

RMRC Genotyping Protocol-PCR

No.	RMRC13243	Strain Name	C57BL/6-Tg(Ins-UBC-GFP-Ins)2Narl
	RMRC13244		C57BL/6-Tg(Ins-UBC-GFP-Ins)5Narl
	RMRC13245		C57BL/6-Tg(Ins-UBC-GFP-Ins)7Narl
	RMRC13246		C57BL/6-Tg(Ins-UBC-GFP-Ins)12Narl
	RMRC13247		C57BL/6-Tg(Ins-UBC-GFP-Ins)13Narl
	RMRC13248		C57BL/6-Tg(Ins-UBC-GFP-Ins)14Narl

A. Primer

	Primer name	Sequence
1	GFP 3688F	5'- AACTCCAGCAGGACCATGTGATCG -3'
2	GFP 4069B	5'- GCACCATCTTCTTCAAGGACGAC -3'
3	β -actinF	5'- GAACATGGCATTGTTACCAACTGG -3'
4	β -actinB2	5'- AAGGGAGTCCTATGGGAGAACG -3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		11.2	μ l	1	95	120	1
PCR buffer	10x		1.5	μ l	2	95	35	35
dNTP	10	mM	0.3	μ l	3	60	35	35
Primer 1	20	μ M(ρ mol/ μ l)	0.3	μ l	4	72	50	35
Primer 2	20	μ M(ρ mol/ μ l)	0.3	μ l	5	72	600	1
Primer 3	20	μ M(ρ mol/ μ l)	0.3	μ l	6			
Primer 4	20	μ M(ρ mol/ μ l)	0.3	μ l	7			
DNA	--		0.5	μ l	8			
Taq	5	U/ μ l	0.3	μ l	9			
Mg ²⁺		mM		μ l				
Total			15	μ l				

C. PCR Product

	Primer set		Product size	Target region
1	GFP3688F	GFP4069B	382 bp	
2	β -actinF	β -actinB2	245 bp	

D. Gel Photo

