

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	33002
<b>MMRRC Strain Name</b>	B6SJL-Tg(Zp3-GFP, -Khdc1b*)1Molx/Mmnc
<b>Gene Name(s)</b>	Zona pellucida glycoprotein 3 (Zp3), KH domain containing 1B (Khdc1b)
<b>Breeding Protocol(s)</b>	Backcross to C57SJLF1/J
<b>Protocol Date</b>	1/7/14

### MMRRC 33002 PCR Reaction

**Thermal Cycler:**

94°C for 45 sec  
60°C for 45 sec  
72°C for 60 sec  
30x

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

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

	<b>1X</b>
DNA	2.0
Qiagen 10X Buffer	2.0
Qiagen 25 mM MgCl <sub>2</sub>	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 <sub>2</sub> O	10.7
Qiagen Q solution	4.0

**Primer sequences 5' to 3':**

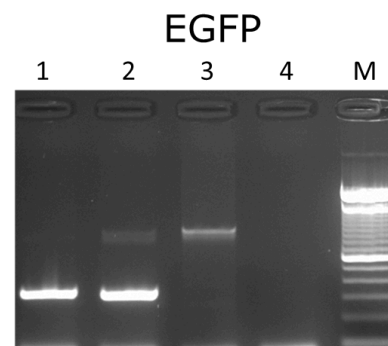
EGFP forward primer: CCT ACG GCG TGC AGT GCT TCA GC

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, 2: EGFP+; Lane 3: WT; Lane 4: H<sub>2</sub>O  
M: 100 bp DNA ladder Invitrogen