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-*Irf7*^{m1Btlr}/Mmcd *(inept)*, MMRRC #032796-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): *Inept* 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. 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: Inept (F)	ACCCTGGAAGCATTTCGGTCGTAG
2: Inept (R)	TGCCCAAGCAGTTCCAAAAGTTCTC
3: Inept _seq (F)	TGCACAGATCTTCAAGGTGG
4: Inept _seq (R)	CCAAGGCTCTGACTTAGCAG

Electrophoresis Protocol:

% Agarose: __2____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.613	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 613 nucleotides (NCBI Mouse Genome Build 37.1, Chromosome 7, bases 148,450,721 to 148,451,333) is amplified:

accctggaag catttcggtc gtagggatct ggatgaagaa gatgcacaga tcttcaaggt
ggtacccagt cctgccctct ttataatctg ccagaactcc tgggggaatc cagcctcaac
ccatgctctc tcctctcctt caggcctggg ctgtggcccg agggaggtgg ccacctagtg
gagttaacct gccaccccca gaggctgagg ctgctgagcg aagagagcga agaggctgga
agaccaactt ccgctgtgca ctccacagca cagggcgttt tatcttgcgc caagacaatt
caggggatcc agttgatccg cataaggtgt acgaacttag ccgggagctt ggatctactg
gtgagcagtg ccacaaacag ggatgggcca gggaggtaca tggcctctgt cattttggtg
gtttcaatg gtttgatttgt ggtttttgaa actagttgta gattcagttc ctctccttcc
aaagtgtgt gtgtgtcgggg tggaggggga gtgcacactt cctgctaagt cagagccttg
ggctgagtg aggtacctggt ggtttgggaa cagagggtat gagaaataga gaacttttgg
aactgcttg ggca

Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is indicated in red.