

MMRRC UNC – Genotyping Protocol

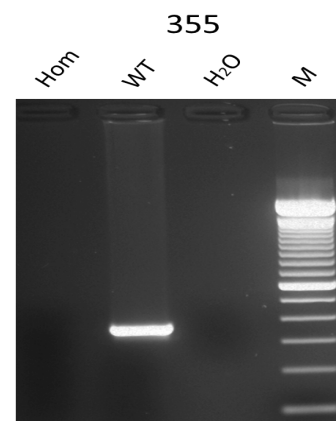
MMRRC Strain ID	355
MMRRC Strain Name	129S1.129P2(Cg)-Zfa-ps ^{tm1Ems} /Mmnc
Gene Name(s)	Zinc finger protein (Zfa)
Breeding Protocol(s)	Sib-mating
Protocol Date	10/14/13

MMRRC #355 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM 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: 58°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 30x from step 2 to step 4
 Step 6: 72°C for 7 min



Primers: WT F (355) + WT R (355)
 M: 100 bp DNA ladder (Invitrogen)

Taq: Apex and Chromataq 5X Buffer

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

WT F (355): GTGGCAGATGAAGCAGATGA

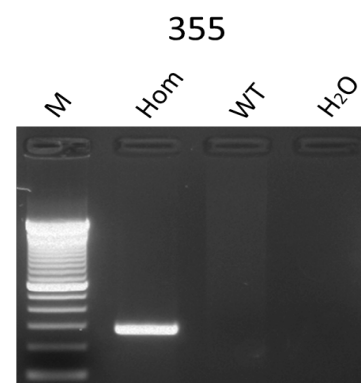
WT R (355): AGTGCTCATCATGGCGAC

Mut F (355): CTTGGGTGGAGAGGCTATTC

Mut R (355): AGGTGAGATGACAGGAGATC

Bands expected: WT F (355) + WT R (355), WT 364 bp
 Mut F (355) + Mut R (355), Mut 280 bp

Run on 2% agarose gel in TAE.



Primers: Mut F (355) + Mut R (355)
 M: 100 bp DNA ladder (Invitrogen)