

## NIH-0656 Genotyping Strategies

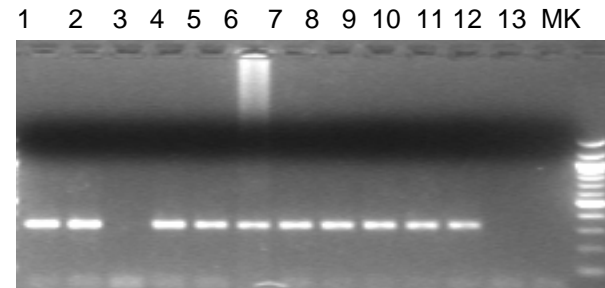
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

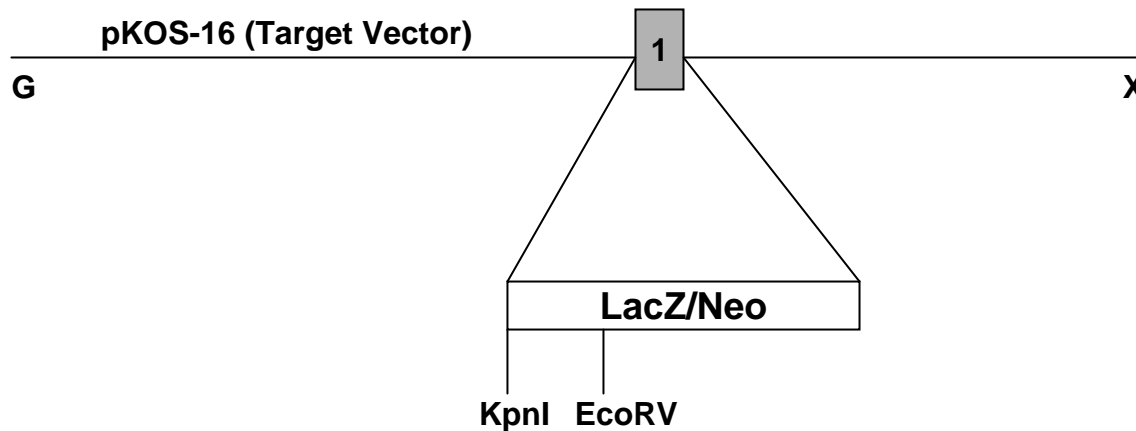
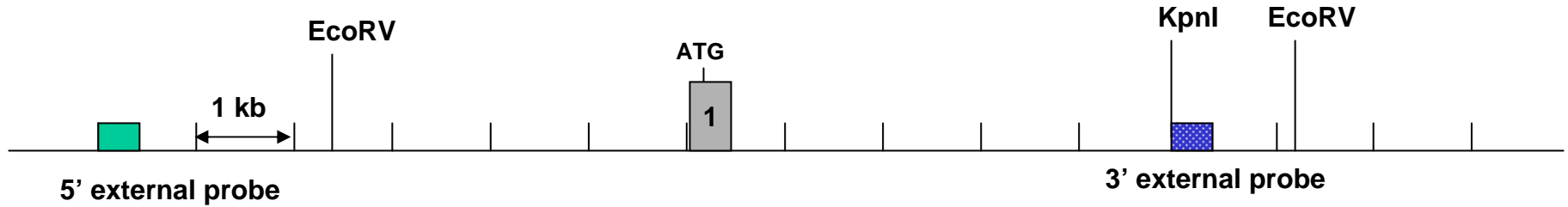
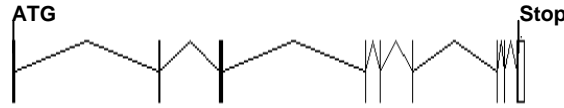
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0656-35, 324 bp	
Recommended Wt PCR: Primer 0656-22 and Primer 0656-35, 214 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0656-35	ACTCTATTTCAACTGAAGTC
Primer 0656-22	CTCAGCATAGTGCCACG

Well	Sample	Genotype
1	9	het
2	17	het
3	18	wt
4	33	het
5	54	het
6	54	het
7	69	het
8	123	hom
9	124	hom
10	125	hom
11	<b>ES DNA</b>	het
12	<b>wt lysate</b>	wt
13	<b>water</b>	no amp



