

NIH-0076 Genotyping Strategies

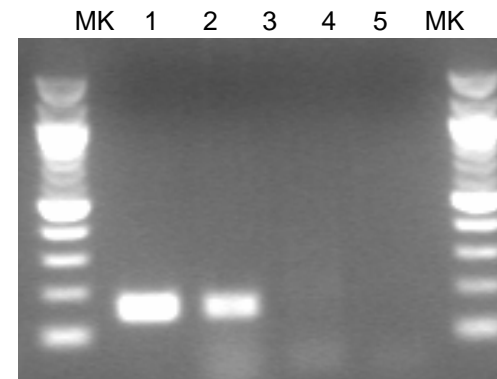
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

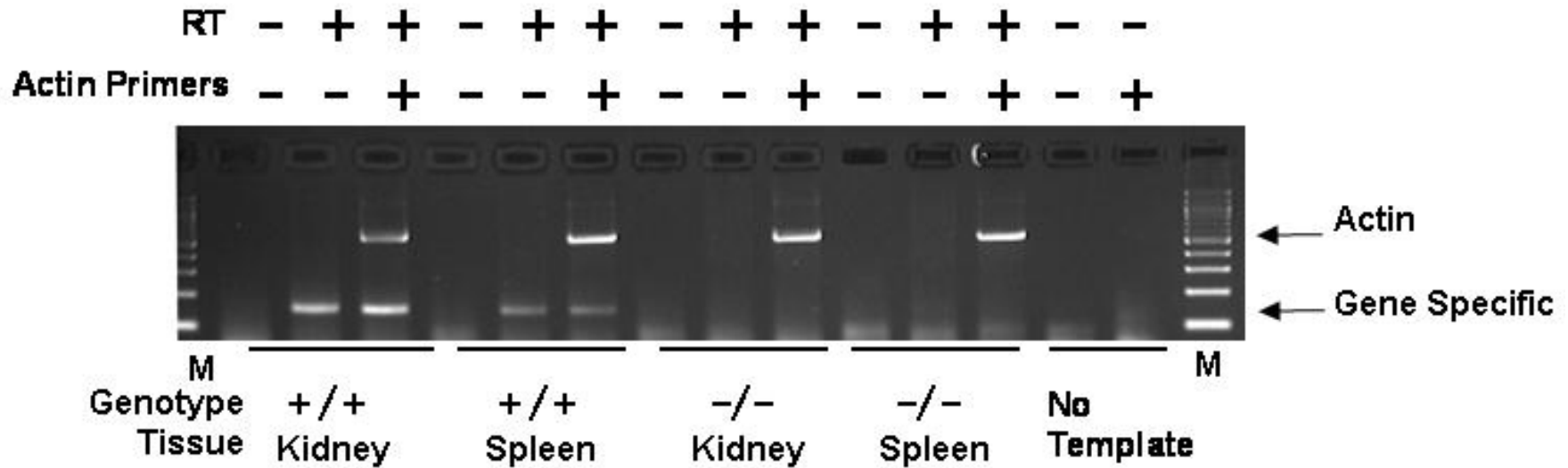
Primer Sequences (5' to 3')	
Mutant PCR: Primer 0076-5' and Primer LTR-rev, 153 bp	
Recommended Wt PCR: Primer 0076-5' and Primer 0076-3', 179 bp	
Primer 0076-5'	TGCCATGCCTCTACCCCATGAGC
Primer LTR-rev	ATAAACCCCTCTTGACGTTGCATC
Primer 0076-3'	TGACCCGTAATTGAGCGCTAAACC

Well	Sample	Genotype
1	219	Het
2	ES DNA	Het
3	wt lysate	wt
4	water	no amp

Mutant PCR



QC Expression



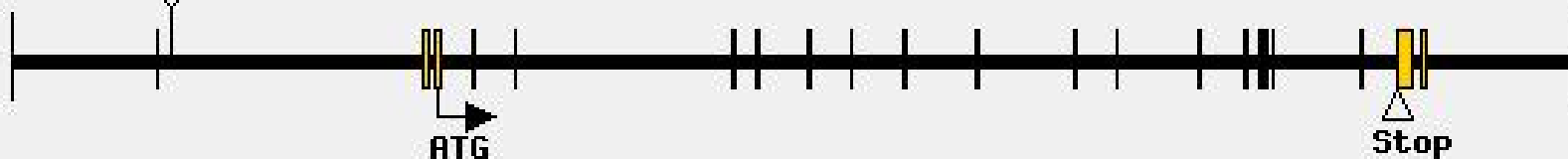
PCR 40 cycles
Primers: 1&2

Mouse ID: 113

QC Image

Accession: NM_138600

LTR TRAPPING CASSETTE LTR



5'

