

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	34760
<b>MMRRC Strain Name</b>	B6.129S1- <i>Cebpb</i> <sup>tm1Es</sup> /Mmnc
<b>Gene Name(s)</b>	CCAAT/enhancer binding protein (C/EBP), beta ( <i>Cebpb</i> )
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	6/27/13

### MMRRC #34760 PCR Reaction

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 70°C for 30 sec  
 Step 4: 72°C for 30 sec  
 Step 5: Step 2 to 4; Cycles: 35  
 Step 6: 72°C for 7 min

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

	<b>1X</b>
ddH <sub>2</sub> O	13.25
5X Buffer	5.0
25mM MgCl <sub>2</sub>	1.5
10mM dNTPs	0.5
10 uM Primer Forward	0.5
10 uM Primer Reverse	0.5
Denville Taq (5U/μl)	0.5
DMSO	1.25
DNA	2

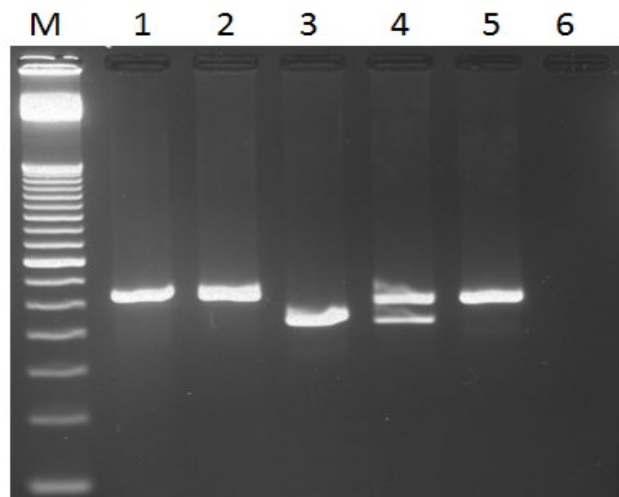
**Primer sequences 5' to 3':** Primers are 10uM with respect to each primer

CRP2 -F (34760): 5'---GAGCCACCGCGTCCTCCAGC---3'

CRP2 -R (34760): 5'---GGTCGGTGCGCGTCATTGCC---3'

**Bands:** WT 223 bp  
 MUTANT ~275 bp

Run on 3% agarose gel in TAE.



M: 50 bp DNA ladder (Invitrogen); Lane 1, 2 and 5: Hom; Lane 3: WT; Lane 4: Het; Lane 6: ddH<sub>2</sub>O