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**Week 8: DNA and Biotechnology**

**Part 1:Connect Resources on Biotechnology**

A. Take notes on vocabulary or information from the simulation “DNA biology and technology: isolation of DNA”.

* DNA exists in all cells but is housed inside the nucleus of eukaryotic cells. In order to extract it, associated proteins and membranes must be removed.
* DNA is released by cell lysis in a sodium chloride solution that makes the DNA more readily condense when later exposed to alcohol.
* Detergent in the solution emulsifies membrane lipids and proteins, keeping them from interacting with DNA.
* Protein-digesting enzymes in meat tenderizer degrade proteins, which then releases the DNA.
* Cold ethanol causes DNA to precipitate (solidify and become visible) while preserving fragile hydrogen bonds, thus preventing damage to the strands.
* DNA isolation is an important procedure that allows researchers to study how DNA functions.

B. Take notes on vocabulary or information from the simulation “DNA biology and technology: gel electrophoresis”.

* Individuals with different genetic phenotypes may have different sized DNA fragments when the DNA is cut by enzymes.
* DNA fragments can be separated by size using an electrophoresis gel.
* DNA fragments are negatively charged and when placed into an electrophoresis gel will travel through an electrical field towards the positive pole.
* Electrophoresis buffer floods the gel and allows for the creation of an electric field.
* Different DNA fragment sizes will be separated as smaller pieces will move more rapidly.
* Loading dye allows for the visualization of the location of the DNA fragments in the gel.
* Ethidium bromide binds to the DNA and will fluoresce under UV light showing the location of DNA fragments.
* Ethidium bromide binds to the DNA and will fluoresce under UV light showing the location of DNA fragments. Other less toxic alternatives, like SYBR Green I, are commonly used as well. Any of these chemicals have a similar effect, to allow visualization of the DNA bands in the gel.

~~C. Take notes on vocabulary or information from the simulation “Evidence of evolution: molecular evidence”.~~

D. Take notes on vocabulary or information from the simulation “Bacterial genetics: PCR”.

* Living organisms organize genetic information using DNA. This is the main blueprint or storehouse of information needed by cells to carry out basic cellular functions.
* DNA is a molecule made up of units called nucleotides. Nucleotides have 3 parts, a deoxyribose sugar with a phosphate connected to one end, and one of four nitrogen-containing bases (adenine, thymine, cytosine, guanine) on the other side of the deoxyribose sugar. These are linked together to form a chain that connects to an opposite chain, forming a double-stranded DNA molecule.
* The nitrogen-containing bases form hydrogen bonds with a counterpart base on the opposite chain forming the classic double helix structure of DNA. Because the base pairing is specific for each base, cells can make copies of DNA by using one strand as a template to create a new, opposite strand. We can take advantage of this characteristic to work with DNA in the laboratory.
* The sequence of nitrogen-containing bases in the DNA provides the information to create specific products, like enzymes, that carry out cell metabolism. Changes in these sequences are mutations that may or may not change how the information is processed.
* Detecting the pattern of nitrogen-containing bases allows for identification of DNA to a particular individual. These sequences can be manipulated in the lab to produce desirable products or learn more about the organism itself.
* To acquire enough DNA for analysis, the PCR technique is used. To start, a template DNA strand is needed.
* Several key materials are needed for PCR. A heat stable polymerase such as Taq polymerase builds the new DNA molecules from free deoxynucleotides added to the reaction. The process is carried out at high temperatures so a heat stable polymerase is used, generally taken from thermophilic (heat loving/heat thriving) bacteria.
* Also needed are primers, short DNA fragments with a known sequence used to direct the polymerase to specific areas on the template DNA strand that flank the target DNA sequence.
* Prepared samples are placed in a thermocycler, a machine that repeats three temperature changes needed for each PCR cycle.
* The PCR cycle has 3 steps:
  + **Denaturation** - Heating to 95°C is used to break the hydrogen bonds between nitrogenous bases and separate the strands of DNA.
  + **Priming** – After cooling, the primers attach to their complementary sites on the separated DNA strands.
  + **Extension** – The sample is warmed back up to 72°C and the polymerase begins synthesizing new strands starting at the primers.
* These steps are repeated over and over till the desired amount of DNA is synthesized.

