

NIH-1150 Genotyping Strategies

Reaction Components	Vol (ul)
10X MGB Buffer	5
25mM MgCl ₂	N/A
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	34.5
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

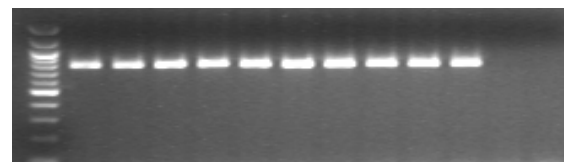
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 1150-96, 800 bp	
Recommended Wt PCR: Primer 1150-28 and Primer 1150-30, 308 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 1150-96	GAATAGGAAAGTCAAGGAAGC
Primer 1150-28	GTTCTTCAGAGAGGCGTTCC
Primer 1150-30	TGATCCAGGTGGCGAGAATG

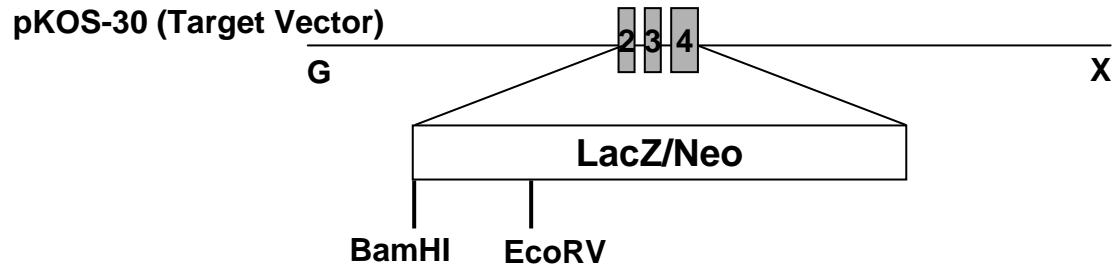
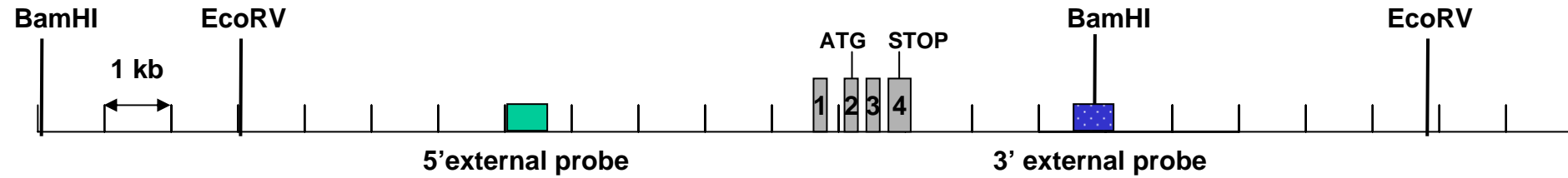
Well	Sample	Genotype
1	14	het
2	18	het
3	22	het
4	35	het
5	39	het
6	41	het
7	42	het
8	54	het
9	63	het
10	ES DNA	het
11	wt lysate	wt
12	water	no amp

Mutant PCR



MK 1 2 3 4 5 6 7 8 9 10 11 12



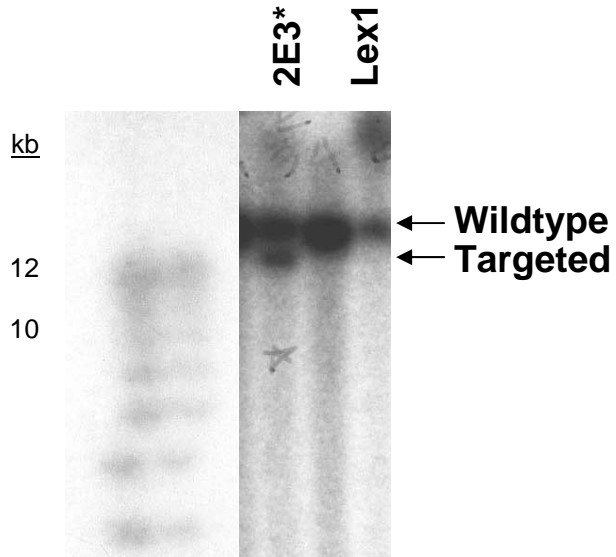
Targeting Strategy



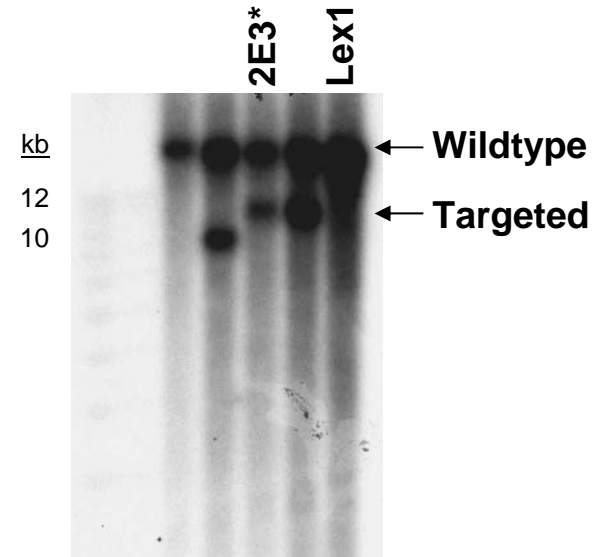
Southern Strategies

Probe	5' external 	3' internal 
Enzyme	BamHI	EcoRV
Wildtype	15.9 kb	18.0 kb
Targeted	13.1 kb	11.3 kb

Southern Data



5' internal probe
BamHI digests
Wildtype 15.9 kb
Targeted 13.1 kb



3' external probe
EcoRV digests
Wildtype 18.0 kb
Targeted 11.3 kb

*** Clone Injected**



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-1150 (LEXKO-579)

Reference accession(s): NM_025498

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS-30 FTVneo
 KOS clone(s) pKOS-30

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	101/93	102/103
Restriction Enzyme for Genomic Digest:	BamHI	EcoRV
Predicted Wild-type Band (kb):	15.9 kb	18.0 kb
Predicted Mutant Band (kb):	13.1 kb	11.3 kb
Probe Size:	608 bp	841 bp

Primer sequences:

Southern probes

1150-101 5' – AAGAAGACCCCTTTAATGAG
1150-93 5' – CTCAGCTTTTGGAGATCCCA
1150-102 5' – CTGATCCAGCGCATTAGACC
1150-103 5' – GGAGTCTTCAATCCTGACTC

TCGCTGATTAATATGATGAAAACGGCAACCCGTGGTTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCGTATGAACGGTCTG
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