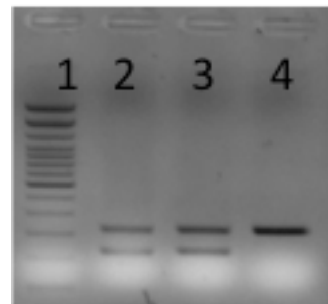


## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	68230
<b>MMRRC Strain Name</b>	B6.129X1- <i>Aft3</i> <sup>tm1Dron</sup> /HaiMmnc
<b>Gene Name(s)</b>	activating transcription factor 3
<b>Breeding Protocol(s)</b>	Backcross and random intra-strain mating
<b>Protocol Date</b>	5/4/2022

### PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	13
10X Buffer	2.5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	2
10uM Primer 2	1
10uM Primer 3	1
Taq	1
DNA	2



1. 100bp Ladder (Invitrogen)
2. Heterozygous Sample
3. Heterozygous Sample
4. Wild-type Control

#### **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

#### **Taq: Apex Taq and Apex 10X Buffer**

Bands: WT: 329bp    MUTANT: 236bp

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

Aft(68230)1: AGAGCTTCAGCAATGGTTTGC  
 Aft(68230)2: TGAAGAAGGTAAACACACCGTG  
 Aft(68230)3: ATCAGCCTCTGTTCCAC