

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Siglecgtm1Lex/Mmucd **MMRRC:** 032572-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.

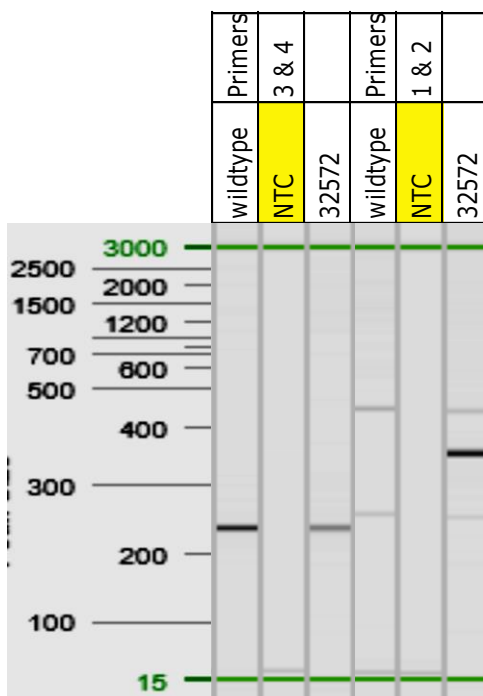
Strategy:

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. DNA372-12	GAGGCATTGCTGCTGTTCCCCT	Estimated Running Time: 90 min.	
2. Neo3a	GCAGCGCATCGCTTCTATC	Primer Combination	Band (bp)
3. DNA372-5	AGCAACATACCCTGGACCTCTC	1 & 2	354
4. DNA372-8	CTGCAATGAAGACAGGGAAGT	3 & 4	237
			Genotype
			mutant
			wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ477	Date of Submission:	7/20/05
Lexicon Contract Name:	DNA372	Mutation Type:	☒ Standard Knock out
LexVision Name:	MAS257N1		☐ Conditional
Reference accessions:	NM_172900	Is this gene X-linked?	No

Required Materials: ☒ pKOS clone DNA(s) __pKos-14_____
☒ Target Vector DNA __pKos-14 TV Neo_____
☒ Targeted ES Cell DNA __1E10_____
☒ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	13+16	17+18
Restriction Enzyme for Genomic Digest:	EcoRV	EcoRV
Predicted Wild-type Band (kb):	7.2	6.4
Predicted Mutant Band (kb):	8.3	7.4
Probe Size:	373	290

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:	DNA372-5	5' Primer Name:	Neo3a
3' Primer Name:	DNA372-8	3' Primer Name:	DNA372-12
Predicted Wild-type Band (bp):	237	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	354

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**

DNA372-13 5' – GGGCTAACTTAACAGTCATGTA
 DNA372-16 5' – TTTCATGATCTGAGCAAACAGA
 DNA372-17 5' – GGGCCCTGAATCTTGATTGTAG
 DNA372-18 5' – ATAGGCCAGTCTGAGCATGTCC

PCR Genotyping

DNA372-5 5' – AGCAACATACCCTGGACCTCTC
 DNA372-8 5' – CTGCAATGAAGACAGGGAAC TG
 DNA372-12 5' – GAGGCATTGCTGCTGTTCCCT
 Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

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Genomic Locus: (The deleted sequence represents nt18103-21723-in the sequence below. KOS14 used to generate the TV represents nt12522-23492- in the sequence below.)

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Selection Cassette:

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Targeted Locus:

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