TEACHING THE GENOME GENERATION™

GEL ELECTROPHORESIS PROTOCOL

BEFORE YOU BEGIN

Set up and turn on the Lonza gel system and laptop (if using) to ensure all components are functional.

PREREQUISITES & GOALS

STUDENT PREREQUISITES

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 change reaction (PCR)
- The reasons for differences in DNA fragment length in PCR products
- 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 the GEL ELECTROPHORESIS PROTOCOL is to ensure amplification was successful from the PCR PROTOCOL and visualize DNA samples to aid in determining genotypes

STUDENT LEARNING GOALS

1. Determine if the PCR process 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.

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.

The following samples can be examined with the Lonza gel system:

- Amplified DNA products from the PCR PROTOCOL
- Digested products from the RESTRICTION DIGEST PROTOCOL (OXTR or CYP2C19)

Many teachers may choose to use standard gel electrophoresis equipment in their classrooms instead of the Lonza gel 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 setup 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

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

PROVIDED BY JAX

For these materials please contact ttgg@jax.org.

Micropipettes & tips (size P20)

Deionized water (diH2O)

DNA ladder

Positive controls (known genotypes)

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

1.2% gel for ACE, ACTN3, TAS2R38
2.2% gel for CYP2C19 or OXTR

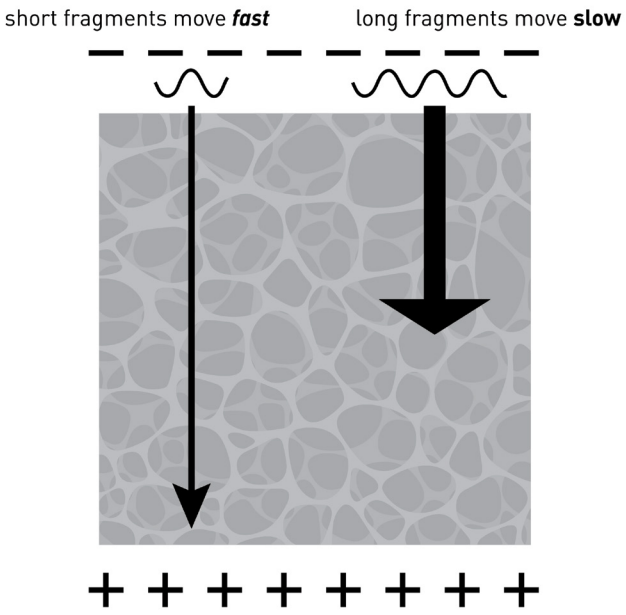
Laptop with software (optional)

STUDENTS WILL WORK COLLECTIVELY TO LOAD THE GELS

PROTOCOL STRUCTURE

ALL STEPS 30 minutes

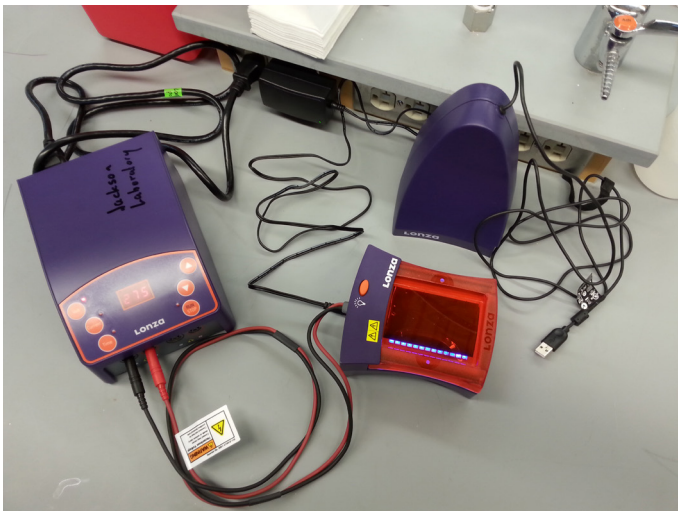
PCR-amplified DNA fragments will move through the gel (repelled by negative electrical charge and attracted to positive electrical charge) based on their length.



PROCEDURE

☐ STEP 1

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



☐ STEP 2

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

> NOTE: DO NOT use Lonza gels >3 months beyond their expiration date. Over time the buffer can evaporate and change concentration, and the dye can degrade, preventing detection of DNA bands 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 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.

