GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other: Any

Protocol: **NAME OF PCR:** MMRRC Strain #30332-UCD, C57BL/6J-*Gja8*^{L1}

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 *(e.g.,* different concentration of MgCl2, etc): 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	94	0.5	35
3. Annealing } steps 2-3-4 will cycle in sequence	55	0.5	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: L1(F)	CGGCACAGATGAGGCACTTGATAG
2: L1(R)	TGTGGCAGACATAGGTCCTTAGCAG
3: L1_seq(F)	GAGATCATCTCAGAGTTGCACTG
4: L1_seq(R)	CATAGGTCCTTAGCAGTGTGCC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.64	
(i.e. 3&4)		
(i.e. 1&2&3)		

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES

L1 genotyping is performed by amplifying the region co
by sequencing of the amplified region to detect the single nucleotide change. The following sequence of 638 nucleotides (from Genbank genomic region **NC_000069** for linear genomic sequence of *Gja8*) is amplified:

5433 cggcacag atgaggcact tgatagaagc

5461 tgttggatac tatgattgtt ccatcagttc caaaaggaaa gtcactccaa gagctaggaa

5521 agagatcatc tcagagttgc actgtggcca attagatttt gccttctgct tccttggtag

5581 tgagcaatgg gcgactggag tttcctggga aacatcttgg aagaggtgaa tgagcactcc

5641 actgtcatcg gcagagtctg gctcacagtg ctcttcatct tccgcatcct catcctcggg

5701 acagcagcgg agtttgtgtg gggcgatgag caatctgatt ttgtatgcaa cacccagcag

5761 ccaggctgtg agaatgtctg ctacgatgag gcctttccca tctcacacat ccgcctctgg

5821 gtgctgcaga tcatcttcgt ctccactcca tcgctgatgt acgtggggca cgcggtacac

5881 cacgttcgca tggaggagaa gcgaaaggac cgtgaagctg aggagctctg tcagcagtcg

5941 cgcagcaacg ggggtgagag ggtaccaatc gccccagacc aggccagcat ccggaagagc

6001 agcagcagta gcaaaggcac caagaagttc cggctggagg gcacactgct aaggacctat

6061 gtctgccaca

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.