

## NIH-0767 Genotyping Strategies

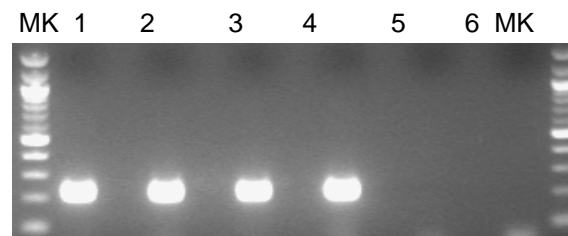
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

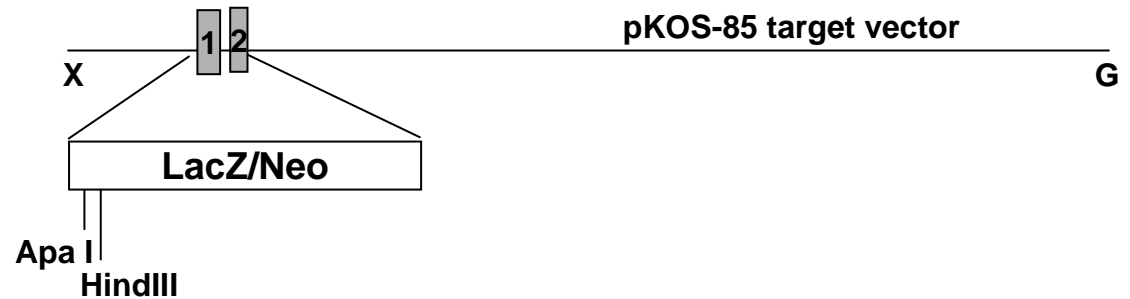
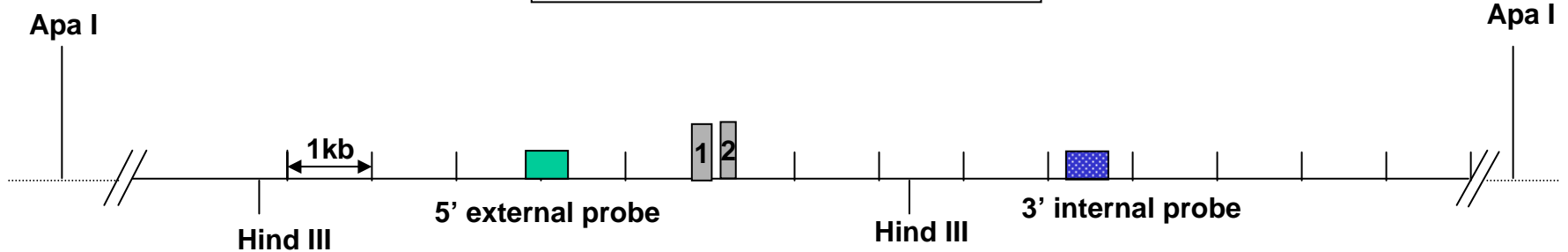
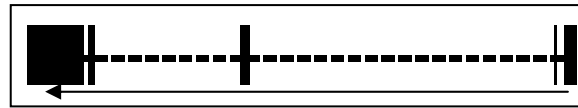
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0767-r, 230 bp	
Recommended Wt PCR: Primer 0767-2 and Primer 0767-3, 305 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0767-r	ACCATTCTACTGACTTGTTAGACTC
Primer 0767-2	GGAGACTCAGTTCTGGTGG
Primer 0767-3	GGAGCTGATAGAAGTTCAGG

Well	Sample	Genotype
1	270	het
2	272	het
3	275	het
5	<b>ES DNA</b>	het
6	<b>wt lysate</b>	wt
7	<b>water</b>	no amp

