

"CYP2C9 promoter variable number tandem repeat polymorphism (pVNTR) regulates mRNA expression in human livers" by D. Wang, X. Sun, Y. Gong, B.E. Gawronski, T.Y. Langaee, M.H.A. Shahin, S.I. Khalifa and J.A. Johnson, Drug Metabolism and Disposition

Supplemental Table 1. Primers and PCR conditions

1. Genotyping using Snapshot

PCR primers

SNP1 & SNP2	forward reverse condition	TGTCCCACTGTAAGGTTTTTCAGG TGCCCTTGTTTCGTTGTTTCTATT Denville PCR mix, 60°C annealing, ext 2min
SNP3 & SNP4	forward reverse condition	CAAAGAGAAAACACCAGACCCATA CACCTGTGGATATGATGATGAGAC Denville PCR mix, 60°C annealing, ext 1 min
SNP5 & SNP6	forward reverse condition	AGTATCATTTATATTAGCACC TGCATTTTATATTTGGATCTA Denville PCR mix, 56°C annealing, ext 1.5 min
SNP7, SNP9-SNP11	forward reverse condition	GGTCTTTTCAACGAAGACTAATGGAGT CAAGCCCTAGCAACAAATAATC Takara Primestar HS, 68°C annealing, ext 1.5 min
SNP12	forward reverse condition	TTTCAAAGCCTACTCTAATCCACC CACATCTCAGTCCAAATGATCAGG Sigma PCR mix, 60°C annealing, ext 1min
SNP13 - SNP18	forward reverse condition	AAGGTCTAGGAAGGAGCCGC GCCACACAGTCATAGCTGG Sigma PCR mix, 60°C annealing, ext 1.5 min
SNP19 & SNP 20	forward reverse condition	TCCCTCCTAGTTTCGTTTCTCTTC AAGGTCAGTGATATGGAGTAGGGT Sigma PCR mix, 55°C annealing, ext 1min
SNP21	forward reverse condition	TCTGGTTAGAATTGATCCTCTGGT ACAAATCACAATTCACAAGCAG Sigma PCR mix, 55°C annealing, ext 1min
SNP22 - SNP24	forward reverse condition	TGTTTGGATACCTTCATGATTCA GGAGTTGCAGTGTAGGAGAAACA Sigma PCR mix, 55°C annealing, ext 1min
SNP25	forward reverse condition	CACTGTTCTGAATGCCTGTGTACA AAGAATTGGATTAACCCCAAAGT Sigma PCR mix, 60°C annealing, ext 1min

SNP26 - SNP28	forward	AGGAGTAACTGCTCTCTGTGTTTGCTA
	reverse	TGAAACATAGGAACTCTCCGTAAT
	condition	Sigma PCR mix, 55°C annealing, ext 1min

Primer extension primers

SNP1	TGTGTTCTTCTACGACTCATTTTCTTTTA
SNP2	GCACATAATACGGAACTACAAAAAGTA
SNP3	CCCCATAAAATGAGTTAAGAATAGAAGAATT
SNP4	TTTTTTTTTTTAAATAAAAGATTAGCAAATTGCATCCAAT
SNP5	GATATAAATAAATACACAAATATTTTCATGTTTCATG
SNP6	TCGAGACCATCCTGGCTAACA
SNP7	TTTTACTACAATGAAGGTATAATCCATGAAATAAAGAAT
SNP9	CATTGGGGTTTTAGTTTGCAGGTC
SNP10	TTTTTTTTTTTTGTTTACCACATTATCTGGTAGTTGTGCTC
SNP11	AAGGCTACATACTGTATGATTCCAACC
SNP12	TTTTTTTTTTGGAGTATCAACATTAAGCCCTCCA
SNP13	TTTTTTTTTTTTTTCATGAGTCAGGGACCAAGTTA
SNP14	TTTTTTTTTTTTTTTTTTTTTTTTTGTCTTTCTTGCCTGTATAAAGG
SNP15	TCAAGGCTCAGCTTCCTCATTC
SNP16	TTTTTTTTTTGTCCCTTTGAATCTCTCAATTACCT
SNP17	TTTTTTTGTGATTTCCCTACCTCCCATCTT
SNP18	CGTTTCACTTCTGCAGTGATGGA
SNP19	TTTTTTTTTTTTTTTTTTTTTTGGGAAGAGGAGCATTGAGGAC
SNP20	TTTTTTTTTTTTTTTTTTTTTTTTTCTCAACTCCTCCACAAGGCAG
SNP21	TTGCTTCTGATGAAATGGAGA
SNP22	TGCATGCAGGGGCTCC
SNP23	GTGCACGAGGTCCAGAGATAC
SNP24	CAGGCTGGTGGGAGAAG
SNP25	GCCCAATAATTAGGATGTATCATGA
SNP26	TTTTTGCGGCACAGAGGCAAA
SNP27	TTTTTTTTTTTTTCTTCTACCAGCTGTGCTTCATT
SNP28	TTTTTTTTTAATGCCTTTTCTCACCTGTATCT

note: for multiplex purpose, different number of Ts were added to the 5' to adjust the length of primers

