GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129S5-*Atraid*$^{Gt(OST23679)Lex}$/Mmucd **MMRRC #** 031640-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		94	0:20	
3. Annealing	steps 2-3-4 will cycle in sequence	64	0:30	**12x**
4. Elongation		72	0:35	
5. Denaturation		94	0:20	
6. Annealing	steps 5-6-7 will cycle in sequence	58	0:30	**25x**
7. Elongation		72	0:35	
8. Amplification		72	2:00	**1**
9. Finish		10	∞	n/a

Primers:

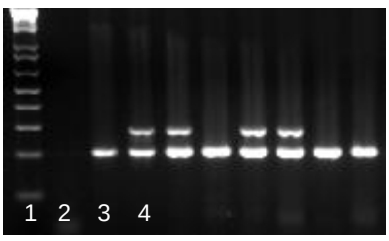
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

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: DNA142
LexVision Name: HSPCO_1
Genentech ID: UNQ214

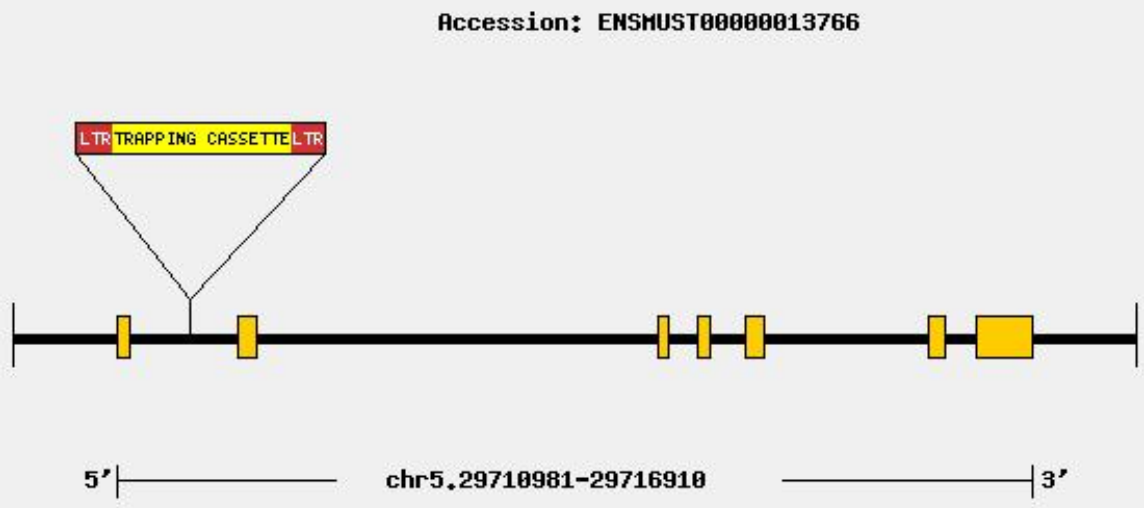
Mouse Accession(s): ENSMUST00000013766
Omnibank Clone: OST23679, VICTR 23

Mutation Description:

Listed below is a portion of the mouse genomic sequence (from mouse chr.5) surrounding the gene trap insertion site in the Omnibank mouse line HSPCO_1 (OST23679). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

5'
CCTGCGGGGATGGTGTTGTCCAGTACTCACGTAGTGCAGGCCACTAGCACCTTCGCCCTGCCCACATTCA
GAGTCCTTTGAAATTTGATCTCCTTGCTTTTGAATAATTTTAAATAGTGCTTCCCAGACGAGTCCCAGGG
TTCTTCTGCCAGCTCTCCTTGCACGAATGCTGTGCGTGGTGCAATGGCACGACTATCTATCTATCTATCT
ATCTATCTATCTATCTATCTATCTATCTATCTATCT*ATCTATCTATCTAAATATATAGTGATGCT
TGAGTACGCTGTGTACCTGCAGCCCTTTAACACTTGCCAAACCATGCCTGTCAGGTGGAGAAGTCTCTGG
AGAGGACACAGTATAGTCAAGTAATAATGCACAAACACGAGGATCTTCTCTCGTACAGTGCGCTGATTTG
GTGCGGGGGTTTTAAGGGAAAAAAATGGGTTCTGTTTACAAAAGGCACTTTTCCGCTTTGCATTATGGGA
ATTCTGGAAAG 3'

Mutation Illustration:



DNA142 – HSPCO_1 PCR to Confirm Genotypes for F1 Hets

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM $MgCl_2$	3.5
10mM dNTPs	1.5
HSPCO_1 5' Primer	0.75
LTR Rev Primer	0.75
5 U/ul Taq polymerase	0.4
Water	33.1
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Cycling Conditions: Touchdown

Step	Temp	Time	Note
1	94C	10'	
2	94C	15"	
3	65C	30"	Decrease 1C/cycle
4	72C	30"	Go to 2, 10 cycles
5	94C	15"	
6	55C	30"	
7	72C	30"	Go to 5, 30 cycles
8	72C	5'	

Primer sequences (5' to 3'):

HSPCO_1 5'	TAAATAGTGCTTCCCAGACGA
LTR Rev	ATAAACCCTCTTGCAGTTGCATC

Well	Sample	Genotype	Well	Sample	Genotype
1	150	wt	13	165	het
2	151	het	14	167	het
3	152	het	15	168	wt
4	153	wt	16	170	het
5	154	wt	17	175	het
6	155	het	18	C57 wt	wt
7	157	het	19	ES DNA	het
8	158	het	20	water	no amp
9	159	het			
10	160	het			
11	163	het			
12	164	het			

Mutant band: 236bp