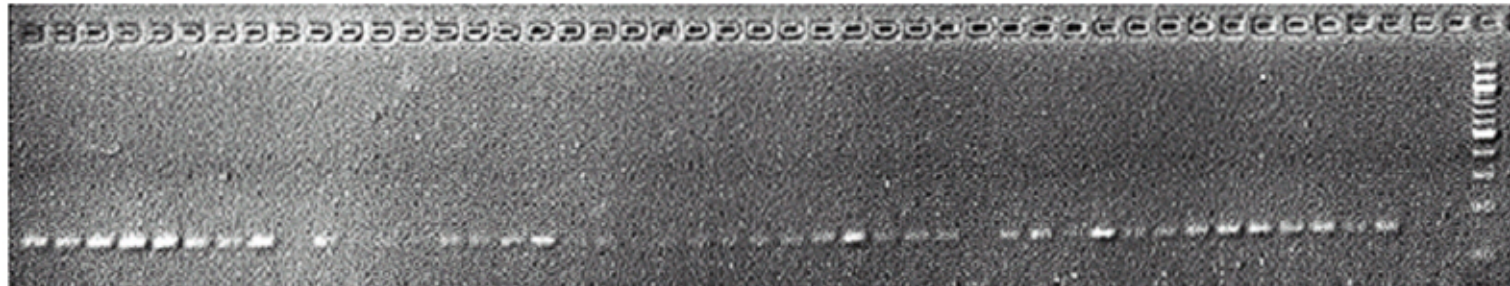
chr18.56895733-56942444

3'

RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2

1 2 3 4 5 6 7 8 9 10 11 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47



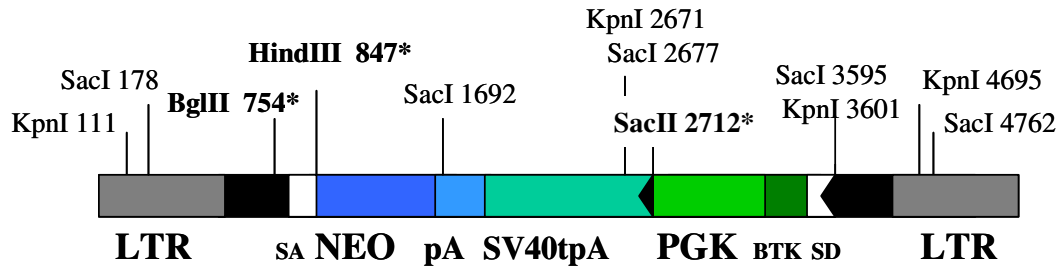
10/29/2002

Note: mouse ES cDNA used as positive (+) control. Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

1	Brain	19	Ovary	37	Mammary Gland
2	Thymus	20	Gall Bladder	38	Placenta
3	Spleen	21	Lymph node	39	9.5 day Embryo
4	Lung	22	Cerebellum	40	12.5 day Embryo
5	Kidney	23	Esophagus	41	Fetal Brain
6	Liver	24	Prostate	42	Fetal Lung
7	Testis	25	Tongue	43	Fetal Liver
8	Adrenal Gland	26	Thyroid	44	Fetal Kidney
9	Pancreas	27	Bone Marrow	45	(-) Control
10	Salivary Gland	28	Spinal Cord	46	(+) Control
11	Stomach	29	Trachea	47	100 bp ladder/marker
12	Small Intestine	30	Aorta		
13	Colon	31	Whole Blood		
14	Skeletal Muscle	32	Eye		
15	Heart	33	Pituitary gland		
16	Uterus	34	Skin		
17	Adipose	35	Nasal Epithelium		
18	Bladder	36	Whole Bone (femur)		

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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