PROTOCOL 4: GEL ELECTROPHORESIS

TEACHER VERSION

BEFORE YOU BEGIN

Set up and turn on the LONZA system and laptop to ensure all components are functional.
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 (for ACE, OXTR and CYP2C19 analyses).

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 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.

The following samples can be examined with the LONZA system:

- PCR products of PROTOCOL 2a (ACE, ACTN3, TAS2R38 and/or OXTR)
- PCR products of PROTOCOL 2b (CYP2C19)
- Restriction enzyme digested products of PROTOCOL 4 (OXTR, CYP2C19)

Many teachers may choose to use standard gel electrophoresis equipment in their classrooms instead of the LONZA system. Please see the suggestions at the end of the protocol for using standard agarose gels.
CURRICULUM INTEGRATION

Use the planning notes space provided to reflect on how this protocol will be integrated into your classroom. You’ll find every course is different, and you may need to make changes in your preparation or set-up depending on which course you are teaching.

Course name: 

1. What prior knowledge do the students need?

2. How much time will this lesson take?

3. What materials do I need to prepare in advance?

4. Will the students work independently, in pairs, or in small groups?

5. What might be challenge points for students during this lesson?
MATERIALS

REQUIRED LAB MATERIALS

Markers for labeling
Gloves
Amplified DNA samples from PROTOCOL 2

PROVIDED BY JAX
For these materials please contact ttgg@jax.org

Micropipettors & tips (1000, 200 & 20)
Deionized water (diH2O) in spray bottle
Pre-mixed 1:10 dilution DNA ladder with dye
Positive controls (3 genotypes)
LONZA System (gel cassettes [either 12+1 well single tier or 16+1 double tier, see right], dock, camera hood and power supply)
1.2% gel for ACE, ACTN3 and TASR. 2.2% gel for CYP or OXTR.
Laptop with software

STUDENTS WILL WORK COLLECTIVELY TO LOAD THE GELS
12+1 single tier
16+1 double tier

PROTOCOL STRUCTURE

ALL STEPS 30 minutes

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

short fragments move fast
long fragments move slow
PROTOCOL 4: GEL ELECTROPHORESIS
TEACHER VERSION

PROCEDURE

☐ STEP 1
Set up and turn on the LONZA system and laptop so it is ready to go once the gels are loaded.

☐ 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.

PLANNING NOTES
□ **STEP 5** -
Obtain PCR samples from PROTOCOL 2 and restriction digest samples from PROTOCOL 3 (if applicable) and an equal number of new 0.2 mL strip tubes.

**NOTE:** For OXTR and CYP2C19, you will want to run both the undigested PCR product (from PROTOCOL 2) and the digested PCR product (from PROTOCOL 3) for each sample next to one another on the gel.

□ **STEP 6** - For ACE and ACTN3 only
Make a 1:2 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, 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 (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 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 an 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.

<table>
<thead>
<tr>
<th>Base Pairs</th>
<th>Mass (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 500/517</td>
<td>97</td>
</tr>
<tr>
<td>- 400</td>
<td>38</td>
</tr>
<tr>
<td>- 300</td>
<td>29</td>
</tr>
<tr>
<td>- 200</td>
<td>25</td>
</tr>
<tr>
<td>- 100</td>
<td>48</td>
</tr>
</tbody>
</table>

Expected results vary depending on the gene of interest. See attached gel sheets at the end of this protocol.

**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.

If this is your end point:
Any used gels can be poured in the sink and flushed with water for 5 minutes.

Any unused reagents can be stored and returned to JAX.

Discard all DNA samples and spit tubes.

All equipment should be boxed up in the boxes provided, labeled with labels provided and arrange for a Fed-Ex pick up to return to JAX at the end of your implementation.
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
ACTN3 variant: nonsense mutation

The ACTN3 gene has at least one single nucleotide polymorphism (SNP) variant that is very common in all humans. The variant (allele R577X) changes arginine to a stop codon at the 577th amino acid.

This variant is not detectable by gel electrophoresis. Therefore, if amplified properly, all PCR products for the ACTN3 PCR experiments should produce one band.

