

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36780
MMRRC Strain Name	129S.129P2- <i>Ryr1</i> ^{tm1.1Msnr} /Mmnc
Gene Name(s)	ryanodine receptor 1, skeletal muscle (<i>Ryr1</i>)
Breeding Protocol(s)	Backcross to 129/SvEv
Protocol Date	8/5/13

MMRRC #36780 PCR Reaction

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

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 62°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 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

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F (36780): TGCCTTGAGTCTGTTGGCGG

R (36780): CGGACTCTCGGTCCTTTTCC

Bands expected: WT, Het and Homo all 342 bp

Reaction requires *Hinf*I restriction digest

***Hinf*I restriction digestion mix for each PCR sample**

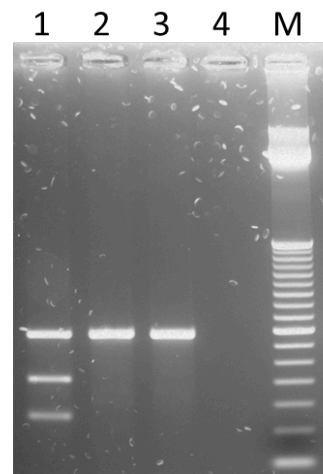
- 10 μl H₂O
- 4 μl NEB Buffer 4 or 2, 10X
- 1 μl *Hinf*I

Add 15ul Mix to each 25ul PCR product. Incubate at 37°C for 3 hours - overnight. Then 80°C for 20 minutes

Expected Results: WT 342bp

Het; 342 bp, 200bp, and 142bp

Homo: 200bp, and 142bp



Lane 1: Het; Lane 2-3: WT; Lane 4: H₂O; M: 50 bp DNA ladder Invitrogen

Run on 2% agarose gel in TAE.