPPEAFFRHLL
SATDYLQYLKEHHPEDAEDRLENVEELLRAAKEAQDLQEFLDRVALTARADQDGGRGVAL
MTLHNAKGLEFPW FLVGVEEGLLPHQSSLSTLEGLEEERRLFYVGVTRAQDRLYLSYAR
EREVYGRREPRRMSRFLEEVPEGLYLPHDPYRQGAQPKPAPRAQGAFRGGEKWHPRFGP
GTVVAASGDEVTVHFEGVGLKRLSLKYADLRPA

>tr | K7QWX5 | K7QWX5_THEOS DNA helicase OS=Thermus oshimai JL-2 GN=Theos_2419 PE=4 SV=1 (SEQ ID NO: 86)
MASSLSKAELVPTPEQEKALHLYRSRQDFKLVAVAGSGKTTTLRLMAESFPRRHIAYLAF
NRAMKEEARRKFPPNTRVFTLHALAYRRTVPGTPYEAKFRLGNGQVRPVHVRERLQVDPL
LAYWRSGLERFIRSGDPEPLPRHLPRDWRKTVEARGPSGFAEVERAVKGVALLWKAMRD
PKDPFPLSHDGYVRIWREEGAGGDPPAGVILVDEAQDLDPNFLTVLSGWRGKAQQVFVGD
PRQQIYGWRGAVNAMGEIDLPESPLTWSFRFGEPLASFVQAVTARQTQGLVPLVGRAGWA
TEVHVNLFPTPPFTILTRSNLGLVTALLEGAQLFSLQKEEAHWGGVEELVWLLTDLQAI
KEGGERPRPHPELLGISKWEEVESLAEYS IVLNRLLRLAKEYDLEALAHKIAQLHGPEEG
AKLVLSTAHKAKGREWDRVLLWEDFYWVAAYRWFFPNTAPPPSEPSPEFLEEENI FYVAM
TRARLGLHISLPEALAEEEAKRILDRLSQGVPSGEDRGEDERGETLPAPFTGPTPVSPKE
ATFPLPSLYDRLLSEALNGGRDPLLHLLRDDLARLSALSPTPLPPEVAQALWERARPEEA
LGAIREGLGAMWREDPYELLRAINALALLGGRNPRKLAKILGDRFPGGEEAEDLLFVARA
RKRELMGRSLAEFWRGLGASVRHPLLKAYARARS

>tr | K7QTS9 | K7QTS9_THEOS DNA helicase OS=Thermus oshimai JL-2 GN=Theos_0356 PE=4 SV=1 (SEQ ID NO: 87)
MRLYVASAGTGKTETLMGELKALLEGGVPLRRVAAVSFTRKSAEELRLRVRRLLEAHREA
FWAREALREVHGALFTTLHGFMAEALRHTAPFLGLDPDFRVMDGFLAQALFLEEARSLLF
LEGHPEAPELLELLEALYEKRSLAEAFTPLPGAEGLLALYERVLARYRARTQEVLGPGDL
EAKALLLLRHPEALGRVAERFSHLLVDEFQDVNPLQGRFLRALEEAGVRWAVGDPKQSI
YLFRNARVEVFLRARAAAEEVRALSRTHRHAKQWELLNRFTTRFFRAEEGNRVEGVREA
EGRVEVHWVLGKLEEARRAEARLLAQRLLALRAEGIPFGEMAVLVRARTSLPPLEKALRA
AGVPFVRGRGQSFFARPEVRDLYHALRLALAERPYALEDRLSLLAFLRSPFLGLDLSELE
EALRAEDPWPLLPKGVQEALEGLRALALLPPLEALRRLARDEGFLRRISRRARANLDTLL
LLAAGARFPTLEDLLLWLALRAKDPESVELPEGGGGVTLLTVHGAKGLEWPWALYDVSR
GPSERPPPLLVDEEGRVALKGTEAYRALLKEAERAEREEALRLLYVALSRARDLLLITGS
TSQRPGPWAEALQALGLGPDAQDPWVETHPLEAIPPLPPIPQAPQDPRPAPYTPWRGEPR
ARPPVYSPSAHLKAEAEPLEVLGEGEALPEWARAVGTLVHYAIARHLDPEDEGAMGGLLR
QEVALAFGEGEREALLEEVRALLRAYRSLLSGALPPLEARAEDHAELPLLLPHKGTVWYG
VLDRLYRVGDRWYLDDYKTDQKVRPEAYRFQLALYRKAVLEAWGVEAEARLVYLRHRQW
PLSPAELEAALEGL

>tr | D1AF88 | D1AF88_THECD DNA helicase OS=Thermomonospora curvata (strain ATCC 19995 / DSM 43183 / JCM 3096 / NCIMB 10081) GN=Tcur_4104 PE=4 SV=1 (SEQ ID NO: 88)
MSSSQVTGRPTTVKDAEIAVEQRRVDQAHARLEEMRAEAQAMIEEGYRQALAGTKGSLVD
RDAMVYQAALRVQALNVADDGLVFGRLDLADGQTRYIGRIGVRTRDHEPMVIDWRAPAAE
AFYRATPEDPQGWRRRVLHTRGRTWDLEDDLLDPSAADSLTIVGDGAFIASLARTREG
TMRDIVATIQREQDEVIRAPADGTVLVRGAPGTGKTAVALHRVAYLLFRHRRRFGSRGVL
WGPNRRFTAYIERVLPSLGEGSATLRSLGDLVEGVSATVHDPPELAALKGSAAMAPVLR
RAVTDHPPGAPDKLRWHGGWVELGRPQLDKLRTSLHRRSTGSVNASRRRVAEALLDAL
WERYVHTGGTEPEPDEPVQGELALWEGILAEGGLAPLDEQDRPSSPADRTSREAFVKNVR
EQRAFTDFLTAWWPIRRPLDVLRSLGDAARLRRAAGRDLDRAQVELLAASWRRALAGDPP
TLSYQDIALLDEIDALLGPPPQPSRATAREEDPYWDGIDILTGEWADEDWEPGLQELT

TTIERLERARRVDDEVADVRPEYAHIWDEAQDLSPMQWRMLGRRGRQATWTIVEDPAQS
AWEDLEEARKAMEAALDGPAARRGRSRRPRRRPRHEYELTTNYRNTTEIAAVSARVLRLA
LPEARPARAVRSSGHRPVIDLVPEEELQAAARRAVRTLLEQVEGTIGVIVPLPGDAWGES
DRRALSAGFPERVQVLDVLEAKGLE FDAAVI CAPE TIAAQS PRGLRVL YVAVS RAT QRL T
VLTADPVWRRRLAGGESAR

>tr IF8A884 |F8A8 84__THEID DNA helicase OS=Thermodesul fatator indicus (strain DSM 15286 / JCM 11887 / CIR29812) GN=Theiri_0 6G7 PE=4 SV=1 (SEQ ID NO: 89)
MTSISLDQYQE QAVKAK GN TLWAG PGAG K TRV L LA KA IHLLEQGIDPE KVL ILTFTIK T
TQELKERLAS IGIKGVKVDTFHALAYDLLKAKGIKPRLATEEELKALARDLSKRKGLSLK
DFRKALDKGENHYRSLWEEALKLHGLYDFSLLLKEATGHYLQQEKVYLLIDEFQDLNPEL
TSFLKT F T KA E FFLVGDPAQAI YGFRGACPQVI KE FVD YLAPQ IYFLKKS YRVPEKVLNF
AETLRETQGFPLEPLEAVQKGGNRLGLSFNKPFNEAKGVAKLVSELLGGLQMEASQRGLA
PPE I A ILSRVRTLLNP IKEA F IKFG IP FQVP SENLKEE I SA IE SLSD IAKS IKS LKE LEA
YLAEGPSSVKEAWLESQSLEGFLFRLEMLKTFAS IS IRKDGVPLLTIHEAKGLEFKWIL
VGAEDGLLPFTLLEDYDLAEEKRVAYVAVTRAQESFYFTQVKTGRFLYGHKLSGKVSPFF
ETLPIKEKSSKTKPKARQKKLFG

>tr IA0A087LEB0 |A0A0 87LEB0__GEOSE Uncharacteri zed protein OS=Geobacillus stearothermophilus GN=GT94_17890 PE=4 SV=1 (SEQ ID NO: 90)
M T ISV IDE L LEKNKQNMNK TAKDAVE A QL IAYAKKEVKKLQE IRPHPYFGRLDFEDE FGR
ETIY IGKKGLEKDGEL IWDWRTDLGRL YNAYQGVQKT FQ IGKENRPVT IHGKRG I V IKN
GKVIKVT D I GKS E 11ENDNGEKVKYMDD YLKE I L TNTEEAHRLRD 11A S IQAEQDE I 1RL
PLKDTI IVQGAAGSGKSTIALHRISYLLYQYHEQVKPKDILILAPNEI FLSYIKDIVPEI
EIEGIEQRTFYDWASTYFTDVHDIPDLHEQYVHI YGSTEKEDLIKIAKYKGSLRFKKLLD
D FVE Y IGN TM IPHGDW IE SG V ILSKEEIHQ FYHAKE H LPLNVRMKE VKE F 11NWRNE Q I
NIRKQQIEDEFEEAYRKWW 7 TLPEGEERIKAVYEALEKAKQLRMKI FQEKMQHEISLIVKK
MENIPALLMYKSVFQKKVFEKFHPDIDEELLSLLLKNGRQIKQERFMYEDIAPLI YLDAK
INGKKLQYEH I V IDEAQDYS PFQLAIMKDYAKSMT ILGD IAQG I F S FYGLDRWEE IE SYV
FKEKEFKRLHLQTSYRSTKQIMDLANRVLLNSNYDFPLVIPVNRPGDVPTIKKVES IGEL
YDEIVNS IRI FEEKGYKKIAILTASKQGAIDTYDQLMRRQITQMEVITEGHQALKEKIVI
IPSYLVKGLEFDAVI IEDVSDETFKDETQHAKMLYMS ITRAHHDLHLFYRGNISPLLEER
DPSAPPKPRKSFADWLITDINDPYVEPQVEAVKRVKKEDMIRLFDDEEEEFVEEAFEDDR
ERYYD FHAW LKVWRRWAEMRKQ LDEKS

>tr | B5Y6N2 |B5Y6N2_COPPD DNA helicase OS=Coprothermobacter proteolyticus (strain ATCC 35245 / DSM 5265 / BT) GN=pcrA PE=4 SV=1 (SEQ ID NO: 91)
MALPQENLIPPSPSHNHLTLSLRSHIGGFFI YNEDVDSVDLSKLNEAQKQAVTAPPKPLA
I IAGPGSGKTRVLTYRALFAVKEWHLPPERILAITFTNKAADELKERLGRLIPEGDRI FA
ATMHS FAARMLRY FAPYAG ISQNFVI YDDDDSKGL IED ILKQMNMD TKR FRPNDVLNH IS
AA.KARMFDCNTFPEFIRQRYGSWGYYFDTVHQVFMTYERLKEQSQALDFDDLIMVLAQRM
EDRPELREMIAGLFDLVMVDEFQDTNFAQYQMLLYMTNPHYSGMNNVTIVGDPDQS IYGF
RAAEYYNIKRFIDDYNPEVVFLDLNYRSNRTIVDSASALINDSPSALFERKLES IKGAGN

KLILRRPFDDADAAITAAFEVQRLHKMGIPYEEIAVLMRTRALTARVERE FATRNIQYHI
IGGVPFFARREIKDILAYLRLSRNAMDRVSLKRILTMKKRGFGTASLEKLFNFAEENKLT
LLEAMKAAVESLLFKKLSMNDYLESLYTLIQTIQEIAEPSQAI YLVMEQENLLDHFRS IS
KSEEEYIERTENVKQLIS IAEESADMDDFLQRSALGTRENNGGVEGVAISTVHGVKGLEF
QAVILYYVTDGFFPHSLSVTTAEKEEERRLLYVAMTRAKEHLI FYVPYKQPWGNGFEQMA
RPSPFLRS IPKELWDGKPNE IESLYAPYSPQQKWSE

>tr|D7BJL0 |D7BJL0_MEISD DNA helicase OS=Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) GN=Mesil_3574 PE=4 SV=1 (SEQ ID NO: 92)
MNDPIRHKEGPALVFAGAGAGKTRTLTQRVKWLVEEGEDPYS ITLVTFTNKAAGEMKERI
ARLVEAPLAEAVWVGTFHRFCLQSLQVYGREIGLEKVAVLDSAAQRKLAERI IAGLFPAK
PPRGFTPMAALGAVSRAANSGWDDIQLATMYADLTEKIVNFRWAYEEAKKGLGALDYDDL
LLRGVRLLKLSEGAARMVRRRAAYLMVDEFQDTNGVQLELVRAIAPGTSPNLMWGDPDR
SIYGWRGANYRTILEFRQHYPGAAVYGLYTNYRSQAGWEVANRIIAQNATRKPEMQEAH
LPQSEEPFLLVAKNRWEEAHFVAQAVE FYRGQGIALEEMAVLMRANFLSRDLEQALRLRG
IPYQFTGGR.SFFERREIQLGMAVLKVLANPKDSLAVAALVEEMVEGAGPLGIQKVLEAAK
AANLSPLEAFRNPAMVKGLRGKEVQAEAMRLAEVLQDQVARLAAEAPEYHALLKETLDRL
GFEAWLDRLGEESEQVYSRKANLDRLLQGMQEWQEVNPGAPLQDLVGTLLLEAGDTPAEE
GQGVHLMTVHASKGMEFRVVFVIGLNEGLFPLSKASSSFEGLEEERRLMYVAVTRAKEVL
HLSYAADGWSRFAQEARVPVEEYDPRLGWSGRQNQQALKALLEIA

>tr|E8MZN5 |E8MZN5_ANATU DNA helicase OS=Anaerolinea thermophila (strain DSM 14523 / JCM 11388 / NBRC 100420 / UNI-1) GN=pcrA PE=4 SV=1 (SEQ ID NO: 93)
MDSLEHLNPQQRAAVTASAGPVLVLAGPGSGKTRVLTFRIGYLLSQLGVAPHHILAVTFT
NKAAREMQSRVEKLLGHSLQGMWLGTFHAICARILRREQQYLPLDANFVI FDEDDQQALI
KRALRDLNLDEKLYRPTSVHAAISNAKNNLILPEDYPTATYRDEWARVYKRYQELLVSS
NAVDFDDLLL YAWKLLNEFSTVREQYARRFEHI LVDEFQDTNLAQYELVKLLAS YHRNLF
WGDEDQS IYRWRGADYRNVLRFEEDFPDRQKILLEQNYRSTQRVLDAAQAVINRNRNRT
PKRLKSTPEHGEGEKLVLYEAVDDYGEAAFWDTIQQLVAGGKARPGDFAIMYRTNAQSR
LLEEAFLRAGVPYRLVGAMRFYGRREVKDMIAYLRLVQNPADEASLGRVINVPPRGIGDK
SQLALQMEAQRTGRSAGLILMELGREGKDSPHWQALGRNASLLADFGSLLGEWHRLKDEI
SLPSLFQRILNDLAYREYIDDNTEEGQSRWENVQELLRIAYEYEEKGLTAFLENLALVSD
QDTLPEIWEAPTLLTLHAAKGLEFPIVFITGLDDGLIPHNRSLDDPEAMAEERRLFYVGL
TRAKKRVYLVRAAQRS TYGSFQDSIPSRFLKDI PADLIQQDGRGRRMGRSWQSESRRSWD
DNYAGTWGSRPERAKPSHAPILQPRFKPGMRVKHPSWGEGLWDSRIQDEDETVDIFFDS
VGFKRVIASIANLEILS

>tr|L0INW7 |L0INW7_THETR ATP-dependent exoDNAse (Exonuclease V), alpha subunit/helicase superfamily I member OS=Thermoanaerobacterium thermosaccharolyticum M0795 GN=Thethe_02902 PE=4 SV=1 (SEQ ID NO: 94)
MDINGQI IKLNRNKTQGTLKLTNGQKIKFKINSDSVKPI FLYEYYKFKGNMIEDTLI IDD
lYGIANDININDFTELFPSVAHDKINNICNRFNVLHVGNLIDLINDENFITVVNDTIGEE
KATIFLSNLQKIKDRQEYIDVWD11KKTNPTFDINVPIKIVNALKYRASMNN ITVSQLIK

ESPWI IEQLDI FDS ITERKKIAENIATHYGLSNDSNKAVISYAIAMTNNYIQQGHSYIPY
YTLVSRVSNSLKLDFNKVNDTLKFLPNDNKSGYLIRDNKYKDEIENEYNSDKKIGYSVYL
PKI FHMEKYIADI ISSI LKKKS T INKIELQKNLKLYRSENKL IFSKEQEEAI FS ISDNKI
TV IT GGA G TGKT TV IKA IIDLVNKMGYTPVVLAPTGIASQRVAPNVGSTIHKYARI FDTY
DPVFDEIEENKENNSGKVI IVDEMSMITVPVFAKLLSVTLDADSFI FVGDPNQLPPIGAG
GVFEAL IELGNKNINNINTWLNQS FRSKNS IVKNAQNILEDKP IYEDDNLN11EAKSWN
KIADEVVNLIRKLLDNGVQYSDIMVLSSKRGEGKNGVSLLNERIRKEI FNNKGKYAVGDI
VITTRNDYDNKSS YFRSKELKKYINS IRHEERPT IFNGTVGVIKDI SDNEVI IEYNTPMP
VEAKYNl'IEELDWYIEYGFAI TVHKAQGGQAKYI IFASDEPRNI SREMLYTAI TRCKNGKV
FLIGGENEDWKIKKEHSFVLSKLKYRILDNIHQQEKESKINSKIVLINQ

>tr iD3PR99 |D3PR99_MEIRD DNA helicase OS=Meiothermus ruber
(strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) GN=K649_05745
PE=4 SV=1 (SEQ ID NO: 95)
MSDLLSSLNPSQQEAVLHFEGPALWAGAGSGKTRTWHRIAYLLRERRVYPAEILAVTF
TNKAA GEMKERLEKLVGRPARDLWVSTFHAAAVRILRTYGEYVGLRPGFV IYDEDDQNTL
LKEVLKELELEAKPGPFRAMIDRIKNRGAGLAEYMREAPDFIGGVPKDAAAEVYRKYQSG
LRMQGALDFNDLLLLTIELFEQHPEVLHKVQQRARFIHVDEYQDTNPVQYKLTRLLAGER
PNLMWGDPDQS IYGFRSADINNILDFTKDYPGARVIRLEENYRSSSS ILRVANAVIEKN
ALRLEKVLRPTRPGGEPVRLYRAPNAREEAAFVAREIVKLGNFQQIAVLYRTNAQSRLLE
EHLRRANVPVRLVGAVGFFERREIKDLLAYGRVAVNPADS INLRRIVNTPPRGIGATTVS
RLVEHAQKTGTTVFEAFRVAEQVISRPQQVQAFVRLLDELIEAAFESGPTAFFQRVLEQT
GFREALKQEPDGEDRLQNVEELLRAAQDWEEEEGGSLSDFLDSVALTAKAEEPQGDAPAE
AVTLMTLHNAKGLEFPTVFLVGLEENLLPHRNSLHRLEDLEEERRLFYVGITRAQERLYL
SYAEERETYGKREYTRPSRFLEDIPQDLLKEVGAFGDSEVRVLPQARPEPKPRTQLAEFK
GGEKVRHPKFGSGTVVAAMGGEVTVMFPGVGLKRLAVKFAGLERLE

>tr iD3PLL2 |D3PLL2_MEIRD DNA helicase OS=Meiothermus ruber
(strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) GN=K649_10770
PE==4 SV=1 (SEQ ID NO: 96)
MKVRVASAGTGKTASLVLRYLELIAKGTPLRRIAGVTFTRKAADELRVRVAAAIEEVLQT
GRHLSFVASGGSRAAFQEAAREIAGATLSTIHGFMAQCLRLAAPLLHLDPDFSMLGDWEA
QAI FEEEWQTLRYLAQDAHHPLFGLVSDELTEPLLHLFSRRSQAEVFEPAAGEANQHLLQ
VYQTVYAAYEARLGANLLSPSELERKALELARNDRAMKRVLERVRVLLVDEYQDVNPVQG
AFFAALEQARLPIEIVGDPKQS IYAFRNADVSVFRKALREGKSEPPLTHSYRHSRVLVRF
LNGLTGYLAKEGLGFGLEEAPPVEGVRPEQGRLEVHWWGELPLEELRKQEARVLAGRLA
ALRGPIEYSQMAVLVRSYGSVRFLEEALAEAQIPYVLLQGRGYYERQEVRDLYHALRAAL
DPRGLSLAVFLRSPFGQHTEAGPLKPLELPQIEGVLRADDPLGRLAQHWPSVYERLRQIQ
AQVRLMAPLEVLKFLIRAPLMDGRPYHDFLEPRARENVDALLFYFAPRPPQNLEGLLERL
ELLSRQADAGDVPQSGEGVQILTVHQAKGLEWPLVAVFDLGRMNVHRPQPLYLGQGPNGG
DGGRLRRWVALPETPQFEAFRQQVKLQEEEESYRLLYVAASRARDTLLLTASASHGQPEG
WGKVLEAMNLGPASKPYHRPDFHLQTWPYQPAPPVRVLSQPAPLQPSPWVDARFEPEPFP
PLFSPSALKRLEAEPLPLPDPEEGEAVPGRARAIGTLVHYAIGQNWRPDNPQHLANLEAQ
EVMFPFGPDERRGIMAEVQALLEHYQELLGRALPWPRDEDYPEFAVALPLGSTVWQGVID
RLYRVGQQW YLEDYKTDQEMRPERYLVQLG IYLAAI RQAWQ IE PEVRLVYLRFGWVERLD
KAI LEAALGE IMPKGEGLRR

>tr|Q9RTI9|Q9RTI9_DEIRA DNA **helicase OS=Deinococcus radiodurans** (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051 / NBRC 15346 / NCIMB 9279 / R1 / VKM B-1422) GN=DR_1775 PE=1 SV=1 (SEQ ID NO: 97)

MTSSAGPDLLQALNPTQAQAADHFTGPALVIAGAGSGKTRTLI YRIAHLIGHYGVHPGEI
LAVTFTNKAAAEMRE RAGHLVPGAGDLWMSTFHSAGVRI LRTYGEHI GLRRGFVI YDDDD
QLDI IKEVMGS IPGIGAETQPRVIRGI IDRAKSNLWTPDDLDRSREPFISGLPRDAAAEA
YRRYEVRKKGQNAIDFGDLITETVRLFKEVPGVLDKVQNKAKFIHVDEYQDTNRAQYELT
RLLASRDRNLLWGDPDQS IYKFRGADIQNILDFQKDYPDAKVYMLEHNYRSSARVLEAA
NKL IENNTERLDKTLKPVKEAGQPVT FHRATDHRAEGDYVADWLTRLHGEGRAWSEMAI L
YRTNAQS RVI EESLRRVQ IPAR IVGGVG FYDRRE IRD ILAYARLALNPADDVALRR 11GR
PRRGIGDTALQKLMEWARTHHTSVLTACANAAEQNILDRGAHKATEFAGLMEAMSEAADN
YEPAAFLRFVMETSGYLDLLRQEGQEGQVRLENLEELVSAAEEWSQDEANVGGSIADFLD
DAALL SSVDDMRTKAENKGAPE DAVTLMTLHNAKGLE FPWF IVGVE QGLL PSKGAIAE G
PSGIEEERRLFYVGITRAMERLLMTAAQNRMQFGKTNAAEDSAFLEDIEGLFDTVDPYGQ
PIEYRAKTWKQYRPTVPAATTAVKNTSPLTAELAYRGGEQVKHPKFGEGQVLAVAGVGER
QEVTVHFASAGTKKLMVK FANLTKL

>tr IM1E5C5 |M1E5C5_9FIRM DNA helicase OS=Thermodesul fobium narugense DSM 14796 **GN=Thena_1375 PE=4** SV=1 (SEQ ID **NO:** 98)

MDLNLNEDQKRAVYSDSRALLIVAGAGTGKTRVLTTRAARLIKENPDARYLLLTFTKKAA
REMTTRVRELIEEDTKNRLYSGTFHSFCSN11RRRSERVGLTNDFVIIDESDSLD LMKKV
FSRIYSKEKIDSLI FKPKDILSLYSYARNNNQDFIEIVQRKYKYVNFEDIKKI ISLYELN
KKERNYLDFDDLLMYGLLAIKTLEKSPFDEVLVDEFQDTNQIQAEMLYYFYDLGSRISAV
GDDAQS IYSFRGAYYENMFNFIKRLDAEKI ILSSNYRSTQQILDIANS IIQSSYSS IKKE
LVANVRLKENVKPKLVIVSDDWEEARYVAREMQKFGEKGLKVAALYRAAYIGRNLESQLN
SMGIKYS FYGGQKLTE SAHAKDFMS FLRVFVNPKDE IALIRILKMFPG IGEKKAEKI KDA
VISGDNLKKALSKEKNLEELNI FFDKLFKITDWHDLLELVFDFYKDIMNRLYPENYEERE
EDL IKFMDMS SNYDNLVE YLEAFTLDPVEKSE FDNNNVI LSTIHSAKGLE FDWFLLSVI
ESVYPHFRAQSTDEIEEERRLFYVAITRAKQRLI FTFPRHSKKSRGYFAKNTISPFLREK
DNYLEVFIAR

>tr|Q5SIE7 |Q5SIE7_THET8 DNA helicase OS=Thermus thermophilus (strain HB8 / ATCC 27634 / DSM 579) GN=TTHA1427 PE=4 SV=1 (SEQ ID NO: 99)

MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVT
FTNKAAEEMRERLRGLVPGAGEVWVS TFHAAALRI LRVYGERVGLRPGFWYDEDDQTAL
LKEVLKELALSARPGPIKALLDRAKNRGVGLKALLGELPEYYAGLSRGRLGDVLVRYQEA
LKAQGALDFGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEE
ANLMAVG DPDQGIYSFRAAD IKN ILDFTRDYP EARV YRL EENYRSTEA ILRFANAV IVKN
ALRLEKALRPVKRGGEPVRLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLL
EQALAGRG IPARWGGVGFFERAEVKDLLAYARLALNPLDAV SLKRVLNT PPRG IGPATW
ARVQLLAQEKGLPPWEALKEAARTFSRPEPLRHFVALVEELQDLVFGPAEAFFRHLLEAT
DYPAYLREAYPEDAEDRLENVEELLRAAKEAEDLQDFLDRVALTAKAEEPAEAEGRVALM
TLHNAKGLEFPWFLVGVEEGLLPHRNSVSTLEGLEEERRLFYVGITRAQERLYLSHAEE
REVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPPHRPRPGAFRGGERWHPRFGPG

TVVAAQGDE VTVHFEGFGLKRL SLKYAE LKPA

>tr jB5YD55 |B5YD55_DICT6 DNA helicase OS=Dictyoglomus thermophilum (strain ATCC 35947 / DSM 3960 / H-6-12) GN=DICTH 0581 PE===4 SV=1 (SEQ ID NO: 100)
MNNQFDSEKKIFIIPSRKKKEFLERIEKDLNEEQRKVVLEADGPSLVIAGPGSGKTRTIV
YRVGYLVALGYSPKNIMLLTFTNOAARHMINRTQALIRESIEEIWGGTFHHVGNRILRVY
GKI IGINEQYNILDREDSLDLIDECLEELFPEENLGKGILGELFSYKVNTGKNWDEVLKI
KAPQI IDKIEIVQKVFERYEKRKRELNVLDYDDLLFFWYRLLLESEKTRKILNDRFLYIL
VDE YQDTNWLQGE IIRLTREENKNI LWGDDAQS IYSFRGATIENI LSFPE IFPGTRI FY
LVFNYRSTPE IINLANE IIKRNTRQYFKE IKPVLKSGSKPKLVWVRDDEEEAQFWEVIK
ELHKEGVKYKDIGVLFRSNYHSMAVQMELTLQGIPYEVRGGLRFFEQAHIKDMISLLKIL
FNPQDE ISAQRFFKL FPG IGRAYAKKL SQVLKE SKDFDK IFQMQ FSGRT LEGLR ILKNIW
DKIKVIPVQNFSEILRVFFNEYYKDYLERNYPDFKDREKDVDQLILLSERYDDLEKFLSE
LTLYTYAGEKLLEEEEEEKDFWLSTIHQAKGLEWHAVFILRLVQGDFPSYKSMDNIEEE
RRL FYVAVT RAKRE LYVITYLTRKVKDMNVFTKPS IFLEELPYKELFEEWIVQRE I