### Mutant PCR



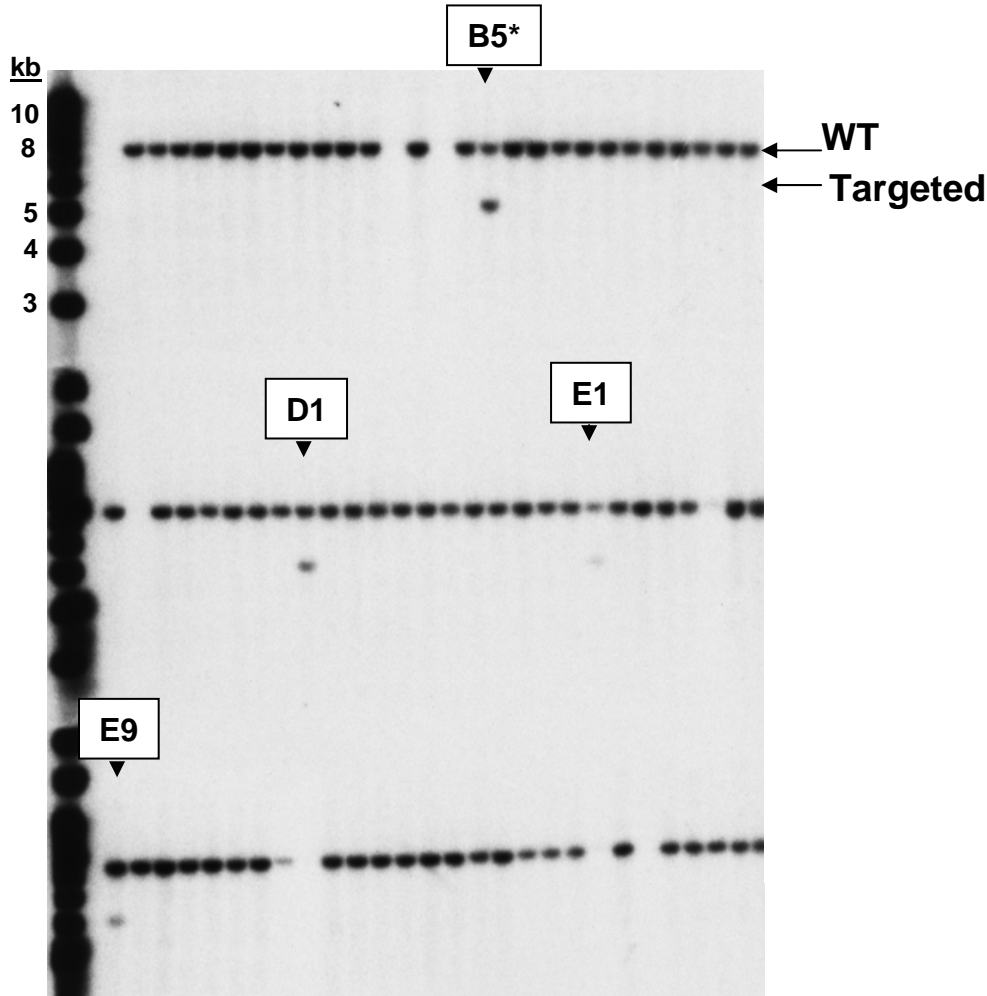
# Targeting Strategy



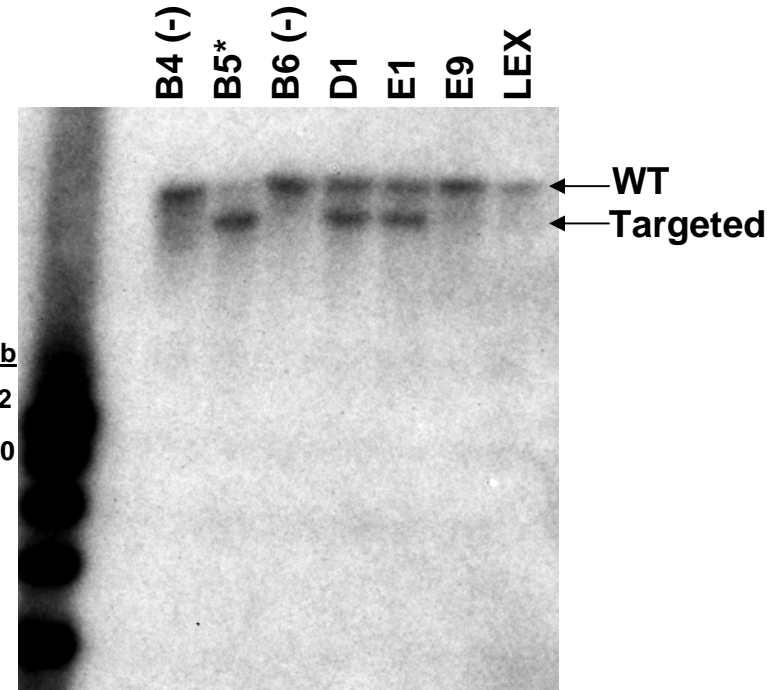
## Southern Strategies

Probe	5' external	3 internal
Enzyme	HindIII	Apal
Wildtype	8.0 kb	28.0 kb
Targeted	5.0 kb	20.0 kb

