

DNA POLYMERASE (PART 2)

Dr: Luu Phuc Loi, PhD.

Goals:

- Understand how DNA polymerase catalyzes the addition of deoxynucleoside triphosphates (dNTPs) to a growing DNA strand.
- Learn experimental assays for detecting and characterizing DNA polymerase activity.

Objectives:

After completing the materials for this unit, you should be able to:

- Recognize the structure of nucleic acids and key features of the structure of DNA polymerase.
- Describe the factors that confer high accuracy to DNA polymerase activity.
- Predict if a DNA-binding protein binds to the major or minor groove of DNA given the protein function.
- Summarize how critical residues of DNA polymerase and metal ions facilitate catalysis.
- Illustrate how a tautomeric state will result in the addition of the wrong base.
- Select the best experiment to test DNA polymerase activity for a given hypothesis and experimental constraints.
- Define processivity in both general terms and specifically in regards to DNA polymerase.
- Troubleshoot negative results from a hypothetical DNA polymerase processivity assay.
- Interpret data from DNA replication experiments.
- Analyze protein structures to infer functional information.

The Chemistry of DNA Replication

1. The Substrate: Primer–Template Junction (PTJ)

The primer provides a free 3' hydroxyl (3'-OH) group. The template is a longer single-stranded DNA adjacent to the primer. Reaction: The 3'-OH attacks the α -phosphate of an incoming dNTP, releasing pyrophosphate (PP).

2. Why Triphosphates? (Thermodynamics)

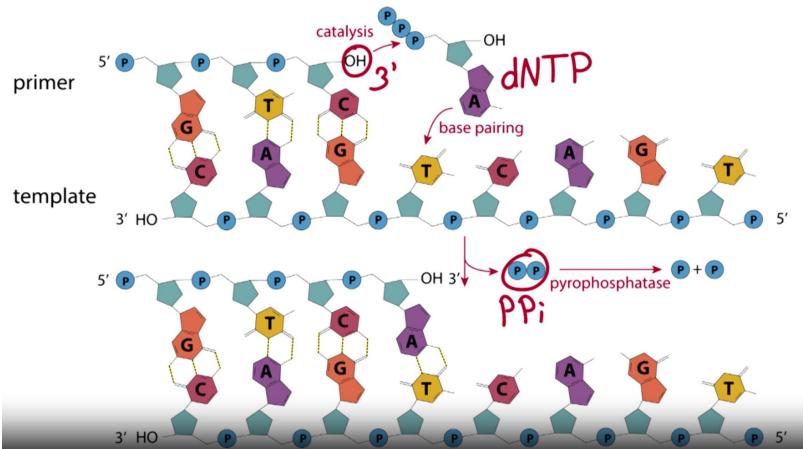
The reaction is driven by hydrolysis of pyrophosphate (PP) into two inorganic phosphates. Energy change: Standard $\Delta G \approx -3.5$ kcal/mol; with pyrophosphatase action, $\Delta G \approx -7$ kcal/mol. This makes DNA synthesis essentially irreversible and ensures forward progression.

3. DNA Polymerase Requirements

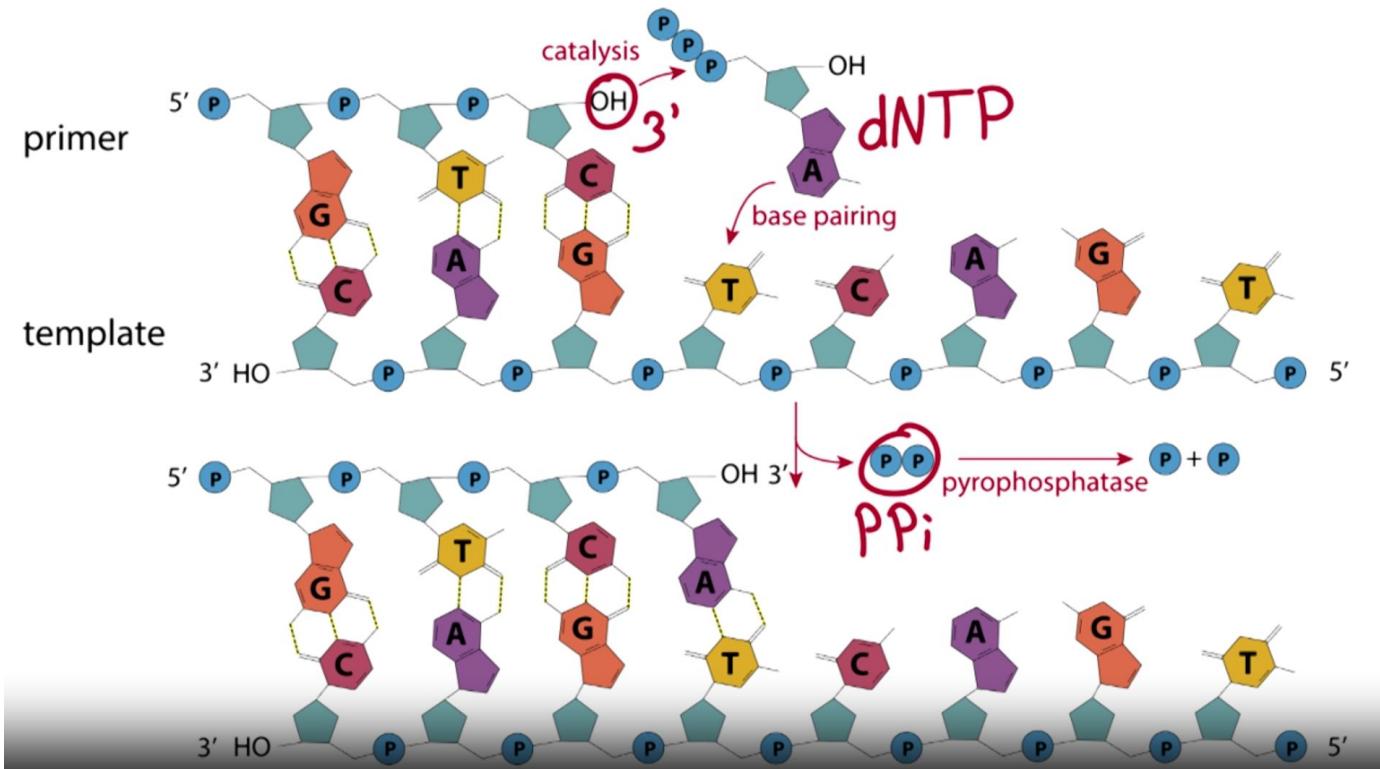
A 3'-OH primer annealed to a single-stranded DNA template (PTJ), all four dNTPs (deoxynucleotide triphosphates), and correct base pairing between the incoming dNTP and the template.

4. Defining the “5' to 3” Direction

The chemistry requires extension at the 3'-OH of the primer. Key concept: The primer is always the strand being elongated.



The Chemistry of DNA Replication



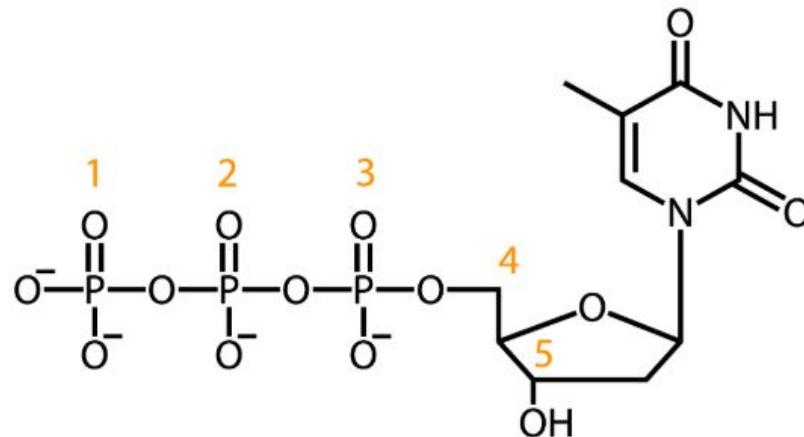
Test Yourself: The Chemistry of DNA Replication

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Test Yourself: The Chemistry of DNA Replication

0.0/1.0 point (graded)

Using the dropdown options, identify each phosphate group, the specified carbons, and which nucleotide is depicted in the following image.



- Which number in the structure corresponds to the alpha (α) phosphate?
- Which number in the structure corresponds to the beta (β) phosphate?
- Which number in the structure corresponds to the gamma (γ) phosphate?
- Which number in the structure corresponds to the 3' carbon?
- Which number in the structure corresponds to the 5' carbon?
- Which nucleotide is depicted?

