

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	41840
MMRRC Strain Name	129.Cg- <i>Map2k1</i> ^{tm1Chrⁿ} <i>Map2k2</i> ^{tm1Chrⁿ} /Mmnc
Gene Name(s)	mitogen-activated protein kinase kinase 1, mitogen-activated protein kinase kinase 2
Breeding Protocol(s)	Sib-mating
Protocol Date	12/11/19

PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

2,3 Primer Reactions

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 57C, 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex and Chromataq 5X Buffer

Bands: MUT: 270bp (2k1), 280bp (2k2)

WT: 178bp (2k1), 480bp (2k2)

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

2k1

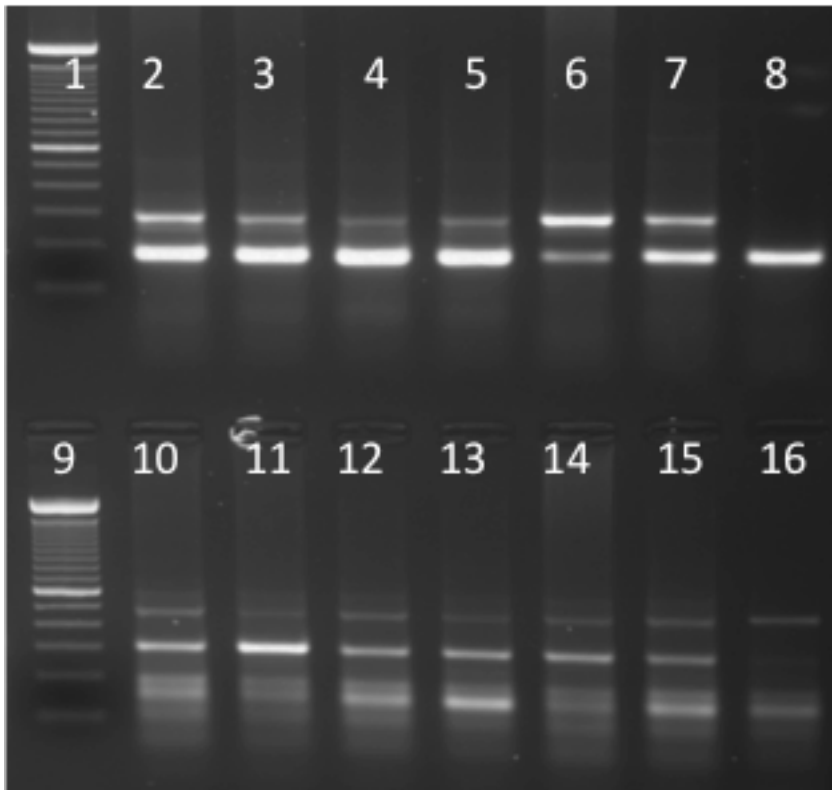
Map2k12(41841)372: ACT GAA CCG TGT CCA GAA GG (Common)

Map2k12(41841)373: AAA ACG CTG GGA TCC CGG AT (WT)
Map2k12(41841)374: TCC CAG CTG GAC GTA AAC TC (MUT)

2k2

Map2k12(41841)294: TGG CGC CTA CCG GTG GAT GT (MUT)
Map2k12(41841)280: TGT ATG GGA CTT GGG GAT GT (WT)
Map2k12(41841)281: TTG GTA GCA ACA GGG TAG GG (Common)

Run on 2.0% agarose gel in TAE.



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| 1. 100bp Marker (Invitrogen) | 12. Map2k2 Heterozygous Sample |
| 2. Map2k1 Heterozygous Sample | 13. Map2k2 Heterozygous Sample |
| 3. Map2k1 Heterozygous Sample | 14. Map2k2 Heterozygous Sample |
| 4. Map2k1 Heterozygous Sample | 15. Map2k2 Heterozygous Sample |
| 5. Map2k1 Heterozygous Sample | 16. Map2k2 Wild-type Control |
| 6. Map2k1 Heterozygous Sample | |
| 7. Map2k1 Heterozygous Sample | |
| 8. Map2k1 Wild-type Control | |
| 9. 100bp Marker (Invitrogen) | |
| 10. Map2k2 Heterozygous Sample | |
| 11. Map2k2 Heterozygous Sample | |