Answer key

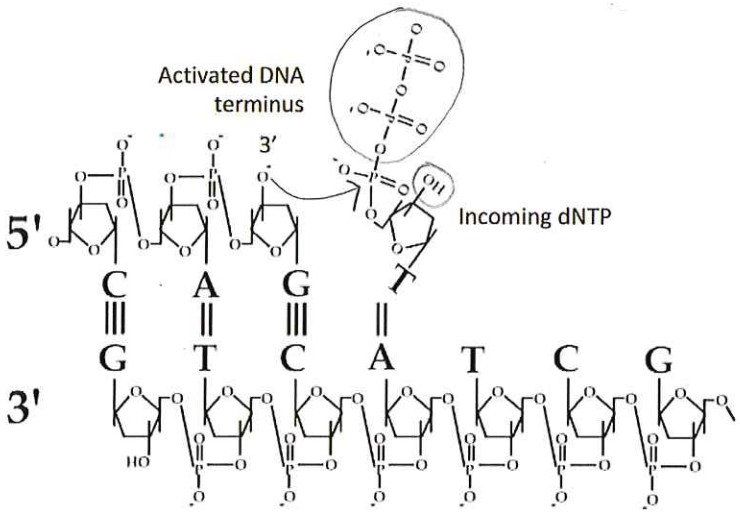
BB2950 Molecular Biology, Quiz 3: 11-16-17, 25 points total
PLEASE WRITE YOUR NAME ON THE BACK OF EACH PAGE

1. *(3 points)* The schematic below shows the reaction mechanism of DNA polymerization. The 3' OH has been activated by deprotonation.

A. Draw an arrow from this activated 3' O^- to the atom on the incoming dNTP that it will form a new bond with.

B. Circle the portion of the incoming dNTP that will be released after the new phosphodiester bond is formed.

C. Circle the new 3' OH that will then be activated for addition of the next nucleotide.



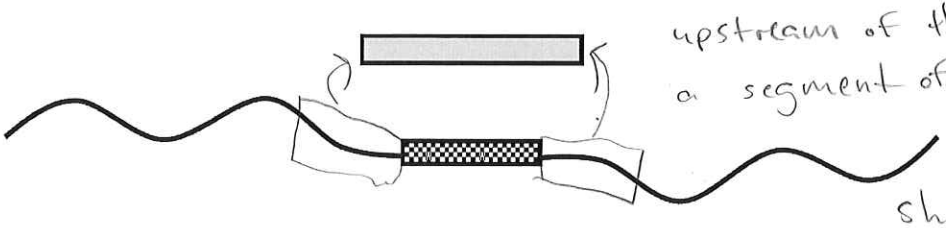
2. *(6 points)* DNA Pol I and DNA Pol III both have exonuclease activities. Indicate the directionality (or directionalities) of the exonuclease activity (or activities) of each polymerase, and state the function of each of these activities in the cell.

Pol I 5'→3': degrades RNA primers

Pol I 3'→5': Proofreading (removing misincorporated nts)

Pol III 3'→5': Proofreading

3. *(3 points)* In the schematic below, the black line represents a yeast chromosome, the checkered bar represents a gene that you wish to delete, and the gray bar represents an antibiotic resistant gene. Assume that all elements shown are double-stranded DNA. What sequences do you need to add to the antibiotic resistance gene in order to carry out gene replacement by homologous recombination?



A segment of sequence upstream of the checkered gene should be added upstream of the resistance gene, and a segment of sequence downstream of the checkered gene should be added downstream of the resistance gene.

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4. *(6 points)* For each of the following, indicate whether it is a protein, an RNA, or a DNA, and briefly state its role in CRISPR-Cas9 genome editing:

sgRNA – RNA, targets Cas9 to a particular location in the genome (base-pairs with the targeted genomic DNA)

PAM – DNA, a sequence motif that must be present on the targeted DNA in order to be bound by Cas9

Cas9 – Protein, cuts both strands of the genomic DNA being targeted.

Repair template – DNA, introduces a segment of DNA sequence that will be inserted into the double-strand break.

5. (4 points) Below is the structure of dATP.

A. Circle and label the 3' carbon.

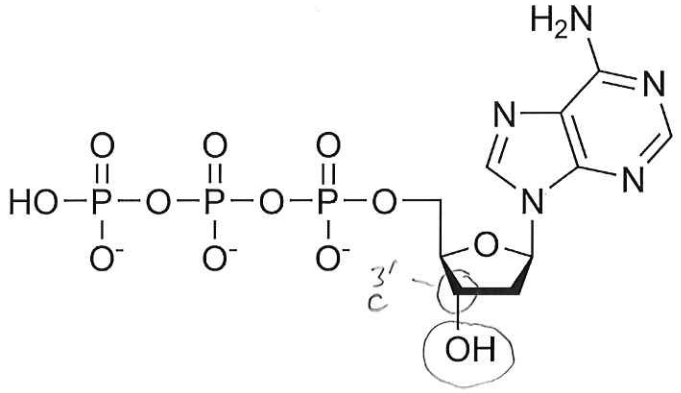
B. Circle the atom or group that would be different if this was ddATP.

C. What would be different about that atom or group?

There would be no OH (H only)

D. What effect will this have on DNA polymerization?

No additional nts can be added (polymerization will stop after this ddNTP is incorporated.



6. *(3 points)* Give a hypothetical scenario in which you would choose to use Sanger sequencing rather than Illumina sequencing, and briefly explain why.

Many possible correct answers. Any scenario in which only one or a small number of molecules need to be sequenced (individual genes or plasmids rather than whole chromosomes/genomes.

Cheaper and easier for small-scale projects. Also longer reads, higher accuracy.