

**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
- Select No Compression in the save options.
- Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.
- 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		
Richard Proia		
Email		
proia@nih.gov		
Institution		
NIDDK		
Address		
9000 Rockville Pike		
City	State	Zip
Bethesda	MD	20892
Lab Contact		
Galina Tuymetova		
Email		
tuymetog@nidk.nih.gov		
Telephone	FAX	
3014964391		
Strain Name		MMRRC Stock Number
B6.Cg-Sptlc1^{tm1.1Rlp}/Mmucd		042294

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NAME OF PCR: Lox SPTLC1 **MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	30
10x Buffer	5
MgCl ₂ (stock concentration is mM)	3
Betaine (stock concentration is 5M) <i>Optional</i>	
dNTPs (stock concentration is 10mM)	5
DMSO <i>Optional</i>	
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	0.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	5
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	TOTAL VOLUME OF REACTION:
	50 µL

Comments on protocol:

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Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5 m	1
2. Denaturation	94	1m	
3. Annealing steps 2-3-4 cycle in sequence	59	1m	35x
4. Elongation	72	1m	
5. Amplification	72	3m	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 2%	V:
1. SDL2	GGGTTCTATGGCACATTTGGTAAG	Estimated Running:Time: 20 min.	
2. LOX2r	CTGTTACTTCTTGCCAGTGAC	Primer Combination	Band
3.		1 AND 2	350 bp
4.		1 AND 2	425 bp
5.			bp
			Genotype
			WT
			LOX

Please size gel images to fit in this space

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Protocol / Gel Comments:

Gel pictures:

