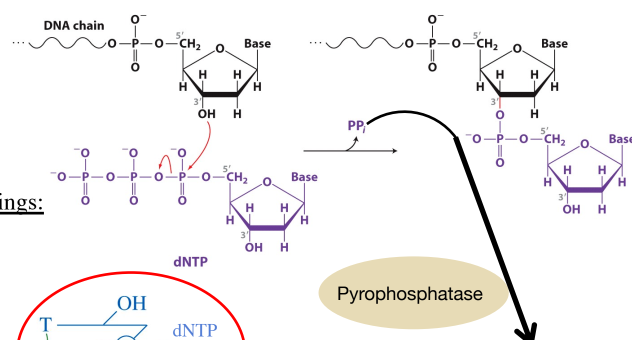
DNA Replication

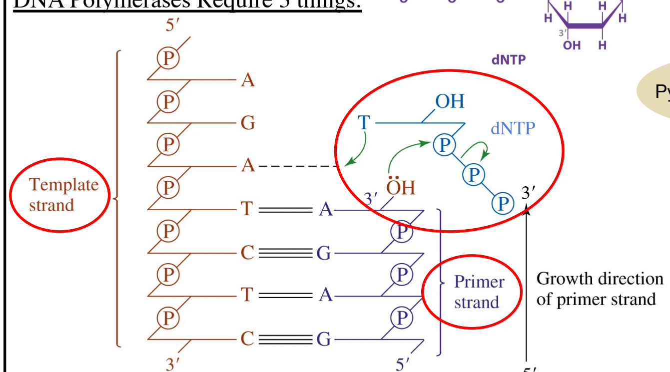
Nucleic acid function: Central Dogma

DNA Replication

Addition at 3' end



DNA Polymerases Require 3 things:



2 P_i

$\Delta G°' \approx -3$ kcal/mole

$\Delta G°' \approx -6$ kcal/mole

$\Delta G°' \approx -9$ kcal/mole

Comparison of Polymerases

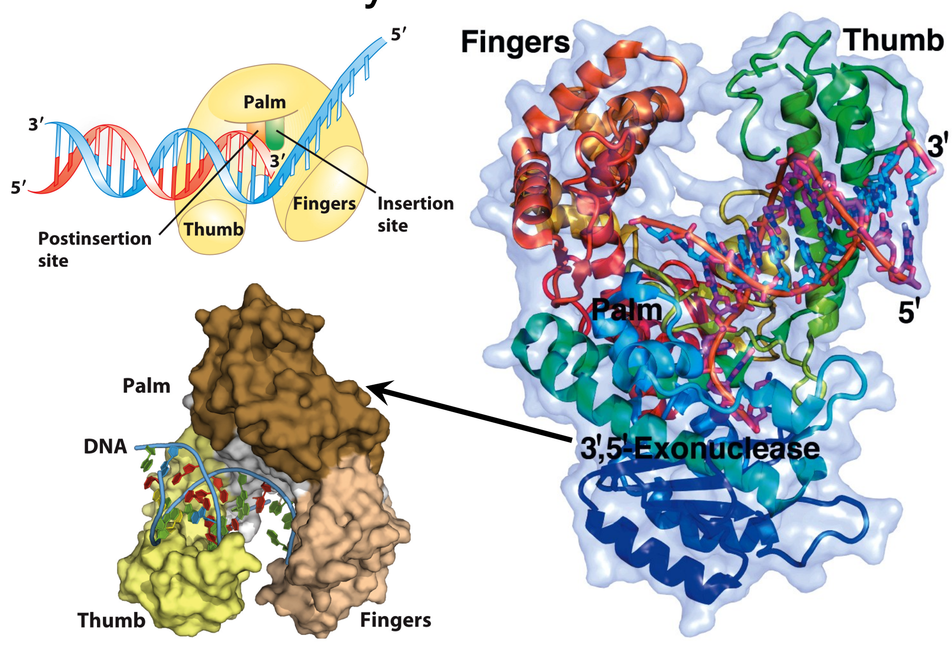
TABLE 25-1 Properties of *E. coli* DNA Polymerases

