

RMRC Genotyping Protocol-PCR

No.	RMRC13197	Strain Name	C57BL/6-Tg(aP2-luc)68Narl/Narl
No.	RMRC13198	Strain Name	C57BL/6-Tg(aP2-luc)73Narl/Narl

A. Primers

No.	Primer name	Sequence
1	LucF	TCACGCAGGCAGTTCTATGAGG
2	LucR	GAGGTGGACATCACTTACGCTGAG
3	Il2F	CTAGGCCACAGAATTGAAAGATCT
4	Il2R	GATGGTGGAAATTCTAGCATCATCC

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of cycles
H ₂ O	—	10.2 μ l	1	94	180	1
PCR buffer	10 x	1.5 μ l	2	94	30	35
Formamide	—	0.5 μ l	3	60	30	35
dNTP	10 mM	0.3 μ l	4	72	30	35
Primer 1	20 μ M (μ mol/ μ l)	0.3 μ l	5	72	180	1
Primer 2	20 μ M (μ mol/ μ l)	0.3 μ l				
Primer 3	10 μ M (μ mol/ μ l)	0.3 μ l				
Primer 4	10 μ M (μ mol/ μ l)	0.3 μ l				
gDNA	50-250 ng/ μ l	1 μ l				
Taq	5 U/ μ l	0.3 μ l				
Total		15 μ l				

C. PCR Product

No.	Primer set		Product size	Target region
1	LucF	LucR	511 bp	Luc
2	Il2F	Il2R	324 bp	Wild type control

D. Gel Photo

