GS Gene Linker® UV Chamber

Instruction Manual

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

Warranty Statement

Bio-Rad Laboratories GS Gene Linker chamber is warranted against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad will repair or replace the defective parts free of charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Damage caused by accident or misuse.
3. Damage caused by disaster.
4. Corrosion due to use of improper solvents or detergents.
5. Repair or modification performed by anyone other than Bio-Rad Laboratories or authorized agent.
6. Use of spare parts supplied by anyone other than Bio-Rad Laboratories or authorized agent.

The following components are not covered under warranty:

1. Fuses
2. Bulbs

Note: There are no user-serviceable parts or components inside the unit. Unauthorized service or repair may void the warranty.

For any inquiry regarding instrument operation, call Bio-Rad Technical Service at 1-(800) 4BIORAD, or contact your local representative.

For any request for repair service, determine the model number and serial number of your instrument, the purchase order number and invoice number, and in the U.S. call Bio-Rad Instrument Service at 1-(800) 876-7614.

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Section 1
Introduction

The GS Gene Linker UV chamber is useful for all molecular biology applications requiring UV irradiation. The GS Gene Linker chamber has three different operational modes: Program, Energy, and Time. The Program mode has preset energy or time parameters for specific applications. You can use one of these preset programs, or you can set your own desired energy level or time using the Energy mode or the Time mode. This manual provides information on how to operate the GS Gene Linker chamber along with valuable application guidelines.

Section 2
Specifications

2.1 Safety  

Definition of Symbols

 Caution, risk of UV exposure  Caution (refer to accompanying document)

Warning

This instrument is intended for laboratory use only.

This product conforms to the “Class A” standards for electromagnetic emissions for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

Lamps

The lamps inside this instrument emit short wave UV radiation. Overexposure to direct or reflected UV light can cause severe damage to the eyes and skin. Never look into an illuminated UV lamp without proper eye protection.

Ozone may be formed near UV lamps. Excessive ozone exposure can cause eye irritation and discomfort in the respiratory tract. Operate the GS Gene Linker chamber in an adequately ventilated area if ozone is detected by measurement or odor.

Sensor

The UV emission sensor is located on the right-hand inside wall. The UV sensor detects only the energy output in the range of 200-400 nm, and will automatically turn off the bulbs when the desired amount of cumulative UV energy has been delivered. The UV sensor also measures maximum energy output from the bulbs, and the Replace Bulb light will illuminate when the energy output is too low (approximately 30%). For these reasons, the sensor should remain clear and clean for accurate energy readings (refer to Section 5 for maintenance).

Safety Features

Power Switch The power switch can be used at any time to stop any of the operations.

Start/Stop The Start/Stop button will stop any of the on-going operations. If pressed again the operation will start at the beginning of the cycle.

Open Door The GS Gene Linker chamber will not operate when the chamber door is open. If the door is open, the Door Open light will be on. When the door is properly closed the Door Open light will turn off. The chamber will stop operating when the door is opened, but will resume operation when the door is closed.

Chamber The sealed chamber protects you from any UV radiation. The viewing window will protect your eyes and body from UV radiation.

Maximum Operation The GS Gene Linker chamber is equipped with an automatic shut off after 999 seconds of energy emission if the detector is covered, or 24 minutes if the radiometer check is not turned off.

2.2 Specifications

Dimensions	Width x Depth x Height
Outside	42.7 x 30.5 x 26.4 cm (16.8 x 12 x 10.4 inches)
Inside	31.7 x 24.1 x 15.2 cm (12.5 x 9.5 x 6 inches)
Weight	10 kg (22 lb)

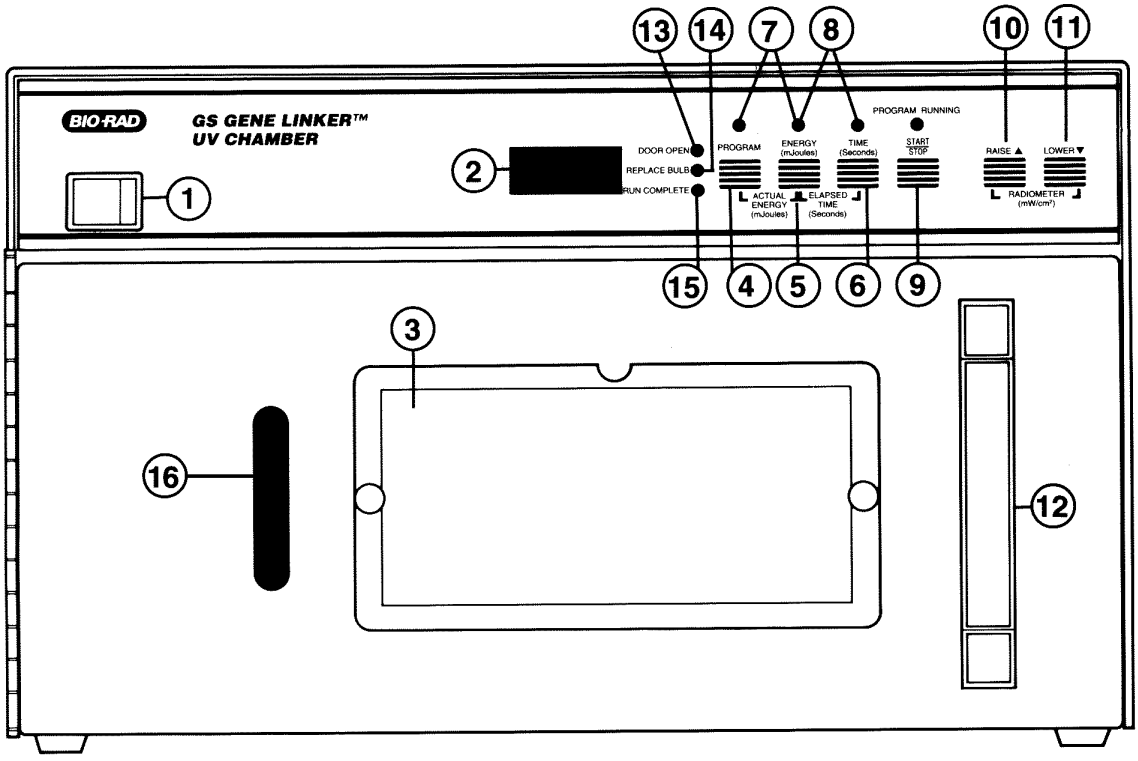
Functional	
Input voltage range	100 VAC/50 Hz/1 amps 120 VAC/60 Hz/1 amps 220 VAC/50 Hz/0.5 amps 240 VAC/50 Hz/0.5 amps
Fuses	2.0 amp Slow-Blow (100/120 V) or 1.0 amp Slow-Blow (220/240 V) Type T
Environmental	
Operating	50 ° F (10 °C) to 90 °F (32 °C) temperature 30–80% humidity
Storage	32 ° F (0 °C) to 140 °F (60 °C) temperature 10–90% humidity

UV Energy Source	
Germicidal bulbs	(5) G8T5 format, minibipin
Output	253.7 nm energy maximum

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference in which case the user will be required to correct the interference at his own expense.

