General

DNA is remarkably stable chemically. In order to obtain purified DNA from cells and to study it, the DNA must first be separated from the rest of the cellular material. This involves destruction of the cell membrane (and/or cell wall), elimination of structural materials, and separation of proteins and RNA from the DNA. Because the extra cellular material differs among the major groups of organisms, the techniques for this are varied. We will be working with *E. coli*, which is a prokaryote and unicellular, removing its cell wall with the enzyme lysozyme. We will then chemically alter (denature) the membranes and proteins with the powerful detergent SDS. Then we will eliminate the cellular RNA with the enzyme ribonuclease, the proteins with the enzyme protease. Finally, we will remove these with chloroform and cause the DNA to precipitate out of solution with alcohol.

In today's lab, we will first learn how to use pipettes, then we will complete the DNA extraction with liquid bacterial cultures. We discard our *E. coli* DNA after the extraction, because there are too many impurities in it. The purpose of this laboratory is therefore to become familiar with the scientific instruments and principles used in DNA extraction.

Part 1: Using pipettes

**Introduction:** One of the most widely used tools in modern biology is the pipette. Today we will familiarize ourselves with the use of pipettes. You will need to remember these guidelines to properly complete our subsequent laboratory exercises.

**Large glass pipettes (1 ml, 5 ml, 10 ml)**

We will usually use reusable glass pipettes. These pipettes have been autoclaved and are therefore sterile. They are stored in metal boxes, which will be placed at the edge of the common tables in the middle of the lab. Do not put your fingers into these sterile boxes, and *never* place a pipette back into the box.

DO NOT use your pipette like a straw (mouth pipetting). To draw the solution into the pipette, we have specially modified syringes with clear polyvinyl fittings at the bottom. To use the pipette, select the syringe of appropriate volume and press the syringe onto the top of the pipette until it is firmly in place. Then you can place the pipette into the solution and remove the desired volume.

When you are finished with a reusable glass pipette, put it into the soaking tank for later washing.

**Activity 1 - using the 1 ml and 5 ml pipettes**

1a. Using the 1 ml pipette, transfer 0.2 ml of water from a beaker into a 2.5 ml eppendorf tube. Pipette 0.8 ml of water into a second tube. Mark the level of the water on each tube.

1b. Using the 5 ml pipette, transfer 4 ml of water from a beaker into a new plastic tube. Pipette 1 ml of water into a second tube. Note the height of the measured volumes in each tube.

**Pipette man pipettes (0.2 ml or 200 microliters, 0.02 ml or 20 microliters)**

These pipettes are used for measuring small volumes. They are superficially very similar, but be careful not to confuse them. You can ruin an experiment easily that way. The 0.02 ml pipette is labeled P20, and the 0.2 ml pipette is labeled P200. Become familiar with their other differences by comparing them.
Before using the pipette, press the plunger down and release it slowly. You should feel that it is easy to press at first, but then there is a stop with a stiffer spring. However, you can press further against this stiffer spring until you reach the bottom. Try it a few times until you are sure that you feel this.

**Setting the volume** - Before measuring your volume, you need to set it, using the dial on the side of the pipette. This dial indicates a range between '000' and '200' for both pipettes! So it is critical that you choose your pipette carefully.

**Obtaining the pipette tip** - When the volume is set, you need a disposable pipette tip. We have plastic boxes with arrays of these tips. The same tips are for both pipettes, and the tips may be either yellow or colorless. To get a tip, open the box, and press the pipette firmly into one of the tips. If you don't press it firmly enough, it will fall off later. Then you can pull the pipette, with tip, out of the box. **Keep your fingers out of the box.**

**Obtaining the sample volume** - Press the plunger down until you reach the first stop, then place the pipette tip into the solution. Release it slowly. (Do not put it so far down that the tip is completely immersed. With some solutions, this will ruin the pipette until it can be cleaned, which is very time consuming.) Now withdraw your pipette from the solution and you should see that some of it is in the pipette tip.

**Dispensing the sample volume** - now place the pipette tip into the container to which you will add your solution. Press the plunger down all the way, past the first stop to the bottom. Hold the plunger down until you have withdrawn the pipette tip completely from the solution.

**Part 2: Isolation of DNA from E. coli**

**Activity 2- DNA extraction. You will work in pairs.**

1. Pipette 10 ml (with a glass pipette) of overnight bacterial culture into a 15 ml disposable polypropylene tube. Cap it tightly and bring it to the clinical centrifuge. Your instructor will run the centrifuge at maximum speed for 10 min.

   [This procedure separates the cells from the liquid medium in which they are growing.]

2. Remove the tube from the centrifuge and carefully pour out the "spent" media into the waste media container (NOT DOWN THE DRAIN). Pipette 1 ml of SET buffer (with a glass pipette) into the tube, vortex to resuspend the cells and transfer the solution to a 12 X 75 mm (5 ml) polypropylene tube (with the same pipette you used to measure the SET). Wait for the instructor to bring you the lysozyme, which is on ice. Use the pipetteman to add 50 microliters (µl) of lysozyme and incubate the culture for 15 min. at 37° C.

   [During this time, the cell walls are being 'digested' by the lysozyme.]

3. Add 100 µl of 10% SDS, mix by inversion several times, and add 10 µl of ribonuclease (it's on ice). Incubate for 15 min. at 37° C.

   [Now the cellular materials are being denatured and the RNA is digested.]

4. Add 10 µl of protease (it's on ice) and incubate for 15 min. at 50° C.

   [This denatures the proteins further.]
5. Add 20 µl of 5 molar NaCl. Cap tightly. Thoroughly mix by inversion. Add 2 ml of chloroform (it's in the hood), cap tightly, mix by inversion, and centrifuge 10 min. in the clinical centrifuge at max. speed.

[The non-polar organic substances will move into the chloroform solution, but the DNA will stay in the aqueous (or water) solution.]

6. Using a 1 ml pipette, carefully remove the upper, aqueous phase (from above the lower organic, chloroform, phase) to a clean 12 X 75 mm glass tube. (At your convenience, discard the chloroform in the organic waste.)

[The organic substances are discarded with the chloroform, leaving DNA and some other materials in the aqueous phase that you collected.]

7. Carefully overlay 2.5 ml of 95% ethanol above the transferred aqueous phase. Then take a fused, bent Pasteur pipette and gently mix the interface between the two layers. The DNA should "spool out" on the end.

[The DNA does not remain dissolved in the ethanol solution, but rather it 'precipitates' out of it. This is why you can see it on the bent Pasteur pipette.]

Notes: Normally the enzymatic digestions would be more extensive and the organic extraction would involve phenol instead of chloroform. For reasons of time and safety, the above procedure has been modified, sacrificing purity of the DNA. What you see at the end of your extraction is a viscous material that consists of millions of strands of DNA. These stick to each other to some extent, and this gives the material its 'stringy' consistency. You could not see the DNA strands themselves, even under an electron microscope.