	Pol I	Pol II	Pol III
Mass (kD)	103	90 (α_4)	130 *
Molecules/cell	400	?	10–20
Turnover number[a]	20	5	1000
Structural gene	*polA*	*polB*	*polC*
Conditionally lethal mutant	+	−	+
Polymerization: 5′ → 3′	+	+	+
?→ Exonuclease: 3′ → 5′	+	+	+
?→ Exonuclease: 5′ → 3′	+	−	−
Processivity	100	10,000	500,000 [b]

[a]dNTP polymerized sec^{-1} at 37 °C. [b]not including Okasaki fragments

*In a complex with 10 proteins (26 subunits) of >900 kD. Core is trimer of α, θ, ε

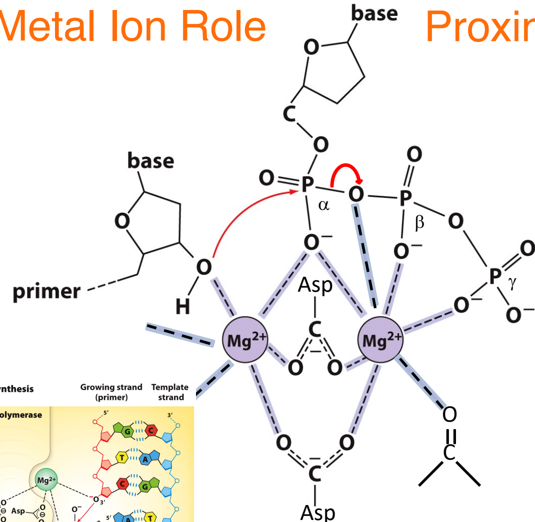
DNA Polymerase Structure

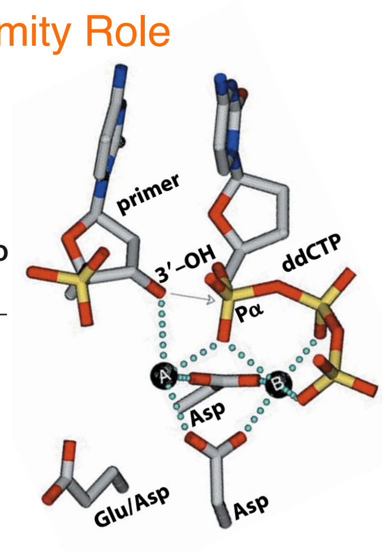


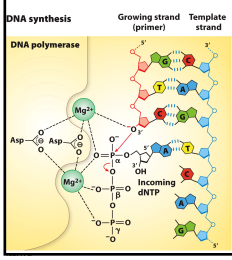
DNA/RNA polymerase mechanism

Metal Ion Role

Proximity Role





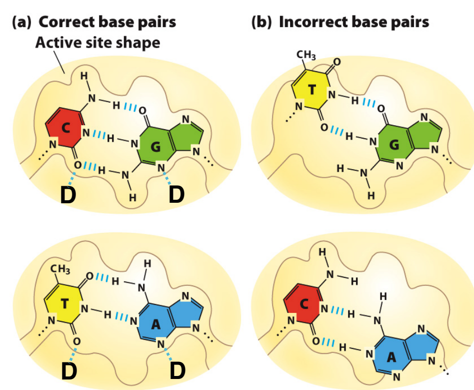


This is just the chemistry step, there are other more important enzymatic steps…

Replication/polymerase Fidelity

Geometry of Base Pairing Accounts for High Fidelity

- Errors in replication for *E. coli*: $1/10^9 - 1/10^{10}$ bp
  - 3×10^6 bp/genome x 10^{-10} mistakes/bp = 0.0003 mistakes/genome
  - 1 mistake per 1,000–10,000 replications
- DNA polymerase active site excludes base pairs with incorrect geometry
  - At BOTH the insertion site and the post insertion site
  - BUT, DNA polymerases still insert wrong base 1/10,000 times.
  - Repair mechanisms fix these errors.



