Genes, Mutations and Diseases: Understanding the Origins of Genetic Disorders through Experiential Learning

A five week series of lessons and experiments designed for cohesiveness as unit, but can be done invidually.

Unit Product of ASBMB HOPES 2013 Grant by:
Edwin Li, Brian Forster, and Caitlin Fritz, St. Joseph University &
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ASBMB American Society for Biochemistry and Molecular Biology

SJU

Genes, Mutations and Diseases: Understanding the Origins of Genetic Disorders through Experiential Learning

Lesson 1

Nucleotides, Nucleic Acids, DNA and genes.

Overview

This activity walks students, step-by-step, through an overview of genetics, from the basics of DNA all the way up through the science behind genetic disorders. Sickle cell anemia is used as an example throughout the module to demonstrate the connections between genes, mutations and disease.

Quick Guide and Materials:

- **Age Range:** High School
- **Preparation Time:** ~ 3 hours
- **In Class Time:** ~ 1.5 hours
- **Duration:** 1 week
- **Materials List:** Isolation & Visualizing DNA and Nucleotide Molecule Kits

www.asbmb.org/912activity/lffj/lesson1

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Activity Outline

Sequence	Activity	Timing
A.	Lecture Slides 1-9	10 min
B.	Isolate DNA	30 min
C.	Lecture Slide 11	5 min
D.	Build a DNA Molecule	20 min
E.	Lecture Slide 10	5 min
F.	Visualize DNA	25 min

Activity Breakdown Below

Materials

Isolating and Visualizing DNA Kit

Laboratory Preparation for 10 groups - 3 students per group

- Strawberries (10)
- Ziplock bags (10)
- Cheesecloth (10 pieces that will fit over beaker)
- Scissors (10)
- 200 mL beaker (10)
- 15 mL conical tube or test tubes (10)
- Plastic bulb pipets (10)
- Glass stir rods (10)
- Water bath set to 60°C
- DNA Extraction buffer (10 mL per group)
  -To make 500 mL: Combine 50 mL shampoo, 1 gram salt, 450 mL water
- 95% Ethanol or Isopropanol (kept on ice till needed) – (3 mLs per group)
- Microcentrifuge Tubes (10)
- Deionized water (1 mL aliquots)
- 1X TAE Running Buffer (Tris-Acetate-EDTA)
  - You can purchase 50X stock from most supply companies and dilute down to 1X (bio-rad.com cat# 1610743EDU).
  - Recipe: 242 g Tris Base; 57.1 mL Glacial Acetic Acid; 100 mL 0.5 M EDTA
- Pipet tips
- Tip waste container

Nucleotide Molecule Kit

Laboratory Preparation for 6 kits - 3 Normal sequences (N-kits) and 3 Mutant/Sickle sequences (S-kits).

- 12 strands of 4 ft pom-pom garland (michaels.com cat# 10241838) (to represent backbone – sugar and phosphate)
- 204 1.5 in (38 mm) styrofoam balls (to represent nitrogenous base)
- 204 magnets (carolina.com cat# 955013)
- 272 safety Pins (to represent phosphodiester bonds)
- Green paint (to indicate phosphate group)
- Sharpie to label Styrofoam balls.

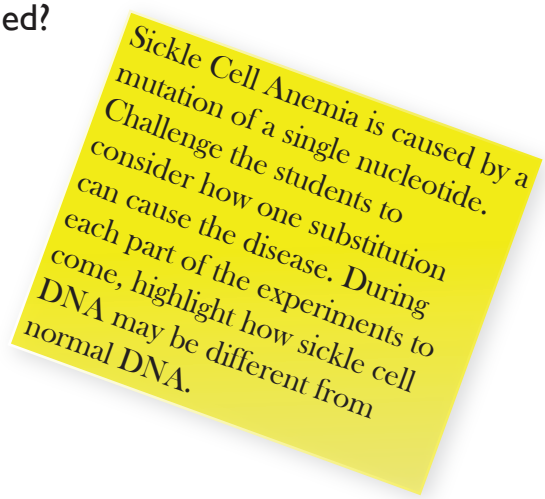
- 1% (w/v) agarose gel stained with SYBRSAFE® stain (lifetechnologies.com cat# S33102).
- Agarose Gel Electrophoresis Apparatus &
- Power Supply Box
- UV light table
- DNA Loading Dye (neb.com cat#B7024)
- DNA Ladder (neb.com cat# N3232)
- Sample of Bovine Serum Albumin in solution (protein sample for gel electrophoresis)
- Micropipettor that can pipet between 3 – 20 µL

Lecture Slides and Handouts

Lecture Material

Lecture- What are genetic diseases and how can they be studied?

By definition, a disease is a deviation from the normal state of the body. Some diseases are genetic in nature. Genetics is the study of inheritance. The gene, comprised of deoxyribonucleic acid (DNA), is the fundamental unit of heredity that is passed on from one generation to the next.
Example found at: www.asbmb.org/912activity/



Handouts

Printable handouts contain protocols, pictures, questions for student during the experiment and further explanation of the science behind certain steps.

Instructor manual found at: www.asbmb.org/912activity/
(contains answer key and reference pictures)
Printable student lab instructions/notebook is found at: www.asbmb.org/912activity/

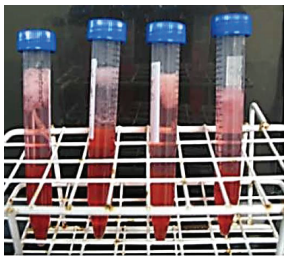
Isolate and Visualize DNA

All living organisms contain DNA within their cells.
Experimental question: Can DNA be isolated from cells?

