

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-Pik3cd^{Gt(OST451938)Lex}/Mmucd **MMRRC:** 011755-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

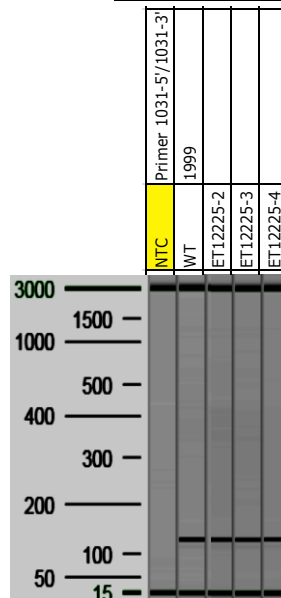
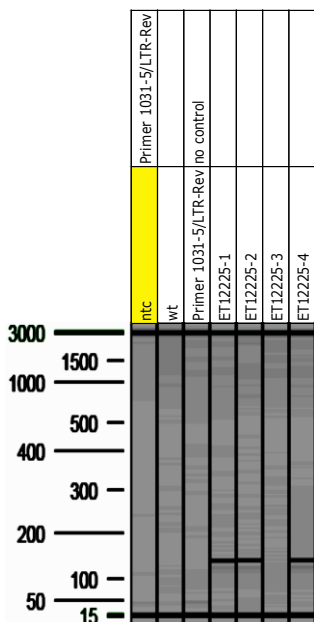
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Primer 1031-5'	CCGTAGAACTGGAGTTAGAGACG
2. Primer LTR-rev	ATAAACCCCTCTTGCAGTTGCATC
3. Primer 1031-3'	CTGCAGTGTCAGATCTGCCTGATG

Electrophoresis Protocol:

Agarose: 1.5% V: 90		
Estimated Running Time: 90 min.		
Primer Combination	Band (bp)	Genotype
1 & 3	123	Wild-type
1 & 2	143	Mutant



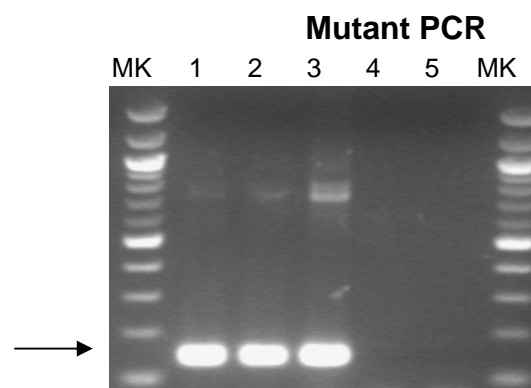
NIH-1031 Genotyping Strategies

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

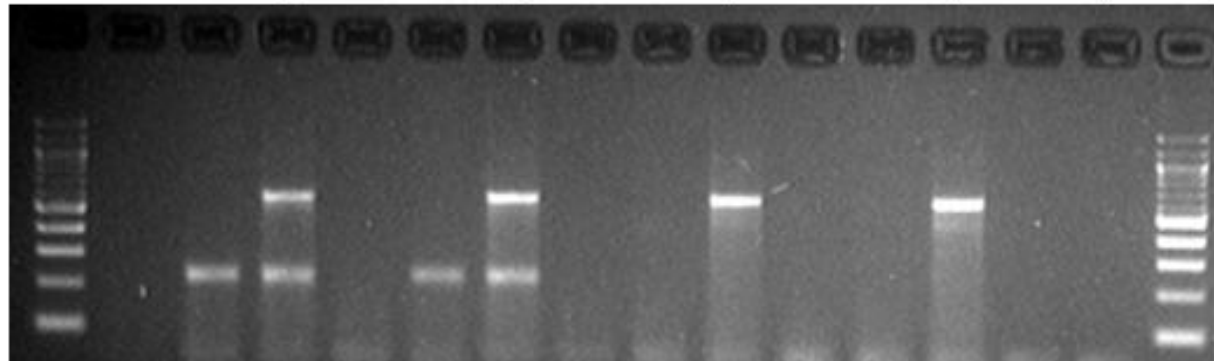
Primer Sequences (5' to 3')	
Mutant PCR: Primer 1031-5' and Primer LTR-rev, 143 bp	
Recommended Wt PCR: Primer 1031-5' and Primer 1031-3', 123 bp	
Primer 1031-5'	CCGTAGAACTGGAGTTAGAGACG
Primer LTR-rev	ATAAACCCCTCTTGCA GTTGCATC
Primer 1031-3'	CTGCAGTGTCAGATCTGCCTGATG

Well	Sample	Genotype
1	233	het
2	242	het
3	ES DNA	het
4	wt lysate	wt
5	water	no amp



