Supplementary Table 1. References for *CYP2A6* genotyping assay primers

Assay	Step	Primer Name	Sequence (5' to 3')	Reference for published primers	Reference for published assays
**9, *31*	1st	2A65Pr1F	ACCTAGACTTAATCTTCCCGTATAC	1 & 2 & 19 & 20	
	1st	2A6in1R	CCCAAGATCCTGTCTTTCTGAT	19 & 20	
**9, *31*	2nd	2A6-460F	ATCCTCCACAACAGAAGACCCCTAA	17 & 19	
**9*	2nd	2A6-17RA	ACGGCTGGGGTGGTTTGCCTTTA	13 & 19	*9: 13
	2nd	2A6-17RC	ACGGCTGGGGTGGTTTGCCTTTC	13 & 19	
**31*	2nd	2A6ex1RW	CAGCAAGGCCACCAGAAGCAT	20	*31: 20
	2nd	2A6ex1RV	CAGCAAGGCCACCAGAAGCAG	20	
**2, *24, *25, *26*	1st	2A61F	GCTGAACACAGAGCAGATGTACA	18	
	1st	2A61R	GGAGGTTGACGTGAACTGGAAGA	18	
**2, *24, *25*	2nd	E3R-1	AACGCGCGCGGGTTCCTCGT	21	
**2*	2nd	2A62wtF	CTCATCGACGCCCT	18	*2: 18
	2nd	2A62v1F	CTCATCGACGCCCA	18	
**24*	2nd	2A6ex2Fwt	GCCACCTTCGACTGGG	8	*24: 8
	2nd	2A6ex2Fv	GCCACCTTCGACTGGC	8	
**25*	2nd	2A6in2ex3FW	CACCTCCCCAGGCGTGGTAT	8	*25: 8
	2nd	2A6in2ex3FV	CACCTCCCCAGGCGTGGTAC	8	
**26*	2nd	2A6ex2Fwt	GCCACCTTCGACTGGG	8	*26: 8
	2nd	2A6ex3R1711w	GCAGGGTGGCGATGGA	8	
	2nd	2A6ex3R1711v	GCAGGGTGGCGATGGC	8	
**20, *23, *27*	1st	2A6exin3F	GGCACTGGCGGTGAGCAG	2	
	1st	2A6in5R	GGCCTGTGTCATCTGCCT	2	
**20, *27*	2nd	2A6in3F	CTGCCTCCTGGAATTCTGAC	2	
**20*	2nd	2A6ex42144Rw	ACAGTGACAGGAACTCTT	20	*20: 14
	2nd	2A6ex42144Rv	ACAGTGACAGGAACTCTG	20	
**27*	2nd	2A6*4171w-M	GGAAGATTCCTAGCATCCTG	8	*27: 8
	2nd	2A6*4171v-M	GGAAGATTCCTAGCATCCTT	8	
**23*	2nd	5M13FOR-H2	GCA GTT GGC AGG TTG TGG TAG G	20	*23: 2
	2nd	2A6ex42161Fw-M	CAA AGA GTT CCT GTC ACT GTC GC	20	
	2nd	2A6ex42161Fv-M	CAA AGA GTT CCT GTC ACT GTC GT	20	
**12, *34*	1st	2AinIF-L	GATCTTGGGATGTCCAGCTCC	19	
	1st	2A6in5R	GGCCTGTGTCATCTGCCT	2	
**12, *34*	2nd	2A6inex5R	GGTGTACCTCCTGCATGC	19	
**12*	2nd	2A6in1/ex2	ACCTCCATCAGATCAGTGAGC	19	*12: 19
	2nd	2A7in1/ex2	GCCTCCATCAGTTCAGTGAGT	19	
