

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Cd320^{Gt(OST110882)Lex}/Mmucd MMRRC # 032186-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	64	0:30	
4. Elongation	72	0:35	
5. Denaturation	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:

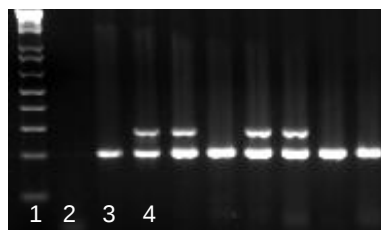
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

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. H₂O
3. Wild-type +/+
4. Neo +

Lexicon Gene Trap Protocol attached.

Lexicon Contract Name: DNA279
LexVision Name: AM2RPT_1
Genentech ID: UNQ198



Mouse Accession(s): NM_019421
Omnibank Clone: OST110882, VICTR 48

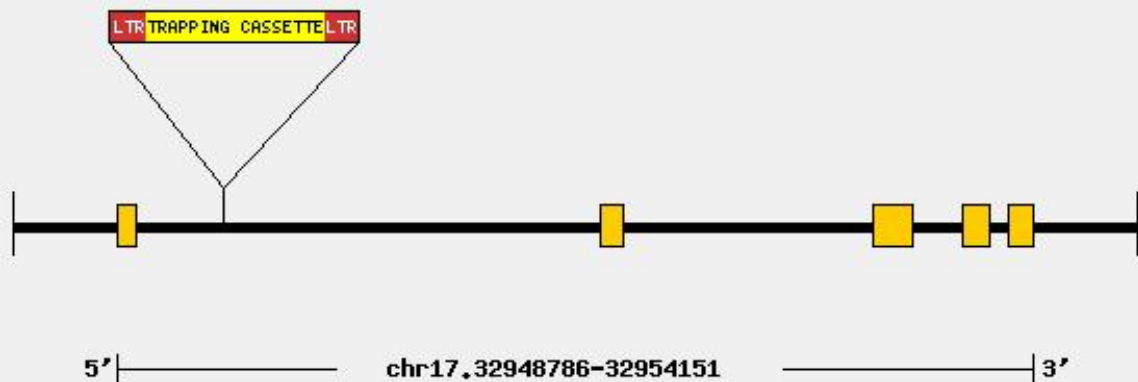
Mutation Description:

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 AM2RPT_1 (OST110882). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

```
5'  
AGCTCAATGTGGGCTGGAAGGCTTTTGTTCGATTTTGACCTCTCACCACGCAGGGCTGGTCACCCGGT  
ACCTTCTTGCAGCAGGTATGCCTTCCCGAAACCTTCAGCCCTGAGGCTGCAGTTCTGTGGCTGTTCTTTC  
AGCACCCAGAGCTTCAGTTTTTTCCTTGATTCCCTGCCTCAGAGAATGGAAGGAAGAGCCCTGGAGTGTAT  
TTCCCTGTGAACCCCAATCTTGCCAGGAACCTGTCCCG*GGCAGGACGAGGAGGCCCGATTGTGACCA  
CTGGCCACTACTGACCATCCACTCTGTATGAGAATAGGTCTTAAGTGTTCAGGTTTTTTTGGTTTGT  
TGTTTTTGTTCGTTTTTAGGTTTTTATTATGTTTTGATTTTTTTTTTTTTTCCAAACAGGGTCTCA  
CTTTATAGCTCTGGCTGTCTGCAACTTGCTATATAGACTAGGCTGGGCTTGAAGTCTAGAGATCCACCT  
CCCAAGTACTG  
3'
```

Mutation Illustration:

Accession: NM_019421



DNA279 – AM2RPT_1 PCR to Confirm Genotypes for Shipping

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3
10mM dNTPs	1
LTR2 Primer	1.25
AM2RPT_1 3' Primer	1.25
5 U/ul Taq polymerase	0.5
Water	33
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
AM2RPT_1 3'	TCAGTAGTGGCCAGTGGTCACAATC

Well	Sample	Genotype
1	140	het
2	141	het
3	143	het
4	147	het
5	148	het
6	150	het
7	152	het
8	wt lysate	wt
9	water	no amp

Mutant band: 395 bp

