

GENOTYPING BY PCR PROTOCOL MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC



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
13807-REG		D7
15		C8
16		D8
17		C9
18		D9
19		C10
20		D10
21		C11
22		D11
23		C12
ntc		D12

NIH-0813 Genotyping Strategies

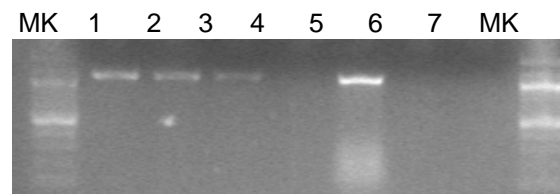
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

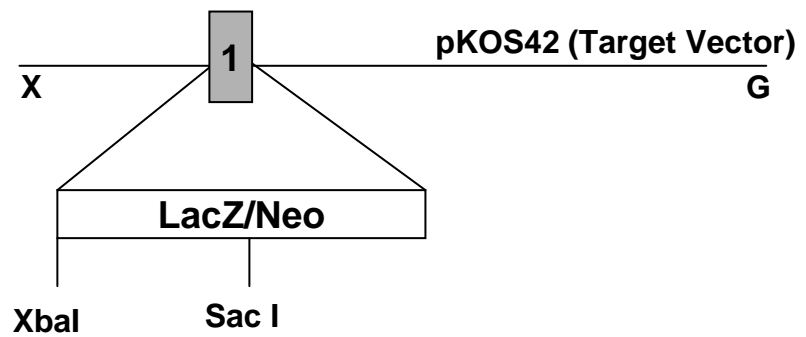
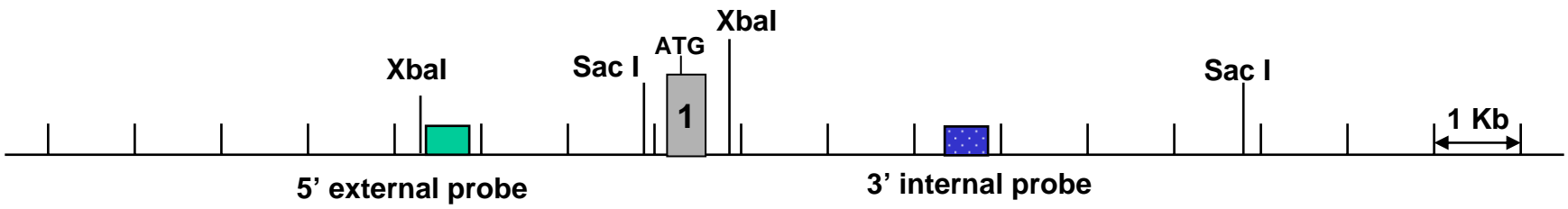
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0813-37, 1kb	
Recommended Wt PCR: Primer 0813-4 and Primer 0813-6, 186 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0813-37	TGGCATGACTCCTATGAG
Primer 0813-4	AAGAACACAGCCCATGTGC
Primer 0813-6	TCTTGTTTCATCTCATAGATTTCG

Well	Sample	Genotype
1	36	het
2	55	het
3	57	het
4	58	wt
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



