

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	260
MMRRC Strain Name	STOCK Tg(Ppp2r2d-EGFP)BK52Gsat/Mmnc
Gene Name(s)	Protein phosphatase 2, regulatory subunit b, delta isoform (Ppp2r2d)
Breeding Protocol(s)	Backcross to Swiss Webster (Taconic)
Protocol Date	7/29/13

MMRRC #260 PCR Reaction

	<u>1X</u>
ddH ₂ O	13.8
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
50 μM each EGFP F/R	0.4
50 μM each Actin F/R	0.8
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 95°C for 5 min
 Step 2: 95°C for 45 sec
 Step 3: 60°C for 45 sec
 Step 4: 72°C for 1 min
 Step 5: 30x from step 2 to step 4
 Step 6: 72°C for 10 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 50 μM with respect to each primer

EGFP F: CCT ACG GCG TGC AGT GCT TCA GC

EGFP R: CGG CGA GCT GCA CGC TGC GTC CTC

Actin PCR control/forward primer: GAT GAC GAT ATC GCT GCG CTG GTC G

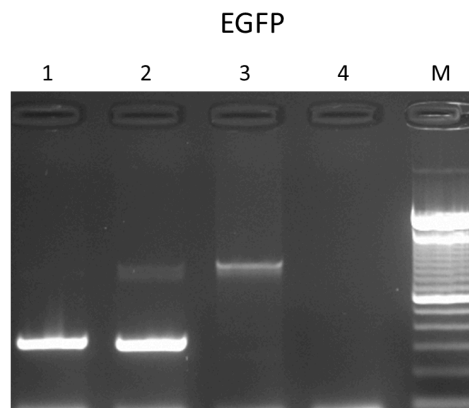
Actin PCR control/reverse primer: GCC TGT GGT ACG ACC AGA GGC ATA CAG

Bands expected: Actin ~1kb (DNA control)

EGFP ~300bp

Run on 1% agarose gel.

Note: 1 kb band of Actin was not shown in some reactions.



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