**Fluorescent Tags: "You keep using that phrase. Do you know what it means?"**

by Kari Rhoades, 2020 CTI Fellow
Mallard Creek High School

This curriculum unit is recommended for:
Project Lead the Way: Principles of Biomedical Sciences, PLTW: Human Body Systems, PLTW: Medical Interventions, PLTW: Biomedical Innovations, AP Biology, grades 9-12

**Keywords:** Fluorescence, fluorescent tags, biochemistry, CTE, medicine, biology, biomedical sciences, luminescence, microscopy, microscope, imaging

**Teaching Standards:** See Appendix 1 for teaching standards addressed in this unit.

**Synopsis:**
This curriculum unit is designed to teach students about fluorescence and its applications in biomedical sciences. It introduces the underlying chemical principles of fluorescence and explores how light is produced at the atomic level. Students are then guided through various applications of fluorescence in biomedical research and diagnosis. The students will extract fluorescent dye from highlighters and observe the properties of the dye under ultraviolet light and various laser colors as an introduction to fluorescence. They will then look at how the rings on purines and pyrimidines can be modified to make them fluoresce to be used in cycle sequencing. They will model the use of fluorescent cDNA to determine protein expression in normal and cancer cells. Finally, they will use SYBR safe, a fluorescent DNA stain to produce DNA profiles for use in diagnosing familial hypercholesterolemia. Lessons in the unit will be a combination of lecture and laboratory work.

*I plan to teach this unit during the coming year to* **75** students in Project Lead the Way: Principles of Biomedical Sciences and PLTW: Medical Interventions in grades 9-12.

*I give permission for Charlotte Teachers Institute to publish my curriculum unit in print and online. I understand that I will be credited as the author of my work.*

## Introduction

Students think it is cool when they see things glow in the classroom setting, but do they really know what causes something to glow? As a 29 year science educator, I know that the physics and chemistry of luminescence is not directly addressed in the science standards at any grade level. As a *biomedical* sciences educator, I know that my future doctors, nurses, radiologists and more will be using fluorescent technologies extensively in research and patient care. It is important that they have an understanding of the "Science of Glow" so that they can appropriately apply fluorescent technologies, and someday be able to apply them in novel ways. For their more immediate educational need of this knowledge, fluorescent tags are frequently employed in many of the lessons and activities they will complete over the course of the biomedical sciences program.

### Rationale

Fluorescent dyes and molecule tags are used extensively in biomedical research and medical diagnostics. Fluorescent stains for microscopy are used to observe specific protein structures in cells to observe changes in drug trials, disease progression, and phenotype changes due to mutation. Fluorescent contrast dyes are used in endoscopies and angiograms. Fluorescent tags are attached to various biomolecules to study drug metabolism and in genetic studies. Fluorescence in situ hybridization (FISH) is a procedure used to locate specific DNA sequences on chromosomes.

A student entering a course of study that will lead to entering a medical profession will be using a wide variety of protocols that rely on the natural fluorescent properties of biomolecules and bioactive compounds, or their association with fluorophores that have been artificially introduced to the study environment. Throughout the four biomedical courses in PLTW, students use these molecules primarily to visualize DNA in the contexts of cycle sequencing, DNA microarrays, and gel electrophoresis. Students are asked to make use of fluorescent biomolecules throughout the PLTW curriculum, but there is not a specific strategy in the prescribed curriculum that explains how these fluorescent markers are produced, or how they produce light. An understanding of the principles of fluorescence will help them to understand what is happening at the molecular level in these procedures, which is important in applying these procedures outside the classroom and in novel situations, including actual interactions with a patient and in their own research.

### School/Student Demographics

Mallard Creek High school is an urban high school in the Northeast Learning Community of Charlotte Mecklenburg Schools, and serves students in grades 9-12. The school has won designation as one of "America's Best Urban Schools" by the National Center for Urban School Transformation (operated by San Diego State University) in both 2014 and 2017, citing that the school had demonstrated through multiple indicators "that your school is achieving impressive academic results for every demographic group you serve" (1). The student population is approximately 2400. According to U.S. News & World Report, the total minority enrollment is 86%, and 41% of students are economically disadvantaged, with 35% of students participating in

AP classes (2). Our graduation historically hovers around 95%. Each subject area in the school has a general department which is divided up by grade level or specific course within that subject area.

The classes I teach are mixed grades in the Biomedical Science Program within the Career and Technical Education department of my high school. Our biomedical curriculum is provided through Project Lead the Way (PLTW), a nationwide STEM (Science, Technology, Engineering and Math) program that has a computer sciences arm, engineering arm, and biomedical arm aimed at high school students. The courses in this program are classified as "honors" classes. I teach two of the courses for the biomedical arm of the program: "Principles of Biomedical Science" and "Medical Interventions". Most of the students I teach are above average academically, and a majority have chosen to take these classes because they are interested in careers in medicine. There are some lower achieving students that choose to take the class.

## Unit Goals

Each course offered by Project Lead the Way is a "canned" curriculum. Lessons are designed and sequenced by PLTW, and the organization administers a standardized end-of-course exam. Scores are reported to the district and state, and are used as the final exam score by Charlotte-Mecklenburg Schools. The curriculum is dense and rapid paced. For that reason, it can be difficult to diverge from the curriculum as written. However, this novel unit exploring fluorescence in biomedical applications can unify several optional lessons that offer strong support to objectives mandated by the curriculum.

The main goal of this unit is to familiarize students with fluorescence and how it is used in biomedical sciences. Students will develop practical skills by performing actual clinical tests that use fluorescent dyes to diagnose disease and design treatment plans for patients.

Students will study the underlying chemical and physical principles of fluorescence, how tags can be added to individual nucleotides for DNA and RNA analysis, and how fluorescent dyes are used in various biomedical research and diagnostic applications. (National Health Standards 1.21 Foundation: Standard 1, 1.23 Foundation: Standard 1)

Students will conduct student-designed experiments and model contact tracing of the spread of a pathogen using a fluorescent powder (National Science Education Standard A: Science as Inquiry)

Short term, my goals are to prepare these students to take the PLTW Principles of Biomedical Science end of course exam, which is prepared and securely administered through PLTW. Students are eligible for credit through University of Iowa and other colleges and universities based on their EOC scores. Long term, I want them to be able to move through the rest of the four-year PLTW program successfully and accumulate college credits so that they can be well armed for the college application process and be accepted into the programs of their choice. Since most of these students elected to take this course specifically because they want a medical career, the work they do in this course can be invaluable!

## Content Research

The Principles of Fluorescence

Fluorescence occurs when atoms absorb energy and then release it as light. The light emitted is noted for its bright, neon-like colors as seen in fluorescent highlighters, paints and fabric dyes. Fluorescence is essentially a subatomic process that occurs due to unique arrangements of shared electrons between atoms.

Electrons are arranged in layers around the nucleus of an atom by energy. Electrons of lower energy are closer to the nucleus, and those of higher energy are further from the nucleus. The electrons in the highest energy level of an atom are called valence electrons and can be used to form bonds with other atoms. Each energy level can be divided into orbitals, which electrons occupy. There are different types of orbitals (s, p, d and f), but the p-orbital is of the most interest in describing the phenomenon of fluorescence, especially in molecules.

When atoms bond with each other to form larger units (molecules), orbitals on the atoms overlap, allowing electrons to be shared and move between atoms, which holds them together. These bonds are covalent. A single bond is formed when two electrons in s orbitals (or sigma orbitals) are shared between two atoms. A double bond forms when two pairs of electrons in p orbitals are shared. A pattern of alternating single and double bonds in a molecule forms a "conjugated" system in which electrons in π-orbitals can move easily from one atom to another, which provides a stable, low energy state. (3)

Electrons can move between orbitals and energy levels as their energy fluctuates. Electrons strive for a stable, low energy arrangement referred to as the "ground state." However, there are constant adjustments as energy from the environment is absorbed by the electrons and emitted. In a π-conjugated system, the longer the pattern of alternating sigma and π-bonds the more electrons that are available to absorb and emit energy. During fluorescence, electrons in the ground state will absorb light energy, exciting them and causing them to "jump" to higher energy levels. These electrons are said to be in an "excited state." However, this state is unstable, and the electrons must emit the extra energy to return to the ground state. Electrons emit the extra energy as packets of energy called photons, which are perceived as fluorescence. The wavelength of the photon determines the color of fluorescence seen. Many conjugated molecules with alternating single and double bonds are especially good at emitting fluorescence. (4) All this electron jumping happens quickly, and only occurs if the molecule is exposed to an electromagnetic energy source. Once the external energy source is removed, the electrons relax to their ground state, and the fluorescence subsides.

