

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

NAME OF PCR: **B6;129S5-Esam<sup>Gt(OST369358)Lex</sup>/Mmucd**

MMRRC # **032286-UCD**

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

| Reagent/ Constituent                                                                                                              | Volume (μL)     |
|-----------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Water                                                                                                                             | 10.275          |
| 10x Buffer                                                                                                                        | 2.5             |
| MgCl <sub>2</sub> (stock concentration is 25mM)                                                                                   | 1.7             |
| Betaine (stock concentration is 5M) <i>Optional</i>                                                                               | 6.5             |
| dNTPs (stock concentration is 10mM)                                                                                               | 0.5             |
| DMSO <i>Optional</i>                                                                                                              | 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 <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other: | 1.0             |
| <b>TOTAL VOLUME OF REACTION:</b>                                                                                                  | <b>25.000μL</b> |

### 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<sub>2</sub> to increase reaction or decrease non specific amplifications.

### Strategy:

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

### Primers:

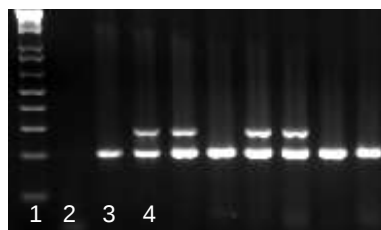
| Name        | Nucleotide Sequence (5' - 3') |
|-------------|-------------------------------|
| 1: Neo TD F | CTTGGGTGGAGAGGCTATTC          |
| 2: Neo TD R | AGGTGAGATGACAGGAGATC          |
| 3: Tcrd F   | CAAATGTTGCTTGTCTGGTG          |
| 4: Tcrd R   | GTCAGTCGAGTGCACAGTTT          |

### 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<sub>2</sub>O  
3. Wild-type +/-  
4. Neo +

Lexicon Gene Trap Protocol attached.

**Lexicon Contract Name: DNA145**  
**LexVision Name: SEC377T1**  
**Genentech ID: UNQ220**



Mouse Accession(s): ENZMUST00000002011, NM\_027102  
Omnibank Clone: OST369358, VICTR 48

## 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 SEC377T1 (OST369358). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk \*.

```
5'
GCCAGTGGGGGTGGGGTGGGGTGGGGTGGGGTGGTGGGGCGGGGCCTAGGGTGAGACTGAGGATAGAAGA
AACACGGTGTGAGTAAACAAAACCTTTTTCTTTTTTGGTCTTATACTATATACCCAGCTGGCCCTCAAAA
CTCACAGAGGCATGCTGCCTCTGCTTCCTGAGTGCTGAGATTAAAGGCAGGGGACACCGTCCCTGCATC
ACCAACTTAAAAAGCTTCCCATTGTTGTGAGCTGACATCTT*CTGGGCTTGAGATTGTCAAGTACAGGCCA
CATCTCTTCCTGGATCCATCTGCCATTATCAGCCCTCCTCCTCATGTTTGTGTAGAGTGGGCTTGTA
CAGGCCATGGTGCTAGTACAGAGTTCAGAGATAAACTTTCAGGAGTCACTTCTTTCCTTCTATCTTTATG
TGACTTCCAGGGACTGAACTCAGATCATTAGCCTTGGTGGCAATGCTTTTTTTTTTTTTTCCCAACAATG AGCCATCTTGC 3'
```

## Mutation Illustration:

Accession: NM\_027102

