GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

NAME OF PCR: C57BL/6J-*Irak4*^{m1Btlr}*Ticam1*^{m1Btlr}/Mmcd, MMRRC #030625-UCD, (*otiose/Lps2* dble mut)

****In order to do genotyping for #030625, two separate genotyping protocols must be performed, the protocol for *Lps2* and the protocol for *otiose*.**

Protocol: Use this for both *otiose* and *Lps2* PCR reactions:

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*: *Lps2* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide deletion. The same primers are used for PCR amplification and for sequencing. *Otiose* 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 for *Lps2* PCR:

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	55	0.50	30
4. Elongation	72	0.75	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: Lps2(F)	ACAGTCCCAATCCTTTCCATCAGC
2: Lps2(R)	AGGATTCAGATTGGAGTCCCACAGTC
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.476	
(i.e. 3&4)		
(i.e. 1&2&3)		

Strategy for *otiose* PCR:

Steps	Temp (°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	40
3. Annealing	55	0.50	40
4. Elongation	68	1	40
5. Amplification (i.e., 72°C, 10 min)	68	10	**1**
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: otiose(F)	CTTGCTGTCATCTGAATAATTGACTGATTTG
2: otiose(R)	GCTGTAGATGTCAGATTTGGGTGTTATTTCTC
3: otiose_seq(F)	GATATCCTAGGCAAGAAGCATG
4: otiose_seq(R)	AAGCCGTTGTGCCCACGATTC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.404	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 476 nucleotides all within one exon (from Genbank Accession **NM_174989**) is amplified:

```
2111            acagtcccaa tcctttccat cagcctcctc cccagcccca cagactccag
2161 gacctcagcc tctcattatt caccatgccc agatggttca gctgggtgtc aacaatcaca
2221 tgtggggcca cacaggggcc cagtcatctg atgacaagac tgagtgttcg gagaacccct
2281 gtatgggccc tctgactgat cagggcgaac cccttcttga gactccagag tgaccaggtt
2341 ggaccccacc tagatggcta gagtgacaag attggacttc acctgggtcc ttaaaatgat
2401 agtggaggaa gggaacctcg cctgggtccc cagagtagcc agaggactta gcttgggctc
2461 cacagtggct attagttgga cccagcttga gaccccagag gcagggaaga ccacacctat
2521 aaatcaggcc tgggaaacat gcagaaaccc catttgaaca gactgtggga ctccaatctg
2581 aatcct
```

Primer binding sites are underlined; the deleted G is shown in red text.

The following sequence of 404 nucleotides (from Genbank genomic region **NC_000081** for linear DNA sequence of *Irak4*) is amplified:

```
17498                                          ctt gctgtcatct gaataattga
17521 ctgatttgaa tatatttcag tcttctctac tgcacatata aaatgacaca caatgtgaca
17581 tgtgcgtata taatatataa tatatagtat gtagaatgca tgatatatat atatatatat
17641 cacattatat acctcttcct aagaacctgt tggatatcct aggcaagaag catgttttcc
17701 accttcaaat ttaatttgtt ttccagtgca aatatcttac tagacaaaga ctttactgcc
17761 aaaatatctg actttgggct tgcacgggct tcggcaaggc tagcgcagac ggtcatgacc
17821 agccgaatcg tgggcacaac ggcttacatg gcacccgaag ctttgcgggg agaaataaca
17881 cccaaatctg acatctacag c
```

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