MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29869
MMRRC Strain Name	STOCK *Onecut1*$^{tm1.1Mga}$/Mmnc
Gene Name(s)	one cut domain, family member 1
Breeding Protocol(s)	Random intra-strain mating
Protocol Date	5/3/2023

Onecut-1 PCR Reaction

	1X
ddH_2O	15
10X Buffer	2.5
25mM MgCl2	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1.0
DNA	2

Thermal Cycler:
94 for 5 min
94 for 30s
58 for 30s
72 for 30s
steps 2-4, 35x
72 for 10 min

Taq: **Apex and 10X Buffer**

Bands: MUTANT: ~590bp
WT: ~550bp

Primer sequences 5’ to 3’:
OnecutF 5' GTCGTCGA**CCTCTCTCCTGTCTCCCTCAGTATCC**
OnecutR 5' ATAAGCGGCCGC**CCTCCCTCTCTCTTTCCATC**

Run on 2.0% agarose gel in TAE.

For R26R Gene:

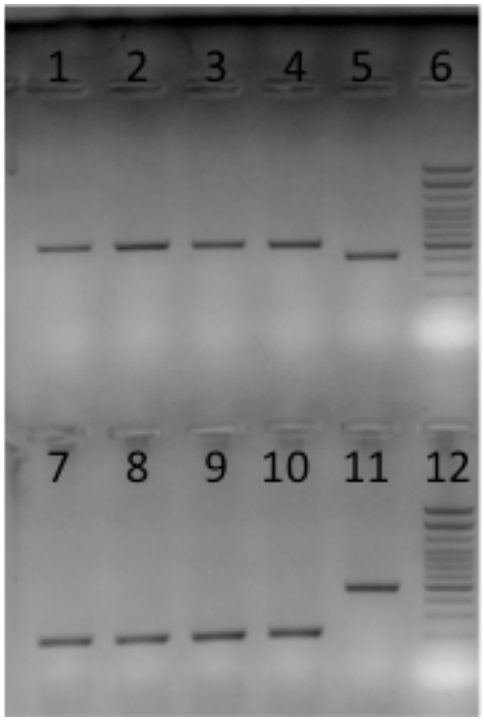
	1X
ddH_2O	14
5X Buffer	2.5
25mM MgCl2	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	1.0
DNA	2

Thermal Cycler:
Step 1: 94C, 5min
Step 2: 94C, 45sec
Step 3: 55C, 45sec
Step 4: 72C, 60sec
Step 2 to 4 Cycles: 30
Step 5: 72C, 7min

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

Bands: WT: 600bp MUTANT: 325bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer
R26RFor(MUT): 5' GCGAAGAGTTTGTCCTCAACC
R26RRev: 5' AAAGTCGCTCTGAGTTGTTAT
R26RFor(WT): 5' GGAGCGGGAGAAATGGATATG



Onecut-1 Reaction

1. Homozygous Sample
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Wild-type Control
6. 100bp Ladder (Invitrogen)

R26R Reaction

7. Homozygous Sample
8. Homozygous Sample
9. Homozygous Sample
10. Homozygous Sample
11. Wild-type Control
12. 100bp Ladder (Invitrogen)