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-*Sgk3*^{m1Btlr}/Mmcd (*woolly*), MMRRC Strain #032233-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*:** *Woolly* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition. 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 } steps 2-3-4 will cycle in sequence	94	0.50	30
3. Annealing	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:

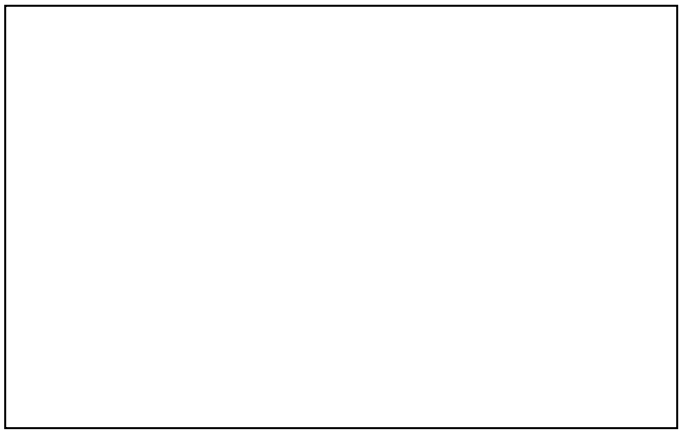
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Woolly (F)	AAGGAATCGCTATTTCTGATACCACCAC
2: Woolly (R)	GGAAATGCCTGTATTAACTCATCCCCG
3: Woolly _seq (F)	CACAACTTTTTGTGGTACACCAG
4: Woolly _seq (R)	GGGTGTCTATTTCTAAAACTGCC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.838	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 838 nucleotides (NCBI Mouse Genome Build 37.1, Chromosome 7, bases 9,875,785 to 9,876,632) is amplified:

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                                          aaggaa tcgctatttc tgataccacc
acaacttttt gtggtacacc agaggtaaga aatatgtctg atcattgata taatttataa
gatataaata caagtcatac cctacatcta cagaagaggt aaaataaaac ctgttagagc
tcccagcttt ctagggactt agagtgtaca gtgggtattt ttcactggtt gtgacagcct
caagacccaa ctcatttgaa aatctagata gcacttcttt agtgtgagta acagtaccta
aaatttgttc ccttttcaga tcctaaattg tgtcttgtgt tcactgggct gctgtaatgt
ttccattttt tctgtagtac cttgcacctg aagtaatcag aaaacagccc tatgacaaca
ctgtggactg gtggtgcctg ggcgctgttc tgtatgagat gctgtacggg ctggtatgtg
tttctgttcc tcattgtcca ctgcaccatt gatgacatag taattagtaa tcctgaagat
gcctaggtgg taccaacaat gccttgttaa atgagtctga aaccatttta ggaatactta
cataaagaaa ggcttcatta ctttcaaaca ccaatatgta atttcggtgt tcctttaggc
atttcttggg gcagttttag aaatagacac ccagtcacaa tgttgaactt tgttgtttcc
tttcattttg tttacatcag acatcacctg cctaatttcc gaaagatgga aataaaagag
ttttccgtta ttaacatatt gctattacgt cttagaagat gatgctttgg ggttttgcct
tccattctgt ttttt cgggg atgagttaat acaggcattt cc
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Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is indicated in red.