TEACHING THE GENOME GENERATION™

RESTRICTION DIGEST PROTOCOL

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 change reaction (PCR)
- What restriction enzymes are and how they work
- How the sequence variants in OXTR and CYP2C19 are affected by restriction enzyme digestion
- The purpose of the RESTRICTION DIGEST PROTOCOL is to use restriction enzymes to aid in the determing of genotype

STUDENT LEARNING GOALS

1. Perform restriction digestion of PCR products of CYP2C19 and/or OXTR.
2. Describe the possible genotypes for individuals with the CYP2C19 and/or OXTR genes.
3. Predict what each genotype will look like after gel electrophoresis and why.

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

Markers for labeling

Amplified DNA samples from the PCR PROTOCOL

PROVIDED BY JAX

For these materials please contact ttgg@jax.org.

Micropipettes & tips (size P20)

0.2 mL tubes in strips

Tube holders/racks

Restriction enzymes (on ice)

Thermal cycler

Mini-microcentrifuge

WORKSTATION NEEDS

Distribute these materials to each workstation.

Micropipettors and tips

0.2 mL tubes in strips

Tube holders

Markers for labeling

Crushed ice/ice bath

Restriction enzymes (on ice)

Amplified DNA samples

PROTOCOL STRUCTURE

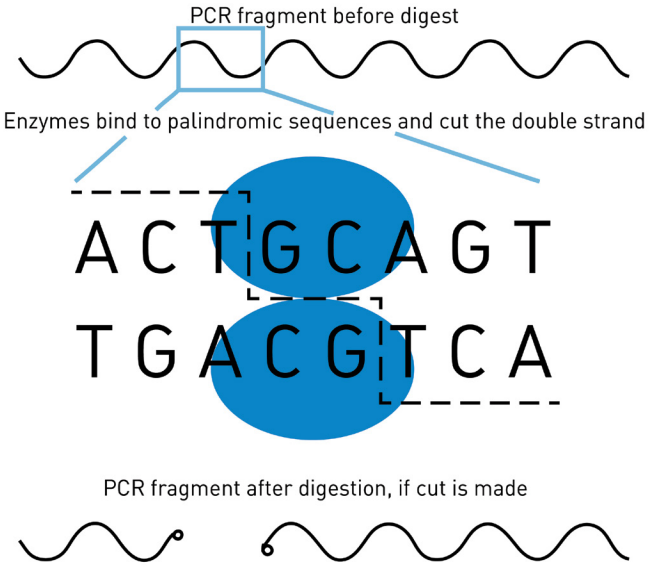
STEPS 1-6 15 minutes

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

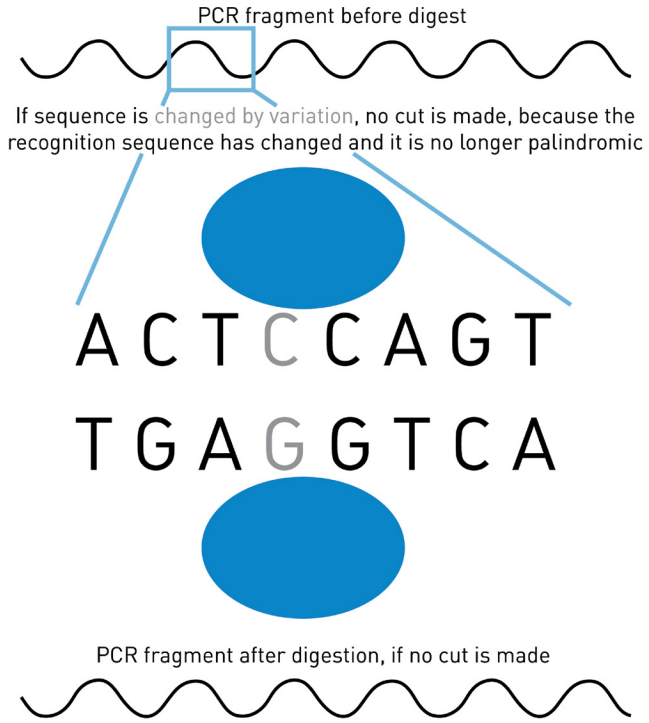
STEPS 7-12 2 minutes to start, 40 minute incubation period

Students do not need to be present.

