

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	235
MMRRC Strain Name	STOCK Tg(Pde1c-EGFP)S45Gsat/Mmnc
Gene Name(s)	Phosphodiesterase 1C (Pde1c)
Breeding Protocol(s)	Backcross to Swiss Webster (Taconic)
Protocol Date	7/29/13

MMRRC #235 PCR Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5.0
25 mM MgCl ₂	1.5
10 mM dNTPs	0.5
10 μM Primer Common	1
10 μM Primer WT-R	1
10 μM Primer Mut-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: Step 2 to 4; Cycles: 35
 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.

Common (235): TGTTTGAAGGGAAAAAGAACAGGAACAG

WT-R (235): CTCGAATTCTTCAATTCCTTGGTTGGA

Mut-R (235): TGCAGATGAACTTCAGGGTCAGCTT

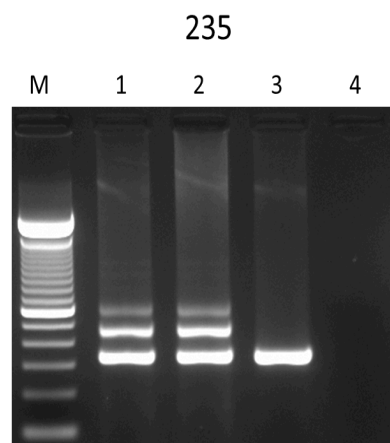
Bands expected:

WT: primers Common (235) + WT-R (235): 331 bp

MUTANT: primers: Common (235) + Mut-R (235): 443bp

Note: There may be a 600 bp unexpected band.

Run on 2 % agarose gel in TAE.



Lane 1, 2: Het; Lane 3: WT; Lane 4: H₂O; M: 100 bp DNA ladder (Invitrogen)