Epigenetics: The Biochemistry of DNA

Beyond the Central Dogma

Since the Human Genome Project was completed, the newly emerging field of epigenetics is providing a basis for understanding how heritable changes, other than those in the DNA sequence, can influence phenotypic variation. Epigenetics, essentially, affects how genes are read by cells and subsequently how they produce proteins. The interest in epigenetics has led to new findings about the relationship between epigenetic changes and a host of disorders including cancer, immune disorders, and neuropsychiatric disorders. The field of epigenetics is quickly growing and with it the understanding that both the environment and lifestyle choices can directly interact with the genome to influence epigenetic change. This unit is designed to provide a detailed look at the influence of epigenetics on gene expression through developmental stages, tissue-specific needs, and environmental impacts. Students will complete this unit with the understanding that gene regulation and expression is truly "above the genome" and well beyond the simplified mechanism of the Central Dogma of Biology.

University of Florida Center for Precollegiate Education and Training

EPIGENETICS: THE BIOCHEMISTRY OF DNA

UF Center for Precollegiate Education and Training
UNIVERSITY of FLORIDA

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This curriculum was developed in the laboratory of:

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The focus of our laboratory is to analyze mechanisms regulating gene expression during erythroid cell differentiation. The beta-globin genes are regulated by a locus control region (LCR). The LCR is composed of several DNase I hypersensitive (HS) sites that together mediate chromatin structure alterations and high-level transcription throughout erythroid development. The human beta-globin gene locus consists of five genes that are expressed in a developmental stage specific manner in erythroid cells. During development the different proteins encoded by the beta-globin gene locus (ε, Aγ, Gγ, δ, and βglobin) dimerize with a-globin subunits to form hemoglobin. The beta-type globin genes are expressed at extremely high levels in erythroid cells which is mediated by the LCR.

Results from our previous work suggest that the individual LCR HS elements interact to generate a higher order structure, referred to as the LCR holocomplex, and that this complex communicates in a stage-specific manner with individual globin genes. We also found that the LCR recruits transcription complexes and proposed that the LCR serves as the primary site of transcription complex recruitment and assembly in the beta-globin gene locus. We use transgenic mice and cell culture to identify and functionally characterize cis-regulatory DNA elements and trans-acting components involved in the regulation of the beta-globin genes. We utilize artificial DNA binding domains to modulate and characterize the function of transcription factor binding sites in the beta-globin gene locus. We also use a variety of molecular techniques, including chromatin immunoprecipitation (ChIP), ChIP-sequencing, shRNA mediated knockdown, and overexpression of dominant negative transcription factors to analyze transcription factor function and globin gene regulation.

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AUTHOR'S NOTE

Summer-session professional development is the time I use to recharge and reenergize my teaching. Through attendance at university-based PD programs, I am able to bring real-world science back to share with my students, learn new skills to share with them, make connections with fellow like-minded educators from around the state, and just have fun learning.

The driving force for this curriculum model was my desire to learn more about the epigenome. I teach an Honors Genetics class to juniors and seniors and recognize that my epigenetics unit was lacking in content and depth of knowledge. My genetics courses as a college student were delivered just prior to the inception of the Human Genome Project and long before epigenetics was a major focus of research. I have done a cursory unit on epigenetic influence and current research in epigenetics but did not feel my unit was thorough/complete. I was fortunate to be placed in an epigenetics laboratory at the University of Florida, Cancer and Genetics Research Center in the Summer of 2016.

Dr. Bungert and his staff are studying the mechanisms for gene expression of the beta-globin gene. Although this lab was not a translational lab, not actively searching for a treatment, the research performed in the lab could eventually be used to develop treatment for a variety of hemoglobin disorders, such as sickle cell disease and beta thalassemia. By using the beta-globin gene as a model of gene expression, I was introduced to the world of the epigenome and the true intricacies of how gene expression really occurs. It is so much more than the Central Dogma!

INTRODUCTION

This curriculum model is designed as a full learning unit on Epigenetics. Students should have solid foundational understanding of the Central Dogma, the impact of base pair mutations, and how mutations impact the folding/misfolding of proteins before embarking on the study of the Epigenome in order to fully grasp the control of epigentics on gene expression.

This module does not use a single disease or disorder as the focal point of the learning module; current research is demonstrating a better understanding of the impact on the epigenome on a variety of conditions – cancer, autism, anxiety/post-traumatic stress disorder, schizophrenia, and even sexual orientation. My hope is, as students move through the lessons, they build a better understanding of gene expression that is much more than just transcription and translation of a sequence of base pairs. The impact of development, the environment, experience, and heredity all play important roles in the expression of specific genes. Research is showing that epigenetics can also be the target for new treatments for a variety of diseases that plague humans today.

TIPS ABOUT THIS CURRICULUM

Science Subject: Honors Genetics; level also appropriate for AP and IB Biology

Grade and ability level: 10-12 students in advanced biology

Science concepts: chemical structure and organization of DNA in eukaryotic cells, control of gene expression, translation of mRNA and protein folding, specificity of tissue function in multicellular organisms.

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

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.

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

STANDARDS: Indicates the NGSSS covered in each lesson.

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

TIME ESTIMATE: Indicates total amount of time needed for the lesson.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Master Vocabulary List also provided.

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.

MATERIALS: Items needed to complete the lesson. Number required for completion of the activity.

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. All references and resources are also collected in one list.

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.

LESSON SUMMARIES

LESSON 1: Organization of Eukaryotic DNA

Students will review the organization of eukaryotic DNA as a mechanism of stability to ensure genetic material is organized and condensed to fit inside the nucleus of the cell. This organizational pattern is essential in understanding the machinery of gene expression as not all DNA is easily accessible for transcription. The DNA-Histone paper modeling activity will provide a hands-on experience in understanding the coiling/uncoiling ability of DNA and histone interaction and briefly introduce methylation and acetylation as a driving force of chromatin organization. To save class time, a Flipped homework assignment to view videos as an introduction to epigenetics will prepare students for the vocabulary and specifics of the epigenome.

LESSON 2: Introduction to Epigenetics and Gene Expression

Working in groups, students will review vocabulary from the video Flipped homework assignment and perform a Word Sort to reinforce the vocabulary and components of epigenetic influence. This activity will provide a "quick look" opportunity for teachers to assess understanding, clarify, and correct misconceptions. Level of student understanding will determine when the teacher progresses to the next phase of the lesson which provides specific details on eukaryotic gene expressions and epigenetics, delivered in a traditional lecture format. The lecture will provide opportunities for understanding through outlines of information, opportunities for diagraming and modeling using the beta-gene locus as an example, and teacher-guided questions and discussion. The final activity is a web interactive on gene control; this lesson can be done in-class (time permitting) or as a final homework assignment.

LESSON 3: Tissue Specific Gene Expression

Students describe developmental changes in organisms and the influence of the epigenome on transcription of necessary proteins for different phases of growth and development. Small group discussion and white board presentation of findings will highlight tissue-specific and developmental-specific gene expression. An in-class and homework assignment from the text will allow students to explore Bioinformatics to learn more about gene expression; *Exploring the Genome – Tissue-Specific Gene Expression*. This is a reading activity and web-based tutorial.

LESSON 4: Epigenetics, Disease, and Therapy

Students will understand more about the impact of epigenetic influence in the progression of disease and approach to therapy by performing a jigsaw activity using a basic science journal article that introduces the link between epigenetic influence and disease. Student groups are provided a copy of the article that has been broken down into segments to perform a jigsaw activity for presentation. This will be followed with a guide to reading primary source journal articles on epigenetic treatment of disease using the high-interest topics of schizophrenia, cancer, anxiety and PTSD, and sexual orientation. Students will select 2 to read as a homework assignment and complete a guide to reading the articles to understand the research presented.

LESSON 5: Epigenetic Impact of Cancer Therapy

Students will model how scientists use DNA microarrays to determine levels of gene expression in breast cancer patients. Provided with patient history, commonly used drugs to treat breast cancer, and drug interactions and limitations based on specific gene expression, students will determine the best (and worst) course of action for treating each patient and present their findings in a laboratory report.

LESSON SEQUENCING GUIDE

Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum.

45 minute periods

	Day 1	Day 2	Day 3	Day 4	Day 5
Week 1	Lesson 1 Organization of Eukaryotic DNA *45 minutes*	Lesson 2 Introduction to Epigenetics and Gene Expression *45 minutes*	Lesson 2 Introduction to Epigenetics and Gene Expression *45 minutes*	Lesson 3 Tissue Specific Gene Expression 45 minutes	Lesson 3 Tissue Specific Gene Expression 45 minutes
H/W	Intro to Epigenetics	Work ahead Review Ppoint	Gene Control	Exploring Genomics	Exploring Genomics
Week 2	Lesson 4 Epigenetics, Disease, and Therapy *45 minutes*	Lesson 4 Epigenetics, Disease, and Therapy *45 minutes*	Lesson 5 Epigenetic Impact of Cancer Therapy *45 minutes*	Lesson 5 Epigenetic Impact of Cancer Therapy *45 minutes*	
H/W	Journal Read	Laboratory Preparation		Study	

VOCABULARY

Acetylation: A process that adds an acetyl group to DNA that removes the positive charge on the histone proteins and loosens the interaction between DNA and histones to increase the availability of the gene to transcription factors.

c-DNA: Complimentary DA is a double-stranded DNA synthesized from a single stranded RNA template in a reaction catalyzed by reverse transcriptase; often used to make a copy of eukaryotic genes in prokaryotes.

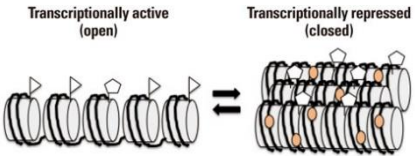
Chemotherapy drug: Any chemical used to treat disease; the chemical agents are selectively toxic to rapidly dividing cells and tissues, specifically for cancer.

Chromatin: The complex of DNA, RNA, histones, and non-histone proteins that make up uncoiled chromosomes, characteristic of the eukaryotic interphase nucleus.

Chromatin remodeling: A process in which the structure of chromatin is altered by a protein complex that results in a change in the transcriptional state of genes in an altered region.

***Cis*-regulatory:** AKA cis-acting; A non-coding DNA sequence that regulates the expression of nearby genes located on the same chromosome; often acts as binding sites for transcription factors; located upstream of gene for transcription.

Closed chromatin: AKA heterochromatin; the heavily staining, late-replicating regions of chromosomes that are prematurely condensed in interphase. DNA sequences are difficult to access for transcription by mRNA.



CpG islands: Areas of ~200 base pairs with greater than 50% GC; associated with promoter regions of transcriptionally active genes.

Differentiation: A process where a cell changes from one cell type to another during cell growth; it occurs numerous times during the development of a multicellular organism.

DNA: Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

Downstream: Toward the 3' end of the gene/chromosome.

Enhancer: A DNA sequence that enhances transcription and the expression of structural genes; can act over a distance of thousands of base pairs and can be located upstream, downstream, or internal to the gene they affect.

Enzyme: Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in biological cells need enzymes in order to occur at rates sufficient for life.

Epigenetics: The study of modification in an organism's gene function or phenotypic expression that are not attributable to alterations in the nucleotide sequence (mutations) of the organism's DNA.

Fluorescent tags: Also known as a label or probe; a molecule that is attached chemically to aid in the labeling and detection of a biomolecule.

Genotype: The genotype is the genetic makeup of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

Histone: Positively charged proteins complexed with DNA in the nucleus; rich in the basic amino acids arginine and lysine, and function in coiling DNA to form nucleosomes.

Histone code: Various chemical modifications applied to histone tails; influence DNA-histone interactions and promote or repress transcription.

Hormone (therapy): (HT) refers to the use of a hormone or combination of hormones as a therapeutic approach to treating cancer; specifically breast cancer that expresses receptors for hormones.

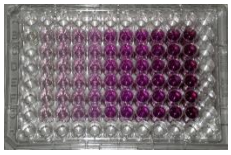
Hybridization: A fragment of DNA or RNA of variable length that can tagged/labeled to detect the presence of nucleotide sequences in a given sample for detection.

Locus/Loci: The site or place on a chromosome where a particular gene is located.

Locus Control Region (LCR): A long-range *cis*-regulatory element that enhances expression of linked genes at distal chromatin sites. It functions in a copy number-dependent manner and is tissue-specific, as seen in the selective expression of β-globin genes in erythroid cells.

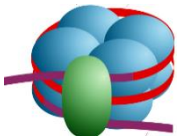
Methylation: Enzymatic transfer of methyl groups to DNA; associated with gene expression and with epigenetic phenomena.

Microarray: Used in quantitative assays of DNA-DNA or DNA-RNA binding to measure profiles of gene expression, often to compare gene expression between normal and abnormal (cancer) cells.



Monoclonal Antibody: specific antibodies that are made as a possible treatment for cancer; these antibodies will bind only to cancer cell-specific antigens and induce an immune response against the target cancer cells.

Nucleosome: In eukaryotes, a nuclear complex consisting of four pairs of histone molecules wrapped by two turns of a DNA molecule; the major structure associated with the organization of chromatin in the nucleus.



Open Chromatin: AKA Euchromatin; loose chromatin easily accessible for transcription to occur.

Phenotype: An organism's observable characteristics or traits. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

Promoter: An upstream regulatory region of a gene to which RNA polymerase binds prior to the initiation of transcription.

RNA: Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

RNA polymerase: An enzyme that catalyzes the formation of an RNA polynucleotide strand using the base sequence of a DNA molecule (gene) as a template.

***Trans*-regulatory:** AKA *trans*-acting; a gene product that acts to regulate the expression of a target gene;

Transcription: DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Transcription Factors: Proteins containing DNA-binding abilities which attaches to specific DNA sequences in preparation for transcription of a gene.

Translation: RNA → Protein; In translation, messenger RNA (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.

Upstream: toward the 5' end of the gene/chromosome.

NEXT GENERATION SUNSHINE STATE STANDARDS – SCIENCE

Benchmarks	1	2	3	4	5	6
SC.912.L.14.6 Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.	X	X	X	X	X	X
SC.912.L.16.2 Discuss observed inheritance patterns caused by various modes of inheritance, including dominant, recessive, codominant, sex-linked, polygenic, and multiple alleles.		X				X
SC.912.L.16.3 Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.	X	X	X			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	X		X
SC.912.L.16.5 Explain the basic processes of transcription and translation, and how they result in the expression of genes.	X	X	X			X
SC.912.L.16.6 Discuss the mechanisms for regulation of gene expression in prokaryotes and eukaryotes at transcription and translation level.	X	X	X	X	X	X
SC.912.L.16.9 Explain how and why the genetic code is universal and is common to almost all organisms.	X	X	X	X		X
SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.	X	X		X	X	X
SC.912.L.16.12 Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).				X		X
SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.	X	X	X	X		X
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
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: 1. pose questions about the natural world, 2. conduct systematic observations, 3. examine books and other sources of information to see what is already known, 4. review what is known in light of empirical evidence, 5. plan investigations, 6. use tools to gather, analyze, and interpret data, 7. pose answers, explanations, or descriptions of events, 8. generate explanations that explicate or describe natural phenomena (inferences), 9. use appropriate evidence and reasoning to justify these explanations to others, 10. communicate results of scientific investigations, and 11. evaluate the merits of the explanations produced by others.				X	X	X
5SC.912.N.1.2 Describe and explain what characterizes science and its methods.	X	X		X	X	X
SC.912.N.1.3	X	X		X		X

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.						
SC.912.N.1.5 Describe and provide examples of how similar investigations conducted in many parts of the world result in the same outcome.				X	X	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	X	X	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.2.4 Explain that scientific knowledge is both durable and robust and open to change. Scientific knowledge can change because it is often examined and re-examined by new investigations and scientific argumentation. Because of these frequent examinations, scientific knowledge becomes stronger, leading to its durability.	X	X	X	X		X
SC.912.N.2.5 Describe instances in which scientists' varied backgrounds, talents, interests, and goals influence the inferences and thus the explanations that they make about observations of natural phenomena and describe that competing interpretations (explanations) of scientists are a strength of science as they are a source of new, testable ideas that have the potential to add new evidence to support one or another of the explanations.				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	X	X	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	

BACKGROUND INFORMATION

General background information is given here. More detail is provided in the individual lessons as needed as well in the student information in lesson one.

C.H. Waddington is credited with coining the term epigenetics in the 1940's to describe how environmental influences in developmental events can affect the phenotype of the adult. He showed that environmental alterations during development induced alternative phenotypes in organisms with identical genotypes. Using Drosophila, Waddington found that wing vein patterns could be altered by administering heat shocks during pupal development. Adults with these environmentally induced changes were used to establish strains that showed the alternative phenotype without the need for continued environmental stimulus. He called this phenomenon "genetic assimilation." Interactions between the environment and the genome during certain stages of development produce phenotypic effects that are heritable.

In the 1970s, Holliday and Pugh proposed that changes in programs of gene expression during development may depend on the methylation of specific bases in DNA, and that altering these methylation patterns might affect the resulting phenotype. In addition, the progressive restriction of developmental pathways during differentiation may be explained by a program of methylation that silences gene sets at specific stages of embryogenesis. Several factors, including Waddington's work, the methylation model of Holliday and Pugh, and the discovery that expression of genes from both maternal and paternal genomes is required for normal development all helped set the stage for the birth of epigenetics and epigenomics as fields of research (Klug, 2012)

Since the Human Genome Project was completed, the newly emerging field of epigenetics is providing us with a basis for understanding how heritable changes, other than those *in* the DNA sequence, can influence phenotypic variation. Epigenetics, essentially, affects *how* genes are read by cells, and subsequently how they produce proteins. Currently, DNA methylation is one of the most broadly studied and well-characterized epigenetic modifications. Other major modifications include chromatin remodeling, histone modifications, and non-coding RNA mechanisms. The interest in epigenetics has led to new findings about the relationship between epigenetic changes and a host of disorders including various cancers, mental retardation associated disorders, immune disorders, neuropsychiatric disorders and pediatric disorders.

