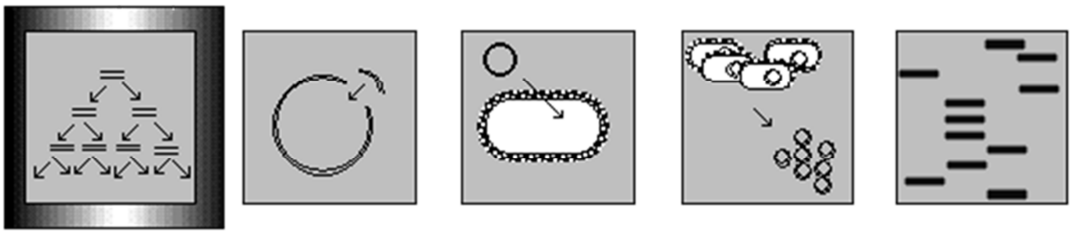
DAY 1 - PCR



Introduction

Reading: Dale et al., 3rd Ed: p109–119.

In this lab, you will set up and analyse a **Polymerase Chain Reaction (PCR).** PCR, invented in 1984, has become essential to almost every aspect of molecular research and clinical diagnosis. The *Drosophila* genome project, like that for humans, is completed and we therefore have access to the complete sequence of the *Nrg* gene from normal flies. We have designed primers for you that will anneal to the sequence either side of our target region and amplify specifically this DNA. You will have a go at designing PCR primers while your reactions are in the PCR machine.

To perform PCR, the following are placed in a plastic eppendorf tube:

- a DNA template (which can be either genomic or cDNA, derived from RNA)
- a FORWARD primer, which complements the reverse strand
- a REVERSE primer, which complements the forward strand
- excess nucleotide monomers ("dNTPs")
- a suitable buffer
- *Taq* DNA polymerase

The ingredients are mixed and placed in a computer-controlled heating block. The reaction runs over approximately 3 hours.

Today, PCR is so widely performed that the basic reagents can be purchased ready-mixed. Your PCR "Master Mix" already contains the dNTPs, buffer and *Taq* polymerase, and so only requires primers and template to be added. It also contains a green dye as a visual aid. You will set up a PCR, and while this is running, you will attempt to design some primers. When the PCR has finished, you will run the products out on a gel, quantify the product and purify the DNA. If the reactions succeed we will save the DNA for next time, when we will attempt to clone it into the plasmid cloning vector pBluescript.

Method

1. Setting up the PCR

Before starting, make sure you know how to dispense liquids **accurately** with a Gilson pipette (Appendix 2)! It is important that you work cleanly, or you will end up doing PCR with *human* genomic DNA as your template!

Each pair should set up **two** PCRs. Make sure you use thin-walled PCR tubes for this:

Tube 1: *Drosophila* genomic DNA template

Tube 2: No DNA negative control (adjust the final volume with **sterile** distilled water as required).

The ingredients and volumes are listed in the table below:

PCR Recipe:		Tube 1	Tube 2
	PCR Master Mix (dNTPs + *Taq* pol + buffer + dye)	25 µl	25 µl
	Template (*Drosophila* genomic DNA)	2 µl	
	Forward primer (10nM)	2 µl	2 µl
	Reverse primer (10nM)	2 µl	2 µl
	Sterile distilled water	19 µl	21 µl
	Final volume	**50 µl**	**50 µl**

Close the tubes, and write your initials on the top of each using a marker pen. Make sure you also distinguish which contains the template and which is the control. Mix the contents by flicking the tube and shake down or spin **very briefly at low speed** (less than 4K for a few seconds only) to collect the contents to the bottom of the tubes. Put the tubes into PCR machine when you are ready.

2. Running the PCR

Load the tubes into the PCR machine, around the temperature sensor tube. Your demonstrator will start the reaction, which will last around 3 hours. You can watch the progress of the temperature cycling on the display panel of the PCR machine

For this reaction, we will use the following programme:

Start		95°C	5 min	to ensure the genomic DNA is fully denatured
Then 5 X	denaturing	95°C	1 min	these 5 cycles have an extended denaturing as the original
	annealing	60°C	30 sec	genomic DNA will take a long time to denature after each
	extension	72°C	1 min	synthesis step
Followed by 25 X	denaturing	95°C	30 sec	in these 25 cycles the denaturing time has been reduced
	annealing	60°C	30 sec	as by now "short", easily denatured PCR products will
	extension	72°C	1 min	represent significant amounts of the available template
And finally		72°C	5 min	to ensure that all products are completely extended

While you are waiting for the PCR to finish, answer the questions at the end of this section and then work through '**Exercise 1: PCR and Primer Design** '.

3. After the PCR

We will now:-

A. Run some of the PCR product and the control reaction on an agarose gel to check the success of the PCR.

B. Save 5µl of the PCR product for running on a gel on a later day of the lab.

C. Purify the remaining PCR product from the other reagents, using a commercial DNA clean–up kit.

Each of these steps is described in the next section.

Questions

Q1. The template used in your PCR reaction can either be genomic DNA (gDNA) or complementary DNA (cDNA). What is the difference between these?

Q2. What does *Taq* DNA polymerase do in a PCR?

Q3. Thin-walled tubes cost ten times as much as conventional eppendorfs. Why do you think they are important for PCR?

Q4. Use the sequence and primer information of the gene (see **Exercise 1: PCR and Primer Design**, Figure 2) to calculate the expected size of the PCR fragment.

Q5. What is the function of the heated lid in the PCR machine?