

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Cxcl16tm1Lex/Mmucd MMRRC: 032260-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.

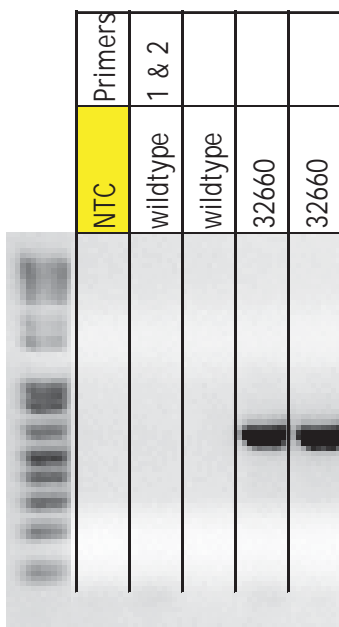
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	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. MEM741.11	GTGTACACACTTAGCTTGACC	Estimated Running:Time: 90 min.	
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)
3. MEM741.3	GAGCTTGGCACGGATCAGC	1 & 2	560
4. MEM741.4	CGGATGTGATCCAAAGTACC	3 & 4	460
			Genotype
			mutant
			wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2759	Date of Submission:	10.804
Lexicon Contract Name:	DNA358	Mutation Type:	<input checked="" type="checkbox"/> Standard Knock out
LexVision Name:	MEM741N1		<input type="checkbox"/> Conditional
Reference accessions:	NM_023158	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS58_____
 X Target Vector DNA __FTV.DNA358.58_____
 X Targeted ES Cell DNA __1E4_____
 X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	3' External
Name of Probe:	12/13	15/18
Restriction Enzyme for Genomic Digest:	Bgl II	HindIII
Predicted Wild-type Band (kb):	9.4 kb	6.5 kb
Predicted Mutant Band (kb):	14 kb	10.3 kb
Probe Size:	569 bp	710 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:	MEM741.3	5' Primer Name:	Neo3A
3' Primer Name:	MEM741.4	3' Primer Name:	MEM741.11
Predicted Wild-type Band (bp):	460 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	560 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**

MEM741.12 5' – CAAGGAGTTCGTCTACTTCC
MEM741.13 5' –GTACTGCAACGCCTTCATC
MEM741.15 5' – CAAGTCTTCTGAAACTCCAG
MEM741.18 5' –CTAAGAGGCTTCCAGAGTC

PCR Genotyping

MEM741.3 5' –GAGCTTGGCACGGATCAGC
MEM741.4 5' –CGGATGTGATCCAAAGTACC
MEM741.11 5' –GTGTACACACTTAGCTTGACC
Neo3A 5' –GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

TGAGGCGGGGCTTTGGACCCTTGTCTCTTGCCTTTCTTCTTTCTTGTGGCGCTGCTGACCCTGCCAGGTTAGTCGGGG
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Genomic Locus: (the deleted sequence represents nt-18742-19497-in the sequence below)

CCCTAGGTCTGGAGTTACAGATGCGAGTGGTTTGGAGCCAGCTTTCCAGCCATTTGTTTTTGTCTTTTGGACAGGATCT
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Selection Cassette: IRES BetaGal MC1 Neo

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Targeted Locus:

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