TEACHER VERSION

TEACHING THE GENOME GENERATION

PROTOCOL 1: DNA EXTRACTION

BEFORE YOU BEGIN

Have you discussed informed consent with your students? This key feature of the TtGG curriculum is vitally important in their decision whether to provide a saliva sample for downstream processing. We strongly encourage you go over this important subject prior to beginning the laboratory experiments.

The Jackson Laboratory

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SUMMARY:

STUDENT PRE-REQUISITES

Prior to implementing this lab, students 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)

STUDENT 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 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, collect the unlabeled DNA tubes and put generic labels (1,2,3,...n or A,B,C,...X) on the tubes 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

Distribute these materials to 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

PROTOCOL STRUCTURE

STEP 1	8 minute video on proper pipetting technique *(critical for good results)*
	Break point
STEPS 2-8	20 minutes
	Break point: samples can be stored at 4°C for several weeks
STEP 9	90 minutes minimum *(incubation period – students need not be present)*
	Break point: samples can be stored at 4°C for several weeks
STEPS 10-30	60-70 minutes
	Break point: sample can be dried overnight
STEPS 31-32	5 minutes

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 students do not do this part well, the results of all downstream activities may be compromised.

BREAK POINT IF NEEDED

☐ STEP 2

Obtain an Oragene-DNA saliva kit.

☐ STEP 3

Each student needs to 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.

WHY: This proprietary buffer lyses cells and stabilizes the DNA in solution.

☐ 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.

☐ STEP 7

Obtain a 1.5 mL microcentrifuge tube. Label the tube with the student's 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.

WHY: This heating step will denature the proteins in the solution.

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.

Samples can be stored at 4°C (refrigerator) for several weeks.

☐ STEP 10

Return each student's 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.

☐ STEP 12

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

WHY: The purifier reagent and cold temperature will precipitate cell debris (proteins, lipids, etc.) from the solution. DNA will remain in solution.

☐ 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).

WHY: Centrifugation pellets (or physically separates) the precipitated cell debris from the DNA in solution.

☐ 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

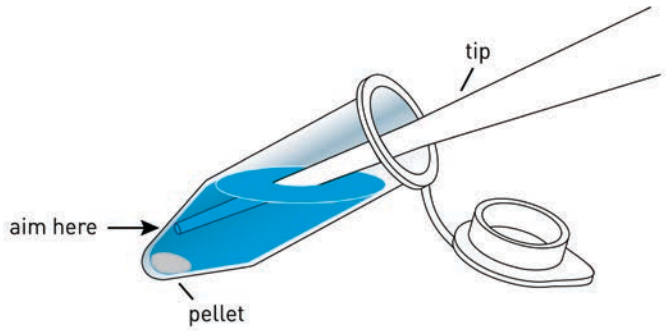
b. The pellet contains impurities. The DNA is in the supernatant (liquid phase) and must be transferred into a fresh tube.

☐ STEP 16

Obtain and label a fresh 1.5 mL tube.

☐ STEP 17

Using the P1000 micropipettor, carefully transfer 400 µL of the clear supernatant into the tube. THE CLEAR SUPERNATANT CONTAINS THE DNA.



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.

Samples can be stored at 4°C for 1-2 days.

☐ STEP 18

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

WHY: DNA is hydrophilic, so adding ethanol will allow DNA to precipitate out of the solution.

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.

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).

WHY: The precipitated DNA is physically separated from the solution by centrifugation.

☐ 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. 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. 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 diagram above). This will allow easier access to extract the supernatant.

☐ STEP 24

Dial the P1000 micropipettor to 800 µL and carefully remove and discard the supernatant. THE PELLET CONTAINS YOUR DNA.

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 reduce the risk of pellet disturbance and allow for a more precise extraction of the supernatant. Dial the pipettor to 200 µL.

☐ STEP 25

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

WHY: The DNA pellet needs to be washed with ethanol again to ensure all impurities are removed.

☐ 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 the 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 open overnight as well.

WHY: All ethanol needs to evaporate or the DNA will not go back into solution fully; residual ethanol may hinder downstream reactions.

BREAK POINT IF NEEDED.

Samples can be dried overnight if needed and stored at 4°C (refrigerator) for several weeks.

☐ 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.

WHY: Molecular Biology-grade water is very pure and will not inhibit downstream reactions, unlike other mineralized or ionized water.

☐ 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.

The DNA sample can be stored at -20° C (freezer) for up to 5 years.

Collect student samples and remove any labels on the tubes by wiping off the marker with left-over ethanol.

Re-label tubes with arbitrary letters or numbers to preserve anonymity.

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

Sources of Potential Error:

The most common error for PROTOCOL 1 is incorrect micropipetting or accidental loss of the pellet.

Clean up:

Discard all tubes except the hydrated DNA sample.

All used tips and tubes can be disposed of in the trash.

NEED HELP?

Email the experts – *ttgg@jax.org*