And the pdf article needs to be added to lesson 6.

The Salty Enigma

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Curriculum Team:

Lesson 4 Adapted from Bio Rad

Lesson 5 Incorporates HHMI's Biointeractive Website

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Additional information regarding the Bench to Bedside or Summer Research Experience is available at http://www.cpet.ufl.edu/bench.

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Introduction

Cystic Fibrosis as a genetic disorder is one of the more tragic results of inheritance. Up until the mid-1900's the life expectancy for a person with cystic fibrosis was ten years old. One of the most difficult concepts regarding CF is the wide variations of single mutations that can create just as many phenotypic changes to the Channel protein it affects. In some, the channel is present, but fails to open - in others the channel is so malformed that it disintegrates from the membrane altogether. Studying the impact on the many epithelial tissue systems impacted by Cystic Fibrosis became a focus for this case study into Cystic Fibrosis. Biotechnology has come up with a plethora of venues to attack the many versions of CF protein malfunctions - chemicals seem to work on some in activating and opening a channel that is present but not functioning. Our interests were drawn to actually reprogramming the gene itself through a gene therapy called CRISPR. CRISPR technology as a gene editing tool is a promising and cutting edge solution for many genetic issues. It is hoped that it may eradicate CF's abbreviated life expectancy and allow those afflicted to live a long, healthy and normal life.

Author's Note

In this unit, students will follow a case of a baby with a autosomal recessive mutation of the F508 gene on the 7th chromosome. This mutation causes cystic fibrosis. The students will journey along with the parents in the genetic counseling, understanding what will happen to their baby, testing for a particular mutation and deciding whether or not the new CRISPR gene editing technology will be effective on their child.

The Salty Enigma case study was developed as a part of CPET's 2018 Summer Research Experience. This project was supported by a grant from the National Institute of General Medical Sciences, the National Institutes of Health to the UF Center For Precollegiate Education and Training. 'As part of a 2 ½ week summer institute, Dr. Qui-Xing Jiang's Molecular Physiology and Biophysics lab graciously hosted two researchers as summer interns - the authors of this lesson. Their focus on protein conformational changes in the phospholipid bilayer and their interactions with the cell served as an inspiration for this unit on a genetic disorder caused by a malformed protein in the epithelial cells of humans affecting multiple systems of the body. This lesson contains many current topics in genetics, biotechnology, protein structure determination, and cell signaling and making the cross connections between those topics become synthesized for the students.

Tips about this Curriculum

Lesson Plan Format: *All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:*

KEY QUESTION(S): Identifies key questions the lesson will explore.

OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list.

MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.

ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.

PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.

EXTENSIONS: (ACTIVITIES/LITERATURE)
There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available.

STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.

TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.

Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in teacher-driven, lecture format, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

Groups: Most of the lessons are carried out in groups. While it isn't necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

Inquiry-based: The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

Technology: Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

Content: This unit provides an opportunity to synthesize discrete content facts into an authentic context. Students take concepts learned such as inheritance and genetic counseling, and put them in the context of what causes a genetic disorder and what that change in a gene will do to a person. The lessons aren't designed to teach students all forms of genetic disorders, but rather take one and look at it from diagnosis to the cellular responses that are disrupted from the mutation of the gene. We are focusing on *why* these ideas are important and *how* researchers can use current biotechnology to treat/cure them.

Implementation notes: This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

Extensions: There are many opportunities to expand the lessons presented here. Extensions to the information presented in each lesson will be offered in this section. If

time allows and you would like to pursue the Genetic Disorders, biotechnologies available, etc. - utilize these sections of the lesson.

Science Subject: Biotechnology, AP Biology, could easily be adapted for an honors Biology as well.

Grade and ability level: 9-12 students in all levels of biology and biotechnology

Science concepts: Genetics, Inheritance, Mutations, Protein structure and function, modeling scientific concepts, biotechnology

Lesson Summaries

Lesson One: Genetic Counseling

Activity 1 Summary: Students role play parents visiting a genetic counselor to get help determining whether their young daughter has cystic fibrosis. The role play is a version of Think-Pair-Share in which each student is armed with information that they must share with each other through questioning to reach a conclusion about the likelihood of a cystic fibrosis diagnosis and what should be the next step(s) for the parent. Students will build a family pedigree based on phenotype and create some Punnett squares.

Activity 2 Summary: This part of the lesson is to fill the gaps from the inquiry lesson in Activity 1. Students will watch 2 informative video clips about what Cystic Fibrosis is, how it is inherited and how it impacts multiple systems in the body. Making connections between the one nitrogen base in a DNA strand mutating ultimately creating this deadly genetic disorder is the main learning goal of this activity.

Lesson Two: CTFR Integral Proteins – what are they and how do they work?

Activity 1: Students fold paper (origami) and assemble the parts into a model of a protein channel. Students watch a short animation (1:34) of a sodium-potassium pump and make comparisons between the origami model and the model in the video animation. The teacher may opt to have students do this lesson individually, with a partner, or with a group (it should be noted that each origami protein requires eight sheets of colored paper so availability of a specific quantity may be a factor in the decision of grouping students). This lesson is written assuming students will work with a partner in order to speed up the process of creating the origami model and leave more time for discussion. Teachers may also opt to hang completed origami proteins in the classroom or allow the students to keep their own model.

Activity 2: Students will learn about the CFTR Protein and how it works through a PREZI that takes them through both G-Protein Signal Transduction Pathways and Gating of the CFTR. Upon understanding the notes, students will make connections and demonstrate comprehension through a Labeling Activity.

Lesson Three: Doctors Test and Results

Students watch a YouTube video illustrating how to build simple finger electrodes. Students will build the finger electrodes, take their own skin conductivity measurement and add their measurement to the class data set. Students will calculate the average skin conductivity measurement in units of microsiemens and determine an error range. Students will use this data to determine if mock patients have a normal or abnormal level of skin conductivity and use that information to predict whether or not the patients have cystic fibrosis.

Lesson Four: Western Blot

This lesson is still under construction (sorry)

Lesson Five: Understanding the differences between Electron Microscopy and X-ray Crystallography

Students watch four very short videos (14 minutes total) explaining the process and advantages of cryo-electron microscopy over x-ray crystallography in determining protein structure and read a journal opinion article on the same topic. Students answer worksheet questions from the videos and article during class or as homework. The teacher can opt to allow students to do the video questions in class and allow time for class discussion and give the journal article questions as a homework assignment or to do both video and journal article questions in class.

Lesson Six:

Activity 1: In this lesson, students will go on a webquest watching small video clips that give a thorough explanation of the technology as well as journey through an HHMI Biointeractive simulation of CRISPR. They will see CRISPR actually read and open a piece of DNA, cut it and then add the new gene sequence to the strand in a computer simulation. Then they will choose different scientists video clips to see the many applications it is currently being used for.

Activity 2: To close our Case Study the students will be given a recent scientific article where CRISPR is being tested on Cystic Fibrosis genes. The scientific Article is very high level reading - teachers may choose to forgo this step or help the students through the context of the article - AP Students should be able to identify and extract important data from the information and make a claim whether this technology could be used to help the CF baby in our Case. They will need to justify their claim with supporting information from the article.

Lesson Sequencing Guide

Since the classroom teacher knows his or her students best, the sequencing of lessons and the amount of time spent on each should be altered to meet the needs of each individual setting. Below is a suggested pacing guide that can be used when planning to use this curriculum, assuming 45-minute class periods.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Week 1	Lesson 1 Visiting a Genetic Counselor Activity 1: (45 minutes)	Lesson 1 Activity 2: (45 minutes)	Lesson 2 Activity 1: How does the CFTR Protein work? (45 minutes)	Lesson 2 Activity 2: Modeling a Protein Channel (Origami Activity) (45 min)	Lesson 3 Building a "Sweat Test" Device (45 minutes)
	Day 6	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Week 2	Lesson 4 Testing for Specific Proteins (45 minutes) Begin Western Blot	Lesson 4 Cont'd Part 2 of Western Blot (45 Min)	Lesson 4 Cont'd Part 3 of Western Blot (45 min)	Lesson 5 Electron Microscopes vs Crystallography (45 min)	Lesson 6 Coming to Grips with CRISPR (45 minutes)
	Day 11	**Day 12**			
Week 3	Lesson 6 Coming to Grips with CRISPR (45 min)	Summative Assessment (45 Min)			

The length of these lessons can vary based on the prior knowledge of the students. Some activities can be assigned as homework to conserve in-class time.

Vocabulary

We still need to add the vocab from lesson 4 The Western Blot which is still under construction

Active Site: Location on a protein where a ligand attaches and activates the protein

***Agonist* -** any substance that triggers activation when it binds to something

***Anion*:** a negatively charged ion, i.e., one that would be attracted to the anode in electrolysis

Autosomal Recessive Mutation - A genetic condition that appears only in individuals who have received two copies of an **autosomal** gene, one copy from each parent. The gene is on an autosome, a nonsex chromosome.

***cAMP pathway* -** a G-protein signaling transduction pathway where cAMP is a secondary messenger.

***Cell Membrane*:** the semipermeable membrane surrounding the cytoplasm of a cell

***Cilia*:** minute hairlike organelles, identical in structure to flagella, that line the surfaces of certain cells and beat in rhythmic waves, providing locomotion to ciliate protozoans and moving liquids along internal epithelial tissue in animals

***Concentration gradient* -** the difference in concentrations of ion particles inside and outside the cell

***Conductivity*:** the degree to which a specified material conducts electricity, calculated as the ratio of the current density in the material to the electric field that causes the flow of current. It is the reciprocal of the resistivity.

CRISPR -stands for "clusters of regularly interspaced short palindromic repeats." a segment of DNA containing short repetitions of base sequences, involved in the defense mechanisms of prokaryotic organisms to viruses, that is used as a genetic engineering tool with its associated protein to edit the base pairs of a gene.

***CRISPR-cas9 Complex* -** made of the protein Cas9 (or "**CRISPR**-associated"), CRISPR DNA and the target DNA it is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA recognizing a target sequence and replacing a specific set of nucleotides with new ones.

***Crystalline*:** formed by crystallization; having the regular arrangement of the atoms in a space lattice

Deletion - is a mutation (a genetic aberration) in which a part of a chromosome or a sequence of DNA is lost during DNA replication.

***Diffraction*:** the process by which a beam of light or other system of waves is spread out as a result of

passing through a narrow aperture or across an edge, typically accompanied by interference between the waveforms produced

***Diffusion*:** the net passive movement of particles (atoms, ions or molecules) from a region in which they are in higher concentration to regions of lower concentration

***Electrolyte*:** a liquid or gel that contains ions and can be decomposed by electrolysis

Endonuclease -are enzymes, used extensively in molecular biology, that cleave double-stranded DNA at specific sequences.

***Epithelial Cells*:** one of the closely packed cells forming the epithelium

Epithelial tissue: thin tissues that cover all the exposed surfaces of the body. They form the external skin, the inner lining of the mouth, digestive tract, secretory glands, the lining of hollow parts of every organ such as the heart, lungs, eyes, ears, the urogenital tract, as well as the ventricular system of the brain and central canals of the spinal cord.

***Epithelium*:** one or more layers of densely packed **cells**. In vertebrates, it lines the outer layer of the skin (epidermis), the surface of most body cavities, and the lumen of fluid-filled organs, such as the gut or intestine.

Frameshift - A type of gene mutation wherein the addition or deletion of nucleotide(s) causes a shift in the reading frame of the codons in the mRNA, thus, may eventually lead to the alteration in the amino acid sequence at protein translation

G-Protein Coupled Receptor - The protein complex in the cell membranes of epithelial cells with an external active site and an attached g-protein-GDP component

Gating -the process of changing conformation to open channel in a integral protein.

Genetic Counseling - Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease.

Gene Therapy - the transplantation of normal genes into cells in place of missing or defective ones in order to correct genetic disorders.

Genetic Screening - the sequencing of human DNA in order to discover genetic differences, anomalies, or mutations that may prove pathological.

Genome - the complete set of genes or genetic material present in a cell or organism.

Genotype - the genetic makeup of an organism regarding a single trait, set of traits, or an entire

complex of traits.

Homeostasis - the tendency of organisms to auto-regulate and maintain their internal environment in a stable state

Hydrophobicity**:** the property of repelling water rather than absorbing it or dissolving in it

Ligand - the external signal that attaches to an active site and begins the signal pathways. (ex. Hormones)

Mucus**:** a slimy substance, typically not miscible with water, secreted by mucous membranes and glands for lubrication, protection, etc.

Nonsense Mutation - a mutation in which a sense codon that corresponds to one of the twenty amino acids specified by the genetic code is changed to a chain-terminating (premature STOP) codon.

Phenotype - the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Pedigree - A diagram showing the lineage or genealogy of an individual and all the direct ancestors, usually to analyze or follow the inheritance of trait.

Phosphatidylinositol Pathway - a G-Protein Signal Transduction Pathway where the signal triggers 2 responses - the activation of PKC and the release of Calcium ions from the Endoplasmic Reticulum.

Phospholipid bilayer**:** a two-layered arrangement of phosphate and lipid molecules that form a cell membrane, the hydrophobic lipid ends facing inward and the hydrophilic phosphate ends facing outward.

Phosphorylation - process that involves the addition of phosphate to an organic compound.

Protein**:** minute hairlike organelles, identical in structure to flagella, that line the surfaces of certain cells and beat in rhythmic waves, providing locomotion to ciliate protozoans and moving liquids along internal epithelial tissue in animals

Protein Kinase - is a kinase enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation).

Secondary Messenger - one of the triggers in a cascade pathway like cAMP.

Signal Transduction - process of cell signaling where a chemical signal is received from outside the cell

and transmitted into the cell.

***Viscosity*:** the state of being thick, sticky, and semifluid in consistency, due to internal friction

X-ray: an electromagnetic wave of high energy and very short wavelength, which is able to pass through many materials opaque to light.

Next Generation Sunshine State Standards – Science

Standard	Lesson					
	1 Inquiry Lab Pedigree	2 Prot. Channel Activities	3 Sweat Test Tech Lab	4 Protein Identification	5 EM vs Xray Crys.	6 CRISPR
SC.912.L.14.2 Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier (passive and active transport)		X	X			
SC.912.L.14.11 Classify and state the defining characteristics of epithelial tissue, connective tissue, muscle tissue, and nervous tissue		X	X			
SC.912.L.14.29 Define the terms endocrine and exocrine			X			
SC.912.L.15.15 Describe how mutation and genetic recombination increase genetic variation.						
SC.912.L.16.2 Discuss observed inheritance patterns cause by various modes of inheritance, including dominant, recessive, codominant, sex-linked, polygenic, and multiple alleles.	X					
SC.912.L.16.4 Explain how mutations in the DNA sequence may or may not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.	X	X				X
SC.912.L.16.9 Explain how and why the genetic code is universal and is common to almost all organisms.						
SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.		X		X	X	
SC.912.L.18.3 Describe the structures of fatty acids, triglycerides, phospholipids, and steroids. Explain the functions of		X			X	

lipids in living organism. Identify some reactions that fatty acids undergo. Relate structure and function of cell membranes.						
SC.912.L.18.4 Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.		X		X	X	
SC.912.L.18.10 Connect the role of adenosine triphosphate (ATP) to energy transfers within a cell.		X				
SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science and do the following:			X		X	
SC.912.N.1.3 Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.			X			
SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.			X		X	
SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.	X		X	X	X	X
SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.		X				X
SC.912.N.4.1 Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.	X			X		X
SC.912.CS-CP.1.3 Analyze and manipulated data collected by a variety of data collection techniques to support a hypothesis.			X			
SC.912.CS-CP.1.4 Collect real-time data from sources such as simulations, scientific and robotic sensors, and device emulators, using this data to formulate strategies or algorithms to solve advanced problems.			X			

National Next Generation Science Standards

Standard	LESSON					
	1 Inquiry Lab Pedigree	2 Prot. Channel Activities	3 Sweat Test Tech Lab	4 Protein Identification	5 EM vs Xray Cryst.	6 CRISPR
HS-LS 1-1	X	X		X		
HS-LS 1-2		X				X
HS-LS 1-3		X				
HS-LS 3-1	X	X				
HS-LS 3-2	X	X				
HS-LS 3-3	X		X	X		X
HS-LS 3-4		X				X
Core Idea Structure & Function		X		X		
Core Idea Inheritance of traits	X					
Core Idea Variation of Traits	X					
Scientific Eng.Practices *Developing and Using Models*		X	X			
Scientific Eng.Practices *Ask ??s & Define a Problem*	X			X		
Scientific Eng.Practices *Analyzing & Interpreting Data*	X		X	X		
Scientific Eng.Practices *Engaging in Argument from Evidence*						

Scientific Eng.Practices *Planning and carrying out investigations*	X		X	X		
Scientific Eng.Practices *Constructing Explanations and designing solutions*		X	X			
CrossCutting Concepts ***Systems and System Modeling***		X	X			X
CrossCutting Concepts ***Structure and Function***		X				X
CrossCutting Concepts ***Stability and Change***		X				
CrossCutting Concepts ***Cause and Effect***		X				X
CrossCutting Concepts ***Scale, Proportion & Quantity***	X	X				
Nature of Science Con. *Scientific Investigations use a variety of methods*	X		X	X		X
Nature of Science Con. *Science is a human endeavor*			X			X

Topic 1: HS. Structure and Function

HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

HS-LS1-3. Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

Disciplinary Core Ideas for HS Structure and Function

L S1.A Structure and Function

HS-LS 1-1 Systems of specialized cells within organisms help them perform the essential functions of life.

HS-LS 1-1 All cells contain genetic information in the form of D N A molecules. Genes are regions in the

DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.

HS-LS 1-2 Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level.

HS-LS 1-3 Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (positive feedback) or discourage (negative feedback) what is going on inside the living system.

Scientific Engineering Practices for HS Structure and Function

Developing and Using Models

HS-LS 1-2 Develop and use a model based on evidence to illustrate the relationships between systems or components of a system

Planning and Carrying Out Investigations

HS-LS 1-3 Plan and conduct an investigation to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly.