Isolate DNA Procedure (Part 1)

1. Place a strawberry in Zip-loc bag. Press the air out and seal the bag. Mash the strawberry for 2 minutes to break open the cells.
2. Add 10 mL of DNA extraction buffer (detergent, salt, water) to the bag. The detergent helps break open the ells and the salt helps remove any protein that is bound to the DNA. Press the air out and seal the bag. Mash for 1 minute.
3. Place a piece of cheesecloth on top of a beaker and secure it with a rubber band. Cut the end of the Zip-loc bag and allow the liquid to filter through the cheesecloth into the beaker. Transfer approximately 2-3 mL of the liquid from the beaker to a clean 15 mL conical tube or test tube.
4. Slowly pour about 2-3 mL of ice-cold ethanol along the side of the conical. The ethanol should form a layer on top of the filtered extract. This will help clump DNA. We are able to see DNA because it is not soluble in ethanol.

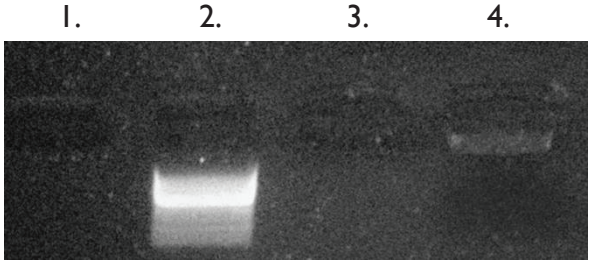
5. Dip a glass-rod to the ethanol-extract boundary and twirl gently. (image on right)
6. Place the DNA in a clean microcentrifuge tube. Add 70% ethanol to rinse the DNA. Once the DNA dries, they will add approximately 100 µL of DNA elution buffer. Incubate the DNA @ 60 °C for approximately 20 minutes.



Tip: While waiting- activity can proceed to Sequence C-D

Visualize DNA Procedure (Part 2)

7. After approximately 20 minutes, take out isolated DNA. DNA is soluble in water. By placing the extract in water, the DNA will dissolve in water. Do not worry if all of the DNA dissolved.
8. Add 20 µL of liquid from the DNA extraction to 3 µL of running dye. The dye will help sink the DNA into the gel.
9. When the entire class' DNA samples have been loaded into the gel, electrophoresis will begin. After approximately 15 minutes, the gel will be placed on the UV table. Any DNA present will appear as bands and will glow.
10. The instructor will also load three additional lanes into the gel: one with a sample of DNA, a second lane that contains water and a third lane that contains a protein sample.



1. Water Only
2. DNA Ladder (Control)
3. Protein Sample (BSA)
4. Strawberry DNA sample

Build a DNA Molecule

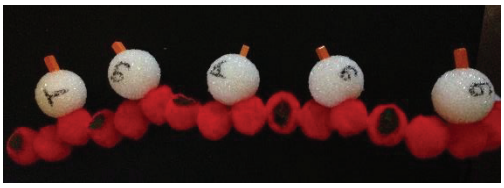
Preparation of Nucleotide Molecule Kits

Each kit contains: 1 DNA Template Strand and 17 DNA nucleotides in a container. Entire kit should be "N" or "S."

DNA Template strand

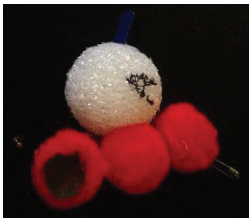
1. Take a strand of pom-pom garland and lay it out on a table.
2. At every third pom-pom in the garland, glue on a Styrofoam ball (need to get 17 balls onto the garland).
3. Glue one magnet onto each styrofoam ball
4. Paint the first pom-pom green. Then repeat at every third pom-pom.
5. Once everything is dried, write in the following sequence in order:
   - For N-kit strands: T-G-A-G-G-A-C-T-C-C-T-C-T-T-C-A-G
   - For S-kit strands: T-G-A-G-G-A-C-A-C-C-T-C-T-T-C-A-G

6. When you finish, should have the following (picture is showing a portion of the template sequence).



DNA Nucleotides (17 nucleotides per kit)

1. Take a strand of pom-pom garland and cut it after every third pom-pom.
2. Glue a styrofoam ball onto the middle pom-pom
3. Glue one magnet onto each styrofoam ball
4. Paint the first pom-pom green.
5. Add a safety pin to the pom-poms on each end.
6. Once everything is dried, write on the styrofoam ball one of the four nitrogenous bases as needed:
   - For N-kit strands: A, C, T, C, C, T, G, A, G, G, A, G, A, A, G, T, C
   - For S-kit strands: A, C, T, C, C, T, G, T, G, G, A, G, A, A, G, T, C
7. When you finish, you should have the following (picture is showing one nucleotide)



Build a DNA Molecule Procedure

Models in science are used to describe ideas, understand biological processes and make predictions.
Objective: Build a model to learn about the composition and structure of DNA.

In groups, work to build a model of DNA. The magnets and safety pins will serve as the hydrogen and phosphodiester bonds, respectively. Based on the model, describe:

1. Structure of the nucleotide
2. Base pair rules
3. Shape of the model (helical vs unwound)
4. How normal DNA differs from Sickle/Mutant DNA

Contact Information

Regarding the lessons or curriculum, please contact Dr. Edwin Li (eli@sju.edu)
Regarding the lab exercises, troubleshooting or technical issues, please contact Dr. Brian Forster (bforster@sju.edu)

For additional lessons in this unit and others, please visit www.asbmb.org/912activity/