

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31722
MMRRC Strain Name	STOCK <i>Fbx15</i> ^{L5Jcs32} +/+ <i>Rw</i> / <i>Mmnc</i>
Gene Name(s)	F-box and leucine-rich repeat protein 5 (<i>Fbx15</i>)
Breeding Protocol(s)	Random Intra-Strain Mating (cross het/white rump x het/white rump. Homo lethal for -/- and WR/WR)
Protocol Date	11/4/13

MMRRC# 31722 PCR Reaction

Thermal Cycler

Denaturing: 94°C for 45 sec
 Annealing Temp.: 55°C for 45 sec
 Extension: 72°C for 1.5 min
 Cycles: 35
 Taq: **Apex and Qiagen 10X Buffer**

Bands: MUTANT: 282bp
 WT: cuts with *Nla*III to 168bp & 114bp

	<u>1X</u>
DNA	1.0
10X Buffer	3.0
10mM dNTPs	0.2
20 uM Primer 1 & 2	1.0
Taq	1.0
ddH ₂ O	15.8
25mM MgCl ₂	2.0
Q solution	6.0

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

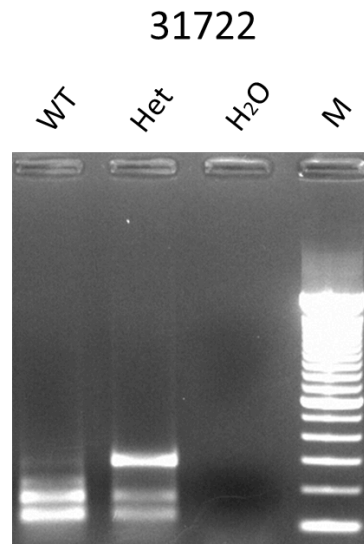
L: GAACCATTTGTTCTGCCTC

R: CAACAGCACATTTCTCCTTC

Restriction Digest:

4ul Buffer 4
 0.4ul BSA
 1ul *Nla*III
 5.2ul HOH
 Add 10ul of mix/reaction, total volume 40ul
 37C for 8 hours, 65C for 20 min, 4C forever

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)