

RMRC Genotyping Protocol-PCR

No.	RMRC13303	Strain Name	C57BL/6-Smad4 ^{tm1a(GEMMS)Narl} /Narl
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A. Primers

No.	Primer name	Sequence
1	Smad4LF	TACCTTTAGTGCAGCAGTTTCAGCC
2	Smad4LR	TCACTTCTGTGAAGCAGTAGTTCCC

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	Smad4LF	Smad4LR	250 bp	Wildtype
2	Smad4LF	Smad4LR	353 bp	Mutant

D. Gel Photo

