

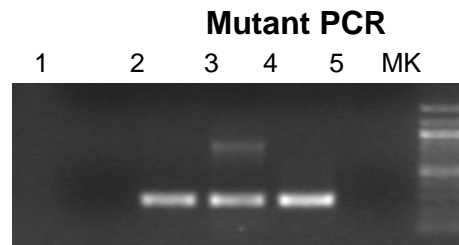
NIH-0768 Genotyping Strategies

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	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

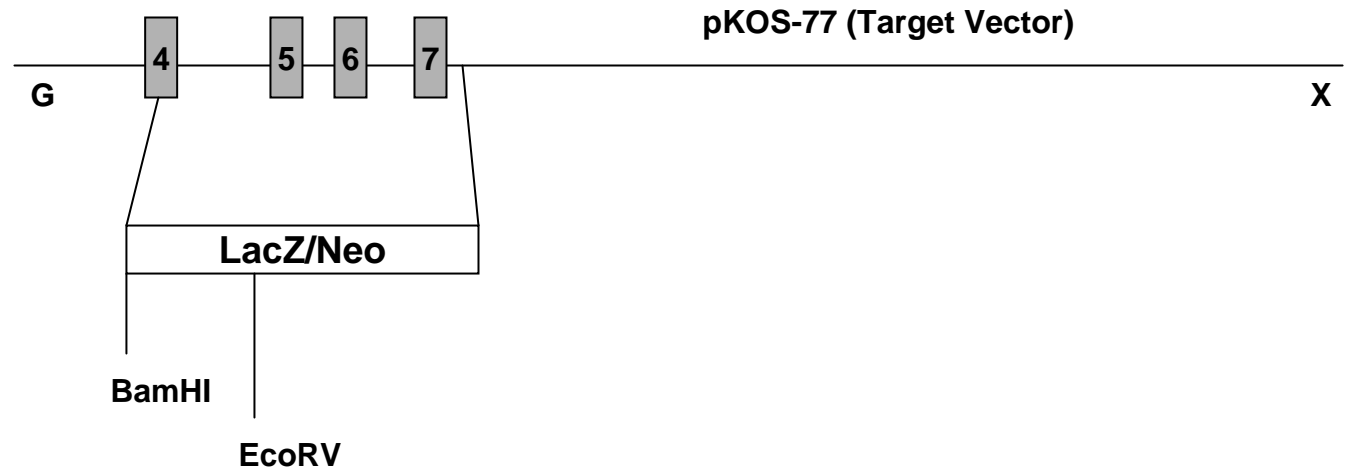
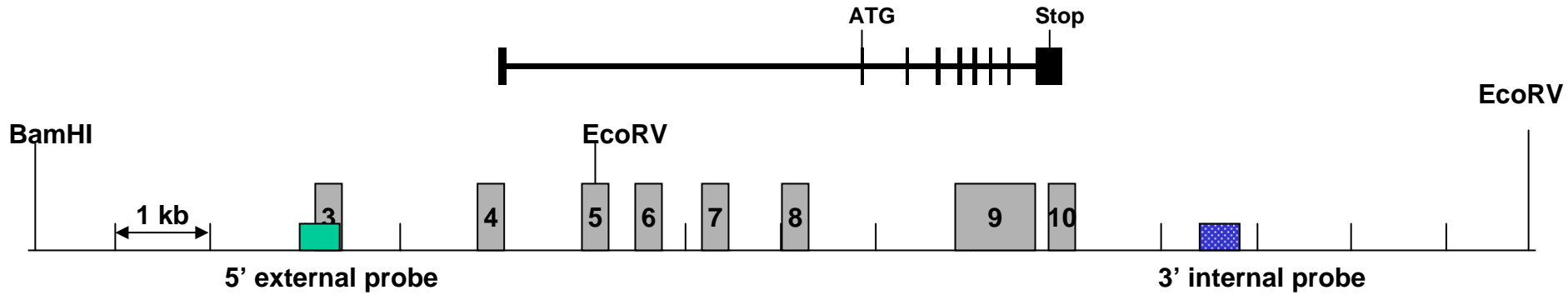
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0768-98, 302 bp	
Recommended Wt PCR: Primer 0768-10 and Primer 0768-50, 252 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0768-98	GAAGTTCTGCACAGTGTCTAGC
Primer 0768-10	GGTACACGTGCCATATGCG
Primer 0768-50	AAACATCACACCTGAGCTGTGC



Well	Sample	Genotype
1	40	wt
2	41	het
3	ES DNA	het
4	target vector	het
5	water	no amp



