STUDENT VERSION

TEACHING THE GENOME GENERATION

PROTOCOL 5: PREP FOR SEQUENCING

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)
- The variants of ACTN3 and/or CYP2C19 Exons 4 and 5
- The connection between the bands on the gel electrophoresis and the sequential gene variants
- The purpose of using ExoSAP-IT
- The purpose of PROTOCOL 5 is to prep DNA samples for genotyping via DNA sequencing.

LEARNING GOALS

1. Prepare amplified DNA products for DNA sequencing.
2. Predict what each sequence will be for the different genotypes.

NOTES

This protocol uses the Invitrogen USB ExoSAP-IT system to remove the residual single stranded, unused PCR primers and free nucleotides from the PCR reaction (PROTOCOL 2). The ExoSAP system uses Exonuclease I (Exo), which digests residual single-stranded DNA primers and any extraneous single-stranded DNAs produced in the PCR, and Shrimp Alkaline Phosphatase (SAP), which dephosphorylates remaining dNTPs (free nucleotides) from the PCR reaction so they do not interfere with the sequencing reaction.

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 of 4

1.5 mL tubes

Tube holders/racks

Mini-microcentrifuge

Thermal cycler

Molecular biology grade water

(F)orward sequencing primer (4 µM)

(R)everse sequencing primer (4 µM)

Invitrogen ExoSAP-IT enzyme (on ice)

WORKSTATION NEEDS

These materials should be at each workstation

Micropipettors and tips

0.2 mL tubes in strips

1.5 mL tubes

Molecular biology grade water

(F)orward and (R)everse primers

Tube holders and markers for labeling

Crushed ice/ice bath

Exo-SAP IT enzyme (on ice)

Amplified DNA samples

PROCEDURE

☐ STEP 1

Obtain 0.2 mL strip tubes and label them with the DNA sample numbers/letters.

NOTE: Do not use the negative control sample in this procedure, only samples that demonstrated positive amplification during gel electrophoresis should be processed.

☐ STEP 2

Using the P20 micropipettor, transfer 5 µL of the amplified DNA sample from PROTOCOL 2 to a fresh tube.

☐ STEP 3

Using the P20 micropipettor, add 2 µL of ExoSAP-IT to the new tube.

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

☐ STEP 4

Tightly cap the tubes and flick to mix.

☐ STEP 5

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

BREAK POINT IF NEEDED.

Expected result is to have one tube per DNA sample with 7 µL of reaction solution (should be red in color).

NOTES

☐ STEP 6

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

EXOSAP: Digests free nucleotides and primers

Cycling conditions

1.	Digestion	37° C	15 min.
2.	Protein degradation	85° C	15 sec.
3.	Final hold	4° C	forever

☐ STEP 7

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

BREAK POINT - the reaction will proceed for 16 minutes.

☐ STEP 8

Remove the samples after the protocol is complete, stop the program and turn the machine off.

BREAK POINT IF NEEDED.

Expected result is to have one tube per DNA sample with 7 µL of reaction solution (should be red in color). Nothing should look different about the solution after the reaction.

NOTES

☐ STEP 9 - **CRITICAL STEP**

Obtain two new 0.2 mL strip tubes per DNA sample to be sequenced.

☐ STEP 10 - **CRITICAL STEP**

Label one tube as the forward sequencing reaction and one as the reverse sequencing reaction for each sample.

EXAMPLE: 4-F would be the label for DNA sample #4 to be sequenced with the Forward primer.

☐ STEP 11

Using the P20 micropipettor, add the following to the FORWARD reaction tube:

- ☐ 10 µL of molecular biology grade water
- ☐ 3 µL of ExoSAP-IT/DNA mix that has been incubated
- ☐ 2 µL of (F)orward sequencing primer

☐ STEP 12

Using the P20 micropipettor, add the following to the REVERSE reaction tube:

- ☐ 10 µL of molecular biology grade water
- ☐ 3 µL of ExoSAP-IT/DNA mix that has been incubated
- ☐ 2 µL of (R)everse sequencing primer

☐ STEP 13

Tightly cap the tubes and flick to mix.

☐ STEP 14

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

NOTES

Expected result is to have two tubes per DNA sample with 15 µL of reaction solution (should be pink in color).

☐ STEP 15

Fill out the DNA sample submission form with the rest of your classmates.

The DNA sample is now ready for sequencing at The Jackson Laboratory.

NOTES