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TEACHING THE GENOME GENERATION™

PCR PROTOCOL

BEFORE YOU BEGIN

DESIGN YOUR PCR EXPERIMENT BEFORE PROCEEDING BY CONSULTING THE EXAMPLE WORKSHEET AT THE END OF THIS PROTOCOL.

The Example Worksheet is a sample of what each student should prepare PRIOR to beginning. The page after that is a blank Student Worksheet that should be completed PRIOR to starting the protocol. See NOTES on page 2 for further details.

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
- 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

STUDENT 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. Students should always prepare a Negative Control Sample that contains no DNA but all the other components of the PCR mix. This will test whether the 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. Remind students to observe the volume in the tip at every pipetting step to make sure they have obtained and dispensed the desired amount.
5. Use fresh tips during every solution transfer to avoid contaminating the stock DNA, primers and reagents.

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

Ice bath or crushed ice

Refrigerator

Markers for labeling

DNA samples from the DNA EXTRACTION PROTOCOL

PROVIDED BY JAX

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

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

Thaw frozen reagents 10 minutes prior to use. Distribute these materials to 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

PROTOCOL STRUCTURE

STEPS 1-6 25 minutes

Break point: samples can be stored at 4 ºC for several days

STEPS 7-12 25 minutes

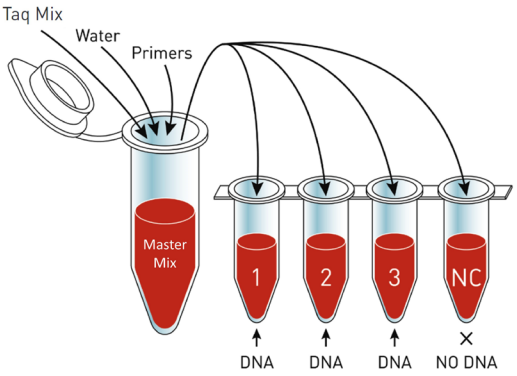
Break point: samples can be stored at 4 ºC for up to 48 hours

STEP 13-15 2 minutes to start

Amplification will take a couple of hours — teacher and students do not need to be present

PROCEDURE

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



☐ STEP 1

Complete Table 1 – Quantities of Reagents to Add to Master Mix from your Student Worksheet.

WHY: It is best practice to combine all common reagents for a set of reactions to minimize pipetting error and ensure consistency.

☐ STEP 2

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

☐ STEP 3

Create a PCR master mix 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

PLANNING 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 this PCR PROTOCOL 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

This PCR master mix can be stored for several days at 4 °C (refrigerator).

☐ STEP 7

PCR will be run in 0.2 mL microcentrifuge tubes. Label each PCR individual reaction tube in the strip of tubes. Carefully label on side of tubes, not on caps.

NOTES:

a. Students may need to use more than one strip depending on the number of samples.

b. Cut off unused tubes with scissors to avoid waste.

PLANNING NOTES

c. Labeling of tubes will need to be a collective effort throughout the class to not confuse samples between groups.

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). The class can use the number or letter scheme of their choice.

☐ STEP 8

Using the P200 micropipette, add 23.0 µL of the PCR master mix to each individual labeled PCR reaction tube.

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

☐ STEP 9

Using the P20 micropipette, add 2 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.

PLANNING NOTES

Strip tubes loaded into mini-microcentrifuge

☐ STEP 12

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

WHY: It is very important that the reaction solution is collected at the bottom of the tube. If the solution is in separate small bubbles in the tubes, reagents might not be mixed in proper ratios which can cause aberrant PCR products.

NOTE: The head of the mini-microcentrifuge may need to be changed to accommodate the strip tubes. If the end tab of the strip hits the top of the microcentrifuge and prevents spinning, they may need to be bent down or removed.

BREAK POINT IF NEEDED

Samples can be stored for up to 48 hours at 4 °C (refrigerator).

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

PLANNING NOTES

☐ STEP 13

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

WHY: While PCR temperature programs are generally the same, annealing temperatures are different depending on the nucleotide composition of the primers and the elongation time can vary depending on the length of the target genomic region.