Multiplex groups:

group 1: SNP1+SNP3+SNP4

group2: SNP2+SNP5+SNP6

group3: SNP7+SNP9

group4: SNP10+SNP11+SNP12

group5: SNP13+SNP14+SNP15+SNP16+SNP17+SNP18

group6: SNP19+SNP20+SNP21+SNP22+SNP23

group7: SNP24+SNP26+SNP27+SNP28

2. Genotyping using allele specific PCR

SNP8	forward (common)	CTTGATTTAAAAATGGGCAACAGAT
	reverse (wt)	CAGGTCCGTAGTGACGTTTAATCAT
	Reverse (SNP)	CAGGTCCGTAGTGACGTTTAATCAC
	condition	Cyber green mix, 60°C annealing, ext 1min

3. Genotyping using FAM labeled primer

promoter repeat	forward (FAM)	TGTAGTCCCAGGTTGTCAAGAGG
	reverse	CCAGTCTCTGTCTTTTCATCTCATTC
	condition	Sigma Jumpstart PCR mix, 60°C annealing, ext 1 min
	PCR products	long=512~522, medium=453~461, short=417~434

4. Sequencing

PCR primers

Promoter fragment 1 -6343 to -3518	forward	AGGGAACCAGAGAAAGAAGGACA
	reverse	TGGATTTTAGCCATTGTATAAGTGTGTAG
	condition	Takara prime star HS, 68°C annealing, ext 3min
Promoter fragment 2 -3646 to -44	forward	CTCACCCAAGAAGAAATACAGATGG
	reverse	CCTTTTATAAACTCCATGCTAATTCG
	condition	Takara prime star HS, 68°C annealing, ext 4min

Sequencing primers

fragment 1

seq 1	AGGGAACCAGAGAAAGAAGGACA
seq 2	GAAAAGTCTCATCATCATATCCACAGG
seq 3	AGTATCATTTATATTAGCACC
seq 4	TGGATTTTAGCCATTGTATAAGTGTGTAG

fragment 2

seq 5	CTCACCCAAGAAGAAATACAGATGG
seq 6	TTCAAAAGCCTACTCTAATCCACC
seq 7	AAGGTCTAGGAAGGAGCCGC
seq 8	GTTAGAATCCCTGTTAAAAATGACCAGTAA
seq 9	CCTTTTATAAACTCCATGCTAATTCG

5. Primers for cloning

reporter gene, 5P -4512 to -3025	forward (add XhoI site)	CCGCTCGAGCGGTCTTTTCAACGAAGACTAATGGAGT
	reverse (add NcoI site)	CATGCCATGGTGG CAAGCCCTAGCAACAAATAATC
	condition	Takara prime star HS, 68°C annealing, ext 1.5 min
reporter gene, 3P -3646 to -48	forward (add XhoI site)	CCGCTCGAGCCTCACCCAAGAAGAAATACAGATGG
	reverse (add NcoI site)	CATGCCATGGTGG CCTTTTATAAACTCCATGCTAATTCG
	condition	Takara prime star HS, 68°C annealing, ext 4 min
repeat region topo clon	forward	GGTCTTTTCAACGAAGACTAATGGAGT
	reverse	TGGATTTTAGCCATTGTATAAGTGTGTAG
	condition	Takara prime star HS, 68°C annealing, ext 1.5 min
CAR V1 cDNA	forward	AGATCAGAGGAAAACCAGCAACAG
	reverse	CCAGTGTATCCAGGGTGTCCA
	condition	Sigma Jump start PCR mix, 60 C annealing, ext 1.5 min
HNF1 α cDNA	forward	GGCAGCCGAGCCATGGTTTC
	reverse	GCCCAGGTGCCGTGGTACTG

	condition	Takara prime star HS, 68°C annealing, ext 2 min
HNF4 α cDNA	forward	GGCGTGGAGGCAGGG
	reverse	TTAGAACAGTGACTGGCACGTG
	condition	Sigma Jump start PCR mix, 60 C annealing, ext 1.5 min
CEBPA	forward	CTGCCATGCCGGGAG
	reverse	AGATCCGGCGACCCAA
	condition	Sigma Jump start PCR mix, 60 C annealing, ext 2min
PXR v1 cDNA	forward	GGAGCCGCTTAGTGCCTACA
	reverse	GAGGTAGCAATGAAAAGACTCAGGAAG
	condition	Takara prime star HS, 68°C annealing, ext 2 min
RXR α cDNA	forward	CGGGCATGAGTTAGTCGCA
	reverse	CAACACATCTCTTAGGCAGAGCA
	condition	Takara prime star HS, 68°C annealing, ext 2 min
GATA4 cDNA	forward	CATATTATCGTTGTTGCCGTCG
	reverse	GATTACGCAGTGATTATGTCCCC
	condition	Takara prime star HS, 68°C annealing, ext 2 min