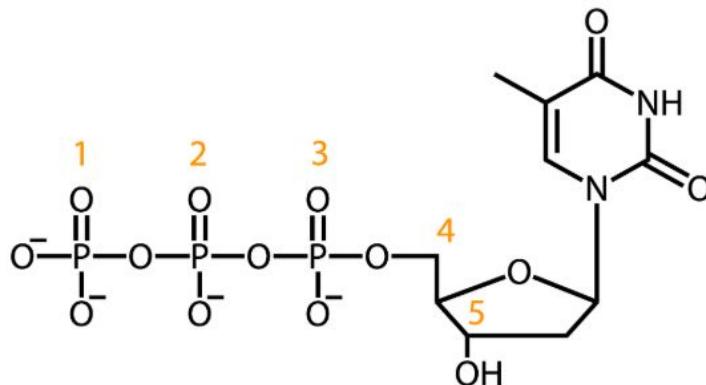
Test Yourself: The Chemistry of DNA Replication

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Test Yourself: The Chemistry of DNA Replication

1.0/1.0 point (graded)

Using the dropdown options, identify each phosphate group, the specified carbons, and which nucleotide is depicted in the following image.



- Which number in the structure corresponds to the alpha (α) phosphate? ✓ Answer: 3
- Which number in the structure corresponds to the beta (β) phosphate? ✓ Answer: 2
- Which number in the structure corresponds to the gamma (γ) phosphate? ✓ Answer: 1
- Which number in the structure corresponds to the 3' carbon? ✓ Answer: 5
- Which number in the structure corresponds to the 5' carbon? ✓ Answer: 4
- Which nucleotide is depicted? ✓ Answer: dTTP

Explanation

The structure shown is a dTTP, which is evident by the single ring and the arrangement of the double bonds. The phosphates from left to right are: gamma, beta, and alpha. The alpha phosphate is always bound to the 5' carbon on the deoxyribose.

The DNA Polymerase Active Site

1. How Many Reactions Can It Catalyze?

- **Reaction Diversity:** Depending on the incoming nucleotide, there are at least **4 distinct reactions**.
- **Contextual Complexity:** Considering the adjacent base on the primer, there are at least **16 possible combinations**.
- **The Question:** Does the enzyme need multiple active sites to handle this variety?

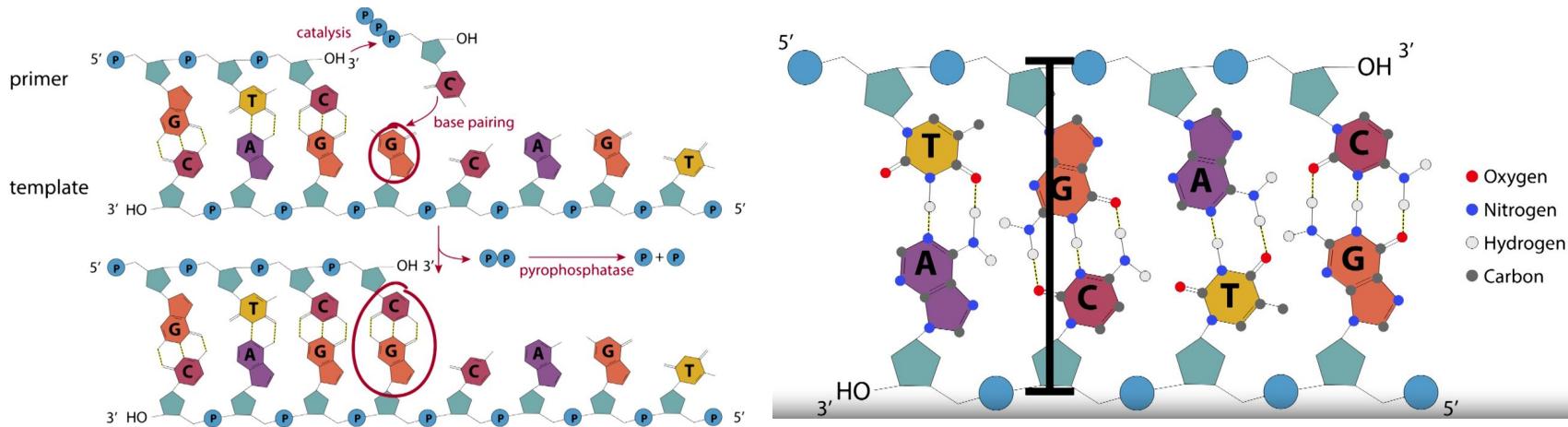
2. The Power of a Single Active Site

- **The Reality:** DNA polymerase catalyzes all these variations using **only one active site**.
- **The "Braille" Mechanism:** The enzyme identifies correct substrates not by the chemical identity of the base, but by the **uniform geometry** of the DNA double helix.
- **Geometric Uniformity:** * All four standard base pairs (A-T, T-A, G-C, C-G) have essentially the **same dimensions**.
 - This structural regularity allows a single "binding pocket" to accommodate any correct base pair.

3. Precision Through Positioning

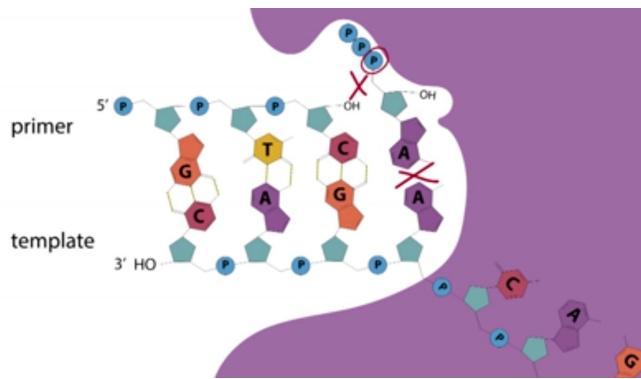
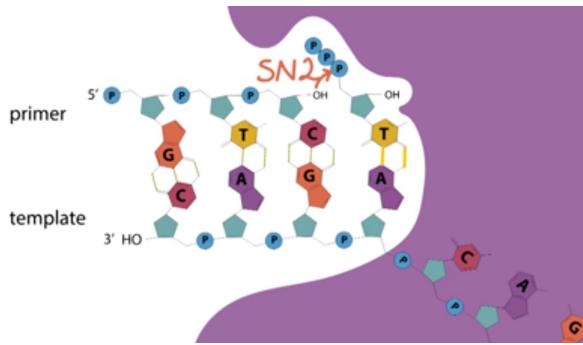
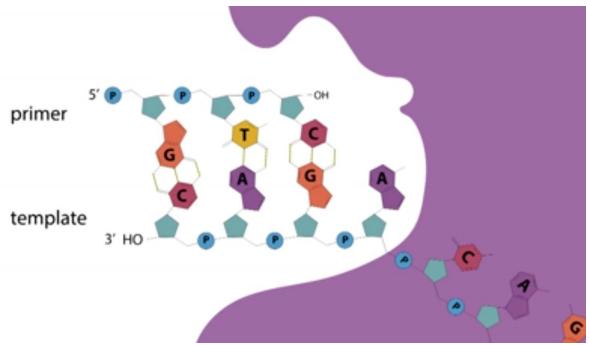
- **Correct Pairing:** Proper base pairing aligns the **α -phosphate** of the dNTP perfectly with the **3' OH** of the primer for an **SN2 nucleophilic attack**.
- **Mismatch Consequences:**
 - Mismatched nucleotides fail to position the phosphate group correctly for catalysis.
 - The reaction rate becomes so slow that the mispaired dNTP simply dissociates (floats out) before a bond can form.
- **Selection Criteria:** The enzyme ensures accuracy through both **binding affinity** and **temporal control** (speed of reaction).

The DNA Polymerase Active Site



https://drive.google.com/file/d/1nFYcv62jAUqYc-btVetlFSGmhzV4vdcE/view?usp=drive_link

The DNA Polymerase Active Site



Test Yourself: The DNA Polymerase Active Site

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Test Yourself: The DNA Polymerase Active Site

0.0/1.0 point (graded)

In the mechanism of DNA polymerase catalysis, how does the replicative DNA polymerase ensure that the correct base pair is made? Select the best answer.

- accomplishing slow catalysis in the presence of the correct base pair
- accomplishing fast catalysis in the presence of the correct base pair
- interacting with and recognizing the correct incoming dNTP

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Test Yourself: The DNA Polymerase Active Site

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- interacting with and recognizing the correct incoming dNTP



Explanation

DNA polymerase cannot distinguish a correct from incorrect dNTP by interacting with the incoming dNTP alone. When the correct base pair forms between the template nucleotide and the incoming dNTP, the $3'OH$ of the primer DNA is positioned near the alpha phosphate group of the incoming dNTP facilitating quick catalysis. In the absence of a correct base pair, the alpha phosphate is not in close proximity to the $3'OH$ resulting in much slower catalysis. The dNTP usually falls out of the active site by the time catalysis would occur. Thus, the correct base pairing is the result of temporal regulation.

The Structure of DNA Polymerase

1. The "Hand" Architecture

- **Right-hand Model:** Most DNA polymerases share a common architecture resembling a right hand.
- **Key Domains:**
 - **Fingers:** Move significantly during the catalysis reaction.
 - **Thumb:** Remains relatively still, holding the DNA in place.
 - **Palm:** The site of catalysis.

