

## PCR lab      Genetics 362

In today's lab, you will set up a polymerase chain reaction (PCR) using the plant DNA from last week as a template. You will be amplifying a region of DNA called the internal transcribed spacer (ITS). After the PCR reaction is finished, you will have many DNA copies of this region. The reaction will take two hours to run in the thermocycler, so you won't analyze your results until next week, when you will run your PCR product on an agarose gel. The following week, you will sequence your PCR product. DNA sequence from this particular genomic region is commonly used to determine how different species are related to each other.

### Components of a PCR reaction:

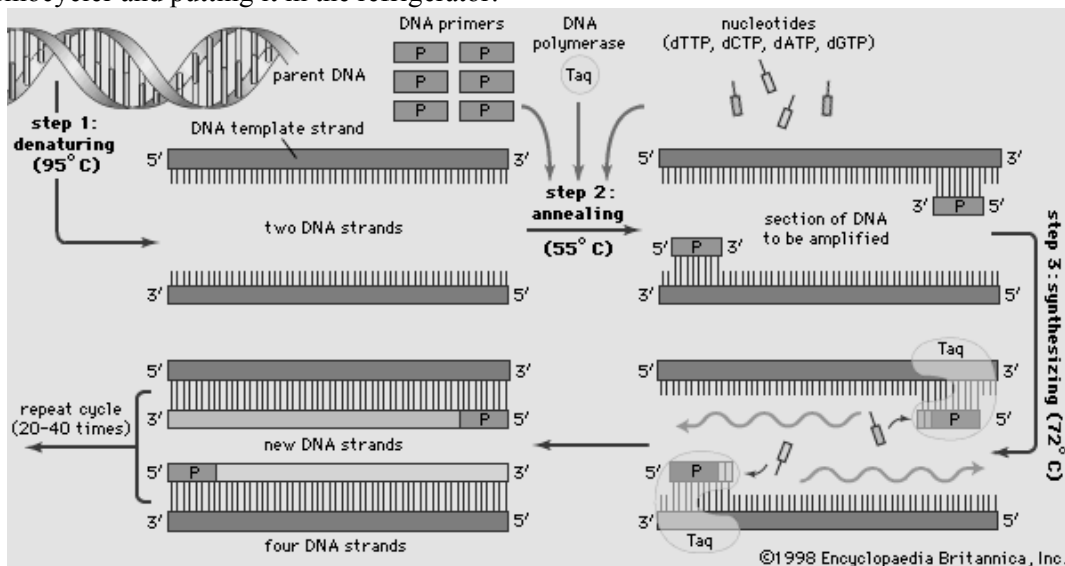
PCR is *in vitro* DNA replication. Some of the same components needed for DNA replication *in vivo* are also required *in vitro*. For instance, **template** DNA is required, **primers** are required for strand extension (addition of new nucleotides), **nucleotides** (dNTPs) are needed for the growing DNA strands, and **DNA polymerase** carries out strand extension (adds the nucleotides). We also need to include a buffer to stabilize the pH, and  $Mg^{++}$ , which most enzymes, including DNA polymerase, require in order to function.

All of the necessary components except template and primer are already in the PCR tube. They have been dried down into a small bead. This is an extremely convenient way set up PCR, which minimizes human error. All you need to do is add water, DNA template (your plant DNA) and primer. Then you place your reaction on the thermocycler. The thermocycler will do the rest of the work, cycling through temperatures to stimulate the various stages of DNA replication.

The temperatures and times used in a PCR reaction vary according to the region that you are attempting to amplify. We will use the following conditions:

### Thermocycling conditions:

- 1) **95°C 5 minutes** This denatures the two strands of genomic DNA (splits them apart).
- 2) **94°C 30 seconds** This denatures the newly synthesized strands at the beginning of each cycle
- 3) **52°C 30 seconds** This allows the primers to anneal (bind) to the DNA where it is complementary
- 4) **72°C 1 minute** This is the optimal temperature for polymerase activity, so new DNA is synthesized
- 5) **go to 2 and repeat 35 times**      Steps 2-4 are repeated over and over to synthesize many copies of DNA
- 6) **72°C 10 minutes** This allows any strands that may not have been synthesized to completion to finish extension
- 7) **4°C forever**      This step refrigerates your sample until someone gets around to taking it off of the thermocycler and putting it in the refrigerator.



## Protocol:

### Obtain:

Your DNA extraction  
One PCR tube (everybody can have their own)  
A tube of water  
A 100ul or 200ul pipettor, and a 2ul or 10ul pipettor

Hydrate your DNA:

- Add:**  
**50ul DNA hydration solution to your dried DNA extraction.**  
The DNA will dissolve in the liquid. DNA hydration solution contains a buffer to stabilize the pH and help preserve the DNA.
  - Flick the tube** with your finger for about 5 minutes go help the DNA dissolve.
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Set up PCR:

- Label your PCR tube.** The best place to label it is on the little tab on the cap with an extrafine sharpie. If you use sharpie on the side of the tube or the top of the cap, it is likely to rub off in the thermocycler.

In the next step, pipette very carefully to ensure that your volumes are accurate. Change the pipettor tips between solutions to make sure you don't contaminate any of the solutions.

- Add to your PCR tube:**  
23ul sterile, deionized water (the small tubes of water)  
1ul of your hydrated plant DNA  
0.5ul of primer 1 (ITS4)  
0.5ul of primer 2 (ITS-18S)
- Close the lid** of the PCR tube. Make sure it is totally closed.
- Mix** the contents of the tube by flicking with your finger until the white foamy stuff is totally dissolved. If a vortex is available, you can use the vortex. It may take a few minutes to dissolve your sample.
- Give your reaction to your TA.** She will quickly spin it down in the centrifuge and place it in the thermocycler. When all of the reactions are ready, she will start the thermocycler.

**PCR Questions:**

1) What is the role of each of the following components of PCR?  
template

primers

polymerase

nucleotides (deoxynucleotide triphosphate, dNTPs)

2) If we used different primers, how would the results of the PCR reaction change?

3) Draw the steps of PCR, and show how this allows us to amplify a specific region of DNA.