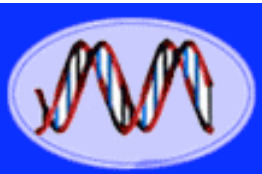
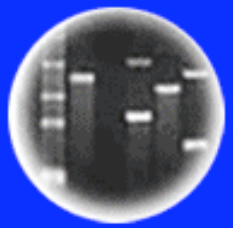


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

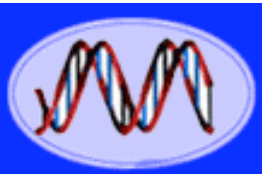
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70AL Spring 2011
Gene Discovery Laboratory

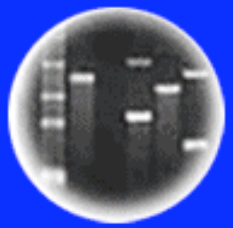
How To Sequence DNA?

4/4/11



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

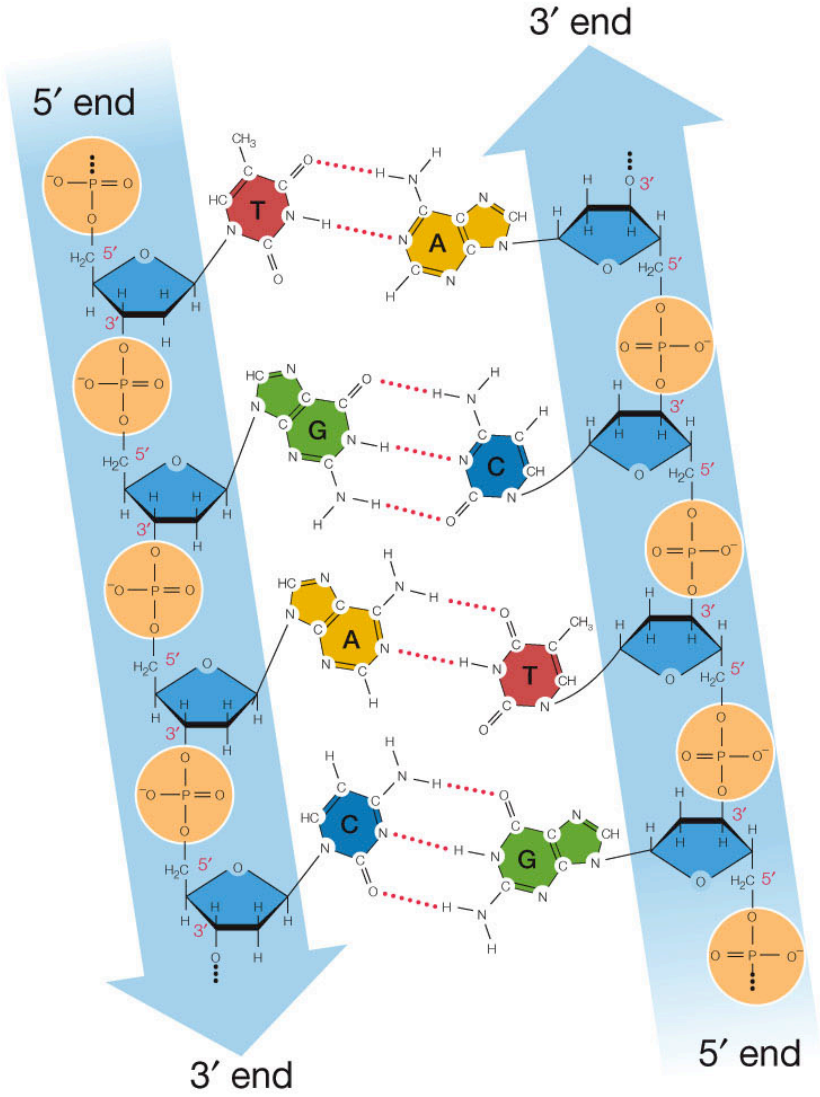
Announcements

1. Read Chapters
   a. Arabidopsis Seed Development
   b. Plant Life Cycle
   c. What it Means to Be a Scientist
   d. Sanger & Hood Articles

2. Review Last Week's Lecture

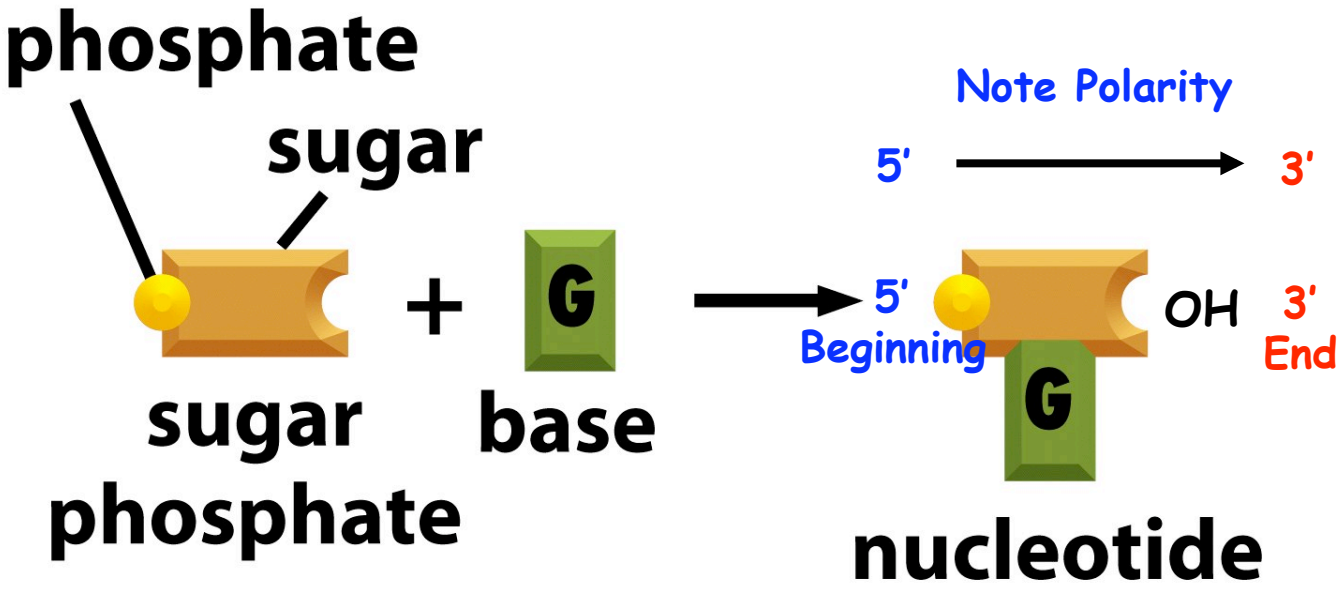
3. Think About Research

Recall…..The Structure of DNA



1. Double Helix
2. Phosphate-Sugar Backbone
3. Bases H-Bonded in Interior
4. Complementary Base Pairing
5. Anti-Parallel Chains
6. 10 Bases/Turn Helix
7. 34A/Turn
8. 3.4A/Base Pair
9. No Sequence Constraint
10. Synthesis 5′ to 3′

Recall……Nucleotides Have Polarity
Based on What is Bonded to the Five-Carbon Sugar
Phosphate on 5'Carbon and OH on 3'Carbon



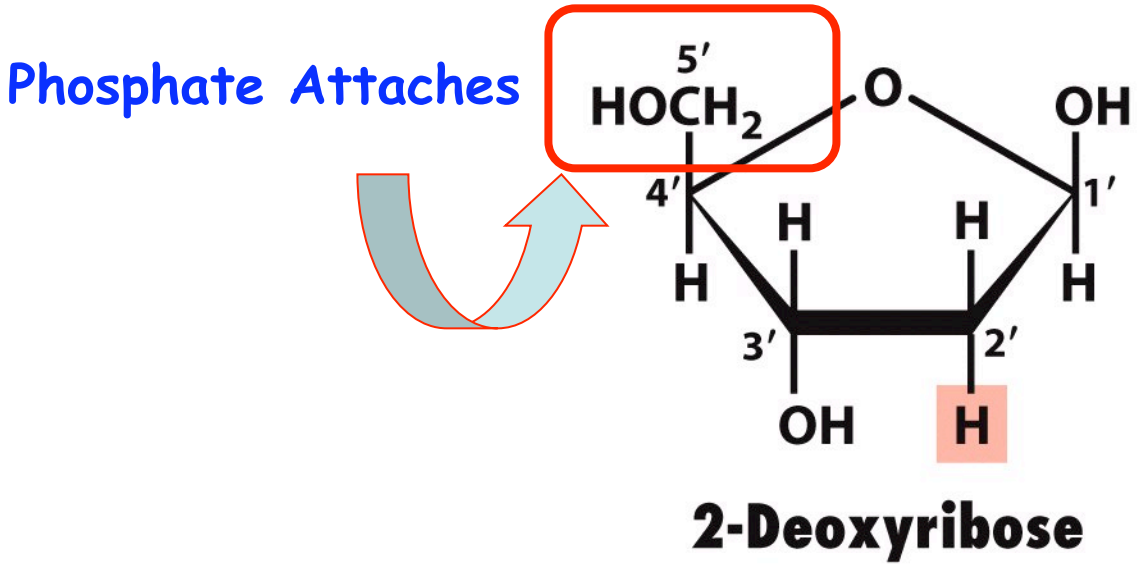
The Sugar is the HUB

DNA Sequence Defined By Nucleotide Order

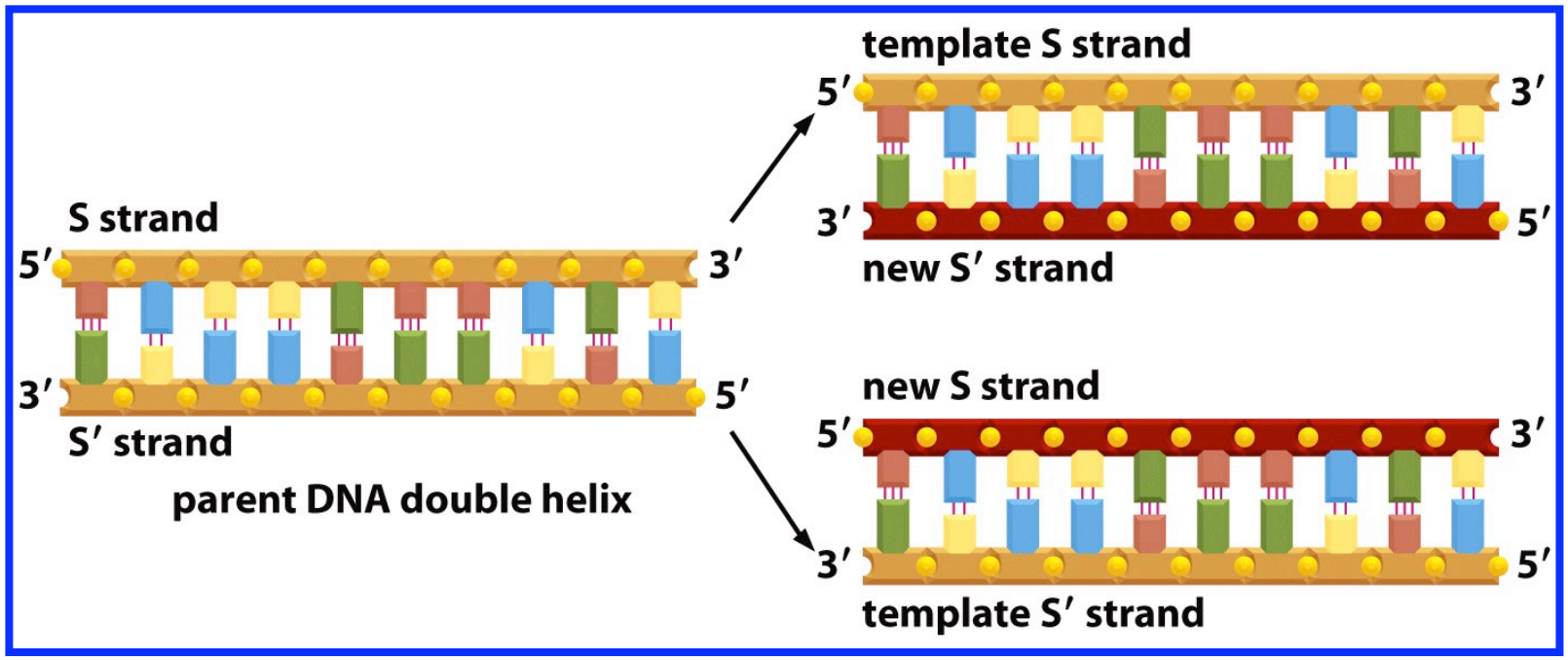
DNA Sequence = Functional Uniqueness = Biology

Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Recall…Structure and Polarity of the Deoxyribose Sugar



Recall…..DNA Replication Occurs Semi-Conservatively

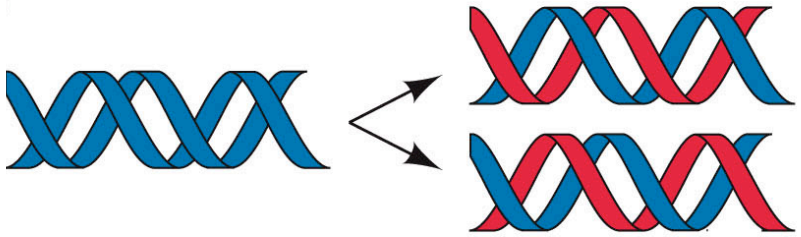


1. DNA Structure Allows DNA Sequence to Be Maintained by Complementary Base Pairing
2. Each Strand Serves as a Template for the Synthesis of a Complementary Strand
3. New DNA Molecules are Precise Copies of Parental DNA - Each Containing One Newly Synthesized Complementary Strand

Meselson & Stahl, PNAS, 1957

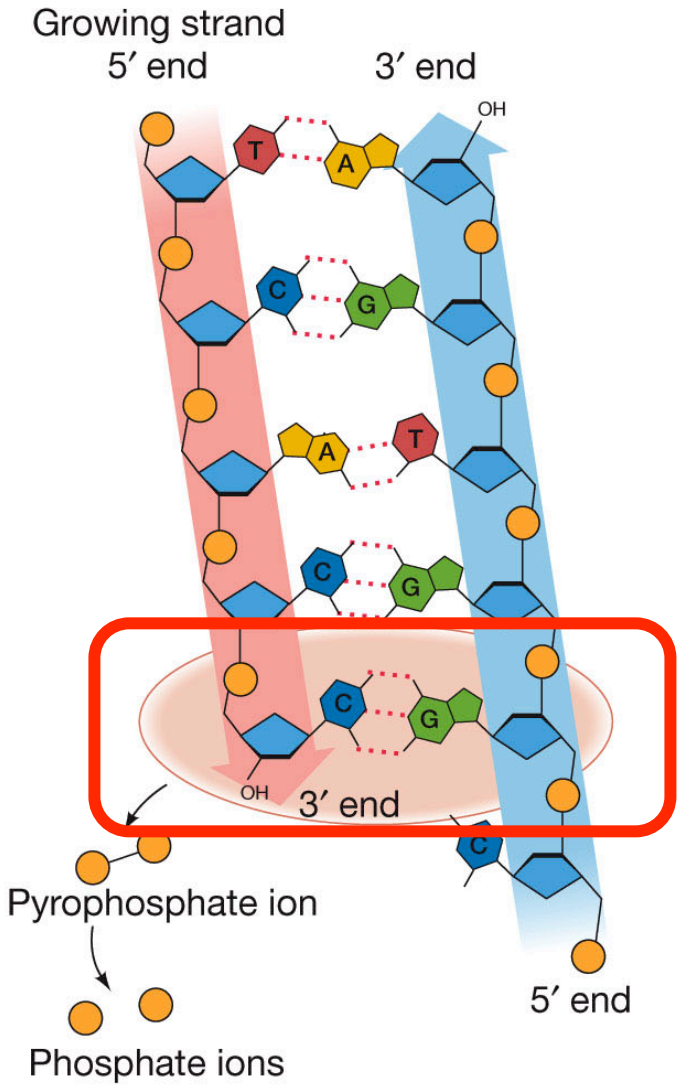
Requirements For DNA Synthesis

- Template
- All Four Nucleotides
- Origin of Replication (in vivo)
- Short Primer to Initiate Synthesis
- DNA Polymerase

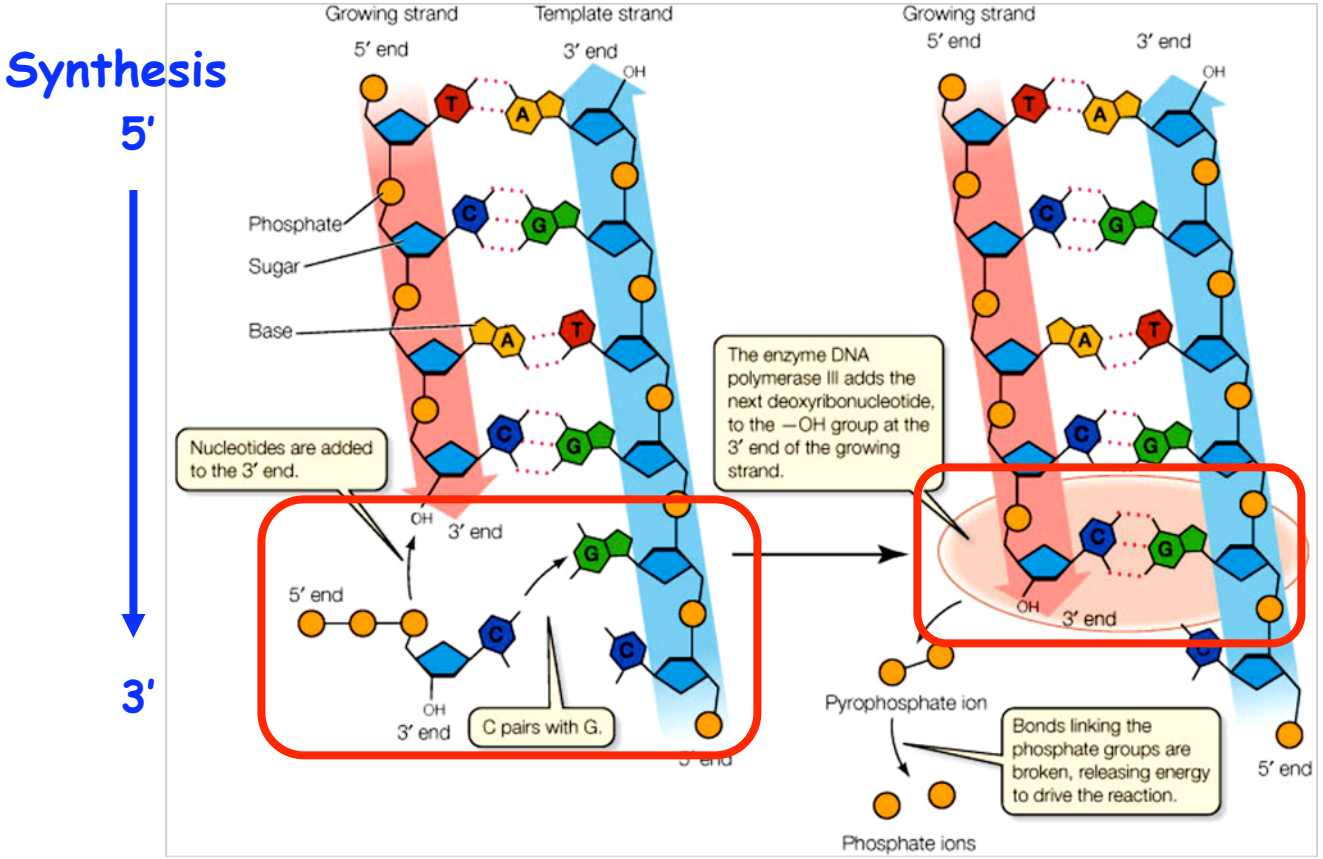


DNA Synthesis Is Always in a 5′ to 3′ Direction-Part Two

1. New Base Pair
2. New Phosphodiester Bond
3. DNA Elongated By 1bp
4. New 3′OH End
5. Diphosphate Released (Pyrophosphate)



DNA Sequence of One Strand is a Template for the New Strand

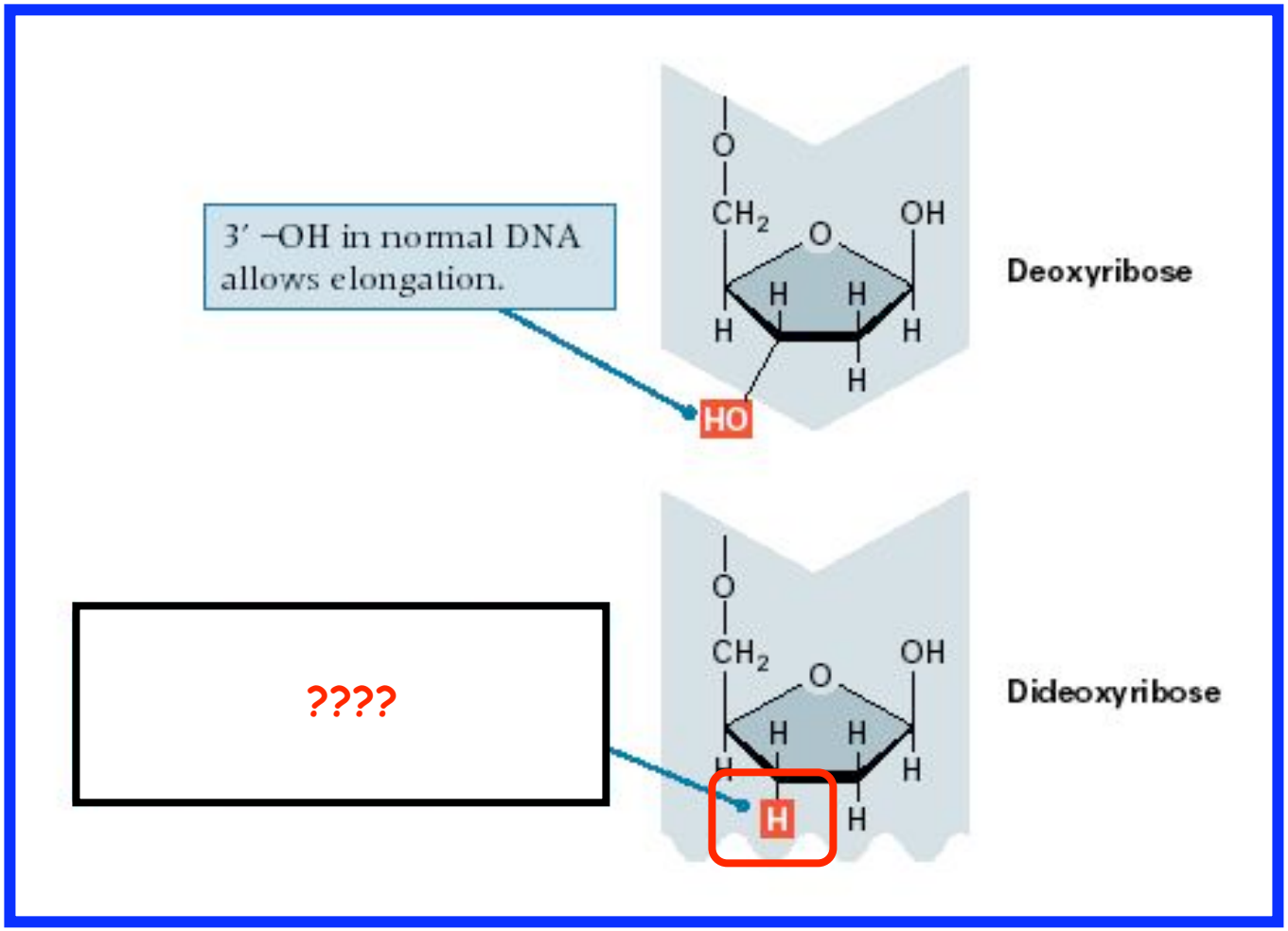


Sequence is Specified by Complementary Bases

Note: 5′ (P) & 3′ (OH)

5′ to 3′ Polarity Specifies Sequence

What Happens to DNA Synthesis if the 3' Carbon of the Deoxyribose Sugar Does Not Have an -OH Group?



