

## NIH-0470 Genotyping Strategies

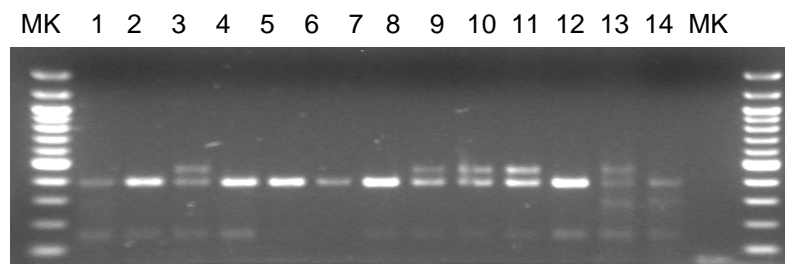
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	96C	17"	
2	57C	25"	
3	72C	30"	35 cycles

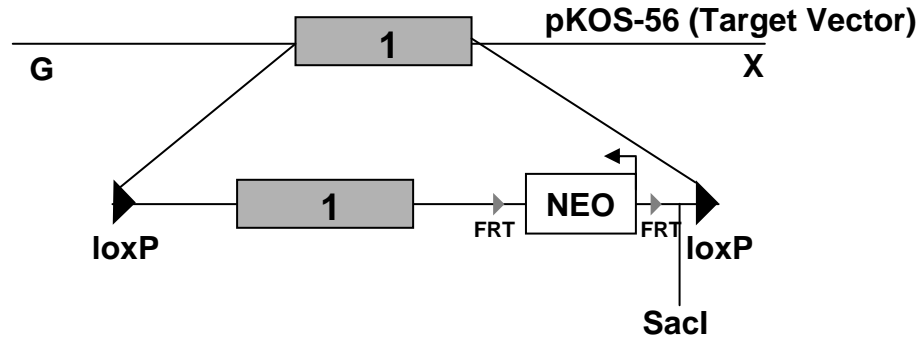
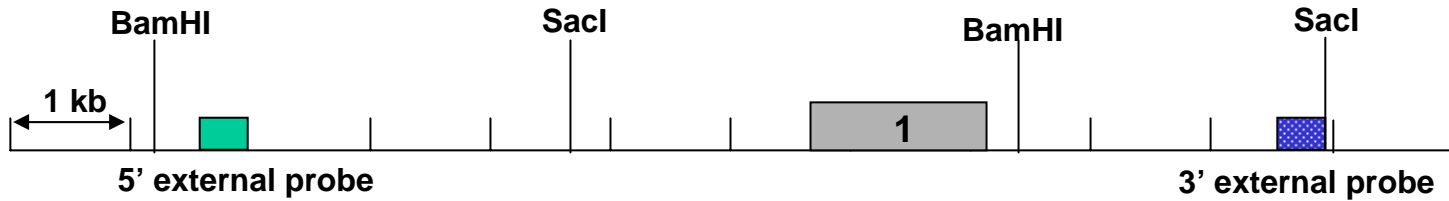
Primer Sequences (5' to 3'):	
Cre excised PCR: Primer 0470-51 and Primer 0470-3', 386 bp	
LoxP Mutant PCR: Primer 0470-51 and Primer 0470-52, 495 bp mutant, 425 bp wt.	
Primer 0470-51	GGAGTGTGGATGAGTTGAAG
Primer 0470-3'	CCTGAGAAGCCCAAGCTCA
Primer 0470-52	CAACACTTTCAGCTCCAGTC

Well	Sample	Genotype
1	603	wt
2	604	wt
3	605	het
4	606	wt
5	607	wt
6	608	wt
7	609	wt
8	610	het
9	611	het
10	612	het
11	613	wt
12	<b>het</b>	het
13	<b>wt lysate</b>	wt
14	<b>water</b>	no amp



