NIH-0687 Genotyping Strategies

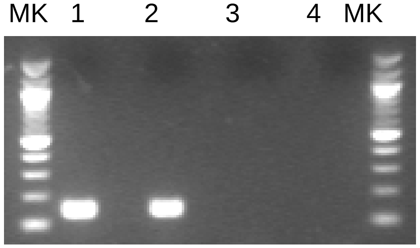
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

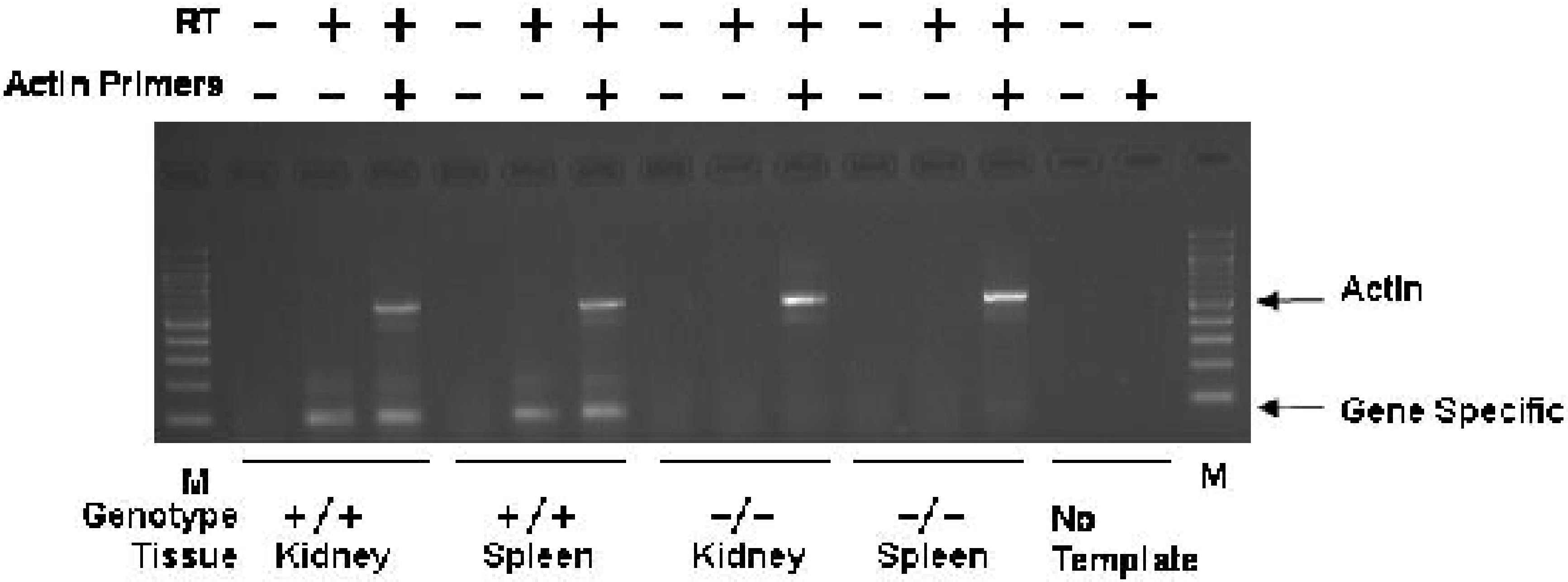
Primer Sequences (5’ to 3’):	
Mutant PCR: Primer 0687-5' and Primer LTR-rev, 149 bp	
Recommended Wt PCR: Primer 0687-5' and Primer 0687-3', 131 bp	
Primer 0687-5'	CACATGCGATCATTCCATAACTGG
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 0687-3'	CTTCCTCTGTTCTCAGATGTTTGG

Well	Sample	Genotype
1	232	het
2	**ES DNA**	het
3	**wt lysate**	wt
4	**water**	no amp

