GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

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 **Zena Werb**		
Email **zena.werb@ucsf.edu**		
University of California San Francisco		
513 Parnassus Ave., HSW 1323		
City **San Francisco**	State **CA**	Zip **94143-0452**
Lab Contact **Mark Owyong**		
Email **Mark.owyong@ucsf.edu**		
Telephone **415-476-4758**	FAX	
Strain Name STOCK *Sulf2*$^{Gt(XST155)Byg}$/WerbMmucd		MMRRC Stock Number #037625-UCD

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **Sulf 2-xst** **MMRRC:** 037625-**UCD**

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

Reagent/Constituent	Volume (µL)
Water	19.8
10x Buffer	2.5
$MgCl_2$ (stock concentration is mM)	n/a
Betaine (stock concentration is 5M) *Optional*	n/a
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	n/a
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0.5
Primer 4. (stock concentration is 20µM)	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
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**25 µL**

Comments on protocol:

-

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? No ☐	94	5:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 cycle in sequence	59 (↓1ºC/cycle)	1.00	**35x**
4. Elongation		72	1:45	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. **ODL180 wt**	**TGG GTG CAT AGT TGT AAC TCG**
2. **ODL225 wt**	**GAT TAG CCT GTC TGT CTC GAG C**
3. **ODL369 ko**	**CGC TGT TGA GAT CCA GTT CGA TGT**
4. **ODL371 ko**	**TGG GTT GGG TGC ATA GTT GTA ACT**
5.	
6.	
7.	

Argarose: **1.5%** V: **90**
Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 and 2	~800	wt
3 and 4	~1200	KO

Please size gel images and comments to fit within this space

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

This page is not protected and freely editable.

Protocol / Gel Comments:

We no longer use 10X buffer and dNTP. Instead we use 12.5μL of JumpStart REDTaq ReadyMix (http://www.sigmaaldrich.com/catalog/product/sigma/p1107?lang=en&region=US) for Taq-polymerase. No picture of gel.

Gel pictures:

Please size gel images and comments to fit within this space