TEACHING THE GENOME GENERATION

DNA EXTRACTION PROTOCOL (LONG)

The Jackson Laboratory
Leading the search for tomorrow's cures

PREREQUISITES & GOALS

PREREQUISITES

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 the DNA EXTRACTION PROTOCOL is to extract human DNA and make the sample ready to amplify in the PCR PROTOCOL
- 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. Identify ethical issues with DNA sample collection.
4. Extract and see a pellet of DNA.
5. Use lab equipment, including the centrifuge and the vortex.

ETHICAL ISSUES

This protocol uses saliva and cheek cells therein as a source for extracting purified human DNA. This DNA collection stage is 100% voluntary. There are personal, cultural, religious, and privacy based reasons why students may not want to participate.

All experiments in these labs are demonstrations; none of the genotyping performed on the human samples are in any way diagnostic. Although you may want to know your own personal genotype or DNA sequence, samples should remain anonymous. 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,etc. or A,B,C,etc.) should be returned to you prior to starting subsequent procedures.

MATERIALS

REQUIRED LAB MATERIALS

Ice bath or crushed ice

Refrigerator

Markers for labeling

PROVIDED BY JAX

DNA Genotek Oragene DNA collection kits

Oragene-DNA "OG-L2P" purifier solution

Micropipettes & tips (sizes P1000, P200 & P20)

1.5 mL tubes

Tube holders/racks

Centrifuge

Ethanol (100% and 70%)

Molecular biology grade water

Heat Block

Vortex

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: Micropipetting skill is a critical component of the lab. If you do not do this part well, the results of all subsequent 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 micropipette 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.

BREAK POINT IF NEEDED

☐ STEP 9

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

NOTE: Samples can incubate overnight at 50°C if necessary, but this is not recommended.

BREAK POINT IF NEEDED

☐ STEP 10

Retrieve your sample.

☐ STEP 11

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

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

NOTES

☐ 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 a pellet on the bottom or side of the tube, on the same side as the hinge.

NOTES:

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

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

NOTES

☐ STEP 16
Obtain and label a fresh 1.5 mL tube.

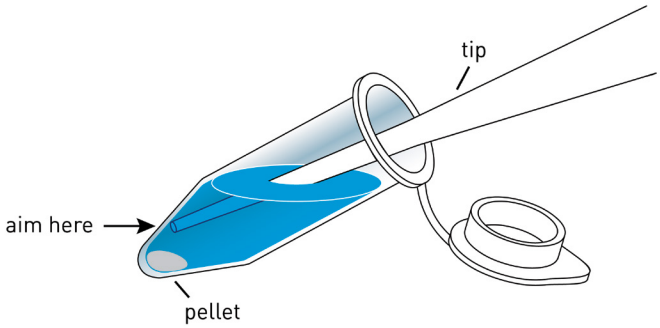
☐ STEP 17
Using the P1000 micropipette, carefully transfer 400 µL of the clear liquid supernatant (containing the DNA) into the tube.

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 µL of room temperature 100% ethanol to the 400 µL of the supernatant.

NOTE: The volume of ethanol added must be equal to the volume of supernatant, so if you have less than 400 µL of supernatant, add less ethanol.

☐ STEP 19
Mix the tube gently by inversion ~10 times.

NOTES

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

☐ 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. Now the pellet contains the DNA. The pellet is very small and will not always be visible, this is okay. Proceed to next steps.

b. If the pellet is disturbed, re-centrifuge.

c. Now 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

☐ STEP 24

Dial the P1000 micropipettor to 800 µL 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 micropipette 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 µL.

☐ STEP 25

Using the P1000 micropipette, add 250µL 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 micropipette, remove all supernatant without disturbing the DNA pellet.

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

☐ STEP 29

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

NOTES

☐ 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.

BREAK POINT IF NEEDED

☐ STEP 31

After the pellet is dry, use the P200 micropipette 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 POLYMERASE CHAIN REACTION (PCR) PROTOCOL

NOTES