2.3 Components

1. **Power switch**	Power switch to turn the GS Gene Linker chamber On or Off.
2. **LED display**	Selected Program, Energy, or Time is displayed here.
3. **Reference chart**	Quick reference on preset programs.
4. **Program**	Program button is used to select the program mode.
5. **Energy**	Energy button is used to select the energy mode. Energy is measured in milliJoules.
6. **Time**	Time button is used to select the time mode. Time is measured in seconds.
7. **Actual energy**	Program light and Energy light are both on when the cumulative energy is displayed on the LED.
8. **Elapsed time**	Energy light and Time light are both on when the elapsed time is displayed on the LED.
9. **Start/Stop**	Start/Stop button is used to start the irradiation cycle or to stop a cycle.
10. **Raise**	Increases selected mode.
11. **Lower**	Decreases selected mode.
12. **Handle**	To open and close chamber door.
13. **Door light**	Door Open light indicates the chamber door is open.
14. **Bulb light**	Replace Bulb light indicates the UV bulbs need replacement.
15. **Run light**	Run Complete light is on when the irradiation cycle has ended.
16. **Window**	Viewing window



Section 3
Operating Instructions

3.1 Set Up

1. Plug the chamber power cord into the appropriate electrical outlet.
2. Press the power switch On. The digital display should be **C-L**. If the display shows **ERR**, the chamber is malfunctioning and needs repair. Contact your local Bio-Rad representative, or call Bio-Rad Instrument Service at 1-800 876-7614.

3.2 Start/Stop

The Start/Stop button is used to start a given irradiation cycle. When a cycle is started, the Start/Stop button can be used to stop the cycle. The cycle parameters will remain in the temporary memory and can be restarted at the beginning of the cycle by pressing the Start/Stop button again.

The light above the Start/Stop button will turn on when in use and turn off after the irradiation cycle has ended.

3.3 Modes

The GS Gene Linker chamber can be operated in 3 different modes: Program, Time, or Energy. The mode is indicated by the light above these buttons. The following instructions describe how to set and use each of these modes.

Program

Each Program is set in either energy or time. To see the preset program value, press the Energy button to display the energy for this program, or press the Time button to display the time for this program. The LED will display - - - when the Time or Energy parameter is not preset. Pressing the Raise or Lower buttons while viewing the set Time or Energy switches out of the Program mode and into the indicated mode.

1. Place the material to be irradiated inside the GS Gene Linker chamber. Use filter paper or plastic wrap to support membranes. Close the chamber door.
2. Press the Program button. Select the desired program by pressing the Raise or Lower button.
3. Start the selected program by pressing the Start/Stop button. The light above the Start/Stop button will be on during the irradiation. The LED will display Actual Energy or Elapsed Time with the two lights above the buttons indicating which units are being displayed.
4. The GS Gene Linker unit will automatically stop after reaching the set time or the set energy level. At the end of the program, the GS Gene Linker chamber will sound a tone for a few seconds. This tone can be stopped by pressing any button. The light above the Start/Stop button will be off and the Run Complete light will be on. The LED will display either energy or time indicated by the light above the button.

Energy

1. Place the material to be irradiated inside the GS Gene Linker chamber. Close the chamber door.
2. Press the Energy button. Select the desired energy level by pressing the Raise or Lower button.
3. Start the selected energy level by pressing the Start/Stop button. The light above the Start/Stop button will be on during the irradiation. The LED will display the Actual Energy with the light above the Program and Energy buttons on.
4. The GS Gene Linker unit will automatically shut down after reaching the set energy level. At the end of the irradiation cycle, the GS Gene Linker chamber will sound a tone for a few seconds. This tone can be stopped by pressing any button. The light above the Start/Stop button will be off and the Run Complete light will be on. The LED will display cumulative energy.

Time

1. Place the material to be irradiated inside the GS Gene Linker chamber. Close the chamber door.
2. Press the Time button. Select the desired irradiation time by pressing the Raise or Lower button.
3. Start the selected time by pressing the Start/Stop button. The light above the Start/Stop button will be on during the irradiation. The LED will display the Elapsed Time and the light above the Time and Energy buttons will be on.
4. The GS Gene Linker unit will automatically shut down after reaching the set time. At the end of the irradiation cycle the GS Gene Linker chamber will sound a tone for a few seconds. The tone can be stopped by pressing any button. The light above the Start/Stop button will be off and the Run Complete LED will be on. The LED will display elapsed time.

3.4 Monitoring

Any time during or after an irradiation cycle, the elapsed time or cumulative energy can be determined.

1. To determine the elapsed time, press both the Energy button and the Time button simultaneously. The elapsed time in seconds will appear on the LED.
2. To determine the cumulative energy, press both the Energy button and the Program button simultaneously. The cumulative energy in milliJoules will appear on the LED.
3. Press the Energy or Time button alone and the LED will display the set values.

3.5 Quick Reference Chart

The GS Gene Linker chamber quick reference chart reproduced below, is located behind a plexiglass shield on the chamber door. This chart can be used as an applications guide. Bio-Rad will publish updated charts as new programs or protocols are developed. To receive these updates, it is important for you to be listed in the GS Gene Linker customer data base. Contact your Bio-Rad representative to be listed.

GS Gene Linker UV Chamber Quick Reference Chart

Make sure material is in the chamber and the door is properly closed. Turn ON power. The LED display should read **C-L**. Select operation mode by pressing the Program, Energy, or Time button. Press the Raise or Lower button to set the desired program, energy level or time. Press the Start/Stop button to start the irradiation cycle. At the completion of the cycle, a tone will sound and the UV light will automatically turn off.

Application	Conditions	Program* (LED Reading)	Setting
Crosslinking	Dot blot/NaOH NH_4OAc dry Zeta-Probe	C-L	125 mJoule
Nicking	Pulsed field gels	nic	60 mJoule
Sterilization	UV resistant material	Str	90 sec
Crosslinking	Dot blot/damp Zeta-Probe	C1	30 mJoule
Crosslinking	Southern dry membrane	C2	50 mJoule
Crosslinking	Southern damp membrane	C3	150 mJoule
Crosslinking	Dot blot/NaOH NH_4OAc dry membrane 312 nm	C4	250 mJoule

*Program (LED Reading)	Section
C-L	4.1
nic	4.2
Str	4.1
C1	4.1
C2	4.3
C3	4.3
C4	[312 nm bulbs (to be released)]

Section 4
Methods

Bio-Rad Laboratories has developed protocols for UV crosslinking of nucleic acids to nylon membrane, UV-induced nicking of DNA prior to transfer to membrane, and UV sterilization. We have an ongoing research program to develop new protocols for molecular biology applications. Our studies indicate that the energy required for optimal crosslinking of nucleic acid to membrane is dependent upon several parameters. These include the type of membrane (charged vs. neutral nylon), the transfer buffer, whether the membrane is wet or dry, as well as the application (dot blot vs. genomic Southern). The following protocols are recommended, depending upon the application.

4.1 DNA Crosslinking

Slot or Dot Blot/damp Zeta-Probe membrane

1. Denature the DNA in 0.4 N NaOH final concentration.
2. Assemble the microfiltration apparatus according to the manufacturer's recommendations. Use a positively charged nylon membrane like Zeta-Probe® GT membrane.
3. Apply the DNA samples directly onto the membrane.
4. Remove the membrane from the apparatus and place the damp membrane inside the GS Gene Linker chamber. Use filter paper or plastic wrap to support the membrane.
5. Follow the instructions in Section 3.3, Program mode. Select the program with the LED reading **C1** (30 mJ). Start the irradiation cycle by pressing the Start/Stop button. Proceed with preferred hybridization protocol.

Slot or Dot Blot/NaOH NH_4OAc/dry Zeta-Probe Membrane

1. Denature the DNA sample by adding NaOH and EDTA to a final concentration of 0.4 N NaOH and 10 mM EDTA. Heat the sample to 100 °C for 10 minutes.
2. Neutralize the DNA sample by adding an equal volume of cold 2 M ammonium acetate (NH_4OAc), pH 7.0.
3. Assemble the microfiltration apparatus according to the manufacturer's recommendations. Use a positively charged nylon membrane like Zeta-Probe GT membrane.
4. Apply the DNA samples directly onto the membrane.
5. Remove the membrane from the apparatus and place the damp membrane between 2 filter papers. Allow the membrane to dry. Remove the top filter paper and place membrane inside the GS Gene Linker chamber.
6. Follow the instructions in Section 3.3, Program mode. Select the program with the LED reading **C-L** (125 mJ). Start the irradiation cycle by pressing the Start/Stop button. Proceed with preferred hybridization protocol.

