

RMRC Genotyping Protocol-PCR

種原編號	RMRC13067	品系名稱	B6;129-Accn3<tm1(fGFP-WGA)Ccc>
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A. Primer

	Primer name	Sequence
1	Wt-for (primer1)	5'-gggttccttcaagccctac-3'
2	Wt-rev (primer2)	5'-ggagccaagacaggtgaaag-3'
3	Ko-rev (primer3)	5'-gaacttcagggtcagcttgc-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		14	μl	1	95	180	1
PCR buffer	10x		2	μl	2	95	90	35
dNTP	10	mM	0.3	μl	3	55	60	
Primer 1	20	μM(μmol/μl)	0.5	μl	4	72	60	
Primer 2	20	μM(μmol/μl)	0.5	μl	5	72	600	1
Primer 3	20	μM(μmol/μl)	0.5	μl	6	4	---	1
DNA	--		2	μl	7			
Taq	2	U/μl	0.2	μl	8			
Mg ²⁺	2	mM (in 10xbuffer)	0	μl	9			
Total			20	μl				

C. PCR Product

	Primer set		Product size	Target region
1	Primer1	Primer2	366 bp	Wildtype Exon1
2	Primer1	Primer3	520bp	eGFP-f

D. Gel Photo