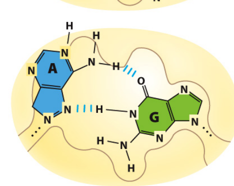
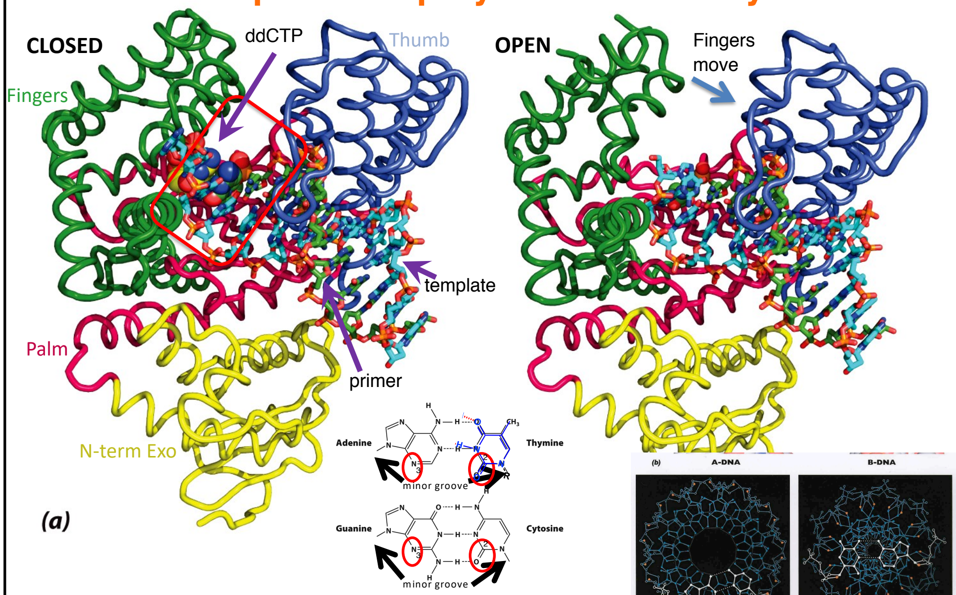


Figure 25-6

Replication/polymerase Fidelity

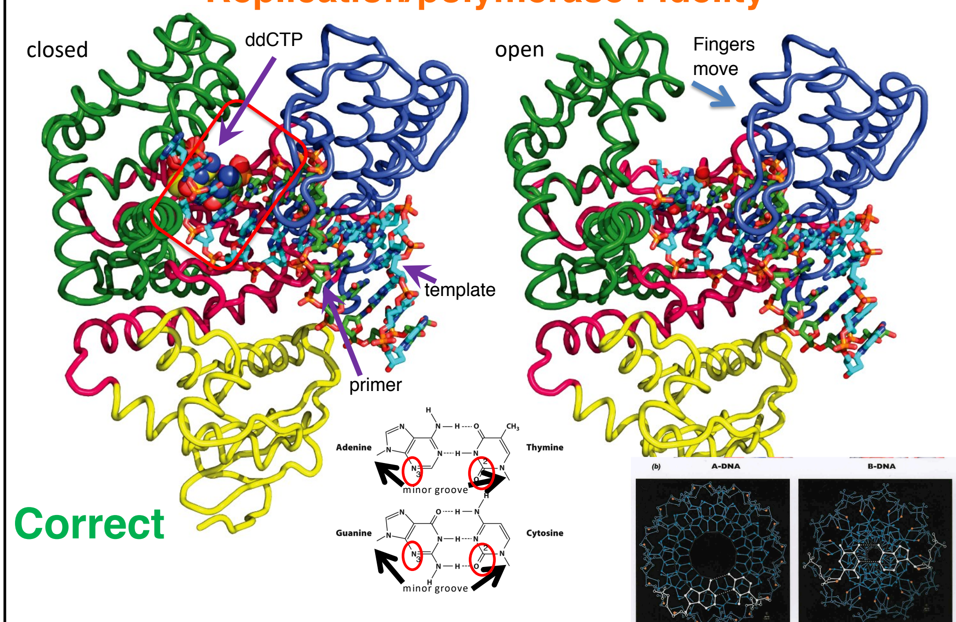


$\leftarrow E^{*(open)} \bullet dNTP^{(incorrect)}$

$E + dNTP \rightarrow E^{*(open)} \bullet dNTP \rightarrow E^{**(closed)} \bullet dNTP^{(incorrect)} \rightarrow E^{***(translocation)} \bullet PP_i \rightarrow E + PP_i$

binding slow chemistry Movement (5'→3')

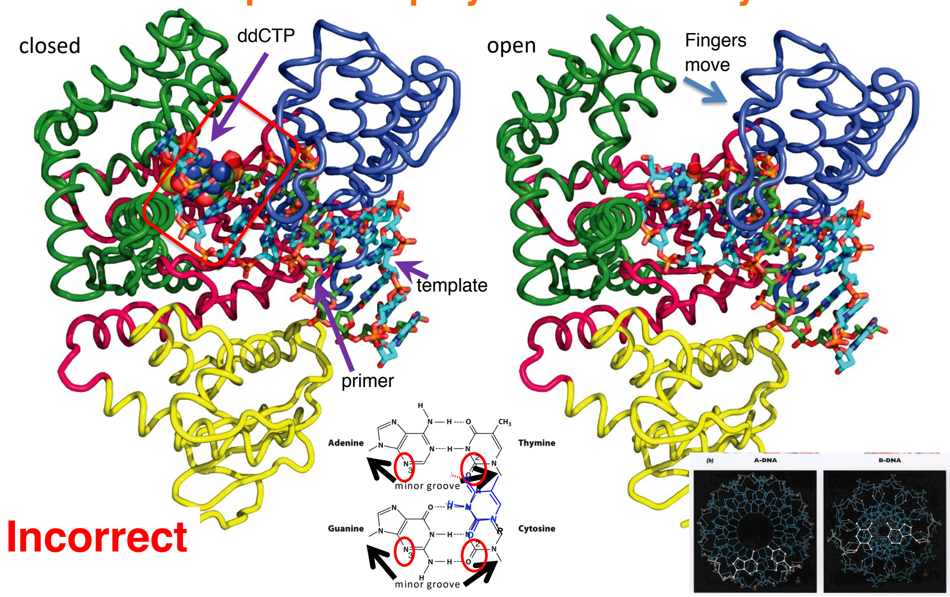
Replication/polymerase Fidelity



$E + dNTP \rightarrow E^{*(open)} \bullet dNTP \rightarrow E^{**(closed)} \bullet dNTP^{(correct)} \rightarrow E^{***(translocation)} \bullet PP_i \rightarrow E + PP_i$

binding slow chemistry Movement (5'→3')

Replication/polymerase Fidelity



$\leftarrow E^{*(open)} \bullet dNTP^{(incorrect)}$

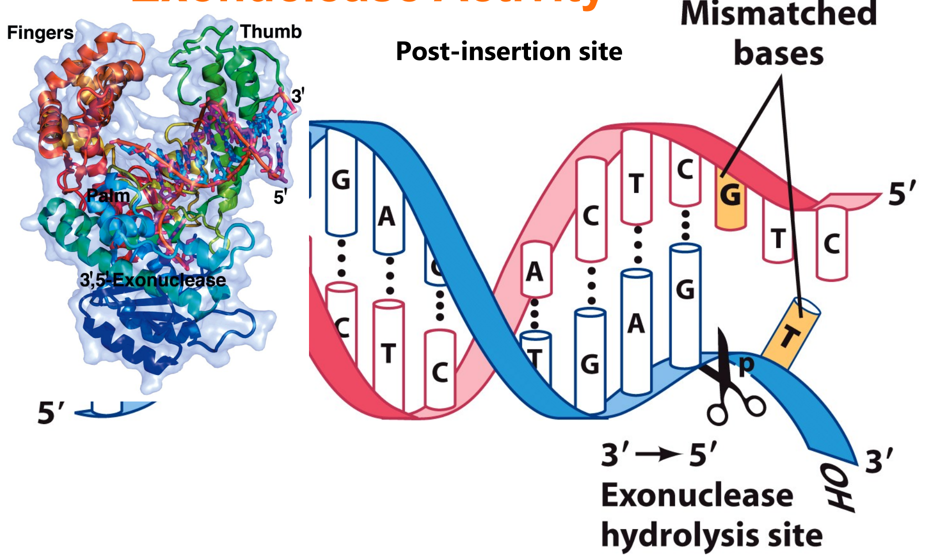
Incorrect put in every 10,000

$E + dNTP \rightarrow E^{*(open)} \bullet dNTP \rightarrow E^{**(closed)} \bullet dNTP^{(incorrect)} \rightarrow E^{***(translocation)} \bullet PP_i \rightarrow E + PP_i$

binding | slow | chemistry | Movement (5'→3')

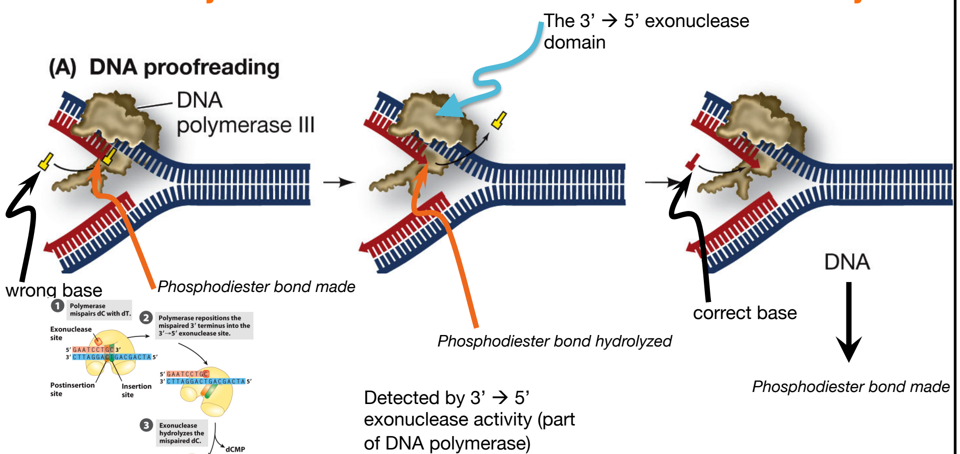
Replication/polymerase Fidelity

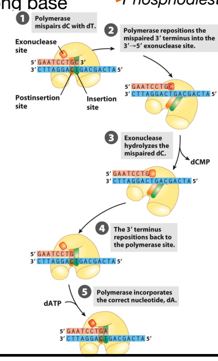
DNA Polymerases have 3' → 5' Exonuclease Activity



Replication/polymerase Fidelity

DNA Polymerases have 3’ → 5’ Exonuclease Activity





Replication Fidelity: Proofreading

Replication/polymerase Fidelity

Polymerases make mistakes in synthesis (polymerase 5’→3’ activity) = 1/10,000

DNA polymerases have proofreading ability (3’→5’ exonuclease activity) = 1/10,000

After replication fork, any mistakes are corrected by **mismatch repair** mechanism. This misses = 1/100

Overall for Replica-tion = $1/10^{10}$

In humans with:
3×10^9 bp/haploid genome x $1/10^{10}$ mistakes/bp = 0.3 mistakes/haploid genome

Plus DNA can be damaged in living cells. These damages are repaired by other mechanisms called Post-replication DNA Repair:

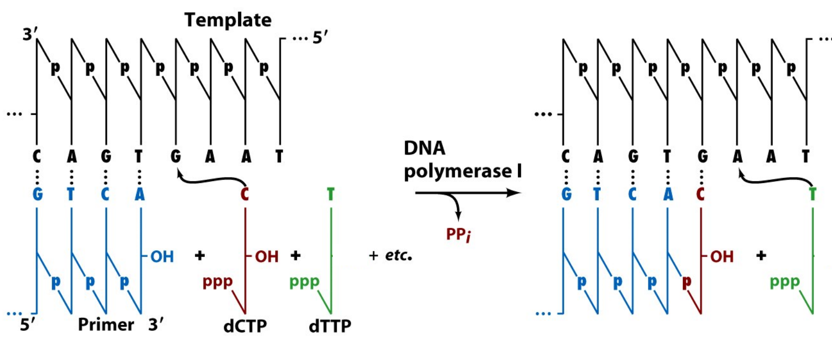
- **Direct reversal of modification**
- **Base-excision repair**
- **Nucleotide excision repair**

