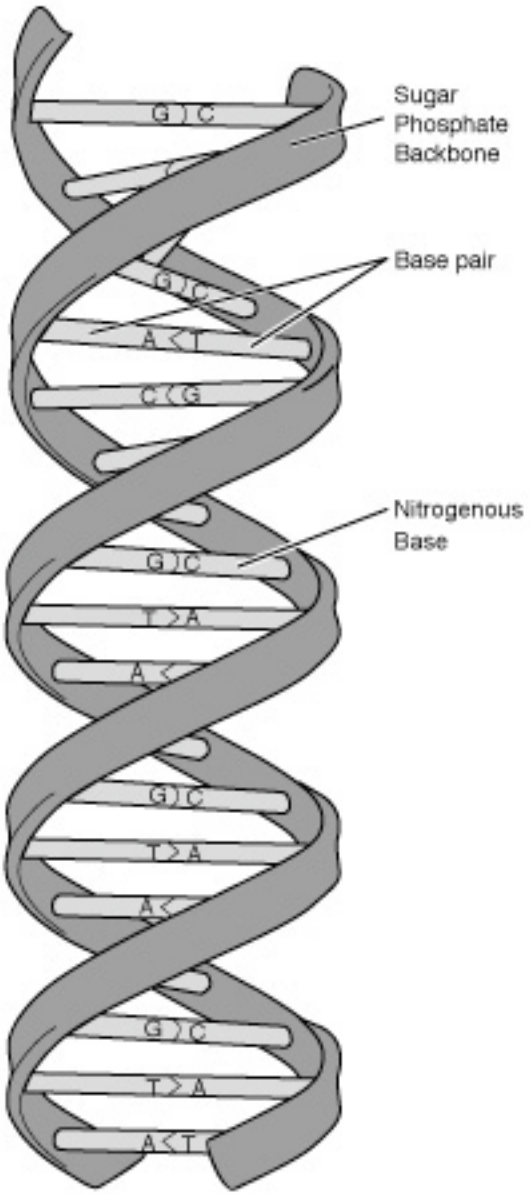
UC 260

Law, Ethics and the Life Sciences

Laboratory Manual

Developed by the faculty and student assistants in UC 260, University of Michigan
Fall 2003



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Laboratory safety

In laboratory activities using human body samples, it is important to follow safety guidelines to minimize the risk of transmitting serious disease. Please follow the following guidelines, developed by the National Association of Biology Teachers,* in all UC 260 laboratory activities.

1. Follow instructions carefully when collecting samples and do not deviate from the written protocol. If you have questions about procedures, ask your laboratory instructor.
2. Do not use samples brought from home or obtained from an unknown source.
3. Do not collect samples if you are ill or from anyone who might have a serious communicable disease.
4. Wear proper safety apparel: latex or plastic gloves, safety glasses or goggles, and lab coat or apron.
5. Supernatants and samples may be disposed of in public sewers (down lab drains).
6. Wash your hands thoroughly at the end of the lab period.
7. Do not store samples in a refrigerator or freezer used for food.

* NABT, "The Use of Human Body Fluids and Tissue Products in Biology," News & Views, June 1996.

Experiment 1 – DNA Isolation

Objectives.

- practice basic techniques for isolating, amplifying, and characterizing mtDNA
- use mtDNA evidence to draw conclusions about the rate of genetic change

Introduction. During the course of three laboratory periods you will isolate, amplify, and study a sample of your own DNA. Before embarking on this Experiment, you need to consider how it could impact you personally. We are asking each student to work with her or his own DNA sample through isolation and amplification. You will then have an option whether to compare your own DNA with other DNA samples to reach conclusions about the rate of genetic change in mtDNA. **You always have an option to discuss any laboratory requirement with your GSI or the course instructor (nsteneck@umich.edu) if you have any concerns.**

Who you are physically–the color of your eyes, your height, the number of fingers you have, and so on–is determined by the DNA in the nucleus of your cells. This Experiment is not concerned with your nuclear DNA. Instead, it explores DNA–called "mitochondrial DNA or mtDNA–in the material that surrounds the nucleus. [Your GSI will outline the science in more detail in the first lab meeting.]

You inherit your mtDNA from your mother, in principle unchanged. However, for reasons that will be explained in laboratory and in your reading, mtDNA does slowly change over time. That rate of change provides a valuable tool for determining how many generations separate one individual from another or the degree of "relatedness" between two individuals. Thus, information about mtDNA can be very useful.