The fluorescent properties of molecules can be exploited in medical research and diagnostics. Fluorescent molecules are used to mark nucleic acids and proteins of interest to sequence pathogens, identify genetic differences in normal cells and cancer cells. Fluorescent properties of pigments of the eye can be observed under blue light and green light to diagnose retinal abnormalities (5). Changes in protein expression can alter autofluorescence and exogenous fluorescent tag uptake in cancer cells (6).

Fluorescent Dyes in Cycle Sequencing

Cycle sequencing is used to produce copies of a genome or portions of a genome from an organism. It can be used to produce genetic profiles for novel pathogens, such as SARS-CoV-2, or identification of unknown organisms in infectious outbreaks. The sequences obtained can then be compared to a library of genetic sequences, such as the National Library of Medicine's Basic Local Alignment Search Tool (BLAST) to identify the pathogen or its most similar genetic match. Cycle sequencing uses polymerase chain reaction (PCR), but with a twist. In regular PCR, genetic sequences are copied and amplified in their entirety using normal, run-of-the-mill nucleotides. In cycle sequencing, fluorescently tagged nucleotides are used along with normal nucleotides. Nucleic acids are extracted from the organism of interest. Short complementary DNA sequences called primers are used to frame the sequence on the organism's DNA to be copied. The DNA is heated to break the hydrogen bonds that hold the base pairs together across the double helix. The DNA is cooled to allow the primers to hybridize to the unzipped sides. The temperature of the mixture is then raised to allow loose nucleotides to match to the exposed bases on the unzipped strands. If regular nucleotides match to the strand, the strand will elongate. However, if a fluorescently tagged nucleotide is added to the strand, it blocks further nucleotides from being added. This occurs due to the placement of the fluorophore. Taq polymerase is the enzyme the binds the new nucleotides into position. The fluorophore basically acts as a roadblock, interfering with the ability of the Taq polymerase to move past the fluorophore and bind the next nucleotide in place (7) (see Figure 1). The new DNA fragment will always end with a fluorescently tagged nucleotide.

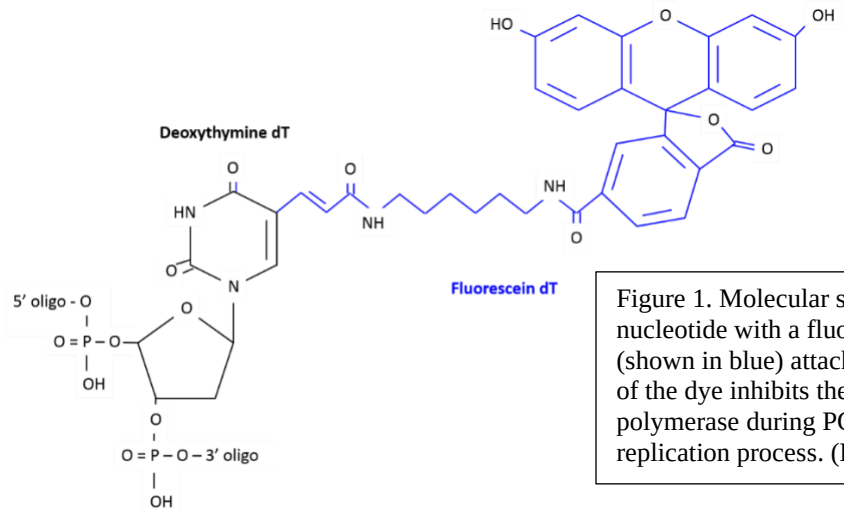



Figure 1. Molecular structure of a thymine nucleotide with a fluorescein dye molecule (shown in blue) attached to it. The placement of the dye inhibits the function of Taq polymerase during PCR and stops the replication process. (Figure by Kari Rhoades)

The addition of a florescent nucleotide is random, and over many cycles, it results in thousands of fragmented copies of the sequence of interest of different lengths, each of which ends with a fluorescently tagged nucleotide. PCR is completed with the binding of the new nucleotides in place with Taq polymerase. The fragments are then run through gel electrophoresis, which causes them to separate by length. Shorter fragments of DNA move through the gel faster than longer ones, and there should be fragments that are all just one nucleotide longer than the next as you move up the gel. The gel is then passed through a

laser array, and the tagged nucleotides fluoresce, sending a signal to a computer, which converts the signal to a chromatogram. The chromatogram peaks can then be read as the base sequence for the original DNA from the organism of interest (see figure 2).

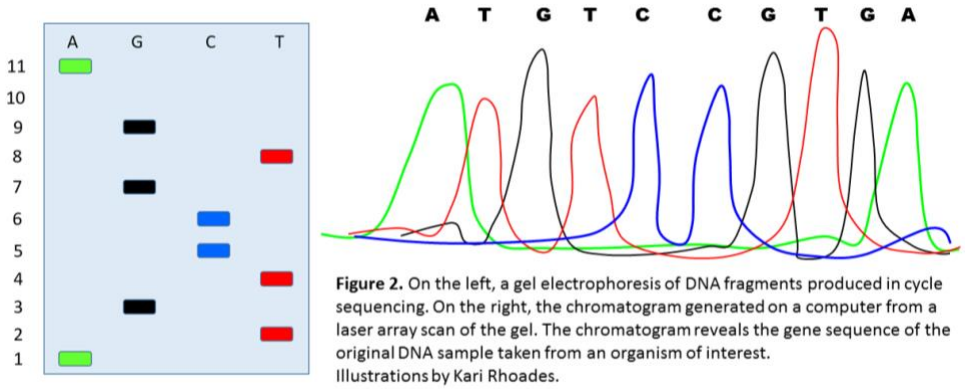



**Figure 2.** On the left, a gel electrophoresis of DNA fragments produced in cycle sequencing. On the right, the chromatogram generated on a computer from a laser array scan of the gel. The chromatogram reveals the gene sequence of the original DNA sample taken from an organism of interest. Illustrations by Kari Rhoades.

The most commonly used fluorescent dyes used in cycle sequencing are referred to as the "big four", and are FAM (blue), JOE (green), ROX (red) and TAMRA (orange/yellow). (See Table 1). These dyes are attached to the nucleosides at the pyrimidine or purine. (See example in Figure 1). All of these dyes have an absorbance that includes 488 nm in their ranges, which allows use of a single laser illuminator to use on the gel.

| Diminutive | Fluorescent Dye | Absorbance | Emission | Color |
|---|---|---|---|---|
| FAM | 6-Carboxyfluorescein (6-FAM) | 495 | 517 | Blue |
| JOE | 4,5-dichloro-dimethoxy-fluorescein | 520 | 548 | Green |
| ROX | 6-Carboxyl-X-Rhodamine | 535 | 608 | Red |
| TAMRA | Carboxytetramethylrhodamine-deoxythymidien | 565 | 580 | Orange |

Table 1. The "Big Four" dyes used in DNA sequencing. (8)

When discussing this procedure in the biomedical classroom, it should be stressed that the fluorescent tags serve two purposes. First, when a fluorescent nucleotide is added to a sequence under assembly, it blocks further nucleotides from being added. This effectively produces fragmented sequences of random lengths. Secondly, the fluorescent nucleotides will light up when passed under the laser array, sending a signal to the computer.

Fluorescence and Microarrays

A microarray is a protocol that is used to determine what proteins are being expressed by cells (9). Microarrays are one tool available to oncologists in crafting a personalized treatment plan for cancer patients. Every cancer patient's disease is unique, and responses to

a particular treatment vary from patient to patient, making it challenging to treat. Mutations in cancer cells alter the proteins that they express compared to normal cells. Treatment can be planned by producing protein profiles for a patient's normal cells and cancer cells using microarrays.

