

## NIH-0846 Genotyping Strategies

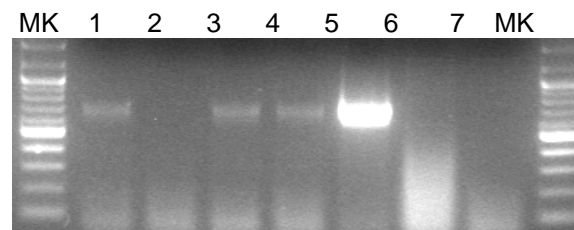
Reaction Components	Vol (ul)
2X Premix G	25
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	N/A
Primer 20 uM	2.5
Primer 20 uM	2.5
5 U/ul Taq polymerase	0.5
Water	11
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	95C	3'	
2	95C	30"	
3	61C	30"	
4	72C	40"	Go to 2, 40 cycles

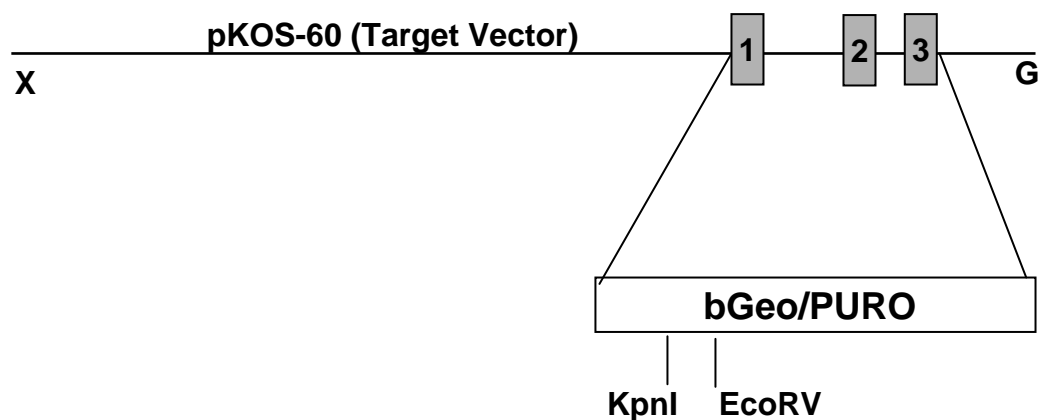
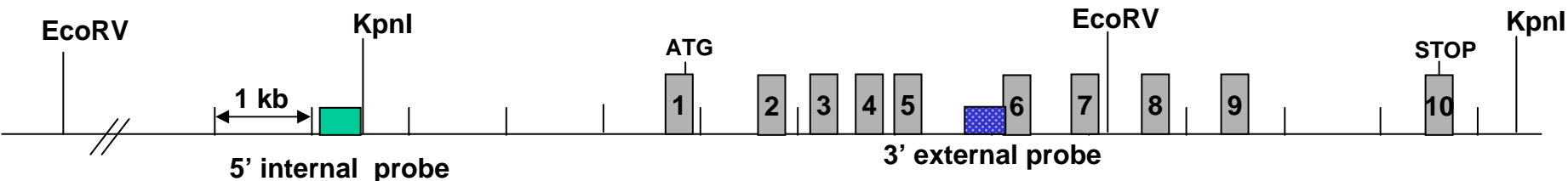
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Puro JA and Primer 0846-lower, 665 bp	
Recommended Wt PCR: Primer 0846-25 and Primer 0846-15, 379 bp	
Primer <del>Puro JA</del>	<del>GAGGAATTGCATCGCATTGTCT</del>
Primer 0846-lower	AGGGTAAAAGTCTCCGTGTTCTG
Primer 0846-25	GGAGCATCACAGAAGCAAAG
Primer 0846-15	CATAGGAGGTAACACAGGTG

Well	Sample	Genotype
1	14	het
2	15	wt
3	16	het
4	17	het
5	<b>ES DNA</b>	het
6	<b>wt lysate</b>	wt
7	<b>water</b>	no amp