**34*	2nd	2A6in4F	CAACCGGCCTCCTGCATA	19	*34: unpublished
	2nd	2A7in4F	CAACCCGCCTCCTGCATG	19	refer to Supplementary Table 2
**1B, *17, *28, *35*	1st	2A6in6F1	ATTTCCTGCTCTGAGACC	5	
	1st	2A6R13	GCCTCCCATAGTGCTATAATTAACA	11	
**1B*	2nd	2A6R12	ATT GTC TTT CAA AGT AGC TGT GT	9	*1B: 4
	2nd	2A6*1Bwt	ACT GGG GGC AGG ATG GC	5	
	2nd	2A6*1Bvar	AAT GGG GGG AAG ATG CG	5	
**17*	2nd	2A6in7AS	CTGAGATTTCTGTCCCTAT	6	*17: 6
	2nd	2A6*17Fwt-M	GAGATCCAAAGATTTGGAGCCG	20	
	2nd	2A6*17Fv-M	GAGATCCAAAGATTTGGAGCCA	20	
**28*	2nd	2A6in7F1	ACCCACATTAGAAGCTTTCTAGA	5	*28: 8
	2nd	2A6ex8R2wt	TCTTCTTAAACTGCCCCTTC	8	
	2nd	2A6ex8R2v	TCTTCTTAAACTGCCCCTTG	8	
**35*	2nd	2A6R12	ATT GTC TTT CAA AGT AGC TGT GT	9	*35: 10
	2nd	2A6in8ex9F6458w	TCCTCAGGAAAGCGGA	10	
	2nd	2A6in8ex9F6458v	TCCTCAGGAAAGCGGT	10	
**4H*	1st	2Aex7F	GGC CAA GAT GCC CTA CAT G	4	
	1st	2A6R13	GCCTCCCATAGTGCTATAATTAACA	11	
**4H*	2nd	2A6R12	ATTGTCTTTCAAAGTAGCTGTGT	9	*4H
	2nd	2A6in7F1	ACCCACATTAGAAGCTTTCTAGA	5	
	2nd	2A7in7F1	CCCCATTAGAAGCTTTCTACTCA	8	
**4B*	1st	2A6/74B-S	CAAGATATATCCACACTCCTATG	unpublished	
	1st	2A6Rdup	AATTCCTGGATTGACAAGAG	9	
**4B*	2nd	2A6Rdup-2	ACAAAAGTTGTGCAATAGCA	unpublished	*4B: unpublished
	2nd	2A6FR4.2KS	GATAAAGAAAATGTGGATAGAATGG	unpublished	genotyping discussed in 9
	2nd	2A7FR5.4KS	GATAAAGAAAATGTGGTACATTC	unpublished	
**7, *8, *10*	1st	2A6in6F1	ATTTCCTGCTCTGAGACC	5	
	1st	2A6R6	TAA TTG GGT TGT TTT CTA TTG AGT	12	
**7*	2nd	2A6R0	AGGTCATCTAGATTTTCTCCTACA	5	*7: 16
	2nd	2A6*7Fwt-M	TCCCAGTCACCTAAGGAAAT	12	
	2nd	2A6*7Fv-M	TCCCAGTCACCTAAGGAAAC	12	
**8*	2nd	2A6R0	AGGTCATCTAGATTTTCTCCTACA	2 & 5	*8: 16
	2nd	2A6*8wtF	GCTTTGCCACGATCCCACG	16	
	2nd	2A6*8vF	GCTTTGCCACGATCCCACT	16	
**10*	2nd	2A6*7Fwt-M	TC CCA GTC ACC TAA GGA AAT	12	*10: 12
	2nd	2A6*7Fv-M	TC CCA GTC ACC TAA GGA AAC	12	
	2nd	2A6*8Rwt-L	GGAAGCTCATGGTGTAGTTTC	12	
	2nd	2A6*8Rv-L	GGAAGCTCATGGTGTAGTTTA	12	
**1v2A*	1st	2Aex7F	GGCCAAGATGCCCTACATG	7	

