POLYMERASE CHAIN REACTION USING TAQ BEADS

DNA template: Use cDNA from Reverse Transcription reaction or plasmid DNA for amplification in PCR.

Reagents Needed:

Forward and reverse primers (10µM)
puReTaq Ready-To-Go PCR beads in 0.5ml tubes (Amersham Biosciences Cat # 27-9558-01)

Protocol

1. Resuspend the PCR bead in 18 µl of RNase/ DNase free H_20. Let bead dissolve.
2. Add 5 ul of cDNA from RT , If using plasmid DNA, add 100ng (if amount exceeds 5µl, resuspend bead in less dH_2O, the object being to have a 25µl total volume reaction)
3. May/ may not need to include positive control (ask)
4. Negative control : Resuspend bead in 23 ul dH_2O (no DNA)
5. Add 1 µl (10µM) each of **forward** and **reverse** primers.
6. Vortex gently and spin down the contents of the tube.
7. Cycle on the PCR program on the thermal cycler in 5S-19 (PCR program): program is preset except for entering your particular melting temp (step c)…can be setup to run overnight.
   a. 80˚C for 5 minutes
   b. 94˚C for 1 minute
   c. X˚C for 1 minute (use temp 5 degrees lower than lowest melting temp of your
   d. 72˚C for 1 minute
   e. Repeat b-d for 27 cycles
   f. 72˚C for 10 minutes
   g. 4˚C ∞

DNA Gel:

1. Pour a 1% agarose gel w/ ethidium bromide in gel (5 µl/ 100 ml gel; 10 mg/ml EtBr stock) with a 10-lane comb (typical). Use 1x TAE buffer for reservoir.
2. Add 5 ul of **DNA LB WITHOUT dye** to each PCR product vial. Don't heat samples!
3. Load your samples on gel. Include wells with
   a. 8 ul of Invitrogen 100bp ladder prepared solution
   b. Dye only control well with Loading buffer WITH dye (20 ul water + 5 ul LB w/ dye)
4. Run gel at 100 V for ~90-120 min.

Take a photo of your gel using camera in Dr. Shewchuck's lab.
Determine size of PCR product using the ladder.