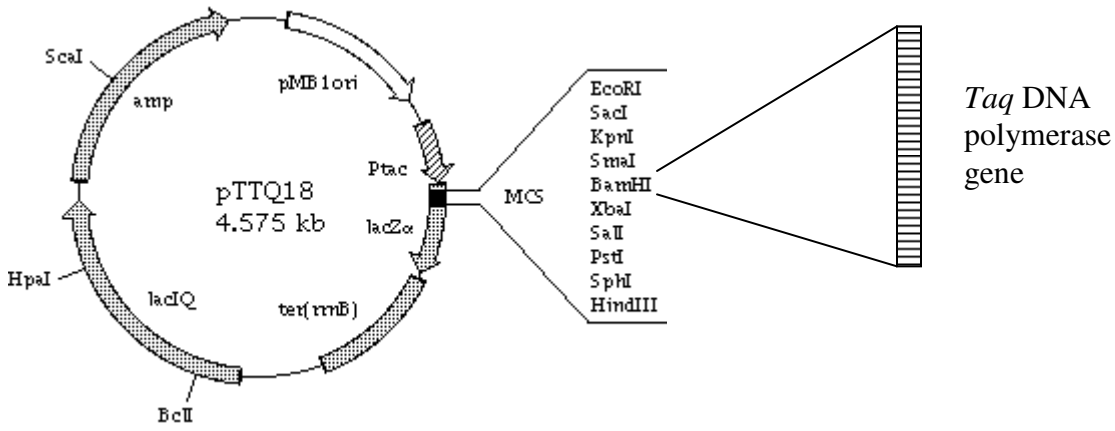
Recombinant *Taq* DNA polymerase purification



In today's lab we will purify recombinant *Taq* DNA polymerase and assay for its function. The recombinant protein will be purified from an *E. coli* strain containing the pTTQ18 plasmid shown above that contains the *Taq DNA polymerase I* gene cloned behind a strong and repressible promoter called Ptac. Ptac consists of the -10 and -35 regions of the Trp operon promoter and the LacO element from the *lac* operon. pTTQ18 also contains a *lacI* gene (why?) and an ampicillin marker gene. We are using this plasmid instead of the pGEM/Taq plasmid you constructed because pTTQ18 is a better plasmid for expression due to its strong promotor.

Protein expression and purification

1. 200 ml LB media containing ampicillin was inoculated with *E. coli* containing the pTTQ18 plasmid.
2. The culture was grown at 37 C with shaking at 250 rpm to an A_{600} of ~0.3; IPTG was then added to 0.2 mM; the culture was further incubated with shaking for another 12 hours.
3. **Students start here:** Obtain 30 ml of cells per group in a 50 ml tube and spin 5' at 4300 rpm; discard supernatant and resuspend cell pellet in 2 mL of buffer A (50 mM Tris-HCl, pH 7.9, 50 mM dextrose, 1 mM EDTA) containing 4 mg/mL lysozyme* and incubate 5 minutes at room temperature.
4. Add 2 mL of buffer B (10 mM Tris-HCl, pH 7.9, 50 mM KCl 0.5% Tween 20 detergent, 0.5% NP40 detergent) and incubate the mixture for 30 minutes at 75 C in a water bath. Mix cells by inverting a few times every 5 minutes.
5. Transfer cell extract to purple-top centrifuge tube and spin at 12,000 rpm for 10 minutes at 10 C in the high speed centrifuge (in room beside Cell lab) and an SS34 rotor to pellet denatured proteins.
6. Pour supernatant into a 15 ml tube and mix with an <u>equal volume</u> of storage buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.5 mM DTT, 1% Triton X-100 detergent) containing 50% glycerol. Give tube to instructor for safe keeping.

Assay for DNA polymerase activity

Set up 4 PCR reactions on ice where you use the following *Taq* DNA polymerase preparations:

1. your purified protein fraction for the DNA polymerase
2. use a 1:10 dilution of your purified protein fraction (make in clean tube using sterile water)
3. use only water, no enzyme (negative control)
4. use commercially-prepared *Taq* polymerase (positive control)

PCR ingredients:

- 2.5 ul 10X PCR buffer
- 2.5 ul dNTPs (2.5 mM stock)
- 2 ul of primer mix: forward primer #85 (5 pmol/ul) reverse primer #86 (5 pmol/ul)
- 1 ul template DNA #264; use water for tube #3, the negative control.
- 15 ul water
- 2.0 ul of *Taq* DNA polymerase as indicated above

In addition to the 3 PCR tubes above, set up another reaction (tube #4) using purchased *Taq* DNA polymerase as a positive control:

NOTE: The template and primers used here should produce a 350 bp DNA fragment if amplification is successful. Next week, you will perform agarose gel electrophoresis to determine if your purified protein fraction indeed contains DNA polymerase activity.

***Interesting tidbit of history:** Lysozyme is an enzyme consisting of 129 amino acid residues with a molecular weight of 14.4 kDa. This enzyme can be found in egg white, tears, human breast milk and other secretions. It is responsible for breaking down the polysaccharide walls of many kinds of bacteria and thus it provides some protection against infection. Bacteria build a cell wall of carbohydrate chains, interlocked by short peptide strands, that braces the cell membrane against the cell's high osmotic pressure. Lysozyme breaks these carbohydrate chains, destroying the structural integrity of the cell wall, causing the bacteria to burst under their own internal pressure. Alexander Fleming discovered lysozyme during a deliberate search for medical antibiotics. Over a period of years, he added everything that he could think of to bacterial cultures, looking for anything that would slow their growth. He discovered lysozyme by chance: One day, when he had a cold, some of his nasal drippings fell into culture plate and, much to his surprise, it killed the bacteria. He had discovered one of our own natural defenses against infection. A solution of lysozyme can be applied topically (actually currently used in disinfecting solution for body piercings, so I'm told); however it cannot rid the entire body of infection, because it is too large to travel between cells. Fortunately, Fleming continued his research, finding a true antibiotic drug five years later: *penicillin*.

Questions

1. Draw what you think next weeks agarose gel should look like, assuming your purified protein contains active *Taq* DNA polymerase? Label the lanes (all 4 tubes) and include a marker lane.

2. What is the function of 75 C incubation step of the purification protocol? How does it help in purifying the DNA polymerase?

3. What is the function of IPTG in step 2? IPTG (MW = 238) is often sold has a stock solution at 24 mg/ml. How many ml of this stock should be added to the culture in step 2?

4. Draw and label the promoter used in the pTTQ18 plasmid? What is the function of all the other genetic elements labeled in the pTTQ18 plasmid.

5. If each microliter of a commercial *Taq* DNA polymerase preparation costs $1.20, and assuming your protein fraction has similar specific activity, how much could you sell your preparation for? (we often find that our preparation is 10 times more concentrated that what we can buy – how does this change your profits?)

Following week:

Did you successfully purify *Taq* DNA polymerase last week?

1. Prepare and pour a 1.5% agarose gel
2. Add 5 ul of DNA Loading Buffer to your PCR samples 1, 2, and 3. #4 already contains loading buffer (green in this case) from the commercial *Taq* polymerase stock.
3. Load 5 ul your PCR sample into the gel and electrophorese for 20-30' at 200V.
4. Take a picture of the gel. Did it work? How does your polymerase preparation compare to the commercial enzyme?
5. You will need this information for doing the next week's CSI lab.