1x2A	1st	2A7R11	GTGCAGAGGTTTTTGTGTGACTG	15	
	2nd	2A7R12	TTTGTCTTCCAAAGTAGCTGTGC	15	*1x2A: 15
	2nd	2A7in7F1	CCCCATTAGAAGCTTTCTACTCA	8	
	2nd	2A6in7F1	ACCCACATTAGAAGCTTTCTAGA	5	
1x2B	1st	2A6F3	TAG ACA GAT TCT TAA AAA GCA CCT	9	
	1st	2A 6/7R	CTG GAT TCT TGG GCA TTC AAC CA	9	
1x2B	2nd	2A6F0	TGA GTA CAA AAC TTC TAG AAG ATA AT	9	*1x2B: 9
	2nd	2A6R dup	AAT TCC TGG ATT GAC AAG AG	9	
	2nd	2A7R dup	AAT TCC TGG ATT GAC GAG AC	9	

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Supplementary Table 2. Previous published versions of *CYP2A6* Endpoint PCR Assay Primers

Assay[a]	Step	Previous Primer	Location	F/R	Sequence (5' to 3')	Reason for Change
**23*	2nd	2A6in3F	Intron 3 (1969)	F	CTGCCTCCTGGAATTCTGAC	Reverse primers on top of **27* variant
	2nd	2A6ex42161AW	Exon 4 (2182)	R	GGAAGATTCCTAGCATCATGCG	
	2nd	2A6ex42161AV	Exon 4 (2182)	R	GGAAGATTCCTAGCATCATGCA	
**12*	1st	2AF	5' flanking (-238)	F	GCA CCC CTC CTG AAG TAC CAC	Modified to accommodate **34* assay
	1st	2A6ex3R1	Ex3/In3 (1826)	R	GTC CCC TGC TCA CCG CCA	
	2nd	2A6ex3R2	Exon 3 (1688)	R	CGC TCC CCG TTG CTG AAT A	
	2nd	2A61F-L	5' flanking (-155)	F	TGG CTG TGT CCC AAG CTA GGC A	
	2nd	2A71F-L	Exon 1 (351)	F	TGG CTG TGT CCC AAG CTA GGT G	
**1B*	1st	2A6in6F1	Intron 6 (4892)	F	ATTTCCTGCTCTGAGACC	2A6R11 less specific for *CYP2A6*
	1st	2A6R11	3' flanking (8515)	R	CAT CAA GCC CTG CCG TAT	
**17, *28, *35*	1st	2A6in6F1	Intron 6 (4892)	F	ATTTCCTGCTCTGAGACC	2A6R6 close to *4H crossover
	1st	2A6R6	3' flanking (8019)	R	TAATTGGGTTGTTTTCTATTGAGT	
**35*	2nd	2A6R0	3' flanking (7783)	R	AGGTCATCTAGATTTTCTCCTACA	2A6R0 will not bind if **4H* positive sample
	2nd	2A6in8ex9F6458W	In8Ex9 (6443)	F	TCCTCAGGAAAGCGGA	
	2nd	2A6in8ex9F6458V	In8Ex9 (6443)	F	TCCTCAGGAAAGCGGT	
**4H*	1st	2Aex7F	Exon 7 (5010)	F	GGC CAA GAT GCC CTA CAT G	2A6R11 less specific for *CYP2A6*
	1st	2A6R11	3' flanking (8515)	R	CAT CAA GCC CTG CCG TAT	