>tr jF6DJA4 |F6DJA4 THETG DNA helicase OS=Thermus thermophilus (strain SG0.5JP17-16) GN=Ththel6_2124 PE=4 SV=1 (SEQ ID NO: 101)
MLSPFGGEEETKAIPLEEEILLAWRVFSAALPPNFLAPVSASLHTLVREAEGKEGAELEA
YAWERLEELARTSWKDAIQSFLEVAAEKPEVLRAGLLWFRTWNRLSPEEREXALYRKAER
FKPTAELASKASFLQGPPPPPKPLSPSVQAARSSPPRFTPTPEQEEAVRAFLSREDMKLV
AVAGSGKTTTLRLMAQSAPKERLLYVAFNRSVRDEAERTFPGNVEVLTLHGLAHRHWRG
SGAYQRKLAARNGRVT PGDVLEALE LPRER YALAYV IRS TLEAFLRS ASEVP TPAH IPPE
YREVLQRRDKDPFSERYVLKAVRLIWKLMQDPDDSFPLSFDGFVKIWAQAGAKIRGYDAV
LVDEAQDLSPVFLQVLEAHRGELRRVYVGDPRQQIYGWRGAVNAMDKLDAPERKLTWSFR
FGEDLARGVRRFLAHVGSPIELHGKAPWDTEVSLARPEPPYTALCRTNAGAVEAVTSFLL
EEGREGARVFWGGVDEIAWLLRDAHLLKVGGEREKPHPELALVENWEELEELAKEVNHP
QARl'ILVRLARRYDLLELARLLKHAQADEEGKADLWS TLHKAKGREWDRWLWGDFI PVW
DEKVREFYRKQGALDELKEEENWYVALTRARRFLGLDQLPDLHERFFQGEGLVKPPSVS
PLSVGGAGVSADLLRELEVRVLAKLEDRLKEVAEVLAALLVEEASKAVAEAMREMGLLGE
EG

>tr |F6DIL2 |F6DIL2__THETG DNA helicase OS=Thermus thermophilus (strain SG0.5JP17-16) GN=Ththel6 1438 PE=4 SV=1 (SEQ ID NO: 102)
MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVT
FTNKAAEEMRERLRGLVPGAGEVWVSTFHAAALRILRVYGERVGLRPGFWYDEDDQTAL
LKEVLKELALSARPGPIKALLDRAKNRGVGLKALLGELPEYYAGLSRGRLGDVLVRYQEA
LKAQGALDFGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEE
ANLMAVGDPDQG IYSFRAAD IKNI LDFTRDYPEARVYRLEENYRS TEAI LRFANAVI VKN
ALRLEKALRPVKRGGEPVRLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLL
EQALAGRGIPARWGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATW
ARVQLLAQEKGLPPWEALKEAARTFPRAEPLRHFVALVEELQDLVFGPAEAFFRHLLEAT
DYPTYLREAYPEDAEDRLENyEELLRAAKEAEDLQDFLDRVALTAKAEEPAEAEGKVALM

TLHNAKGLEFPVVFLVGVEEGLLPHRNSLSTLEGLEEERRLFYVGITRAQERLYLSHAEE
REVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPPHRPRPGAFRGGERWHPRFGPG
TWAAQGDE VTVHFEGVGLKRL SLKYAE LKPA

>tr IF6DJ67 | F6DJ67 _ THETG DNA helicase OS=Thermus thermophilic (strain SG0.5JP17-16) GN=Ththel 6__2 078 PE=4 SV=1 (SEQ ID NO: 103)
MEANLYVAGAGTGKTYTLAERYLGFLEEGLSPLQWAVTFTERAALELRHRVRQMVGERS
LGHKE RVLAELEAAP IGTLHALAARVCREFPEEAGVPADFQVME DLEAAL LLEAWLEEAL
LEALQDPRYAPLVEAVGYEGLLDTLREVAKDPLAARELLEKGLGEVAKA LRLEAWRALRR
RMEELFHGERPEERYPGFPKGWRTEEPEWPDLLAWAGEVKFNKKPWLEYKGDPALERLL
KLLGGVKEGFSPGPADERLEEVWPLLRELAEGVLARLEERRFRARRLGYADLEVHALRAL
EREEVRAYYRGRFRRLLVDE FQDTNPVQVRL LQALFPDLRAWTVVGDPNQS IYSFRRADP
KVMERFQAEAAKEGLRVRRLEKSHRYHQGLADFHNRFFPPLLPGYGAVSAERKPEGEGPW
VFHFQGDLEAQARFI AQEVGRLLSEGFQVYDLGEKAYRPMSLRDVAVLGRTWRDLARVAE
ALRRLEVPAVEAGGGNLLETRAFKDAYLALRFLGDPKDEEALVGLLRSPFFALTDGEVRR
LAEARGEGETLWEVLEREGDLSAEAERARETLRGLLRRKALEAPSRLLQRLDGATGYTGV
AARLPQGRRRVKDWEGTLDLVRKLEVGSEDPFLVARHLRLLLRSGLSVERPPLEAGEAVT
LLTVHGAKGLEWPWFVLNVGGWNRLGSWKNNKTKPLFRPGLALVPPVLDEEGNPSALFH
LAKRRVEEEEKQEENRLLYVAATRASERLYLLLSPDLSPDKGDLDPQTLIGAGSLEKGLE
ATEPERPWSGEEGEVEVLEERIQGLPLEALPVSLLPLAARDPEAARRRLLGEPEPEGGEA
WEPDGPQETEEEVPGGAGVGRMTHALLERFEAPEDLEREGRAFLEESFPGAEGEEVEEAL
RLART FLTAEVFAP YRGNAVAKE VPVALE LLGVRLE GRADRVGE DWVLD YKT DRGVDAKA
YLLQVGVYALALGKPRALVADLREGKLYEGASQQVEEKAEEVLRRLMGGDRPEA

>tr | G8N9P8 |G8N9P8_9DEIN DNA helicase OS=Thermus sp . CCB_US3_UF1 GN=TCCBUS 3UF1_1 7030 PE=4 SV=1 (SEQ ID NO: 104)
MDAFPSGKPLDEAWLSSLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLMARRGV
YPSEI LAVT FTNKAAEEMRERLKAMVKGAGELWVS TFHAAALRI LRFYGERVGLKPGFW
YDEDDQTALLKEVLKELGVSAKPGPIKALLDRAKNRGEPPERLLADLPEYYAGLSRGRLL
DVLHRYQQALWAQGALDFGDILLLALKLLEEDPEVRKRVRKRARFIHVDEYQDTSPVQYR
LTKLLAGEEANLMAVGDPDQGI YSFRAADIKNILQFTEDFPGAKVYRLEENYRSTERILR
FANAVIVKNALRLEKTLRPVKSGGEPVRLFRARDAREEARFVAEEVLRLGPPYDRVAVLY
RTNAQSRLLEQALASRGIGARWGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTP
PRGIGPATVEKVQAIAQEKGLPLYEALKVAAQVLPRPEPLRHFLALMEELMDLAFGPAEA
FFRHLLEATDYPAYLKEAYPEDLEDRLENVEELLRAAREAEGLMDFLDKVALTARAEEPG
EAGGKVALMTLHNAKGLEFPWFLVGVEEGLLPHRSSVSTLEGLEEERRLFYVGVTRAQE
RLYLSYAEEREVYGRPEASRPSRFLEEVEEGLYEEYDPYRLPPPKPVPPPHRAKPGAFRG
GEKWHPRFGLGTWAASGDEVTVHFDGVGLKRLSLKYADLRPA

>tr | Q lJ014 | Q lJ014_DEIGD DNA helicase OS=Deinococcus geothermalis (strain DSM 11300) GN=Dgeo_0868 PE=4 SV=1 (SEQ ID NO: 105)
MPDLPASSLLAQLNPNQAQAANHYTGPALVIAGAGSGKTRTLVYRIAHLIGHYGVDPGEI
LAVT FTNKAAAEMRE RARH LVEGADRL WM STFHSAGVRI LRAYGEHI GLKRGFVI YDDDD
QLD ILKE IMGS IPGIGAE THPRVLRG ILDRAKSNLLT PADLARHPE PFISGLPRE VAAEA

YRRYEARKKGQNAIDFGDLITETVRLFQEVPAVLERVQDRARFIHVDEYQDTNKAQYELT
RLLASRDRNLLWGDPDQSIYRFRGADIQNILDFQKDYLDAKVYMLEQNYRSSARVLTIA
NKLIENNAERLEKTLRPVKEDGHPVLFHRATDQRAEGDFVAEWLTRLHAEGMRFSDMAVL
YRTNAQSRVIEESLRRVQIPAKIVGGVGFYDRREIKDVLAYARLAINPDDDVALRRI IGR
PKRGIGDTALERLMEWARVNGTS ILTACAHAQELNILERGAQKAVEFAGLMHAMSEAADN
DEPGPFLRYVIETSGYLDLLRQEGQEGQVRLENLEELVSAAEEWSRENEGTIGDFLDDAA
LLSSVDDMRTKQENKDVPEDAVTLMTLHNAKGLEFPWFIVGTEEGLLPSKNALLEPGGI
EEERRLFYVGITRAMERLFLTAAQNRMQYGKTLATEDSRFLEEIKGGFDTVDAYGQVIDD
RPKSWKEYRPTESARPGAVKNTSPLTEGMAYRGGEKVRHPKFGEGQVLAVAGLGDRQEVT
VHFPSAGTKKLLVKFANLTRA

>tr iQ745W4 !Q745W4 __THET2 DNA helicase OS=Thermus thermophilics (strain HB27 / ATCC BAA- 163 / DSM 7039) GN=TT_P0191 PE=4 SV=1 (SEQ ID NO: 106)
MALRPTEEQLKAVEAYRS GQDLKVVAVAGS GKT TTLRLMAEAT PGKRGL YLAFNRS VQQE
AARKFPRNVRPYTLHALAFRMAVARDEGYRAKFQAGKGHLPAQAVAEALGLRNPLLLHAV
LGTLEAFLRSEAASPDPGMIPLAYRTLRAGTKTWPEEEAFVLRGVEALWRRMTDPKDPFP
LPHGAYVKLWALSEPDLSFAEALLVDEAQDLDPI FLKVLEAHRGRVQRVYVGDPRQQI YG
WRGAINAMDRLEAPEARLTWSFRFAETLARFVRNLTALQDRPVEVRGKAPWATRVDAALP
RPPFTVLCRTNAGWGAVWTHEVHRGRVHWGGVEELVHLLRDAALLKKGEKRTDPHPD
LAMVETWEELEALAEAGYAPAYGVLRLAQEHPDLEALAAYLERAWTPVEVAAGVWSTAH
KAKGREWDRVVLWDDFYPWWEEGAAARVNWGS DPAHLEEENLL YVAATRARKHLS LAQ IR
DLLEAVDRMGVYRVAEEATRAYLLLSAEVLRGVATDPRVPAEHRVRALKALGYLERGEEA
LDSPGKPGGQG

>tr | Q72IS0 !Q72IS0_THET2 DNA helicase OS=Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039) GN=uvrD PE=4 SV=1 (SEQ ID NO: 107)
MSDALLAPLNEAQRQAVLHFEGPALWAGAGSGKTRTWHRVAYLVARRGVFPSEILAVT
FTNKAAEEMRERLRGLVPGAGEVWVS T FHAAALRI LRVYGERVGLRPGFWYDEDDQTAL
LKEVLKELALSARPGPIKALLDRAKNRGVGLKALLGELPEYYAGLSRGRLGDVLVRYQEA
LKAQGALDFGDILLYALRLLEEDEEVLRLVRKRARFIHVDEYQDTSPVQYRFTRLLAGEE
ANLMAVGDPDQG I Y S FRAAD I KNI LDFTRDYPEARVYRLEENYRS TEAT L R FANAVI VKN
ALRLEKALRPVKRGGEPVRLYRAEDAREEARFVAEEIARLGPPWDRYAVLYRTNAQSRLL
EQALAGRGIPARWGGVGFFERAEVKDLLAYARLALNPLDAVSLKRVLNTPPRGIGPATW
ARVQLLAQEKGLPPWEALKEAARTFPRPEPLRHFVALVEELQDLVFGPAEAFFRHLLEAT
DYPAYLRE 7\YPEDAEDRLENVEELLRAAKEAEDLQDFLDRVALTAKAEEPAEAEGRVALM
TLHNAKGLEFPVVFLVGVEEGLLPHRNSVSTLEGLEEERRLFYVGITRAQERLYLSHA.EE
REVYGRREPARPSRFLEEVEEGLYEVYDPYRRPPSPPPHRPRPGAFRGGERVVHPRFGPG
TVVAAQGDE V TV H F E G FGLKRL S LKYAE LKPA

>tr jF2NK7 8 |F2NK7 8_MARHT DNxA helicase OS=Marini thermus hydrothermalis (strain DSM 14884 / JCM 11576 / T1) GN=Marky 1312 PE===4 SV=1 (SEQ ID NO: 108)
MDLLRDLNPAQREAVQHYTGPALVVAGAGSGKTRTVVHRIAYLIRHRGVYPTEILAVTFT
NKAAGEMKE RLARMV G PAARE LWV S T FHSAALRI LRVYGE Y IGLK P G FVV Y DEDDQLALL

KEVXGGLGLETRPQYARGVTDRIKNP^lWSVDAFLREAEDWVGGLPKEQi4AAVYQAYEAR>l
RALGAVDFNDLLLKVIGLFEAHPEVLHRVQQRARFIHVDEYQDTNPAQYRLTRLLAGAER
NLMWGDPDQS I YGFRNAD I HNI LNFEKDYPDARVYRLEENYRSTEAI LRVANAVIEKNA
LRLEKTLRPVRSGGDPVFLYRAPDHREEAAFVAREVQRLKGRGRRLDEIAVLYRTNAQSR
VLEEAFRRQNLGVRIVGGVGFYERREVKDVLAYARAAVNPADDLAVKRVLNVPARGIGQT
SLAKLSQLAETARVSFFEALRRAGEVLARPQAQAVQRFVALIEGLANAAYDTGPDAFLRL
VLAETGYADMLRREPDGEARLENLEELLRAAREWEEQHAGTIADFLDEVALTARAEEPEG
EVPAEAVTLMTLHNAKGLEFPWFIVGVEEGLLPHRSSTARVEDLEEERRLFYVGITRAQ
ERLYLTLSEERETYGRREAVRASRFLEDIPEAFLQPLSPFGEPLGAGREPVAVRPTRRSS
AAGGFRGGEKVRHPRFGQGLWAASGEGDRQEVTVHFAGVGLKKLLVKYAGLERIE

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[00259] All patents, patent applications, patent application publications and other publications that are cited herein are hereby incorporated by reference as if set forth in their entirety.

[00260] It should be understood that the methods, procedures, operations, composition, and systems illustrated in figures may be modified without departing from the spirit of the present disclosure. For example, these methods, procedures, operations, devices and systems may comprise more or fewer steps or components than appear herein, and these steps or components may be combined with one another, in part or in whole.

[00261] Furthermore, the present disclosure is not to be limited in terms of the particular embodiments described in this application, which are intended as illustrations of various embodiments. Many modifications and variations can be made without departing from its scope and spirit. Functionally equivalent methods and apparatuses within the scope of the disclosure, in addition to those enumerated herein, will be apparent to those skilled in the art based on the foregoing descriptions.

CLAIMS

What is claimed is:

1. A modified heiicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid,

wherein said first amino acid is at least about 30 **A** from said second amino acid when the heiicase is in an inactive conformation, and said first amino acid is less than about 20 **A** from said second amino acid when the heiicase is in an active conformation, and

wherein a side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conformationally-constrained heiicase.

2. The modified heiicase of claim 1, wherein said modified heiicase is a Super Family 1 (SFl) heiicase or a Super Family 2 (SF2) heiicase.

3. The SFl heiicase of claim 2, wherein said SF1 heiicase is an SFl A or an SFIB heiicase.

4. The modified heiicase of claim 3, wherein the first subdomain comprises a 1A subdomain or a IB subdomain and the second subdomain comprises a 2B subdomain.

5. The modified heiicase of any one of claims 1-4, wherein the first amino acid is less than about 15 **A,** about 10 **A,** about 9 **A,** about 8 **A,** about 7 **A,** about 5 **A,** or about 4 **A** from the second amino acid when the heiicase is in an active conformation.

6. The modified heiicase of any one of claims 1-5, wherein the first amino acid is less than about 20 A from the second amino acid when the heiicase is in an active conformation.

7. The modified heiicase of any one of claims 1-6, wherein the first amino acid is at least about 50 **A,** about 55 **A,** about 60 **A,** about 65 **A,** about 70 **A,** about 75 **A,** about 80 **A** or about 85 **A** from the second amino acid when the heiicase is in an inactive conformation.

8. The modified helicase of any one of claims 1-7, wherein the first amino acid is at least about 30 Å from the second amino acid when the helicase is in an inactive conformation.

9. The modified helicase of any one of claims 1-8, wherein said helicase is selected from the group consisting of a Rep helicase, a UvrD helicase and a PerA helicase.

10. The modified helicase of any one of claims 1-9, wherein said helicase is a Rep helicase or a PcrA helicase.

11. The modified helicase of any one of claims 1-10, wherein said helicase is a Rep helicase.

12. The modified helicase of any one of claims 1-10, wherein said helicase is a PcrA helicase.

13. The modified helicase of claim 11, wherein said Rep helicase or said UvrD helicase is from *E. coii.*

14. The modified helicase of claim 12, wherein the PcrA helicase is from *B. stearothermophilus.*

15. The modified helicase of any one of claims 1-8, wherein the first amino acid is at any one of positions 84-116 or 178-196 of the modified helicase amino acid sequence, and the helicase is a Rep, PcrA or UvrD helicase, or homolog thereof.

16. The modified helicase of claim 15, wherein the first amino acid is at any one of positions 92-116 or 178-196 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

17. The modified helicase of claim 15, wherein the first amino acid is at any one of positions 84-108 or 169-187 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

18. The modified helicase of claim 15, wherein the first amino acid is at any one of positions 90-114 or 175-193 of the modified helicase amino acid sequence, and the helicase is a **IJvrD** helicase, or homolog thereof.

19. The modified helicase of claim 17, wherein the first amino acid is at position 178 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

20. The modified helicase of claim 16, wherein the first amino acid is at position 187 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

21. The modified helicase of any one of claims 1-8, wherein the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 13, and the helicase is a Rep helicase, or homolog thereof.

22. The modified helicase of any one of claims 1-8, wherein the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 14, and the helicase is a Rep helicase, or homolog thereof.

23. The modified helicase of any one of claims 1-8, wherein the second amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 15, and the helicase is a Rep helicase, or homolog thereof.

24. The modified helicase of any one of claims 1-8, wherein the second amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 16, and the helicase is a Rep helicase, or homolog thereof.

25. The modified helicase of any one of claims 1-8, wherein the second amino acid residue is at any one of positions 388-411, 422-444 and 518-540 of the modified helicase amino acid sequence, and the helicase is a Rep, PcrA or UvrD helicase, or homolog thereof

26. The modified helicase of claim 25, wherein the second amino acid is at any one of positions 397-411, 431-444 or 526-540 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

27. The modified helicase of claim 25, wherein the second amino acid is at any one of positions 388-402, 422-435 or 519-531 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

28. The modified helicase of claim 25, wherein the second amino acid is at any one of positions 393-407, 427-440 or 523-540 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

29. The modified helicase of claim 27, wherein the second amino acid is at position 400 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

30. The modified helicase of claim 26, wherein the second amino acid is at position 409 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

31. The modified helicase of any one of claims 1-8, wherein said first subdomain and said second subdomain comprise no more than a total of two cysteine residues.

32. The modified helicase of any one of claims 1-8, wherein the first amino acid and the second amino acid are each independently an unnatural amino acid or a natural amino acid.

33. The modified helicase of any one of claims 1-8, wherein one or more of an amino acid of the helicase is substituted with an unnatural amino acid or a natural amino acid.

34. The modified helicase of claim 33, wherein the natural amino acid is cysteine or homocysteine.

35. The modified helicase of any one of claims 1-8, wherein said helicase comprises a sequence selected from SEQ ID NOs:4 and 12.

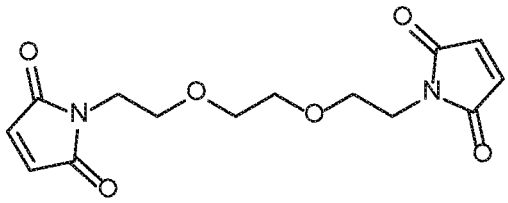
36. The modified helicase of any one of claims 1-8, wherein the first amino acid is covalently crosslinked to the second amino acid by a disulfide bond.

37. The modified helicase of any one of claims 1-8, wherein the first amino acid is covalently crosslinked to the second amino acid by a chemical crosslinker.

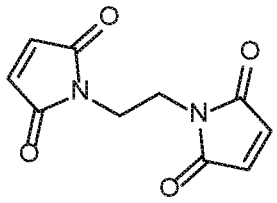
38. The modified helicase of claim 37, wherein the chemical crosslinker is a bis-maleimide crosslinker.

39. The modified helicase of any one of claims 37-38, wherein the chemical crosslinker has a length of from about 6Å to about 25A.

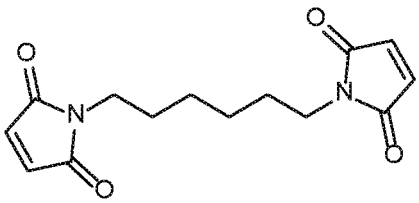
40. The modified helicase of any one of claims 37-39, wherein the chemical crosslinker is selected from the group consisting of



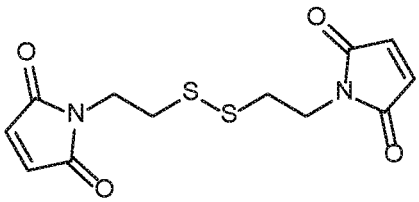
1-[2-[2-[2-(2,5-dioxopyrrol-1-yl)ethoxy]ethoxy]ethyl]pyrrole-2,5-dione,



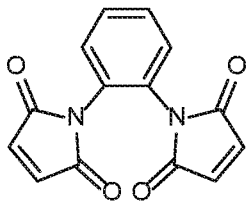
1-[2-(2,5-dioxopyrrol-1-yl)ethyl]pyrrole-2,5-dione,



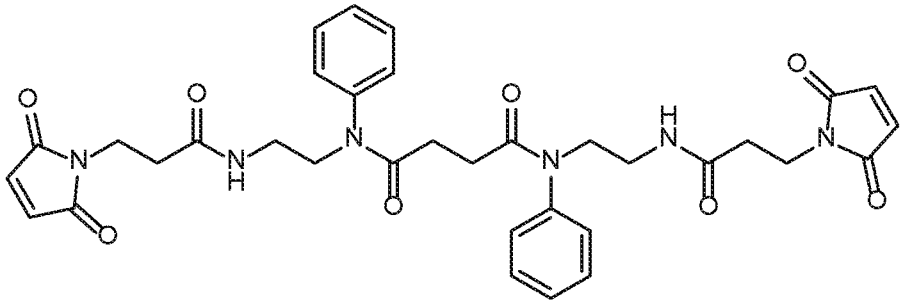
1-[6-(2,5-dioxopyrrol-1-yl)hexyl]pyrrole-2,5-dione,



1-[2-[2-(2,5-dioxopyrrol-1-yl)ethyldisulfanyl]ethyl]pyrrole-2,5-dione,

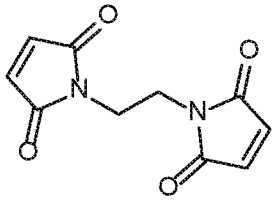


1-[2-(2,5-dioxopyrrol-1-yl)phenyl]pyrrole-2,5-dione, and



N,N'-bis[2-[3-(2,5-dioxopyrroM-yl)propanoy lamino]ethyl]-N,N'-diphenylbutanediamide.

41. The modified helicase of any one of claims 37-39, wherein the chemical crosslinker is

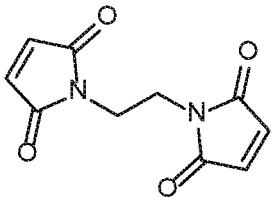


l-[2-(2,5-dioxopyrrol-l-yl)ethyl]pyrrole-2,5-dione.

42. A modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid,

wherein said first amino acid is at least about 30 Å from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 A from said second amino acid when the helicase is in an active conformation, and

wherein a side chain of the first amino acid is chemically crosslmked to a side chain of the second amino acid using



1-[2-(2,5-dioxopyrrol-l -yl)ethyl]pyrrole-2,5-dione

to form an active, conformationally-constrained helicase.

43. A modified Rep, PerA or UvrD helicase or homolog thereof, comprising a first subdomain having a first amino acid at any one of positions 84-116 and a second subdomain having a second amino acid at any one of positions 388-411, 422-444 and 518-540,

wherein a side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conformationally-constrained Rep, PerA or UvrD helicase, or homolog thereof.

44. A modified Rep helicase or homolog thereof comprising an amino acid at position 178 covalently crosslinked to an amino acid at position 400 to form an active, conformationally-constrained Rep helicase or homolog thereof.

45. A modified Rep helicase or homolog thereof comprising an amino acid at position 187 covalently crosslinked to an amino acid at position 409 to form an active, conformationally-constrained helicase.

46. A modified helicase comprising a first subdomain having a first amino acid and a second subdomain having a second amino acid,

wherein said first amino acid is at least about 30 Å from said second amino acid when the helicase is in an inactive conformation, and said first amino acid is less than about 20 Å from said second amino acid when the helicase is in an active conformation, and

wherein a side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a chemical crosslinker to form an active, conformationally-constrained helicase, and

wherein one or more of an amino acid of the helicase is substituted with an unnatural amino acid or a natural amino acid.

47. A method of making an active, conformationally-constrained helicase comprising:

selecting in a helicase a first amino acid in a first subdomain that is at least about 30 Å from a second amino acid in a second subdomain when the helicase is in an inactive

conformation, and the first amino acid is less than about 20 Afrom the second amino acid when the helicase is in an active conformation, and

covalently crosslinking the first amino acid to the second amino acid when the helicase is in an active conformation to form an active, conformationally-constrained helicase.

48. The modified helicase of claim 47, wherein said modified helicase is a Super Family 1 (SF1) helicase or a Super Family 2 (SF2) helicase.

49. The SF1 helicase of claim 48, wherein said SF1 helicase is an SF1A or an SF1B helicase.

50. The modified helicase of claim 49, wherein the first subdomain comprises a 1A subdomain or a 1B subdomain and the second subdomain comprises a 2B subdomain.

51. The modified helicase of any one of claims 47-50, wherein the first amino acid is less than about 15 A, about 10 A, about 9 A, about 8 A, about 7 A, about 5 A, or about 4 Afrom the second amino acid when the helicase is in an active conformation.

52. The modified helicase of any one of claims 47-51, wherein the first amino acid is at least about 30 A, about 35 A, about 40 A, about 45 A, about 50 A, about 55 A, about 60 A, about 65 A, about 70 A, about 75 A, about 80 Aor about 85 Afrom the second amino acid when the helicase is in an inactive conformation.

53. The modified helicase of any one of claims 47-52, wherein said helicase is selected from the group consisting of a Rep helicase, a UvrD helicase and a PerA helicase.

54. The modified helicase of any one of claims 47-52, wherein said helicase comprises a sequence selected from SEQ ID NOs:4 and 12.

55. The modified helicase of any one of claims 47-52, wherein the first amino acid is covalently linked to the second amino acid by a disulfide bond.

56. The modified helicase of any one of claims 47-52, wherein the first amino acid is covalently linked to the second amino acid by a chemical crosslinker.

57. A method of catalyzing an unwinding reaction of a double-stranded DNA, comprising contacting the double-stranded DNA with the conformationally-constrained helicase of claim 1.

58. The method of claim 57, wherein the conformationally-constrained helicase comprises SEQ ID NO:4 or SEQ ID NO: 12,

59. The method of any one of claims 57-58, wherein the conformationally-constrained helicase is chemically crosslinked.

60. The method of any one of claims 57-59, wherein the linker comprises an alkyl having a length in the range from C7 to C23.

