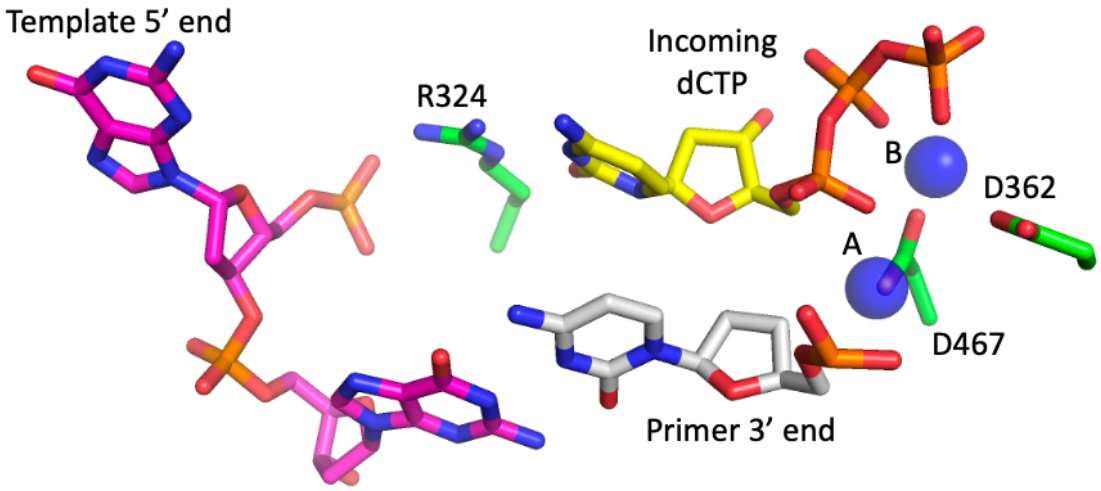
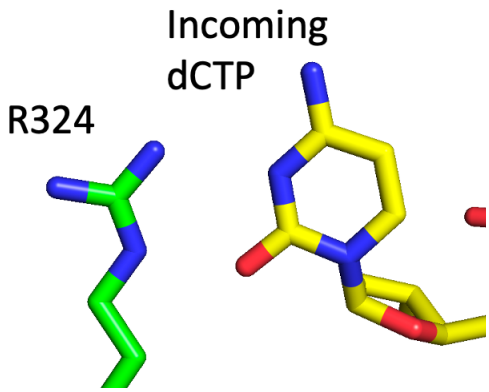
Discussion Problems – Winter 2026 - Week 4 (Polymerases)
Due on January 26 at 11:59pm
Please do all parts of the question your group is assigned to (i.e., group "1A" should do all parts of question 1).



Group 1

Rev1 is a specialized DNA polymerase which can only incorporate a C opposite to a G (it does not incorporate G, A or T in front of a G, and does not incorporate any nucleotides opposite to an A, T or C). The crystal structure of Rev1 in complex with a primer-template duplex and with dCTP has been obtained.



The template (magenta) contains a G at its 5'end, and the primer (gray) is only one nucleotide smaller than the template. A view of the active site is shown on the top. P atoms are colored in orange. A and B are two divalent cations (blue). A more detailed view of the molecular environment surrounding the "incoming dCTP" (yellow) is also shown on the right.

A- Why were researchers able to capture the polymerase in this configuration? (What prevents the substrates from being used by the enzyme?)

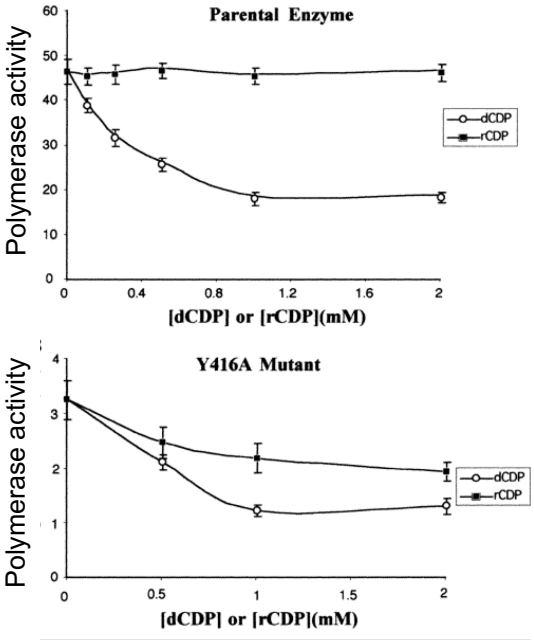
B- What aspect of the active site of the Rev1 DNA polymerase is similar to other DNA polymerases?

C- Assuming Rev1 uses a steric gate mechanism, where would the amino acid involved as a steric gate be located?

D-Using the two pictures of the active site, explain the specificity of Rev1 for dCTP and compare the base selection mechanism to the mechanisms used by the DNA polymerase studied in class.

