

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: MMRRRC Address: 2795 2nd Street, Suite 400, Davis, CA 95618 Contact: Reneé Araiza
 Telephone: 530-754-MMRRRC FAX: 530-757-3284 email: mmrrc@ucdavis.edu

DNA Extraction Method: NaOH _____ Proteinase K _____ Other __Any__

NAME OF PCR: C57BL/6J-*Irak4*^{m1Btlr}*Ticam1*^{m1Btlr}/Mmcd, MMRRRC #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 (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?...CHECK HERE [x]	94	2	1
2. Denaturation	94	0.50	30
3. Annealing	55	0.50	30
4. Elongation			
5. Amplification (i.e., 72°C, 10 min)	72	7	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

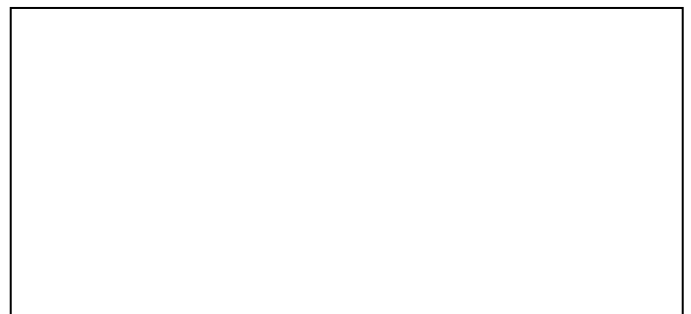
Primer Name	Nucleotide Sequence (5' - 3')
1: <i>Lps2</i> (F)	ACAGTCCCAATCCTTTCCATCAGC
2: <i>Lps2</i> (R)	AGGATTCAGATTGGAGTCCCACAGTC
3:	
4:	

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	94	0.50	40
3. Annealing	55	0.50	40
4. Elongation			
} steps 2-3-4 will cycle in sequence			
5. Amplification (i.e., 72°C, 10 min)	68	10	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

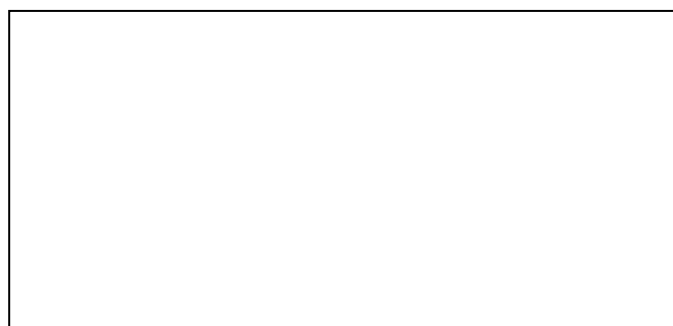
Primer Name	Nucleotide Sequence (5' - 3')
1: otiose(F)	CTTGCTGTCATCTGAATAATTGACTGATTTG
2: otiose(R)	GCTGTAGATGTCAGATTTGGGTGTTATTTCTC
3: otiose_seq(F)	GATATCCTAGGCAAGAAGCATG
4: otiose_seq(R)	AAGCCGTTGTGCCACGATTC

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 gagaaccctc
2281 gtatggggcc tctgactgat cagggcgaac cccttcttga gactccagag tgaccaggtt
2341 ggaccccacc tagatggcta gagtgacaag attggacttc acctgggtcc ttaaaatgat
2401 agtggaggaa gggaacctcg cctgggtccc cagagtagcc agaggactta gcttggggctc
2461 cacagtggct attagttgga ccagcttga gacccagag gcaggggaaga ccacacctat
2521 aatcaggcc tgggaacat gcagaaacc catttgaaca gactgtggga ctccaatctg
2581 aatcct

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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:

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17498      ctt gctgtcatct gaataattga
17521 ctgatttgaa tatatttcag tcttctctac tgacatata aatgacaca caatgtgaca
17581 tgtgcgata taatataaa tatatagat gtagaatgca tgatatatat atatatatat
17641 cacattatat acctcttctc aagaacctgt tggatatacct aggcaagaag catgttttcc
17701 accttcaaat ttaatttggt ttccagtgca aatatcttac tagacaaaga ctttactgcc
17761 aaatatctg actttgggct tgacgggct tggcaaggc tagcgcagac ggtcatgacc
17821 agccgaatcg tgggcacaac ggcttacatg gcacccgaag ctttgcgggg agaaataaca
17881 cccaaatctg acatctacag c

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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.