PLANNING NOTES

☐ STEP 5

Obtain amplified DNA samples from the PCR PROTOCOL (all genes) and enzyme digested samples from the RESTRICTION DIGEST PROTOCOL (CYP2C19 and OXTR only)

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

☐ STEP 6

As a template for the loading procedure, have students label the diagram on the last page of this protocol with well numbers and indicate which samples will be loaded into each well.

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. Ensure each gel is labeled 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.

NOTE: Any extra PCR product (amplified DNA samples) can be stored at -20° C (freezer) for up to 5 years.

PLANNING NOTES

☐ 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 power supply:

a. Press VOLT
b. Press arrow keys to set voltage at 200 V
c. Press TIME
d. Press arrow keys to set time for 5-10 minutes
e. Press RUN

☐ STEP 10

When the timer goes off, 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.

☐ 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

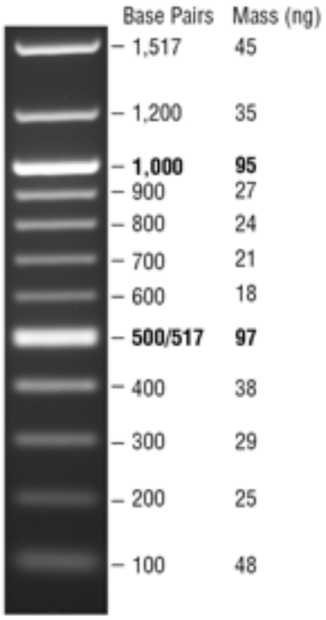
Click on the camera icon on the right side of software interface.

PLANNING NOTES

☐ STEP 14

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 15

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

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

PLANNING NOTES

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:

Any unused gels, tubes and tip boxes should be stored and returned to JAX.

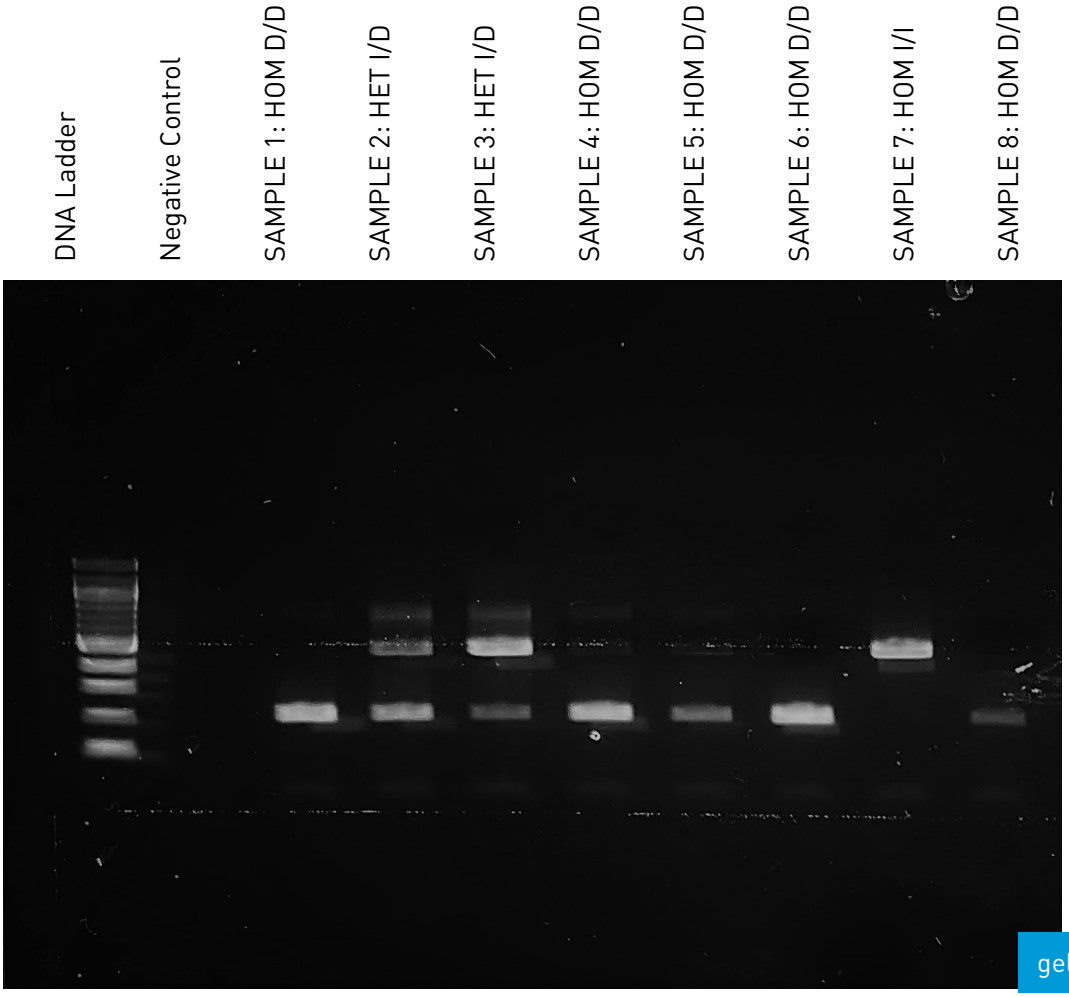
If unexpected or no results are obtained, you may want to start again from the PCR PROTOCOL.

