

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	15499
<b>MMRRC Strain Name</b>	B6;129P2- <i>Ezh2</i> <sup>tm1Tara</sup> /Mmnc
<b>Gene Name(s)</b>	Enhancer of zeste homolog 2 ( <i>Ezh2</i> )
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	10/4/13

### DNA Extraction

Digest tail snips using NaOH Method

#### Lysis Buffer

25mM NaOH  
0.2mM EDTA  
pH 12

#### Neutralization Buffer

40mM Tris-HCl  
pH 5

1. Add 50ul of lysis buffer to the tail
2. Heat at 95C for 45 minutes
3. Cool down and add 50ul of neutralization buffer

### MMRRC# 15499 PCR Reaction

	<b><u>1X</u></b>
ddH <sub>2</sub> O	16.25
10X Buffer	2.5
50mM MgCl <sub>2</sub>	1.25
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 95°C for 2 min  
Step 2: 94°C for 30 sec  
Step 3: 56°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 Apex 10X Buffer**

**Bands:**

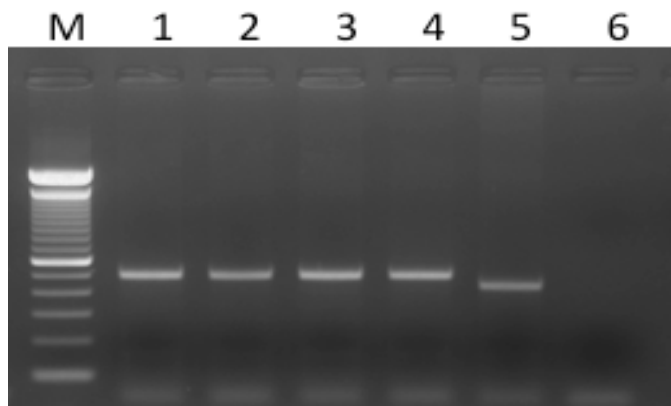
MUTANT: 470bp  
WT: 430bp

**Primer sequences 5' to 3':**

Enx1 3' of loxP: CTG CTC TGA ATG GCA ACT CC  
Enx1 5' of loxP: TTA TTC ATA GAG CCA CCT GG

Run on 2% agarose gel.

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Lane 1-4: Flox; Lane 5: WT; Lane 6 :  
H<sub>2</sub>O; M: 100 bp DNA ladder  
(Invitrogen)