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 **Dr. Youske Mukoyama**		
Email **mukoyamay@nih.gov**		
Institution **NIH/NHLBI**		
Address **9000 Rockville Pike Bldg. 10 Rm. 6C104**		
City **Bethesda**	State **MD**	Zip **20892**
Lab Contact **Krista Gill**		
Email **gillkr@nih.gov**		
Telephone **301-451-1662**	FAX **301-480-4581**	
Strain Name **Sema3G**		MMRRC Stock Number

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

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

NAME OF PCR: Sema3G **MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Wildtype:	
DNA	2
10uM TUF	1
10uM TUR	1
Promega GoTaq	12.5
H2O	8.5
Mutant:	
DNA	2
10uM SU	1
10uM LacInZRev	1
Promega GoTaq	12.5
H20	8.5
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 EACH REACTION:**	**25.000 µL**

Comments on protocol:

-

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	98	1:00	**1**
2. Denaturation	96	0:30	
3. Annealing steps 2-3-4 cycle in sequence	55	0:30	30**x**
4. Elongation	72	0:30	
5. Amplification	72	2:00	**1**
6. Finish	4	∞	n/a

Primers:

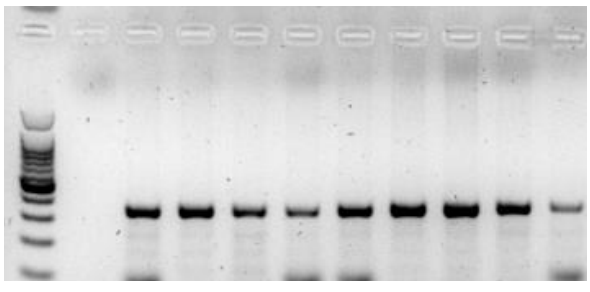
Name	Nucleotide Sequence (5' - 3')
1. TUF	GCGGCTCTCCTACAGAGGTACTG
2. TUR	TGTCCTAGAGCCACGGACATTC
3. SU	TAGCTGCACGGGCATTGAGC
4. LacInZRev	GTCTGTCCTAGCTTCCTCACTG
5.	

Electrophoresis Protocol:

Argarose: V:

Estimated Running:Time: min.

Primer Combination	Band	Genotype
1/2	86 bp	WT
3/4	345 bp	Mut
	bp	



PCR protocol provided by Donating Investigator

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER
sacoord@mmrrc.org
800-910-2291 North America, +1-530-757-5710 International

Protocol / Gel Comments:

Gel pictures: