

RMRC Genotyping Protocol-PCR

No.	RMRC13228	Strain Name	C57BL/6-Trim5 ^{tm1b(GEMMS)Narl}
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A. Primers

No.	Primer name	Sequence
1	Trim5KF	TCGCCTTCTTGACGAGTTCTTC
2	Trim5LR	TGCTTGAATGCTGCTGTGCTC

B. Reaction Conditions

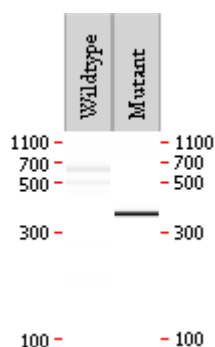
Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 µl	1	94	110	1
Primer 1	20 µM (pmol/µl)	0.3 µl	2	94	15	35
Primer 2	20 µM (pmol/µl)	0.3 µl	3	60	15	35
gDNA	50-250 ng/µl	1 µl	4	68	30	35
OneTaq*	2X Master Mix	7.5 µl	5	68	180	1
Total		15 µl				

*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	Trim5KF	Trim5LR	360 bp	Mutant

D. Gel Photo



Heterozygote should be positive in both mutant and wild type tests and homozygote only has positive result in mutant test.

A. Primers

No.	Primer name	Sequence
1	Trim5LF	GGTACACGGATCATACATGCAGG
2	Trim5LR	TGCTTGAATGCTGCTGTGCTC

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of cycles
H ₂ O	–	10.8 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				
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	Trim5LF	Trim5LR	210 bp	Wildtype

D. Gel Photo

