

RMRC Genotyping Protocol-PCR

No.	RMRC13403	Strain Name	B6;129-Cyp4v3 ^{em1Sphcc} /Narl
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A. Primer

	Primer name	Sequence(5'→3')
1	Cyp4v3-F	GAA TTT GTC CCT CCC TCC TCT GC
2	Cyp4v3-R	CCT CTG AGT AAC GTC ACA CAG TG
3	Cyp4v3-exon7-F	CGT CTG CAC TGT TCC TCA TTA C
4	Cyp4v3-intron7-R	CAG GTA GAT ACT GGG ACT TGA ACC

B. Genomic extraction :

- Phenol-Chloroform extraction commercial extraction kit _____
 Lysis buffer Direct PCR Buffer other _____

C. Reaction Conditions

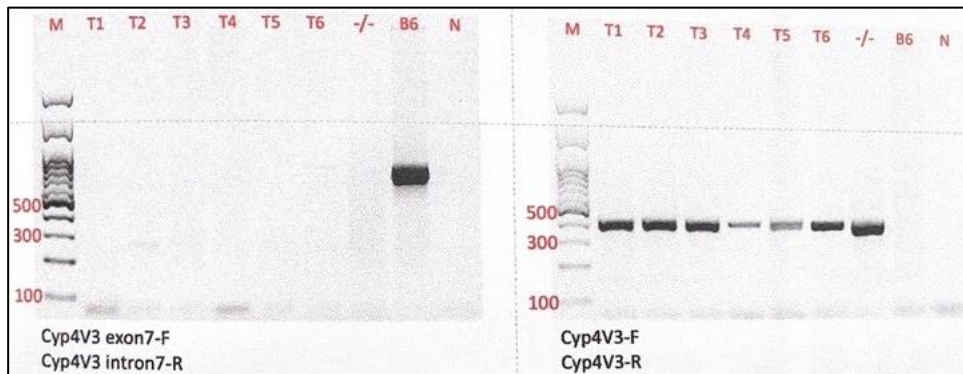
Component	Concentration		Volume		Step	Tempera- ture, °C	Time, sec	Number of Cycles
H ₂ O	--		20.5	μl	1	94	180	1
PCR buffer	10x		3	μl	2	94	30	35
dNTP	10	mM	0.6	μl	3	64	35	35
Primer 1	10	μM(ρmol/μl)	0.8	μl	4	72	35	35
Primer 2	10	μM(ρmol/μl)	0.8	μl	5	72	180	1
DNA	--		2	μl	6			
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺	25	mM	1.8	μl	8			
Total			30	μl	9			

The information of Taq, including company name and catalog number : GeneDireX (MB101-0500)

D. PCR Product

	Primer set		Product size	Target region
1	Cyp4v3-F	Cyp4v3-R	366 bp	
2	Cyp4v3-exon7-F	Cyp4v3-intron7-R	724 bp	

E. Gel Photo



Number	Genotype
T1	-/-
T2	-/-
T3	-/-
T4	-/-
T5	-/-
T6	-/-
B6	+/+