

## MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	226
MMRRC Strain Name	B6.129S4- <i>Fn1</i> <sup>tm1Bwg</sup> /Mmnc
Gene Name(s)	Fibronectin 1 (Fn1)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	10/1/15

### MMRRC #226 PCR Reaction

	<b><u>1X</u></b>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

**2, 2 Primer Reactions (WTF + R) for the Wild-type and (MutF + R) for the Mutant reaction**

#### **Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 45 sec

Step 3: 55°C for 45 sec

Step 4: 72°C for 60 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

**Taq: Denville Taq (5U/ul) and Chromataq 5X Buffer**

**Bands:** WT: ~570bp MUTANT: ~900bp

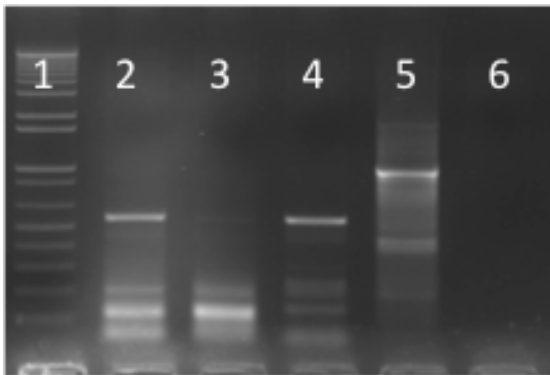
**Primer sequences 5' to 3'**: Primers are 10uM with respect to each primer

Fn1(226)R: GTACGTAACCAATGCTCGGT

Fn1(226)MutF: AATGCCTGGCTTAAGAGGGT

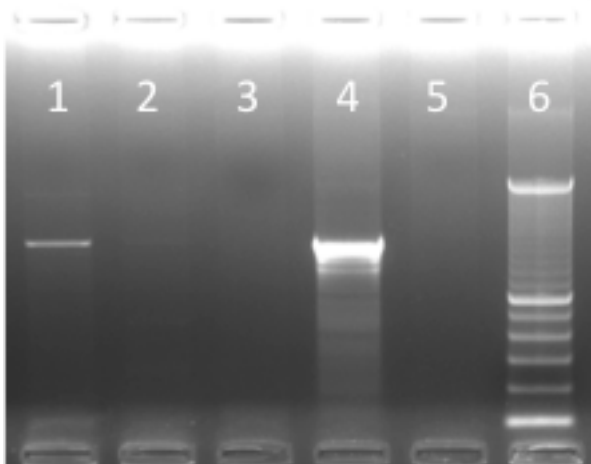
Fn1(226)WTF: AGATGGCTCAGAGGTTAGGAG

### Wild-type Reaction



1. 1kb+ Marker
2. Wild-type/Heterozygous Sample
3. Wild-type/Heterozygous Sample (Light Band)
4. Wild-type Control
5. Homozygous Positive Control
6. Negative Control

### Mutant Reaction



1. Mutant Sample
2. Mutant Sample (Light Band)
3. Wild-type Control
4. Mutant Positive Control
5. Negative Control
6. 100bp Marker