

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Sulf1Gt(OST352220)Lex/Mmucd **MMRRC: 032608-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:

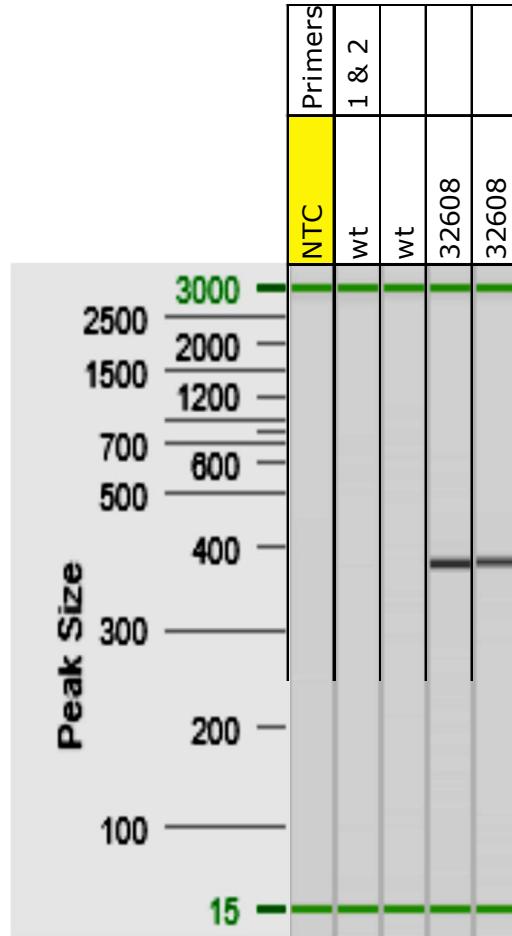
Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 32608-F	CAAAGAGGTTTGCTGAGAAATAGTGTC	Estimated Running Time: 90 min.		
2. LexGT-R	CCCTCAGCAGTTTCTAGAGAACC	Primer Combination	Band (bp)	Genotype
3. 32608-wtR	CTGGGGGAACCTTCACACTCC	1 & 2	390	mutant
		1 & 3	292	wildtype

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Lexicon Contract Name: DNA509
LexVision Name: ENZ596T1
Genentech ID: UNQ14964



Mouse Accession(s): NM_172294
OmniBank Clone: OST352220, VICTR48

Mutation Description:

Listed below is a portion of the mouse genomic sequence ([from mouse chr.1](#)) surrounding the gene-trap insertion site in the OST352220 OmniBank ES cell line. The sequence below includes 250 nucleotides of sequence on either side of the gene-trap insertion site, which is denoted with an asterisk *****.

```
5'
ACAGGCTGTCGCCGCAAAGACTTGGAATTAAGTCTGAGAAGGAATGACCAGAGGCGGCTTGGGTAAACTG
AGTCTTGATCCTCAGGACATAAGCTAAAAGCTTGCCATGGCAGTATTCAGCTATAGCCACAGTATGCGCG
CAGAGACGAGAGTCTTGAGTTCTTCAGTCAGCCAGTATAGCAGAATCAAAGAGGTTTGCTGAGAAATAG
TGTCCACGAGGTGATGAGACATCTGAATGACACCCGAC*CTCCAACCTCAGCCTCTAGCCTCCGTATA
AACCACAGAGAGAGAGAGAGAGACAGAGACAGAGACAGAGACAGAGACAGAGACAGAGACAGAGACAGAG
ACAGACAGAGACAGACAGGCAGACAGACAGACAGAGACAGACAGAGACAGACAGACAGACAGACAGACAG
ACAGACAGAGACAGAGACAGACAGAGAACTGAGTGTGGAGTGTGTGAAGTCCCCCAGAGGGGCCCA GGGACAAGGAA
3'
```

Mutation Illustration:

Accession: NM_172294

