

TEACHING THE
GENOME
GENERATION

PROTOCOL 1: DNA EXTRACTION

PRE-REQUISITES & GOALS

PRE-REQUISITES

Prior to implementing this lab, you should understand:

- The central dogma of how DNA bases code for mRNA and then for proteins
- The purpose of PROTOCOL 1 is to extract human DNA and make the sample ready to amplify in PROTOCOL 2
- How the saliva kit is extracting DNA
- Units of measurement (μL)

LEARNING GOALS

1. Complete lab procedures necessary to collect DNA samples.
2. Perform proper micropipetting technique.
3. Extract and see a pellet of DNA.
4. Identify ethical issues with DNA sample collection.
5. Use lab equipment including the centrifuge and the vortex.

ETHICAL ISSUES

The protocol uses saliva and cheek cells as a source for extracting purified human DNA. All experiments in the course are demonstrations; none of the genotyping performed on the human samples are in any way diagnostic. For a large number of ethical reasons it is very important to allow the DNA collection stage to be 100% voluntary. There are personal, cultural, religious, and privacy based reasons why students may not want to participate. Further, it is imperative that the samples collected are not labeled by name, number or category of any kind. The goal is to keep samples anonymous and not be able to match sample to person. At the end of this protocol, unlabeled DNA tubes will be collected and a tube with a generic labels (1,2,3,...n or A,B,C,...X) will be returned to you prior to starting subsequent procedures.

MATERIALS

REQUIRED LAB MATERIALS

Ice bath or crushed ice

Refrigerator

Markers for labeling

DNA Genotek Oragene DNA collection kit

Oragene-DNA “OG-L2P” purifier solution

Micropipettors & tips (1000, 200 & 20)

Centrifuge

1.5 mL tubes

Heat Block

Vortex

Tube holders/racks

Molecular biology grade water

Ethanol (100% & 70%)

WORKSTATION NEEDS

These materials should be at each workstation

DNA collection kit and purifier solution

Micropipettors and tips

1.5 mL tubes

Ethanol (100% and 70%)

Molecular biology grade water

Tube holders

Markers for labeling

Crushed ice/ice bath

PROCEDURE

□ STEP 1

Watch: Using a Micropipette - University of Leicester
www.youtube.com/watch?v=uEy_NGDfo_8&sns=em
 and/or perform the MICROPIPETTING EXERCISE.

NOTE: Micro-pipetting skill is a critical component of the lab. If you do not do this part well, the results of all downstream activities may be compromised.

BREAK POINT IF NEEDED

□ STEP 2

Obtain the Oragene-DNA/saliva kit.

□ STEP 3

Produce enough saliva to fill the sample tube up to the black line with fluid — not bubbles. (Yes, you spit into the tube.)

□ STEP 4

Once the saliva is in the tube, close the cap. This releases the required buffer.

□ STEP 5

After the buffer is released, the funnel cap should be removed and discarded and the small circular cap should be put on the tube.

□ STEP 6

Mix the saliva in the sample tube by inversion and gentle shaking for a few seconds.

NOTE: DNA in Oragene-DNA buffer is stable at room temperature and can be kept as backup until all procedures are complete.

NOTES

□ STEP 7

Obtain a 1.5 mL microcentrifuge tube.
Label the tube with your initials.

□ STEP 8

Use the P1000 micropipettor to transfer 500 µL of the mixed Oragene-DNA/saliva sample into the tube.

NOTES:

- a. If there is mucus-like material in the collection tube, try to avoid sucking up the viscous mucus component.
- b. There may be color in the solution. For instance, if you collect saliva while drinking coffee the solution will be brown. It is best to collect saliva when not eating or drinking.
- c. If the sample is not being utilized in subsequent steps immediately, the remainder of the sample can be stored at room temperature or frozen for long term storage.

□ STEP 9

Incubate the sample tubes at 50° Celsius in the heat block provided by JAX for a minimum of 90 minutes.

NOTES:

- a. Samples are stable at room temperature if they can not be incubated right away.
- b. Samples can incubate overnight at 50° C if necessary, but this is not recommended.

BREAK POINT IF NEEDED.

□ STEP 10

Retrieve your respective sample.

□ STEP 11

Using a P20 micropipettor, add 20 µL Oragene-DNA “OG-L2P” purifier to each sample and mix by vortexing for a few seconds.

NOTES

NOTE: OG-L2P purifier tubes should be labeled with your initials.

□ STEP 12

Incubate on ice for 10 minutes. (While waiting, read ahead and prepare tubes for Step 16)

□ STEP 13

Place tubes in centrifuge with the hinge of the tube along the outer edge of the rotor (see photo). The tubes need to be balanced in the centrifuge. Tubes with water can be used if needed.

NOTE: Make sure tubes are labeled before they enter the centrifuge.



