

RMRC Genotyping Protocol-PCR

No.	RMRC158	Strain Name	B6.129-Asic1<tm1Lien>
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A. Primer

	Primer name	Sequence
1	Left loxP-F	5'-TCCTCTCCCAAACACACAC-3'
2	Left loxP-R	5'-GAGTTCCCTCCAGATGTGAG-3'
3	Right loxP-F	5'-AGGCCTGCAAACACTGTCATCT-3'
4	Right loxP-R	5'-GTTGCATCTTGAGCCTCCTC-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		9.5	μl	1	95	180	1
2X PCR premix	2X		12.5	μl	2	95	30	35
				μl	3	55	30	35
Primer 1	10	μM(ρmol/μl)	1	μl	4	72	40	35
Primer 2	10	μM(ρmol/μl)	1	μl	5	72	60	1
DNA	--		1	μl	6	4	∞	1
				μl	7			
				μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	Left loxP-F	Left loxP-R	410 bp	5' loxP
2	Right loxP-F	Right loxP-R	406 bp	3' loxP

D. Gel Photo

