GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: MMRRC Address: 2795 2nd Street, Suite 400, Davis, CA 95618 Contact: Reneé Araiza
Telephone: 530-754-MMRRC FAX: 530-757-3284 email: mmrrc@ucdavis.edu

DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR: C57BL/6J-*Tomt*^{m1Btlr}/Mmcd (*add*), MMRRC #030528-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol: *Add* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transversion. The same primers are used for PCR amplification and for sequencing. The lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs).

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [**x**]	94	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0.25	35
3. Annealing	60	0.3	35
4. Elongation	72	1	35
5. Amplification (i.e., 72^{o}C, 10 min)	72	5	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Add(F)	GCAGGAGGTTGCAAAGAGCACAGGC
2: Add(R)	GTGATCTCTGCCTCCCTCAGCACCTG
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.678	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 678 nucleotides (from Genbank genomic region **NC_000073** for linear genomic sequence of *Tomt*) is amplified:

3899 gc
3901 aggaggttgc aaagagcaca ggctgaactc aaatcccagt tctgctgtgt ctttggcaaa
3961 ggacagcctc tcagacatgt ttgtataagc gtggaaattg cataaataag tctgcacaaa
4021 tgacaggcaa atggtacatc taggtttccc cgccagcctc cctctatcct aggacccagg
4081 tagggacaat gtcccctgcc attgcactgg cattcctgcc actcgtggta acactgctgg
4141 tgagataccg gcaccatttc cgactgctgg tgcgtacagt cctgctgaga ggatttcgag
4201 actgcctgtc aggacttcgg attgaggagc **g**ggctttcag ctatgtgctc acccatgccc
4261 tgcctggaga ccctggtcac atcctcacca cgcttgacca ttggagcagc tgctgcgagt
4321 acctgagcca catgggccct gttaaaggtg agtgttcctt tccctaccct tctgtttgag
4381 aaataaaccc aagacggtga agaaaaacct cgcatggccc actgcttacc tgggtaaatt
4441 aggaagtttc gtgattgcgt tatgacattt tccccccctc atttagaaat gaggagtctg
4501 gaccaagtta ttcagttatc cagcagacat ttattgccac tcttttgtgc caggtgctga
4561 gggaggcaga gatcac

Primer binding sites are underlined; the mutated G is highlighted in red.