

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Agpat6^{Gt(OST103559)Lex}/Mmucd MMRRC # 032136-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) <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 ☐ 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 MgCl₂ 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	} 12x
3. Annealing } steps 2-3-4 will cycle in sequence	64	0:30	
4. Elongation	72	0:35	
5. Denaturation	94	0:20	} 25x
6. Annealing } steps 5-6-7 will cycle in sequence	58	0:30	
7. Elongation	72	0:35	
8. Amplification	72	2:00	1
9. Finish	10	∞	n/a

Primers:

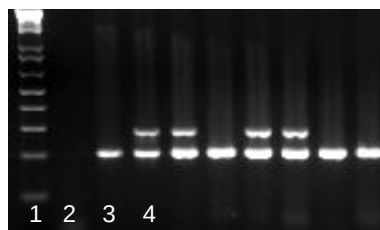
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

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. H₂O
3. Wild-type +/+
4. Neo +

Lexicon Gene Trap Protocol attached.

Lexicon Contract Name: DNA420
LexVision Name: ENZ375T1
Genentech ID: UNQ551



Mouse Accession(s): ENSMUST00000033949, AK083589
Omnibank Clone: OST103559, VICTR 48

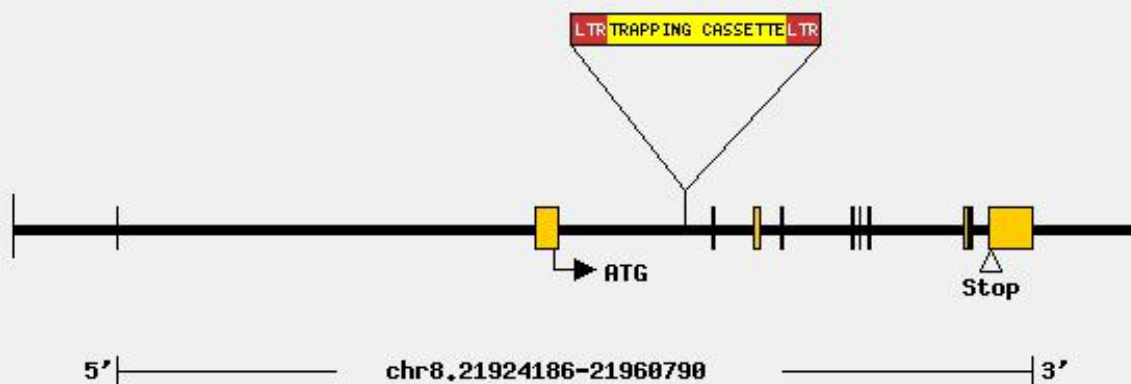
Mutation Description:

Listed below is a portion of the mouse genomic sequence ([from mouse chr.8](#)) surrounding the gene trap insertion site in the Omnibank mouse line ENZ375T1 (OST103559). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *****.

```
5'
CTACCTAAGCAAGGCCTGCCTCCCAGCAACCACACCCCAAAGAAAAGTAACACATTCTTCCCTAGCCGCC
ATCAATCTGCCAGTAGCTAGCTCCTCATAGCTCCTCAGCTAGGGGTGGAGTCTCGTGAGCCTTTTCTGTG
TCCATGCTGCAGTGTGACTGCCTTGATTTGGTGACCATAGCCACAGTGAGCGCATGAGTGCAGCAGCAGC
CACACCACCCCGAGCAGGCAGCCCTTCACAGCGCTGTCTT*ACTCTCCATCATCTGCAATGCTCCTTGAG
ACCTTGAGGATATGGATAGGCGCCCGACATAGGGCTGATAATCAGACAGTCATTTAGTCTCAGCACATTG
ACCCGCGTGAATCTCTCTATTAATTGTTGTCTAAAGATAGACACATTTTCTACGAAAGCCGAAGGGGAAG
GACCACAGCTATAACGGCAGCAAACACCCAGCATTCTAGAGCTAGGAGAACTCAGTGGAGAGACTCCTGC AGCAAACAGTA
3'
```

Mutation Illustration:

Accession: AK083589



DNA420 – ENZ375T1 PCR to Confirm Genotypes for shipping

Reaction Components Vol (ul)

10X Sigma Buffer	5
25mM MgCl ₂	5
10mM dNTPs	1.25
LTR2 primer	1.25
ENZ375T1 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:

LTR2	AAATGGCGTTACTTAAGCTAGCTTGC
ENZ375T1 3'	GCGGGTCAATGTGCTGAGACTAAA

Well	Sample	Genotype
1	186	het
2	198	het
3	204	het
4	205	het
5	206	het
6	ES DNA	het
7	wt lysate	wt
8	water	no amp

Mutant band: 154 bp