2. Interactions in the Palm

- **Binding Site:** The palm binds the **primer-template junction (PTJ)** and the newly synthesized DNA.
- **The 45-Degree Bend:** The template DNA is bent at a 45° angle immediately after the active site.
 - **Function:** This ensures only the next single nucleotide is positioned correctly for base pairing, preventing interference from upstream bases.

3. Non-Sequence Specific Recognition

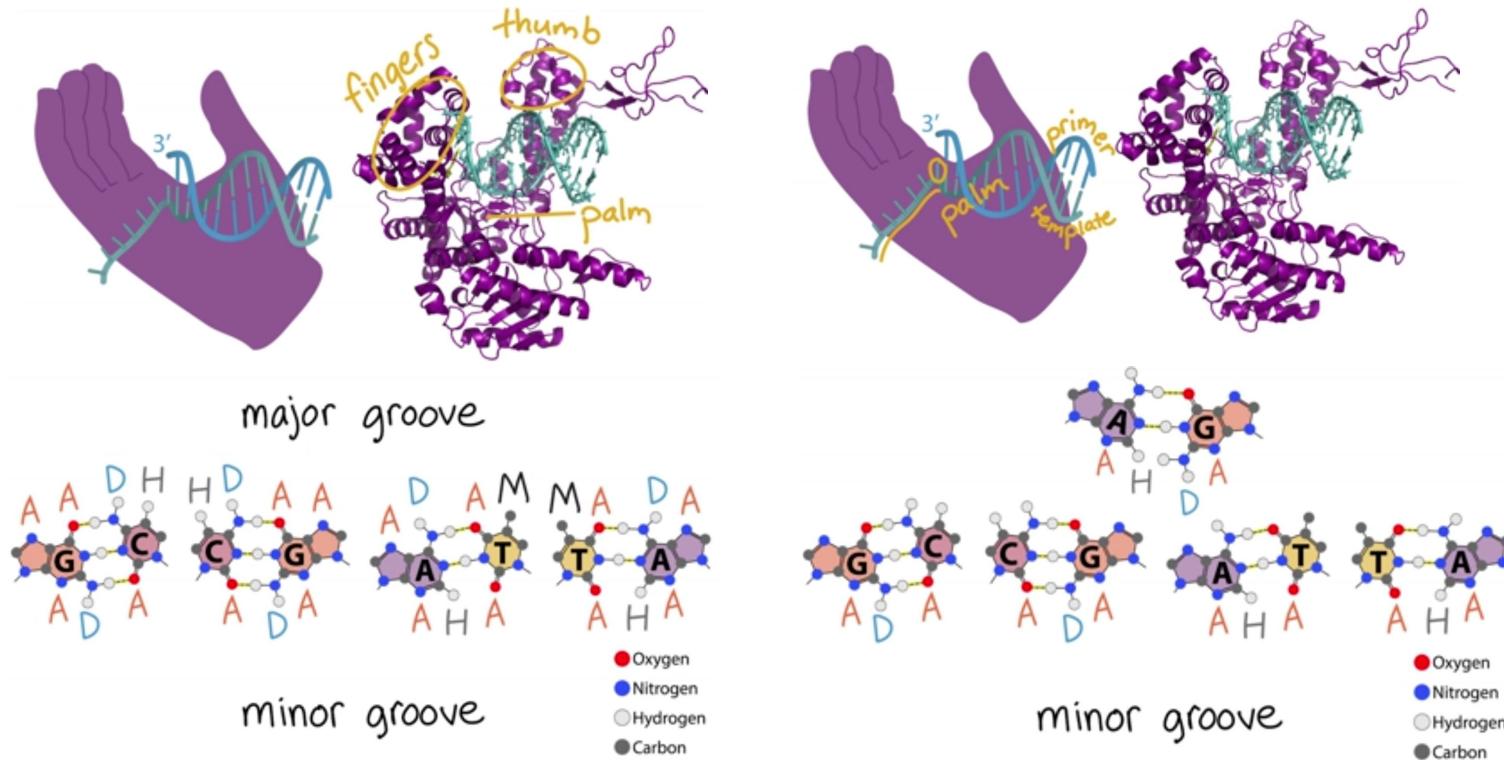
How does the enzyme bind any DNA sequence (GATC, AGAA, etc.)?

- **Backbone Binding:** It interacts with the phosphate backbone, which is constant regardless of sequence.
- **Minor Groove Interactions:**
 - Unlike the Major Groove (which contains sequence-specific information), the **Minor Groove** presents identical hydrogen bond acceptors for all four canonical base pairs.
 - This allows the polymerase to bind DNA non-specifically while demanding correct base pairing.

4. Quality Control of Base Pairing

- **Detection Mechanism:** The enzyme monitors the precise positioning of hydrogen bond acceptors in the minor groove.
- **Mismatch Detection:** Non-canonical pairs (e.g., A-G, C-C) distort these positions.
- **Enzyme Response:** By detecting these distortions, the polymerase can "sense" an incorrect insertion and respond accordingly.

The Structure of DNA Polymerase



Test Yourself: The Structure of DNA Polymerase

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Test Yourself Part A: The Structure of DNA Polymerase

0.0/1.0 point (graded)

Use the dropdown options to complete the sentences describing the major and minor grooves of DNA.

1

The groove of DNA is information rich, allowing proteins binding to this groove to distinguish between A:T and T:A base pairs and between G:C and C:G base pairs, respectively. An example of a protein that needs to interact with this groove to perform its function is 2

3

The groove of DNA is less information rich, only allowing proteins binding to this groove to distinguish between A:T/T:A base pairs and G:C/C:G base pairs. An example of a protein that interacts primarily with this groove to perform its function is 4

Select option

1. major/minor
2. BamHI endonuclease/ DNA polymerase/ topoisomerase type I
3. major/minor
4. DNA polymerase/ EcoRI restriction endonuclease/ Lac repressor

Test Yourself: The Structure of DNA Polymerase

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Test Yourself Part A: The Structure of DNA Polymerase

1.0/1.0 point (graded)

Use the dropdown options to complete the sentences describing the major and minor grooves of DNA.

The ✓ Answer: major groove of DNA is information rich, allowing proteins binding to this groove to distinguish between A:T and T:A base pairs and between G:C and C:G base pairs, respectively. An example of a protein that needs to interact with this groove to perform its function is ✓ Answer: BamHI endonuclease.

The ✓ Answer: minor groove of DNA is less information rich, only allowing proteins binding to this groove to distinguish between A:T/T:A base pairs and G:C/C:G base pairs. An example of a protein that interacts primarily with this groove to perform its function is ✓ Answer: DNA polymerase.

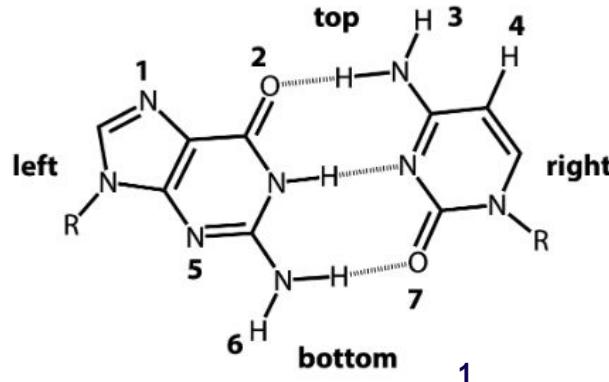
Explanation

BamHI recognizes a specific sequence of bases (5'-GGATCC-3') and cuts DNA within the recognition site. Similarly, *EcoRI* recognizes a specific sequence of bases (5'-GAATTC-3') and cuts DNA within its recognition site. To be able to do this, these enzymes must distinguish between A:T and T:A, or G:C and C:G and thus must bind in the major groove of DNA. DNA polymerase recognizes if a correct base has been inserted by the geometry of the base pair and cannot distinguish between A-T, T-A, G-C or C-G and therefore cannot recognize a specific sequence of base pairs, and interacts primarily with the minor groove. DNA topoisomerase I can make a covalent bond with the 5'OH and a noncovalent bond with the 3'OH groups of any nucleotide regardless of the specific base. And the Lac repressor protein binds in a sequence-specific manner to operator sequences in the *lac* operon.

Test Yourself Part B: The Structure of DNA Polymerase

0.0/1.0 point (graded)

Use the dropdown options to label the different grooves, bases, and chemical groups for the following DNA base pair.



Select option

1. major/minor
2. major/minor
3. adenine/ cytosine/ guanine/thymine
4. adenine/ cytosine/ guanine/thymine

- 2 The groove at the **top** of the image is the groove, whereas the groove at the **bottom** of the image is the groove. The base on the **left** is **3**, whereas the base on the **right** is **4**

For the chemical groups of the base pairs (numbered 1-7), identify them as:

- hydrogen bond donors (D)
- hydrogen bond acceptors (A)
- nonpolar hydrogens (H)
- methyl groups (M)

Select option

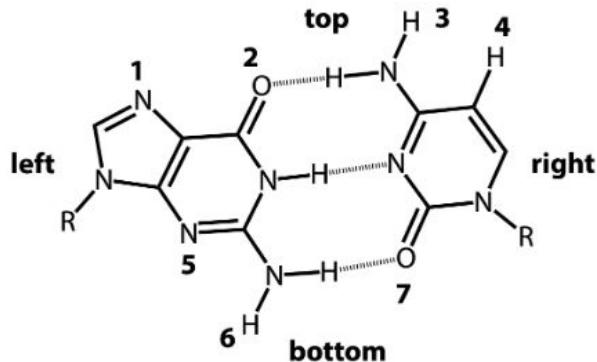
1 -> 7 : A/ D/ H/ M

1.
2.
3.
4.
5.
6.
7.

