

RMRC Genotyping Protocol-PCR

No.	RMRC13135	Strain Name	B6.129-(Cg)-Gt(ROSA)26Sor ^{tm4(ACTB-tdTomato,-EGFP)Luo/JNarl}
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A. Primer

	Primer name	Sequence
1	oIMR7318	CTC TGC TGC CTC CTG GCT TCT
2	oIMR7319	CGA GGC GGA TCA CAA GCA ATA
3	oIMR7320	TCA ATG GGC GGG GGT CGT T

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		3.36	μl	1	94	180	
PCR buffer	10x		1.2	μl	2	94	30	34
dNTP	2.5	mM	0.96	μl	3	61	60	34
oIMR7318	20	μM(μmol/μl)	0.6	μl	4	72	60	34
oIMR7319	20	μM(μmol/μl)	0.4	μl	5	72	120	
oIMR7320	20	μM(μmol/μl)	0.8	μl	6	10	-	
DNA	--		2	μl	7			
Taq	5	U/μl	0.06	μl	8			
Mg ²⁺	25	mM	0.96	μl	9			
DNA Loading Dye	5	mM	1.66	μl	10			
Total			12	μl	11			

C. PCR Product

	Primer set	Product size	Target region
1		250 bp	Mutant
2		250 bp and 330 bp	Heterozygote
		330 bp	Wildtype

D. Gel Photo

