

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30
MMRRC Strain Name	STOCK <i>Figla</i> ^{tm1Dean} /Mmnc
Gene Name(s)	Factor in the germline alpha (<i>Figla</i>)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	8/26/13

MMRRC #30 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 65°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

WT-F: CTGGCCCCTTCGTGGTACCACC

WT-R: GGGCCTGTGAAGGGTCAGCCC

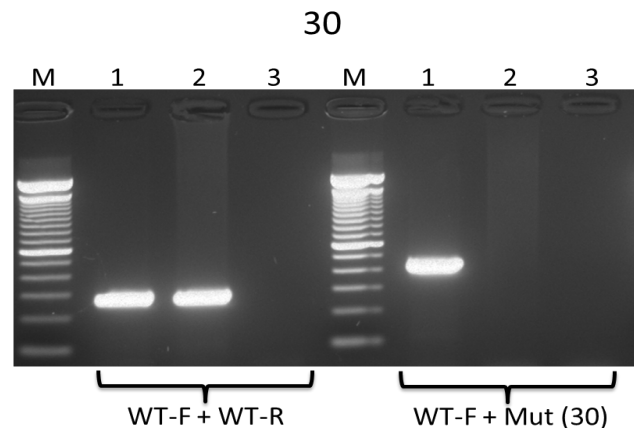
Mut (30): CATTGTACGTCCTGCACGAC

Bands expected:

(WT-F + WT-R) WT 290bp

[WT-F + Mut (30)] Mut ~480bp

Run on 2% agarose gel in TAE.



Lane 1: Het; Lane 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder Invitrogen