GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6;129S5-Egfl6tm1Lex/Mmucd **MMRRC:** 032277-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

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/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:

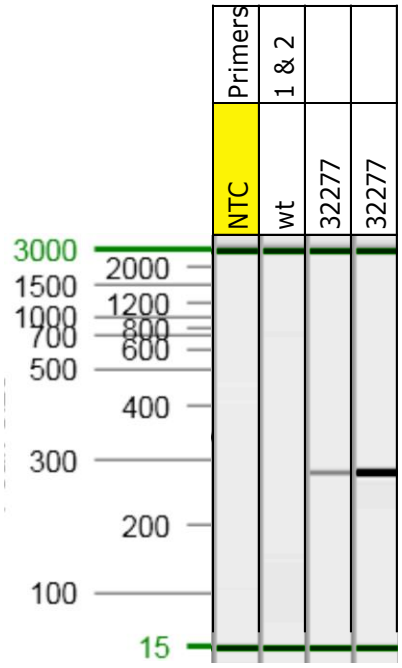
Name	Nucleotide Sequence (5' - 3')
1. DNA474-4	GCGGTGTCCTGGTGAAATG
2. Neo3a	GCAGCGCATCGCCTTCTATC
3. DNA474-31	CTCGTGGGTCGTCCTG
4. DNA474-32	ACACTCACCTGGTCCCTACT

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	300	mutant
3 & 4	363	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ281	**Date of Submission:**	2-17-04
Lexicon Contract Name:	DNA474	**Mutation Type:**	X Standard Knock out
LexVision Name:	SEC294N1		☐ Conditional
Reference accessions:	NM 019397	**Is this gene X-linked?**	YES

Required Materials:
X pKOS clone DNA(s) __KOS 79_____
X Target Vector DNA __TV79_____
X Targeted ES Cell DNA __2A11_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' Internal**
Name of Probe:	23+24	25+26
Restriction Enzyme for Genomic Digest:	HincII	ApaI
Predicted Wild-type Band (kb):	5kb	18kb
Predicted Mutant Band (kb):	6kb	11.7kb
Probe Size:	158bp	512 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:	31	5' Primer Name:	Neo3a
3' Primer Name:	32	3' Primer Name:	4
Predicted Wild-type Band (bp):	363bp	Predicted Wild-type Band (bp):	NONE
Predicted mutant band (bp)	none	Predicted mutant band (bp)	300bp

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
DNA474-23 5' –CAGCAGTGCCAATGAGGTTAC
DNA474-24 5' – TCAGCCAACCCAGTTACGAC
DNA474-25 5' – CCAGTTTGTCCCTAGGCT
DNA474-26 5' – CACTGGGATTTGAGATCTTATT

PCR Genotyping
DNA474-4 5' – GCGGTGTCCTGGTGAAATG
DNA474-31 5' – CTCGTGGGTCGTCCTG
DNA474-32 5' – ACACTCACCTGGTCCCTACT
Neo3A 5' - GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

AGCCCACCCCCGGCCAGTATGCAGCCGCCCTGGGGCCTGGCGCTTCCTCTGCTGCTCCCCTGGGTGACAGGTGGAGTAG
GGACCAGGTGAGTGTT

Genomic Locus: ***(the deleted sequence represents nt 10602-10696 in the sequence below)***

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Selection Cassette: IRES/LacZ/MC1Neo

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