NIH-0617 Genotyping Strategies

Reaction Components	Vol (ul)
2X Premix G	25
25mM MgCl2	3.5
10mM dNTPs	N/A
Primer 20 uM	2.5
Primer 20 uM	2.5
5 U/ul Taq polymerase	0.5
Water	11
Total mix volume	45
Tail lysate extracted	2.5
Total reaction volume	47.5

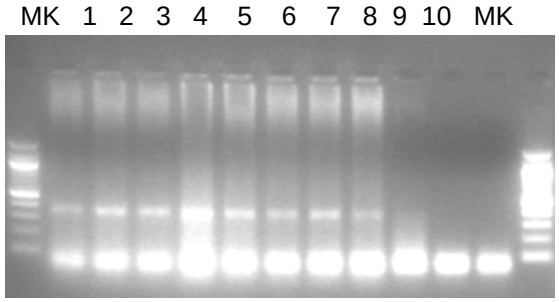
Step	Temp	Time	Note
1	95C	1'	
2	95C	17"	
3	58C	30"	
4	72C	40"	Go to 2, 40 cycles

Primer Sequences (5' to 3'):	
Mutant PCR: Primer 0617-5' and Primer LTR-rev, 385 bp	
Recommended Wt PCR: Primer 0617-5' and Primer 0617-3', 446 bp	
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 0617-5'	TGCTCTGAAGGACTCGTGACTGG
Primer 0617-3'	TAAGCGAAAGGATTCACTAATTC

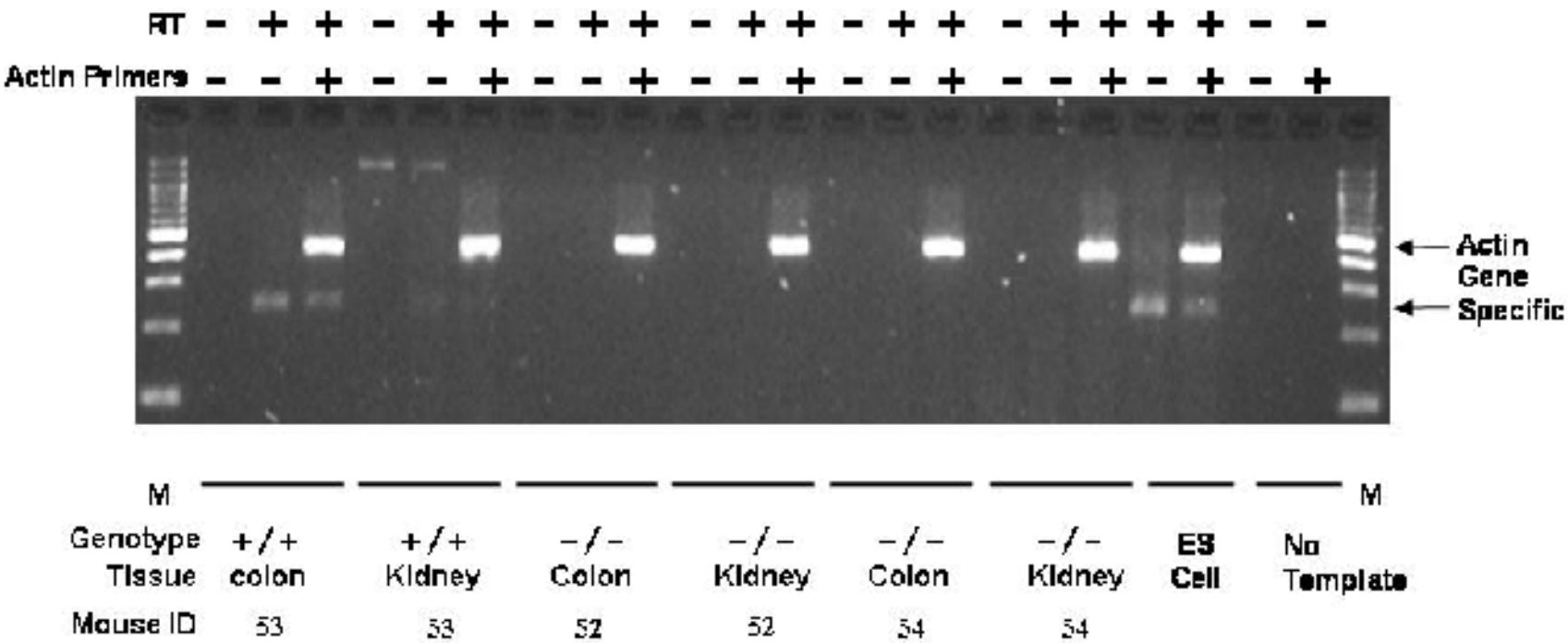
Samples were phenol-chloroform extracted

