Standard PCR Conditions in the Aguirre Lab

Introduction: The Polymerase Chain Reaction (PCR) is one of the most useful methods in molecular biology. PCR allows researchers to make millions of copies of a small fragment of the genome (≤1000bp) for downstream applications (e.g., sequencing).

PCR reactions involve taking DNA extracted from organisms and combining it with the following reagents to amplify a predetermined gene: nucleotides (dntps) - the A, C, T, and G that make up DNA, a DNA polymerase called Taq - The protein that does the work, magnesium - A cofactor for the polymerase, primers - Single stranded DNA fragments typically 18-22 bp long that are complementary to the DNA fragment to be amplified and mark the beginning and end of said fragment, PCR buffer - Maintains appropriate conditions for the reaction, and molecular biology grade water - filler. In our lab, we also typically add Bovine Serum Albumin (BSA) - Binds impurities that can interfere with the PCR reaction. The reaction mix is then run on a thermocycler, a machine that can quickly and very precisely change the temperature of the reaction.

The PCR reaction typically consists of three phases: 1) Denaturation (~90-95°C): When the genomic double-stranded DNA strands separate, 2) Annealing (~45-65°C): When the primers bind to the template DNA, and 3) Extension (~72°C): When the polymerase makes copies of the template DNA (Fig. 1). The annealing temperature is the most variable because it depends on the primer/template sequence. These phases repeat over and over in a series of 30-40 cycles on the thermocycler. Below are the reagent concentrations and cycling conditions that we typically use:

Reagents: We run 10-50µl reactions depending on how much PCR product we want. To just see how the PCR reaction works, 10 µl is enough for an agarose gel. For Sanger sequencing, we use 50µl reactions and for Nanopore sequencing, we use 20-30µl reactions. The following is for a 10µl reaction:

Stock Concentration	**Volume (Conc.) in 10 ul Reaction**
Molecular Biology Grade Water	3.9 µl
BSA (2 units/µL)	2 µl (0.4 U/µl)
Buffer (10X)	1 µl (1X. Components: 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3@25°C)
dntps (10 mM)	0.25 µl (250 µM)
Extra Mg (50 mM)	0.1 µl (0.5 mM; so with Mg in buffer, final Mg concentration = 2mM)
Forward Primer (10 µM)	0.35 µl (0.35 µM)
Reverse Primer (10 µM)	0.35 µl (0.35 µM)
Taq (5 units/µl)	0.05 µl (0.25 Units)
DNA	2 µl (~4 to >50ng depending on DNA concentration)

Cycling conditions: Our standard PCR protocols on the thermocycler start with a letter "K" for Kingsley because the protocol was taken from the first "Molecular Biology of the Threespine Stickleback" course offered at Stanford University ~2003 and organized by Dr. David Kingsley. The number that follows indicates the annealing temperature. So K50 is the protocol below with an annealing temperature of 50°C.

- One cycle:
  - 95°C for 1 min 45 sec
  - Annealing temperature for 45 s
  - 72°C for 45 sec
- Four cycles:
  - 94°C for 45 s
  - Annealing temperature for 45 s
  - 72°C for 45 sec
- Thirty cycles:
  - 92°C for 30 s
  - Annealing temperature for 45 s
  - 72°C for 45 sec
- Final extension of 72°C for 7 min.
- 4°C Forever

VENDORS: Where we get our reagents from and the costs.

Reagent	Vendor – Catalog Number	Cost
Molecular Biology Grade Water (100ml – 6 pack)	VWR - 95000-094	47.36
Bovine Serum Albumin (BSA)[1]	NA	NA
Buffer (10X)	New England Biolabs. Comes with Taq. See below	NA
dntps	VWR - 101414-958	$176
Magnesium (Mg)[2]	NA	NA
Primers	Thermo Fisher Scientific	~$10-25 depending on length and amount
Taq (2000 U)	New England Biolabs: M0273L	$289
Strip Tubes	USA Scientific - 1402-1700	$92.90

[1]We bought BSA >10 years ago and keep a stock solution in the lab.
[2]We bought Mg >10 years ago and keep a stock solution in the lab.

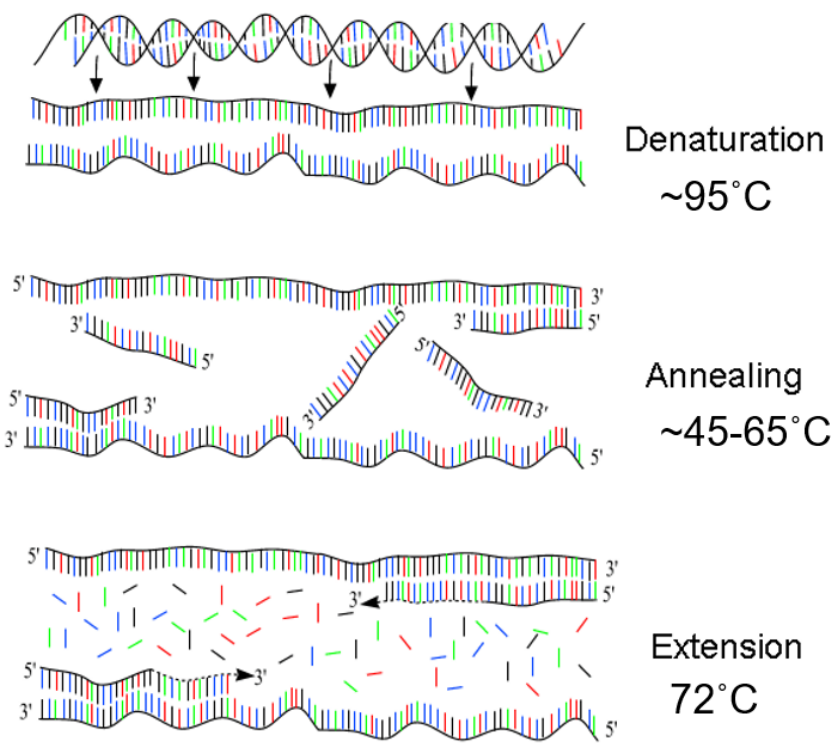

Fig. 1. Phases of a typical PCR reaction.