A microarray is protocol starts with selecting the proteins that will be looked for. Let's say that a patient has lung cancer. Specific proteins are associated with changes in normal lung cells and cancerous lung cells, but again, these vary from patient to patient. A glass plate is spotted with complementary DNA (cDNA) for the proteins of interest selected from a cDNA library and produced by polymerase chain reaction (PCR) (see figure 3). Next, some of the patient's normal lung tissue is biopsied, along with some of their cancerous lung tissue. Messenger RNA is extracted from both samples. Messenger RNA (mRNA) is a copy of a gene code from the DNA made in the nucleus of a cell to send to its ribosomes for protein synthesis. We are able to determine which proteins these cells are actively making, or not making by the mRNA that is being transcribed. The mRNA is converted to cDNA and amplified via polymerase chain reaction (PCR). The engineered cDNA from the cells is made with modified nucleotides, such as aminoallyl-UTP (aa-UTP), that have had a florescent dye added to the amino group of the nitrogen base (9). In the case of the cDNA from the cancer specimen, nucleotides with red fluorescent tags are added. For the normal specimen cDNA, nucleotides with green fluorescent tags are used. The prepared cDNA from both specimens is then dripped onto the spots on the prepared microarray plate. If the specimen cDNA is complementary to the cDNA on the microarray plate, it will hybridize (see figure 4). The plate is then viewed under an ultraviolet light, and the color of fluoresce in each spot is noted. Red fluorescence indicates that the protein associated with the cDNA on the spot is being produced primarily by the cancerous cells, green indicates that the protein is being produced by the normal cells, yellow is a combination of the red and green both fluorescing, indicating that the protein is produced by both specimens, and no fluorescence means that neither specimen was producing the protein of interest (see figure 5) (10).

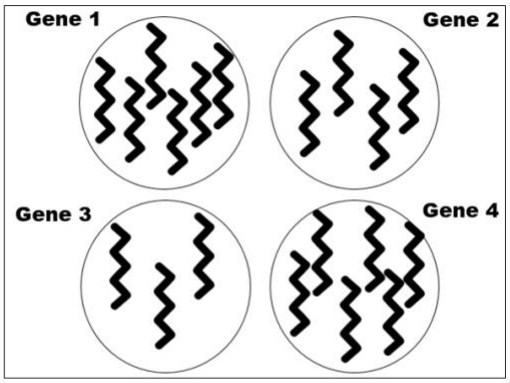



**Figure 3:** cDNA selected from a library attached to microspots on a glass microarray plate.

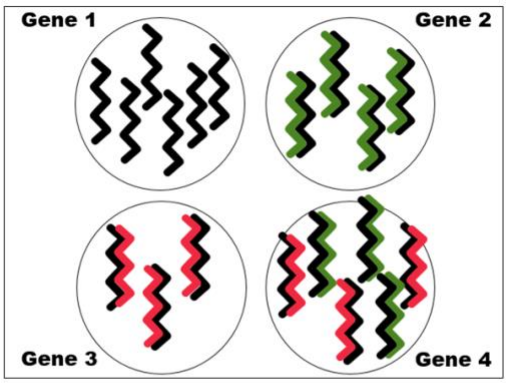



**Figure 4:** cDNA from cancer cells (red) and normal cells (green) dripped onto each spot on microarray plate. If the cDNA from the cells hybridizes with the cDNA on the microspots, the cells are producing the proteins associated with the cDNA.

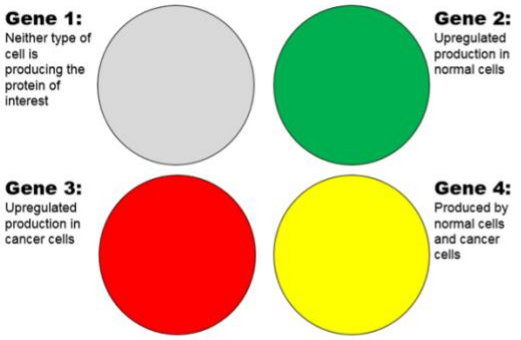



**Figure 5:** Key for fluorescence colors of microspots when viewed under ultraviolet light.

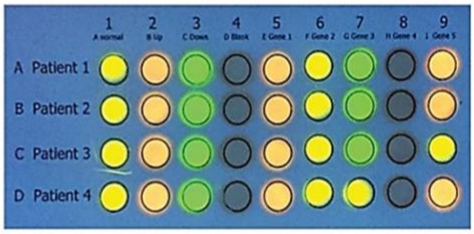



**Figure 6:** Results for a modified microarray carried out on a paper card. Photo by Kari Rhoades

Figure 6 shows the results of a modified microarray made for the classroom. The four patients have all been diagnosed with lung cancer, but analysis of columns 5 through 7 indicates that their cells are not all expressing the same proteins. This information can be used by an oncologist to determine a course of treatment. For example, a certain chemotherapy drug that targets the action of gene 3 may work well for patient 4, for whom both types of cells are expressing the associated protein, but not for patients 1, 2 and 3, for whom only the normal cells express the associated protein. The fluorescent tags used in this procedure are the primary method of discovery.

Fluorescence in Gel Electrophoresis Visualization

Electrophoresis of nucleic acids is an important diagnostic tool in genetic counseling. Applications include paternity testing, verifying the presence of genetic mutations such as ΔF508, a deletion mutation that is the most common cause of cystic fibrosis, and mutations in BRCA-1, BRCA-2, the genes generally known to be associated with breast cancer.

In preparation for gel electrophoresis, sequences of interest on the DNA sample are targeted with restriction enzymes, which cut the DNA at specific sites in the sequence. The pieces of DNA are “restriction fragment length polymorphisms”, or RFLPs. The RFLPs are then inserted into an agarose gel, and then immersed in a buffer solution through which an electric current flows. The RFLPs have a negative charge, and flow with the current toward the positive terminal of the power source. Shorter RFLPs move more quickly than longer ones, and the RFLPs sort into distinctive patterns based on base-pair lengths. The gel is immersed in a stain, and the RFLPs take up the stain for visualization.

Electrophoresis was first used to separate nucleic acid fragments in the 1960s, but to visualize the fragments required radioactive labeling of the nucleic acids, or staining with ethidium bromide, a known mutagen. Safer pigment stains were developed in the 1990s, but these stains are not very “sensitive”, and if there is not a significant amount of sample, the RFLPs will not show up. Easy-to-use fluorescent nucleic acid stains have become available in the last decade. These stains have the advantages of being less toxic/mutagenic, and being very sensitive. The gels are visualized under UV or blue light transilluminator, and the

fragments fluoresce brightly. The most commonly used fluorescent gel stain in high school labs is SYBR Safe by ThermoFisher Scientific (11). SYBR Safe is a cyanin dye, and emits green light with a wavelength of about 524 nm. It has aromatic rings that facilitate fluorescence (12). (See figure 7)

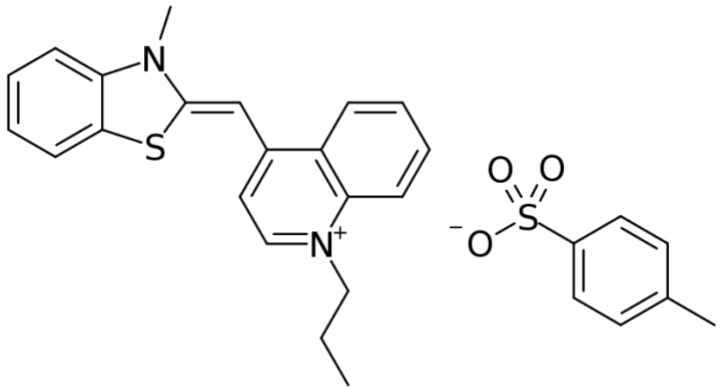



Figure 7. Chemical structure of SYBR Safe. (Public domain)

DNA stains generally adhere to DNA in one of two ways. The first is intercalative, in which stain molecules embed themselves between base pairs in the DNA ladder. Ethidium bromide binds to DNA by intercalation. The second method involves the stain molecules tucking themselves into the minor groove of the DNA ladder. The minor groove is the narrow trench formed along the double helix where the two sides of the backbone run closer together. Hoechst stain is a fluorescent dye that attaches to DNA in this manner (see Figure 8). The mechanism by which SYBR binds to DNA is not completely understood, but a study by Zipper (13) indicates that the mechanism of SYBR Green I binding is likely by insertion into the minor groove.

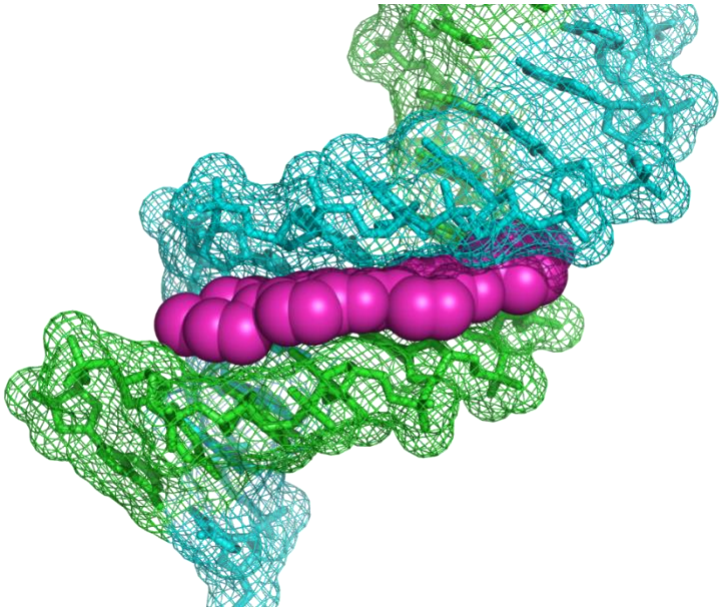


Figure 8. Hoechst stain (magenta) embedded in the minor groove of double stranded DNA (blue and green). Illustration by Zephyris at the English language Wikipedia, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=10690812

**Instructional Implementation**

Lesson 1: Fluorophore Fun!

*Learning Goals: The student will –*

- Explain how fluorescence occurs.
- Devise experiments to observe and apply fluorescence in a clinical setting.
- Model the spread of a pathogen through a population with fluorescent powder.

*Instructional Input:*
Students will be introduced to essential vocabulary (link). The teacher will present a slide show and give a lecture explaining the atomic arrangements and events that cause fluorescence. Students will be shown various clinical and research applications for fluorophores, and be introduced to higher level experiments they will do in the unit employing fluorophores.

*Modeling:*
The teacher will demonstrate fluorescence with glow sticks, and discuss the fluorophores responsible for the different colors.

*Check for Understanding:*
Students will draw Bohr models of atoms and label the nucleus, energy levels and valence electrons. Students will illustrate the movement of an electron between its ground state and excited state, including the emission of a photon. Students will write an accompanying paragraph explaining how their diagram illustrates the production of fluorescence, and give more details about the unique chemical structure of fluorophores.

*Guided Practice:*
Students will work in groups to carry out various activities demonstrating fluorescence and some of its practical applications at activity stations set up in the classroom.
Activity 1: Extracting fluorescent ink from highlighters (14) (Link)
Activity 2: Glow paint (15) (Link)
Activity 3: Contact tracing with Glo Germ (16) (Link)

*Independent Practice:*
Students will research three applications of fluorescence in biomedical sciences of their choosing. Students will make a table to visually organize four key pieces of information: What category of medical intervention the application belongs to, description of the procedure, how the fluorescence is detected in the procedure, and why the procedure is employed.

*Time:*
Approximately one 90 minute block period.

Lesson 2: Fluorescent Gels

*Learning Goals: The student will -*

- Describe how fluorescent stains are used in DNA visualization in gel electrophoresis.
- Modeled the placement of SYBR Green I stain in the minor groove of DNA.
- Perform marker analysis to determine the presence of a genetic mutation associated with breast cancer.
- Stain and visualize a gel with SYBR Safe stain.

*Instructional Input:*
Intro: Students will be introduced to essential vocabulary. (Link)

Part I:

- Students will review the principles of gel electrophoresis.
- The teacher will explain how DNA gel stains attach to DNA by intercalation and by binding to the minor groove.
- Students will review the principles of fluorescence. The teacher will explain the properties of SYBR Safe DNA stain, and how it is used to visualize DNA in the lab that they will perform in part II of this lesson.

Part II: The students will participate in a lecture/discussion of genes associated with increased risk of cancer, and how gel electrophoresis can be conducted to determine if a patient has these genes. Emphasis will be placed on BRCA 1 and 2. This lecture will be based on curriculum content provided by PLTW for Medical Interventions lesson 3.2.3.

*Modeling:*
Part I: The teacher will show students a 3D model of DNA and point out the major and minor grooves. The teacher will share pictures indicating the attachment of Hoechst stain to DNA in the minor groove.

Part II: The teacher will demonstrate how to make a standard curve using marker DNA and use the standard curve to determine base pair lengths of RFLPs in a patient sample to verify the presence or absence of BRCA genes in a patient sample.

*Check for Understanding:*
The class will participate in a Kahoot to verify that they have grasped the basic concepts of fluorescence, gel electrophoresis and genetic markers. (Link)

*Guided Practice:*

- Part I: Students will build DNA models from the materials list (link) using the protocol provided in the student resources. (Link)
- Part II:
    - Students will carry out the lab protocol for Ward's® Detection of Hereditary Breast Cancer Kit and as prescribed in the PLTW curriculum for Medical Interventions lesson 3.2.3 (Link)

- Students will make a standard curve from their marker DNA results as described in the PLTW curriculum for Medical Interventions lesson 3.2.3.

*Independent Practice:*
Students will conduct a gel electrophoresis on members of a fictional family to determine if member have the BRCA2 mutation that is associated with increased risk of breast cancer. Students will stain the gels with SYBR Safe and analyze the gel results with a blue-light transilluminator. The students will take pictures of their gels and use rulers and graph paper to make a standard curve from the marker DNA. Students will then examine patient RFLP patterns to determine which of the patients have RFLP patterns that indicate the BRCA2 mutation. Students will respond to a set of reflection questions and conclusion questions that are provided in the PLTW curriculum for Medical Interventions lesson 3.2.3.

Lesson 3: Cycle Sequencing and Fluorescence

*Learning Goals: The student will -*

- Describe the role of fluorescently tagged nucleotides in cycle sequencing.
- Describe how bioinformatics, the collection, classification, storage, and analysis of biochemical and biological information using computers, can be used to identify disease pathogens.
- Use publicly available molecular databases to search for DNA sequences and identify pathogens.

*Instructional Input:*
Students will be introduced to the essential vocabulary (link). The teacher will present a slide show to review the molecular characteristics of nucleotides and fluorophores. New information will include how the fluorophores are bound to the nucleotides used in the cycle sequencing process.

*Modeling:*
The teacher will use models of fluorophore-nucleotide conjugated molecules to demonstrate the “Fluorescent Stop Signs” activity.

Check for Understanding: Students will respond to the analysis and conclusion prompts at the end of the student handout for the “Fluorescent Stop Signs” activity.

*Guided Practice:*
Students will be given copies of the “Fluorescent Stop Signs” activity and the “Nucleotide Resource Sheet”. Following the instructions on the handouts, students will model the process of cycle sequencing by making paper nucleotides and copying a short template DNA sequence. (Link)

*Independent Practice:*
Students will complete PLTW Medical Interventions lesson 1.1.3.

*Time:*
Approximately 2.25 hours across two 90 minute block class periods. Lecture and student nucleotide activity day 1, PLTW Medical Interventions lesson 1.1.3 on day 2.

Lesson 4: Microarrays and Fluorescence

*Learning Goals:*

- Students will recognize that DNA microarrays measure the amount of mRNA for genes that is present in a cell sample.
- Students will perform a DNA microarray to analyze gene expression patterns.
- Students will describe the role of fluorescence in the visualization of microarray results.

*Instructional Input:*
Students will be introduced to essential vocabulary. (Link)
Students will participate in lecture and class discussion on the underlying principles of a DNA microarray as the teacher presents a slide deck. (Link)

*Modeling:*
Teacher will demonstrate the use of the micropipettor prior to the lab activity.

*Check for Understanding:*
Students will complete post lecture/lab questions and review microarray concepts on a simulation. (Link)

*Guided Practice:*
Students will carry out the lab protocol for the microarray provided by Edvotek for kit #235. The teacher will circulate the room to answer questions, do spot checks for understanding, and to ensure that students are adhering to the protocol. (Link)

*Independent Practice:*

- Students will watch an animation and take a short quiz. They will email the results to the instructor. (Link)
- Students will make a paper model of a DNA microarray and present it to a partner or to the teacher in a video. The model should have 3D moving parts that they can manipulate as they describe the process. (Link)

*Time:*
Approximately 2.25 hours, lecture day 1 (25 minutes) with animation and quiz, lab day 2 with time to begin working on paper model of microarray.

