

# THE POLYMERASE CHAIN REACTION (PCR)

## OBJECTIVE:

To amplify a region of ribosomal DNA from a small amount of genomic DNA.

## INTRODUCTION:

The polymerase chain reaction (PCR) was developed in 1983 by Kary Mullis, a nucleic acids chemist (then at Cetus). Since the first published reports of PCR, in 1985, over a thousand papers and several manuals have been published using PCR or describing other applications of this technology (see, e.g., Erlich et al., 1988; McMahon et al., 1987; Oste, 1988; Sakai et al., 1988; Wrischnik et al., 1987). It is a very powerful molecular biology method, because of its simplicity and its sensitivity. It can also be used to amplify single stranded DNAs, by asymmetric priming (White, et al., 1990), that can be used as templates for DNA sequencing. Through cycles of heating and cooling, it is possible to amplify a single DNA molecule into several thousands to several million in a few hours. Initially, while simple, the method was labor intensive, since during each cycle of heating and cooling, more enzyme (DNA polymerase) had to be added, since it was inactivated by the heating step. With the recent switching to a heat stable DNA polymerase (*Taq* polymerase, isolated from the thermophilic bacterium, *Thermus aquaticus*), and automated thermal cyclers, the amount of time needed to perform the amplifications is limited to the time necessary to set up the reaction mixes (usually about 1 hour).

While one of the biggest advantages of PCR is its ability to amplify even a single DNA molecule, this is also probably its most serious disadvantage. Since the method does not necessarily discriminate between the DNA of interest and a contaminating DNA molecule, either molecule may be amplified. This means that contamination must be kept at a minimum, otherwise, contaminating DNAs will be amplified, either giving invalid or confusing results.

Essentially, the method is as follows: a heat stable polymerase (*Taq*), two synthesized primer DNAs (each about 20 bp in length, one homologous to the left end of the region to be amplified and one homologous to the opposite DNA strand on the right end of the region to be amplified), deoxynucleotides, a buffer and a small amount of the test DNA (usually around 10 ng) are mixed. The mixture is taken through 20-40 cycles of heating (to 94°C, to denature the DNA), cooling (usually between 50°C to 60°C, to anneal the primers to the test DNA, and to begin polymerization of new DNA) and heating (to 72°C, to complete polymerization of the new DNA strands). Initially, longer strands of DNA predominate in each cycle, but after a few cycles, the majority of amplification products are ones that span from the first primer to the second primer. The DNA can be amplified from a few copies to 10<sup>5</sup> to 10<sup>6</sup> in 3-6 hours. We will be amplifying ribosomal DNA, which already exists in hundreds to thousands of copies, so that amplification of this DNA is further facilitated.

