

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6N.129S5-Sigirrtm1Lex/Mmucd **MMRRC: 032569-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. DNA152A-12	TCAGGGTCTCATGATGGAG	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA152A-17	GTGCTTGGATACAGGCCAG	1 & 2	345	mutant
4. DNA152A-18	TCTGGGCTTCCAGGAATCC	3 & 4	325	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ301	Date of Submission:	11.12.04
Lexicon Contract Name:	DNA152A	Mutation Type:	X Standard Knock out
LexVision Name:	MEM550N1		☐ Conditional
Reference accessions:	NM_023059	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS 15_____
X Target Vector DNA __pTV 15neo_____
X Targeted ES Cell DNA __1H11_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	13/14	15/16
Restriction Enzyme for Genomic Digest:	BamHI	XbaI
Predicted Wild-type Band (kb):	5.2	20
Predicted Mutant Band (kb):	3.9	15.4
Probe Size:	236 bps	229 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:	DNA152A-17	5' Primer Name:	Neo3a
3' Primer Name:	DNA152A-18	3' Primer Name:	DNA152A-12
Predicted Wild-type Band (bp):	325	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	345

Primer sequences:**Southern probes**

DNA152A-13 5' – CATGATGCACGGGTATACT
DNA152A-14 5' – CAAGCTTCCAGGATGTGAC
DNA152A-15 5' – CACACTTCCTTGGAGCCTTG
DNA152A-16 5' – CTGTGGTGCTAGCTATGGT

PCR Genotyping

DNA152A-12 5' – TCAGGGTCTCATGATGGAG
DNA152A-17 5' – GTGCTTGGATACAGGCCAG
DNA152A-18 5' – TCTGGGCTTCCAGGAATCC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

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GCCCATATCCCAGCAGCCAGACCATGAGATCACGGTGGCAGCTTCCAGGGTAG

Genomic Locus: (The deleted sequence represents nt 10109-13618 in the sequence below. KOS15 used to generate the TV represents nt 8838-17187 in the contig below.)

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[illegible]

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Selection Cassette: LacZ/Neo

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[illegible]

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