

RMRC Genotyping Protocol-PCR

No.	RMRC13341	Strain Name	B6.129-Scube1 ^{tm2Rbya} /Narl
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A. Primer

	Primer name	Sequence
1	E64	5'-cct agg tga gcg gtg ggt aat-3'
2	E65	5'-ggg tgc ctg agc gac ata aga c-3'
3	E91	5'-gag gca ggt gga tct ttg tga gt-3'
4		

B. Reaction Conditions

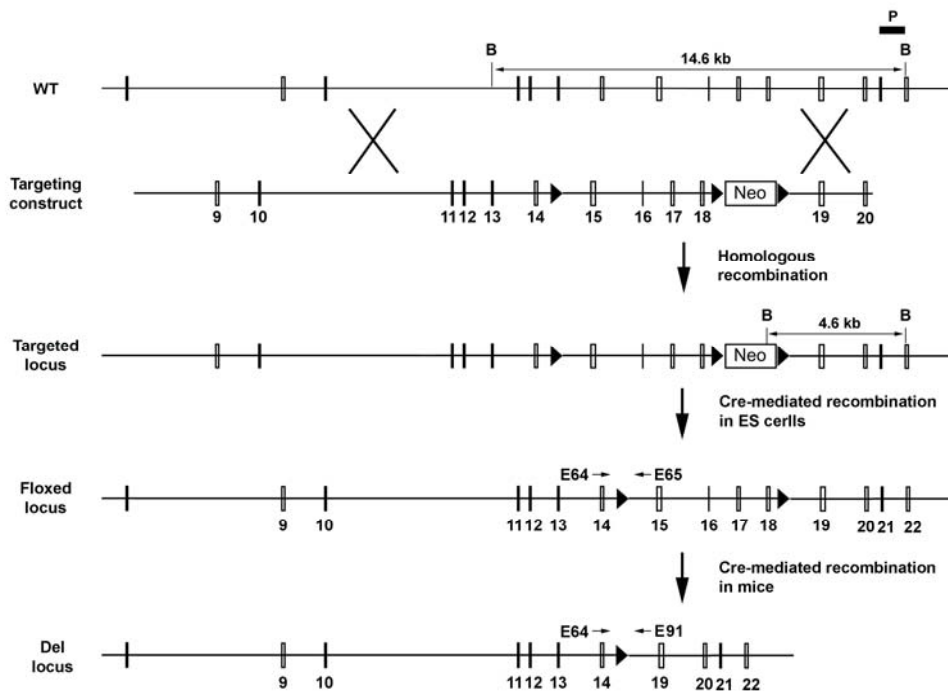
Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H ₂ O	--		14	μl	1	94	300	
PCR buffer	10x		2	μl	2	94	30	
dNTP	10	mM	0.5	μl	3	57	30	
E64	10	μM(ρmol/μl)	0.5	μl	4	72	30	Go to step 2 for 35 cycles
E65	10	μM(ρmol/μl)	0.5	μl	5	72	300	
DNA	100 ng/μl		2	μl	6	4	forever	
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺		mM		μl	8			
Total			20	μl	9			

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H ₂ O	--		14	μl	1	94	300	
PCR buffer	10x		2	μl	2	94	30	
dNTP	10	mM	0.5	μl	3	68	30	
E64	10	μM(ρmol/μl)	0.5	μl	4	72	60	Go to step 2 for 35 cycles
E91	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 ²⁺		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	E64	E65	403 bp	As shown in below
2	E64	E91	~400 bp	As shown in below

D. Map of targeting strategy



E. Gel photo