QC Expression

RT	-	+	+	-	+	+	-	+	+	-	+	+	-	-
Actin Primers	-	-	+	-	-	+	-	-	+	-	-	+	-	+



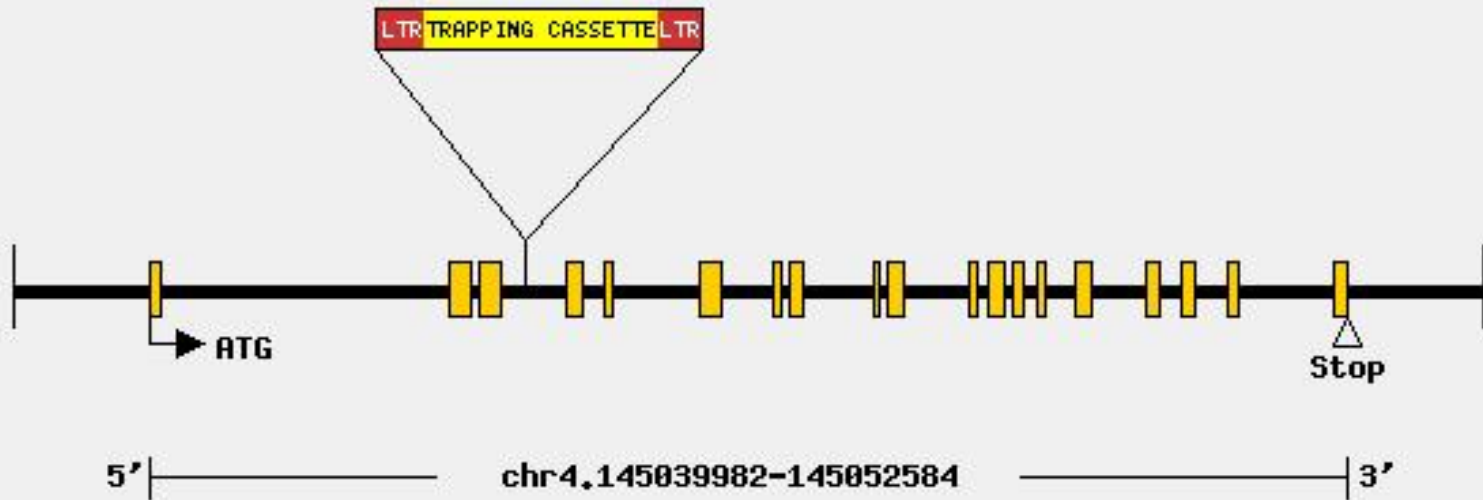
M									M
Genotype	+/+	+/+	-/-	-/-	No				
Tissue	Spleen	Thymus	Spleen	Thymus	Template				

PCR 35 cycles
Primers: 1&2

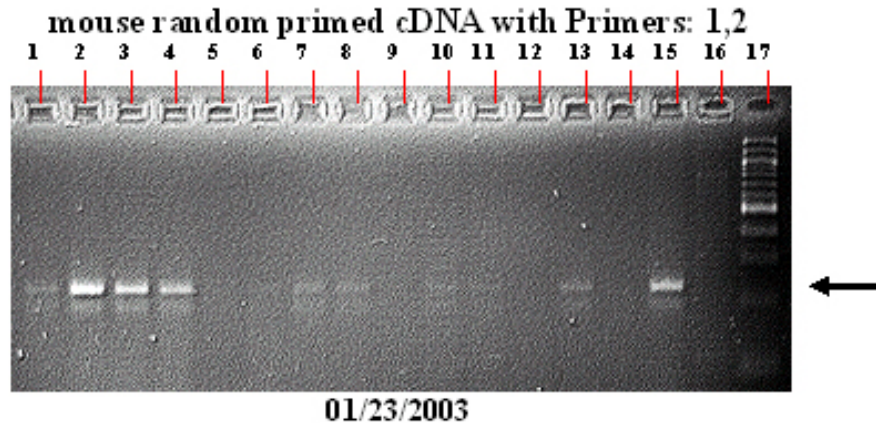
Mouse ID: 107

QC Image

Accession: NM_008840



RT-PCR WT Expression

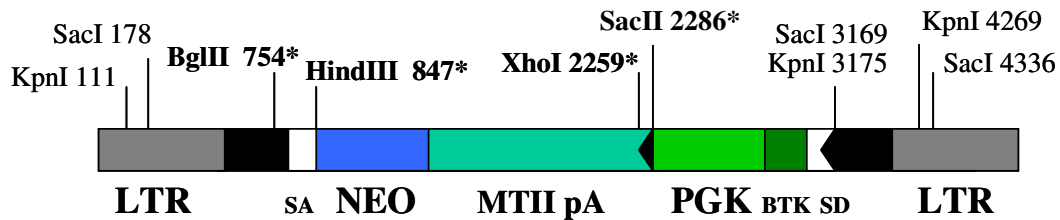


Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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

VICTR 48 MTII Omnibank Vector



Total Size: 4748 nucleotides

Non-Cutters: ApaI, BclI

* Unique sites

Location of components in VICTR 48 MTII:

LTR (viral long terminal repeat): 1-590, 4159-4748

SA (splice acceptor): 755-847

NEO: 867-1684

MTII pA: 1688-2217

frt sites: 2305-2352; 3185-3232

PGK promoter: 2377-2893

BTK exon: 2928-3152

>VICTR 48MTII

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TGAAAGACCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
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