

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Cacfd1tm1Lex/Mmucd **MMRRC: 031660-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:

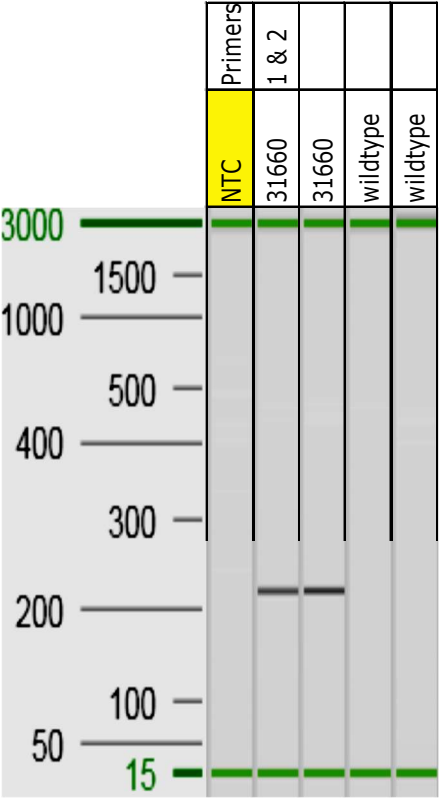
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. UNQ3071-36	CGCAACAAC TGGCAGTAAGC	Estimated Running:Time: 90 min.		
2. Neo3A	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. UNQ3071-32	CCCGCTCCCTGGACCCGATACT	1 & 2	238	Mutant
4. UNQ3071-33	CGCAGCGTTTAGCATGAGCGGCT	3 & 4	156	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ3071	Date of Submission:	10-6-04
Lexicon Contract Name:	DNA308	Mutation Type:	X Standard Knock out
LexVision Name:	MEM737N1		☐ Conditional
Reference accessions:	XM_194006	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __KOS20, KOS48, KOS62_____
☐ Target Vector DNA __pKOS48TV_____
☐ Targeted ES Cell DNA __1A10_____
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	21+22	29+30
Restriction Enzyme for Genomic Digest:	ApaLI	ApaLI
Predicted Wild-type Band (kb):	12.3 kb	12.3 kb
Predicted Mutant Band (kb):	6.1 kb	8.8 kb
Probe Size:	596 bps	588 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:	33	5' Primer Name:	Neo3A
3' Primer Name:	32	3' Primer Name:	36
Predicted Wild-type Band (bp):	156 bps	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	238 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**

21 5' – CTGTGCAGAGTCAGTGGTGCA
 22 5' – GTACACAACCTGCAGACTCTCC
 29 5' – CAGGGCCTATGGCATGGTTC
 30 5' – CCTGCAGAAGCCTCTGAGTC

PCR Genotyping

33 5' – CGCAGCGTTTAGCATGAGCGGCT
 32 5' – CCCGCTCCCTGGACCCGATACT
 Neo3A 5' – GCAGCGCATCGCCTTCTATC
 36 5' – CGCAACAACCTGGGCAGTAAGC

Genomic Sequence Deleted:

GCGCAGCGTTTAGCATGAGCGGCTCGGGCGCCGCCGGAGCGGCCGCGGGCCCAGCCCCGCCGGCACAGGAAGAGGGC
ATGACTTGGTGGTACCGCTGGCTGTGCCGCCTGGCGGGAGTGCTAGGAGCGGTGTGTGAGTATCGGGTCCAGGGAGCG
GGGTCCCCGGG

Genomic Locus: (the deleted sequence represents nt8,784-8,950-in the sequence below)

GGGAAATTCATACCTTATGTGCTCCCACTCCCGGTTAACCTCTGGGGAAGGGATATTATGCAGCATTGTTGGGCCTTATTTT
GTCCAATGAAAACACCCCCATCAGGAGGGTATTTCAGCTAAAGCAAAAAATATCATGGCAAAGATGGGTATATAAAGAAG
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[illegible]

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Selection Cassette:

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[illegible]

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