## Appendix I: Teaching Standards

### *National Science Education Standards*

**Unifying Concepts and Processes:** As a result of activities in grades K-12, all students should develop understanding and abilities aligned with the following concepts and processes:

- Systems, order, and organization
  - Types and levels of organization provide useful ways of thinking about the world.
- Evidence, models, and explanation
  - Evidence consists of observations and data on which to base scientific explanations.
  - Scientific explanations incorporate existing scientific knowledge and new evidence from observations, experiments, or models into internally consistent, logical statements.
  - Models are tentative schemes or structures that correspond to real objects, events, or classes of events, and that have explanatory power.
- Form and function
  - The form or shape of an object or system is frequently related to use, operation, or function

### *National Health Standards*

- 1.21 Foundation: Standard 1: Academic Foundation: Understand human anatomy, physiology, common diseases and disorders, and medical math principles.
  - Describe common diseases and disorders of each body system (such as: cancer, diabetes, dementia, stroke, heart disease, tuberculosis, hepatitis, COPD, kidney disease, arthritis, ulcers).
  - a. Etiology b. Pathology c. Diagnosis d. Treatment e. Prevention
- 1.22 Foundation: Standard 1: Academic Foundation: Understand human anatomy, physiology, common diseases and disorders, and medical math principles.
  - Discuss research related to emerging diseases and disorders (such as: autism, VRSA, PTSD, Listeria, seasonal flu).
- 1.23 Foundation: Standard 1: Academic Foundation: Understand human anatomy, physiology, common diseases and disorders, and medical math principles.
  - Describe biomedical therapies as they relate to the prevention, pathology, and treatment of disease.

## Appendix II: Materials Lists

Materials List Lesson 1:

- Activity 1: Highlighters of various colors, water, beakers, UV light, gloves, scissors, pliers (for teacher use)
- Activity 2: Liquid laundry detergent, weigh boats, water-based paint, paper, paint brushes, blacklight, pipet, small beakers
- Activity 3: Glo Germ powder (Link to ordering information)

Materials List Lesson 2:

- For lecture: Slides (Link)

- For Part I: Modeling DNA and SYBR Green I attachment: toothpicks, gum-drops (or suitable substitute), glow dough, straw or dowel rod, paper cup (Link)
- For Part II Wet Lab: Ward's Detection of Hereditary Breast Cancer Kit (Link), blue-light transilluminator and/or UV light, distilled water, gel chambers, micropipettors, micropipette tips, personal protective equipment, SYBR Safe DNA gel stain

Materials List Lesson 3:
Copies of student handout (Link), copies of Nucleotide Resource Sheet (Link), pink, blue, yellow and green highlighters, UV lamp, camera, scissors, tape

Materials List Lesson 4:
- For lecture: Microarray slide deck (Link)
- For the lab: Automatic micropipettes and tips, distilled water, beakers or flasks, Edvotek kit # 235 (Includes instructions, simulated patient DNA and RNA samples, controls, microarray cards, plastic bags to incubate membrane, microcentrifuge tubes, pipets.)
- Edvotek kit #235 instructions (Link)
- For the model: Glue, scissors, construction paper, paperclips, video recording device.

## Appendix III: Vocabulary Lists

Vocabulary List Lesson 1:

| | |
|---|---|
| **Electron** | A negatively charged particle of an atom that orbits the nucleus. |
| **Covalent Bond** | A bond that forms between atoms when electron orbitals overlap, allowing two atoms to share electrons. |
| **Fluorescence** | The emission of light by a substance that has absorbed light or other electromagnetic radiation. |
| **Fluorophore** | A fluorophore is an organic molecule with the ability to absorb light at a particular wavelength and then emit it at a higher wavelength. |
| **Photon** | A packet of energy that is released by an electron when relaxes down to a lower energy level. A photon is perceived as a wavelength of light. |
| **Energy Level** | A fixed amount of energy that a system (electrons, etc.) can have. |
| **Orbital** | A region around the nucleus of an atom that electrons inhabit. |
| **Ground State** | Describes an atom in which the electrons are in their lowest energy state, and the atom is in its most stable configuration. |
| **Excited State** | Describes an electron that has absorbed energy and jumped to a higher energy level. |

Vocabulary List Lesson 2:

| | |
|---|---|
| **Deoxyribonucleic Acid (DNA)** | A double-stranded, helical nucleic acid molecule capable of replicating and determining the inherited structure of a cell's proteins. |
| **Gel Electrophoresis** | The separation of nucleic acids or proteins, on the basis of their size and electrical charge, by measuring their rate of movement through an electrical field in a gel. |
| **Helix** | Something spiral in form. |

| | |
|---|---|
| **Minor Groove** | A shallow "furrow" in the molecular structure of a DNA double helix, which measures 1.2 nm across and extends the entire length of DNA as long as the molecule remains in a normal or right-handed DNA conformation. |
| **Allele** | Any of the alternative forms of a gene that may occur at a given locus |
| **BRCA** | Either of two tumor suppressor genes (BRCA1 and BRCA2) that in mutated form tend to be associated with an increased risk of certain cancers and especially breast and ovarian cancers |

Vocabulary List Lesson 3:

| | |
|---|---|
| **Bioinformatics** | The collection, classification, storage, and analysis of biochemical and biological information using computers especially as applied in molecular genetics and genomics |
| **Genome** | All of an organism's genes; an organism's genetic material |
| **Pathogen** | A specific causative agent of disease |
| **Primer** | A molecule (a short strand of RNA or DNA) whose presence is required for formation of another molecule (a longer chain of DNA) |

Vocabulary List Lesson 4:

| | |
|---|---|
| **Biopsy** | The removal and examination of tissue, cells, or fluids from the living body. |
| **Cancer** | A malignant tumor of potentially unlimited growth that expands locally and systemically. |
| **DNA Microarray** | A microarray of immobilized single-stranded DNA fragments of known nucleotide sequence that is used especially in the identification and sequencing of DNA samples and in the analysis of gene expression (as in a cell or tissue). |

## Appendix IV: Student Resources

Student Resources Lesson 1:
Station instructions:
Activity 1: Extracting fluorescent ink from highlighters

1. Put on gloves. Remove the ink column from one of the highlighters, and use the scissors to cut along the length of the plastic tube encasing the ink column. You will find that the column is filled with fibers soaked in the fluorescent ink.
2. Soak the column in 50 mL of water in a beaker for about 5 minutes. Move the column around and turn it upside down to get as much ink as possible to mix with the water.
3. Turn out the lights and use the UV light to observe the liquid. Try mixing it with more water, or with other colors to see how it affects the degree of glow in your beaker.
4. Clean up your mess, but leave some of your original ink mixture for the next lab group to observe and experiment with!

Activity 2: Glow paint

1. Put on gloves.
2. Pour a small amount of liquid laundry detergent into a beaker. Observe it under a UV lamp. What do you see?
3. Pour 15 mL of white paint into a weigh boat. Observe it under a UV lamp. What do you see?
4. Using a pipet, add 1mL of the liquid laundry detergent to the paint and stir it to mix thoroughly.
5. Shine the UV lamp on the paint mixture. If the glow of the paint is too faint, add small amounts of additional laundry detergent until you are satisfied. Warning: too much and your paint will be too thin to get a good glow on your final product!
6. Using a paint brush and a piece of construction paper, paint the word "fluorophore" and write its definition. You may then decorate the paper with your own drawings. Maybe of a Bohr model of an atom or fluorescent molecule that you look up!
7. Observe your painting under UV light and take a picture. Give your picture to the instructor, then clean up and dispose of materials as instructed.
8. Research: Why do some laundry detergents fluoresce? Cite your source.

Activity 3: Contact tracing with Glo Germ

1. Obtain a small amount of Glo Germ powder. Rub it all over the palms of your hands.
2. For the rest of the class period, go about your normal business. Try not to wipe your hands on your clothing. It will wash out in cold water, but if you forget and wash your clothes in hot water it will stay permanently.
3. About 10 minutes before the end of class, wash your hands thoroughly. Use a blacklight or UV lamp to observe objects and places that you made contact with.
4. Reflection: What does this tell you about the importance of you washing your own hands, and others washing their hands?

