

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30471
MMRRC Strain Name	B6;129S7-Sox8 ^{tm1Mkob} /Mmnc
Gene Name(s)	SRY (sex determining region Y)-box 8
Breeding Protocol(s)	Backcross
Protocol Date	9/18/19

PCR Reaction

	<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 (Common + WT) and (Common + Mut)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 60C, 30sec

Step 4: 72C, 45sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

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

Bands: MUTANT: 553bp WT: 299bp

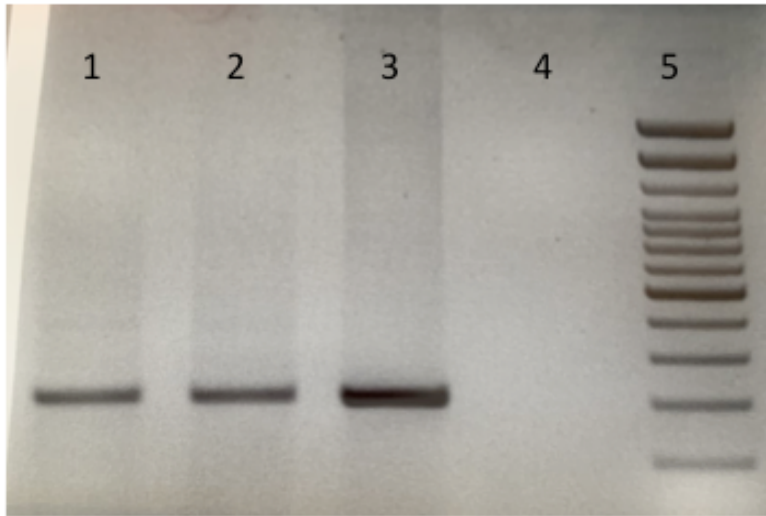
Primer sequences 5' to 3':

Common: GAGGACAAAGATTGGGTCCTGC

WT: GAAGCGTTCGTCTGCTGCC

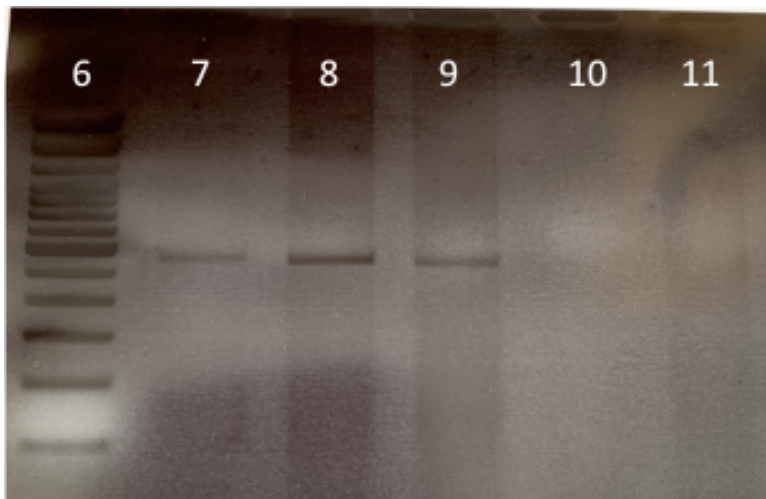
Mut: GATGAAACGCCGAGTTAAACGC

Run on 2% agarose gel.



Wild-type Reaction

1. Heterozygous Sample
2. Heterozygous Sample
3. Wild-type Control
4. Negative Control
5. 100bp Marker (Invitrogen)



Mutant Reaction

6. 100bp Marker (Invitrogen)
7. Heterozygous Sample
8. Heterozygous Sample
9. Heterozygous Sample
10. Wild-type Control
11. Negative Control