

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 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 (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 (°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:

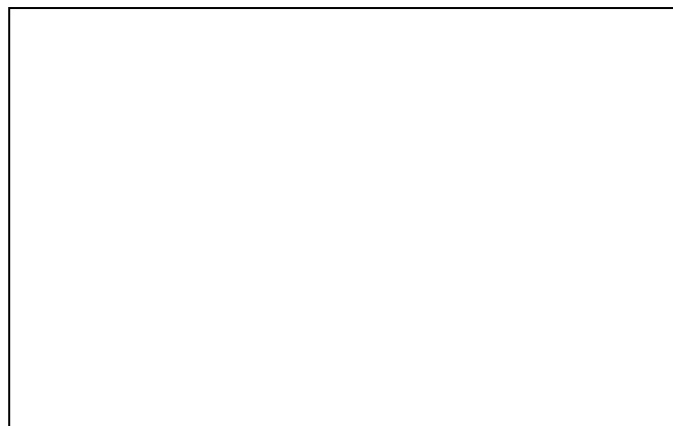
Primer Name	Nucleotide Sequence (5' - 3')
1: Inept (F)	ACCCTGGAAGCATTTTCGGTCGTAG
2: Inept (R)	TGCCCAAGCAGTTCCAAAAGTTCTC
3: Inept_seq (F)	TGCACAGATCTTCAAGGTGG
4: Inept_seq (R)	CCAAGGCTCTGACTTAGCAG

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 catttcgggc gtagggatct ggatgaagaa gatgcacaga tcttcaaggt
ggtagccagt cctgccctct ttataatctg ccagaactcc tgggggaatc cagcctcaac
ccatgctctc tcctctcctt caggcctggg ctgtggcccg agggaggtgg ccacctagt
gagttaacct gccaccccca gaggctgagg ctgctgagcg aagagagcga agaggctgga
agaccaactt ccgctgtgca ctccacagca cagggcgttt tatcttgccg 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 cagaggggtat gagaaataga gaacttttgg
aactgcttg ggca

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