a: Refer to Table 1 and Supplementary Table 3 for current assay primers

F: Forward primer; R: Reverse primer

Supplementary Table 3. Current Versions of *CYP2A6* Endpoint PCR Assay Primers

Assay	Step	Primer Name	Location	F/R	Sequence (5' to 3')
**9, *31*	1st	2A65Pr1F	5' flanking (-1417)	F	ACCTAGACTTAATCTTCCCGTATAC
	1st	2A6in1R	5' flanking (324)	R	CCCAAGATCCTGTCTTTCTGAT
**9, *31*	2nd	2A6-460F	5' flanking (-466)	F	ATCCTCCACAACAGAAGACCCCTAA
**9*	2nd	2A6-17RA	5' flanking (-26)	R	ACGGCTGGGGTGGTTTGCCTTTA
	2nd	2A6-17RC	5' flanking (-26)	R	ACGGCTGGGGTGGTTTGCCTTTC
**31*	2nd	2A6ex1RW	Exon 1 (36)	R	CAGCAAGGCCACCAGAAGCAT
	2nd	2A6ex1RV	Exon 1 (36)	R	CAGCAAGGCCACCAGAAGCAG
**2, *24, *25, *26*	1st	2A61F	Exon 1 (144)	F	GCTGAACACAGAGCAGATGTACA
	1st	2A61R	Exon 4 (2199)	R	GGAGGTTGACGTGAACTGGAAGA
**2, *24, *25*	2nd	E3R-1	Intron 3 (1882)	R	AACGCGCGCGGGTTCCTCGT
**2*	2nd	2A62wtF	Exon 3 (1786)	F	CTCATCGACGCCCT
	2nd	2A62v1F	Exon 3 (1786)	F	CTCATCGACGCCCA
**24*	2nd	2A6ex2Fwt	Exon 2 (579)	F	GCCACCTTCGACTGGG
	2nd	2A6ex2Fv	Exon 2 (579)	F	GCCACCTTCGACTGGC
**25*	2nd	2A6in2ex3FW	In2/Ex3 (1653)	F	CACCTCCCCAGGCGTGGTAT
	2nd	2A6in2ex3FV	In2/Ex3 (1653)	F	CACCTCCCCAGGCGTGGTAC
**26*	2nd	2A6ex2Fwt	Exon 2 (579)	F	GCCACCTTCGACTGGG
	2nd	2A6ex3R1711w	Exon 3 (1726)	R	GCAGGGTGGCGATGGA
	2nd	2A6ex3R1711v	Exon 3 (1726)	R	GCAGGGTGGCGATGGC
**20, *23, *27*	1st	2A6exin3F	Ex3/In3(1804)	F	GGCACTGGCGGTGAGCAG
	1st	2A6in5R	Intron 5 (3638)	R	GGCCTGTGTCATCTGCCT
**20, *27*	2nd	2A6in3F	Intron 3 (1969)	F	CTGCCTCCTGGAATTCTGAC
**20*	2nd	2A6ex42144Rw	Exon 4 (2158)	R	ACAGTGACAGGAACTCTT
	2nd	2A6ex42144Rv	Exon 4 (2158)	R	ACAGTGACAGGAACTCTG
**27*	2nd	2A6*4171w-M[a]	Exon 4 (2182)	R	GGAAGATTCCTAGCATCCTG
	2nd	2A6*4171v-M[a]	Exon 4 (2182)	R	GGAAGATTCCTAGCATCCTT
**23*	2nd	5M13FOR-H2	Intron 4 (2260)	R	GCA GTT GGC AGG TTG TGG TAG G
	2nd	2A6ex42161Fw-M[c]	Exon 4 (2139)	F	CAA AGA GTT CCT GTC ACT GTC GC
	2nd	2A6ex42161Fv-M[c]	Exon 4 (2139)	F	CAA AGA GTT CCT GTC ACT GTC GT
**12, *34*	1st	2AinIF-L	Intron 1 (316)	F	GATCTTGGGATGTCCAGCTCC
	1st	2A6in5R	Intron 5 (3638)	R	GGCCTGTGTCATCTGCCT
**12, *34*	2nd	2A6inex5R	Ex5/In5(3559)	R	GGTGTACCTCCTGCATGC
**12*	2nd	2A6in1/ex2	In1/In2(436)	F	ACCTCCATCAGATCAGTGAGC
	2nd	2A7in1/ex2	In1/In2(954)	F	GCCTCCATCAGTTCAGTGAGT
**34*	2nd	2A6in4F	Intron 4 (2573)	F	CAACCGGCCTCCTGCATA
	2nd	2A7in4F	Intron 4 (2977)	F	CAACCCGCCTCCTGCATG
**1B, *17, *28, *35*	1st	2A6in6F1	Intron 6 (4892)	F	ATTTCCTGCTCTGAGACC
	1st	2A6R13	3' flanking (8548)	R	GCCTCCCATAGTGCTATAATTAACA
**1B*	2nd	2A6R12	3' flanking (8278)	R	ATT GTC TTT CAA AGT AGC TGT GT
	2nd	2A6*1Bwt	3' flanking (6719)	F	ACT GGG GGC AGG ATG GC
	2nd	2A6*1Bvar	3' flanking (7110)	F	AAT GGG GGG AAG ATG CG
**17*	2nd	2A6in7AS	Intron 7 (5426)	R	CTGAGATTTCTGTCCCTAT
	2nd	2A6*17Fwt-M[a]	Exon 7 (5044)	F	GAGATCCAAAGATTTGGAGCCG
	2nd	2A6*17Fv-M[a]	Exon 7 (5044)	F	GAGATCCAAAGATTTGGAGCCA
**28*	2nd	2A6in7F1	Intron 7 (5200)	F	ACCCACATTAGAAGCTTTCTAGA
	2nd	2A6ex8R2wt	Exon 8 (5769)	R	TCTTCTTAAACTGCCCCTTC
	2nd	2A6ex8R2v	Exon 8 (5769)	R	TCTTCTTAAACTGCCCCTTG
	2nd	2A6R12	3' flanking (8278)	R	ATT GTC TTT CAA AGT AGC TGT GT