   Note: When using a neutral membrane select program **C3** (150 mJ).

Southern Transfer

1. Depurinate the DNA by soaking the gel in 0.25 N HCl for 10-15 minutes.
2. Denature the DNA by placing the gel in a bath of 0.5 N NaOH for 30 minutes.
3. Neutralize the gel by soaking it in 0.5 M Tris-HCl, pH 7.4, 1 M NaCl for 30 minutes.

4. Transfer the DNA onto a nylon membrane using 10x SSC or 10x SSPE as the transfer buffer. The membrane can be a neutral nylon or a positively charged nylon membrane like Zeta-Probe GT membrane.

5. After transfer, rinse the membrane in 2x SSC for 5 minutes. The membrane can be damp or dry.

6. Follow the instructions in Section 3.3 Program mode. Select the program with the LED reading **C2** (50 mJ) if the membrane is dry, or **C3** (150 mJ) if the membrane is damp. Start the irradiation cycle by pressing the Start/Stop button. Proceed with preferred hybridization protocol.

4.2 Sterilization

The GS Gene Linker chamber has a sterilization cycle preset. Follow the instructions in Section 3.3 Program mode. Select the third program with the LED reading **Str** (90 sec). Start the irradiation cycle by pressing the Start/Stop button. The GS Gene Linker chamber will sterilize only the exposed areas on any object in the chamber.

4.3 DNA Nicking[25]

The transfer efficiency of large DNA (>20 kb) from agarose gels is poor unless the DNA is nicked prior to transfer to nylon membrane. The DNA can be cleaved either by HCl depurination or by UV-irradiation. The depurination reaction is harder to control and is extremely sensitive to temperature. Exposure to short wavelength UV light (254 nm) is a more reliable method for nicking DNA in pulsed field gels before transfer. For optimal results, the following protocol must be followed rigorously.

1. Stain the gel with 1.0 µg/ml ethidium bromide (EtBr) for exactly 30 minutes with constant agitation. Do not destain the gel prior to nicking. Use a fresh dilution of EtBr for each gel.

2. Place the gel on a glass tray for transporting the gel to the GS Gene Linker chamber.

3. Place the gel inside the chamber and close the door.

4. Follow the instructions in Section 3.3 Program mode. Select the second program with the LED reading **nic** (60 mJ).

5. Start the irradiation cycle by pressing the Start/Stop button. The gel can be photographed, but exposure to UV radiation must be minimized (< 10 seconds). The gel can be destained prior to photography if desired.

6. Soak the gel in 0.4 N NaOH, 1.5 M NaCl for 15 minutes.

7. Transfer the DNA onto Zeta-Probe GT membrane using two liters of 0.4 N NaOH, 1.5 M NaCl as the transfer solvent.

8. Set up the capillary transfer as follows, from bottom to top:

   A. Corning Pyrex glass dish (28 x 18 x 4 cm).

   B. A plexiglass or plastic box for support, about 3 cm high and small enough to fit in the glass dish (*e.g.*, Eppendorf yellow pipette tip rack).

   C. Glass Plate (16 x 20 cm).

   D. Three sheets of blotting paper as wick (18 x 30 cm) (S&S, GB002).

E. Agarose gel (well side down).

F. Zeta-Probe membrane cut to the same size as the gel and pre-wetted with distilled water.

G. Three sheets of blotting paper (18 x 15 cm) (S&S, GB002).

H. A stack of paper towels 10 cm thick.

9. Transfer the DNA for 24–48 hours.

10. Carefully remove the paper towel and blotting papers. Remove the membrane together with the gel, turn over the membrane and gel, lay them gel side up, and mark the location of the wells and the orientation marker on the top of the gel. The position of the wells can be accurately marked on the membrane by using a fine point permanent alcohol marker pen, cutting through the bottoms of the wells.