Test Yourself Part B: The Structure of DNA Polymerase

1.0/1.0 point (graded)

Use the dropdown options to label the different grooves, bases, and chemical groups for the following DNA base pair.



The groove at the **top** of the image is the groove, whereas the groove at the **bottom** of the image is the groove. The base on the **left** is whereas the base on the **right** is

For the chemical groups of the base pairs (numbered 1-7), identify them as:

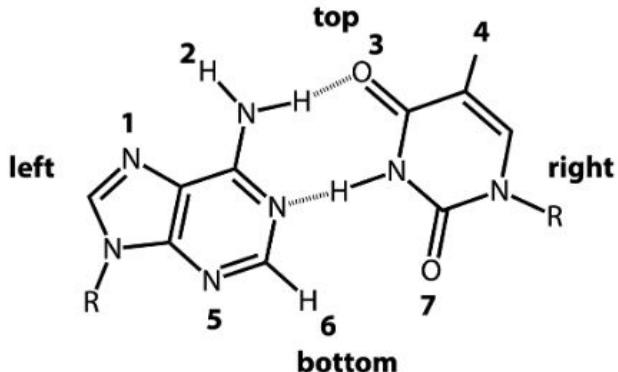
- hydrogen bond donors (D)
- hydrogen bond acceptors (A)
- nonpolar hydrogens (H)
- methyl groups (M)

- | | |
|-----------------------------------|-------------------------------------|
| 1. <input type="text" value="A"/> | <input checked="" type="checkbox"/> |
| 2. <input type="text" value="A"/> | <input checked="" type="checkbox"/> |
| 3. <input type="text" value="D"/> | <input checked="" type="checkbox"/> |
| 4. <input type="text" value="H"/> | <input checked="" type="checkbox"/> |
| 5. <input type="text" value="A"/> | <input checked="" type="checkbox"/> |
| 6. <input type="text" value="D"/> | <input checked="" type="checkbox"/> |
| 7. <input type="text" value="A"/> | <input checked="" type="checkbox"/> |

Test Yourself Part C: The Structure of DNA Polymerase

0.0/1.0 point (graded)

Use the dropdown options to label the different grooves, bases, and chemical groups for the following DNA base pair.



Select option

1. major/minor
2. major/minor
3. adenine/ cytosine/ guanine/thymine
4. adenine/ cytosine/ guanine/thymine

The groove at the **top** of the image is the **Select an option** groove, whereas the groove at the **bottom** of the image is the **Select an option** groove. The base on the **left** is **Select an option** whereas the base on the **right** is **Select an option**

For the chemical groups of the base pairs (numbered 1-7), identify them as:

- hydrogen bond donors (D)
- hydrogen bond acceptors (A)
- nonpolar hydrogens (H)
- methyl groups (M)

1. **Select an option**
2. **Select an option**
3. **Select an option**
4. **Select an option**
5. **Select an option**
6. **Select an option**
7. **Select an option**

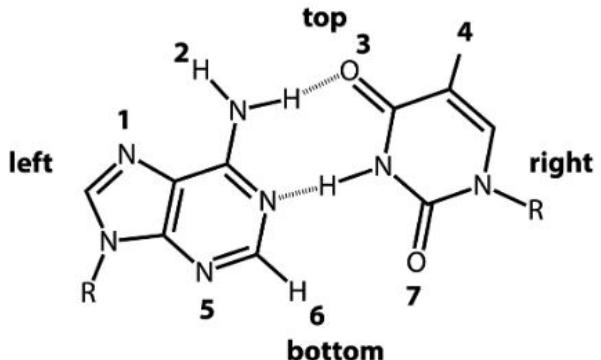
Select option

1 -> 7 : A/ D/ H/ M

Test Yourself Part C: The Structure of DNA Polymerase

1.0/1.0 point (graded)

Use the dropdown options to label the different grooves, bases, and chemical groups for the following DNA base pair.



The groove at the **top** of the image is the ✓ groove, whereas the groove at the **bottom** of the image is the
minor ✓ groove. The base on the **left** is ✓ whereas the base on the **right** is
thymine. ✓

For the chemical groups of the base pairs (numbered 1-7), identify them as:

- hydrogen bond donors (D)
- hydrogen bond acceptors (A)
- nonpolar hydrogens (H)
- methyl groups (M)

1. A	✓
2. D	✓
3. A	✓
4. M	✓
5. A	✓
6. H	✓
7. A	✓

Test Yourself Part D: The Structure of DNA Polymerase

0.0/1.0 point (graded)

You are designing a protein that binds to a specific sequence of DNA by binding the major groove of DNA. You want the protein to bind to the three base pair stretch **G:C, A:T, C:G**. Which of the following arrangements of chemical groups should your protein be designed to bind? Select the best answer.

Hint: You can use your answers to the other questions to help you determine the answer.

AADH

MADA

AADH

ADA

AHA

ADA

HDAA

ADAM

AADH

AADH

ADAM

HDAA

Test Yourself Part D: The Structure of DNA Polymerase

1.0/1.0 point (graded)

You are designing a protein that binds to a specific sequence of DNA by binding the major groove of DNA. You want the protein to bind to the three base pair stretch **G:C, A:T, C:G**. Which of the following arrangements of chemical groups should your protein be designed to bind? Select the best answer.

Hint: You can use your answers to the other questions to help you determine the answer.