61. The method of any one of claims 57-59, wherein the linker comprises an alkyl having a length in the range from C8 to CI3.

62. A method of performing isothermal DNA amplification, comprising combining:
a DNA template;
the conformationally-constrained helicase of claim 1; and
amplification reagents;
under conditions compatible for performing isothermal DNA amplification.

63. The method of claim 62, wherein the conformationally-constrained helicase comprises SEQ ID NO:4 or 12.

64. The method of any one of claims 62-63, further comprising the use of a DNA-dependent DNA polymerase selected from a group consisting of E. coli DNA Pol I, E. coli DNA Pol I Large Fragment, Bst 2.0 DNA Polymerase, Bst DNA Polymerase, Bst DNA Polymerase Large Fragment, Bsu DNA Polymerase I Large Fragment, T4 DNA Polymerase, T7 DNA polymerase, PyroPhage® 3173 DNA Polymerase and phi 29 DNA Polymerase.

65. The method of any one of claims 62-64, wherein the conformationally-constrained helicase is chemically crosslinked.

66. The method of any one of claims 62-65, wherein the chemical crosslinker comprises a length in the range from about 6Å to about 25A.

67. The method of any one of claims 62-66, wherein the chemical crosslinker comprises an alkyl having a length in the range from C7 to C23.

68. The method of any one of claims 62-66, wherein the chemical crosslinker comprises an alkyl having a length in the range from C8 to C13.

69. A kit for performing helicase dependent amplification, comprising:
the conformationally-constrained helicase of claim 1; and
amplification reagents.

70. The kit of claim 69, wherein the conformationally-constrained helicase is selected from SEQ ID NOs: 4 and 12.

71. The kit of any one of claims 69-70, further comprising a DNA-dependent DNA polymerase.

72. The kit of any one of claims 69-71, wherein the DNA-dependent DNA polymerase is selected from a group consisting of E. coli DNA Pol I, E. coli DNA Pol I Large Fragment, Bst 2.0 DNA Polymerase, Bst DNA Polymerase, Bst DNA Polymerase Large Fragment, Bsu DNA Polymerase I Large Fragment, T4 DNA Polymerase, T7 DNA polymerase, PyroPhage® 3173 DNA Polymerase and phi29 DNA Polymerase.

73. An isolated nucleic acid encoding the modified helicase of claim 1.

74. The isolated nucleic acid of claim 73, wherein said isolated nucleic acid is selected from the group consisting of SEQ ID NOs: 2, 3, 10 and 11.

75. A modified *E coli.* Rep helicase or homolog thereof comprising

a first subdomain having a first amino acid, a second subdomain having a second amino acid, and an axis vector defined by the alpha carbon of ILE371 from which the vector originates and the alpha carbon of SER280 or the alpha carbon of ALA603, wherein theta is an angle of rotation of said first amino acid and said second amino acid around the axis vector,

wherein a first theta between said first amino acid and said second amino acid is between about 60 degrees and about 155 degrees when the helicase is in an inactive conformation, and a second theta between said first amino acid and said second amino acid is between about 355 degrees and about 25 degrees when the helicase is in an active conformation, and

wherein a side chain of the first amino acid is covalently crosslinked to a side chain of the second amino acid with a linker to form an active, conformationally-constrained helicase.

76. The modified *E coli.* Rep helicase or homolog thereof of claim 75, wherein the first theta is about 133 degrees.

77. The modified *E coli.* Rep helicase or homolog thereof of claim 75 or 76, wherein the second theta is about 0 degrees.

78. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-77, wherein the axis vector is defined by the alpha carbon of ILE371 and the alpha carbon of SER280.

79. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the first amino acid is at any one of positions 84-108 or 169-187 of the modified helicase amino acid sequence.

80. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the first amino acid is at position 178 of the modified helicase amino acid sequence.

81. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 13.

82. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the first amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 14.

83. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the second amino acid is at any one of positions 388-402, 422-435 or 519-531 of the modified helicase amino acid sequence.

84. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the second amino acid is at position 400 of the modified helicase amino acid sequence.

85. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the second amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 15.

86. The modified *E coli.* Rep helicase or homolog thereof of any one of claims 75-78, wherein the second amino acid is present in an amino acid sequence having at least 20% amino acid sequence identity to SEQ ID NO: 16.

87. The modified helicase of any one of claims 1-8 or 75-78, wherein the first amino acid is at any one of positions 60-82 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

88. The modified helicase of any one of claims 1-8 or 75-78, wherein the first amino acid is at any one of positions 68-79 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

89. The modified helicase of any one of claims 1-8, wherein the first amino acid is at any one of positions 69-89 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

90. The modified helicase of any one of claims 1-8, wherein the first amino acid is at any one of positions 77-87 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

91. The modified helicase of any one of claims 1-8, wherein the first amino acid is at any one of positions 67-87 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

92. The modified helicase of any one of claims 1-8, wherein the first amino acid is at any one of positions 75-85 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

93. The modified helicase of any one of claims 1-8 or 75-78, wherein the second amino acid is at any one of positions 509-536 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

94. The modified helicase of any one of claims 1-8 or 75-78, wherein the second amino acid is at any one of positions 519-525 of the modified helicase amino acid sequence, and the helicase is a Rep helicase, or homolog thereof.

95. The modified helicase of any one of claims 1-8, wherein the second amino acid is at any one of positions 516-534 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof

96. The modified helicase of any one of claims 1-8, wherein the second amino acid is at any one of positions 526-532 of the modified helicase amino acid sequence, and the helicase is a PcrA helicase, or homolog thereof.

97. The modified helicase of any one of claims 1-8, wherein the second amino acid is at any one of positions 513-531 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

98. The modified helicase of any one of claims 1-8, wherein the second amino acid is at any one of positions 523-529 of the modified helicase amino acid sequence, and the helicase is a UvrD helicase, or homolog thereof.

99. The modified helicase of claim 13, wherein said helicase comprises one cysteine residue.

100. The modified helicase of claim 99, wherein said helicase is from a bacterium selected from the group consisting of Deinococcus geothermalis, Meiothermus sp., Marinithermus hydrothermalis, Marinithermus hydrothermalis and Oceanithermus profundus.

101. The modified helicase of claim 13, wherein said helicase comprises one cysteine residue or no cysteine residues.

102. The modified helicase of claim 99, wherein said helicase is from a bacterium selected from the group consisting of Thermococcus sp. EXT9, Thermococcus sp. IRI48, Thermococcus sp. IRI33, Thermococcus sp. AMT7, Thermococcus nautili, Thermococcus onnurineus (strain NAl), Thermococcus kodakarensis (strain ATCC BAA-918 / JCM 12380 / KOD 1) (Pyrococcus kodakaraensis (strain KOD 1)), Thermococcus sibiricus (strain MM 739 / DSM 12597), Thermococcus paralvinellae, Thermus aquaticus Y51MC23, Thermus aquaticus Y51MC23, Thermus aquaticus Y51MC23, Thermus sp. RL, Thermus sp. RL, Thermus sp. 2.9, Salinisphaera hydrothermalis C41B8, Thermus filiformis, Meiothermus ruber, Thermus sp. NMX2.A1, Thermus thermophilus JL-18, Thermus scotoductus (strain ATCC 700910 / SA-01), Thermus scotoductus (strain ATCC 700910 / SA-01), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Oceanithermus profundus (strain DSM 14977 / NBRC 100410 / VKM B-2274 / 506), Thermus oshimai JL-2, Thermus oshimai JL-2, Thermus oshimai JL-2, Thermomonospora curvata (strain ATCC 19995 / DSM 43183 / JCM 3096 / NCJJV1B 10081), Thermodesulfatator indicus (strain DSM 15286 / JCM 11887 / CIR29812), Geobacillus stearothermophilus (Bacillus stearothermophilus), Coprothermobacter proteolyticus (strain ATCC 35245 / DSM 5265 / BT), Meiothermus silvanus (strain ATCC 700542 / DSM 9946 / VI-R2) (Thermus silvanus), Anaerolinea thermophila (strain DSM 14523 / JCM 11388 / NBRC 100420 / UNI-1), Thermoanaerobacterium thermosaccharolyticum M0795,

Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Meiothermus ruber (strain ATCC 35948 / DSM 1279 / VKM B-1258 / 21) (Thermus ruber), Deinococcus radiodurans (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051 / NBRC 15346 / NCIMB 9279 / R1 / VKM B-1422), Thermodesulfobium narugense DSM 14796, Thermus thermophilus (strain HB8 / ATCC 27634 / DSM 579), Dictyoglomus thermophilum (strain ATCC 35947 / DSM 3960 / H-6-12), Thermus thermophilus (strain SG0.5JP17-16), Thermus thermophilus (strain SG0.5JP17-16), Thermus thermophilus (strain SG0.5JPI7-16), Thermus sp. CCB_US3_UF1, Deinococcus geothermalis (strain DSM 11300), Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039), Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039), and Marinithermus hydrothermalis (strain DSM 14884 / JCM 11576 / T1).

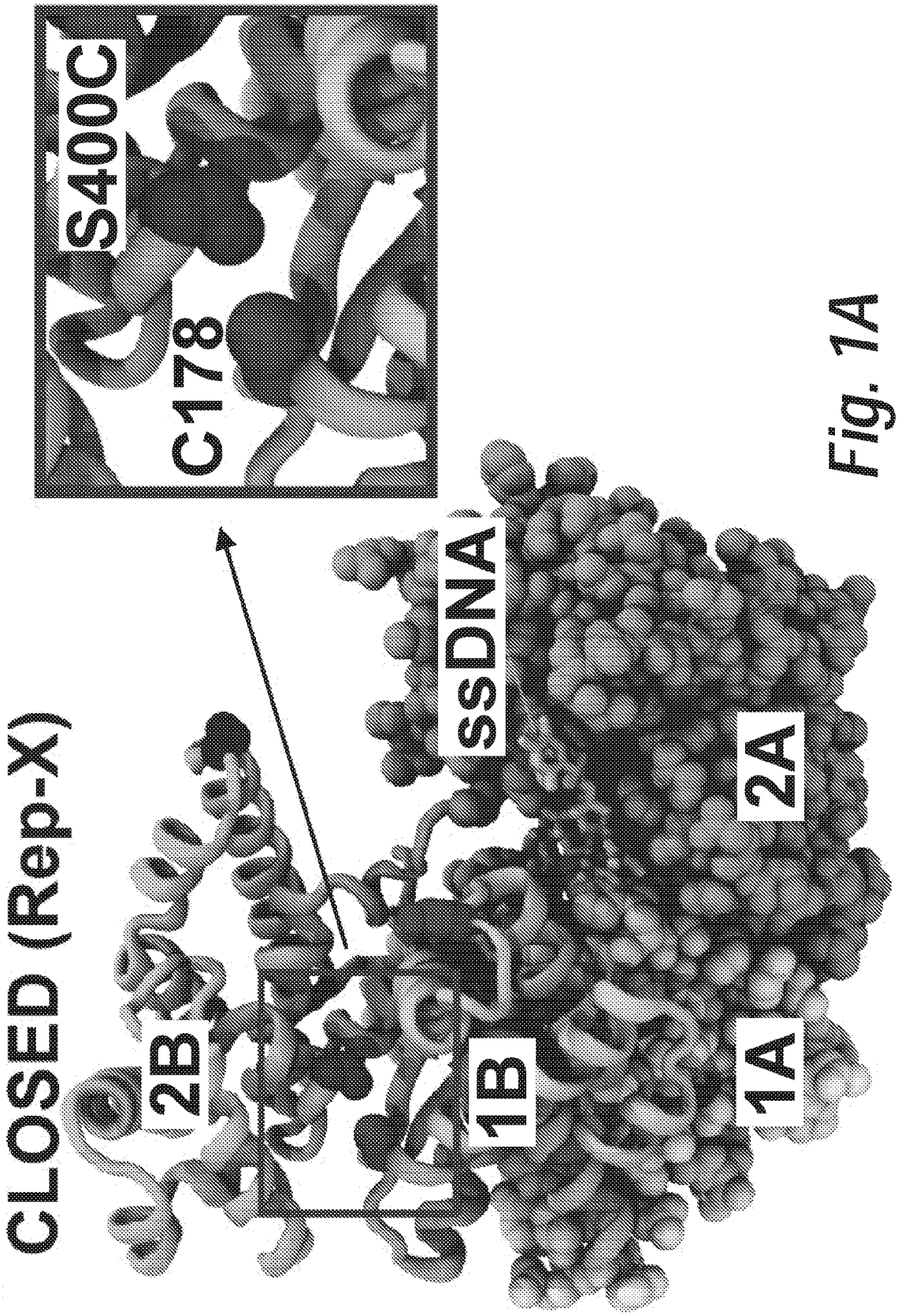


Fig. 1A

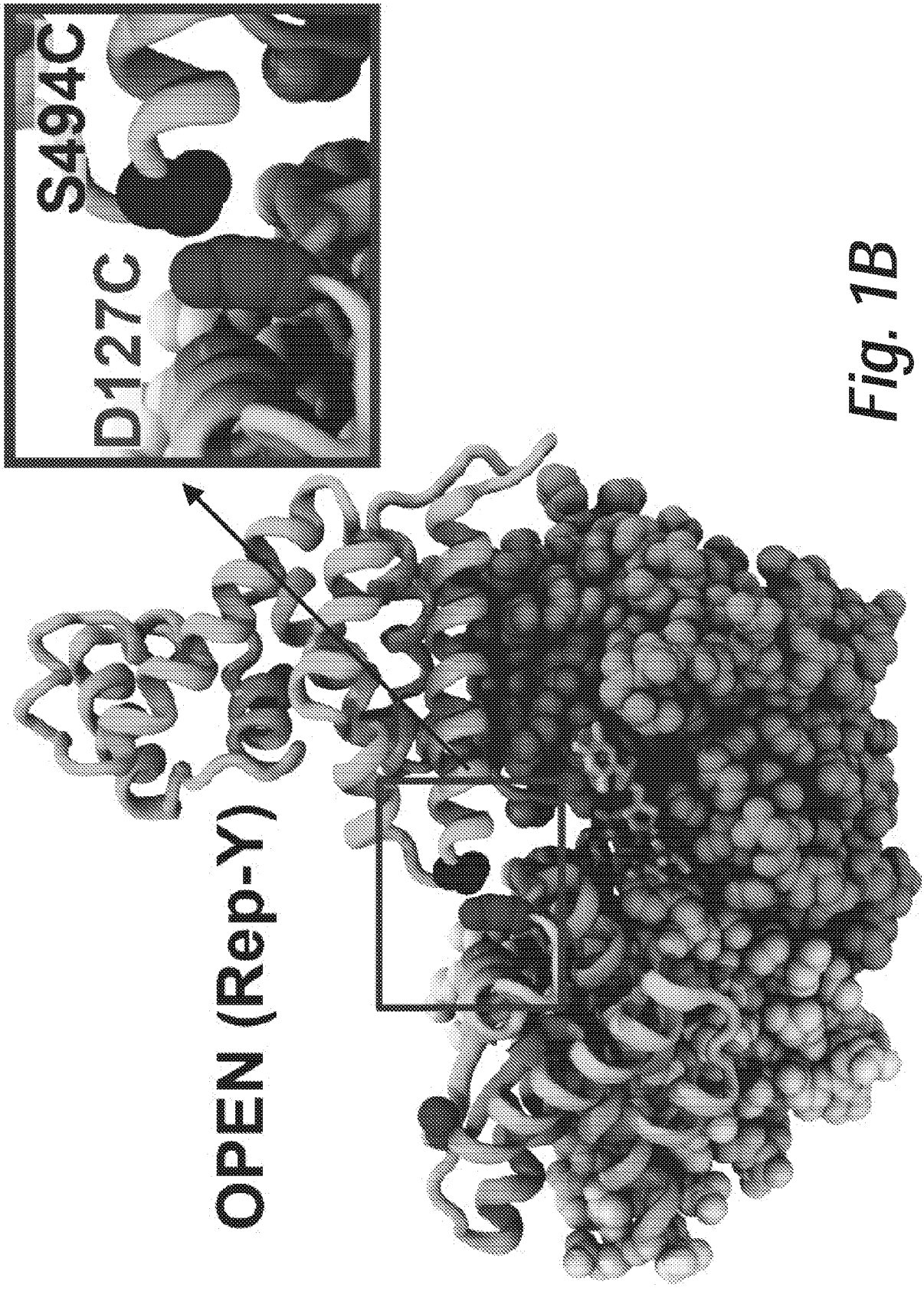


Fig. 1B

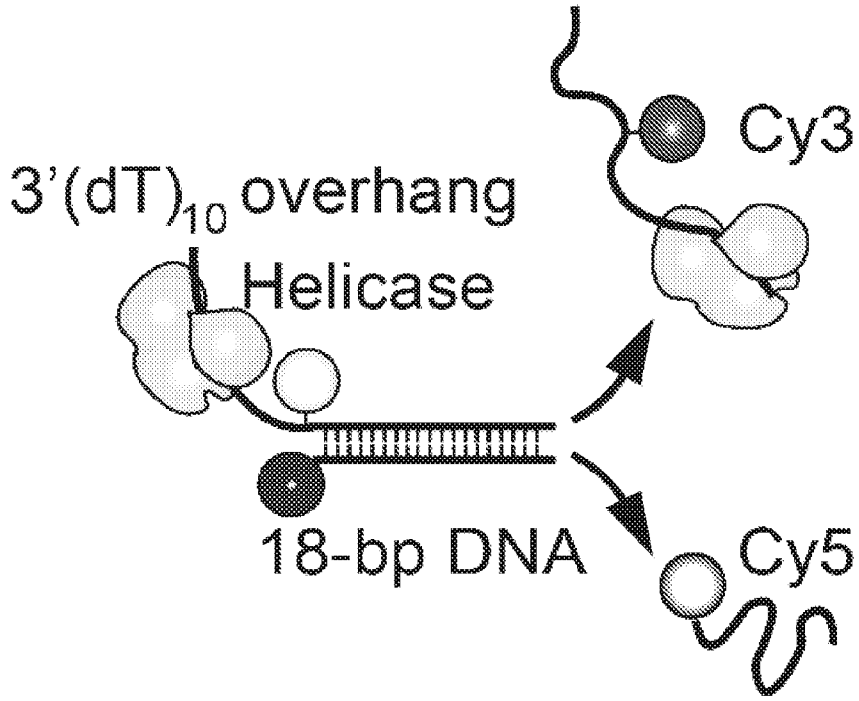

Cy3
3'(dT)10 overhang
Helicase
18-bp DNA
Cy5

Fig. 1C

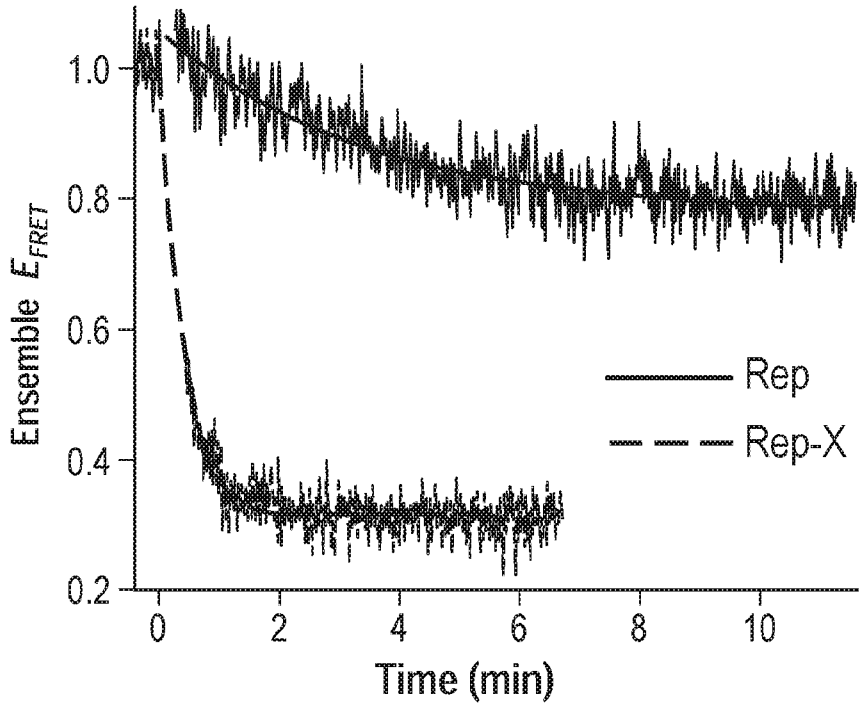

1.0
0.8
0.6
0.4
0.2
Ensemble E_{FRET}
0
2
4
6
8
10
Time (min)
Rep
Rep-X

Fig. 1D

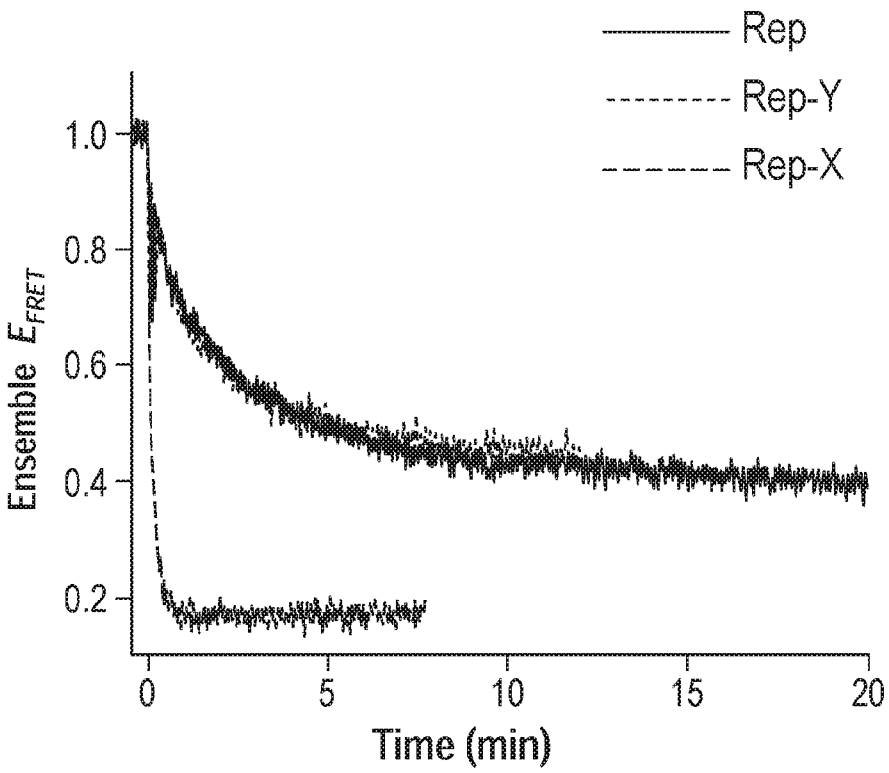


Fig. 1E

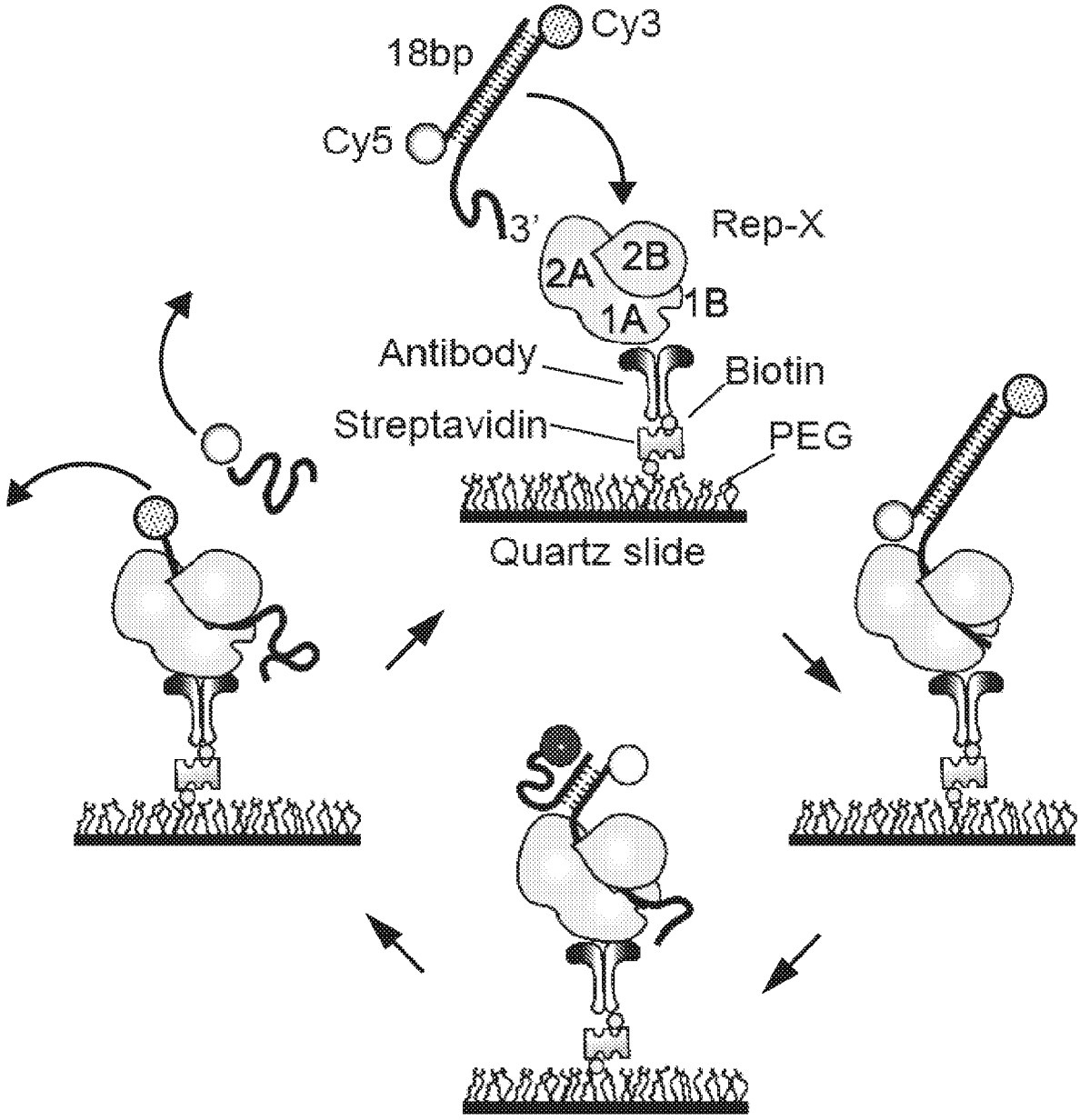

18bp
Cy3
Cy5
3'
Rep-X
2A
2B
1A
1B
Antibody
Biotin
Streptavidin
PEG
Quartz slide

Fig. 2A

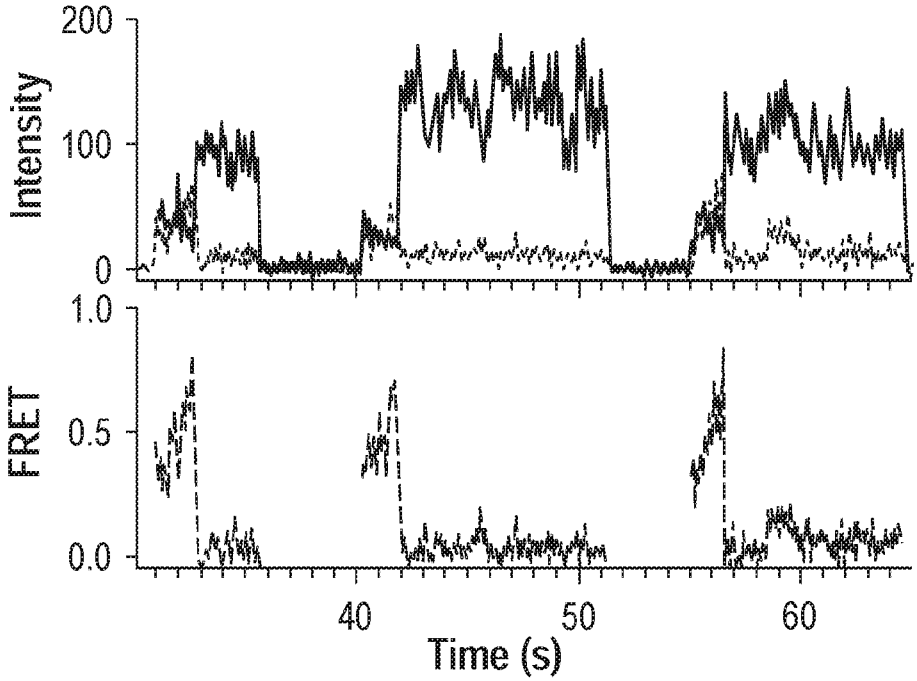

Intensity
200
100
0
FRET
1.0
0.5
0.0
40
50
60
Time (s)

