

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34558
MMRRC Strain Name	129S6(B6)- <i>Map3k4</i> ^{tm1Glj}
Gene Name(s)	mitogen-activated protein kinase kinase kinase 4 (Map3k4)
Breeding Protocol(s)	Sib-mating (heterozygous x heterozygous)
Protocol Date	1/23/14

MMRRC# 34558 PCR Reaction

2,2 Primer reactions (M41168F + 22R1) for wild-type and (NeoR + 2003R) for mutant reaction

Note: In heterozygous samples, light 1.6 and 2.3 kb bands may be seen in the wild-type reaction

Thermal Cycler:

94°C for 30 sec
 61°C for 1 min
 72°C for 3 min
 32x

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

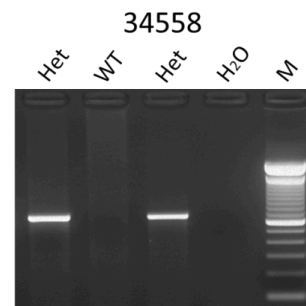
Taq: Apex and Chromataq 5X Buffer

Bands: MUTANT: ~600bp
 WT: ~1kb

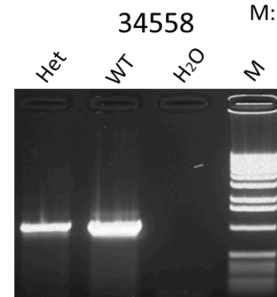
Primer sequences 5' to 3':

M41168F: CTG CAT CAG TGT TGA CAC AGG GGA
 22R1: GTG AAG CTC CAC GCC AAA ATA CCG
 NeoR 5082: CCG ACT GCA TCT GCG TGT TCG
 2003R: GGG ATG TGA GCG ACT CTG GCC TTA

Run on 2.0% agarose gel in TAE.



34558
 Primers: NeoR + 2003R
 M: 100 bp DNA ladder (Invitrogen)



34558
 Primers: M41168F + 22R1
 M: 1Kb DNA ladder (Invitrogen)