

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: _____ **B6;129S5-C1qtnf6tm1Lex/Mmucd** _____ **MMRRC: 031616-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.

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. UNQ581-19	CCACATTTTGGATTCCAGGG	Estimated Running Time: 90 min.		
2. Neo3A	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. UNQ581-18	GATGTCCTCTGATGATACGG	1 & 2	262	mutant
		1 & 3	351	wildtype

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	NTC	Primers
	wildtype	1 & 2
	31616	
	31616	
	wildtype	
	wildtype	
	31616	
	wildtype	
	wildtype	



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ581	Date of Submission:	4-19-04
Lexicon Contract Name:	DNA091	Mutation Type:	X Standard Knock out
LexVision Name:	SEC434N1		☐ Conditional
Reference accessions:	ENSMUST00000023075, NM_028331	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS76_____
X Target Vector DNA __PKOS76TV_____
X Targeted ES Cell DNA __1F5_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	16+17	11+12
Restriction Enzyme for Genomic Digest:	SpeI	BamHI
Predicted Wild-type Band (kb):	15.4 kb	6.4 kb
Predicted Mutant Band (kb):	11.9 kb	4.3 kb
Probe Size:	395 bps	504 bps

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:	19
Predicted Wild-type Band (bp):	351 bps	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	263 bps

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**

16 5' – TGGGATCAGGAAGTGAGCAG
 17 5' – CCTCATGCTGTCTAGAAAGG
 11 5' – GTGCTCTCTCTAAAGTGTCC
 12 5' – GGAGTCTTCTAACATCAGGG

PCR Genotyping

18 5' – GATGTCCTCTGATGATACGG
 19 5' – CCACATTTTGGATTCCAGGG
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GGATAGCCAGCCTGGGGTTCCTCTGGGCAGTATTCCTGCTTCCTCTTGTGTTTGGGGTCCCCACAGAGGAGACTACCTTT
GGAGAATCTGTGGCCTCCCATCTCCCCAAAGGCTGTGACGATGCTGTGACCCGAGGACCTGATGTCTCTGATGATA
CGGTCCAGGCCCTGTTTCCCTTATGTCCTGCCTGAAGTCAGGCCGTACATCAACATTACTATCCTAAAGGGTGAGTTA
CCACACTGGGCAGGGTGTGGGCCAAATTGGGAGGGAAGAAAGGACGAAGGGAGGGAGGGAGCAAGGGAGGGAGAGG
GGGAGGGAGGGACAGAAGAAAACAGGGCAGGTCTTGCCTAGGCCCTGTCTGCCTTCCATCTGCATGTACACAGGACC
TAG

Genomic Locus: (the deleted sequence represents nt13656 to 14051 in the sequence below)

AAAATGGCTGCTCCTGCTGCATCCTCCTGCTGCATCCAGTGTTCAAGTGCAGGGCCCTGCATGCAGCCCTGCACCTGGG
AGATGTGGCTTCAGATGCAGATTTCTCATCACCAGTCTGTCCCGAGGAGGAGGAGTGGTCCAGGCACCCACTCACATT
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[illegible]

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Selection Cassette: LacZ/Neo

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