Maxam-Gilbert Chemical Chain-Breakage DNA Sequencing

1. Chemicals Break Phosphodiester Bonds at Specific Bases Randomly
2. Base Sequence Determined By Chemical Used To Break DA Into Fragments
3. Base broken is at the End of a DNA Fragment
4. Fragments Sized By Gel Electrophoresis
5. Single-Stranded DNA Labeled at One End (e.g., 3' End) Providing Direction For Sequence

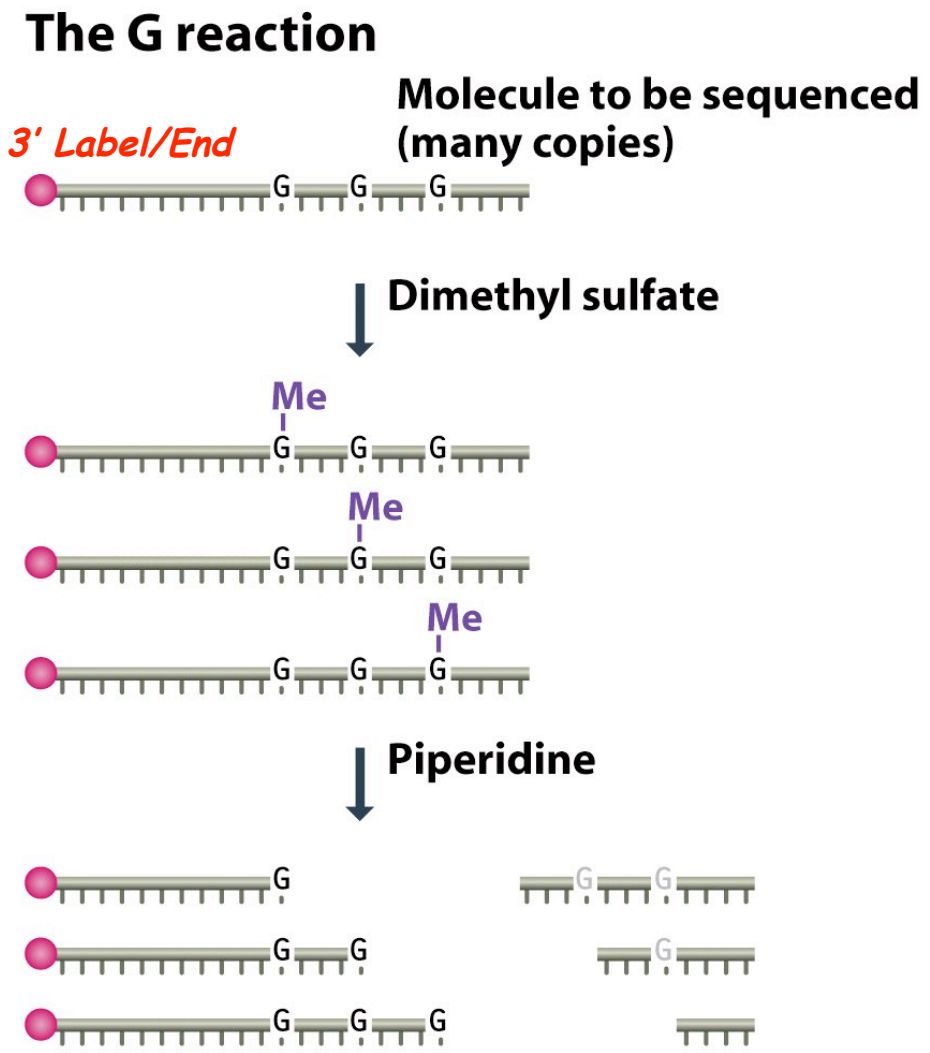


Figure 4.8b *Genomes 3* (© Garland Science 2007)

Using Gel Electrophoresis To Separate DNA Fragments By One Nucleotide Spacings and Read DNA Sequence After Autoradiography

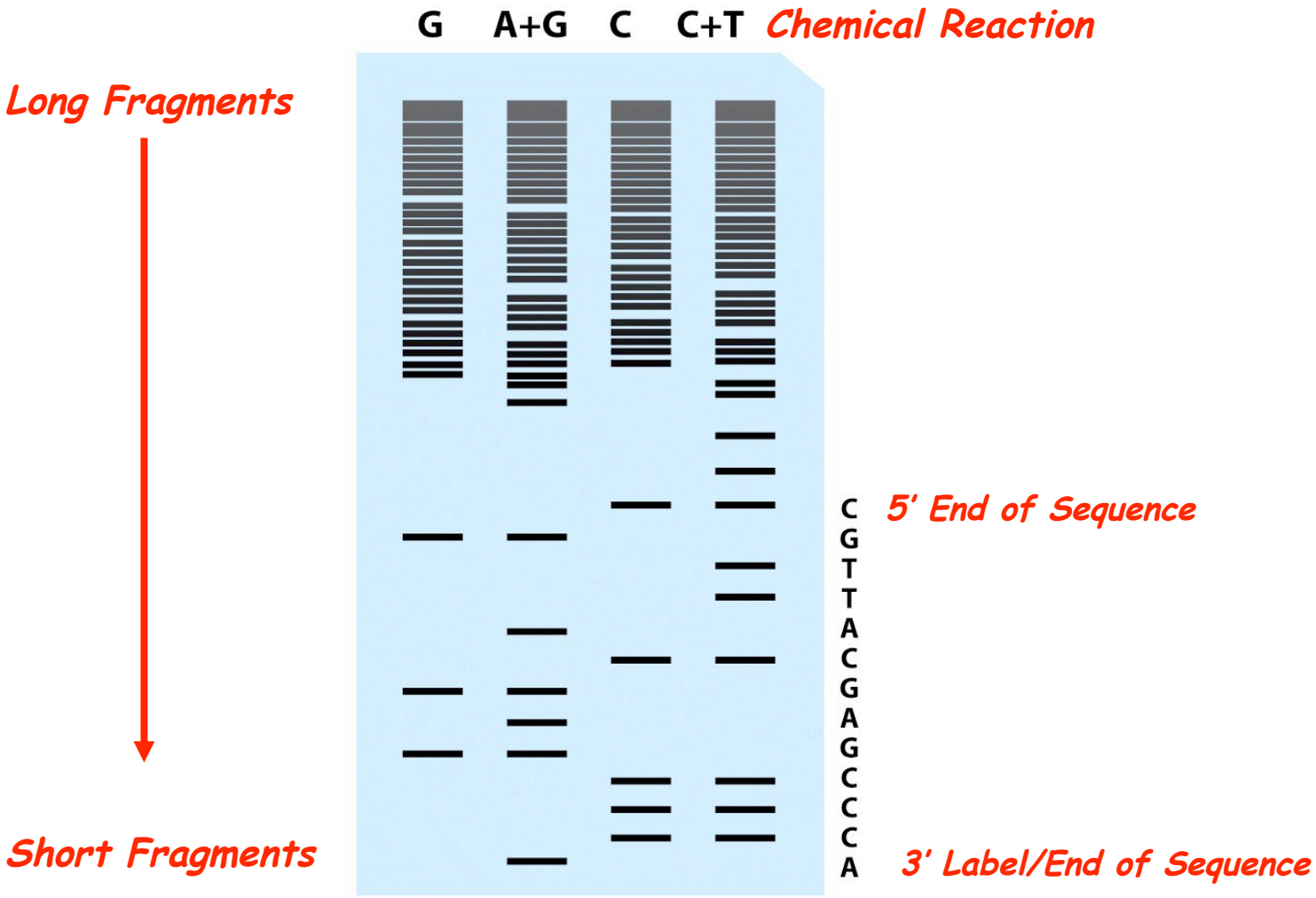


Figure 4.8c *Genomes 3* (© Garland Science 2007)

Recall…..Sizing DNA Fragments Using Gel Electrophoresis

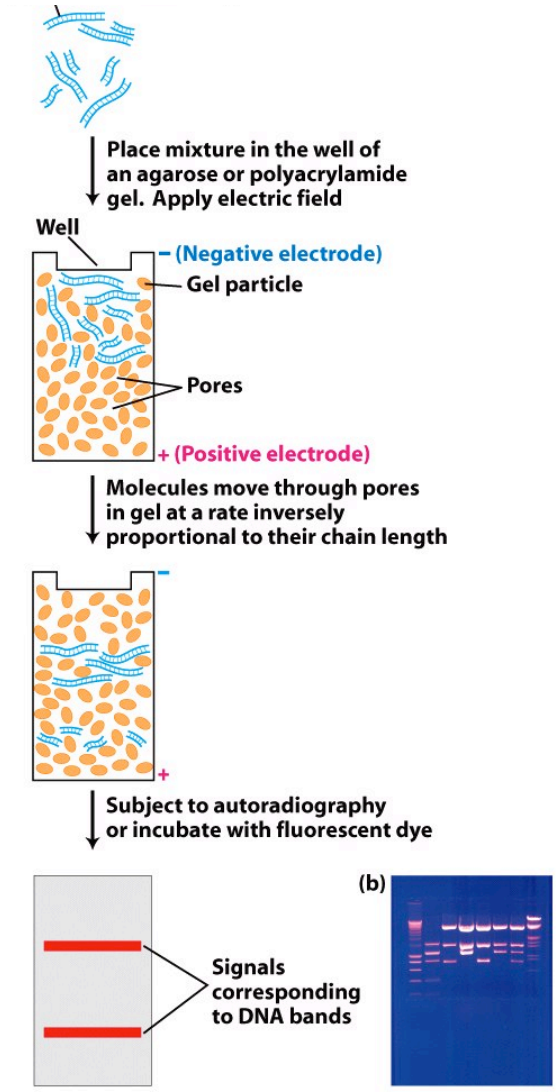
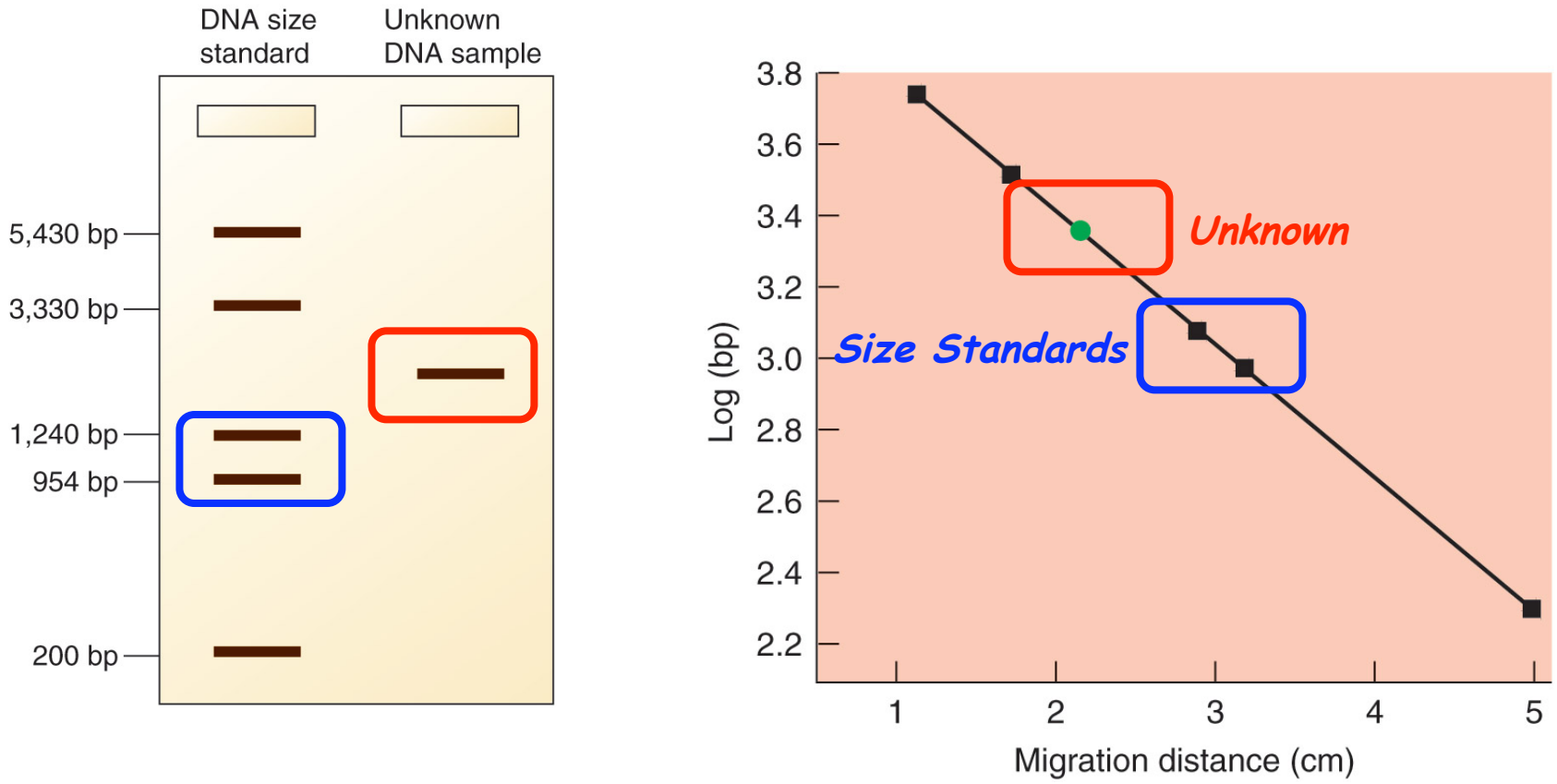