The section of mtDNA chosen for this experiment does not code for any specific traits or processes. In other words, *as far as scientists know*, the section of mtDNA you are going to study has no relationship to who you are physically. Since it is not in the nucleus it did not play a formative role in developing the person you have become. Moreover, it conveys no specific information about possible physical conditions, your likelihood to develop a particular disease, your race, your ethnicity, or any other specific information about you, other than your relationship to your mother, your siblings, and your ancestors, traced back through the female line. Since you will be studying only your own DNA in this Experiment, you will not be able to draw any conclusions about your parents or immediate ancestry on the basis of this experiment alone.

Even so, simply isolating and studying your own DNA is a personal process, one that for one reason or another you might not want to undertake. Therefore, there are two ways in which you can complete this laboratory. We would like all students to have the practical experience of isolating and amplifying their own DNA to learn how easy it is to gain personal information about you. Thus, we would like all students to complete Laboratories 1-3, as outlined below. However, whether you then enter your own mtDNA data or data from a sample of mtDNA we provide you into the Coldspring Harbor database is up to you. Either option is acceptable and will allow you to complete the Experiment. We will ask you to make this decision at the beginning of the third laboratory period. If at any time before then you have questions or concerns, talk with your GSI or the main course instructor (nsteneck@umich.edu).

L A B O R A T O R Y 1.1A: PIPET EXERCISE

Equipment & Supplies

- 10-μl and 20-μl pipettes and tips
- Microcentrifuge tube
- Water reservoir (i.e. beaker filled with water)

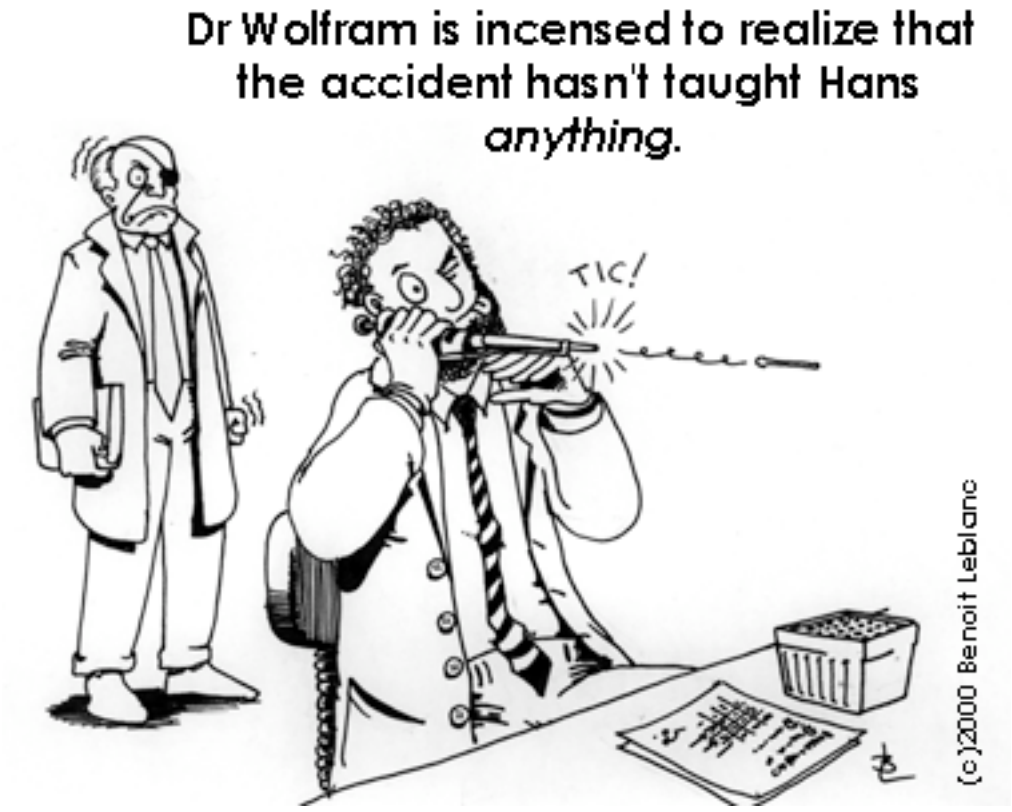
The GSI will demonstrate the pipette's basic components and operations. The student should be comfortable adjusting the pipette volume, putting a tip on the pipette, withdrawing solution, dispensing solution, and ejecting a used tip.

Student Procedure

1. With the 10-μl pipette, transfer 5 μl from the water reservoir to a micro centrifuge tube.
2. Next, transfer 7 μl from the water reservoir to the same microcentrifuge tube.
3. Lastly, transfer 8 μl from the water reservoir to the microcentrifuge tube.
4. With the 20-μl pipette, withdraw all 20 μl that <u>should</u> be in the microcentrifuge tube.
5. Record observations.