Constructing Explanations and Designing Solutions

HS-LS 1-1 Construct an explanation based on valid and reliable evidence obtained from a variety of sources and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.

Crosscutting Concepts For HS- Structure and Function

Systems and System Models

HS-LS 1-2 Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions— including energy, matter, and information flows—within and between systems at different scales.

Structure and Function

HS-LS 1-1 Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem.

Stability and Change

HS-LS 1-3 Feedback (negative or positive) can stabilize or destabilize a system.

Connections to Nature of Science For HS- Structure and Function

HS-LS 1-3 Scientific Investigations Use a Variety of Methods. Scientific inquiry is characterized by a common set of values that include: logical thinking, precision, open-mindedness, objectivity , skepticism, replicability of results, and honest and ethical reporting of findings.

Topic 3: HS. Inheritance and Variation of Trait s

HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and /or (3) mutations caused by environmental factors.

DISCIPLINARY CORE IDEAS for HS-Inheritance and Variation of Traits

L S1 .A: Structure and Function

HS-LS 3-1 All cells contain genetic information in the form of DNA molecules. Genes are regions in the D N A that contain the instructions that code for the formation of proteins.

L S3 .A: Inheritance of Traits

HS-LS 3-1 Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA . The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function.

L S3 .B: Variation of Trait

HS-LS 3-2 In sexual reproduction, chromosomes can sometimes swap sections during the process of meiosis (cell division), thereby creating new genetic combinations and thus more genetic variation. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited.

Science and Engineering Practices For HS- Inheritance and Variation of Traits

Asking Questions and Defining Problems

HS-LS 3-1 Ask questions that arise from examining models or a theory to clarify relationships.

Developing and Using Models

HS-LS 3-4 Use a model based on evidence to illustrate the relationships between systems or between components of a system.

Analyzing and Interpreting Data

HS-LS 3-3 Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and problems, using digital tools when feasible.

Engaging in Argument from Evidence

HS-LS 3-2 Make and defend a claim based on evidence about the natural world that reflects scientific knowledge, and student-generated evidence.

Cross Cutting Concepts for HS- Inheritance and Variation of Traits

Cause and Effect

HS-LS 3-1/HS-LS 3-2 Empirical evidence is required to differentiate between cause and correlation

and make claims about specific causes and effects.

Scale, Proportion, and Quantity

HS-LS 3-3 Algebraic thinking is used to examine scientific data and predict the effect of a change in one variable on another (e.g. linear growth vs. exponential growth).

Systems and System Models

HS-LS 1-4 Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions—including energy , matter, and information flow s— within and between systems at different scales.

Connections to Nature of Science

Science is a Human Endeavor

HS-LS 3-3 Technological advances have influenced the progress of science and science has influenced advances in technology .

HS-LS 3-3 Science and engineering are influenced by society and society is influenced by science and engineering.

AP BIOLOGY Curriculum Framework Standards

Standard	LESSON					
	1 Inquiry Lab Pedigree	2 Prot. Channel Activities	3 Sweat Test Tech Lab	4 Protein Identification	5 EM vs Xray Crys.	6 CRISPR
2.A.2. Organisms capture, use, and store energy in biological processes such as growth, reproduction and maintaining homeostatic processes.		X				
2.B.1. Cell membranes are selectively permeable due to their structure.		X				
2.B.2. Growth and homeostasis is maintained by the constant movement of molecules across membranes.						
2.B.3. Eukaryotic cells maintain internal membranes that partition the cell into specialized regions.		X				
2.C.1. Positive feedback mechanisms amplify responses and processes in biological organisms.		X				
2.C.2. Organisms use negative feedback mechanisms to maintain their internal environments and respond to external environmental changes.		X				
2.D.3. Biological systems are affected by disruptions to their homeostasis.	X	X	X			
3.A.1 DNA, and in some cases RNA, is the primary source of heritable information.	X					X
3.A.3 Mendelian genetics provides a basic understanding of the underlying causes of the pattern traits from parent to offspring.	X					
3.B.1 Cells can be activated, produce new products, and retain their activated state through gene regulation.		X				X

3.B.2 A variety of intercellular and intracellular signal transmissions mediate gene expression.		**X**				
3.C.1 Changes in genotype can result in changes in phenotype.	**X**	**X**		**X**	**X**	**X**
3.D.3. Signal transduction pathways link signal reception w/ cellular response.		**X**				
4.A.1. The subcomponents of a biological polymer and their sequence determine the properties of that polymer.		**X**		**X**	**X**	
4.A.2. Interactions of subcellular structures, including a repertory of eukaryotic organelles possessing specialized functions, provide essential cellular functions and activities.		**X**				
4.B.1. Interactions between molecules affect their structure and function.		**X**		**X**		

Background information:

According to the National Human Genome Research Institute (NHGRI), cystic fibrosis (CF) is the most common fatal genetic disease in the United States. It is a homozygous recessive hereditary disease that affects more than 30,000 people in the United States and more than 70,000 worldwide. The disease as most people are familiar with it, is a manifestation a mutated version of a single gene, the cystic fibrosis transmembrane conductance regulator (CFTR). While approximately 2,000 different mutations of this gene are recognized, only about 242 of these mutations are known to be disease causing. These disease causing mutations alter the structure, and therefore the function, of the CFTR anion channel protein. Mutated versions of this protein severely limit or prevent the ability of specific anions, such as chloride and thiocyanate, from moving in and out of the cell. The inability of these anions to cross the cellular membrane result in the health issues we commonly recognize as symptoms of cystic fibrosis. Most people are familiar with the fact that cystic fibrosis affects the lungs. Since homeostasis cannot be achieved, water does not diffuse from inside the cell into the extracellular matrix. Therefore, the mucus secreted by specific apical epithelial cells becomes too thick. This makes it difficult for gas exchange across the alveolar-capillary membrane and breathing becomes laborious and inefficient. In the lungs, the mucous layer traps bacteria and other invading pathogens, but cannot be removed by cilia that are effectively immobilized by the sticky mucus. This allows bacteria to grow virtually unchecked and infections to become common occurrences. Other mucus secreting organs or tissue negatively affected by cystic fibrosis are the pancreas, digestive and reproductive systems, and the skin.

We believe it is important to note that cystic fibrosis does not affect an individual's cognitive abilities or intellect.

Life Expectancy and Treatment

There is no cure for cystic fibrosis, but because of early diagnosis and improved treatment, the life expectancy for someone with cystic fibrosis has improved from about ten years in 1962 to 37 years today, with many people living into their 50s. By constantly monitoring for infection, treating infection early, and daily physiotherapy to remove mucus build-up in the lungs, cystic fibrosis can be managed. Patients must also follow a strict regimen of exercise, monitor salt intake, treat and prevent intestinal blockage, and make sure they are receiving adequate nutrition.

Prevention

Cystic fibrosis is not a disease that is contagious from one person to another. You cannot 'catch' cystic fibrosis from an affected individual. Since the root cause of cystic fibrosis lies in a defective gene of the gamete, if both egg and sperm carry the same mutation, cystic fibrosis cannot be avoided. Many couples that find they are carriers for disease causing mutations use genetic counselors to help them determine whether or not adoption might be the best option for growing their family based upon their unique odds of passing a mutated gene on to the next generation.

Lesson 1: Visiting a Genetic Counselor, investigating the salty enigma.

LESSON 1

TITLE: Visiting a Genetic Counselor, investigating the salty enigma.

KEY QUESTION(S):

How can a pedigree help make inferences?

What is the role of a Genetic Counselor?

What happens to a person with Cystic Fibrosis?

How does biotechnology play a role in this genetic disorder?

***SCIENCE SUBJECT:** Biology (Honors and or AP), Biotechnology

***GRADE AND ABILITY LEVEL:**

Activity 1: 9-12, may be scaffolded for different levels of students

Activity 2: 9-12, This activity requires some synthesis of old information so it would work better for a higher level student, scaffolding may be required for lower level students Honors 9th grade Biology, AP Biology or Biotechnology Students.

SCIENCE CONCEPTS: Making inferences, Pedigree Analysis, Genetic Counseling, Genotype affecting Phenotype, mutation affecting phenotype, scientific tools to identify and screen for genetic disorders

OVERALL TIME ESTIMATE:

Activity 1: (1-50 min class period)

Activity 2: (45 min) as a class or homework assignment

LEARNING STYLES:

Activity 1: Visual, auditory, and kinesthetic

Activity 2: Visual and auditory (video clips)

LESSON VOCABULARY:

Autosomal Recessive Mutation	Genetic Counseling	Genotype
Deletion	Gene Therapy	Homeostasis
Frameshift	Genetic Screening	Nonsense Mutation

Phenotype Pedigree

LESSON SUMMARY:

Activity 1 Summary: Students role play parents visiting a genetic counselor to get help determining whether their young daughter has cystic fibrosis. The role play is a version of Think-Pair-Share in which each student is armed with information that they must share with each other through questioning to reach a conclusion about the likelihood of a cystic fibrosis diagnosis and what should be the next step(s) for the parent. Students will build a family pedigree based on phenotype and create some Punnett squares.

Activity 2 Summary: This part of the lesson is to fill the gaps from the inquiry lesson in Activity 1. Students will watch 2 informative video clips about what Cystic Fibrosis is, how it is inherited and how it impacts multiple systems in the body. Making connections between the one nitrogen base in a DNA strand mutating ultimately creating this deadly genetic disorder is the main learning goal of this activity.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The student will be able to…

Activity 1:

1. Explain the role of a genetic counselor
2. Explain the need for genetic counselors
3. Use information to develop a family pedigree
4. Use a family pedigree to make inferences

Activity 2:

1. Recognize how a single mutation can cause a change in the homeostasis of an entire organism
2. Identify the inheritance pattern of a genetic disorder

NEXT GENERATION SUNSHINE STATE STANDARDS:

SC.912.L.16.2, SC.912.L.16.4**,** SC.912.N.17, SC.912.N.4.1

NEXT GENERATION SCIENCE STANDARDS (http://www.nextgenscience.org/next-generation-science-standards)

HS-LS 1-1 HS-LS 1-3 HS-LS 3-1 HS-LS 3-2

COMMON CORE STANDARDS (http://www.corestandards.org/)

AP BIOLOGY CURRICULUM FRAMEWORK

2.D.3 3.A.1 3.A.3 3.C.1

MATERIALS:

ESSENTIAL:

For Activity 1:

- Role Playing Handouts for each team
- Worksheet for each team
- Scrap paper
- Colored pencils, markers, highlighters, or crayons (one set for each team)

For Activity 2:

- Access to internet to play the video clips - Overhead projector, Ipad, Laptop, SmartPhone.
- Copies of the What is Cystic Fibrosis Handout per student or pair

PRIOR KNOWLEDGE:

Students should understand the difference between phenotype and genotype. Students should know how to build a Punnett square and use it to predict probabilities.

BACKGROUND INFORMATION:

Genetic counselors are not exclusively about sequencing the genome of a patient and then translating their genetic information into terms that can be easily understood, although that may be a small part of the job. Genetic counselors can play multiple roles in a patient's life. A genetic counselor can help patients understand a disease, help a patient to make testing decisions, and help a patient make treatment decisions as well as help a patient understand how to live with a specific disease. Genetic counselors may build a family ***pedigree*** (a

branched map showing related individuals and their descendants) based upon ***genotype*** (the allele set that represent traits inherited from parents) or ***phenotype*** (the physical characteristics expressed by the genotype), depending on what information is available and what the family can afford. In an effort to diagnose, and treat this condition, meeting with a ***genetic counselor***, who is a specially-trained professional is recommended. They can help people learn about genetic conditions, find out their chances of being affected by or having a child or other family member with a genetic condition, and make informed decisions about testing and treatment. In ***genetic screening***, people are actually tested to see if they have or are predisposed to have a gene or genetic condition. Current biotechnology often employs the use of gene therapy as well, ***gene therapy*** is an experimental technique that inserts a gene into a patient's cells to correct faulty gene sequences. This technique is new to the treatment of Cystic Fibrosis.

Cystic Fibrosis is caused by an ***autosomal recessive mutation***, meaning it can be hidden by a dominant allele (recessive) and it has mutated a gene found on a chromosome from pairs 1-22 (autosomal chromosomes), in this case it is found on chromosome 7. The gene F508 has a ***deletion*** of one nitrogen base in the gene sequence (a base is missing), this will cause a ***frameshift*** to the bases in the gene where the whole sequence shifts to the left. All codons after the deletion will be a new combination and no longer code the same message it had before. In the case of Cystic Fibrosis this causes a new codon combination which signals a premature stop code in the gene sequence shortening the message. The result of this is a misfolded integral protein channel in the cell membranes of epithelial cells. Without a functioning channel many body systems are affected. This ion channel balances ***homeostasis*** of respiratory, secretory, and intestinal cells and systems. Homeostasis (maintaining the same condition) is disrupted as fluids and salts do not balance properly resulting in system failures.

ADVANCE PREPARATION:

Activity 1:

- Decide whether to have students work in groups of three or four (the fourth student could record the information gathered during the role play)
- Review the TED video on the role of a genetic counselor
- Print off copies of handouts (one per group)
- Print off copies of the worksheet (one per group)
- Print off a copy of 'What is cystic fibrosis' handout (one per group)

Activity 2:

- Ensure students have access to internet and can watch the video clips to answer the worksheet
- Make enough copies of the worksheet for each student or group

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Activity 1: Genetic Counselor/Pedigree (1 - 50 min class period)

1. Ask student if they have ever heard of a genetic counselor. Ask if they know what a genetic counselor does. Have them suggest possible job requirements, degree requirements, and job descriptions for a genetic counselor. (5 minutes)

2. Play the TED genetic counselor video for the class https://www.youtube.com/watch?v=7yIW0L9dLCQ (8:54)

3. Ask the class again to the suggest possible job requirements, degree requirements, and job descriptions for a genetic counselor. Guide the discussion to a better understanding genetic counseling as a profession. (5 minutes)

4. Assign the students to groups of three or four.

5. Pass out the role playing information sheets, the final worksheet, scrap paper, and colored pencils. The student playing the role of the mother gets only the mother handout, the student playing the role of the father gets only the father handout, and the student playing role of the genetic counselor gets both the genetic counselor handout and the 'What is cystic fibrosis' handout.

6. Go over the directions of the role playing assignment with the class making sure to remind them that no other group member should be able to see what is on their information sheet.

7. Allow the class time to build their pedigree and complete their assignment. (30 minutes or the remainder of the class period)

8. If time allows the teacher may choose to discuss the predicted genotypes for the two family members on the worksheet (#4 and #16) and have students explain why they predicted one genotype over another based on the information available.

Activity 2: What is Cystic Fibrosis? Worksheet (40 min to complete including video time - 10 min to review)

1. Distribute a copy of the worksheet per student.

2. Tell the students that the Genetic Counselor is sending the parents home with information to understand this disorder better. Completing this assignment will help fill any gaps from the first inquiry activity and give a more thorough understanding of exactly what Cystic Fibrosis is, how it affects a person, and what treatments may be offered for this baby.

3. Students will watch the two video clips (approx 15 min) and answer the questions. Working in pairs will allow students an opportunity to synthesize prior knowledge more effectively by bouncing ideas off one another

4. Teacher should circulate during the question answering time to help make connections to prior knowledge and check for understanding.

5. When all students are finished, go through the questions - giving focus on ones with more than one answer possible - have the students explain why they chose their answer for those particular examples

(ex. Predict why this disease is most common in those of Northern European Caucasian descent - students can come up with some very unique and valid options for this question eliciting great discussions)**.**

ASSESSMENT SUGGESTIONS:

Activity 1: Much of this lesson has many possible outcomes which would make it difficult to assess student work based on an absolute correct answer. Obviously, the student groups should conclude the patient (Ashley) is likely to have cystic fibrosis based on her symptoms, the family history, the age of frequent deaths of family members.

- Participation in role play
- Reasonable family pedigree
- Completed worksheet

Activity 2: Participation grade may be given. Worksheet can be graded for student understanding and synthesis of past and current topics as evidenced by the higher level question responses. If you go over all the questions it is difficult to give more than a participation grade for the work, unless you collect it before you go over the questions.

EXTENSIONS:

ACTIVITIES:

LITERATURE:

RESOURCES/REFERENCES:

For Activity 1:

- Haven, J. (2017, February 9). *Jaclyn Haven What is Genetic Counseling [Video File]*. Retrieved from https://www.youtube.com/watch?v=7yIW0L9dLCQ
- Woods, B. (2018, March 29). *This Kentucky Coal Town is Fighting for Survival Long after the War on Coal is Over. CNBC News.* Retrieved from https://www.cnbc.com/2018/03/29/the-kentucky-coal-town-fighting-to-survive-after-coal-mining-closings.html

For Activity 2:

- *Therapies for cystic fibrosis.*. (2010, April 12). Retrieved from http://www.cff.org/treatments/Therapies/.
- *Types of mutations.* (n.d.). Retrieved from http://www.ebi.ac.uk/2can/disease/genes5.html.

- Zieve, D., & Kaneshiro, N. (2010, July 14). *Cystic fibrosis.* Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000107.htm.

TEACHER PAGES:

For Activity 1:

Student Handout

Teacher Pedigree Key

For Activity 2:

Student Worksheet Handout

Teacher Key Worksheet Handout

Activity 1 Student Worksheet

Role Playing Activity

Background: Genetic counselors are not exclusively about sequencing the genome of a patient and then translating their genetic information into terms that can be easily understood, although that may be a small part of the job. Genetic counselors can play multiple roles in a patient's life. A genetic counselor can help patients understand a disease, help a patient to make testing decisions, and help a patient make treatment decisions as well as help a patient understand how to live with a specific disease. Genetic counselors may also build a family pedigree based upon genotype or phenotype, depending on what information is available and what the family can afford.

The Hughes family has a six-month-old daughter, Ashley Kate. The family doctor has recommended the family see a genetic counselor as he suspects that Ashley may have cystic fibrosis.

Your job is to build a family tree based upon phenotype and predict possible genotypes for the cystic fibrosis gene which we will call 'F' (dominant, wild-type) and 'f' (recessive, disease causing mutation). To do this you will make a Punnett square for at least three of the family members.

Finally, based on the information you have gathered, you will decide as a group if Ashley likely has cystic fibrosis and, if so, whether the family should move forward with testing for Ashley.

Genetic Counseling Session Worksheet

Name(s) of group members: _____

Attach your color-coded family tree with predicted genotypes for the Hughes family to the back of this paper. Write your predicted genotypes for the cystic fibrosis gene under each family member on the tree.

Write your conclusions based on your genetic counseling session below.

We believe Ashley is likely / not likely (circle one) to have cystic fibrosis for the following reasons:

We believe the family should / should not (circle one) proceed with further testing to confirm this diagnosis for the following reasons:

Predict the genotypes and explain how you arrived at your prediction for the family members listed below.
Family Member #4 predicted genotype is _____.
Justification:

Family Member #16 predicted genotype is _____.
Justification:

Mom: Jessica Hughes
Family History
(do not share this paper with any other members of your group)

I was born in Hazard, Kentucky in 1992

Family has lived in Hazard, Kentucky for generations

Family has, mostly, worked at the TECO Coal Corp. mine in one form or another until it closed finally and forever in 2012

My mother was born, Lisa Hamilton, in Hazard, Kentucky in 1967. She had me when she was 25. I am an only child. My mom was raised in foster homes while she was growing up and we don't know anything about her birth parents.

My dad was born, Michael Anderson, in Hazard Kentucky in 1962. He worked in the mines from the time he was 19 until they closed in 2012. He was 50 at that time. He's been doing odd jobs since then, but never anything permanent or even full time. Dad had two brothers that died when they were really little, like one was five or something and one was younger I think. My grandmother always said they were born with the black lung from living so close to the coal mine. I never really asked her much else about them because it always made her so sad. Daddy never spoke much about his brothers. I don't think he remembers them much because he was the youngest so he was pretty much just a baby when they died. I don't know why he wasn't born with the black lung. Even working in the mines for 31 years he never got the black lung.

My fraternal grandfather, Robert Anderson, was also born in Hazard, but in 1927. He's 91 years old now and still alive and doing great for a man his age. He always said his oldest boys died of a curse from the Knockers. I don't think he really believed in knockers, but that's what he always said. He had a sister that died the same way when my grandpa was a teenager. He said she suffocated to death and that she'd always been a sickly child. I think she was 7 when she died, but I'm not sure. Grandpa's parents were born in Hazard, too, but I never knew them, they died before I was born.

Ashley is 6 months old. It seem like she gets an upper respiratory infection at least once a month. She was nearly hospitalized for it once. It seems she is always on antibiotics. We thought it was just allergies or asthma. She hasn't met any of the weight milestones for her age since she was born. She was 6 lbs and 5 oz when she was born. She has met all her developmental milestones and is already crawling. I nursed her for the first month, but then we switched to formula because she wasn't gaining enough weight. I thought it was my fault. But she didn't seem to gain any weight when we

switched to formula either. She is constipated a lot and seems to get bad gas pains. Then she won't eat. And, this is probably silly, Matt thinks it is, but when I kiss her I notice a really salty taste on my lips. Matt says all sweat is salty, but I think Ashley tastes more salty than Matt when he's been working in the heat on cars all day. Ashley does drink a lot. I give her a lot of prune juice because she's always constipated.

Dad: Matthew Hughes
Family History
(do not share this paper with any other members of your group)

I was born in Hazard, Kentucky in 1990

My family has lived in Hazard, Kentucky for generations

My family has, mostly, worked at the TECO Coal Corp. mine in one form or another until it closed finally and forever in 2012

Melissa and I met in town at a party and got married two years ago. We wanted to have a big family, but now we aren't sure. Ashley is almost a year old and has been sick more than she hasn't. I guess it's still the black lung or something which is kind of weird since the mine has been closed since 2012. I don't know if we should have any more kids or if we should move out of town if we want to have healthy ones. Though my brother has kids and they are all really healthy. Maybe we should adopt. I just don't know. They can't seem to figure out what is wrong with Ashley and it's really not getting any better. And I don't like to say this in front of Melissa and god knows I love Ashley more than my own life, but every time she gets sick we end up further in debt. I'm an auto mechanic with my dad. Business is kind of slow and we might have to move so I can get a job in a bigger town just to make ends meet. It would be hard to leave Hazard though, in some ways. It's our roots, you know?

I have one living sibling, a brother, Joe. He's older than me. He was born in 1984. He's married now and has three kids, identical twin girls and a boy. They couldn't be healthier. They are always running around outside getting into regular kid kind of trouble. Last week, my nephew, he's 13 now, ran his first 15K. We all went to cheer him on. He's really a great kid.

My dad is still alive and healthy. His name is Jim Hughes and he was born in 1958. He doesn't have any brothers or sisters. His mom was real young when she had him, like 17 or something. She died shortly thereafter and his dad never remarried. Just raised my dad on his own while working in the mines. He's 60 now. Not my dad, my dad's dad. He works as an auto mechanic since the mines closed.

My mom was born, Melissa Parker in 1970. She's actually the only one in my family, I think, that wasn't born in Hazard. She was born in the next town over because her parents didn't think the hospital in Hazard was good enough at the time. She has one sister, my Aunt Amy, and one brother, my Uncle Dan. Uncle Dan moved away and we don't hear much about him, but Aunt Amy still lives in town. She never married. She is a secretary/bookkeeper at an insurance company in town. She's never had any health problems that I know of. Uncle Dan was still healthy last we heard. I think he

might be addicted to opioids , but my parents don't talk about him much.

My maternal grandparents are both still alive. My grandma Barb was born in 1944 and still lives in a small house in Hazard, the same one mom grew up in. My grandpa Bill was born in 1947. He is in a nursing home now, hospice. He has skin cancer. Well, it started as skin cancer, but now it's pretty much everywhere. My grandma visits him every day except Sunday and brings him his favorite soup, lentil, every Friday. I don't know much about my maternal grandfather's family. They actually might not even be from around here. I know my grandma had a sister that died when she was two and a brother that died when he was about the same age, I guess. My grandma was 12 or so when they died, they didn't die at the same time, of course. Whatever they died of, I don't know, but grandma always said that those babies suffered a lot and were always sick. I know it broke my grandma's heart. She wears a locket with the babies' picture in it every day.

I've never been sick a day in my life, well, apart from a cold here or there, but Ashley is sick all the time. It's really exhausting. My wife can't work because she has to stay home with Ashley and we are in the red as far as a budget goes. I just don't make enough money to meet all of our expenses. We don't have health insurance.

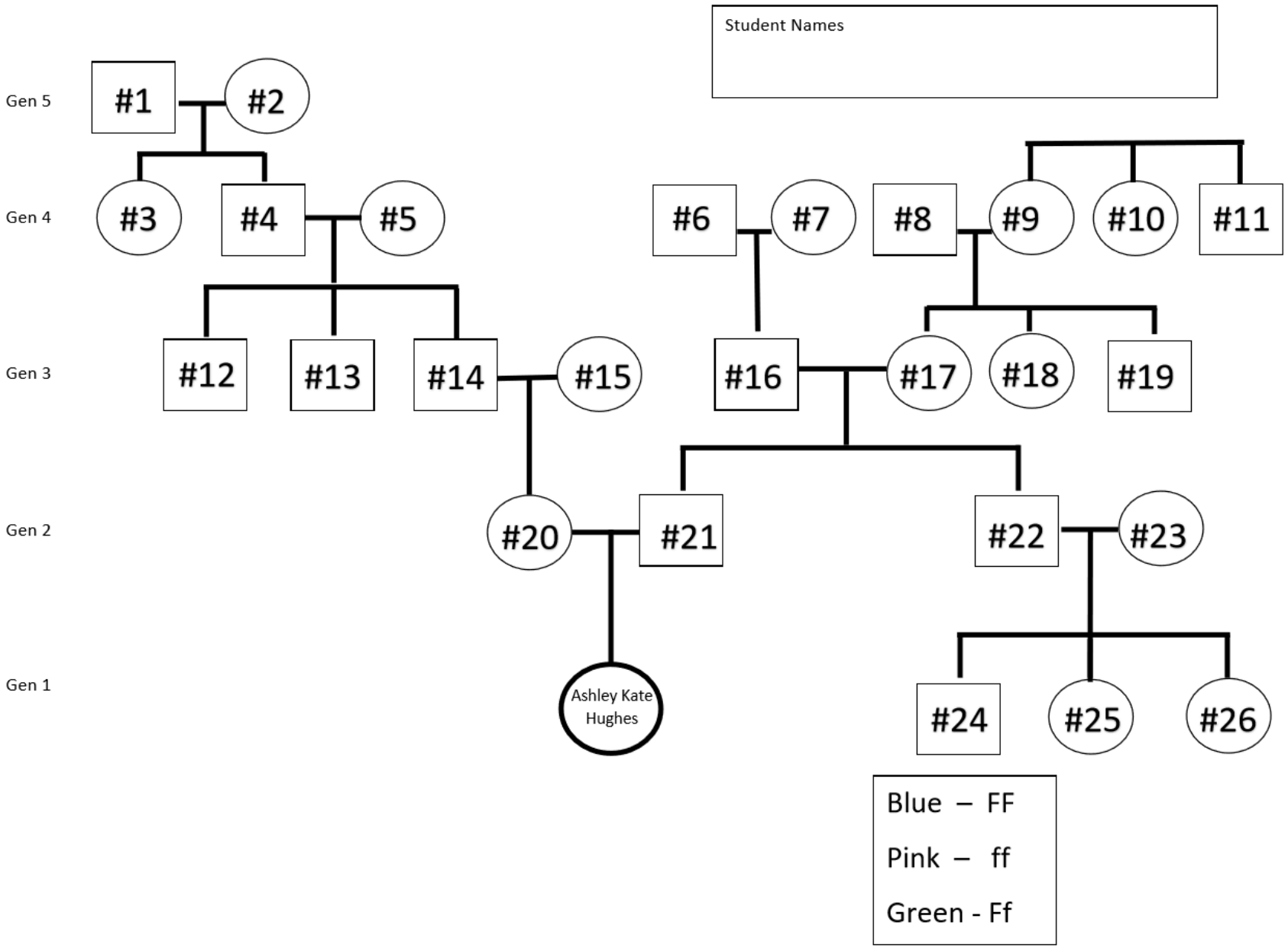
Genetic Counselor

So I'm _____ and I'm your genetic counselor. Your doctor recommended that you see me because he suspects your daughter may have cystic fibrosis and that would mean that each of you are carriers. I have the family tree that you put together for me. Thank you for that.

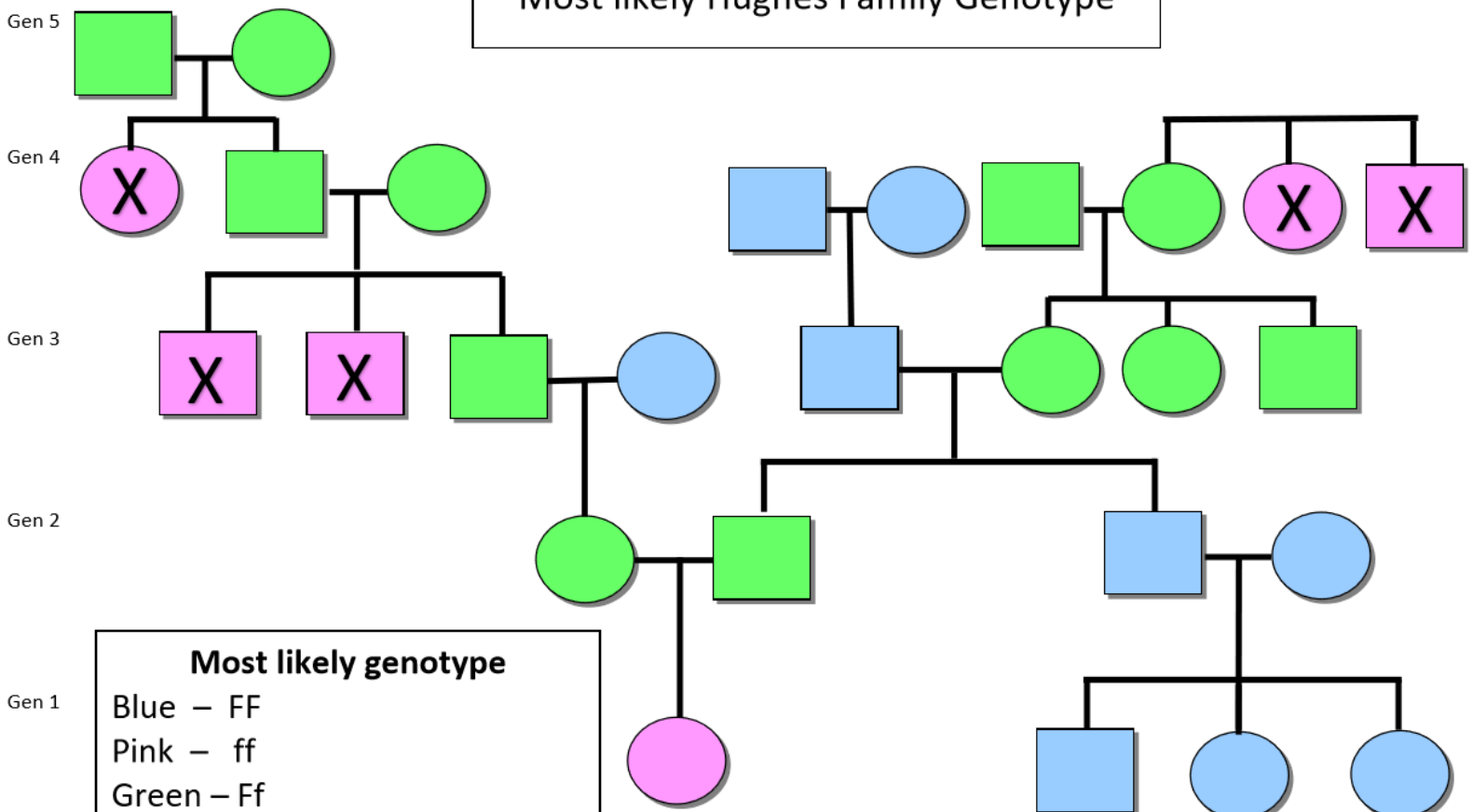
Let's see if we can build a pedigree based on phenotypes and predict some genotypes to determine if there is a probable history of the disease and the probabilities that either of you, your daughter, or any future children may have the cystic fibrosis or be carriers of cystic fibrosis.

I also have a pamphlet about cystic fibrosis that we can go through together and you can take with you to share with your family.

Ask questions of the parents and build as much of a family tree as possible. Look for abnormalities such as high death rates and ages of death. Together, with the family, build a possible genotype pedigree for the Hughes family for the cystic fibrosis gene, F (dominant, wild type) and f (recessive mutation).


Student Names
Gen 5
Gen 4
Gen 3
Gen 2
Gen 1
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19
#20
#21
#22
#23
Ashley Kate Hughes
#24
#25
#26
Blue – FF
Pink – ff
Green - Ff

Most likely Hughes Family Genotype

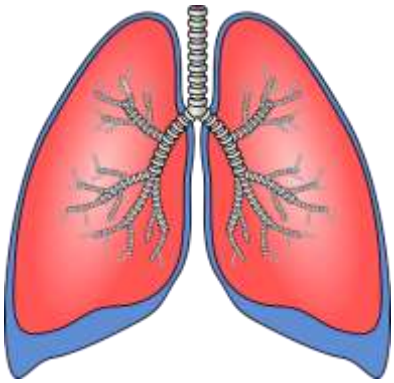


Most likely genotype

Blue – FF
Pink – ff
Green – Ff
Yellow - Cannot be determined

Died before the age of 13 - X

Activity 2: STUDENT HANDOUT What is Cystic Fibrosis?



What is Cystic fibrosis?

Cystic fibrosis (CF) is a genetic disease that changes the

code on the 7th chromosome. This creates a recessive condition which has the

Cystic Fibrosis phenotype – people with the dominant condition do not get CF. Therefore it is a hereditary genetic disease a child must get from both parents and it causes chronic buildup of abnormally thick mucus in the lungs' passages and the digestive tract. In addition, the pancreas absorbs nutrients in a destructive way, causing repeated infection and sickness. A fault with the cilia in the affected passageways allows for these illnesses to take hold much easier, and the body will far overproduce mucus due to it trying to fight disease to a normal extent. Watch more about Cystic fibrosis at the following links…

5:42 video What is Cystic Fibrosis

8:28 video Cystic Fibrosis

Use the information and your prior knowledge to help answer the questions below in red.

Where did Cystic Fibrosis Originate?

Cystic fibrosis is caused by a deletion mutation – we classify it as a ***nonsense mutation***.

What is a nonsense mutation?

It is found on chromosome seven of the human genome.

What gene is affected?

The ***deletion*** created a string of codons that are different from the original code due to a ***frameshift*** in the gene.

How can one deletion of one nitrogen base cause so many problems for a child?

As an ***autosomal recessive*** gene it can often skip multiple generations and causes defects to several systems of the body including the secretory, respiratory and digestive systems. Cystic fibrosis is experienced by billions of people without even realizing it, as for the disease to actually take hold, the defective genes must be passed to a child by both parents. It is most often experienced by Caucasians, particularly those with ancestry in Northern Europe.
Predict why this disease is most common in those of Northern European Caucasian descent.

Syptoms

Symptoms of cystic fibrosis include frequent infections, a decreased absorption of nutrients, infertility, failure of several organs including the pancreas, liver, and/or digestive tract. Other symptoms include malnutrition and poor growth.

Babies can experience lack of weight gain and have fewer stools in the diapers in addition to chronic lung and respiratory infections.

Children will suffer from frequent infections and can exhibit asthmatic breathing conditions, low weight, chronic exhaustion, repeating bouts of pneumonia, and persistent coughing.

The effects are not always severe. There are more mild phenotypes where cystic fibrosis symptoms can be significantly reduced. Some children will not even be diagnosed due to a lack of detection until they are 18 years old. People exhibiting this limited form of cystic fibrosis, despite not knowing about it, will live longer lives.

Living with Cystic Fibrosis

There are few treatments available for CF patients today. Most are ineffective. Airway clearing techniques along with antibiotics help minimize the mucous in the airways and prevent bacterial infections that the alveoli usually help with. New leaps are being made in genetic technology to help isolate and correct the gene defects.

What do we call a biotechnology that manipulates or may introduce new/corrective genes into a host's genome?

Genetic screening and counseling are, however, very effective tools in both in deciding whether or not to have children or giving the parents a heads up to prepare for the treatments needed by a newborn if pre-diagnosed with CF. The effectiveness in successful recognition of cystic fibrosis ranges from 60 to 90% of cases.

Compare ***genetic counseling*** to genetic screening – how are each important for parents?

In the 1960's when the sweat test first started to diagnose children – the life expectancy was 10 years old. Today, with modern treatments and early detection, most patients with CF live fairly healthy, active lives until adulthood; though there is a serious decrease in quality of life as they age due to the repeated infections of lung disease. A baby born with CF with proper care and medical treatments can expect to live to the age of 35 before dying due to complications from Cystic Fibrosis. Modern medical technology has allowed those afflicted with cystic fibrosis to live longer and healthier lives, and the average lifespan of victims increases year by year.

Significance

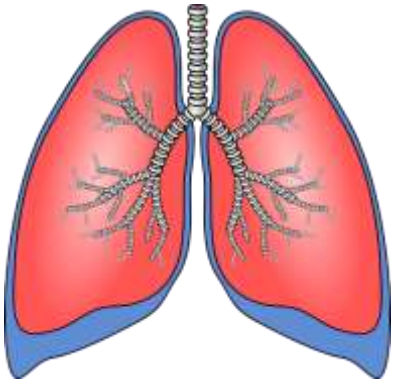
There is no real way of preventing it Cystic Fibrosis. CF is significant to the study of Science especially Biotechnology and AP Biology because of the large variations in mutations that lead to a lack of a single protein. This protein forms a critical channel in the cell membrane and without it has a profound effect on the entire organism.

Explain why this lack of a single protein can have such a large impact on a person with CF. (please include the term ***homeostasis*** in your answer)

Sources

- *Therapies for cystic fibrosis.*. (2010, April 12). Retrieved from http://www.cff.org/treatments/Therapies/.
- *Types of mutations.* (n.d.). Retrieved from http://www.ebi.ac.uk/2can/disease/genes5.html.
- Zieve, D., & Kaneshiro, N. (2010, July 14). *Cystic fibrosis.* Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000107.htm.

Adapted from Waldowski and Small



TEACHER KEY - What is Cystic Fibrosis?

What is Cystic fibrosis?

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Use the information and your prior knowledge to help answer the questions below in red.

Where did Cystic Fibrosis Originate?

Cystic fibrosis is caused by a deletion mutation – we classify it as a nonsense mutation.

What is a nonsense mutation? A nonsense mutation is where there is a change in a base in the DNA that changes an active codon to a premature STOP codon - shortening the message often forming an incomplete or nonfunctional protein.

It is found on chromosome seven of the human genome.

What gene is affected? Student may put CFTR gene OR the F508 gene.

The deletion created a string of codons that are different from the original code due to a frameshift in the gene.

How can one deletion of one nitrogen base cause so many problems for a child? There are several valid reasons a student may come up with - a frameshift will occur messing up the entire gene. The deletion will make something not function in the child, etc. Accept all logical answers.

As an autosomal recessive gene it can often skip multiple generations and causes defects to several systems of the body including the secretory, respiratory and digestive systems. Cystic fibrosis is experienced by billions of people without even realizing it, as for the disease to actually take hold, the defective genes must be passed to a child by both parents. It is most often experienced by Caucasians, particularly those with ancestry in Northern Europe.
Predict why this disease is most common in those of Northern European Caucasian descent.The hope with this question is that students will make a connection between where a mutation originates in a world population and how this will dictate that the mutation would be most commonly found in those areas or in areas that are emigrants from the place of the mutation's origin with past social structures encouraging same ethnic marriages, the gene would mainly be passed down within those ethnic groups - Accept all logical responses.

Symptoms

Symptoms of cystic fibrosis include frequent infections, a decreased absorption of nutrients, infertility, failure of several organs including the pancreas, liver, and/or digestive track. Other symptoms include malnutrition and poor growth.

Babies can experience lack of weight gain and have fewer stools in the diapers in addition to chronic lung and respiratory infections.

Children will suffer from frequent infections and can exhibit asthmatic breathing conditions, low weight, chronic exhaustion, repeating bouts of pneumonia, and persistent coughing.

The effects are not always severe. There are more mild phenotypes where cystic fibrosis symptoms can be significantly reduced. Some children will not even be diagnosed due to a lack of detection until they are 18 years old. People exhibiting this limited form of cystic fibrosis, despite not knowing about it, will live longer lives.

Living with Cystic Fibrosis

There are few treatments available for CF patients today. Most are ineffective. Airway clearing techniques along with antibiotics help minimize the mucous in the airways and prevent bacterial infections that the alveoli usually help with. New leaps are being made in genetic technology to help isolate and correct the gene defects.

What do we call a biotechnology that manipulates or may introduce new/corrective genes into a host's genome?Gene Therapy, Genetic Engineering, Transgenics, CRISPR

Genetic screening and counseling are, however, very effective tools in both in deciding whether or not to have children or giving the parents a heads up to prepare for the treatments needed by a newborn if pre-diagnosed with CF. The effectiveness in successful recognition of cystic fibrosis ranges from 60 to 90% of cases.

Compare genetic counseling to genetic screening – how are each important for parents?In **genetic counseling**, specially-trained professionals help people learn about **genetic** conditions, find out their chances of being affected by or having a child or other family member with a **genetic** condition, and make informed decisions about **testing** and treatment. In **genetic screening**, people are actually tested to see if they have or are predisposed to have a gene or genetic condition.

In the 1960's when the sweat test first started to diagnose children – the life expectancy was 10 years old. Today, with modern treatments and early detection, most patients with CF live fairly healthy, active lives until adulthood; though there is a serious decrease in quality of life as they age due to the repeated infections of lung disease. A baby born with CF with proper care and medical treatments can expect to live to the age of 35 before dying due to complications from Cystic Fibrosis. Modern medical technology has allowed those afflicted with cystic fibrosis to live longer and healthier lives, and the average lifespan of victims increases year by year.

Significance

There is no real way of preventing it Cystic Fibrosis. CF is significant to the study of Science especially Biotechnology and AP Biology because of the large variations in mutations that lead to a lack of a single protein. This protein forms a critical channel in the cell membrane and without it has a profound effect on the entire organism.

Explain why this lack of a single protein can have such a large impact on a person with CF. (please include the term homeostasis in your answer)

Because this protein channel is found in many epithelial tissues, a defect in this protein channel will cause issues in the secretory, intestinal, respiratory systems where Chloride Ions will not travel correctly causing excessive fluid and mucous to interfere with maintaining homeostasis of salts and other ions, as well as fluids throughout the body.

Sources

- *Therapies for cystic fibrosis.*. (2010, April 12). Retrieved from http://www.cff.org/treatments/Therapies/.
- *Types of mutations.* (n.d.). Retrieved from http://www.ebi.ac.uk/2can/disease/genes5.html.
- Zieve, D., & Kaneshiro, N. (2010, July 14). *Cystic fibrosis.* Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000107.htm.

Adapted from Waldowski and Small

Lesson 2: CTFR Integral Proteins – what are they and how do they work?

LESSON 2

TITLE: *CTFR Integral Proteins – what are they and how do they work?*

KEY QUESTION(S):

- What would happen to a signal transduction pathway if a protein misfolded?
- How does a protein make conformational changes when gating?
- What roles do enzymes play in cell signaling?
- How do cells communicate with each other?

***SCIENCE SUBJECT:** Biology (Honors and or AP), Biotechnology

***GRADE AND ABILITY LEVEL:**

Activity 1:Origami Protein Channels 9-12, may be scaffolded for different levels of students

Activity 2: Understanding cell signaling and channel gating with a CFTR Protein 11-12, This activity requires some synthesis of old information and higher order thinking skills so it would work better for an honors/AP level student, scaffolding may be required for lower level students Honors 9th grade Biology, AP Biology or Biotechnology Students.

SCIENCE CONCEPTS: Modeling a protein channel, G-Protein Signaling Pathways, CFTR mutations affecting gating

OVERALL TIME ESTIMATE:

Part 1: Origami Lesson (1 class period)

Part 2: CFTR and How it Works - (this will probably take 2 class periods to get through)

LEARNING STYLES:

Part 1 Origami - Kinesthetic, Visual

Activity 2 Visual, Auditory, Kinesthetic

LESSON VOCABULARY:

Part 1:

Anion, **Cell Membrane, Cilia, Diffusion, Electrolyte, Epithelial Cells, Epithelium, Homeostasis, Mucus, Phospholipid bilayer, Protein, Viscosity**

Part 2: Active Site, Agonist, cAMP pathway, Concentration gradient, Gating, G-Protein Coupled Receptor, Ligand, Phosphatidylinositol Pathway, Phosphorylation, Protein Kinase, Secondary messenger, Signal Transduction

LESSON SUMMARY:

Part 1: Students fold paper (origami) and assemble the parts into a model of a protein channel. Students watch a short animation (1:34) of a sodium-potassium pump and make comparisons between the origami model and the model in the video animation. The teacher may opt to have students do this lesson individually, with a partner, or with a group (it should be noted that each origami protein requires eight sheets of colored paper so availability of a specific quantity may be a factor in the decision of grouping students). This lesson is written assuming students will work with a partner in order to speed up the process of creating the origami model and leave more time for discussion. Teachers may also opt to hang completed origami proteins in the classroom or allow the students to keep their own model.

Part 2: Students will learn about the CFTR Protein and how it works through a PREZI that takes them through both G-Protein Signal Transduction Pathways and Gating of the CFTR. Upon understanding the notes, students will make connections and demonstrate comprehension through a Labeling Activity.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

Part 1:

The student will be able to…

1. Explain how the shape of a protein relates to its function and how function can be affected if a protein is misfolded
2. Explain that the shape of a protein can be dynamic
3. Use origami to model a protein channel and observe its change in conformation
4. Compare and contrast a paper model of a protein channel to an actual protein channel

Part 2:

The student will be able to …

1. Explain what type of channel the CFTR Protein is and what makes it unique.

2. Compare and Contrast the 2 G-Protein Signal Transduction Pathways.
3. Explain how a cell uses chemical messengers to communicate intra/extracellularly.
4. Understand factors that affect gating in a channel protein.

NEXT GENERATION SUNSHINE STATE STANDARDS:

SC.912.L.14.2 SC.912.L14.11 SC.912.L.16.4 SC.912.L18.1
SC.912.L18.2 SC.912.L.18.4 SC.912.L18.10 SC.912.N.1.7
SC.912.N.3.5

NEXT GENERATION SCIENCE STANDARDS (http://www.nextgenscience.org/next-generation-science-standards)

HS-LS 1-1 HS-LS 1-2 HS-LS 1-3 HS-LS 3-1

HS-LS 3-2 HS-LS 3-4

Core Idea - Structure and Function

Scientific Engineering Practices - Developing and Using Models, Constructing Explanations and developing solutions

Cross Cutting Concepts - System Modeling, Structure and Function, Stability and Change, Cause and Effect, Scale, Proportion and Quantity

COMMON CORE STANDARDS (http://www.corestandards.org/)

AP BIOLOGY CURRICULUM FRAMEWORK

2.A.2 2.B.1 2.B.3 2.C.1 2.C.2 2.D.3 3.B.1

3.B.2 3.C.1 3.D.3 4.A.1 4.A.2 4.B.1

MATERIALS:

Part 1:

- · 8 pieces of colored paper per pair of students
- · Laptop, phone, or other Internet capable device for purposes of following a YouTube video
- · Worksheet for each pair of students

Part 2:

- · Ability to show a Prezi online - you will need sound for a video clip embedded in the prezi
- Students will need access to all the colors in the activity table
- · Copies of the Activity Handout G Protein Signal Transduction Pathways for each student

BACKGROUND INFORMATION:

Part 1:

Modeling is especially important when trying to help students visualize the microscopic world. This lesson instructs students to fold and assemble a paper model (origami) of a membrane protein that is capable of alternating between two conformations (open and closed). Students should already be familiar with the structure of the four biological macromolecules (and their monomers) as well as the concept of polarity and how it relates to water solubility.

In all cells, molecules need to be able to move in and out to allow for ***homeostasis***. Molecules that can be used as energy, like glucose, need to be able to move into the cell and other molecules, like waste products, need to be able to move out of the cell. Some molecules can move in or out of a cell by directly passing through the ***phospholipid bilayer*** in a process called ***diffusion***. Other molecules, such as those that might be too big or too polar for diffusion or those that need to have their concentrations inside and outside the cell strictly regulated, require special membrane ***proteins*** to allow passage via a molecular channel. Some of these protein channels remain open and specific molecules can pass through unaided, while other channels rest in an open or closed conformation. Channels that rest in a closed position must be triggered to open by specific chemical signals from within the cell or outside the cell. For example, a membrane protein called cystic fibrosis transmembrane conductance regulator (CFTR) allows ***anions*** like chloride and thiocyanate anions to move out of epithelial cells into the ***mucous*** layer that coats them. This increase of anions in the mucous layer actively encourages the movement sodium cations to exit the cell via diffusion in order to reduce the buildup of negative charges in the mucous layer that lines the outside of the epithelial cells. This, in turn, increases the ***electrolyte*** concentration in the mucous layer and triggers the exodus of water molecules from inside the cell into the mucous layer in order to reduce the salt concentration. The ***viscosity*** of the mucous layer must be low enough to allow ***cilia*** to move within it, but high enough to stick to the surface of the epithelial cells so that it can act as a protective barrier from environmental insults like bacteria or toxins. If the CFTR protein is misfolded and doesn't allow anions to leave the cell, the mucous layer remains thick and viscous. The thick mucus

traps bacteria, but is not easily removed eliminated by cilia and remains clogging the epithelial cells and providing a good environment for infection to develop. This is essentially what happens in people who have cystic fibrosis.

Part 2:

One of the most difficult concepts to teach is Cell Signaling pathways. CFTR Proteins utilize 2 different G-Protein ***Signal transduction*** pathways. The first pathway is called the cAMP (Cyclic Monophosphate) Signal Transduction pathway. The cAMP uses an external signal (***ligand***) which is a molecule (ex. hormones) that acts as an ***agonist***, a substance that triggers a response when it binds to a receptor ending the pathway with a ***Protein Kinase***. When you see the prefix "kin" it means to move or activate - the kinase is an enzyme that will help the CFTR change conformation (shape) into an open channel. The protein kinase molecules ***phosphorylate*** (it will add a phosphate group) to the CFTR's regulatory domain which triggers a change in the conformation to occur. The second pathway is called the ***Phosphatidylinositol Pathway.*** Essentially these two begin the same way but the signal is sent down a different pathway triggering 2 additional responses that are needed for the CFTR Protein to open. We get a second type of protein kinase (both are needed for gating) and a change in the ***concentration gradient*** of Calcium by an increase in Calcium ions being released from the Endoplasmic Reticulum. A concentration gradient is created by the net difference of ion concentrations inside compared to outside the cell. Calcium ions are used by the body to accelerate and activate signaling pathways. CFTR requires the calcium to complete the gating process. The ***G-Protein Coupled Receptor*** is a folded protein embedded in the cell membrane that initially receives the signals. Receptors are like an inbox that allows these chemical messages to attach, then relays the message internally by releasing aG-Protein bonded to a GTP into the cytoplasm which will trigger a ***secondary messenger*** from another protein into the cytoplasm. This secondary messenger triggers a series of signals to go between molecules one by one as they each receive the signal until the final signal triggers the desired response. In our case the response is ***gating*** (to open the Gated Channel) of a CFTR molecule. Signal transduction is how a cell moves a signal through its membrane or between intermolecular structures. The signals are usually in molecular form, examples are hormones, cAMP, etc.

Once the signals are released and the cell has the PKA and PKC kinases along with the increase in calcium ions - gating can occur in the CFTR. To open the gate requires a PKC to attach, which will trigger a new binding site to open allowing the PKA to attach. This regulates the CFTR and "unlocks" it to be able to open. This process now requires ATP to help with the conformational change. 2 ATP molecules attach to sites on the CFTR causing they to move together and attach, this opens the channel and allows chloride ions to leave the cell.

ADVANCE PREPARATION:

Part 1:

- Print off a copy of the student worksheet for each pair students
- Reserve computers or other Internet device capable of viewing YouTube videos
- Gather paper and scissors for origami

Part 2:

- **Print off a copy of the Activity Handout G-Protein Signal Transduction Pathway Worksheet**
- **Be able to access the internet and show a Prezi (you may need to download the Prezi program to use it with some equip)**
- **Sound needs to be accessed - the prezi has an embedded video clip to play**
- **Tell students or have access to sets of crayons, colored pencils, highlighters, etc to complete coloring pages.**

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Part 1:

30 minutes (reserving class time to discuss the answers to the questions on the worksheet)

1. (Optional) Assign students to a group or a partner.
2. Have students login to their assigned computer.
3. Pass out the required eight sheets of colored paper per student that will be creating the model.
4. Allow students time to complete their origami model and answer the questions on the worksheet.
5. Collect the worksheets

Remaining class time (15 minutes)

6. Direct the same questions from the worksheet to the class and discuss the suggested answers to each question to determine the best response.
7. Exit Ticket: Print off the exit tickets and randomly pass them out to each student. The teacher could opt to allow the use of notes or not to allow the use of notes. Have them turn in their ticket when the bell rings.

Part 2:

(1 to 2 45 min classes)

1. Prezi Teaching tool on the CFTR Protein and How it Works can be accessed at

   https://prezi.com/p/a_gp6nkeu1b7/

   This may take up to 2 class periods to get through depending on the level of student - some may need longer time to understand what is happening in this complex set of information.

(30 min - makes a good homework assignment)

2. **Activity handout on G Protein Signal Transduction Pathways**

ASSESSMENT SUGGESTIONS:

Part 1:

- Participation grade may be given
- Worksheets may be turned in for a grade based on correction or completion
- Teachers may assign one of the worksheet questions as an exit ticket

Part 2:

- **Check Point Questions in Prezi**
- **Activity Handout emphasis on the questions on the first page more than the coloring**

EXTENSIONS:

ACTIVITIES:

LITERATURE: Article about CRISPR as a possible treatment for CFTR https://www.ncbi.nlm.nih.gov/pubmed/24315439/

RESOURCES/REFERENCES:

Ask a Biologist (2013, January 22). *Paper Protein Activity Part 1: Amino Acid* [Video File]. Retrieved from https://www.youtube.com/watch?v=3OCd570EQb8

Ask a Biologist (2013, January 22). *Paper Protein Activity Part 2: Protein Channel* [Video File]. Retrieved from https://www.youtube.com/watch?v=rXS4UQNxBjY

New England Blogger (2017, October 30) Origami Membrane Protein [Blog Post]. Retrieved from https://kaiserscience.wordpress.com/2017/10/30/origami-membrane-protein/

Prakash, B.G. (2015, April 25). Sodium - Potassium Pump Animated Lecture [Video File]. Retrieved from https://www.youtube.com/watch?v=xweYA-IJTqs

https://youtu.be/6IbP1A Cystic Fibrosis Mechanism and treatment

https://www.news-medical.net/health/Cystic-Fibrosis-and-Salty-Skin.aspx How a CFTR Channel works

Part 1: Origami Lesson

Student Worksheet:

Background Information:

In all cells, molecules need to be able to move in and out to allow for ***homeostasis***. Molecules that can be used as energy, like glucose, need to be able to move into the cell and other molecules, like waste products, need to be able to move out of the cell. Some molecules can move in or out of a cell by directly passing through the ***phospholipid bilayer*** in a process called ***diffusion***. Other molecules, such as those that might be too big or too polar for diffusion or those that need to have their concentrations inside and outside the cell strictly regulated, require special membrane ***proteins*** to allow passage via a molecular channel. Some of these protein channels remain open and specific molecules can pass through unaided, while other channels rest in an open or closed conformation. Channels that rest in a closed position must be triggered to open by specific chemical signals from within the cell or outside the cell. For example, a membrane protein called cystic fibrosis transmembrane conductance regulator (CFTR) allows ***anions*** like chloride and thiocyanate anions to move out of epithelial cells into the ***mucous*** layer that coats them. This increase of anions in the mucous layer actively encourages the movement sodium cations to exit the cell via diffusion in order to reduce the buildup of negative charges in the mucous layer that lines the outside of the epithelial cells. This, in turn, increases the ***electrolyte*** concentration in the mucous layer and triggers the exodus of water molecules from inside the cell into the mucous layer in order to reduce the salt concentration. The ***viscosity*** of the mucous layer must be low enough to allow ***cilia*** to move within it, but high enough to stick to the surface of the epithelial cells so that it can act as a protective barrier from environmental insults like bacteria or toxins. If the CFTR protein is misfolded and doesn't allow anions to leave the cell, the mucous layer remains thick and viscous. The thick mucus traps bacteria, but is not easily removed eliminated by cilia and remains clogging the epithelial cells and providing a good environment for infection to develop. This is essentially what happens in people who have cystic fibrosis.

Instructions:

Paper Protein Activity Part 1: Amino Acid (5:00)

https://www.youtube.com/watch?v=3OCd570EQb8

Paper Protein Activity Part 2: Protein Channel (4:56)

https://www.youtube.com/watch?v=rXS4UQNxBjY

Sodium – Potassium Pump Animated Lecture

https://www.youtube.com/watch?v=xweYA-IJTqs (1:39)

Once you have folded and assembled your membrane protein, watch the third video titled "Sodium – Potassium Pump Animated Lecture", then answer the following questions using complete sentences.

1. What does each sheet of paper represent?
2. How are these similar or different from the real thing?
3. How is the assembled origami membrane protein similar or different from an actual membrane protein?

EXIT TICKET

Name _____ Date _____ Period _____

How is the assembled origami protein similar to an actual membrane protein?

EXIT TICKET

Name _____ Date _____ Period _____

How is the assembled origami protein different from an actual membrane protein?

G Protein

Signal Transduction Pathways

And the CFTR Protein Channel

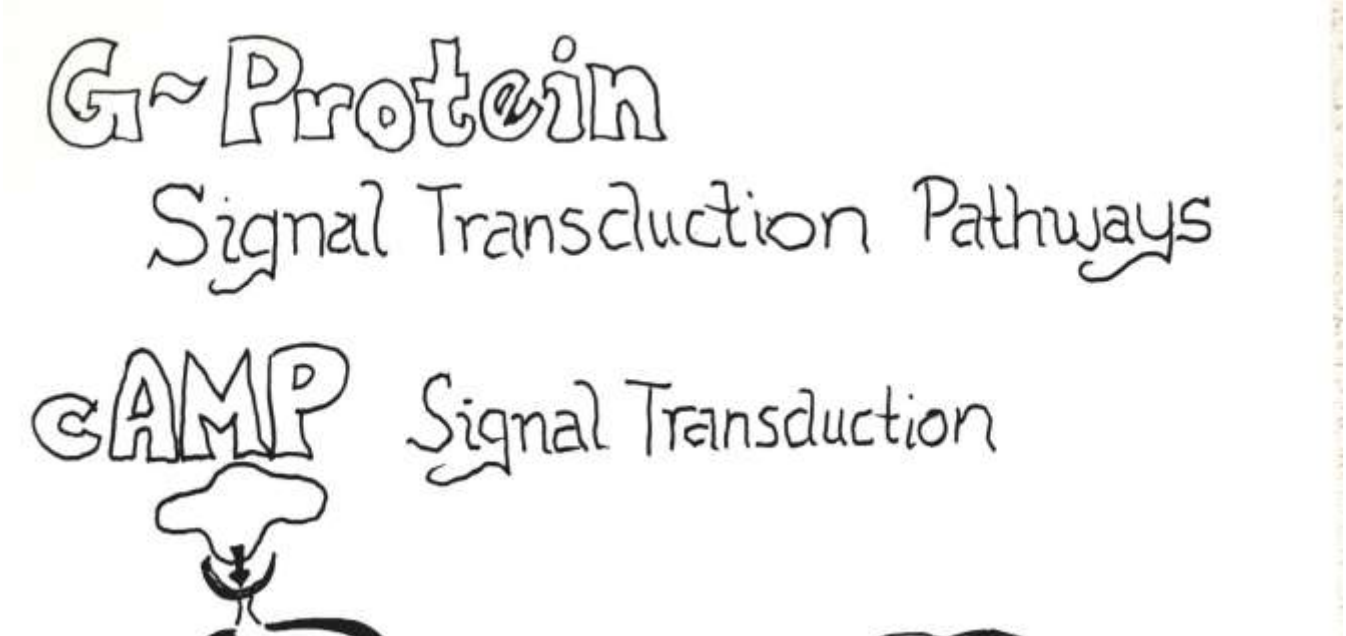
Procedure:

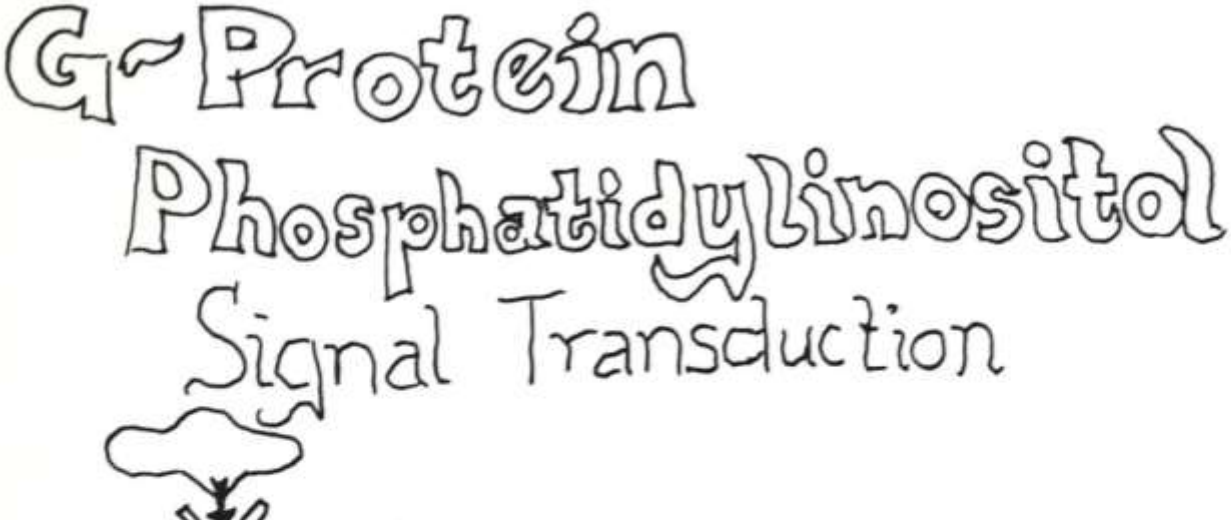
1. Label all key structures in the 2 pathways.
2. For each of the structures, follow the color key in the table.

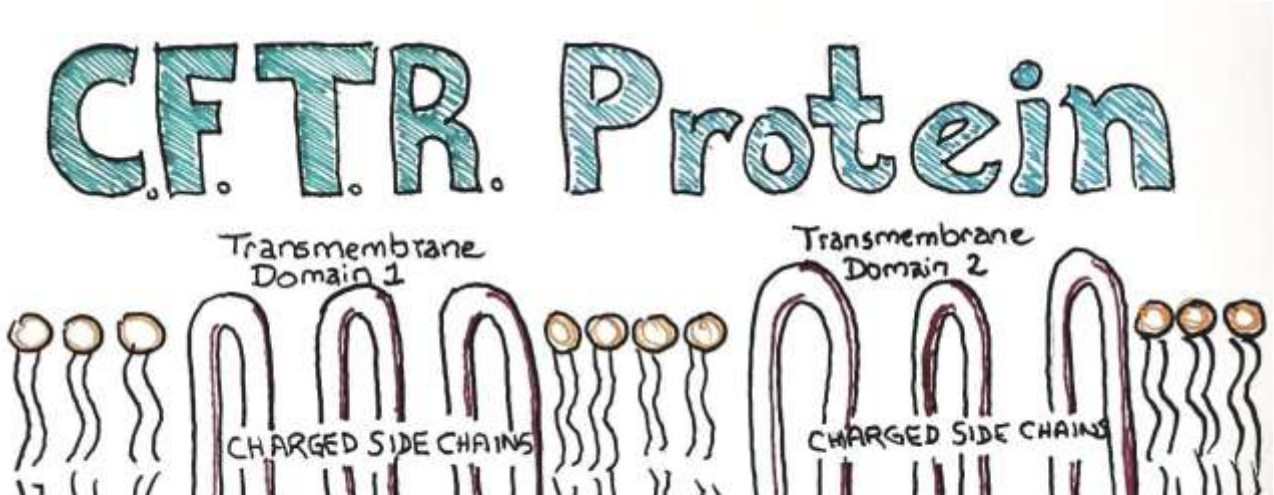
Active Sites	Highlight Yellow
Adenylate cyclase	Light Green
Alpha R G-Protein Subunit	Dark Purple
Alpha Q G-Protein Subunit	Light Purple
ATP molecules	Yellow
Enzymes	Draw a square around them
GDP molecules	Light Blue
Glycerol bonded to 2 fatty acids	Grey
GPCR – G Protein Coupled Receptor	Dark Green
GTP molecules	Dark Blue
Hydrophillic parts of Phospholipid Bilayer	Orange
IP_3 – Inositol Trisphosphate	Aqua
Ligands	Red
Pip_2 – Phosphatidylinositol bisphosphate	Pink Polka Dots
Phospholipase C	Light Green Stripes
PKA – Protein Kinase A	Dark Brown
PKC – Protein Kinase C	Light Brown
Signal Molecules	Circle them
Structure changing ion levels in the cytoplasm	Highlight Green

3. What 3 things are necessary from the signaling pathways to trigger a CFTR Protein?
4. Why are the extracellular-ligand signals called agonists?
5. Compare AND contrast the 2 G-Protein Signal Transduction Pathways.

6. **Making Connections: What will happen to the water potential of the cell if the CFTR is malfunctioning and blocks Chloride Ions from leaving the cell?**




G-Protein
Phosphatidylinositol
Signal Transduction


C.F.T.R. Protein
Transmembrane Domain 1
Transmembrane Domain 2
CHARGED SIDE CHAINS
CHARGED SIDE CHAINS

7. **The CFTR Protein has several domains that have different functions*. Draw each* of the following into the picture above. *Tell what it will do for the protein.***

   A. **ATP molecules (2 of them will attach – draw 2)**

      Function:

   B. **Protein Kinase C**

      Function:

   C. **Protein Kinase A**

      Function:

8. **Imagine that all the molecules have attached and the NBD1 and NBD2 move together to touch. What would the shape of this protein look like after they come together? Draw your vision below. Show how Chloride Ions would move in your "Open" Channel.**

(Student Handout: Lesson 2 Activity 2)

Lesson 3: Sweat it Out!

LESSON 3

TITLE: Sweat it Out!

KEY QUESTION(S):

- How can conductivity of sweat be measured?
- How can a conductivity measurement be converted to a concentration measurement?

- How can skin conductivity values be used to determine the health of an individual?

SCIENCE SUBJECT: Biology (Standard, Honors, or AP), Biotechnology, Physics (Standard or Honors)

GRADE AND ABILITY LEVEL: 9 – 12

SCIENCE CONCEPTS: electrical conductivity

OVERALL TIME ESTIMATE: One 45 minute class period

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

Conductivity: the degree to which a specified material conducts electricity, calculated as the ratio of the current density in the material to the electric field that causes the flow of current. It is the reciprocal of the resistivity.

Epithelial tissue: thin tissues that cover all the exposed surfaces of the body. They form the external skin, the inner lining of the mouth, digestive tract, secretory glands, the lining of hollow parts of every organ such as the heart, lungs, eyes, ears, the urogenital tract, as well as the ventricular system of the brain and central canals of the spinal cord.

LESSON SUMMARY:

Students watch a YouTube video illustrating how to build simple finger electrodes. Students will build the finger electrodes, take their own skin conductivity measurement and add their measurement to the class data set. Students will calculate the average skin conductivity measurement in units of microsiemens and determine an error range. Students will use this data to determine if mock patients have a normal or abnormal level of skin conductivity and use that information to predict whether or not the patients have cystic fibrosis.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The student will be able to…

1. Follow directions to build a set of finger electrodes
2. Take and record measurements using the finger electrodes
3. Analyze data to make inferences regarding healthy/disease parameters

NEXT GENERATION SUNSHINE STATE STANDARDS:

SC.912.L.14.2

SC.912.L.14.11

SC.912.N.1.1

SC.912.N.1.3

SC.912.N.1.6

SC.912.N.1.7

SC.912.P.10.13-16????

MATERIALS:

Per individual:

- 'eSense: Skin Response' app
- Electrical tape
- Velcro
- Cotton Makeup Pads
- Copy of data and calculations table

Per lab station:

- Scissors
- Knives or wire cutters
- Aluminum foil
- Isopropyl Alcohol 70%

PRIOR KNOWLEDGE REQUIRED:

Students should already have some practice in using significant figures in calculations, converting between units, a familiarity with the International System of Units (SI) base units, and understand the difference between base units and derived units.

BACKGROUND INFORMATION:

When most people think of cystic fibrosis they think of it being a disease of the lungs, but cystic fibrosis affects all ***epithelial tissues***, not just the epithelial tissue of the lungs. The channels responsible for the transport of ions across the cell membrane in apical epithelial cells are obstructed, impeding the flow of ions in and out of the cell. The net effect is that people with cystic fibrosis have a higher concentration of salt in their sweat than healthy people. Because of this, measuring the chloride ion concentration in sweat is widely recognized as the gold standard for a cystic fibrosis diagnosis. However, a positive test will not provide information as to which of the approximately 242 different mutations are responsible for the disease in any individual.

Sweat tests for cystic fibrosis should be done in an accredited laboratory by a qualified technician. Even so, it is often difficult to collect enough sweat to measure accurately. Because of these two limitations, researchers have suggested that a simple skin conductance measurement can accurately mimic the results of a sweat test. This simple test would allow a diagnosis to be made in regions that have minimal health care facilities.

https://www.ncbi.nlm.nih.gov/pubmed/20920895

Students may work individually or in groups of varying sizes that will depend primarily on the teacher's budget to purchase the required supplies.

ADVANCE PREPARATION:

1. Have students download the free app ‘eSense: Skin Response’ as homework the night before
2. Purchase required supplies that can’t be found in the classroom
3. Organize all required supplies in bins for each lab station so that each person (or group) making the galvanic skin response electrodes has all the supplies they need. Items like scissors may be shared at a lab station.
4. Print off a copy of the worksheet and data table for each student or each student group

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Explain to the students that they are going to build electrodes that will give a good approximation of chloride ion concentration in their sweat and that, aside from gene sequencing, this remains a simple and effective positive test for cystic fibrosis.
2. Pass out the required supplies
3. Allow students time to build their electrodes, take their readings, and complete their data & calculations table worksheet.

Making Galvanic Skin Response Electrodes

https://www.youtube.com/watch?v=ljVQpwVHpOo&app=desktop (6:51)

ASSESSMENT SUGGESTIONS:

- Participation and/or completion of a set of finger electrodes
- Data, Calculations & Analysis sheet based upon completion or correction

RESOURCES/REFERENCES:

Science of CFTR Function

https://www.hopkinscf.org/what-is-cf-teen/science-of-cf-teen/cftr-teen/function-teen/

"High-sweat Na+ in cystic fibrosis and healthy individuals does not diminish thirst during exercise in the heat"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3197340/

How cystic fibrosis affects the sweat glands

https://myhealth.alberta.ca/Health/pages/conditions.aspx?hwid=hw185029

"Getting into your skin: epithelial tissue"

https://www.dummies.com/education/science/anatomy/getting-into-your-skin-epithelial-tissue/

Statistical Definitions (standard deviation & variance)

https://www.mathsisfun.com/data/standard-deviation.html

Galvanic Skin Response: The Complete Pocket Guide

https://imotions.com/blog/galvanic-skin-response/

Making Galvanic Skin Response Electrodes

https://www.youtube.com/watch?v=ljVQpwVHpOo&app=desktop (6:51)

A Practical Guide to Conductivity Measurement

http://www.mbhes.com/conductivity_measurement.htm

Normal Skin Conductivity Range

https://glneurotech.com/FAQ/skin_conductance.html

What should I know to use (Electrodermal Activity) EDA data in my experiment?

https://support.empatica.com/hc/en-us/articles/203621955-What-should-I-know-to-use-EDA-data-in-my-experiment-

Sweat Chloride Test

https://labtestsonline.org/tests/sweat-chloride-test

Patient Registry Annual Data Report (2015) for the Cystic Fibrosis Foundation

https://www.cff.org/Our-Research/CF-Patient-Registry/2015-Patient-Registry-Annual-Data-Report.pdf

Abnormal electrochemical skin conductance in cystic fibrosis

https://www.ncbi.nlm.nih.gov/pubmed/20920895

https://www.cff.org/Care/Clinical-Care-Guidelines/Diagnosis-Clinical-Care-Guidelines/Sweat-Test-Clinical-Care-Guidelines/

eSense Skin Response: Mindfield Biosystems Ltd (free app)

https://itunes.apple.com/us/app/esense-skin-response/id496503504?mt=8

Supplies:

3.5 mm TRRS plug: Tip Ring Ring Sleeve

https://www.amazon.com/Cerrxian-Terminal-Headphone-Converter-Adapter/dp/B06W2K9XMM/ref=pd_sim_23_3?_encoding=UTF8&pd_rd_i=B06W2K9XMM&pd_rd_r=9906d023-7a21-11e8-b657-fff5d677929c&pd_rd_w=fFA4l&pd_rd_wg=qwcKt&pf_rd_i=desktop-dp-sims&pf_rd_m=ATVPDKIKX0DER&pf_rd_p=7967298517161621930&pf_rd_r=BPYF7EVEVV9QX3A6HKMZ&pf_rd_s=desktop-dp-sims&pf_rd_t=40701&psc=1&refRID=BPYF7EVEVV9QX3A6HKMZ

Results:

Parameters may need to be adjusted once we build the electrode and see what kind of values we are capable of getting.

From https://www.ncbi.nlm.nih.gov/pubmed/20920895

"ESC measurements on hands and feet were significantly different in CF patients (on feet: 75±10μSi), as compared with control subjects (62±13μSi, $p<0.0001$); dESC was also significantly different and more discriminative in CF patients (on feet: 34±24μSi), as compared with control subjects (93±24μSi, $p<0.0001$). dESC measurement provided a diagnostic specificity of 1 and a sensitivity of 0.93."

Student Worksheet:

Data, Calculations, and Analysis for a Skin Conductivity Test for Cystic Fibrosis Diagnosis

DATA & CALCULATIONS TABLE:

Collect your data, in microsiemens (μs), using your finger electrodes. Use the 'helpful links' below to help you convert μs to ppm, then to molarity and to mmol/L. For your conversions, assume the solute you are looking for is the chloride ion, Cl-.

Name	Conductivity (μS)	Parts per million (ppm)	Molarity (M)	mmol/L	
Ashley Hughes	82.0	52.5	1.48		
Matthew Hughes	61.0	39.0	1.1		
Jessica Hughes	59.0	37.8	1.1		
Yours					
Group Mean					
Class Mean					

What is the range of conductivity from the people in your group?

What is the range of conductivity from the people in the class?

Helpful Links:

How to find the mean

https://www.mathsisfun.com/mean.html

Converting conductivity to molarity

https://sciencing.com/convert-conductivity-concentration-6925703.html

https://www.khanacademy.org/math/probability/data-distributions-a1/summarizing-spread-distributions/a/range-and-interquartile-range-worksheet

TEACHER NOTES:

microsiemens can be converted to ppm by multiplying by 0.64

ppm can be converted to molarity by dividing by the molar mass of the solute (chloride, 35.45 g/mol)

TEACHER PAGES:

Lesson 4: Blot it Western Style

(Lesson Still Under Development)

Students will be able to…

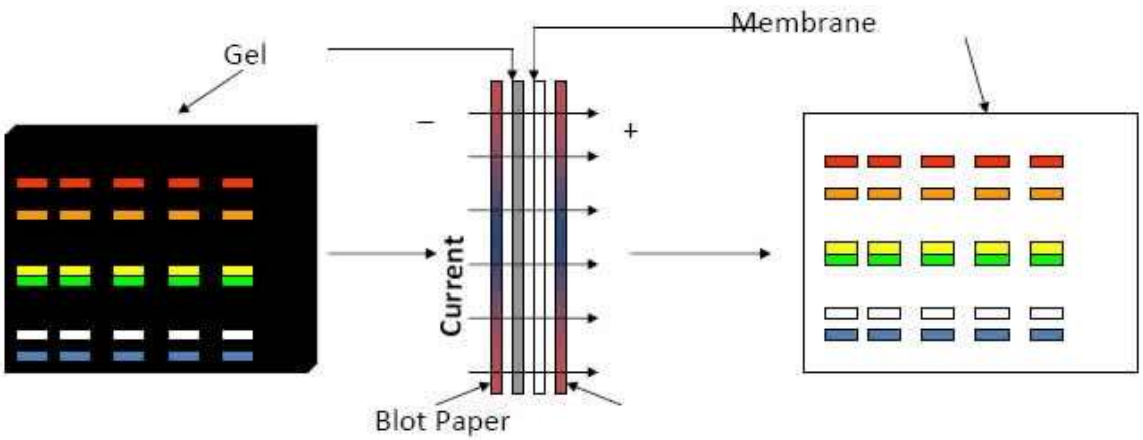
- Explain the concept of immunoblotting
- Recognize the specificity of immunoblotting with respect to our immune system and to laboratory procedures

OVERALL TIME ESTIMATE: 3-5 class periods

Lesson Summary:

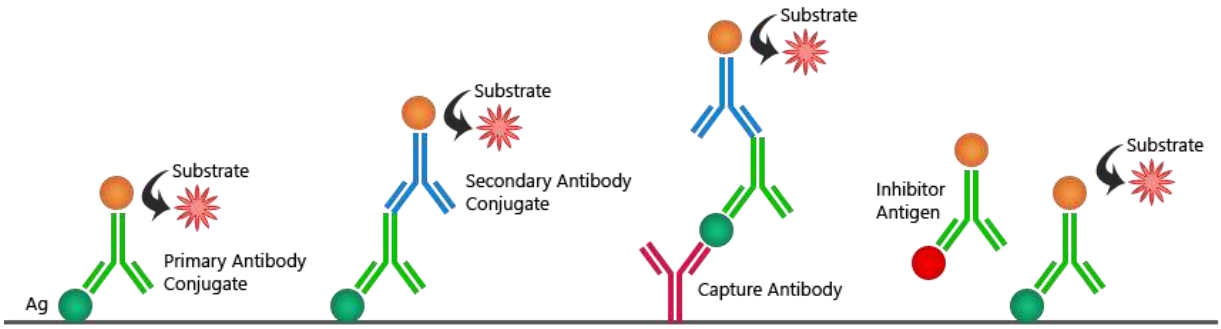
We hope to make the Bio-Rad Protein Profiler and Western Blot kits match the scenario of being able to identify which specific mutation of the CFTR gene that Ashley has. The background to the lab will be

designed for students to conclude that Ashley has the specific mutation that will most benefit from gene therapy using CRISPR-cas9.



ALTERNATIVE:

An alternate scenario to Western blot would be an ELISA (fewer class periods (1-2), cheaper, less complicated) to determine the specific version of CFTR



Lesson 5: Battle of the Super Powers

LESSON 5

TITLE: Battle of the Super Powers

KEY QUESTION(S):

1. Why is it important to understand the three-dimensional structure of a protein?

2. What is the importance of protein crystallization and what are some of the difficulties associated with crystallizing a protein?
3. What is x-ray crystallography and how can it help researchers determine protein structure?
4. Why are membrane proteins notoriously difficult to crystallize?
5. What is cryo-electron microscopy and how does it help researches circumvent the crystallization problem of membrane proteins?

SCIENCE SUBJECT: Biology (Standard, Honors, or AP), Biotechnology, Physics (Standard or Honors)

GRADE AND ABILITY LEVEL: 9 – 12

SCIENCE CONCEPTS: crystallography, x-ray crystallography, cryo-electron microscopy, protein structure, membrane proteins

OVERALL TIME ESTIMATE: one 45 minute class period (with the possibility of completion as homework)

LEARNING STYLES: Visual, auditory

VOCABULARY:

Crystalline: formed by crystallization; having the regular arrangement of the atoms in a space lattice

Diffraction: the process by which a beam of light or other system of waves is spread out as a result of passing through a narrow aperture or across an edge, typically accompanied by interference between the waveforms produced

Hydrophobicity: the property of repelling water rather than absorbing it or dissolving in it

X-ray: an electromagnetic wave of high energy and very short wavelength, which is able to pass through many materials opaque to light

LESSON SUMMARY:

Students watch four very short videos (14 minutes total) explaining the process and advantages of cryo-electron microscopy over x-ray crystallography in determining protein structure and read a journal opinion article on the same topic. Students answer worksheet questions from the videos and article during class or as homework. The teacher can opt to allow students to do the video questions in class and allow time for class discussion and give the journal article questions as a homework assignment or to do both video and journal article questions in class.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

Students will be able to:

1. Explain how protein structures can be computationally and/or mathematically derived from x-ray crystallography and cryo-electron microscopy images.
2. Describe advantages and disadvantages to both x-ray crystallography and cryo-electron microscopy in determining protein structure.
3. Explain why it is important to understand the three-dimensional structure of a protein.

NEXT GENERATION SUNSHINE STATE STANDARDS:

SC.912.I.18.1

SC.912.I.18.3

SC.912.I.18.4

SC.912.N.1.1

SC.912.N.1.6

SC.912.N.1.7

SC.912.CS-CS.1.3

SC.912.CS-CS.1.4

MATERIALS:

- Computers with Internet access
- Copies of student worksheets

PRIOR KNOWLEDGE REQUIRED:

Students should understand what properties make a structure crystalline (as opposed to amorphous) and that proteins, like almost any other molecule, can also be crystallized. Students should also be able to explain how proteins are folded and that their folding results in a specific three-dimensional shape that directly relates to the protein's function. Students should understand that misfolded proteins are often the cause of human disease. Students should understand the different natures of biological macromolecules, especially with respect to hydrophobicity, and that the phospholipid bilayer has both polar and nonpolar components.

BACKGROUND INFORMATION:

The function of a protein, like any substance, is a direct result of its three-dimensional structure. If researchers understand how a biological protein functions with respect to its structure (i.e. where an enzyme fits in a protein to elicit a specific biological response), then they can potentially design drugs that would interact with that protein and alter its function in a way that improves someone's quality of life. This could mean anything from reducing pain to lowering cholesterol levels to allowing someone with cystic fibrosis to breathe easier.

Figuring out exactly what the three-dimensional structure of a protein is has traditionally been the role of x-ray crystallography. This method uses ***x-rays*** to produce a ***diffraction*** pattern that can then be analyzed on a computer allowing a researcher to determine the most likely three-dimensional structure of a protein. One of the most challenging aspects of x-ray crystallography has been growing crystals that are of high enough quality (a near perfect internal ***crystalline*** structure free of impurities to make x-ray diffraction a reliable option.

Many proteins are notoriously difficult or impossible to crystallize due to their lack of rigidity or ***hydrophobicity***. Membrane proteins, which comprise approximately one quarter of all the proteins in our body and are involved in cellular mechanisms like respiration and signal transduction, fall into this category. Researchers have recently turned to a technology called cryo-electron microscopy to help determine the three-dimensional structure of difficult to crystallize proteins like membrane proteins. Cryo-electron microscopy does not require crystals, but rather flash freezes a solution of proteins and uses an electron beam to obtain hundreds to thousands of images of the protein in various orientations.

Using a computer, researchers can select the best images and combine the different orientations into a single three-dimensional structure. Jacques Dubochet, Joachim Frank, and Richard Henderson won the 2017 Nobel Prize in chemistry for their development of this powerful technique.

ADVANCE PREPARATION:

- Print off one copy of the student worksheets per student
- Reserve computers or computer lab with Internet access
- (Optional) have students bring their own ear buds

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Assign students to a computer.
2. Pass out the worksheet.
3. Allow students time to complete the assignment.
4. If students are not able to finish the assignment during the class period, the teacher can opt to allow them to finish it for homework.

ASSESSMENT SUGGESTIONS:

- Grade random questions for correctness
- Grade worksheet for completion

RESOURCES/REFERENCES:

Borman, S. (2017, October 4). Cryo-electron microscopy innovators win 2017 Nobel Prize in Chemistry. *Chemical and Engineering News.* Retrieved from https://cen.acs.org/articles/95/web/2017/10/Cryo-electron-microscopy-innovators-win-2017-Nobel-Prize-in-Chemistry.html

Carpenter, E.P., Beis, K., Cameron, A.D.,& Iwata, S. (2008). Overcoming the Challenges of Membrane Protein Crystallography. *Current Opinion in Structural Biology, Volume 18* (Issue 5), pages 581-586. https://doi.org/10.1016/j.sbi.2008.07.001

Vinothkumar, K.R. (2015). Membrane protein structures without crystals, by single particle electron cryomicroscopy. *Current Opinion in Structural Biology, Volume 33*, pages 103-114. https://doi.org/10.1016/j.sbi.2015.07.009

The Royal Institution. (2013, October 9). *Celebrating Crystallography - An animated adventure* [Video File]. Retrieved from https://www.youtube.com/watch?v=uqQlwYv8VQI

UC San Francisco (UCSF). (2015, May 28). *What is Cryo-Electron Microscopy (Cryo-EM)?* [Video File]. Retrieved from https://www.youtube.com/watch?v=Qq8DO-4BnIY

Lander, G. (2011, August 17). *A 3 minute introduction to CryoEM* [Video File]. Retrieved from https://www.youtube.com/watch?v=BJKkC0W-6Qk

CEN Online (2017, October 4). the 2017 *Nobel Prize in Chemistry: Cryo-electron microscopy explained* [Video File]. Retrieved from https://www.youtube.com/watch?v=026rzTXb1zw

STUDENT PAGES:

Battle of the Super Powers

Scientists have been growing crystals for x-ray crystallography for just over 100 years when it was discovered that shooting a monochromatic beam of x-rays at a crystalline substance produces a precise diffraction pattern. This is how Raymond Gosling and Rosalind Franklin obtained the famous diffraction image of DNA in 1951 (figure 1). But, by itself, this image doesn't seem to look like much, especially not like a double helix. However, in 1913 William Bragg and his son, Lawrence Bragg, realized that the angle of incidence of monochromatic x-rays hitting parallel planes of atoms and the distance between those planes could be mathematically described in an equation now called Bragg's law (figure 2). This equation allowed scientists to use these strange looking diffraction patterns like those in photo 51 to mathematically determine the three-dimensional structure of the molecules that make up the crystal.

Crystals are required because a three-dimensional array of hundreds of billions of molecules can amplify a signal produced by a single molecule would be too weak to measure.

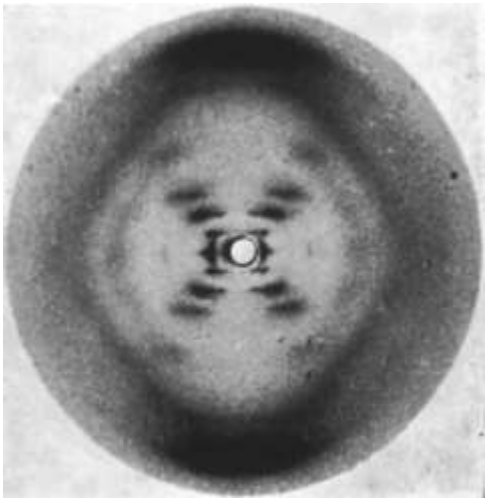


Figure 1. The famous photo 51 of the DNA double helix taken by Raymond Gosling under the direction of Rosalind Franklin in 1951.

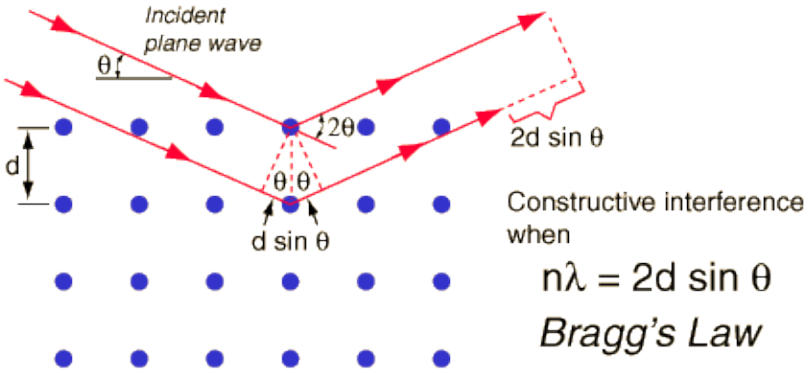


Figure 2. Bragg's Law

Watch the following four videos to answer the questions below.

Celebrating Crystallography – An animated adventure

https://www.youtube.com/watch?v=uqQlwYv8VQI (3:05)

What is Cryo-Electron Microscopy (Cryo-EM)?

https://www.youtube.com/watch?v=Qq8DO-4BnIY (2:36)

A 3 minute introduction to cryoEM (this video has no sound)

https://www.youtube.com/watch?v=BJKkC0W-6Qk (2:58)

The 2017 Nobel Prize in Chemistry: Cryo-electron microscopy explained

https://www.youtube.com/watch?v=026rzTXb1zw (4:59)

1. How did 1962 Nobel Prize winner in chemistry, Max Perutz, describe x-ray crystallography?

2. In x-ray crystallography, what is the diffraction pattern a result of?

3. What does the Bragg's law equation do?

4. What is the most famous result of x-ray crystallography?

5. What is a biological example of an application of crystallography?

6. What is an 'out of this world' example of an application of crystallography?

7. For x-ray crystallography to work, what is required of the proteins?

8. Why is it so challenging to get some proteins to crystallize?

9. What are the basic steps involved in determining a 3D protein structure?

10. How is the sample used in x-ray crystallography different from the sample used in cryo-electron microscopy?

11. How does the computer determine a 3D structure from 2D images?

12. What are some advantages of cryo-electron microscopy over x-ray crystallography in determining the three-dimensional structure of proteins?

13. What is vitrified water and why is it used for cryo-electron microscopy?

Read the following article and complete the accompanying worksheet

Title of article: _____

Author(s): _____ Date of Publication: _____ Type of Article: _____

Name of Scientific Journal: _____

1. What are the two biggest advantages of cryo-electron microscopy?

2. What are three specific processes or tools that allowed cryo-electron microscopy to rapidly expand to become the important field it is today?

3. How do scientists get around the poor signal to noise ratio obtained by the limited electron dose requirement to minimize radiation damage to the crystal?

4. Since many membrane proteins are comprised of multiple subunits or domains that can dissociate during freezing, it can be advantageous to combine what kind of images?

5. What necessary component is a difficulty for both x-ray crystallography and cryo-electron microscopy?

6. What are two added difficulties that membrane proteins present to imaging?

7. What are some current limitations to cryo-electron microscopy?

   Answer: Proteins smaller than 64 kDaltons cannot be oriented reliably; as the size of the molecule decreases, the difficulty in obtaining high-resolution structures increases; also membrane proteins that are structurally heterogeneous such as the rotary ATPase's may require many more particles and may still only give lower resolution maps

8. In your own words, what is meant by the term ‘map’ used throughout this article?

9. Do you think that x-ray crystallography will become obsolete? Explain.

10. Why is it so important to be able to determine the three-dimensional structure of a protein?

TEACHER PAGES:

Answer Key

Battle of the Super Powers

Scientists have been growing crystals for x-ray crystallography for just over 100 years when it was discovered that shooting a monochromatic beam of x-rays at a crystalline substance produces a precise diffraction pattern. This is how Raymond Gosling and Rosalind Franklin obtained the famous diffraction image of DNA in 1951 (figure 1). But, by itself, this image doesn’t seem to look like much, especially not like a double helix. However, in 1913 William Bragg and his son, Lawrence Bragg, realized that the angle of incidence of monochromatic x-rays hitting parallel planes of atoms and the distance between those planes could be mathematically described in an equation now called Bragg’s law (figure 2). This equation allowed scientists to use these strange looking diffraction patterns like those in photo 51 to mathematically determine the three-dimensional structure of the molecules that make up the crystal.

Crystals are required because a three-dimensional array of hundreds of billions of molecules can amplify a signal produced by a single molecule would be too weak to measure.

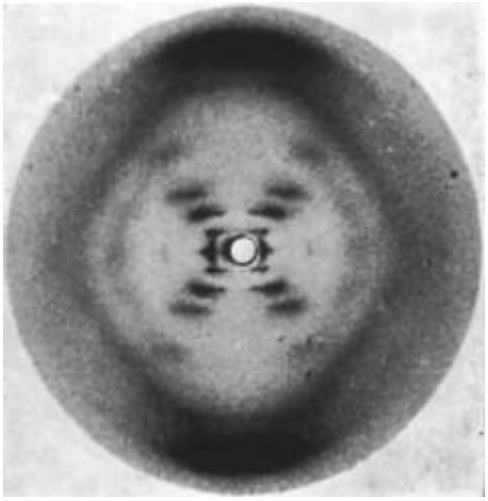


Figure 1. The famous photo 51 of the DNA double helix taken by Raymond Gosling under the direction of Rosalind Franklin in 1951.

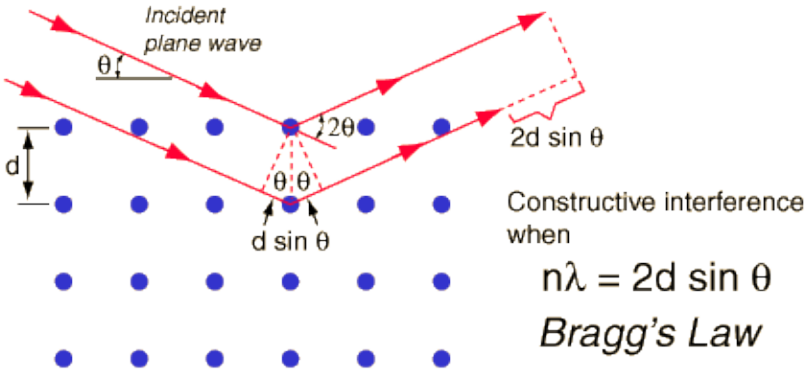


Figure 2. Bragg's Law

Watch the following four videos to answer the questions below.

Celebrating Crystallography – An animated adventure

https://www.youtube.com/watch?v=uqQlwYv8VQI (3:05)

What is Cryo-Electron Microscopy (Cryo-EM)?

https://www.youtube.com/watch?v=Qq8DO-4BnIY (2:36)

A 3 minute introduction to cryoEM (this video has no sound)

https://www.youtube.com/watch?v=BJKkC0W-6Qk (2:58)

The 2017 Nobel Prize in Chemistry: Cryo-electron microscopy explained

https://www.youtube.com/watch?v=026rzTXb1zw (4:59)

1. How did 1962 Nobel Prize winner in chemistry, Max Perutz, describe x-ray crystallography?

   Answer: structural analysis

2. In x-ray crystallography, what is the diffraction pattern a result of?

   Answer: the splitting of x-ray beams by the atoms in a crystal

3. What does the Bragg's law equation do?

   Answer: provides a mathematical relationship between the spots in a diffraction pattern and the specific arrangement of atoms in a crystal

4. What is the most famous result of x-ray crystallography?

   Answer: the determination of the DNA double helix structure

5. What is a biological example of an application of crystallography?

   Answer: understanding how our immune system fights off viruses

6. What is an 'out of this world' example of an application of crystallography?

   Answer: Curiosity Rover performing x-ray analysis of soil on Mars

7. For x-ray crystallography to work, what is required of the proteins?

   Answer: that they are packed together into a stable, organized crystal

8. Why is it so challenging to get some proteins to crystallize?

   Answer: because they are too floppy or wiggly

9. What are the basic steps involved in determining a 3D protein structure?

   Answer: collect images → select specific protein images → process data → build 3D model

10. How is the sample used in x-ray crystallography different from the sample used in cryo-electron microscopy?

    Answer: x-ray crystallography requires a highly ordered sample while cryo-electron microscopy requires a random arrangement of molecules suspended in fluid that has been flash frozen

11. How does the computer determine a 3D structure from 2D images?

    Answer: it sums selected images from specific orientations and then computationally combines the sum of many orientations into a three-dimensional image

12. What are some advantages of cryo-electron microscopy over x-ray crystallography in determining the three-dimensional structure of proteins?

    Answer: proteins do not need to be crystallized, proteins can remain inside their membranes which helps them to maintain their correct biological structure

13. What is vitrified water and why is it used for cryo-electron microscopy?

    Answer: glass-like and randomly ordered rather than crystal like and ordered; the crystalline pattern of frozen water interferes with the electron microscope images

Read the following article and complete the accompanying worksheet

Title of article: Membrane protein structures without crystals, by single particle electron cryomicroscopy

Author(s): Kutti R Vinothkumar

Date: 2015

Type of Article: Opinion

Name of Scientific Journal: Opinions in Structural Biology

1. What are the two biggest advantages of cryo-electron microscopy?

   Answer: don't need a large amount of protein; don't need to crystallize a protein

2. What are three specific processes or tools that allowed cryo-electron microscopy to rapidly expand to become the important field it is today?

   Answer: the development of rapid freezing of specimens in thin, aqueous films, the introduction of field emission guns, the development of better vacuums

3. How do scientists get around the poor signal to noise ratio obtained by the limited electron dose requirement to minimize radiation damage to the crystal?

   Answer: they average many different projections

4. Since many membrane proteins are comprised of multiple subunits or domains that can dissociate during freezing, it can be advantageous to combine what kind of images?

   Answer: high electron dose images with low electron dose images; high electron dose images offer a high signal to noise ratio that can allow specific features to be clearly visible.

5. What necessary component is a difficulty for both x-ray crystallography and cryo-electron microscopy?

   Answer: choice of detergent and concentration of detergent can both add to the background noise and affect a membrane protein's ability to remain in their native conformation.

6. What are two added difficulties that membrane proteins present to imaging?

   Answer: they are dynamic and can exhibit multiple conformations in solution

7. What are some current limitations to cryo-electron microscopy?

   Answer: Proteins smaller than 64 kDaltons cannot be oriented reliably; as the size of the molecule decreases, the difficulty in obtaining high-resolution structures increases; also membrane proteins that are structurally heterogeneous such as the rotary ATPase's may require many more particles and may still only give lower resolution maps

8. In your own words, what is meant by the term ‘map’ used throughout this article?

   Answer: Answers may vary; an electron density topography image of the molecule in being observed

9. Do you think that x-ray crystallography will become obsolete? Explain.

   Answer: Answers may vary; probably not, at least until the problem of good resolution at low dosage remains, they could complement each other and be used to verify structures or fill in structural gaps that one method cannot adequately image

10. Why is it so important to be able to determine the three-dimensional structure of a protein?

    Answer: knowing the location and structure of pockets and indentations in proteins may allow researchers to develop drugs that could fit and, therefore, alter protein function in a way that benefits an individual or group of individuals.

Lesson 6: Coming to Grips with CRISPR

LESSON 6

TITLE: Coming To Grips with CRISPR

KEY QUESTION(S):

What are common applications for CRISPR technology?

What are the necessary components to activate a Cas-9 Complex?

Explain how gene therapy using CRISPR can eliminate a mutation from a genome.

***SCIENCE SUBJECT:** Biology (Honors and or AP), Biotechnology

***GRADE AND ABILITY LEVEL:**

Activity 1: 9-12, may be scaffolded for different levels of students

Activity 2: 9-12, This activity requires some synthesis of old information and higher order thinking skills so it would work better for an honors/AP level student, scaffolding may be required for lower level students Honors 9th grade Biology, AP Biology or Biotechnology Students.

SCIENCE CONCEPTS: Making inferences, Justifying a claim with data, biotechnology, CRISPR technology, Gene therapy techniques

OVERALL TIME ESTIMATE:

Part 1: The CRISPR webquest handout (1 - 50 min period)

Part 2: Reading the Scientific Article - making a claim and writing a paragraph justification of the claim (30 min)

LEARNING STYLES:

Part 1 (Webquest): Visual & auditory Activity 2 (Justification Paragraph): Visual

LESSON VOCABULARY:

CRISPR	CRISPR-cas9 Complex	Genome
Endonuclease	Gene Therapy	

LESSON SUMMARY:

In this lesson, students will go on a webquest watching small video clips that give a thorough explanation of the technology as well as journey through an HHMI Biointeractive simulation of CRISPR. They will see CRISPR actually read and open a piece of DNA, cut it and then add the new gene sequence to the strand in a computer simulation. Then they will choose different scientists video clips to see the many applications it is currently being used for. To close our Case Study the students will be given a recent scientific article where CRISPR is being tested on Cystic Fibrosis genes. The scientific Article is very high level reading - teachers may choose to forgo this step or help the students through the context of the article - AP Students should be able to gleen important data from the information and make a claim whether this technology should be used to help the CF baby in our Case. They will need to justify their claim with supporting information from the article.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The Students will

Be able to explain how gene therapy can correct mutations and change phenotypic expression of a gene.

Recognize the importance of justifying a claim with evidence from scientific sources.

Understand the steps involved in the CRISPR biotechnology through a computer modeling simulation.

Distinguish different applications of the same technology in science.

NEXT GENERATION SUNSHINE STATE STANDARDS:

SC.912.L.16.4 **SC.912.N.1.7** **SC.912.N.3.5** **SC.912.N.4.1**

NEXT GENERATION SCIENCE STANDARDS (http://www.nextgenscience.org/next-generation-science-standards)

HS-LS 1-2 HS-LS 3-3 HS-LS 3-4

CrossCutting Concepts: Systems and Modeling, Cause and Effect, Structure and Function

Nature of Science Connection: Scientific Investigations use a variety of methods, Science is a human endeavor

COMMON CORE STANDARDS (http://www.corestandards.org/)

AP BIOLOGY CURRICULUM FRAMEWORK

3.A.1 **3.B.1** **3.C.1**

MATERIALS:

- **Students will need access to the internet and headphones to listen to videos on a device.**
- **Copies of Webquest with the Scientific Article attached to the back. (a copy per student)**

BACKGROUND INFORMATION:

It is important to keep up with current science, especially in biology. The field of Biotechnology is full of complex procedures and techniques that are ever changing. CRISPR is rising to the top of the pack - leading the way in genetic correction and manipulation. ***CRISPR*** (clustered regularly interspaced short palindromic repeats) is a genome editing tool that contains a short piece of DNA with repeating base sequences. Originally it was discovered from studying a bacterial immune defense system (the bacteria would use it to cut up DNA of invading organisms). When scientists combined it with a protein (cas9) it become a powerful enzyme called the ***CRISPR-cas9 Complex***. Together it acts as an ***endonuclease*** when activated, its role is to slice DNA with precision and introduce a corrected or a new form of a gene into the ***genome*** (an entire set of genes for an organism). It has been so successful in the lab it now has integrated into the ***gene therapy*** world as the mechanism for inserting and changing genes within cells to help people with genetic disorders or even cancer mutations.

ADVANCE PREPARATION:

- Make sets of handouts for each student
- Make sure they bring headphones to listen to the videos
- Ensure every student has access to the internet to complete the webquest

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. **Give each student the handouts - the webquest and the scientific article attached together.**
2. **Discussion Question: What kind of technology might help this baby with CF?**
3. **Read the first paragraph with the students until you get to the "Mission" of the assignment (5 min)**
4. **Once they have their assignment, they proceed individually through the webquest. (45 min)**

5. **The final question is about the article, this makes an excellent solo homework assignment (30-45 min) OR if time allows have them read the article that night and the next day put the students into small 2-3 person groups and have them discuss ideas for a claim together citing information from what they have read. As a group they come up with a paragraph to tell the parents with their justifications.**

ASSESSMENT SUGGESTIONS:

The major assessment for this assignment comes from the 10 questions about the steps of the CRISPR technology more than the facts learned from the video clips. Those 10 questions should hold more weight in the grading process.

This should be weighed heavily in the assessment process of this assignment. The justification paragraph is on its own a very important assessment tool to a students ability to interpret scientific data and analyze someone else's research. To Justify the student must find supportive evidence to their claim that "shows they are right". A strong answer for this question will include valid information from the article that was interpreted by the student correctly and supports their claim. This evidence can not be opinion or emotionally based, only from the actual experiment in the research and the results. Interpretation of the results is subjective at times by the students - you may find they end up on both sides of whether or not to use the technology - this leads into excellent class discussions about what to do next. We highly recommend you take the time to allow those discussions to occur to challenge the "justifications" being made - it will strengthen a students ability to support or refute claims in future assignments.

EXTENSIONS:

ACTIVITIES:

LITERATURE: Article about CRISPR as a possible treatment for CFTR https://www.ncbi.nlm.nih.gov/pubmed/24315439/

RESOURCES/REFERENCES:

CRISPR and How It Works Video Clip https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr

Biologists explaining CRISPR to 5 different levels of students from Child to doctorate

https://youtu.be/sweN8d4_MUg

Interactive about CRISPR – animation of how it works – 20 different scientist clips that support how it is used.

http://media.hhmi.org/biointeractive/click/CRISPR/?_ga=2.126412680.402022488.1529892175-722829706.1529510601

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https://www.google.com/search?rlz=1C1CHXU_enUS655US655&biw=1242&bih=577&tbs=sur%3Afmc&tbm=isch&sa=1&ei=BmcyW4KxJYr3zgLnwaLABA&q=DNA+scissors&oq=DNA+scissors&gs_l=img.3..0l2j0i30k1j0i5i30k1j0i8i30k1j0i24k1l5.17262.20416.0.20771.14.10.0.0.0.0.167.852.8j2.10.0....0...1c.1.64.img..8.6.564...0i7i30k1j0i67k1j0i8i7i30k1.0.Wy6pJsttvrE

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TEACHER PAGES:

Student Handout: Coming to Grips with CRISPR

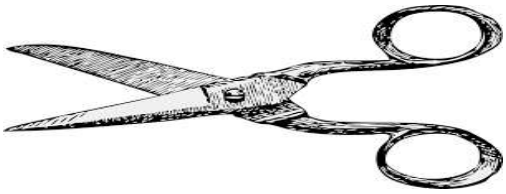
Scientific Article *Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients (PDF)*

Teacher Key: Coming to Grips with CRISPR

Student Handout: Coming to Grips with CRISPR

Purpose: The purpose of this activity is to introduce the CRISPR technology; understand how it works and how scientists are trying to apply it in science.

Our parents of the CF baby are desperately searching for treatments that could reduce or eliminate the symptoms associated with CF. Using biotechnology to solve scientific problems is part of ground breaking science. A biotechnology that is on the rise as a molecular solution for many genetic issues is CRISPR. With this technology we have created a simple, powerful tool for editing genomes. Scientists can use it to easily alter DNA sequences modifying a gene's function – or lack of function. The protein Cas9 (or "**CRISPR**-associated") is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA.



Your mission is to gather information on this biotechnology and help the parents make an informed decision about using it by understanding more about CRISPR.

Click all three links to help understand the basics of CRISPR technology…

Link 1: CRISPR What is it and how it works

Link 2: Biologist Explaining CRISPR to 5 different levels of Students - Child to Doctorate

Link 3: UF CRISPR Research in the Labs

What did you learn?

