

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	236
<b>MMRRC Strain Name</b>	STOCK Tg(Cdh3-EGFP)BK102Gsat/Mmnc
<b>Gene Name(s)</b>	Cadherin 3(Cdh3)
<b>Breeding Protocol(s)</b>	Backcross to Swiss Webster (Taconic)
<b>Protocol Date</b>	4/12/13

### MMRRC # 236 PCR Reaction

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primers	1/1/1
Taq	0.5
ddH <sub>2</sub> O	12.0
25mM MgCl <sub>2</sub>	2.0

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 51°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

**Bands:**

MUTANT: 241bp

WT: 369bp

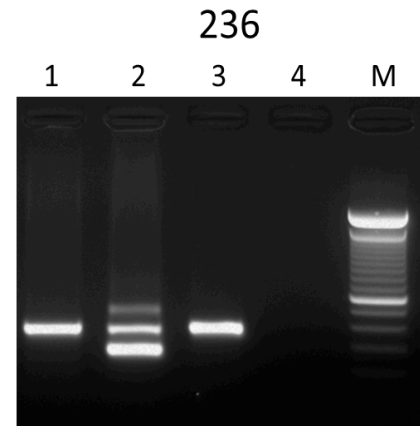
**Primer sequences 5' to 3':**

Com-F (236): 5'-GGGACCTTGTGACGTTGGACCAAT-3'

WT-R (236): 5'- TGCCTGGTTCTGTACACCCATTCTTG-3'

Mut-R (236): 5'-CACGCTGAACTTGTGGCCGTTTAC-3'

Run on 2% agarose gel in TAE.



Lane 1, 3: WT; Lane 2: Het; Lane 4: H<sub>2</sub>O  
M: 100 bp DNA ladder (Invitrogen)