NIH-0456 Genotyping Strategies

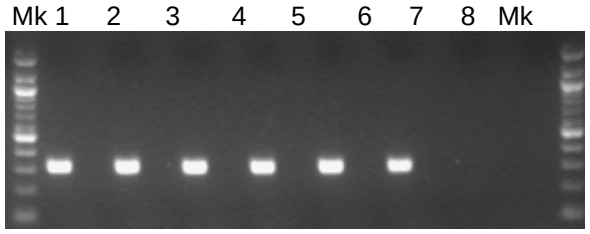
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

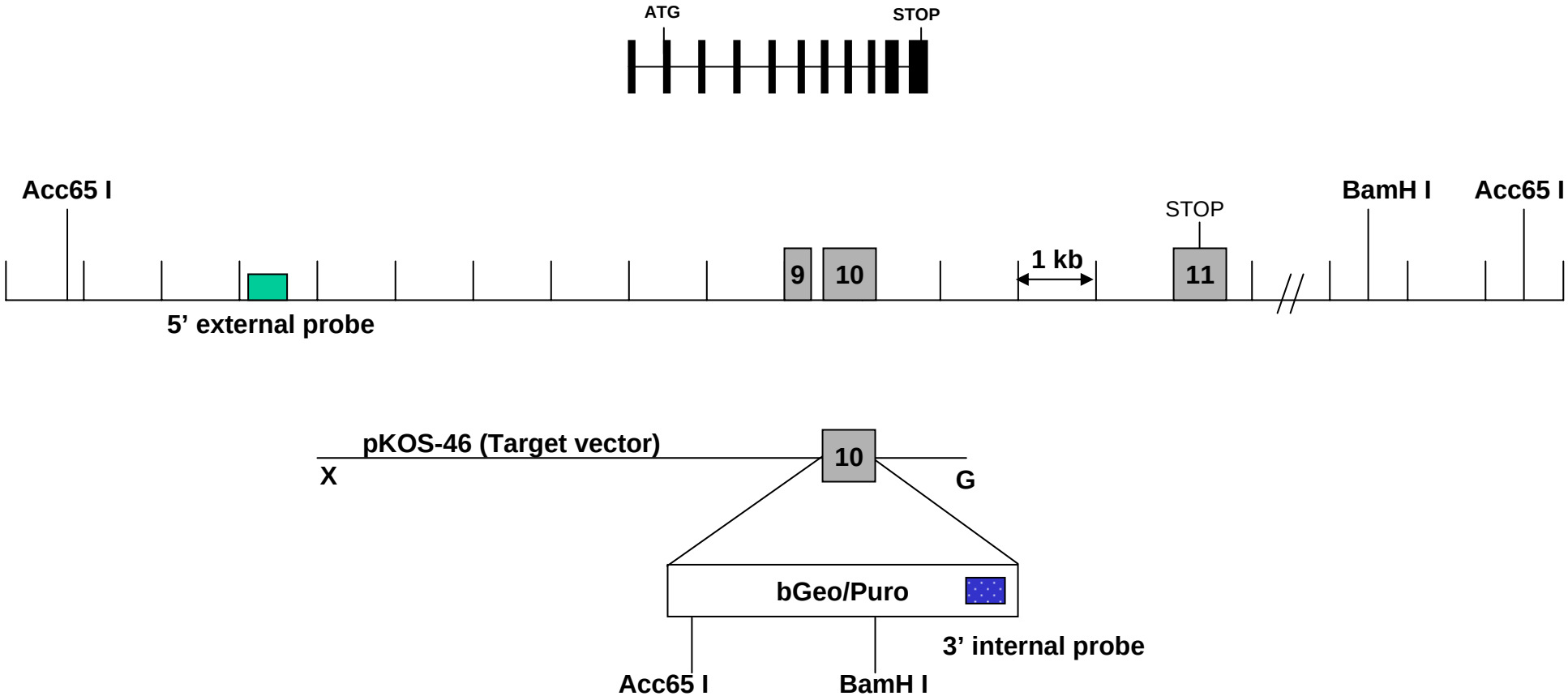
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 0456-f and Primer GT-IRES, 304 bp	
Recommended Wt PCR: Primer 0456-100 and Primer 0456-101, 381 bp	
Primer 0456-f	TTGTGACTGCTTCTGTGTTTGTCC
Primer GT-IRES	CCCTAGGAATGCTCGTCAAGA
Primer 0456-100	TACCATGGGGGCTTCCGCTGTTAC
Primer 0456-101	TTACAGCCACTGGTGTTACCTAAG

Well	Sample	Genotype
1	288	het
2	298	het
3	314	het
4	321	het
5	334	het
6	410	het
7	**wt lysate**	wt
8	**water**	no amp

