NIH-1032 Genotyping Strategies

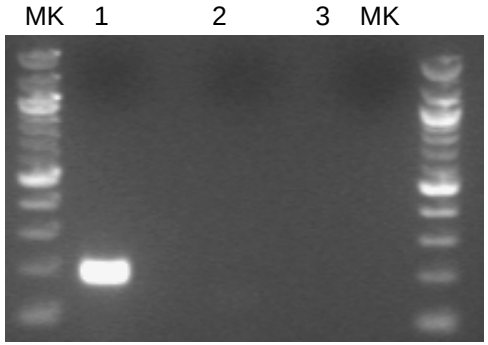
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

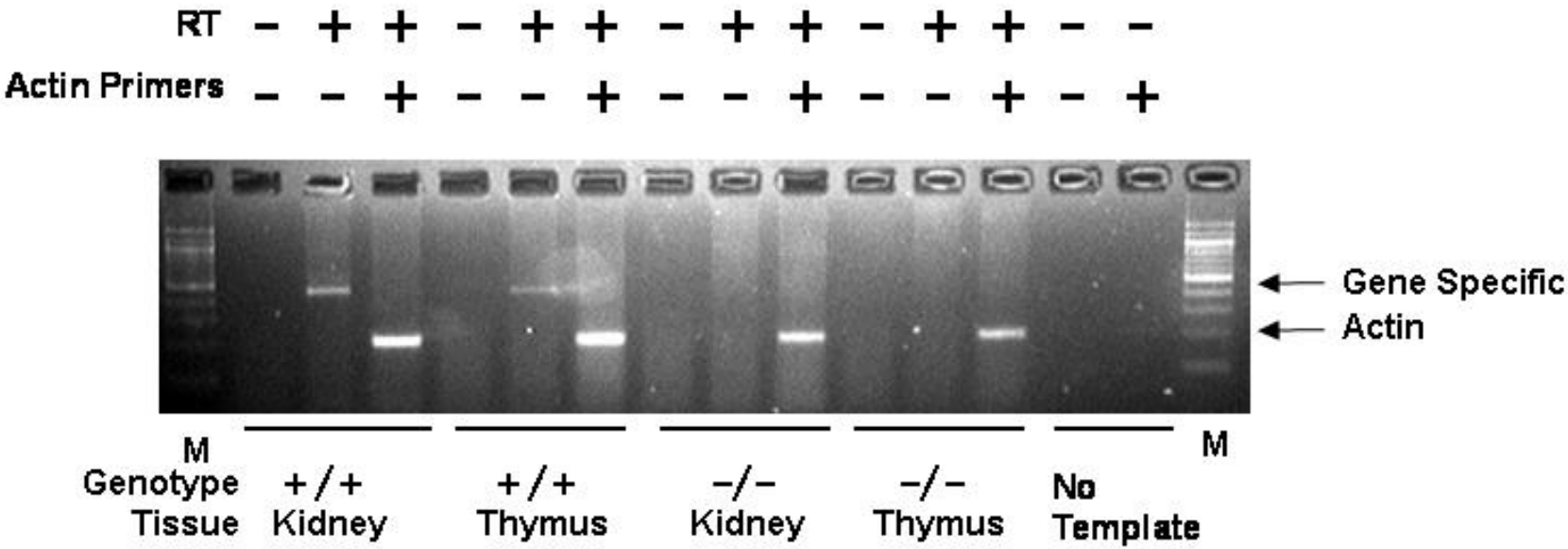
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 1032-5' and Primer LTR-rev, 194 bp	
Recommended Wt PCR: Primer 1032-5' and Primer 1032-3', 140 bp	
Primer 1032-5'	GATTACAGAACTCGGCATATCCAG
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 1032-3'	GTGCCATGATTCTGCTAAGCTAGC