Well	Sample	Genotype
1	135	het
2	137	het
3	138	het
4	145	het
5	149	het
6	163	het
7	164	het
8	167	het
9	**ES DNA**	het
10	**wt lysate**	wt
11	**water**	no amp

Mutant PCR

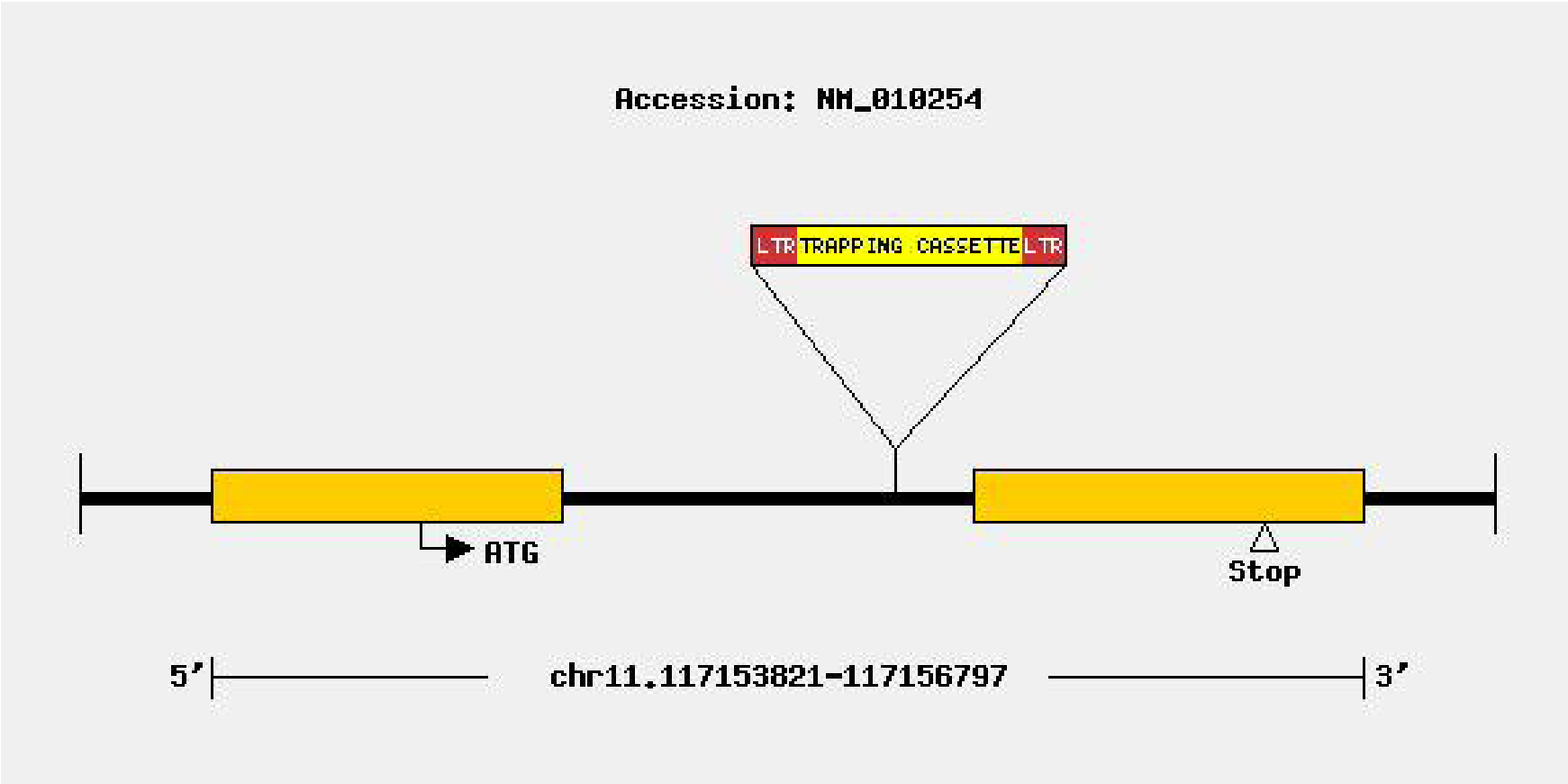


QC Expression

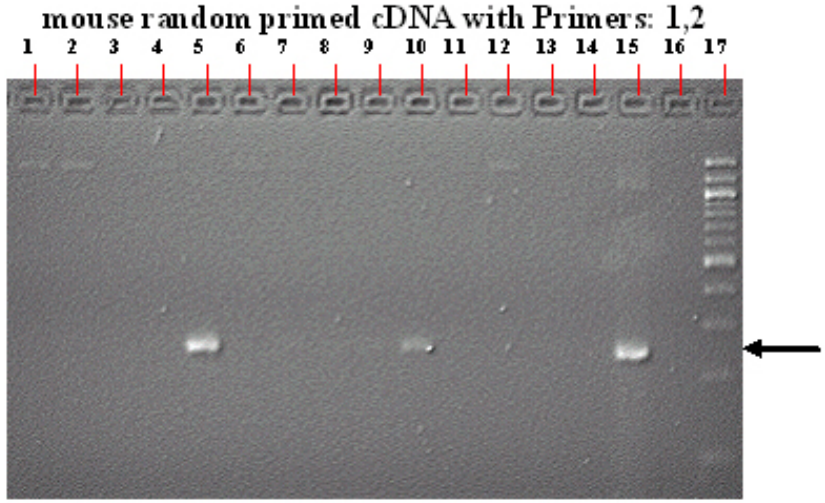


PCR 35 cycles
Primers: 1&2

QC Image


Accession: NM_010254
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr11.117153821-117156797
