

RMRC Genotyping Protocol-PCR

No.	RMRC13330	Strain Name	C57BL/6- <i>Slitrk1</i> ^{tm1c(GEMMS)<i>Narl</i>/Narl}
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A. Primers

No.	Primer name	Sequence
1	Slitrk1NF	GAATTAAGCCAGCTCAGTAAAAG
2	Slitrk1NR	ACGAAGTTATAAGCTTGAAGTTCCT

B. Reaction Conditions

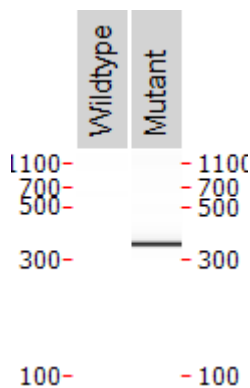
Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 μl	1	94	80	1
Primer 1	20 μM (pmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (pmol/μl)	0.3 μl	3	60	15	35
gDNA	50-250 ng/μl	1 μl	4	68	30	35
OneTaq*	2X Master Mix	7.5 μl	5	68	60	1
Total		15 μl				

*M0488 OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer from New England Biolabs.

C. PCR Product

No.	Primer set		Product size	Target region
1	Slitrk1NF	Slitrk1NR	350 bp	Mutant

D. Gel Photo



A. Primers

No.	Primer name	Sequence
1	Slitrk1DF	GAGGGAGATTTCACTGGTCAGCAC
2	Slitrk1DR	ATTGCCAGAGGACAGTCATCTTCC

B. Reaction Conditions

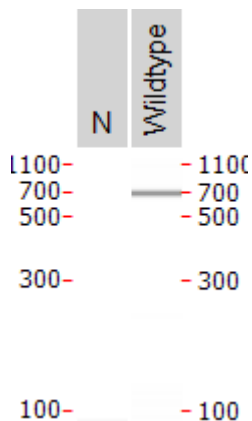
Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 µl	1	94	80	1
Primer 1	20 µM (µmol/µl)	0.3 µl	2	94	15	35
Primer 2	20 µM (µmol/µl)	0.3 µl	3	60	15	35
gDNA	50-250 ng/µl	1 µl	4	68	30	35
OneTaq*	2X Master Mix	7.5 µl	5	68	60	1
Total		15 µl				

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C. PCR Product

No.	Primer set		Product size	Target region
1	Slitrk1DF	Slitrk1DR	710 bp	Wildtype

D. Gel Photo



The first genotype protocol only can distinguish wild type and knockout allele but not for heterozygote and homozygote.

If you would like to separate heterozygote from homozygote, you have to do both protocols above mentioned.