GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE RESOURCE & RESEARCH 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.

Please fill in form electronically by tabbing through the text fields.
The first 2 pages are protected with gray text fields available as needed to describe your protocol. The last page in not protected to allow for pictures and or comments that will not fit on the protected form.
Thank you!

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 **Bruce Hamilton**			
Email **bah@ucsd.edu**			
Institution **University of California San Diego**			
Address **9500 Gilman Drive**			
City **La Jolla**		State **CA**	Zip **92093**
Lab Contact **Dorothy Concepcion**			
Email **dconcepcion@ucsd.edu**			
Telephone **(858) 822-2298**	FAX		
Strain Name **nur12 Chr17-129SvImJ on BALB/c**			MMRRC Stock Number 41446

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE RESOURCE & RESEARCH CENTER

sacoord@mmrrc.org
800-910-2291 North America, +1-530-757-5710 International

NAME OF PCR: _____ **MMRRC # 041446-**

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	7.61
10x Buffer (contains 15mM $MgCl_2$)	2
dNTPs (stock concentration is 10mM)	0.36
Primer 1 (stock concentration is 20µM) forward	0.2
Primer 2 (stock concentration is 20µM) reverse	0.2
Primer 3 (stock concentration is µM)	
Primer 4 (stock concentration is µM)	
Taq Polymerase 5units/ul	0.18
Additives / Other (if applicable): BSA 10mg/ml	0.15
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	5
TOTAL VOLUME OF REACTION:	**5.330 µL**

Comments on protocol *(e.g.,* different concentration of $MgCl_2$, etc):

25mM MgCl2

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	3:00	**1**
2. Denaturation	95	0:15	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:15	} **35**
4. Elongation	72	0:30	
5. Amplification (i.e., 72°C, 10 min)	72	6:00	**1**
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: D15Mit226	ATCTCACGCTTCTCCCTTCA TGATGCTTGGATCTTGTGGA
2: D15Mit209	TTGTGCTTCACTAGATGTAGACCA TTTTATAGTTGCACATAAGCAGCA
3: D15Mit107	CAACACTTATACACTTGTGTCAGGG TCATGGTTGGAACAGCAGAC
4: D15Mit245	#4: ACCAATACACTTCATGCAAACG CAGTGACCGTAGGTTCATTAACC
5: D15Mit161	#5: Primer 1 Sequence TCTGTTTTGTTTGTTCGTTTGC Primer 2 Sequence TAAAATCTCCCTGTATACAAGTCTGTG

Electrophoresis Protocol:

Agarose: 3%Superfine **V:** 275

Estimated Running Time: 40 **min.**

Primer Combination	Band	Genotype

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE RESOURCE & RESEARCH CENTER

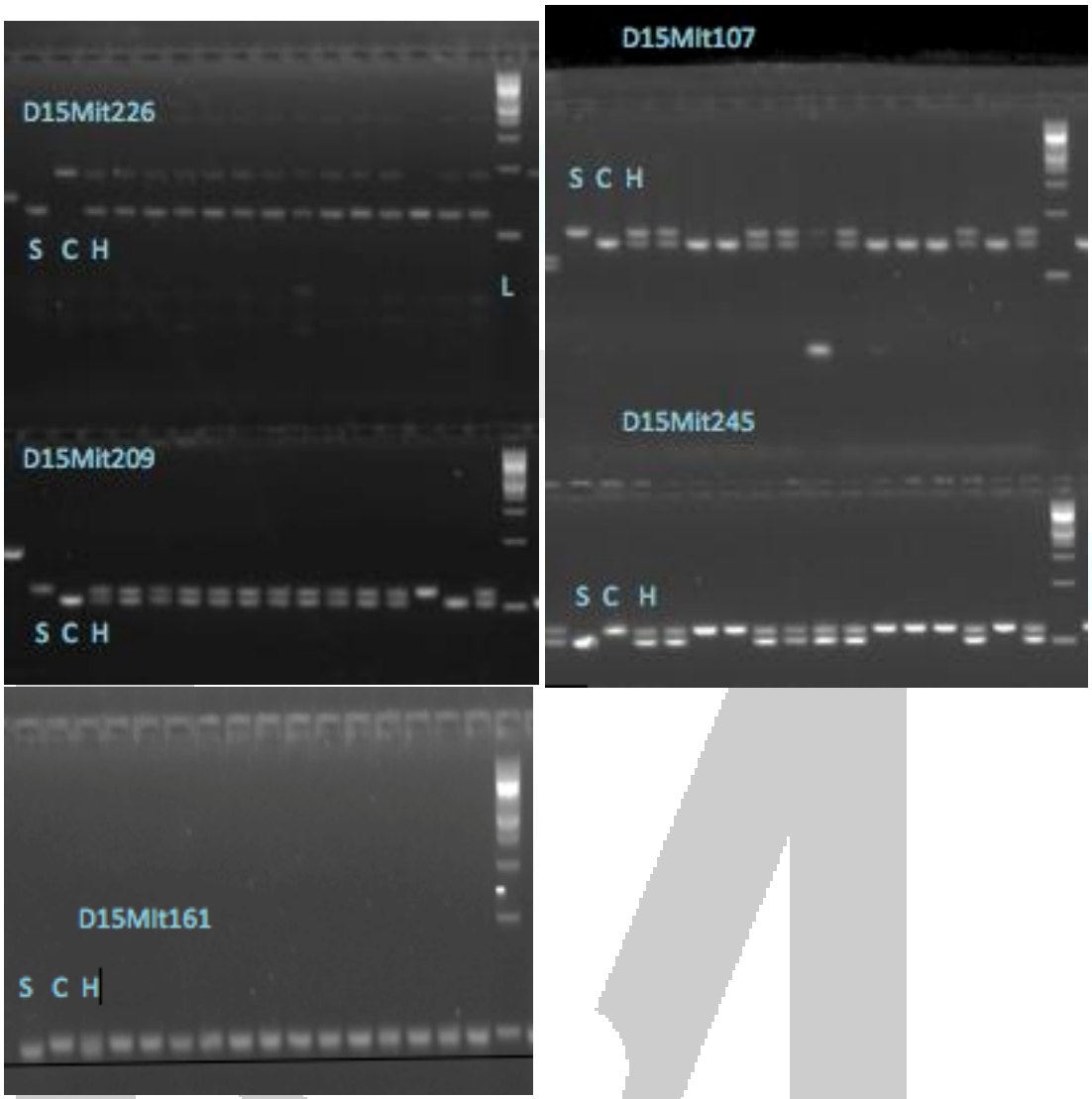
sacoord@mmrrc.org
800-910-2291 North America, +1-530-757-5710 International

	bp	
	bp	
	bp	

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE RESOURCE & RESEARCH CENTER

sacoord@mmrrc.org
800-910-2291 North America, +1-530-757-5710 International

Protocol / Gel Comments: Ladder= 100bp; S= 129SvIm/J; C=Balb/c; H= heterozygote



Lanes:
1. 1Kb+ ladder
2. H_2O
3. Wild-type +/+ sample
4-5. Hom -/- samples

Sample Gel:

PCR protocol provided by Donating Investigator