GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: **NAME OF PCR: C57BL/6J-*Tap2*^{m1Btlr}/Mmcd (*ganymede*), MMRRC Strain #032642-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 25mM)	0.4
Primer **1** (stock concentration is 20 uM)	1
Primer **2** (stock concentration is 20 uM)	1
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	2.5
Additives if applicable: H2O	39.1
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): *Ganymede* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide insertion.

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	30 sec	29
3. Annealing	56	30 sec	29
4. Elongation	72	1	29
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: ganymede_PCR(F)	5'- TCTTCCAGGAGACCAAGACAGGTG -3'
2: ganymede_PCR(R)	5'- TATAGATCCAGTGGGCCTCCAACC -3'
3: ganymede_seq(F)	5'- AGGTGAACCTGGCATCTGG -3'
4: ganymede_seq(R)	5'- GTCCATCAGTGAATGACTGAAC -3'

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	765 bp	
(i.e. 3&4)		
(i.e. 1&2&3)		

Mutation site (red) and flanking sequence:

WT **tgagaaggtgtacaacccccgccatcaggta**
ganymede **tgagaaggtgtacaaccccccgccatcaggta**

The following sequence of 765 nucleotides (from Genbank genomic region NC_000083 for linear genomic sequence of *Tap2*, sense strand) is amplified.

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4299                                              tc ttccaggaga ccaagacagg
4321 tgaacctggc atctgggtct tgggtgctcg gccctttctg gagtcctcag gtctcctgtc
4381 tgcctccctg ctggagcctg gcagttttct cttagagcag ggtagaggtc tagcccagtc
4441 tctttgtaaa ggctgagggg atgagtcagc agggagaccc agaggaaggt tttgggttcc
4501 catcatcctt tctgccctcc ccaggggagc tgaactcgag gctgagctct gacacctctc
4561 tgatgagccg ctggctccct ttcaatgcca atatcctgct gcggagcctg gtgaaggtgg
4621 tggggctcta cttcttcatg ctccaggtat cgccccgact caccttcctc tccctgctgg
4681 acctgcccct cacgatagca gctgagaagg tgtacaaccc ccgccatcag gtatgtgtgc
4741 atgtcacagt gccctgagag aaggcaaaca gacaagcaga cagacaaata agtagatagg
4801 taggtaggta gatagatggg tggatggata ggtaggtagt agacagacag acagacagac
4861 agacaaataa gtagataggt aggtagatag ataaataggt aggtaggtag atagatagat
4921 tgatagacag ataatcggta gacagacaga acacctctta tcagtcccta cagtccattc
4981 tatgcttgca caacattttg ctagttcagt cattcactga tggacatttg agttgaggag
5041 gttggaggcc cactggatct ata
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Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the stretch of 5 Cs into which an extra C is inserted is indicated in red.