

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Slc39a8tm1Lex/Mmucd **MMRRC: 032588-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.15
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
Primer 3. (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:

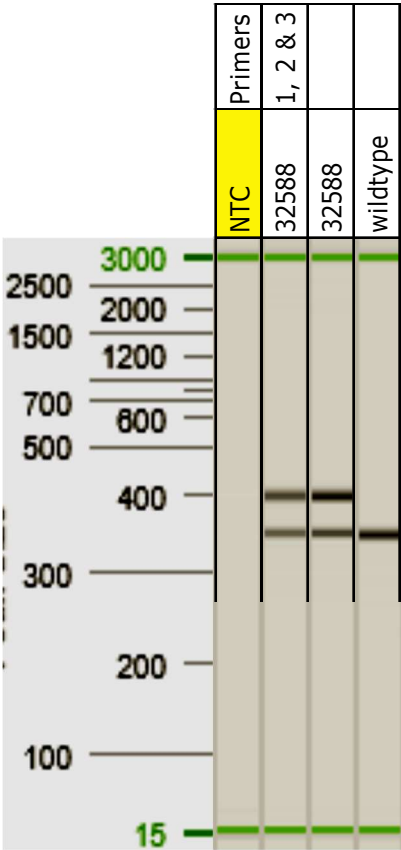
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA496-27	CTTCGTGGGCTTAAGCATCCAG	Estimated Running Time: 90 min.		
2. Neo3bExt	GGTGTGGCGGACCGCTATCAGGAC	Primer Combination	Band (bp)	Genotype
3. DNA496-3	AATGACCATACTCACTTCAGG	1 & 2	403	mutant
		1 & 3	348	wildtype

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Sample ID	Assay	Well
ntc		A1
wt		B1
10466		A2
2		B2
3		A3
4		B3
5		A4
6		B4
7		A5
8		B5
9		A6
ntc		B6
wt		A7
32358		B7
3		A8
4		B8
5		A9
6		B9
7		A10
8		B10
9		A11
10		B11
11		A12
12		B12
13		C1
14		D1
15		C2
ntc		D2
wt		C3
31665		D3
3		C4
4		D4
5		C5
6		D5
7		C6
ntc		D6
wt		C7
13807-REG		D7
15		C8
16		D8
17		C9
18		D9
19		C10
20		D10
21		C11
22		D11
23		C12
ntc		D12



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ11645	Date of Submission:	2/25/2005
Lexicon Contract Name:	DNA496	Mutation Type:	X Standard Knock out
LexVision Name:	TRA406N1		☐ Conditional
Reference accessions:	ENSMUST00000029810, NM_026228	Is this gene X-linked?	NO

Required Materials:

- x pKOS clone DNA(s) __pKOS92__
- x Target Vector DNA __pKOS92TV neo__
- x Targeted ES Cell DNA __2A12__
- x Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	13/14	Neo 2/5
Restriction Enzyme for Genomic Digest:	BclI	NsiI
Predicted Wild-type Band (kb):	15.1 kb	---
Predicted Mutant Band (kb):	11.5 kb	14.5 kb
Probe Size:	287 bp	609 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:	DNA496-3	5' Primer Name:	Neo3bExt
3' Primer Name:	DNA496-27	3' Primer Name:	DNA496-27
Predicted Wild-type Band (bp):	348 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	403 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

DNA496- 13 5' – TCTGGATTAAAGAGGAACGG
 DNA496-14 5' – TTCAGCTGCTGTAAGATCGC
 Neo 2 5' – CCTCAGAAGAACTCGTCAAG
 Neo 5 5' – GGCAGCGCGGCTATCGTG

PCR Genotyping

DNA496-3 5' – AATGACCATACTCACTTCAGG
 DNA496-27 5' – CTTCGTGGGCTTAAGCATCCAG
 Neo3bExt 5' – GGTGTGGCGGACCGCTATCAGGAC

Genomic Sequence Deleted:

Genomic Locus: (The deleted sequence represents nt 12012 – 14367 in the sequence below. KOS-92 used to generate the TV represents nt8510 – 19415 in the sequence below.)

GCTTCTCAATAGAGCTCGTCACTCATCACCATCTGGTGTCTCTGCAGGCATTACGAATTTAGATGTCTGAAATGCACTGGCCACCCTGCTGCCGAATGTC
CTACCAAGGACCAACATCCGCTTTCCATTTGCCCGGAGGCTTTTAGTCTCATGTTTCTTATGATTTCTTCTGTCATACAACCTTCTCCCTCTCATAAAGATTTCTTA
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[illegible]

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Selection Cassette:

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