□ STEP 14

Centrifuge at room temperature for 5 minutes at 13,000 rpm (15,000 x g).

□ STEP 15

Remove from centrifuge and look for pellet on the bottom of the tube on the side of the hinge.

NOTES:

- a. If a pellet doesn't form or is just a viscous mess, re-centrifuge for up to 15 minutes

NOTES

A large grid of small grey dots for taking notes.

- b. The pellet contains impurities. The DNA is in the supernatant (liquid phase) and must be transferred into a fresh tube.

□ STEP 16

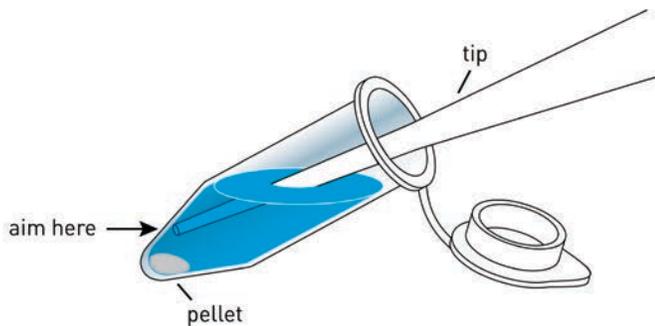
Obtain and label a fresh 1.5 mL tube.

□ STEP 17

Using the P1000 micropipettor, carefully transfer 400 μ L of the clear supernatant (containing the DNA) into the tube.

NOTES:

- a. For a more precise supernatant transfer, the P200 can be used with two draws.
- b. Do not disturb the pellet. However, if the pellet is disturbed, re-centrifuge and transfer again.
- c. Discard the tube with the pellet in the trash.



BREAK POINT IF NEEDED.

□ STEP 18

Add 400 μ L of room temperature 100% ethanol to the 400 μ L of the supernatant (ethanol amount must be equal to the supernatant.)

□ STEP 19

Mix the tube gently by inversion 10 times.

NOTE: The DNA may precipitate out and appear as a clot of fibers or cloudiness.

NOTES

A large grid of dots for taking notes.

□ STEP 20

Let stand for 5 minutes at room temperature to allow the DNA to fully precipitate.

□ STEP 21

Centrifuge at room temperature for 2 minutes at 13,000 rpm (15,000 x g) following the previous instructions for proper balancing (STEP 13).

□ STEP 22

Remove the tube and locate the position of the DNA pellet. It should be at the inside bottom portion of the tube below the hinge. (In circle area of tube, below hinge, see photo.)



NOTES:

- a. A pellet will not always be visible, this is okay. Proceed to next steps.
- b. If the pellet is disturbed, re-centrifuge.
- c. The supernatant contains impurities (not the DNA) and will be discarded in a later step.

□ STEP 23

Rotate the tube so the DNA pellet and hinge are on the upper wall of the tube (see photo previous photo). This will allow easier access to extract the supernatant.

NOTES

A large grid of small grey dots for taking notes.

□ STEP 24

Dial the P1000 micropipettor to 800 μ L and carefully remove and discard the supernatant.

NOTES:

- a. It is not necessary to remove all of the supernatant in this step.
- b. Do not disturb the DNA pellet.
- c. The P200 micropipettor can be used with multiple draws to lessen the chance of pellet disturbance and a more precise extraction of the supernatant. Dial the pipettor to 200 μ L.

□ STEP 25

Using the P1000 micropipettor, add 250 μ L of 70% ethanol to wash the DNA pellet.

□ STEP 26

Centrifuge at room temperature for 2 minutes at 13,000 rpm (15,000 x g) following the previous instructions for proper balancing (STEP 13).

□ STEP 27

Remove the tube and locate the position of the DNA pellet.

□ STEP 28

Using the P200 micropipettor, remove all supernatant without disturbing the DNA pellet.

NOTE: To remove all of the supernatant, it will take two draws with the micropipettor.

□ STEP 29

Carefully turn tube upside down over a paper towel to allow excess drops of ethanol to run out.

□ STEP 30

Keep tube open, place it on its side and store at room temperature to allow ethanol to evaporate completely for 20-30 minutes. Tubes can be left overnight as well.

NOTES

BREAK POINT IF NEEDED.

□ STEP 31

After the pellet is dry, use the P200 micropipettor to add 100 µL of molecular biology grade water to dissolve the DNA pellet.

□ STEP 32

Wait 5 minutes and vortex for 10 seconds or until the pellet has dissolved. The expected concentration of the fully hydrated DNA is 20-200 ng/µL.

Expected result is to have one tube with 100 µL of hydrated DNA per sample.

Turn in samples to your teacher and receive a randomized DNA sample to continue to PCR.

The DNA sample is now ready for PROTOCOL 2 – POLYMERASE CHAIN REACTION (PCR)

NOTES