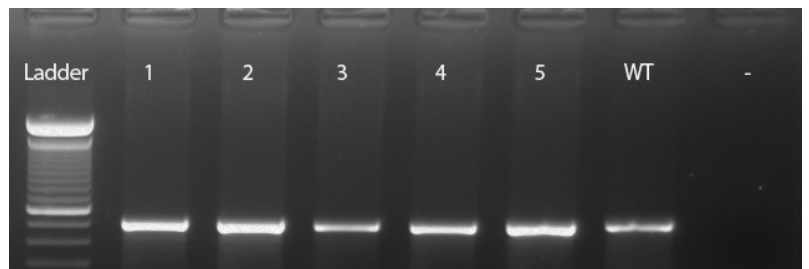


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11674
MMRRC Strain Name	B6;129S5- <i>Stub1</i> ^{tm1Lex} /Mmnc
Gene Name(s)	STIP1 homology and U-Box containing protein 1 (<i>Stub1</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	12/20/13

MMRRC #11674 PCR Reaction

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



WT Reaction

2,2 Primer Reactions: (1+2) for wild-type and (R+PGK) for the mutant reaction.

Thermal Cycler:

Step 1: 94°C for 5 min

Mutant Reaction

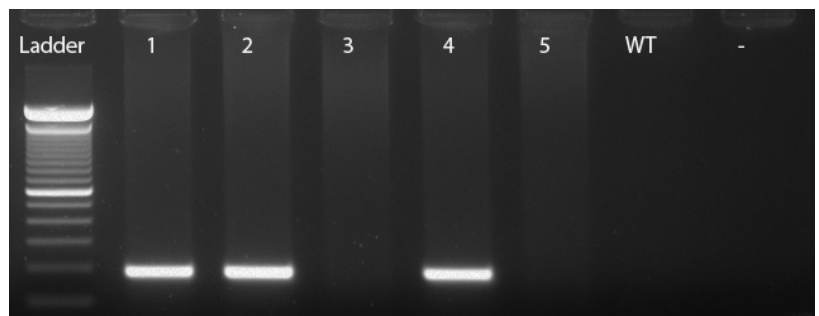
Step 2: 94°C for 45 sec

Step 3: 60°C for 45 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min



Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 187bp

WT: 500bp

Primer sequences 5' to 3':

NIH1616-1: GATCCGCCACCGGAACTTC

NIH1616-2: CGCAGCTACTTCTATAGCAAC

NIH1616-R: GATGTGGCCATCATCTTCATGACC

NIH1616-PGK: GTGGATGTGGAATGTGTGCGAGGCC



Run on 2.0% agarose gel in TAE.