DISCUSSION ASSIGNMENT #10: Lecture 17 (some Lec18-PCR)

READ THIS FIRST! Important notes about DQ 10

- Both parts of Lecture 17 (DNA mutations AND repair) will be covered on Exam2.
- Lec 18 (PCR) may be covered as well. It will depend on our progress
- This DQ is due on 3/21 by 11pm. This is the night *before spring break begins*.
- We *will* have a zoom DQ for this set, but it will be *after spring break*. (3/29)
- This DQ set has questions about Lec 18 (PCR), you do not have to answer/submit them for a grade. They are simply here as a study tool.
- The questions you DO have to answer/submit are those associated with Lecture 17.

Lecture 17 (parts 1-2): DNA mutations, damage and Repair mechanisms

<u>Natural</u> mutations can occur (1) during/end of replication or other cell processes, (2) spontaneously in a cell or (3) due to the environment.

1.) List and briefly describe different <u>types</u> of mutations. (different types of point and chromosomal). Also discuss heritable vs non-heritable mutations (This does not mean simply defining them- think cell type, effects on individual, effects on offspring, effects on evolution)

2.) Describe, in detail, causes for mutations that occur *during/end of replication*. For each, use detail to describe how the mutation would occur, classify the type of mutation that results, and the effect it may have on the cell

- a.) What are two causes for mutations during replication
- **b.)** What is one cause for mutations <u>at the end of</u> replication.
- **3.)** Describe, in detail, causes for mutations that occur *due to byproducts of cell processes*.
 - **a.)** Identify the damaging by-product and how it actually causes mutation (details!)
 - **b.)** List at least three cell processes that result in this molecule.
 - c.) What are the effects of this type of mutation on the cell? And then what happens?

4.) Describe, in detail, causes for mutations that occur *spontaneously during a cell's life*. For each, use detail to describe what the mutation is, classify the type of mutation that results, and the effect it may have on the cell

- a.) Tautomerization: (in addition to prompts above), what is a tautomer? specific examples?
- **b.)** Depurination: (in addition to prompts above), specific examples?
- c.) Deamination: (in addition to prompts above), specific examples?

5.) The environment also results in production of DNA damaging molecule(s), and can also damage DNA, directly

a.) List different environmental factors that produce ROS at a "natural rate" of mutation.

b.) List different environmental factors that are deemed "mutagenic". Your answer should include a definition of "mutagen"

c.) List different environmental factors that directly affect DNA at a "natural rate" of mutation.

- 6.) Discuss, compare/contrast environmental radiation
 - **a.)** Describe the difference between ionizing vs non-ionizing radiation; provide examples.
 - **b.)** Describe the *direct* effects each has on DNA molecules (describe the damage)
 - c.) Describe how each type of damage effects the cell: what happens (or cannot happen)?

7.) Describe chemical mutagens- these are typically implemented in a lab, and rarely are we as organisms exposed to this in our natural environment.

a). How is a base "replaced"? What does this mean, how does this happen? Examples.

b.) How is a base "changed"? What does this mean, how does this happen? Examples.

c.) How/why does a mutagen insert itself into DNA? What does this mean? Examples.

<u>v.</u>					
	Mechanism name	subtype	Type of mutation(s)/damage it fixes. Also include a potential but specific cause for such a mutation/damage	Description of mechanism (include name of molecules and role, in chronological order)	
	proofreading				
	telomere repair				
	mismatch repair				
	DNA damage repair	Direct Reversal Base Excision Nucleotide Excision DSB repair (2 types)			
	Apoptosis				

8.) Fill in the table regarding DNA repair

QUESTIONS BELOW ARE FOR PCR. USE QUESTIONS AS STUDY TOOL. You do not need to complete/submit in your DQ10

<u>User-friendly (IMO) resources (all less than 7 min, some are even just 2min)</u> PCR animation, no verbal explanation (CanalDivulgacion) <u>https://www.youtube.com/watch?v=iQsu3Kz9NYo</u>

PCR explained with cartoon (DNA Learning Center) https://www.youtube.com/watch?v=JRAA4C2OPwg

PCR recap, RT-PCR and specific example (2:25-end Osmosis) <u>https://www.youtube.com/watch?v=gubLAtn2o4s</u>

1.) Answer the following about PCR.

a.) What is PCR, in general?

b.) Name/Describe briefly what happens in the three steps of one round/cycle of PCR? In your explanation, be sure to identify the temperature of each step and why these temperatures are chosen.

2) Address the following about PCR primers.

a.) Explain what a primer is, what it is made out of, and its role in the PCR.

b.) Describe where, on DNA, a scientist will design their primers and why they would choose this locus (place).

c.) Explain how primers affect the annealing temperature a scientist would choose in PCR.

3. Explain what *Taq* Polymerase is, where it can be found in nature, and why it is a common polymerase used for PCR. Also list its two disadvantages?

4. Draw 3 cycles of PCR. Distinguish between the original template, the region of interest to be amplified, products that are "too long" (denote with #), products that are the desired length (denote with *). Always include directionality, and at the completion of each cycle, circle the # of double stranded products that are the desired length. (Colored pencils/pens will be useful here.)

5. Make a table comparing/contrasting DNA replication (that occurs naturally) and PCR. Be sure to include relevant molecules and compare/contrast how they each function in their respective processes. Break it into "Initiation vs Denaturing & Annealing" and "Elongation vs Extension". Explain why PCR doesn't need "Termination" machinery and why there is no "end of replication problem" in PCR

6. Address the following about PCR applicability.

a.) List and describe 4 different reasons/application for PCR use in laboratory settings.
b.) For one of your reasons (in part a), provide a detailed research objective that a scientist would be addressing. Saying "for crime scene investigations" is not enough. Instead, describe <u>how</u> the scientist would conduct the experiment and what clues/pieces of evidence (in the PCR results) they use/look for to make a conclusion. For each, draw/label a mock gel that shows the results of the PCR with controls, a negative result and two different results
c.) Repeat part b for another application you listed in part a.

7. Explain why/when a scientist would use PCR coupled by sequencing analysis vs gel electrophoresis.