TEACHING THE

# GENOME GENERATION

## *PROTOCOL 4: GEL ELECTROPHORESIS*

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 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 PROTOCOL 4 is to determine genotype and/or check for gene amplification.

## LEARNING GOALS

1. Determine if the sample has amplified the DNA by the presence of bands on the gel.
2. Understand 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.

## NOTES

There are several types of gel electrophoresis systems, and you may use any to analyze the PCR samples. If you choose to use the recommended system from LONZA, know that it is a small footprint, pre-cast gel system.Unlike traditional systems, it requires a very small amount of sample to be loaded. The system integrates a gel dock, camera hood and power supply and runs in 2-7 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. This system is a faster and safer substitute to the conventional electrophoresis systems, though those could also be used if you already have one.

# MATERIALS

## REQUIRED LAB MATERIALS

Markers for labeling

Amplified DNA samples from PROTOCOL 2

Micropipettors & tips (1000, 200 & 20)

1.2% gel for ACE

Deionized water (diH2O) in spray bottle

Laptop with software

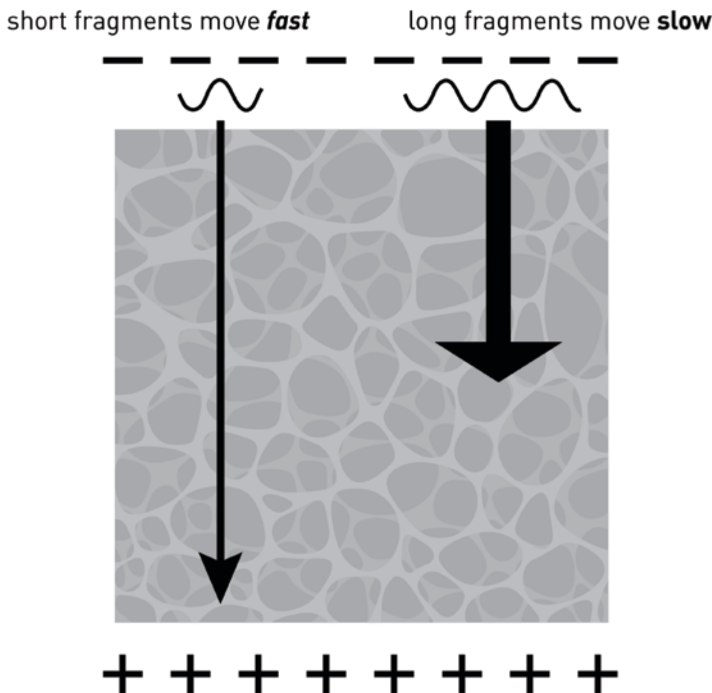
Gloves

Positive controls of each genotype

LONZA System (gel cassettes [either 12+1 well single tier or 16+1 double tier, see right], dock, camera hood and power supply)

Pre-mixed 1:10 dilution DNA ladder with dye

YOU WILL WORK WITH YOUR CLASSMATES TO LOAD THE GELS




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

# PROCEDURE

## ☐ STEP 1

Set up and turn on the LONZA system and laptop so they are ready to go once the gels are loaded.

You may be using another gel electrophoresis system, so directions may differ based on your system.

## ☐ STEP 2

Open a fresh gel cassette package from the LONZA system and insert gel cassette into gel dock by sliding into place. Then remove white 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 Kimwipe or 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 Sharpie just below the well.

## ☐ STEP 5

Obtain PCR samples from PROTOCOL 2 and an equal number of new 0.2 mL strip tubes.

## ☐ STEP 6

Make a dilution of the amplified PCR sample by adding 3 µL of molecular biology water and 3 µL of amplified sample in the new labeled tubes.

## NOTES

## ☐ STEP 7

Label the diagram on the last page of this protocol with well number and the loaded sample name as a template for the loading procedure.

NOTES:

a. Each DNA sample must have its own well.

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

d. Label your gel (to differentiate from the gel of classmates). You can write on the orange frame of LONZA gel with a black sharpie (not on the flat viewing field).

e. Do not skip wells to ensure sufficient space for all samples.

## ☐ STEP 8

Using a P20 micropipettor, load 3 µL of each diluted PCR product, the negative control, provided positive controls and pre-mixed DNA ladder into separate wells.

NOTE: If using the 16+1 double tier gel, place the FlashGel Mask provided (white plastic strip) underneath of the second tier of wells in the gel dock. This makes the gel wells easier to see when loading samples.

## ☐ STEP 9

Attach high voltage cables (red and black) to power portals (red and black, respectively).

## NOTES

## ☐ STEP 10

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 4 minutes for two tier gel or 5 minutes for one tier

e. Press LIGHT button icon on the dock system

f. Press RUN

## NOTES

## One tier LONZA Gel template (12+1)

## Two tier LONZA Gel template (16+1)