The field of epigenetics is quickly growing and with it the understanding that both the environment and lifestyle choices can directly interact with the genome to influence epigenetic change. These changes may be reflected at various stages throughout a person's life and even in later generations. For example, epidemiology studies have provided evidence that prenatal and early postnatal environmental factors influence the adult risk of developing various chronic diseases and behavioral disorders. Studies have shown that children born during the period of the Dutch famine from 1944-1945 have increased rates of coronary heart disease and obesity after maternal exposure to famine during early pregnancy compared to those not exposed to famine, associated with less DNA methylation of insulin-like growth factor II gene.

Dr. Bungert's epigenetics lab is analyzing the mechanisms (chromatin remodeling and histone modifications) that regulate gene expression during red cell (eryhthrocycte) differentiation using the MEL (murine erythro-leukemia) cell line as a model system. The beta-globin genes are regulated by a locus control region (LCR) and is composed of several hypersensitive sites that mediate chromatin structure alterations and transcription of appropriate βglobin throughout red cell development. The human beta-globin gene consists of five genes that are expressed in a specific order during different developmental stages in erythrocytes. During development, the different proteins encoded by the beta-globin gene locus (embryonic, fetal, gamma and adult beta) with alpha-globin subunits to

form hemoglobin. For diseases like sickle cell anemia and beta-thalassemia, the hope is by altering the chromatin structure of the beta-globin locus, patients suffering from a hemoglobinopathy could express earlier developmental forms of the beta-globin gene (fetal) to reduce or eliminate symptoms of their condition.

LESSON ONE: ORGANIZATION OF EUKARYOTIC DNA

KEY QUESTION(S): What is the ultrastructure of DNA? How is this organization vital to cell structure and function?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 45-60 minutes + homework time

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

Acetylation: A process that adds an acetyl group to DNA that removes the positive charge on the histone proteins and loosens the interaction between DNA and histones to increase the availability of the gene to transcription factors.

Chromatin: The complex of DNA, RNA, histones, and non-histone proteins that make up uncoiled chromosomes, characteristic of the eukaryotic interphase nucleus.

Chromatin remodeling: A process in which the structure of chromatin is altered by a protein complex that results in a change in the transcriptional state of genes in an altered region.

Closed chromatin: AKA heterochromatin; the heavily staining, late-replicating regions of chromosomes that are prematurely condensed in interphase. DNA sequences are difficult to access for transcription by mRNA.

DNA: Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

Histone: Positively charged proteins complexed with DNA in the nucleus; rich in the basic amino acids arginine and lysine, and function in coiling DNA to form nucleosomes.

Methylation: Enzymatic transfer of methyl groups to DNA; associated with gene expression and with epigenetic phenomena.

Nucleosome: In eukaryotes, a nuclear complex consisting of four pairs of histone molecules wrapped by two turns of a DNA molecule; the major structure associated with the organization of chromatin in the nucleus.

Open chromatin: AKA Euchromatin; loose chromatin easily accessible for transcription to occur.

LESSON SUMMARY: Students will review the organization of eukaryotic DNA as a mechanism of stability to ensure genetic material is organized and condensed to fit inside the nucleus of the cell. This organizational pattern is essential in understanding the machinery of gene expression as not all DNA is easily accessible for transcription. The DNA-Histone paper modeling activity will provide a hands-on experience in understanding the coiling/uncoiling ability of DNA and histone interaction and briefly introduce methylation and acetylation as a driving force of chromatin organization. To save class time, a Flipped homework assignment to view videos as an introduction to epigenetics will prepare students for the vocabulary and specifics of the epigenome.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Describe the eukaryotic organization of DNA as both a stable arrangement and as an influence for gene expression.
2. Define epigenetics as a study of how gene expression occurs
3. Explain the significance of genetic and environmental factors on the health of an individual.

4. Describe chromatin structure in terms of being “open” or “closed” in reference to methylation or acetylation.

STANDARDS:
SC.912.L.14.6
SC.912.L.16.5
SC.912.L.16.6
SC.912.L.16.9
SC.912.L.16.10
SC.912.L.18.1
SC.912.N.1.3
SC.912.N.1.6
SC.912.N.1.7
SC.912.N.3.5

MATERIALS:

- 1 copy of *Student Worksheet – Lesson 1 Part 1 Review of Eukaryotic DNA and Chromatin Remodeling*
- 1 copy of *Student Worksheet – DNA-Histone Paper Modeling*
    - Tape, paper clips, scissors
- 1 copy of *Student Worksheet: Flipped Video Homework Notes and Questions*

BACKGROUND INFORMATION: Teachers are encouraged to review *Background Information* links provided at the end of the Lesson Overview and to view both Flipped Video assignments along with students.
Teachers may elect to watch both homework video clips as a class, but this will extend the unit beyond 10 days.

ADVANCE PREPARATION:

1. Photocopy Student Worksheets:
    a. *Lesson 1/Part 1 Review of Eukaryotic DNA and Chromatin Remodeling* – 1 per student
    b. *DNA-Histone Paper Modeling* - 1 per student partner group
    Student Pages – DNA-Histone Paper Model or Web link provided
    Page A, B, C - teacher instructions.
    For students, copy 1, 2, 5, 6, 7, 8 for Lesson 1 and 3, 4 for Lesson 2
    http://teach.genetics.utah.edu/content/epigenetics/print/DNA%20Histone%20ModelFinal.pdf
    c. *Flipped Video Homework Notes and Questions* – 1 per student
    Nova – Epigenetics *http://www.pbs.org/video/1525107473/*
    Epigenome at a Glance *http://learn.genetics.utah.edu/content/epigenetics/intro/*
2. Prepare overhead or smartboard copy of labeling diagram for review and Summary diagram for class discussion.
3. Select partner groups and indicate on desks prior to students entering the class. Pair based on performance of previous assessment/understanding of DNA organization.
4. Gather tape, paper clips, scissors for each working partner group.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (2 minutes) Pass out *Lesson 1/Part 1 - Review of Eukaryotic DNA and Chromatin Remodeling* worksheet and Introduce labeling activity for Organization of Eukaryotic DNA.

2. (5 minutes) Ask student partner groups to complete the labeling activity, using the word bank provided, and to complete the summary box to describe the importance of this organization as a mechanism of stability. Teacher moves around room to check for mistakes/clarify misconceptions.

3. (10 minutes) Tell the students they are going to have a chance to learn more about the importance of DNA organization as a mechanism of gene expression/transcription. Students will use their devices or a textbook to complete the back of the provided worksheet, *Chromatin Remodeling*, as a quick intro to acetylation and methylation of DNA. While students are completing the reading activity, teacher distributes student copies of *DNA-Histone Paper Modeling* activity sheets.

4. (25 minutes) Students work in partner groups to construct their own small segment of DNA–Histone complexes. Students are urged to work quickly to assemble their INACCESSIBLE DNA models.
URGE STUDENTS to select ONE segment of DNA and color this segment with a highlighter to represent a necessary GENE SEGMENT prior to the assembly of their DNA-Histone Model.
While students are completing the paper modeling activity, teacher moves around room to check for mistakes/clarify misconceptions and pass out the homework assignment, *Flipped Video Homework Notes and Questions.*

5. (3 minutes) Completed DNA-Histone models from each group are organized into continuous chains and left for class discussion on Day 2.

FORMATIVE ASSESSMENT SUGGESTIONS:

- Student worksheet can be checked for completion.
- DNA-Histone Paper Models can be used to correct misconceptions

RESOURCES/REFERENCES/BACKGROUND INFORMATION:

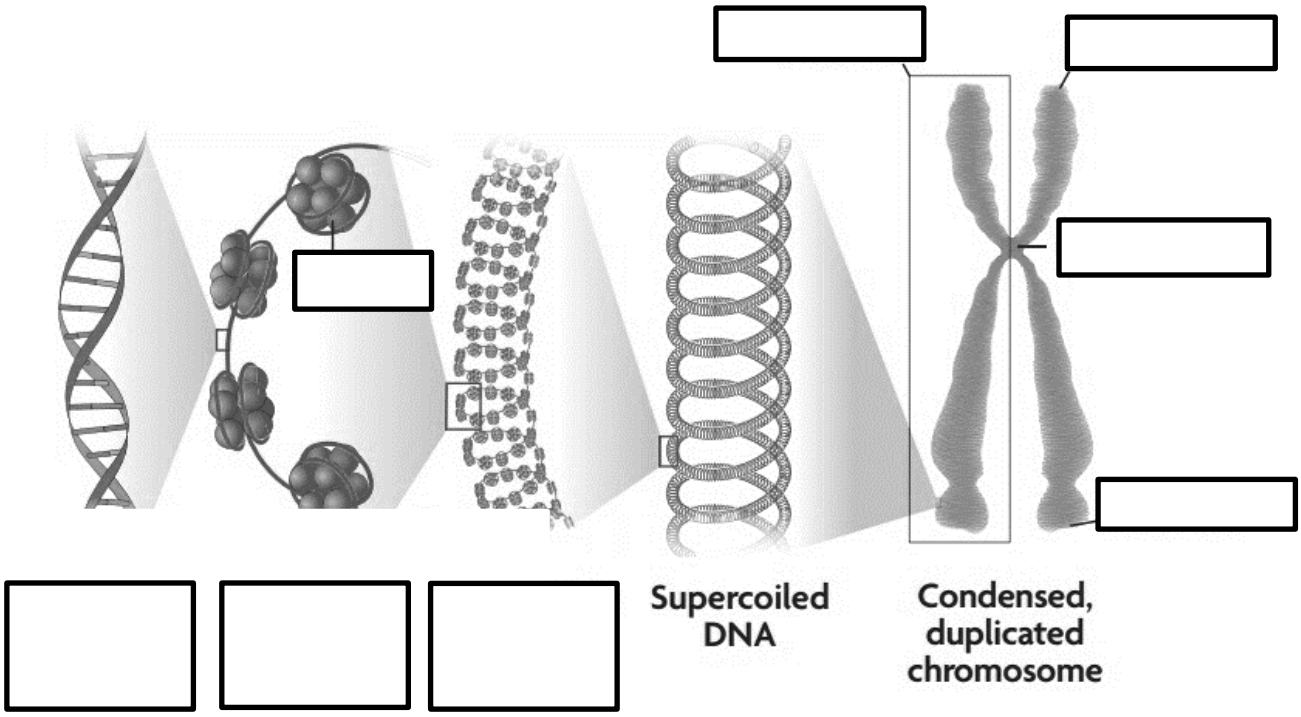
- Teach.genetics – Epigenetics http://teach.genetics.utah.edu/content/epigenetics/
- Gene Ed – Intro to Epigenetics https://geneed.nlm.nih.gov/topic_subtopic.php?tid=35
- What is Epigenetics? http://www.whatisepigenetics.com/
- Ted Talk – Epigenetics and the Influence of our Genes - http://ed.ted.com/on/9AqzZAzx
- DNA-Histone Paper Model Tutorial http://teach.genetics.utah.edu/content/epigenetics/

Lesson 1/Part 1: Eukaryotic Organization of DNA – A Review

Purpose: Students will review the organization of eukaryotic DNA as a stable organization for storage of DNA in the nucleus and introduce this organization as a mechanism for gene expression

Use the following terms to label the diagram below.

Centromere	Chromatin	DNA/Double helix
Histone	Nucleosome	Sister Chromatid
Telomere		



Describe the importance of this organizational structure in providing STABILITY to the eukaryotic genome.

CHROMATIN REMODELING

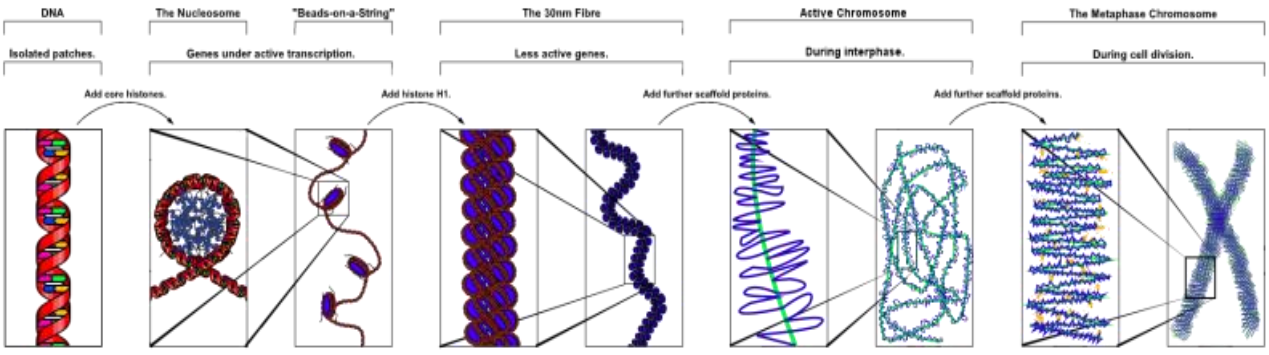
READ the passage in the online version of the textbook, page 303, Chromatin Remodeling, and complete the following statements as our introduction to Epigenetics.

- The various proteins that function in _____ and regulatory roles during the processes of _____ and _____ must interact directly with DNA.
- To accomplish these protein-DNA interactions, chromatin must be induced to _____ _____, a process called _____ remodeling.
- To allow replication and gene expression, chromatin must _____ its compact structure and _____ regions of DNA to regulatory proteins and there must also be a mechanism to _____ the process during periods of inactivity.
- Describe acetylation:

- Describe methylation:

- Summarize the final paragraph of column 1, page 304

Summary Diagram courtesy of "Chromatin Structure". Via Embryology - https://embryology.med.unsw.edu.au/embryology/index.php/File:Chromatin_Structure.png#/media/File:Chromatin_Structure.png



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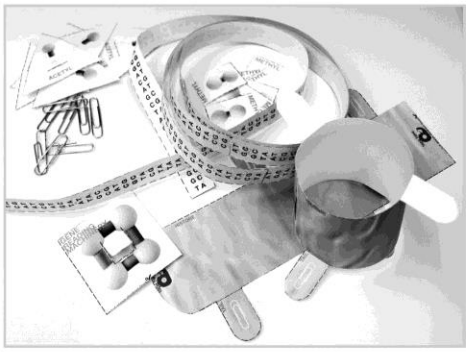
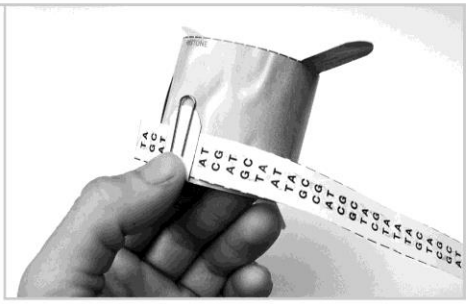
DNA and Histone Model

How do molecules control gene expression?



MAKING DNA **INACCESSIBLE**

Cut out all of the molecules on pages 5-8, assemble the DNA ribbon and histone spools. Gather 8 paperclips.



In the cell, DNA is wound around spool-like molecules called histones. Attach one end of the DNA ribbon to a histone. Fold one of the histone tails over the DNA ribbon to help hold it in place. Secure it with a paperclip.

Attach the remaining histones along the DNA ribbon at a distance of 2 strips of DNA apart (roughly 16 cm).

Hold the first histone upright in one hand. Wind the DNA ribbon clockwise around it roughly two times or until you bump in to the next histone. Fold all of the histone tails over the DNA ribbon to help hold it in to place and secure with a paperclip.

In a real cell, a length of DNA wraps around a histone roughly 1.7 times and histone tails wrap around the wound DNA similarly.

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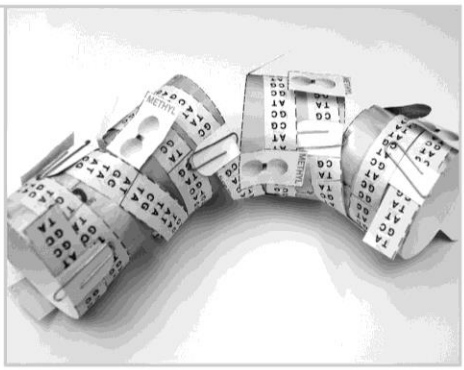
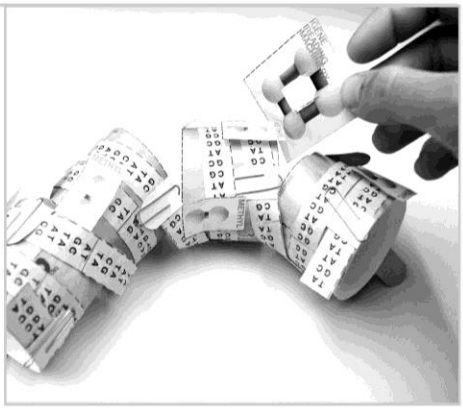
DNA and Histone Model MAKING DNA INACCESSIBLE cont.

Trying not to fold or bend the DNA ribbon, wind it around the next histone. Again, fold the histone tails around the DNA ribbon and secure with a paperclip. Repeat until all of the DNA ribbon has been wound. The histones should begin to stack on top of one another as you wind.