Products should be:

This variant is not detectable by gel electrophoresis of PCR products.

All genotypes:
single band at ~480 bp.

Negative Control:
lane should be blank
CYP2C19 variant: frameshift mutation

The CYP2C19 gene has a common single nucleotide polymorphism (SNP) in Exon 5 that generates an alternative mRNA splice site and results in a translational frameshift (allele “m1”). The presence of this variant can be detected by restriction enzyme digestion prior to gel electrophoresis. Therefore, it is recommended that PROTOCOL 3 be completed before PROTOCOL 4. Digested products of PROTOCOL 3 are run side by side with the original PCR products of PROTOCOL 2. Without doing PROTOCOL 3, only a single band of DNA will appear on the gel if the PCR amplification is successful and you will not be able to distinguish the various genotypes.

Products should be:

- All genotypes (undigested): single band at ~320 bp
- Homozygous allele “m1”: single band at ~320 bp
- Heterozygous wild type and allele “m1”: three bands, at ~110, ~210 and ~320 bp
- Homozygous wild type allele: two bands at ~110 and 210 bp
- Negative Control: lane should be blank
OXTR variant: silent mutation

The OXTR gene has a common single nucleotide polymorphism (SNP) within an intron of the gene. Since the variant is in the intron it has no effect on the protein for which the gene codes. The variant (allele “A”) creates a site for the BamHI restriction enzyme to cut the DNA segment.

Therefore, it is recommended that PROTOCOL 3 be completed before PROTOCOL 4. Digested products of PROTOCOL 3 are run side by side with the original PCR products of PROTOCOL 2. Without doing PROTOCOL 3, only a single band of DNA will appear on the gel if the PCR amplification is successful and you will not be able to distinguish the various genotypes.

Products should be:

All genotypes (undigested):
- single band at ~435 bp

Homozygous allele “A”:
- two bands at ~120 and 310 bp

Heterozygous wild type (“G”) and allele “A”:
- three bands, at ~120, ~310 and ~435 bp

Homozygous wild type “G” allele:
- one band at ~435 bp

Negative Control:
- lane should be blank
TAS2R38 variants: missense mutations

The TAS2R38 gene has three single nucleotide polymorphism (SNP) variants that occur at high frequency in human populations. The variants (haplotype CCG) changes the amino acid coding of the single exon gene.

This variant is not detectable by gel electrophoresis. Therefore, if amplified properly, all PCR products for the TAS2R38 PCR experiments should produce one band.

Products should be:

This variant is not detectable by gel electrophoresis of PCR products.

All genotypes: single band at ~1200 bp.

Negative Control: lane should be blank
Gel template

DNA Ladder
PROTOCOL MODIFICATION

While the Lonza system for gel electrophoresis has the advantage of being fast and efficient (no gels to pour and band migrations in 5 minutes!), we understand that many teachers may already have standard gel electrophoresis equipment in their classrooms. Please see the suggestions below for preparing, running and visualizing the PCR samples using standard agarose gels.

Standard Gel Electrophoresis Protocol:

1. Prepare a 1.2% agarose gel using either TAE or TBE buffer.

2. Load DNA sample and DNA ladder.

NOTES:

a. Required DNA sample volume will vary based on concentration of samples and size of well, but a good target point is 100 ng, usually between 5 – 20 µL.

b. Since you are analyzing PCR products, dilution may be required to achieve proper band separation if DNA concentrations are very high. You may wish to run both diluted and undiluted samples side by side on the gel as a comparison. We suggest a 1:2 dilution to start, but additional dilution may be necessary, depending on the robustness of the PCR.

c. Remember to add a gel loading dye to the DNA samples, to facilitate samples entering gel wells and also for easy tracking of sample movement through the gel over time.

3. Run the gel to achieve band migration and separation using 80-150 V until the dye line is approximately 75-80% of the way down the gel. The higher the voltage, the shorter time it will take for the bands to migrate; however, your bands will be clearer if you run the gel at a lower voltage.