

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-lfnetm1Lex/Mmucd **MMRRC: 032369-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. DNA349-9	CGAAATTTCACTGAAGAACC	Estimated Running Time: 90 min.		
2. neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA349-2	AATCCACCGCTGTATGTTCTTTG	1 & 2	425	mutant
4. DNA349-5	CCTTTTGCCGTATTCTATTATG	3 & 4	489	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ360	Date of Submission:	6-27-05
Lexicon Contract Name:	DNA349	Mutation Type:	X Standard Knock out
LexVision Name:	SEC540N1		☐ Conditional
Reference accessions:	NM_177348	Is this gene X-linked?	NO

Required Materials: ☐ pKOS clone DNA(s) __pKOS-4_____
 ☐ Target Vector DNA __pKOS-4 TVneo_____
 ☐ Targeted ES Cell DNA __1G3_____
 ☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	15/16	21/22
Restriction Enzyme for Genomic Digest:	NheI	Bgl II
Predicted Wild-type Band (kb):	13.5 kb	9.8 kb
Predicted Mutant Band (kb):	18.2 kb	15.3 kb
Probe Size:	333 bp	346 bp

PCR Strategies:

For standard knockouts, give wildtype and mutant-specific strategies

For conditionals, give 5' loxP and cre-excision strategies

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	DNA349-2	5' Primer Name:	Neo3a
3' Primer Name:	DNA349-5	3' Primer Name:	DNA349-9
Predicted Wild-type Band (bp):	489 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	425 bp

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:**Southern probes**

DNA349-15 5' – CTGTCATTGTCTCTGCCTCATT
DNA349-16 5' – GTTACTTCTTTTATTTGCCTTACG
DNA349-21 5' – GGCCACTTCTCCCTCCTC
DNA349-22 5' – GCCCATATCACCAAAGCAGTC

PCR Genotyping

DNA349-2 5' – AATCCACCGCTGTATGTTCTTTG
DNA349-5 5' – CCTTTTGGCCGTATTTCTATTATG
DNA349-9 5' – CGAAATTTCACTGAAGAACC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

TGGTTCACAGACAGCTCCCTGAAACGGTGTTGCTGCTCTTGGTTTCTTCCACTATCTTCTCCCTAGAACCGAAACGGATT
CCCTTCCAATTGTGGATGAACAGAGAAAGCCTACAACCTTCTGCAAGCTCGTCAGTCCAGCAGTGTCTAG
CACACAGGAAGAATTTCTGCTTCTCAGCAGCCTGTGAGTCTCACCAGTACCAAGAGGGACAGGTGCTGGCTGTTGT
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GTACAGCAGCTGTGCTGGATCATTGTCCAGACAGAAATCCACCGCTGTATGTTCTTTGTGTTTCAGGTTACAAACATGG
CTGAGCAGACAAGACCCAGACCCCTGAACACTGAGAA

Genomic Locus: (The deleted sequence represents nt-20002-20589 in the sequence below. KOS4 used to generate the TV represents nt-15117-25565 in the sequence below.)

CAGATGTGTTCTGTGCTGATTCTAAATACAGTCAAGGTGATAGTGAACATTAACCTCCACACTGCCTTTCCCCTTCATC
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[illegible]

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Selection Cassette:

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