GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-*Ide*$^{Gt(neo)486Lex}$/Mmucd **MMRRC #** 011719-UCD

Protocol: ***(PCR protocol as provided in Donator's publication)***

Reagent/Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM) Wt forward	0.5
Primer 2. (stock concentration is 20µM) Wt reverse	0.5
Primer 3. (stock concentration is 20µM) LTR reverse	0.5
Primer 4. (stock concentration is 20µM) LTR2	0.5
Taq Polymerase 5Units/µL	0.2
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

- Reagents and Strategy used for MMRRC-UCD PCR reactions.
- 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 $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	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. Wt forward	ATCTGTGTCAGGAGGAGGGAC
2. Wt reverse	CAGGGTAGGGAAGTCAAGGTTAC
3. LTR-rev	ATAAACCCTCTTGCAGTTGCATC
4. LTR2	AAATGGCGTTACTTAAGCTAGCTTGC

Electrophoresis Protocol:

Agarose: 1% **V:** 90

Estimated Running Time: 90 **min.**

Primer Combination	Band	Genotype	
1 and 2	479 bp	Wild-type	} Simplex reactions, run as two separate reactions
3 and 4	177 bp	Mutant	
1 and 3	177 bp	Mutant (1)	} Mutant PCR strategies using one of wt oligos
2 and 4	453 bp	Mutant (2)	
1+2+3	479 bp	Wild-type	} Multiplex reaction, Separate these on a 1% agarose gel
	177 bp	Mutant	

Once confident that the wild-type and mutant strategies are working separately, you can try to run them as a multiplex reaction:

NIH-0754 Genotyping Strategies

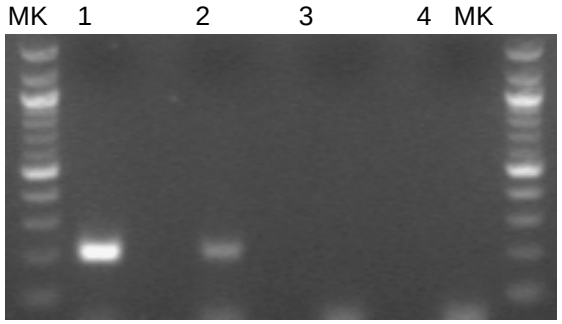
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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 0754-5' and Primer LTR-rev, 214 bp	
Recommended Wt PCR: Primer 0754-5' and Primer 0754-3', 310 bp	
Primer LTR-rev	ATAAACCCTCTTGCAGTTGCATC
Primer 0754-5'	CTAATGAAACTGGGAGGGTTGG
Primer 0754-3'	ACATACTTCCCAGAGCATAGGACG

Well	Sample	Genotype
1	44	het
2	**ES DNA**	het
3	**wt lysate**	wt
4	**water**	no amp