### Mutant PCR



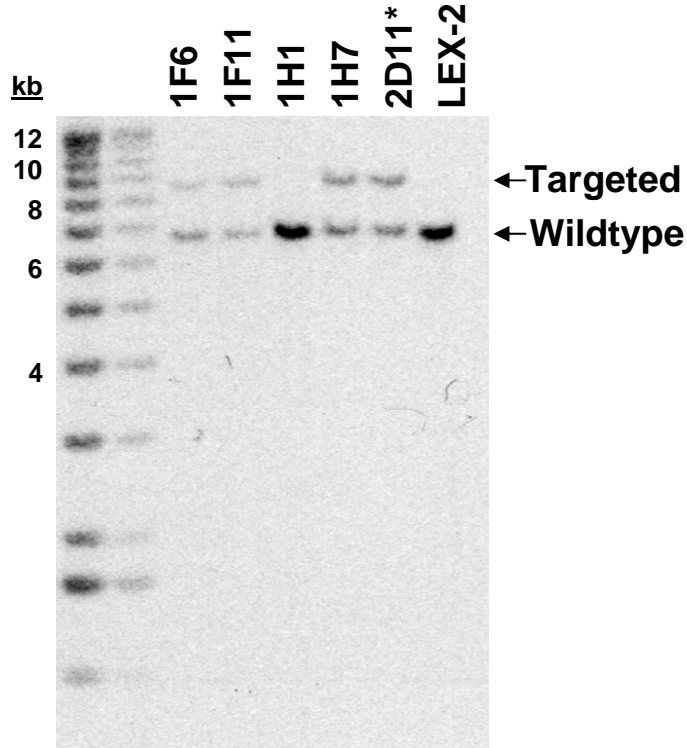
# Targeting Strategy



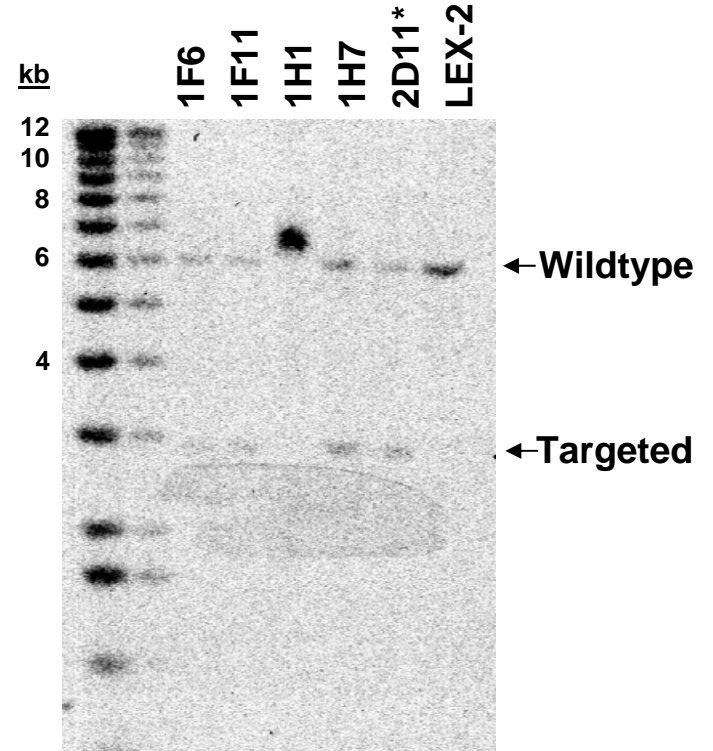
## Southern Strategies

Probe	5' external 	3' external 
Enzyme	BamHI	SacI
Wildtype	6.8 kb	6.0 kb
Targeted	8.8 kb	2.9 kb

# Southern Data



**5' external probe**  
**BamHI digests**  
**Wildtype 6.8 kb**  
**Targeted 8.8 kb**



**3' external probe**  
**SacI digests**  
**Wildtype 6.0 kb**  
**Targeted 2.9 kb**

**\* clone achieving germline transmission**



**Lexicon Genetics Incorporated  
Molecular Genetics Project Materials**

Catalog Number: NIH-0470 (LEXKO-202)

Reference accession(s): NM\_010162

Standard KO or Conditional: Conditional

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Materials Submitted: x Target Vector pKOS-56TVneo  
x KOS clone(s) pKOS-56

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**Southern Blot Genotyping Strategies:**

