GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6;129S5-*Acsbg2*$^{Gt(OST346159)Lex}$/Mmucd** **MMRRC #** **031669-UCD**

Protocol: **Neo Tcrd Duplex used for Lexicon/Genentech gene trap lines.**

Reagent/ Constituent	Volume (µL)
Water	10.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM) Neo TD F	0.5
Primer 2. (stock concentration is 20µM) Neo TD R	0.5
Primer 3. (stock concentration is 20µM) Tcrd F	0.5
Primer 4. (stock concentration is 20µM) Tcrd R	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25.000µL**

Comments on protocol:

- This protocol only indicates the presence or absence of internal Neo vector; it does not distinguish heterozygous vs. knockout, nor is it specific to any single construct. TCRD is an internal control to verify DNA is present.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	3:00	**1**
2. Denaturation	steps 2-3-4 will cycle in sequence	94	0:20	**12x**
3. Annealing		64	0:30	
4. Elongation		72	0:35	
5. Denaturation	steps 5-6-7 will cycle in sequence	94	0:20	**25x**
6. Annealing		58	0:30	
7. Elongation		72	0:35	
8. Amplification		72	2:00	**1**
9. Finish		10	∞	n/a

Primers:

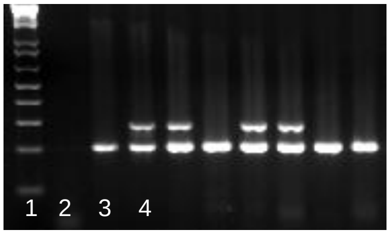
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

Electrophoresis Protocol:

Agarose: 2% **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
200 bp	WT
200 bp / 280 bp	Neo positive



Lanes:
1. 1Kb+ ladder
2. H_2O
3. Wild-type +/+
4. Neo +

Lexicon Gene Trap Protocol attached.

Lexicon Contract Name: DNA192
LexVision Name: ENZ761T2
Genentech ID: UNQ2443

Mouse Accession(s):ENSMUST00000043289, A.chr17-000007.0.0.5590897
Omnibank Clone: OST346159, VICTR48

Mutation Description:

Listed below is a portion of the mouse genomic sequence (from mouse chr.17) surrounding the gene trap insertion site in the Omnibank mouse line ENZ761T2 (OST346159). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

5'
CTACAGTCCTGGGTCAGTGGGTGAACAAAGGCTCTTCTCGAAAGCCCACTGGCCTGGCTAAGGTGGCGAG
TGACTGGTTCAGAGGGAGACCTGTTTTAACACAGAGACATGGCAAGCAACAGAGATACTGCACATCACAC
ACCCAGATGGATGGCATGTGAGCTGTGACTGTCTCTGTGTACACACCCATGTGTAGGCACATCCATACAC
AAATCAGACTTACAATCAGCAGACCACCTGGAACTTCCTC*CTGTCACCCTGCGCTCAGAGGGCTCAAGA
TCAAGGTCTTTTTTTTTTTTTTTAAAGATTTATTTATTTTATTTATGAGTACACTGTAGCTGTCTTCAGA
CACACCAGAAAAGGGCATTAGATCTCATTATGGGTGGTTGTGAGCCACCATGTGGTTGCCGAGAATTGAA
CTCAGGACCTCTGGTAGAGCAGTCAGTGCTCTTACCCTCTGAGCCATCTCTCCAGCCCAAGATCAAGATC
TTAAAGGGAAA
3'

Mutation Illustration: