

## RMRC Genotyping Protocol-PCR

No.	RMRC13353	Strain Name	B6.129- <i>Cdk12<sup>tm1Fmj</sup>Trp53<sup>em1Fmj</sup></i> /Narl
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### A. Primer

	Primer name	Sequence
1	Cdk12 Flx-F	G TTCAGACAGTGT CAGACCACCTGGAGAAGC
2	Cdk12 Y3-R	CCTCTGACCTCCCAATGTGTGCATGACAC
3	Trp53 5VF1-16323	ACGTGGTTGGTTACCTCTGC
4	Trp53 3VF1-20245	AGTGGTTGTGTGACCTTGTC
5	Trp53 3VR1-20720	CACCATCACCATGAGACAGG

### Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	--		15.9	μl	1	95	5 min	1
PCR buffer	10x		2	μl	2	95	30	
dNTP	10	mM	0.2	μl	3	60	30	40
Primer 1	10	μM(ρmol/μl)	0.2	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.2	μl	5	72	3 min	1
DNA	--		1	μl	6	16	∞	
Taq	5	U/μl	0.5	μl	7			
Mg <sup>2+</sup>		mM	0	μl	8			
Total			20	μl	9			

### B. PCR Product

	Primer set		Product size	Target region
1	Cdk12 Flx-F	Cdk12 Y3-R	438 bp	Cdk12 WT allele
2	Cdk12 Flx-F	Cdk12 Y3-R	563 bp	Cdk12 Flx allele
3	Trp53 3VF1-20245	Trp53 3VR1-20720	475 bp	p53 WT allele
4	Trp53 5VF1-16323	Trp53 3VR1-20720	572 bp	p53 KO allele

### C. Gel Photo

1.2% agarose gel,

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