Figure 5-19
Molecular Cell Biology, Sixth Edition

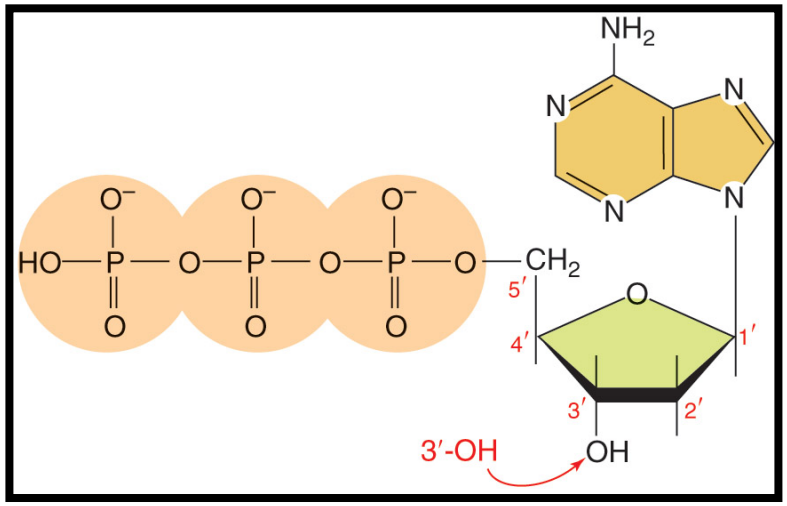
Determining DNA Sizes Relative to the Sizes of Known Standards



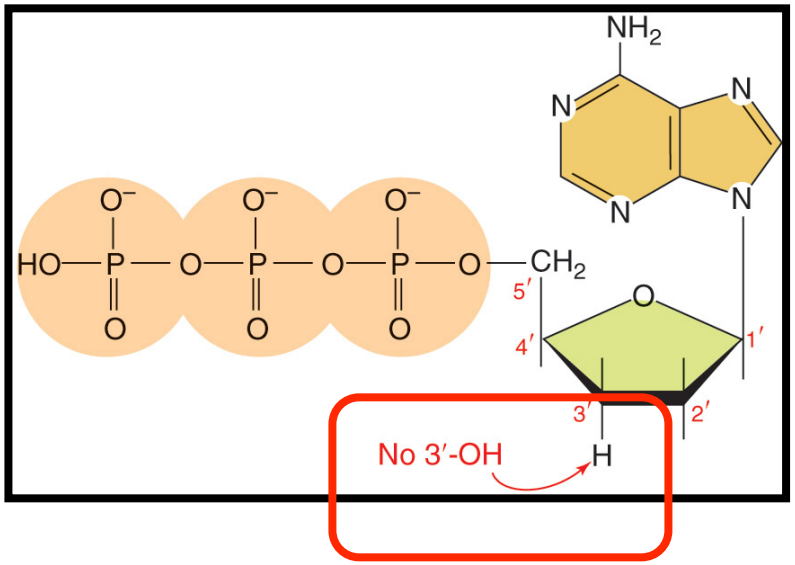
A DNA size standard and a DNA of unknown size were placed in the left and right lanes, respectively.

Using Sanger Chain-Terminating DNA Synthesis to Sequence DNA

Deoxyadenosine Triphosphate (dATP)



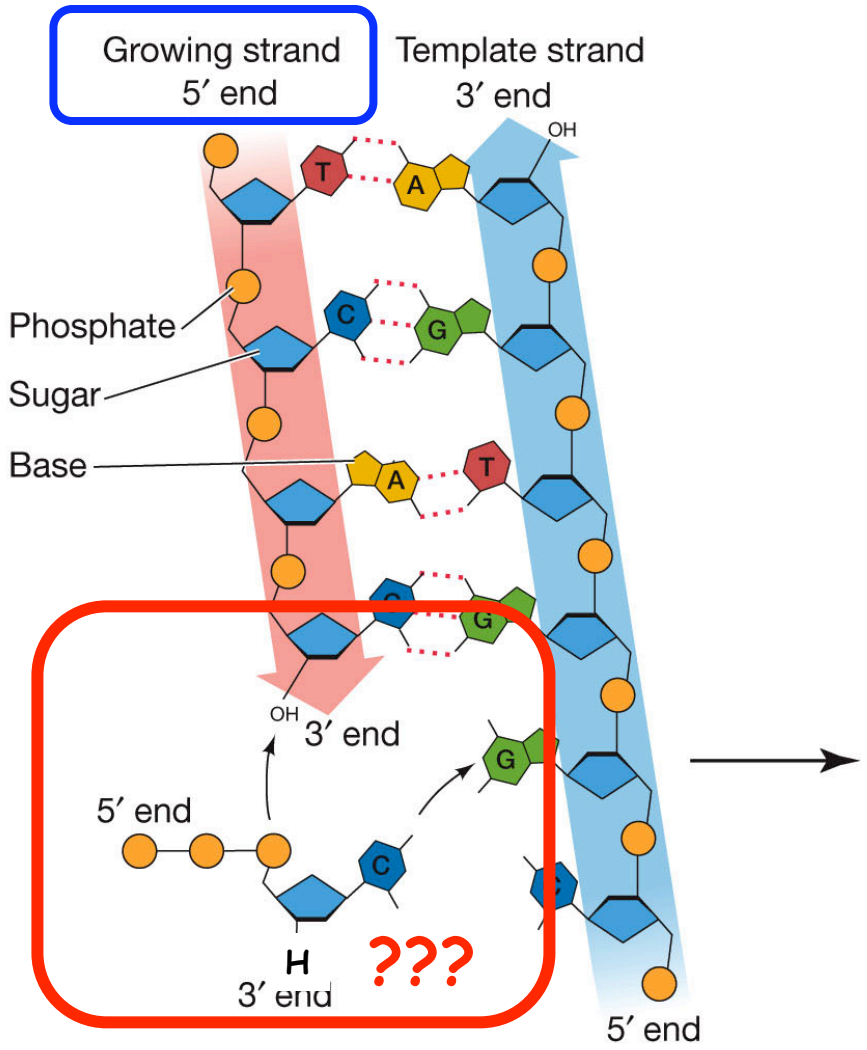
Dideoxyadenosine Triphosphate (ddATP)



Sanger Sequencing Requires Requires:

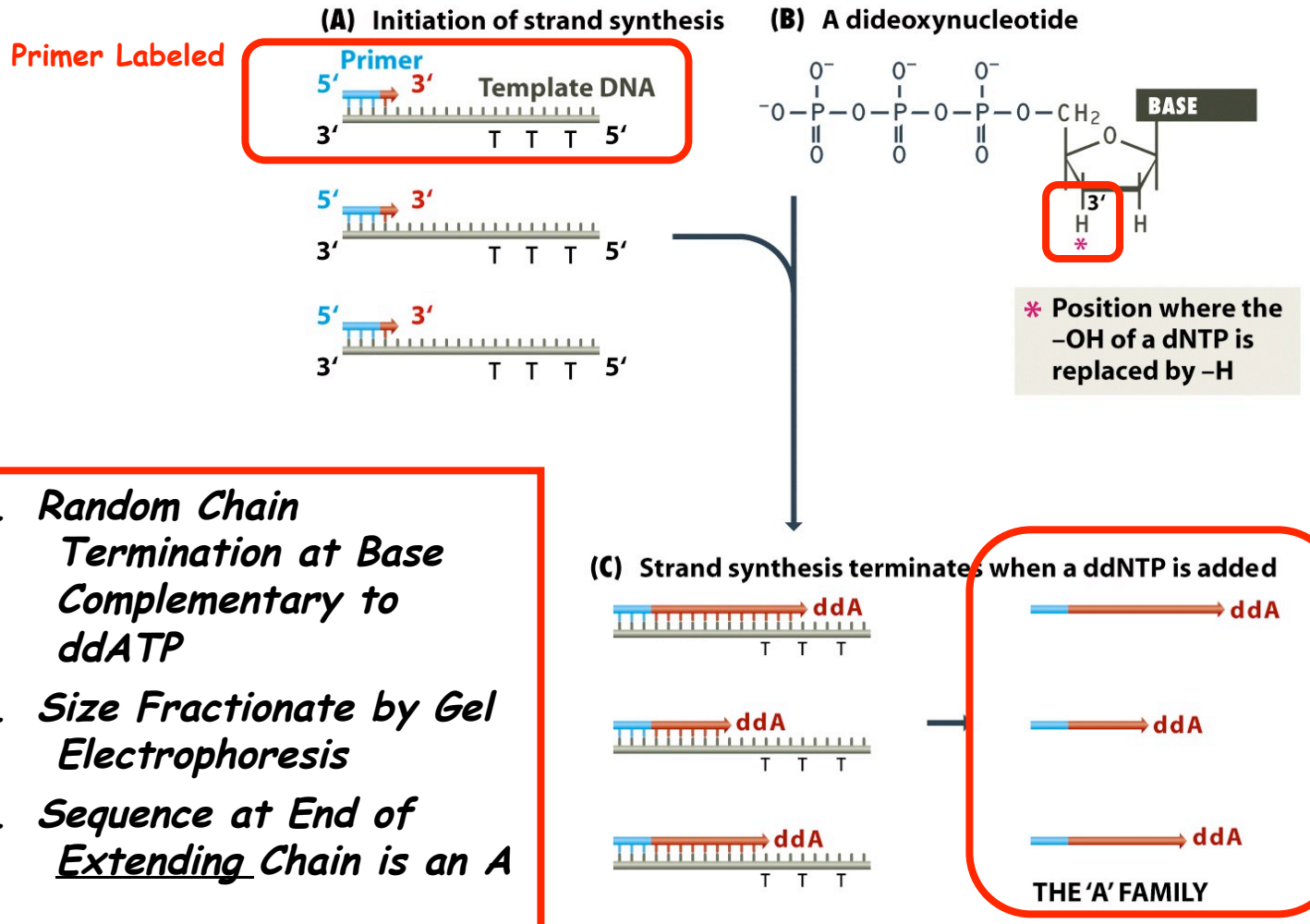
1. Template (specific DNA fragment)
2. All Four dXTPs and ddXTPs
3. Short Primer To Initiate Synthesis
4. DNA Polymerase

What Happens to DNA Synthesis if the Complemetary Nucleotide is a ddXTP?



Recall…The Sugar is the Hub and Sets the Polarity of the DNA Molecule

Sanger Chain-Terminating DNA Sequencing-The "Old Way"

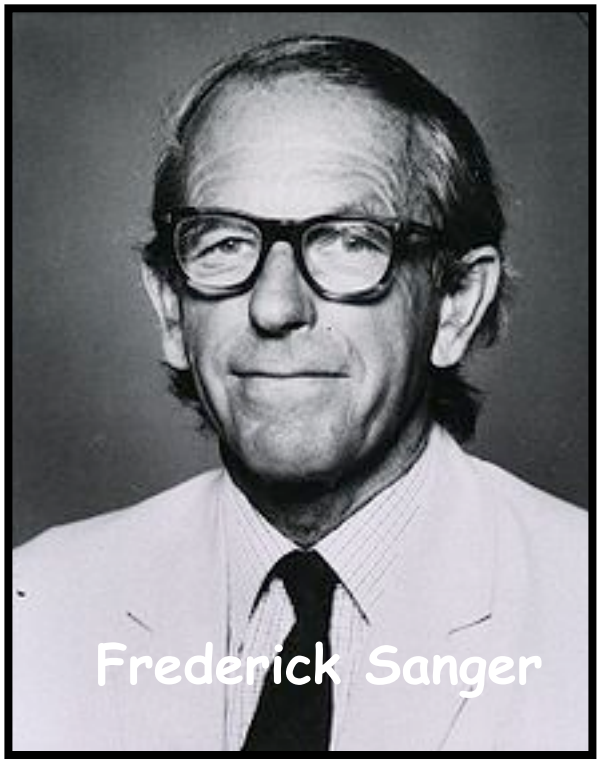


1. Random Chain Termination at Base Complementary to ddATP
2. Size Fractionate by Gel Electrophoresis
3. Sequence at End of Extending Chain is an A

Size By Gel Electrophoresis & Detect Using Autoradiography

DNA sequencing with chain-terminating inhibitors PNAS 74, 5463-5467, 1977

F. Sanger, S. Nicklen, and A. R. Coulson



Nobel Prize For Chemistry in 1958 - Protein Sequencing
Nobel Prize For Chemistry in 1980 - DNA Sequencing

