PFU PCR REACTIONS

1. Set up following PCR reaction:

   10 μl 10x Pfu buffer
   8 μl dNTPs (2.5 mM stock)
   2.5 μl Primer 1 (100 ng/μl)
   2.5 μl Primer 2 (100ng/μl)
   1μl Pfu enzyme
   xμl template (use around 10ng)
   yμl H_2O

   Make to 100μl with the H_2O

2. Run reaction in PCR machine. Typical cycles may be:

   94°C - 1min
   53°C - 1min
   72°C - 1min 30 sec

   for 30 cycles. End on a 10 min 72°C extension and a 4 °C soak.

3. Run on gel to check reaction. If cloning, purify PCR product from gel and. A-tail the product as it will be blunt ended.

NOTES

1. Extension times are normally longer for Pfu than Taq.
2. If amplification doesn't work first time, add extra $MgCl_2$.
3. Annealing temperatures will vary.
4. Need to resolve Pfu product on a gel as template will contaminate ligation and will transform a lot better than any ligated target vector