Generic PCR Protocol

Background This protocol is designed for use with PfuUltra (Stratagene). Note that the 10x PFUUltra reaction buffer already contains Mg^{++}. If you're using another polymerase (*e.g.* Invitrogen's Platinum Pfx) that does not include Mg^{++} in the reaction buffer, you'll need to add it in.

Oligos I always design my oligo's to have a $\geq$60 °C T_m, calculated based on a 50 mM salt concentration, and a 250 pM oligo concentration. My oligo concentrations are rarely 250 pM, but I never correct the T_m's for this.

Mix

1.25 μl 10x PfuUltra reaction buffer
0.25 μl 40 mM dNTP mix, (10 mM each)
0.25 μl forward primer, 100 pmoles/μl
0.25 μl reverse primer, 100 pmoles/μl
0.25 μl Template DNA
0.25 μl PfuUltra Hotstart (Stratagene)
10.0 μl sterile H_2O
12.5 μl total

PCR Program

1. 5 min @ 95°C
2. Repeat 30x
   (a) 30 s @ 95°C
   (b) 30 s @ 60°C
   (c) 1 min+1 min/1 kb template @ 72°C
3. hold @ 4°C

Trouble Shooting

If no product is seen, try repeating the protocol with 5% DMSO in the reaction mix. DMSO disrupts base pairing, facilitating strand separation in GC rich regions of DNA and reducing the propensity of the DNA to form secondary structure. The end effect, is a little DMSO will often get you past issues with poor primer design and/or difficult templates.