Sanger Chain-Terminating DNA Sequencing Reactions

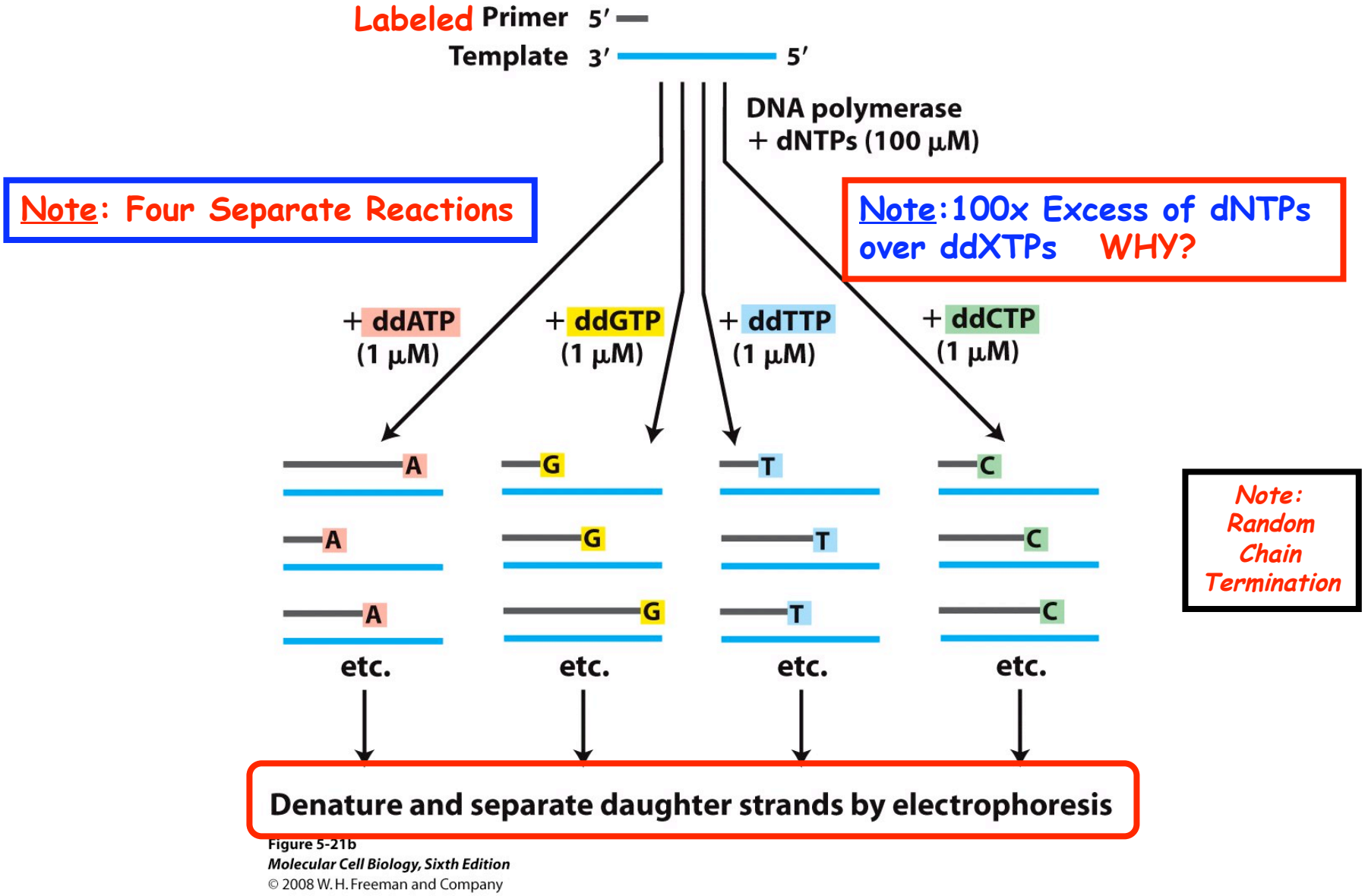
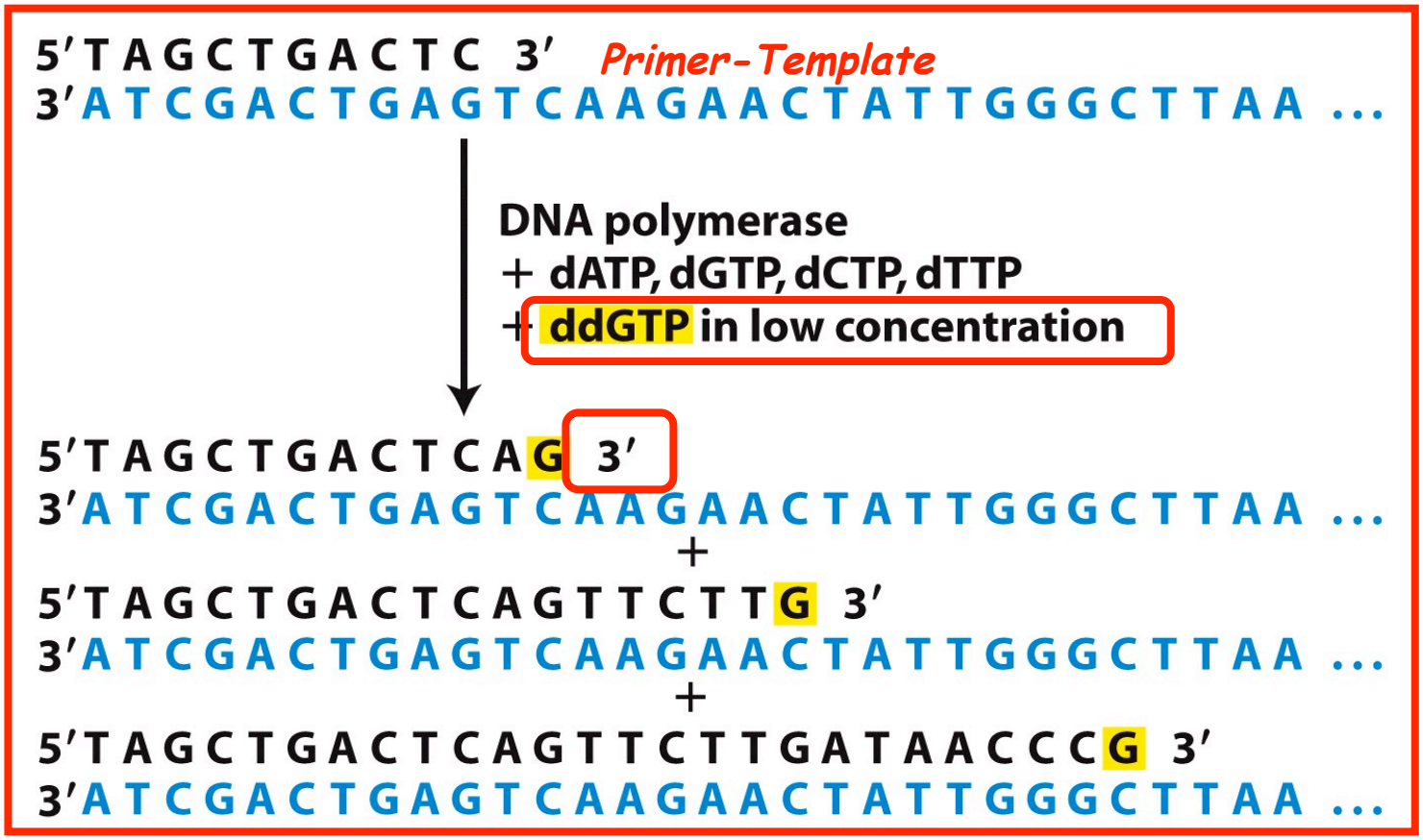


Figure 5-21b
Molecular Cell Biology, Sixth Edition

Sanger Chain-Terminating DNA Sequencing

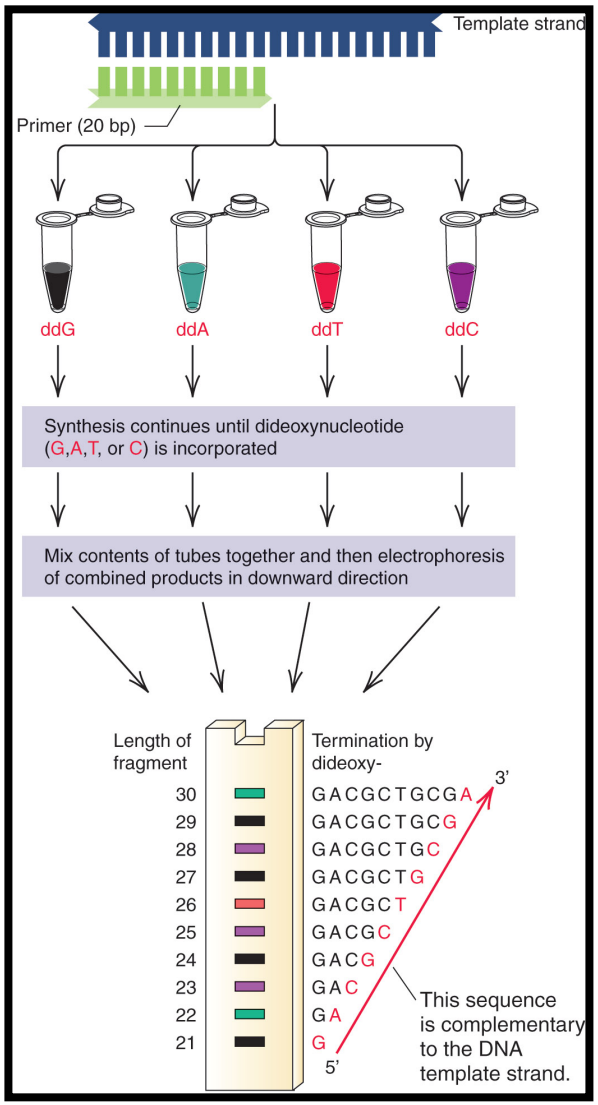


Note: Random Chain Termination

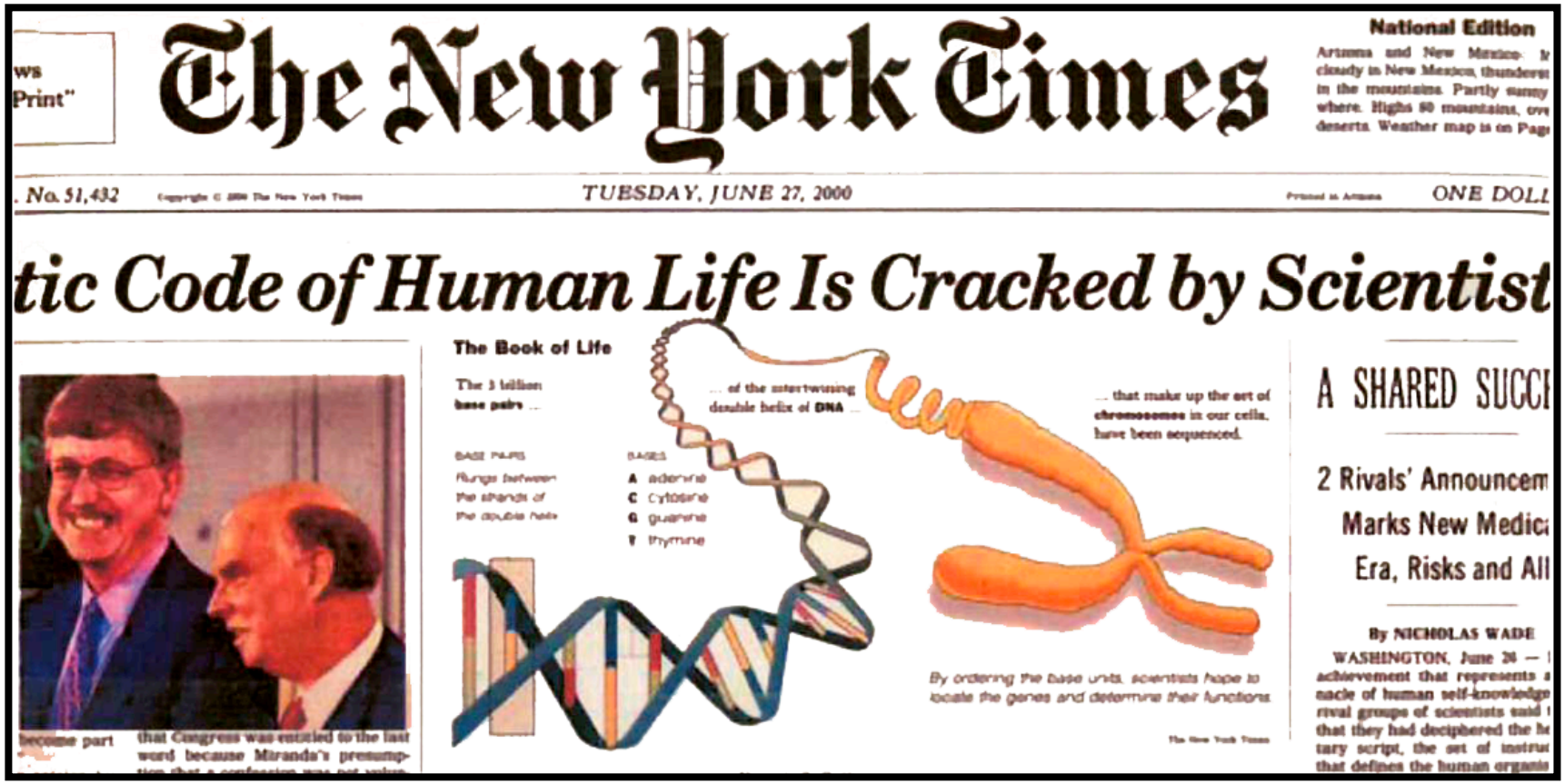
Problems With Maxam-Gilbert and "Old Time" Sanger Sequencing for Genome Sequencing

- Manual Process That is Labor Intensive
  - Gels
  - Base Detection
  - Chemical Reactions & DNA Synthesis
- Low Throughput (i.e.,thousands of base sequences)
- Slow
- Use of Radioactivity & Autoradiography to Detect Gel Fragments
- Separate Reactions or Synthesis For Each Base
- Need Specific Cloned DNA Fragments
- Cannot Automate
- Expensive ($5-$10 per base or more!)