When DNA is wound tightly around histones, there tends to be a lot of methyl molecules bound to it. The methyl molecules cover the DNA, making it unreadable to gene reading machinery. Use tape to attach the methyl molecule cut outs to exposed areas of your DNA ribbon.



Genes become active when gene reading molecules attach and move down a length of accessible DNA, "reading" the DNA code as they go along. Try to attach and move the Gene Reading Machinery cut-out to any length of the DNA ribbon that is not spooled around a histone or covered by methyl. Can the machinery read any significant stretch of DNA? Would this be an active, or inactive gene?



Remove the methyl molecules and de-construct your model if moving to the next step: MAKING DNA ACCESSIBLE

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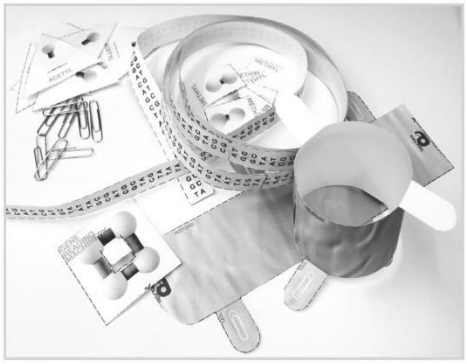
DNA and Histone Model

How do molecules control gene expression?



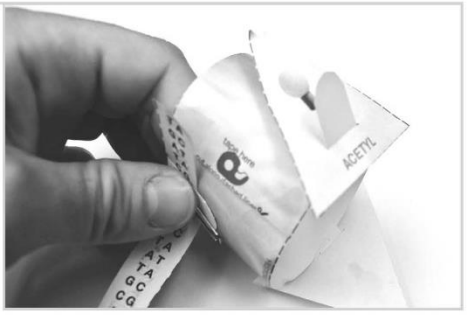
MAKING DNA **ACCESSIBLE**

Cut out all of the molecules on pages 5-8, assemble the DNA ribbon and histone spools. Gather 8 paperclips.

DNA is wound around spool-like molecules called histones. At times, acetyl molecules bind to histone tails. Attach two acetyl molecules to each histone at different locations. To attach the molecules, pull a histone tail trough the cut in the center of the acetyl molecules. Now your histones are "acetylated".

Attach an acetylated histone to one end of your DNA ribbon, secure it with a paperclip.

Attach the remaining acetylated histones along the length of the DNA ribbon at distances of 2 DNA strips apart (roughly 16 cm).



Hold the first histone upright in one hand. Wind the DNA ribbon clockwise around it two times or until the first histone touches the next one.

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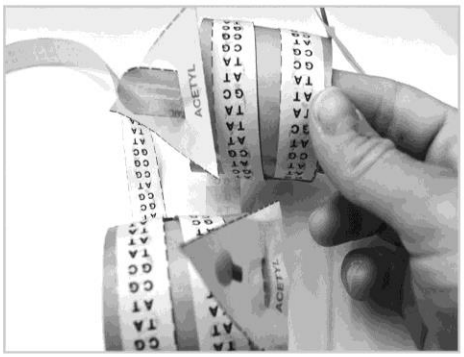
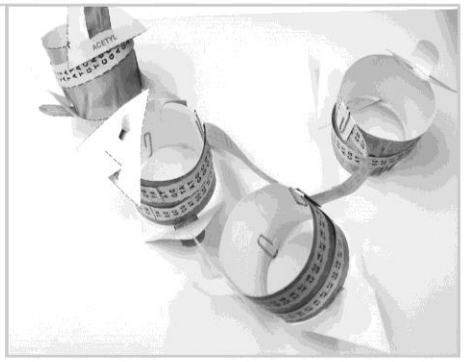
DNA and Histone Model MAKING DNA ACCESSIBLE cont.

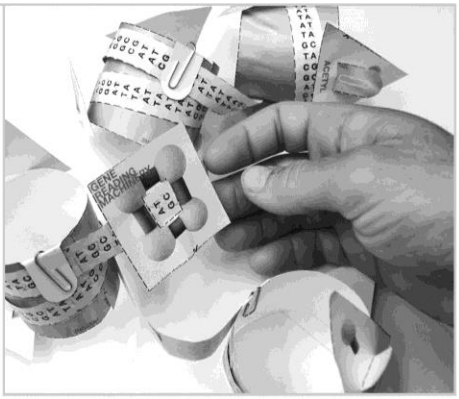
In a real cell, the addition of acetyl molecules cause the histones to distance themselves from one another. Be sure that no part of the neighboring histones, including the acetyl molecules are touching. If they are, unwind the DNA ribbon a little bit to put some space between the histones. Secure the DNA ribbon with a paperclip.



Wind the DNA ribbon clockwise around the next histone. Again, be sure that no part of neighboring histones are touching then secure the DNA ribbon with a paperclip. Repeat until the DNA ribbon has been wound around all the histones. The histones and DNA should be spooled loosely, with some space between histones.



Genes become active when gene reading molecules attach and move down a length of accesible DNA, "reading" the DNA code as they go along. Try to attach and move the Gene Reading Machinery cut-out to any length of the DNA ribbon that is not spooled around a histone. Can the machinery read any significant stretch of DNA? Would this be an active, or inactive gene?



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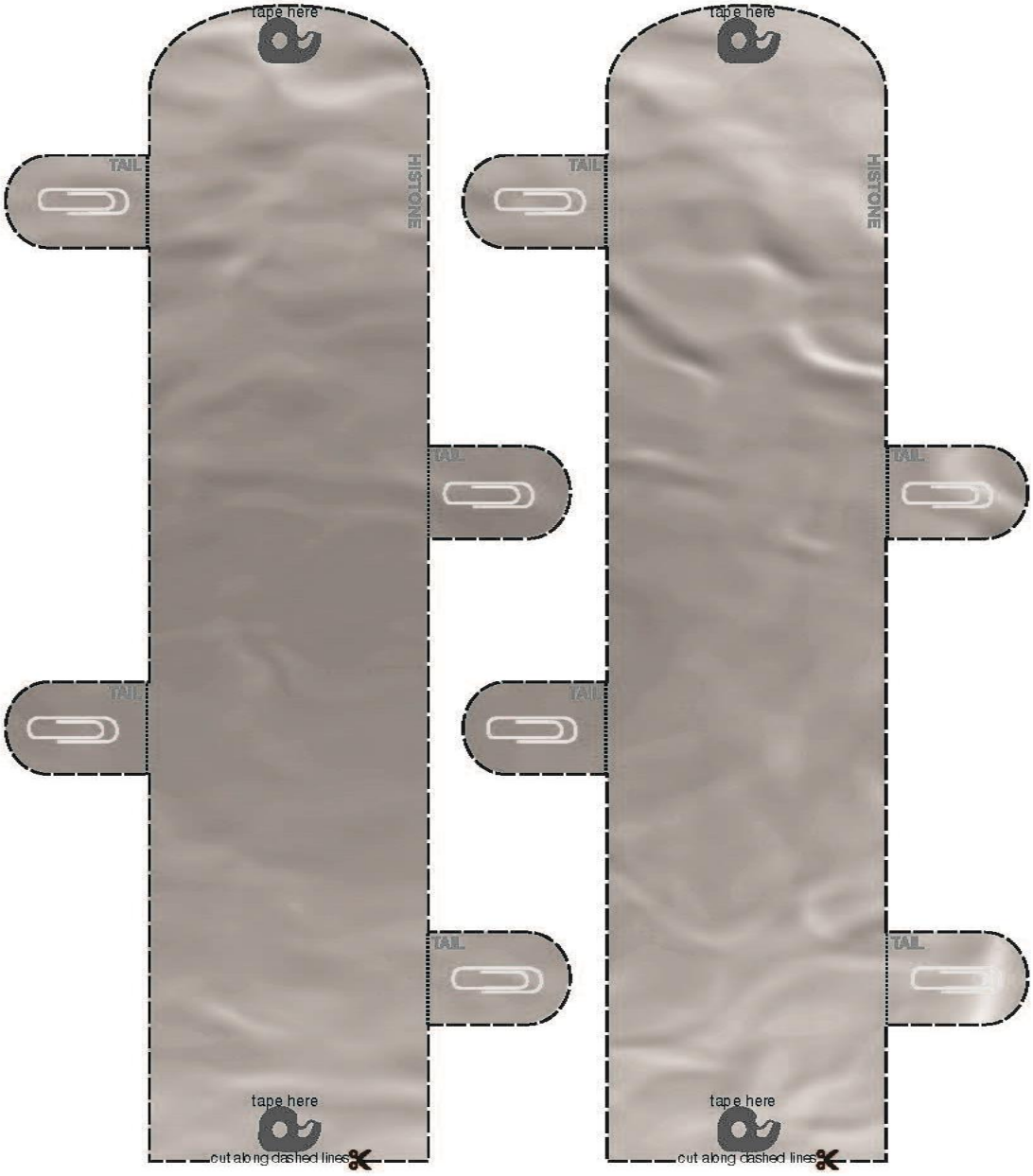
HISTONE SPOOLS - Set 1

Tape the ends of each histone together to form spools.



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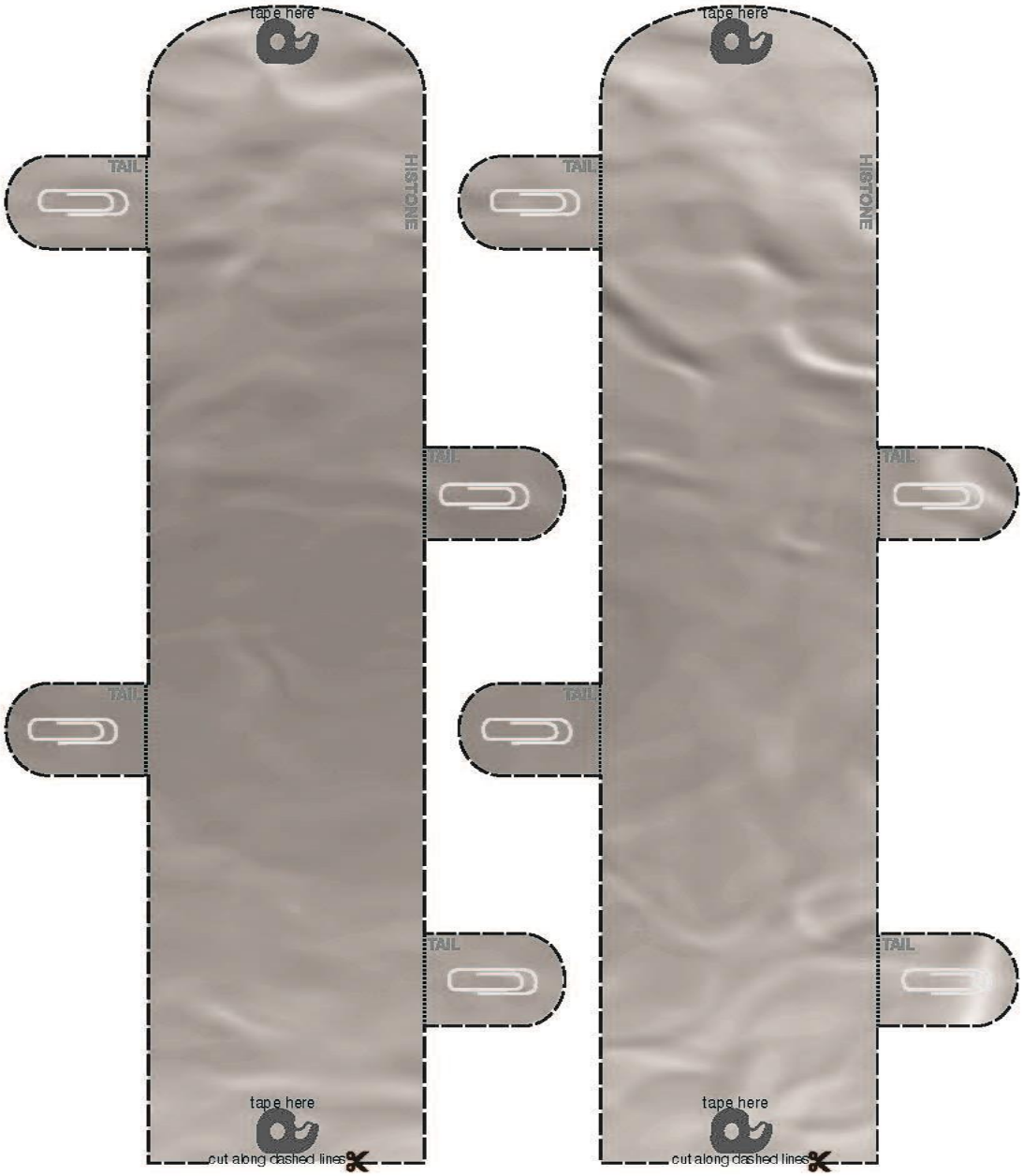
HISTONE SPOOLS - Set 2

Tape the ends of each histone together to form spools.



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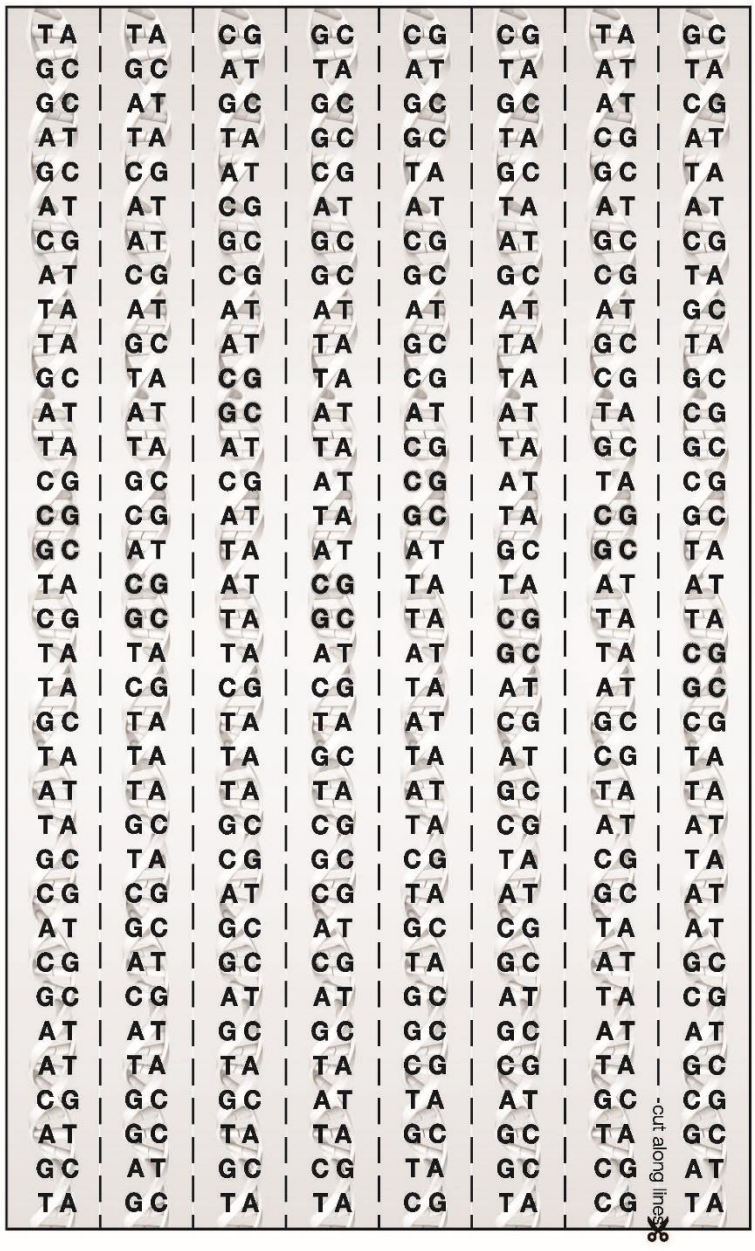
DNA Strips

Tape the short ends of the DNA strips together to form one long DNA ribbon.