AADH
MADA
AADH



ADA
AHA
ADA



HDAA
ADAM
AADH



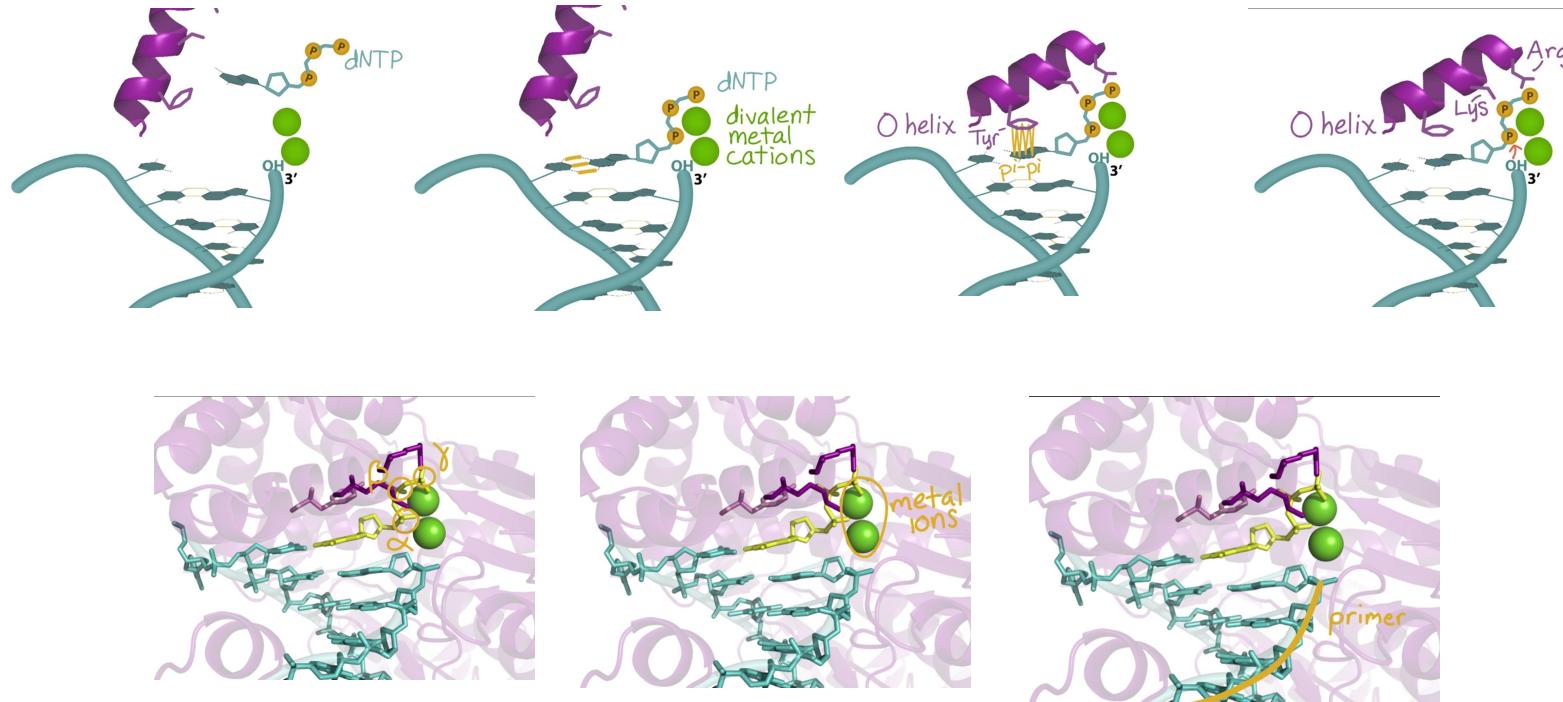
AADH
ADAM
HDAA



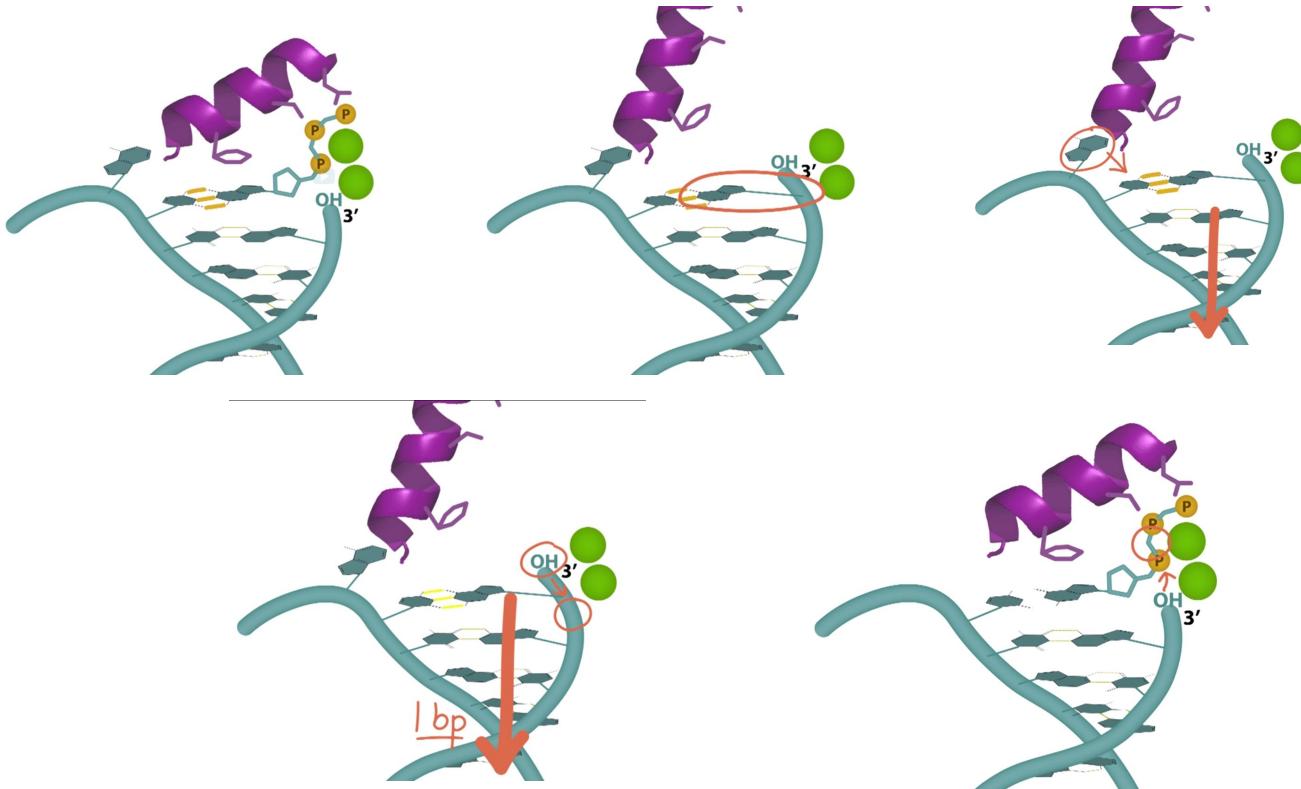
Explanation

The AADH, ADAM, and HDAA chemical group combination matches that of a G:C, A:T, C:G sequence of the major groove. The ADA, AHA, and ADA chemical group combination represents a minor groove chemical group combination, which does not distinguish between G:C and C:G or A:T and T:A, respectively. The other two combinations are incorrect for the major groove.

Catalysis by the Fingers of DNA Polymerase



Catalysis by the Fingers of DNA Polymerase



Test Yourself: Catalysis by the Fingers of DNA Polymerase

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Test Yourself: Catalysis by the Fingers of DNA Polymerase

0.0/1.0 point (graded)

The arginine and lysine of the O-helix of the DNA polymerase as well as the metal ions present in the active site play key roles in DNA polymerase catalysis. Which of the following statements accurately describe the function of these residues and cations within the O-helix? Select all that apply.

You may want to refer to this amino acid chart.

- Arginine, lysine, and the metal ions are positively charged.
- Metal ions interact with and help position the alpha, beta, and gamma phosphate groups of the incoming dNTP for catalysis.
- The lysine is critical for making stacking interactions with the base of the incoming dNTP.
- The arginine and lysine of the O-helix interact with and help position the phosphate groups of the incoming dNTP for catalysis.

Test Yourself: Catalysis by the Fingers of DNA Polymerase

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Test Yourself: Catalysis by the Fingers of DNA Polymerase

1.0/1.0 point (graded)

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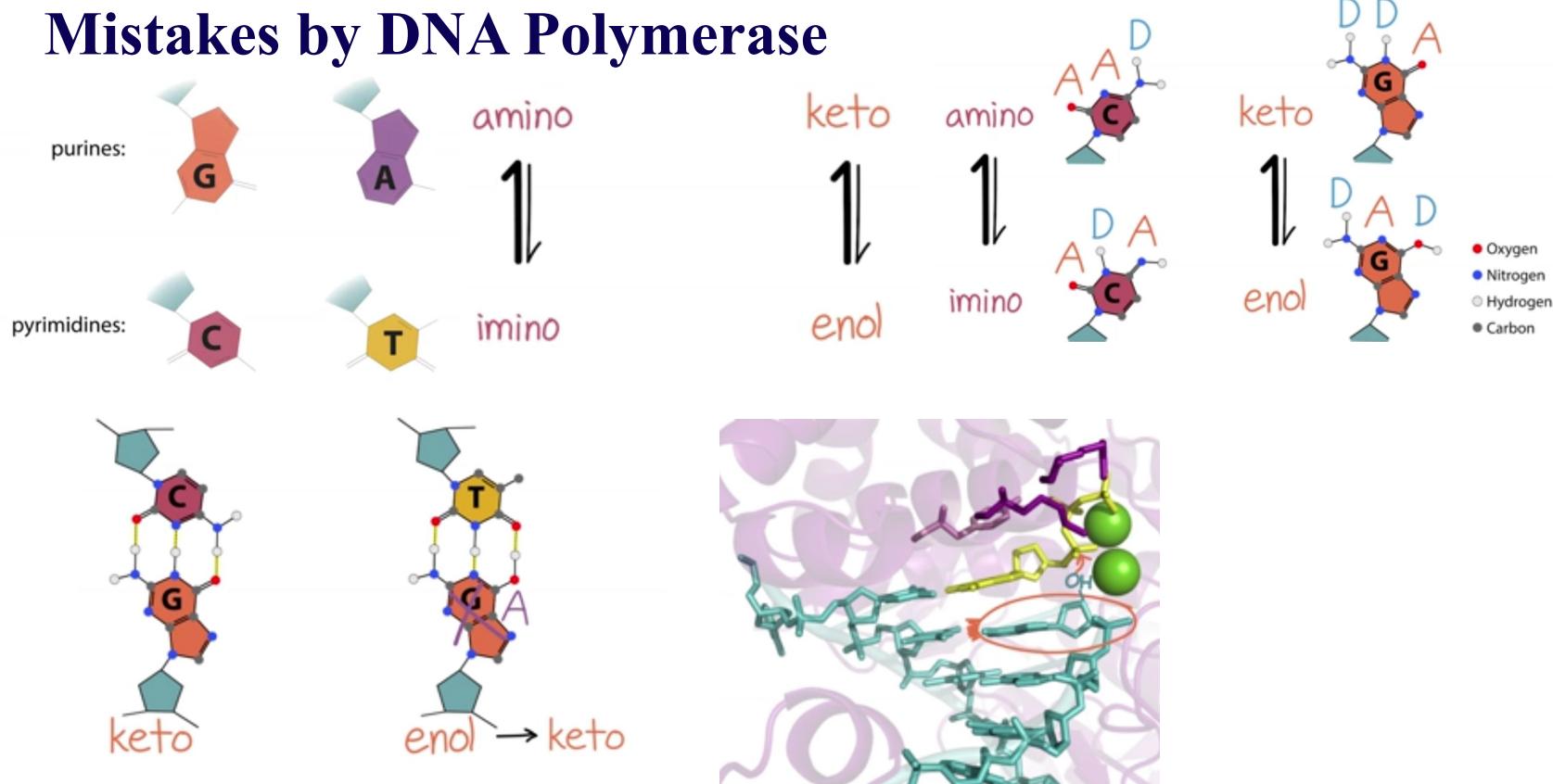
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- The lysine is critical for making stacking interactions with the base of the incoming dNTP.
- The arginine and lysine of the O-helix interact with and help position the phosphate groups of the incoming dNTP for catalysis.



