

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	37370
MMRRC Strain Name	CAn.129(Cg)- <i>Deaf1</i> ^{tm1.2Mico} /Mmnc
Gene Name(s)	deformed epidermal autoregulatory factor 1 (Drosophila) [Deaf1]
Breeding Protocol(s)	Sib-mating
Protocol Date	5/13/15

MMRRC #37370 PCR Reaction

	1X
ddH ₂ O	11
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primers F/R	1/1
10uM MUT Primers F/R	1/1
Taq	0.5
DNA	2

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 45 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

Bands: WT: 174bp MUTANT: ~360bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Deaf1(37370)For: TAGAATTCGATCTATGGTGGGTGTG

Deaf1(37370)Rev: CATACTGGGGCACACCTAATTTAG

Deaf(37370)MutF: TTGGGATTTGGGGTCAAGG

Deaf(37370)MutR: AGTGGGTGTAGTGGTTAAGG

Run on 2% Agarose Gel

1. Homozygous Sample
2. Homozygous Sample
3. Wild-type Control
4. Negative Control
5. 100 bp Marker