E. Take notes on vocabulary or information from the simulation “Bacterial genetics: DNA profiling”.

* The nitrogen-containing bases form hydrogen bonds with a counterpart base on the opposite chain, forming the classic double helix structure of DNA. Because the base pairing is specific for each base, cells can make copies of DNA by using one strand as a template to create a new, opposite strand. We can take advantage of this characteristic to work with DNA in the laboratory.
* The sequence of nitrogen-containing bases in the DNA provides the information to create specific products, like enzymes, that carry out cell metabolism. Changes in these sequences are mutations that may or may not change how the information is processed.
* Detecting the pattern of nitrogen-containing bases allows for identification of DNA to a particular individual. These sequences can be manipulated in the lab to produce desirable products or learn more about the organism itself.
* Individual organisms can be identified by slight differences in their nucleotide sequence. This can either be done by sequencing a long strand of DNA or by a quicker method referred to as DNA profiling. In this method, DNA is cut with enzymes that recognize specific sequences and then the fragments are separated on a gel to reveal a specific pattern for an individual. This pattern is sometimes referred to as a DNA fingerprint, since it is unique for genetically different individuals.
* DNA profiling can be used to identify individuals for crime scene investigations, determining paternity of a child, and identifying victims from disasters. It can also be used to track the causative agent of an epidemic.
* Restriction enzymes (restriction endonucleases) are produced by bacteria to cut the DNA of viruses that infect them. We can use these natural enzymes in the lab to cut DNA samples to create DNA profiles. Different restriction enzymes recognize specific sequences of DNA to cut.
* Living organisms organize genetic information using DNA. This is the main blueprint, or storehouse, of information needed by cells to carry out basic cellular functions.
* DNA is a molecule made up of units called nucleotides. Nucleotides have 3 parts, a deoxyribose sugar with a phosphate connected to one end, and one of four nitrogen-containing bases (adenine, thymine, cytosine, guanine) on the other side of the deoxyribose sugar. These are linked together to form a chain that connects to an opposite chain, forming a double-stranded DNA molecule.

**Part2: Textbook chapter 22 sections 1&2**

**A. DNA Structure and Replication (Section 22.1)**

1. What three molecules is a nucleotide composed of?

A phosphate, sugar, and a nitrogenous base.

2. Using fig 22.1b on pg 474 as a guide, what types of molecules make up the backbone (exterior parts of the ladder) of DNA? What molecules are the rungs of the ladder made up of?

The backbone of DNA is the phosphate groups and deoxyribose sugars, while the rungs are the nitrogenous bases.

3. What type of bond joins the nitrogen bases together?

Hydrogen bonds.

4. Using fig 22b on pg 474 as a guide, the base A (Adenine) is always paired with

base \_\_\_\_\_ ( Guanine ).

5. Using fig 22b on pg 474 as a guide, the base C (Cytosine) is always paired with

base \_\_\_\_\_ ( Thymine ).

6. Fill in the table below. Pair the correct bases of DNA together to make a new strand of DNA, as would occur in S phase of the cell cycle during DNA replication. The first three of the new strand are done for you.

|  |  |
| --- | --- |
| Old Strand | C A T G A G C C A T T G A A T C G G C T T A G C T C G T A T C T G |
| New Strand | G T A C T C G G T A A C T T A G C C G A A T C G A G C A T A G A C |

7. What are the 5 major events of DNA replication from page 476?

The helicase unwinds double-stranded DNA by breaking the hydrogen bonds.

New nucleotides are fit into place by the process of complementary base pairing.

DNA synthesis occurs, leading strand follows the helicase enzyme, lagging strand waits for DNA to unwind and forms new short fragments of DNA.

