NIH-1075 Genotyping Strategies

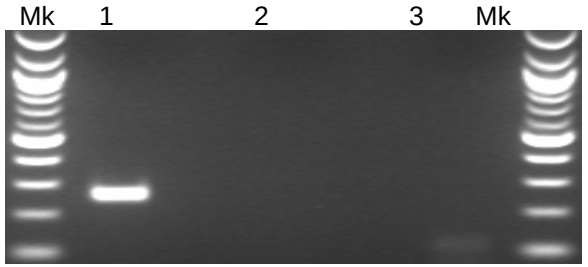
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

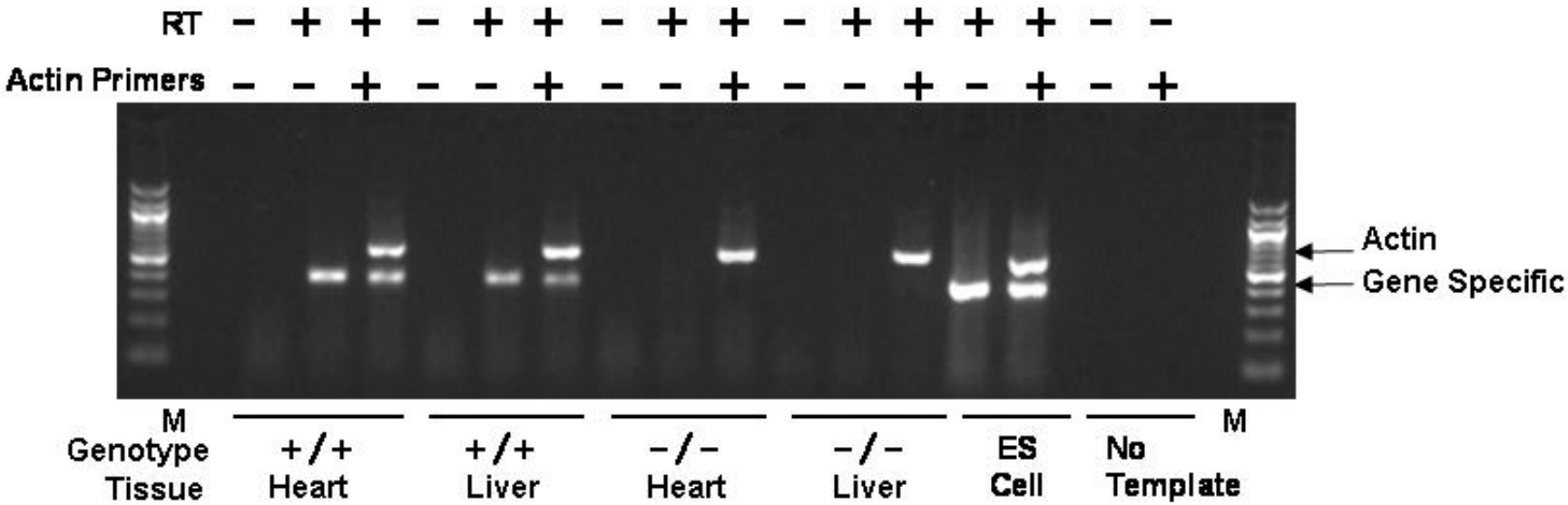
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 1075-5' and Primer LTR-rev, 266 bp	
Recommended Wt PCR: Primer 1075-5' and Primer 1075-3', 437 bp	
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 1075-5'	TGATGTCTACAGCTAACTCTGC
Primer 1075-3'	AACTCTGAGCAAGGCAGAGTAAGC

