NIH-0670 Genotyping Strategies

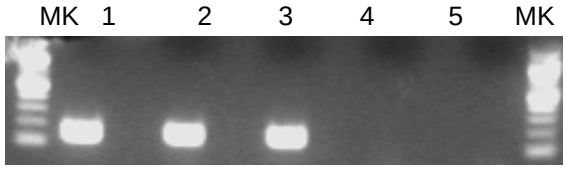
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

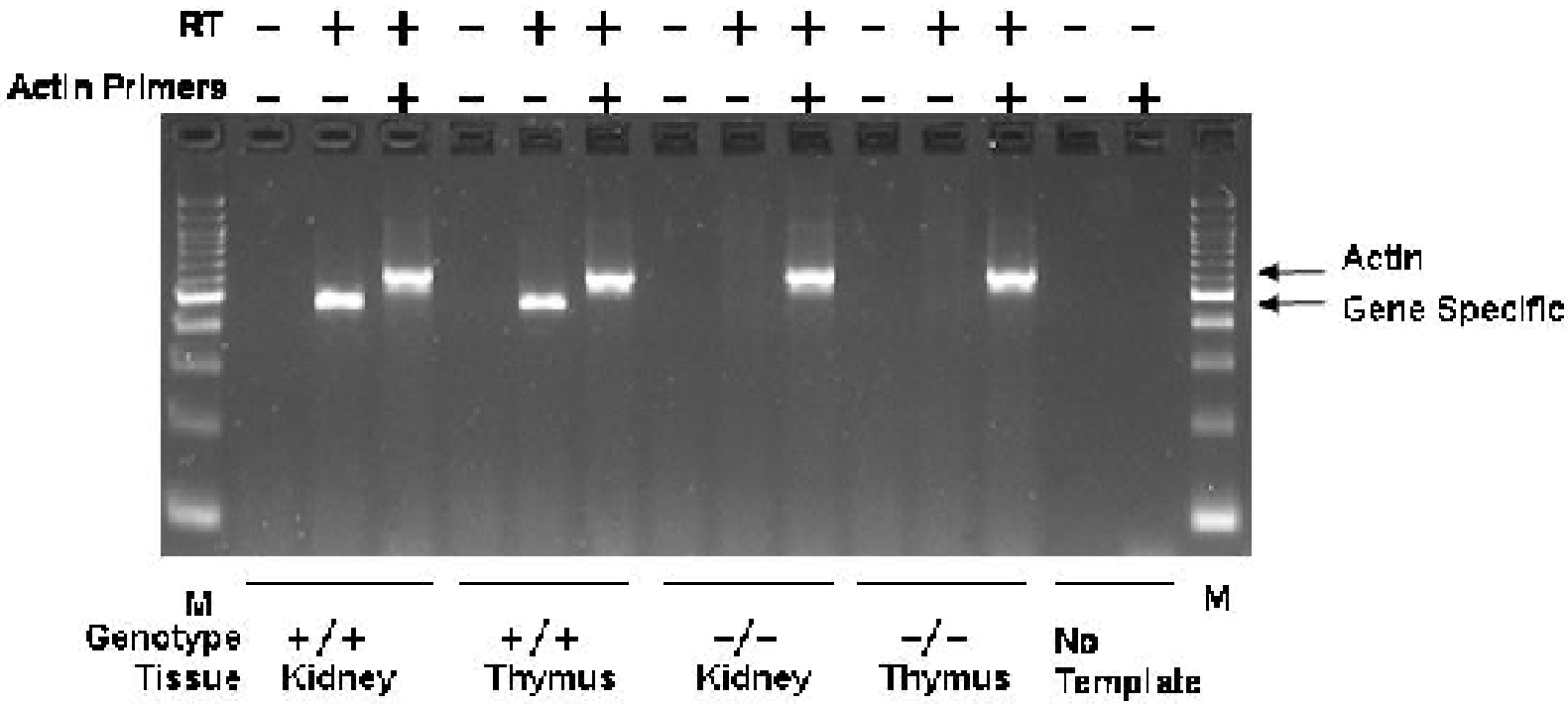
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 0670-5' and Primer LTR-rev, 140 bp	
Recommended Wt PCR: Primer 0670-5' and Primer 0670-3', 135 bp	
Primer 0670-5'	GGAGAAGGGACATTTTTAGGTTG
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 0670-3'	CAATTGCTTGAGTTAGGCCACAGG

