

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	9994
MMRRC Strain Name	B6.SJL-dblr/Mmnc
Gene Name(s)	Doubleridge (dbl)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	5/21/12

MMRRC# 9994 PCR

	<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

2,2 Primer Reaction: (F1+ R1) for wild-type reaction and (F1+R2) for mutant 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

Taq: **Apex 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.