NIH-0818 Genotyping Strategies

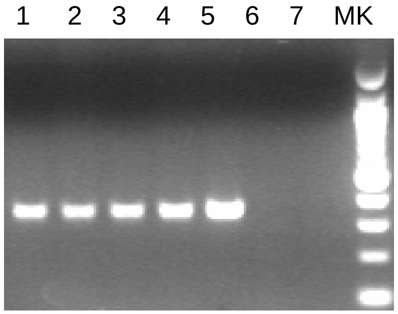
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl2	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

Step	Temp	Time	Note
1	96C	17"	
2	63C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	57C	15"	
6	72C	15"	Go to 4, 29 cycles

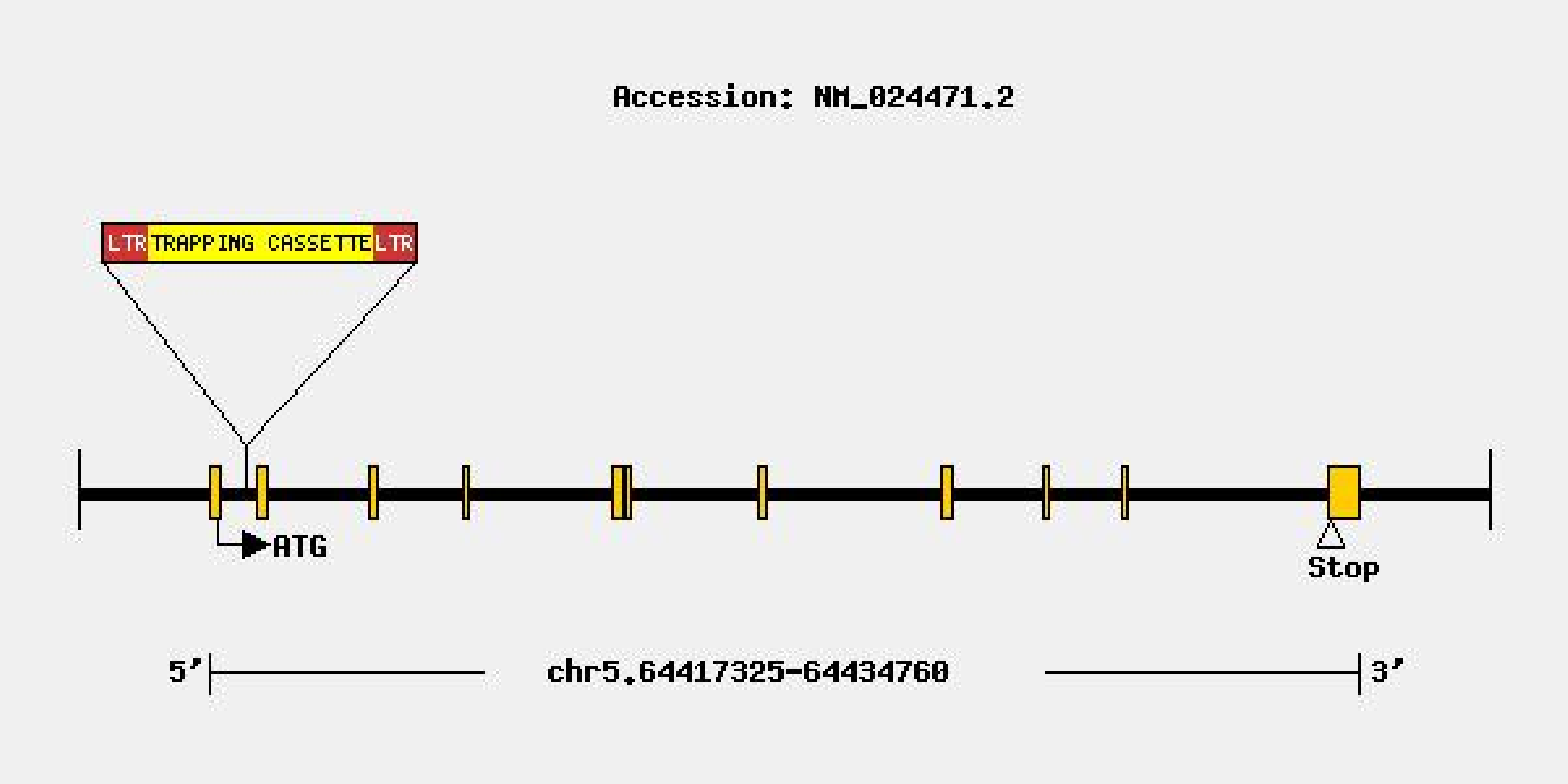
Primer Sequences (5’ to 3’):	
Mutant PCR: Primer LTR-2 and Primer 0818-lower, 353 bp	
Recommended Wt PCR: Primer 0818-5' and Primer 0818-lower, 351 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0818-5'	AATCCGATCATCCAGGAGTCAGAGG
Primer 0818-lower	TGCTGCCTCGGCCTCTACCT

Well	Sample	Genotype
1	44	het
2	48	het
3	49	het
4	51	het
5	**ES DNA**	het
6	**wt lysate**	wt
7	**water**	no amp

Mutant PCR

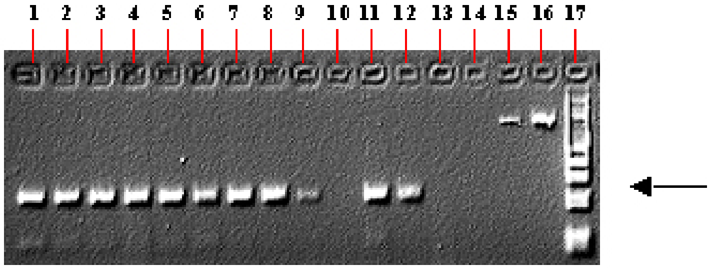


QC Image


Accession: NM_024471.2
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr5.64417325-64434760
3'

RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



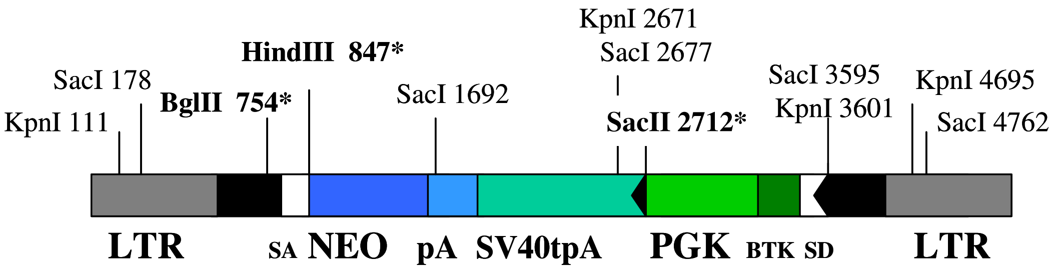
01/22/2005

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) Kidney
8) Liver
9) Skeletal Muscle
10) Bone
11) Stomach, Small Intestine & Colon
12) Heart
13) Adipose
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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