

## RMRC Genotyping Protocol-PCR

No.	RMRC13340	Strain Name	B6.129-Scube1 <sup>tm3Rbya</sup> /Narl
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### A. Primer

	Primer name	Sequence
1	F1	5'-cct agg tga gcg gtg ggt aat-3'
2	R1	5'-ggg tgc ctg agc gac ata aga c-3'
3	F2	5'-gcc aga gga aga aag gaa gcg cga-3'
4	R2	5'-ctc att tgt atg cta caa ggg gta-3'

### B. Reaction Conditions

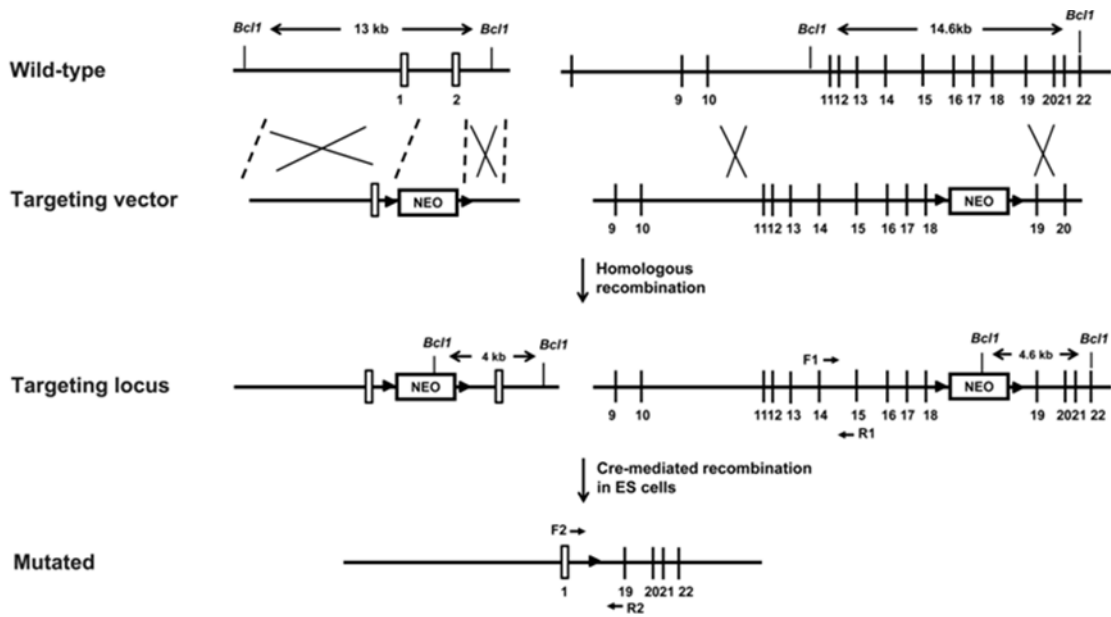
Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H <sub>2</sub> O	--		14	μl	1	94	300	
PCR buffer	10x		2	μl	2	94	30	
dNTP	10	mM	0.5	μl	3	57	30	
F1	10	μM(ρmol/μl)	0.5	μl	4	72	30	Go to step 2 for 35 cycles
R1	10	μM(ρmol/μl)	0.5	μl	5	72	300	
DNA	100 ng/μl		2	μl	6	4	forever	
Taq	5	U/μl	0.5	μl	7			
Mg <sup>2+</sup>		mM		μl	8			
Total			20	μl	9			

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H <sub>2</sub> O	--		14	μl	1	94	300	
PCR buffer	10x		2	μl	2	94	30	
dNTP	10	mM	0.5	μl	3	60	30	
F2	10	μM(ρmol/μl)	0.5	μl	4	72	60	Go to step 2 for 35 cycles
R2	10	μM(ρmol/μl)	0.5	μl	5	72	300	
DNA	100 ng/μl		2	μl	6	4	forever	
Taq	5	U/μl	0.5	μl	7			
Mg <sup>2+</sup>		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	F1	R1	403 bp	As sown in below
2	F2	R2	811 bp	As sown in below

D. Map of targeting strategy



E. Gel photo