### Mutant PCR



# Targeting Strategy

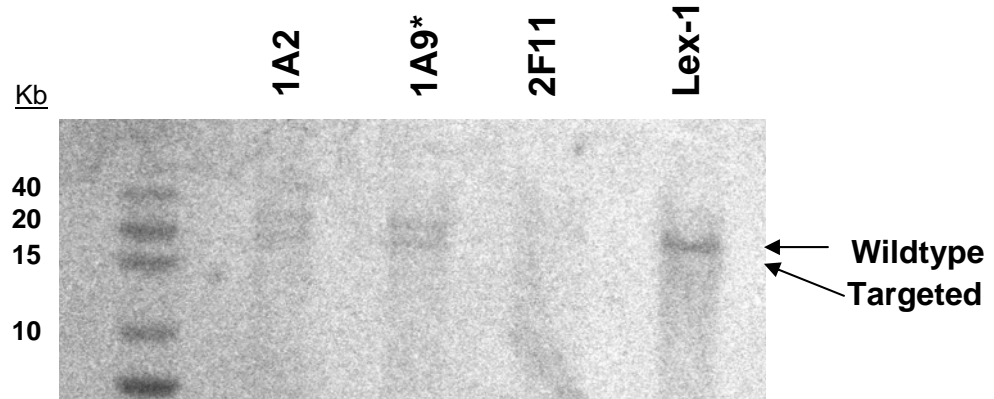


## Southern Strategies

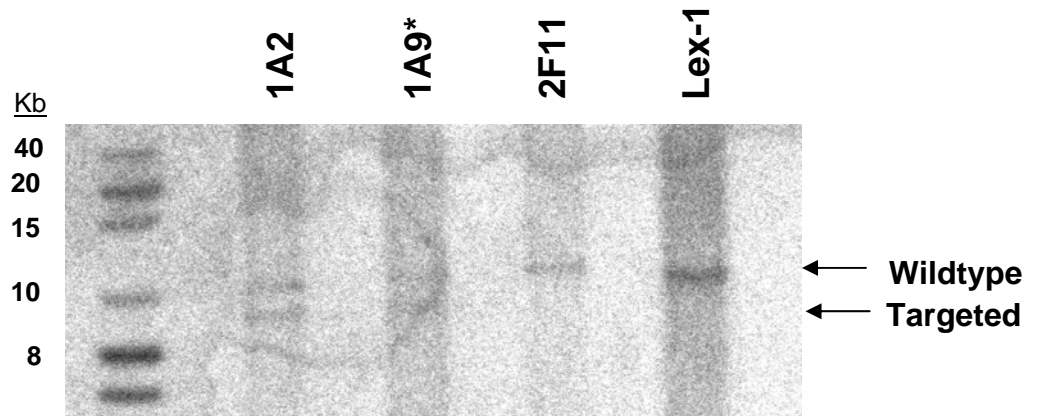
Probe	5' external 	3' external 
Enzyme	KpnI	EcoRV
Wildtype	24 kb	10 kb
Targeted	18.9 kb	8.9 kb

# Southern Data

**5' external probe**  
**KpnI digests**  
 WT 24 kb  
 Targeted 18.9 kb



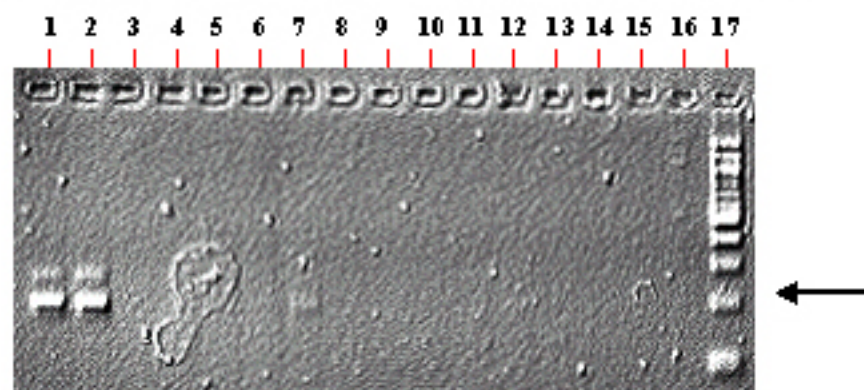
**3' external probe**  
**EcoRV digests**  
 WT 10 kb  
 Targeted 8.9 kb



\* clone injected

## RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 5,6



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

### Mouse cDNA Tissues

- 1) Brain
- 2) Spinal Cord
- 3) Eye
- 4) Thymus
- 5) Spleen
- 6) Lung
- 7) Kidney
- 8) Liver
- 9) Skeletal Muscle
- 10) Bone
- 11) Stomach, Small Intestine & Colon
- 12) Heart
- 13) Adipose
- 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-0656 (LEXKO-467)

Reference accession(s): BB179652, AK138366

Standard KO or Conditional: Standard

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Materials Submitted:  Target Vector pKOS-16TVneo  
 KOS clone(s) pKOS-16

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**Southern Blot Genotyping Strategies:**

	<u>5' External</u>	<u>3' External/ Internal</u>
Name of Probe:	41/42	37/38
Restriction Enzyme for Genomic Digest:	KpnI	EcoRV
Predicted Wild-type Band (kb):	24	10.0
Predicted Mutant Band (kb):	18.9	8.9
Probe Size:	335	375

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**Primer sequences:**

**Southern probes**

0656-37 5' – TAAGTAAATGTCAAGTGATGC  
0656-38 5' – AAAACAAACATGCATGATGCC  
0656-41 5' – AACTGCCACAGTATGTTATG  
0656-42 5' – ACAGCCAAAGGTAGATTAT

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## Genomic Sequence Deleted:

ATGAGGAGACTGAGTTTGTGGTGGCTGCTGAGCAGGGTCTGTCTGTTGCTGCCTCCGCCCTGTGCACTGGTGTGGCCGGGGTGCCAGCTCCTCCTCG  
CACCCGCAACCCTGCCAGATCCTCAAGCGCATCGGACACGGGTGAGGGTGGGCGGGTGCACCTTGCAACCCTGGACCACGGCCCCACGCGCAGCCA  
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CGGGAGAGTCAAGTGAGAGGAG

## KOS clone sequence: *(note: pKOS-16 was used to generate the TV and that is the sequence included here)*

GATCATCTGTGGAATATCCCAGGCATGTAGAAGTCACTCAAATAAAGTCTCTAAAATGATGATCTCACAATCTTGAACCTTCTGAAAATTTCCCT  
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ATATTAATTAGTATTTAGATGATC

**Selection cassette sequence:** (note: linker sequences may vary and are not provided)

**LacZ/Neo**

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