Well	Sample	Genotype
1	**ES DNA**	het
2	**wt lysate**	wt
3	**water**	no amp

Mutant PCR



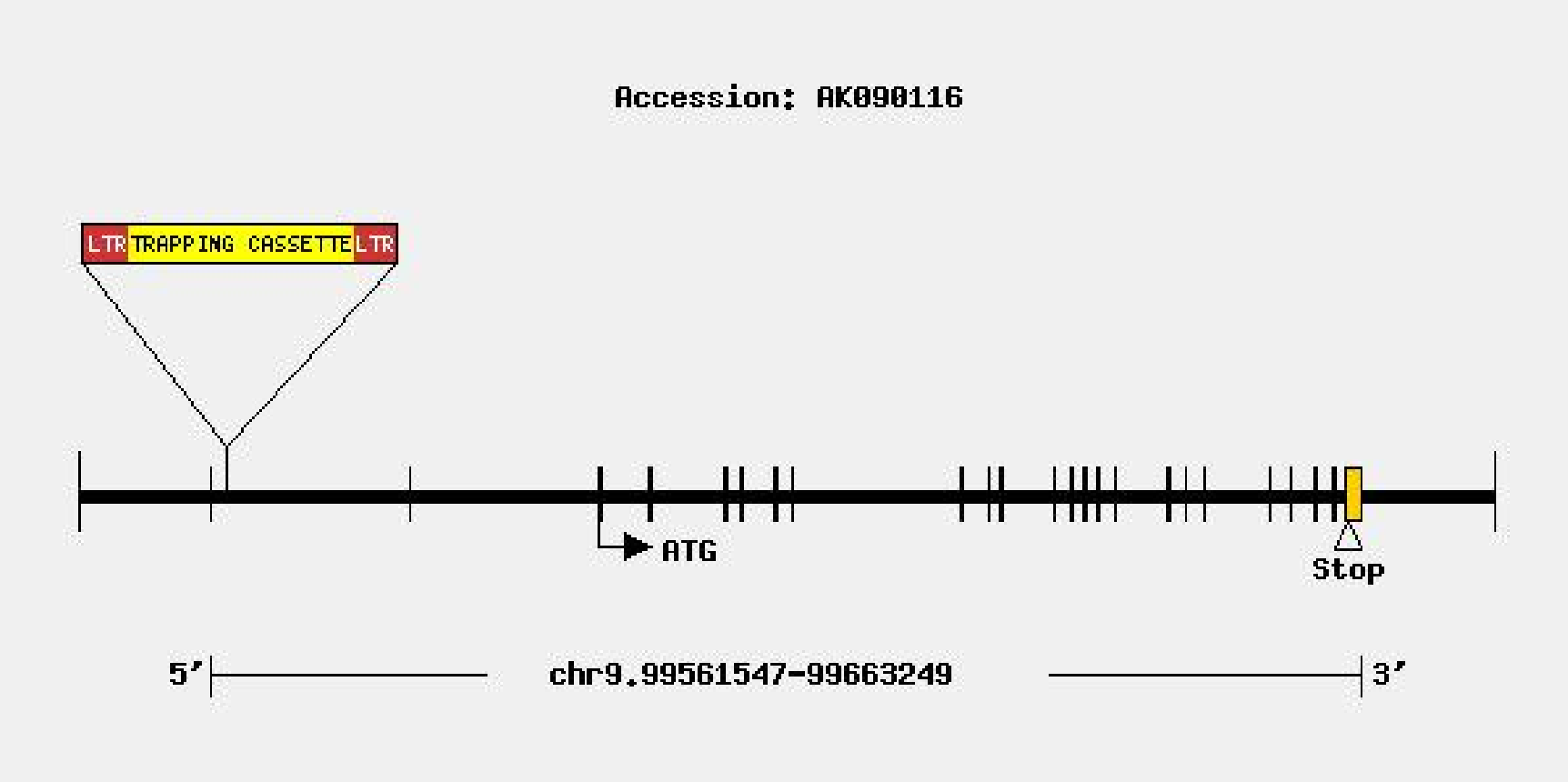
QC Expression



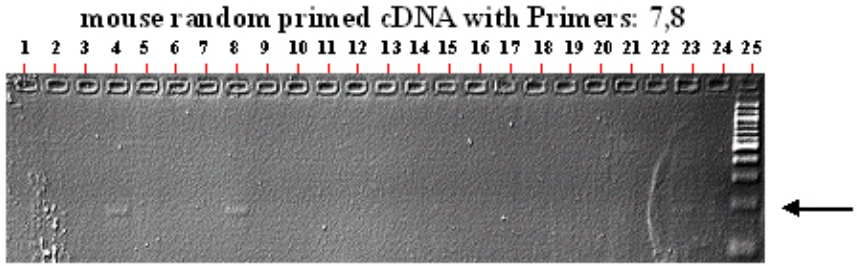
PCR 35 cycles
Primers: 9&10

Mouse ID: 103

QC Image


Accession: AK090116
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr9.99561547-99663249
3'

RT-PCR WT Expression



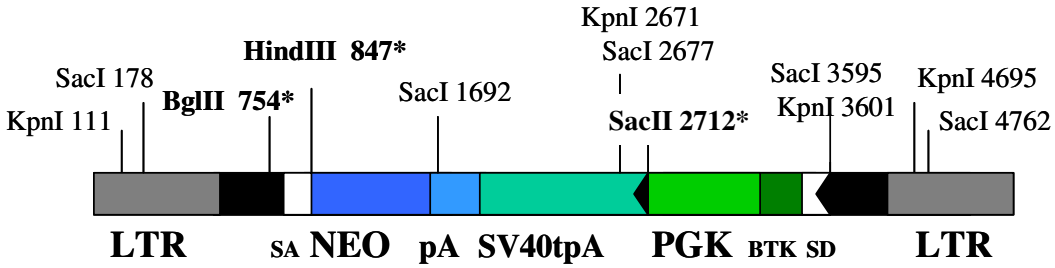
03/24/03

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) Internal Test
8) Kidney
9) Liver
10) Internal Test
11) Skeletal Muscle
12) Bone
13) Stomach, Small Intestine & Colon
14) Adipose
15) Internal Test
16) Internal Test
17) Internal Test
18) Heart
19) Internal Test
20) Internal Test
21) Blank-TBD
22) (-) Control
23) (+) Control- ES cell cDNA
24) (+) Control- Genomic/Lex1 DNA
25) 100 bp ladder/marker

VICTR 48 Omnibank Vector



Total Size: 5174 nucleotides
Non-Cutters: ApaI, XhoI, XmnI
* Unique sites

Location of components in VICTR 48:
LTR (viral long terminal repeat): 1-590, 4585-5174
SA (splice acceptor): 755-847
NEO: 867-1684
pA: 1688-1874
pA (SV40 poly adenylation sequence): 1875-2691
frt sites: 2733-2780, 3613-3661
PGK promoter: 2805-3321
BTK exon: 3356-3580

>VICTR 48
TGAAAGACCCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
CGGGTACCCGGGCGACTCAGTCAATCGGAGGACTGGCGCGCCGAGTGAGGG
GTTGTGGGCTCTTTTATTGAGCTCGGGGAGCAGAAGCGCGCGAACAGAAGCG
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