

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	43908
<b>MMRRC Strain Name</b>	B6J.C3-Sst1 <sup>C3HeB/Fej</sup> Krmn/Mmnc
<b>Gene Name(s)</b>	Sp110 nuclear body protein
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	10/25/19

### PCR Reaction

	<b><u>1X</u></b>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

**This strain requires 5, 2 primer reactions**

#### **Thermal Cycler:**

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 57C, 30sec

Step 4: 72C, 45sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

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

Bands: D1Mit10: WT: 139bp MUTANT: 144bp

D1Mit49: WT: 128bp MUTANT: 122bp

D1Mit80: WT: 144bp MUTANT: 134bp

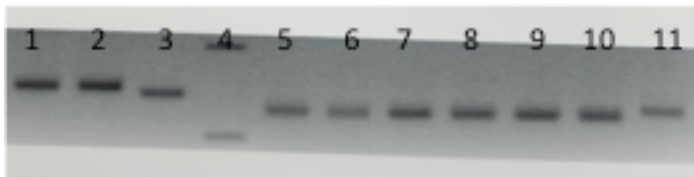
D1Mit334: WT: 98bp MUTANT: 90bp

D1Mit439: WT: 124bp MUTANT: 128bp

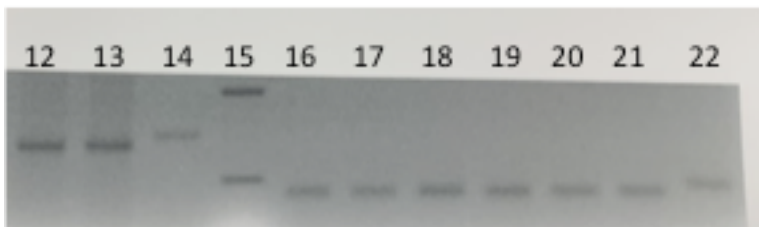
Gel: Prepare a large 3% agarose gel and let chill at 4°C overnight.

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

Sst(43908)334F	CAAGAGATGAAAAAAAAAATCAGC
Sst(43908)334R	CAGATGCAAACCCCATTTT
Sst(43908)80F	CATAGACAAGTCTCTGAACCATGG
Sst(43908)80R	ACTACATAGCCAATAGCCCTGG
Sst(43908)439F	GTGTTGGAAGGAGAAAGTTTGC
Sst(43908)439R	GTTTCATGAGTTCATGCGTTCA
Sst(43908)49F	GAAACCCTACCTTGAAGCCC
Sst(43908)49R	ATTGTGTGATTATGTGAATGAGGG
Sst(43908)10F	AAACCATGCAGGTACTGATATGG
Sst(43908)10R	GAAGAAATTA ACTGAGAGCAAGGC

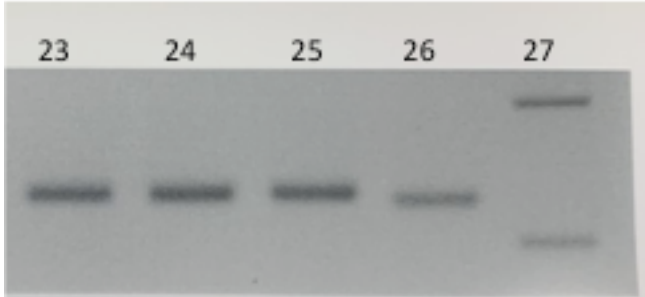


1. D1Mit10 Homozygous Sample
2. D1Mit10 Homozygous Sample
3. D1Mit10 Wild-type Control
4. 100bp Marker (Invitrogen)
5. D1Mit49 Homozygous Sample
6. D1Mit49 Homozygous Sample
7. D1Mit49 Homozygous Sample
8. D1Mit49 Homozygous Sample
9. D1Mit49 Homozygous Sample
10. D1Mit49 Homozygous Sample
11. D1Mit49 Wild-type Control



12. D1Mit80 Homozygous Sample
13. D1Mit80 Homozygous Sample
14. D1Mit80 Wild-type Control
15. 100bp Marker (Invitrogen)
16. D1Mit334 Homozygous Sample
17. D1Mit334 Homozygous Sample

- 18. D1Mit334 Homozygous Sample
- 19. D1Mit334 Homozygous Sample
- 20. D1Mit334 Homozygous Sample
- 21. D1Mit334 Homozygous Sample
- 22. D1Mit334 Wild-type Control



- 23. D1Mit439 Homozygous Sample
- 24. D1Mit439 Homozygous Sample
- 25. D1Mit439 Homozygous Sample
- 26. D1Mit439 Wild-type Control
- 27. 100bp Marker (Invitrogen)