Mutant PCR



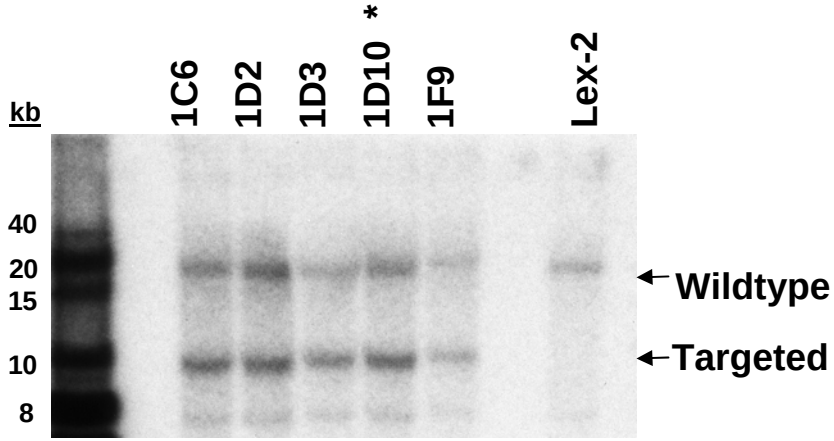
Targeting Strategy



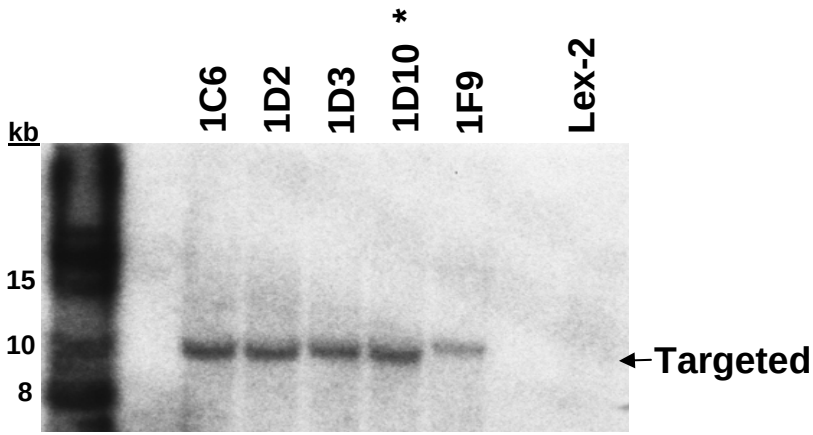
Southern Strategies

Probe	5’ external	3’ internal
Enzyme	Acc65 I	BamHI
Wildtype	20.0 kb	---
Targeted	10.2 kb	10.7 kb

Southern Data



5' external probe
Acc65 I digests
Wildtype: 20.0 kb
Targeted: 10.2 kb

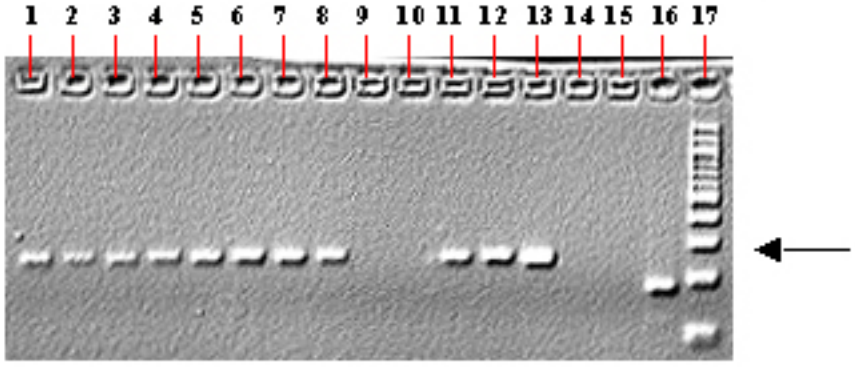


3' internal probe
BamHI digest
Targeted: 10.7 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



02/13/2004

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-0456** (LEXKO-940)

Reference accession(s): AK077302

Standard KO or Conditional: Standard

Materials Submitted: ☐ Target Vector pKOS-46TVPuro
☐ KOS clone(s) pKOS-46

Southern Blot Genotyping Strategies:

	5' External	**3' Internal**
Name of Probe:	26/27	Neo 2/5
Restriction Enzyme for Genomic Digest:	Acc65I	BamHI
Predicted Wild-type Band (kb):	20.0 kb	---
Predicted Mutant Band (kb):	10.2 kb	10.7 kb
Probe Size:	260 bp	609 bp

Primer sequences:

Southern probes

0456-26 5' – TACACATGGCAAGCTTTCCTATC
0456-27 5' – CACCAATGTCGCACCTATGA
Neo2 5' – CCTCAGAAGAACTCGTCAAG
Neo5 5' – GGCAGCGCGGCTATCGTG

Genomic Sequence Deleted:

ATGTGCCGGGAGCTGCCCCAGTCCATTGTCTACAAATACATGAGCATCCGATCTGACAGGTCCGTGCCTTCAGACATCTTCCAGATACAGGCAACAATG
ATTTATGCAAACACCATTAATACTTTTCGGATTAAATCTGGAAATGAAAATGGAGAGTTCTACCTACGAGTAAGTATGCTTAGGTAACACCAGTGGCTG
TAAAGGTGGCCATTTGC

KOS clone sequence: *(note: pKOS-46 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)*

Bgeo/Puro

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