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STUDENT VERSION

TEACHING THE GENOME GENERATION™

GEL ELECTROPHORESIS PROTOCOL

The Jackson Laboratory
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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 chain reaction (PCR)
- The reasons for differences in DNA fragment length
- The process of gel electrophoresis, including DNA charge and migration
- How DNA fragment length is reflected in the outcome of gel electrophoresis
- The meaning of genotype, including terms heterozygous and homozygous
- The purpose of GEL ELECTROPHORESIS PROTOCOL is to ensure amplification was successful from the PCR PROTOCOL and visualize DNA samples to aid in determining genotypes

NOTES

You will be using a small footprint pre-cast gel electrophoresis system from Lonza. The system integrates a gel dock, camera hood and power supply and runs in 5-10 minutes. DNA bands can be seen in real time as they migrate through the gel. The system includes PC-based software for gel image capture which can be saved and shared. Images can also be saved by taking a picture with a smartphone. This system is a faster and safer substitute to the conventional electrophoresis systems.

LEARNING GOALS

1. Determine if the PCR reaction has successfully amplified the DNA at the targeted locus by the presence of bands at the correct size(s) on the gel.
2. Describe how genotype can be determined using laboratory procedures.
3. Visualize and compare the presence of DNA bands in multiple samples.
4. Demonstrate human genetic variation by performing genotyping assays for several common human alleles.
5. Determine the genotypes of various DNA samples based upon the banding patterns present in the gel.

MATERIALS

REQUIRED LAB MATERIALS

Markers for labeling

Amplified DNA samples from the PCR PROTOCOL (all genes) and enzyme digested samples from the RESTRICTION DIGEST PROTOCOL (ACTN3, CYP2C19, OXTR and TAS2R38)

Micropipettes & tips (size P20)

Deionized water (diH2O)

DNA ladder

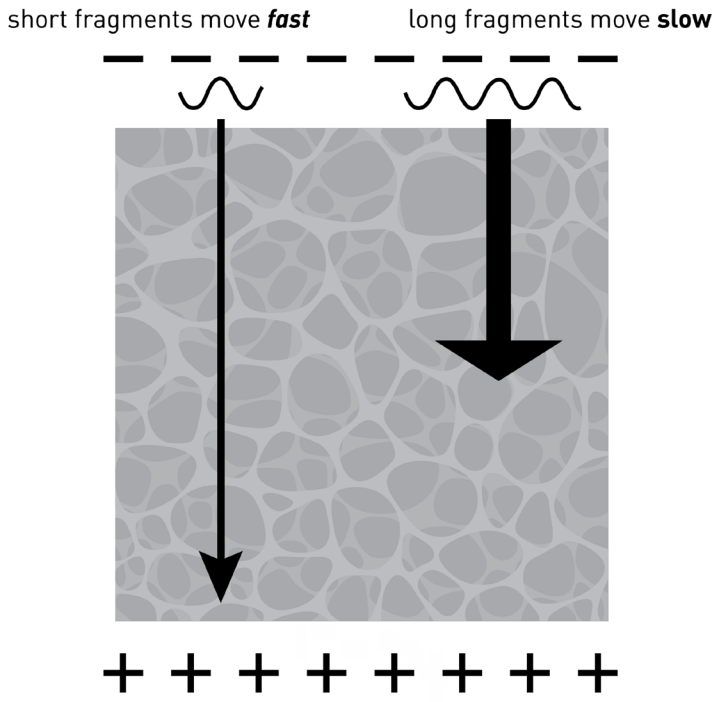
Positive controls

Lonza system (gel cassettes, dock, camera hood and power supply)

2.2% gel for running PCR products and digests

Laptop with software (optional)

WORK WITH YOUR CLASSMATES TO LOAD THE GELS



PCR-amplified DNA fragments will move through the gel (repelled by negative electrical charge and attracted to positive electrical charge) based on their length.

PROCEDURE

☐ STEP 1

Set up and turn on the Lonza system (and laptop, if using) so they are ready to go once the gels are loaded.

If you are using a different gel electrophoresis system, your teacher may provide modified directions.

☐ STEP 2

Open a new gel cassette package from the Lonza system. Remove the white sticker seals from gel cassette.

☐ STEP 3

Flood wells with deionized water (using the small squirt bottle), then tip to drain excess water and blot orange plastic (not the wells) dry with a paper towel.

> NOTE: Wells should have water in them but should not be overflowing.

☐ STEP 4

Number the wells by writing on the plastic with a marker just below each well.

☐ STEP 5

Obtain amplified DNA samples from the PCR PROTOCOL (all genes) and enzyme digested samples from the RESTRICTION DIGEST PROTOCOL (ACTN3, CYP2C19, OXTR, and TAS2R38).

☐ STEP 6

Make a template of your gel before you load it, so you have a record of which gel well corresponds to which DNA sample. Using the gel diagram on the last page of this protocol, lable each well with the gel well number and the sample name.

NOTES

NOTES:

a. Each DNA sample must have its own well.

b. Use a separate well for the DNA ladder, and a separate well for the negative control.

c. Label your gel uniquely to differentiate from the gel of classmates. You can write on the frame of the Lonza gel with a marker (not on the flat viewing field).

d. Avoid skipping wells to ensure sufficient space for all samples.

☐ STEP 7

Using a P20 micropipette, load 3 µL of each sample (PCR product, digest product, negative control, provided positive controls and DNA ladder) into separate wells.

☐ STEP 8

Attach the high voltage cables (red and black) from the Lonza casset dock to the power portals (red and black, respectively) on the Lonza power supply.

☐ STEP 9

Set the following on the Lonza power supply:

a. Press VOLT

b. Press arrow keys to set at 200 V

c. Press TIME

d. Press arrow keys to set for 5-10 minutes

e. Press RUN

☐ STEP 10

When the timer goes off, use the light to check the gel for progress and determine if a longer run time is needed. If not, turn off the power supply.

NOTE: The light can be turned on briefly to monitor the progress of the bands through the gel, however the light should be turned off promptly to avoid degradation of the dye before the run is complete. Keep an eye on the progress of the smallest band in the ladder to ensure no bands are run off the gel.

NOTES

☐ STEP 11

Turn on the light and take a picture of the gel. Use a smartphone or camera to capture an image of your gel. Alternatively, follow the steps below to use the Lonza camera hood system by attaching the USB cable to the laptop.

☐ STEP 12

Double click on the FlashGel Capture icon on desktop. A rendering of the Lonza setup appears and your DNA bands can be seen.

☐ STEP 13

Click on the camera icon on the right side of software interface.

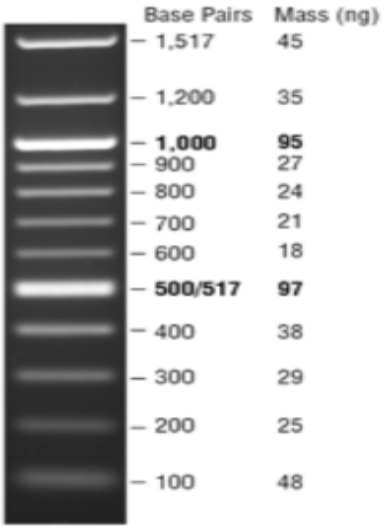
☐ STEP 14

Save the photo with you or the gel's name and date.

> NOTE: If you do multiple gels they start to look alike, so unique naming is critical.

☐ STEP 15

Analyze results by determining the number of bands in each lane and estimating band size based on the DNA ladder.



NOTES

GEL TEMPLATE