

NIH-1038 Genotyping Strategies

Reaction Components	Vol (ul)
5X GoTaq Buffer	10.0
25mM MgCl ₂	3.5
10mM dNTPs	1.0
Primer 20 uM	1.0
Primer 20 uM	1.0
5 U/ul Taq polymerase	0.5
Water	28.0
Tail lysate (1:20 dilution)	5.0
Total reaction volume	50.0

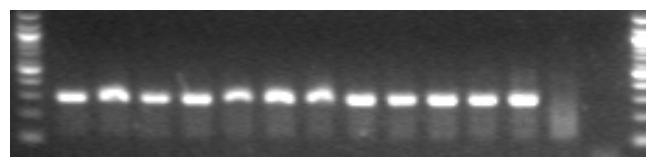
Step	Temp	Time	Note
1	94C	4'	
2	94C	15"	
3	65C	30"	Decrease 1C/cycle
4	72C	40"	Go to 2, 10 cycles
5	94C	15"	
6	55C	30"	
7	72C	40"	Go to 5, 30 cycles

Primer Sequences (5' to 3'):	
Mutant Oligos: 1038-5' and LTR-rev, 309 bp	
Recommended Wt Oligos: 1038-5' and 1038-3', 334 bp	
1038-5'	CTCACTTTGTAGACTAGGCTAGC
LTR-rev	ATAAACCCCTCTTGCAGTTGCATC
1038-3'	GACAGTAAAGCCAGGCCTTGTGC

Well	Sample	Genotype
1	1	het
2	8	het
3	23	het
4	24	het
5	26	het
6	28	het
7	32	het
8	38	het
9	39	het
10	40	het
11	41	het
12	es DNA	het
13	wt lysate	wt
14	water	no amp

Mutant PCR

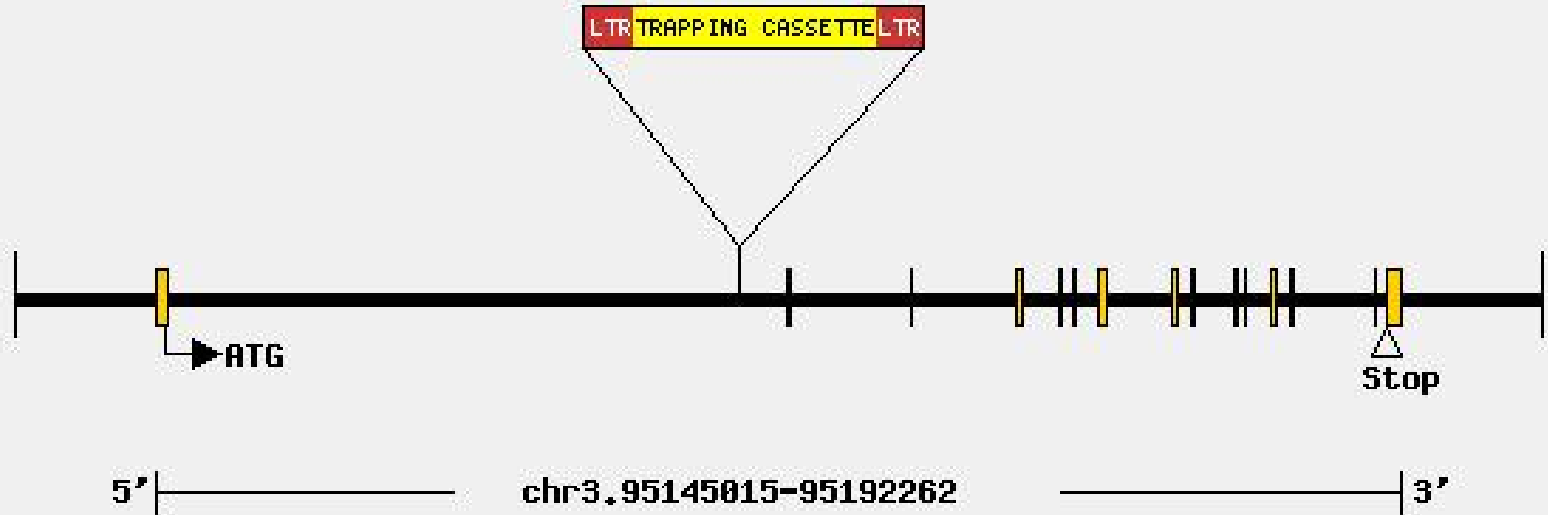
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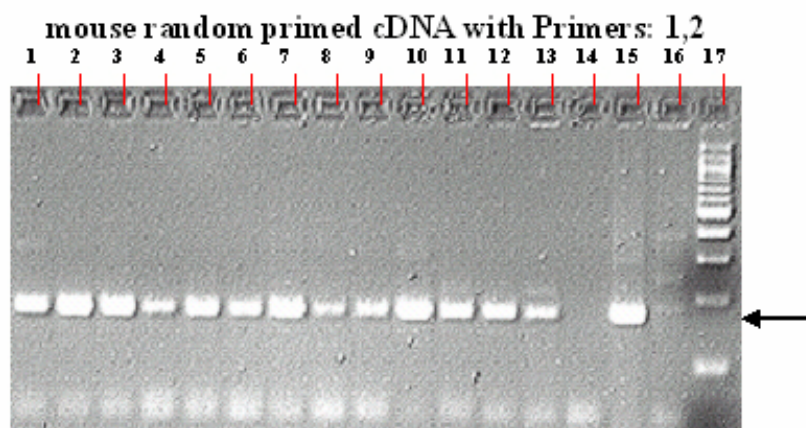
*2% Agarose, NEB 100 bp ladder

QC Image

Accession: NM_008847.2



RT-PCR WT Expression Analysis

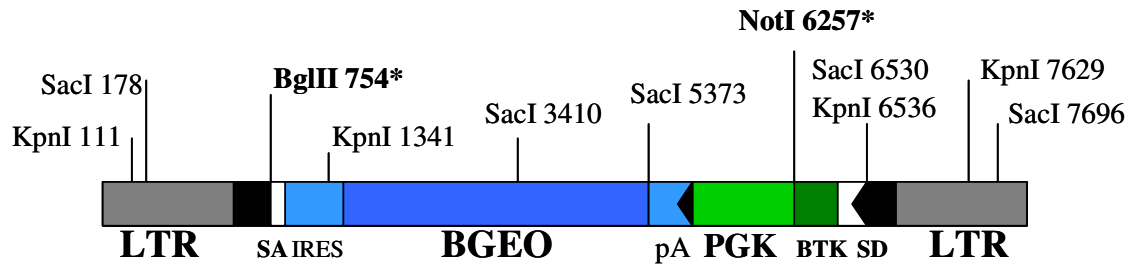


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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

VICTR 37 Omnibank Vector



Total Size: 8108 nucleotides

Non-Cutters: SacII, DraI, XhoI

* Unique sites

Location of components in VICTR 37:

LTR (viral long terminal repeat): 1-590, 7519-8108

SA (splice acceptor): 759-888

IRES: 885-1469

Bgeo: 1471-5365

pA: 5370-5655

frt sites: 5670-5717, 6546-6593

PGK promoter: 5738-6254

BTK exon: 6289-6514

>VICTR 37

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