

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-*Pi16*^{Gt(OST292194)Lex}/Mmucd **MMRRC:** 032520-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	11.275
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)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

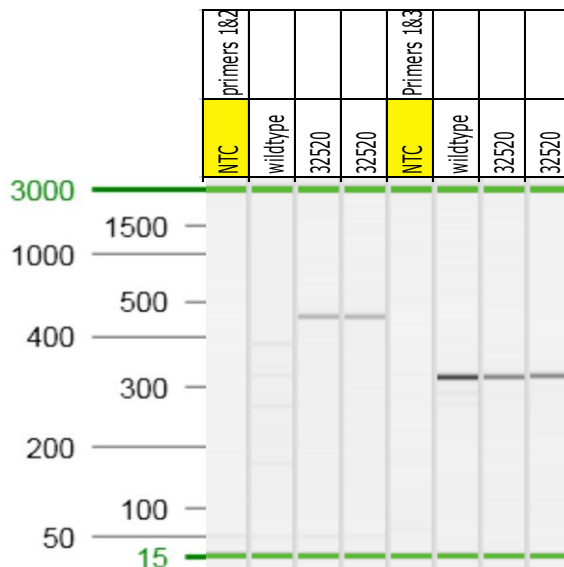
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 32520-wtF	CCAACACTTGGGAGGCAGAGG	Estimated Running:Time: 90 min.		
2. 32520-mutR2	AGCTCAATAAAAGAGCCCACAACC	Primer Combination	Band (bp)	Genotype
3. 32520-R2	CAGCATTGGGAAGAGAAAACAGTCC	1 & 2	451	Mutant
		1 & 3	323	Wild-type



Lexicon Contract Name: DNA151
LexVision Name: SEC482T2
Genentech ID: UNQ289



Mouse Accession(s): ENSMUST00000024808, NM_023734
Omnibank Clone: OST292194, 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 SEC482T2 (OST292194). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

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5'  
GGCAGATTTCTGAGTTCAAGGCCAGCCTGGTCTACAGAGTGAGTTCCAGGACAGCCAGGGCTACACAGAG  
AAACCCTATCTTGAAAAACCAAAAAAAAAAAAAAGTTTCTAAAAACACTGTGTAGGCAGAAAGCCTACTT  
TCTCACTGGAGAAAGGTTTCTGGAACCTTCTGACAGTGTTCCTGGCCTCTCTTAGATAACCTTGGCTTAC  
CACTCAGTTCCGGATGCTTCTGCAAAGTTCAGTCTTGGGG*AGTGACGCTAGCCTTAGTTTTAAAGGGAC  
TGTTTTCTCTTCCCAATGCTGATTGTACAACCGATTACACCAGAAACCAAAAGGGGGGGGGGGATCAACA  
GTCCCCTCCCATTTCCAGTGCCTTTTTGAGCTGGTATTTTTAGCCCTAATCTTAAGGAAATACAATCACC  
CACCCAAGCTGGGAAGTTGAAGGTCCCAGCACCTCCCTGCCGGGACATCCCAGCCCACCCTCACCCAAG  
ATACTGGTGAC 3'
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Mutation Illustration:

Accession: NM_023734