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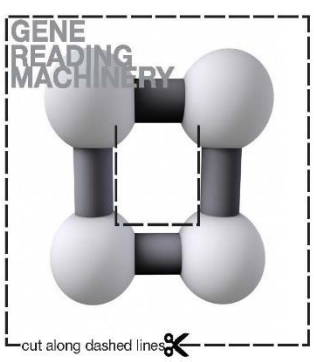
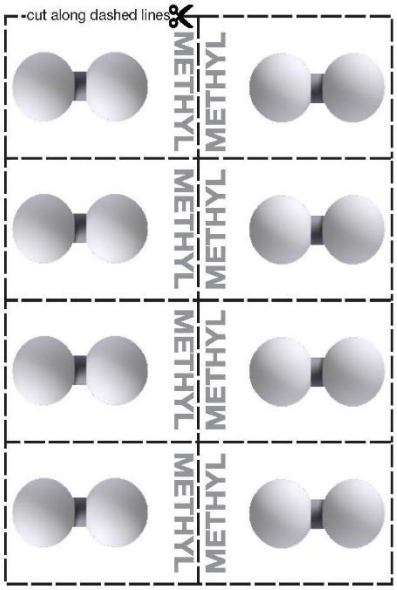
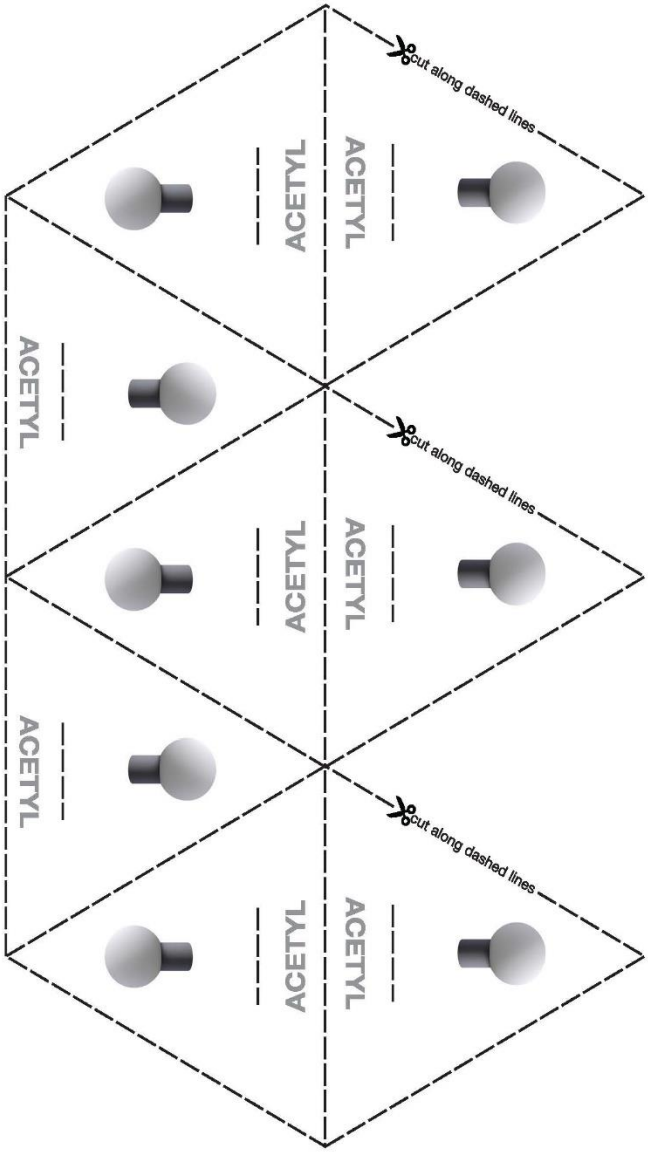
Methyl, Acetyl and Gene Reading Machinery

Cut out and slit along interior dashed lines.







Flipped Assignment – *Epigenetics at a Glance* and *Nova - Epigenetics*

Purpose: To familiarize the student with the relationship between Eukaryotic Organization of DNA, the biochemistry of the genome, and the process of gene expression.

Video 1: Epigenetics at a Glance
http://learn.genetics.utah.edu/content/epigenetics/intro/
Navigate to the web page, watch the video as many times as needed to complete the notes and questions below.

1. What are proteins around which DNA wraps?

2. What are DNA and the histone proteins covered with?

3. What is the epigenome? What differentiates the genome from the epigenome?

4. Are tightly wrapped genes considered "readable" or unreadable?" Explain.

5. Using the visual provided in the video, what makes genes accessible and easier to read?

6. What are some example of epigenetic tags that we experience through our lifetime that may alter gene expression?

7. Sketch a tightly wrapped segment of DNA and a loosely wrapped segment of DNA. What impacts these configurations?

Video 2: Nova – Epigenetics

http://www.pbs.org/wgbh/nova/body/epigenetics.html

Navigate to the web page, watch the video as many times as needed to complete the notes and questions below. The introduction is interesting but the main focus of epigenetics begins at minute 3:04.

1. At minute 4:00, they discuss the similarities between the fat and skinny mouse. The agouti gene is mentioned and is considered "on" all of the time in the fat mouse. How do you think this gene expression is affecting the fat mouse differently than the skinny mouse?

2. How do methyl groups impact gene expression?

3. Give the translated definition of Epigenetics provided.

4. How does the epigenome impact development?

5. How do researchers feel the epigenome can change during a lifetime?

6. How is the coat color of the mice linked to expression of the agouti gene?

7. Why are twins an important experimental model for epigenetics?

8. Compare the similarities of the overlapping genes in the 6-year-olds vs the 70-year-olds. What did this Spanish study suggest? Why are identical twins more likely to be similar when young but different as they age? Explain.

9. What is the link between epigenetics and cancer?

10. How can we impact the health of our epigenome?

LESSON TWO: INTRODUCTION TO EPIGENETICS AND GENE EXPRESSION

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure:
  Day 1 - 45 minutes + homework time
  Day 2 - 45 minutes + homework time

LEARNING STYLES: Visual, auditory, and kinesthetic.

VOCABULARY:

***Cis*-regulatory:** AKA cis-acting; A non-coding DNA sequence that regulates the expression of nearby genes located on the same chromosome; often acts as binding sites for transcription factors; located upstream of gene for transcription.

Downstream: toward the 3' end of the gene/chromosome.

Enhancer: A DNA sequence that enhances transcription and the expression of structural genes; can act over a distance of thousands of base pairs and can be located upstream, downstream, or internal to the gene they affect.

Enzyme: Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life.

Histone code: Various chemical modifications applied to histone tails; influence DNA-histone interactions and promote or repress transcription.

Locus/Loci: The site or place on a chromosome where a particular gene is located.

Locus Control Region (LCR): a long-range *cis*-regulatory element that enhances expression of linked genes at distal chromatin sites. It functions in a copy number-dependent manner and is tissue-specific, as seen in the selective expression of β-globin genes in erythroid cells.

Promoter: An upstream regulatory region of a gene to which RNA polymerase binds prior to the initiation of transcription.

RNA: Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

RNA polymerase: An enzyme that catalyzes the formation of an RNA polynucleotide strand using the base sequence of a DNA molecule (gene) as a template.

***Trans*-regulatory:** AKA *trans*-acting; a gene product that acts to regulate the expression of a target gene;

Transcription: DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Transcription Factors: Proteins containing DNA-binding abilities which attaches to specific DNA sequences in preparation for transcription of a gene.

Upstream: toward the 5' end of the gene/chromosome.

LESSON SUMMARY: Working in groups, students will review vocabulary from the video/Flipped homework assignment and perform a Word Sort to reinforce the vocabulary and components of epigenetic influence. This activity will provide a "quick look" opportunity for teachers to assess understanding, clarify, and correct misconceptions. Level of student understanding will determine when the teacher progresses to the next phase of the lesson which provides specific details on eukaryotic gene expressions and epigenetics, delivered in a traditional lecture format. The lecture will provide opportunities for understanding through outlines of information, opportunities for diagraming and modeling using the beta-gene locus as an example, and teacher-guided questions and discussion. The final activity is a web interactive on gene control; this lesson can be done in-class (time permitting) or as a homework assignment.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Describe the molecules involved in the transcription of genes to increase/decrease gene expression.
2. Using the beta-globin gene locus as a model, identify promoters, enhancers, locus control regions (LCR), transcription factors in the process of transcription.
3. Describe the effect that promoters and enhancers have on up-regulating or down-regulating gene expression.
4. Consider the role technology has played in the rapid advances in biomedical science during the last twenty years

STANDARDS:

SC.912.L.14.6
SC.912.L.16.2
SC.912.L.16.5
SC.912.L.16.6
SC.912.L.16.9
SC.912.L.16.10
SC.912.L.18.1
SC.912.N.1.2
SC.912.N.1.5
SC.912.N.1.6
SC.912.N.1.7
SC.912.N.3.5

MATERIALS:

1. *Vocabulary of Gene Expression Word Sort Cards* – 1 set per partner group
   2-Column Notes for organization of vocabulary – 1 per student
2. *Student Outline – Eukaryotic Gene Expression and Chromatin Modification*
   Teachers may elect to copy the 2-Column Notes for vocabulary and the outline together.
3. *Homework Directions – Web-Interactive on Gene Control*
   http://learn.genetics.utah.edu/content/epigenetics/control/

BACKGROUND INFORMATION: Teachers should familiarize themselves with the Word Sort and feel comfortable with the vocabulary to address any misconceptions; Pay attention to Notes Pages prior to delivering the PowerPoint and student completion of diagrams; place PowerPoint on teacher web page for students to reinforce difficult concepts, review diagrams, and make corrections.

ADVANCE PREPARATION:

1. Check DNA-Histone Models from previous class session for good construction according to directions provided.
2. Display the "Discussion Points" from the DNA-Histone Model Activity for student discussion when class begins.
3. Prepare the Word Sort for each student pair or group: Print one set single sided, copy and cut a set for each student pair or group. Paper clip each set or place in envelope.
   For extended use, consider cardstock and/or laminating.
4. Photocopy *2-Column Notes – Word Sort Vocabulary* – 1 per student.
5. Photocopy *Student Outline – Eukaryotic Gene Expression and Chromatin Modification* – 1 per student.
6. Photocopy *Homework Directions – Web-Interactive on Gene Control* – 1 per student.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

DAY 1

1. (5 minutes) Return paper models to students as they enter class.
   Explain to students that they will now modify their construction to illustrate how chemistry controls gene expression. Refer to the "Discussion Points" displayed in the room and ask students to discuss with their partner if the "gene segment" they highlighted prior to construction of their model is accessible or inaccessible and discuss what makes this chemical accessibility/inaccessibility possible.

2. (15 minutes) Using the *Accessible DNA* instructions provided, have students work to alter the Inaccessible construction from the previous lesson. Tell students to pay special attention to the molecules they are removing and adding to alter this biochemical structure of DNA and to note that the base pair sequence is not changing. Teacher should walk around room to correct mistakes and answer questions.

3. (5 minutes) Instruct students to make a single continuous strand of DNA by assembling one model to another at a free DNA end to construct a larger DNA-Histone Model. Discuss the significance of this in the scheme of an entire genome of billions of base pairs. As students are constructing the class model, move around the room and pass out the *Vocabulary of Gene Expression Word Sort* envelopes and *2-Column Notes.*

4. (10 minutes) Instruct students to work with a partner to complete the word sort and fill out the vocabulary *2-Column Notes* to keep as a reference sheet. Teacher should move around room to address mistakes during the word sort and use this as a formative assessment.

5. (10 minutes) Transition to PowerPoint Presentation using beta-globin gene as a model for gene expression. Students should have copy of *Student Outline – Eukaryotic Gene Expression and Chromatin Modification* to accompany the lesson.

6. As students leave room, encourage to work ahead in the *Eukaryotic Gene Expression and Chromatin Modification* PowerPoint lesson and complete all diagrams prior to the next class.

DAY 2

7. (10 minutes) With posted diagram of the beta-globin gene locus, ask students what they learned about the beta-globin gene from the PowerPoint homework assignment.
   Prompting questions:
   What is hemoglobin used for?
   What is the significance/importance of shape and the function of the beta-globin gene?
   What disease(s) could result due to a faulty beta-globin gene?

8. (30 minutes) Use the remainder of class time to complete the PowerPoint lesson on *Eukaryotic Gene Expression and Chromatin Modification.*

9. As students leave the room, provide a copy of *Homework Directions – Web-Interactive on Gene Control*

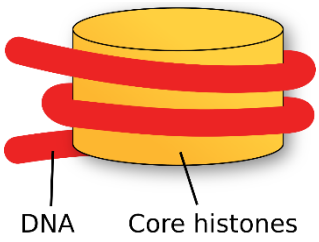
RESOURCES/REFERENCES:

- Bozeman Biology – Gene Expression https://www.youtube.com/watch?v=3S3ZOmleAj0
  Great overview/review for teacher or student use to reinforce concepts of gene expression and regulation.
- Web Reference – What is Epigenetics – focus on DNA methylation and Chromatin Remodeling
  http://epigeneticsnetwork.ca/about/

Lesson 2 –Word Sort 2-Column Notes

TERM	DEFINITION/SKETCH

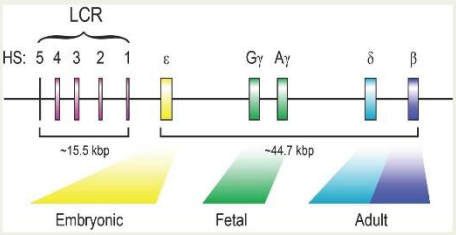
ACETYLATION	A process that adds an acetyl group to DNA that removes the positive charge on the histone proteins and loosens the interaction between DNA and histones to increase the availability of the gene to transcription factors.
METHYLATION	Enzymatic transfer of methyl groups to DNA; associated with gene expression and with epigenetic phenomena.
PROMOTER	An upstream regulatory region of a gene to which RNA polymerase binds prior to the initiation of transcription.
ENHANCER	A DNA sequence that enhances transcription and the expression of structural genes; can act over a distance of thousands of base pairs and can be located upstream, downstream, or internal to the gene they affect.
CpG ISLANDS	Areas of ~200 base pairs with greater than 50% GC; associated with promoter regions of transcriptionally active genes.
HISTONE	Positively charged proteins complexed with DNA in the nucleus; rich in the basic amino acids arginine and lysine, and function in coiling DNA to form nucleosomes.
HETEROCHROMATIN	AKA Closed chromatin; the heavily staining, late-replicating regions of chromosomes that are prematurely condensed in interphase. DNA sequences are difficult to access for transcription by mRNA.

EUCHROMATIN	AKA Open chromatin; loose chromatin easily accessible for transcription to occur.
EPIGENETICS	The study of modification in an organism's gene function or phenotypic expression that are not attributable to alterations in the nucleotide sequence (mutations) of the organism's DNA.
TRANSCRIPTION FACTORS	Proteins containing DNA-binding abilities which attaches to specific DNA sequences in preparation for transcription of a gene.
LCR	A long-range *cis*-regulatory element that enhances expression of linked genes at distal chromatin sites. It functions in a copy number-dependent manner and is tissue-specific, as seen in the selective expression of β-globin genes in erythroid cells.
BETA-GLOBIN	A single unit of the tetramer hemoglobin protein used by erythrocytes to carry oxygen to cells; the focus of epigenetic control of gene expression during developmental phase and a target for therapy for sickle cell disease and thalassemia.
NUCLEOSOME	

EUKARYOTIC GENE EXPRESSION AND CHROMATIN MODIFICATION

Honors Genetics
Susan Chabot
Lemon Bay High School
2016-2017

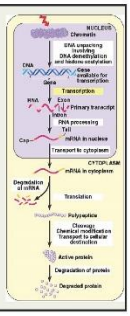
Beta-globin Gene Locus



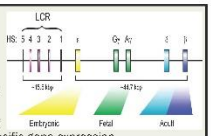
Objectives

Students will:

- Identify the main components of the beta-globin gene locus as a mechanism for understanding control of eukaryotic gene expression.
- Describe the impact of methylation and acetylation on gene expression.
- Differentiate between heterochromatin and euchromatin/inaccessible and accessible DNA.
- Describe the location and function of promoters, enhancers, and locus control regions in gene expression.
- Evaluate the impact of methylation and acetylation on DNA-histone complexes and gene expression.



Beta-globin Gene Locus

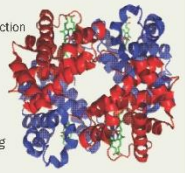


The Players:

- LCR – Locus Control Region – regulatory DNA elements that control multiple genes.
- HS – Hypersensitive Sites – comprise the LCR and allow for looping of the gene locus for specific gene expression.
- Beta-globin (β) chains: expressed during specific developmental phases. Expression depends on accessibility of gene segment due to degree of methylation
  - ε: expressed during embryonic development (<8 weeks)
  - γ: expressed during fetal development (8 weeks – infancy)
  - δ: expressed prior to birth (fetal – death)
  - β: expressed at birth (birth – death)

Beta-globin Gene Locus

- The beta-globin gene is responsible for the production of the beta chain of the hemoglobin molecule.
  - *Located on Chromosome 11*
  - *Hemoglobin is a tetramer, used to transport oxygen in erythrocytes.*
- The beta-globin locus (*red*) provides a model for studying epigenetic control of gene expression.
  - *Experiences major epigenetic changes during transcription of beta-globin as development occurs.*
  - *There are 5 different forms of the beta-globin chain produced during different developmental stages.*
- The type of beta-globin produced is determined through accessibility of the beta-globin locus.



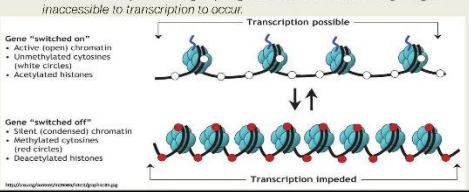
Objectives

Students will:

- Identify the main components of the beta-globin gene locus as a mechanism for understanding control of eukaryotic gene expression.
- Describe the impact of methylation and acetylation on gene expression.
- Differentiate between heterochromatin and euchromatin/inaccessible and accessible DNA.
- Describe the location and function of promoters, enhancers, and locus control regions in gene expression.
- Evaluate the impact of methylation and acetylation on DNA-histone complexes and gene expression.

