

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	274
MMRRC Strain Name	STOCK Tg(Colgal2-EGFP)BN20Gsat/Mmnc
Gene Name(s)	collagen beta(1-O)galactosyltransferase 2 (Colgal2)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	9/30/13

MMRRC# 274 PCR Reaction

	<u>1X</u>
DNA	1.0
Qiagen 10X Buffer	2.0
Qiagen 25 mM MgCl ₂	0.5
Qiagen 10mM dNTPs	0.4
50 uM EGFP F/R	0.4
50 uM Actin F/R	0.8
Taq	0.2
ddH ₂ O	<u>10.7</u>
Qiagen Q solution	4.0

Thermal Cycler:

Step 1: 94⁰C for 45 sec

Step 2: 60⁰C for 45 sec

Step 3: 72⁰C for 60 sec

Step 4: Step 1 to 3; Cycles: 30

Taq: **Apex and 10X Qiagen Buffer**

Bands: MUTANT: Actin ~ 1kb (control), EGFP ~300bp
WT: No Band

Primer sequences 5' to 3':

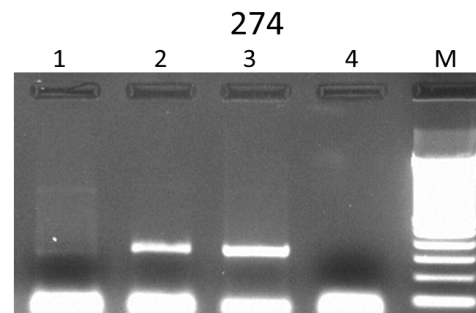
EGFP forward primer: CCT ACG GCG TGC AGT GCT TCA GC

EGFP reverse primer: 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

Run on 2.0% agarose gel in TAE.



Lane 1: WT; Lane 2-3: EGP⁺; Lane 4: H₂O
M: 100 bp DNA ladder Invitrogen