Updated 02/09/2024

TEACHING THE GENOME GENERATION™

DNA EXTRACTION PROTOCOL (QUICK)

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 that you go over this important subject prior to beginning the laboratory experiments.

PREREQUISITES & GOALS

STUDENT PREREQUISITES

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 the DNA EXTRACTION PROTOCOL is to extract human DNA and make the sample ready to amplify in the PCR PROTOCOL
- Units of measurement (µL)

STUDENT LEARNING GOALS

1. Complete lab procedures necessary to collect DNA samples.
2. Identify ethical issues with DNA sample collection.

NOTE: This quick DNA extraction does save time, but does produce a more impure DNA sample. DNA samples cannot be stored upon completion of this protocol and must be immediately used for the PCR PROTOCOL.

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 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.

Although most students will want to know their own personal genotype or DNA sequence, don't give in to their pleadings. 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, etc. or A, B, C, etc.) on the tubes prior to starting subsequent procedures.

CURRICULUM INTEGRATION

Use the planning notes space provided to reflect on how this protocol will be integrated into your classroom. You'll find every course is different, and you may need to make changes in your preparation or setup depending on which course you are teaching.

Course name:

1. What prior knowledge do the students need?

2. How much time will this lesson take?

3. What materials do I need to prepare in advance?

4. Will the students work independently, in pairs, or in small groups?

5. What might be challenge points for students during this lesson?

MATERIALS

REQUIRED LAB MATERIALS

Refrigerator

Markers for labeling

Toothpicks (flat-head type)

PROVIDED BY JAX

Provided for TtGG-trained teachers, contact ttgg@jax.org.

0.2 mL tubes with 50 µL of X-tract buffer

Tube holders/racks

Thermal cycler

WORKSTATION NEEDS

Distribute these materials to each workstation.

0.2 mL tubes with X-tract buffer

Tube holders

Markers for labeling

PROTOCOL STRUCTURE

STEPS 1-5	5 minutes
STEP 6-7	20 minutes

There are no break points in this protocol; samples must be used immediately.

PROCEDURE

☐ STEP 1

Obtain one 0.2 mL tube with 50 µL of X-tract buffer.

☐ STEP 2

Obtain a flat-headed toothpick and with one end, instruct students to gently rub the inside of their cheeks for 5 seconds.

☐ STEP 3

Dip the cheek-end into the buffer.

☐ STEP 4

Swirl the toothpick in the buffer for 5 seconds.

☐ STEP 5

Remove the toothpick from the solution and cap the tube tightly.

☐ STEP 6

Program the themal cycler (if needed) to run the XTRACT program. The thermal cycler provided by JAX has been pre-programmed to run the extraction protocol.

WHY: This heating step will lyse cells and denature proteins in solution.

XTRACT: Isolates DNA from saliva samples in X-tract buffer

Thermal cycler program conditions:

1. Cell lysis and protein degradation 95° C 20 min.
2. Final hold 4° C forever

PLANNING NOTES

☐ STEP 7 using PTC 1000

1. Turn on the thermal cycler using the switch in back and wait for the machine to run a self-test.
2. Check that tubes are tightly capped to avoid evaporation, place the tubes in the thermal cycler and close the lid.
3. With the cursor blinking on RUN, hit PROCEED.
4. Select the appropriate protocol and PROCEED.
5. Prompt will ask if you want to enable the heated lid, hit PROCEED.

☐ STEP 7 using T-100

1. Turn on the thermal cycler using the switch in back.
2. Check that tubes are tightly capped to avoid evaporation, place the tubes in the thermal cycler and close the lid.
3. On the touch screen select SAVED PROTOCOLS.
4. Select the appropriate protocol and hit RUN.

☐ STEP 7 using miniPCR

1. Plug the miniPCR block into both the computer and power outlet, turn on the thermal cycler using the switch in back.
2. Check that tubes are tightly capped to avoid evaporation, place the tubes in the thermal cycler block and close the lid.
3. Open the miniPCR software on the computer.
4. Double click the appropriate protocol. Select the miniPCR block to run the program on and click OK.
5. After two minutes of the program running, you can unplug the miniPCR block from the computer (keeping it plugged into the power oulet) and it will still run the desired program. Plug into the computer at any point to watch the temperature cycling on the software.
6. Repeat with each miniPCR block to run each.

BREAK POINT

PLANNING NOTES

The reaction will proceed for 20 minutes.

Once the protocol has completed, it will hold a constant temperature of 4° C until samples are removed (except the miniPCR platform). Upon completion of the XTRACT protocol, samples must be used for PCR immediately.

☐ STEP 8

Remove the samples after the protocol is complete, stop the program and turn the machine off.

Expected result is to have one tube per DNA sample with 50 µL of clear solution.

SAMPLES MUST BE USED IMMEDIATELY.

WHY: Unlike the "long" spit kit-based DNA extraction where impurities and other compounds are separated from the DNA, all of those debris are still contained within this "quick" sample. If left overnight, the DNA will degrade and you will get poor results in downstream protocols.

The samples are now ready for POLYMERASE CHAIN REACTION (PCR) PROTOCOL

Clean up:

Discard all toothpicks.

PLANNING NOTES

NEED HELP?
Email the experts: *ttgg@jax.org*