

RMRC Genotyping Protocol-PCR

No.	RMRC13209	Strain Name	C57BL/6-Nme7 ^{tm1LjN6}
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A. Primer

	Primer name	Sequence
1	Knockout	Forward: CGTGGGAGCATCCAATCACTCTGTA Reverse: GCCTGTCTCTTCTGTAAATTGCCC
2	Wild type	Forward: AATCTGTCTCTGTCACTCATAGCTG Reverse : CGAGGAGAGGAAATCAGAAGTTAGT

B. Reaction Conditions

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, sec	Number of Cycles
H ₂ O	--		11	μl	1	95	5 min	1
PCR buffer	5x (Phire F524)		4	μl	2	95	30 sec	35
dNTP	2.5	μM	1.7	μl	3	55	30 sec	35
Primer 1	25	μM(μmol/μl)	1	μl	4	72	15 sec	35
Primer 2	25	μM(μmol/μl)	1	μl	5	72	1 min	1
DNA	--		1	μl	6	4	∞	
Taq	--	U/μl (Phire F122L)	0.3	μl	7			
Mg ²⁺	--	mM	--	μl	8			
Total			20	μl	9			

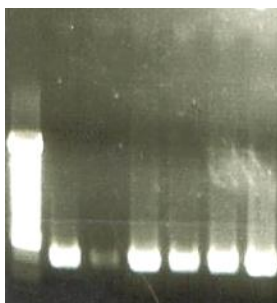
C. PCR Product

	Primer set		Product size	Target region
1	Knockout	KO	699 bp	
2	Wild type	WT	500 bp	

D. Gel Photo

WT

M 1 2 3 4 5 6



KO

M 1 2 3 4 5 6

