

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	32241
MMRRC Strain Name	B6.129S- <i>Map3k1</i> ^{tm1Gjl} /Mmnc
Gene Name(s)	Mitogen-activated protein kinase kinase kinase 1 (Map3k1)
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	7/22/13

MMRRC #32241 PCR Reaction

	<u>1X</u>
ddH ₂ O	8.5
5X Buffer	5.0
25mM MgCl ₂	1.5
10mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Denville Taq (5U/μl)	0.5
5 M Betaine	5
DNA	2

Thermal Cycler:

Step 1: 95°C for 5 min
 Step 2: 95°C for 30 sec
 Step 3: 63°C for 30 sec
 Step 4: 72°C for 1 min
 Step 5: 36x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Denville Taq (5U/μl) and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

MEKK1 WT forward primer: GCCCTGCCCATCTACTTCCCCGTC

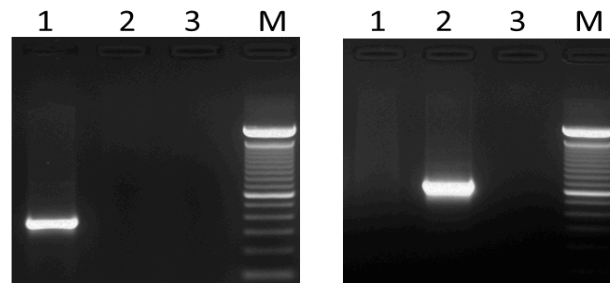
MEKK1 KO forward primer: CGGAGAACCTGCGTGCAATCCATC

MEKK1 Universal reverse primer: GCCTGTACATGTCCGCAAACAAT

Bands expected: Mutant: ~630 bp
 Wild type: 396 bp

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Run on a 2% agarose gel.



WT: MEKK1 WT forward +
 MEKK1 Universal reverse

Mut: MEKK1 KO forward +
 MEKK1 Universal reverse

Lane 1: WT; Lane 2: Hom; Lane 3: H₂O; M: 100 bp DNA ladder (Invitrogen)

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