Unit 6 Progress Check: FRQ

1. Read each question carefully. Write your response in the space provided for each part of each question. Answers must be written out in paragraph form. Outlines, bulleted lists, or diagrams alone are not acceptable and will not be scored.

CD3 is a signaling protein that is typically found only in the plasma membrane of immune system T lymphocytes. CD3 is composed of several different polypeptides, including a gamma chain, CD3γ. Scientists analyzed the promoter of the CD3γ chain gene for regulatory sequences that might have positive or negative effects on expression of the gene.

The scientists cloned fragments of the CD3γ gene that included the first transcribed nucleotides plus up to 789 nucleotides of upstream regulatory sequences into plasmids in which the gene for the firefly enzyme luciferase immediately follows the fragments. The plasmids were then introduced into a line of T lymphocytes (Figure 1), and the cells were allowed to grow for a short while. Because the regulatory sequences of the CD3γ gene immediately precede the luciferase gene in the plasmids, the activity, either positive or negative, of the regulatory sequences affected the amount of luciferase gene expression by the T lymphocytes. Luciferase catalyzes a reaction that results in the release of light and is responsible for the bioluminescence (light flashes) of fireflies. By quantifying the bioluminescence, or luciferase activity, in the cells, the scientists were able to determine the effects of each CD3γ gene fragment cloned into the plasmids (Figure 2) on expression of the gene.

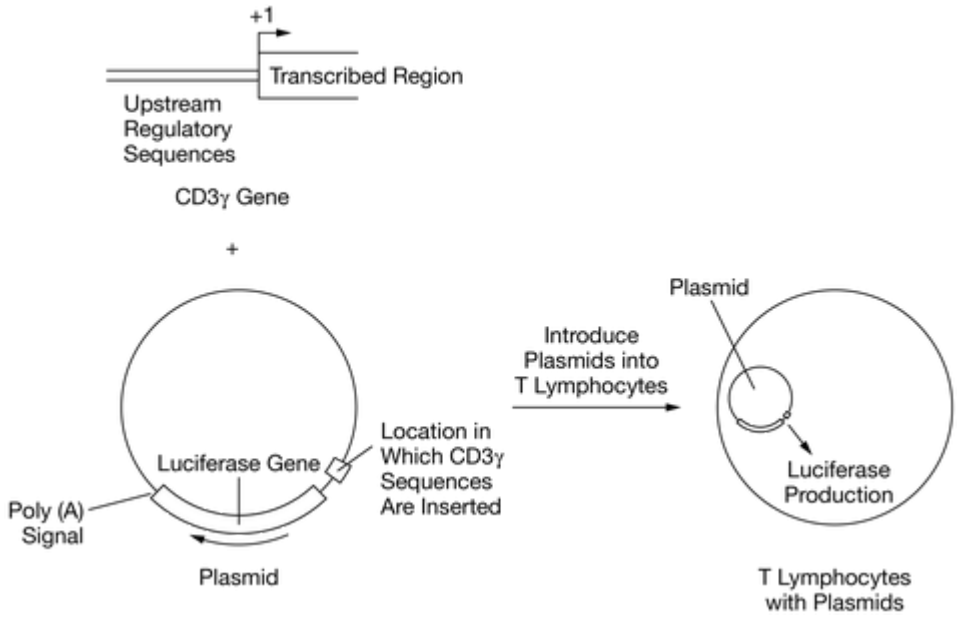

Figure 1. Summary of experimental procedure. A series of plasmids containing fragments of the CD3γ upstream regulatory sequences cloned immediately before the luciferase gene were constructed. Each type of plasmid was introduced into T lymphocytes. The amount of luciferase produced by the lymphocytes was dependent on the regulatory sequences present in each plasmid.

