

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-Gsdma3^{m1Btr}/Mmcd (Fuzzy), MMRRC #031044-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl ₂)	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: Fuzzy 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. The lab uses JumpStart[®] REDTaq[®] ReadyMix[®] (P1107- Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs).

Strategy:

Steps	Temp (°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°C, 10 min)	72	7	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Fuzzy (F)	CCTGACCTTTGCATGACACATTTTCCCACAC
2: Fuzzy (R)	GGCAGGCAGCTTGGATGATCTTCCCAGT
3: Fuzzy _seq (R)	GTTGAGGACCCTAAGCCTCTAAG
4:	

Electrophoresis Protocol:

% Agarose: _____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	2.48	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 2480 nucleotides (from Genbank genomic region [NC_000077](#) for linear DNA sequence of *Gsdma3* plus 1314 additional nucleotides taken from [NCBI m37 mouse assembly](#) Chromosome 11: 99246568:99251097) is amplified:

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10565      cctgac cctttgcatga cacattttcc cacacaaaaa gaaaaacgtc tcaggccaca
10621 ctgggtacag agtgcctaac ttaaccctgg gctacagaat gagattctgt ctcaaaaaca
10681 aacagaaagg ccatggcttc tctcaaaact gctttgaaag attagaattt gtttggtgtg
10741 tgttttttag atgaggactc actatgtagc cctggctggc ctggaactca ctgagtagag
10801 cactctggcc tcgaactcac aaaggttcat ataactctgc cttccaaaat gctgagatta
10861 aagggtgtgtg tcactttacc cagtcaaaaa atacagcttt tttaaaagtt aatgaccagc
10921 aaagggtgtat gttatagagc aggtgttcag ctgtggacag atggacggtg ggatggatga
10981 atggacaaat gagcatatga atgaatagat agatggatgg atgctgtgat gcatggatta
11041 attgatcaga gtgtgctaac actatttgta gtctgtagt cctctgtac cagcaacaca
11101 tcgttttttt ggttttggtt ctttggtttg tttggttttt ttctttcttt ctctgctttt
11161 ccctccagag ctaactgaag aacaactgaa gattctagta aaatccttgg agaaaaagat
11221 cttaccagtg caactgaagc tgggtgagaaa aagtgcacaga ttacaggggg agtttagggt
11281 ggggcaggga tgggcgctag aacttgtggg aacatgcaga cctactgtgc tcacaggacc
11341 cagccttatt cctagagagg tcttctcctc tccttagagg cttaggggtcc tcaacctgct
11401 ctggtaggag ggagaaaggc tttctagttt cttacttcct gttaccttgg tcccttgtag
11461 gttgaaagca ccttgagagc gaacttcctg caagataaag aggggtgttt cccctgcaa
11521 cctgatctgc tctcctccct cggggaggag gaactgacct taacggaagc actggtggga
11581 ctaagcggcc tggaagtcca gagatcaggc cccagtagc cgtgggatcc agacactcgc
11641 cacaaccttt gtgcctcta tgctggcctc tccctccttc acctgctaag caggaaatct
11701 aatgcactta cttattgtgc tctatcttaa cactccaaga cactacagag cctccagtct
11761 gaggataact gaaatgcctg ctcactctgc ctctcgatat gtctctctcc ttctgtcctc
11821 cagctcagca cccactaagc ccactctcct atgcttaaat cctgaagtta ctgaaagatc
11881 ccgagagaga gagtaaaaca ttatgtaagc ccctagactt gtgtccaaaa tagacctcac
11941 taatggcagg gagtaaaagt taaactcggg gctggagaga tggctcagcg gttaagagca
12001 ctgactgctc ttccagagat cctgagttca aattccagag atcctgagtt caaatcccag
12061 caaccacatg gtggctcaca accatccata acaatatctg gcgccctctt ctggagtgtc
12121 tgaagacacc tacagtgtac ttacatataa taaataaata attttaaaaa aaagttaaac
12181 tagtccatt gttccaggat gtactggcca caaagtagaa gtttaacttg ctccattgtt
12241 tcaggaaatg tttgtacaga atactccttg aacccttatg cttgccaaat gctatagccc
12301 catgccagggt gtttgcggtt tctgccttta taaaccctg accccttgag ctcggtcga
12361 ctctctacc cctgcgtgtg atacaagttg tccccagtg actggccttt cccgattaaa
12421 cctcttgccg tttgcatcaa gactggtctc tcgtgagttc ttgggcggtg cgtcatccgg
12481 gacttgagtg aggttccttt ccgttttggg ggactttcaa tacagaatca ttcaaaccct
12541 tattactttg agtcacttca ttaagaagg agaggtaagg cctgaggttg tggtaatggt
12601 ggttgggtgtg gtcattgatg tggtagacaa ctttaatccc agcactcatg aggcagaggc
12661 agaagaatct cttagtttca agttcagcct gggctacaga atgctgtcca ggacaccag
12721 ggctacccaa gacaaccctg tcttgaaaac aacaacaacc acatttatat cctagtcaaa
12781 tcattcccat gtgatcctct gaaaaaaaaa tatggcctta aaagaataaa tggaaaacat
12841 tatgtcaatc ctaggacaag ctgtcttctg gtttttctgt aaggagaatc tgtcatttgt
12901 cccaggtaaa gaaacagaca tgatccatac atacacatta ctgctcacac aggccccccc
12961 cccctgaaaa atgcttccag agctcagagg gttagaattc agaacatctg ctgtacactg
13021 ggaagatcat ccaagctgcc tgcc

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