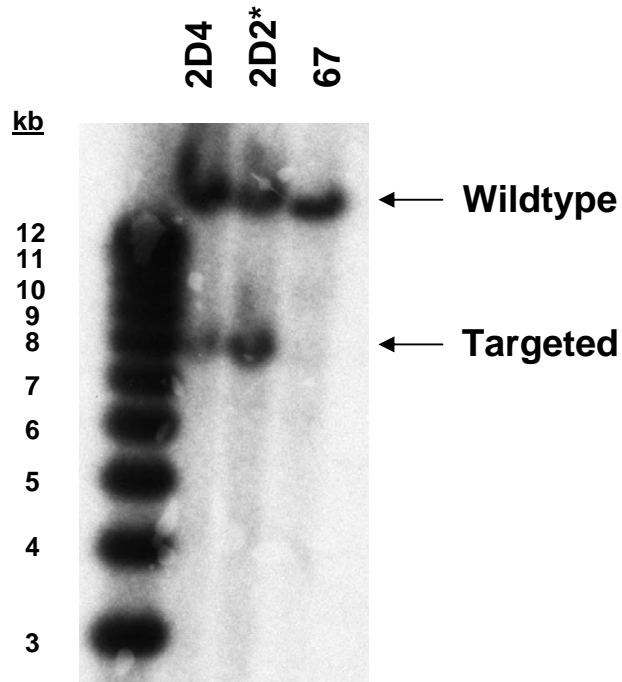
Targeting Strategy



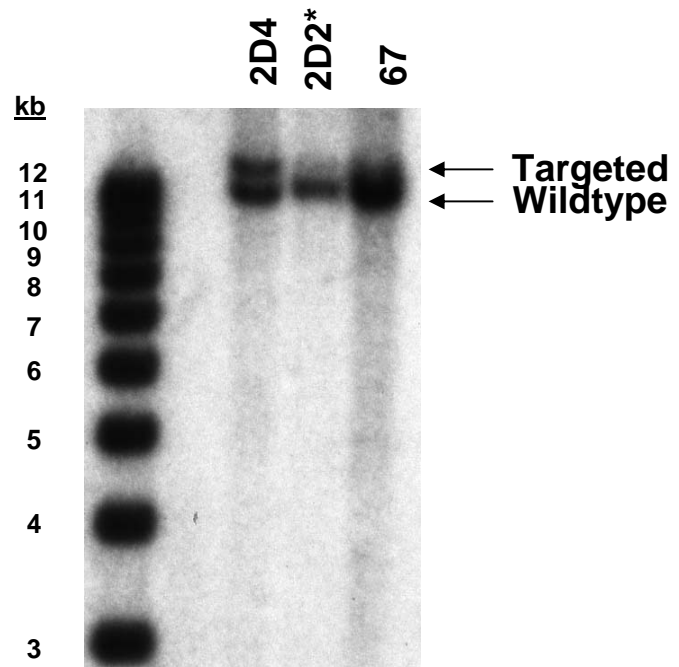
Southern Strategies

Probe	5' external 	3' internal 
Enzyme	BamHI	EcoRV
Wildtype	14.9 kb	13.5 kb
Targeted	7.8 kb	15.1 kb

Southern Data



5' external probe
 BamHI digests
 WT 14.9 kb
 Targeted 7.8 kb



3' external probe
 EcoRV digests
 WT 13.5 kb
 Targeted 15.1 kb

* Clone achieving germline transmission



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0768 (LEXKO-899)

Reference accession(s): NM_021887

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS-77TVneo
 KOS clone(s) pKOS-77

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	66/52	121/106
Restriction Enzyme for Genomic Digest:	BamHI	EcoRV
Predicted Wild-type Band (kb):	14.9	13.5
Predicted Mutant Band (kb):	7.8	15.1
Probe Size:	391	503

Primer sequences:

Southern probes

0768-52 5' – CTGGCTCCCAGCTCATAATCC
0768-66 5' – TCCCAGCCTGTGACTCTGCC
0768-106 5' – ACCCTGCCTTGCAAATGAGCAG
0768-121 5' – GCTCTGGTTCTGTAGCTAGTAC

Genomic Sequence Deleted:

ACAGGTCTGGCCACAACACCACATATATGGTACACGTGCCATATGCGCTTGTCTCAATTCTGTCCGATGAAGTTTTCATTGTCAATGTGACGGACC
AGTCTGGCAACAACCTCCAAGAGTGTGGCAGCTTTGTCTGGCTGAGAGCAGTAAGTACCATGAGCCCGAAGCAGAGAGGACCTAGGACCTTTCTCTG
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KOS clone sequence: (note: pKOS-77 was used to generate the TV and that is the sequence included here)

GATCACAAAATGGGAGGCTGTAGTCTGACTTGTCTTCCCTCATAGACCTTCTGGGTTGGTAGCCCTGGGATGGACAGTTCACAGTGACCTTCTAAGC
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