

RMRC Genotyping Protocol-PCR

No.	RMRC13014	Strain Name	BALB/cByJ-Tg(Pgk1-EGFP)01Narl
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A. Primer

	Primer name	Sequence
1	5GFP	tgcccatcctggctcgagctgga
2	3GFP	cacgaactccagcaggacctg
3	CP-1_F	gccagcttgagttacagaatgg
4	CP-1_R	taaacagactccacagcctcca

CP-1_F & _R as PCR control primer set.

B. Reaction Conditions

Component	Concentration		Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		17.5 µl	1	95°C	300	1
PCR buffer	--			2	95°C	40	34
dNTP	--	mM		3	58°C	40	34
5GFP	10 µM	µM(ρmol/µl)	0.5 µl	4	72°C	40	34
3GFP	10 µM	µM(ρmol/µl)	0.5 µl	5	72°C	120	1
CP-1_F	10 µM	µM(ρmol/µl)	0.25 µl	6			
CP-1_R	10 µM	µM(ρmol/µl)	0.25 µl	7			
DNA	5~100 ng		1 µl	8			
Taq	--	U/µl		9			
Mg ²⁺	--	mM		10			
Total			20	µl			

Use Bioneer corp. premix. Content is as below,

Component	Reaction size	20 µl reaction
Top DNA polymerase		1 U
Each: dNTP (dATP, dCTP, dGTP, dTTP)		250 µM
Tris-HCl (pH 9.0)		10 mM
KCl		30 mM
MgCl ₂		1.5 mM
Stabilizer and tracking dye		

C. PCR Product

	Primer set		Product size	Target region
1	5GFP	3GFP	637 bp	EGFP
2	CP-1_F	CP-1_R	494 bp	control

D. Gel Photo

