STUDENT VERSION

TEACHING THE GENOME GENERATION

PROTOCOL 3: RESTRICTION DIGEST

The Jackson Laboratory
Leading the search for tomorrow's cures

PRE-REQUISITES & GOALS

PRE-REQUISITES

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 PROTOCOL 3 is to determine genotype

LEARNING GOALS

1. Perform restriction digestion of PCR products of CYP2C19 Exon 4 and 5, 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

Gloves

Amplified DNA samples from PROTOCOL 2

PROVIDED BY JAX

Micropipettors & tips (1000, 200 & 20)

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

PROCEDURE

☐ STEP 1

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

☐ STEP 2

Using the P20 micropipettor, transfer 10 µL of each of the amplified DNA samples from PROTOCOL 2 to the fresh tubes.

☐ STEP 3

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

FOR CYP2C19 EXON 5: SmaI

FOR OXTR: BamHI

NOTE: Enzymes must be kept on ice at all times.

☐ 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.

BREAK POINT IF NEEDED.

Expected result is to have one tube per DNA sample (plus negative control) with 11 µL of reaction solution (should be red in color).

NOTES

FOR CYP2C19 EXON 5

☐ 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 (should be red in color). Nothing should look different about the solution after the restriction digestion.

The samples are now ready for PROTOCOL 4 – GEL ELECTROPHORESIS

NOTES

FOR OXTR

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 (should be red in color). Nothing should look different about the solution after the restriction digestion.

The samples are now ready for PROTOCOL 4 – GEL ELECTROPHORESIS

NOTES