ACE: PCR cycling conditions to amplify the ACE variant locus

1.	Initialization	94 °C	2 min.
2.	Denaturation	94 °C	30 sec.
3.	Annealing	54 °C	30 sec.
4.	Extension	72 °C	40 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72 °C	5 min.
7.	Final hold	4 °C	forever

ACTN: PCR cycling conditions to amplify the ACTN3 variant locus

1.	Initialization	94 °C	2 min.
2.	Denaturation	94 °C	20 sec.
3.	Annealing	56 °C	20 sec.
4.	Extension	72 °C	30 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72 °C	3 min.
7.	Final hold	4 °C	forever

PLANNING NOTES

CYP: PCR cycling conditions to amplify the CYP2C19 variant locus

1.	Initialization	94 °C	5 min.
2.	Denaturation	94 °C	20 sec.
3.	Annealing	53 °C	15 sec.
4.	Extension	72 °C	15 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72 °C	5 min.
7.	Final hold	4 °C	forever

OXTR: PCR cycling conditions to amplify the OXTR variant locus

1.	Initialization	94 °C	2 min.
2.	Denaturation	94 °C	30 sec.
3.	Annealing	65 °C	30 sec.
4.	Extension	72 °C	40 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72 °C	5 min.
7.	Final hold	4 °C	forever

TAS2R: PCR cycling conditions to amplify the TAS2R38 locus

1.	Initialization	94 °C	2 min.
2.	Denaturation	94 °C	30 sec.
3.	Annealing	61 °C	30 sec.
4.	Extension	72 °C	30 sec.
5.	Back to #2 30X (Exponential Amplification)		
6.	Final extension	72 °C	5 min.
7.	Final hold	4 °C	forever

PLANNING NOTES

☐ STEP 14 using T100

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 press RUN.

Once the protocol has completed, it will hold a constant temperature of 4 °C until samples are removed. It is best to remove the samples and turn off the machine within 24 hours. However, samples can be left over the weekend, if necessary.

☐ STEP 14 using miniPCR

1. Plug the miniPCR block into both the computer and power outlet, and 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. Open the miniPCR software.
4. If the appropriate protocol does not exist, create a new protocol using the PCR template. Input the name of the protocol, times and temperatures indicated above for each step. Save the new protocol.
5. Double click the appropriate protocol. Select the miniPCR block to run the program on and click OK.
6. After two minutes of the program running, you can unplug the miniPCR block from the computer (keeping it plugged into the power outlet) and it will still run the desired program. Plug into the computer at any point to watch the temperature cycling on the software.
7. Repeat with each miniPCR block to run each.

The miniPCR platform cannot perform a 4 °C hold. Once the protocol has completed, remove the samples and turn off the machine.

PLANNING NOTES

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.

Samples can be stored for up to a week at 4 °C (refrigerator) or can be stored at -20 °C (freezer) for up to 5 years.

Expected result is to have one reaction tube per DNA sample (plus negative control) with 25 µL of PCR mix. Nothing should look different about the solution after the PCR run.

The samples are now ready for the RESTRICTION DIGEST PROTOCOL (ACTN3, CYP2C19, OXTR & TAS2R38 genes) and the GEL ELECTROPHORESIS PROTOCOL (all genes)

Sources of Potential Error:

The most common error for the PCR PROTOCOL is incorrect micropipetting leading to improper master mix ratios or insufficient volumes.

Clean up:

Discard all used tubes and tips in the trash except the DNA sample and amplified reactions.

PLANNING NOTES

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

EXAMPLE WORKSHEET

Use this sheet as a guide for the Student Worksheet

In this example, a group of 4 students (with 4 unique DNA samples) are working together to amplify the portion of the ACE gene with the TtGG variant of interest.

Name: _____

Date: _____

Number of DNA samples to be used: ___4___

Gene of interest: _____ACE____

___4___ DNA samples + 1 for Neg. Control = __5__ **tubes**

___5___ **tubes** + 1 for error = __6__ **(MULTIPLIER for master mix)**

TABLE 1 — Quantities of Reagents to Add to Master Mix

Component	µL per reaction	MULTIPLIER	µL in master mix
Taq Polymerase Mix	12.5	6	**75.0**
Molecular biology grade H_2O	9.5	6	**57.0**
Primer Mix (eg ACE-F/R)	1	6	**6.0**
Total PCR master mix volume	**23.0 µL**	**6**	**138.0 µL**

For 4 DNA samples (a "6 reaction" PCR master mix) you would mix:

- 75.0 µL of Taq Polymerase Mix + 57.0 µL of Water + 6.0 µL of the Primer Mix to yield the total PCR master mix volume of 138.0 µL.
- This 138.0 µL would then be distributed across the 5 reaction tubes (23 µL each).
  - » Individual DNA samples (2 µL each) are added to 4 of the reaction tubes.
  - » No DNA is added to the 5th reaction tube, which will serve as the negative control.
  - » Remainder left in PCR master mix tube is to allow for pipetting error and does not go in a reaction tube.

STUDENT WORKSHEET

To be completed prior to beginning the protocol.

Name: _____

Date: _____

Number of DNA samples to be used: _____ _____ DNA samples + 1 for Neg. Control = ____**tubes**

Gene of interest: _____ _____ **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 PCR master mix volume	**23.0 µL**		**µL**

TABLE 2 — Labels for PCR Microcentrifuge Tubes

PCR Strip Tube Label	DNA Sample	Primer Mix