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.

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Telephone **631-444-7488**	FAX **631-444-6313**	
Strain Name **P38 delta null**	MMRRC Stock Number **43950**	

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NAME OF PCR: **P38 Delta Null** **MMRRC:** **0-CTR**

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

Reagent/Constituent	Volume (µL)
Water	7.5
10x Buffer WE USE PROMEGA MASTER MIX (see comments)	12.5
$MgCl_2$ (stock concentration is mM)	0
Betaine (stock concentration is 5M) *Optional*	0
dNTPs (stock concentration is 10mM)	0
DMSO *Optional*	0
Primer 1. (stock concentration is 10µM) P38_Forward	1.0
Primer 2. (stock concentration is 10µM) P38_Reverse	2.0
Primer 3. (stock concentration is 10µM) P38_NEO	1.0
Taq Polymerase 5Units/µL	0
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:**	**25.000 µL**

Comments on protocol:

-

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	2.:00	**1**
2. Denaturation	95	:30	
3. Annealing steps 2-3-4 cycle in sequence	60	:30	40**x**
4. Elongation	72	1:00	
5. Amplification	72	10:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. P38_Forward	CCCTTGAGCCATAGATCCTGG
2. P38_Reverse	CATGAGCTTGAGATGCTCTCT
3. P38_NEO	GGCGATGCCTGCTTGCCGAAT
4.	
5.	

Electrophoresis Protocol:

Argarose: 1% V:

Estimated Running:Time: 30 min.

Primer Combination	Band	Genotype
1/2/3	500 bp	Wildtype
1/2/3	750/800 bp	Null
	bp	

Please size gel images to fit in this space

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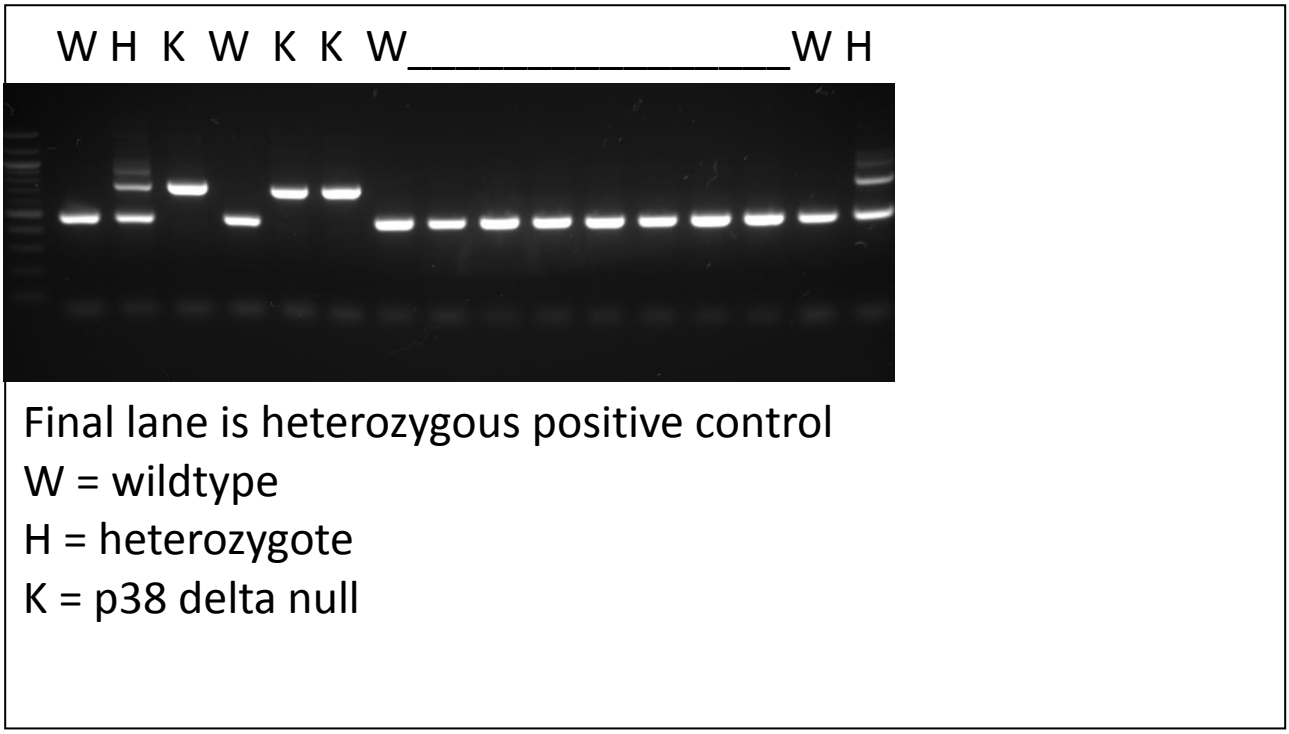
Protocol / Gel Comments:

For the PCR reaction we use GoTaq® Colorless Master Mix from Promega (catalog # M7133).

Gel pictures:



Final lane is heterozygous positive control
W = wildtype
H = heterozygote
K = p38 delta null