Recommendations for Clinical *CYP2D6* Genotyping Allele Selection

A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and European Society for Pharmacogenomics and Personalized Therapy

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AMP PGx Working Group

Recommendations for Clinical Pharmacogenetic Testing: Defining a Minimum Set of Variants that should be included in Genotyping assays

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- **Ann M. Moyer,** Mayo Clinic, CAP representative
- **Stuart A. Scott**, Stanford University Medical Center
- **Ron van Schaik,** Erasmus MC University Medical Center, ESPT and DPWG representative
- **Michelle Whirl-Carrillo,** Stanford University, CPIC/PharmGKB

AMP Association for Molecular Pathology

AMP PGx Working Group *CYP2D6*

4th deliverable: consensus expert opinion recommendations for clinical *CYP2D6* testing

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Recommendations for Clinical *CYP2D6* Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy

Victoria M. Pratt [*, †, ✉], Larisa H. Cavallari [*, ‡], Andria L. Del Tredici [*, §], Andrea Gaedigk [*, ¶], Houda Hachad [*, ‖], Yuan Ji [*, **], Lisa V. Kalman [*, ††], Reynold C. Ly [*, ‡‡], Ann M. Moyer [*, §§], Stuart A. Scott [*, ¶¶, ‖‖], R.H.N. van Schaik [*, ***, †††], Michelle Whirl-Carrillo [*, ‡‡‡], Karen E. Weck [*, §§§]

AMP Association for Molecular Pathology

AMP PGx *CYP2D6* Working Group:
Expert consensus recommendation/opinion development

- **Tier 1 -** Minimum "must-test" alleles
  - Well-characterized effect on the function of the protein and/or gene expression
  - Appreciable minor allele frequency in a patient population
    - *CYP2D6*: ≥1% for Tier 1
    - *CYP2D6*: ≥0.1% for Tier 2
  - Available reference materials
  - Technical feasibility to detect variant in a clinical laboratory (NEW for CYP2D6)
- **Tier 2 -** Extended panel
  - Meet at least one but not all of the criteria for inclusion in Tier 1

Note:

- We evaluated alleles up to *114
- Technical feasibility was added due to the complexity of *CYP2D6*

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AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

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AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Characteristic variant associated with altered function

Multiple systems of nomenclature provided

See Table 1 in Manuscript

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
		rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
		rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
		rs3892097	1846G>A	[illegible]	[illegible]	[illegible]	[illegible]>A	(splicing defect)	Yes	0.5-18.5%
		CYP2D6 full gene deletion								
*6	No function	rs5030655	1707delT	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

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Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function					del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function					C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function								Yes	1.6-5.4%
*6	No function					del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function					_421281	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function					G>A, C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.7870C>T, g.9200G>C	G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

Included because
- c.886C>T (2850C>T) and c.1457G>C (4180G>C) are present in other haplotypes
- Often interrogated by labs to differentiate haplotypes

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Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	r				42130692G>A, 42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	r				42129770G>A, 42127941G>A, 42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

Defining variant also present in *109 and *115

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Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	r r r				42129770G>A, 42127941G>A, 42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	r rs61736512+ rs1058164, rs16947, rs1135840	1659G>A, 1661G>C, 2850C>T, 4180G>C	1660G>A, 1662G>C, 2851C>T, 4181G>C	g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

Note that c.100C>T is present in many alleles

AMP Association for Molecular Pathology

AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	r r r rs1135840			g.9200G>C	42127608C>T, 42525132_425251 delinsGAT, 42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

Note that c.320C>T also present in *40, *58, and *64

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AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function							N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles								Yes	variable

- Many platforms only test c.1012G>A due to difficulty in testing c.406_408delinsATC
- c.1012G>A is present in *70 and *109

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AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	29[illegible] 28[illegible]						[illegible]s	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							[illegible]s	variable

- Duplications may be detected by a single probe
  - May not always distinguish between duplications and hybrid alleles
  - Functional effect depends on allele duplicated
    - Single or duplicated *4 allele has no activity
    - Duplicated *1 has > activity than single *1

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AMP Guidelines for *CYP2D6* clinical testing – Tier 2

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*7	No function	rs5030867	2935A>C	2936A>C	g.7955A>C			s324Pro	Yes	0-0.6%
*8	No function	rs5030865, rs16947, rs1135840	1758G>T, 2850C>T, 4180G>C	1759G>T, 2851C>T, 4181G>C	g.6778G>T, g.7870C>T, g.9200G>C	g.42126611C>G	c.1457G>C	y169Ter, g.Arg296Cys, p.Ser486Thr	No	0-0.1%
*12	No function	rs5030862, rs16947, rs1135840	124G>A, 2850C>T, 4180G>C	124G>A, 2851C>T, 4181G>C	g.5143G>A, g.7870C>T, g.9200G>C			p.Gly42Arg, Arg296Cys, Ser486Thr	No	0-1.7%
*14	Decreased function	rs5030865, rs16947, rs1135840	1758G>A, 2850C>T, 4180G>C	1759G>A, 2851C>T, 4181G>C	g.6778G>A, g.7870C>T, g.9200G>C			Gly169Arg, Arg296Cys, Ser486Thr	Yes	0-0.3%
*15	No function	rs774671100	137_138insT	137_138insT	g.5156dup			Leu47fs	Yes	0-0.6%
*21	No function	rs72549352, rs16947, rs1135840	2579_2580insC, 2850C>T, 4180G>C	2580_2581insC, 2851C>T, 4181G>C	g.7599dup, g.7870C>T, g.9200G>C			Arg269fs, Arg296Cys, Ser486Thr	Yes	0-0.4%
*31	No function	rs267608319, rs16947, rs1135840	4042G>A, 2850C>T, 4180G>C	4043G>A, 2851C>T, 4181G>C	g.9062G>A, g.7870C>T, g.9200G>C			Arg440His, Arg296Cys, Ser486Thr	Yes	0-0.8%
*40	No function	rs72549356, rs28371706, rs16947, rs1135840	1863_1864ins TTTCGCCCCTTTCGCCCC, 1023C>T, 2850C>T, 4180G>C	1864_1865ins TTTCGCCCCTTTCGCCCC, 1022C>T, 2851C>T, 4181G>C	g.6875_6883 TTTCGCCCC[3], g.6041C>T, g.7870C>T, g.9200G>C	g.42128934_42128942AAAG GGGCG[3], g.42129770G>A, g.42127941G>A, g.42126611C>G	c.514_522 TTTCGCCCC[3], c.320C>T, c.886C>T, c.1457G>C	p.172_174FRP[3], p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-1.3%
*42	No function	rs72549346,	3260_3261insGT,	3261_3262insGT,	g.8279_8280dup,	g.42127532_42127533dup,	c.1088_1089dup,	p.Gln364fs, p.Arg296Cys, p.Ser486Thr	No	0-0.5%
*49							C>T,	p.Phe120Ile, p.Pro34Ser, p.Ser486Thr	No	0-1.1%
*56		rs1135840				g.42126611C>G	57G>C	p.Arg344Ter, p.Ser486Thr	Yes	0-0.2%
*59	Decreased function	rs79292917, rs16947, rs1135840	2939G>A, 2850C>T, 4180G>C	2940G>A, 2851C>T, 4181G>C	g.7959G>A, g.7870C>T, g.9200G>C	g.42127852C>T, g.42127941G>A, g.42126611C>G	c.975G>A, c.886C>T, c.1457G>C	p.Pro325= (splicing defect), p.Arg296Cys, p.Ser486Thr	Yes	0-0.7%
Hybrid genes	no function	variable								

Do not meet MAF for Tier 1

Do not have reference materials; *12 and *49 may be promoted to Tier 1 if reference materials become available in the future

Meets standard criteria for Tier 1, but is defined by an in-frame insertion of 18 base pairs that is difficult to detect; therefore, assigned to Tier 2 based on technical difficulty

Tier 2 – Hybrid Alleles

- Hybrid alleles considered nonfunctional, but included because they may complicate *CYP2D6* testing, especially when only 1 position is queried

Allele	Hybrid type	5' UTR†	exon 1†	intron 2†	intron 5†	intron 6†	exon 9†
*13	2D7-2D6	no	no	yes/no	yes/no	yes/no	yes‡,‖

With only 1 CNV probe, could mistakenly think a gene duplication is present

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Tier 2 – Hybrid Alleles

- Hybrid alleles considered nonfunctional, but included because they may complicate *CYP2D6* testing, especially when only 1 position is queried

Allele	Hybrid type	5' UTR†	exon 1†	intron 2†	intron 5†	intron 6†	exon 9†
*4.013 (*4N)	2D6-2D7	yes	yes	yes	yes	yes	no
*36	2D6-2D7	yes	yes	yes	yes	yes	no
*68	2D6-2D7	yes	yes	no‡	no‡	no‡	no
*83	2D6-2D7	yes	yes	yes	yes	yes	no

However, for a *CYP2D6-CYP2D7* hybrid (which is more common than a *13 *CYP2D7-CYP2D6* hybrid), this may not be a problem if the probe is located in exon 9

