

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36773
MMRRC Strain Name	B6.Cg- <i>Calca</i> ^{tm1.1(EGFP/HBEGF)Mjz} /Mmnc
Gene Name(s)	calcitonin/calcitonin-related polypeptide, alpha; targeted mutation 1.1, Mark Zylka (Calcatm1.1(EGFP/HBEGF)Mjz)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	5/19/14

MMRRC #36773 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

Note: 2,2 Primer Reaction (CGRP2+CGRP3) for wild-type and (CGRP3+EGFP2) for the mutant reaction.

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C 30 sec

Step 3: 65°C 30 sec

Step 4: 72°C 60 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7min

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

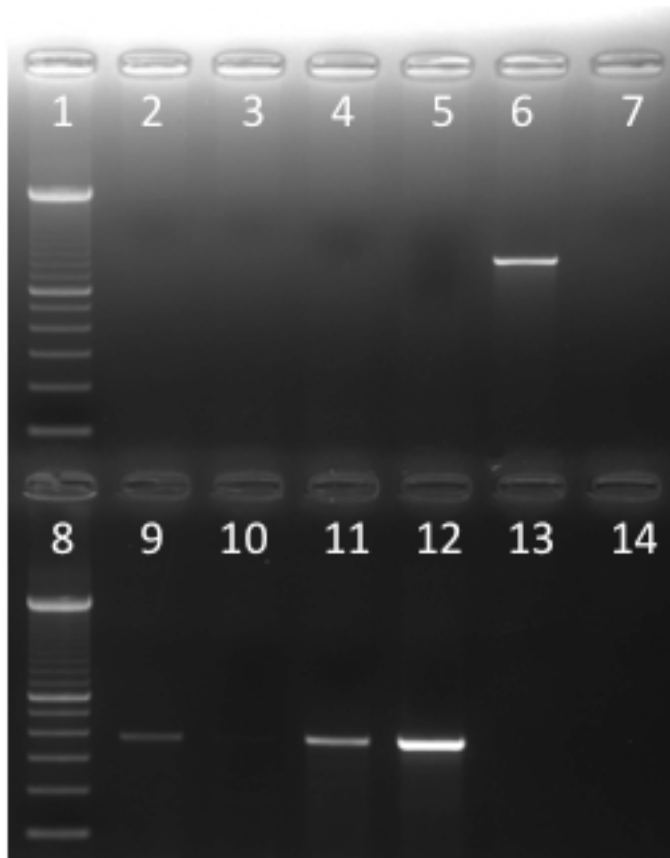
Bands: WT: 830bp MUTANT: 369bp

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

CGRP2 : CAG CTC CCT GGC TTT CAT CTG C

CGRP3: AAA TGT CGG GGA GTC ACA GGC

EGFP2: CCG TAG GTC AGG GTG GTC ACG AGG



Wild-type Reaction

1. 100bp Marker
2. Homozygous Mutant
3. Homozygous Mutant
4. Homozygous Mutant
5. Homozygous Mutant
6. Wild-type Control
7. Negative Control

Mutant Reaction

8. 100bp Marker
9. Homozygous Mutant
10. Homozygous Mutant (Band is faint but present)
11. Homozygous Mutant
12. Homozygous Mutant
13. Wild-type Control
14. Negative Control