

## MMRRC UNC – Genotyping Protocol

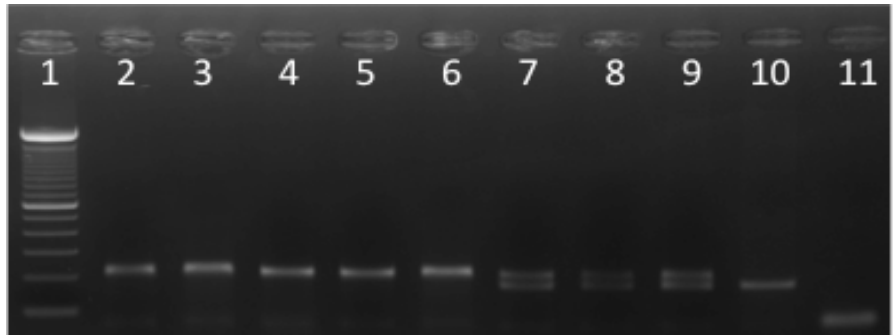
<b>MMRRC Strain ID</b>	37350
<b>MMRRC Strain Name</b>	B6.129(FVB)- <i>Deaf1</i> <sup>tm1.1Mico</sup> /Mmnc
<b>Gene Name(s)</b>	deformed epidermal autoregulatory factor 1 (Drosophilia) [Deaf1]
<b>Breeding Protocol(s)</b>	Intra-strain mating
<b>Protocol Date</b>	11/17/14

### MMRRC #37350 PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM WT Primer	1
10uM Mut Primer	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 95°C for 5min  
 Step 2: 95°C for 30 sec  
 Step 3: 58°C for 30 sec  
 Step 4: 72°C for 45 sec  
 Step 2 to 4 Cycles: 35  
 Step 5: 72°C for 7 min



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

**Bands:** MUTANT: 226bp WT: 174bp

**Primer sequences 5' to 3':**

Deaf1(37350)For: TAGAATTCGATCTATGGTGGGTGTG  
 Deaf1(37350)Rev: CATACATGGGGCACACCTAATTTAG

Run on 2% agarose gel.

1. 100bp Marker
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Homozygous Sample
6. Homozygous Sample
7. Heterozygous Sample
8. Heterozygous Sample
9. Heterozygous Sample
10. Wild-type Control
11. Negative Control