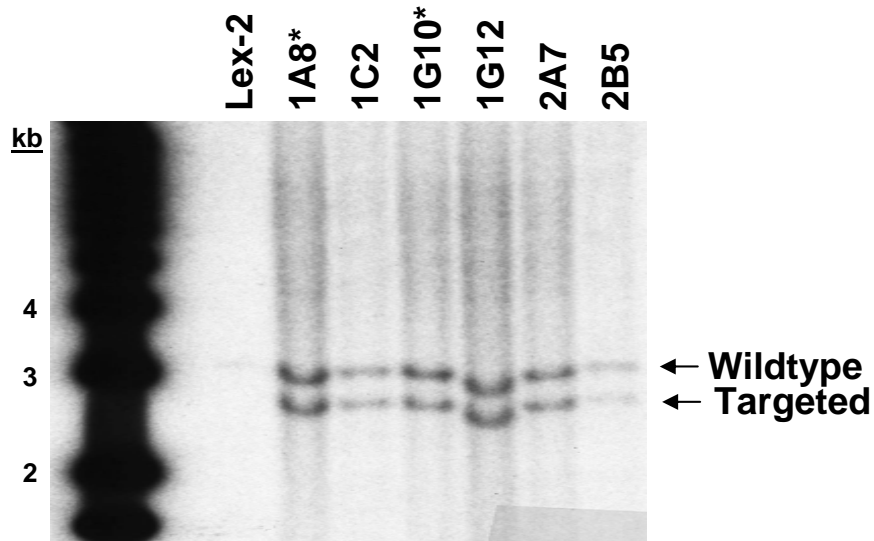
Targeting Strategy



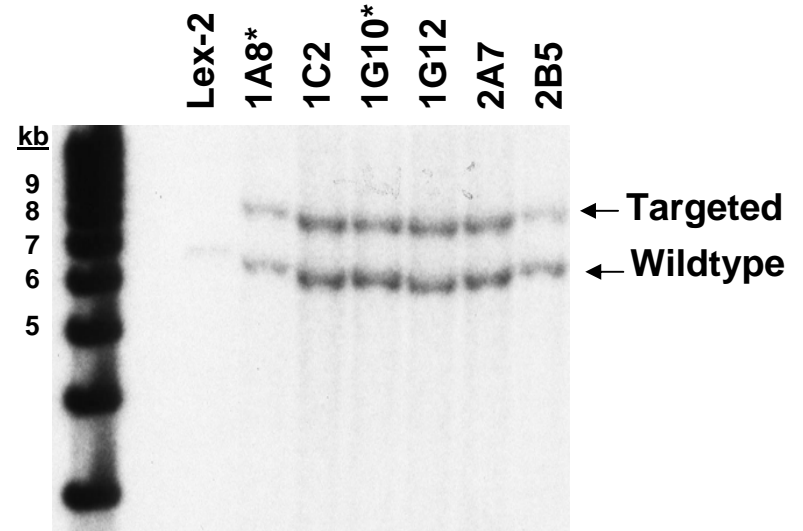
Southern Strategies

Probe	5' external 	3' internal 
Enzyme	XbaI	SacI
Wildtype	3.1 Kb	6.9 Kb
Targeted	2.7 Kb	9.1 Kb

Southern Data



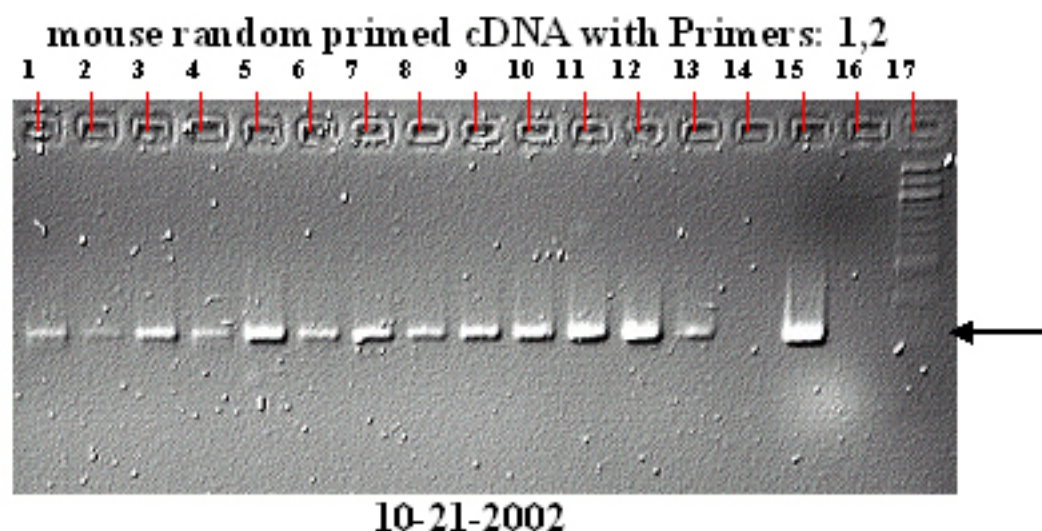
5' External Probe
XbaI Digest
WT = 3.1 Kb
Targeted = 2.7 Kb



3' Internal Probe
SacI Digest
WT = 6.9 Kb
Targeted = 9.1 Kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis



Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

- 1) Brain
- 2) Thymus
- 3) Spleen
- 4) Lung
- 5) Kidney
- 6) Liver
- 7) Testis
- 8) Bone
- 9) Small Intestine & Colon
- 10) Skin Fibroblast
- 11) Heart
- 12) Adipose
- 13) Blood
- 14) (-) Control
- 15) (+) Control- ES cell cDNA
- 16) (+) Control- Genomic/Lex1 DNA
- 17) 100 bp ladder/marker



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0813 XXXXXXXXXX

Reference accession(s): NM 010698

Standard KO or Conditional: Standard KO

Materials Submitted: X Target Vector pKOS-42TVneo
X KOS clone(s) pKOS-42

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	59+60	62+63
Restriction Enzyme for Genomic Digest:	XbaI	SacI
Predicted Wild-type Band (kb):	3.1	6.9
Predicted Mutant Band (kb):	2.7	9.1
Probe Size:	142 bp	292 bp

Primer sequences:

Southern probes

0813-59 5' – CTCCACAAAGTTGTCCTCAG
0813-60 5' – GTCCCTTCTTTACCTATG
0813-62 5' – GAAAGCAGTTCACAGGAGG
0813-63 5' – CCAAAGTAGCTAACCGAC

ATTGCCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCCTAGGGGTCTTTCCCCTC
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