

RMRC Genotyping Protocol-PCR

種原編號	RMRC13086	品系名稱	C57BL/6- <i>Cyp11a1</i> ^{tm8Bcc}
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A. Primer

	Primer name	Sequence
1	SFKO(S)	GGCATTCACTGTAGAATGAC
2	SFKO(AS)	AGGCCTCTCTACACAATCAG
3	NEO5'	GAATATGATCGGAATTCCTCG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		11.4	μl	1	95	600	
PCR buffer	10x		2	μl	2	95	30	
dNTP	2	mM	2	μl	3	55	60	
Primer 1	5	μM(ρmol/μl)	1	μl	4	72	90	→2#35
Primer 2	5	μM(ρmol/μl)	1	μl	5	72	300	
DNA	--		0.5	μl	6	6	pause	
Taq	5	U/μl	0.1	μl	7			
Mg ²⁺	25	mM	2	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	SFKO(S)	SFKO(AS)	WT:327 bp Mutant: can't be amplified	Intron 1
2	NEO5'	SFKO(AS)	Mutant:320 bp WT: can't be amplified	Neo cassette, Intron 1

D. Gel Photo