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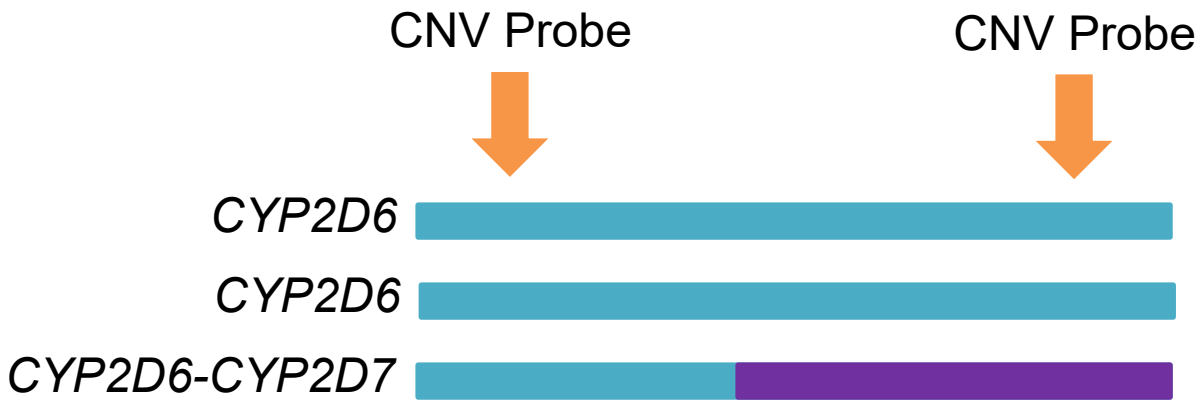
Tier 2 – Hybrid Alleles

- When one probe is used, differentiating between a duplication and hybrid is not possible; however, when 2 probes are used, this is feasible



Allele	Hybrid type	5' UTR†	exon 1†	intron 2†	intron 5†	intron 6†	exon 9†
*4.013 (*4N)	2D6-2D7	yes	yes	yes	yes	yes	no
*13	2D7-2D6	no	no	yes/no	yes/no	yes/no	yes‡,‖
*36	2D6-2D7	yes	yes	yes	yes	yes	no
*68	2D6-2D7	yes	yes	no‡	no‡	no‡	no
*83	2D6-2D7	yes	yes	yes	yes	yes	no

However, for a *CYP2D6-CYP2D7* hybrid (which is more common than a *13 *CYP2D7-CYP2D6* hybrid), this may not be a problem if the probe is located in exon 9

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Tier 2 – Hybrid Alleles

Reference chart for common hybrid alleles – note there may be variation in the switch location between *CYP2D6* and *CYP2D6*

Allele	Allele Functional Status†	Reference Material Available	Multiethnic Allele Frequency	Hybrid type	5' UTR†	exon 1†	intron 2†	intron 5†	intron 6†	exon 9†
**4.013 (*4N)*	no function			*2D6-2D7*	yes	yes	yes	yes	yes	no
**13*	no function	Yes	0 to 0.4%	*2D7-2D6*	no	no	yes/no	yes/no	yes/no	yes‡,‖
**36*	no function	Yes	0 to 1.2%	*2D6-2D7*	yes	yes	yes	yes	yes	no
**68*	no function	Yes	not available	*2D6-2D7*	yes	yes	no‡	no‡	no‡	no
**83*	uncertain function	Yes	not available	*2D6-2D7*	yes	yes	yes	yes	yes	no

†Citations for assignment of function can be found at https://www.pharmvar.org, last accessed 11/13/2020; ‡signal present on copy number analysis for this allele, ǂit cannot be excluded that rare/undefined hybrids switch in different regions affecting the copy number call in that region; ‖ a hybrid with a switch to CYP2D6 past exon 9 has been described in tandem arrangements; these are technically also CYP2D7-2D6 hybrids and are producing /no/ calls across all regions tested.

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Alleles Considered, but Not Included in a Tier

- *11 - c.181-1G>C (883G>C) – CPIC frequency data suggested MAF up to 0.1% in European population; however, this variant in gnomAD has MAF of 0.025% in non-Finnish European population, frequency too low
- *69 – no function allele, but difficult to discriminate from other haplotypes
  - Includes c.100C>T, c.886C>T, c.985_39G>A, and c.1457G>C which are also present in *2, *10, and *41
  - If 4 variants are heterozygous, can't differentiate *1/*69 from the common *10/*41 diplotype, unless testing for c.352+7A>G (unique to *69), but both are predicted to result in an intermediate metabolizer phenotype
- CYP2D6 "enhancer" (c.63-2604G>A), 116kb downstream of *CYP2D6* thought to modulate expression levels; however, evidence is conflicting and limited

Detection rate

- Tier 1
  - ≥78% of African-American
  - ~84% of European Caucasian
  - ~85% of East Asian
- Tier 2
  - ≥80% African-American
  - ~85% European Caucasian
  - ~87% East Asian

- Tier 1
  - *2, *3, *4, *5, *6, *9, *10, *17, *29, *41, duplications
- Tier 2
  - *7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56, *59, hybrids

Non wild-type variant only in calculation (does not include all possibly duplications)

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Please email Dr. Victoria Pratt (vpratt@iu.edu), Chair of the AMP PGx Working Group, for feedback and suggestions!

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