

NIH-0671 Genotyping Strategies

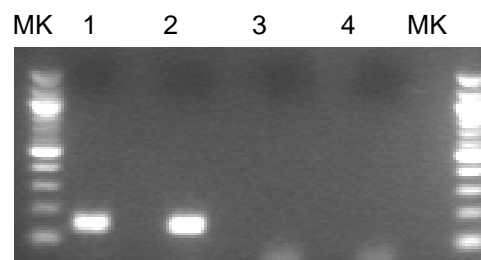
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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 0671-3', 155 bp	
Recommended Wt PCR: Primer 0671-5' and Primer 0671-3', 168 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0671-3'	TTCCTATTTTCCCCTTTCTCTTTGC
Primer 0671-5'	ACAGTTTCTGCTTTGAAGGCACGC

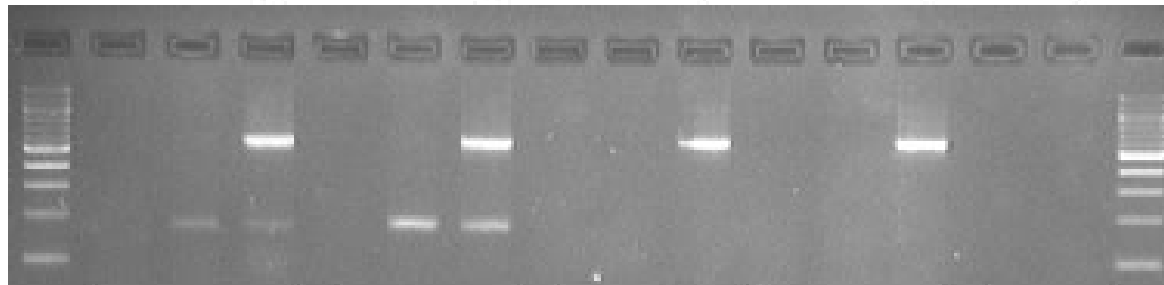
Well	Sample	Genotype
1	277	het
2	ES DNA	het
3	wt lysate	wt
4	water	no amp

Mutant PCR



QC Expression

RT	-	+	+	-	+	+	-	+	+	-	+	+	-	-
Actin Primers	-	-	+	-	-	+	-	-	+	-	-	+	-	+



← Actin
← Gene Specific

M									M
Genotype	+/+	+/+	-/-	-/-	No				
Tissue	Brain	Thymus	Brain	Thymus	Template				

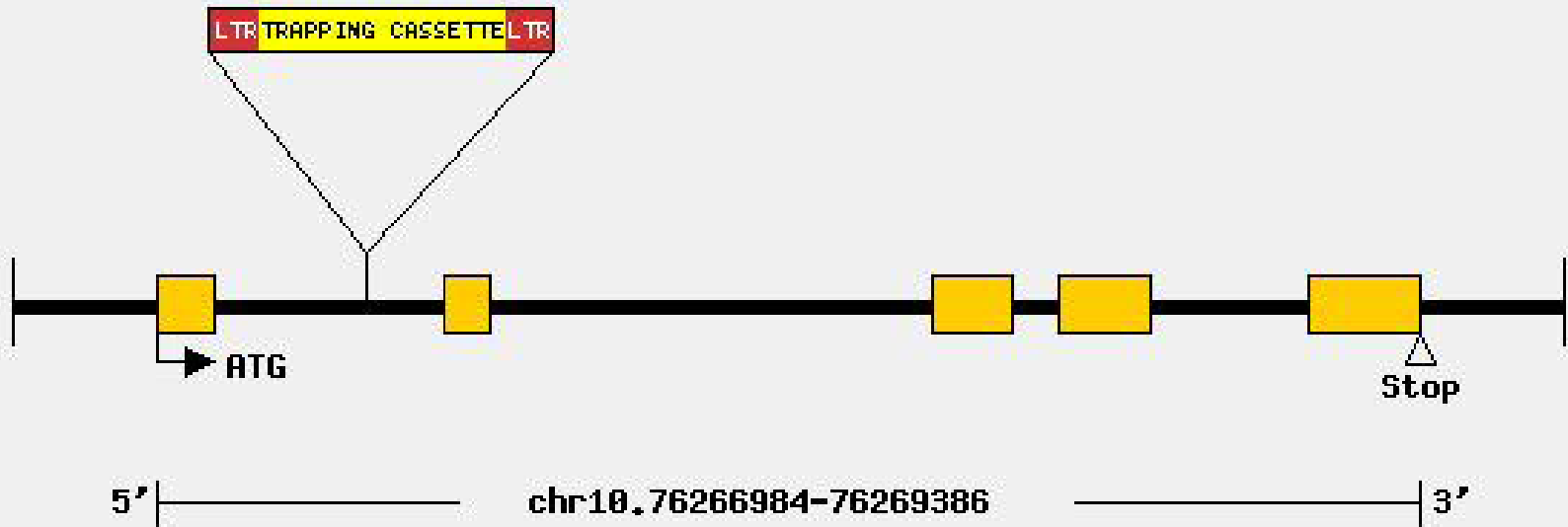
PCR 30 cycles

Mouse ID: 175

Primers: 1&2

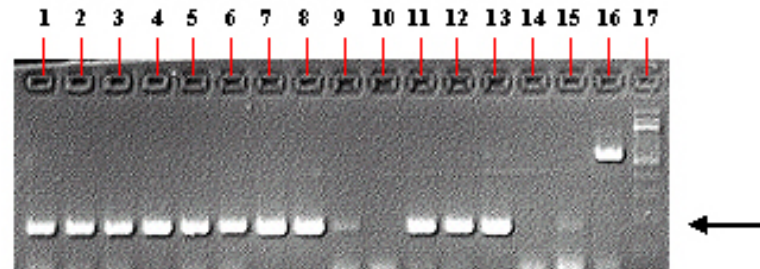
QC Image

Accession: NM_010361



RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



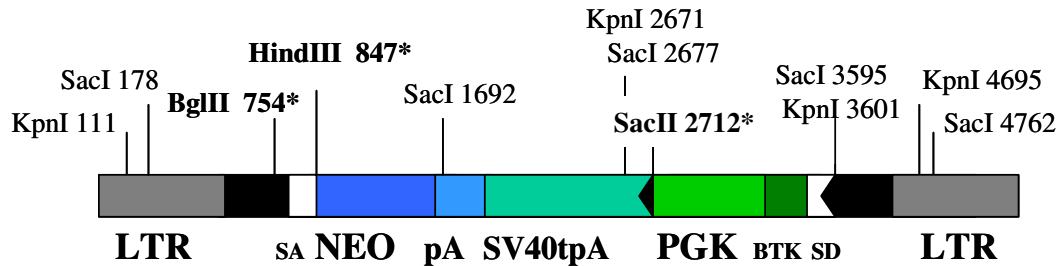
02/19/2004

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 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

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TGAAAGACCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
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