

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34759
MMRRC Strain Name	FVB.129S1(B6)- <i>Cebpb</i> ^{tm1Rcsm} /Mmnc
Gene Name(s)	CCAAT/enhancer binding protein (C/EBP), beta (<i>Cebpb</i>)
Breeding Protocol(s)	Backcross to FVB/N (Charles River)
Protocol Date	6/27/13

MMRRC #34759 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

	1X
ddH ₂ O	13.25
5X Buffer	5.0
25mM MgCl ₂	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

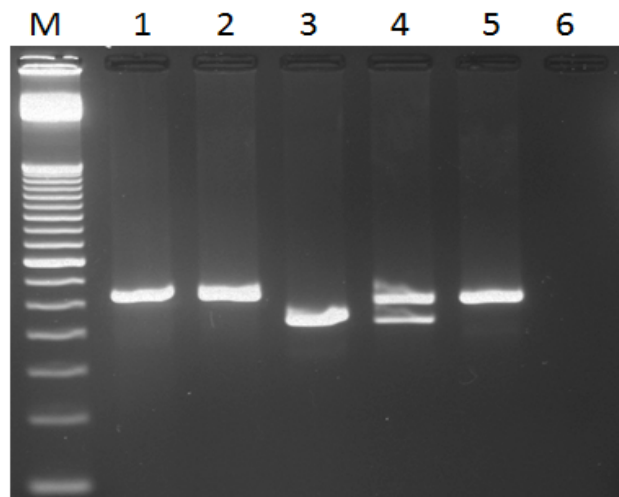
Primer sequences 5' to 3': Primers are 10uM with respect to each primer

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: ddH₂O