

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	16824
<b>MMRRC Strain Name</b>	B6;129X1- <i>Cebpa</i> <sup>tm1Gonz</sup> /Mmnc
<b>Gene Name(s)</b>	CCAAT/enhancer binding protein (C/EBP), alpha
<b>Breeding Protocol(s)</b>	Random Intra-strain Mating
<b>Protocol Date</b>	3/3/14

### MMRRC #16824 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 60°C for 30 sec  
 Step 4: 72°C for 45 sec  
 Step 5: 35 × from step 2 to step 4  
 Step 6: 72°C for 7 min

	<b><u>1X</u></b>
ddH <sub>2</sub> O	8.5
5X Buffer	5.0
25 mM MgCl <sub>2</sub>	1.5
10 mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Denville Taq (5U/μl)	0.5
5 M Betaine	5
DNA	2

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

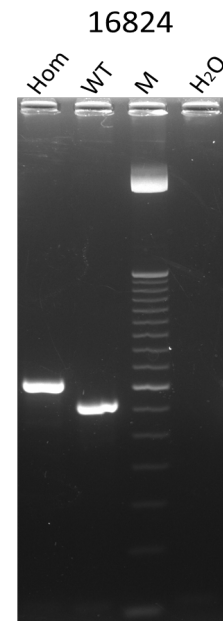
**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

P1 (16824): CCA CTC ACC GCC TTG GAA AGT CAC A

P2 (16824): CCG CGG CTC CAC CTC GTA GAA GTC G

**Bands expected:** WT: 311 bp  
 Floxed band: ~355 bp

Run on 2% agarose gel in TAE.



M: 50 bp DNA ladder (Invitrogen)