PROCEDURE



The ability for a restriction enzyme to cut a PCR product depends on whether the genetic variant creates or abolishes a restriction enzyme recognition site.



PLANNING NOTES

☐ STEP 1

Obtain a 0.2 mL tube strip and label each tube with the PCR amplified DNA sample numbers.

☐ STEP 2

Using the P20 micropipette, transfer 10 µL of each of the amplified DNA samples (and negative control) from the PCR PROTOCOL to individually labeled 0.2 mL tubes.

NOTE: Negative control samples are also processed through this procedure.

☐ STEP 3

Using the P20 micropipette, add 1 µL of restriction enzyme to each new tube that contains PCR product.

FOR CYP2C19: use the SmaI enzyme
(pronounced smah-one)

FOR OXTR: use the BamHI enzyme
(pronounced bam-aich-one)

NOTE: Enzymes must be kept on ice.

WHY: Restriction enzymes, derived from bacteria, have different DNA sequence recognition sites. The SmaI and BamHI enzymes are specific to the site of the allelic variants being studied in the CYP2C19 and OXTR genes, respectively.

☐ STEP 4

Check that tubes are tightly capped and gently flick the tube to mix.

☐ STEP 5

Place tubes in the mini-microcentrifuge outfitted with the strip tube head. Balance with tubes on both sides.

☐ STEP 6

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

NOTE: If the end tabs of the strips hit the top of the mini-microcentrifuge and prevent spinning, they may need to be bent down or removed.

PLANNING NOTES

BREAK POINT IF NEEDED

The DNA sample 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 11 µL of reaction solution.

For the remaining steps, choose the appropriate procedure for the gene of interest.

WHY: Restriction enzymes also have different environmental conditions that are optimal for enzymatic activity. You must choose the procedure appropriate for the enzyme you are working with.

FOR CYP2C19 (Small)

☐ STEP 7

Check that tubes are tightly capped to avoid evaporation.

☐ STEP 8

Incubate tubes at room temperature for 30 minutes.

BREAK POINT

The reaction will proceed for 30 minutes.

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.

PLANNING NOTES

Expected result is to have one tube per DNA sample (plus negative control) with 11 µL of reaction solution. Nothing should look different about the solution after the restriction digestion.

The samples are now ready for the GEL ELECTROPHORESIS PROTOCOL

FOR OXTR (BamHI)

☐ STEP 7

The thermal cycler provided by JAX has been pre-programmed with the restriction digestion protocol.

CUT: Digests PCR products

Cycling conditions

1.	Digestion	37° C	30 min.
2.	Protein degradation	85° C	10 min.
3.	Final hold	4° C	forever

☐ STEP 8 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 hit **PROCEED**.
5. Prompt will ask if you want to enable the heated lid, hit **PROCEED**.

PLANNING NOTES

☐ STEP 8 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 press RUN.

☐ STEP 8 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.

BREAK POINT

The reaction will proceed for 40 minutes.

Once the protocol has completed, it will hold a constant temperature of 4 °C until samples are removed (except the miniPCR platform). It is best to remove the samples and turn off the machine within 24 hours of completing the run to avoid excessive condensation accumulation on the machine.

PLANNING NOTES

☐ STEP 9

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 11 µL of reaction solution. Nothing should look different about the solution after the restriction digestion.

The samples are now ready for the GEL ELECTROPHORESIS PROTOCOL

Sources of Potential Error:

The most common errors for the RESTRICTION DIGEST PROTOCOL include:

- incorrect micropipetting
- using the wrong restriction enzyme for the gene of interest
- not keeping the enzymes on ice which reduces cutting activity

Clean up:

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

PLANNING NOTES

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