PCR protocol for genotyping *Gck*^{K414E} mouse line

PCR reagents:

1. oligo primers at 20 µM

   GK primers

   GKP2 5'-TGT CTC AAT TTG CTG TGT CCT CCA-3' (top)
   GKP8 5'-ATG TGT GAG TGT GCC AAT AT GAG T-3' (bottom)

   (GKP2 + GKP8 yelds a **636bp WT** (wild type allele) band and a **741bp TM** (targeted mutant allele) band.

2. Perkin Elmer PCR buffer with $MgCl_2$
3. 1.25mM dNTP premix (dNTP premix is made by using 100 mM NEB dNTP's. The premix contains 250 µl of each dNTP - A, C, G, &T - and 19 ml sterile water and is stable at -20°C. I freeze this in 1 ml aliquots and thaw and refreeze as needed).
4. Perkin Elmer Amplitaq Gold
5. genomic DNA samples diluted to 50 ng/µl with sterile water

PCR reaction mixture:

15.5 µl sterile water
2.5 µl 10X PCR buffer
4 µl dNTP premix
0.75 µl primer GKP2
0.75 µl primer GKP8
1 µl dil. DNA template
0.2 µl Amplitaq Gold
25 µl total volume

Cycling conditions:

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

Analysis of PCR products:

Load 10 µl aliquots of reactions + 2 µl gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.