If this is your end point:

Discard all used gels, tubes, tips and reagents, as well as unused reagents.

All equipment should be boxed up in the boxes provided, labeled with labels provided and arrange for a FedEx pick up to return to JAX at the end of your implementation. Check your email from a message from the JAX team with more details.

PLANNING NOTES



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 (insertion allele "I") and one without the insertion (deletion 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.

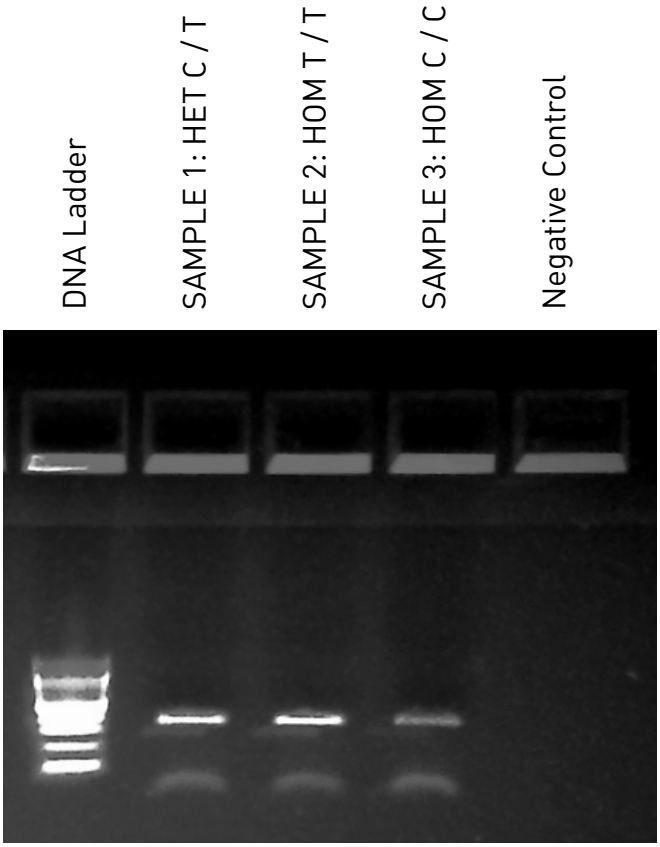
Products should be:

Homozygous insertion allele "I": single band at ~500 bp

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

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

Negative Control: lane should be blank



gel of ACTN3 PCR products

ACTN3 variant: nonsense mutation

This assay examines a single nucleotide polymorphism (SNP) in exon 16 of the ACTN3 gene. This SNP location corresponds to the 577th amino acid in the protein sequence. The nucleotide at this position is typically either a C or a T. The C variant results in an arginine (R) for amino acid 577, so this allele is called 577R. The T variant results in a stop codon (X) instead of an amino acid (R577X), producing a shortened protein.