Using Fluorescent ddXTPs to Visualize DNA Sequence And Size of DNA Fragments on a Gel Continuously



What Was the "Game Changer" That Allowed Genomes To Be Sequenced For the First Time?



ws
Print"

The New York Times

National Edition

. No. 51,432 TUESDAY, JUNE 27, 2000 ONE DOLL

tic Code of Human Life Is Cracked by Scientist

The Book of Life

... that make up the set of chromosomes in our cells, have been sequenced.

By ordering the base units, scientists hope to locate the genes and determine their functions.

A SHARED SUCCI

2 Rivals' Announcem
Marks New Medic
Era, Risks and All

By NICHOLAS WADE

WASHINGTON, June 26 —

become part

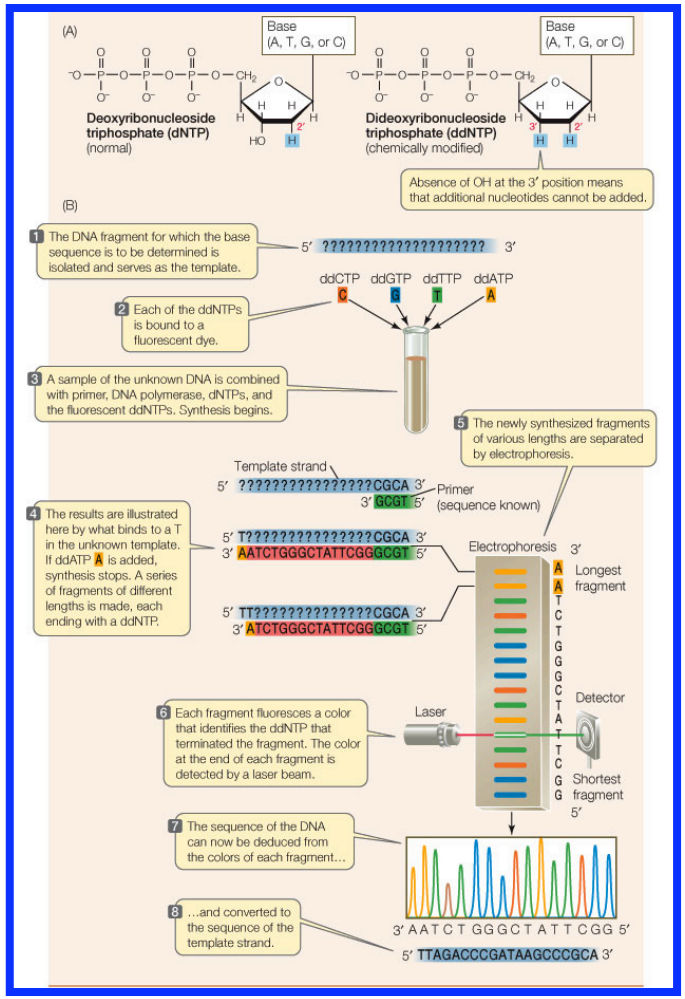
that Congress was entitled to the last word because Miranda's presump-

Automated Sanger Dideoxy DNA Sequencing

Fluorescence detection in automated DNA sequence analysis

Lloyd M. Smith, Jane Z. Sanders, Robert J. Kaiser, Peter Hughes, Chris Dodd, Charles R. Connell*, Cheryl Heiner*, Stephen B. H. Kent & Leroy E. Hood

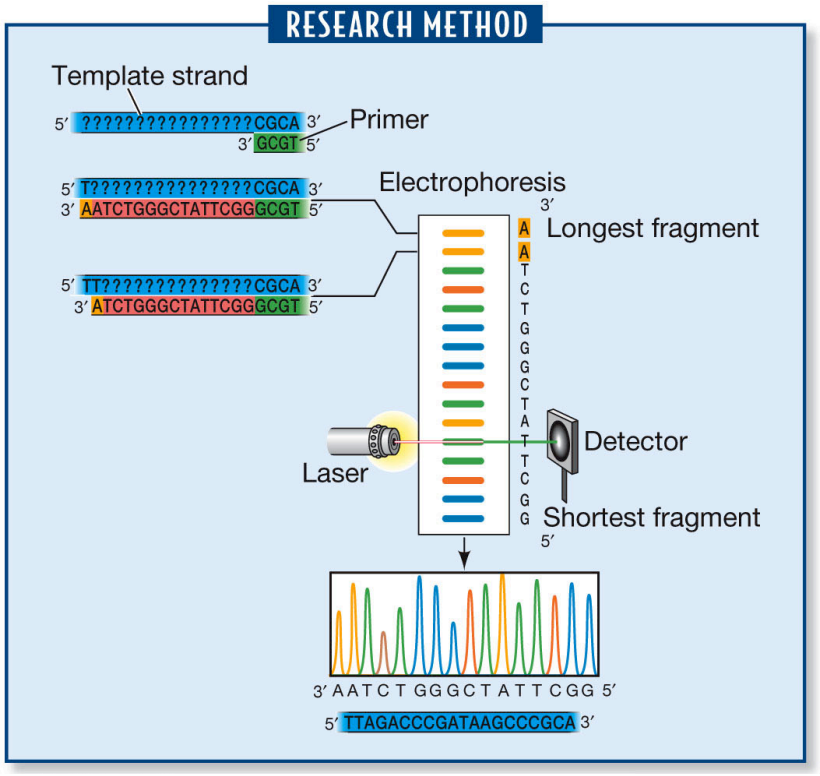
Division of Biology, California Institute of Technology, Pasadena, California 91125, USA
* Applied Biosystems, Inc., Foster City, California 94404, USA



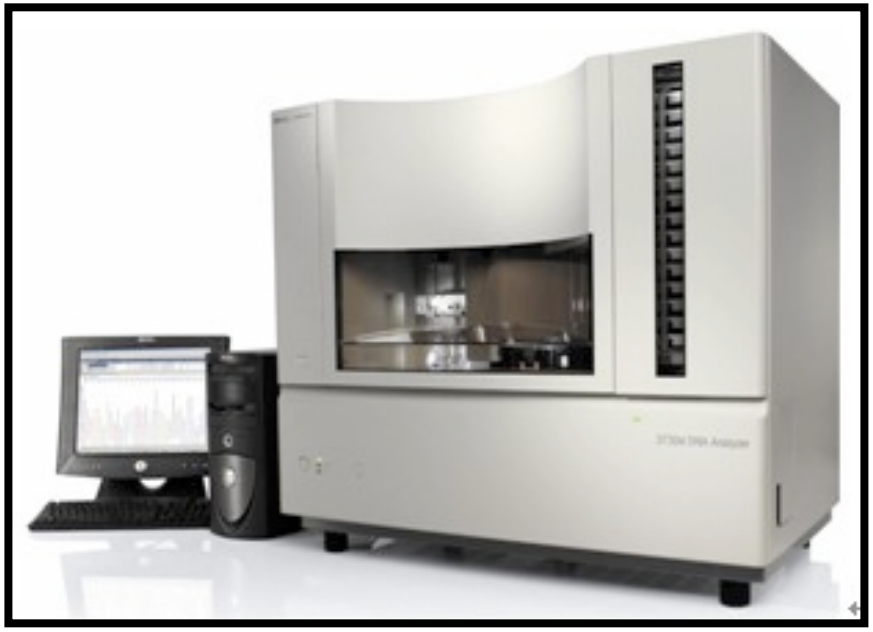
Nature 321, 674-679 (1986)

The Breakthroughs……

- Fluorescent ddXTPs
- Computer Automation
  - Size DNA
  - Detect Specific Bases
- Bioinformatic Analyses
  - BLAST
  - GenBank



<u>However</u>: Still Need Genome Libraries, Cloned DNA Fragments, Manual DNA Synthesis, & Lots of $$$. DNA Sequencing Reads ~500 Bases.



Applied Biosystems DNA Sequencer

A DNA Sequencing "Factory"

- 100 lanes/machine
- 500 bp/reaction/lane
- 3 hour run time
- ~ 50 kb/run
- ~ 400 kb/machine/day

Need Lots of Machines to Sequence a Genome!!

Using Fluorescent ddXTPs to Visualize DNA Sequence And Size of DNA Fragments on a Gel Continuously