DNA ligase seals any breaks in the sugar-phosphate backbone.

The 2 double-helix molecules are identical to each other and to the original DNA.

**B. RNA Structure (Section 22.1)**

1. What are the three molecular components of an RNA nucleotide?

Pentose sugar, pyrimidine, and single strand.

2. List the nitrogen containing bases used in RNA.

Adenine, guanine, cytosine, uracil.

3. Fill in the following table to show the complementary DNA bases for RNA bases. The base pairing rules are very similar to what you did in the DNA replication process, but when RNA is made during a process called transcription, RNA is synthesized based on complimentary binding to a DNA template which is very compatible. This is why you are being asked to base pair the RNA to the DNA.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RNA Bases | C (Cytosine | U (Uracil) | A (Adenine) | G (Guanine) |
| DNA Bases | G (guanine) | A (Adenine) | U (uracil) | C (cytosine) |

4. Fill in the following comparison table between DNA and RNA, using Table 22.1 as your guide.

|  |  |  |
| --- | --- | --- |
|  | **DNA** | **RNA** |
| Sugar | Deoxyribose | ribose |
| Bases | Adenine, Guanine, Thymine, and Cytosine | Adenine, guanine, cytosine, uracil |
| Strands | Double stranded with base pairing | Single strand |
| Double Helix | Yes | No |

5. What are the different types of RNA, and what are their roles?

Messenger RNA (mRNA) carries genetic information from the DNA to the ribosomes.

Ribosomal RNA (rRNA) joins with specific proteins to form the large and small subunits of ribosomes.

Transfer RNA (tRNA) transfers amino acids to the ribosomes.

**C. Gene Expression (Section 2)**

1. What are the monomers of proteins, and how many of these subunits exist in nature?

Amino acids, there are plenty

2. What is the shape of the protein dependent upon?

Sequence of amino acids

3. What are some of the roles of proteins in the human body?

Build and rebuild tissue, make enzymes, hormones, bones, muscle, cartilage, skin, etc…

4. What are the two steps of gene expression?

Transcription and translation

5. Complete the table below for transcription of DNA into RNA, using the base pairing rules from part B. The first four have been done for you.

|  |  |
| --- | --- |
| DNA | C A T G A G C C A T T G A A T C G G C T T A G C T C G T A T C T G |
| mRNA | G U A C T C G G T A A C T T A G C C G A A T C G T G C A T A G A C |

6. Why is it said that DNA has a triplet code and what are these three base sequences referred to as during the process of transcription?

The codons have a three base sequence. The reason that there are three bases in the code is because it allows for 64 possible codons. During transcription, these are referred to codons.

7. Using figures 22.6 as your guide,what are the start codons? What are the stop codons?

Start codons: AUG

Stop codons: UAA, UAG, UGA

8. What are the steps involved in the formation of mRNA?

1. RNA pol binds to promoter. 2. Nucleotides are added to mRNA strand by RNA pol. 3. Terminates when RNA crosses the gene's termination sequence.

9. What is involved in the processing of mRNA, and what is the purpose of each of these features?

1. One end of the mRNA is capped by the addition of an altered guanine nucleotide. The other end is given a tail, by the addition of multiple adenine nucleotides.
2. The introns are removed, and the exons are joined to form a mature mRNA molecule consisting of continuous exons.

The purpose of these features is to produce mature mRNA.

10. On the ribosome, what are the A, P, and E sites for?

The A sites are for accepting incoming mRNA, P sites are for the mRNA to bind with tRNA, and E sites are for the pair to exit.

