TEACHING THE
GENOME GENERATION

RESTRICTION DIGEST PROTOCOL

The Jackson Laboratory
Leading the search for tomorrow's cures

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 change reaction (PCR)
- What restriction enzymes are and how they work
- How the sequence variants in OXTR and CYP2C19 Exons 4 and 5 are affected by restriction enzyme digestion
- The purpose of the RESTRICTION DIGEST PROTOCOL is to use restriction enzymes to aid in the determing of genotypes

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.

MATERIALS

REQUIRED LAB MATERIALS

Ice bath or crushed ice

Markers for labeling

Amplified DNA samples from the PCR PROTOCOL

PROVIDED BY JAX

Micropipettes & tips (size P20)

0.2 mL tubes in strips

Tube holders/racks

Restriction enzymes (on ice)

Thermal cycler

Mini-microcentrifuge

WORKSTATION NEEDS

These materials should be at each workstation

Micropipettors and tips

0.2 mL tubes in strips

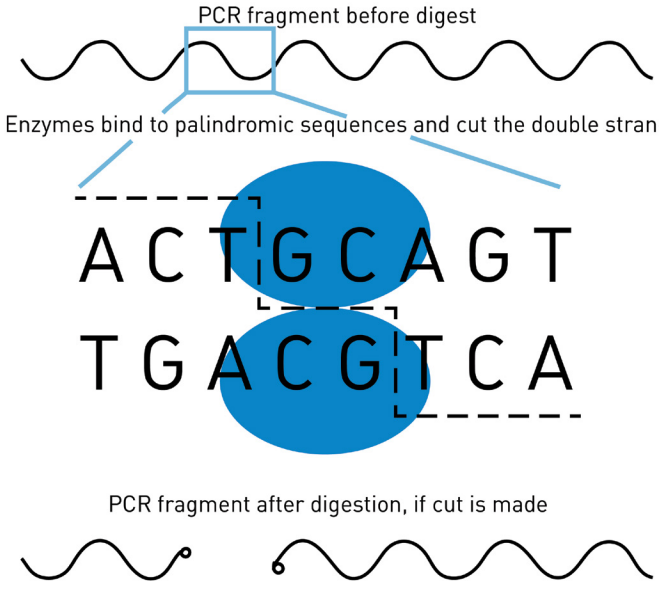
Tube holders

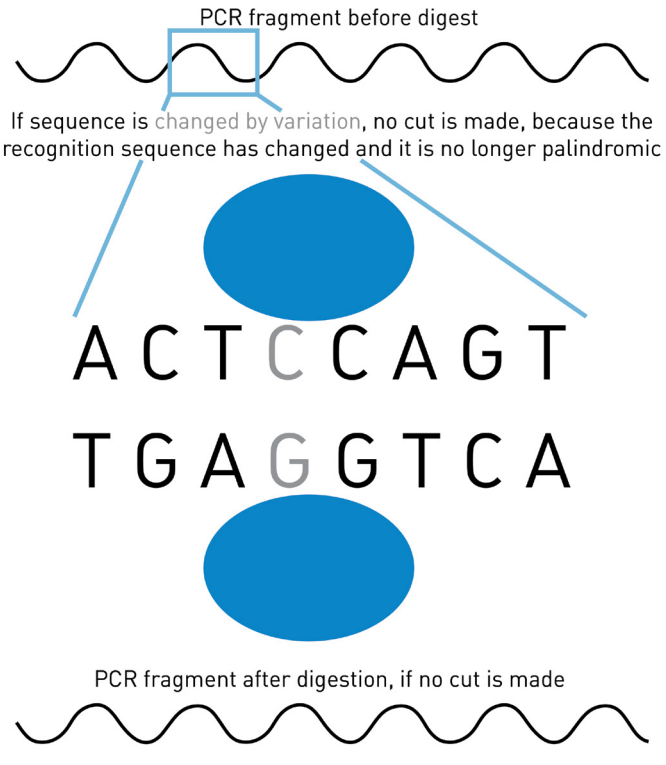
Markers for labeling

Crushed ice/ice bath

Restriction enzymes (on ice)

Amplified DNA samples





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.

PROCEDURE

☐ 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 PCR amplified DNA samples (and negative control) from the PCR PROTOCOL to individually labeled 0.2 mL tubes.

☐ 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.

☐ 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.

NOTES

BREAK POINT IF NEEDED.

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.

FOR CYP2C19 (SmaI)

☐ 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.

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

NOTES

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

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

BREAK POINT

The reaction will proceed for 40 minutes.

☐ STEP 9

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 (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

NOTES