GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6;129S5-Tnfrsf19Gt(OST294987)Lex/Mmucd **MMRRC:** 032686-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	15

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1x**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**30X**
7. Elongation		72	0:40	

Primers:

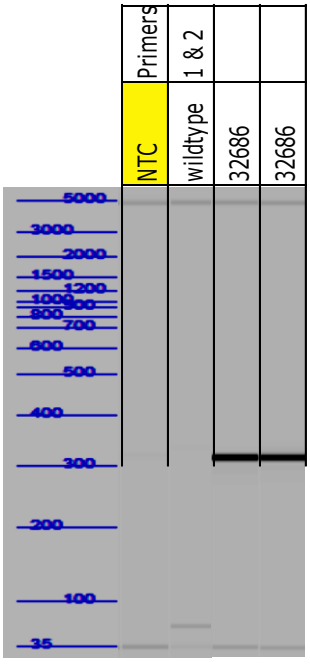
Name	Nucleotide Sequence (5' - 3')
1. 32686-mutR	CGGGCTTGGATTCATATTGAC
2. Primer LTR-2	AAATGGCGTTACTTAAGCTAGCTTGC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	306	mutant



PCR protocol developed by MMRRC at University of California, Davis

Lexicon Contract Name: DNA264
LexVision Name: MEM487T2
Genentech ID: UNQ1888

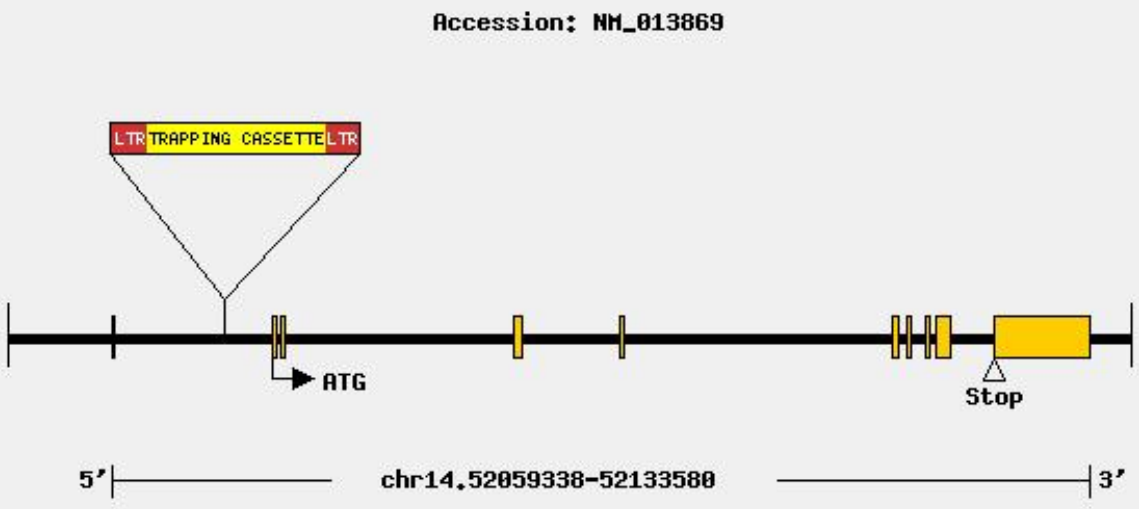
Mouse Accession(s): ENSMUST00000040839, NM_013869
Omnibank Clone: OST294987, VICTR48

Mutation Description:

Listed below is a portion of the mouse genomic sequence (from mouse chr.14) surrounding the gene trap insertion site in the Omnibank mouse line MEM487T2 (OST294987). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

5'
GTTTATCTGGAGTTAGCCTACACAGGGGATAATGCACTTCTCAAATCATAGGTTGCCAAAAAAGAAGCCC
ACTGCCTGGTTTAGGATACTTCCATTAAACTGTTGGTTGTGAGTGTCCTGGAGACCCTCAAACAAAACTG
GCTTTTGCCATTGCTCTTAGTTGCCCAACAGGACTTGTTTTAAGGACACAAATGATGAAGATGCCATATA
TGTTGGTCATACTACATGGAGTAATCAAGTAAATACTGAC*CAGGAAACTTTTTCTCCACTGGTTAGCTT
TCACAGTGCTGGAAGGGGCTATGTCGACTGCTGGGGAGGAAGTCTTCAACTCTCTTACCCAGCTGGGAGT
CCAGTGAGCTCCATTAACAACTGCTATGATGAGATATGGTAGGATATAATAGTGGTGTGGATGTTGTGGA
GGTAACCAATAGTTGTTAAACTTATATGTAAGGTCTGCTCCACAATATGAAATGCATCCCTAGTATTGTC AATATGAATCC
3'

Mutation Illustration:



DNA264 – MEM487T2 PCR to Confirm Genotypes for F1 Hets

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM $MgCl_2$	3.5
10mM dNTPs	1.5
LTR2 Primer	1.5
MEM487T2 3' Primer	1.5
5 U/ul Taq polymerase	0.3
Water	31.7
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Cycling Conditions:

Step	Temp	Time	Note
1	96C	17"	
2	64C	30"	
3	72C	35"	Go to 1, 40 cycles

Primer sequences (5' to 3'):

LTR2	AAATGGCGTTACTTAAGCTAGCTTGC
MEM487T2 3'	CGGGCTTGGATTCATATTGAC

Well	Sample	Genotype
1	189	**het**
2	193	**het**
3	204	**het**
4	223	**het**
5	wt lysate	**wt**
6	ES DNA	**het**
7	water	no amp

Mutant band: 306 bp