

RMRC Genotyping Protocol-PCR

No.	RMRC13001	Strain Name	FVB/NCr1-Tg(Pgk1-EGFP)01Narl
No.	RMRC13002	Strain Name	C57BL/6J-Tg(Pgk1-EGFP)03Narl

A. Primer

	Primer name	Sequence
1	5GFP	tgcccatcctggtcgagctgga
2	3GFP	cacgaactccagcaggaccatg
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 μ l	μ l	1	95°C	300	1
PCR buffer	--			μ l	2	95°C	40	34
dNTP	--	mM		μ l	3	58°C	40	34
5GFP	10 μ M	μ M(ρ mol/ μ l)	0.5 μ l	μ l	4	72°C	40	34
3GFP	10 μ M	μ M(ρ mol/ μ l)	0.5 μ l	μ l	5	72°C	120	1
CP-1_F	10 μ M	μ M(ρ mol/ μ l)	0.25 μ l	μ l	6			
CP-1_R	10 μ M	μ M(ρ mol/ μ l)	0.25 μ l	μ l	7			
DNA	5~100 ng		1 μ l	μ l	8			
Taq	--	U/ μ l		μ l	9			
Mg ²⁺	--	mM		μ l	10			
Total			20	μ l				

Use Bioneer corp. premix. Content is as below,

Component	Reaction size	20 μ l reaction
Top DNA polymerase		1 U
Each: dNTP (dATP, dCTP, dGTP, dTTP)		250 μ M
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

