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-*Krt33a*$^{Plsh\text{-}3Btlr}$/Mmcd (*spikey*), MMRRC Strain #030961-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: *Spikey* 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:

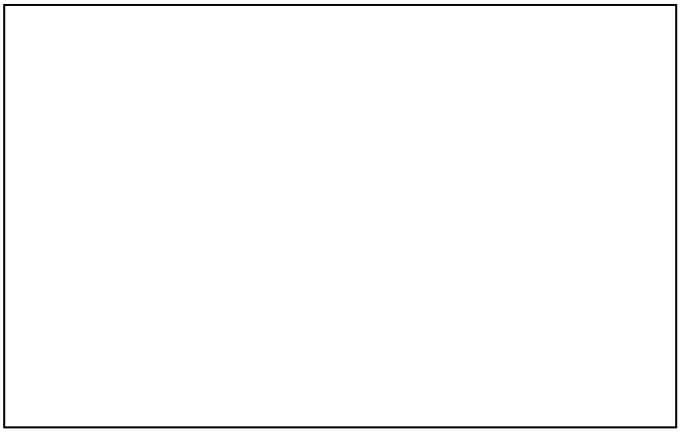
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Spikey(F)	TTGCCTATTGCAGACAGAGGAGTTG
2: Spikey (R)	ACTGATGTTGTTTCACATGCTTGCC
3: Spikey _seq(F)	TCAATGCCCTGGAGATCGAG
4: Spikey _seq(R)	GAGTATTGTAGGCTCCCCAAGAC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.19	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 1193 nucleotides (from Genbank genomic region **NC_000077** for linear DNA sequence of *Krt33a*) is amplified:

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3736                ttgcc tattgcagac agaggagttg aacaagcagg tggtgtccag
3781 ctcagagcag ctgcagtcct gccaggccga gatcatcgag ctgagacgca cagtcaatgc
3841 cctggagatc gagctgcagg cccagcatga actggtgtgt agtgtctaga ctgctgctga
3901 gcagtgtgga gttgggaggc agagtcactg gggtgtcctt ggggcttctc tgtctctgtc
3961 tctgtctctg tctctgtctc tgtctctgtc tgtctctctt cttagctctt gaagcctgtg
4021 acttctctgg aagtcatgca gaaaccttca gagggaacag ctctgtgaca gcctttgtct
4081 tctcccccac agagaaactc tctggagaac accctgacag agagtgaggc tcgctacagc
4141 tcccagctgt cccaggtgca gtgcctgatc accaatgtgg agtcccagct tggtgagatc
4201 cgggctgacc tggagcgtca gaaccaggag taccaagtgc tgctggacat tcggtctcgt
4261 ctggagtgtg agatcaacac gtacaggggc ctgctggaga gcgaggactg caagtgagta
4321 tggctgcatg acttttcctt ggggtgagct gttttcttgg cgggagttaa tttacccctt
4381 tgcacttcta cctggtgaca tccaaaggaa aggccctagc ctgactgtgt ttctagtgtc
4441 ttcctgtgtg ttgaactcac tctaccctaa ctttctccaa tcatttcctg tctccaggct
4501 cccttgcaat ccctgtgcca caaccaatgc ttgtgataag cccattgggc cctgtgtccc
4561 taacccttgt gtcacacgac ctcgatgtgg accttgcaac acttttgtgc gttagagacg
4621 ccattgccag aaggggacaa ggataccagt tacatcgtaa tgccacccct tcccaagtcc
4681 tcaaagagtc accgcactgg acaaagacgg accagttctt ggcttcagtt caagcctaga
4741 gtgtattttc tcatttgatc cttgggcaca ggagatgcaa agtctgtctt ggggagccta
4801 caatactcac acaaagccat atttacatat gcaatgctgt gctttaataa aacttctcta
4861 aatgtgactg taaataactc cgttgagtgt ggaggatctg gttggcaagc atgtgaaaca
4921 acatcagt
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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.