**35*	2nd	2A6in8ex9F6458w	In8Ex9 (6443)	F	TCCTCAGGAAAGCGGA
	2nd	2A6in8ex9F6458v	In8Ex9 (6443)	F	TCCTCAGGAAAGCGGT
**4H*	1st	2Aex7F	Exon 7 (5010)	F	GGC CAA GAT GCC CTA CAT G
	1st	2A6R13	3' flanking (8548)	R	GCCTCCCATAGTGCTATAATTAACA
**4H*	2nd	2A6R12	3' flanking (8278)	R	ATTGTCTTTCAAAGTAGCTGTGT
	2nd	2A6in7F1	Intron 7 (5200)	F	ACCCACATTAGAAGCTTTCTAGA
	2nd	2A7in7F1	Intron 7 (5594)	F	CCCCATTAGAAGCTTTCTACTCA
**4B*	1st	2A6/74B-S	3' flanking (10679)	F	CAAGATATATCCACACTCCTATG
	1st	2A6Rdup	3' flanking (13776)	R	AATTCCTGGATTGACAAGAG
**4B*	2nd	2A6Rdup-2	3' flanking (13216)	R	ACAAAAGTTGTGCAATAGCA
	2nd	2A6FR4.2KS	3' flanking (10770)	F	GATAAAGAAAATGTGGATAGAATGG
	2nd	2A7FR5.4KS	3' flanking (12450)	F	GATAAAGAAAATGTGGTACATTC
**7, *8, *10*	1st	2A6in6F1	Intron 6 (4892)	F	ATTTCCTGCTCTGAGACC
	1st	2A6R6	3' flanking (8019)	R	TAA TTG GGT TGT TTT CTA TTG AGT
**7*	2nd	2A6R0	3' flanking (7783)	R	AGGTCATCTAGATTTTCTCCTACA
	2nd	2A6*7Fwt-M[a]	Exon 9 (6539)	F	TCCCAGTCACCTAAGGAAAT
	2nd	2A6*7Fv-M[a]	Exon 9 (6539)	F	TCCCAGTCACCTAAGGAAAC
**8*	2nd	2A6R0	3' flanking (7783)	R	AGGTCATCTAGATTTTCTCCTACA
	2nd	2A6*8wtF	Exon 9 (6582)	F	GCTTTGCCACGATCCCACG
	2nd	2A6*8vF	Exon 9 (6582)	F	GCTTTGCCACGATCCCACT
**10*	2nd	2A6*7Fwt-M[a]	Exon 9 (6539)	F	TC CCA GTC ACC TAA GGA AAT
	2nd	2A6*7Fv-M[a]	Exon 9 (6539)	F	TC CCA GTC ACC TAA GGA AAC
	2nd	2A6*8Rwt-L	Exon 9 (6620)	R	GGAAGCTCATGGTGTAGTTTC
	2nd	2A6*8Rv-L	Exon 9 (6620)	R	GGAAGCTCATGGTGTAGTTTA
**1x2A*	1st	2Aex7F	Exon 7 (5010)	F	GGCCAAGATGCCCTACATG
	1st	2A7R11	3' flanking (8800)	R	GTGCAGAGGTTTTTGTGTGACTG
**1x2A*	2nd	2A7R12	3' flanking (8687)	R	TTTGTCTTCCAAAGTAGCTGTGC
	2nd	2A7in7F1	Intron 7 (5594)	F	CCCCATTAGAAGCTTTCTACTCA
	2nd	2A6in7F1	Intron 7 (5200)	F	ACCCACATTAGAAGCTTTCTAGA
**1x2B*	1st	2A6F3	3' flanking (7076)	F	TAG ACA GAT TCT TAA AAA GCA CCT
	1st	2A 6/7R	3' flanking (13821)	R	CTG GAT TCT TGG GCA TTC AAC CCA
**1x2B*	2nd	2A6F0	3' flanking (7735)	F	TGA GTA CAA AAC TTC TAG AAG ATA AT
	2nd	2A6R dup	3' flanking (13776)	R	AAT TCC TGG ATT GAC AAG AG
	2nd	2A7R dup	3' flanking (15131)	R	AAT TCC TGG ATT GAC GAG AC

