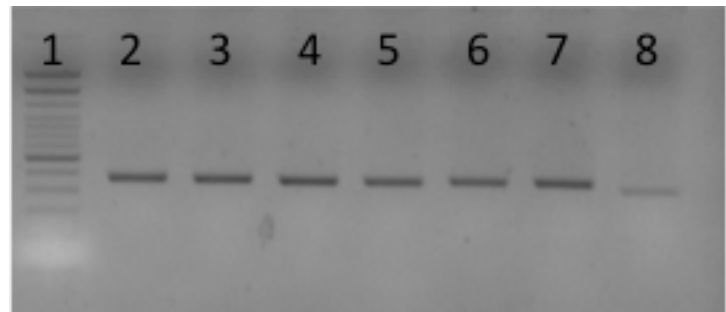


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	66517
MMRRC Strain Name	B6.129P2- <i>Dcaf1</i> ^{tm1.1Yxi} /Mmnc
Gene Name(s)	DDB1 and CUL4 associated factor 1
Breeding Protocol(s)	Backcross and random intra-strain mating
Protocol Date	11/17/2020

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



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

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

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 434bp MUTANT: 468bp

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

Vpr(66517)R: GCT GCC AAC TAT GGG TGC

Vpr(66517)F: CAG TTA GAG AGT GAC TTT GGA CG