Fig. 2B

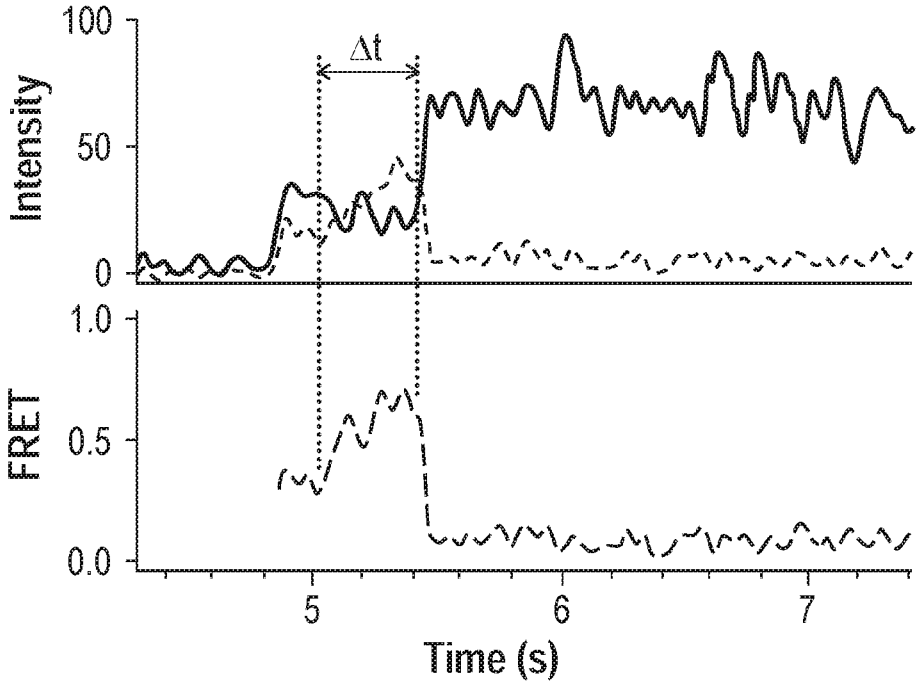

Intensity
100
50
0
Δt
FRET
1.0
0.5
0.0
5
6
7
Time (s)

Fig. 2C

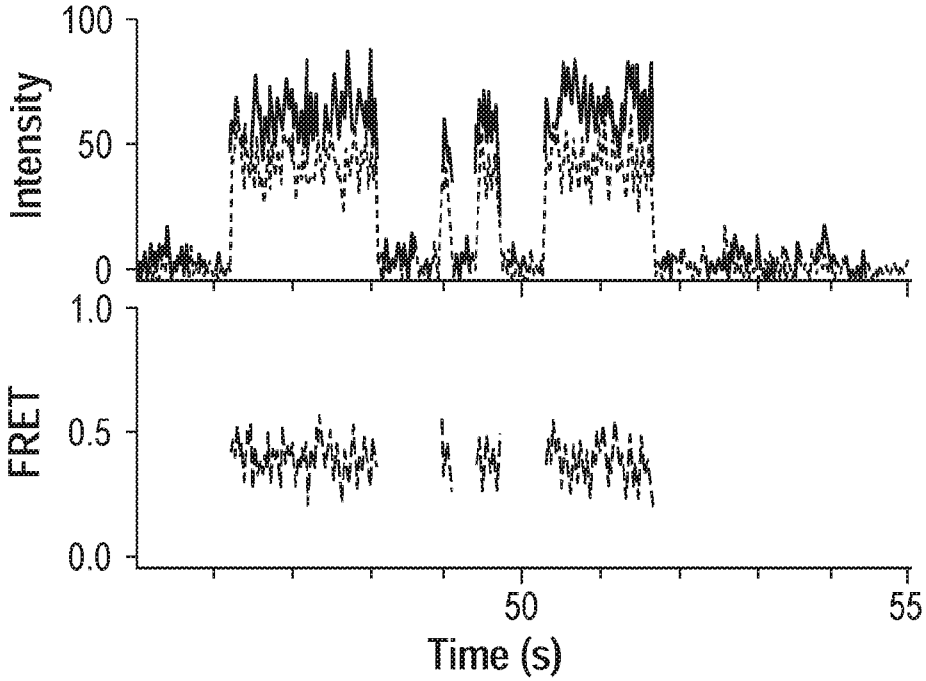

Intensity
100
50
0
FRET
1.0
0.5
0.0
50
55
Time (s)

Fig. 2D

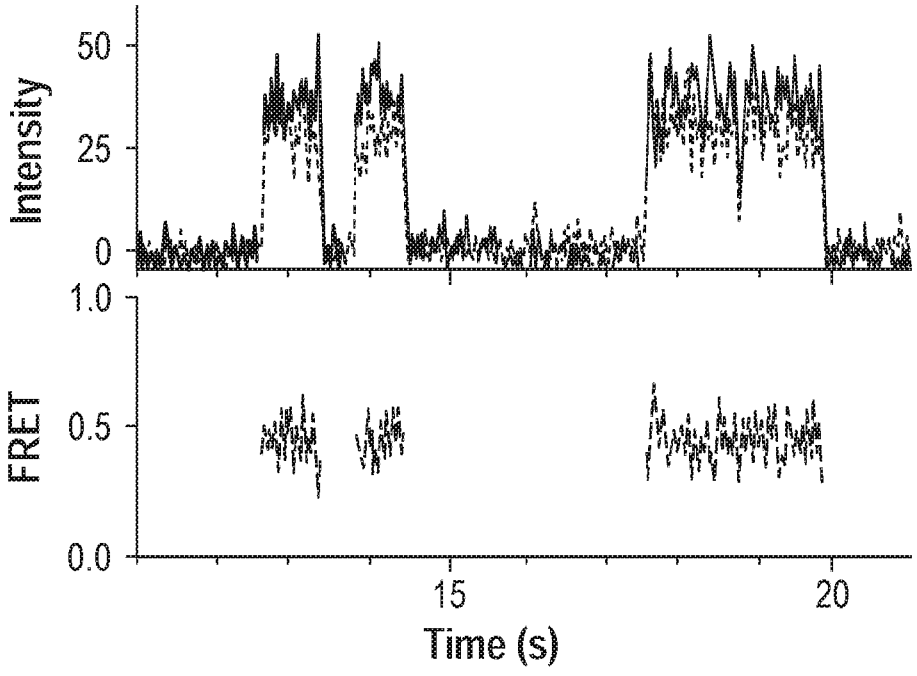

Intensity
50
25
0
FRET
1.0
0.5
0.0
15
20
Time (s)

Fig. 2E

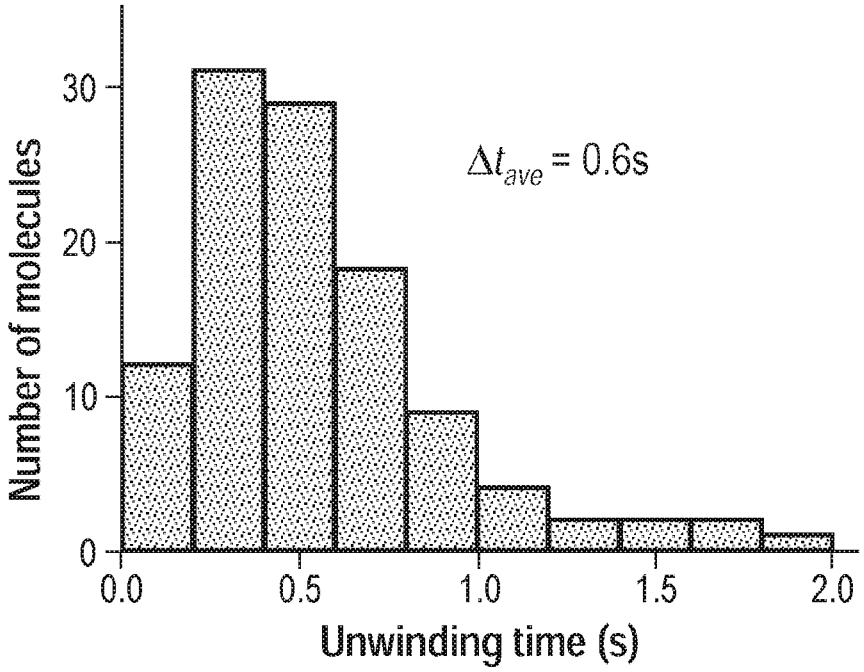

Number of molecules
30
20
10
0
Δt_{ave} = 0.6s
0.0
0.5
1.0
1.5
2.0
Unwinding time (s)

Fig. 2F

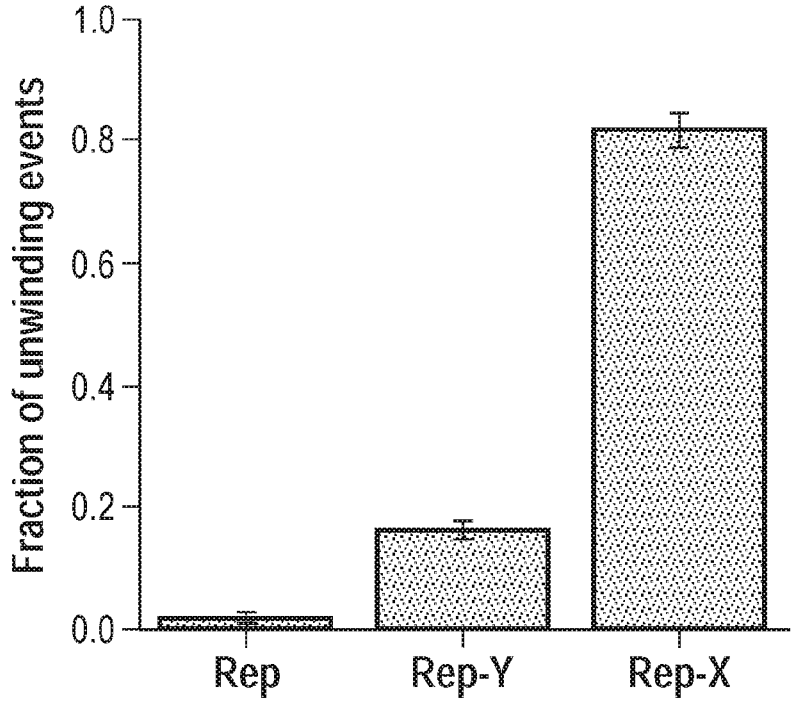

Fraction of unwinding events
1.0
0.8
0.6
0.4
0.2
0.0
Rep
Rep-Y
Rep-X

Fig. 2G

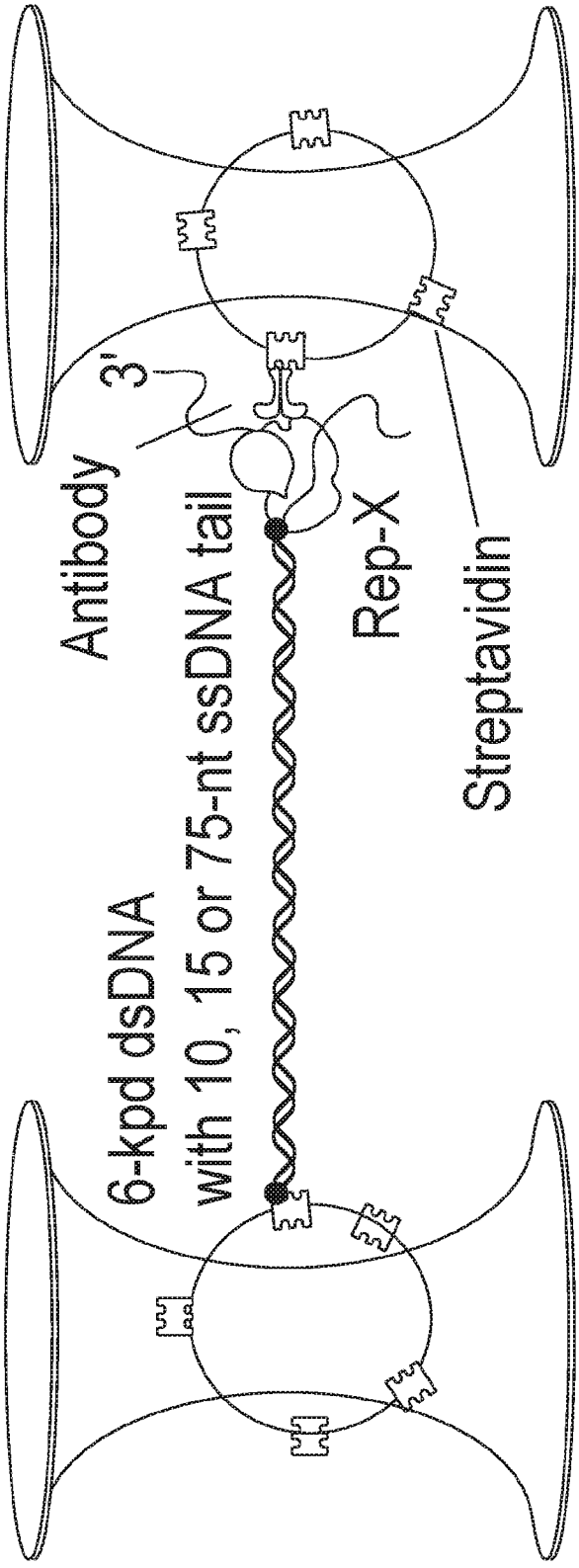

3'
Antibody
6-kpd dsDNA
with 10, 15 or 75-nt ssDNA tail
Rep-X
Streptavidin

Fig. 3A

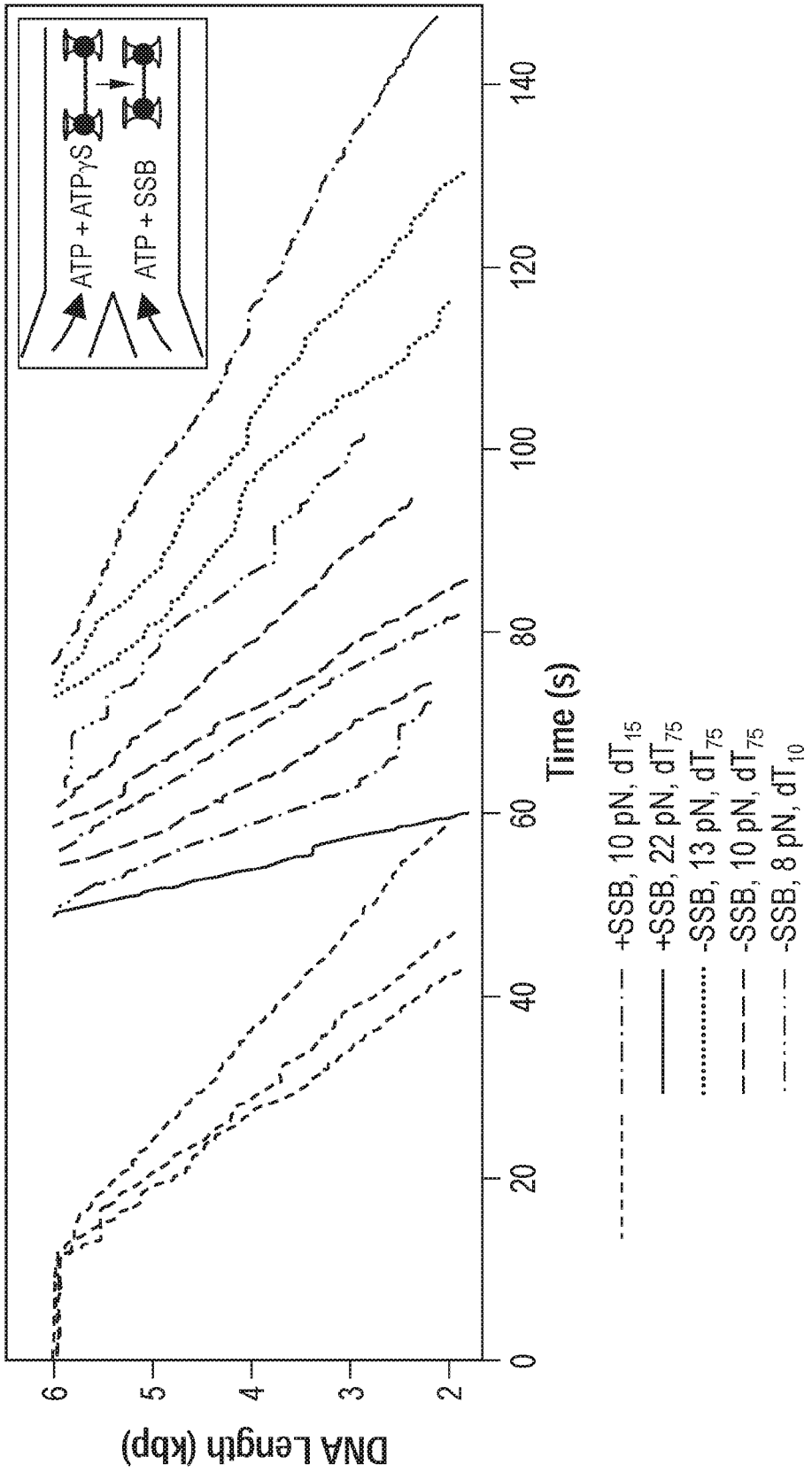

DNA Length (kbp)
Time (s)
ATP + ATPγS
ATP + SSB
+SSB, 10 pN, dT15
+SSB, 22 pN, dT75
-SSB, 13 pN, dT75
-SSB, 10 pN, dT75
-SSB, 8 pN, dT10

Fig. 3B

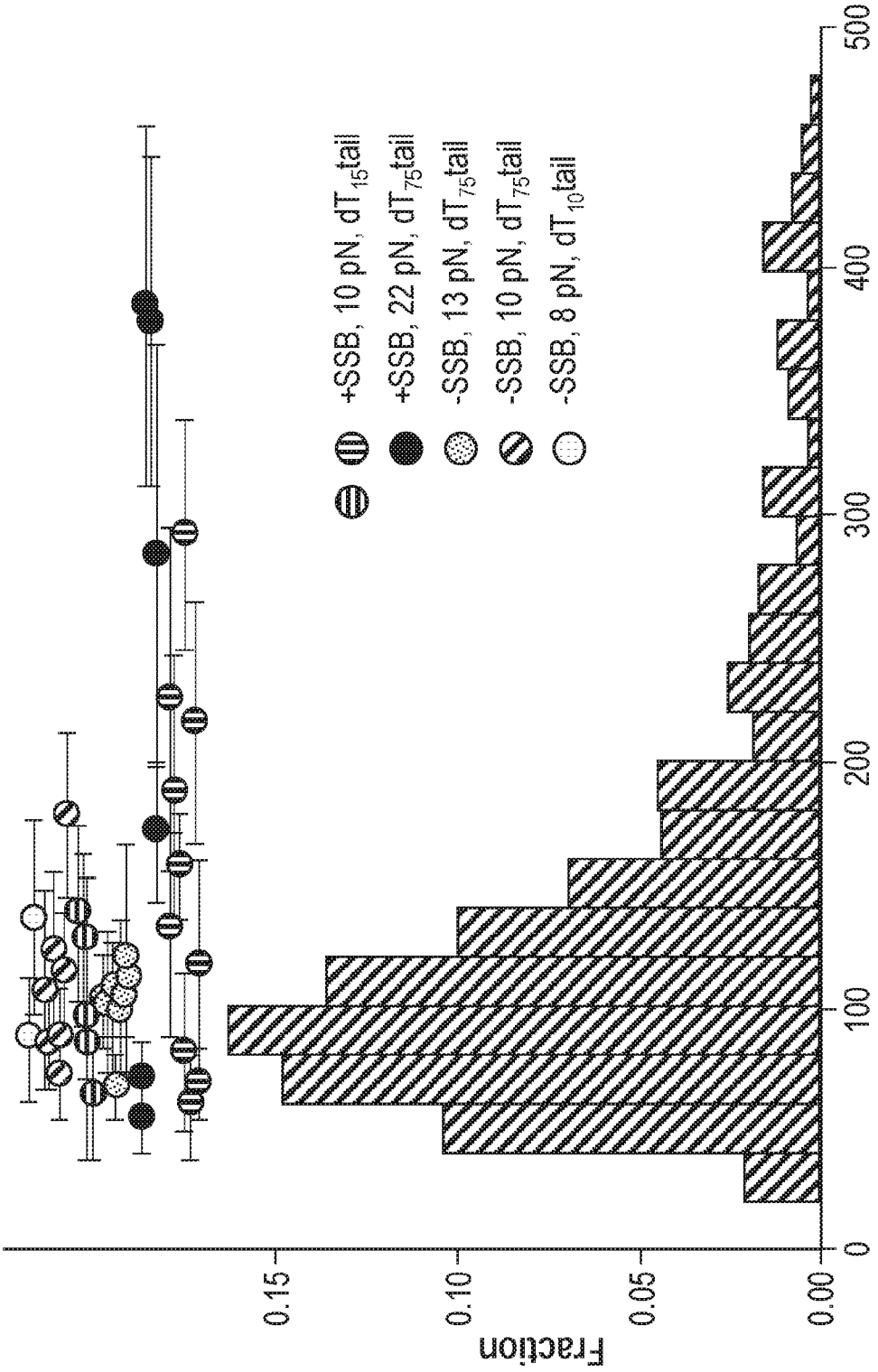

+SSB, 10 pN, dT15tail
+SSB, 22 pN, dT75tail
-SSB, 13 pN, dT75tail
-SSB, 10 pN, dT75tail
-SSB, 8 pN, dT10tail
Fraction
0.00
0.05
0.10
0.15
Unwinding velocity (bp/s)
0
100
200
300
400
500

Fig. 3C

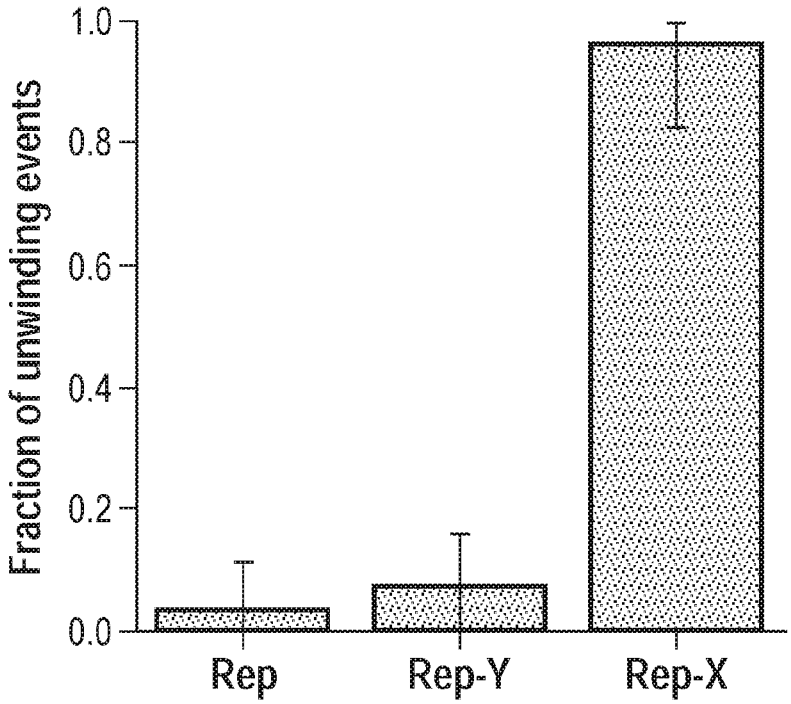

Fraction of unwinding events
1.0
0.8
0.6
0.4
0.2
0.0
Rep
Rep-Y
Rep-X

Fig. 3D

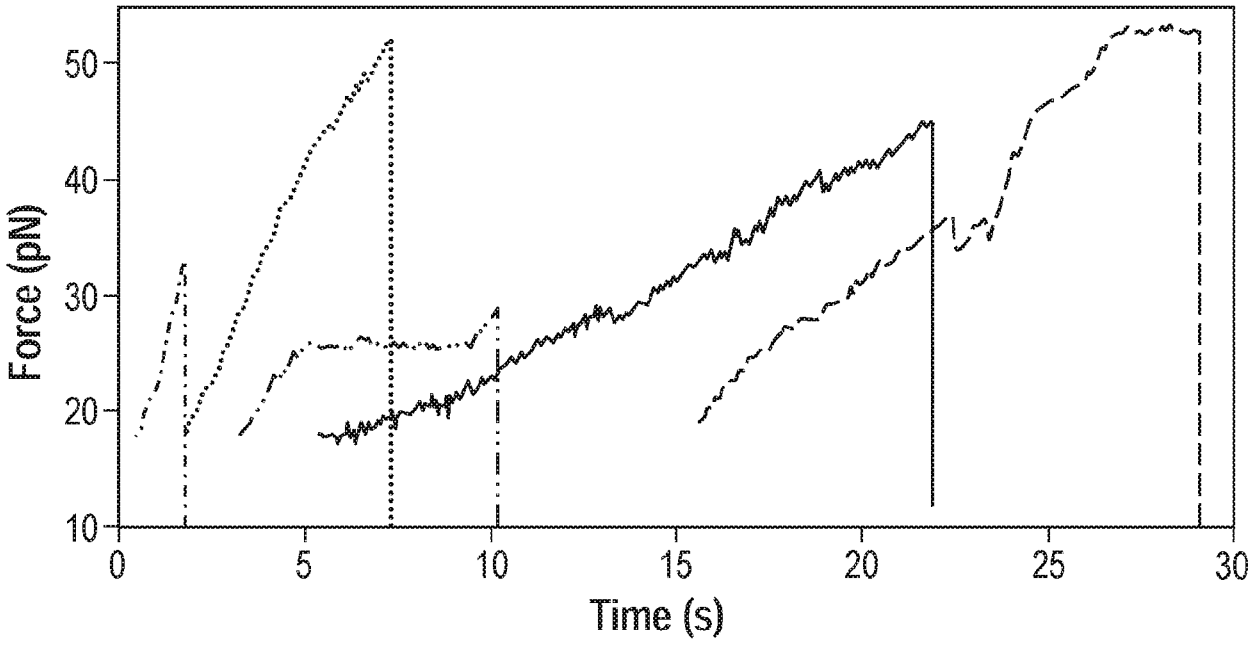

Force (pN)
50
40
30
20
10
0
5
10
15
20
25
30
Time (s)

