

RMRC Genotyping Protocol-PCR

No.	RMRC13313	Strain Name	B6.129- <i>Cdk12</i> ^{tm1.1Fmj} /Narl
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A. Primer

	Primer name	Sequence
1	Flx-F	GTTCAGACAGTGTCTCAGACCACCTGGAGAAGC
2	Del-3 F	CTTCCTGCCTCCTCTTCATCATCAGGTATTG
3	Y3 R	CCTCTGACCTCCCAATGTGTGCATGACAC

Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		76.5	μl	1	95	5 min	1
PCR buffer	10x		10	μl	2	95	30	
dNTP	10	mM	1	μl	3	58	30	40
Primer 1	10	μM(μmol/μl)	1	μl	4	72	30	
Primer 2	10	μM(μmol/μl)	1	μl	5	72	3 min	1
DNA	--		10	μl	6	16	∞	
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺		mM	0	μl	8			
Total			100	μl	9			

B. PCR Product

	Primer set		Product size	Target region
1	Flx-F	Y3 R	563 bp	Flx allele
2	Del-3 F	Y3 R	495 bp	KO allele

C. Gel Photo

1.5% agarose gel,

Marker: 1 Kb plus DNA Ladder (Thermo Fisher Scientific)