Mutant PCR



QC Expression

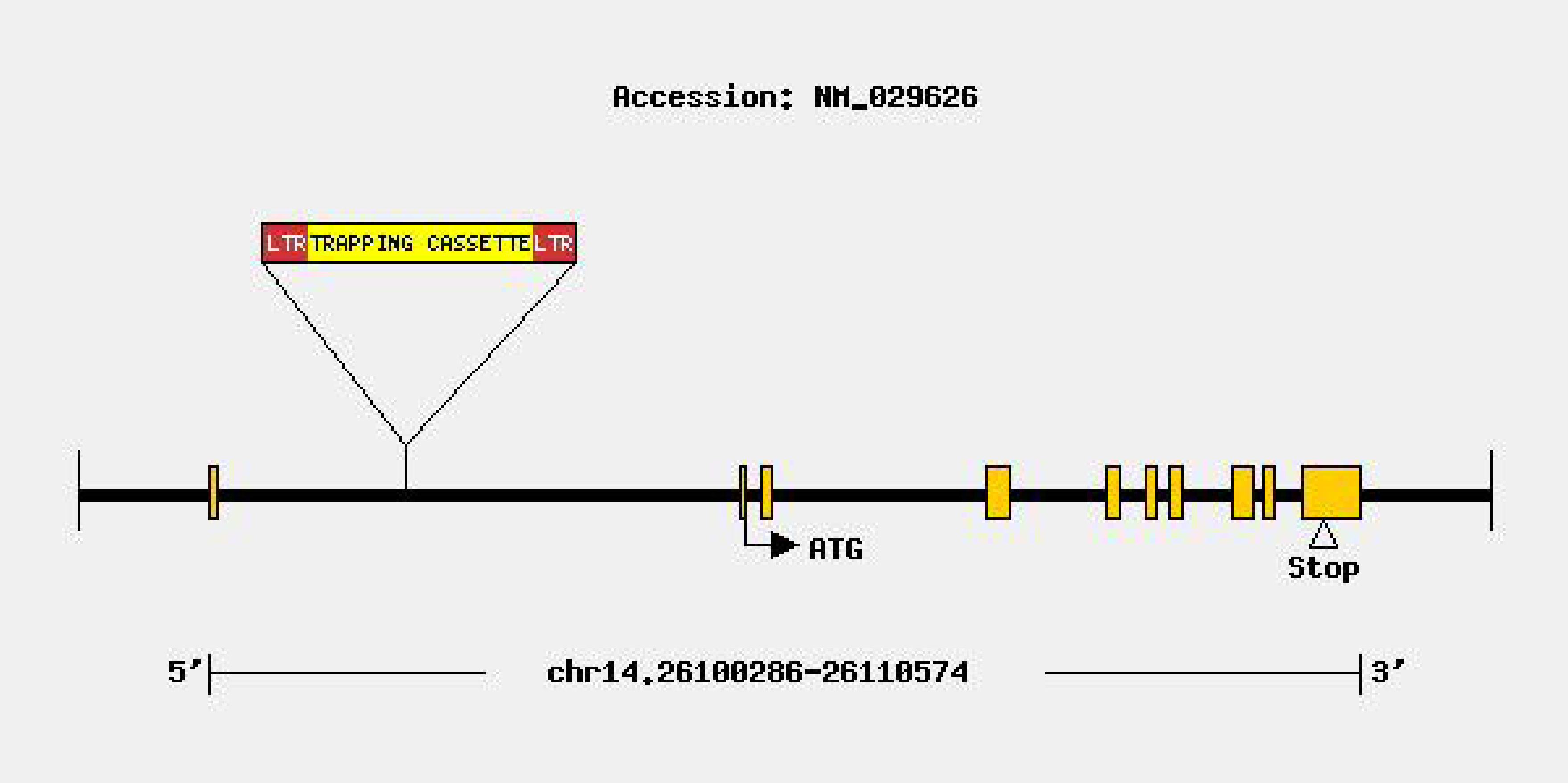


PCR 35 cycles

Mouse ID: 63

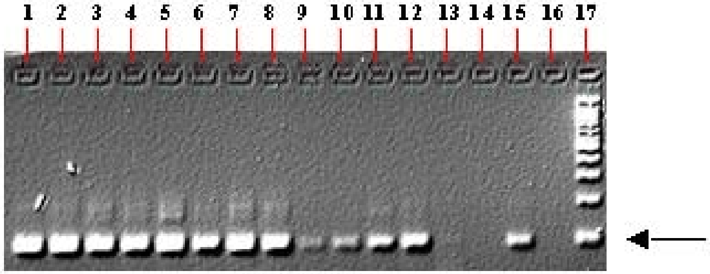
Primers: 1&2

QC Image


Accession: NM_029626
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr14,26100286-26110574
3'

RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



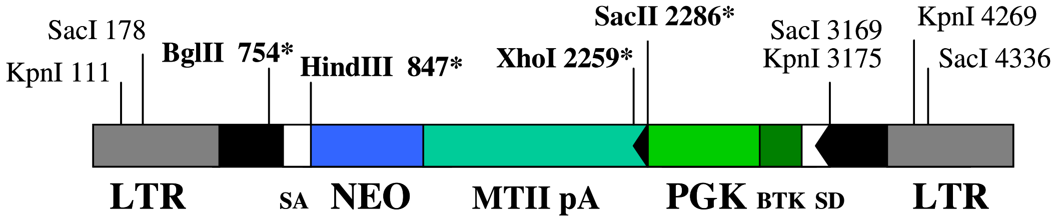
05/27/2004

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 MTII Omnibank Vector



Total Size: 4748 nucleotides
Non-Cutters: ApaI, BclI
* Unique sites

Location of components in VICTR 48 MTII:
LTR (viral long terminal repeat): 1-590, 4159-4748
SA (splice acceptor): 755-847
NEO: 867-1684
MTII pA: 1688-2217
frt sites: 2305-2352; 3185-3232
PGK promoter: 2377-2893
BTK exon: 2928-3152

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>VICTR 48MTII
TGAAAGACCCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
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