

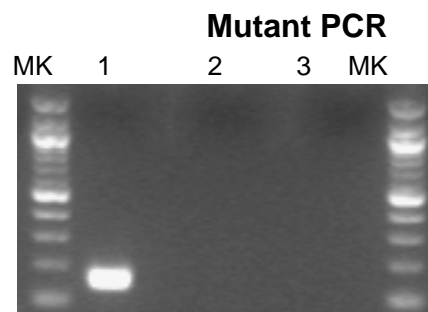
NIH-1010 Genotyping Strategies

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

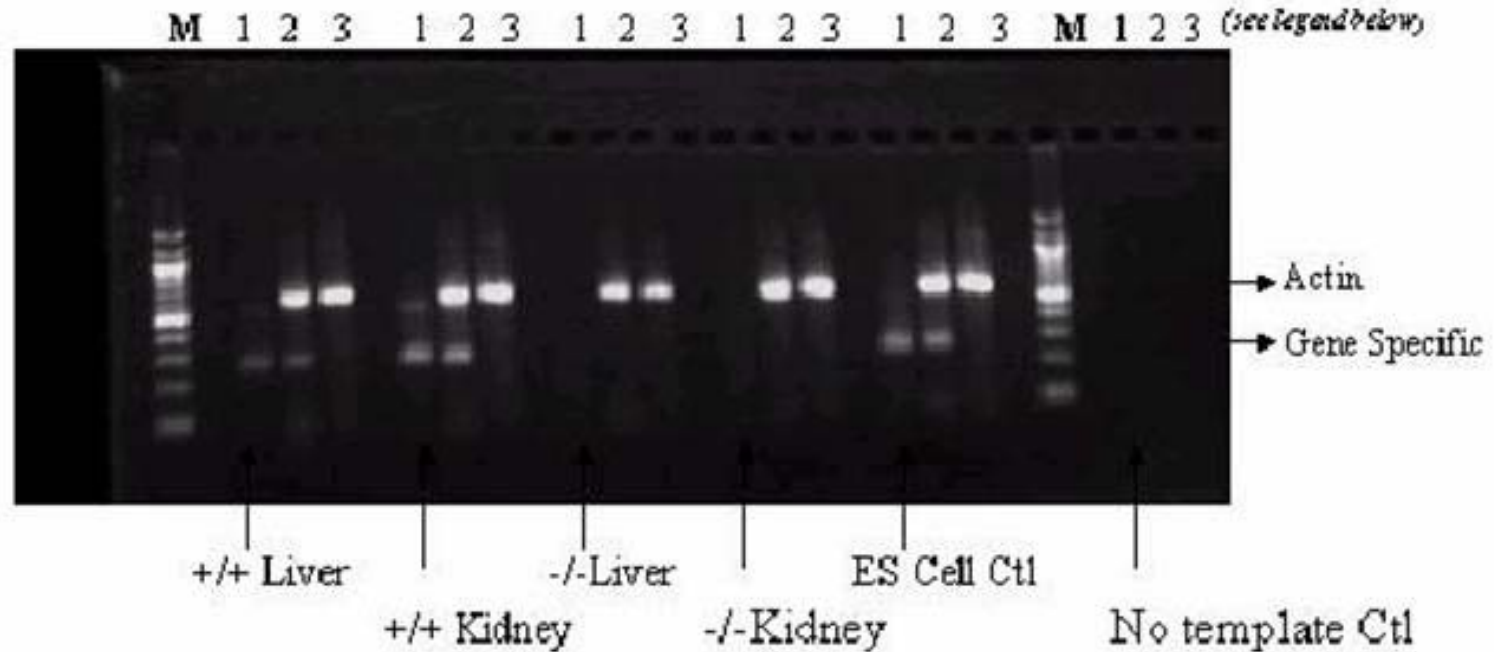
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer 1010-5' and Primer LTR-rev, 159 bp	
Recommended Wt PCR: Primer 1010-5' and Primer 1010-3', 134 bp	
Primer LTR-rev	ATAAACCCCTCTTGCAGTTGCATC
Primer 1010-5'	GGTGAATCTCTGTGAGTTTGAAGC
Primer 1010-3'	TTACAGACAGCCACTCACAAGACG

