

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36295
MMRRC Strain Name	129S6- <i>Sfxn3</i> ^{tm1Mdf} Mmnc
Gene Name(s)	sideroflexin 3 (Sfxn3)
Breeding Protocol(s)	Backcross to 129SvEv
Protocol Date	6/27/13

MMRRC #36295 PCR Reaction

NOTE: This is a 2, 2 –primer reaction

Thermal Cycler:

Step1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: Step 2 to 4 Cycles: 35

Step 6: 72°C for 7 min

Taq: **Apex and chromataq Buffer**

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

Primers are 10uM with respect to each primer

Primer sequences 5' to 3':

P 1: AGGGCTAGCACAGGCATCTA

P 2: TCTCCCCAGACTTGCTGACT

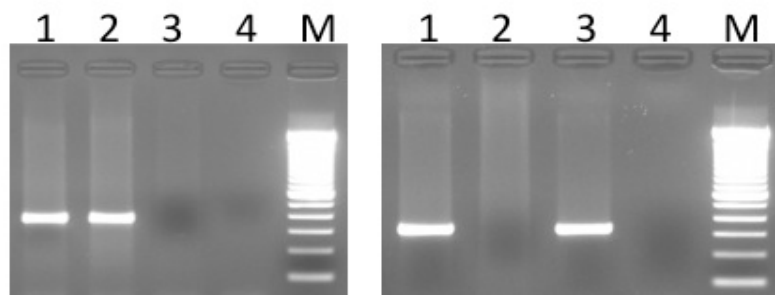
P 3: CTAAAGCGCATGCTCCAGAC

Bands: P 1+ P 2; WT ~380bp

P 2 + P 3; KO ~340bp

Run on 1% agarose gel in TAE.

36295



WT: Primer P1 + P2

Mut: Primer P2 + P3

Lane 1; Het; Lane 2: WT; Lane 3: Hom; Lane 4: ddH₂O;
 M: 100 bp DNA ladder Invitrogen