

**GENOTYPING BY PCR PROTOCOL  
MUTANT MOUSE REGIONAL RESOURCE CENTER**

[sacoord@mmrrc.org](mailto: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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<b>Dr. Caton</b>		
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<b>Caton@wistar.org</b>		
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<b>610-329-8472</b>		
Strain Name		MMRRC Stock Number
<b>HA104</b>		

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**NAME OF PCR:** HA104

**MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	18.9
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	2.5
Betaine (stock concentration is 5M) <i>Optional</i>	0
dNTPs (stock concentration is 5mM)	1
DMSO <i>Optional</i>	0
Primer 1. (stock concentration is 20µM)	.05
Primer 2. (stock concentration is 20µM)	.05
Primer 3. (stock concentration is 20µM)	0
Primer 4. (stock concentration is 20µM)	0
Taq Polymerase 5Units/µL	.05
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1
<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>	
<b>TOTAL VOLUME OF REACTION:</b>	<b>26.050 µL</b>

**Comments on protocol:**

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**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float:right">HOT START? <input type="checkbox"/></span>	50	1 m	
2. Denaturation	72	3 m	
3. Annealing <span style="float:right">steps 1-2-3 cycle in sequence</span>	94	1 m	35x
4. Elongation	50	1 m	1
5. Amplification	72	10 m	1
6. Finish	4	∞	n/a

**Primers:**

**Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')	Argarose: 1%	V: 100
1. HA uni-5'	CCA GCA GAT TTC ATC GAC TAT G	Estimated Running:Time: 30 min.	
2. HA uni-3'	CCA GTA ATA GTT CAT CCT CCC	<b>Primer Combination</b>	<b>Band</b>
3.		1 & 2	400 bp
4.			bp
5.			bp
			<b>Genotype</b>
			+

*Please size gel images to fit in this space*

*PCR protocol provided by Donating Investigator*

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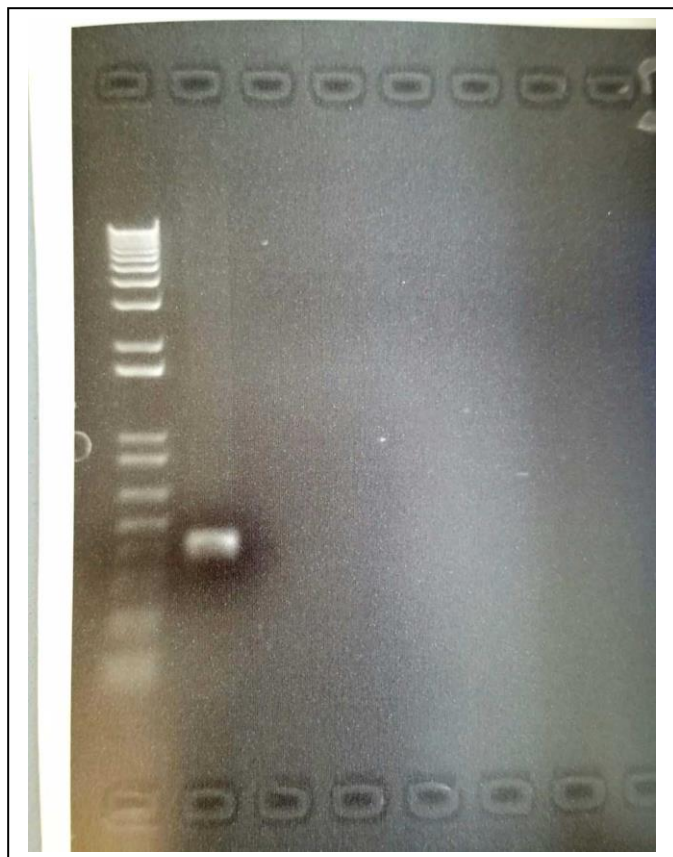
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**Protocol / Gel Comments:**

A negative result is indicated by the absence of a band.

**Gel pictures:**



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