

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	34253
<b>MMRRC Strain Name</b>	STOCK <i>Haus3<sup>L5Jcs31</sup> Rw/Mmnc</i>
<b>Gene Name(s)</b>	HAUS augmin-like complex, subunit 3; L5 John C Schimenti 31 (Haus3L5Jcs31)
<b>Breeding Protocol(s)</b>	Intra-Strain Mating
<b>Protocol Date</b>	7/22/13

### MMRRC #34253 PCR Reaction

**Note: Genomic DNA prepared by “HotSHOT” — NaOH digestion buffer. Biotechniques. 2000. 29(1): 52**

Lysis Buffer

25mM NaOH

0.2mM EDTA

pH 12

1. Add 50ul of lysis buffer to the tail

2. Heat at 95C for 45 minutes

3. Cool down and add 50ul of neutralization buffer

Neutralization Buffer

40mM Tris-HCl

pH 5

### WT Reaction

**Thermal Cycler:**

Step 1: 95°C for 5 min

Step 2: 95°C for 30 sec

Step 3: 52°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: 35x from step 2 to step 4

Step 6: 72°C for 10 min

	<b><u>1X</u></b>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Taq	0.5
DNA	2

**Taq: Apex and Chromataq 5X Buffer**

**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

L31RwL1: CCATGAACACCTGGAGGAAG

Haus3 wt-MAMA: CCACGATACAAAGCATCACATA

**Bands expected:** 150 bp

## Mutant Reaction

### Thermal Cycler:

Step 1: 95°C for 5 min  
Step 2: 95°C for 30 sec  
Step 3: 58°C for 30 sec  
Step 4: 72°C for 30 sec  
Step 5: 28x from step 2 to step 4  
Step 6: 72°C for 10 min

### Taq: Apex and Chromataq 5X Buffer

**Primer sequences 5' to 3'**: Primers are 10 µM with respect to each primer.

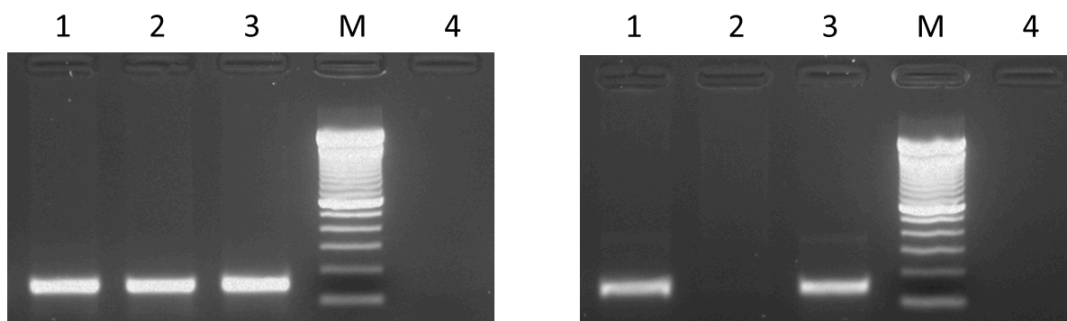
L31RwL1: CCATGAACACCTGGAGGAAG

Haus3 mut-MAMA: CCACGATACAAAGCATCACATG

**Bands expected:** 150 bp

Run on 2% agarose gel in TAE.

34253



WT Primers: L31RwL1 + Haus3 wt-MAMA

Mut Primers: L31RwL1 + Haus3 mut-MAMA

Lane 1, 3: Het; Lane 2: WT; Lane 4: H<sub>2</sub>O; M: 100 bp DNA ladder