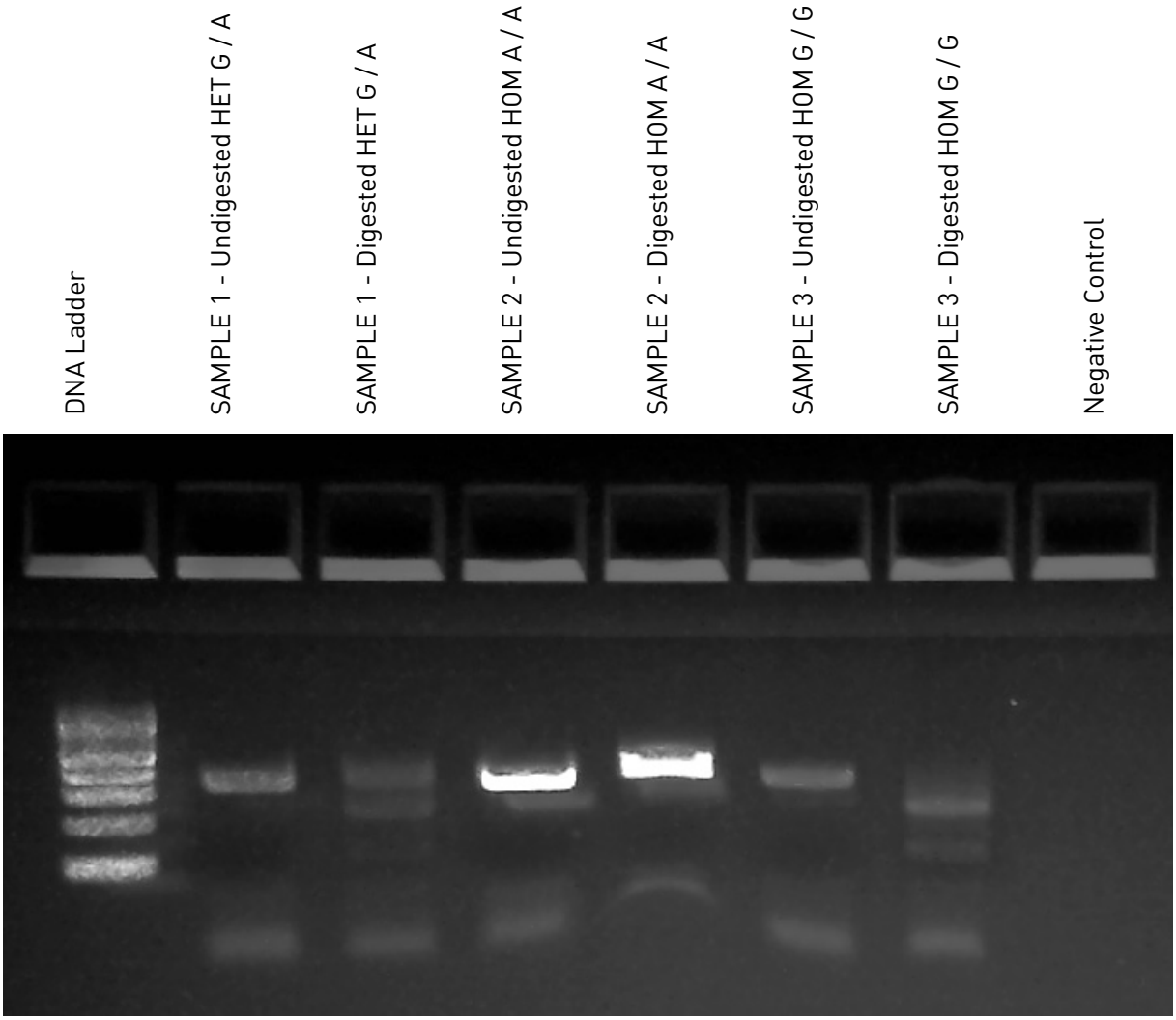
This variant is not detectable by gel electrophoresis. Therefore, all PCR products for the ACTN3 PCR experiment should produce one band of the same size. It is important to still check the PCR was successful before proceeding to the SEQUENCE ANALYSIS PROTOCOL.

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



gel of CYP2C19 PCR products and SmaI digested amplicons

CYP2C19 variant: frameshift mutation

This assay examines a single nucleotide polymorphism (SNP) in exon 5 of the CYP2C19 gene. The nucleotide at this position is typically either a G or an A. The A allele produces an mRNA splice site in the middle of exon 5, resulting in a translational frameshift.