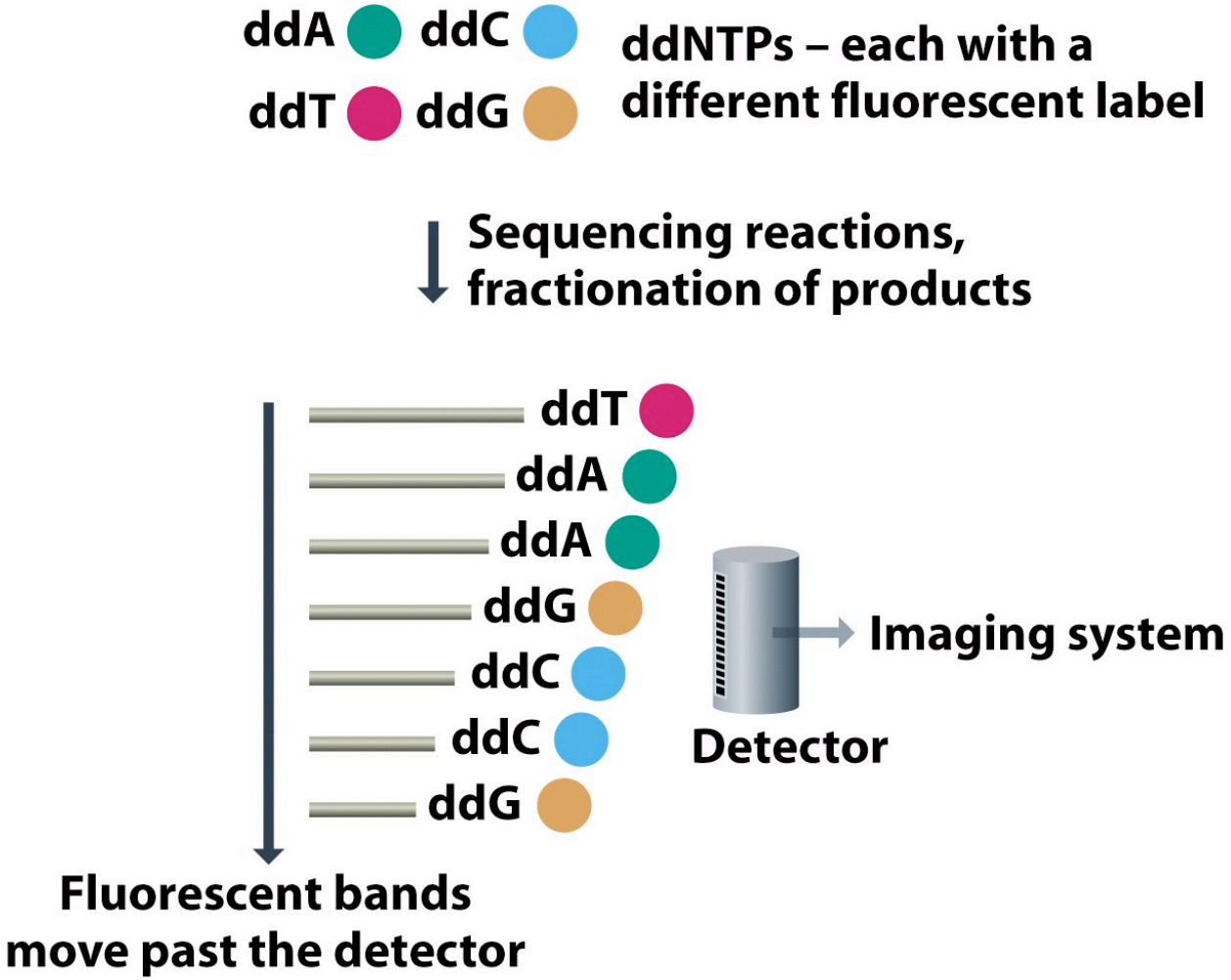
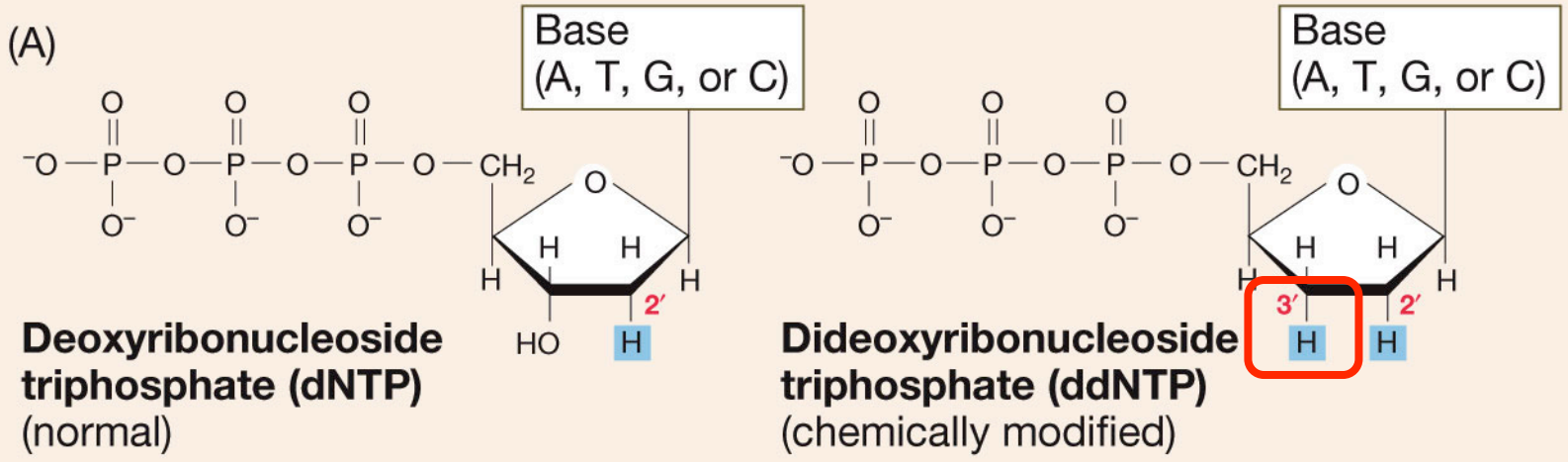
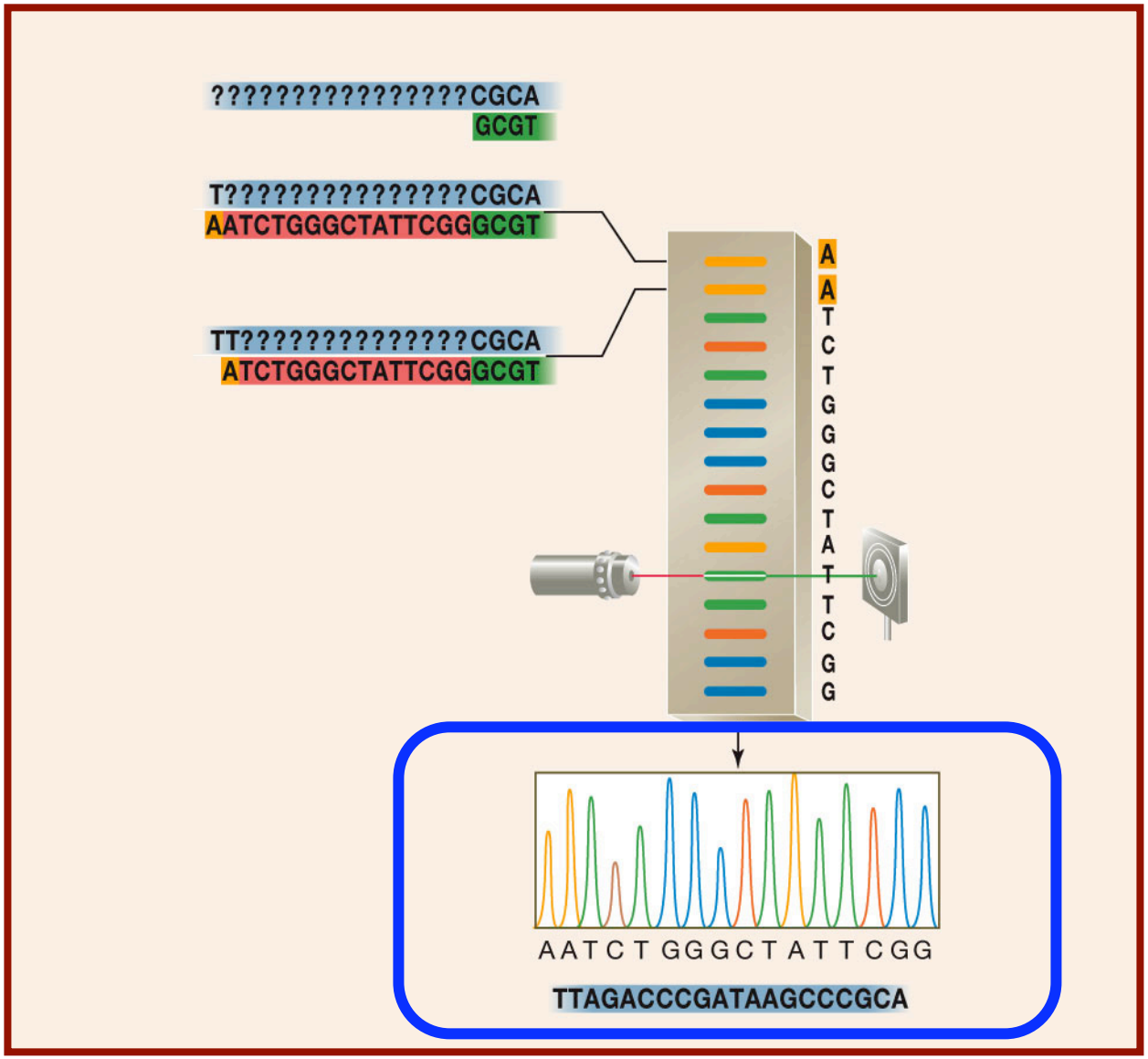


Figure 4.3a *Genomes 3* (© Garland Science 2007)

