

NIH-1197 Genotyping Strategies

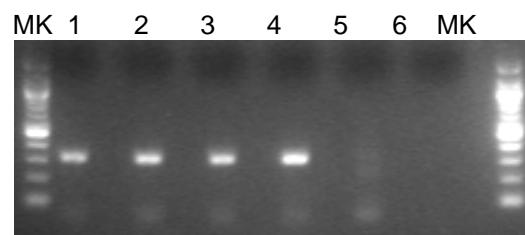
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl ₂	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

Step	Temp	Time	Note
1	96C	17"	
2	63C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	57C	15"	
6	72C	15"	Go to 4, 29 cycles

Primer Sequences (5' to 3'):	
Mutant PCR: Primer 1197-upper and Primer LTR-rev, 318 bp	
Recommended Wt PCR: Primer 1197-upper and Primer 1197-3', 314 bp	
Primer 1197-upper	GATCTTAAGTTCCAGCGAGACA
Primer LTR-rev	ATAAACCCCTCTTGCAAGTTGCATC
Primer 1197-3'	GCCTCACTGTGATATTACAGGTCC

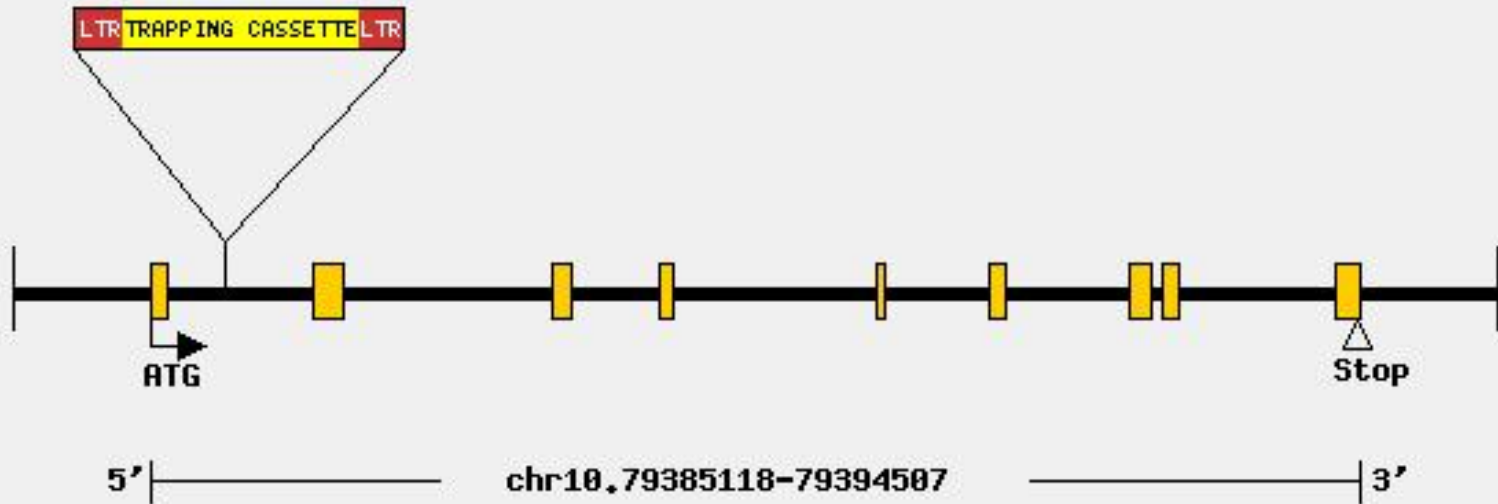
Well	Sample	Genotype
1	217	het
2	218	het
3	228	het
4	ES DNA	het
5	wt lysate	wt
6	water	no amp

Mutant PCR



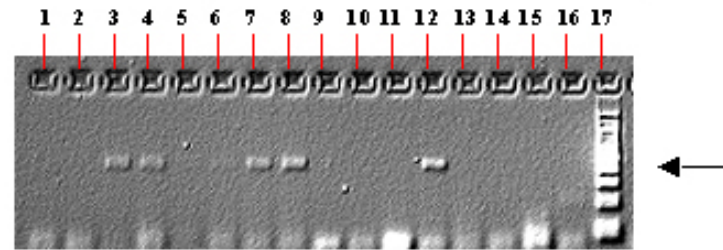
QC Image

Accession: NM_030262.2



RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



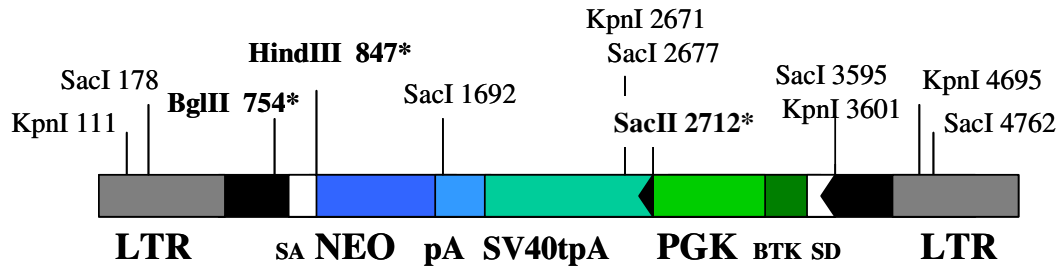
08/11/2004

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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