

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Ms4a6dtm1Lex/Mmucd **MMRRC: 032483-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.

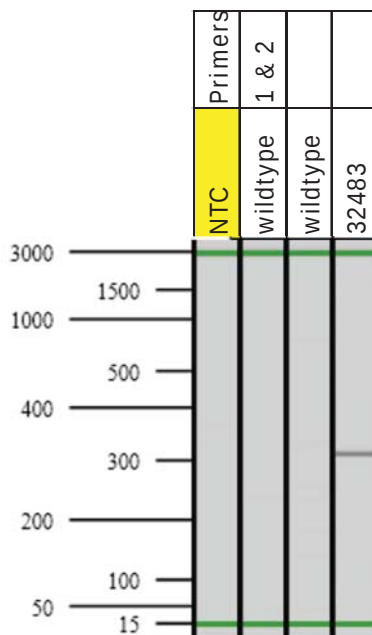
Strategy:

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. DNA459-21	TTCACATGTCCAGACTGTAAGC	Estimated Running Time: 90 min.		
2. GT Ires	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. DNA459-7	TTGCGCTGTAATTCAATGACC	1 & 2	325	mutant
4. DNA459-8	TAAGCTAAGACCATCAGTTCC	3 & 4	388	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ15965	Date of Submission:	7-12-05
Lexicon Contract Name:	DNA459	Mutation Type:	X Standard Knock out
LexVision Name:	MEM623N1		☐ Conditional
Reference accessions:	NM_026835	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __pKOS45, pKOS79, and pKOS82_____
☐ Target Vector DNA __pKOS82. TV_____
☐ Targeted ES Cell DNA __1B2_____
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	17+18	19+20
Restriction Enzyme for Genomic Digest:	Sph I	Sph I
Predicted Wild-type Band (kb):	6.5 kb	12.2 kb
Predicted Mutant Band (kb):	9.7 kb	10.5 kb
Probe Size:	318 bp	238 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:	DNA459-7	5' Primer Name:	DNA459-21
3' Primer Name:	DNA459-8	3' Primer Name:	GT Ires
Predicted Wild-type Band (bp):	388 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	325 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**

DNA459-17 5' – TGAACCAAGCCTACCAGGG
 DNA459-18 5' – CCTCTGTGTTGTTGATTCCG
 DNA459-19 5' – TGGGTCTTTGGATGTGATCC
 DNA459-20 5' – TAAACTCACAGCAGATGTACC

PCR Genotyping

DNA459-7 5' – TTGCGCTGTAATTCAATGACC
 DNA459-8 5' – TAAGCTAAGACCATCAGTTCC
 DNA459-21 5' – TTCACATGTCCAGACTGTAAGC
 GT Ires 5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

AGGAACCAAGGATCAGACTGAGAAAXCCCATAGTTAAAGCTCTTGGGGTTTCTGAGAGCTCAGCTGGAAGTGACTGGGTGACAAGGCGCACAGGCTC
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Genomic Locus: (*The deleted sequence represents nt16261-19217 in the sequence below. KOS82 used to generate the TV represents nt-12527-21519 in the sequence below.*)

[illegible]

[illegible]

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Selection Cassette:

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Targeted Locus:

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