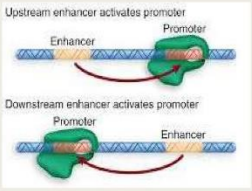
Methylation and Acetylation

- As we learned in our *DNA Histone Model* Activity
  - *Addition of acetyl functional groups relaxes the chromatin making gene accessible to transcription to occur.*
  - *Addition of methyl function groups tightens the chromatin making the gene inaccessible to transcription to occur.*



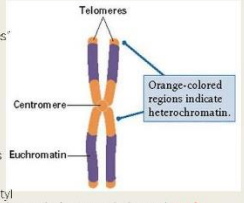
Promoters and Enhancers

- In addition to the LCR and HS, many genes also rely on the influence of promoters and enhancers to fine-tune the level of gene expression.
- Simplified:
  - *Promoters recruit and position RNA polymerase for transcription.*
  - *Enhancers assist promoters by repositioning genes toward the chromatin surface (accessibility).*



Heterochromatin and Euchromatin

- Refers to the banding pattern that occurs when chromosomes are stained for visualization.
- Euchromatin
  - *Often referred to as "coding regions" of DNA*
  - *Loosely associated with histones*
  - *Transcriptionally active*
- Heterochromatin
  - *Often referred to as "non-coding regions" of DNA*
  - *Tightly condensed around histones*
  - *Transcriptionally inactive*
- It is the combination of methyl and acetyl groups that organizes the hetero- or euchromatin for transcription and can be changed!



Objectives

Students will:

- Identify the main components of the beta-globin gene locus as a mechanism for understanding control of eukaryotic gene expression.
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- Differentiate between heterochromatin and euchromatin/inaccessible and accessible DNA.
- Describe the location and function of promoters, enhancers, and locus control regions in gene expression.
- **Evaluate the impact of methylation and acetylation on DNA-histone complexes and gene expression.**

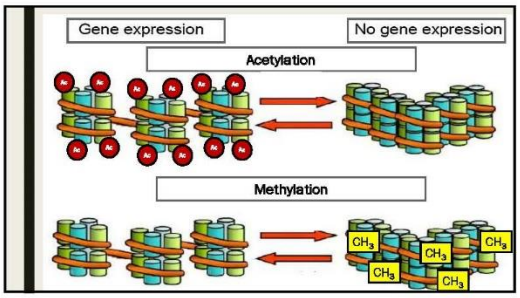
Objectives

Students will:

- Identify the main components of the beta-globin gene locus as a mechanism for understanding control of eukaryotic gene expression.
- Describe the impact of methylation and acetylation on gene expression.
- Differentiate between heterochromatin and euchromatin/inaccessible and accessible DNA.
- **Describe the location and function of promoters, enhancers, and locus control regions in gene expression.**
- Evaluate the impact of methylation and acetylation on DNA-histone complexes and gene expression.

Impact of Methylation and Acetylation on Gene Expression

- On the diagram provided, indicate the following:
  - *Acetylation*
  - *Location of acetyl groups*
  - *Methylation*
  - *Location of methyl groups*
  - *Gene Expression*
  - *No Gene Expression*


Gene expression
No gene expression
Acetylation
Methylation
CH3
CH3
CH3
CH3
CH3

Lesson 2 – Outline Eukaryotic Gene Expression and Chromatin Modification

Beta-globin Gene Locus

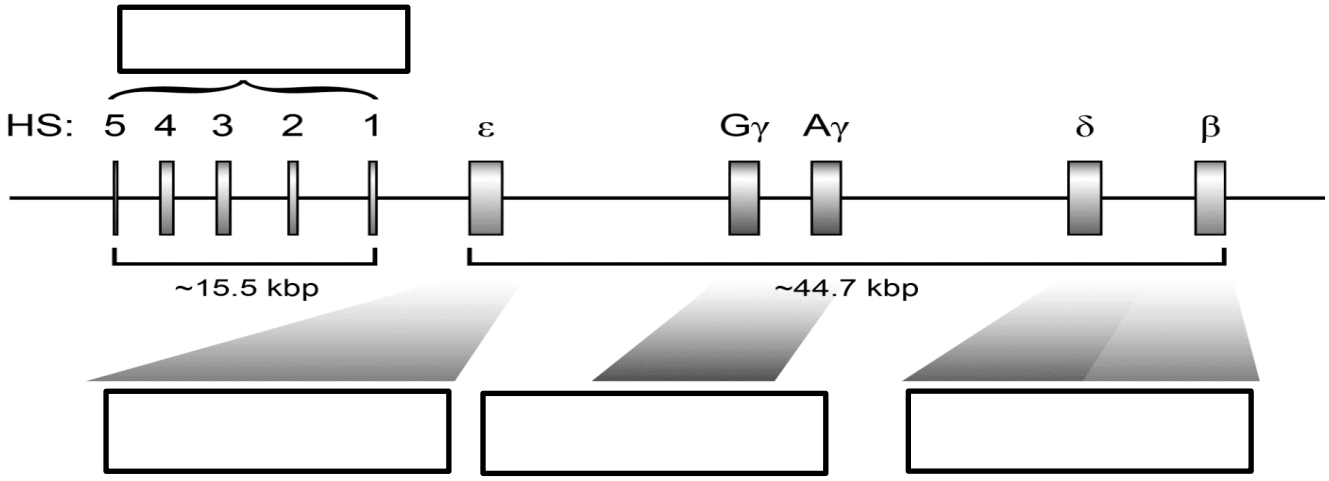
- What is the function of the beta-globin gene?

- Why is the beta-globin gene a model for epigenetic gene expression?

- How many forms of the beta-globin chain are produced?

- What determines which form of beta-globin is produced?

- Label the beta-globin gene locus and color-code each segment of the gene as shown.



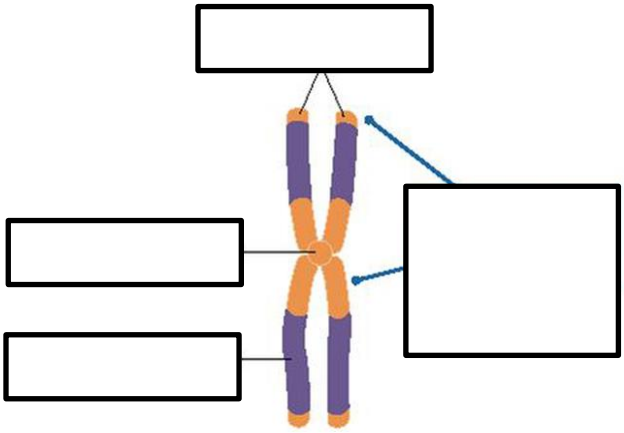
- The Players
  - LCR
  - HS
  - Beta-globin segments
    - ε:
    - γ:
    - δ:
    - β:

Impact of Methylation and Acetylation on Gene Expression

- When a gene is “switched on:”
  -
  -
  -
  - SKETCH

- When a gene is “switched off:”
  -
  -
  -
  - SKETCH

- Complete the diagram below to differentiate between Heterochromatin and Euchromatin



  - Euchromatin
  - Heterochromatin

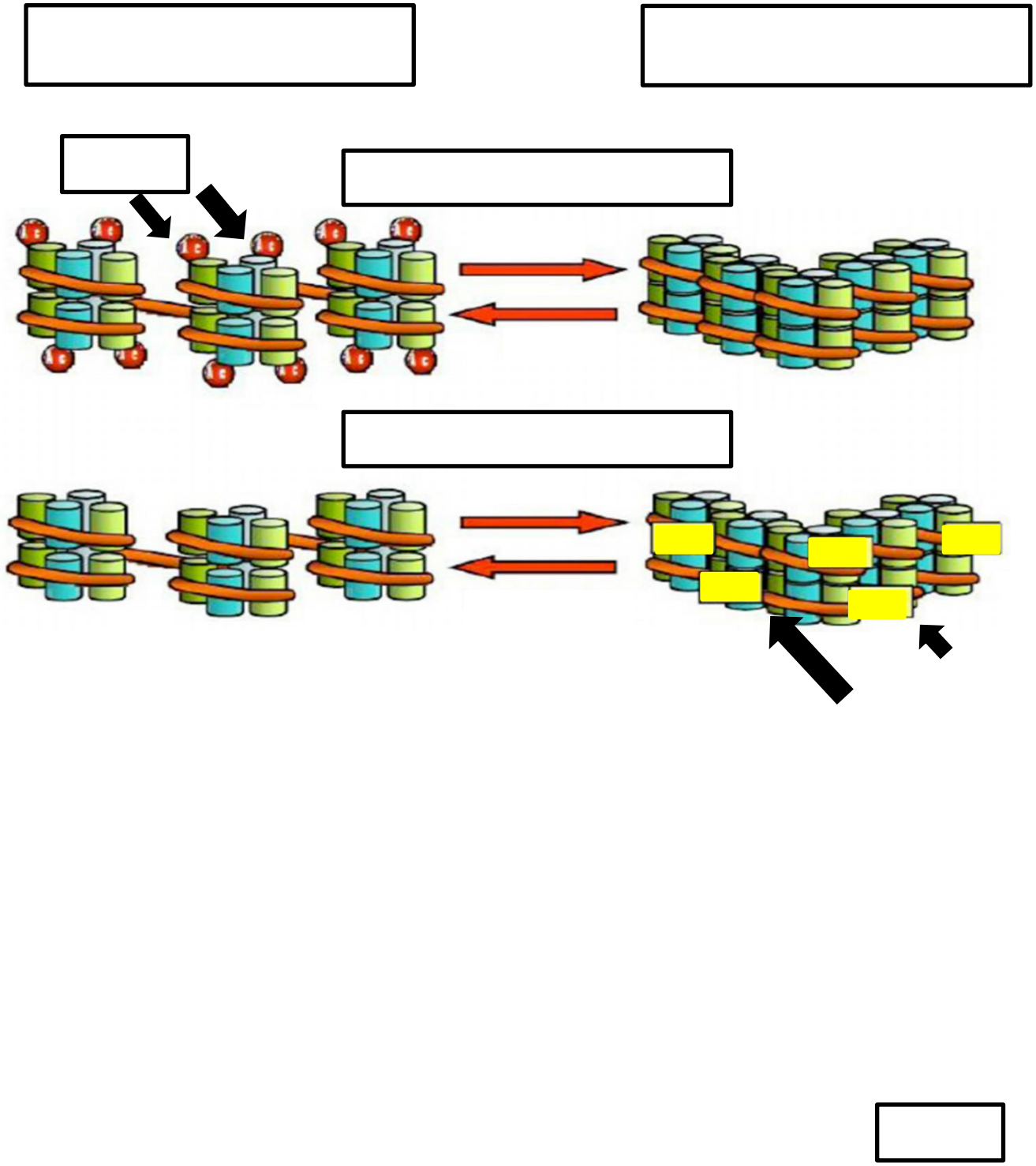
  - It is the combination of methyl and acetyl groups that organizes the heterochromatin and euchromatin for transcription and …

    _____!

Promoters and Enhancers

- How do Promoters and Enhancers work to influence gene expression?

Impact of Methylation and Acetylation on Gene Expression



Web-Interactive – Gene Control

Navigate to the following web page http://learn.genetics.utah.edu/content/epigenetics/control/

1. Introduction:
   Signals from the outside world can work through the epigenome to change a cell's gene expression.
   In the activity, you act as the signal. As you turn the control knob, epigenetic tags come and go to change the shape of the gene.
   Notice what happens to the mRNA and protein levels when you manipulate the epigenetic tags on the gene. Gene, mRNA, and protein production are linked. They change together.
   In this simulation, we are using the gene for Green Fluorescent Protein. When expressed, cells that transcribe and translate this gene will produce a green protein that fluoresces.

2. PLAY! Turn the knob, playing close attention to:

   a. The shape of the GFP gene.

   b. The amount of GFP mRNA produced.

   c. The amount of GFP produced.

   d. The brightness/dimness of the cell

3. What do each of the colored structures in the *GFP gene* window represent?

   a. Round blue objects with small spikes –

   b. Small green circles –

   c. Small red circles -

4. What happens when we “turn down” gene expression?

   a. The GFP gene

   b. GFP mRNA

   c. GFP expression

5. What happens when we “turn up” gene expression?

   a. The GFP gene

   b. GFP mRNA

   c. GFP expression

6. Is this currently being done in science &/or medicine?

7. What is the relationship between Gene Control and Cancer? Summarize

LESSON THREE: TISSUE-SPECIFIC GENE EXPRESSION

KEY QUESTION(S): How does reading the genome produce different cells and tissues? How do some cells produce some proteins but other cells produce different proteins? What aspect of the genome allows for developmental changes over the lifespan of an organism?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure:
  - Day 1: 45 minutes
  - Day 2: 45 minutes

LEARNING STYLES: Visual and auditory

VOCABULARY:

Acetylation: A process that adds an acetyl group to DNA that removes the positive charge on the histone proteins and loosens the interaction between DNA and histones to increase the availability of the gene to transcription factors.

Chromatin remodeling: A process in which the structure of chromatin is altered by a protein complex that results in a change in the transcriptional state of genes in an altered region.

Closed chromatin: AKA heterochromatin; the heavily staining, late-replicating regions of chromosomes that are prematurely condensed in interphase. DNA sequences are difficult to access for transcription by mRNA.

CpG islands: Areas of ~200 base pairs with greater than 50% GC; associated with promoter regions of transcriptionally active genes.

Enhancer: A DNA sequence that enhances transcription and the expression of structural genes; can act over a distance of thousands of base pairs and can be located upstream, downstream, or internal to the gene they affect.

Histone: Positively charged proteins complexed with DNA in the nucleus; rich in the basic amino acids arginine and lysine, and function in coiling DNA to form nucleosomes.

Locus/Loci: The site or place on a chromosome where a particular gene is located.

Methylation: Enzymatic transfer of methyl groups to DNA; associated with gene expression and with epigenetic phenomena.

Nucleosome: In eukaryotes, a nuclear complex consisting of four pairs of histone molecules wrapped by two turns of a DNA molecule; the major structure associated with the organization of chromatin in the nucleus.

Open Chromatin: AKA Euchromatin; loose chromatin easily accessible for transcription to occur.

Promoter: An upstream regulatory region of a gene to which RNA polymerase binds prior to the initiation of transcription.

Transcription: DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Transcription Factors: Proteins containing DNA-binding abilities which attaches to specific DNA sequences in preparation for transcription of a gene.

LESSON SUMMARY: Students describe developmental changes in organisms and the influence of the epigenome on transcription of necessary proteins for different phases of growth and development. Small group discussion and white board presentation of findings will highlight tissue-specific and developmental-specific gene expression. An in-class and homework assignment from the text will allow students to explore Bioinformatics to learn more about gene expression; *Exploring the Genome – Tissue-Specific Gene Expression*. This is a reading activity and web-based tutorial.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Describe the relationship between cells having the same genome and cell differentiation.
2. Identify reasons gene expression differ during developmental stages of an organism's lifespan.
3. Describe the impact epigenetics plays on tissue-specific gene expression.

STANDARDS:

SC.912.L.14.6
SC.912.L.16.3
SC.912.L.16.4
SC.912.L.16.5
SC.912.L.16.6
SC.912.L.16.9
SC.912.L.18.1
SC.912.N.1.6
SC.912.N.2.4
SC.912.N.3.5

MATERIALS:

- Copies of *Notes – Crash Course Biology Evolutionary Development*, one per student
- Copies of *Exploring Genomics – Tissue Specific Gene Expression* text assignment, one per student
- Small white boards or large Post-it notes
- Markers

BACKGROUND INFORMATION: Virtually all cells in a eukaryotic organism contain a complete genome; however, only a subset of genes is expressed in any particular cell type. For example, some white blood cells express genes encoding certain immunoglobulins, allowing these cells to synthesize antibodies that defend the organism from infection and foreign agents. However, skin, kidney, and liver cells do not express immunoglobulin genes. Pancreatic islet cells synthesize and secrete insulin in response to the presence of blood sugars; however, they do not manufacture immunoglobulins. In addition, they do not synthesize insulin when it is not required. The mechanism for controlling which genes are read by specific cells/tissues or genes that are read during specific development stages is controlled through epigenetic factors like methylation and acetylation. Eukaryotic cells regulate their growth and division to occur at appropriate places in the body and at appropriate times during development. The loss of gene regulation that controls normal cell growth and division may lead to developmental defects or cancer.

Eukaryotic gene regulation is one of the most rapidly advancing fields in genetic research. Our ever-increasing understanding about the mechanisms that regulate gene expression in eukaryotes is contributing knowledge that is fascinating and often surprising. It is also leading to practical applications, such as potential therapies for many human diseases (Klug, 2012).

ADVANCE PREPARATION:

- Make copies:
  - *Video Outline – Crash Course Biology #17 – Evolutionary Development*
  - Homework Assignment – *Exploring Genomics – Tissue Specific Gene Expression*
- Download or Load You Tube Video – Crash Course Biology #17 Evolutionary Development
- Download or Load Ted Talks video – Epigenetics and the influence of our genes
- Day 1: Display slides to prompt discussion on Tissue-specific gene expression
- Day 2: Review process for BLAST search http://blast.ncbi.nlm.nih.gov/Blast.cgi

RESOURCES:

- TED TALK – Epigenetics and the Influence of our Genes
  https://www.youtube.com/watch?v=JTBg6hqeuTg
- Crash Course Biology – Evolutionary Development
  https://www.youtube.com/watch?v=9sjwlxQ_6LI
- Genetics of Development video
  http://www.learner.org/courses/biology/textbook/gendev/index.html#

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:
Day One (45 minutes)

1. (5 minutes) As students enter the room, display Slide 1 of *Lesson 3 Discussion Prompts* and have students answer questions on their own.
    a. How is your genome like a book?
    b. Do all of your cells have the same copy of your genetic code?
    c. Are all genes used in all cells of your body? Why or why not?
    d. Are all genes used in all cells during your entire lifetime? Why or why not?

1. (10 minutes) Ask students to share their answers to the Discussion Prompts displayed and help to clarify misconceptions about the genetic code.

2. (15 minutes) Have students work with a partner to white board/Post-It the 2^{nd}, 3^{rd}, 4^{th} Discussion Prompts, providing detail on their display to fully explain their answer to others.

