

MMRRC UNC – Genotyping Protocol

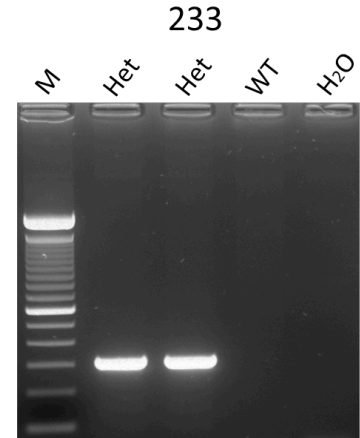
MMRRC Strain ID	233
MMRRC Strain Name	STOCK Tg(Zic4-EGFP)AK178Gsat/Mmnc
Gene Name(s)	Zinc Finger protein of the cerebellum 4 (Zic4)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	4/7/14

MMRRC #233 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: 35x from step 2 to step 4
 Step 6: 72°C for 7 min



Primers: Com-F3 (233) + Mut-R3 (233)
 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.

Com-F3 (233): CACAGACTCAGCCCTGCTGCCTTT

WT-R3 (233): GGTCAGGTTTCATGCCGCCATAAC

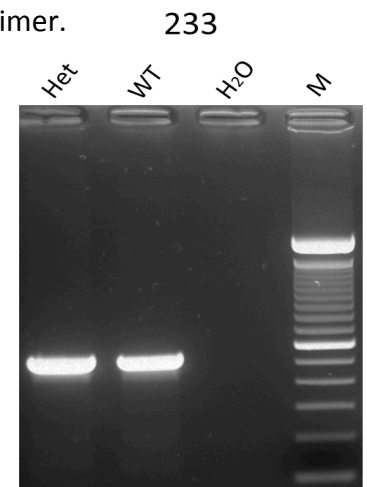
Mut-R3 (233): GAACTTGTGGCCGTTTACGTGCG

Bands expected:

WT: Com-F3 (233) + WT-R3 (233), 529 bp

MUTANT: Com-F3 (233) + Mut-R3 (233), ~300bp

Run on 2% agarose gel in TAE.



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