11. What are the 3 major components of a tRNA molecule?

Anticodon, amino acid, and a 3’ acceptor end

12. Using fig 22.6, complete the table below. You may use the three letter abbreviation for the amino acids.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| mRNA Codons | AUG | CCC | GAU | GUU | GAG | UUG | UCU |
| tRNA Antiocodons | UAC | GGG | CUA | CAA | CUC | AAC | AGA |
| Amino Acid | Methionine | proline | Aspartic  acid | valine | Glutamic  acid | letucine | serine |

13. What are the 3 steps of translation, and what is involved in each step?

1. Initiation: mRNA binds to the smaller of the two ribosomal subunits. Then, the larger ribosomal subunit associates with the smaller one, forming the translation complex.
2. Elongation: The polypeptide lengthens, one amino acid at a time, about five amino acids per second. An incoming tRNA arrives at the A site and then receives the growing peptide chain from the outgoing tRNA. The ribosome moves laterally down the mRNA strand one codon at a time, so that again the P site is filled by a tRNA-peptide complex. The spent tRNA, now at the E site, exits the ribosome. The A site is now available to receive another incoming tRNA, as the complex has moved down one codon. In this manner, the peptide grows and the linear structure of a polypeptide is made. The particular shape of a polypeptide begins to form as the linear structure is established.
3. Termination: The termination of synthesis occurs when one of the three stop codons enters the A site. Termination requires a protein called a release factor, which can bind to a stop codon and cleave the polypeptide from the last tRNA. The ribosome then dissociates into its two subunits and falls off the mRNA molecule. THe individual portions of the translation complex can then re-form at the beginning of the mRNA strand to repeat this process and make another polypeptide.

14. List the 5 levels of control that can be applied to influence the rate and amount of output of gene expression (p. 483-484).

1. Pretranscriptional control
2. Transcriptional control
3. Posttranscriptional control
4. Translational control
5. Posttranslational control

15. What is the purpose of transcription factors?

After the right combination of transcription factors and other associated regulatory proteins bind to DNA, an RNA polymerase attaches to the DNA and begins transcription.

~~17.4 Isolation of DNA and Biotechnology~~

~~Complete the genes in a bottle handout. Each person can do their own. You may take it with you when you leave J.~~

~~17.5 Detecting Genetic Disorders~~

~~1. How do the red blood cells in people with sickle-cell disease defer?~~

~~2. What problems can this disease cause in a person?~~

~~3. The sickle-shaped red blood cells are caused by what?~~

~~4. Using fig 17.11 on pg 241, list the following genotypes for each of the following:~~

~~Normal:~~

~~Have the sickle-cell disease:~~

~~Have the sickle-cell trait:~~

~~5. Using fig 17.11 on pg 241, answer the following questions:~~

~~In what one base does~~ *~~Hb~~~~A~~* ~~differ from~~ *~~Hb~~~~S~~*~~?~~ *~~Hb~~~~A~~* ~~\_\_\_\_\_\_\_~~ *~~Hb~~~~S~~* ~~\_\_\_\_\_\_\_~~

~~What are the codons that contain this base?~~ *~~Hb~~~~A~~* ~~\_\_\_\_\_\_\_~~ *~~Hb~~~~S~~* ~~\_\_\_\_\_\_\_~~

~~What is the amino acid difference?~~ *~~Hb~~~~A~~* ~~\_\_\_\_\_\_\_~~ *~~Hb~~~~S~~* ~~\_\_\_\_\_\_\_~~

~~6. Detection of sickle-cell disease can be done through protein gel electrophoresis. Using fig 7.12 on pg 242, answer the following questions:~~

~~Which lane contains only~~ *~~Hb~~~~S~~*~~, signifying that the individual is~~ *~~Hb~~~~S~~~~Hb~~~~S~~*~~?~~

~~Which lane contains only~~ *~~Hb~~~~A~~*~~, signifying that the individual is~~ *~~Hb~~~~A~~~~Hb~~~~A~~*~~?~~

~~Which lane contains both~~ *~~Hb~~~~A~~*~~and~~ *~~Hb~~~~S~~*~~, signifying that the individual is~~ *~~Hb~~~~A~~~~Hb~~~~S~~*~~?~~

