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: C57BL/6J-*Kcnn2*^{m1Btlr}/Mmcd (*jitter*), MMRRC #030543-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 *(e.g.,* different concentration of MgCl2, etc): *Jitter* 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. Use primers 1 & 2 for amplification and 3 & 4 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	94	0.50	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	0.50	30
4. Elongation	72	1	30
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: Jitter(F)	GAGCAACTTCCTCTAGGCTTTAGCG
2: Jitter(R)	GCAAGGCTACTCACTTCCTCTGAC
3: Jitter_seq(F)	CCTCTAGGCTTTAGCGAGTCAG
4: Jitter_seq(R)	AGAAAGATCTGCCTGTTGCC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.17	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 1172 nucleotides (from Genbank genomic region **NC_00008**4 for linear DNA sequence of *Kcnn2*) is amplified:

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 795                gagcaa cttcctctag gctttagcga gtcaggtacc ttgtttcagt
 841 ccttggtggt gcgtagcccg cagccaaccc acgagttttc ccttgttcct ggctcctctc
 901 acggagcatt atggccaaat aacctgcatc ccagagccaa aacagattgc cccctcccga
 961 gtcccctacc ttgaattcag gtccacgtgc tcggaactga cttttatggc ctggaaagag
1021 attggaagtg cttctttctt aaagtgttcc atctaactgt gttgcaggcg tcgctgtatt
1081 ctttagctct gaaatgcctt atcagtctct ccacgatcat cctgcttggt ctgatcatcg
1141 tgtaccacgc cagggaaata caggtaacac aggctccact gttttctgaa taaccagaag
1201 ccatgcaggc agcataggag aaaagcaaga cagcaagggg cctttaccaa gcagctgtgt
1261 ccttgcttga ggttacagaa gacacatgca ctgttatctc agcacctgac ctgtccttcc
1321 agaaagctaa acaaacaaac aagtcaacac agcaggcaa caggcagatc tttctgagat
1381 atatttgata gaatcttaag attttcccca acttcttcag gctggtacac ttctaccaga
1441 caaatgtttt taaaggagcg gtacacaata tcctgaatct gtgcagcagt gtgtgttctt
1501 gtttaagaca ccttttaaa aggagtaagt cattagggga ggaggccttg ccaatctgga
1561 ttgcctataa ttataatata agaaaattgg ttcactgctt ctatcaacat gggaagagca
1621 cctcccctcc ccaaacttct aacgcatttt acaacagctt atttagttca taaagcctgt
1681 gtaggtatta gtgtagtccg agcattttaa atgtcacttg agagtatttg gagtgggtaa
1741 tgatagacga aagcacactt ggtcgaattt ttaatattag gacagtatat agattcttca
1801 gtgcagtcct cacgatggtg gcattttgag attcttccag aggtcctctg tggtcctctg
1861 ctgtgcttta agcctgggtc actttgactt gaagcctgga agatagagaa tgagtctttg
1921 acttcgtttt cagatggaaa cagtcagagg aagtgagtag ccttgc
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.