GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
- *Choose File, Save As*
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- *Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.*

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI **Pampee Young**		
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Strain Name CAG-L4SL-SPRR3	MMRRC Stock Number 43824	

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NAME OF PCR: SPRR3 LSL WT (No transgene at allele) **MMRRC:** 0-CTR

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	6.5
2x Buffer	12.5
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	5
The total volume is auto-calculated based on volumes entered, right click the total and update field to	**TOTAL VOLUME**

Comments on protocol:

- We use NEB OneTaq Quick-Load 2X Master Mix for this protocol. It may need to be optimized for standard PCR reagents. 0.5uL of both forward and reverse primers are used at 10uM stock concentrations.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	95	2:00	**1**
2. Denaturation		95	0:30	
3. Annealing	steps 2-3-4 cycle in sequence	60	0:30	35**x**
4. Elongation		72	1:00	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. LSL WT F	ACTCTACTGGAGGAGGACAAACTGGTCAC
2. LSL WT R	TTGTTCCCTTTCTGCTTCATCTTGCTGA
3.	
4.	
5.	

Electrophoresis Protocol:

Agar 1.8% V:100

Estimated 30 min.

Primer	Band	Genotype
1/2	326 bp	+/+ OR +/LSL
1/2	No band	LSL/LSL
	bp	

NAME OF PCR: SPRR3 LSL TG (positive for Sprr3 transgene) **MMRRC:** 0-CTR

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	6.5
2x Buffer	12.5
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	5
The total volume is auto-calculated based on volumes entered, right click the total and update field to	**TOTAL VOLUME**

Comments on protocol:

- We use NEB OneTaq Quick-Load 2X Master Mix for this protocol. It may need to be optimized for standard PCR reagents. 0.5uL of both forward and reverse primers are used at 10uM stock concentrations.

Strategy:

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5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. LSL TG F	CTGACTCTCAACATTCTACTCCTCC
2. LSL TG R	GCTGACAAGGTTGCTTCACTTGATC
3.	
4.	
5.	

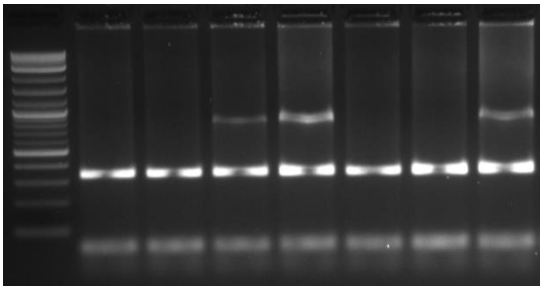
Electrophoresis Protocol:

Agar 1.8% V: 100

Estimated 30 min.

Primer	Band	Genotype
1/2	722 bp	LSL/LSL or +/LSL
1/2	No band	+/+
	bp	

Please size gel images to fit in this space



Upper band (722) indicates presence of transgene. Lower band (326) indicates a wildtype allele. These are all either homozygous WT (one band) or heterozygous for the Sprr3 transgene (two bands). Homozygous transgenics are embryonic lethal.

These PCR reactions were prepared separately but run on the same program and dual-loaded into the gel (10ul of each reaction per well).