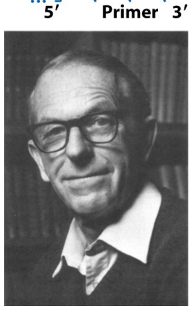
DNA Replication

- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Exonuclease Activities
  - Mismatch repair
  - Post-replication repair
- DNA Sequencing
- PCR

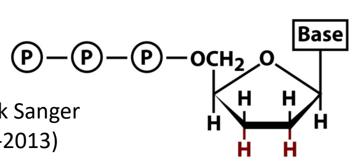
I have included slides we did not cover, but these are also available on the website linked to the Videos on Nucleic Acids. Please learn.

DNA Sequence Determination (Sanger)





Frederick Sanger
(1918-2013)



2′,3′-Dideoxynucleoside triphosphate

DNA Sequencing

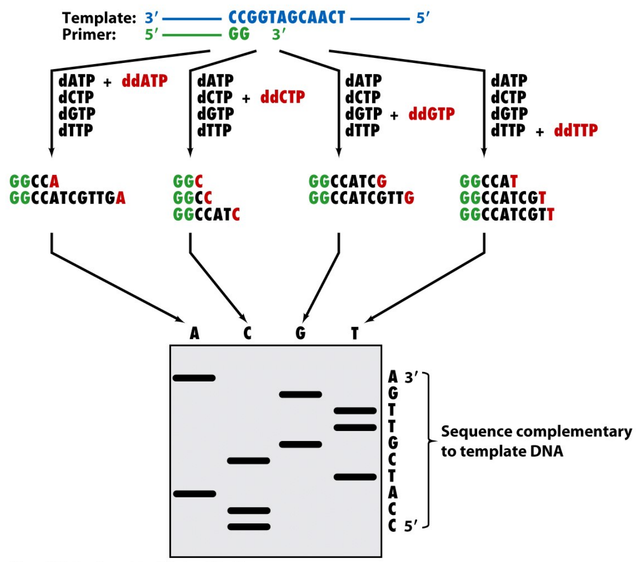


Figure 3-21 Fundamentals of Biochemistry, 2/e

Visualize the DNA Sequence (I)

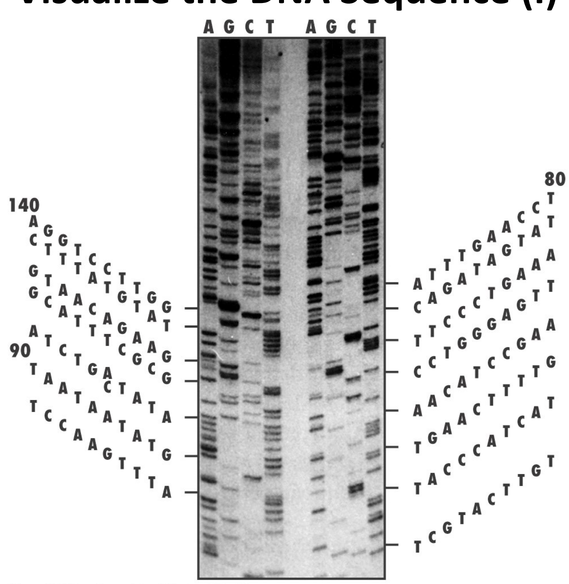
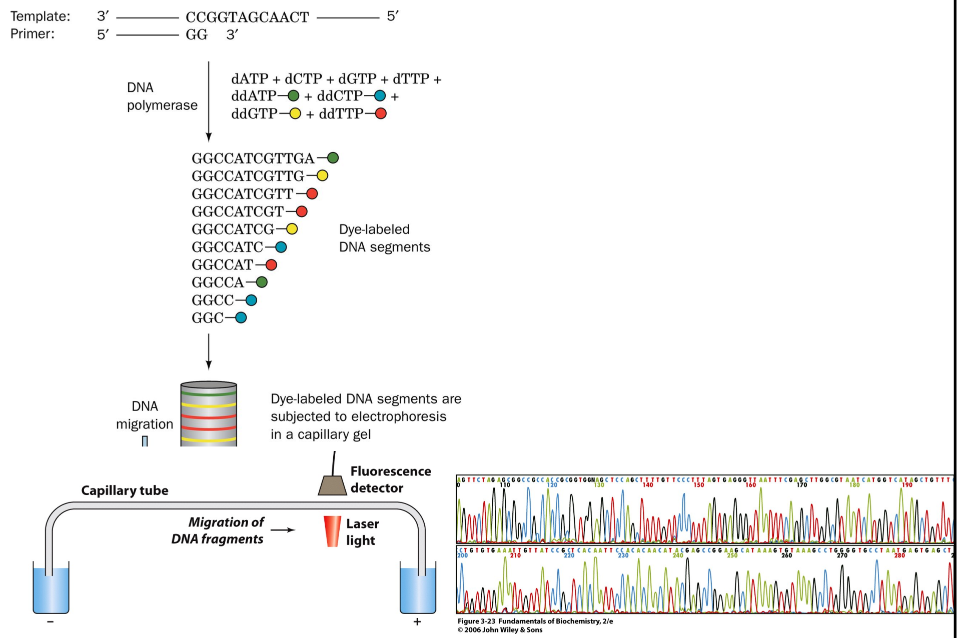