Student Resources Lesson 2:

Student handout for Part I

## Fluorescent DNA Stains

Introduction:

Gel electrophoresis separates RFLPs in a gel, but how are we going to see them to evaluate them? In this lesson, we will be using SYBR Safe DNA stain, a green fluorescing stain that will be visualized with a blue-light transilluminator. But how does SYBR Safe actually stain the DNA? In this activity you will demonstrate the methods by which DNA stains bind by building a rudimentary 3D model of DNA and using fluorescent modeling dough to represent SYBR Safe stain.

Materials:

- Toothpicks
- Gumdrops (or suitable substitute)
- Straw or dowel rod
- Paper cup

- Glow dough
- UV light source

Instructions:

1. Preparation: Review the structure of DNA. Be sure that you remember the placement of deoxyribose sugar, phosphate groups, and bases. Consult the 3D model provided by the teacher and the pictures from the lecture to guide you. Review the methods of DNA stain binding. Be sure that you can explain how stain can be intercalated in the base pairs or bound to the minor groove.
2. Design:
    a. With a partner, determine how you want to represent the specific parts of DNA with your materials. These parts must include deoxyribose sugar, phosphate groups and bases.
    b. Discuss how to best use the materials to produce a double helix that has a major and minor groove. DNA has 10 base pairs per turn, so with a 20 base pair model, you should have a single twist in the center of your model. Note: you may use the straw or dowel rod as a central support to build your helix around. And prop it upright using the cup as a pedestal. If this proves problematic, you do not have to use it, but it will improve the neatness and presentation of your final product.
    c. Plan how the glow dough will be employed to represent SYBR Safe in your DNA model.
3. Execution: Build your DNA model, adjusting as necessary to produce a major and minor groove. Attach your "SYBR Safe" glow dough model. Use the UV light to observe it!
4. Check for accuracy: Ask another group to look at your model. Ask them if they can easily identify the different parts to determine if your representation is accurate.
5. Presentation: Rehearse an explanation of your model that explains the structure of DNA, and the mechanism of attachment for the SYBR Safe stain. Ask the instructor to visit your work area and give a brief, informal presentation of your model. Your instructor may ask you to record a video.

Student Resources Lesson 3:

Student handout:

## Fluorescent Stop Signs

Introduction:

Fluorophores are molecules that glow and are used in many contexts in biomedical research. In this activity, you will model how fluorophores attached to nucleotides are an important component in cycle sequencing. Cycle sequencing uses polymerase chain reaction (PCR) to copy an unknown sequence of original template DNA in order to determine the order of its adenine, thymine, guanine and cytosine. This sequence can then be compared to a DNA library to reveal the gene it codes for, or what organism it comes from. The template DNA is then mixed with regular loose nucleotides, fluorescently tagged loose nucleotides and Taq

polymerase. Under PCR conditions, the template DNA is matched with loose nucleotides, which are then bound together with Taq polymerase. However, if one of the fluorescent nucleotides is matched to the template DNA, it stops the production of the new copy of DNA. This results in fragmented copies of the template DNA, which all end in a fluorophore tagged nucleotides. These fragments are then separated by length using gel electrophoresis. The gel is read in a laser array, which lights up and sends a signal to a computer every time a fluorescent tag is detected. This produces a chromatogram in the computer program. In this activity, you will model what happens during the PCR procedure in cycle sequencing. You will observe the effects of the fluorophore tagged nucleotides on the copying process, and their use in visualizing the final DNA sequence.

Materials:

- Nucleotide Resource Sheet
- Pink, blue, yellow and green highlighter
- Scissors
- Tape
- Camera

Set-Up Instructions:

1. Get a "Nucleotide Resource Sheet" and other materials from your instructor.
2. For the "Fluorescent Nucleotides": Use the highlighters to color the "Fluorescent Nucleotides". Notice that these nucleotides have a tag sticking out of one side of the nucleotide. This will inhibit the action of Taq polymerase during PCR. Cut the individual nucleotides apart from the sheet.
3. For the "Template DNA": Cut out the "Template DNA Strand" as one piece.
4. For the "Normal Nucleotides": Cut the strips of nucleotides out, then cut the individual nucleotides apart at the dotted lines.

Activity Instructions:

1. One lab partner will act as the "Taq Polymerase". This partner will read the DNA Template and call out the nucleotide that matches up with the Template DNA Strand in order.
2. The other lab partner will hand free nucleotides to the "Taq Polymerase" partner when called for from the Taq Polymerase partner.
3. Starting from the 3' end, the Taq Polymerase partner calls for the complementary nucleotide for the first base in the Template DNA strand. In this case, the first base in the template is "C", so the Taq Polymerase partner will ask for a "G".
4. The free nucleotide partner will hand the nucleotide requested to the Taq Polymerase partner, and the Taq Polymerase partner will begin taping together nucleotides as they are handed to them to make a complementary copy of the template strand.
5. HOWEVER, the free nucleotide can act "sus"! They can hand the Taq Polymerase partner a normal nucleotide, or a fluorescent one!
    a. If a normal nucleotide is added, the Taq Polymerase partner continues taping on the new nucleotides.
    b. If a fluorescent nucleotide is added, the tag sticking off the side acts as a barrier and blocks a new nucleotide from being added. The end of each DNA

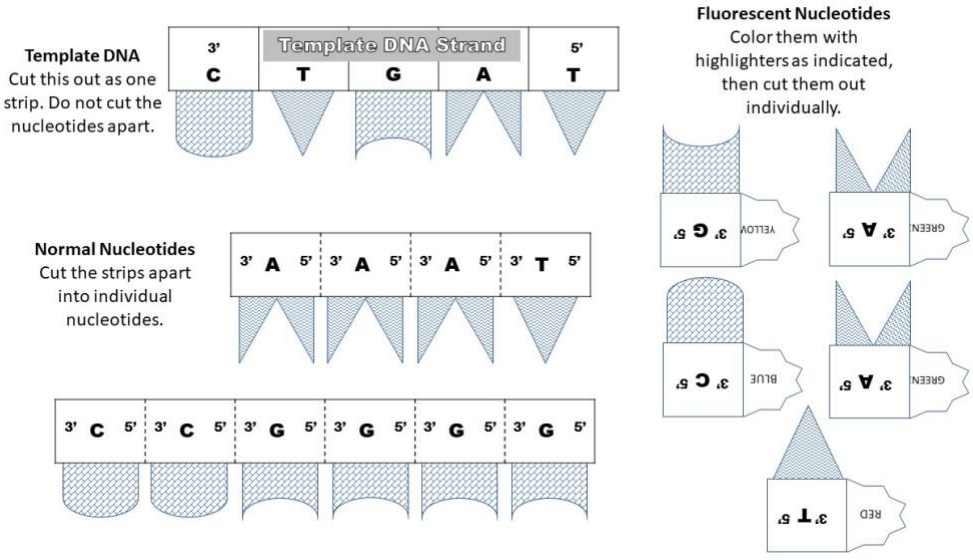
fragment will have a fluorophore tag. Each time a fluorescent tag is added, the fragment is done. Tape them together and then start a new copy of the Template DNA.

6. Continue this process until you run out of free nucleotides. You should end up with five fragments of different lengths.
7. Arrange the five fragments in order from shortest to longest.
8. View your fragments under UV Light. You should see that your tagged nucleotides glow! Write the order of the glowing nucleotides on the fragments, starting with the shortest.
9. Take a picture of your fragments under UV light and under normal light. You will include these pictures in the document you submit as a check for understanding.

Analysis and Conclusions:

1. What is the order of fluorescent nucleotides on your fragments, from shortest to longest?
2. Compare this sequence to that of the template DNA strand. What do you observe? Explain.
3. Explain how the fluorescent tags on some of the nucleotides produced DNA fragments of different lengths.
4. Look back on step 7 of the instructions. In a real cycle sequencing lab protocol, how would the DNA fragments be separated by length?
5. Make a chromatogram of your fluorescent tag results using highlighters. Label the chromatogram.
6. In a real cycle sequencing lab protocol, why would the fluorescent tags be important in producing a chromatogram?
7. The title of this activity is “Fluorescent Stop Signs”. Explain why this title is appropriate for this activity.

