TEACHING THE GENOME GENERATION

PCR PROTOCOL

BEFORE YOU BEGIN

DESIGN YOUR PCR EXPERIMENT BEFORE PROCEEDING BY CONSULTING THE WORKSHEET AT THE END OF THIS PROTOCOL. A blank Student Worksheet should be completed PRIOR to starting the protocol. See NOTES on page 2 for further details.

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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
- How DNA samples were collected and prepared for PCR
- The steps that occur during the process of polymerase chain reaction (PCR). Watch Polymerase Chain Reaction (PCR) *www.youtube.com/watch?v=2KoLnIwoZKU*
- How PCR reflects cellular DNA replication
- The use of primers in amplifying DNA
- The purpose of the PCR PROTOCOL is to amplify human DNA at a specific genomic region for downstream protocols

LEARNING GOALS

1. Practice essential molecular biology techniques.
2. Implement mathematical formulas for preparing solutions.
3. Use lab equipment including the thermal cycler.

NOTES

1. You should always prepare a Negative Control sample that contains no DNA but includes all other components of the PCR mix. This will test whether your reagents are contaminated with DNA or if previous PCR products are lurking around the lab.
2. Cleanliness and care in setting up the PCR experiment are absolutely necessary for useable results.
3. Use extreme caution as you do all of your pipetting. Do not allow yourself to be distracted during this process.
4. Observe the volume in the tip at every pipetting step to make sure you have obtained and dispensed the desired amount.
5. Use fresh tips during every solution transfer to avoid contaminating the stock DNA, primers and reagents.

MATERIALS

REQUIRED LAB MATERIALS

Ice bath or crushed ice

Refrigerator

Markers for labeling

DNA samples from the DNA EXTRACTION PROTOCOL

PROVIDED BY JAX

Micropipettes & tips (sizes P200 & P20)

1.5 mL tubes

0.2 mL PCR tubes in strips

Thermal cycler

Taq Polymerase Mix

PCR Primer Mix (F and R, 10 µM)

Tube holders/racks

Mini-microcentrifuge

Molecular biology grade water

Vortex

WORKSTATION NEEDS

Set out frozen reagents 10 minutes prior to use to defrost. These materials should be at each workstation.

Micropipettors and tips

1.5 mL tubes

0.2 mL PCR tubes in strips

Taq Polymerase Mix (on ice)

PCR primer mix

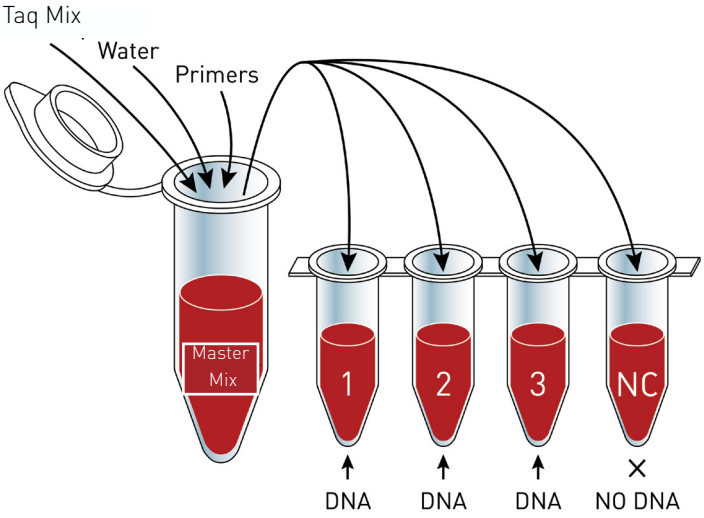
Molecular biology grade water

Tube holders and markers for labeling

DNA samples

PROCEDURE

Each group will make one PCR master mix to use in multiple individual reactions.



☐ STEP 1

Use Table 1 – Quantities of Components to Add to Each Tube from your Student Worksheet to determine how much of each component needs to be added to create the master mix.

☐ STEP 2

Obtain a 1.5 mL microcentrifuge tube and label it "master mix"

☐ STEP 3

Create your PCR cocktail using the quantities from Table 1 of the Student Worksheet.