Mutant PCR



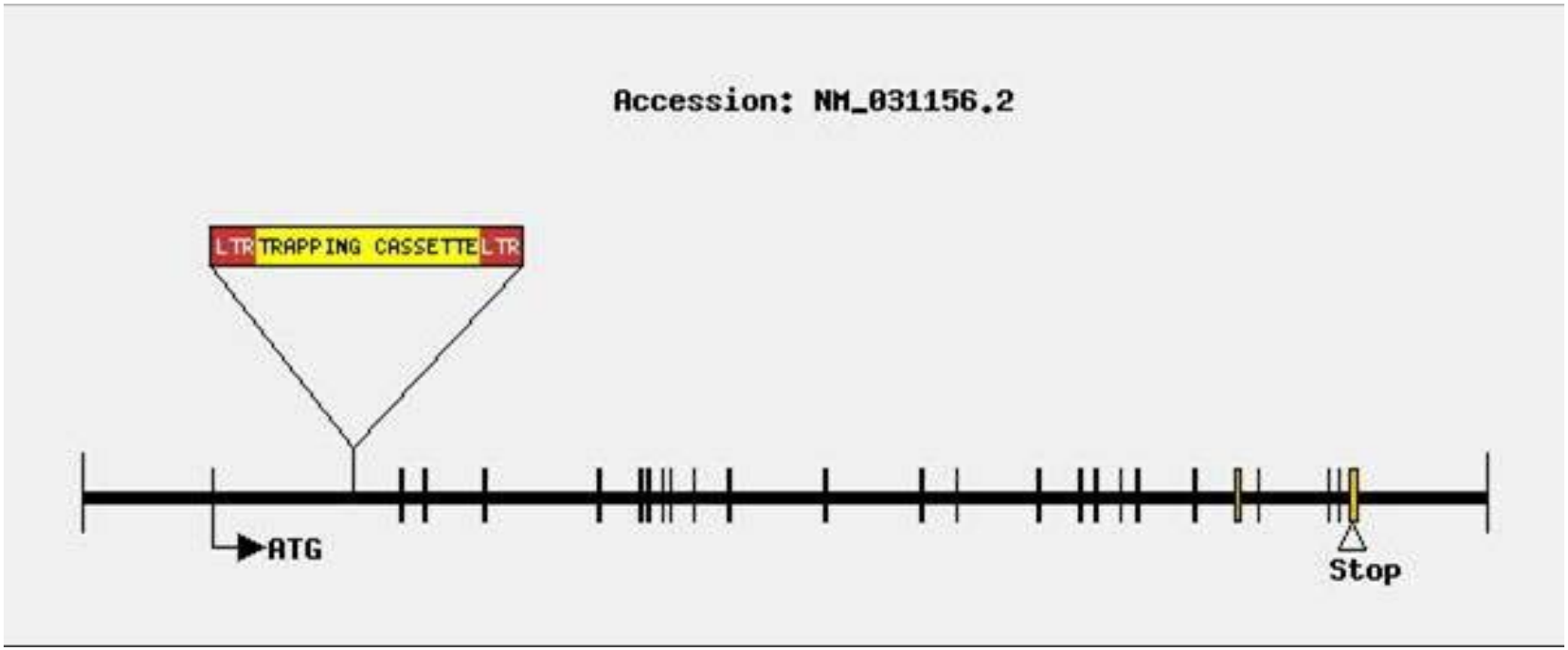
QC Expression

Tissue	Kidney	Liver	Kidney	Liver	ES cell	*Negative*
Genotype	+/+	+/+	-/-	-/-		*Control*

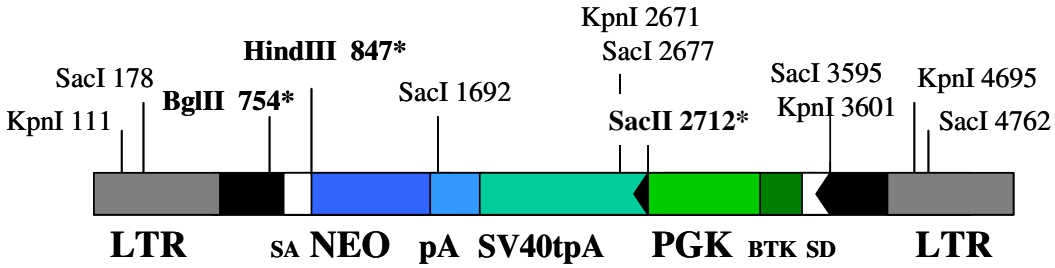
Fig legend; (per each template RT)
1:Gene Specific Primer 2: GSP with no RT templ 3: GSP + actinIC 4: actin
M: 100bp ladder

Mouse ID # 138

QC Image



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
TGAAAGACCCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
GAACAGCGAGACCACAAGTCGGATGCAACTGCAAGAGGGTTTATTGGATACA
CGGGTACCCGGGCGACTCAGTCAATCGGAGGACTGGCGCGCCGAGTGAGGG
GTTGTGGGCTCTTTTATTGAGCTCGGGGAGCAGAAGCGCGCGAACAGAAGCG
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