

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	9993
MMRRC Strain Name	C3Fe.SJL(B6)-dblr/Mmnc
Gene Name(s)	Doubleridge (dblr)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	6/15/16

MMRRC #9993 PCR Reaction

Thermal Cycler:

94°C for 5 min
 94°C for 30 sec
 57°C for 30 sec
 72°C for 45 sec
 35x
 72°C for 7 min

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

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

Bands: MUTANT: 350bp
 WT: 537bp

Primer sequences 5' to 3':

F1: GTT TCA GCC CCA AAG ACT GCA TAG
 R1: TTC ATT GAC GCT TTC CTT TCC AAG
 R2: GAA TGT TGA GAG TCA GCA GTA GCC

Run on 2.0% agarose gel in TAE.

1. Heterozygous Sample
2. Wild-type Sample
3. Homozygous Sample
4. Wild-type Sample
5. Wild-type Control
6. Negative Control
7. 100bp Marker