Fig. 3E

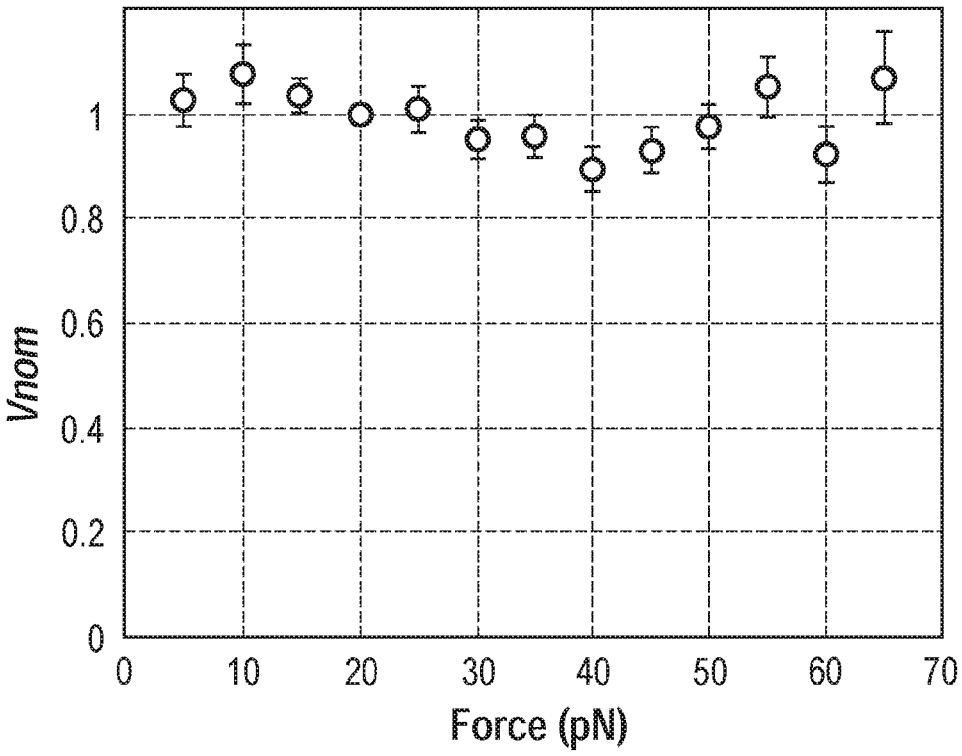


Fig. 3F

Supplementary Table S2. Conserved Cys247 in Motif III of PcrA

		Motif Ia	TxGx Motif	
Rep	*Escherichia coli*	KIAHLIRGCGYQARHIAAVTFTNKAAREMKERVGQTLGRK-EARGLMISTFHTLGLDIIK		93
UvrD	*Escherichia coli*	RIAWLMSVENCSPYSIMAVTFTNKAAAEMRHRIGQLMGT--SQGGMWVGTFHGLAHRLLR		99
UvrD	*Mycobacterium tuberculosis*	RIAYLMAARGVGVGQTLAITFTNKAAAEMRERVVGLVGE--KARYMWVSTFHSTCVRILR		112
UvrD	*Mycoplasma capricolum*	KIAYLIEKQNIDPSRILAVTFTNKAAKEMKERVLQITNN--SFKSPFISTFHSWCSKVLR		99
UvrD	*Deinococcus radiodurans*	RIAHLIGHYGVHPGEILAVTFTNKAAAEMRERAGHLVPG---AGDLWMSTFHSAGVRILR		101
PcrA	***Geobacillus sterothermophilus***	**RIAYLMAEKHVAPWNILAITFTNKAAREMKERVQSLLGG--AAEDVWISTFHSMCVRILR**		**101**
PcrA	*Bacillus subtilis*	RIAYLMAEKHVAPWNILAITFTNKAAREMKERVESILGP--GADDIWISTFHSMCVRILR		101
PcrA	*Staphylococcus aureus*	RIAYLLDEKDVSPYNVLAITFTNKAAREMKERVQKLVGD--QAEVIWMSTFHSMCVRILR		97
PcrA	*Leuconostoc citreum*	RIAHLVQDLNVFPWRILAITFTNKAAREMRERIAAILSED-VARDIWVSTFHALAVRILR		100
PcrA	*Fructobacillus fructosus*	RVAHLIEDLDVRPWRILAITFTNKAAREMRERIAKLVDPE-QAQAVWVSTFHALAVRILR		100
PcrA	*Staphylococcus epidermis*	RIAYLLDEKDVSPYNILAITFTNKAAKEMKARVEHLVGE--EAQVIWMSTFHSMCVRILR		97
PcrA	*Carnobacterium maltaromaticum*	RIAYLIEEKQVNPWNILAITFTNKAAKEMKERVNRLNVR--GGNDVWVSTFHSMCVRILR		100
PcrA	*Alloiococcus otitis*	RIAYLIQEKGVNPWNILAITFTNKAAGEMKDRVQKLVSQ--GGSGVWVSTFHSMCVRILR		100
PcrA	*Mitsuokella multacida*	RIANLLA-QGVAPYSILAITFTNKAATEMRERVDRMIGD--AAKDVWLSTFHSFCARFLR		99
PcrA	*Alkaliphilus metalliredigens*	RIAYLVEELGVSPYHILSITFTNKAAREMKERIHQLLGD--GFRDLWVSTFHSSCVRILR		95
PcrA	*Desulfotomaculum reducens*	RIAQILS-QGVRPYNILAITFTNKAANEMRARVENLVPQ--AAKDLWVTTFHSACLRILR		95
PcrA	*Listeria fleischmannii*	RIAYLIKEREVNPYNILAITFTNKAAREMKARVTGLLGG--IAESIWMSTFHSMCVRILR		101
PcrA	*Sporosarcina newyorkensis*	RIAYLVVEKQVYPSNILAITFTNKAAREMRERIDGLLGAG-SGERMWVSTFHSMCVRILR		102
PcrA	*Kurthia massiliensis*	RIAYLVIEKAVNPWNILAITFTNKAAREMRDRIDGLLGNG-SGQQMWVSTFHSMCVRILR		102
PcrA	*Marinococcus halotolerans*	RIAYLIREKMIPPYATLAITFTNKAAKEMKERVGKLVGP--VAEDIWMSTFHSMCVRILR		102
PcrA	*Planococcus antarcticus*	RIAYLVLEKQVYPSNILAITFTNKAAREMRNRIDGLLGHG-TGQRMWASTFHSMCVRILR		102
PcrA	*Lysinibacillus fusiformus*	RIAYLVIEKEVYPSKILAITFTNKAAREMRDRIDGILGNG-TGDSMWVSTFHSMCVRILR		102
PcrA	*Oceanobacillus iheyensis*	RIAYLMGEKDVSPRNILAITFTNKAAREMKERVSKLVGP--QGEYMWVSTFHSMCVRILR		101
PcrA	*Virgibacillus sp.*	RIAYLLEEKDVAARNILAITFTNKAAREMKERVRRLVGS--ESDQMWVSTFHSMCVRILR		101
PcrA	*Caldibacillus debilus*	RIAYLIAEKGVNPYNILAITFTNKAAREMKERVEKIIGG--TAAEVWISTFHAMCVKILR		118
PcrA	*Halobacillus halophilus*	RIAYLLSEKDVAPRNILAITFTNKAAREMKDRVESLVGK--DGEKIWMSTFHSMCVRILR		101
PcrA	*Gracilibacillus halophilus*	RIAYLLGEKEVSPRNILAITFTNKAAREMKERITDLVGS--RGADMWVSTFHSMCVRILR		101
PcrA	*Bacillus cereus*	RIAYLLGEKGVAPWNILAITFTNKAAREMRERIDTLVGP--EAEDIWISTFHSMCVRILR		100
PcrA	*Macrococcus caseolyticus*	RIAYLIDEKDVSPYKILAITFTNKAAKEMKERVKKIIGD--ESDVIWISTFHSMCVRILR		97
PcrA	*Laceyella sacchari*	RVAYLLAEKGIHPWNVLAITFTNKAAREMKERIGQLVGP--MAEEIWISTFHSMCVRMLR		101
PcrA	*Brevibacillus laterosporus*	RISYLVGYKQAEPWSILAITFTNKAAREMKERVERMVGRDAGADDIWISTFHSLCVRILR		102
PcrA	*Paenibacillus sp.*	RIAYLIATRKAPPWAILAITFTNKAAREMQDRVSRLVGGP-EGRDIWVSTFHSMCVRILR		109
PcrA	*Thermobacillus composti*	RIAYLIGKKRVAPWSILAITFTNKAAREMQERVEALV-GP-GASDIWVSTFHSMCVRILR		103
PcrA	*Amphibacillus xylanus*	RIAYLLDEKDVSPRNILAITFTNKAAREMKDRVTNLIGP--EADQMWVSTFHSMCVRILR		102

Fig. 4A

Motif II (Walker)
Motif III
Rep
UvrD
UvrD
UvrD
UvrD
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
PcrA
Escherichia coli
Escherichia coli
Mycobacterium tuberculosis
Mycoplasma capricolum
Deinococcus radiodurans
Geobacillus sterothermophilus
Bacillus subtilis
Staphylococcus aureus
Leuconostoc citreum
Fructobacillus fructosus
Staphylococcus epidermis
Carnobacterium maltaromaticum
Alloiococcus otitis
Mitsuokella multacida
Alkaliphilus metalliredigens
Desulfotomaculum reducens
Listeria fleischmannii
Sporosarcina newyorkensis
Kurthia massiliensis
Marinococcus halotolerans
Planococcus antarcticus
Lysinibacillus fusiformus
Oceanobacillus iheyensis
Virgibacillus sp.
Caldibacillus debilus
Halobacillus halophilus
Gracilibacillus halophilus
Bacillus cereus
Macrococcus caseolyticus
Laceyella sacchari
Brevibacillus laterosporus
Paenibacillus sp.
Thermobacillus composti
Amphibacillus xylanus
256
262
287
267
269
265
265
261
264
264
264
261
264
264
259
265
268
268
266
268
268
265
283
265
265
265
266
261
265
266
273
267
266

Fig. 4B

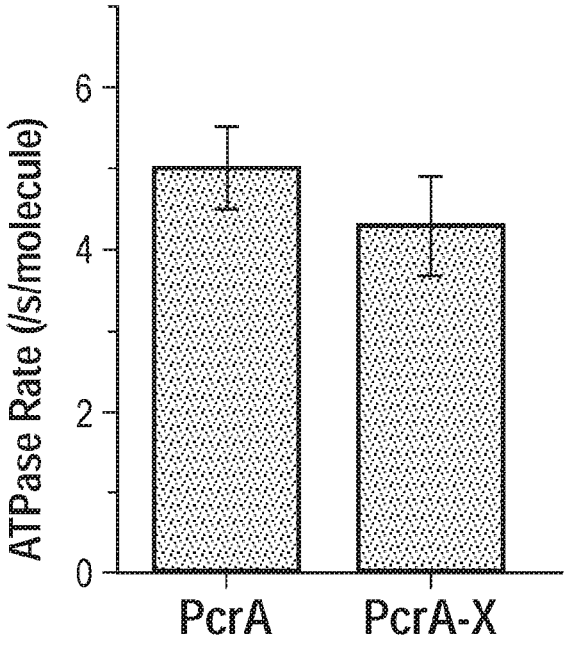

ATPase Rate (/s/molecule)
0
2
4
6
PcrA
PcrA-X

Fig. 5A

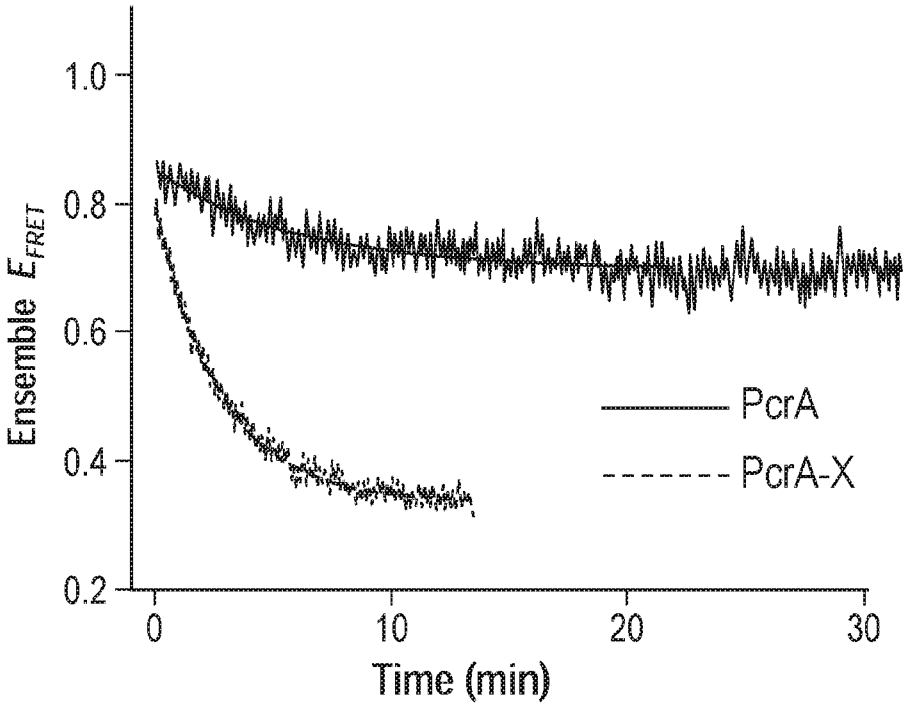

Ensemble E_{FRET}
1.0
0.8
0.6
0.4
0.2
0
10
20
30
Time (min)
PcrA
PcrA-X

Fig. 5B

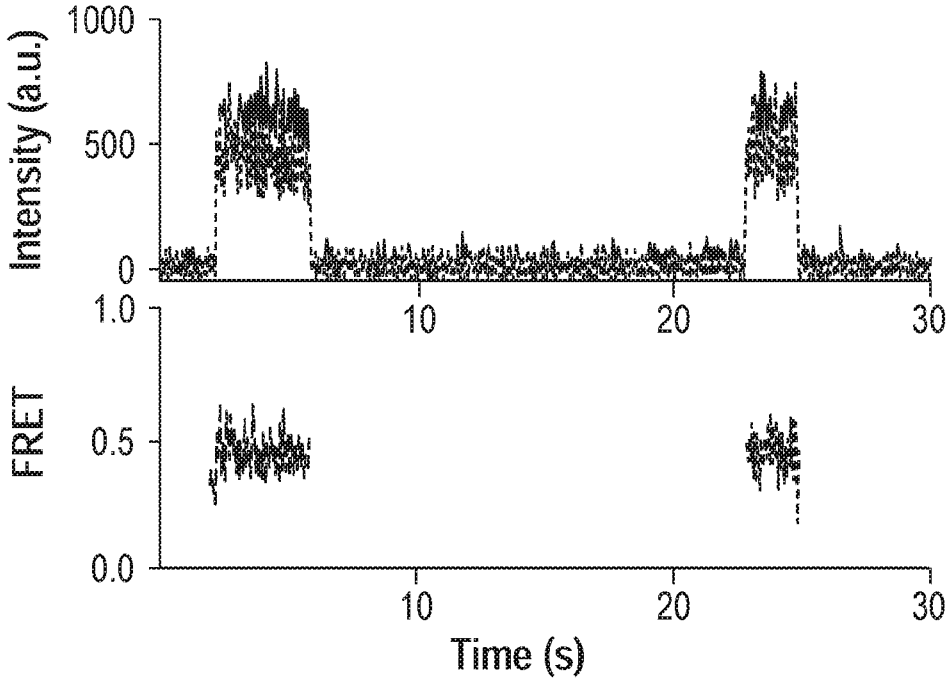

Intensity (a.u.)
1000
500
0
1.0
0.5
0.0
FRET
10
20
30
10
20
30
Time (s)

Fig. 6A

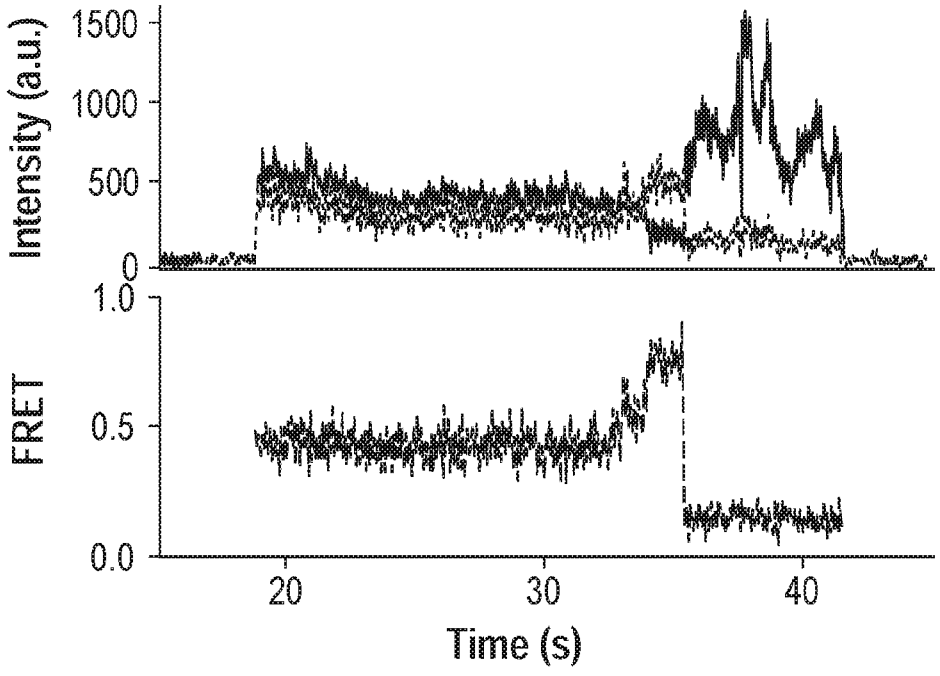

Intensity (a.u.)
1500
1000
500
0
1.0
0.5
0.0
FRET
20
30
40
Time (s)

Fig. 6B

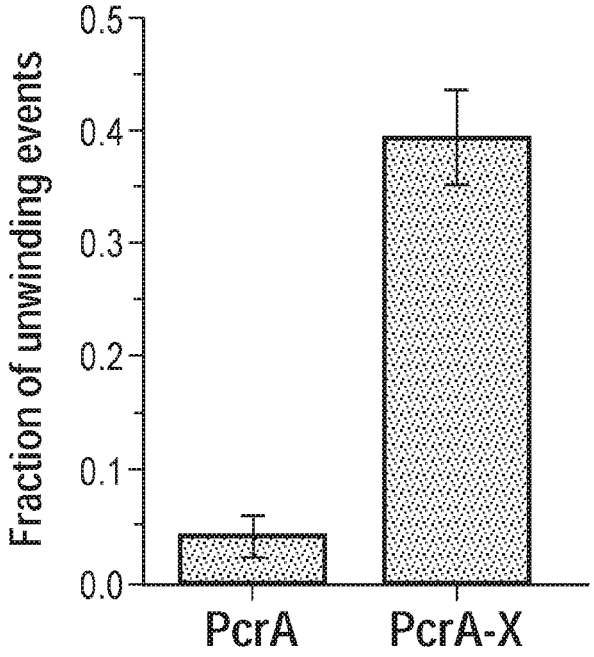

Fraction of unwinding events
0.5
0.4
0.3
0.2
0.1
0.0
PcrA
PcrA-X

Fig. 6C

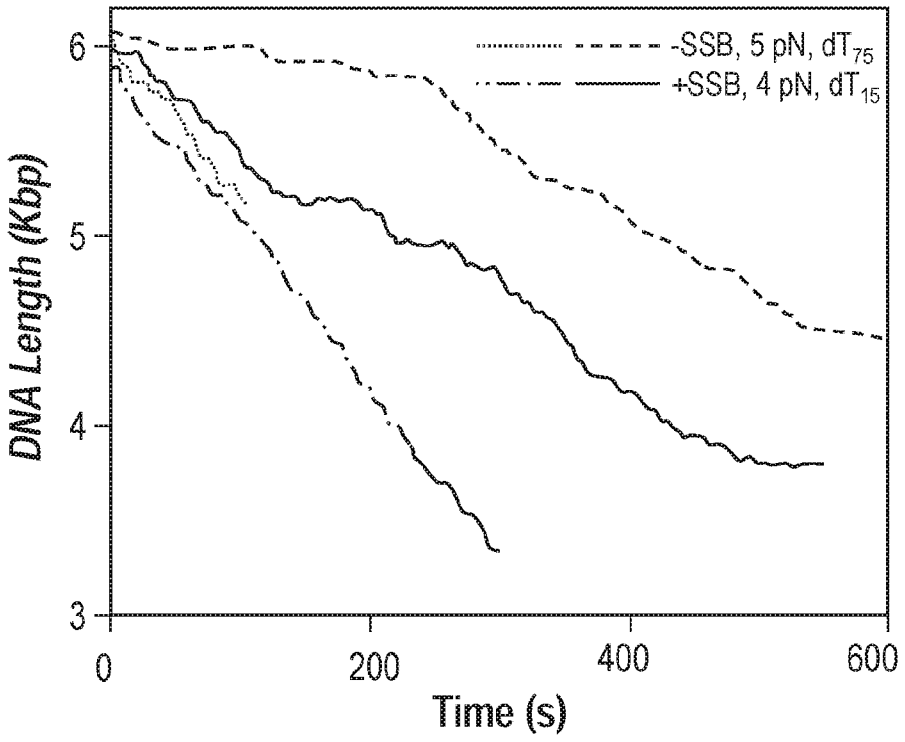

-SSB, 5 pN, dT75
+SSB, 4 pN, dT15
DNA Length (Kbp)
6
5
4
3
0
200
400
600
Time (s)

Fig. 6D

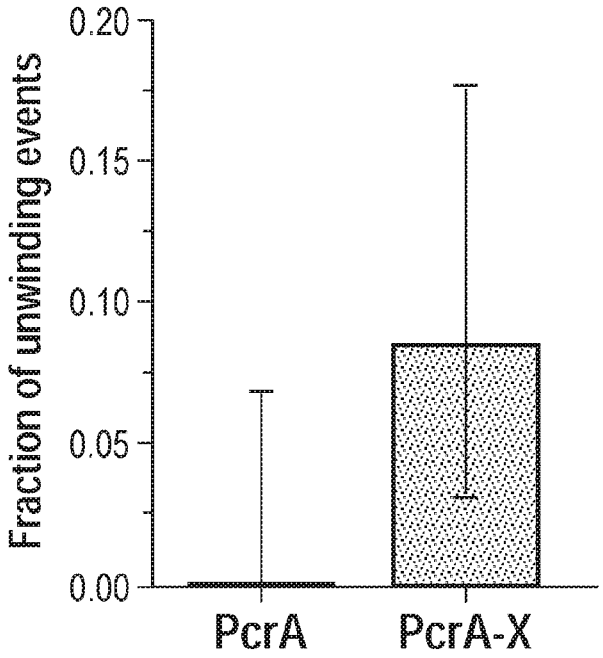


Fig. 6E

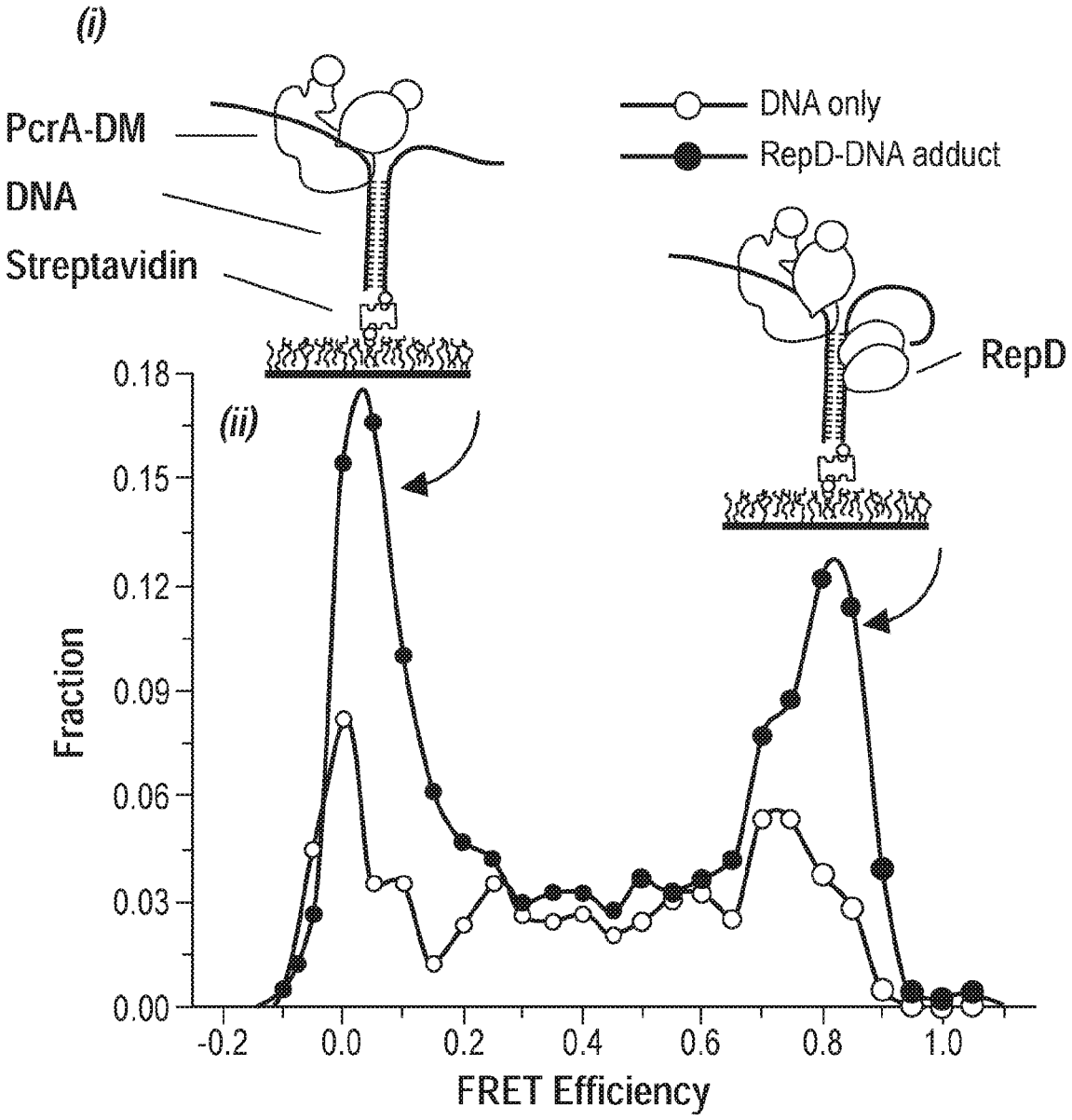

(i)
PcrA-DM
DNA
Streptavidin
DNA only
RepD-DNA adduct
RepD
(ii)
Fraction
0.18
0.15
0.12
0.09
0.06
0.03
0.00
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
FRET Efficiency

Fig. 6F

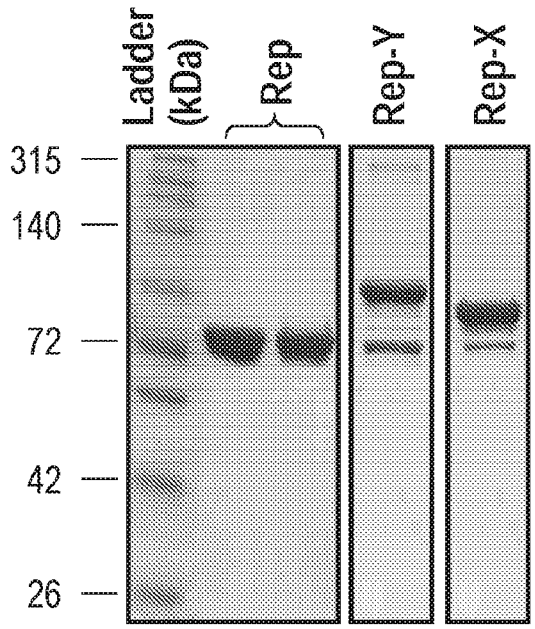

Ladder (kDa)
Rep
Rep-Y
Rep-X
315
140
72
42
26

Fig. 7A

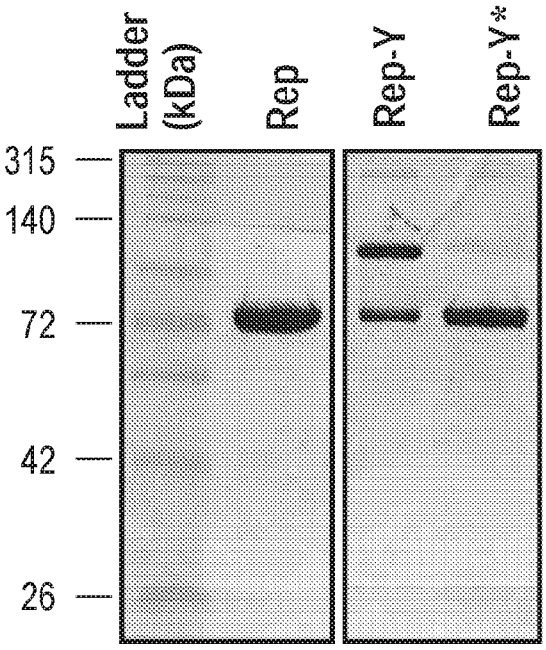

Ladder (kDa)
Rep
Rep-Y
Rep-Y*
315
140
72
42
26

Fig. 7B

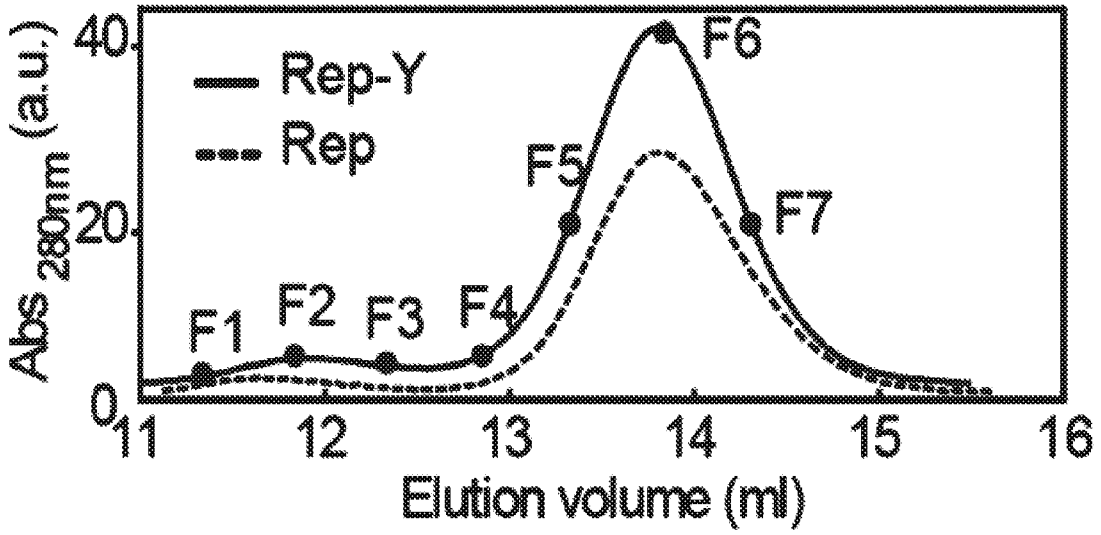

Abs 280nm (a.u.)
40
20
0
Rep-Y
Rep
F1
F2
F3
F4
F5
F6
F7
11
12
13
14
15
16
Elution volume (ml)