Well	Sample	Genotype
1	177	het
2	179	het
3	180	het
4	**wt lysate**	wt
5	**water**	no amp

Mutant PCR



QC Expression

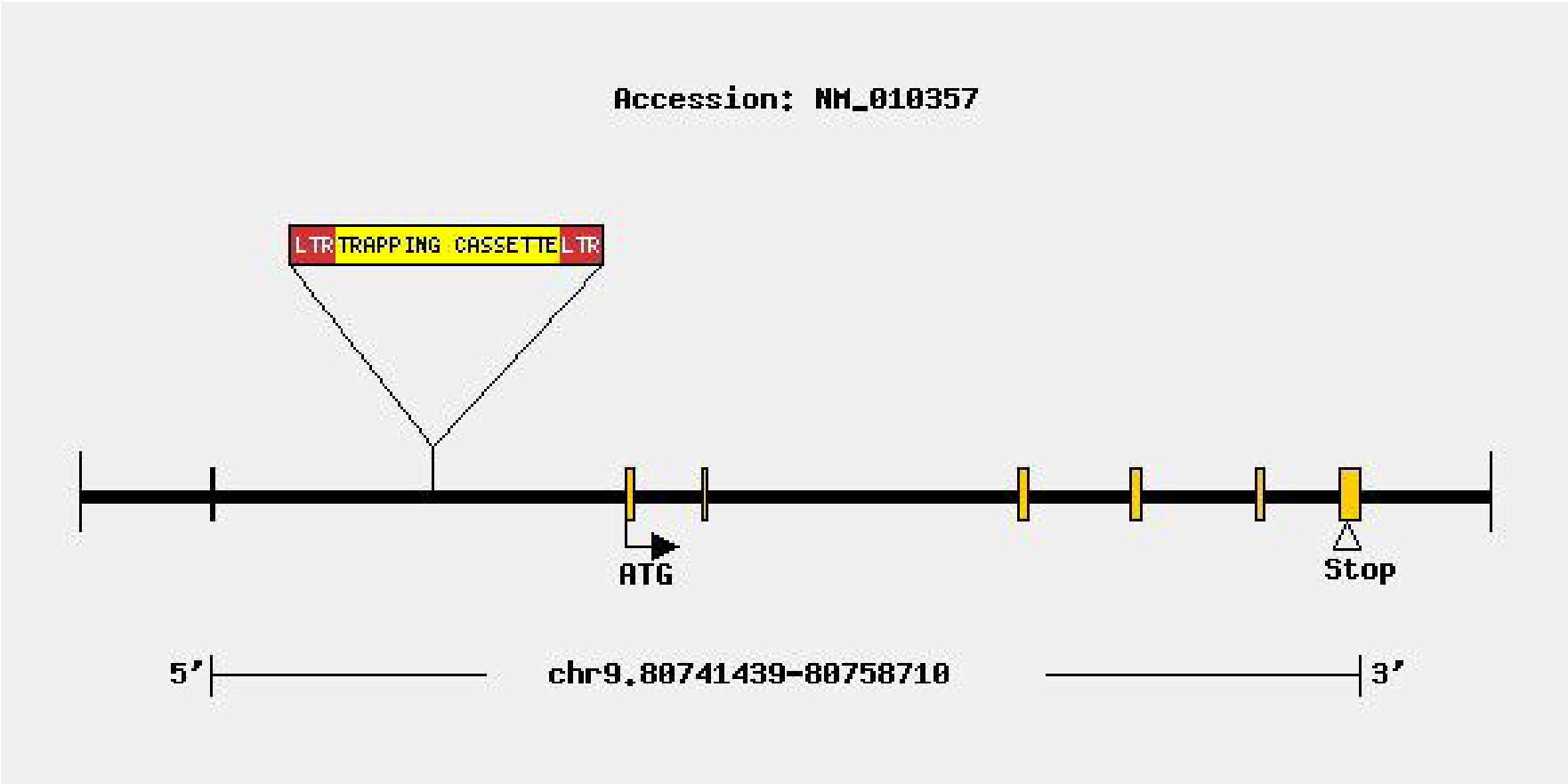


PCR 35 cycles

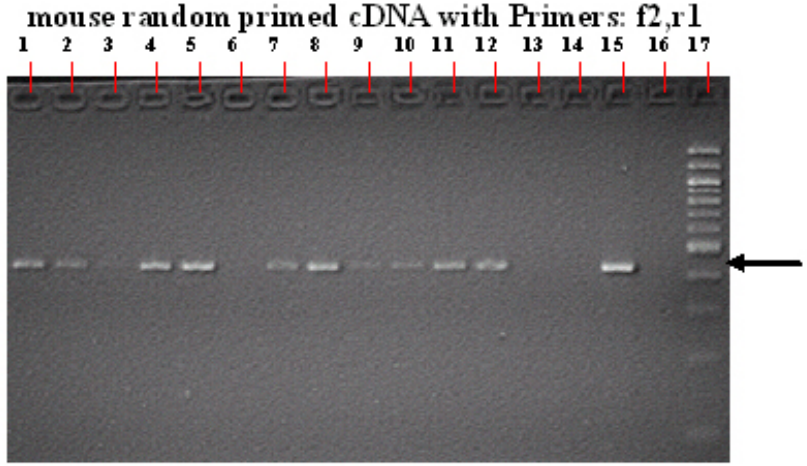
Mouse ID: 96

Primers: 1&2

QC Image


Accession: NM_010357
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr9.80741439-80758710
3'

RT-PCR WT Expression



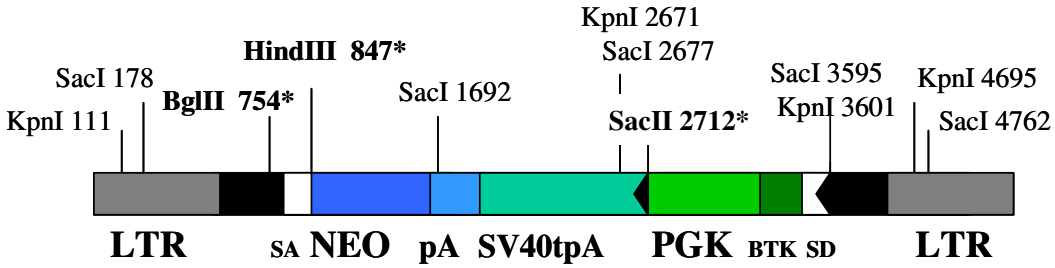
01/30/2002

Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

1) Brain
2) Thymus
3) Spleen
4) Lung
5) Kidney
6) Liver
7) Testis
8) Stomach
9) Small Intestine
10) Colon
11) Heart
12) Adipose
13) Skin
14) (-) Control
15) (+) Control- ES cell cDNA
16) (+) Control- Genomic/Lex1 DNA
17) 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
AGAAGCGAACTGATTGGTTAGTTCAAATAAGGCACAGGGTCATTTCAGGTCC
TTGGGGCACCCTGGAAACATCTGATGGTTCTCTAGAAACTGCTGAGGGCTGG
ACCGCATCTGGGGACCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCT
TACCACAGATATCCTGTTTGGCCCATATTCAGCTGTTCCATCTGTTCTTGGCCC
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GCTGTTCCATCTGTTCCTGACCTTGATCTGAACTTCTCTATTCTCAGTTATGTA
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