

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Tmc2tm1Lex/Mmucd **MMRRC:** 032627-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	6.6
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
Primer 3. (stock concentration is 20μM)	0.45
Primer 4. (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.

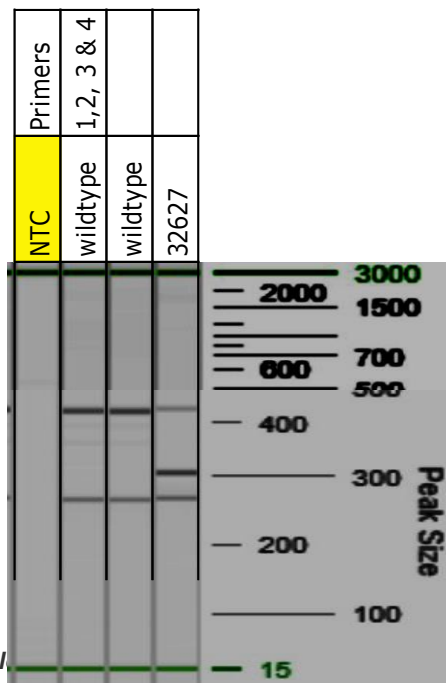
Strategy:

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. DNA434-3	GCATGGCTCTCTGCACAGGTGA	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA434-23	GAGGCCTGTTGAGTAGCACC	1 & 2	338	Mutant
4. DNA434-4	GGAGGAGTGTCTGGATAGATTG	3 & 4, 1 & 4	294, 483	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ907	Date of Submission:	10/19/04
Lexicon Contract Name:	DNA434	Mutation Type:	X Standard Knock out
LexVision Name:	MEM634N1		☐ Conditional
Reference accessions:	NM_138655	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __KOS 53_____
X Target Vector DNA __KOS 53 TV_____
X Targeted ES Cell DNA __1B1_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	DNA434-19/20	DNA434-21/22
Restriction Enzyme for Genomic Digest:	EcoR I	EcoR I
Predicted Wild-type Band (kb):	13.6 kb	13.6 kb
Predicted Mutant Band (kb):	7.0 kb	6.6 kb
Probe Size:	297 bp	356 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:	DNA434-4	5' Primer Name:	neo3A
3' Primer Name:	DNA434-23	3' Primer Name:	DNA434-3
Predicted Wild-type Band (bp):	294 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	338 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**

DNA434-19 5' – CAGTATTGACAAAGAAGCCAGAAA

DNA434-20 5' – CAGCCACATCAACTTAAAAACATC

DNA434-21 5' – CACGCACCCTCTTTCCTTTGT

DNA434-22 5' – GTTTGCCATCATCCTCATTCTCA

PCR Genotyping

DNA434-3 5' – GCATGGCTCTCTGCACAGGTGA

DNA434-4 5' – GGAGGAGTGTCTGGATAGATTG

DNA434-23 5' – GAGGCCTGTTGAGTAGCACC

neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

ATGAGCCCCCAGTTAAAGAGCTTGGACGAGGAAGGTGAGTCTTCATCTTCATCCTCATGGGTGCTACTCAACAGGCCTC
TGGGCACAG

Genomic Locus: (the deleted sequence represents nt-10001-10088-----in the sequence below)

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[illegible]

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Selection Cassette:

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Targeted Locus:

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