Fig. 7C

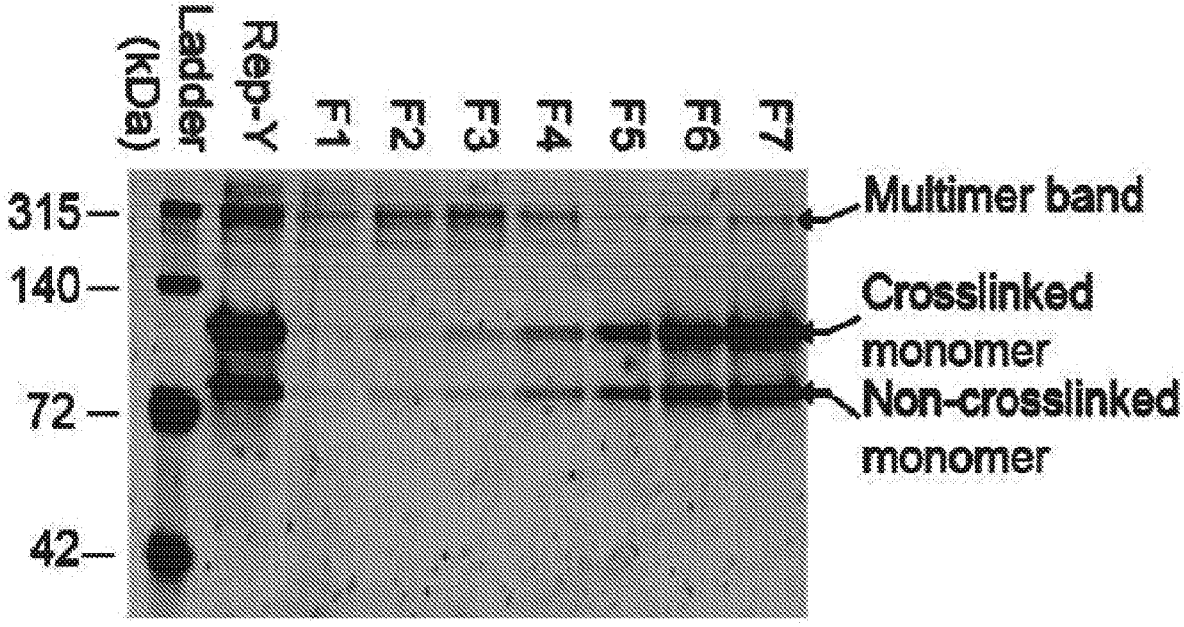

Ladder (kDa)
Rep-Y
F1
F2
F3
F4
F5
F6
F7
315
140
72
42
Multimer band
Crosslinked monomer
Non-crosslinked monomer

Fig. 7D

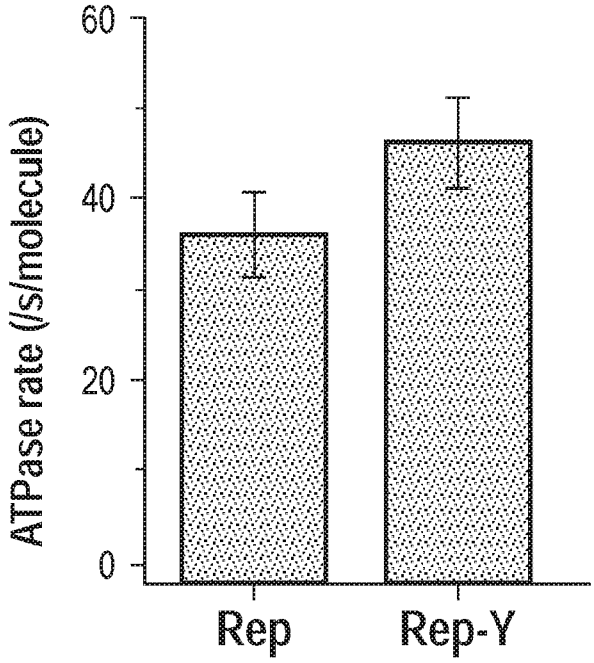


Fig. 7E

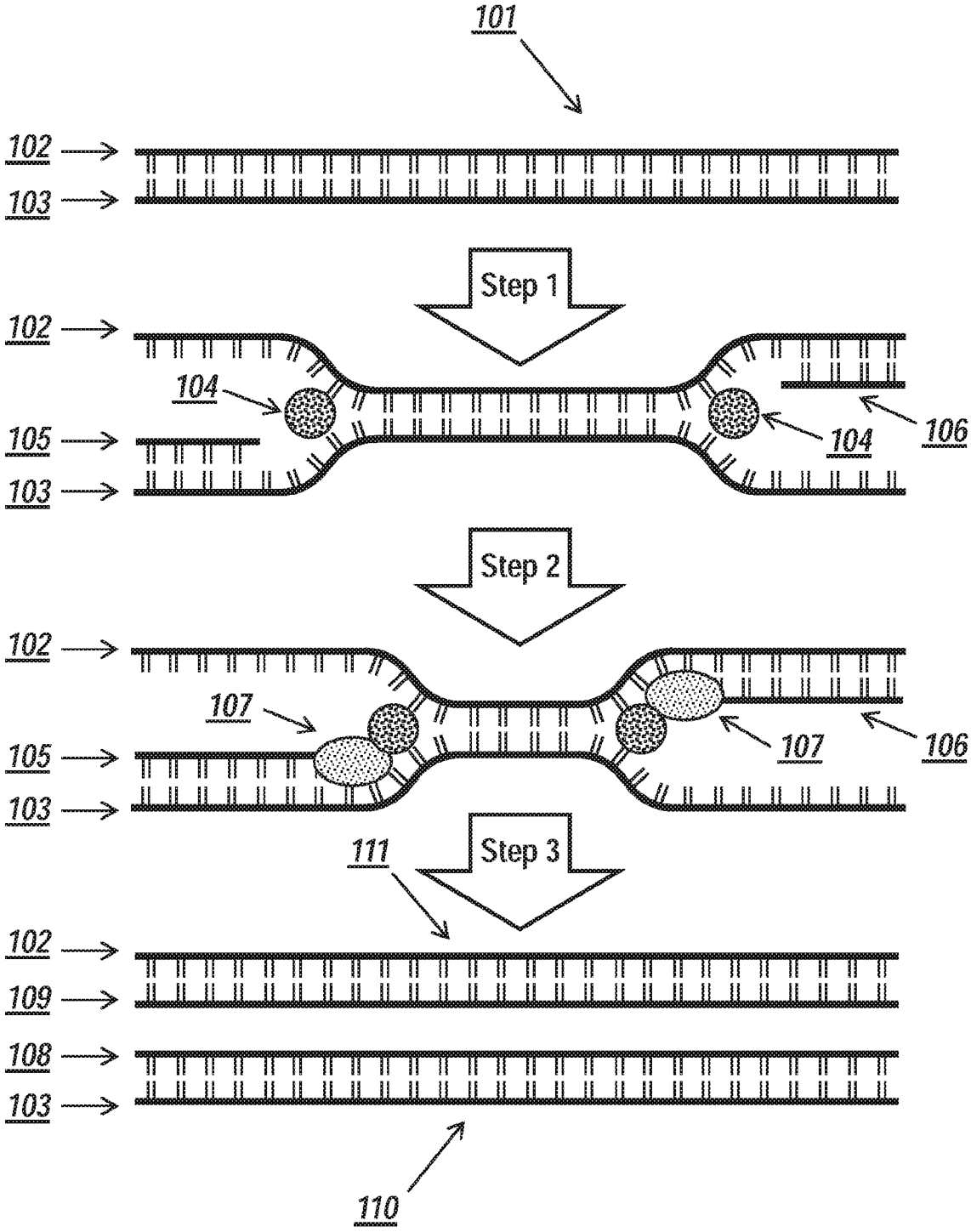

101
102
103
Step 1
102
104
105
103
104
106
Step 2
102
107
105
103
107
106
111
Step 3
102
109
108
103
110

Fig. 8

Target Residues

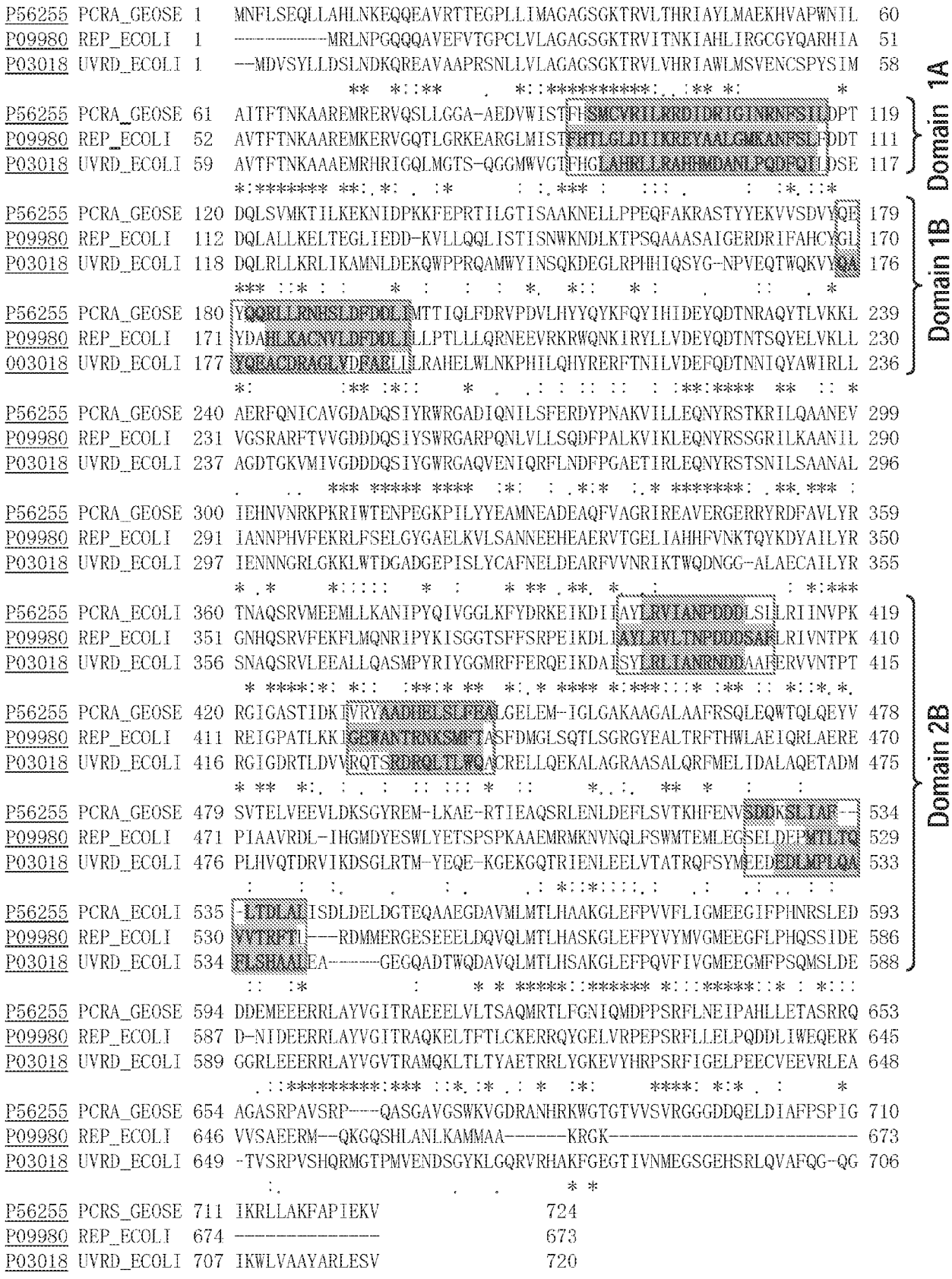


Fig. 9A

Domain 1A
Target Residues

P09980	REP_ECOLI	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	119
O51889	REP_BUCAP	60	AHEIKVRLAKHLNLLQIKKMIISTFHSLGLEIIKKEINTLKFNSNFSLFDERDQMMLLKK	119
P57654	REP_BUCAI	60	AYEMRIRLSKYLNIPEIKKIIISTFHSLGLEIIKKEIDALELNNNFTLLDEKDQILLLKK	119
P44804	REP_HAEIN	60	AREMKERVAHSIGKEQSKGLLVSTFHTLGFDILKREYKALGFKSNMTLFDEHDQFALLKE	119
Q9L6S1	REP_SALTY	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A077ZIR6	A0A077ZIR6_TRITR	77	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	136
S3IEG5	S3IEG5_9ENTR	60	AREMKERVSQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQTALLKE	119
J1R585	J1R585_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLTIIKREFAALGMKSNFSLFDDTDQVALLKE	119
K8ABZ8	K8ABZ8_9ENTR	41	AREMKERVAQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQMALIKE	100
A0A060VJ91	A0A060VJ91_KLEPN	121	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	180
A0A090V5M6	A0A090V5M6_ESCVU	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKD	119
A0A083YZC2	A0A083YZC2_CITAM	60	AREMKERVGQTLGRKEARGLMISTFHTLGLEVIKREYAALGMKSNFSLFDDTDQVALLKD	119
A0A0J6D7T8	A0A0J6D7T8_SALDE	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A085ITL8	A0A085ITL8_RAOPL	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
E7T4Q1	E7T4Q1_SHIBO	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKVNFSLFDDTDQLALLKE	119
A0A085GMM2	A0A085GMM2_9ENTR	60	AREMKERVAQTLGRKEARGLLISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQTALLKD	119
A0A085HAK1	A0A085HAK1_9ENTR	41	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	100
D4BE16	D4BE16_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDVIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0H5PMJ7	A0A0H5PMJ7_SALSE	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0J1JQT3	A0A0J1JQT3_CITFR	60	AREMKERMAQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0J8VI05	A0A0J8VI05_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLEIIKREYNALGMKANFSLFDDTDQMALIKE	119
F5S3F4	F5S3F4_9ENTR	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
D2ZMA5	D2ZMA5_9ENTR	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A084ZTW9	A0A084ZTW9_9E	60	AREMKERVAQTLGRKEARGLMISTFHTLGLEIIKREFAALGMKSNFSLFDDTDQVALLKE	119
A0A038CLT3	A0A038CLT3_RAOOR	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
Q8Z385	Q8Z385_SALTI	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
Q83IX8	Q83IX8_SHIFL	60	AREMKERVGQTLGRKEAHGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	119
A0A0D5WYP4	A0A0D5WYP4_9ENTR	60	AREMKERVSQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQTALLKE	119
A0A0H3FM31	A0A0H3FM3	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0H2WUK6	A0A0H2WUK6_SALPA	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0H3H1F3	A0A0H3H1F3_KLEOK	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
X7I146	X7I146_CITFR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0H3CTF5	A0A0H3CTF5_ENTCC	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
D2TH67	D2TH67_CITRI	60	AREMKERVGQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQLALLKE	119
Q329V6	Q329V6_SHIDS	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	119
W6J7C4	W6J7C4_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLNIIKREFAALGMKSNFSLFDDTDQVALLKD	119
I2BE87	I2BE87_SHIBC	60	AREMKERVAQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQLALIKD	119
B5EZ38	B5EZ38_SALA4	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0F5SGU2	A0A0F5SGU	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDVIKREYAALGMKSNFSLFDDTDQVALLKE	119
G9YY11	G9YY11_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A090UXU3	A0A090UXU3_9ENTR	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDVIKREYAALGMKSNFSLFDDTDQVALLKE	119
A9MJ31	A9MJ31_SALAR	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
Q3YVI6	Q3YVI6_SHISS	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	119
D3RHB6	D3RHB6_KLEVT	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
Q57HT8	Q57HT8_SALCH	93	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDSDQVALLKE	152
B5RFS5	B5RFS5_SALG2	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A089Q204	A0A089Q204_9ENTR	60	AREMKERVSQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQTALLKE	119
A0A0H3BNR1	A0A0H3BNR1_SALNS	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
C9Y4T0	C9Y4T0_SICTZ	60	AREMKERVAQTMGRKEARGLMISTFHTLGLEIIKREYVALGMKSNFSLFDDTDQMALIKE	119
B7LU77	B7LU77_ESCF3	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDDTDQLALLKE	119
A0A0H3TAW8	A0A0H3TAW8_SALEN	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
G2S5J6	G2S5J6_ENTAL	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A0A0F7JC30	A0A0F7JC30_SALET	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119
A7MQI4	A7MQI4_CROS8	60	AREMKERVAQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQMALIKE	119
L0M8J0	L0M8J0_ENTBF	60	AREMKERVAQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKD	119
A0A0K0HFU2	A0A0K0HFU2_SALBC	60	AREMKERVGQTLGRKEARGLMISTFHTLGLEIIKREYAALGMKSNFSLFDDTDQTALLKE	119
A8ACT1	A8ACT1_CITK8	60	AREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKSNFSLFDDTDQVALLKE	119

Fig. 9B

Domain 1B
Target Residues

P09980	REP_ECOLI	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKACNYLDFDDLI	187
Q51889	REP_BUCAP	120	ICSKS-IKNDTKLLKKLVFMISFWKNKFLTPLQVQLSAQSNLEKDFAFFYKQYTFHLRKS	178
P57654	REP_BUCAI	120	ICKKE-IKNNIQLLKKLNFMISYWKNKFLTPLQVQLLAKSSQEKDFAYVYEQYTNYLYKA	178
P44804	REP_HAEIN	120	LTADV-LKEDKDLLRELISVISNWKNDLISPKQAFALARDAKYQTFAKCYERYATQIRTY	178
Q9L6S1	REP_SALTY	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A077ZIR6	A0A077ZIR6_TRITR	137	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKAC	195
S3IEG5	S3IEG5_9EN	120	LTEGL-LENDKVLLQQLISTISNWKNSLLTPAQAAAQAKGERDRIFAHCYGLYDTHLKSC	178
J1R585	J1R585_9ENTR	120	LTEGL-IDDDKAVLQQLISTISNWKNDLLTPPQAAAQAKGERDRIFAHCYGLYDSHMKSC	178
K8ABZ8	K8ABZ8_9ENTR	101	LTEGL-IENDKVLLQQLISTISNWKNDLLSPPQAAARAIGERDRIFAHCYSLYDAHLKAC	159
A0A060VJ91	A0A060VJ91_KLEPN	181	LTEGL-IEDDKVVLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	239
A0A090V5M6	A0A090V5M6_ESCVU	120	LTEGL-IEDEKTILQQLISTISNWKNDLMTPAQAAAQARGERDRIFAHCYSLYDAHMKAC	178
A0A083YZC2	A0A083YZC2_CITAM	120	LTEGL-IEDDKVILQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0J6D7T8	A0A0J6D7T8_SALDE	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A085ITL8	A0A085ITL8_RAOPL	120	LTEGL-IDDDKVVLQQLISTISNWKNDLQTPAQAAAGAKGERERIFAHCYGLYDGHMKAC	178
E7T4Q1	E7T4Q1_SHIBO	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKAC	178
A0A085GMM2	A0A085GMM2_9ENTR	120	LTEGL-LEDDKTLLQQLISTISNWKNDLMSPSQAAAQAKGERDRIFAHCYGLYDTHLKSC	178
A0A085HAK1	A0A085HAK1_9ENTR	101	LTEGL-IEDDKTVLQQLISTISNWKNDLMNPEQAAASAKGERDRIFAHCYGLYNDHLKAC	159
D4BE16	D4BE16_9ENTR	120	LTEGL-IEDDKLILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
A0A0H5PMJ7	A0A0H5PMJ7_SALSE	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0J1JQT3	A0A0J1JQT3_CITFR	120	LTEGL-IEDDKLILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
A0A0J8VI05	A0A0J8VI05_9ENTR	120	LTEGL-VENDKSLLQQLISTISNWKNDLLNPLQAAAQAKGERDRIFAHCYGLYDAHLKAC	178
F5S3F4	F5S3F4_9ENTR	120	LTEGL-IEDDKVLLQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
D2ZMA5	D2ZMA5_9ENTR	120	LTEGL-IEDDKTVLQQLISTISNWKNDLMTPSQAAAIAKGERDRIFAHCYGLYDAHMKAC	178
A0A084ZTW9	A0A084ZTW9_9ENTR	120	LTEGL-IDDDKTLLQQLISTISNWKNDLLTPAQAAAQAKGERDRIFAHCYGLYDAHMKAC	178
A0A038CLT3	A0A038CLT3_RAOOR	120	LTEGL-IDDDKVVLQQLISTISNWKNDLQTPAQAAAGAKGERERIFAHCYGLYDGHMKAC	178
Q8Z385	Q8Z385_SALTI	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
Q83IX8	Q83IX8_SHIFL	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKAC	178
A0A0D5WYP4	A0A0D5WYP4_9ENTR	120	LTEGL-LENDKVLLQQLISTISNWKNGLLSPAQAAAQAKGERDRIFAHCYGLYDTHLKSC	178
A0A0H3FM31	A0A0H3FM31_ENTAK	120	LTEGL-IEDDKVVLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0H2WUK6	A0A0H2WUK6_SALPA	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0H3H1F3	A0A0H3H1F3_KLEOK	120	LTEGL-IEDDKVVLQQLISTISNWKNDLQTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
X7I146	X7I146_CITFR	120	LTEGL-IEDDKLILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
A0A0H3CTF5	A0A0H3CTF5_ENTCC	120	LTEGL-IEDDKVLLQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
D2TH67	D2TH67_CITRI	120	LTEGL-LEEDKVLLQQLISTISNWKNDLQTPAQAAAGAKGERDRIFAHCYGLYDAHLKAC	178
Q329V6	Q329V6_SHIDS	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAEAKGERDRIFAHCYGLYDAHLKAC	178
W6J7C4	W6J7C4_9ENTR	120	LTEGL-IDDDKVVLQQLISTISNWKNDLLTPPQAAARANGERDRIFAHCYGLYDAHMKAC	178
I2BE87	I2BE87_SHIBC	120	LTEGL-LENDKVIYQQLISTISNWKNDLMGPDQAAAMARGERDRIFAHCYRLYYDHLKAC	178
B5EZ38	B5EZ38_SALA4	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0F5SGU2	A0A0F5SGU2_CITAM	120	LTEGL-IEDDKLILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
G9YY11	G9YY11_9ENTR	120	LTEGL-LEDDKVLLQQLISTISNWKNDLMTPAQAAAQAKGERDRIFAHCYSLYDAHMKAC	178
A0A090UXU3	A0A090UXU3_9ENTR	120	LTEGL-IEDDKLILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHMKAC	178
A9MJ31	A9MJ31_SALAR	120	LTEGL-IDDDKVVLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
Q3YVI6	Q3YVI6_SHISS	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPSQAAASAIGERDRIFAHCYGLYDAHLKAC	178
D3RHB6	D3RHB6_KLEVT	120	LTEGL-IEDDKVVLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
Q57HT8	Q57HT8_SALCH	153	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	211
B5RFS5	B5RFS5_SALG2	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A089	A0A089Q204_9ENTR	120	LTEGL-LENDKVLLQQLISTISNWKNGLLSPAQAAAQAKGERDRIFAHCYGLYDTHLKSC	178
A0A0H3BNR1	A0A0H3BNR1_SALNS	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
C9Y4T0	C9Y4T0_SICTZ	120	LTEGL-VENDKVLLQQLISTISNWKNDLLSPPQAAARAIGERERIFAHCYSLYDAHLKAC	178
B7LU77	B7LU77_ESCF3	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAEAKGERDRIFAHCYGLYDSHLKAC	178
A0A0H3TAW8	A0A0H3TAW8_SALEN	120	LTEGL-IEDDKVVLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
G2S5J6	G2S5J6_ENTAL	120	LTEGL-IEDDKVLLQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYSLYDAHMKAC	178
A0A0F7JC30	A0A0F7JC30_SALET	120	LTEGL-IEDDKVLLQQLISTISNWKNDLKTPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A7MQI4	A7MQI4_CROS8	120	LTEGL-VENDKVLLQQLISTISNWKNDLLSPPQAAARAIGERERIFAHCYSLYDAHLKAC	178
L0M8J0	L0M8J0_ENTBF	120	LTEGL-IEDDKVQLQQLISTISNWKNDLKSPAQAAAGAKGERDRIFAHCYGLYDAHMKAC	178
A0A0K0HFU2	A0A0K0HFU2_SALBC	120	LTEGL-IEDDKILLQQLISTISNWKNDLKTPAQAAASAIGERDRIFAHCYGLYDAHMKAC	178
A8ACT1	A8ACT1_CITK8	120	LTDGL-IEDDKVILQQLISTISNWKNDLMTPAQAAASAKGERDRIFAHCYGLYDAHLKAC	178

