

NIH-0402 Genotyping Strategies

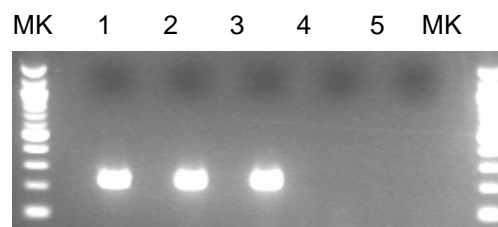
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

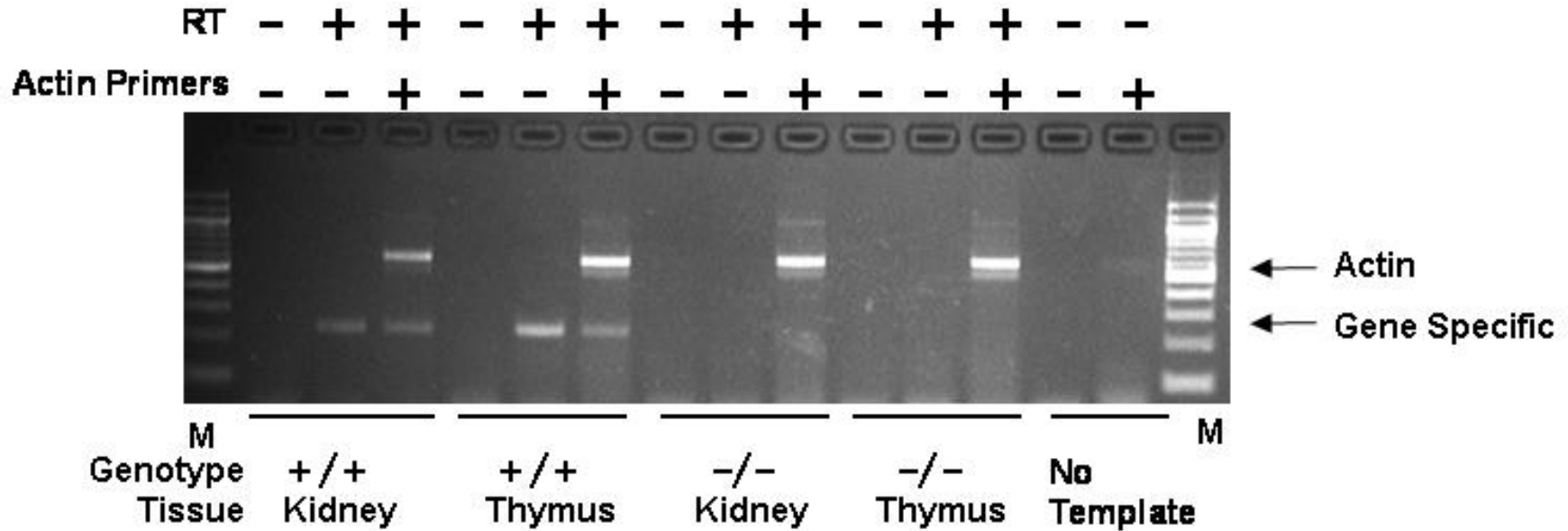
Primer Sequences (5' to 3')	
Mutant PCR: Primer LTR-2 and Primer 0402-3', 224 bp	
Recommended Wt PCR: Primer 0402-5' and Primer 0402-3', 272 bp	
Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC
Primer 0402-3'	ATGAGGAAATGGCCTAAGGACACC
Primer 0402-5'	AACTACCAAGGAAAATAGTAGCAACC

Well	Sample	Genotype
1	229	het
2	232	het
3	ES DNA	het
4	wt lysate	wt
5	water	no amp