# Southern Data



5' external probe  
 Hind III digests  
 Wildtype 8.0 kb  
 Targeted 5.0 kb



3' internal probe  
 Apal digests  
 Wildtype 28kb  
 Targeted 20kb

\*Clone achieving germline transmission



**Lexicon Genetics Incorporated  
Molecular Genetics Project Materials**

Catalog Number: NIH-0767 XXXXXXXXXX

Reference accession(s): NM\_021782

Standard KO or Conditional: Standard

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Materials Submitted: x Target Vector pKOS-85TVneo  
x KOS clone(s) pKOS-85

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**Southern Blot Genotyping Strategies:**

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	39 + 40	41 + 42
Restriction Enzyme for Genomic Digest:	HindIII	Apal
Predicted Wild-type Band (kb):	8.0	28
Predicted Mutant Band (kb):	5.0	20
Probe Size:	413 bp	370 bp

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**Primer sequences:**

**Southern probes**

0767-39 5' – TCTGTTGGCTTGTACTGCTG  
0767-40 5' – GATCTACCTGCTTGTCACAC  
0767-41 5' – AGGAGCTCTTGGATCAGAGT  
0767-42 5' – CCTGCAGTCCAGTCTAAG

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**Genomic Sequence Deleted:**

GCATGGAGAGGACCCCTTGTCTGTCTGGTAGTCATCTTCTTGGGGACAGTGGCCCATAAATCAAGCCCCCAAGGGCCAGA  
TCGCCTCCTGATTAGACTTCGTCACCTTATTGACATTGTTGAACAGCTGAAAATCTATGAAAATGACTTGGTAAGGCCA  
TGTTTGTCACAATGCAATTTAAATAATATTTTTTAATTAATGCAAAAGGAGCCTTTTACTTGTATCACCAACTCAGAAC  
TAATACTCTTTGGTTAATTTCTTAGGATCCTGAACTTCTATCAGCTCCACAAGATGTAAAGGTAAGATCGGTT

**KOS clone sequence:** (note: *pKOS-85* was used to generate the TV and that is the sequence included here)

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

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCC TCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAAATAAGGCCGGTGTGCGTTTTGTCTATATGTTATTTTCCACCAT ATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTTCCCTC TCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT CTGTAGCGACCCCTTTCAGGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAG ATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCT CAAGCGTATTCAACAAGGGGGTGAAGGATGCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCA CATGCTTTACATGTGTTTGTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA AACACGTGATAAGCTTGCCACAACCATGGAAGATCCCGTCTGTTTTACAACGTGACTGGGAAAAACCTTGGCGTTAC CCAACTAATCGCCTTGACACATCCCTTTCCGACGCTGGCGTAATAGCAGAAGAGGCCCGCACCGATCGCCCTTCC CAACAGTTGCGCAGCTGAATGGCGAATGGCGCTTTGCTGGTTTTCCGGCACCAGAAGCGGTGCCGAAAGCTGGCTG GAGTGCATCTTCTGAGGCCGATACTGTGCTGCTCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCCATCTACA CCAACGTGACCTATCCATTACGGTCAATCCGCGTTTTGTTCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTT AATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTTTTTTGATGGCGTTAACTCGGCGTTTTATCTGTGGT GCAACGGGCGCTGGGTGCGTTACGGCCAGGACAGTCGTTTTGCGCTGTAATTTGACCTGAGCGCATTTTTACGCGCCGG AGAAAACCGCCTCGCGGTGATGGTGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAG CGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATG ATGATTTACGCCGCGCTGACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTTT TTTATGGCAGGGTGAACCGCAGGTGCGCAGCGGCACCGCGCTTTCCGGCGGTGAAATTATCGATGAGCGTGGTGGTTAT GCCGATCGCGTCACACTACGTCTGAACGTGCAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCG GTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTGCGTTTTCCGCGAGGTGCGGATT GAAAATGGTCTGCTGCTGTAACGGCAAGCCGTTGCTGATTGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATG GTCAGGTGATGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACCTTTAACGCCGTGCGCTGTT CGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGA AACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCG AATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCTGCTGGGGAATGAATCAGGCCACGGCGCTAATCA CGACGCGCTGTATCGCTGGATCAAACTGTGCTGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCAC GGCCACCGATATTTTGGCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATC AAAAAATGGCTTTCTGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCACGCGATGGGTAACAGTCTTG

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