Fig. 9C

Domain 2B
Target Residues

P09980	REP_ECOLI	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
O51889	REP_BUCAP	359	EKTFLKKNIPYDISTNSSFFSRPEIKDLI	SYLRLIVNPDDNHAF	IRILNIPHRQIGLTTL	418
P57654	REP_BUCAI	359	EKALIKENIPYNISEKSSFFSRPEIKDLI	SYLRVVINRDDNHAF	MRIVNIPSRQIGKTTL	418
P44804	REP_HAEIN	359	EKVLMQNRIPYKISGGTSFFSRAEIKDMM	AYLRLVVNQDDDAAF	LRIVNTPKREIGTATL	418
Q9L6S1	REP_SALTY	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A077ZIR6	A0A077ZIR6_TRITR	376	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	435
S3IEG5	S3IEG5_9ENTR	359	EKMLMQNRIPYHISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
J1R585	J1R585_9ENTR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNTEDDSAF	LRIVNTPKREIGSATL	418
K8ABZ8	K8ABZ8_9ENTR	340	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	399
A0A060VJ9	A0A060VJ91_KLEPN	420	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	479
A0A090V5M6	A0A090V5M6_ESCVU	359	EKFLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A083YZC2	A0A083YZC2_CITAM	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A016D7T8	A0A0J6D7T8_SALDE	359	EKFLMQNRIPYKISGGTSFFSRLEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A085ITL8	A0A085ITL8_RAOPL	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
E7T4Q1	E7T4Q1_SHIBO	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A085GMM2	A0A085GMM2_9ENTR	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	MRIVNTPKREIGSATL	418
A0A085HAK1	A0A085HAK1_9ENTR	340	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	399
D4BE16	D4BE16_9ENTR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLSNPDDDSAF	LRIVNTPKREIGSATL	418
A0A0H5PMJ7	A0A0H5PMJ7_SALSE	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0J1JQT3	A0A0J1JQT3_CITFR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLSNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0J8VI05	A0A0J8VI05_9ENTR	359	EKLLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
F5S3F4	F5S3F4_9ENTR	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGSATL	418
D2ZMA5	D2ZMA5_9ENTR	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A084ZTW9	A0A084ZTW9_9ENTR	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A038CLT3	A0A038CLT3_RAOOR	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
Q8Z385	Q8Z385_SALTI	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
Q83IX8	Q83IX8_SHIFL	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0D5WYP4	A0A0D5WYP4_9ENTR	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H3FM31	A0A0H3FM31_ENTAK	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H2WUK6	A0A0H2WUK6_SALPA	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H3H1F3	A0A0H3H1F3_KLEOK	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	MRIVNTPKREIGSATL	418
X7I146	X7I146_CITFR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLSNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H3CTF5	A0A0H3CTF5_ENTCC	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
D2TH67	D2TH67_CITRI	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
Q329V6	Q329V6_SHIDS	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
W6J7C4	W6J7C4_9ENTR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNTDDDSAF	LRIVNTPKREIGSATL	418
I2BE87	I2BE87_SHIBC	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPRREIGPATL	418
B5EZ38	B5EZ38_SALA4	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0F5SGU2	A0A0F5SGU2_CITAM	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLSNPDDDSAF	LRIVNTPKREIGPATL	418
G9YY11	G9YY11_9ENTR	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A090UXU3	A0A090UXU3_9ENTR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLSNPDDDSAF	LRIVNTPKREIGPATL	418
A9MJ31	A9MJ31_SALAR	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGSATL	418
Q3YVI6	Q3YVI6_SHISS	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
D3RHB6	D3RHB6_KLEVT	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
Q57HT8	Q57HT8_SALCH	392	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	451
B5RFS5	B5RFS5_SALG2	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	41
A0A089Q204	A0A089Q204_9ENTR	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H3BNR1	A0A0H3BNR1_SALNS	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
C9Y4T0	C9Y4T0_SICTZ	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPGDDSAF	LRIVNTPKREIGPATL	418
B7LU77	B7LU77_ESCF3	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0H3TAW8	A0A0H3TAW8_SALEN	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
G2S5J6	G2S5J6_ENTAL	359	EKMLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A0A0F7JC30	A0A0F7JC30_SALET	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A7MQI4	A7MQI4_CROS8	359	EKMLMQNRIPYRISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
L0M8J0	L0M8J0_ENTBF	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGAATM	418
A0A0K0HFU2	A0A0K0HFU2_SALBC	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418
A8ACT1	A8ACT1_CITK8	359	EKFLMQNRIPYKISGGTSFFSRPEIKDLI	AYLRVLTNPDDDSAF	LRIVNTPKREIGPATL	418

Fig. 9D

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Domain 2B

Target Residues

P09980	REP_ECOLI	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQRLAEREPIAAVRD	477
O51889	REP_BUCAP	419	NKLEELASKKNKSLFQISNDIEIKKILRERTVKKIKDFIYWIKKIIK-LSLLKEDIILDK	477
P57654	REP_BUCAI	419	KKLEEWANKKHVSLFQASNNIEIKKFLNENTIKKIKNFISKIEKFTA-WSCLKPSNIIDD	477
P44804	REP_HAEIN	419	QKLGELAQEKHISLFEAIFEFELIQRITPKAYDSLQKFGRWIVELNDEIQRSEPERAVRS	478
Q9L6S1	REP_SALTY	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A077ZIR6	A0A077ZIR6_TRITR	436	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	494
S3IEG5	S3IEG5_9ENTR	419	QKLGEWATQRNKSLLTASFDMGLSQTLSGRGLESLQRFTHWLREVAT-LAEREPVAAVRD	477
J1R585	J1R585_9ENTR	419	QKLGEWAMLRNKSLFAASFDVGLNQTLTGRGYESLTRFTQWLGDVQQ-LSEREPIAAVRD	477
K8ABZ8	K8ABZ8_9ENTR	400	QKLGEWATQRNKSLFTASFDMGLSQSLTGRGYESLTRFTQWLQEVAV-LSEREPVAAVRD	458
A0A060VJ91	A0A060VJ91_KLEPN	480	QKLGEWAMGRNKGLFTASFDMGLSQTLTGRGYESLTRFTHWLREIQQ-LAEREPVNAVRD	538
A0A090V5M6	A0A090V5M6_ESCVU	419	QKLGEWANSRNKGLFAASFDMGLTQTLSGRGYESLTRFTHWLSEVQR-LSEREPIAAVRD	477
A0A083YZC2	A0A083YZC2_CITAM	419	QKLGEWAMTRNKSLFTASFDLGLSQTLTGRGYDSLTRFTHWLGEVQR-LAEREPIAAVRD	477
A0A0J6D7T8	A0A0J6D7T8_SALDE	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A085ITL8	A0A085ITL8_RAOPL	419	QKLGEWAMTRNKSLFTASFDMGLNQTLTGRGYDALTGFTQWLADIQR-LAEREPVAAVRD	477
E7T4Q1	E7T4Q1_SHIBO	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	477
A0A085GMM2	A0A085GMM2_9ENTR	419	QKLGAWAMQRNKGLFLSSFDMGLSQTLSGRGLESLQRFTHWLREVAT-LAEREPIAAVRD	477
A0A085HAK1	A0A085HAK1_9ENTR	400	QKLGEWAMTRNKSLLTASFDMGLSHTLTGRGYESLTRFTHWLREIQQ-LSEREPIAAVRD	458
D4BE16	D4BE16_9ENTR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0H5PMJ7	A0A0H5PMJ7_SALSE	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0J1JQT3	A0A0J1JQT3_CITFR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0J8VI05	A0A0J8VI05_9ENTR	419	QKLGEWAMSRNKSLFTASFDMGLSQTLTGRGYESLTRFTHWLGEVAR-LSEREPVAAVRD	477
F5S3F4	F5S3F4_9ENTR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDALTRFTHWLGEVQR-LAEREPVAAVRD	477
D2ZMA5	D2ZMA5_9ENTR	419	QKLGEWAMTRSKSMFTASFDMGLSQTLTGRGYDNLTRFTHWLGEVQR-LAEREPVAAVRD	477
A0A084ZTW9	A0A084ZTW9_9ENTR	419	QKLGEWASTRNKSLFTASFDVGLTQTLSGRGYDALTRFTHWLGEIQR-LSEREPVAAVRD	477
A0A038CLT3	A0A038CLT3_RAOOR	419	QKLGEWAMTRNKSLFTASFDMGLNQTLTGRGYDALTGFTQWLADIQR-LAEREPVAAVRD	477
Q8Z385	Q8Z385_SALTI	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
Q83IX8	Q83IX8_SHIFL	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	477
A0A0D5WYP4	A0A0D5WYP4_9ENTR	419	QKLGEWAMQRNKGLFTASFDMGLSQTLSGRGLDALQRFTHWLRDVAT-LAEREPVAAVRD	477
A0A0H3FM31	A0A0H3FM31_ENTAK	419	QKLGEWAMSRNKGLFTASFDMGLSQTLTGRGYESLTRFTHWLREIQQ-LAEREPVNAVRD	477
A0A0H2WUK6	A0A0H2WUK6_SALPA	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0H3H1F3	A0A0H3H1F3_KLEOK	419	KKLGEWAMGRNKSMFTASFDMGLTQTLNGRGYESLTRFTHWLREIQQ-LSEREPIAAVRD	477
X7I146	X7I146_CITFR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0H3CTF5	A0A0H3CTF5_ENTCC	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEVQR-LAEREPVAAVRD	477
D2TH67	D2TH67_CITRI	419	QKLGEWAMTRNKSLFTASFDLGLAQTLTGRGYESLTRFTHWLGEVQR-LAEREPVAAVRD	477
Q329V6	Q329V6_SHIDS	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	477
W6J7C4	W6J7C4_9ENTR	419	QKLGEWAMLRNKSLFAASFDVGLNQTLTGRGYDALTRFTHWLGEVQR-LSEREPIAAVRD	477
I2BE87	I2BE87_SHIBC	419	QKLGEWALQRNKSMFTASFDLGLSQTLTGRGYEALTRFTHWLGEVAR-LSEKEPVAAVRD	477
B5EZ38	B5EZ38_SALA4	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A0F5SGU2	A0A0F5SGU2_CITAM	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGDIQR-LAEREPVAAVRD	477
G9YY11	G9YY11_9ENTR	419	QKLGEWAMTRSKSMFTASFDMGLSHLLPGRGYESLTRFTHWLGEVQR-LSEREPIAAVRD	477
A0A090UXU3	A0A090UXU3_9ENTR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A9MJ31	A9MJ31_SALAR	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEIQR-LAEREPIAAVRD	477
Q3YVI6	Q3YVI6_SHISS	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	477
D3RHB6	D3RHB6_KLEVT	419	QKLGEWAMGRNKGLFTASFDMGLSQTLTGRGYESLTRFTHWLREIQQ-LAEREPVNAVRD	477
Q57HT8	Q57HT8_SALCH	452	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	510
B5RFS5	B5RFS5_SALG2	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A0A089Q204	A0A089Q204_9ENTR	419	QKLGEWAMQRNKGLFTASFDMGLSQTLSGRGLDSLQRFTHWLRDVAT-LAEREPVAAVRD	477
A0A0H3BNR1	A0A0H3BNR1_SALNS	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
C9Y4T0	C9Y4T0_SICTZ	419	QKLGEWANQRNKSLFTASFDMGLAQTLSGRGYESLTRFTSWLQDVAV-LSEREPVAAVRD	477
B7LU77	B7LU77_ESCF3	419	KKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR-LAEREPIAAVRD	477
A0A0H3TAW8	A0A0H3TAW8_SALEN	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
G2S5J6	G2S5J6_ENTAL	419	QKLGEWAMTRNKSLFTASFDMGLSQTLTGRGYDSLTRFTHWLGEVQR-LAEREPVAAVRD	477
A0A0F7JC30	A0A0F7JC30_SALET	419	QKLGEWAMTRNKSLFTASFDMGLSQKLTGRGYDSLTRFTHWLGEIQR-LAEREPVAAVRD	477
A7MQI4	A7MQI4_CROS8	419	QKLGEWANQRNKSLFTASFDMGLAQTLTGRGYESLTRFTSWLQEVAV-LSEREPVAAVRD	477
L0M8J0	L0M8J0_ENTBF	419	QKLGEWAMTRNKSLFAASFDMGLTQTLTGRGYESLTRFTQWLAEVQR-LSEREPIAAVRD	477
A0A0K0HFU2	A0A0K0HFU2_SALBC	419	QKLGEWAMTRSKSLFTASFDMGLSQTLTGRGYDALTRFTHWLAEIQR-LAEREPVAAVRD	477
A8ACT1	A8ACT1_CITK8	419	QKLGEWAMTRNKSLFTASFDVGLNQTLTGRGYDSLTRFTHWLGEIQR-LSEREPIAAVRD	477

Fig. 9E

Domain 2B Target Residues

P09980	REP_ECOLI	478	LIHGMDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
O51889	REP_BUCAP	478	IINDIKYELWLTKILKEPKKIKTSINNIYTLSNWLKEMLRGNEFEKPMNLLQIVKKMTLR	537
P57654	REP_BUCAI	478	IVDDLEYEKWLSKFLKDPNKIKNSINNVHTLSQWFKNMIKGDDFEKPMTLFQIVTRMTIR	537
P44804	REP_HAEIN	479	MLSAIHYEEYLYEYATSPKAAEMQSKNVATLFDWVADMLKGDETNEPMNLNQVVTRLTLR	538
Q9L6S1	REP_SALTY	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A0A077ZIR6	A0A077ZIR6_TRITR	495	LIHGMDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	554
S3IEG5	S3IEG5_9ENTR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEASDLDEPMTLVQVVTRFTLR	537
J1R585	J1R585_9ENTR	478	LIHGIDYESWLFETSPSPKAAEMRMKNVNTLFGWMTEMLEGSELDEPMTLAEVVTRFTLR	537
K8ABZ8	K8ABZ8_9ENTR	459	LIRGIDYEAWLFETSPSPKAAEMRMKNVNQLFSWMTEMLEGTDLDEPMTLTQVVTRFTLR	518
A0A060VJ91	A0A060VJ91_KLEPN	539	LIRGIDYESWLYETSPSPKAAEMRMKNVNQLFTWMTEMLEGSEIDEPMTLTQVVTRFTLR	598
A0A090V5M6	A0A090V5M6_ESCVU	478	LIHGIDYESWLFETSPSPKAAEMRMKNVNTLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
A0A083YZC2	A0A083YZC2_CITAM	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVSRFTLR	537
A0A0J6D7T8	A0A0J6D7T8_SALDE	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A0A085ITL8	A0A085ITL8_RAOP	478	LIRGVDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSDIDEPMTLTQVVTRFTLR	537
E7T4Q1	E7T4Q1_SHIBO	478	LIHGMDYEFWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
A0A085GMM2	A0A085GMM2_9ENTR	478	LIHGIDYESWLYETSPSQKAAEMRMKNVNTLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
A0A085HAK1	A0A085HAK1_9ENTR	459	LIRGIDYESWLYETSASPKAAEMRMKNVNQLFSWMTEMLEGSDIDEPMTLTQVVTRFTLR	518
D4BE16	D4BE16_9ENTR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNTLFGWMTEMLEGSELEEPMTLTQVVTRFTLR	537
A0A0H5PMJ7	A0A0H5PMJ7_SALSE	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A0A0J1JQT3	A0A0J1JQT3_CITFR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNTLFSWMTEMLEGSELEEPMTLTQVVTRFTLR	537
A0A0J8VI05	A0A0J8VI05_9ENTR	478	LIHGIDYEAWLYETSPSQKAAEMRMKNVNQLFSWMTEMLEGTELDEPMTLTQVVTRFTLR	537
F5S3F4	F5S3F4_9ENTR	478	LIHGIDYESWLYETSASPKAAEMRMKNVNQLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
D2ZMA5	D2ZMA5_9ENTR	478	LIHGIDYESWLYETSASPKAAEMRMKNVNQLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
A0A084ZTW9	A0A084ZTW9_9ENTR	478	LIHGIDYESWLFETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
A0A038CLT3	A0A038CLT3_RAOOR	478	LIRGVDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSDIDEPMTLTQVVTRFTLR	537
Q8Z385	Q8Z385_SALTI	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
Q83IX8	Q83IX8_SHIFL	478	LIHGMDYESWLYETSPSTKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
A0A0D5WYP4	A0A0D5WYP4_9ENTR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSDLDEPMTLTQVVTRFTLR	537
A0A0H3FM31	A0A0H3FM31_ENTAK	478	LIRGIDYESWLYETSPSPKAAEMRMKNVNQLFTWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
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A0A0H3H1F3	A0A0H3H1F3_KLEOK	478	LIRGVDYESWLYETSPSPKAAEMRMKNVNTLFTWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
X7I146	X7I146_CITFR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNTLFSWMTEMLEGSELEEPMTLTQVVTRFTLR	537
A0A0H3CTF5	A0A0H3CTF5_ENTCC	478	LIHGIDYESWLYETSASPKAAEMRMKNVNQLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
D2TH67	D2TH67_CITRI	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
Q329V6	Q329V6_SHIDS	478	LIHGMDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
W6J7C4	W6J7C4_9ENTR	478	LIHGIDYESWLFETSASPKAAEMRMKNVNTLFGWMTEMLEGSELDEPMTLAEVVTRFTLR	537
I2BE87	I2BE87_SHIBC	478	LIHGIDYESWLFETSPSPKAAEMRMKNVNQLFTWMTEMLEGSDLEEPMTLTQVVTRFTLR	537
B5EZ38	B5EZ38_SALA4	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A0A0F5SGU2	A0A0F5SGU2_CITAM	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNTLFSWMTEMLEGSELEEPMTLTQVVTRFTLR	537
G9YY11	G9YY11_9ENTR	478	LIHGIDYESWLFETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELEEPMTLTQVVTRFTLR	537
A0A090UXU3	A0A090UXU3_9ENTR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNTLFSWMTEMLEGSELEEPMTLTQVVTRFTLR	537
A9MJ31	A9MJ31_SALAR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELNEPMTLTQVVTRFTLR	537
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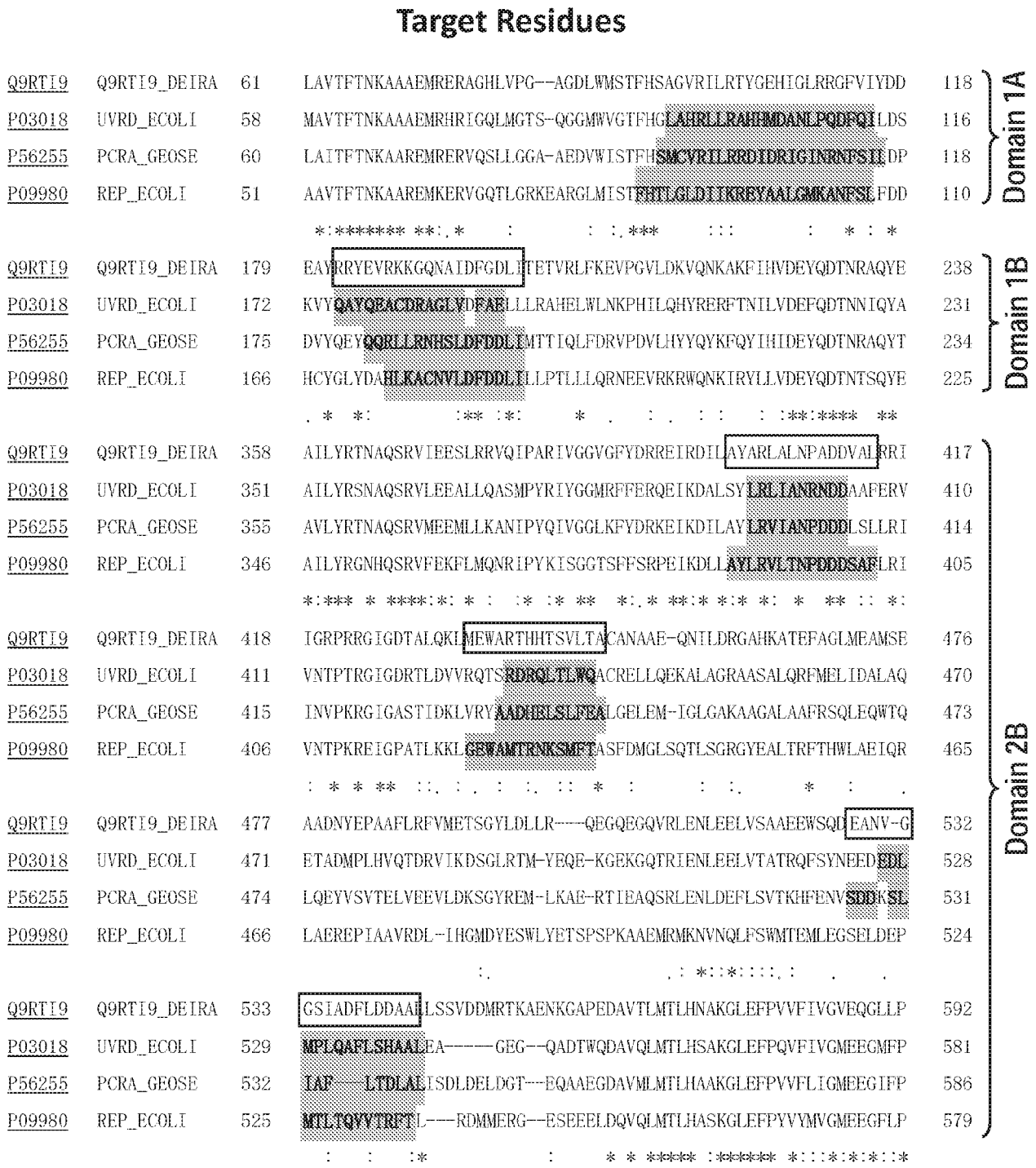
D3RHB6	D3RHB6_KLEVT	478	LIRGIDYESWLYETSPSPKAAEMRMKNVNQLFTWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
Q57HT8	Q57HT8_SALCH	511	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	570
B5RFS5	B5RFS5_SALG2	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A0A089Q2Q4	A0A089Q2Q4_9ENTR	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSDLDEPMTLTQVVTRFTLR	537
A0A0H3BNR1	A0A0H3BNR1_SALNS	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
C9Y4T0	C9Y4T0_SICTZ	478	LIRGIDYEAWLFETSPSPKAAEMRMKNVNQLFSWMTEMLEGTDLDEPMTLTQVVTRFTLR	537
B7LU77	B7LU77_ESCF3	478	LIHGMDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEPMTLTQVVTRFTLR	537
A0A0H3TAW8	A0A0H3TAW8_SALEN	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
G2S5J6	G2S5J6_ENTAL	478	LIHGIDYESWLYETSASPKAAEMRMKNVNQLFSWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
A0A0F7JC30	A0A0F7JC30_SALET	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537
A7MQI4	A7MQI4_CROS8	478	LIRGIDYEAWLFETSPSPKAAEMRMKNVNQLFSWMTEMLEGTDLDEPMTLTQVVTRFTLR	537
L0M8J0	L0M8J0_ENTBF	478	LIHGIDYESWLFETSASPKAAEMRMKNVNTLFGWMTEMLEGSEIDEPMTLTQVVTRFTLR	537
A0A0K0HFU2	A0A0K0HFU2_SALBC	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFGWMTEMLEGSDIDEPMTLTQVVTRFTLR	537
A8ACT1	A8ACT1_CITK8	478	LIHGIDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGNELDEPMTLTQVVTRFTLR	537

Fig. 9F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P56255 PCRA_GEOSE 60 LAITFTNKAAREMRERVQSLLGGA-AEDVWISTFHSMCVRILRRDIDRIGINRNFSILDP 118
P09980 REP_ECOLI 51 AAVTFTNKAAREMKERVGQTLGRKEARGLMISTFHTLGLDIIKREYAALGMKANFSLFDD 110
Domain 1A
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P03018 UVRD_ECOLI 172 KVYQAYQEACDRAGLVDFAELLLRAHELWLNKPHILQHYRERFTNILVDEFQDTNNIQYA 231
P56255 PCRA_GEOSE 175 DVYQEYQQRLLRNHSLDFDDLIMTTIQLFDRVPDVLHYYQYKFQYIHIDEYQDTNRAQYT 234
P09980 REP_ECOLI 166 HCYGLYDAHLKACNVLDFDDLILLPTLLLQRNEEVRKRWQNKIRYLLVDEYQDTNTSQYE 225
Domain 1B
Q9RTI9 Q9RTI9_DEIRA 358 AILYRTNAQSRVIEESLRRVQIPARIVGGVGFYDRREIRDILAYARLALNPADDVALRRI 417
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P56255 PCRA_GEOSE 355 AVLYRTNAQSRVMEEMLLKANIPYQIVGGLKFYDRKEIKDILAYLRVIANFDDDLSLLRI 414
P09980 REP_ECOLI 346 AILYRGNHQSRVFEKFLMQNRIPYKISGGTSFFSRPEIKDLLAYLRVLTNPDDDSAFLRI 405
Q9RTI9 Q9RTI9_DEIRA 418 IGRPRRGIGDTALQKLMEWARTHHTSVLTACANAAE-QNILDRGAHKATEFAGLMEAMSE 476
P03018 UVRD_ECOLI 411 VNTPTRGIGDRTLDVVRQTSRDRQLTLWQACRELLQEKALAGRAASALQRFMELIDALAQ 470
P56255 PCRA_GEOSE 415 INVPKRGIGASTIDKLVRYAADHELSLFEALGELEM-IGLGAKAAGALAAFRSQLEQWTQ 473
P09980 REP_ECOLI 406 VNTPKREIGPATLKKLGEWAMTRNKSMFTASFDMGLSQTLSGRGYEALTRFTHWLAEIQR 465
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P03018 UVRD_ECOLI 471 ETADMPLHVQTDRVIKDSGLRTM-YEQE-KGEKGQTRIENLEELVTATRQFSYNEEDEDL 528
P56255 PCRA_GEOSE 474 LQEYVSVTELVEEVLDKSGYREM-LKAE-RTIEAQSRLENLDEFLSVTKHFENVSDDKSL 531
P09980 REP_ECOLI 466 LAEREPIAAVRDL-IHGMDYESWLYETSPSPKAAEMRMKNVNQLFSWMTEMLEGSELDEP 524
Domain 2B
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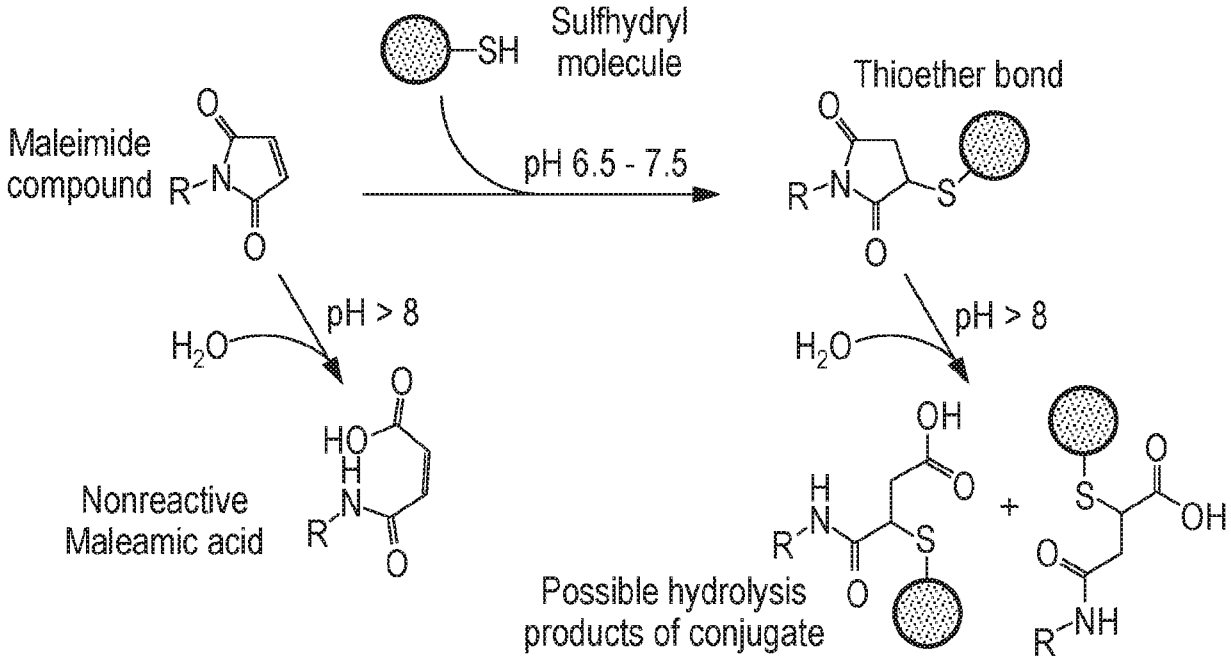
P03018 UVRD_ECOLI 529 MPLQAFLSHAALEA-----GEG--QADTWQDAVQLMTLHSAKGLEFPQVFIVGMEEGMFP 581
P56255 PCRA_GEOSE 532 IAF--LTDLALISDLDELDGT---EQAAEGDAVMLMTLHAAKGLEFPVVFLIGMEEGIFP 586
P09980 REP_ECOLI 525 MTLTQVVTRFTL---RDMMERG--ESEEELDQVQLMTLHASKGLEFPYVYMVGMEEGFLP 579

Fig. 9G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Maleimide compound
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Thioether bond
pH > 8
H2O
Nonreactive Maleamic acid
Possible hydrolysis products of conjugate

