

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30438
MMRRC Strain Name	B6.Cg-Tg(H2-K-DUSP5)21Wjl/Mmnc
Gene Name(s)	histocompatibility 2, K region, dual specificity phosphatase 5
Breeding Protocol(s)	Backcross to B6/J
Protocol Date	10/08/2019

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 Reactions

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: 399bp MUTANT: 303bp

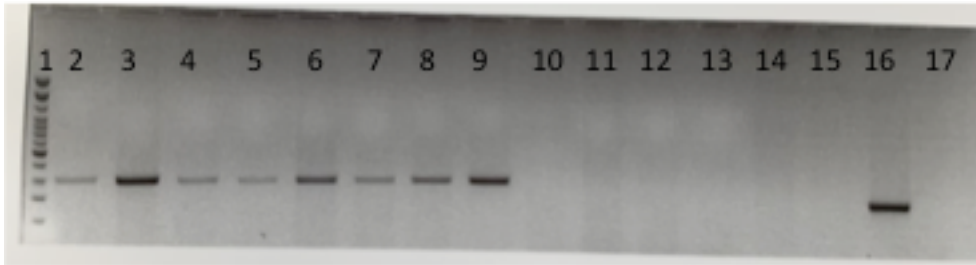
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Dus(30438)WF TAGGACACCCAAGTTAGTTGTTGG

Dus(30438)WR TCCTTGCCTCAACTTGTGAATACC

Dus(30438)MF ACCCACCTACACTACAAGTGGATC

Dus(30438)MR TGTGGAGGGCAGGATCTCAGATTC



1. 100bp Marker (Invitrogen)
2. Wild-type Reaction: Wild-type Sample
3. Wild-type Reaction: Wild-type Sample
4. Wild-type Reaction: Wild-type Sample
5. Wild-type Reaction: Wild-type Sample
6. Wild-type Reaction: Wild-type Sample
7. Wild-type Reaction: Wild-type Sample
8. Wild-type Reaction: Heterozygous Sample
9. Wild-type Reaction: Wild-type Control
10. Mutant Reaction: Wild-type Sample
11. Mutant Reaction: Wild-type Sample
12. Mutant Reaction: Wild-type Sample
13. Mutant Reaction: Wild-type Sample
14. Mutant Reaction: Wild-type Sample
15. Mutant Reaction: Wild-type Sample
16. Mutant Reaction: Heterozygous Sample
17. Mutant Reaction: Wild-type Control