GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129S5-*Angptl6*$^{Gt(OST285266)Lex}$/Mmucd **MMRRC #** 032148-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:

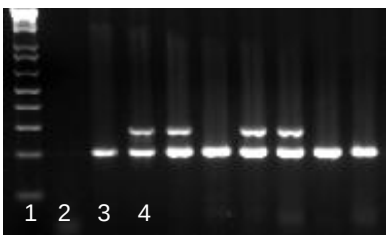
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

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: DNA134
LexVision Name: SEC273T1
Genentech ID: UNQ152

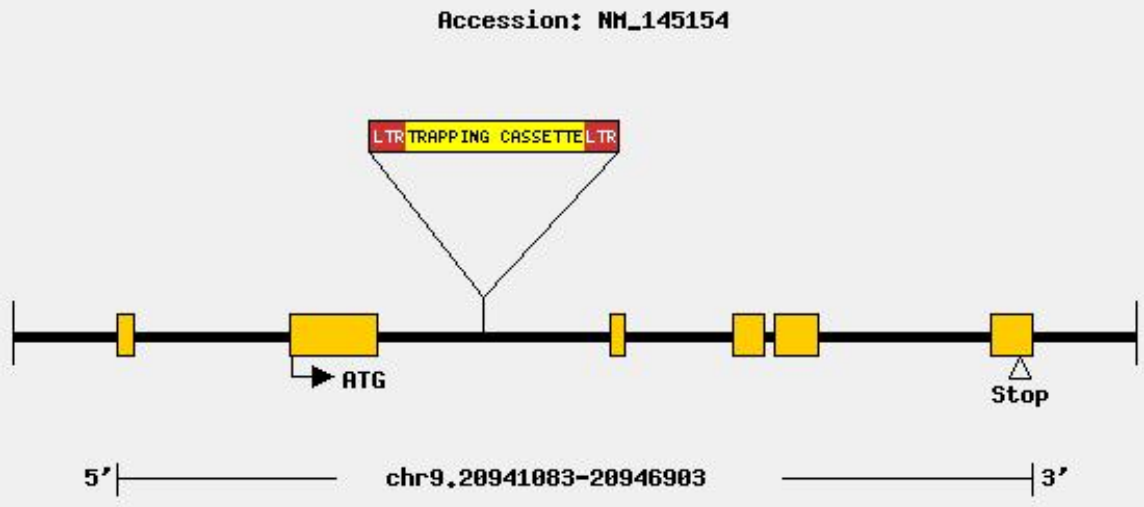
Mouse Accession(s): ENSMUST00000043726, NM_145154
Omnibank Clone: OST285266, VICTR48

Mutation Description:

Listed below is a portion of the mouse genomic sequence (from mouse chr.9) surrounding the gene trap insertion site in the Omnibank mouse line SEC273T1 (OST285266). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

5'
GATGACTGTGAGCCACCTTGCTGGGAATTGAATCTGGGTTTTCTGGAAGAACAGGCAATGCTCTTAACCG
CTGAGTCAGCTCTCCAGTCCCTAAGACAATATATTCTTGTAACTACATGGGACTTATGAATTTTGGGCAA
TTTCTGTCACCAGCCTTTTTGGTTTTGTTTGCTTGTTTTCTTTTTTCTTTTATTGAGATAGGGTCTCATG
TAGCCCAGGCTAGGTTCTAGCATGTAGCTCAGGCTAGGTT*CTAGCTCCTTGTGTAGTTGTGAATGACCT
TGATTCTCATGTTCTGCCTCAGCGCAAATGACAGACATGGGACAACAAACAGCCAGGTTGGCTGGTTTTA
CATTCCTATTGATTTTGGGGTCCCTGGGACCTCCTATATGTGAGGTGGCTGTTCTGCTGCTGAGCCACAT
CCCCCAGCATTGGGCCTGAATTTTGGCTTAGTGATGGTTCTGTGGATTTTCCTAACTCGTCTGGGGCCCA
CGTGCTCCTGC
3'

Mutation Illustration:



DNA134 – SEC273T1 PCR to Confirm Genotypes for F2 Animals

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM $MgCl_2$	5
10mM dNTPs	1.25
LTR2 Primer	1.25
SEC273T1 3' Primer	1.25
5 U/ul Taq polymerase	0.5
Water	30.75
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Cycling Conditions: Touchdown

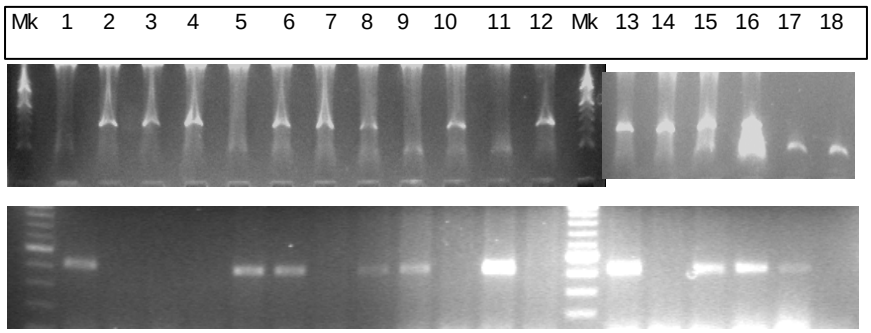
Step	Temp	Time	Note
1	94C	10'	
2	94C	15"	
3	65C	30"	Decrease 1C/cycle
4	72C	30"	Go to 2, 10 cycles
5	94C	15"	
6	55C	30"	
7	72C	30"	Go to 5, 30 cycles
8	72C	5'	

Primer sequences (5' to 3'):

LTR2	AAATGGCGTTACTTAAGCTAGCTTGC
SEC273T1 5'	TTCTGGAAGAACAGGCAATGCTC
SEC273T1 3'	TCCACAGAACCATCACTAAGCCAA

Well	Sample	Genotype
1	71	wt
2	73	hom
3	80	hom
4	81	hom
5	82	wt
6	84	het
7	86	hom
8	87	het
9	88	wt
10	91	hom
11	96	wt
12	97	hom
13	98	het
14	99	hom
15	102	het
16	es DNA	het
17	wt lysate	wt
18	water	no amp

Mutant band: 266bp (LTR2 + SEC273T1 3')



Wt band: 425bp (SEC273T1 5' + SEC273T1 3')