

## NIH-0920 Genotyping Strategies

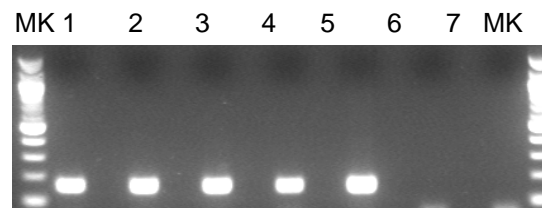
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl <sub>2</sub>	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

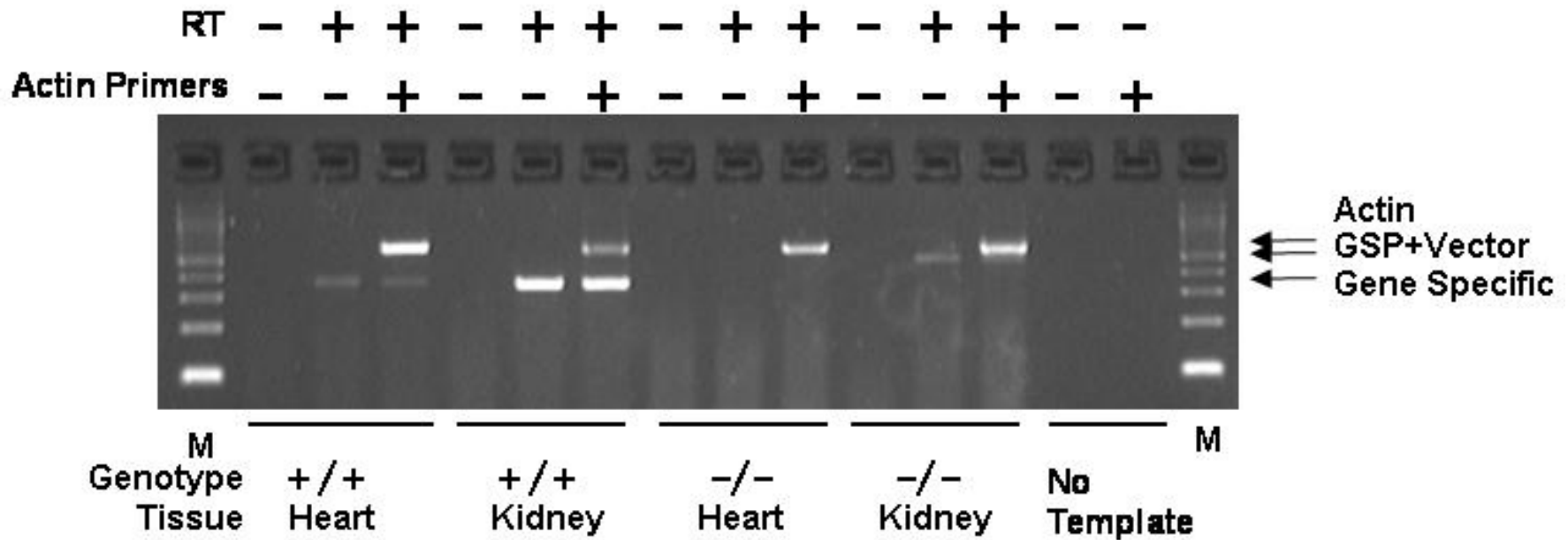
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 0920-5' and Primer LTR-rev, 163 bp	
Recommended Wt PCR: Primer 0920-5' and Primer 0920-3', 115 bp	
Primer 0920-5'	GGCATATATGTGCAGGCAGAATGC
Primer LTR-rev	ATAAACCCCTCTTGCAGTTGCATC
Primer 0920-3'	TCTAGAAATGAGTCTGAACCGGAC

Well	Sample	Genotype
1	228	het
2	235	het
3	236	het
4	239	het
5	<b>ES DNA</b>	het
6	<b>wt lysate</b>	wt
7	<b>water</b>	no amp

### Mutant PCR



# QC Expression



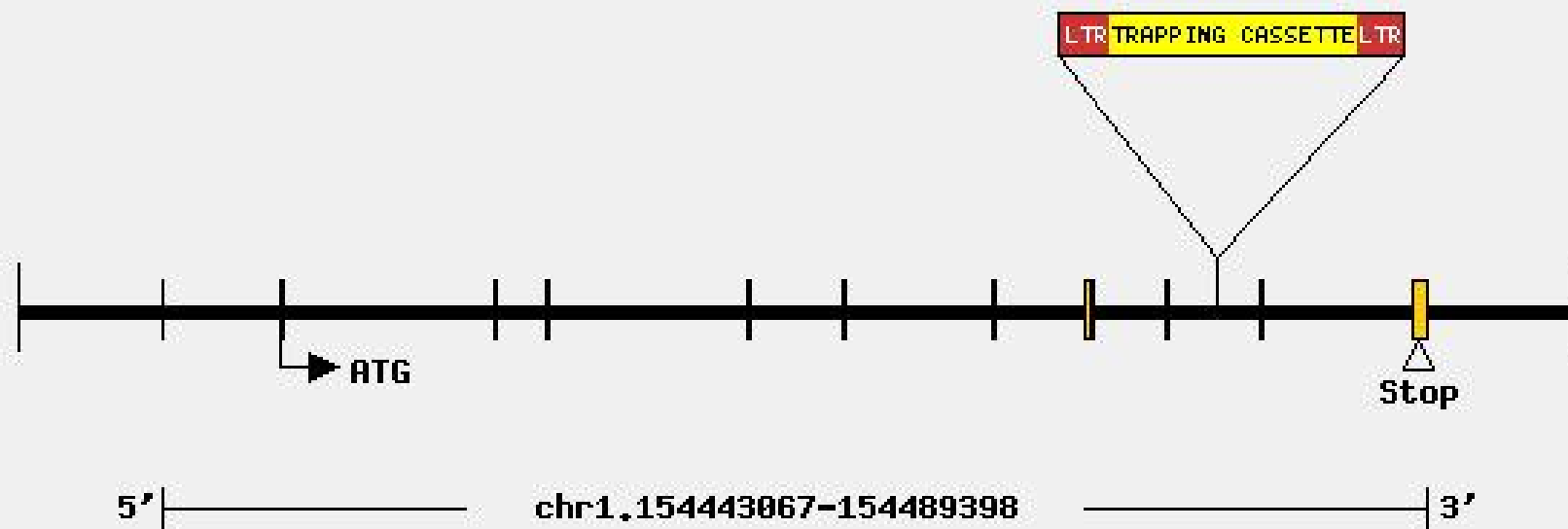
PCR 35 cycles

Mouse ID: 135

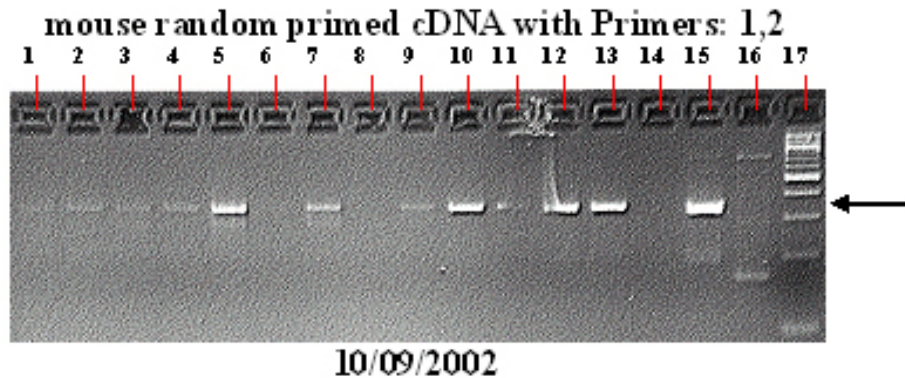
Primers: 1&2

# QC Image

Accession: NM\_028749



# RT-PCR WT Expression

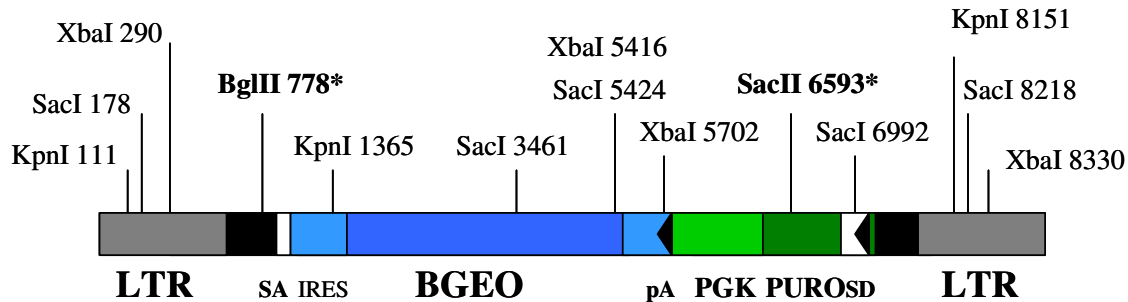


**Note:** Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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

# VICTR 22 Omnibank Vector



**Total Size:** 8630 nucleotides

**Non-Cutters:**

\* Unique sites

## Location of components in VICTR 22:

LTR (viral long terminal repeat): 1-590, 8014-8630

SA (splice acceptor): 783-912

IRES: 909-1493

Bgeo: 1522-5416

pA: 5421-5706

lox p sites: 5726-5796, 7045-7121

PGK promoter: 5840-6356

PURO SD: 6379-7026

>VICTR 22

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