Mutant PCR



QC Expression



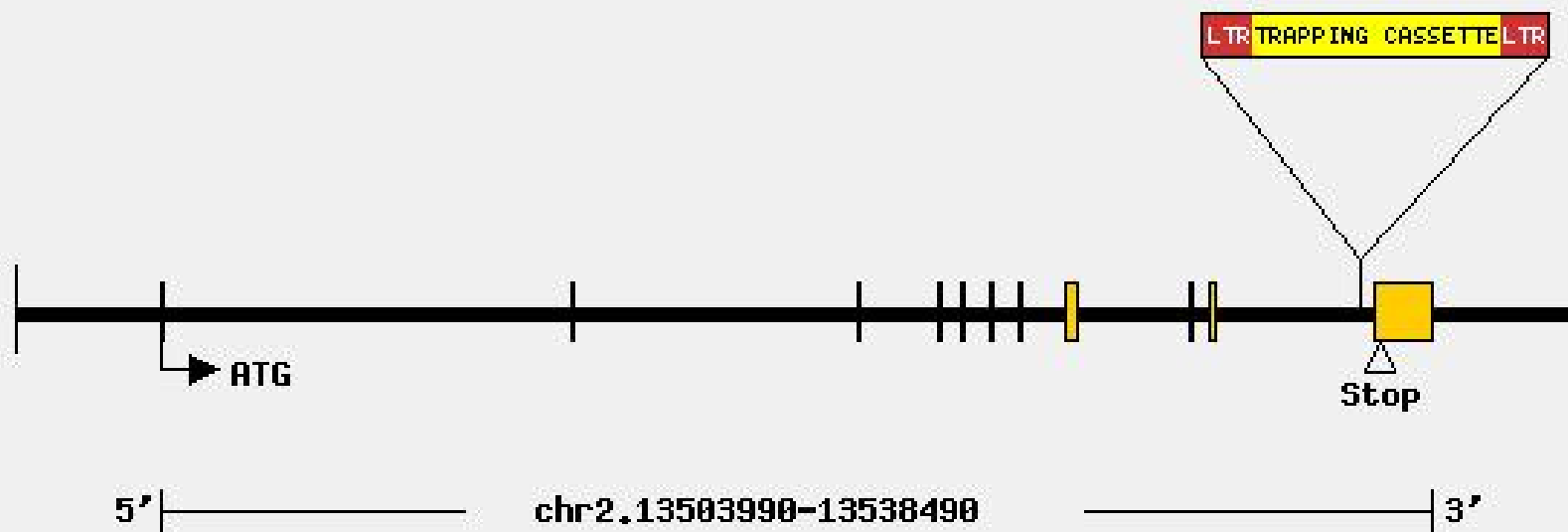
PCR 40 cycles

Mouse ID: 103

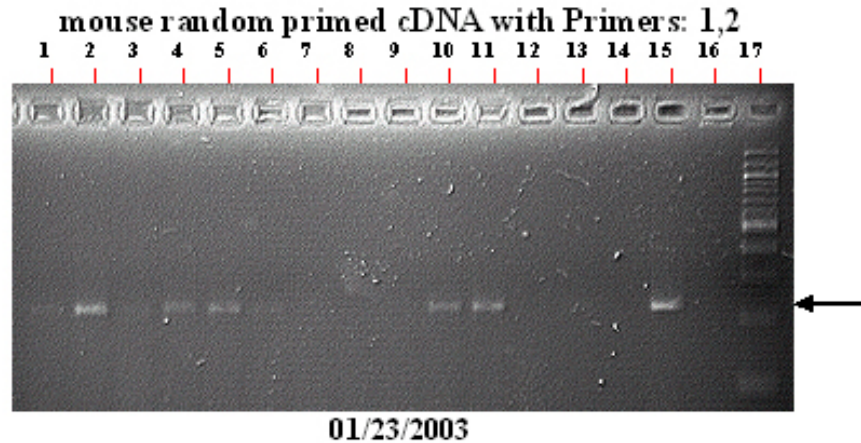
Primers: 1&2

QC Image

Accession: NM_010067.2



RT-PCR WT Expression

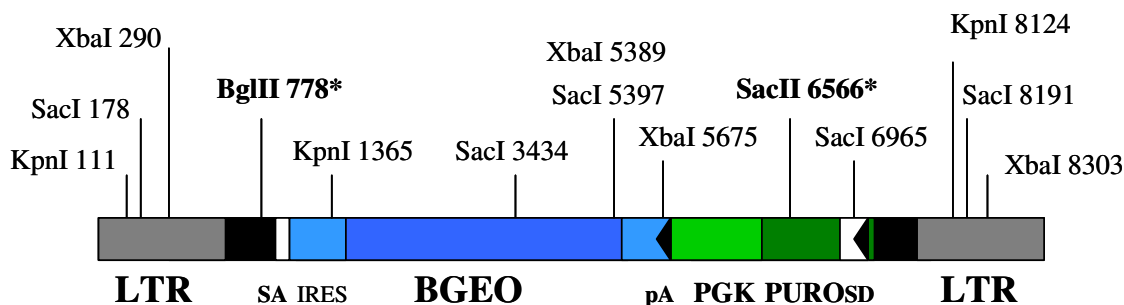


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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) Blood
- 14) (-) Control
- 15) (+) Control- ES cell cDNA
- 16) (+) Control- Genomic/Lex1 DNA
- 17) 100 bp ladder/marker

VICTR 23 Omnibank Vector



Total Size: 8603 nucleotides

Non-Cutters: NotI, NsiI, DraI

* Unique sites

Location of components in VICTR 23:

LTR (viral long terminal repeat): 1-590, 8014-8603

SA (splice acceptor): 783-912

IRES: 909-1493

Bgeo: 1495-5389

pA: 5394-5679

lox p sites: 5701-5776, 7019-7094

PGK promoter: 5785-6329

PURO SD: 6352-6998

>VICTR 23

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