

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Clec1atm1Lex/Mmucd **MMRRC: 032208-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)	

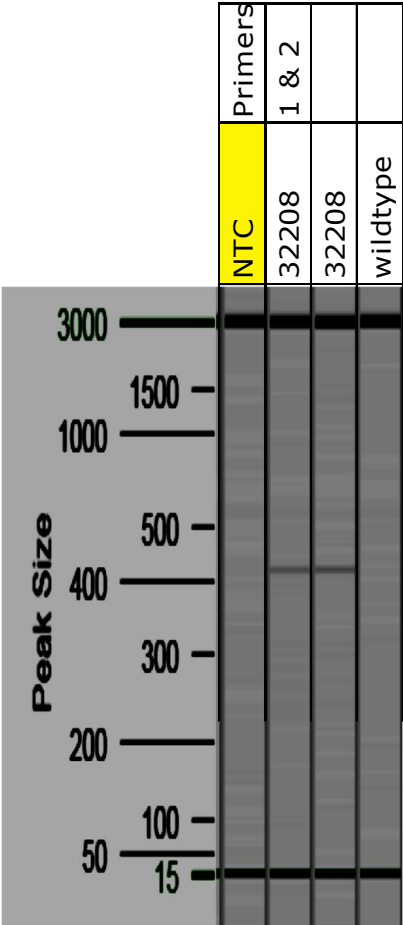
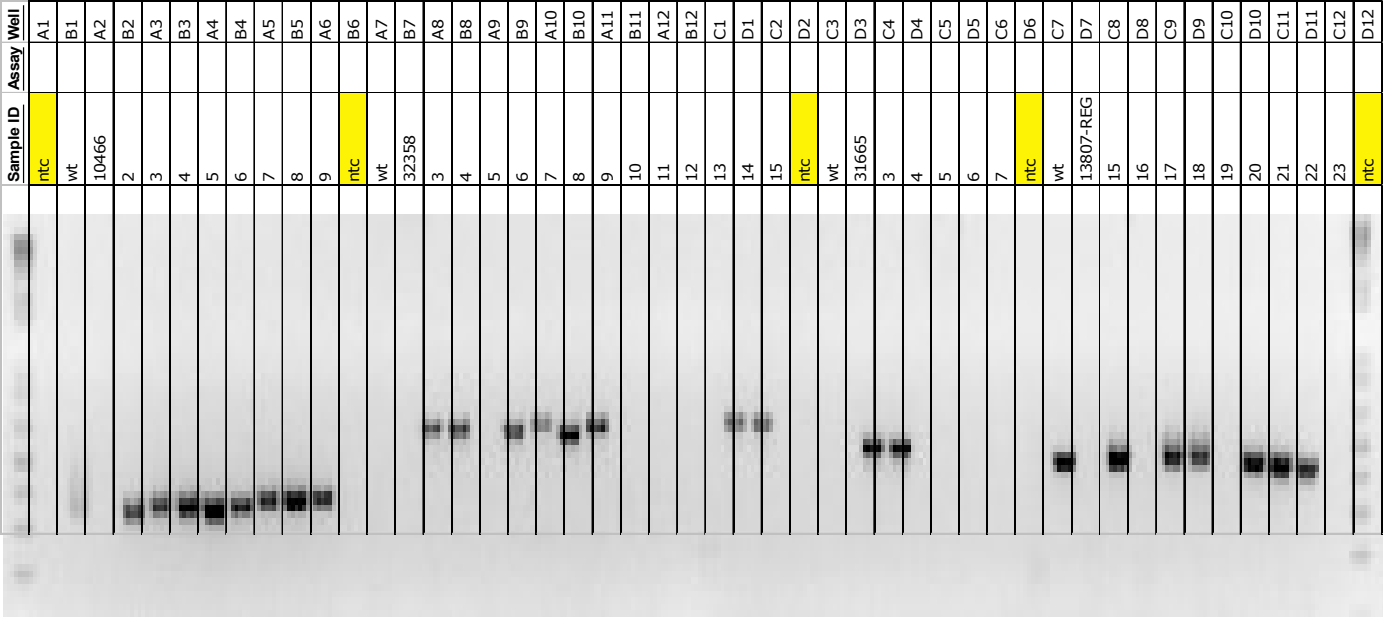
Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA088-up	TCACAACCACCCGTAATGA	Estimated Running Time: 90 min.		
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. DNA088-f	TTGTAAGGAGCCAAGGTTCTGACC	1 & 2	411	mutant
4. DNA088-r	TCCACTTCAGGAATCACTTGTAGC	3 & 4	97	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ569	Date of Submission:	4-3-06
Lexicon Contract Name:	DNA088	Mutation Type:	X Standard Knock out
LexVision Name:	MEM647N1		☐ Conditional
Reference accessions:	NM_175526	Is this gene X-linked?	No

Required Materials:

pKOS clone DNA(s)	<u>N/A</u>
X Target Vector DNA	<u>ScramblerTVpuro</u>
X Targeted ES Cell DNA	<u>1H5</u>
X Genomic Map	

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	13+14	15+16
Restriction Enzyme for Genomic Digest:	EcoRV	BamHI
Predicted Wild-type Band (kb):	13.3	21.5
Predicted Mutant Band (kb):	8.4	10.2
Probe Size:	317 bp	477 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:	DNA088-f	5' Primer Name:	DNA088-up
3' Primer Name:	DNA088-r	3' Primer Name:	GT-Ires
Predicted Wild-type Band (bp):	97	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	411

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**

DNA088-13 5' – CTGAACTGCTGAAGGTGACTC

DNA088-14 5' – GTTCCATGAGGACAAGTCAC

DNA088-15 5' – GCTGCTTGCTGCTACTGAC

DNA088-16 5' – CACACCGAGATCTAGCTGTC

PCR Genotyping

DNA088-f 5' – TTGTAAGGAGCCAAGGTTCTGACC

DNA088-r 5' – TCCACTTCAGGAATCACTTGTAGC

DNA088-up 5' – TCACAACCACCCGTAATGA

GT-Ires 5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

[illegible]

Genomic Locus: *(The deleted sequence represents nt21790-24156-in the sequence below. ScramblerTV represents nt 18178-31684 in the sequence below.)*

[illegible]

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[illegible]

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Selection Cassette:

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[illegible]