

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36932
MMRRC Strain Name	129S6(Cg)-Ryr2 ^{tm1.1Msnr} /Mmnc
Gene Name(s)	ryanodine receptor 2, cardiac (Ryr2)
Breeding Protocol(s)	Backcross to 129SvEv
Protocol Date	9/30/13

MMRRC #36932 PCR Reaction

Note: This genotyping requires *HinfI* restriction digestion.

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 62°C for 60 sec
 Step 4: 72°C for 60 sec
 Step 5: Step 2 to 4; Cycles: 30
 Step 6: 72°C for 7 min

	1X
ddH ₂ O	13
5X Buffer	5
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Forward Primer	1
10 μM Reverse Primer	1
Taq	0.5
DNA	2

Taq: Apex and Chromataq 5X Buffer

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

Ryr2 (36932/3) For: CAAGGCTTAGGAGCTACGAG

Ryr2 (36932/3) Rev: TTCACGTTGCCCTCGTTAC

Bands expected: WT, Het and Homo all 397bp

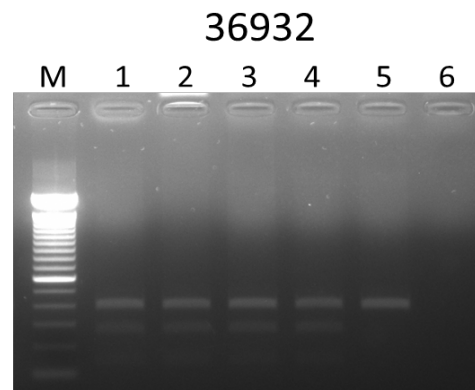
***HinfI* restriction digestion mix for each PCR sample**

10 μl H₂O
 4 μl NEB Buffer 3, 10X
 1 μl *HinfI*

Add 15ul Mix to each 25ul PCR product and incubate at 37°C overnight.

Bands expected: MUTANT: 264 bp and 133 bp
 WT: 397 bp

Run on 2.0% agarose gel in TAE



Lane 1 to 4 Het ; Lane 5: WT; Lane 6: H₂O;
 M: 100 bp DNA ladder (Invitrogen)