

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36513
MMRRC Strain Name	B6.129S6- <i>Fgd2</i> ^{tm1Gava} /Mmnc
Gene Name(s)	FYVE, RhoGEF and PH domain containing 2 (<i>Fgd2</i>)
Breeding Protocol(s)	Sib-Mating (homozygous x homozygous)
Protocol Date	2/26/13

MMRRC # 36513 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 30

Step 6: 72°C for 7 min

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

Bands: MUTANT: ~400bp

WT: 312bp

Primer sequences 5' to 3':

E5s: CCTTCCAGGAGGTGGTCAC

E6as: CTCTGGGCATCTTCGAGGTCT

I3260: GGTTCTCTCTAGATGTGTTTGC

Run on 2% agarose gel in TAE.

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2