

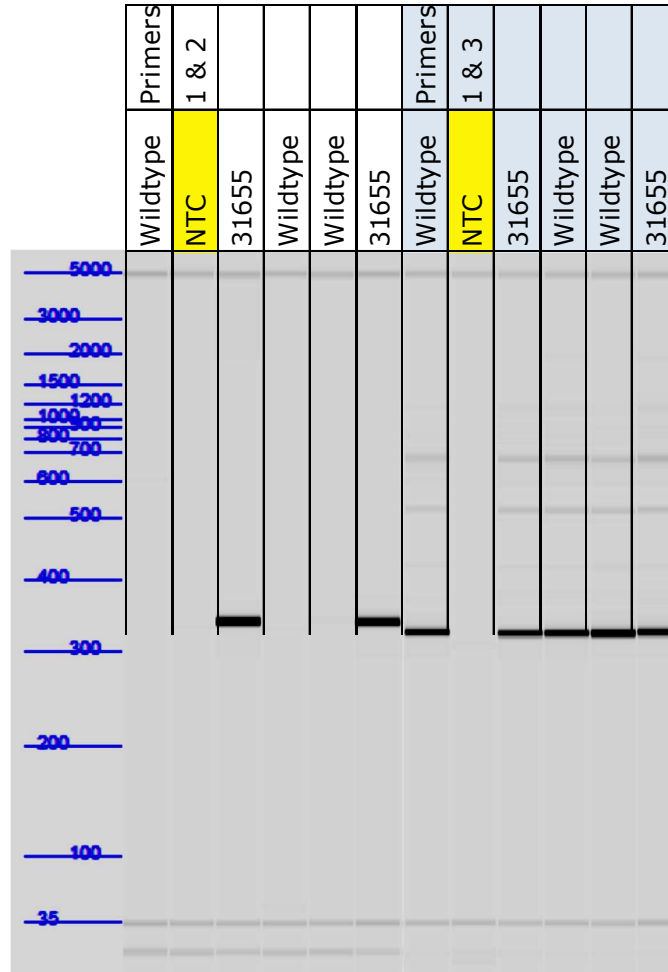


# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC



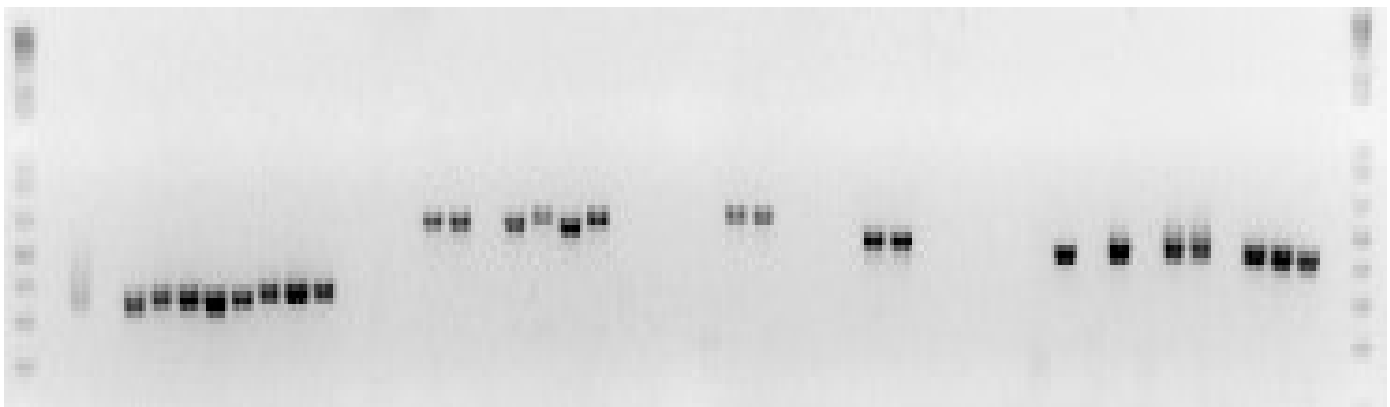
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Sample ID	Assay	Well
ntc		A1
wt		B1
10466		A2
2		B2
3		A3
4		B3
5		A4
6		B4
7		A5
8		B5
9		A6
ntc		B6
wt		A7
32358		B7
3		A8
4		B8
5		A9
6		B9
7		A10
8		B10
9		A11
10		B11
11		A12
12		B12
13		C1
14		D1
15		C2
ntc		D2
wt		C3
31665		D3
3		C4
4		D4
5		C5
6		D5
7		C6
ntc		D6
wt		C7
I3807-REG		D7
15		C8
16		D8
17		C9
18		D9
19		C10
20		D10
21		C11
22		D11
23		C12
ntc		D12





## Lexicon Genetics Incorporated – Genentech Project Materials

<b>Genentech ID:</b>	UNQ730	<b>Date of Submission:</b>	10-8-04
<b>Lexicon Contract Name:</b>	DNA427	<b>Mutation Type:</b>	x Standard Knock out
<b>LexVision Name:</b>	MAS228N1		<input type="checkbox"/> Conditional
<b>Reference accessions:</b>	NM_028732	<b>Is this gene X-linked?</b>	no

**Required Materials:**

- pKOS clone DNA(s) \_\_pKOS52\_\_\_\_\_
- Target Vector DNA \_\_DNA427-FTV52\_\_\_\_\_
- Targeted ES Cell DNA \_\_1D1 & 1D8\_\_\_\_\_
- Genomic Map

**Southern Blot Analysis:**  
*External/Internal Probe Strategies*

	<b><u>5' External</u></b>	<b><u>3' External</u></b>
Name of Probe:	<b>16 + 17</b>	<b>18 + 19</b>
Restriction Enzyme for Genomic Digest:	<b>EcoRI</b>	<b>EcoRI</b>
Predicted Wild-type Band (kb):	<b>16.2</b>	<b>16.2</b>
Predicted Mutant Band (kb):	<b>8.6</b>	<b>7.5</b>
Probe Size:	<b>298 bp</b>	<b>318 bp</b>

**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:	DNA427-1	5' Primer Name:	Neo3a
3' Primer Name:	DNA427-2	3' Primer Name:	DNA427-2
Predicted Wild-type Band (bp):	319	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	337

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**

DNA427-16 5' – CATCAGACTTGGGAGACTTC  
DNA427-17 5' – TCCCAGGCAGAAGACAGAGAA  
DNA427-18 5' – CTGTTCTGCATGCCTGTATG  
DNA427-19 5' – CATTGTCAAGGGACCATGGA

**PCR Genotyping**

DNA427-1 5' – TTGCTCCAAGCGCACTCCAGC  
DNA427-2 5' – AGATGTGCCTGATGAAGGATGG  
Neo3a 5' – GCAGCGCATCGCCTTCTATC

**Genomic Sequence Deleted:**

ATGGGTGTCGCCGCGTCCCAGAGGCCAGCAGCCCGCGCTGGGAACCCTGCTCTTGCTATTTCTGGCTGCATCCAGAGGTAAGATCAA

**Genomic Locus: (the deleted sequence represents nt 955 – 9646 in the sequence below)**

CCCCCTGTCCTGGGCACTCCAGATTTCTTCTAGGCTCTATGTTTGGCTCCTTATACCCTATGGTATCACAACAACTTCCCACCCTGAAGAGAGGTTGC  
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**Selection Cassette:**

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**Targeted Locus:**

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