## STEPS IN THE PROCEDURE:

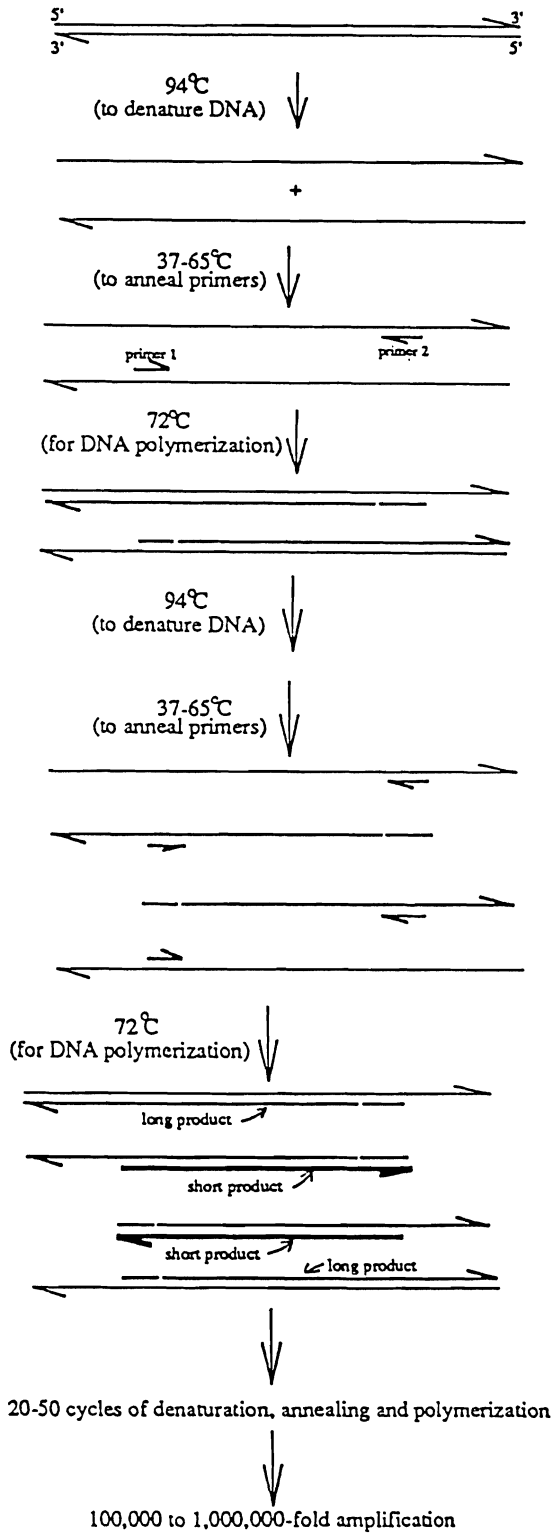
1. Dilute the DNA sample to 5 ng/ $\mu$ l.
2. Mix the following in a 0.5 ml microfuge tube on ice:
  - 10  $\mu$ l Master mix
  - 1.25  $\mu$ l primer #1 (ITS4)
  - 1.25  $\mu$ l primer #2 (ITS5)
  - 2.5  $\mu$ l DNA (10 ng total)
  - 10  $\mu$ l H<sub>2</sub>O
3. Place 35 ml of light mineral oil into each tube. Place the samples in the Thermal Controller (or similar computerized heating block). The following program is suggested: 30 cycles of; 1 min @ 95°C, 1 min. @ 55°C and 2 min @ 72°C. Generally, a 10 min incubation @ 72°C follows the last cycle.
3. Return when samples have gone through the entire sequence of cycles.
4. Run 2  $\mu$ l of each of the amplified samples on a gel, to confirm the amplification.

