

human UGT2 cluster detection

	Primer	Sequence (5'-3')	
A	UGT2A1_1L	TCTTCTGCATCAAGCCACATCA	122bp
	UGT2A1_1R	AGCCAATGACTACCTTCCATTG	
B	UGT2B7_1L	CCAGCAAGAAAGATTGTGATGC	138bp
	UGT2B7_1R	TTCTAACCATGAAGTGGTGGT	
C	UGT2B28_1L	CATTTGAAGCTGGAAAACGAGA	164bp
	UGT2B28_1R	CCTGGGTGGTAAATCTCTGAAA	
D	TRANS_L1	TGGAGGCCATAAACAAAGAAGAC	408bp
	TRANS_R1	CCCCTTGACCCAGAAATTCCA	

rat Ugt2 KO genotyping

	Primer	Sequence (5'-3')	
E	rUgt2 BD250 L	GAAGGTAGCGTCAAGAGTACGAGGA	512bp
	rUgt2 BD250 R	ACCCTGCACACCCTCAGCTT	
F	rUgt2a3 L	GCAGCAAGAGGGCATGAGGT	189bp
	rUgt2a3 R	TGCTGCTTCCACAGTGACA	
G	rUgt2b7int3 L	GCCAGGTTCCACATCACAGT	554bp
	rUgt2b7int3 R	TGGCATCATTTGGTGGCTGT	
H	rUgt2b1ex1 L	TGGAGCCTGTGGAAAAGTGC	569bp
	rUgt2b1ex1 R	CCACAAATGTCATGCGGTCA	

A,B,C	Mix(Taq Gold)					
	MQ	5.45		Cycle	泳動	
	10 × Buffer	1		95°C	10min	10 μ L
	dNTPs	1		94°C	30sec	2% agarose
	20 μ M primer L	0.25	× *	62°C	30sec	100V
	20 μ M primer R	0.25		72°C	30sec	15min
	Taq Gold	0.05		72°C	5min	
	DNA(50ng/ μ L)	2		10°C	∞	
	Total	10 μ L				A:30, B:32, C:35 cycle

D	Mix (LA taq)					
	MQ	2.9		Cycle	泳動	
	2 × GCI buffer	6.25		98°C	1min	13.25 μ L
	dNTPs	2		94°C	10sec	2% agarose
	20 μ M primer L	0.5	× 35	60°C	30sec	100V
	20 μ M primer R	0.5		72°C	3min	15min
	LA taq	0.1		72°C	10min	
	DNA(50ng/ μ L)	1		10°C	∞	
	Total	13.25				

E	Mix(Taq Gold)					
	MQ	5.45		Cycle	泳動	
	10 × Buffer	1		95°C	10min	2% agarose
	dNTPs	1		95°C	30sec	100V
	20 μ M primer L	0.25	× 35	58°C	30sec	20min
	20 μ M primer R	0.25		72°C	1min	
	Taq Gold	0.05		72°C	5min	
	DNA(50ng/ μ L)	2		10°C	∞	
	Total	10 μ L				

F,G,H	Mix(Taq Gold)					
	MQ	5.45		Cycle	泳動	
	10 × Buffer	1		95°C	10min	10 μ L
	dNTPs	1		95°C	30sec	2% agarose
	20 μ M primer L	0.25	× 35	60°C	30sec	100V
	20 μ M primer R	0.25		72°C	30sec	15min
	Taq Gold	0.05		72°C	5min	
	DNA(50ng/ μ L)	2		10°C	∞	
	Total	10 μ L				

Genotyping まとめ

	WT	MAC	KO	KO/MAC
A	×	○	×	○
B	×	○	×	○
C	×	○	×	○
D	×	○	×	○
E	×	×	○	○
F	○	○	×	×
G	○	○	×	×
H	○	○	×	×