# Lab 3: – Polymerase Chain Reaction (PCR)

**Background**

We will use PCR to amplify the 5'-end of the mitochondrial DNA (mtDNA) control region of different duck species. The sequence you will amplify is about 600 base pairs long. You will begin by using the genomic DNA you extracted from tissue last week. Polymerase Chain Reaction (PCR) uses a template to make many copies of target DNA. In a PCR reaction, the DNA is incubated in a test tube with Taq polymerase, dNTPs, Mg2+, and short pieces of DNA called primers that bind to the template. PCR is a technique developed by Kary Mullis and useful for amplifying DNA with high selectivity (but PCR can also be used to amplify DNA under less selective conditions). The Taq polymerase used in PCR is a DNA polymerase that is stable at high temperatures. If you look at the temperatures we will expose the DNA to in the thermal-cycler, you will see that we nearly boil the PCR solution. Under these circumstances, most DNA polymerases would denature and lose their function. Taq polymerase is capable of withstanding high temperatures because it is derived from *Thermus aquaticus*, an archeobacteria that lives in hot springs. Under these hot conditions, Taq polymerase is stable and does not denature.

We also will use oligonucleotide DNA primers. The primers are synthetic sequences of DNA that complement a specific segment of template DNA (in this case, the template DNA is the 5' sequence of the mtDNA control region). The primers bind to the template DNA, and the Taq polymerase synthesizes DNA beginning at the 3' end of the primer. In this lab, we will use the primers provided by your TA and a kit from QIAGEN that contains the Taq, PCR buffer, Mg2+, and dNTPs.

**Materials**

0.2 mL thin-walled PCR tubes
Distilled water
Taq polymerase
PCR buffer
Mg2+
dNTPs mix
Forward and reverse primers pre-mixed by your TA
Pipettes and tips
Template DNA (your extracted DNA from last week)
PCR machine (thermocycler)

**Methods**
Work individually using your own DNA extract and PCR tube. Your TA will do a negative control, which contains everything but DNA extract in a separate PCR tube.

**Procedure**
1. Obtain the tube that contains your DNA.

2. Remove 2 ul of DNA extract and place it in a new 0.2 mL PCR tube.

3. Next add the following in this order to each tube:
- 1 ul Taq polymerase
- 20 ul PCR buffer
- 1 ul Mg2+
- 4 ul dNTPs mix
- 17 ul distilled H20
- 2.5 ul L78 primer (5'-GTTATTTGGTTATGCATATCGTG-3')
- 2.5 ul H774 primer (5'-CCATATACGCCAACCGTCTC-3')