Well	Sample	Genotype
1	ES DNA	het
2	wt lysate	wt
3	water	no amp



QC Expression

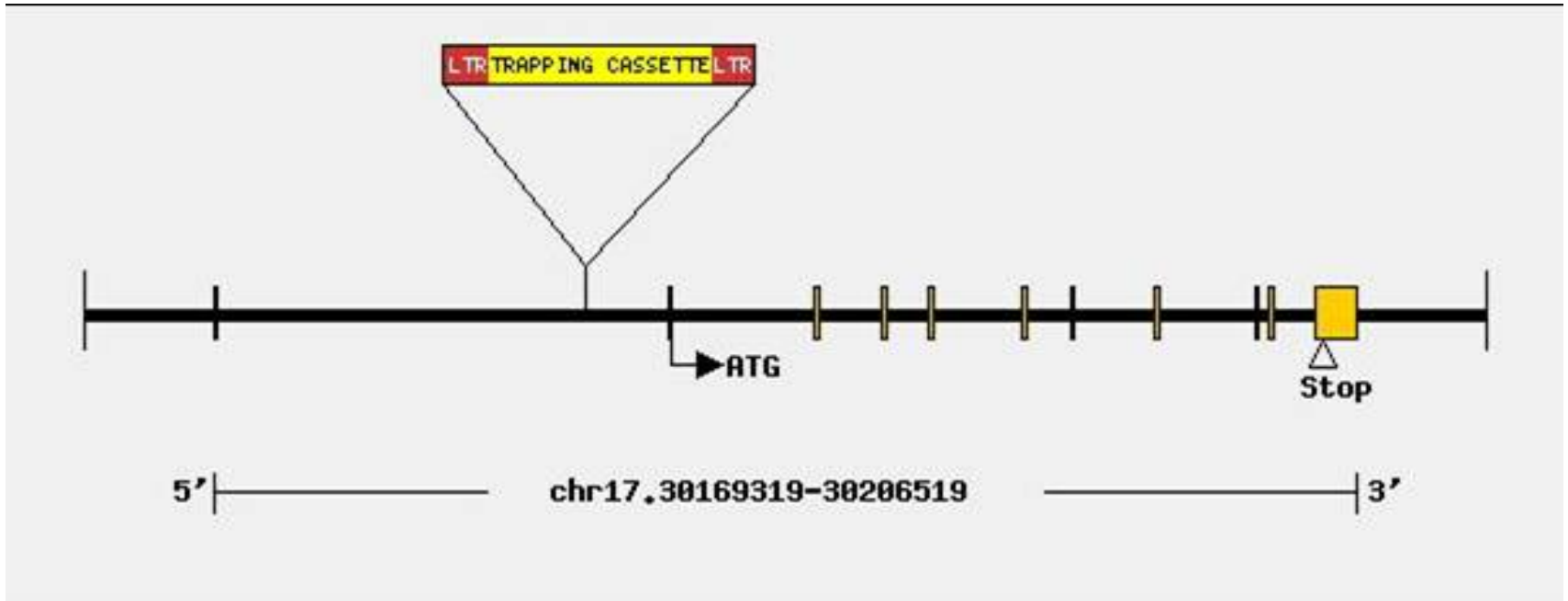


Legend for Gel Picture (per each RT template)

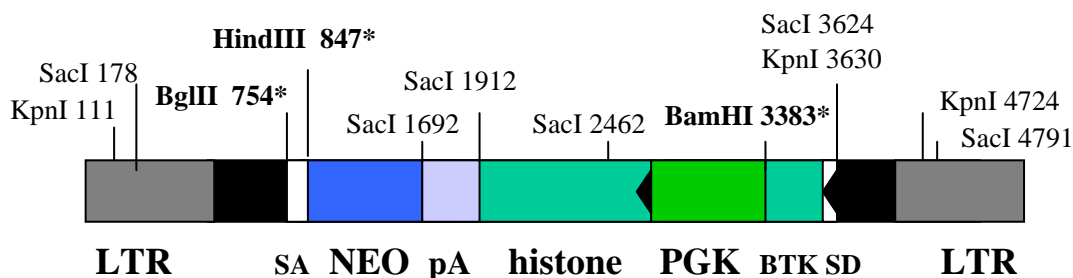
1: Gene Specific Primer pair 2: GSP (+ actin Ctl) 3: actin Ctl

Mouse ID # 83

QC Image



VICTR45 Omnibank Vector



Total Size: 5203 nucleotides

Non-Cutters: BclI, NdeI, SfiI XhoI

* Unique sites

Location of components in VICTR 45:

LTR (viral long terminal repeat): 1-590, 4614-5203

SA (splice acceptor): 755-847

NEO: 867-1684

pA: 1688-1874

pA (histone): 1875-2718

frt sites: 2760-2807, 3640-3687

PGK promoter: 2832-3348

BTK exon: 3383-3607

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