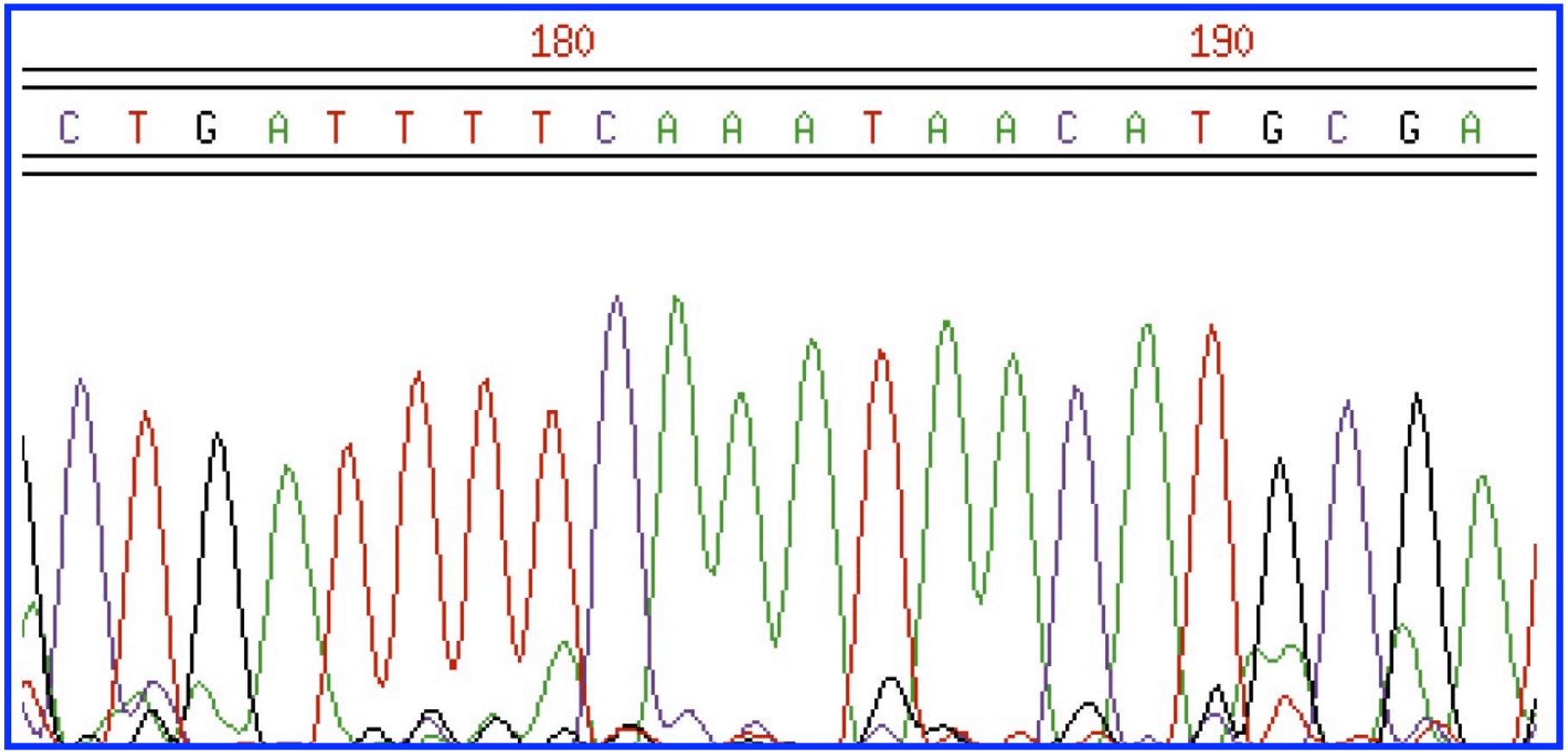
TOOLS FOR INVESTIGATING LIFE



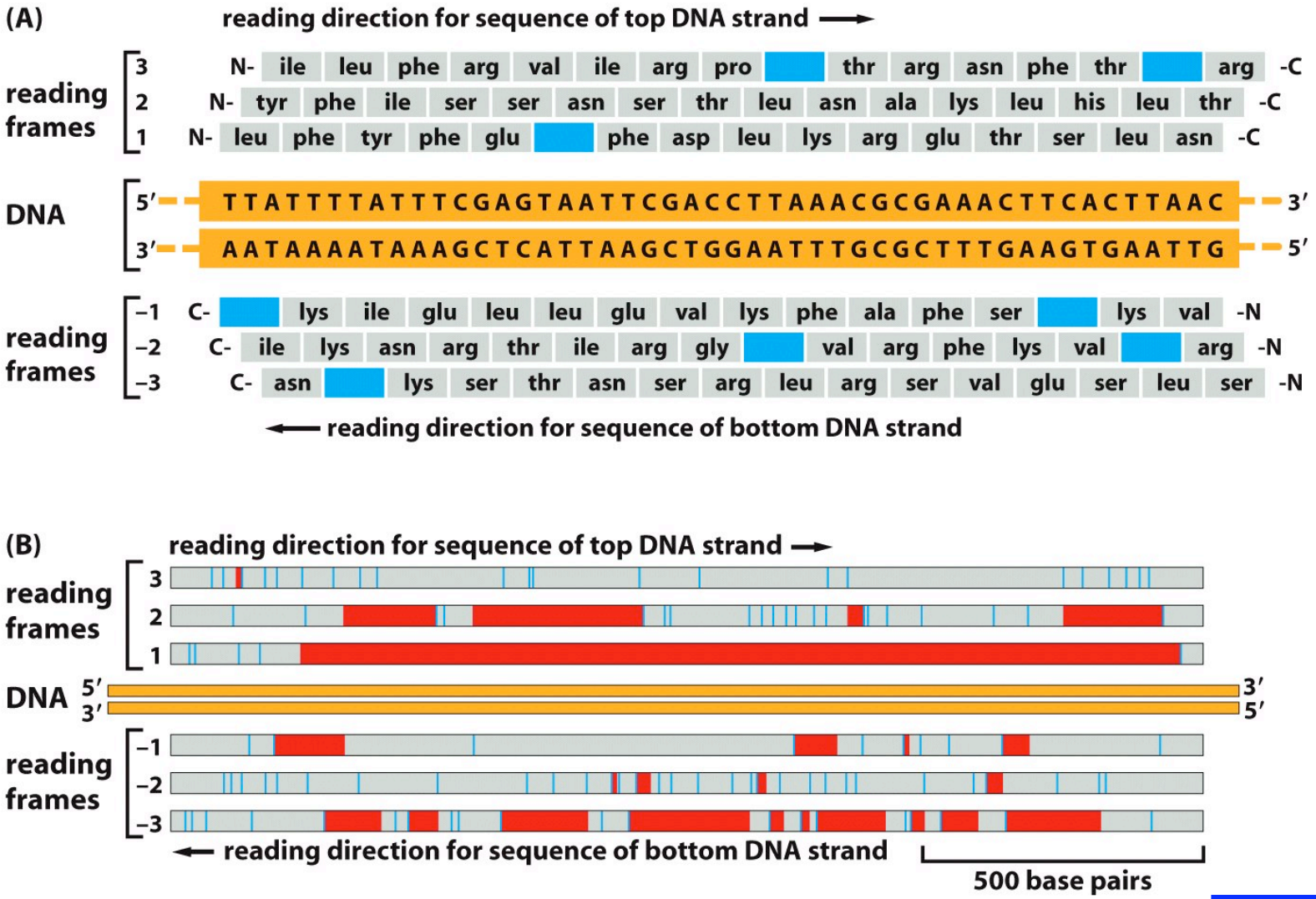
Need Precise Base-Calling Software



Reading the DNA Sequence



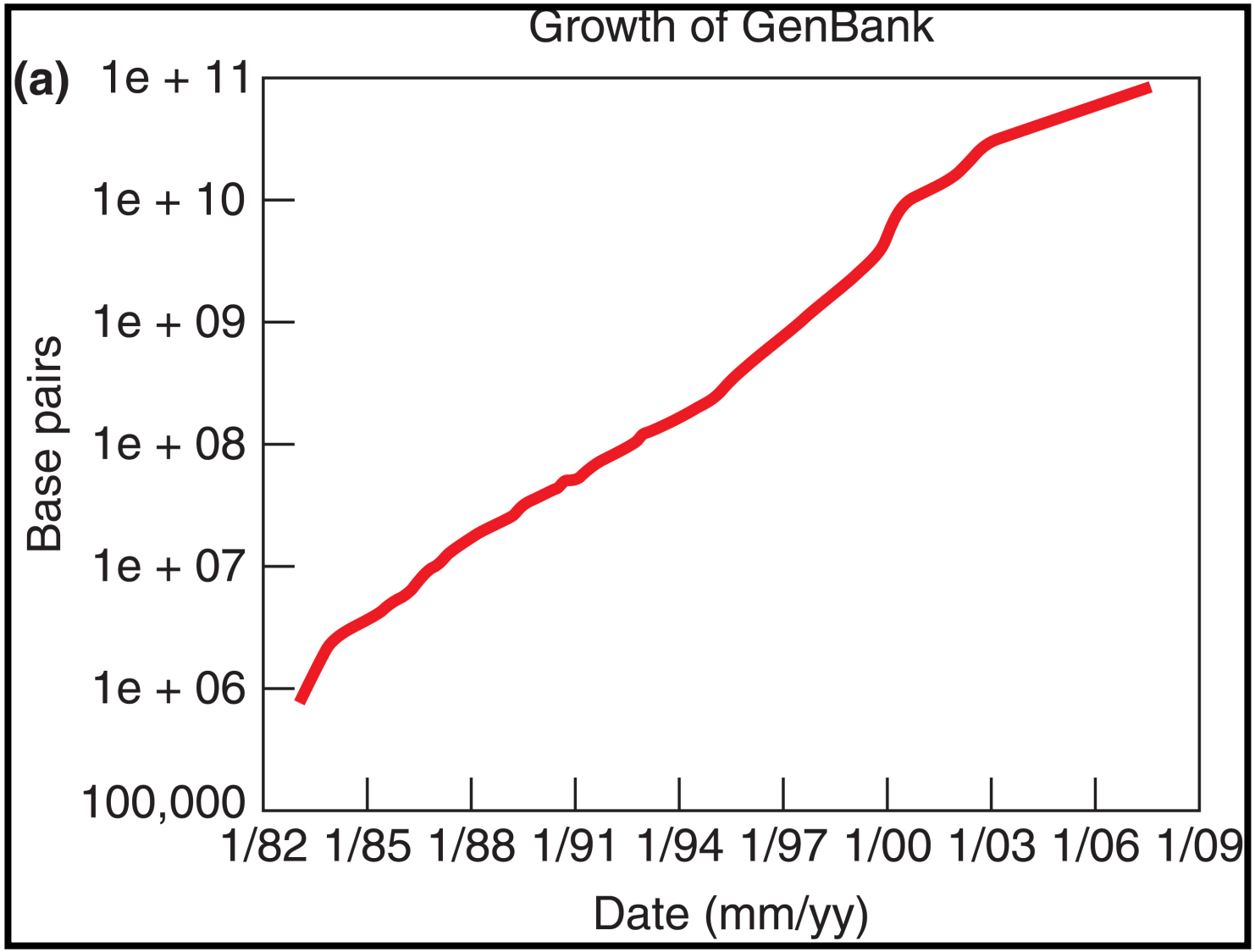
Using Bioinformatics to Identify a Gene



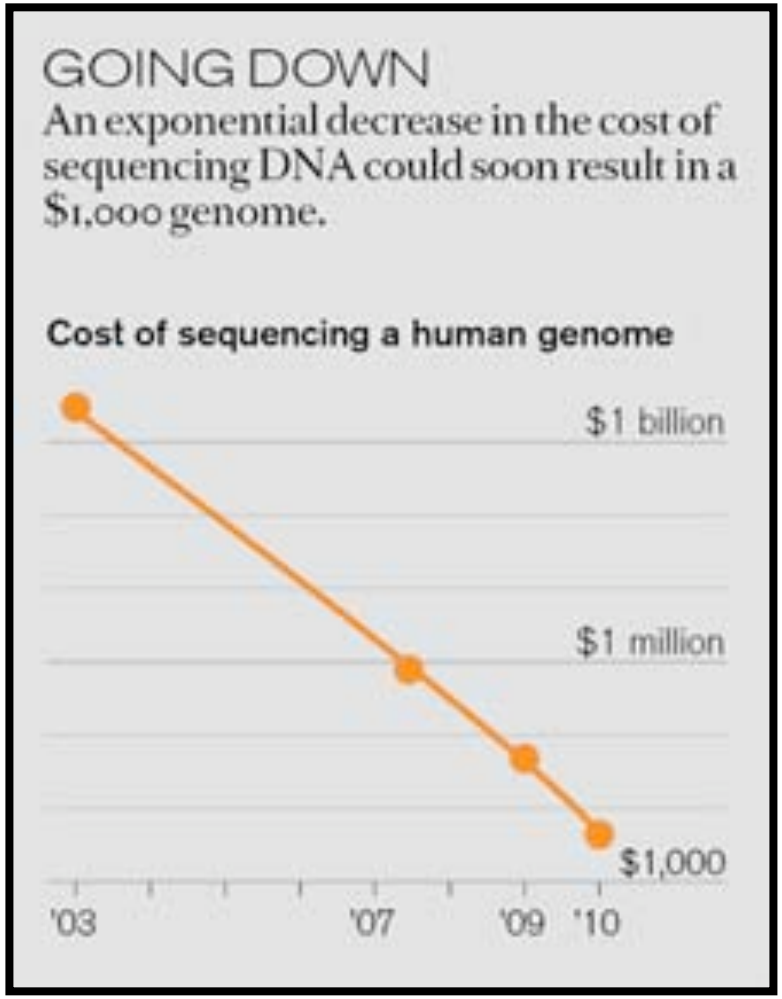
BLAST

Figure 8-52 *Molecular Biology of the Cell* (© Garland Science 2008)

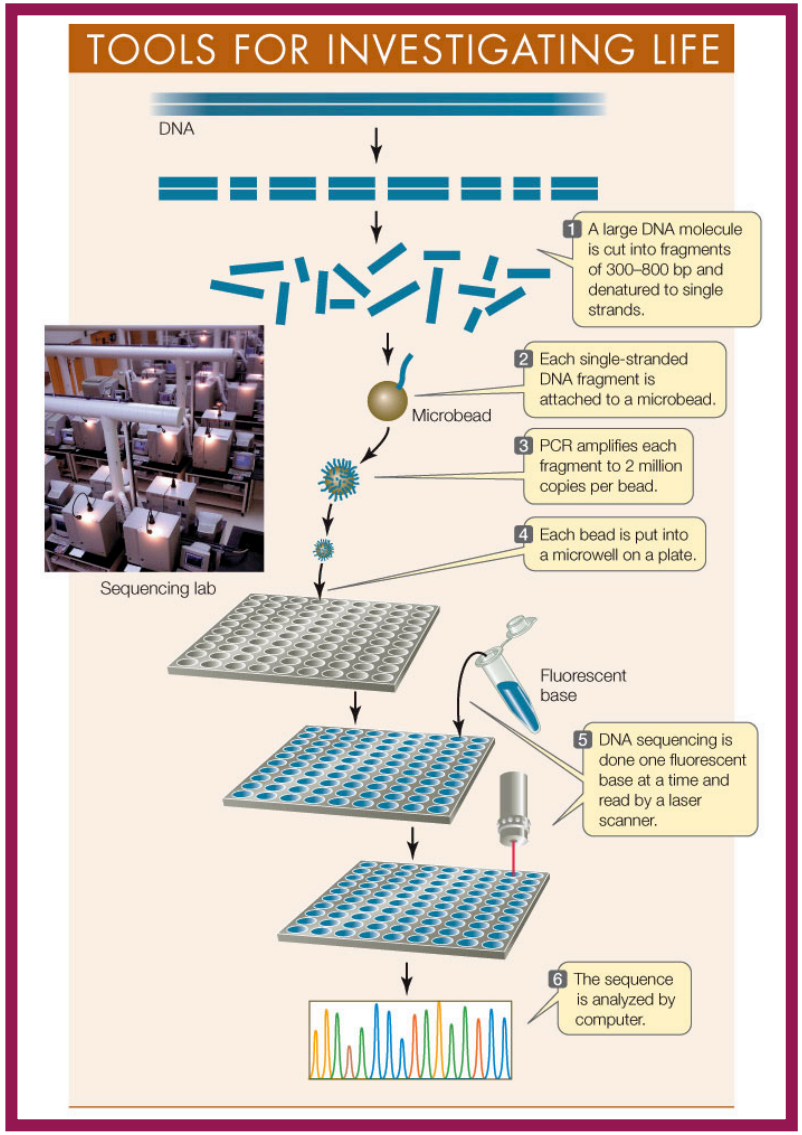
DNA Sequencing Throughput Has Exploded!



Cost of Sequencing is Going Down Precipitously!



NextGen Sequencing Has Lead To An Explosion of Whole Genome Sequencing



- No Cloning
- Random Shearing of DNA
- Attachment to Microbeads
- One DNA Sequence per Microbead
- PCR Amplification
- Sequencing By DNA Synthesis
- Fluorescent Nucleotides
- Sequence Millions of Unique DNA
- Fragments at Same Time
- (Massively Parallel DNA Sequencing)
- Sequence By DNA Synthesis One Nucleotide at a Time
- Uses Nanotechnology & Robotics
- One "Lane" Can Sequence 20 Gb or 160 Gb per Sequencing Run or ~50X the Human Genome (2 hours)

New Sequencing Technologies Have Lead To An Explosion of Whole Genome Sequencing

TABLE 10.1 Number of Species with Finished Whole-Genome Sequences Deposited at the National Center for Biotechnology Information as of February 1, 2010 (2/9/11)

Organism	Whole Genome		In Progress		Total	
Prokaryotes	1058		2354		3412	
Mammals	56		69		125	
Birds	2		12		14	
Fishes	15		17		32	
Insects	36		7		43	
Flatworms	3		2		5	
Roundworms	12		14		26	
Amphibians	1		0		1	
Reptiles	1		0		1	
Other animals	11		18		29	
Plants	25		88		113	
Fungi	129		91		220	
Protists	54		58		112	
Total	1403	1581	2730	9640	11,221	4133

http://www.genomesonline.org/cgi-bin/GOLD/bin/gold.cgi