Solutions to 7.012 Quiz II

Class Average = 68
Median = 71

<table>
<thead>
<tr>
<th>GRADE</th>
<th>RANGE</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>83-100</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>70-82</td>
<td>32</td>
</tr>
<tr>
<td>C</td>
<td>55-69</td>
<td>29</td>
</tr>
<tr>
<td>D</td>
<td>40-54</td>
<td>15</td>
</tr>
<tr>
<td>F</td>
<td>0-39</td>
<td>4</td>
</tr>
</tbody>
</table>
Question 1

a) Shown below is a representation of two replication forks.

![Diagram of replication forks with sites A, B, C, and D]

i) To which site or sites (A, B, C, or D) can the primer 5'-GUUCC-3' bind to initiate replication?

   *A and D*

ii) For sites A and B, answer the following with respect to fork 1. For sites C and D, answer the following with respect to fork 2.

   - What is the direction of elongation (left or right) of the daughter DNA strand?
   - Is DNA synthesis performed in a **continuous** or a **discontinuous** fashion?

<table>
<thead>
<tr>
<th>Site</th>
<th>Direction</th>
<th>continuous or discontinuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Left</td>
<td>continuous</td>
</tr>
<tr>
<td>B</td>
<td>Right</td>
<td>discontinuous</td>
</tr>
<tr>
<td>C</td>
<td>Left</td>
<td>discontinuous</td>
</tr>
<tr>
<td>D</td>
<td>Right</td>
<td>continuous</td>
</tr>
</tbody>
</table>
Question 1, continued

You have just started a UROP in Dr. Ori’s laboratory. Dr. Ori has invented a new machine that allows you to see the DNA of a cell at high resolution while the cell is still alive. Your project is to examine DNA replication in two *E. coli* mutants.

b) When you look at mutant 1, you find regions of newly replicated DNA that have gaps in the sugar phosphate backbone such that the 3’OH of one nucleotide has not formed a bond with the 5’ phosphate of the adjacent one.

- Why might the DNA have this appearance?
  
  Some of the newly replicated DNA is made as Okazaki Fragments. These fragments need to be joined together into one continuous DNA strand by the formation of a covalent bond between the free 3’OHs and 5’ phosphates.

- What enzyme could be missing in this mutant?
  DNA Ligase

c) Mutant 2 is defective in all DNA replication. Mutations in a number of proteins or enzymes would result in a replication defective mutant. Give three functions or activities and the associated enzyme (other than that listed for (b)) that could be lacking in mutant 2.

<table>
<thead>
<tr>
<th>Function or activity</th>
<th>Enzyme name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unwinding the DNA at the ori</td>
<td>Helicase</td>
</tr>
<tr>
<td>Relieving super-coiling of the DNA as the</td>
<td>Topoisomerase</td>
</tr>
<tr>
<td>replication forks progress</td>
<td></td>
</tr>
<tr>
<td>Preventing the two strands from re-annealing</td>
<td>Single-stranded binding protein</td>
</tr>
<tr>
<td>DNA polymerization</td>
<td>DNA polymerase</td>
</tr>
<tr>
<td>Forming the primer to give a free 3’ OH.</td>
<td>Primase</td>
</tr>
</tbody>
</table>

d) You have determined that mutant 3 has a mutant DNA polymerase that results in a much higher error rate than seen in wild type *E. coli*. The error rate in your mutant is $10^{-6}$, while normal error rate is $10^{-9}$. Which of the following activities is the mutant polymerase missing?

- $5’\rightarrow 3’$ recombinase
- $5’\rightarrow 3’$ polymerase
- $5’\rightarrow 3’$ exonuclease
- $3’\rightarrow 5’$ polymerase
- $3’\rightarrow 5’$ exonuclease
Impressed with your problem solving skills, Dr. Ori asks you to help her amplify a gene using the Polymerase Chain Reaction (PCR) method. She has attempted to amplify this gene unsuccessfully three times.

- The first time she did not get any amplified DNA (no product).
- The second time she got DNA of the size and sequence expected, but very little of it (low yield).
- The third time she got a lot of DNA, but it was not the gene she wanted to amplify (amplified wrong region).

You realize that there are three possibilities for these results. The three possibilities are:

1) The PCR machine broke after only completing 10 cycles.
2) She added RNA polymerase instead of DNA polymerase.
3) She had meant to use a primer that was: $$5' \text{GATACCTGAAT} 3'$$
   but had instead used a primer that was: $$3' \text{GATACCTGAAT} 5'.$$

E) Match the result of the failed experiment with the best explanation from above.

<table>
<thead>
<tr>
<th>Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No product</td>
<td>2</td>
</tr>
<tr>
<td>Low yield</td>
<td>1</td>
</tr>
<tr>
<td>Amplified wrong region</td>
<td>3</td>
</tr>
</tbody>
</table>
**Question 2**

The mouse genome contains approximately 25,000 genes. You are interested in one particular gene, neuronatin (Nnat), a gene that is important in brain development.

Below is a schematic showing the Nnat transcription initiation region. Both DNA strands are shown. The RNA polymerase binding site (the promoter) is in **bold italics**. Transcription begins at and includes the T/A base pair shown in **bold**.

