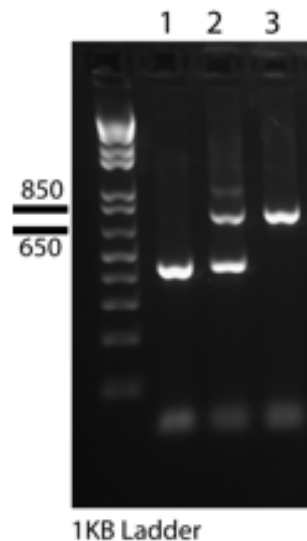


MMRRC UNC – Genotyping Protocol

| | |
|-----------------------------|---|
| MMRRC Strain ID | 42287 |
| MMRRC Strain Name | STOCK <i>Ezh2^{tm1Tara} Ezh1^{tm1Jnw}/MagMmnc</i> |
| Gene Name(s) | enhancer of zeste 2 polycomb repressive complex 2 subunit, enhancer of zeste 1 polycomb repressive complex 2 subunit |
| Breeding Protocol(s) | Sib-mating |
| Protocol Date | 10/7/2020 |

Ezh1 PCR Reaction

| | 1X |
|------------------------|-----------|
| ddH ₂ O | 14.5 |
| 10X Buffer | 2.5 |
| 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 |



1. Homozygous Sample
2. Heterozygous Sample
3. Wild-type Control

Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 30sec
 Step 3: 65C, 30sec
 Step 4: 72C, 30sec
 Step 2 to 4 Cycles: 34
 Step 5: 72C, 7min

Taq: Apex Taq and 10X Buffer

Bands: WT: 750bp MUTANT: 470bp

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

| | |
|------------|-----------------------|
| Ezh1WT | CTCCTGTCCTCATAGCAAGAC |
| Ezh1SA | GTACTCTTAACCACTGGACTG |
| Ezh1LacZ-2 | AAGCGCCATTCGCCATTCAGG |

Ezh2 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

PCR Reaction

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

Thermal Cycler:

Step 1: 95C, 2min

Step 2: 94C, 30sec

Step 3: 56C, 30sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

Taq: **Apex Taq and Apex 10X Buffer**

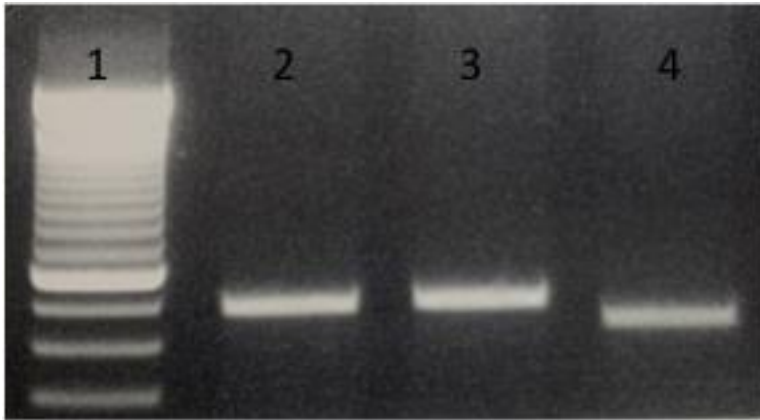
Bands: MUTANT: 470bp WT: 430bp

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.



1. 100bp Marker
2. Homozygous Sample
3. Homozygous Sample
4. Wild-type Control