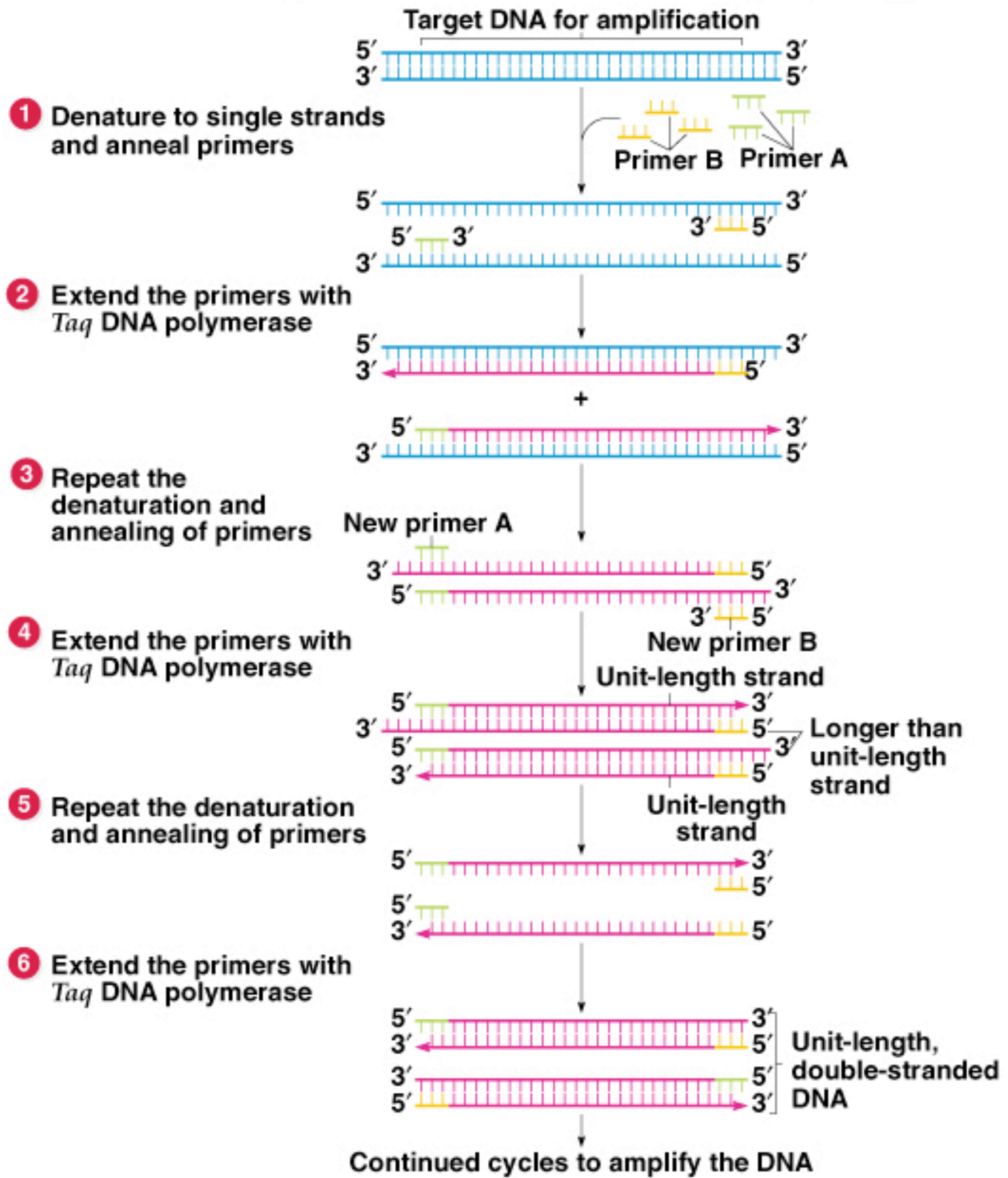
3. Cap your tubes (do not shake or invert).

4. Label the PCR tube as indicated, and place it on ice until your TA instructs you to proceed to the next step.

5. Place the PCR tubes in the ThermalCycler. Once all of the PCR samples are loaded, the TA will initiate the thermal-cycling protocol.

The thermal-cycler protocol is as follows:
Start with 7 minutes at 94 ºC
45 cycles of:
20 seconds at 94 ºC
20 seconds at 52 ºC
1 minute at 72 ºC
After 45 cycles, 7 minutes at 72 ºC
Finally, an infinite hold at 4 ºC

At each cycle, exponential amplification of the template occurs. The 20 seconds at 94 ºC denatures double stranded DNA into single strands. Dropping the temperature down to 52 ºC allows for annealing of the primers (i.e., the DNA primers in solution find the areas they compliment and stick to them). We then bring the temperature back up to 72 ºC. With the DNA primers on the template DNA, Taq polymerase begins synthesizing new DNA, making complimentary strands to our template DNA. Taq polymerase can do this at 52 ºC, but it is slow. Bringing up the temperature to 72 ºC speeds up the process, and repeating the cycles 45 times makes approximately $3.5 \times 10^{13}$ identical copies of the original template DNA (this assumes maximum efficiency).



Original double-stranded DNA containing target sequences
Target DNA for amplification
1 Denature to single strands and anneal primers
Primer B
Primer A
2 Extend the primers with Taq DNA polymerase
3 Repeat the denaturation and annealing of primers
New primer A
New primer B
4 Extend the primers with Taq DNA polymerase
Unit-length strand
Longer than unit-length strand
Unit-length strand
5 Repeat the denaturation and annealing of primers
6 Extend the primers with Taq DNA polymerase
Unit-length, double-stranded DNA
Continued cycles to amplify the DNA