Add the appropriate volume of each reagent to the master mix tube, and check off as reagents are added:

- ☐ Taq Polymerase Mix (then return tube to ice)
- ☐ Molecular biology grade water
- ☐ PCR primer mix

NOTES

NOTES:

a. Use the P200 micropipette for quantities over 20 µL.

b. Use the P20 micropipette for quantities 20 µL or less.

c. Use extreme care in pipetting the amounts accurately as this is critical in the success of the PCR process. Keep in mind that the product of PROTOCOL 2 can be used in three more protocols. If mistakes are made now, they will affect results for all subsequent protocols.

☐ STEP 4

Once the master mix is complete, cap tube tightly and mix by gently flicking the tube.

☐ STEP 5

Place all master mix tubes created by students in the centrifuge, ensuring the centrifuge is properly balanced (recall DNA EXTRACTION PROTOCOL (LONG), STEP 13).

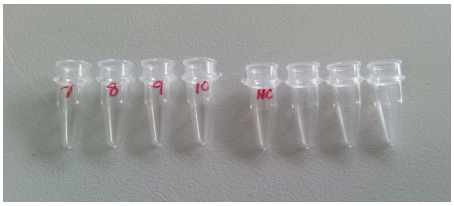
☐ STEP 6

Centrifuge at room temperature for 10 seconds by pressing the "short" button.

BREAK POINT IF NEEDED

☐ STEP 7

PCR will be run in 0.2 mL microcentrifuge tubes. Label each PCR individual reaction tube in the strip of tubes with the correspondnding sample number, and track in Table 2, column 1 of the Student Worksheet. Carefully label on side of tubes, not on caps. (See photos for examples)



NOTES

Tubes are provided in strips – more strips can be used as needed. In this example, there are four samples (numbered 7-10) and a negative control (NC). Extra tubes can be removed with scissors.

☐ STEP 8

Using the P200 micropipette, add 24.0 µL of the PCR maser mix to each of your labeled reaction tubes.

NOTE: Cap the Negative Control tube to avoid accidental addition of DNA.

☐ STEP 9

Using the P20 micropipette, add 1 µL of DNA sample to each sample tube with the corresponding label.

NOTE: DO NOT ADD ANY DNA TO THE NEGATIVE CONTROL TUBE.

☐ STEP 10

Tightly cap the tubes and mix the contents thoroughly by gently flicking the tube.

☐ STEP 11

Place tubes in the mini-microcentrifuge. Balance with tubes on both sides.

☐ STEP 12

Spin the tubes briefly in the mini-microcentrifuge to collect the solution.

BREAK POINT IF NEEDED

Expected result is to have one reaction tube per DNA sample (plus negative control) with 25 µL of PCR mix.

NOTES

☐ STEP 13

The thermal cycler should be pre-programmed for amplification of each specific gene.

Basic PCR Program

PCR Cycling Conditions

1.	Initialization	94° C	2 min.
2.	Denaturation	94° C	20-30 sec.
3.	Annealing	~55° C	30 sec.
4.	Extension	72° C	30-75 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72° C	5 min.
7.	Final hold	4° C	forever

☐ STEP 14

Consult your teacher for proper use of the thermal cycler provided.

BREAK POINT

The PCR run will proceed for about 2 hours.

☐ STEP 15

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

Nothing should look different about the solution after the PCR run.

The samples are now ready for the RESTRICTION DIGEST PROTOCOL (CYP2C19 & OXTR genes only) and the GEL ELECTROPHORESIS PROTOCOL (all genes)

NOTES

STUDENT WORKSHEET

To be completed prior to beginning the protocol

STUDENT VERSION

Name: _____

Date: _____

Number of DNA samples to be used: _____

Gene of interest: _____

___ DNA samples + 1 for Neg. Control = ___**tubes**

___ **tubes** + 1 for error =___ **(MULTIPLIER for master mix)**

☐ TABLE 1 — Quantities of Components to Add to Each Tube

Component	µL per reaction	MULTIPLIER	µL in master mix
Taq Polymerase Mix	12.5		
Molecular biology grade H_2O	9.5		
Primer Mix (eg ACE-F/R)	1		
Total master mix volume	**23.0 µL**		**µL**

☐ TABLE 2 — Labels for PCR Microcentrifuge Tubes

PCR Strip Tube Label	DNA Sample	Primer Mix