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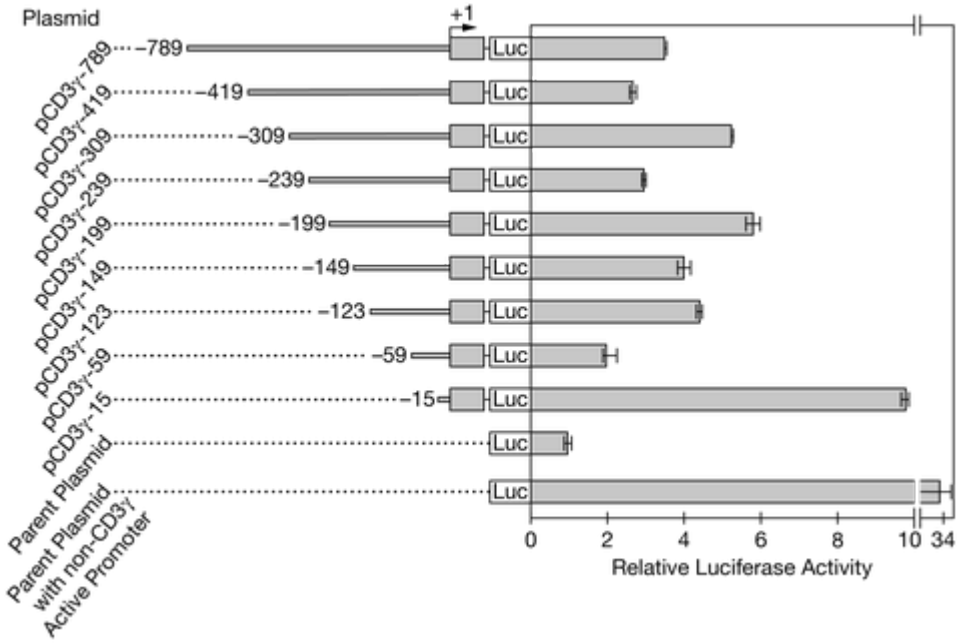

Figure 2. Analysis of the luciferase activity of T lymphocytes containing plasmids with different amounts of the $\mathrm{CD3}\gamma$ regulatory sequence. Names of the plasmids are shown on the left. Included regulatory sequences and the resulting luciferase activity are shown on the right. Error bars represent $\pm 2\mathrm{SE}_{\bar{x}}$. The transcription start site is indicated by $+1$.

(a) **Identify** both the cellular component and the location of the component that is responsible for producing the luciferase protein from **mRNAs** transcribed in the plasmid-containing T lymphocytes. **Explain** what dictates to the lymphocytes the correct order in which amino acids should be linked to form the luciferase protein.

Please respond on separate paper, following directions from your teacher.

(b) **Identify** the independent variable in the experiment described. **Identify** the plasmid that was used as a negative control for luciferase activity. **Justify** including the plasmid with the non-$\mathrm{CD3}\gamma$ active promoter in the experiments.

Please respond on separate paper, following directions from your teacher.

(c) **Identify** the plasmid that must contain the $\mathrm{CD3}\gamma$ core promoter sequence but the fewest or no negative regulatory sequences. Based on the data in Figure 2, **describe** the most likely cause of the variation in luciferase activity among the cells that contain plasmids $\mathrm{pCD3}\gamma\text{-}419$, $\mathrm{pCD3}\gamma\text{-}309$, $\mathrm{pCD3}\gamma\text{-}239$, and $\mathrm{pCD3}\gamma\text{-}199$. **Calculate** the approximate percent increase in luciferase activity

between cells containing plasmid **pCD3γ-59** and cells containing plasmid **pCD3γ-149**. Round to the nearest whole number.

Please respond on separate paper, following directions from your teacher.

(d) **Predict** the most likely observed level of luciferase activity if plasmid **pCDγ3-789** is introduced into nonlymphoid cells such as a line of kidney tissue cells. Provide reasoning to **justify** your prediction.

Please respond on separate paper, following directions from your teacher.

Part A (i)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that luciferase is produced by ribosomes in the cytoplasm OR in the cytosol of the lymphocytes.

Part A (ii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the sequence of nucleotides in the **mRNA** dictates the amino acid sequence of luciferase. Sequential triplets of nucleotides called codons specify each amino acid to be added, in order, to the growing polypeptide chain.

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Part B (i)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the independent variable is the particular plasmid or that it is the CD3γ gamma regulatory sequences introduced into the lymphocytes.