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

Mutant PCR



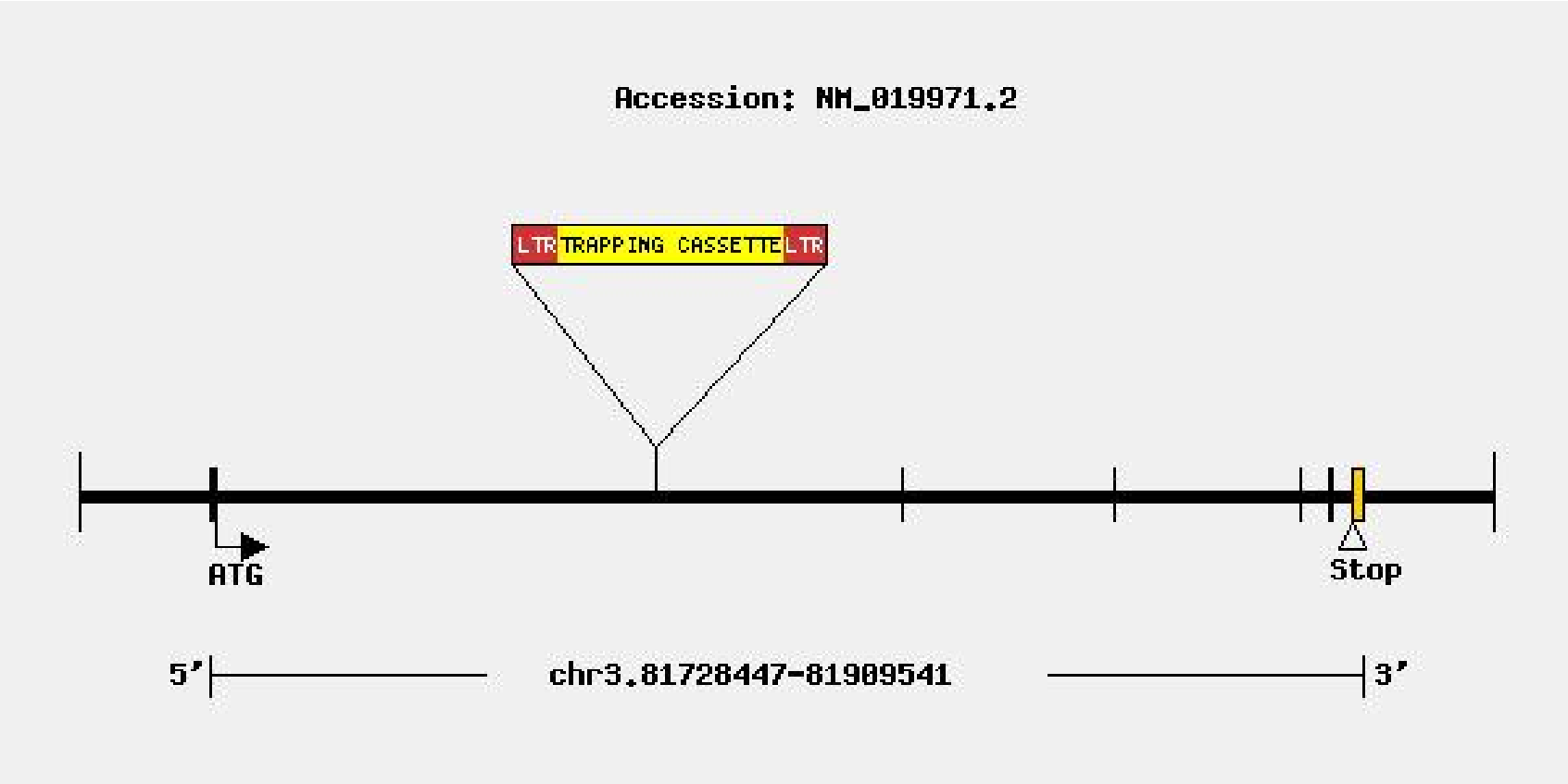
QC Expression



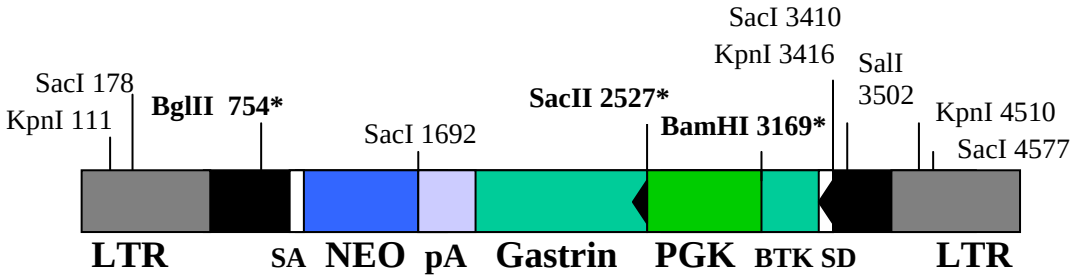
PCR 35 cycles
Primers:S1&AS1

Mouse ID # 56

QC Image


Accession: NM_019971.2
LTR
TRAPPING CASSETTE
LTR
ATG
Stop
5'
chr3.81728447-81909541
3'

VICTR44 Omnibank Vector



Total Size: 4,989 nucleotides
Non-Cutters: ApaI, XhoI
***** Unique sites

Frt sites

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GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
CGGGTACCCGGGCGACTCAGTCAATCGGAGGACTGGCGCGCCGAGTGAGGG
GTTGTGGGCTCTTTTATTGAGCTCGGGGAGCAGAAGCGCGCGAACAGAAGCG
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