Exercise Questions (answer in your lab notebooks)

1. How close were you to the final 20-μl target?
2. How do you think you can improve your pipette technique?



L A B O R A T O R Y 1.1B: DNA ISOLATION BY CHEEK SWAB

Experiment

Procedure is slightly modified from Cold Spring Harbor Laboratory procedure available at http://vector.cshl.org/geneticorigins/mito/laboratory.html

Reagents

- Saline solution (0.9% NaCl), 1 ml
- 10% Chelex, 100 μl

Equipment & Supplies

- 1.5 ml microcentrifuge tube
- 0.5ml microcentrifuge tube
- 100-1,000 μl pipettes and tip
- Sterile Cotton Fiber Tipped Swabs

Shared Items

- Microcentrifuge (to be used at 10 K)
- PCR machine

Student Procedure

1. Open non-cotton end of cotton swab pack.
2. Remove one cotton swab and generously wipe swab against inner cheek.
3. Place used cotton swab in 1.5 ml microcentrifuge tube containing 1 ml 0.9% saline solution.
4. Rinse swab in saline solution.
6. Remove swab and dispose of swab according to the GSI's instructions.
7. Swirl 1.5 ml microcentrifuge tube to mix cells.
8. Label your sample tube, and place it together with other student samples in a balanced configuration in the microcentrifuge. Spin for 1 minute at 10,000 rpm.
9. Carefully pipette off supernatant (liquid on top) into paper cup or sink. Be careful not to disturb the cell pellet at the bottom of the test tube. A small amount of saline will remain in the tube.
10. Resuspend cells in remaining saline by pipetting in and out. (If needed, 30 μl of saline solution may be added to facilitate resuspension.)
11. Withdraw 30 μl of cell suspension, and add to 0.5 ml microcentrifuge tube containing 100 μl of Chelex. Shake well to mix.
12. Label tube, then place it in PCR machine (set at 99 degrees) for 10 minutes.
13. After heating, shake tube. Place in a balanced configuration in a microcentrifuge, and spin for 1 minute.
14. Transfer 30 μl of supernatant (liquid on top containing the DNA) with pipette to clean 0.5 ml microcentrifuge tube. Avoid cell debris and Chelex beads. This sample will be used for setting up one or more PCR reactions.
15. Label your tube and store your sample in the refrigerator for later identification.

Post-Laboratory Questions (answer in your lab notebooks)

1. What do you think the scientific reason is for heating the cell sample in step 12 of the procedure?
2. What is the purpose of the Chelex solution?
3. For parts a and b, give a response and then explain your response.
   a. Do you know for sure that your DNA is in the sample you have stored?
   b. Is it possible prove that your DNA is in the sample?

Background Information 1.1: DNA Extraction

Experiment

- ✓ **The Cell**
    - The basic unit of life
    - Prokaryotes versus eukaryotes
    - Organelles
- ✓ **Nuclear DNA**
    - Encodes biological instructions
    - Double helix
    - Genes (30,000)
    - Chromosomes
    - Human genome
- ✓ **Mitochondrial DNA**
    - Encodes biological instructions for cellular respiration
    - "Powerhouse" of the cell
    - Double-stranded, circular molecule
    - Genes (37)
    - Maternal inheritance of mitochondrial DNA
- ✓ **Laboratory procedure**

L A B O R A T O R Y 1.2: DNA AMPLIFICATION BY PCR

Background Information 1.2: PCR Amplification

- ✓ **Overview of the Polymerase Chain Reaction (PCR)**
  - Method for obtaining large amounts of a specific DNA sequence
  - Developed by Kary Mullis in 1983
  - Based on inherent properties of DNA structure

- ✓ **DNA Structure**
  - Deoxyribonucleic Acid
  - Double Stranded
  - Nucleotides
    - Base (A, T, C, G)
    - Deoxyribose
    - Phosphate
  - Complementary base pairs
  - 5' ⟶ 3' Direction

- ✓ **Reagents**
  - DNA template
  - 5' Primer
  - 3' Primer
  - Deoxynucleotides
  - Polymerase
  - Buffer

- ✓ **Denaturing**
  - **This separates the DNA strands**
  - The hydrogen bonds break at 95°C

- ✓ **Annealing**
  - **Primers bind to specific DNA sequence**
  - Primers are short complementary sequences of DNA, which flank the specific DNA sequence you are interested in studying.
  - Annealing temperatures may vary but are usually around 50-60°C

- ✓ **Elongation**
  - **DNA is amplified in the region between the primers**
  - *Taq* polymerase
    - *Thermus aquaticus:* hot springs bacteria
    - Polymerase is the enzyme used in all cells to copy DNA
    - *Taq* polymerase is stable at very high temperatures.