### Mutant PCR



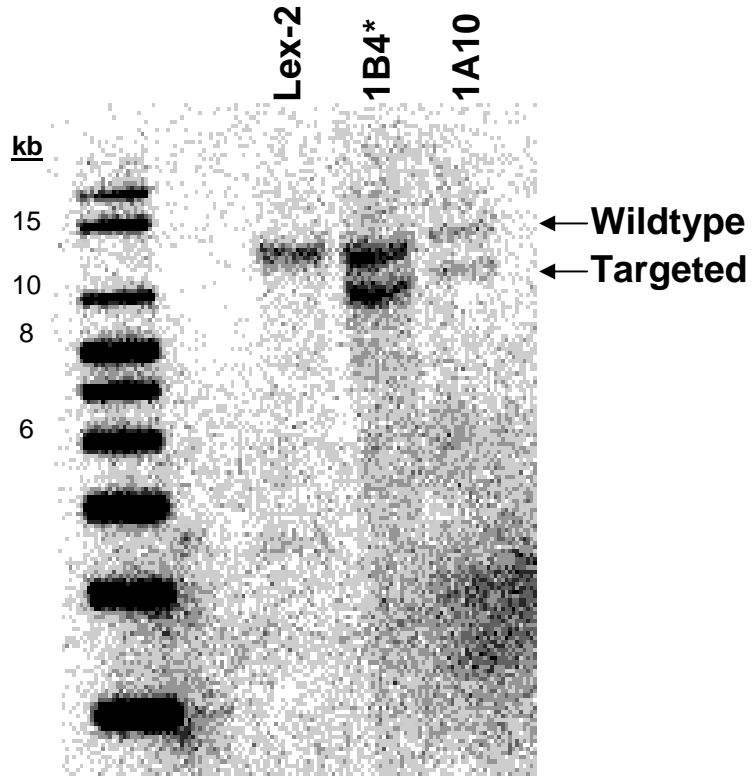
# Targeting Strategy



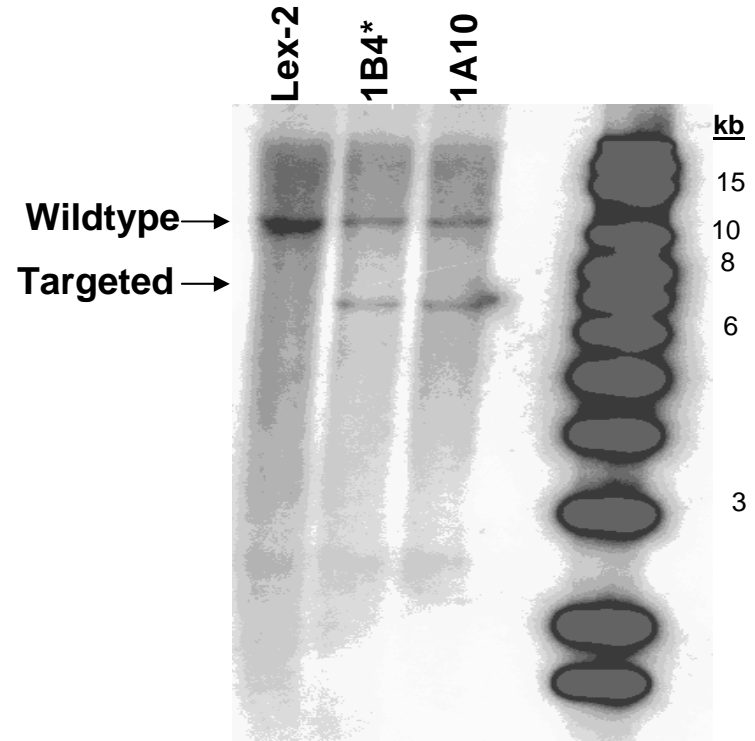
## Southern Strategies

Probe	5' internal 	3' external 
Enzyme	EcoRV	KpnI
Wildtype	12.5kb	11.7kb
Targeted	10.2kb	6.6kb

# Southern Data



5' Internal probe  
EcoRV digests  
Wildtype = 12.5kb  
Targeted = 10.2kb

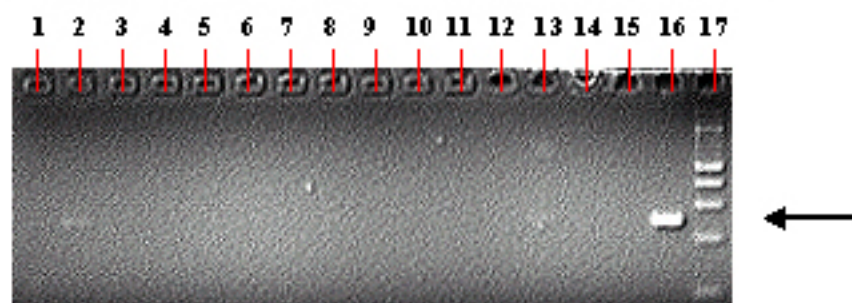


3' external probe  
KpnI digests  
Wildtype = 11.7kb  
Targeted = 6.6kb

\* Clone achieving germline transmission

## RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 3,4



**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/NotI DNA
- 17) 100 bp ladder/marker



**Lexicon Genetics Incorporated  
Molecular Genetics Project Materials**

Catalog Number: NIH-0846 (LEXKO-938)

Reference accession(s): NM\_019471

Standard KO or Conditional: standard

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Materials Submitted: x Target Vector pKOS-60TVpuro  
x KOS clone(s) pKOS-60

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**Southern Blot Genotyping Strategies:**

	<b>5' Internal</b>	<b>3' External</b>
Name of Probe:	23 + 24	20 + 21
Restriction Enzyme for Genomic Digest:	EcoRV	KpnI
Predicted Wild-type Band (kb):	12.5	11.7
Predicted Mutant Band (kb):	10.2	6.6
Probe Size:	488 bp	524 bp

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**Primer sequences:**

**Southern probes**

0846-23 5' – CATAAGCCTGCTTGTTTTACTC  
0846-24 5' – CTGACAGACTGAACCATCAG  
0846-20 5' – GAAAACCTGGTATATGTGCATGC  
0846-21 5' – TCAGAACACTGTCCGTGTTGTG

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**Genomic Sequence Deleted :**

TTTTTAAAGTAAAGACTGTCTGTATGGAGCCACTAGCCATCCTGGCACTGCTGAGCCTACCAATCTGCTCAGCGTATCCT  
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TAATGCTCT

**KOS clone sequence:** (note: pKOS-60 was used to generate the TV and that is the sequence included here)

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

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