TEACHING THE GENOME GENERATION

PROTOCOL 2: PCR

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.

The Jackson Laboratory
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PRE-REQUISITES & GOALS

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
- 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 PROTOCOL 2 is to amplify human DNA 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. You should always use a Negative Control that contains no DNA but includes all other components in addition to your DNA samples. 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

Gloves

DNA samples from PROTOCOL 1

Micropipettors & tips (1000, 200 & 20)

1.5 mL tubes

0.2 mL PCR tubes in strips

Thermal cycler

Sigma RedTaq ReadyMix

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

Sigma RedTaq ReadyMix (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°C for several days

STEPS 7-12 25 minutes

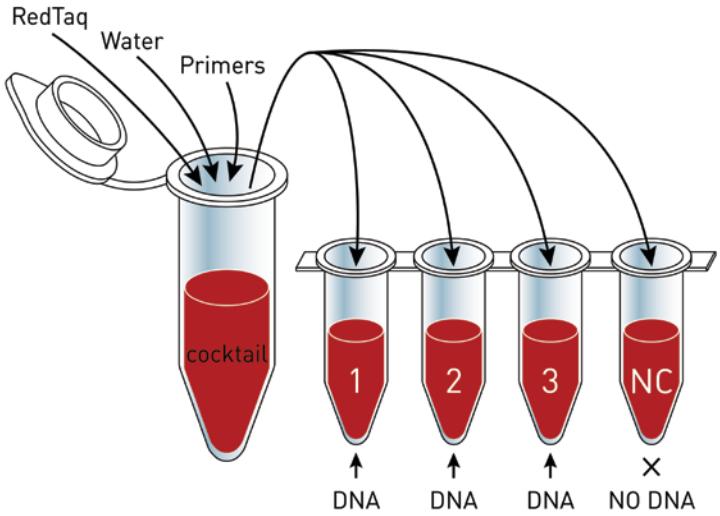
Break point: samples can be stored at 4°C for up to 48 hours

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 Cocktail to create multiple PCR reactions.



☐ STEP 1

Complete Table 1 – Quantities of Reagents to Add to Cocktail 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 micro-centrifuge tube and label it "Cocktail"

☐ STEP 3

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

Check off as reagents are added:

☐ Add the RedTaq to the Cocktail tube (return RedTaq to ice)

☐ Add the Molecular biology grade water to the Cocktail tube

☐ Add the PCR primer mix to the Cocktail tube

NOTES:

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

b. Use the P20 micropipettor 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 will be used in subsequent protocols. If mistakes are made now, they will affect results for all future protocols.

☐ STEP 4

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

☐ STEP 5

Place all cocktail tubes created by students in centrifuge following the previous instructions for proper balancing (PROTOCOL 1, STEP 13).

☐ STEP 6

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

BREAK POINT IF NEEDED

This Cocktail can be stored for several days at 4° C (refrigerator).

☐ STEP 7

Label each PCR reaction tube in the strip of tubes provided (0.2 mL micro-centrifuge tubes). Carefully label on side of tubes, not on caps.

NOTES:

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

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

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 micropipettor, add 24.0 µL of the PCR cocktail to each of your labeled PCR reaction tubes.

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

☐ STEP 9

Using the P20 micropipettor, add 1 µL of DNA sample to each sample tube with the corresponding label. Yes, you can dial down to 1 µL on the P20 for this purpose.

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.

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 micro-centrifuge and prevents spinning, they may need to be bent down or removed.

Strip tubes loaded into mini-microcentrifuge

BREAK POINT IF NEEDED

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

Expected result is to have one tube per DNA sample (plus negative control) with 25 µL of PCR reaction (should be red in color).

☐ STEP 13

The thermal cycler will need to be programmed for amplification of the ACE 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.

Amplifies the ACE loci

PCR Cycling Conditions

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 40X (Exponential Amplification)		
6. Final extension	72° C	5 min.
7. Final hold	4° C	forever

☐ STEP 14

1. Turn on the thermal cycler.
2. Check that tubes are tightly capped to avoid evaporation, place the tubes in the thermal cycler and then close lid.
3. Select the appropriate protocol for amplification of your samples.
4. Run the protocol.

BREAK POINT

The PCR reaction will proceed for several 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 tube per DNA sample (plus negative control) with 25 µL of PCR reaction (should be red in color). Nothing should look different about the solution after the PCR reaction.

The samples are now ready for

PROTOCOL 4 – GEL ELECTROPHORESIS (ACE)

Sources of Potential Error:

The most common error for PROTOCOL 2 is incorrect micropipetting leading to improper cocktail ratios or insufficient volumes.

Clean up:

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

EXAMPLE WORKSHEET

TEACHER VERSION

(Use as guide for Student Worksheet)

In this example, a group of 4 students (4 samples) are working together to amplify the ACE gene

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 cocktail)**

☐ TABLE 1 — Quantities of Reagents to Add to Cocktail

Component	µL per reaction	MULTIPLIER	µL in cocktail mix
Sigma RedTaq ReadyMix	12.5	6	**75.0**
Molecular biology grade H_2O	10.5	6	**63.0**
Primer Mix (eg ACE-F/R)	1	6	**6.0**
Total Cocktail volume	**24.0 µL**	**6**	**144.0 µL**

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

- *75.0 µL of RedTag ReadyMix + 63.0 µL of Water + 6.0 µL of the Primer Mix to yield the total cocktail mix volume of 144.0 µL.*
- *This 144.0 µL would then be distributed across the 5 reaction tubes (24 µL each).*
  - *Individual DNA samples would be 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 cocktail mix tube is to allow for pipetting error and does not go in a reaction tube.*

STUDENT WORKSHEET

STUDENT VERSION

To be completed prior to beginning the protocol

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 cocktail)**

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

Component	µL per reaction	MULTIPLIER	µL in cocktail mix
Sigma RedTag ReadyMix	12.5		
Molecular biology grade H_2O	10.5		
Primer Mix (eg ACE-F/R)	1		
Total Cocktail volume	**24.0 µL**		**µL**

☐ TABLE 2 — Labels for PCR Microcentrifuge Tubes

PCR Strip Tube Label	DNA Sample	Primer Mix