Experiment

Procedure is slightly modified from Cold Spring Harbor Laboratory available at http://vector.cshl.org/geneticorigins/mito/laboratory2.html

Reagents

- PCR tube containing:
  - 45 μl PCR Supermix, containing:
    - deoxyribonucleotide triphosphates (dNTPs)
    - Mg^{2+}
    - Taq Polymerase
    - deionized H20
  - 1.0 μl 5' Primer
  - 1.0 □l 3' Primer
- 3.0 μl Human DNA from Lab 1.1b

Equipment & Supplies

- 1-20 μl pipettes and tips

Shared Items

- Vortex
- PCR machine

Student Procedure

1. Add 3 □l of your DNA to the PCR reaction tube.
2. Vortex for 1-2 seconds. Make sure the cap is securely shut before using the Vortex.
3. Label the cap of your tube with your initials.
4. Store all samples on ice until ready to amplify according to the following profile.
5. The GSI will program thermocycler for 30 cycles according to the following cycle profiles.

Denaturing time and temperature 30 sec - 94°C
Annealing time and temperature 30 sec - 58°C
Extending time and temperature 30 sec - 72°C

Post-Laboratory Questions (answer in your lab notebooks)

1. Draw out a pictorial representation of PCR. Include such steps as the separation of DNA strands, binding of primers, and elongation.
2. a. Suppose you begin a PCR reaction with 1 piece of double stranded DNA. After 28 cycles of replication, how many pieces of double stranded DNA do you now have?
   b. At the end of the entire PCR reaction, are the original DNA strands bound to each other or to new DNA strands? Explain.
3. What exactly is PCR used for and why is it an effective and important technique?
4. What is the role of the DNA primers in PCR?
5. What do you think would happen if you added the wrong primers? Explain.
6. What do you think would happen if you added only 3 out of the 4 total types of dNTPs? Explain.

L A B O R A T O R Y 1.3: ELECTROPHORESIS/"RUNNING A GEL"

Background Information 1.3: Gel Electrophoresis

- ✓ Overview
    - Method to separate different sized pieces of DNA
    - Uses electric current to move DNA through agarose gel
- ✓ Why does it work?
    - DNA has an overall negative charge due to phosphate groups along the backbone
    - Negative charge causes the DNA to migrate toward the anode (positive end)
    - Larger fragments move slower because they have a higher molecular weight.
- ✓ Agarose gel
    - Agarose is the chemical used to make gels for DNA analysis
    - Different percentages can be used depending on the size of your fragment
- ✓ Loading Buffer
    - Contains glycerol to increase the density of the DNA sample
        - Pulls sample to the bottom of the well
    - Contains dye to provide a reference for the extent of migration through gel.
        - Different dyes correspond to different sized fragments of DNA.
- ✓ Visualization
    - The gel contains ethidium bromide (EtBr)
        - Ethidium bromide intercolates with base pairs
        - Dye which absorbs ultraviolet light (UV)
    - Visualize DNA stained with EtBr using UV transilluminator

Experiment

Procedure is slightly modified from Cold Spring Harbor Laboratory available at http://vector.cshl.org/geneticorigins/mito/laboratory3.html

Reagents

- Loading dye
- 123 base pair ladder
- 1.0% agarose gel containing ethidium bromide

    - ❖ Ethidium Bromide is a mutagen! Please wear gloves and avoid contact with skin while loading your gel.

Equipment & Supplies

- 1-20 μl pipettes and tips
- 1.5 ml microcentrifuge tube

Shared Items

- Electrophoresis chamber
- Electrophoresis power supply

Student Procedure

1. Mix 2 μl of loading dye with 8 l of PCR sample in a 1.5 ml microcentrifugure tube.
2. Pipette in and out several times to ensure the sample is mixed.
3. Add 10 μl of the PCR sample/loading dye mixture into your assigned well of a 1% agarose gel. (This will leave enough product if you intend to sequence the mitochondrial control region.)
4. Electrophorese at 120 volts for 60 minutes. Adequate separation will have occurred when dye front has moved at least 3 cm from the wells.

Post-Laboratory Questions (answer in your lab notebooks)

1. Does DNA carry an overall positive, neutral, or negative charge?
2. When placed in a potential difference, DNA migrates toward which electrode?
3. Does fragment size have an effect on how fast DNA migrates through the gel? Explain.
4. Why are we able to "see" the DNA fragments in the gel when it is exposed to UV light?