

NIH-0001 Genotyping Strategies

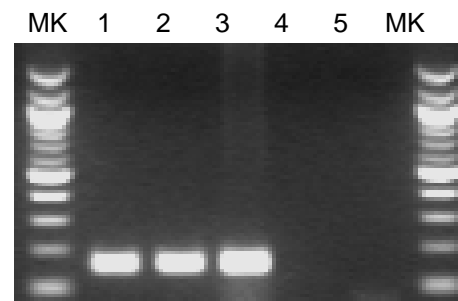
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
10 mM dNTPs	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

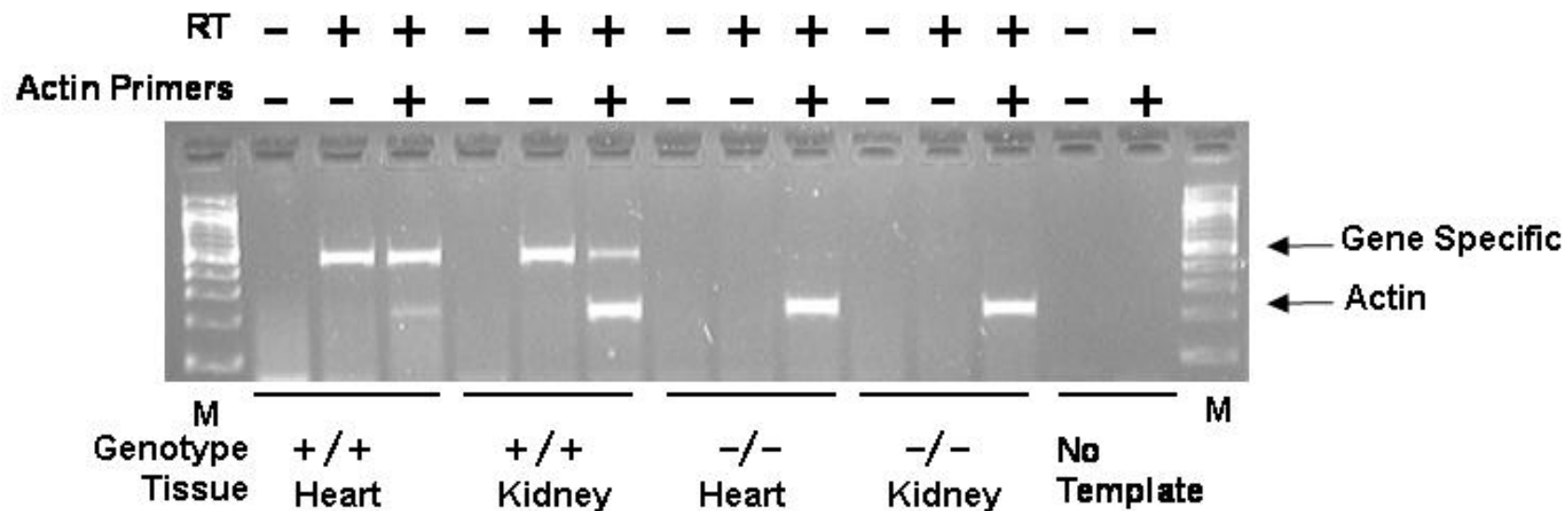
Primer Sequences (5' to 3')	
Mutant PCR: Primer 0001-5' and Primer LTR-rev, 166 bp	
Recommended Wt PCR: Primer 0001-5' and Primer 0001-3', 138 bp	
Primer 0001-5'	CAGTTCTGAAGGCTGAAAGTCTCC
Primer LTR-rev	ATAAACCCCTCTTGCGATTGCATC
Primer 0001-3'	AAGGATGTCCACATGTGGGAAAGG

Well	Sample	Genotype
1	165	het
2	176	het
4	ES DNA	het
5	wt lysate	wt
6	water	no amp