Part B (ii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the plasmid used as a negative control was the parent plasmid or that it was the plasmid without any regulatory sequences inserted.

Part B (iii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the plasmid with the non- CD3γ gamma active promoter is a positive control for the effective introduction of the plasmids into the T lymphocytes and for the successful use of the luciferase assay to measure promoter activity.

Part C (i)

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Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the plasmid that must contain the $CD3\gamma$ core promoter sequence but the fewest or no negative regulatory sequences is $pCD3\gamma\text{-}15$ (because this plasmid gives the greatest luciferase activity).

Part C (ii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the different sequences cloned into the plasmids contain different combinations of positive and negative regulatory sequences.

Part C (iii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that the percent increase is approximately 100%. (The $pCD3\gamma\text{-}59$ bar ends at the 2 and the $pCD3\gamma\text{-}149$ bar ends at the 4 on the axis labeled Relative Luciferase Activity.)

Part D (i)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that there will be little or no luciferase activity if plasmid pCD3γ-789 is introduced into nonlymphoid cells such as a line of kidney tissue cells.

Part D (ii)

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that nonlymphoid cells will not contain the transcription factors that bind to the CD3γ gene regulatory sequences.

2. Read each question carefully. Write your response in the space provided for each part of each question. Answers must be written out in paragraph form. Outlines, bulleted lists, or diagrams alone are not acceptable and will not be scored.

DNA replication in eukaryotes and prokaryotes starts when a complex of proteins binds to an origin of replication (Figure 1A) and separates the two strands of the double helix to form a replication bubble with replication forks at each end (Figure 1B). In order for replication to proceed, the enzyme topoisomerase binds to the DNA immediately in front of the advancing replication forks.

Figure 1. DNA replication starts when the two strands of the double helix separate at an origin of replication. Topoisomerase binding sites are indicated.

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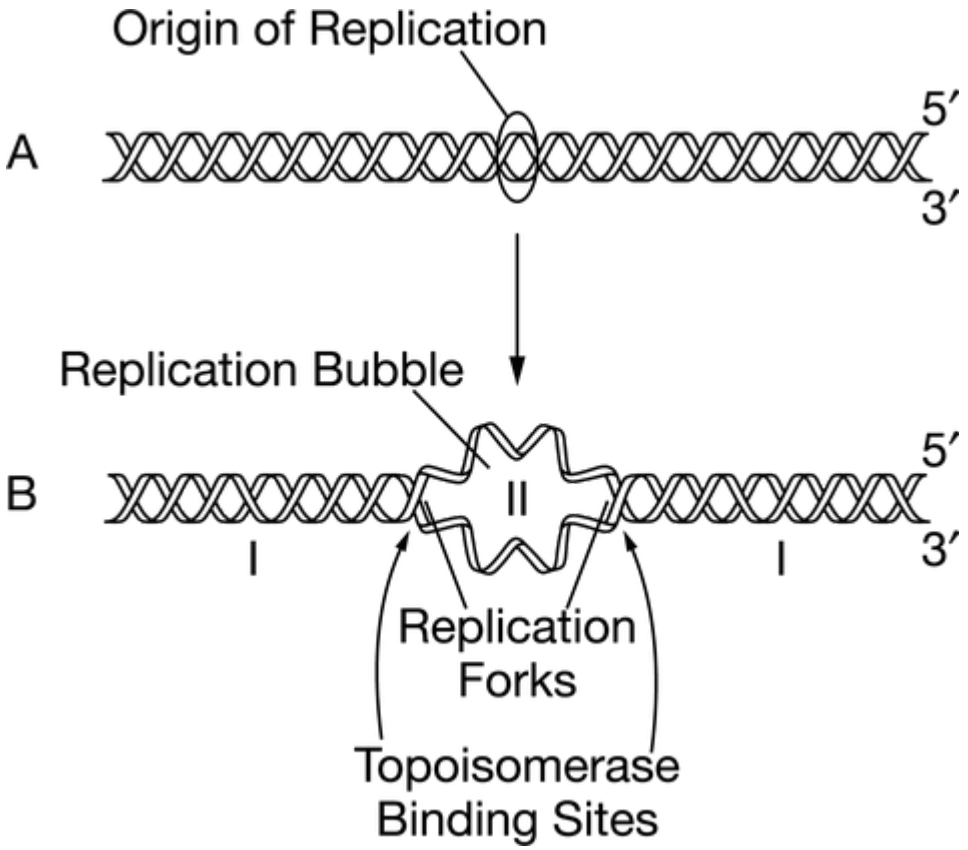

(a) **Describe** how the structure of a prokaryotic chromosome differs from the structure of a eukaryotic chromosome.