a: Mismatch primer at third position from 3'end, underlined in sequence

F: Forward primer; R: Reverse primer

Supplementary Table 4. Concordance between endpoint (Method 1) and SYBR green (Method 2) genotyping assays

	SYBR green (Method 2)		Endpoint PCR (Method 1)		Concordance	Number of Samples[b]	Number of Datasets[c]
	1st amp primers	2nd amp primers	1st amp primers	2nd amp primers			
*CYP2A6*9*	2A65Pr1F 2A6in5R	2A6*9wtR-Sybr-M 2A6*9vR-Sybr-M 125M13BEV-B	2A65Pr1F 2A6in1R	2A6-460F 2A6-17RA 2A6-17RC	100%[a]	189	2 sets
*CYP2A6*31*	2A65Pr1F 2A6in5R	2A6*31wtR-Sybr 2A6*31vR-Sybr 2A61F-L	2A65Pr1F 2A6in1R	2A6-460F 2A6ex1Rw 2A6ex1Rv	100%[a]	92	5 sets
*CYP2A6*24*	2A65Pr1F 2A6in5R	2A6*24wtR-Sybr-M 2A6*24vR-Sybr-M 2A6ex2-505F	2A61F 2A61R	2Aex2Fwt 2Aex2Fv E3R-1	100%[a]	23	3 sets
*CYP2A6*20*	2A65Pr1F 2A6in5R	2A6*20wtR-sybr-M 2A6*20vtR-sybr-M 2A6in3F-L	2A6exin3F 2A6in5R	2A6in3F 2A6ex42144Rw 2A6ex42144Rv	100%[a]	49	5 sets
*CYP2A6*23*	2A65Pr1F 2A6in5R	2A6ex42161Fw-M 2A6ex42161Fv-M-L 5M13FOR-H2	2A6exin3F 2A6in5R	2A6ex42161Fw-M 2A6ex42161Fv-M 5M13FOR-H2	100%[a]	16	3 sets
*CYP2A6*17*	2A6in6F1 2A6R13	2A6*17Fwt-Sybr-M 2A6*17Fv-Sybr-M 2A6in7-17R2	2A6in6F1 2A6R13	2A6*17Fwt-M 2A6*17Fv-M 2A6in7AS	100%	127	5 sets
*CYP2A6*35*	2A6in6F1 2A6R13	2A6*35wtR-Sybr 2A6*35vR-Sybr 2A6in8-6395F	2A6in6F1 2A6R13	2A6in8ex9F6458w 2A6in8ex9F6458v 2A6R12	100%	119	5 sets
*CYP2A6*12*	2Ain1F-L 2A6in5R	2A6in1/ex2-L 2A7in1/ex2-L 2A6R-667	2AinF-L 2A6in5R	2A6in/ex2 2A7in/ex2 2A6inex5R	100%	16	3 sets
*CYP2A6*34*	2Ain1F-L 2A6in5R	2A6-2572F 2A7-2978F 2A6-2862R	2AinF-L 2A6in5R	2A6in4F 2A7in4F 2A6inex5R	100%	16	3 sets
*CYP2A6*1B*	2A6in6F1 2A6R13	2A6*1Bwt 2A6*1Bvar-L 2A6R6944	2A6in6F1 2A6R13	2A6*1Bwt 2A6*1Bvar 2A6R12	100%	35	2 sets

a: SYBR green assays also tested in novel population of ~200 African Americans and ~200 European Americans and genotypes found to be in Hardy-Weinberg Equilibrium

b: All samples were from de-idenified DNA datasets acquired with ethical approval from University of Toronto's IRB.

c: Number of unique datasets that samples were drawn from to test concordance