Explanation

It is the tyrosine of the O-helix, not the lysine, which is critical for making stacking interactions with the base of the incoming dNTP. All other statements are true.

Mistakes by DNA Polymerase



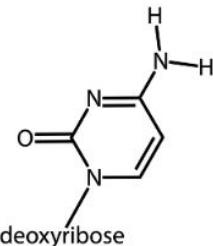
Test Yourself: Mistakes by DNA Polymerase

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Test Yourself Part A: Mistakes by DNA Polymerase

0.0/1.0 point (graded)

For the portion of deoxycytidine triphosphate (dCTP) shown, decide if the base is in the common form or the rare tautomeric state.



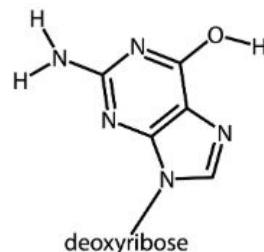
common form

rare tautomer form

Test Yourself Part B: Mistakes by DNA Polymerase

0.0/1.0 point (graded)

For the portion of deoxyguanosine triphosphate (dGTP) shown, decide if the base is in the common form or the rare tautomeric state.



common form

rare tautomer form

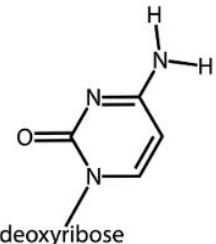
Test Yourself: Mistakes by DNA Polymerase

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Test Yourself Part A: Mistakes by DNA Polymerase

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common form

rare tautomeric form



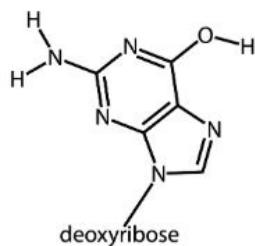
Explanation

This cytosine is in the amino state, which is the common form of this nucleotide.

Test Yourself Part B: Mistakes by DNA Polymerase

1.0/1.0 point (graded)

For the portion of deoxyguanosine triphosphate (dGTP) shown, decide if the base is in the common form or the rare tautomeric state.



common form

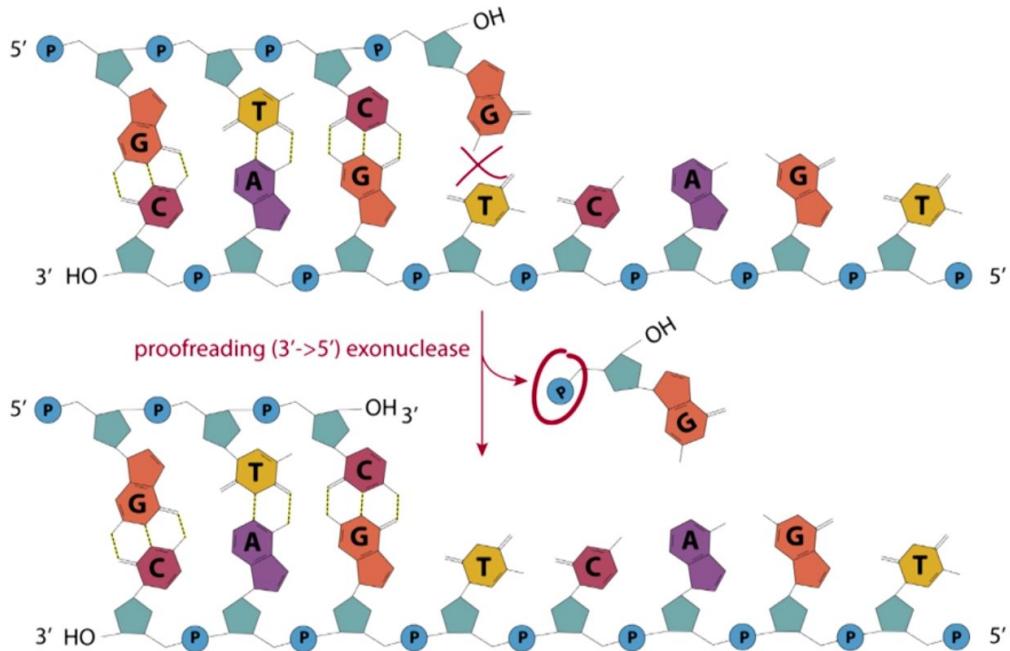
rare tautomeric form



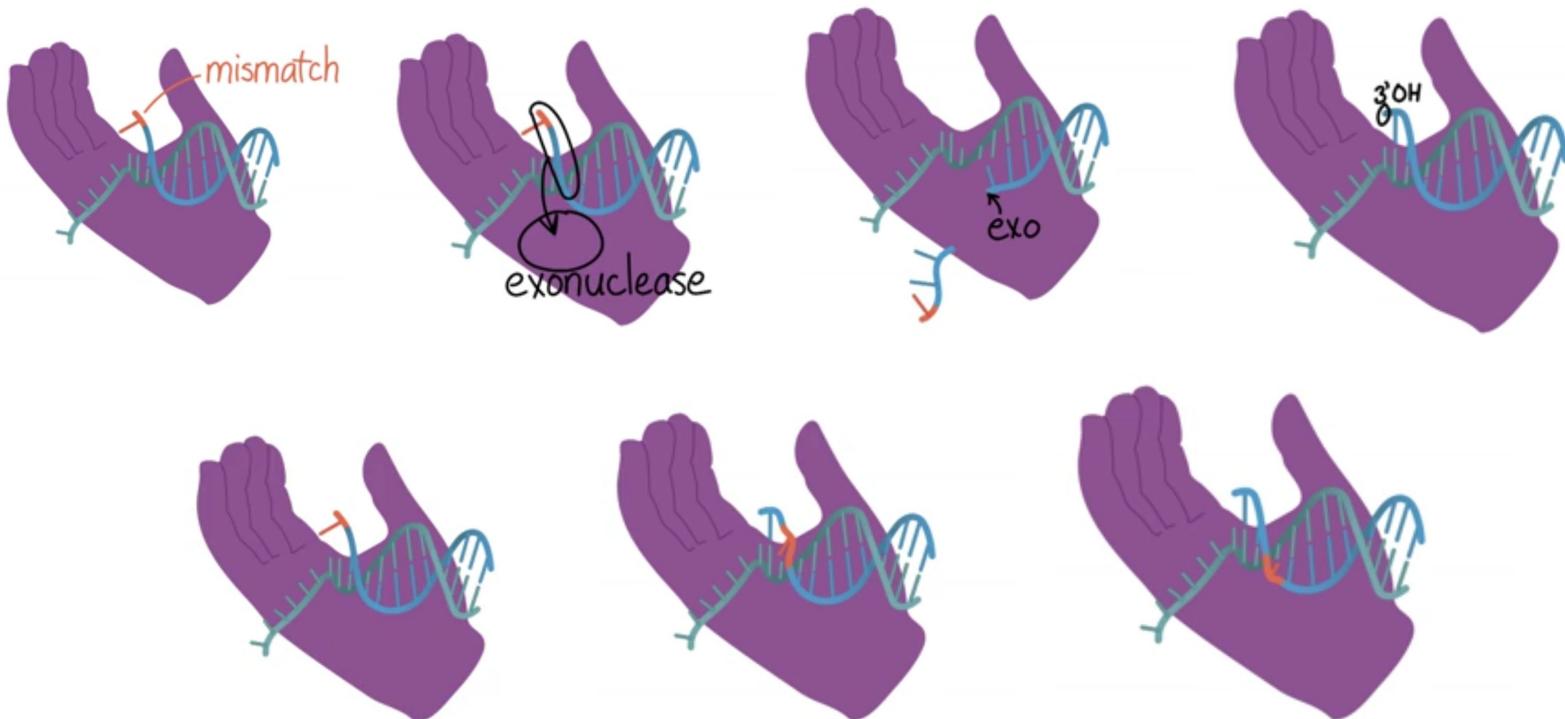
Explanation

This guanine is in the enol state, which is the rare tautomeric form. When a base is in the rare tautomeric form in the template strand of the DNA, DNA polymerase may introduce the incorrect nucleotide in the newly synthesized strand of DNA. This can lead to a mismatch when the template base returns to the common form.

Proofreading Exonucleases



Proofreading Exonucleases



Test Yourself: Proofreading Exonucleases

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Test Yourself: Proofreading Exonucleases

1.0/1.0 point (graded)

Proofreading exonucleases are critical for the fidelity of DNA synthesis. Which of the following statements accurately describe this activity? Select all that apply.