Group 2

The polymerization activity of a DNA polymerase (wild-type version = Parental enzyme) or a version carrying a Tyrosine to Alanine mutation at position 416 (Y416A) is measured in the presence of a DNA template, a primer and all 4 dNTPs (y-axis, x-origin). Numbers represent arbitrary polymerization activity. In separate experiments, the enzymes are also incubated with the same components, but also increasing concentrations of either dCDP or rCDP nucleotides and the dNTP polymerization rates measured and plotted on the y-axis as a function of the concentration of dCDP or rCDP.

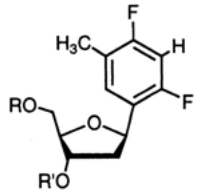


A – Describe and explain the impact of increasing dCDP or rCDP on the activities of the parental enzyme.
(make sure you differentiate description and explanation)

B – Describe and explain the impact of increasing dCDP or rCDP on the activities of the Y416A enzyme.

C – based on these results propose a function for residue 416 in the mechanism of the enzyme – your answer should fit into 1-2 sentences

Group 3



F

Figure 1. Structure of F.

Running start substrate

5'-TAATACGACTCACTATAG
3'-ATTATGCTGAGTGATATCCCTCTNGTCA

Standing start substrate

5'-TAATACGACTCACTATAGGGAGA
3'-ATTATGCTGAGTGATATCCCTCTNGTCA

Figure 2. The two starting substrates.

An experiment was conducted to compare the abilities of two different enzymes, Klenow fragment and Pol η, to synthesize DNA.

Two different substrates were tested, shown in Figure 2: the running start (Lanes 1-3) and the standing start (Lanes 4-6). The "N" in the sequence indicates the position where either a T nucleotide (lanes labeled T) or difluorotoluene nucleotide (lanes labeled F, structure shown in Figure 1) is present in the starting substrate. These starting substrates were incubated with dNTPs as well as either Klenow fragment (shown on the left in Figure 3), Pol η (shown on the right in Figure 3.), or no enzyme, which acts as a control (Lanes 1 and 4 on both templates).

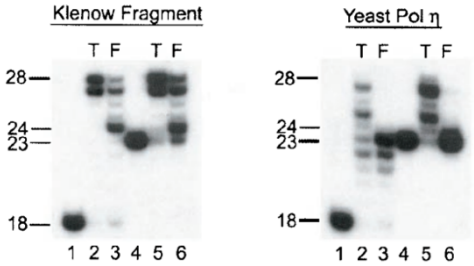


Figure 3. Experimental data.

A – What is the purpose of using F in this experiment?

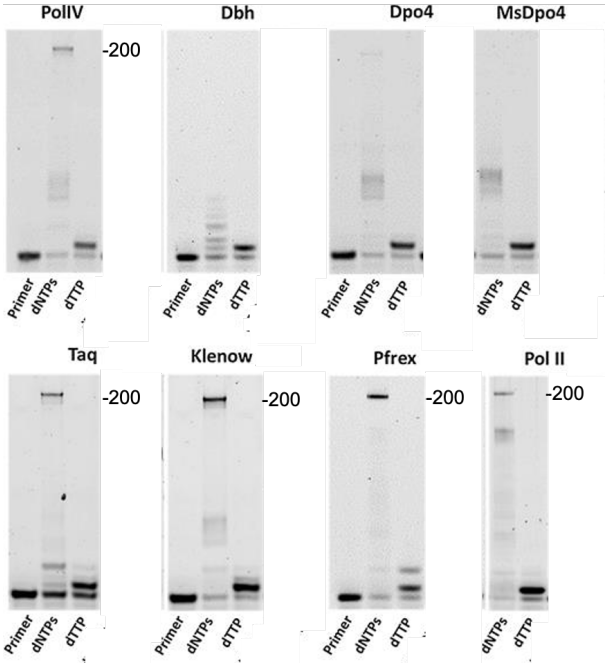
B – For Klenow fragment acting as the enzyme: How well does the F template work compared to T with the running start substrate, and how well does each work with the standing start substrate?

C – For Pol η acting as the enzyme: How well does the F template work compared to T with the running start substrate, and how well does each work with the standing start substrate?

D – Is Klenow or Pol η more processive on the running start substrate? Which lanes did you compare to make this conclusion?

Group 4

In this experiment, researchers analyze the extension of a primer annealed to a 200 nucleotide long template by different DNA polymerases. The first position in the template after the primer is an A. For each DNA polymerase, they incubate the polymerase and the template-primer duplex with either dTTP alone, or with all dNTPs for a short time (1 minute). The migration of the unextended primer is also shown for most polymerases. Only the primer is visible and the intensity of the signal is proportional to the amount of product obtained.



1- Describe the pattern of extension generated by each of these DNA polymerases <u>in the presence of all dNTPs only</u> and compare them to each other. What information does this experiment provides regarding the properties of each of these polymerases?

2- Describe the pattern of extension generated by each of these DNA polymerases in the presence of <u>only dTTP</u> and compare them to each other. Why do you think it was important to perform this experiment considering the results obtained previously?

3 – Assume Dbh is a bacterial DNA polymerase. Propose two modifications in the conditions in which the experiment performed with Dbh is done, which would result in a pattern more similar to the one observed for Taq or Pfrex.