3'

RT-PCR WT Expression

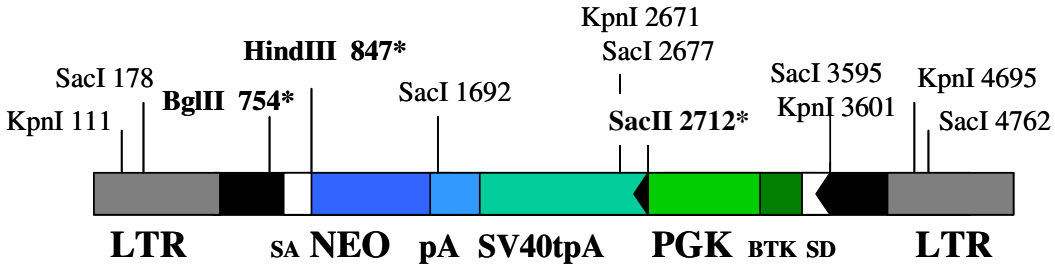


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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
TGAGCCGGGGCAGGAACTGCTTACCACAGATATCCTGTTTGGCCCATATTCA
GCTGTTCCATCTGTTCCTGACCTTGATCTGAACTTCTCTATTCTCAGTTATGTA
TTTTTCCATGCCTTGCAAAATGGCGTTACTTAAGCTAGCTTGCCAAACCTACA
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