The G variant creates a site for the restriction enzyme SmaI to cut the DNA. Therefore, the RESTRICTION DIGEST PROTOCOL must be completed before the GEL ELECTROPHORESIS PROTOCOL. Without performing the digest, each sample will appear as a single band of DNA on the gel and the genotypes will remain unknown. Digested products are run side by side with the products from the PCR PROTOCOL.

Products should be:

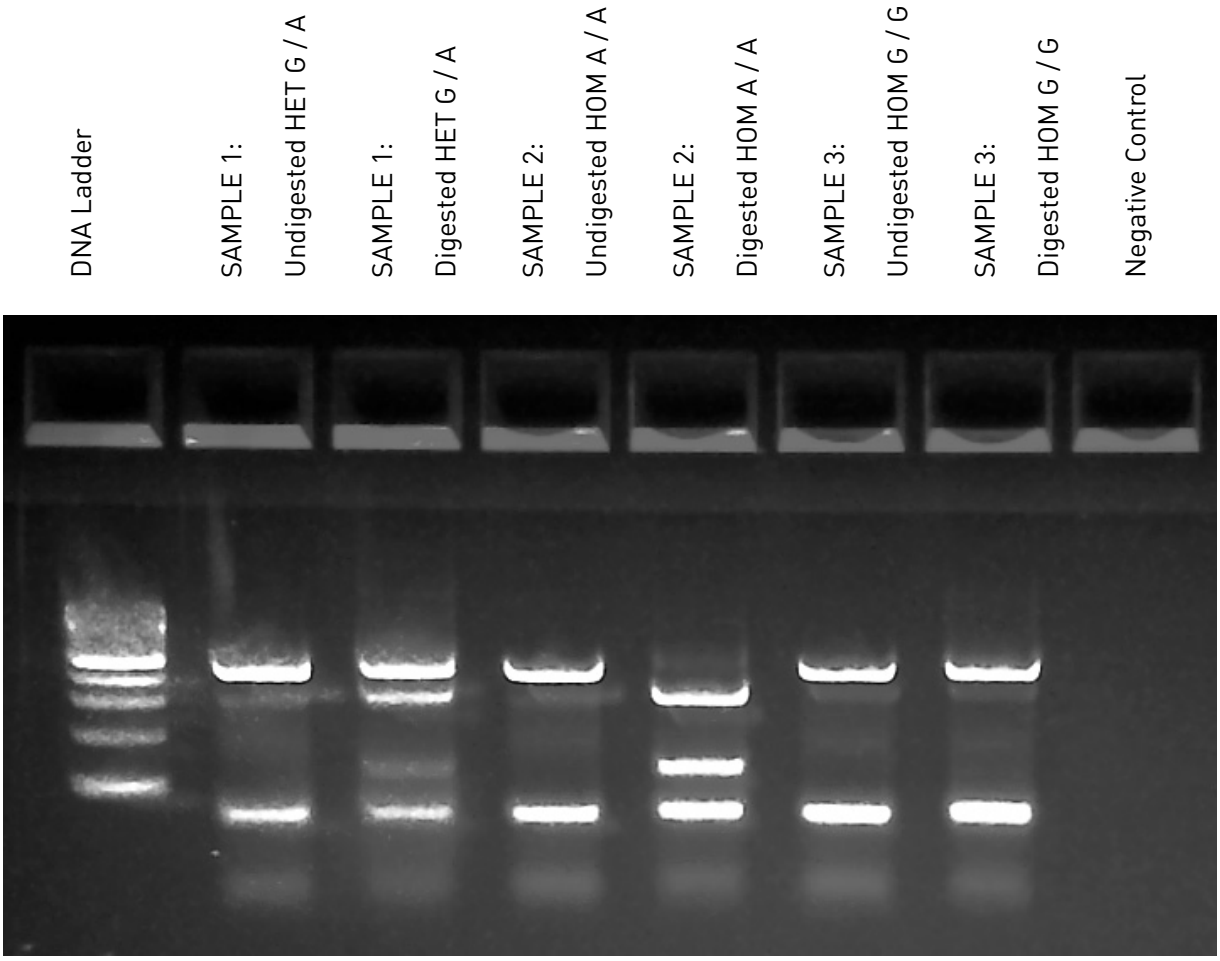
All genotypes (undigested): single band at ~320 bp

Homozygous allele "A": single band at ~320 bp

Heterozygous allele "G" & allele "A": three bands, at ~110, ~210 and ~320 bp

Homozygous allele "G": two bands at ~110 and ~210 bp

Negative Control: lane should be blank



gel of OXTR PCR products and BamHI digested amplicons

OXTR variant: silent mutation

This assay examines a single nucleotide polymorphism (SNP) in intron 3 of the OXTR gene. The nucleotide at this position is typically either a G or an A.

