

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	273
MMRRC Strain Name	STOCK Tg(Htr3a-EGFP)DH30Gsat/Mmnc
Gene Name(s)	5-hydroxytryptamine receptor 3A (Htr3a)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	10/28/13

MMRRC #273 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: 34× 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.

Com-F (273): GCAAGATGTGACCAAGCCACCTATTT

WT- R (273): CAGCCCTCAGCCCTTTGAGACTTAAG

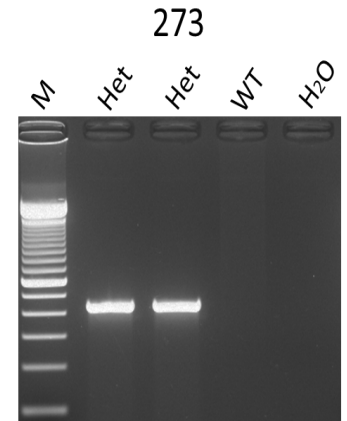
Mut-R (273): TGAAGTTGTGGCCGTTTACGTCG

Bands expected:

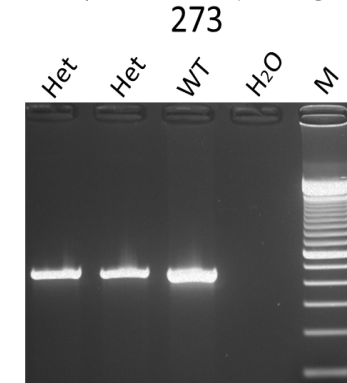
WT: Com-F (273) + WT- R (273), 459 bp

MUTANT: Com-F (273) + Mut-R (273), 402 bp

Run on 2% agarose gel in TAE.



Primers: Com-F (273) + Mut-R (273)
 M: 100 bp DNA ladder (Invitrogen)



Primers: Com-F (273) + WT- R (273)
 M: 100 bp DNA ladder (Invitrogen)