Fig. 10

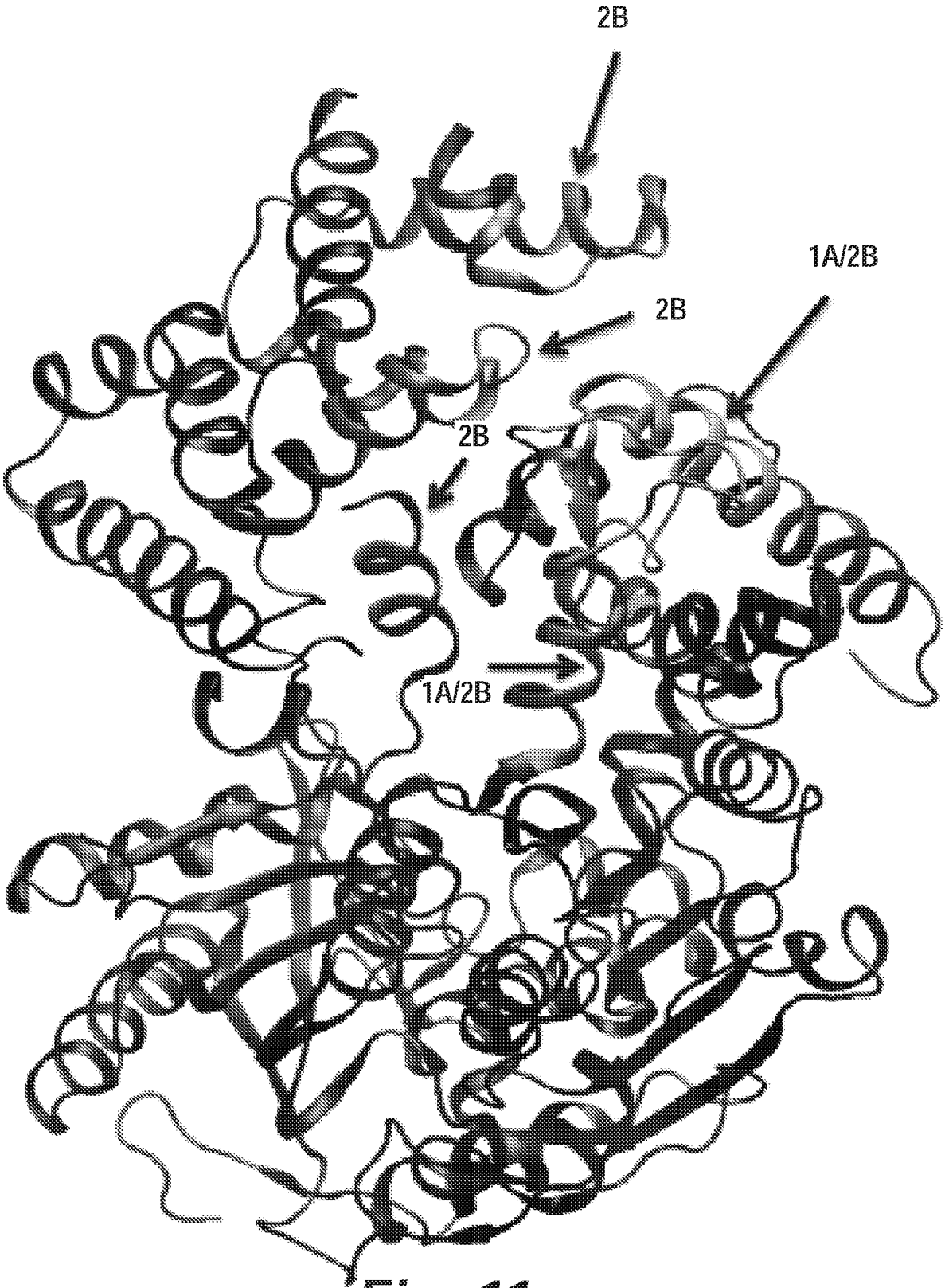


Fig. 11

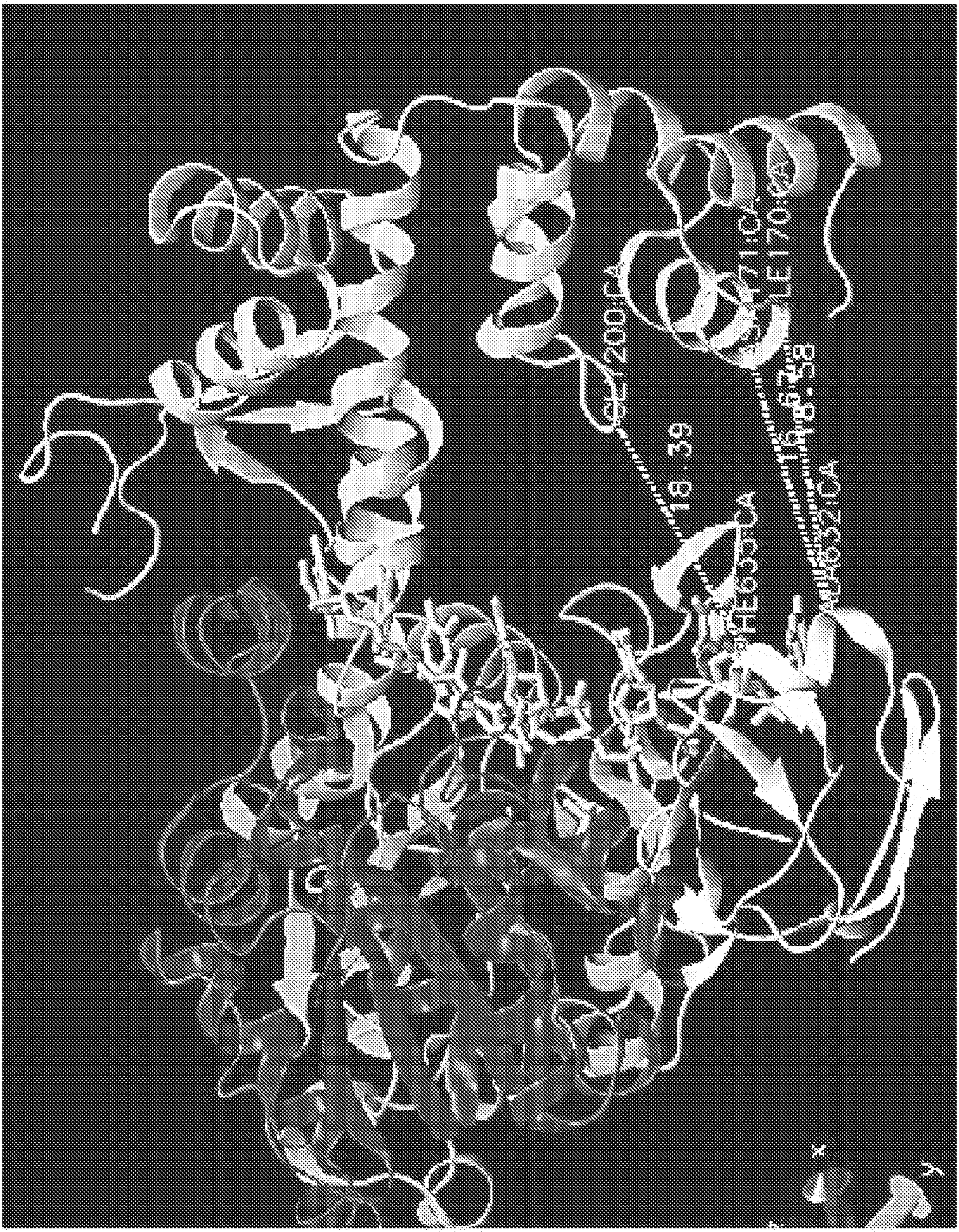


Fig. 12

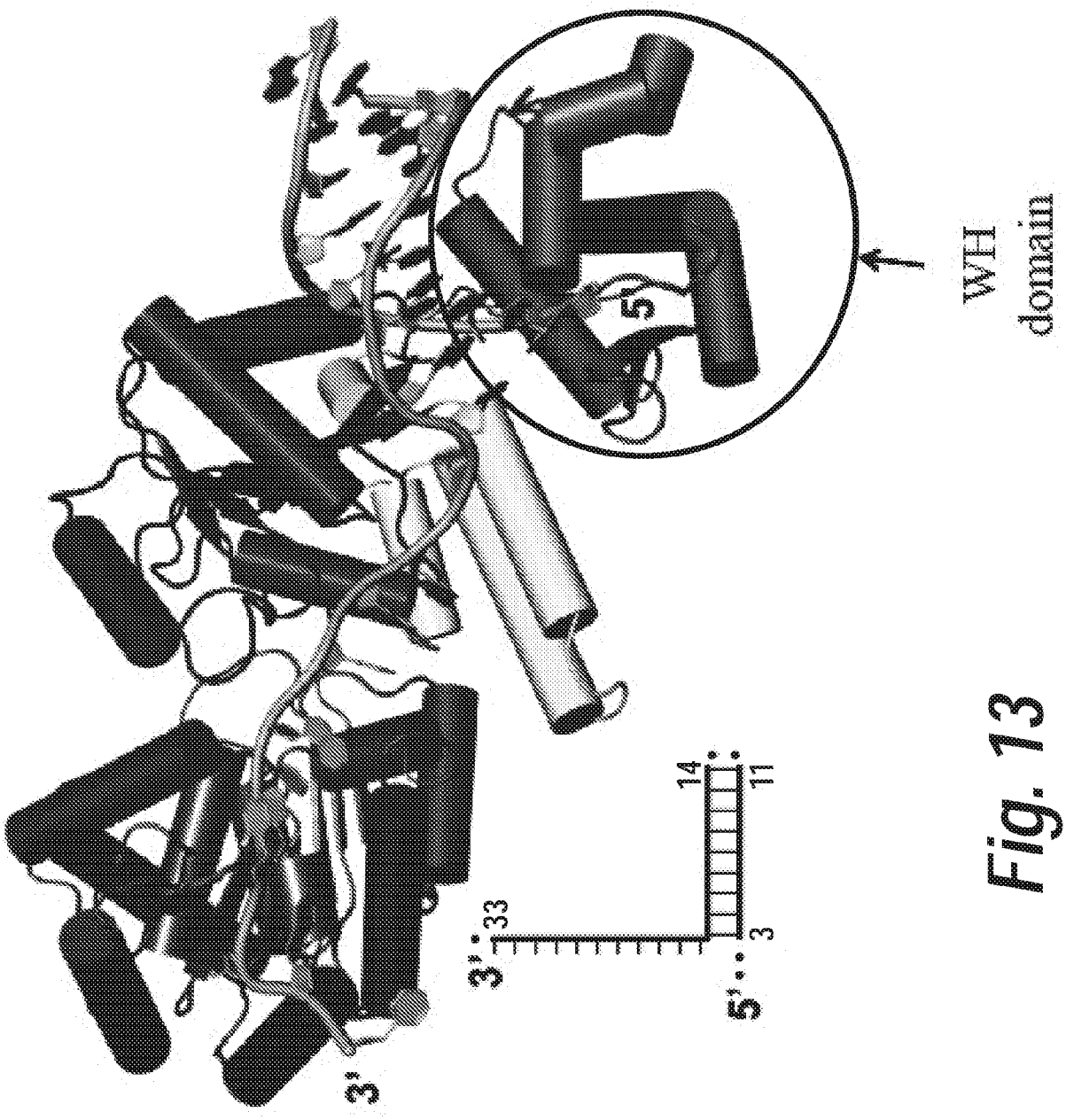
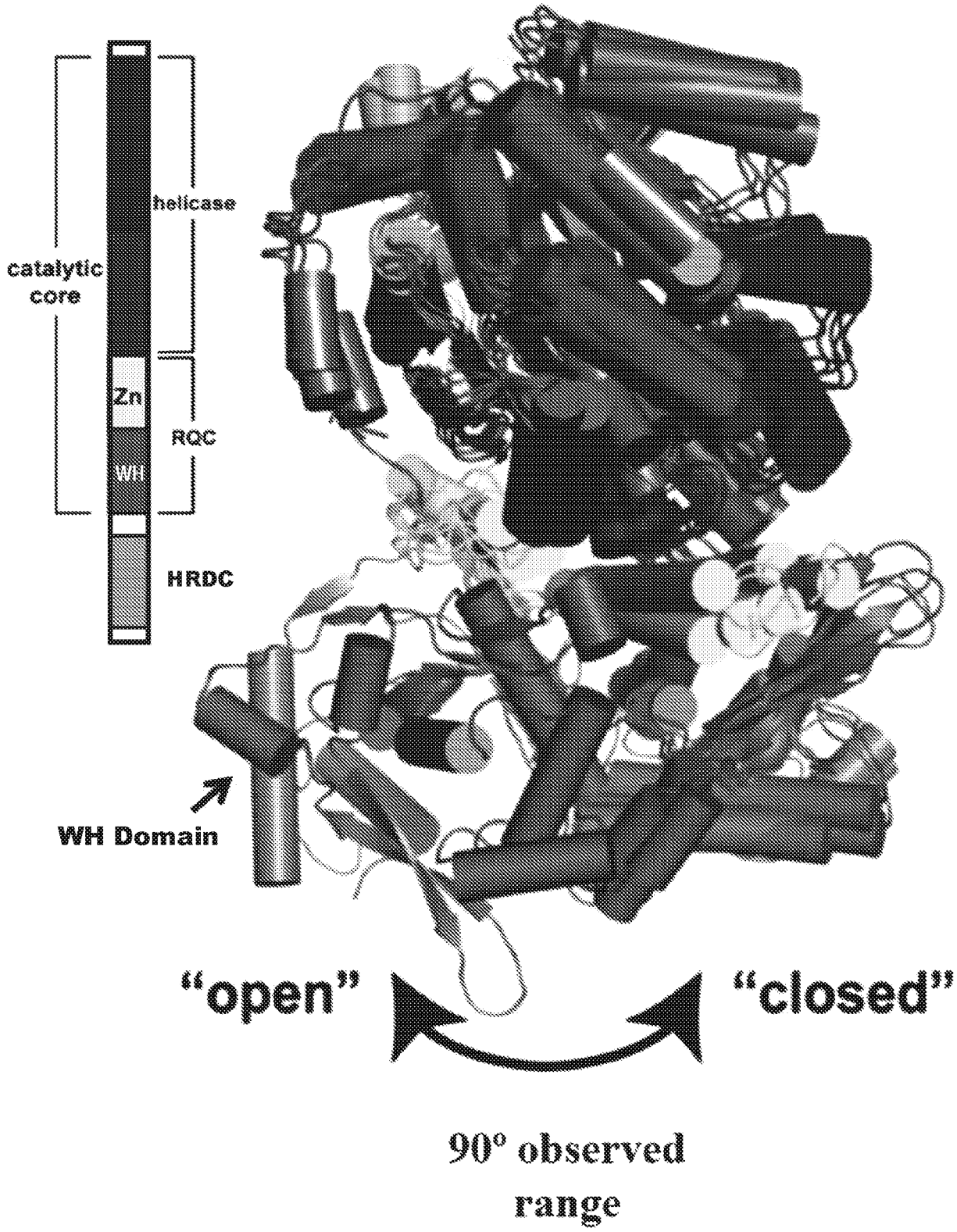


Fig. 13


helicase
catalytic core
Zn
RQC
WH
HRDC
WH Domain
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"closed"
90° observed range

Fig. 14

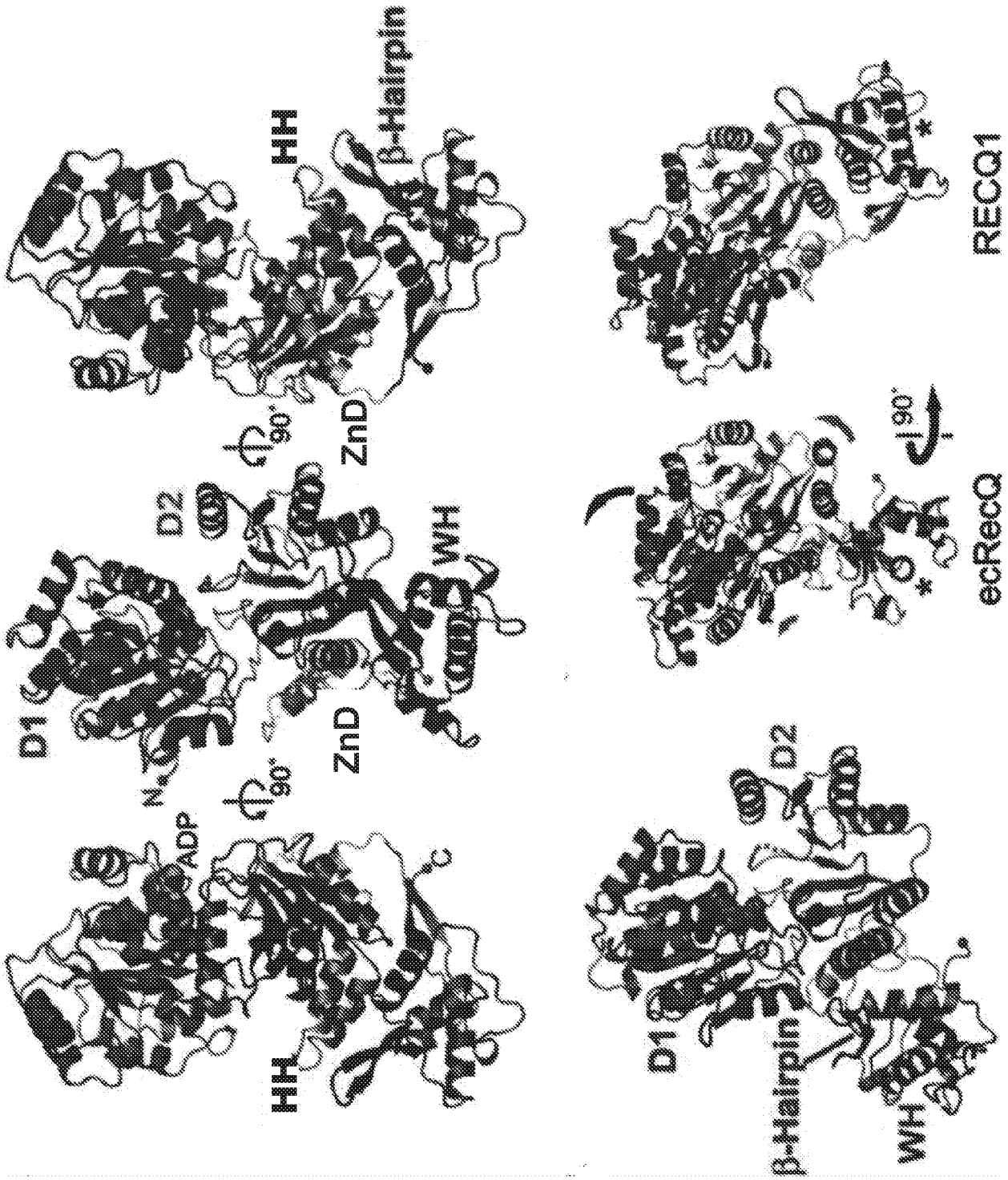

Fig. 15

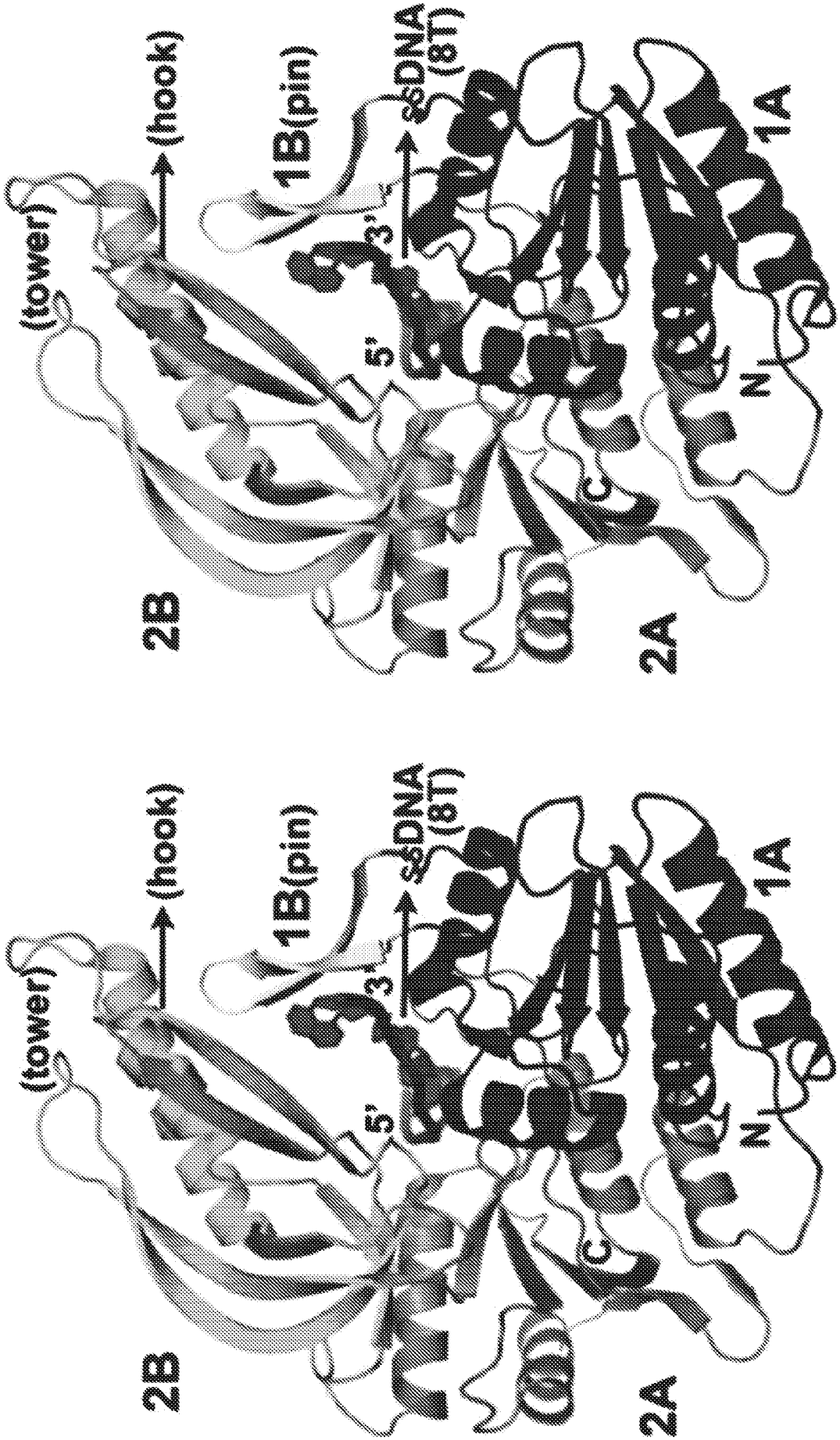
(tower)
(hook)
1B(pin)
ssDNA (8T)
3'
5'
2B
2A
1A
N
C
(tower)
(hook)
1B(pin)
ssDNA (8T)
3'
5'
2B
2A
1A
N
C

Fig. 16

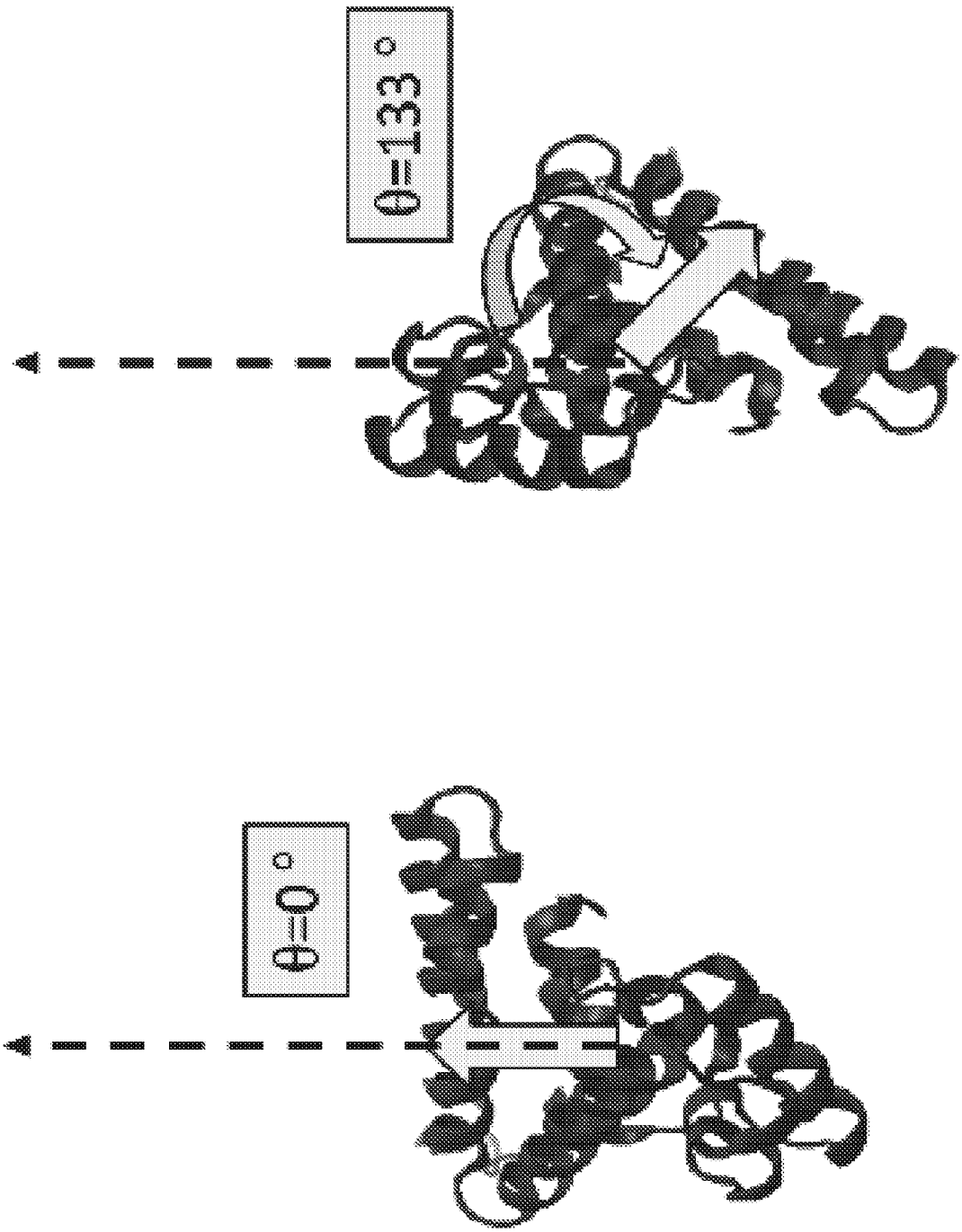

Fig. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20 15/060693

A. CLASSIFICATION OF SUBJECT MATTER
IPC (8) - C12M 1/00 (2016.01)
CPC - C12Q 1/6827 (2016.02)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12M 1/00, 1/34; C12N 9/00, 9/12, 15/09 (2016.01)
CPC - C07K 2319/80; C12Q 1/6827; C12Y 599/01002, 599/01003 (2016.02)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/44A, 44R; 536/23.1 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google PubMed.

Search Terms Used - vector, plasmid, ethylene bismaleimide, helicase, RepA, subdomain, crosslink

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/01 3260 A1 (OXFORD NANOPORE TECHNOLOGIES LIMITED et al) 23 January 2014 (23.01.2014) entire document	1-5, 42, 46-51, 57, 59, 62, 64, 69-71, 73
Y	KOROLEV et al. "Major Domain Swiveling Revealed by the Crystal Structures of Complexes of E. coli Rep Helicase Bound to Single-Stranded DNA and ADP," Cell, 22 August 1997 (22.08.1997), Vol. 90, Pgs. 635-647. entire document	1-5, 42, 46-51, 57, 59, 62, 64, 69-71, 73
Y	RANEY et al. "Structure and Mechanisms of SF1 DNA Helicases," Adv. Exp. Med. Biol. 28 October 2013 (28.10.2013), Vol. 767, Pgs. 1-33. entire document	2-4, 48-50
Y	WO 2014/158665 A1 (ARKEMA INC.) 02 October 2014 (02.10.2014) entire document	42
P. X	ARSLAN et al. "Engineering of a super-helicase through conformational control," Science, 17 April 2015 (17.04.2015), Vol. 348, No. 6232, Pgs. 344-347. entire document	1-5, 42-51, 57-59, 62-64, 69-71, 73-77

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 February 2016	04 MAR 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCTOSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060693

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item l.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 2-4 and 10-12 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060693

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-41, 52-56, 60, 61, 65-68, 72, 78-102
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.