

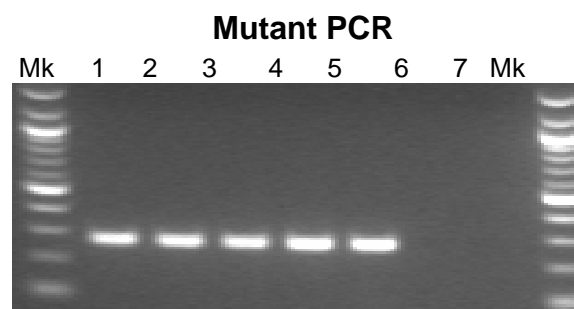
NIH-0274 Genotyping Strategies

Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	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

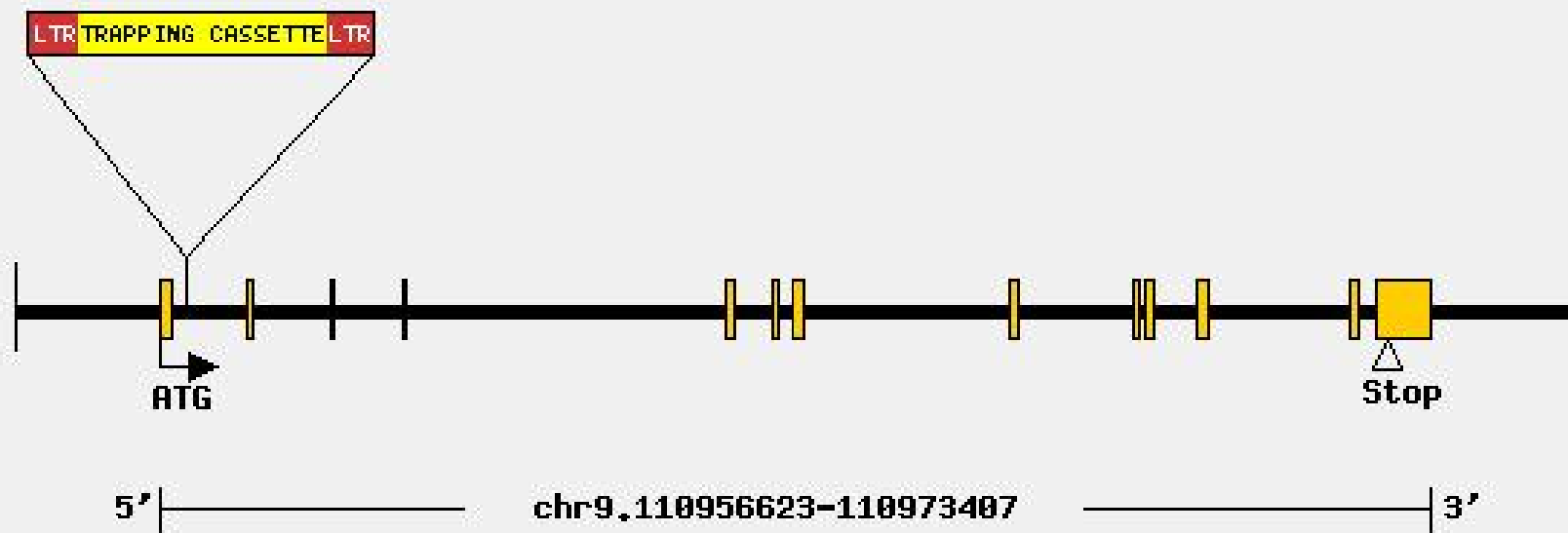
Primer Sequences (5' to 3'):	
Mutant PCR: Primer LTR-2 and Primer 0274-3', 274 bp	
Recommended Wt PCR: Primer 0274-5' and Primer 0274-3', 258 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0274-5'	TTCGGATAGTCCAGGGCTATCC
Primer 0274-3'	TGCCACGTCCCTTTGTTGAGAAGG

Well	Sample	Genotype
1	162	het
2	165	het
3	166	het
4	167	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp

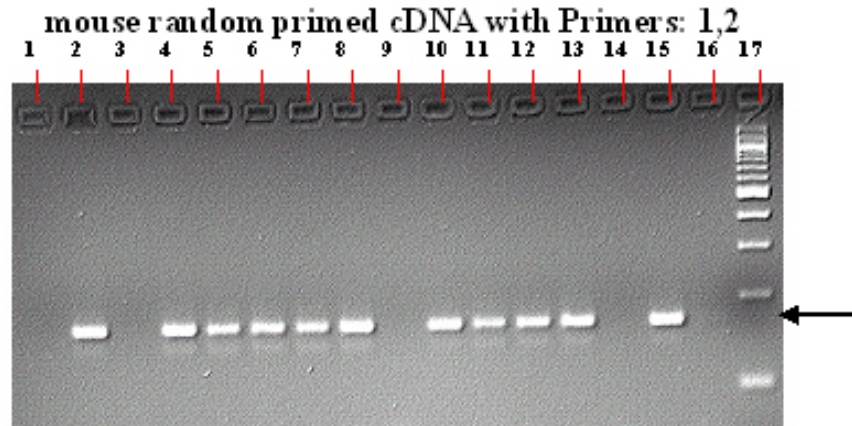


QC Image

Accession: `NM_007658.1`



RT-PCR WT Expression

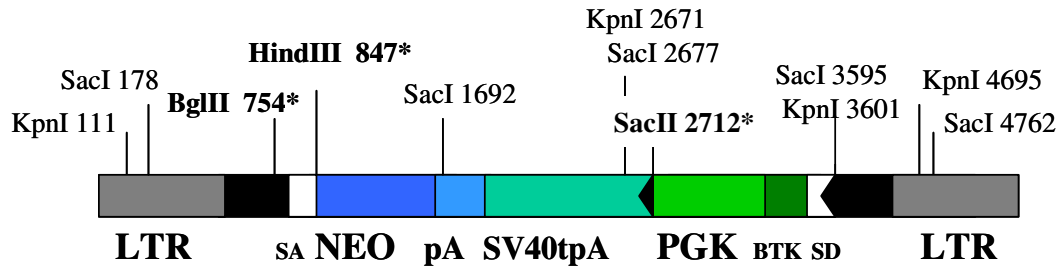


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

- 1) Brain
- 2) Thymus
- 3) Spleen
- 4) Lung
- 5) Kidney
- 6) Liver
- 7) Testis
- 8) Bone
- 9) Small Intestine & Colon
- 10) Skin Fibroblast
- 11) Heart
- 12) Adipose
- 13) Tail
- 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

```
TGAAAGACCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
CGGGTACCCGGGCGACTCAGTCAATCGGAGGACTGGCGCGCCGAGTGAGGG
GTTGTGGGCTCTTTTATTGAGCTCGGGGAGCAGAAGCGCGCGAACAGAAGCG
AGAAGCGAACTGATTGGTTAGTTCAAATAAGGCACAGGGTCATTTTCAGGTCC
TTGGGGCACCCCTGGAAACATCTGATGGTTCTCTAGAACTGCTGAGGGCTGG
ACCGCATCTGGGGACCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCT
TACCACAGATATCCTGTTTGGCCCATATTCAGCTGTTCCATCTGTTCTTGGCCC
TGAGCCGGGGCAGGAACTGCTTACCACAGATATCCTGTTTGGCCCATATTCA
GCTGTTCCATCTGTTTCTGACCTTGATCTGAACTTCTCTATTCTCAGTTATGTA
TTTTCCATGCCTTGCAAATGGCGTACTTAAGCTAGCTTGCCAAACCTACA
GGTGGGGTCTTTCATTCCCCCTTTTTCTGGAGACTAAATAAAATCTTTTATTT
TATCTATGGCTCGTACTCTATAGGCTTCAGCTGGTGATATTGTTGAGTCAAAA
CTAGAGCCTGGACCACTGATATCCTGTCTTTAACAAATTGGACTAATCGATAC
CGTCGATCGACCTCGACAGATCTTAAGCCAGTTTTTCGTACCCTTGACTGCGTT
```

TCATCGATTGCTACTAACATTGCCTTTTCCTCCTTCCCTCCCACAGGTGGAA
GAGCAAGCTTTGATGAGCCGCCACCATGGGATCGGCCATTGAACAAGATGGA
TTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTTCGGCTATGACTG
GGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCG
CAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGA
ACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCT
TGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTAT
TGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGA
GAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCG
GCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTA
CTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCA
GGGGCTCGCGCCAGCCGAACCTGTTCCGCCAGGCTCAAGGCGCGCATGCCCGAC
GGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGG
TGAAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCG
GATCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTG
GCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGAT
TCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCA
ATTCTCTAGAGCTCGGGAGGTAAGTGGAGCGGCCGCAATAAAATATCTTTATT
TTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGATAGTACTAACATACGC
TCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCC
AGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAGGCGGCCCTGCGACT
CTAGAGGATCTGCGACTCTAGAGGATCATAATCAGCCATAACCACATTTGTAG
AGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACAT
AAAATGAATGCAATTGTTGTTGTTAACTTGTTTGTGTTGCAGCTTATAATGGTTA
CAAATAAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGC
ATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCT
GCGACTCTAGAGGATCATAATCAGCCATAACCACATTTGTAGAGGTTTTACTTG
CTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGC
AATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCA
ATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGT
GGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTGCGACTCTAG
AGGATCATAATCAGCCATAACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAA
CCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTG
TTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACA
AATTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAA
ACTCATCAATGTATCTTATCATGTCTGGATCCCCGGGTACCGAGCTCGAAGGC
CGGCCGTTTAAACCAATCGAATTCCCGCGGCTAGACCCAGCTTTCGGAAGTT
CCTATTCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCTCGATATGGTC
GATCGACCTGCAGGAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAG
TCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTG
GCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGGCGCCAACCGGCTCC
GTTCTTTGGTGGCCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGT
TCCCCCGCCCCGAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGC
ACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGG
GTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGC
TCAGAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAG

GGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTT
CAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCCTCATCTCCGGGCCTTTC
GACCTGCAGGCGGCCGCGAATTCAGTACTAGTGCAGCGTACGGATCCGCCG
CCGCCATGGCTCCGGTAGGTCCAGAGTCTTCAGAGATCAAGTCCCACCTTCC
AAGTCCTGGCATCTCACGACGTCTGGGGAGCTACCTGCATTAAGTCAGAACT
GAGGTGGGTTTGGGCTGAGGTAGAGCCTGGGCAGAGGCCATAAATTACTTCTT
GTGGAACCTCTCAAAGGTCGGACAGGAAGCATGGCTGGTTCATATATCTACT
GCCTCGAATCGATGAATTCGAGCTCGGTACCCGGGGATCGAAGTTCCTATTC
GGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCTCGACCTGCAGGCATGC
AAGCTGGGGGGTTCGACGTCGAGAAGGAGTGAGGGCTGGATAAAGGGAGGA
TCGAGGCGGGGTCGAACGAGGAGGTTCAAGGGGGAGAGACGGGGCGGATGG
AGGAAGAGGAGGCGGAGGCTTAGGGTGTACAAAGGGCTTGACCCAGGGAGG
GGGTCAAAGCCAAGGCTTCCCAGGTCACGATGTAGGGGACCTGGTCTGGG
TGTCCATGCGGGCCAGGTGAAAAGACCTTGATCTTAACCTGGGTGATGAGGT
CTCGGTAAAGGTGCCGTCTCGCGGCCATCCGACGTTAAAGGTTGGCCATTCT
GCAGAGCAGAAGGTAACCCAACGTCTTCTTGTGACATCTACCGACTGGTTGT
GAGCGATCCGCTCGACATCTTCCAGTGACCTAAGGTCAAACCTAAGGGAGT
GGTAACAGTCTGGCCCATATTTTCAGACAAATACAGAAACACAGTCAGACAG
AGACAACACAGAACGATGCTGCAGCAGACAAGACGCGCGGCGCGGCTTCGG
TCCCAAACCGAAAGCAAAAATTCAGACGGAGGCGGGAACCTGTTTTAGGTTCT
CGTCTCCTACCAGAACCACATATCCCTCCTCTAAGGGGGGTGCACCAAAGAG
TCCAAAACGATCGGGATTTTTGGACTCAGGTCCGGGCCACAAAACGGCCCC
GAAGTCCCTGGGACGTCTCCAGGGTTGCGGCCGGGTGTTCCGAACTCGTCA
GTTCCACCACGGGTCCGCCAGATACAGAGCTAGTTAGCTAACTAGTACCGAC
GCAGGCGCATAAAATCAGTCATAGACACTAGACAATCGGACAGACACAGAT
AAGTTGCTGGCCAGCTTACCTCCCGGTGGTGGGTCCGTGGTCCCTGGGCAGG
GGTCTCCCGATCCCGGACGAGCCCCAAATGAAAGACCCCCGCTGACGGGTA
GTCAATCACTCAGAGGAGACCCTCCCAAGGAACAGCGAGACCACAAGTCGG
ATGCAACTGCAAGAGGGTTTATTGGATACACGGGTACCCGGGCGACTCAGTC
AATCGGAGGACTGGCGCGCCGAGTGAGGGGTTGTGGGCTCTTTTATTGAGCT
CGGGGAGCAGAAGCGCGCGAACAGAAGCGAGAAGCGAACTGATTGGTTAGT
TCAAATAAGGCACAGGGTCATTTTCAGGTCCTTGGGGCACCTGGAAACATCT
GATGGTTCTCTAGAAACTGCTGAGGGCTGGACCGCATCTGGGGACCATCTGT
TCTTGGCCCTGAGCCGGGGCAGGAACTGCTTACCACAGATATCCTGTTTGGCC
CATATTCAGCTGTTCCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCTT
ACCACAGATATCCTGTTTGGCCCATATTCAGCTGTTCCATCTGTTCCCTGACCTT
GATCTGAACTTCTCTATTCTCAGTTATGTATTTTTCCATGCCTTGCAAAATGGC
GTTACTTAAGCTAGCTTGCCAAACCTACAGGTGGGGTCTTTCA