

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	50726
MMRRC Strain Name	B6J.B6N- <i>Usp20</i> ^{tm1a(EUCOMM)Hmgu} /ShesMmnc
Gene Name(s)	ubiquitin specific peptidase 20
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	1/29/2021

PCR Reaction

	<u>1X</u>
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

2,2 Primer Reaction (CF+WR for Wild-type and CF+MR for Mutant Reaction)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 62C, 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 520bp MUTANT: 420bp

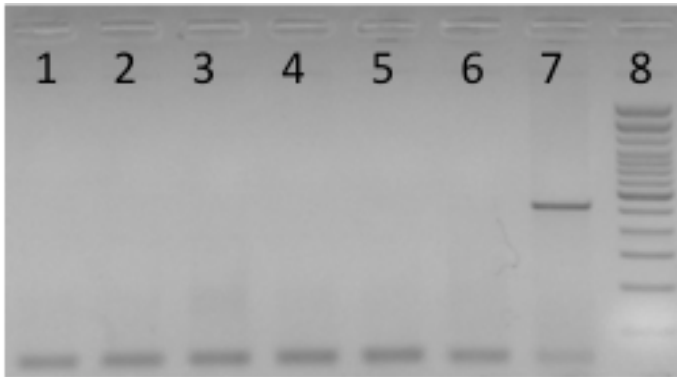
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Usp20(50726)CF CACGCGGATTTCTGAGTTTG

Usp20(50726)WR CAAGCAAGAGGGGAGACCAG

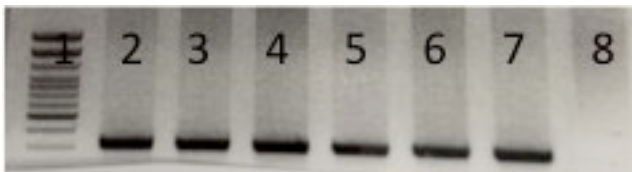
Usp20(50726)MR CACAACGGGTTCTTCTGTTAGTCC

Wild-type Reaction



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

Mutant Reaction



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