3. (10 minutes) Have students share their answers by selecting a spokes-person to present their board for each discussion prompt.

4. (5 minutes) Provide copy of *Crash Course Biology #17 – Evolutionary Development*
   Load You Tube video – Crash Course Biology #17 and begin watching as class.
   Students complete video notes and questions as homework assignment.

Day Two (45 minutes)

5. (5 minutes) As students enter the room, display the following questions to review the Crash Course Video from the previous night's homework.
    a. What are Hox genes?
    b. Why are Hox genes important?
    c. Who is Bill McGinnis?

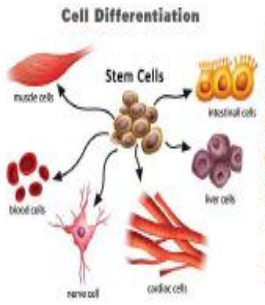
6. (5 minutes) Review answers the questions above and point out that Hox genes drives the early development of every body blueprint for every organism on the earth.

7. (20 minutes) View the Ted Talk video – Epigenetics and Influence of Our Genes as a class. Viewing this video will help in completion of the homework assignment.

8. (15 minutes) Provide copy of text assignment *Exploring Genomics – Tissue Specific Gene Expression.*
    a. Instruct students to begin by reading expectations of assignment.
    b. Display BLAST webpage and instruct students on how to use BLAST search engine.
    c. Students finish for homework.

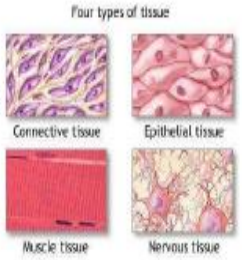
Lesson 3 Discussion Prompts

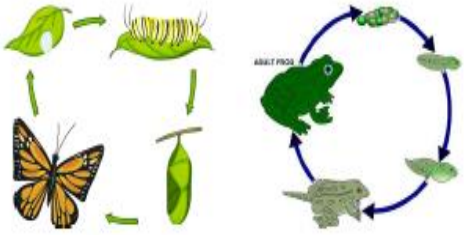
- How is your genome like a book?
- Do all of your cells have the same copy of your genetic code?
- Are all genes used in all cells of the body? Why or why not?
- Are all genes used in all cells during your entire lifetime? Why or why not?



How are these cells alike?
How are they different?
Discuss in your group how genetics plays a role in differentiation.

How are these tissues alike?
Why is it important that these tissues perform different functions?
Does genetics influence these differences?





In your group, discuss the role of genes in different developmental phases of a life cycle.

Video Outline – Crash Course Biology #17 – Evolutionary Development
Link to the following You Tube video and complete the notes and questions below.
https://www.youtube.com/watch?v=9sjwlxQ_6LI

2:08 - Developmental Regulatory Genes

1. Are all genes turned on and off at the same time? Explain the function of developmental regulatory genes in the process of gene expression.

    a. If you need to review protein synthesis, click on the link that pops up at minute 2:15

    b. When do they start working?

    c. What are Gap genes?

    d. What are hox genes? What does Hox stand for?

2. Sketch the Regulatory Genes graphic at minute 3:29 and describe what it means. Hank likens Hox genes to architects that have the plans to make something but don't actually do the building.

4:10 – What activates the first regulatory gene and how do they tell each other to do stuff?

3. Junk DNA is now thought to be regulatory genes. How many Hox genes do they believe now appear in our genome?

4. What chromosomes do these Hox genes appear on?

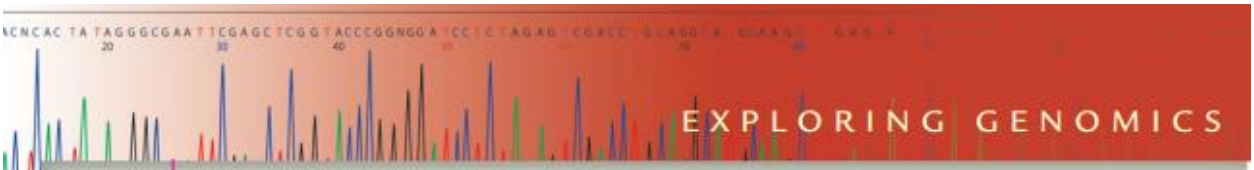
5. Hox genes within a species have exactly the same gene sequences, no differences alleles. Why is this important?

6. Who is Bill McGinnis – look him up and tell me about his research. Why did Hank mention him?

5:32 – Hox genes are also alike from one species to another, like all mammals, all insects, all vertebrates…

7. How similar is the mouse genome to the human genome? Why are they so similar?

Summarize the Biolo-graphy and describe why the experiment was important.



Tissue-Specific Gene Expression

Study Area: Exploring Genomics

In this chapter, we discussed how gene expression can be regulated in many complex ways. Recall that one aspect of gene expression regulation we considered is the way promoter, enhancer, and silencer sequences can govern transcriptional initiation of genes to allow for tissue-specific gene expression. All cells and tissues of an organism possess the same genome, and many genes are expressed in all cell and tissue types. However, muscle cells, blood cells, and all other tissue types express genes that are largely tissue-specific (i.e., they have limited or no expression in other tissue types). In this exercise, we use BLAST to learn more about tissue-specific gene-expression patterns.

Exercise – Tissue-Specific Gene Expression

In this exercise, we return to the National Center for Biotechnology Information site (NCBI) and use the search tool **BLAST, Basic Local Alignment Search Tool**, which you were introduced to in the "Exploring Genomics" exercise for Chapter 10.

1. Access BLAST from the NCBI Web site at http://www.ncbi.nlm.nih.gov/BLAST.
2. The following are GenBank accession numbers for four different genes that show tissue-specific expression patterns.

(*continued*)

Exploring Genomics—continued

You will perform your searches on these genes.

NM_021588.1
NM_00739.1
AY260853.1
NM_004917

3. For each gene, carry out a nucleotide BLAST search using the accession numbers for your sequence query. Refer to the "Exploring Genomics" feature in Chapter 10 to refresh your memory on BLAST searches. Because the accession numbers are for nucleotide sequences, be sure to use the "nucleotide blast" (blastn) program when running your searches. Once you enter "blastn" under the "Choose Search Set" category, you should set the database to "Others (nr etc.)," so that you are not searching an organism-specific database.
4. For the top alignments for each gene, the "Links" column (far right) contains colored boxes labeled U (for UniGene expression data), E (Gene Expression Profiles), and G (Gene Information). Each of these boxes will link you to information about the gene. The UniGene link will show you a UniGene report. For some genes, upon entering UniGene you may need to click a link above the gene name before retrieving a UniGene report. Be sure to explore the "Expression Profile" link under the "Gene Expression" category in each UniGene report. Expression profiles will show a table of gene expression patterns in different tissues.

   Also explore the "GEO profiles" link under the "Gene Expression" category of the UniGene reports, when available. These links will take you to a number of gene-expression studies related to each gene of interest. Explore these resources for each gene, and then answer the following questions:

   a. What is the identity of each sequence, based on sequence alignment? How do you know this?

   b. What species was each gene cloned from?

   c. Which tissue(s) are known to express each gene?

   d. Does this gene show regulated expression during different times of development?

   e. Which gene shows the most restricted pattern of expression by being expressed in the fewest tissues?

LESSON FOUR: EPIGENETICS, DISEASE, AND THERAPY

KEY QUESTION(S): How do you read a scientific journal article for understanding?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes including reading articles
- Student Procedure:
  - Day 1: 45 minutes
  - Day 2: 45 minutes

LEARNING STYLES: Visual and auditory.

VOCABULARY:
Peer-review is the act of having another writer read what you have written and respond in terms of its effectiveness. This reader attempts to identify the writing's strengths and weaknesses, particularly how sound the science is, and then suggests strategies for revising it. The hope is that not only will the specific piece of writing be improved, but that future writing attempts will also be more successful. Peer-review happens with all types of writing, at any stage of the process, and with all levels of writers (Bokor, 2012).

LESSON SUMMARY: Students will understand more about the impact of epigenetic influence in the progression of disease and approach to therapy by performing a jigsaw activity using a basic science journal article that introduces the link between epigenetic influence and disease. Student groups are provided a copy of the article that has been broken down into segments to perform a jigsaw activity and create bullet points on a small white board or large post-it note. Following, a teacher-led presentation will present current research in the relationship between epigenetics and common diseases with additional information on therapeutic approaches to treating disease. This lecture will be followed with a guide to reading primary source journal articles on epigenetic treatment of disease using the high-interest topics of schizophrenia, cancer, anxiety and PTSD, and sexual orientation. Students will select an article to read as a homework assignment and complete a guide to reading the article to understand the research presented.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Identify key developments in the discovery of epigenetics and diseases/disorders.
2. Improve scientific literacy by reading primary sources
3. Read a scientific paper for understanding
4. Recognize that science is ever growing and building on previous discoveries
5. Conclude that research takes place around the globe and through publishing, discoveries are shared

STANDARDS:
SC.912.L.14.6
SC.912.L.16.9
SC.912.L.18.1
SC.912.N.1.2
SC.912.N.1.3
SC.912.N.1.4
SC.912.N.1.5
SC.912.N.2.4
SC.912.N.2.5

MATERIALS:

- Copies of *Epigenetic Influences and* Disease journal article that has been divided for Jigsaw Activity
- Small white boards or Large Post-it notes and markers for Jigsaw Activity
- Copies of *Guide to Reading Scientific Papers*, one per student
- Copies of *Guide to Reading Scientific Papers Worksheet*, one per student
- Copies of introductory journal articles, 2 full class sets or make available for student download from teacher web page.
    - Epigenetics of Breast Cancer
    - Epigenetics of Cancer
    - Epigenetics of Anxiety
    - Epigenetics of Schizophrenia
    - Epigenetics of Sexual Orientation

BACKGROUND INFORMATION:
While epigenetic changes are required for normal development and health, they can also be responsible for some disease states. Disrupting any of the three systems - methylation, histone modification, and RNA-associated silencing - that contribute to epigenetic alterations can cause abnormal activation or silencing of genes. Such disruptions have been associated with cancer, chromosomal instabilities, and mental retardation.

The first human disease to be linked to epigenetics was cancer, in 1983. Researchers found that diseased tissue from patients with colorectal cancer had less DNA methylation than normal tissue from the same patients. Because methylated genes are typically turned off, loss of DNA methylation can cause abnormally high gene activation by altering the arrangement of chromatin. On the other hand, too much methylation can undo the work of tumor suppressor genes, responsible for controlling cell division.

Because so many diseases involve epigenetic changes, it seems reasonable to try to approach through epigenetic treatments. These changes seem an ideal target because they are reversible, unlike DNA sequence mutations. The most popular of these treatments aim to alter either DNA methylation or histone acetylation. Caution in using epigenetic therapy is necessary because epigenetic processes and changes are so widespread. Despite this possible drawback, researchers are finding ways to specifically target abnormal cells with minimal damage to normal cells, and epigenetic therapy is beginning to look increasingly promising (Simmons, 2008).

ADVANCE PREPARATION:

- Make all photocopies
    - Student Handout - *Guide to Reading Scientific Papers*
    - Student Worksheet - *Guide to Reading Scientific Papers Worksheet*
    - Journal articles – class sets depending on student selections. Make available for review the day before to ensure enough copies are made OR make available on teacher web page for student download.
- Divide journal article, *Epigenetic Influence and Disease,* to prepare for Jigsaw Activity. Suggested divisions:
        - Introduction
        - What is Epigenetics? How Do Epigenetics Affect Genes? And Figure 1
        - DNA Methylation
        - Histone Modification
        - RNA-Associated Silencing (may need another resource)
        - Epigenetics and Disease – Some Examples (TABLE ONLY)
        - Epigenetics and Cancer
        - Epigenetics and Mental Retardation
        - Combating Disease with Epigenetic Therapy and Figure 2

- Gather small white boards or large post-it notes for student statements on their portion of the jigsaw.
- Read selected articles

IMPLEMENTATION NOTES:
The in-class activity/jigsaw of the article is designed to introduce the link between epigenetic influence and disease progression and also provides a first-look at a scientific journal article. This activity assumes students have had some exposure to reading a science journal article and can work independently to read assigned articles on their own. The *Guide to Reading Scientific Papers* and *Guide to Reading Scientific Papers Worksheet* has been adopted from *The Pompe Predicament*, Bokor 2012

ASSESSMENT SUGGESTIONS:

1. Student worksheet can be used for assessment.

EXTENSIONS:
ACTIVITIES:

- TedTalk video – Epigenetics and the influence of our genes, Courtney Griffins
  https://www.youtube.com/watch?v=JTBg6hqeuTg
- YouTube Video – Battling Cancer Using Epigenetics
  https://www.youtube.com/watch?v=xQH6mcmBRqk

RESOURCES/REFERENCES:

- Epigenetic Influence and Disease
  http://www.nature.com/scitable/topicpage/epigenetic-influences-and-disease-895#
- Epigenetics and Breast Cancer
  http://www.ncbi.nlm.nih.gov/pubmed/25588111
- Epigenetics and Anxiety
  http://www.ncbi.nlm.nih.gov/pubmed/27189589
- Epigenetics and Schizophrenia
  http://www.ncbi.nlm.nih.gov/pubmed/25476119
- Epigenetics and Sexual Orientation
  http://www.psychologicalscience.org/index.php/publications/sexual_orientation.html
- Epigenetics and Cancer
  http://tcr.amegroups.com/article/view/7940

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Day One (45 minutes) Jigsaw Introduction to Epigentics and Disease

1. (5 minutes) Explain to the class that the first article, *Epigenetic Influence and Disease*, will be used to provide details about the link between epigenetics and disease.

2. Project the first article *Epigenetic Influence and Disease*
   http://www.nature.com/scitable/topicpage/epigenetic-influences-and-disease-895#

3. (10 minutes) Ask student pairs to work together on the assigned part of their article (Jigsaw Activity). After reading their portion of the article, student pairs will create a visual to share with the class the important details of their portion of the article. Remind them of the foundation lessons over the past few days and to draw on those examples to expand their understanding.

4. (20 minutes) Students display their summaries of the article and present these findings to the class in the order that the information is delivered in the article.
   Students are encouraged to take notes and on their own copy of the article as needed and to ask questions for clarification.

5. (5 minutes) Give each student a copy of *Guide to Reading Scientific Papers*.
   Instruct the students to read the *Guide* silently.
   Provide a copy of *Guide to Reading Scientific Papers Worksheet* for homework completion.

6. While students are reading, project a list of the journal articles for students to select for homework reading.

7. (5 minutes) Instruct the students to read the journal article for homework following the suggestions in the *Guide* and complete the *Worksheet* while reading. They should be prepared to discuss their paper in a same article group and a mixed group.

Day Two (45 minutes) Journal Share Groups

1. (5 minutes) Present students with the following quick write prompt as a bell ringer:
   A. In three complete sentences, summarize the journal article you read last night.
   B. List one thing you learned.
   C. List one question you still have.
   Collect the writing prompt as students move to their journal groups.

2. (5 minutes) Introduce the importance of publishing scientific findings. Tell the students that in science, information is primarily shared with others through writing papers that are reviewed by other scientists for accuracy and clarity. These publications are the primary means to share new findings with others across the world and allow researchers to build on prior findings to make new discoveries. It is important for scientists to be good written and oral communicators (Bokor, 2012).

IMPLEMENTATION NOTE: There will be 2 different groupings for the students to participate;
The first grouping will be for students that selected the SAME journal article to read.
The second grouping will be a mixed discussion for students to share their specific article.

3. (5 minutes) Explain to students that they will be sharing their journal findings with students in 2 ways (see Implementation Note above). They should bring their article, their *Worksheet*, and any other supplemental materials/notes that will assist in the discussion. Move to first grouping.

4. (10 minutes) Students work in SAME article groups to discuss the details of their article. They should use their completed *Worksheet* to guide the discussion, paying attention to the following points:
    a. What was the purpose of the study?
    b. What questions were asked?
    c. What were the final answers?
    d. What was unique about the study?
    e. What is the next step?

5. (20 minutes) Students move to mixed grouping with 1 students/article chosen and will share their journal findings with the group, keeping in mind that each student read a different article. Instruct students to share more details than in the last grouping as none of the other students have read the same article.

6. (5 minutes) Regroup and ask students to share something they learned from the second grouping that was new and of interest to them. Suggest that students swap articles of interest with other members in the class for further reading/interest.

TEACHER PAGE: DISCUSSING A SCIENCE JOURNAL ARTICLE

Disease severity in children and adults with Pompe disease related to age and disease duration
http://amdapompe.ehclients.com/downloads/publications/Hagemans_Neurology_280605.pdf

Feel free to use any article. This one was selected to model because it is short and not very complicated, which will allow students to focus on the main parts and how to read an article rather than be burdened with jargon and methods during the introduction.

Things to point out:

1. Read the title: Disease severity in children and adults with Pompe disease related to age and disease duration.
2. Note the journal: Neurology. This is a peer-reviewed and highly respected journal.
3. Multiple authors contributed, indicating collaboration among many individuals. In this case, the funding for the project and the main research lab is indicated by the last name listed, Van der Ploeg.
4. In the bottom left, note the institutions represented, who funded the project, and potential conflict of interest. This information is telling regarding any bias that might be apparent or inadvertent.
5. Also, note the date the paper was originally received: Dec. 21, 2004. This is the date it was submitted to the journal. After going through peer review, suggested modifications are sent to the authors for the chance to revise. The revision is then either approved or denied publishing. In this case, it was accepted on March 23, 2005 and appeared in print in June, 2005.
6. The layout of papers differ. Each journal has its own way of arranging the text on the page, as well as specific sections they do or don't want included.

