NIH-0669 Genotyping Strategies

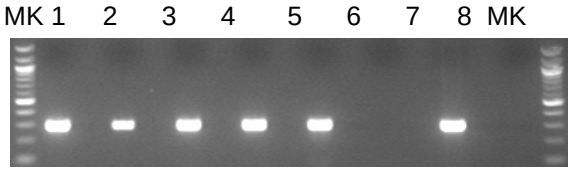
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

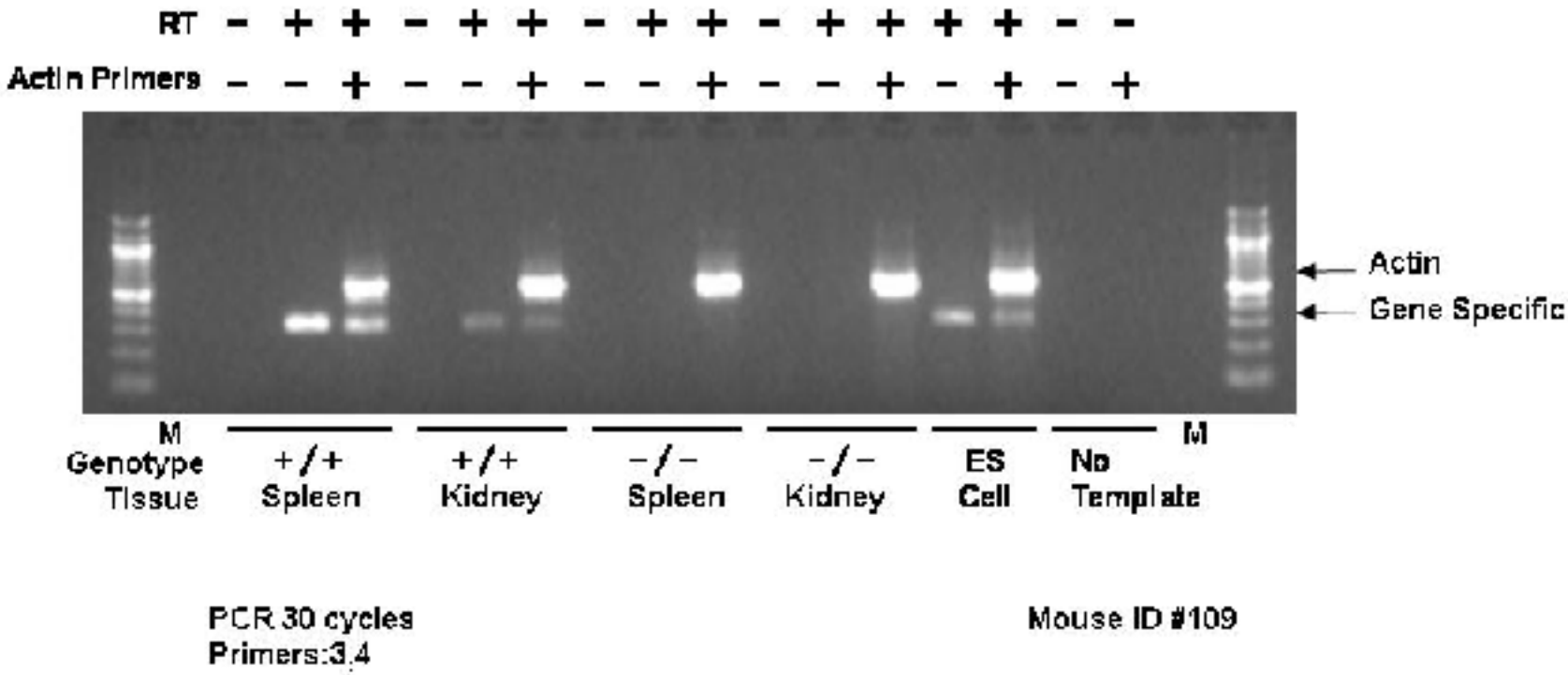
Primer Sequences (5' to 3'):	
Mutant PCR: Primer LTR-2 and Primer 0669-3', 292 bp	
Recommended Wt PCR: Primer 0669-5' and Primer 0669-3', 342 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0669-5'	AGTCACAAGCTGGGTGGCACTTGC
Primer 0669-3'	ACTCCTCCACGCCAGAACCCTC

Well	Sample	Genotype
1	3060	het
2	3061	het
3	3069	het
4	3076	het
5	3077	het
6	**wt lysate**	wt
7	**ES DNA**	het
8	**water**	no amp

Mutant PCR



QC Expression



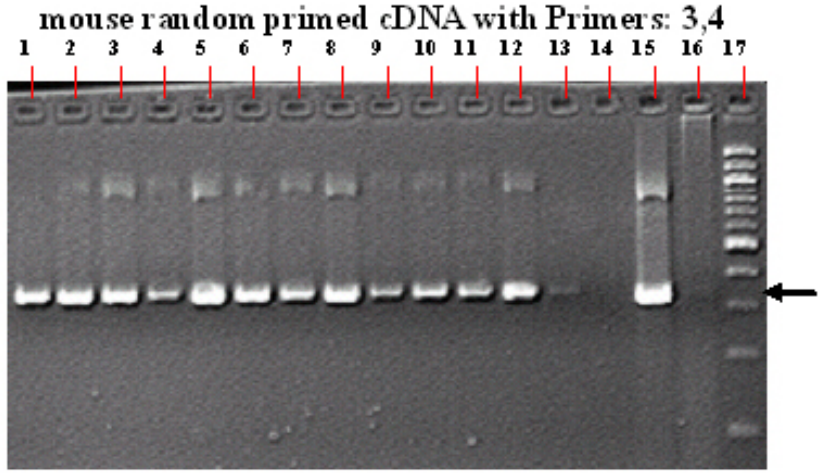
PCR 30 cycles
Primers:3,4

Mouse ID #109

QC Image


Accession: NM_010344
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr8.32527518-32571933
3'

RT-PCR WT Expression



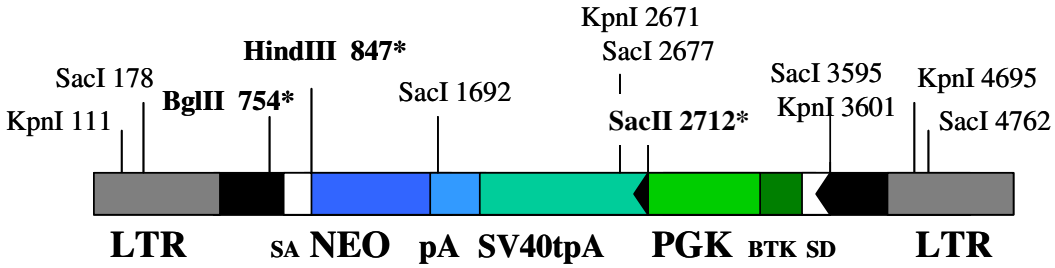
04/04/2001

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
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