

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Ms4a6ctm1Lex/Mmucd **MMRRC: 032482-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 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.

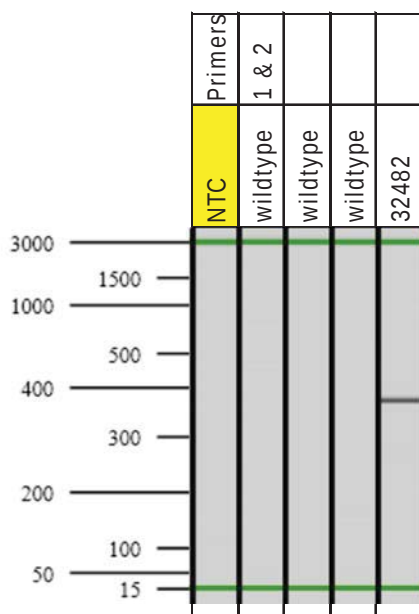
Strategy:

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. DNA369-11	CTAAACTCAAGAGGTCATTGAAG	Estimated Running:Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA369-18	GGACAGAAACGCCTAAAGGT	1 & 2	380	mutant
4. DNA369-19	AGAGAAGGGAGATGGTGACTACTA	3 & 4	204	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ 15982	Date of Submission:	10.21.04
Lexicon Contract Name:	DNA369	Mutation Type:	x Standard Knock out
LexVision Name:	MEM755N1		☐ Conditional
Reference accessions:	ENSMUST00000025579; AF237910	Is this gene X-linked?	No

Required Materials: x pKOS clone DNA(s) __KOS47_____
 x Target Vector DNA __TV47-4_____
 x Targeted ES Cell DNA __2H1_____
 x Genomic Map

Southern Blot Analysis: External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	16+17	7+8
Restriction Enzyme for Genomic Digest:	HindIII	Acc65I
Predicted Wild-type Band (kb):	10.1 kb	12.1 kb (running ~1kb smaller)
Predicted Mutant Band (kb):	5.6 kb	9.9 kb (running ~1kb smaller)
Probe Size:	290 bp	266 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:	18	5' Primer Name:	Neo3a
3' Primer Name:	19	3' Primer Name:	11
Predicted Wild-type Band (bp):	204 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	380 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**

DNA369-16 5' – AATAAGGTCCGACTGGGGAAAGC
 DNA369-17 5' – ACCTGACATCCCCTCACCAGTC
 DNA369-7 5' – GAATGCCACGTAAGTCAATAG
 DNA369-8 5' – TGGTCTTGGCATATACTAGTTTC

PCR Genotyping

DNA369-18 5' – GGACAGAAACGCCTAAAGGT
 DNA369-19 5' – AGAGAAGGGAGATGGTGACTACTA
 DNA369-11 5' – CTAAACTCAAGAGGTCATTGAAG
 Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

ATGATTCCACAGGTAGTGACCAATGAGACCATCACAACGATTTACCCAAATGGAATCAACTTTCCCCAAAAAGACGAGTCCAGCCTACCCAACAGAG
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Genomic Locus: (The deleted sequence represents nt 8699-10162 in the sequence below. KOS47 used to generate the TV represents nt 6679-12088 in the sequence below.)

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Selection Cassette: LacZ/Neo

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Targeted Locus:

[illegible]

[illegible]

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