Use the reading guide with the students, calling attention to each section and highlighting key points.

The **Abstract** provides a nice summary of the paper.

- This one is particularly short, reflective of a short paper. 255 individuals with Pompe completed a survey to gather information about the natural course of the disease.

The **Introduction** gives a history of the topic and discusses what others have found. It also poses the research question(s).

- The author devotes the first paragraph to a description of Pompe. This provides background. They then discuss what the current treatment available is – ERT is just in clinical trials (this is why it is important to note the date). The authors want to help determine the ideal time to administer the treatment, so they need to understand the natural course of the disease, particularly for individuals with late-onset.

Methods and Materials are most meaningful to those in the field who might want to repeat the research or to help clarify results. Skip this section, but note that as you become more experienced with reading primary sources, it can be helpful to return to this section to better understand some of the results and discussion.

- Interesting how tiny the font is for the methods section in this journal, indicating this section is really for those that need to know all the details of carrying out the experiment. Not necessary for our students to understand the paper.

Results are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly. Just a skim of this section will suffice.

- Font size is increased a bit, but not as large as the introduction or conclusions. There are a lot of percentages given, and description of the figures. It does give some nice descriptions of the symptoms and pathologies affected individuals reported, and the age of onset.

The **Discussion/Conclusion** is where the author explains what happened. In this section, the questions should be answered. This is usually where the author reflects on the work and its meaning in relation to other findings and to the field in general.

- General findings are discussed such as disease severity increases with duration. This is a progressive disease, so not unexpected. It wasn't correlated with age however, supporting the diverse set of symptoms that present at all ages. Other than the classic infantile form, all other forms are heterogeneous. They do point out the subset of children who were affected very young, as presenting more severe symptoms earlier and consistently: those who are respirator dependent, are likely to be wheelchair dependent. There concluding remarks suggest ERT should be started as early as possible, before "irreversible damage has occurred" such as muscle weakness requiring a wheelchair or respiratory assistance.

Add your own interpretations to these:

- What was the purpose of the study? Compile and analyze data about the natural course of Pompe disease (how does it progress in individuals not on enzyme replacement therapy?)
- What questions were asked? Is there a relation between age of onset, duration, and symptom severity?
- What were the final answers? Only in those presenting symptoms very young is there an expected course; all others present a mix of symptoms.
- What was unique about the study? Surveyed 255 patients, quite a large sample size for a disease so rare, to record the natural history of the disease with one standard questionnaire. Rather than piecing bits of case reports together for the literature, they were able to standardize the questions and therefore the results.
- What is the next step? Compare to the next generation who receives ERT. Does ERT make a difference in severity and duration? Can those who present symptoms early, be treated with enzyme replacement therapy and delay serious symptoms? For how long?

STUDENT PAGE: GUIDE TO READING SCIENTIFIC PAPERS

Scientific papers can be daunting, full of details and language that is unfamiliar. Scientific papers are best read and considered in small, manageable pieces. Unless you plan to repeat the experiment, you really just need to get the general idea of the questions and answers along with the big idea of the paper. As you become more comfortable with reading journal articles, you will naturally read for more depth and content. When starting out however, the key is knowing what to read, what to skim, and what to skip. Yes. There are parts of a paper that you can skip.

The paper is divided into sections, based generally on the scientific method. Most research papers contain the following sections: Abstract, Introduction, Methods/Materials, Results, Discussion, sometimes Conclusions, and References.

The **Abstract** provides a nice summary of the paper. It might have some unknown words or numbers, but it gives the overall flavor of the paper. It should be read and then re-read at the end.

The **Introduction** gives a history of the topic and discusses what others have found. It also poses the research question(s).

Methods and Materials are most meaningful to those in the field who might want to repeat the research or to help clarify results. Skip this section, but note that as you become more experienced with reading primary sources, it can be helpful to return to this section to better understand some of the results and discussion.

Results are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly. Just a skim of this section will suffice.

The **Discussion/Conclusion** is where the author explains what happened. In this section, the questions should be answered. This is usually where the author reflects on the work and its meaning in relation to other findings and to the field in general.

Re-read the Abstract. Does it make more sense now? It should tie everything together.

Vocabulary. You may need to look words up if you can't figure them out using context clues. You can miss a really important point of the paper if you don't understand the language.

In summary:

- Absolutely read the Abstract, Introduction, Discussion, and then the Abstract again.
- Skim the results.
- Skip the methods/materials.

In the end, you want to be able to answer the following questions with some confidence:

- What was the purpose of the study?
- What questions were asked?
- What were the final answers?
- What was unique about the study?
- What is the next step?

STUDENT PAGE: GUIDE TO READING SCIENTIFIC PAPERS WORKSHEET

Name: _____

Paper name: _____

1. What was the purpose of the study?

2. What questions were asked?

3. What were the final answers?

4. What was unique about the study?

5. What is the next step?

LESSON FIVE: EPIGENETIC IMPACT OF CANCER THERAPIES

KEY QUESTION(S): How can an epigenetic approach to therapy personalize cancer treatment for a patient?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure
    - Day 1: 45 minutes to perform Microarray testing
    - Day 2: 45 minutes to evaluate results and complete research on epigenetic influence and determine appropriate chemotherapy protocol

LEARNING STYLES: Visual and kinesthetic.

VOCABULARY:

Complementary DNA (cDNA): A single strand of DNA synthesized in the lab to complement the bases in a given strand of messenger RNA. Complementary DNA represents the parts of a gene that are expressed in a cell to produce a protein.

Messenger RNA (mRNA): Serves as a template for protein synthesis. mRNA matched/complements a specific sequence of DNA.

Microarray: used in qualitative/quantitative assays of DNA-DNA or DNA-RNA binding to measure profiles of gene expression, often to compare gene expression between normal and abnormal (cancer) cells.

LESSON SUMMARY: Students will model how scientists use DNA microarrays to determine levels of gene expression in breast cancer patients. Provided with patient history, commonly used drugs to treat breast cancer, and drug interactions and limitations based on specific gene expression, students will determine the best (and worst) course of action for treating each patient and present their findings in a laboratory report.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Discuss the role of epigenetics on determining treatment for disease, specifically cancer.
2. Describe that cancer is a disease that differs from person-to-person and treatment must be customized for optimal results.
3. Describe how Microarrays are used to determine gene expression.
4. Perform and Evaluate the results of a Microarray.
5. Explain how understanding gene expression can lead to improved treatments for disease.

STANDARDS:

SC.912.L.14.6
SC.912.L.16.2
SC.912.L.16.9
SC.912.L.16.6
SC.912.L.16.10
SC.912.N.1.1
SC.912.N.1.7
SC.912.N.3.5
SC.912.N.4.1

MATERIALS:

- 96-well Microarray plates
- Microcentrofuge/Eppendorf tubes (8/student pair)
- Microcentrifuge/Eppendorf tube rack
- Disposable pipettes or adjustable micropipettes
- Indelible marker/Sharpie
- 200 mL vinegar (acid pH to represent normal expression)
- 200 mL weak sodium hydroxide (basic pH to represent under-expression)
- 200 mL DI water (neutral pH to represent normal expression)
- 200 mL indicator solution – phenolphthalein or universal indicator. Determine prior to lesson what color change is expected with appropriate indicator and list for students on Table 1.

BACKGROUND INFORMATION:
If a DNA molecule mutates, it may produce faulty proteins. If these proteins are involved in controlling the processes of cell growth and division, the mutation could trigger a cell to become abnormal and divide uncontrollably. For many years, this was the only mechanism known to cause cancer. Treatment of this type of cancer mainly relied on trying to destroy the mutated cells.

Researchers have now discovered that cancer can be triggered by **epigenetic changes** -modifications to the mechanisms associated with DNA that *alter gene expression without mutating the original DNA*. These changes are like switches turning genes on and off. Some epigenetic effects turn on/activate genes that stimulate tumor growth; other effects turn off/silence genes that would normally suppress tumor growth. Since epigenetic changes do not alter the DNA sequence itself, they hold the promise of being chemically reversed with drug (and potentially nutritional) therapies. Cancer may be caused by several different mutations or epigenetic changes that cause genes to be expressed (turned on) and/or silenced (turned off) when they should not be. By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer.

One way scientists try to determine which genes are working abnormally is to use a DNA microarray. These gene-expression "fingerprints" allow a doctor to determine two vital characteristics of the cancer. #1: the genes involved in a patient's cancer and #2: the possible reaction of each patient to different drug treatments. This activity models how doctors use microarrays to determine levels of gene expression in breast cancer patients and then choose treatments based on what they learn.

ADVANCE PREPARATION:

1. Copy *Student Worksheet: Epigenetic Microarray Diagnosis of Cancer and Determination of Therapy* – 1 per student.
   Copy *How DNA Microarrays Work* 1 per student
   Copy *Cancer Therapy Options* – 1 per student pair
   Adapted from Nova Teacher *Ghost in Your Genes* Classroom Activity
2. Prepare labeled patient tubes as indicated, 1 set of tubes for each partner group.
   I usually have 12 partner groups and make 24 tubes just to be prepared.
   a. J-1 = acid pH J-2 = base pH J-3 = neutral pH
   b. B-1 = acid pH B-2 = base pH B-3 = neutral pH
3. Fill labeled tubes with appropriate acid, base, or neutral solutions – 2 per student pair
   Using ***phenolphthalein*** as your indicator will produce the following results:
   a. Tubes labeled "1"/ acids will be clear = normal expression
   b. Tubes labeled "2"/bases will be light pink = under-expressed
   c. Tubes labeled "3"/water will be dark pink = over-expressed
   If you choose to use a different indicator, make sure to pay attention to the color change results.
4. Label tubes "indicator" and fill with selected indicator solution.

5. Prepare student stations/pair groups
    a. IN RACK
       2 each Patient J – tubes #1, 2, 3
       2 each Patient B – tubes #1, 2, 3
       2 indicator tubes
    b. 7 disposable pipettes OR
       p20 adjustable micropipette and tips
    c. 96-well plate
    d. Disposal beaker/bin

PROCEDURE WITH TIME ESTIMATES:

DAY 1: Data Collection and Preliminary Analysis

1. (5 minutes) Tell the students they are now going to take on the role of a laboratory technician. Patient samples have been submitted, and each lab team is to perform the tests and complete the lab report sheet to inform the requesting clinician of the results.

2. (5 minutes) Distribute *Epigenetic Microarray Diagnosis of Cancer and Determination of Therapy* and *How DNA Microarrays Work* to each student.
   Ask students to work in groups of 2 (Twelve groups recommended.)

3. (5 minutes) As students gather at lab stations, ask them to read the *How DNA Microarrays Work,* and *Background information*, *Key Terms*, and *Activity Summary* from *Epigenetic Microarray Diagnosis of Cancer and Determination of Therapy.* Be sure to point out that this is an actual laboratory protocol that oncologists use to determine the best combination therapy for the epigenetic expression of oncogenes &/or tumor suppressor genes for a specific patient.

4. (15 minutes) Tell the students to follow the directions given for the Microarray Assay, steps #1 & 2 and record their results in Table #1.
   + for over-expression
   - for under-expression
   0 for normal expression

5. (5 minutes) Distribute the *Cancer Therapy Options* sheet to each student pair. Explain that this sheet is vital in interpreting their results. Each medication describes the drug classification, mechanism of action, and recommended use/limitations for patient delivery. Not all medications are helpful to a patient; some may inert and other may cause more damage.

6. (10 minutes) Students will use the last 10 minutes to begin interpreting the data, ask questions, and clean up their laboratory station. Remind them they will complete their analysis and evaluation the following day.

DAY 2: Complete Analysis and Interpretation, Research

7. (5 minutes) Have students rejoin their partner with data sheets and *Cancer Therapy Options* sheet. Ask for any questions to clarify the laboratory simulation from the previous day.

8. (40 minutes) Students work with their partner to complete Table 2 – Therapy Recommendations, Think About It question, and Table 3. Table 3 requires an internet connection so students can access a gene databank, such as www.ncbi.nlm.nih.gov/sites/entrez?db=gene. This may be completed as a homework assignment and turned in the following day.

ASSESSMENT SUGGESTIONS:

1. Lab report sheet can be collected.

RESOURCES/REFERENCES:

- Nova Ghost in Your Genes Classroom Activity http://www.pbs.org/wgbh/nova/education/activities/3413_genes.html
- Q&A Epigenetic Therapy http://www.pbs.org/wgbh/nova/body/epigenetic-therapy.html
- Ask the Expert http://www.pbs.org/wgbh/nova/genes/expert.html
- HHMI Dr. Sawyer Cancer Lecture Highlights http://www.hhmi.org/biointeractive/cancer-genetic-disease-video-highlights

Student Page: Epigenetic Microarray Diagnosis of Cancer and Determination of Therapy

Background Information

If a DNA molecule mutates, it may produce faulty proteins. If these proteins are involved in controlling the processes of cell growth and division, the mutation could trigger a cell to become abnormal and divide uncontrollably. For many years, this was the only mechanism known to cause cancer. Treatment of this type of cancer mainly relied on trying to destroy the mutated cells through surgical removal, undirected chemotherapy, and radiation (cut, poison, burn).

Researchers have now discovered that cancer can be triggered by **epigenetic changes** -modifications to the mechanisms associated with DNA that *alter gene expression without mutating the original DNA*. These changes are like switches turning genes on and off. Some epigenetic effects turn on/activate genes that stimulate tumor growth; other effects turn off/silence genes that would normally suppress tumor growth. Since epigenetic changes do not alter the DNA sequence itself, they hold the promise of being chemically reversed with drug (and potentially nutritional) therapies. Cancer may be caused by several different mutations or epigenetic changes that cause genes to be expressed (turned on) and/or silenced (turned off) when they should not be. By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer.

One way scientists try to determine which genes are working abnormally is to use a DNA microarray. These gene-expression "fingerprints" allow a doctor to determine two vital characteristics of the cancer. #1: the genes involved in a patient's cancer and #2: the possible reaction of each patient to different drug treatments. This activity models how doctors use microarrays to determine levels of gene expression in breast cancer patients and then choose treatments based on what they learn.

Key Terms

- **complementary DNA (cDNA):** A single strand of DNA synthesized in the lab to complement the bases in a given strand of messenger RNA. Complementary DNA represents the parts of a gene that are expressed in a cell to produce a protein.

- **DNA microarray:** A collection of microscopic DNA spots attached to a solid surface, such as glass, plastic, or silicon chip, forming an array. Scientists use DNA microarrays to measure gene expression levels.

- **messenger RNA (mRNA):** Serves as a template for protein synthesis. mRNA matched/complements a specific sequence of DNA.

Activity Summary

You will be playing the role of oncologists specializing in breast cancer and will be conducting **Microarray Analyses** on two newly diagnosed breast cancer patients, Mrs. Jones and Mrs. Brown, to determine which therapy will work best for their specific breast cancer.

- Mrs. Jones is a 46-year-old African-American woman with no family history of breast cancer.
- Mrs. Brown is a 63-year-old Caucasian woman who has previously had breast cancer and a maternal history of breast cancer.

Students will model how scientists use DNA microarrays to determine levels of gene expression in breast cancer patients and then select the best treatment for each patient based on the results of the Microarray Analysis.

Procedure

1. Prepare your Microarray plate
    a. You will be using a 96 well plate.
    Rows A and B for Mrs. Jones
    Rows G and H for Mrs. Brown
    b. Remember, the wells of the microarray contain single-stranded sequences of DNA specific to the genes of interest. Each well contains a different gene.
    We will begin by loading our patient's cDNA into the wells
    c. Follow the grid below, adding 30 microliters of the labeled substance into the wells as indicated.

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**	J-1	J-1	J-2	J-2	J-3	J-2	J-3	J-1	EMPTY
	B	J-3	J-1	J-3	J-1	J-2	J-3	J-2	J-3	
Mrs. Brown	**G**	B-3	B-2	B-3	B-2	B-1	B-2	B-1	B-3	
	H	B-1	B-2	B-1	B-2	B-2	B-3	B-2	B-1	

2. Add your mRNA indicator
    a. Once the cDNA of the patients' cancer tissue is loaded into each well, we can detect the over- or under-expression of the genes through fluorescent or color-labeled mRNA tags.
    The result can then be read by computer (high throughput) or visual analysis of the results.
    b. Add 30 microliters of the mRNA indicator into each of the wells for Mrs. Jones and Mrs. Brown.
    c. Indicate the results for each well in Table 1. Follow the key provided.

3. Interpret your results
    a. Use the *Cancer Therapy Options* handout to determine which therapies might be indicated for each patient.
    b. If the genes listed in the "Do not use if" category for each therapy are expressed in the manner indicated, then the patient would react badly or not respond to the treatment.
    c. Record your recommendations on Table 2.

4. What do the wells/genes signify?
    a. The seeded genes used in Microarray testing for cancer therapy are the most common genetic/epigenetic profiles linked to, in this case, breast cancer development.
    b. In Table 3, use the following database source to determine the effect modifications to these genes have on cancer development and/or cancer therapies. Simply type in the number-letter sequence in the search window at the top of the page.
    www.ncbi.nlm.nih.gov/sites/entrez?db=gene

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3	EMPTY
	B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7	
Mrs. Brown	**G**	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3	
	H	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7	

DATA ANALYSIS

TABLE 1:

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**									**+** = over-expressed (_____)
	B									**-** = under-expressed (_____)
Mrs. Brown	**G**									**0** = normal expression (_____)
	H									

TABLE 2:

	Mrs. Jones	**Mrs. Brown**
Therapy Recommendations		

Think About It:

Some genes, such as ERB-B2 and ESR1, have been found to be associated with particular diseases or conditions such as cancer. Other genes, such as the ABC-B2 gene, are not associated with a disease but are involved in resistance to certain drugs or treatments.