~~Questions:~~

~~1. If the codons are AUG, CGC and UAC, what are the anticodons?~~

~~2. Where does protein synthesis take place?~~

~~3. In what part of the cell does translation occur?~~

~~4. During transcription, what type of RNA is formed that carries the codons?~~

**~~Names:~~**

**~~Micropipetting Rules and Exercise~~**

**~~~~**

**~~1. Choose the appropriate device. When you have the option of using a couple of different micropipettes, select the one for which the desired volume is in the center of its range. These ranges are printed on the micropipette knob.~~**

~~P1000: 100-1000 uL~~

~~P200: 20-200 uL~~

~~P20: 2-20 uL~~

~~P2: 0.2-2uL~~

**~~2. Dial the appropriate volume by twisting the knob at the top of the instrument. Do NOT twist above or below the device range as listed. This will ruin the micropipette. Notice that the units of the micropipette are in microliters. Anything in red indicates it is a placeholder of a new unit. For micropipetting exercise, using water, one partner sets and pipettes the volume indicated in Examples A, B, and C and put it in a microcentrifuge tube. The second partner checks the volume by pipetting the same volume.~~**

~~Example A: You need to draw up 500 uL of liquid.~~

~~a. Which micropipette do you choose?~~

~~b. What do you set the dial to?~~

~~Example B: You need to draw up 100 uL of liquid.~~

~~a. Which micropipette do you choose?~~

~~b. What do you set the dial to?~~

~~Example C: You need to draw up 15 uL of liquid.~~

~~a. Which micropipette do you choose?~~

~~b. What do you set the dial to?~~

**~~3. Put a tip on the micropipette from the appropriate sterile tip box, securing the tip well onto the device. Do NOT touch the tip with your gloved hand or allow the tip to touch ANYTHING. Do NOT put the micropipette down onto the bench unless the tip has been removed.~~**

**~~4. Depress the knob to the first stop. This is the point at which you notice tension as you press. Do not press farther than this point. ALWAYS have the micropipette set and depressed to the first stop BEFORE you come into contact with the liquid.~~**

**~~5. Insert the tip into the fluid of the container to the depth that you expect the tip to fill. Do NOT touch the side of the bottle.~~**

**~~6. VERY SLOWLY release the knob to fill the tip. Examine the contents of your tip. If there are any bubbles, you must expel the liquid and do this again.~~**

**~~7. Remove the device from the bottle and place the tip onto the inside of the receptacle tube, touching the side of the tube near the bottom of the container. While carefully watching, SLOWLY depress the knob all the way to the second stop.~~**

**~~8. Look at what was delivered. Does it make sense? Always assess the transfer of the fluid—was it all expelled?~~**

**~~9. Use the ejector to expel the tip from your micropipette into an appropriate receptacle (usually a beaker). Do not reuse micropipettes unless you are transferring the same type of liquid so you do not cross-contaminate. Also do not reuse micropipettes if your tip touches any other object such as your sleeve, the table, etc.~~**

**~~A few reminders:~~**

**~~Always hold the micropipette upright.~~**

**~~For your protection, use gloves when dealing with micropipettes.~~**

**~~Make sure your micropipette tip is on securely.~~**

**~~Do not angle the micropipette sideways/upside down when there are contents in the tip. Do not let the tip touch the table.~~**

**~~Use aseptic technique (close all tip boxes and containers when not in use, do not breathe directly over any containers or substances).~~**

**~~DO NOT DROP THE MICROPIPETTES.~~**

**~~DO NOT DIAL BEYOND THE RANGE OF THE MICROPIPETTE.~~**

~~Directions: Practice micropipetting the volumes 500, 100, and 15 ul of water, carefully, with the appropriate micropipettes, into microcentrifuge tubes. Compare your tube to your partner’s. Then compare with the standards at the front table to see how you did.~~

~~Next, we’ll view plasmid DNA on a gel and compare it to genomic DNA. To do so, each team will mix 2-3ul of plasmid DNA with loading dye. It will then be loaded into the well of the gel prepared at the beginning of the period, following the protocol on the powerpoint.~~