Mutant PCR



QC Expression

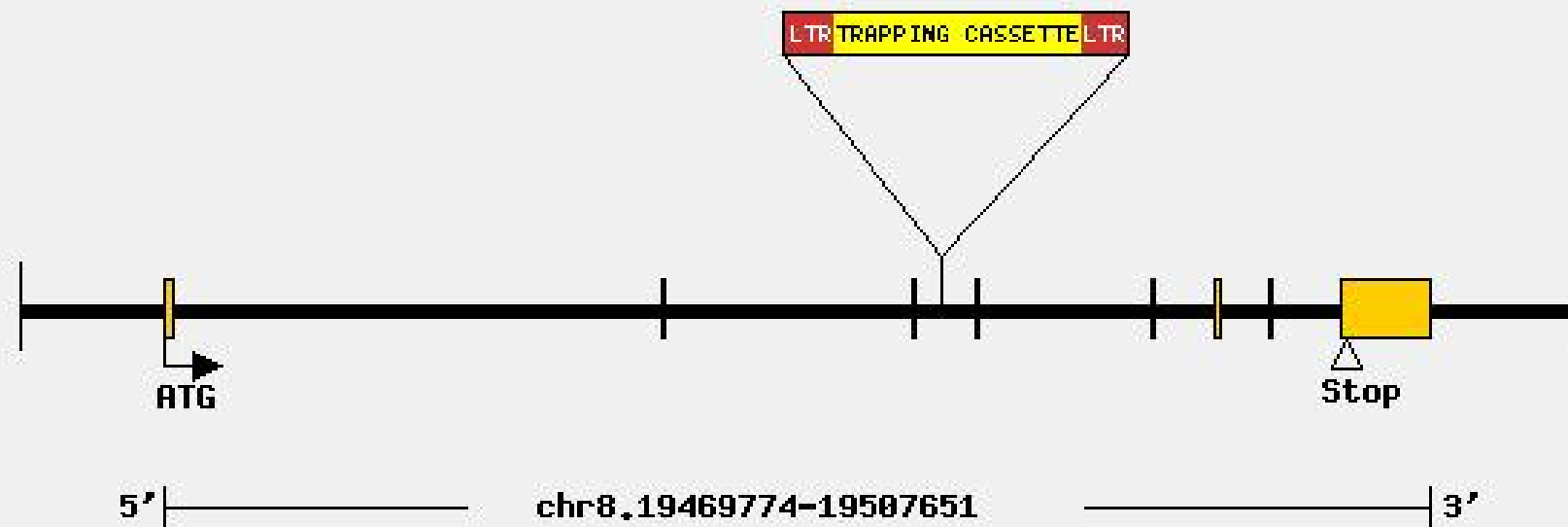


PCR 35 cycles
Primers: 1&2

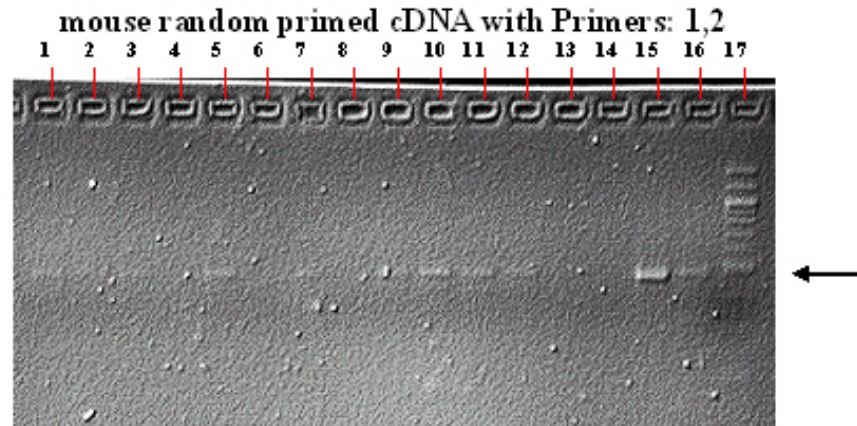
Mouse ID: 95

QC Image

Accession: **NM_026792.2**



RT-PCR WT Expression

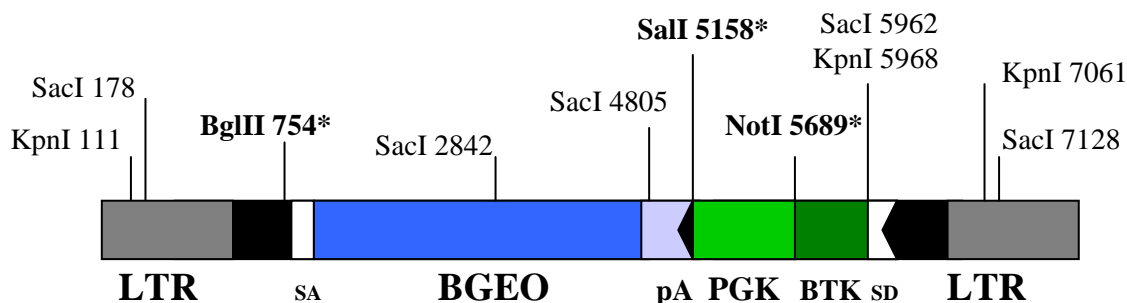


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

- 1) Brain
- 2) Thymus
- 3) Spleen
- 4) Lung
- 5) Kidney
- 6) Liver
- 7) Testis
- 8) Stomach
- 9) Small Intestine & Colon
- 10) Skin Fibroblast
- 11) Heart
- 12) Adipose
- 13) Tail
- 14) (-) Control
- 15) (+) Control- ES cell cDNA
- 16) (+) Control- Genomic/Lex1 DNA
- 17) 100 bp ladder/marker

VICTR40 Omnibank Vector



Total Size: 7,540 nucleotides

Non-Cutters: ApaI, SacII, XhoI, XmnI

* Unique sites

◀ Frt

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