Why would it be useful to test for the expressions of genes like the ABC-B2 gene on a microarray?

Can you provide an example of a drug in this activity that is contraindicated (will not work) or might cause harm to the patient(s)?

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3	EMPTY
	B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7	
Mrs. Brown	**G**	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3	
	H	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7	

TABLE 3:

Gene	Gene Name	Description
ESR1		
ABC-C6		
BCL2		
DPYD		
TOP2A		
GSTP1		
CDC2		
GATA3		
DHFR		
EGFR		
ERB-B2		
ABC-B2		
MT1		
TGFB3		
ANXA		
GRB7		

Teacher Page: Epigenetic Microarray Diagnosis and Determination of Therapy

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**	J-1	J-1	J-2	J-2	J-3	J-2	J-3	J-1	EMPTY
	B	J-3	J-1	J-3	J-1	J-2	J-3	J-2	J-3	
Mrs. Brown	**G**	B-3	B-2	B-3	B-2	B-1	B-2	B-1	B-3	
	H	B-1	B-2	B-1	B-2	B-2	B-3	B-2	B-1	

TABLE 1:

		1	2	3	4	5	6	7	8	9 - 12
Mrs. Jones	**A**	0	0	-	-	+	-	+	0	**+** = over-expressed (_____)
	B	+	0	+	0	-	+	-	+	**-** = under-expressed (_____)
Mrs. Brown	**G**	+	-	+	-	0	-	0	+	**0** = normal expression (_____)
	H	0	-	0	-	-	+	-	0	

TABLE 2:

	Mrs. Jones	**Mrs. Brown**
Therapy Recommendations	Combination of cyclophosphamide, doxorubicin, fluorouracil, and trastuzumab	Combination of cyclophosphamide, doxorubicin, and tamoxifin

Think About It:

Some genes, such as ERB-B2 and ESR1, have been found to be associated with particular diseases or conditions such as cancer. Other genes, such as the ABC-B2 gene, are not associated with a disease but are involved in resistance to certain drugs or treatments.

Why would it be useful to test for the expressions of genes like the ABC-B2 gene on a microarray? Can you provide an example of a drug in this activity that is contraindicated (will not work) or might cause harm to the patient(s)?

If the gene is strongly expressed, it would mean that a particular treatment might not work, or might even be harmful to the person taking that drug.

TABLE 3:

Gene	Gene Name	Description
ESR1	Estrogen Receptor 1	Binds with estrogen hormone; Over-expression of ESR1 will feed estrogen hormone breast cancer tumors
ABC-C6	ATP Binding Cassette	Associated with drug resistance and blood cell cancers
BCL2	B cell leukemia	Blocks apoptosis in some lymphocytes
DPYD	Dihydropyrimidine dehydrogenase	Toxicity risk when receiving fluorouracil chemotherapy
TOP2A	Topoisomerase 2 alpha	Target for anticancer drugs; gene controls transcription and translation
GSTP1	Glutathione S-transferase	Role in susceptibility to cancer
CDC2	Cyclin dependent kinase 1	AKA CDK1: Control cell division; responds to phosphorylation and methylation
GATA3	GATA binding protein 3	Useful marker for METASTATIC breast cancer
DHFR	Dihydrofolate reductase	Role in DNA synthesis; target for treatment of cancer; some toxicity
EGFR	Epidermal growth factor receptor	Mutations associated with lung cancer, promotes tumor angiogenesis (increased blood supply to tumors)
ERB-B2	Avian erythroblastic leukemia viral oncogene	Proto-oncogene; over-expression of this gene has been reported in numerous cancers, including breast.
ABC-B2 AKA TAP1	ATP binding cassette family B	Low/defective gene expression can be higher risk to breast cancer metastasis.
MT1	Melatonin receptor Metallothionein 1A	Expression of gene relative to breast cancer survival
TGFB3	Transforming growth factor beta 3	Cell cycle control of mitosis = proto-oncogene
ANXA	Annexin 1 Aka lipocortin 1	Promotion of apoptosis If over-expressed it's a target to control estrogen fed cancers
GRB7	Growth factor receptor bound protein 7	Over-expression = poor prognosis for breast cancer survival

How DNA Microarrays Work

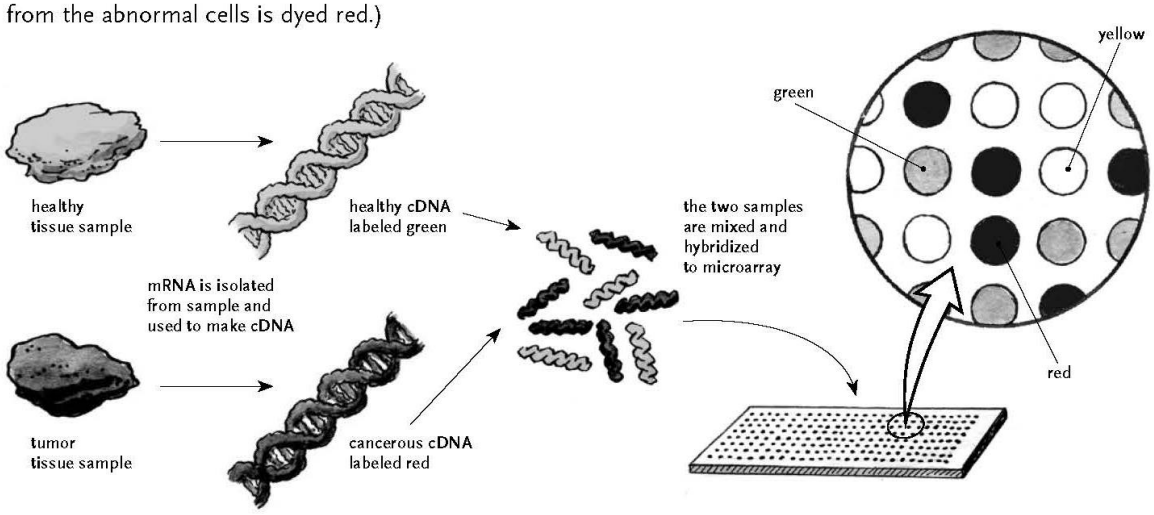
In each type of cell, like a muscle cell or a skin cell, different genes are expressed (turned on) or silenced (turned off). If the cells that are turned on mutate, they could—depending on what role they play in the cell—trigger the cell to become abnormal and divide uncontrollably, causing cancer.

By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer. One way they do this is to use a DNA microarray to determine the expression levels of genes. When a gene is expressed in a cell, it generates messenger RNA (mRNA). Overexpressed genes generate more mRNA than underexpressed genes. This can be detected on the microarray.

The first step in using a microarray is to collect healthy and cancerous tissue samples from the patient. This way, doctors can look at what genes are turned on and off in the healthy cells compared to the cancerous cells. Once the tissues samples are obtained, the messenger RNA (mRNA) is isolated from the samples. The mRNA is color-coded with fluorescent tags and used to make a DNA copy (the mRNA from the healthy cells is dyed green; the mRNA from the abnormal cells is dyed red.)

The DNA copy that is made, called complementary DNA (cDNA), is then applied to the microarray. The cDNA binds to complementary base pairs in each of the spots on the array, a process known as hybridization. Based on how the DNA binds together, each spot will appear red, green, or yellow (a combination of red and green) when scanned with a laser.

- A red spot indicates that that gene was strongly expressed in cancer cells. (In your experiment these spots will be dark pink.)
- A green spot indicates that that gene was strongly repressed in cancer cells. (In your experiment these spots will be light pink.)
- If a spot turns yellow, it means that that gene was neither strongly expressed nor strongly repressed in cancer cells. (In your experiment these spots will be clear.)
- A black spot indicates that none of the patient's cDNA has bonded to the DNA in the gene located in that spot. This indicates that the gene is inactive. (All of the genes in your experiment are active.)



A microarray is an orderly arrangement of rows and columns on a surface like a glass slide. Each of the spots on an array contains single-stranded DNA molecules that correspond to a single gene. An array can contain a few, or thousands, of genes.

Cancer Therapies

Cyclophosphamide

Brand Names: Cytoxan, Neosar

What it is: chemotherapy drug

How it works: Cyclophosphamide acts by transferring one or more saturated carbon atoms to cellular macromolecules. This damages the cancer cell DNA, and slows or stops the growth of the cancer cells.

Do not use if one or more is true: ABC-B2 = +
GSTP1 = +
MT1 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Doxorubicin

Brand Names: Adriamycin, Rubex

What it is: chemotherapy drug

How it works: Doxorubicin inhibits RNA synthesis and causes DNA strand breakage. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
ABC-C6 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Fluorouracil (5-FU)

Brand Name: Adrucil

What it is: chemotherapy drug

How it works: Fluorouracil binds with and deactivates a key enzyme (thymidylate synthetase) in thymidine biosynthesis. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
BCL2 = +
DPYD = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Methotrexate

Brand Names: Mexate, Folex

What it is: chemotherapy drug

How it works: Methotrexate binds to and inactivates the enzyme dihydrofolate reductase (DHFR), and inhibits the synthesis of purine and pyrimidine. This prevents the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
DHFR = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Paclitaxel

Brand Name: Taxol

What it is: chemotherapy drug

How it works: Paclitaxel binds to tubulin and blocks cell division. This slows or stops the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
ERB-B2 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Tamoxifen

Brand Name: Nolvadex

What it is: hormone (antiestrogen)

How it works: Tamoxifen binds to the estrogen receptor, preventing cell growth. It also affects the cycling of the cell in the natural cell cycle.

Do not use if one or more is true: ESR1 = 0 or –
ERB-B2 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Trastuzumab

Brand Name: Herceptin

What it is: monoclonal antibody

How it works: Herceptin binds to the ERB-B2 growth factor receptor and prevents the cell from dividing.

Do not use if one or more is true: ERB-B2 = 0 or –

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Name: _____ Class Pd _____ ID: A

Epigenetics: The Biochemistry of DNA

Multiple Choice
Identify the choice that best completes the statement or answers the question.

____ 1) Which of the following mechanisms is (are) used to coordinate the expression of multiple, related genes in eukaryotic cells?
A) Genes are organized into clusters, with local chromatin structures influencing the expression of all the genes at once.
B) Environmental signals enter the cell and bind directly to promoters.
C) A single repressor is able to turn off several related genes.
D) The genes share a common intragenic sequence, and allow several activators to turn on their transcription, regardless of location.

____ 2) _____ groups are added to the chromatin structure to repress transcription of DNA for protein synthesis.
A) acetyl
B) hydroxyl
C) methyl
D) acid

____ 3) Two potential devices that eukaryotic cells use to regulate transcription are
A) DNA methylation and histone amplification.
B) DNA amplification and histone methylation.
C) DNA methylation and histone modification.
D) histone amplification and DNA acetylation.

____ 4) Modification in an organism's gene function/expression that is not attributed to mutations in the nucleotide sequence is defined as:
A) euploidies
B) epigenetics
C) aneuploidies
D) chromosomal modifications

____ 5) In humans, the embryonic and fetal forms of hemoglobin have a higher affinity for oxygen than that of adults. This is due to
A) identical genes that generate many copies of the ribosomes needed for fetal globin production.
B) pseudogenes, which interfere with gene expression in adults.
C) nonidentical genes that produce different versions of globins during development.
D) histone proteins changing shape during embryonic development.

____ 6) The protein complexes that are responsible for the coiling of chromatin for stability and organization of DNA are _____.
A) antibodies
B) nucleosomes
C) histones
D) enzymes

____ 7) Closed chromatin is also know as:
A) euchromatin
B) open chromatin
C) heterochromatin
D) transcriptionally active

____ 8) Open chromatin is considered to be _____ due to the _____ organization of DNA.
A) transcriptionally inactive/closed
B) transcriptionally active/closed
C) transcriptionally inactive/open
D) transcriptionally active/open

____ 9) Mutations in which of the following genes lead to transformations in the identity of entire body parts?
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B) morphogens
C) egg-polarity genes
D) homeotic genes

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B) chromosomal rearrangements.
C) translocation.
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A) have turned off or slowed down the process of transcription.
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C) induce protein synthesis by not allowing repressors to bind to it.
D) be replicating nearly continuously.

Short Answer

13) Describe how lifetime events/exposure can lead to the formation of "epigentic tags" that may lead to diseases like cancer. Provide a specific example, with details, that you learned about in the video or laboratory activity.

14) Why is the beta-globin chain of hemoglobin a great model for studying epigenetic expression of specific genes? Describe the developmental expression of the globin genes during growth and provide details of expression for different types of the globin locus.

15) Use the Word Bank provided to complete the diagram below

centromere	chromatin	DNA/double helix
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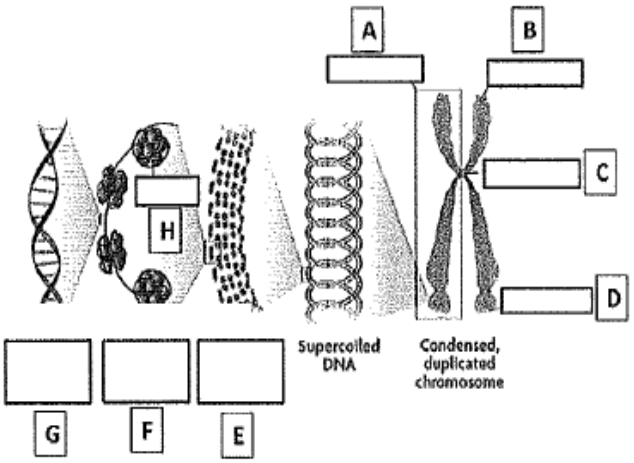

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D: H:

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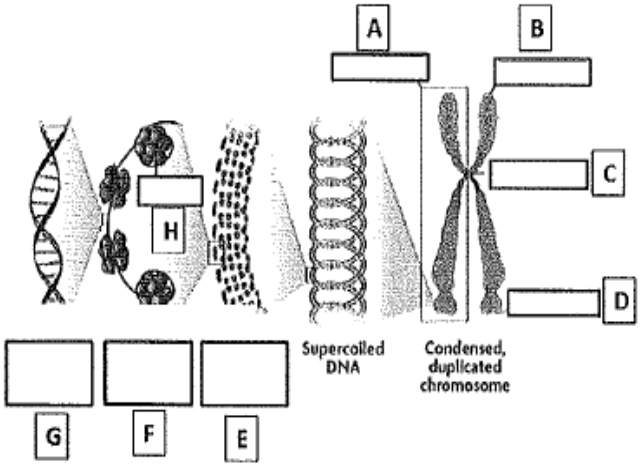

A: E:

B: F:

C: G:

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Exposure to chemicals, starvation, and medications can all alter the chemical tags/(acetyl + methyl) groups that impact the open + closed organization of DNA for transcription of genes.

for example, acetylation of a protooncogene can promote cell division, allowing the ~~DN~~ gene sequences that promote cancer development, to be constantly transcribed and increase the 2rate of cell division.

14) Why is the beta-globin chain of hemoglobin a great model for studying epigenetic expression of specific genes? Describe the developmental expression of the globin genes during growth and provide details of expression for different types of the globin locus.

The beta-globin gene locus is composed of several developmental specific gene segments that are each expressed at different times to allow for proper production of hemoglobin. Embryonic + fetal hemoglobin are produced from conception to just after birth and adult beta globin is produced just after birth. The type of globin expressed is determined by the organization of chromatin around the histones.

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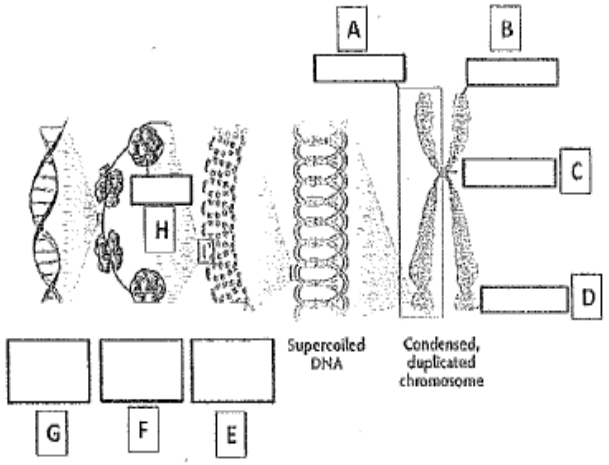

A: Sister chromatid

B: telomere

C: centromere

D: telomere

E: chromatin

F: nucleosome

G: DNA/Double helix

H: ~~nucleosome~~ histone

Name: Key Class Pd _____ ID: B

Epigenetics: The Biochemistry of DNA

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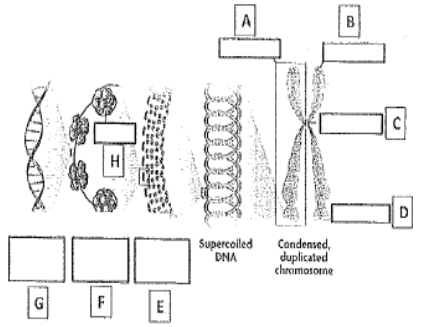

See Key A

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What is Epigenetics?
http://www.whatisepigenetics.com/