Please respond on separate paper, following directions from your teacher.

(b) **Explain** why DNA replication cannot proceed to the regions of the chromosome labeled as I in Figure 1B unless topoisomerase binds ahead of each advancing replication fork in region II.

Please respond on separate paper, following directions from your teacher.

(c) Use the template of a replication fork to **draw arrows** that represent both continuous and discontinuous DNA synthesis. Draw one long arrow to show continuous DNA synthesis and three arrows to show discontinuous DNA synthesis. The arrows should point in the direction of nucleotide addition, and the three arrows showing discontinuous synthesis should be numbered 1, 2, and 3, in the order of fragment synthesis.

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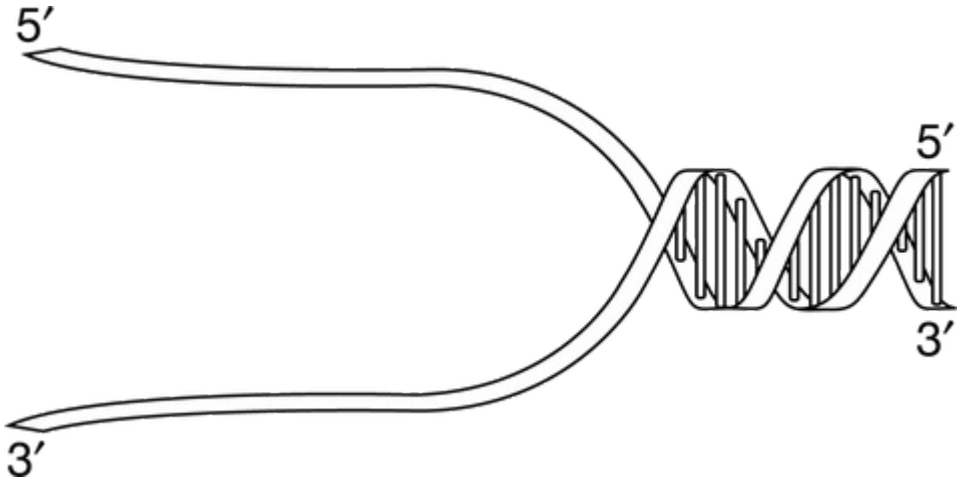

Please respond on separate paper, following directions from your teacher.

(d) Referring to Figure 1B, **explain** why any newly synthesized strand of **DNA** is the result of both continuous and discontinuous **DNA** synthesis.

Please respond on separate paper, following directions from your teacher.

Part A

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that a prokaryotic chromosome is typically circular, whereas a eukaryotic chromosome is linear.

Part B

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

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0	1 ✓

The response indicates that as the DNA helix unwinds, it becomes increasingly tightly coiled or supercoiled ahead of each replication fork (the two regions labeled as I). Topoisomerase relieves the supercoiling so that DNA unwinding and replication can continue (region II).

Part C

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates by the presence of one long arrow drawn along the lower strand of the template and pointing to the right that continuous DNA synthesis occurs along this template strand. The response also indicates by the presence of three arrows drawn along the upper strand of the template and pointing to the left that discontinuous DNA synthesis occurs along this template strand. From left to right, the three arrows should be numbered 1, then 2, and then 3.

Part D

Select a point value to view scoring criteria, solutions, and/or examples and to score the response.

0	1 ✓

The response indicates that because DNA synthesis only occurs in a 5′ to 3′ direction, synthesis of a DNA strand in a replication bubble (region II) is continuous at one replication fork and discontinuous at the other replication fork.