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	31+50	22+40
Restriction Enzyme for Genomic Digest:	BamHI	SacI
Predicted Wild-type Band (kb):	6.8 kb	6.0 kb
Predicted Mutant Band (kb):	8.8 kb	2.9 kb
Probe Size:	407 bp	276 bp

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**Primer sequences:**

**Southern probes**

0470-31 5' -GGGAAAATAGAGGAGGTCTTG  
0470-50 5' -CATTAAGTTGAGCCATTGGAG  
0470-22 5' -CAAGATATCGTGATGGTGTG  
0470-40 5' -CTTCCAGGACAGTGAGAAATG

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**Genomic Sequence Floxed:**

CGTGCATTGTGCAGCCCAAAGATGAAAGACCGAAGGGGAGAAAAGTTAAAGAAAATCGCCACATGCGCTGGATCAGTCC  
ACGGCTTGGGGAAAGGCATCCAGAGAAGGTGGGAGCGGAGAGTTTGAAGTCTTTACAGGCGGGAAGATGGCGGACTG  
GAGCTGAAAGTGTGATTGGGAACTTGGGTGATTCTTGTGTTTATTTACAATCCTCTTGACCCAGGCAGGACACATGC  
AGGCCAAAAACGCTATTTCACTTCTGCTCTCAGCTGGCTCTTGTCTCGCCCTTTTGTTTTATTTTGGAGGCGTGCAGTTTA  
GGGCATCGAGGAGCCACAGCCGGAGAGAAGAGCACAGTGGTCGGAATGGCTTGCACCAGCCAGTCCGGATCATTCT  
GGCCCCGCTTCCCGGACGCTCTGCGCCCTTTCTTTCCCTTGGGATCAATTGGAAAACGAGGATTCCAGCGTGCACATTTCC  
CCCCGGCAGAAGCGAGACGCCAACTCGAGCATCTACAAAGGCAAGAAGTGCCGCATGGAGTCTTCTGATTTCCACC  
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CTTTAGACAGAGACCAGTTATCACCTCAGTATGTGCACAATTTGAGATCCAAAGTGCAGAGTCTCCACTTGTGGAACAA  
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GTCTTGCTCCTCACCACTGCAAGCATGGCAAAGACTGGCAAAGCACAAGGATTCTCGCTGTGACAGAGACAACACC  
GAGTATGAGAAGTGAGTAAGGGCTGCCTCCCTTCGAGGCTCGGCCCTGGGCCCTGGATACCTGAGTCCAGGTGGGAGG  
GTGGGGGATGCGGGTGGAGACAGAAGCCCTCAGT

**KOS clone sequence:** (note: pKOS-56 was used to generate the TV and that is the sequence included here)

CTGACATCCTGCTCTGACTTCCATGAGTATCAGGCTTATAGGTGGTGCACGTACATATATCCAGGCCAAAACATCGCTAC  
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ATCTCTACCTTTACCTGAACCGTTTTATCCTGCTCGGAAAATGACTGAACCTTACTCTTGTTCAGGCGCTCTCTAAATC  
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GATACTTCAACTCCAAGATATCGTGATGGTGTGTAATGTTGTAGGTCTCAAGGGAAGTG

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

The 5' and 3' **Lox P sites** are in **bold**. The 5' and 3' **FRT sites** are in **bold italics**. The floxed region was subcloned into the multi-enzyme cloning site (italics, underlined). The region between the loxP sites was excised upon exposure to Cre recombinase.

**GGCCATAGCGGCCGGCCATAACTTCGTATAGCATAACATTATACGAAGTTATGGCGCGGAGTCGACGATCAAGCTTTCCG**  
***AAGATCTACGTGGCGCGCCCTCGAGCTTTCGGAAGTTCCTATTTCGGAAGTTCCTATTCTCTAGAAAAGTATAGGAACTTCTC***  
GAGATCCGATATCGAATTCCTCCGCCCCAGCTGGTCTTTCCGCCTCAGAAGCCATAGAGCCCACCGCATCCCCAGCA  
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ACAATGCGATGCAATTTCTCATTATTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGG  
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GCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTTCGCTTG  
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GGCCAGCGAGGCC