Figure 3-22 Fundamentals of Biochemistry, 2/e

Chain-Terminator Sequencing Method



Molecular Cloning: Polymerase Chain Reaction

Besides using host to MAKE MORE copies of DNA sequences, they can be made by the **polymerase chain reaction** (**PCR**) technique (*in vitro)*.

PCR is a cyclical process:

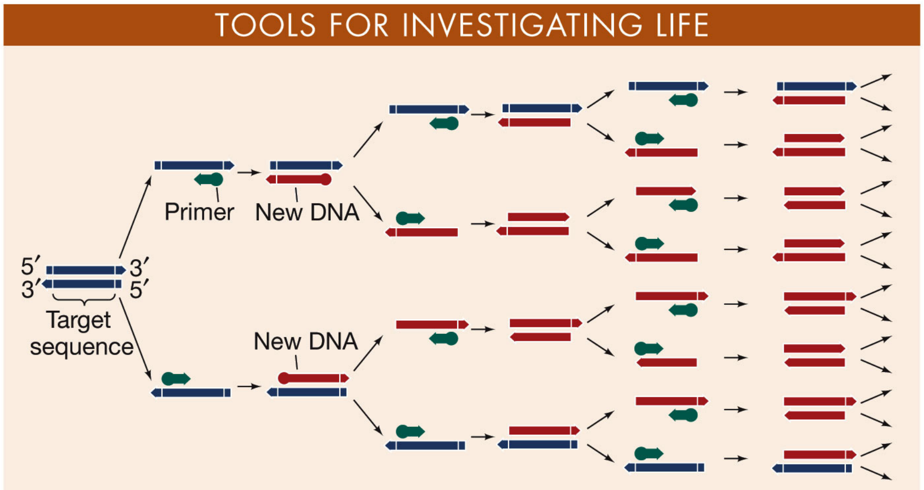
- DNA fragments are **denatured** by heating → single-stranded DNA
- Primers (one complimentary to each strand), plus dNTPs, and DNA polymerase are added → **annealing** and **extension** → new double-stranded DNA
- Repeat

An initial problem with PCR was its temperature requirements.

The key insight was the use of an enzyme that could withstand the heat needed to denature the DNA → DNA polymerase from *Thermus aquaticus, **Taq*** **Polymerase** (insight of Kerri Mullis).

The amplified DNA can then be inserted into plasmids to create recombinant DNA and cloned in host cells. Synthetic DNA can be manipulated to create specific mutations in order to study the consequences of the mutation: Site-directed Mutagenesis.

Process Diagram: Polymerase Chain Reaction (PCR)



Molecular Cloning: Polymerase Chain Reaction