<table>
<thead>
<tr>
<th>5'</th>
<th>51</th>
<th>CACCTGCAGC</th>
<th>AGCACCACGA</th>
<th>AGATGTAACC</th>
<th>GCCGATGATG</th>
<th>AGCAAGTCTG</th>
<th>CCGAGGCTGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>51</td>
<td>GTGGACGTGC</td>
<td>TCGTGCCCTT</td>
<td>TCTACATGGT</td>
<td>CGGCTACTAC</td>
<td>TCGTCAAGAC</td>
<td>GGCTCCGACG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5'</th>
<th>101</th>
<th>TGCCACCTGCG</th>
<th>GCCATGGTTTC</th>
<th>CGAAAGTGGG</th>
<th>CAATGCCGCA</th>
<th>GCCCCGGTGG</th>
<th>CCGTGCCTTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>101</td>
<td>ACGGTGACGC</td>
<td>CGGTACCAAG</td>
<td>GCTTTCAACC</td>
<td>GTTAGGCGGT</td>
<td>CGGGCCACC</td>
<td>GCCAGGGAAT</td>
</tr>
</tbody>
</table>

C  ala  val  ala  ala  met  N

<table>
<thead>
<tr>
<th>5'</th>
<th>151</th>
<th>AGTACCCCT</th>
<th>TGCCACCTAA</th>
<th>GTGCGCATGC</th>
<th>GCCGTTGGG</th>
<th>GATGTTGAGA</th>
<th>AGGAGGGTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>151</td>
<td>TCAATGGGA</td>
<td>AGCGTGGATT</td>
<td>CACCGGTACG</td>
<td>CGCGAACCC</td>
<td>CTACAACTCT</td>
<td>TCCTCCACAC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5'</th>
<th>201</th>
<th>ATGGGGGGCC</th>
<th>CTCCTCATTC</th>
<th>GCCAAGAGAG</th>
<th>AAAAAAT</th>
<th>CCCGTCTGTT</th>
<th>CTTCCACAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>201</td>
<td>TACCACCCGC</td>
<td>GAGGAGTAAG</td>
<td>CGGTTCCTTC</td>
<td>TTTTTTTTA</td>
<td>GGGCAGACAA</td>
<td>GAAAGGTTGT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5'</th>
<th>251</th>
<th>CGACCCCCAC</th>
<th>CTCTGAGGCC</th>
<th>CGCCTCTACA</th>
<th>CGCTCCAGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>251</td>
<td>GCTGGGGGTG</td>
<td>GAGGACTCGG</td>
<td>GCGAGATTGT</td>
<td>GGCAAGGTCT</td>
</tr>
</tbody>
</table>

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**a) What are the first five amino acids encoded for by the Nnat mRNA? Circle only one answer.**

1. met-val-pro-lys-val  
2. **met-ala-ala-val-ala**  
3. ile-ala-pro-ser-ser  
4. met-val-gly-gly-tyr  
5. met-thr-arg-ala-ser  
6. met-pro-gln-pro-arg  
7. leu-arg-met-arg-arg
Question 2, continued

During development, individual cells must communicate with one another to become organized into complex tissues and organs. The Nnat protein is believed to be involved in such cell-cell signaling during brain development. Shown below are the final 16 codons, which code for the last 15 amino acids in the Nnat protein. This region is highly charged as indicated by the notation below the amino acids, and is responsible for the Nnat protein’s ability to signal.

ACC GGG CGG CAG GUG CUG GGG GAG CGC AGG CAG CGA GCC CCC AAC UGA
thre thr gly arg gln val leu gly glu arg arg gln arg ala pro asn *
+                   -   +       +

b) You have isolated several different Nnat mutants and have sequenced each mutant gene to identify the mutation (shown in bold below). For each sequence, give the type of the mutation (silent, nonsense, missense or frameshift) and predict the effect of each mutation on the protein’s ability to signal.

1. ACC GGG CGG CAG GUG CUG GGG GAG CGC AGG CAG A\text{GA} GCC CCC AAC UGA
   • What type of mutation is this? \text{Silent}
   • Predict the effect this mutation will have on the protein’s ability to signal? Why? 
     This mutation will have no affect on the protein’s ability because the protein produced by the wild-type gene is identical to the protein produced by the mutant gene.

2. ACC GGG CGG U\text{AG} GUG CUG GGG GAG CGC AGG CAG CGA GCC CCC AAC UGA
   • What type of mutation is this? \text{Nonsense}
   • Predict the effect this mutation will have on the protein’s ability to signal? Why? 
     This mutation results in a premature stop codon. The protein produced from this mutant gene is truncated prior to the region containing the highly charged amino acids. It will be unable to function because it is missing the region responsible for the protein’s ability to signal.