11. Neutralize the membrane in 0.5 M Tris, pH 7.0 (neutralization buffer) for 5 minutes followed by rinsing briefly in 2x SSC. Transferred DNA can be visualized on the membrane by placing the damp blot on a transilluminator.

12. Dry the membrane by blotting on 3MM or other adsorbent paper and proceed to hybridization. UV crosslinking of the DNA to the membrane is not recommended with this alkaline transfer method.

Discussion

1. The procedure is based on gels approximately 6 mm thick. If thicker gels are used, the staining period may be prolonged to allow diffusion of EtBr into the middle of the gels. DNA that is not stained with EtBr will not be nicked by the UV light and thus will not be transferred from the gel.

2. Presoaking the gel in NaOH prior to transfer decreases background and increases transfer efficiency.

3. Pulsed field gels can also be blotted onto membranes using 10x SSC as the transfer buffer with standard alkaline denaturation followed by neutralization. Alkaline transfer onto nylon membranes gives as good or better sensitivity as standard transfers onto nitrocellulose filters. The alkaline procedure is much simpler and faster. In addition, nylon membranes can be reused many more times than nitrocellulose filters. Some blots may be reused as many as twenty times.

4. DNA separated on the CHEF-DR® II or CHEF Mapper® system can also be vacuum transferred onto nylon membrane in 4 hours using the Model 785 Vacuum Blotter (165-5001, 120 V) and NaOH as the transfer buffer.

5. The DNA is transferred from the back of the gel (the side opposite the wells) onto the membrane because irregularities in the surface of the gel frequently occur during solidification of these high percentage gels (1%). These surface artifacts will interfere with the transfer of the DNAs from the gel. Transfer from the other side of the gel insures smooth surface contact between the gel and the membrane.

6. It is essential to neutralize the membrane after transfer to prevent changing the pH of the hybridization buffer during the hybridization.

7. It is not necessary to bake nylon membranes after alkaline transfer since the DNA should be fixed onto the membrane by NaOH.

8. To monitor the efficiency of the transfer, stain the gel in neutralization buffer for 30 minutes with 1.0 µg/ml EtBr. Photograph the post-transferred gel and compare with the original picture.

Section 5
Cleaning and Maintenance

5.1 Cleaning

Outside - Clean the outside of the GS Gene Linker UV chamber with a damp towel. Do not use solvents or strong detergents.

Inside - Clean the inside aluminum walls of the chamber with ethanol (reagent grade) and a soft towel. The sensor is located on the right-hand inside wall. Clean the sensor with a soft towel and ethanol. Be careful not to scratch the sensor with an abrasive towel.

5.2 Bulb Replacement

The GS Gene Linker chamber is equipped with a radiometer mode. This mode is used to determine the bulb brightness. Enter into the radiometer mode by pressing both the Raise and Lower buttons at the same time. The UV lights will turn on and the LED will display an 'L' along with the UV illuminance measured in mW/cm^2. After 1 minute, the reading should stabilize. If the UV illuminance is ≤ 2 mW/cm^2 ALL 5 bulbs should be replaced. Stop the radiometer mode by pressing any button.

Turn off the GS Gene Linker chamber power and unplug the power cord. Remove the bulb by holding either metal end and turn the bulb 1/4 turn. The bulb will slip out of the socket through the slots on the bottom. Insert the new bulb through the slots and turn the bulb 1/4 turn until the bulb snaps into place.

