GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6J-*Map3k14*^{m1Btlr}/Mmcd, *(lucky)*** **MMRRC #** **031808-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	20.0
10x Buffer (contains 15mM $MgCl_2$)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer 1 (stock concentration is 20µM) Rfur (F)	0.5
Primer 2 (stock concentration is 20µM) Rfur (R)	0.5
Taq Polymerase	0.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	0.5
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- *Lucky* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.
- lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma), 12.5 µl in a 25 µl reaction (includes Taq, buffer, dNTPs).

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	60	0:30	**30x**
4. Elongation		68	1:00	
5. Amplification		68	7:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Rfur (F)	TGA GTG AAA ATG TCC CGT GAG CAG
2: Rfur (R)	TGC ATA CCA AGG GCA GCA ATC AG
3: Rfur_Seq (F)	GCA CCA GAT CTC ATT ATA CGT GG
4: Rfur_Seq (R)	TCA GGG TCT CAC AGC ATA GTC

Electrophoresis Protocol:

Agarose: 1.5% **mV:** 90 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	1140 bp	*lucky*
***Map3k14* sequence below**		

Mutation site (red) and flanking sequence:

WT ggattatgag tat**C**gagaag
lucky ggattatgag tat**T**gagaag

The following sequence of 1140 nucleotides (from Genbank genomic region **NC_000077** for linear DNA sequence of *Map3k14*) is amplified:

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29106      tgagt gaaaatgtcc cgtgagcaga ggatgccgtc ctccctctcc ctccctccca
29161 catctccctt ttccttcagt tctctgcctg aataactttg gaacctgaag cgatttgttc
29221 aacttgaggg attgattcta ttcactttta ataacggtgc ttgcccctgt ctcttcaacc
29281 ttggagttac tgagttatat gtcccattat caaggttgtt ggcgactctg cttctcatcg
29341 caggaattat ctgcctaccc cacccccccag gcagcctatg gtggccacct ttgccattta
29401 agatttaagt atacagcact ctgcctgcag gccagaagag ggcaccagat ctcattatac
29461 gtggttatga gccaccgcgt ggttgctggg aattgaactc aggcgctcca gaagagcaag
29521 cagtcagtgc tcttaacctc tgaggcatct ccccagcccc acctttgtct tcttattccc
29581 ctaaacccct tttggcctgt cttccttatc atcgcgctgt agggctggta gtctctttag
29641 atgtggtggc catccagcca ccagagccct agctattgac tagagagcct tccccactgc
29701 agtcctctgt ccctctgctg cccaggtcac tggtactgac ctcctcctcc tcctcttcaa
29761 ccccagaaac tcaagccagt ggattatgag tatcgagaag aggtccactg gatgacacac
29821 cagcctcggg tgggcagagg ctccttcggc gaggtccaca gaatgaagga caagcagaca
29881 ggcttccagt gtgctgtcaa aaaggtatgc cgaggtgata caccactggg ctggcaccca
29941 aatttccaac actggggagg ctgaggcagg aggatgacca tggatcctag gccagctggg
30001 actatgctgt gagaccctga cttggaaaaa aaaaattaca tagtaggtag aactctgggg
30061 agctgaaagg gaagccacac aggggctgct gccttccacc tcagctttct gtcagcaaca
30121 gtcatggggt atccggggct ctcccttgat ggttggcacc ctagctgctc tcgggtcagc
30181 tggagctgga agggagccgt ctggtgtgtc acatggcctg gcctgattgc tgcccttggt
30241 atgca
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated C is shown in red text.