

RMRC Genotyping Protocol-PCR

No.	RMRC13168	Strain Name	C57BL/6-Tg(TRE-emGFP)4IBMS/BTU
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A. Primer

	Primer name	Sequence
1	TRE-emGFP-F	5'GACGAGGCCACCATGGTGAGC3'
2	TRE-emGFP-R	5'ACCGGTCTTGTACAGCTCGTCCATGCC 3'
3	Ctrl-F(oIMR8744)	5' CAA ATG TTG CTT GTC TGG TG3'
4	Ctrl-R(oIMR8744)	5' GTC AGT CGA GTG CAC AGT TT3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--			μl	1	94	180	
PCR buffer	10x		1	μl	2	94	30	
dNTP	5000	μM	1	μl	3	55	30	25
Primer 1	10	μM(μmol/μl)	1	μl	4	72	30	
Primer 2	10	μM(μmol/μl)	1	μl	5	72	300	
DNA	--			μl	6	4	stop	
Taq	1	U/μl	1	μl	7			
Mg ²⁺	30	mM	1	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	Tg		730 bp	GFP
2	Ctrl		200 bp	Internal control

D. Gel Photo