1. Where did the CRISPR technology come from?
2. How does CRISPR-cas9 Complex compare to other genetic editing tools?

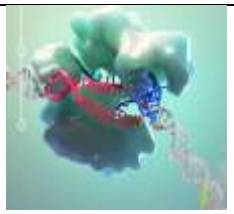
3. What are some ways people are using CRISPR?
4. Explain in your own words a simple way to understand what CRISPR is and how it works.

Now that you have a basic understanding, let's dive into the process itself.

Visit this website: HHMI BIOINTERACTIVE about CRISPR and How it Works

Click on HOW IT WORKS first and work your way through the 4 steps involved - answer the following questions and fill in the chart as you go.

Complete each step in the chart below and identify what is happening.

STEPS	NAME OF THE STEP		In *YOUR OWN WORDS*, ***DESCRIBE*** what happens in each step
1			
2			
3			

4			

Who are the "Players" in the process? - Use the HHMI interactive website to answer the following questions for each step.

Step 1:

1. What does the acronym ***CRISPR*** stand for?
2. CRISPR associated protein 9 (Cas 9) is an ***endonuclease*** - break this word down into its component parts (endo)= (nucle)= (ase) =

   What do you think the meaning of endonuclease is?

3. What is the role of the ***Cas 9 Guide RNA complex***?
4. It targets 3 nucleotide sequences called _____ that are found in the ***genome***.

Step 2:

5. During DNA replication and the central dogma you learned about other enzymes found in

   the nucleus. Compare the role of endonuclease to one of these other enzymes - name the enzyme and justify why you made the comparison.

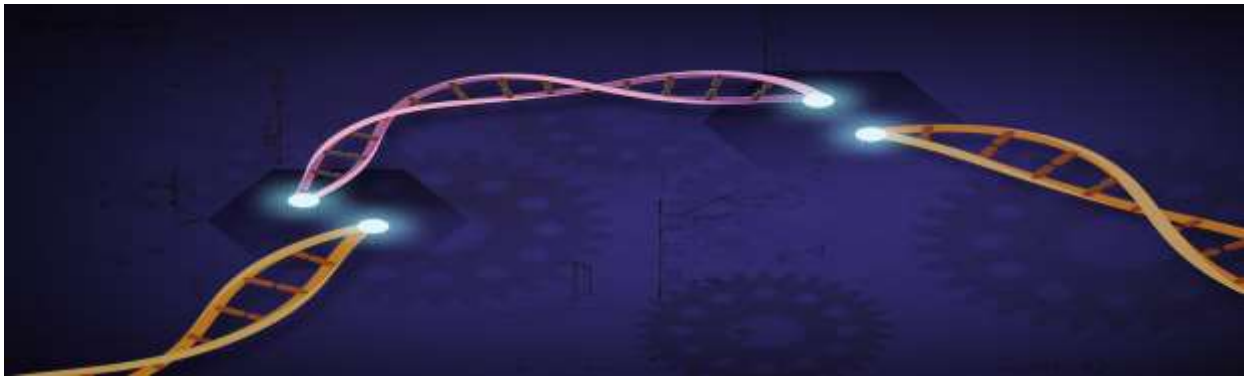
6. What are the components of a ***PAM*** unit?

Step 3:

7. A cleavage site is activated by what?
8. What happens if the 20 nucleotide bases are not an exact match to the code on the DNA strand?

Step 4:

9. Identify 2 ways CRISPR induced DNA breaks can be repaired.



10. What is the major difficulty in using the faster repair mechanism?

Now that you know how it works. Let's see how it is used…

Click on the ***How It's Used*** link - there are 20 videos each telling their own story about CRISPR technology. Choose 3 video clips that interest you and complete the following…

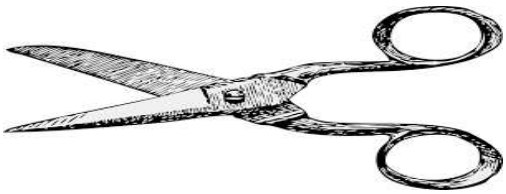
Video Clip Name	Scientist Involved	4 things you learned in the clip
		1. 2. 3. 4.
		1. 2. 3. 4.
		1. 2. 3. 4.

Back to our baby with Cystic Fibrosis. Is this a viable treatment option for this family? None of that research connects CRISPR to Cystic Fibrosis. You still need to know more about how it is used to treat CF. The Genetic Counselor gives you some advice to find out more with the attached article on current research using CRISPR on Cystic Fibrosis. ***Read the article***, ***gather important data points*** (ex. what type of subjects were they testing on, was the data supportive to show successful repair of the protein function, etc) and ***make a claim*** - is it a viable treatment option for our baby? ***JUSTIFY*** your answer from the research and ***cite supporting data*** from the article. ***EXPLAIN*** all of this to the parents in a paragraph including all relevant information to persuade or dissuade them from using this technology, be sure you have accurately cited information to support your claim in your justification from the article.

TEACHER KEY : Coming To Grips with CRISPR

Purpose: The purpose of this activity is to introduce the CRISPR technology; understand how it works and how scientists are trying to apply it in science.

Our parents of the CF baby are desperately searching for treatments that could reduce or eliminate the symptoms associated with CF. Using biotechnology to solve scientific problems is part of ground breaking science. A biotechnology that is on the rise as a molecular solution for many genetic issues is CRISPR. With this technology we have created a simple, powerful tool for editing genomes. Scientists can use it to easily alter DNA sequences modifying a gene's function – or lack of function. The protein Cas9 (or "**CRISPR**-associated") is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA.



Your mission is to gather information on this biotechnology and help the parents make an informed decision by understanding more about CRISPR.

Click all three links to help understand the basics of CRISPR technology…

Link 1: CRISPR What is it and how it works

Link 2: Biologist Explaining CRISPR to 5 different levels of Students - Child to Doctorate

Link 3: UF CRISPR Research in the Labs

What did you learn?

1. Where did the CRISPR technology come from? Archae/bacteria's immune response
2. How does CRISPR-cas9 Complex compare to other genetic editing tools?It is an efficient alternative that also allows targeting more than one sequence at a time.
3. What are some ways people are using CRISPR?It allows us to diagnose, identify and target cancer, quickly create model cells for research, etc
4. Explain in your own words a simple way to understand what CRISPR is and how it works.Accept all logical responses.

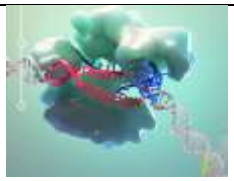
Now that you have a basic understanding, let's dive into the process itself.

Visit this website: HHMI BIOINTERACTIVE about CRISPR and How it Works

Click on HOW IT WORKS first and work your way through the 4 steps involved - answer the following questions and fill in the chart as you go.

Complete each step in the chart below and identify what is happening.

STEPS	NAME OF THE STEP		DESCRIBE what happens in each step
1	Targeting		Scientists introduce the Cas9-guide RNA complex into a cell (in this case, a human cell), where it randomly associates and dissociates with the DNA. Cas9 recognizes and binds to a three-nucleotide sequence motif called PAM that is abundant throughout the genome. **(this should be in student's own words)**

2	Binding		Once it binds to a PAM motif, Cas9 unwinds the DNA double helix. If the DNA at that location perfectly matches a sequence of about 20 nucleotides within the guide RNA, the DNA and matching RNA will bind through complementary base pairing.
3	Cleaving		The DNA-RNA pairing triggers Cas9 to change its three-dimensional structure and activates its nuclease activity. Cas9 cleaves both DNA strands at a site upstream of PAM.
4	DNA Repair		Cells contain enzymes that repair double-stranded DNA breaks. The repair process is naturally error-prone and will lead to mutations that may inactivate a gene. Cleaving DNA at a precise location is one of many applications of the CRISPR-Cas9 technology.

Who are the "Players" in the process? - Use the HHMI interactive website to answer the following questions for each step.

Step 1:

1. What does the acronym ***CRISPR*** stand for?*Clustered Regularly Interspaced Short Palindromic Repeats*

2. CRISPR associated protein 9 (Cas 9) is an ***endonuclease*** - break this word down into its component parts
(endo)= inside (nucle)= nucleus (ase) = enzyme

What do you think the meaning of endonuclease is?an enzyme that stays in the nucleus and cleaves nucleic acids in specific locations

3. What is the role of the ***Cas 9 Guide RNA complex***?to randomly associate with the DNA looking for a PAM to bind with and activate its cleavage site

4. It targets 3 nucleotide sequences called PAMs that are found in the ***genome***.

Step 2:

5. During DNA replication and the central dogma you learned about other enzymes found in

the nucleus. Compare the role of endonuclease to one of these other enzymes - name the enzyme and justify why you made the comparison. DNA Helicase because it unzips the DNA will work here, but RNA Polymerase because it unzips and reads the code then rezips the DNA is a stronger answer. Be sure they have a justification.

6. What are the components of a ***PAM*** unit? 5'- (1 of any of the 4 Nitrogen bases), followed by 2 Guanines - 3' (5'-(N)-G-G-3')

Step 3:

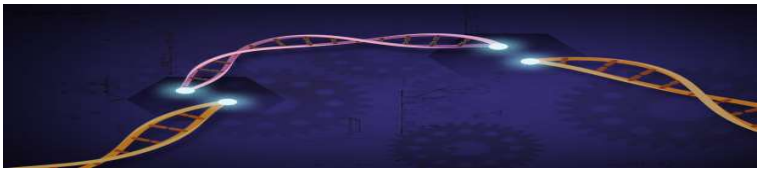
7. A cleavage site is activated by what?The DNA nucleotide bases pairing with the Cas9's target RNA code exactly.

8. What happens if the 20 nucleotide bases are not an exact match to the code on the DNA strand?Cas9 disengages from the DNA, which zips back up into a double helix.Cas9 disengages from the DNA, which zips back up into a double helix.

Step 4:

9. Identify 2 ways CRISPR induced DNA breaks can be repaired. CRISPR-induced double-stranded DNA breaks can be repaired by either nonhomologous end joining (NHEJ) or homology-directed repair (HDR)

10. What is the major difficulty in using the faster repair mechanism? NHEJ is the more frequently used, faster repair mechanism, because the cell does not use a template to join broken DNA ends together. It is, however, an error-prone process that can introduce mutations in the target sequence.



Now that you know how it works. Let's see how it is used…

Click on the ***How It's Used*** link - there are 20 videos each telling their own story about CRISPR technology. Choose 3 video clips that interest you and complete the following…

Answers to this section will vary depending on the video clip and the points they focused on

accept all logical responses that might connect to the "clip name" description.

Video Clip Name	Scientist Involved	4 things you learned in the clip
		1. 2. 3. 4.
		1. 2. 3. 4.
		1. 2.

		3. 4.

Now back to our baby with Cystic Fibrosis. Is this a viable treatment option for this family? None of that research connects CRISPR to Cystic Fibrosis You still need to know more about how it is used to treat CF. The Genetic Counselor gives you some advice and the attached article on current research using CRISPR on Cystic Fibrosis. Read the article, gather important data points (ex. what type of subjects were they testing on, was the data supportive to show successful repair of the protein function, etc) and make a decision - is it a viable option for treatment of our baby? JUSTIFY your answer from the research and cite supporting data from the article. Write a paragraph to the parents with all of this information in it to persuade or dissuade them from using this technology, be sure you have accurate justification cited from the article in your paragraph.

To Justify the student must find supportive evidence to their claim that "shows they are right". A strong answer for this question will include valid information from the article that was interpreted by the student correctly and supports their claim. This evidence can not be opinion or emotionally based, only from the actual experiment in the research and the results. Interpretation of the results is subjective at times by the students - you may find they end up on both sides of whether or not to use the technology - this leads into excellent class discussions about what to do next. We highly recommend you take the time to allow those discussions to occur to challenge the "justifications" being made - it will strengthen a students ability to support or refute claims in future assignments.

Actual URLs for the hyperlinks in the webquest.

CRISPR and How It Works Video Clip https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr

Biologists explaining CRISPR to 5 different levels of students from Child to doctorate

https://youtu.be/sweN8d4_MUg

Article about CRISPR as a possible treatment for CFTR

https://www.ncbi.nlm.nih.gov/pubmed/24315439/

Interactive about CRISPR – animation of how it works – 20 different scientist clips that support how it is used.

http://media.hhmi.org/biointeractive/click/CRISPR/?_ga=2.126412680.402022488.1529892175-722829706.1529510601

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Curriculum Pre-Test/Post Test

The Salty Enigma: Pretest / Post-test

1. Which of the following are ways in which a genetic counselor might help a patient?

    A. recommend a couple don't have biological children if they are carriers of a genetic disease

    B. recommend a specific treatment

C. require a specific genetic test

D. provide the information necessary for a patient to make an informed decision

2. Which of the following are ***NOT*** symptoms of cystic fibrosis?

A. Weakness and shortness of breath

B. persistent coughing and constipation

C. frequent respiratory infections and delayed puberty

D. All of the above

E. None of the above

3. Cystic fibrosis is

A. homozygous dominant

B. homozygous recessive

C. heterozygous dominant

D. heterozygous recessive

4. A segment of DNA containing short repetitions of base sequences involved in the defense mechanisms of prokaryotic organisms against viruses and can be used as a genetic engineering tool is called _____.

A. an endonuclease

B. a secondary messenger

C. CRISPR-cas9

D. a ligand

5. Siemens is ____

A. a derived SI unit for conductivity

B. part of the protein kinase pathway

C. a specific kind of gene therapy that can be used for cystic fibrosis

D. a measure of standard deviation

6. Membrane proteins are difficult to crystallize for all of the reasons below ***except:***

A. they are wiggly

B. they must remain in their lipid layer to retain their native conformation

C. they are so big

D. they are not very soluble

7. Two advantages to cryo-electron microscopy in imaging membrane proteins are:

A. they don't need to be frozen and they don't need to be crystallized

B. they don't need to be crystallized and smaller quantities can be used

C. they don't need to be purified and they don't need to

8, From the perspective of the cell receiving the message, the three stages of cell signaling are…

A. Signal reception, signal transduction and cellular response
B. Signal reception, nucleus disintegration, and new cell generation.
C. The alpha helix, beta helix and q helix
D. Signal reception, cellular response, and cell division.

9. A small molecule that specifically binds to another molecule, usually a larger one…

A. Is called a signal transducer
B. Is called a ligand
C. Seldom is involved in hormone signaling
D. Usually terminates a signal reception.

10. Of the following, a receptor protein in a membrane that recognizes a chemical signal is most similar to …

A. The active site of an alloteric enyme in the cytoplasm that binds to a specific substrate.
B. The RNA specifying the amino acids in a polypeptide.
C. An enzyme with an optimum pH and temperature for activity.

D. Genes making up a chromosome.

11. In general, a signal transmitted via phosphorylation of a series of proteins

A. Brings a conformational change to each protein.
B. Requires binding of a hormone to a cytosol receptor.
C. Requires phosphorylase activity.
D. Allows target cells to change their shape and therefore their activity.

12. Binding of a signaling molecule to which type of receptor leads directly to a change in distribution of ions on opposite sides of the membrane?

A. Receptor tyrosine kinase
B. G-Protein coupled receptor
C. Ligand-gated ion channel
D. Intracellular receptor

Curriculum Evaluations

Content Area Expert Evaluation of "The Salty Enigma"

Thank you for taking the time to evaluate this lesson plan. Please enter your comments below. Expert criticism is always welcome to ensure the science is accurate and current.

Content Area Expert Evaluator Name): _____

Job Title: _____

Questions Regarding the Curriculum
General Comments Regarding the Curriculum

Questions Regarding Each Specific Lesson
Lesson 1: The Genetic Counselor
Lesson 2: CFTR Integral Proteins – What are they and how do they work?
Lesson 3: Sweat It Out!
Lesson 4: Blot It "Western" style
Lesson 5: Battle of the Super Powers (EM vs X-Ray Crystallography)
Lesson 6: Coming to Grips with CRISPR

Educator Feedback Form

Thank you for taking the time to evaluate and provide feedback on this lesson plan. Please enter your comments below. Expert criticism is always welcome to ensure the most efficient use of classroom time maximizing learning..

Educator (Name): _____

Courses Taught: _____

Please Provide a Brief Review of the Overall Curriculum
Feedback Regarding the Curriculum Guide Itself

Your Comments Regarding Each Specific Lesson
Lesson 1: The Genetic Counselor
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Student Feedback Form

Thank you for taking the time to evaluate and provide feedback on this lesson plan. Please enter your comments below. Criticism from the target audience is always welcome to help improve the value of this lesson..

Student's Name: _____

Teacher's Name: _____

EVALUATIVE QUESTIONS ABOUT EACH LESSON
LESSON 1: Visiting a Genetic Counselor. 1. Was the lesson easy to understand? 2. Did role playing the roles of the counselor and parents engage you into this lesson? 3. Did the videos help pull this information together better for you? 4. What was your favorite part of this lesson? 5. What was your least favorite pat? (Comment on your responses in the next section)
LESSON 2: CFTR Proteins – What they are and how they work 1. Did folding the origami model of the protein give you a good visual to understand how the protein makes conformational changes? 2. Were the instructions for forming the model clear? 3. Was the Prezi easy to follow? 4. Did the labeling activity help you put all the steps of cell signaling together?
LESSON 3: Sweat It Out! 1. How difficult was it to build the sweat conductor? 2. Did the instructional videos help in the process? 3. Were you able to connect this to the lesson about Cystic Fibrosis?
LESSON 4: Blot it Out "Western" Style 1. How easy were the procedures to follow? 2. Were you able to get good results? 3. Could you connect this lab to our lesson story about the baby?
LESSON 5: Battle of the Superpowers – EM vs X-Ray Crystallography 1. Did this lesson help you see different ways to see and create protein structures? 2. Were the videos helpful in understanding the different technologies? 3. How difficult was the journal article to read?
LESSON 6: Coming To Grips with CRISPR 1. How easy was the HHMI BioInteractive website to use? 2. Did the animations and explanations help give you a clearer understanding of how CRISPR works? 3. Were the videos helpful in seeing how this technology has MANY different applications?

Your Comments Good and Bad Regarding Each Specific Lesson
Lesson 1: The Genetic Counselor
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