- The proofreading exonuclease can be part of the same polypeptide chain as the DNA polymerase.
- The proofreading exonuclease increases the fidelity of DNA synthesis about 100 fold.
- The proofreading exonuclease degrades single-stranded DNA starting at the 5' end.
- The proofreading exonuclease active site has a higher affinity for the 3' single-stranded DNA resulting from a mismatch at the primer:template junction than the DNA polymerase active site.
- The incorporation of a mismatch by DNA polymerase increases the rate of DNA synthesis.

Test Yourself: Proofreading Exonucleases

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Test Yourself: Proofreading Exonucleases

1.0/1.0 point (graded)

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The proofreading exonuclease degrades single-stranded DNA starting at the 5' end.

The proofreading exonuclease active site has a higher affinity for the 3' single-stranded DNA resulting from a mismatch at the primer:template junction than the DNA polymerase active site.

The incorporation of a mismatch by DNA polymerase increases the rate of DNA synthesis.



Explanation

The presence of a mismatch destabilizes the primer:template junction enough to create a small region of single-stranded DNA at the 3' end that the proofreading exonuclease active site has a 10-fold higher affinity for than the DNA polymerase active site. The proofreading exonuclease will remove a nucleotide or two before the primer:template junction properly reforms to allow DNA polymerase to continue DNA synthesis. The incorporation of a mismatch decreases the rate of DNA synthesis allowing the rate of the proofreading exonuclease activity to increase.

Home study guidance

Incorporation Assay:

https://drive.google.com/file/d/1X6lqVqxb3E4p2N8vXVZxu2edmx_73Mjv/view?usp=sharing

Primer Extension Assay:

https://drive.google.com/file/d/1EYmbJi2E9hvHKMCNHqS_UC_oO71_FuzM/view?usp=sharing

Processivity and the Template Challenge Assay:

https://drive.google.com/file/d/1oQ_hQHwKMM653UtmMUBGjEEG-qbB7pZh/view?usp=sharing

DNA Populations in Experiments:

https://drive.google.com/file/d/15juxoPmRKi_0MaOO1e9aIrSfRMgVwzwK/view?usp=sharing

Home assignment

DNA Polymerase Rate

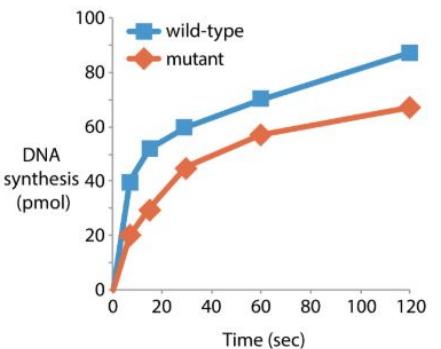
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Assessing DNA Polymerase Rate

0.0/1.0 point (graded)

After correcting your experimental set up, you want to compare how fast your *Taq* polymerase synthesizes DNA relative to a mutant. Remember, the rate of polymerase synthesis is defined as the number of nucleotides incorporated over a period of time.

You decide to pre-incubate a 6 kb single-stranded linear DNA substrate (with a primer annealed) with wild-type *Taq* polymerase or the mutant in the appropriate reaction buffers, leaving out dTTP in each reaction. Then you begin the elongation reaction by adding alpha-radiolabeled dTTP ($dTTP^*$) to each tube, taking samples over time, and ending the reactions by adding EDTA. You then separate the synthesized DNA from the unincorporated labeled nucleotides by gel electrophoresis and analyze the amount of label incorporated into the DNA over time.



From these data, Select an option

Taq polymerase synthesizes DNA faster than Select an option

Taq polymerase.

Home assignment

Examining the Structure of DNA Pol III

Your labmate identified a mutant of *T. aquaticus* that revealed that there is a point mutation in the codon encoding amino acid 671 in the catalytic core of Pol III, changing a glutamic acid (Glu) to a lysine (Lys).

You want to examine whether the site of this amino acid substitution could directly affect DNA binding or catalysis by Pol III. The wild-type *T. aquaticus* structure of Pol III is shown in Appendix 1, which you should already have open in a separate window.

Location of the Substitution

0.0/1.0 point (graded)

By examining the amino acid of interest in wild-type *T. aquaticus*, what domain of DNA Pol III is the site of substitution within? Select the best answer.

Thumb

Fingers

Palm

Home assignment

Physical Consequence of Mutation

0.0/1.0 point (graded)

Could a change from Glu to Lys in this location directly affect the interaction of Pol III with DNA? Use the dropdown options to indicate whether this amino acid change could plausibly alter these interactions. You can use this [amino acid chart](#) as a reference if you need one.

Select an option ▾

Select all reasons that support your answer.

- The amino acid substitution switches the charge of the amino acid from negative to positive, and the DNA backbone is negatively charged.
- The amino acid substitution is within the polymerase active site where DNA binds.
- The amino acid substitution is distant from the active site and unlikely to contact the DNA.
- The amino acid substitution does not significantly change the nature of the amino acid at that site.
- The site of the amino acid substitution is in contact with the incoming dNTP.

Home assignment

Functional Consequence of Mutation

0.0/1.0 point (graded)

Considering the location of the substitution within Pol III, which of the following properties of the polymerase could **possibly** be affected by the substitution? Remember that changes to amino acids in essential locations in enzymes can have many and varied effects on protein function.

Select all that apply.

nucleotide incorporation rate

polymerase processivity

polymerase fidelity

DNA binding affinity

Home assignment

Amino Acid Identification: Removing Misincorporated Bases

0.0/1.0 point (graded)

From what you know about DNA polymerase structure and function, choose an appropriate residue from those highlighted as spheres to answer the following questions. You can investigate an amino acid by hovering over it in the structure. If there is more than one correct answer, submit only one. Enter your answer as a three-letter abbreviation and number of one amino acid (for example: Ala254, or Lys12, or Leu344). You can use this [amino acid chart](#) as a reference if you need one.

What is an amino acid that helps remove misincorporated bases from the end of the nascent DNA strand?

Amino Acid Identification: Binding Phosphates

0.0/1.0 point (graded)

Of the amino acids labeled as spheres in the structure, which help bind the phosphates of the incoming nucleotide? If there are multiple answers, submit only one.

Home assignment

Effect of Amino Acid Substitutions

0.0/1.0 point (graded)

Your goal is to engineer Nsin to include specific amino acid substitutions that improve its function. There are thousands of substitution possibilities, but not all of them would be beneficial to the function of your polymerase. Which of the following amino acid substitutions would likely result in a failure of 5' to 3' polymerase activity? Select all that apply.

Arg → Asp at position 247

Asp → Ala at position 404

Asp → Glu at position 542

Asp → Arg at position 542

Home assignment

Engineering A Better Polymerase - Creating Mutants

 Bookmark this page

Selecting Your Assay

0.0/1.0 point (graded)

In your quest to develop a more processive and faster DNA polymerase, you create three mutant forms of Nsin, A523R, A523K, and Y515R (an amino acid substitution of A to R or K at position 523 and Y to R at position 515, respectively). You purify the three mutant proteins and wild-type Nsin.

You want to compare the activities of your mutant proteins and wild-type Nsin. Your labmate, Alice, is working with a **labeled**, linear oligonucleotide annealed to a circular, single-stranded DNA template. She offers to let you use some of her DNA. With Alice's DNA, which assay could you perform to gain information about your four Nsin DNA polymerases? Be specific by using the term for the assay used in this course. You can include or exclude the word assay in the answer, either way will grade correctly.

Home assignment

Comparing Assays

0.0/1.0 point (graded)

How does the information this assay yields compare to that of your prior incorporation assay that you used for your studies on *Taq*? Choose all that apply.

- The signal detected in the new assay is constant for all products.
- The signal detected in the new assay varies depending on the length of the extended products.
- The new assay is more likely to give precise information about the length products regardless of length.
- The new assay is more likely to give precise information about the length of short products.

Home assignment

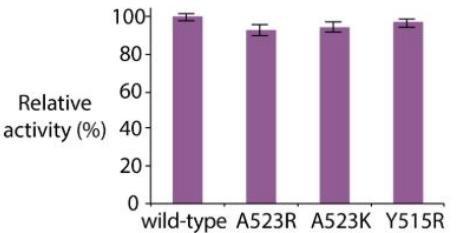
Engineering A Better Polymerase - Analyzing Data

 Bookmark this page

Analyze Assay Data

0.0/1.0 point (graded)

Your labmate, Rajeev, performs an incorporation assay on your four Ns1N DNA polymerases using a 50-nt long template annealed to a 10-nt primer. He measures the amount of radioactivity incorporated for each polymerase and normalizes relative to the wild-type polymerase. He graphs the data shown.



Which of the following statements do the data support? Select the best answer.

- All four polymerases create extended product DNA of approximately the same size.
- All four polymerases incorporate roughly the same number of nucleotides per PTJ binding event.
- All four polymerases incorporate roughly the same amount of labeled nucleotide into product.
- The experiment does not give any meaningful data.

