

Extraction of genomic DNA from buccal epithelial cells

(Modified from Amgen Bruce Wallace Biotechnology Lab Program)

The purpose of this lab is to collect a DNA sample from the cells that line the inside of your mouth and to use this sample to explore one of the most powerful techniques in molecular biology—the Polymerase Chain Reaction (PCR). Although PCR has many applications, it is commonly used to produce many copies of a selected gene segment or *locus* of DNA. In criminal forensics, for example, PCR is used to amplify DNA evidence from small samples that may have been left at a crime scene. A skilled technician can even obtain a DNA sample left by the tongue on the back of a postage stamp used to send a letter. DNA samples obtained in this manner have been used for PCR in several high-profile criminal cases.

To obtain DNA from your buccal (cheek) cells, you'll first need to obtain cheek cells. You'll do this by swishing saline solution in your mouth (this will dislodge some cheek cells), then spitting the solution out into a cup. To separate your cheek cells from the saline solution, you will centrifuge (rapidly spin) the mixture; centrifugation will cause the things that are more dense (e.g. the cheek cells) to be forced to the bottom of the tube while the things that are less dense (e.g. substances dissolved in the saline solution) will remain towards the top of the tube.

Once you have your cheek cells collected, you will transfer them to a solution containing Chelex beads. The Chelex beads will bind divalent magnesium ions (Mg^{++}). These ions often serve as cofactors for nucleases that will degrade your DNA sample and may interfere with the enzyme (*Taq* DNA polymerase) used in the reaction. By removing magnesium ions, the degradation of genomic DNA by nucleases is reduced. This mixture will be placed into boiling water to lyse the cells and liberate the DNA.

The mixture of your released cheek cell DNA, cell debris and Chelex beads is then centrifuged to pellet the cell debris and Chelex, while keeping your cheek cell DNA in the supernatant. This is a quick and easy way to separate DNA from the cell debris. The DNA sample, however, is far from pure as it contains proteins and nucleic acids from organisms that were in your mouth at the time of sampling (mostly bacteria and food). Generally, these contaminants do not inhibit PCR because the process uses specific primers, short segments of DNA about 25 nucleotides in length that can be made to target only human genomic DNA. Therefore, if the supernatant carries some foreign DNA, it should not interfere with the targeting of the human specific primers. A more detailed description of PCR and the role that primers play will be discussed later in this lab.

Genomic DNA Extraction from Buccal Epithelial Cells

Materials Required (per group of four):

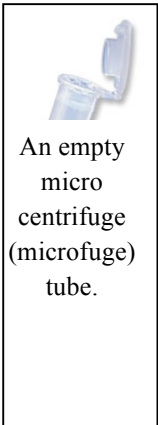
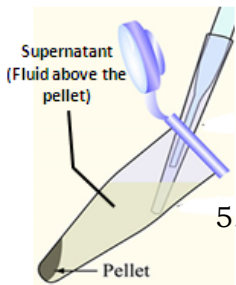
- 4 tubes containing 0.5ml of 10% Chelex
- 4 empty 1.5 ml micro centrifuge tubes
- Sharpie
- 4 paper cups containing saline solution (in back room)
- P1000 pipette w/ tips
- Small beaker for dispensing used pipette tips
- Plastic microtube holder
- Foam microtube holder

Materials Required (for the entire class)

- Microcentrifuge
- Vortexer
- 56°C water bath
- 100°C water bath, or temp. block with sand

Procedure:

1. Obtain your Chelex tube, which contains a 0.5ml of 10% Chelex , Set this aside.
2. Obtain a small paper cup containing saline solution. Pour the saline into your mouth and swish for at least 30 seconds. Spit the saline out into your cup.
3. Using a micropipette, transfer 1.5 ml of your expelled saline, which now contains buccal epithelial cells, to an empty micro centrifuge tube. *Label this tube with your initials.* In the next step, you will separate the cheek cells from the saline solution.
4. Place your tubes in the centrifuge in a balanced manner. Centrifuge your tube at full speed (10-14k rpm) for 2 minutes. When you remove your tube, you should notice a pellet (which contains your cheek cells) at the bottom of the tube. The pellet should be about the size of a match head. If not, then carefully decant the supernatant (see figure to the left). Then, repeat steps 3 and 4.
5. Pour off and discard the supernatant, while being careful not to disturb the pellet (see figure to the left). If any supernatant remains, remove it by using a micropipette.
6. Resuspend (separate) the pellet by flicking the tube with the fleshy part of your forefinger. If one is available, you may also use a Vortexer to resuspend your pellet.
7. Use a micropipette to transfer 500 μ l of the Chelex to the tube containing your cheek cells.
8. When all members of the group have their Chelex tubes prepared, place the tubes in a microtube float, and incubate in a 56^o C water bath for ten minutes. At the 5 minute mark remove the tubes and flick them with the fleshy part of your forefinger (or mix them using a Vortexer). Then, return the tubes to the water bath for the remaining five minutes.
9. Remove the tubes from the 56^o water bath and mix several times by flicking with the fleshy part of your finger. Then transfer the tubes (along with the test tube holder) to a 100^oC water bath for 5 minutes.
10. After 5 minutes remove tubes from the 100^oC bath and resuspend the sample by flicking the tube with the fleshy part of your forefinger. At this point, the cheek cells in the tube should be lysed, resulting in the release of DNA. To separate the DNA from the cell debris and the Chelex beads, centrifuge the tubes for 5 minutes at 8000 x rpm in the microfuge (or mini-fuge for 10 minutes). The less dense DNA should now be in the supernatant; the more dense cell debris and Chelex beads should now be in the pellet.
11. Using a micropipet, transfer the supernatant (liquid) containing your DNA to a clean microfuge tube that is labeled with your initials. **Do not transfer any of the Chelex to the clean tube along with your DNA.** If you do so by



accident, repeat the 5 minutes centrifugation step and transfer the supernatant to a new, clean, labeled microfuge tube. This is the DNA you will use to set up your PCR reaction.