

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	50536
MMRRC Strain Name	STOCK <i>H3f3a^{tm1.2Mag} H3f3b^{tm1.2Mag}/Mmnc</i>
Gene Name(s)	H3.3 histone A, H3.3 histone B
Breeding Protocol(s)	Sib-mating
Protocol Date	3/12/21

PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

Note: 2,2 Primer Reactions (a1F+a1R) for H3f3a and (b1F+b1R) for H3f3b

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 58C, 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 3a: 102bp; 3b: 159bp MUTANT: 3a: 262bp; 3b: 299bp

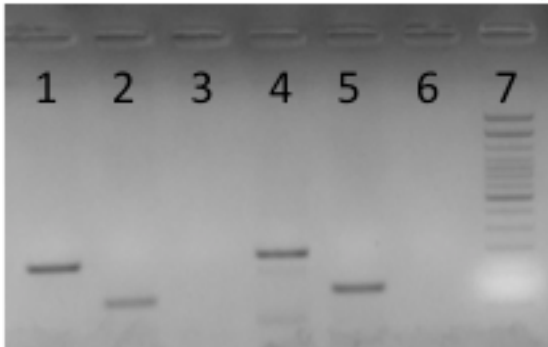
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

H3a(50536)1F: GGCTGTAATTTGACTCGACCTTTCTGT

H3a(50536)1R: CCCCACCATGGCTTTTCGATCCA

H3b(50536)1F: GCAAACAGCTGGCCACCAAGGC

H3b(50536)1R: GAAGGCGTCAACGGTCAGGGAG



H3f3a Reaction

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

H3f3b Reaction

4. Homozygous Sample
 5. Wild-type Control
 6. Negative Control
7. 100bp Marker (Invitrogen)