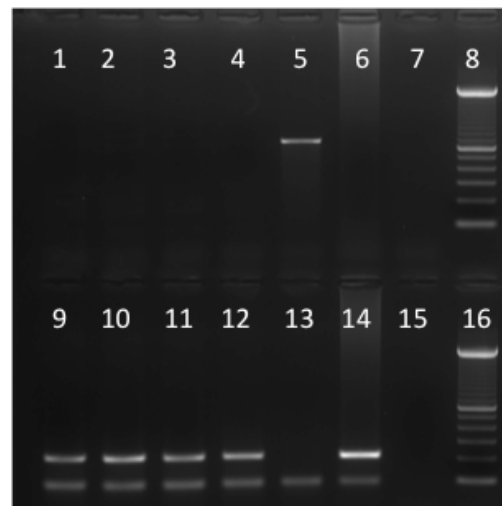


## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	11530
<b>MMRRC Strain Name</b>	B6;129- <i>Hbegf</i> <sup>tm1Dcl</sup> /Mmnc
<b>Gene Name(s)</b>	Heparin-binding EGF-like growth factor (Hbegf)
<b>Breeding Protocol(s)</b>	Intra-Strain Mating
<b>Protocol Date</b>	3/3/11

### MMRRC# 11530 PCR Reaction

	<b><u>1X</u></b>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer F/R+MUT	1.5
Taq	1.0
ddH <sub>2</sub> O	12.75
25mM MgCl <sub>2</sub>	2.0
DMSO	



Wild-type Reaction

1. Homozygous Sample
2. Homozygous Sample
3. Homozygous Sample
4. Homozygous Sample
5. Wild-type Control
6. Homozygous Positive Control
7. Negative Control
8. 100bp Marker

Mutant Reaction

9. Homozygous Sample
10. Homozygous Sample
11. Homozygous Sample
12. Homozygous Sample
13. Wild-type Control
14. Homozygous Positive Control
15. Negative Control
16. 100bp Marker

**Thermal Cycler:**

Denaturing: 94°C for 45 sec  
 Annealing Temp.: 55°C for 45 sec  
 Extension: 72°C for 1 min  
 Cycles: 35

Taq: **Apex and Chromataq 5X Buffer**

**Bands:** MUTANT: between 194bp and 234bp  
 WT: ~660bp

**Primer sequences 5' to 3':**

**HBEGF Wild Type Allele**

Forward (HBEGF-5) 5'-CTG ACA GAC CTT CAA GGG CT  
 Reverse (HBEGF-8) 5'-AAA GGT TCA GAT CTG TCC CT

**Knockout Allele**

Forward (HBEGF-5) 5'-CTG ACA GAC CTT CAA GGG CT  
 Reverse (ES-Neo): 21mer 5'-CCG GTG GAT GTG GAA TGT GTG

Run on 2.0% agarose gel in TAE.