

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Sppl3Gt(OST279815)Lex/Mmucd **MMRRC:** 032604-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	5:00	1x
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	72	0:40	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 32604-LacZF	ATCCTCTGCATGGTCAGGTC	Estimated Running Time: 90 min.		
2. 32604-LacZR	CGTGGCCTGATTCATTCC	Primer Combination	Band (bp)	Genotype
3. 32604-wtF	GGACTGAATGACCAACTAGTATCAGCC	1 & 2	315	mutant
4. 32604-wtR	CCTCTTTGCATCTTTTACGTGCATGG	3 & 4	269	wildtype

This protocol is untested

Lexicon Contract Name: DNA186
LexVision Name: PRT340T2
Genentech ID: UNQ1887



Mouse Accession(s): ENSMUST00000031530
Omnibank Clone: OST445980, VICTR 37

Mutation Description:

Listed below is a portion of the mouse genomic sequence ([from mouse chr.5](#)) surrounding the gene trap insertion site in the Omnibank mouse line PRT340T2 (OST445980). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *****.

5'
TCTCTGTATAGCCCTGGCTGTCCTGGAACCTCACTTTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCGC
CTGCCTCTGCCGCCCAAGTGCTGGGATTAAAGGCGAGCGCCACCACGCCCAGCAGAACCCATGTATTGAA
TGATACGTTGTGGTGTGCTTCTTCTTTATAGTCATAGCATATGCCACTATCATCGTAGAAAGAAACATCT
GAAATAGCTCTAGAGCATTCTGAAGTACGTGTGTCATTTA*ACTGTTTCTGGGTCCTTACATTGTCTTTT
TAAACACCTAAAATAAACATGCTAGGTCTACTCCTTGGGATAGATGTGGCATCACTCTGATCCACCTAGG
CAAGTGCACTTGCTGTGTACGCGCATACATGATTTTCTAAAGATAAATTTCCAGGCATGACGCCTCTCCC
TAGTAAAGGTTTTCTATTTACTGTGTGTGTTCCAAACAGGGTTTCTCTGTGTAGAGCCCTGGCTGTCC
TGGAACTTGCT 3'

Mutation Illustration:

Accession: ENSMUST00000031530

