

RMRC Genotyping Protocol-PCR

No.	RMRC13315	Strain Name	C57BL/6- <i>Smad4</i> ^{tm1c(GEMMS)<i>Narl</i>} / <i>Narl</i>
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A. Primers

No.	Primer name	Sequence
1	Smad4NF	ACTTTTGAGTCCAGTCTCAGTGGCA
2	Smad4KR	CCCAGGATCCATTAGGTGACACTA

B. Reaction Conditions

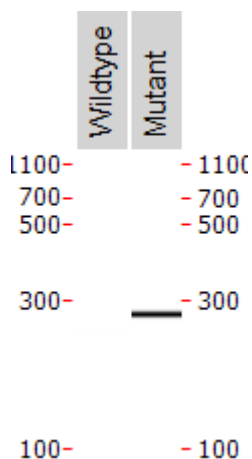
Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 μl	1	94	110	1
Primer 1	20 μM (pmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (pmol/μl)	0.3 μl	3	60	15	35
gDNA	50-250 ng/μl	1 μl	4	68	30	35
OneTaq*	2X Master Mix	7.5 μl	5	68	180	1
Total		15 μl				

*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	Smad4NF	Smad4KR	270 bp	Mutant

D. Gel Photo



The above protocol CANNOT distinguish heterozygote from homozygote.

Only if you do both protocols (above and below) in the meantime are heterozygote and homozygote differentiated.

A. Primers

No.	Primer name	Sequence
1	Smad4DF	TCAGGTGACACACCTGAGCAGCCTCTA
2	Smad4DR	GTCTTCCTGGAGAGGCTATGGAGGT

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	Smad4DF	Smad4DR	251 bp	Wildtype

D. Gel Photo