3. ACC GGG CGG CAG G\text{CG} CUG GGG GAG CGC AGG CAG CGA GCC CCC AAC UGA
   • What type of mutation is this? \text{Missence}
   • Predict the effect this mutation will have on the protein’s ability to signal? Why? 
     The protein produced from this mutant gene is likely to have a normal ability to signal. The mutant protein has an alanine instead of a valine. Valine is not one of the charged amino acids important for signaling and this is a conservative substitution. (Alanine and valine are two similar amino acids).
Question 3

The following is a diagram of an inducible operon in *E. coli*. Enzymes A and B are both required for the breakdown of the sugar xylose. The wild-type operon is regulated by the repressor protein (R), which is continuously produced.

![Diagram of an inducible operon in E. coli.](image)

You have three mutants (m1, m2 and m3), each one the result of a mutation in a single component shown in the diagram. The mutants m1, m2 and m3 exhibit the following phenotypes when grown with or without xylose in the medium.

<table>
<thead>
<tr>
<th></th>
<th>without xylose</th>
<th>with xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme A activity</td>
<td>Enzyme B activity</td>
</tr>
<tr>
<td>wild-type (+)</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>m1</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>m2</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>m3</td>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>

a) A mutation in which single component could produce the phenotype seen in the m2 mutant? Why?

_A mutation in the promoter, P<sub>enzymes</sub>, could produce this phenotype. If the promoter is altered such that it no longer binds RNA polymerase then there is no transcription or translation of enzymes A or B, regardless of xylose concentration._

b) Name three different components that when mutant could produce the phenotype seen in m1 and m3.

*P<sub>R</sub>, R, and O*
Question 3, continued

c) You then construct the following diploids by inserting a second copy of the operon 
\((R^+ P^- O^+ A^- B^-)\) into each mutant. + indicates that the component is wild type, - indicates that the 
component is non-functional.

<table>
<thead>
<tr>
<th>Strain</th>
<th>without Xylose</th>
<th>with Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme A activity</td>
<td>Enzyme B activity</td>
</tr>
<tr>
<td>m1 with (R^+ P^- O^+ A^- B^-)</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>m2 with (R^+ P^- O^+ A^- B^-)</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>m3 with (R^+ P^- O^+ A^- B^-)</td>
<td>low</td>
<td>low</td>
</tr>
</tbody>
</table>

Which one of the three mutants (m1, m2 or m3) has a mutation in the coding region for the 
repressor protein? Briefly explain your reasoning.

Normal regulation is restored when the second copy of the operon, \((R^+ P^- O^+ A^- B^-)\) is provided to m3. This means that m3 is complemented in trans, and the mutation in m3 is a mutation in the coding region for the repressor protein.

d) Mark on the figure below where you would expect to find...

i) Stop codon(s)

ii) Transcription terminator(s)
Question 4

You want to study cysteine biosynthesis in yeast. You have obtained a known cysteine auxotroph (can not make cysteine), \( cysA^- \) from the catalogue. This \( cysA^- \) strain carries a single mutation that disrupts the \( cysA \) gene. You mutagenize a wild type strain and obtain several other cysteine auxotrophs that you call \( cysI^- - cys4^- \).

In the table below, the haploid cells of the \( cysI^- \) strain are missing only the product of the \( cys1 \) gene, the haploid cells of the \( cys2^- \) strain are missing only the product of the \( cys2 \) gene, etc. + indicates the resulting diploids grow in the absence of cysteine.

<table>
<thead>
<tr>
<th></th>
<th>( cysI^- ) strain</th>
<th>( cys2^- ) strain</th>
<th>( cys3^- ) strain</th>
<th>( cys4^- ) strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>( cysA^- ) strain</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a) To clone the \( cysA \) gene you begin by making a yeast genomic library. You could make your library from which of the following yeast strains? Circle all that you could use.

- \( cysA^- \) strain
- Wild type strain
- \( cysI^- \) strain
- \( cys2^- \) strain
- \( cys3^- \) strain
- \( cys4^- \) strain

b) The vector that you choose for the construction of your library should have what three key features.

Your vector should have a selectable marker, an origin of replication, and a cloning site (a unique restriction enzyme site).

c) You cut both your vector and the genomic DNA (from part a) with restriction enzyme, BamHI. You successfully create a genomic library that you use to transform the \( cysA^- \) yeast strain. What type of media could you use to find the yeast cells that have been transformed with the \( cysA \) gene? Circle any or all that apply.

- Rich
- Rich + ampicillin
- Minimal + cysteine
- Minimal
- Minimal + ampicillin + cysteine

Explain your choice.

You are cloning by complementation of the \( cysA^- \) phenotype. Cells with a wild type \( cysA \) gene will be the only ones that grow on minimal media, all other media listed would allow the un-transformed cells to grow.
d) You attempt the isolation of the cysA gene using your library, but repeatedly fail. You make a new library using BglII to cut your both your vector and the genomic DNA and repeat the experiment. Everything else in the protocol is identical. This time you succeed in isolating the cysA gene. Explain why the second library worked when the first library did not.

If the cysA gene has a BamHI site within the coding region, a functional copy of the cysA gene would not be present in the library made with BamHI. The second library made with BglII would contain a complete, functional copy of the cysA gene because the cysA gene does not have a BglII site within the coding region.

*Note, answers asserting that the vector is not cut with BamHI were not corrects as you were previously told that “You successfully create a genomic library that you use to transform the cysA yeast strain.”