

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	15388
MMRRC Strain Name	B6(129S4)- <i>Dicer1</i> ^{tm1Tara} /Mmnc
Gene Name(s)	Dicer 1, ribonuclease type III
Breeding Protocol(s)	Random Intra-Strain Mating
Protocol Date	7/30/14

MMRRC #15388 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
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

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 55°C for 30 sec
 Step 4: 72°C for 60 sec
 Step 2 to 4 Cycles: 35
 Step 5: 72°C for 7 min

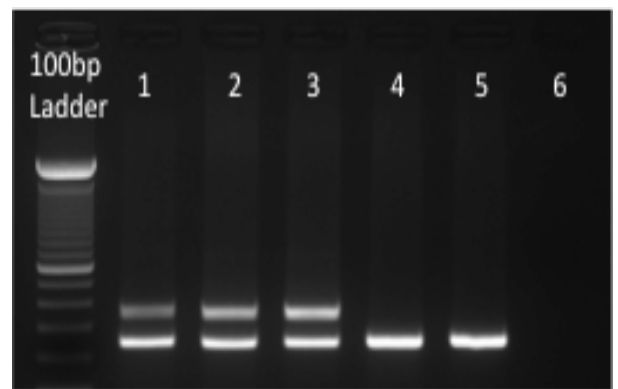
Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: MUTANT: ~350bp
 WT: 244bp

Primer sequences 5' to 3':

15388_FN: GGTTACATGGCTAGACTCAAAGC
 15388_RN: AGGTGCCTTTCGTTTAGGAAC
 15388_FWF: AAAGCAGAACTCTAATGCCCC

Run on 2% agarose gel.



Lane

1 Heterozygous Sample
 2 Heterozygous Sample
 3 Heterozygous Sample
 4 Wild-type Sample
 5 Wild-type Control
 6 Negative Control