

RMRC Genotyping Protocol-PCR

No.	RMRC13227	Strain Name	C57BL/6-Mir122a ^{tm1c(GEMMS)Narl} /Narl
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A. Primers

No.	Primer name	Sequence
1	Mir122aLF	TTCTTAGTTGGACCCCTCACCTG
2	Mir122aLR	ATCCACCTATGCCTGCCTCTTG
3	Mir122aDR	AACCCCAGTTTATAGCTGGTGAGTC

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	—	10.5 μl	1	94	180	1
PCR buffer	10 x	1.5 μl	2	94	30	35
Formamide	—	0.5 μl	3	60	30	35
dNTP	10 mM	0.3 μl	4	72	30	35
Primer 1	20 μM (pmol/μl)	0.3 μl	5	72	180	1
Primer 2	20 μM (pmol/μl)	0.3 μl				
Primer 3	20 μM (pmol/μl)	0.3 μl				
gDNA	50-250 ng/μl	1 μl				
Taq	5 U/μl	0.3 μl				
Total		15 μl				

C. PCR Product

No.	Primer set		Product size	Target region
1	Mir122aLF	Mir122aLR	210 bp	Wildtype
2	Mir122aLF	Mir122aDR	450 bp	Mutant

D. Gel Photo