Home assignment

Your Next Question

0.0/1.0 point (graded)

After looking at Rajeev's data, you would like to test the processivity of the four DNA polymerases Rajeev is using in his assay. If you were comparing two polymerases, A and B, which of the following observations would indicate that A was a more highly processive DNA polymerase than B? Choose the best answer.

- A takes less time to bind to a PTJ than B.
- A creates more replicated DNA product than B, given plenty of PTJs to work on.
- B adds the incorrect dNTP to a PTJ more frequently than A.
- A adds more dNTPs to a PTJ it has bound before falling off of the PTJ than B does.

Home assignment

Making Your Conclusions

0.0/1.0 point (graded)

Now that you had time to think about Brian's assay, you tune back in to his conversation with Rajeev:

- Brian says that his PCR results show that only the A523R and A523K mutant Nsins are significantly active *in vitro*.
- Rajeev disagrees, arguing that the PCR results combined with his incorporation assay data show that all four DNA polymerases are active, but that A523R and A523K are processive while wild-type and Y515R are not.

They turn to you and ask who you think is correct. Before delivering your decision, you think through what the data mean all together. Which of the following conclusions do you make? Select all that apply.

A523R is more processive than wild-type Nsin.

All four polymerases reliably show activity in Rajeev's experiment.

Brian's experiment measures how efficiently the polymerases amplify 4 kb of a 6 kb template.

A523K has a faster rate of incorporation than Y515R.

Home assignment

Pre-incubation Processivity Assay

2 point possible (graded)

[Keyboard Help](#)

Your labmates, Brian and Rajeev, are confused about how to measure the processivity of polymerases. You remember that a processivity assay consists of four steps:

1. a pre-incubation of some of the reagents
2. the main reaction, in which some more reagents are added to the pre-incubation
3. allow the reaction to proceed for sufficient time
4. stop the reaction and separate the products by gel electrophoresis

You take it upon yourself to diagram the first two steps for them. Using the drag-and-drop options in this problem, assemble the reagents in appropriate amounts necessary for the pre-incubation step. The asterisks (*) indicate a radioactive label and the blue boxes indicate places to indicate the amounts of different reagents.

excess

limited

3' * 3'

3' * 3'

3' * 3'

3' * 3'

3' * 3'

3' * 3'

3' * 3'

pre-incubation:

DNA Pol + substrate

polymerase amount

substrate amount

Submit You have used 0 of 3 attempts.

Reset

Home assignment

Processivity Assay Reaction

2 point possible (graded)

[Keyboard Help](#)

Now that you have diagrammed your pre-incubation, diagram your reaction mixture for Brian and Rajeev. Remember that your pre-incubation reagents are already part of your reaction; you do not have to add them again. Using the drag-and-drop options in this problem, assemble the reagents in appropriate amounts necessary for the reaction step of the assay.

The diagram shows five boxes representing different components:

- Top left: "excess" with a DNA strand labeled 3' * 3'.
- Top right: "limited" with a DNA strand labeled * 3'.
- Middle left: A DNA strand labeled * 3'.
- Middle center: A DNA strand labeled 3' *.
- Middle right: A DNA strand labeled * 3'.
- Bottom left: A DNA strand labeled 3' *.
- Bottom center: A DNA strand labeled 3'.
- Bottom right: Two buttons: "labeled dNTPs" and "unlabeled dNTPs".

reaction:

pre-incubation + substrate + reagent added

substrate amount

Submit

You have used 0 of 3 attempts.

Reset

Home assignment

Why Excess, Why Limited?

0.0/1.0 point (graded)

Brian still has questions after your explanation. What is the purpose of having some reagents be limited and some reagents be in excess in your setups above? Select the best answer.

- To ensure that after falling off its initial substrate, DNA polymerase will only find unlabeled substrates.
- To provide enough dNTPs so that the polymerization reaction can reach the end of the template.
- To avoid inhibiting DNA polymerase with an excess of unlabeled double-stranded DNA product.
- To ensure that the DNA template is radiolabeled so you can observe it on an autoradiogram.

Home assignment

Differentiating Polymerase Processivities

0.0/1.0 point (graded)

Rajeev understood your explanation but now is confused about the limitations of the processivity assay. Suppose you have two processive DNA polymerases with different processivities.

- Polymerase A can polymerize **10 kilobases (kb)** before falling off the DNA
- Polymerase B can polymerize **50 kb** before falling off the DNA

You put both polymerases through the processivity assay using a **4 kb** DNA template annealed to a labeled primer as a substrate.

How will each polymerase compare in this autoradiogram of the processivity assay? Select the best answer.

- Polymerase A will show a band representing 4 kb DNA while Polymerase B will show a band representing shorter, faster migrating DNA population.
- Polymerases A and B will both show a band representing 4 kb DNA.
- It is impossible to predict the band pattern of the autoradiogram from the processivities of the polymerases.

Home assignment

Base-pairing and Major and Minor Groove Interactions

Bookmark this page

The Base Pairing of Inosine

0.0/2.0 points (graded)

Your lab also studies other DNA-binding proteins in thermophilic organisms and you would like to understand how they bind DNA. Your supervisor has an experiment in mind and suggests you first review how inosine base pairs with other nucleotides. Which of the following basepairs can form with inosine? Select all that apply.

<input type="checkbox"/> inosine	adenine	
<input type="checkbox"/> inosine	cytosine	
<input type="checkbox"/> inosine	guanine	
<input type="checkbox"/> inosine	thymine	
<input type="checkbox"/> inosine	uracil	

Reading T/A and A/T Base Pairs

0.0/1.0 point (graded)

Your labmate thinks she has discovered a region of thymine-adenine base pairs that the some of these proteins bind. Before experimentally testing binding between this region and a candidate protein, you help her describe the chemical groups that the DNA-binding protein would encounter in this region.

For each base pair given, enter the sequence of chemical groups (from left to right) a protein reading the base pair would see in the major groove and the minor groove.

Use A for hydrogen bond acceptors (electronegative atoms with partial negative charge), D for hydrogen bond donors (atoms with partial positive charge), H for nonpolar hydrogens, and M for methyl groups. Do not separate your letters with spaces or punctuation. For example: DADH or MAM

Chemical Groups in the Major and Minor Grooves of T/A and A/T Base Pairs

	thymine	adenine	adenine	thymine
Major groove				
Minor groove				

Save

Home assignment

Reading I/C and C/I Base Pairs

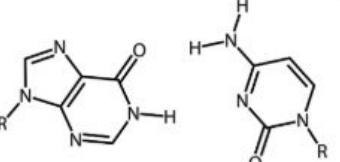
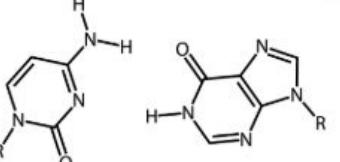
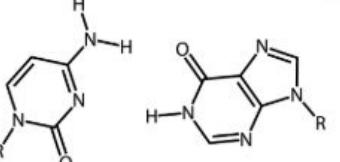
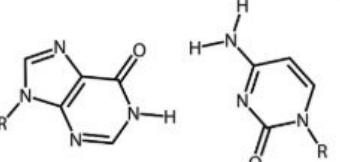
0.0/1.0 point (graded)

Your advisor suggests comparing the candidate binding sequence your lab mate found to an altered sequence at the same site using inosine and cytosine. You both pause and take a moment to repeat the process of describing the hydrogen bonding patterns in the major and minor grooves for this altered sequence.

For each base pair given, enter the sequence of chemical groups (from left to right) a protein reading the base pair would see in the major groove and the minor groove.

Use A for hydrogen bond acceptors (electronegative atoms with partial negative charge), D for hydrogen bond donors (atoms with partial positive charge), H for nonpolar hydrogens, and M for methyl groups. Do not separate your letters with spaces or punctuation. For example: DADH or MAM

Chemical Groups in the Major and Minor Grooves of I/C and C/I Base Pairs

	inosine		cytosine	cytosine		inosine
Major groove						
Minor groove	<input type="text"/>		<input type="text"/>	<input type="text"/>		<input type="text"/>

Save

Home assignment

Major and Minor Groove Interactions

0.0/1.0 point (graded)

You prepare to test the binding of the DNA-binding proteins to the two substrates, the candidate sequence your labmate identified (substrate 1) and the altered sequence your advisor proposed as a comparison (substrate 2). Both substrate sequences are shown with the binding sites underlined.

substrate 1

5'....CGTTTAGTG....3'
3'....GCAAATCAC....5'

substrate 2

5'....CGCCCAGTG....3'
3'....GCIIITCAC....5'

In the following table, the left side summarizes possible results. Use the dropdown options on the right side of the table to choose what each combination of results would suggest about where the DNA-binding protein binds.

Interpreting Binding Results for The Viral Protein

Experiment results	Part of the DNA the protein binds to
Protein binds substrate 1 but not substrate 2.	<input type="button" value="Select an option ▾"/>
Protein binds substrate 1 and substrate 2.	<input type="button" value="Select an option ▾"/>
Protein did not bind either substrate.	<input type="button" value="Select an option ▾"/>

Save

THANKS FOR LISTENING