

## **Ins2.H2B-Apple.hGH PCR Protocol**

The following conditions are used to genotype Ins2.Apple mouse genomic DNA.

### **PCR primers:**

Ins2.F1	5'-GAGGTGCTTTGGTCTATAAAGG-3' (forward)
Apple.R1	5'-GCATGAACTCCTTGATGATGG-3' (reverse)
Ins2.R1	5'-CCAAGTTACTACCACCAAGGAC-3' (reverse)

### **PCR reaction mixture:**

- 15.8 ul sterile water
- 2.5 ul 10X PCR buffer with MgCl<sub>2</sub>
- 4 ul dNTP premix
- 0.75 ul forward primer (20uM)
- 0.75 ul reverse primer (20uM)
- 1 ul dil. DNA template
- 0.2 ul Amplitaq Gold
- 25 ul total volume

dNTP premix: Made using 100 mM NEB dNTP's. The premix contains 250 ul of each dNTP - A, C, G, &T - and 19 ml sterile water and is stable at -20°C.

### **Cycling conditions:**

- 1 cycle - 94° x 6 min.
- 40 cycles - 94° x 1 min., 60° x 30 sec., 72° x 1 min.
- 1 cycle - 72° x 7 min.
- hold at 4°C.

### **Analysis of PCR products:**

Ins2.F1 and Apple.R1 produce a targeted band of 713 bp  
Ins2.F1 and Ins2.R1 produce a wildtype band of 620 bp