TEACHING THE GENOME GENERATION

PROTOCOL 4: GEL ELECTROPHORESIS

BEFORE YOU BEGIN

Set up and turn on the LONZA system or other gel electrophoresis system and laptop to ensure all components are functional.

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PRE-REQUISITES & GOALS

STUDENT PRE-REQUISITES

Prior to implementing this lab, students 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.

STUDENT LEARNING GOALS

1. Determine if the PCR reaction has successfully amplified the DNA by the presence of bands 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.

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 smount 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

STUDENTS WILL WORK COLLECTIVELY TO LOAD THE GELS

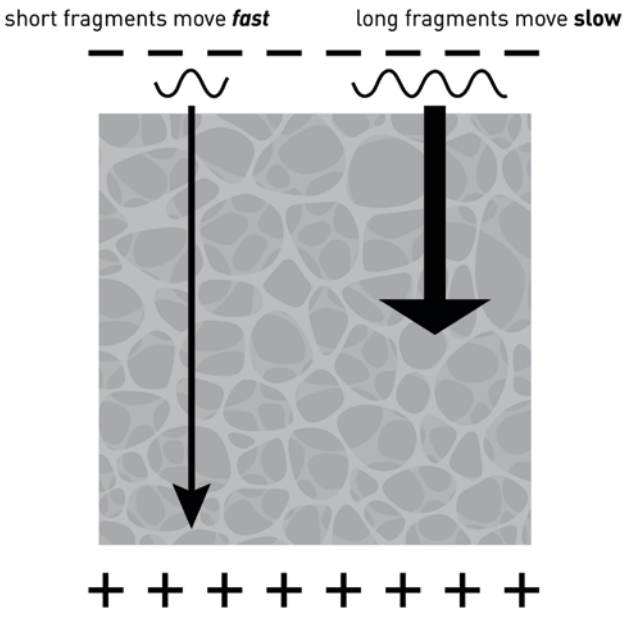
12+1 single tier

16+1 double tier

PROTOCOL STRUCTURE

ALL STEPS 30 minutes

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

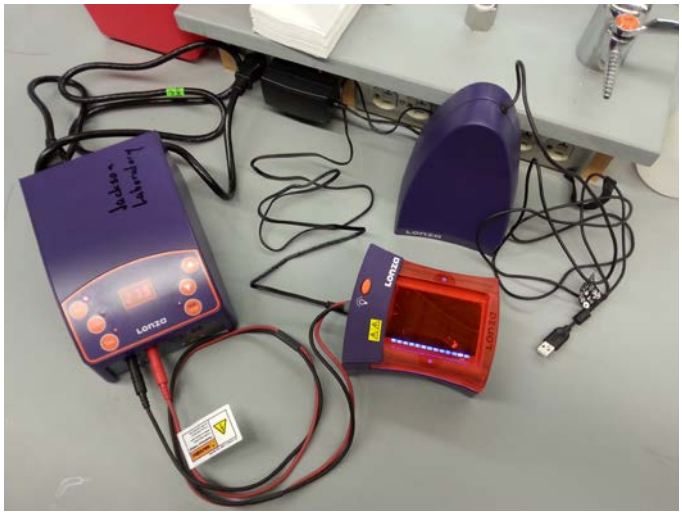


PROCEDURE

☐ STEP 1

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

You are also welcome to use another gel electrophoresis system, but please know the 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.

NOTE: DO NOT use LONZA gels that are beyond their expiration date. The dye can degrade over time preventing DNA bands from detection by eye.

☐ 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) 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.

☐ STEP 7

Label the diagram on the last page of this protocol with well number and the sample names 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.

c. 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).

d. 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, positive controls and pre-mixed DNA ladder into separate wells.

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

The amplified DNA samples can be stored at -20° C (freezer) for up to 5 years.

☐ STEP 9

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

☐ 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

☐ STEP 11

When the smallest band in the ladder has migrated more than half way to the end of the gel or when the timer goes off, turn off the power supply.

☐ STEP 12

Use the LONZA camera hood to capture the image of your gel by attaching the USB cable to the laptop.

NOTE: If the laptop does not work, you can still view the gel on the dock and capture and image with a general camera.

☐ STEP 13

Double click on the FlashGel Capture icon on desktop. A rendering of the LONZA set-up appears and your DNA bands can be seen.

☐ STEP 14

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

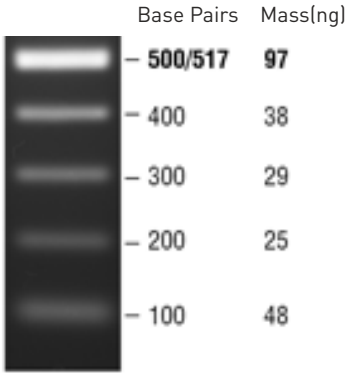
☐ STEP 15

Save the photo with student or gel name and date.

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

☐ STEP 16

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



See attached gel sheets at the end of this protocol for more information.

Sources of Potential Error:

General gel interpretation guidelines if the gel does not look like the examples provided:

- If you only see the ladder, it means that the genes did not amplify during PCR possibly due to incorrect micropipetting.
- Any illumination in the negative control means that there is contamination of your reagents or a sample was loaded into the wrong well.
- If only a portion of the samples produce visible bands then there may have been incorrect micropipetting by some groups or multiple samples may have been loaded into the same well.
- If all lanes are empty, electrical current may have been applied to the gel for too long causing DNA bands to run off the gel, or the ladder may have not be loaded (paired with other sample amplification issues).

Clean up:

If positive results are obtained, discard all used tips and tubes except for the amplified DNA samples.

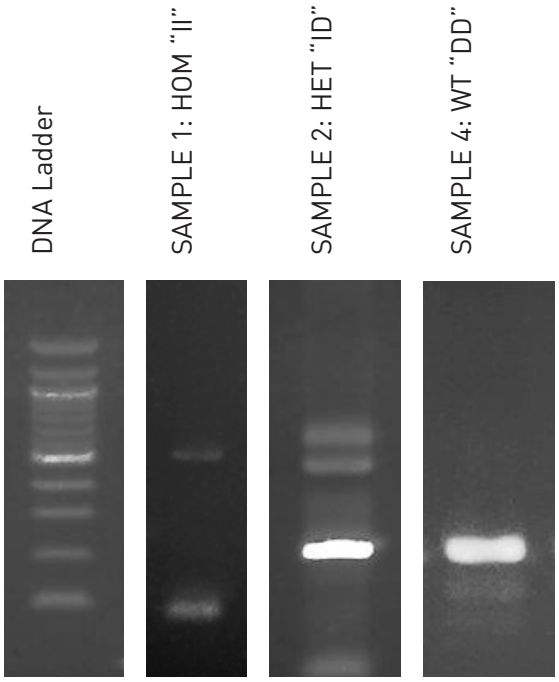
If unexpected or no results are obtained, discard all used tips and tubes except for the original DNA sample as you may want to redo the amplification.

Any used reagents can be poured in the sink and flushed with water for 5 minutes.

Discard all DNA samples and spit tubes.

NEED HELP?

Email the experts – *ttgg@jax.org*



gel of ACE PCR products

ACE variant: Alu insertion in intron

The ACE gene has two common alleles: one with an Alu transposon inserted into an intron (allele "I") and one without the insertion (allele "D"). The insertion makes the DNA segment of this allele longer than the DNA segment of the allele that does not have the Alu repeat. Since the variant is in the intron it has no effect on the protein for which the gene codes.

This assay simply looks for presence or absence of an Alu insertion in the intron of the genomic DNA within the ACE gene through PCR.

Products should be:

Homozygous allele "I":
single band at ~500 bp (allele "I")

Heterozygous alleles "I"and "D":
two bands, at ~500 bp and at ~200 bp

Homozygous Wildtype allele "D":
single band at ~200 bp

Negative Control:
lane should be blank

One tier LONZA Gel template (12+1)

Two tier LONZA Gel template (16+1)