Nucleotide Resource Sheet:



Student Resources Lesson 4:

- Student independent practice: http://highered.mheducation.com/sites/0072556781/student_view0/chapter15/animation_quiz_2.html
- Set-up instructions, student worksheets, answer key and protocol for Edvotek kit #235
- Student check for understanding handout:

Microarray Online

You will complete an online microarray lab. As you progress through the lab, respond to the prompts given below on your own sheet of paper. Link to lab: https://goo.gl/7XaS78

1. Why are tissue samples form healthy AND cancer cells taken from the same patient?
2. Describe the process used to isolate mRNA from the other types of RNA.
3. Why is it necessary to make a cDNA copy? Why is mRNA not used?
4. Draw and label a diagram of the process of how labeled DNA copies are made. Your diagram should be in color.
5. What happens once you apply the DNA from the two samples to the DNA microarray?
6. What does the red color indicate?
7. What does the green color indicate?
8. What does the yellow color indicate?
9. What conclusions can you draw from the microarray data?
10. What are the limitations of DNA microarray technology?

- Independent practice: Microarray model handout:

Microarray Model Project

You will build a model of a DNA microarray with moving parts that you will use to describe the procedure in a presentation to a small group or on a video that you will submit on our online learning management system.

Materials:

- Construction paper
- Glue
- Scissors
- Paper clips or other device for temporarily attaching pieces of your model together.
- Optional: fluorescent highlighters, black light

Required elements of the model:

- 4 microspots with 3D cDNA attached. Identify the genes the cDNA will be specific for. At least one gene must be a tumor suppressor gene. Plan to have one gene be

expressed by your normal cells only, one by your cancer cells only, one that will be expressed by both, and one that will not be expressed by either type of cell.

- cDNA from a cancer cell (must be 3D and detachable)
- cDNA from a normal cell (must be 3D and detachable)
- Some mechanism for attaching and detaching the cDNA on the microspots to the cDNA from your patient cells.

Required elements of presentation:

- Explanation of how cDNA is made.
- Explanation of how fluorescent tags are employed.
- Manipulation of your model AS YOU EXPLAIN what is happening.
- How to analyze results.
- Prediction of what colors each gene will be when observed under UV light. This will depend on the specific genes you chose. Justify your prediction!

Rubric:

| Model Elements | Strong | Satisfactory | Basic | Not Present |
|---|---|---|---|---|
| • 3D parts | 3 | 2 | 1 | 0 |
| • Parts are attachable/detachable | 3 | 2 | 1 | 0 |
| • Specific genes are chosen and named | 3 | 2 | 1 | 0 |
| • Model has four different results | 3 | 2 | 1 | 0 |
| Presentation Elements | | | | |
| • Explanation of how cDNA is made | 6 | 4 | 2 | 0 |
| • Explanation of how fluorescent tags are employed | 3 | 2 | 1 | 0 |
| • Proper use of your model during your presentation. | 3 | 2 | 1 | 0 |
| • Explanation of how to analyze results. | 6 | 4 | 2 | 0 |
| • Prediction of what colors each gene will be when observed under UV light. Justification is provided and is accurate. | 6 | 4 | 2 | 0 |

**Appendix V: Teacher Resources**

Teacher Resources Lesson 1:
Provide materials and read through the student resources for the lesson. For "Activity 1", you will need to remove the ink columns from the highlighters with pliers and have them ready for student use at the activity station.

Teacher Resources Lesson 2:

The Kahoot for the "Check for Understanding" can be found here.

Part I: Modeling SYBR Green I attachment to DNA in the minor groove.
The instructor will need to provide materials to make the DNA models and glow-in-the-dark modeling dough. Modeling dough can be ordered. Do-it-yourself glowing dough can be made, and there are numerous recipes on the internet that incorporate glow-in-the dark paint into a cornstarch and salt mixture. Commercial doughs that glow are also available for purchase. Students will need enough materials to work in pairs or individually. Circulate the room to help students in placement of their toothpick and gum-drop structures to accurately approximate the major and minor grooves in their DNA models.

Part II: Wet Lab.

- Ordering information for Ward's® Detection of Hereditary Breast Cancer Kit
- Ordering information for SYBR™ Safe DNA Gel Stain

The instructor will need to prepare the gels with agarose and buffer provided in the Ward's Detection of Hereditary Breast Cancer Kit, and aliquot patient DNA samples prior to the lab. Before preparing the gels, determine how you would like to incorporate the SYBR Safe gel stain. SYBR Safe can be implemented by two methods. The following protocols are paraphrased from the ThermoFisher literature that comes in the product packaging. (14)

- Method 1: Soak the gel in SYBR Safe™ stain. If using SYBR Safe™ gel stain concentrate, dilute 10,000X in buffer prior to use. Place the gel in a plastic container. Do not use a glass container, as the dye in the staining solution may adsorb to the walls of the container, resulting in poor gel staining. Add diluted SYBR Safe™ DNA gel stain to cover the gel. Incubate for 30 minutes. Protect the gel and staining solution from light by covering it with aluminum foil or by placing it in the dark. Gently and continuously agitate the gel at room temperature. No destaining is required.
- Method 2: Pre-casting SYBR Safe™ Stain in Agarose Gels. Prepare agarose in buffer as per instructions. Allow agarose to cool to ~65°C. Add SYBR Safe (1µL per 10mL agarose solution). Run the gel. Use a running buffer appropriate to the SYBR Safe™ gel stain formulation. No post-staining or destaining is needed. Mix and dispense. Visualize completed gels with a blue light transilluminator or UV light.

Each Ward's kit provides enough DNA to run three gels. Consider this when aliquoting DNA samples. Determine how to divide students into lab groups and plan distribution of gel chambers, pipettors, access to buffer, and power sources accordingly.

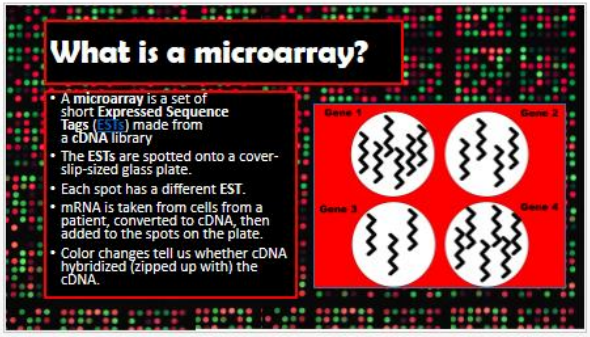
Teacher Resources Lesson 3:
Part I:

- For the “Fluorescent Stop Signs” activity, have students work in pairs.
- Make copies of the “Fluorescent Stop Signs” handout in the student resources. (Link)
- Make copies of the “Nucleotide Resource Sheet” in the student resources. It is recommended that you copy and paste the picture provided into a PowerPoint or Google Slide so that it can be enlarged to a typical 8.5” x 11” sheet of paper. (Link)
- Provide the other materials listed to student pairs. (Link)

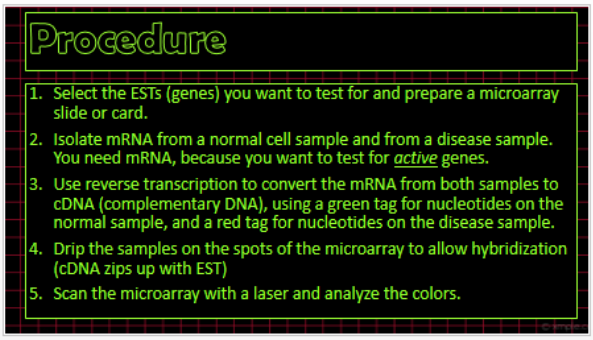
Teacher Resources Lesson 4:

- Ordering information for Edvotek kit #235
- Set-up instructions, student worksheets, answer key and protocol for Edvotek kit #235
- Slide deck for lecture:

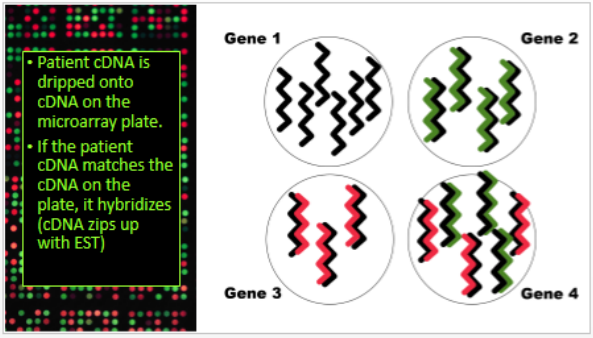
1



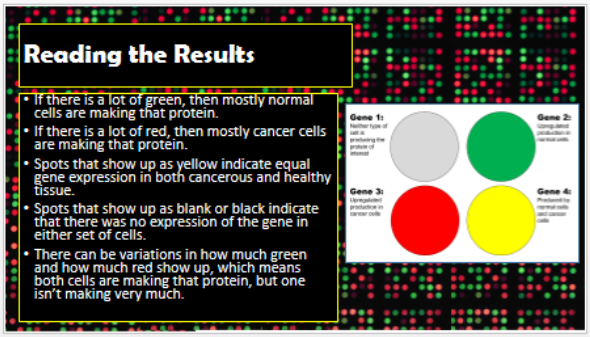
2



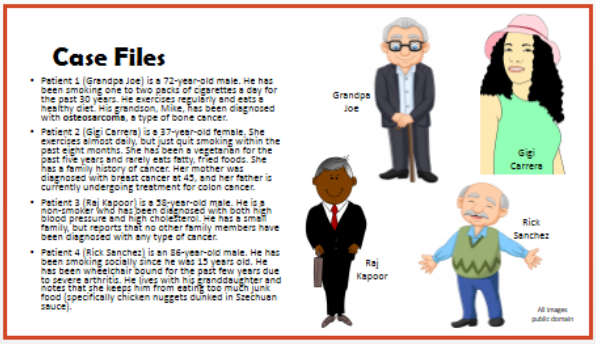
3



4



5



6

**Notes**


1. Rich Haag, “Mallard Creek High among 'Best Urban Schools' – Again,” University City Partners (University City Partners, March 24, 2017), https://universitycitypartners.org/mallard-creek-high-among-best-urban-schools/.
2. U.S. News and World Report, How Does Mallard Creek High Rank Among America's Best High Schools?
3. Libretexts. “1.10: Pi Conjugation.” Chemistry LibreTexts. Libretexts, August 11, 2020. https://chem.libretexts.org/Courses/Purdue/Purdue:_Chem_26505:_Organic_Chemistry_I_(Lipton)/Chapter_1._Electronic_Structure_and_Chemical_Bonding/1.10:_Pi_Conjugation.
4. Walter, Michael. “What Is a Fluorophore? (…and How Does It Work?).” *Charlotte Teacher's Institute - The Science of Glow*. Lecture presented at the Charlotte Teacher's Institute - The Science of Glow, May 5, 2020.
5. Yasir J. Sepah et al, Fundus Autofluorescence Imaging: Fundamentals and Clinical Relevance, 111
6. Aleksander Seiron et al, The role of fluorescence diagnosis in clinical practice, 978
7. Maja Sidstedt, Peter Rådström, and Johannes Hedman, “PCR Inhibition in QPCR, DPCR and MPS—Mechanisms and Solutions,” Analytical and Bioanalytical Chemistry 412, no. 9 (December 2020): pp. 2009-2023, https://doi.org/10.1007/s00216-020-02490-2.
8. Peter ‘tHoen etal, Fluorescent labelling of cRNA for microarray applications.
9. Fluorescent Labeling-What You Should Know
10. Figures 1, 2 and 3 by Kari Rhoades.
11. SYBR™ Safe DNA Gel Stain (ThermoFisher Scientific, February 2, 2016), http://tools.thermofisher.com/content/sfs/manuals/sybr_safe_dna_gel_stain_man.pdf.
12. “SYBR Safe,” Wikipedia (Wikimedia Foundation, June 3, 2020), https://en.wikipedia.org/wiki/SYBR_Safe.
13. H. Zipper, “Investigations on DNA Intercalation and Surface Binding by SYBR Green I, Its Structure Determination and Methodological Implications,” *Nucleic Acids Research* 32, no. 12 (July 2004), https://doi.org/10.1093/nar/gnh101.
14. “Make Glowing Fluorescent Liquid: Science Workshops Devon,” Make Glowing Fluorescent Liquid | Science Workshops Devon | (Sciencedipity, October 9, 2020), https://sciencedipity.co.uk/glowing-fluorescent-liquid-highlighter-pen/.
15. “Program Kit: Blacklight Painting for Young Adults,” Library as Incubator Project (Library as Incubator Project, July 21, 2015), https://www.libraryasincubatorproject.org/?p=17071.
16. “Glo Germ Training Manual,” Glo Germ: visual tool for handwashing training, aseptic techniques, infection control (Glo Germ Company), accessed December 1, 2020, https://www.glogerm.com/index.html.

**Bibliography**


Walter, Michael. “What Is a Fluorophore? (…and How Does It Work?).” *Charlotte Teacher's Institute - The Science of Glow*. Lecture presented at the Charlotte Teacher's Institute - The Science of Glow, May 5, 2020.

Sidstedt, Maja, Peter Rådström, and Johannes Hedman. “PCR Inhibition in QPCR, DPCR and MPS—Mechanisms and Solutions.” *Analytical and Bioanalytical Chemistry* 412, no. 9 (2020): 2009–23. https://doi.org/10.1007/s00216-020-02490-2.

Libretexts. “1.10: Pi Conjugation.” Chemistry LibreTexts. Libretexts, August 11, 2020. https://chem.libretexts.org/Courses/Purdue/Purdue:_Chem_26505:_Organic_Chemistry_I_(Lipton)/Chapter_1._Electronic_Structure_and_Chemical_Bonding/1.10:_Pi_Conjugation.

Sepah, Y. J., Akhtar, A., Sadiq, M. A., Hafeez, Y., Nasir, H., Perez, B., Mawji, N., Dean, D. J., Ferraz, D., & Nguyen, Q. D. (2014). Fundus autofluorescence imaging: Fundamentals and clinical relevance. *Saudi journal of ophthalmology : official journal of the Saudi Ophthalmological Society*, *28*(2), 111–116. https://doi.org/10.1016/j.sjopt.2014.03.008

Sieroń, A., Sieroń-Stołtny, K., Kawczyk-Krupka, A., Latos, W., Kwiatek, S., Straszak, D., & Bugaj, A. M. (2013). The role of fluorescence diagnosis in clinical practice. *OncoTargets and therapy*, *6*, 977–982.https://doi.org/10.2147/OTT.S42074

Haag, Rich. “Mallard Creek High among 'Best Urban Schools' – Again.” University City Partners. University City Partners, March 24, 2017. https://universitycitypartners.org/mallard-creek-high-among-best-urban-schools/.

How Does Mallard Creek High Rank Among America's Best High Schools? (2020, April 20). Retrieved from https://www.usnews.com/education/best-high-schools/north-carolina/districts/charlotte-mecklenburg-schools/mallard-creek-high-14583

't Hoen, Peter A C et al. “Fluorescent labelling of cRNA for microarray applications.” Nucleic acids research vol. 31,5 (2003): e20. doi:10.1093/nar/gng020

Fluorescent Labeling - What You Should Know. (n.d.). Retrieved October 19, 2020, from https://www.promocell.com/cell-culture-basics/fluorescent-labeling/

Nucleic Acid Gel Electrophoresis-A Brief Overview and History: Thermo Fisher Scientific - US. (n.d.). Retrieved October 19, 2020, from https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-separation-overview.html

“SYBR Safe.” Wikipedia. Wikimedia Foundation, June 3, 2020. https://en.wikipedia.org/wiki/SYBR_Safe.

"Oligos Modifications - Hex-5'." Gene Link (TM). Gene Link. Accessed November 16, 2020. http://www.e-oligos.com/eoweb/products/mod_detail.asp?modid=34.

Zipper, H. "Investigations on DNA Intercalation and Surface Binding by SYBR Green I, Its Structure Determination and Methodological Implications." *Nucleic Acids Research* 32, no. 12 (2004). https://doi.org/10.1093/nar/gnh101.

SYBR™ Safe DNA Gel Stain. ThermoFisher Scientific, February 2, 2016. http://tools.thermofisher.com/content/sfs/manuals/sybr_safe_dna_gel_stain_man.pdf.

"Make Glowing Fluorescent Liquid: Science Workshops Devon." Make Glowing Fluorescent Liquid | Science Workshops Devon |. Sciencedipity, October 9, 2020. https://sciencedipity.co.uk/glowing-fluorescent-liquid-highlighter-pen/.

"Program Kit: Blacklight Painting for Young Adults." Library as Incubator Project. Library as Incubator Project, July 21, 2015. https://www.libraryasincubatorproject.org/?p=17071.

"Glo Germ Training Manual." Glo Germ: visual tool for handwashing training, aseptic techniques, infection control. Glo Germ Company. Accessed December 1, 2020. https://www.glogerm.com/index.html.