Section 6
Equipment and Accessories

6.1 GS Gene Linker UV Chamber and Accessories

165-5031	**GS Gene Linker UV Chamber,** 120 VAC/60 Hz; includes five 254 nm bulbs and instruction manual
165-5032	**GS Gene Linker UV Chamber,** 220 VAC/50 Hz; includes five 254 nm bulbs and instruction manual
165-5033	**GS Gene Linker UV Chamber,** 240 VAC/50 Hz; includes five 254 nm bulbs and instruction manual
165-5034	**GS Gene Linker UV Chamber,** 100 VAC/50 Hz; includes five 254 nm bulbs and instruction manual
165-5035	**GS Gene Linker UV Chamber Replacement Bulbs,** 254 nm, 5 bulbs

6.2 Electrophoresis Reagents and Equipment

170-4300	**Sub-Cell DNA Electrophoresis Cell without Casting Tray**
170-4304	**Sub-Cell DNA Electrophoresis Cell with 15x20 cm Casting Tray**
170-4343	**Wide Mini-Sub Cell DNA Electrophoresis Cell Basic Unit**
170-4307	**Mini-Sub Cell DNA Electrophoresis Cell Basic Unit**

Electrophoresis Reagents and Equipment (Continued)

165-5052 **PowerPac 200 Power Supply,** 100/120V

165-5053 **PowerPac 200 Power Supply,** 220/240V

Ultra Pure DNA Agaroses

162-0017 **Low Melt Preparative Grade Agarose,** 25 gm

162-0020 **Low Melt Preparative Grade Agarose,** 250 gm

162-0125 **High Strength Analytical Grade Agarose,** 100 gm

162-0126 **High Strength Analytical Grade Agarose,** 500 gm

162-0133 **Molecular Biology Certified Agarose,** 100 gm

162-0134 **Molecular Biology Certified Agarose,** 500 gm

162-0135 **Chromosomal Grade Agarose,** 25 gm

162-0136 **Chromosomal Grade Agarose,** 100 gm

161-0733 **Premix 10x TBE Buffer,** 1 liter

6.3 Transfer Reagents and Equipment

165-5000 **Model 785 Vacuum Blotter with Regulator,** includes: Vacuum Regulator; Base unit with Vacuum Stage; Porous Vacuum Plate; Reservoir Seal O-ring; Sealing Frame; Assortment Window Gaskets; Vacuum Blotter lid; Instruction manual

165-5001 **Model 785 Vacuum Blotter System:** same as 165-5000 except with vacuum pump (120 V)

165-5002 **Model 785 Vacuum Blotter System:** same as 165-5000 except with vacuum pump (220/240 V)

165-5003 **Model 785 Vacuum Blotter Basic Unit**

170-6545 **Bio-Dot Apparatus**

170-6542 **Bio-Dot Slot Format**

170-3910 **Trans-Blot Electrophoretic Transfer Cell**

162-0190 **Zeta-Probe GT Membrane,** sheets, 9 x 12 cm, 15

162-0191 **Zeta-Probe GT Membrane,** sheets, 10 x 15 cm, 15

162-0192 **Zeta-Probe GT Membrane,** sheets, 15 x 15 cm, 15

162-0193 **Zeta-Probe GT Membrane,** sheets, 15 x 20 cm, 15

162-0194 **Zeta-Probe GT Membrane,** sheets, 20 x 20 cm, 15

162-0195 **Zeta-Probe GT Membrane,** sheets, 20 x 25 cm, 3

162-0196 **Zeta-Probe GT Membrane,** roll 30 cm x 3.3 m, 1

162-0197 **Zeta-Probe GT Membrane,** roll 20 cm x 3.3 m, 1

162-0198 **Zeta-Probe GT Membrane,** roll 30 cm x 30 m, 1

170-3557 **Random Primer DNA Labeling Kit,** 25 reactions

165-0962 **Filter Paper Backing,** 35 x 45 cm, 25 sheets

165-0921 **Filter Paper Backing,** 18 x 34 cm, 25 sheets

Section 7
References

7.1 UV Crosslinking of DNA to Membrane

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