

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-VapbGt(OST284921)Lex/Mmucd **MMRRC:** 032702-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

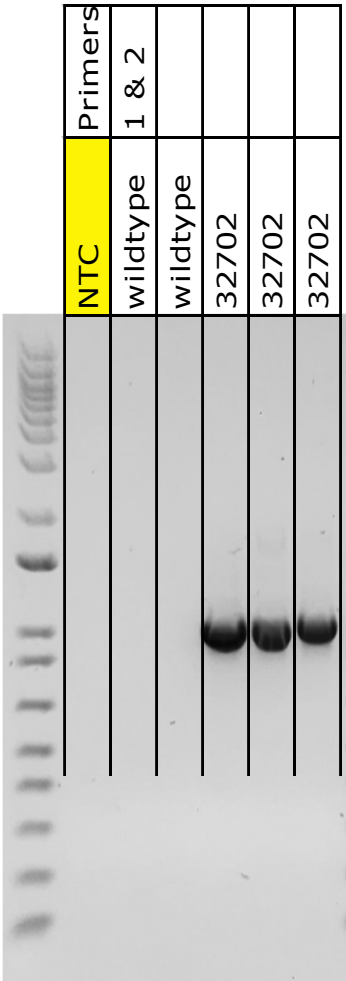
Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 32702-F	CCGTGCACACTTTCTGTGTAGTAC	Estimated Running Time: 90 min.		
2. B-Geo-R	TGCCCAGTCATAGCCGAATA	Primer Combination	Band (bp)	Genotype
3. 32702-wtR	CAAGAGTCTGTGGGTACAGTGG	1 & 2	~900	mutant
32702-wtR is untested		1 & 3	587	wildtype

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Lexicon Contract Name: DNA160
LexVision Name: MEM592T1
Genentech ID: UNQ484



Mouse Accession(s): ENSMUST00000029026, NM_019806
Omnibank Clone: OST284921, VICTR 48

Mutation Description:

Listed below is a portion of the mouse genomic sequence ([from mouse chr.2](#)) surrounding the gene trap insertion site in the Omnibank mouse line MEM592T1 (OST284921). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *****.

```
5'
CGGCCGCCCCGGGAGCTGTCACTCAGCGGCCGGTGCCGGAAGGGACGCGCCCTGAGGGCAGGACACTGT
C CGCTTGAGGCCCCGCATGCACCAGGCGTCTAATGCATGCATGTGGAGTCAGTGCATGGAAACGGTTTGCCT
GAGTACCCCTTCATCAGCTCCCCGAGGCAGCGAGCTGCCGGGGTTACAGCCACGAAGCCCGTGCACAC
TTTCTGTGTAGTACACTTTCTGTATACATCTGTGTGTA**GGTGCATGCCCAGGTGATTAAAAAGGTGC
ACGTAGACAAATATATATGCATGGTATACGCATGTTATAGATAGTGTGTATACATACATGTGCGTACATA
TATGTACATACACACACACAAGTGTGTGTGTATGTACACATGAATGTTTCCATTCACTAATTTTAAGGGC
CACGAGGCAACAGGGTCCATGCCCAGCCTGTTCTTGCTTCGTTGCATGTTTGAGTCATGAGATTGGGCA
CAGGAGGCCTA 3'
```

Mutation Illustration:

Accession: NM_019806

