

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Lrrc4ctm1Lex/Mmucd **MMRRC:** 032444-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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

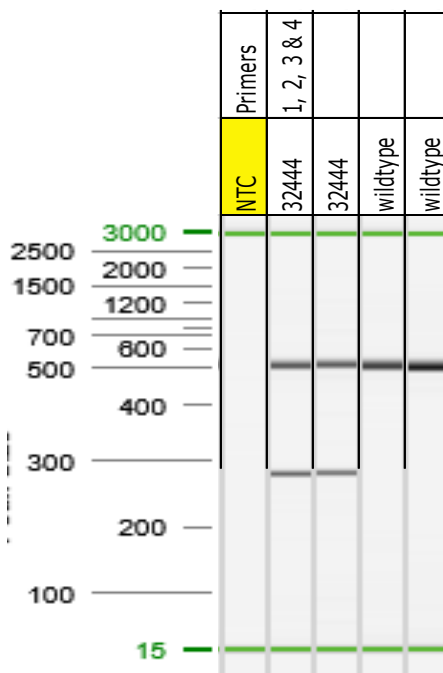
Strategy:

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. DNA054-1	CAGACTGTTTGAAGTCCAGAAG	Estimated Running Time: 90 min.		
2. DNA054-5	GAACAAGATGACCTTACATCC	Primer Combination	Band (bp)	Genotype
3. DNA054-6	CAATAGGGTTGTTCTCAACCAG	1 & 4	289	mutant
4. GT IRES	CCCTAGGAATGCTCGTCAAGA	2 & 3	476	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ292	Date of Submission:	11/05/03
Lexicon Contract Name:	DNA054	Mutation Type:	X Standard Knock out
LexVision Name:	MAS225N1		☐ Conditional
Reference accessions:	ENSMUST00000037320, AK048322	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __KOS48, KOS52_____
 X Target Vector DNA __KOS52-FTV(Puro)_____
 X Targeted ES Cell DNA __2G10_____
 X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External/Internal</u>	<u>3' External/Internal</u>
Name of Probe:	16+17	20+21
Restriction Enzyme for Genomic Digest:	EcoRI	BamHI
Predicted Wild-type Band (kb):	6.6	29.4
Predicted Mutant Band (kb):	4.4	16.1
Probe Size:	527 bp	454 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:	DNA054-5	5' Primer Name:	GT-IRES
3' Primer Name:	DNA054-6	3' Primer Name:	DNA054-1
Predicted Wild-type Band (bp):	476	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	289

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**

DNA054-16	5' – GCACATAATATGTTTCACAATG
DNA054-17	5' – GTTGTACTTAATATAGGCTAAG
DNA054-20	5' – GGTCATCCTTCACGTTTTTAATG
DNA054-21	5' – GCCATACTAATATGGATTAGG

PCR Genotyping

DNA054-5	5' – GAACAAGATGACCTTACATCC
DNA054-6	5' – CAATAGGGTTGTTTCCTCAACCAG
DNA054-1	5' – CAGACTGTTTGAAGTCCAGAAG
GT-IRES	5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

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Genomic Locus: (the deleted sequence represents nt 9,949-12,039 -in the sequence below)

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[illegible]

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Selection Cassette:

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[illegible]

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