

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-Cbln4^{tm1Lex}/Mmucd **MMRRC:** 032175-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	11.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION	25.000 μL

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust

Strategy:

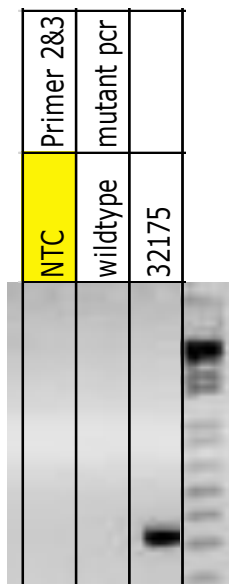
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. UNQ718-18	TCTGCTGATTCTAGTGCTGCC
2. UNQ718-2	TGTGCCAAAGACTACGGGACT
3. Neo3A	GCAGCGCATCGCCTTCTATC

Electrophoresis Protocol:

Argarose: 1.5% V: 90		
Estimated Running Time: 90 min.		
Primer Combination	Band (bp)	Genotype
1 & 3	439	Wild-type
2 & 3	354	Mutant





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ718	Date of Submission:	7-16-04
Lexicon Contract Name:	DNA116	Mutation Type:	X Standard Knock out
LexVision Name:	SEC428N1		☐ Conditional
Reference accessions:	AK032406	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __KOS41_____
X Target Vector DNA __KOS41TV_____
X Targeted ES Cell DNA __3D1_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	9+10	Neo2+5
Restriction Enzyme for Genomic Digest:	BglII	ScaI
Predicted Wild-type Band (kb):	8.0 kb	-
Predicted Mutant Band (kb):	13.0 kb	10.2 kb
Probe Size:	369 bps	609 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:	2	3' Primer Name:	2
Predicted Wild-type Band (bp):	439 bps	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	354 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**

9 5' – AGTGGACTCAACATCTCAAGC

10 5' – GAGTTGAAGTTGTCAGTGTGC

Neo2 5' – CCTCAGAAGAAGCTCGTCAAG

Neo5 5' – GGCAGCGCGGCTATCGTG

PCR Genotyping

18 5' – TCTGCTGATTCTAGTGCTGCC

2 5' – TGTGCCAAAGACTACGGGACT

Neo3A 5' – GCAGCGCATCGCCTTCTATC

Contact Information:

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Genomic Sequence Deleted:

GGGCTCCGCGCGCCGGGCGCTGTCCGTGGTACCAGCCGTTCTGCTGATTCTAGTGCTGCCTGTCTGGGCACAGAATGAC
ACGGAACCGATCGTTCTGGAGGGCAAGTGTCTGGTGGTGTGCGACTCGAACCAGCTACAGACTCCAAGGGATCATCA
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CATCTGAGATGAGCAACAAGACTCGCATCATTTACTTTGATCAGGTCAGACTCC

Genomic Locus: (the deleted sequence represents nt9124-9413-in the sequence below)

[illegible]

TCCCACCCCGCCTGCGTGACTGACCCAAGGCTGTCTGGACAGGATGCTTGGAGTCTTTAGTCACTCAGAGCCAGGCGAG
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Selection Cassette:

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Targeted Locus:

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