## NOTES ON STEPS IN THE PROCEDURE:

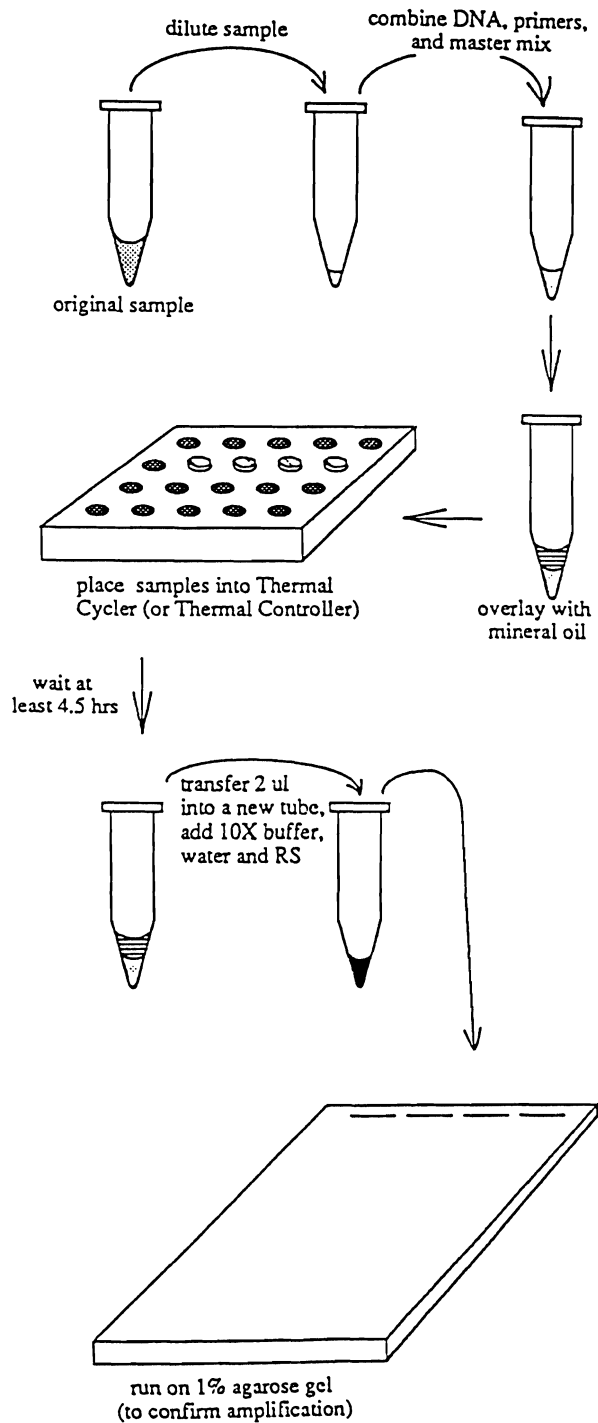
1. Generally, 10 ng of DNA in a total reaction volume of 25 ml is used. Larger reaction volumes are sometimes desired and the reaction mix can be scaled up accordingly. If you want to alter the proportion of DNA added, usually the addition of smaller amounts of DNA work better than larger amounts.[You will be adding 2 ml, or 10 ng, of this DNA into the reaction mixture.]
2. Mix thoroughly. The sequence of ITS4 (5' - 3') is TCCTCCGCTTATTGATATGC, and ITS5 is GGAAGTAAAAGTCGTAACAAGG (White et al., 1990).
3. The mineral oil prevents evaporation of the liquid from the reaction mixture, due to the high temperatures used. To ensure efficient heat transfer between the block and the tube, a few drops of mineral oil can be added to the tube receptacles in the heating block. This prevents evaporation during the heating phases of the process.
4. It will take approximately 4-5 hours to complete the 30 cycles (described above). You should then place your amplified sample in the -20°C freezer until you are ready to run the samples on a gel.

# PCR

## Theory



## Method



5. Generally, 2  $\mu$ l will contain 10-100 ng (or more) of the amplified DNA. This will be easily observed on an ethidium bromide stained agarose gel. Since the fragments we are amplifying are around 500-1000 bp in length, a 1.0 % gel should be used.

## **SOLUTIONS:**

### **Master Mix**

280  $\mu$ l dNTP mix (see below)

175  $\mu$ l 10X buffer (see below)

15  $\mu$ l *Taq* DNA polymerase (5 units/ $\mu$ l) [NOTE: Do not use "sequencing grade" *Taq* DNA polymerase.]

280  $\mu$ l H<sub>2</sub>O (distilled and sterilized)

### **dNTP mix**

125  $\mu$ l dATP solution (10 mM)

125  $\mu$ l dCTP " "

125  $\mu$ l dGTP " "

125  $\mu$ l dTTP " "

500  $\mu$ l H<sub>2</sub>O (distilled, sterile)

### **10X Buffer solution**

100 mM KCl

200 mM Tris-Cl (pH 8.3)

100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>\*

20 mM MgCl<sub>2</sub>

1% Triton X-100

1 mg/ml BSA

### **DNA Molecular Weight Markers**

123 bp or 100 bp ladder (BRL)

[3-5  $\mu$ l of this is used in one lane on the gel.]

### **Primers**

stock solutions of primers should be at 10 $\mu$ M

### **0.1X TE**

### **Gel Apparatus**

(\* Optional)

## REFERENCES:

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- Sakai, R.K., G.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* 239:487-491.
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- Wrischnik, L.A., R.G. Higuchi, M. Stoneking, H.A. Erlich and H.H. Kazazian, Jr. 1987. Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. *Nucleic Acids Res.* 15:529-542.