

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	239
MMRRC Strain Name	STOCK Tg(Otx2-EGFP)BD199Gsat/Mmnc
Gene Name(s)	Orthodenticle homolog 2 (Otx2)
Breeding Protocol(s)	Backcross to Swiss Webster (Taconic)
Protocol Date	3/3/14

MMRRC #239 PCR Reaction

	<u>1X</u>
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

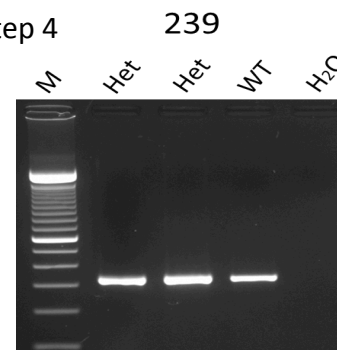
Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

Step 4: 72°C for 45 sec

Step 5: 32x from step 2 to step 4

Step 6: 72°C for 7 min



Primers: TM-Com-F (239) + TM-WT-R (239)
 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.

TM-Com-F (239): ACGCATCTGCAACTCCTTTAAAGCC

TM-WT-R (239): CAAGTCCATACCCGAAGTGGTCAGA

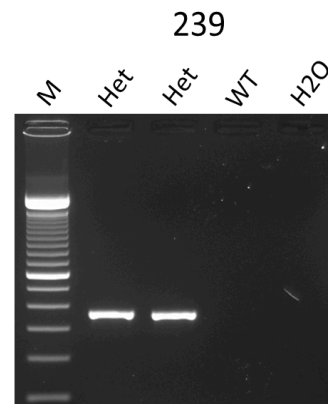
TM-Mut-R (239): TGAACCTGTGGCCGTTTACGTCTG

Bands expected:

WT: TM-Com-F (239) + TM-WT-R (239): 328 bp

Mut: TM-Com-F (239) + TM-Mut-R (239): 341 bp

Run on 1% agarose gel in TAE.



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