The A allele creates a site for the restriction enzyme BamHI to cut the DNA. Therefore, the RESTRICTION DIGEST PROTOCOL must be completed before the GEL ELECTROPHORESIS PROTOCOL. Without performing the digest, each sample will appear as a single band of DNA on the gel and the genotypes will remain unknown. Digested products are run side by side with the products from the PCR PROTOCOL.

Products should be:

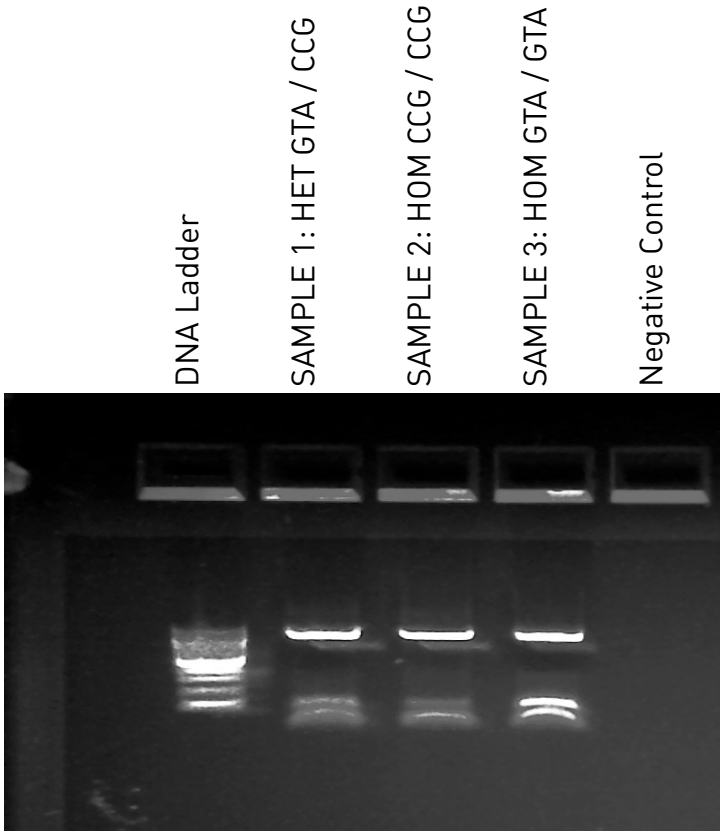
All genotypes (undigested): single band at ~435 bp

Homozygous allele "A": two bands at ~120 and ~310 bp

Heterozygous allele "G" and allele "A": three bands at ~120, ~310 and ~435 bp

Homozygous allele "G" : one band at ~435 bp

Negative Control: lane should be blank



gel of TAS2R38 PCR products

TAS2R38 variants: missense mutations

This assay examines three single nucleotide polymorphisms (SNPs) in the TAS2R38 gene. The SNPs are located at base pairs 145, 785, and 886 (corresponding to amino acids 49, 262, and 296). There are eight possible combinations of these three SNPs, producing eight possible alleles.

These variants is not detectable by gel electrophoresis. Therefore, all PCR products for the TAS2R38 PCR experiment should produce one band of the same size. It is important to still check the PCR was successful before proceeding to the SEQUENCE ANALYSIS PROTOCOL.

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

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: Required DNA sample volume will likley be more than what you load in a Lonza gel. Volume needed will vary based on concentration of samples and size of well, but a good target point is 100 ng, usually between 10 – 20 uL.
3. Run the gel to achieve band migration and separation using 100 -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.
4. Visualize the gel.

   NOTES: Depending on your system you may need to stain the gel after the run, or be able to visualize right away. Follow the recommended directions for the system you are using precisely! The DNA products trapped within the gel will fade over time, making visualization a challenge if not done in a timely manner.

PLANNING NOTES

NEED HELP?
Email the experts: *ttgg@jax.org*