SYLICA 2013
Bowater lectures

Contemporary DNA Sequencing Technologies

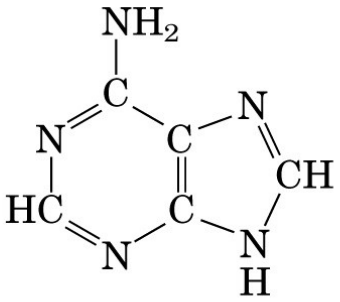
Bowater Lectures in Brno, Feb. 2013

4 lectures on linked topics will be delivered during the coming week:

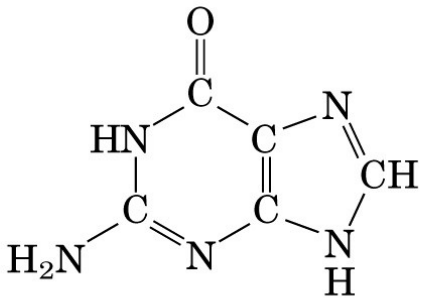
- *Contemporary DNA Sequencing Technologies* – 26/2/2013 @ 10:00
- *Using 'Omic Technologies to Investigate Gene Function* – 26/2/2013 @ 14:00
- *Biophysical Methods to Study Molecular Interactions* – 27/2/2013 @ 10:00
- *Synthetic Biology & Nanotechnology: Tomorrow's Molecular Biology?* – 28/2/2013 @ 10:00

DNA STRUCTURE

Bases

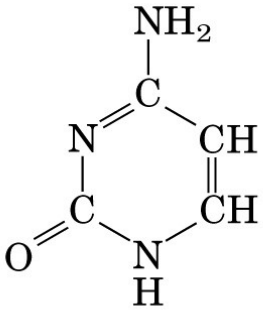


Adenine

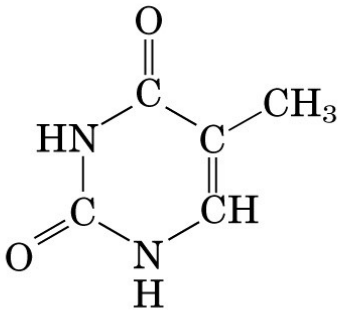


Guanine

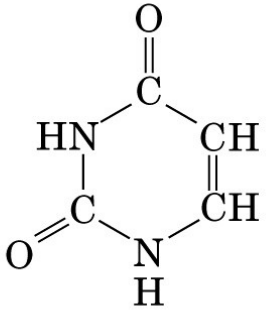
Purines



Cytosine



Thymine
(DNA)



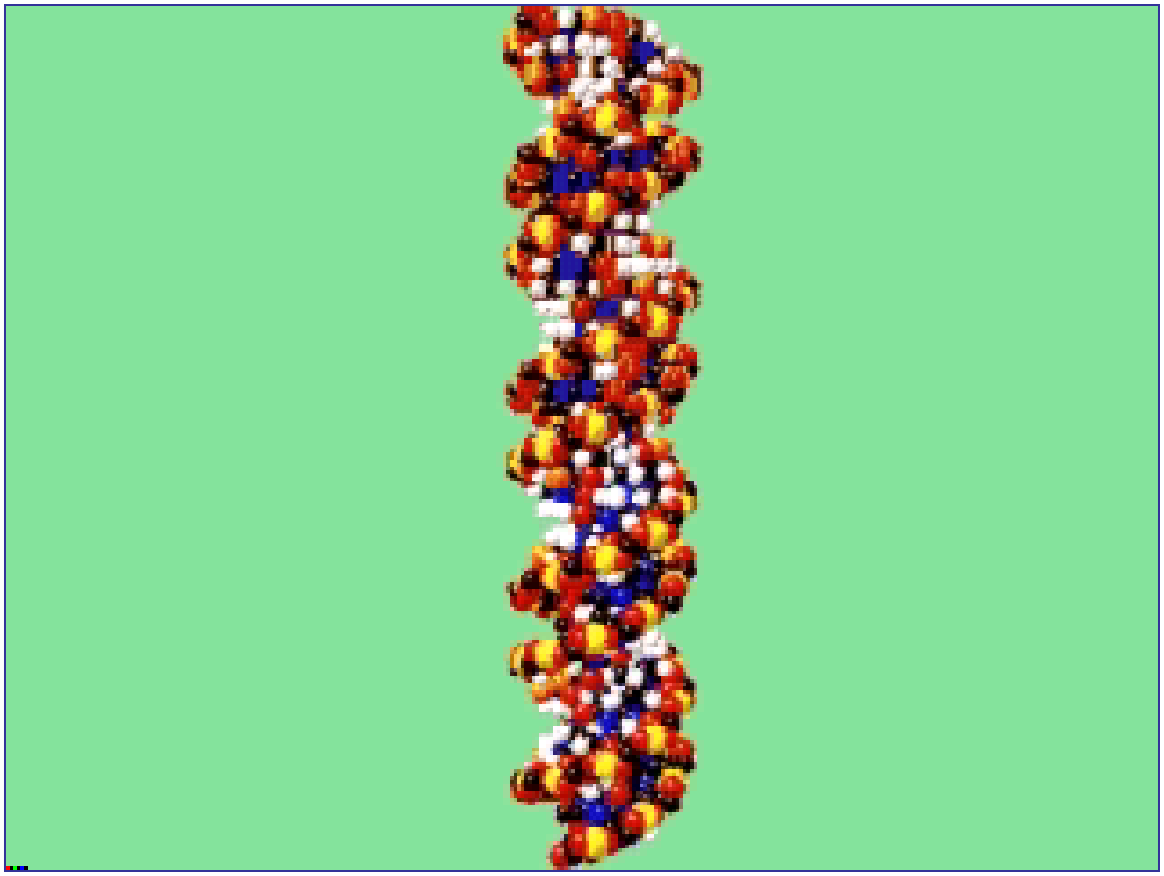
Uracil
(RNA)

Pyrimidines

Nelson & Cox, "Lehninger, Principles of Biochemistry", 5th edn, 2008, p. 272

DNA

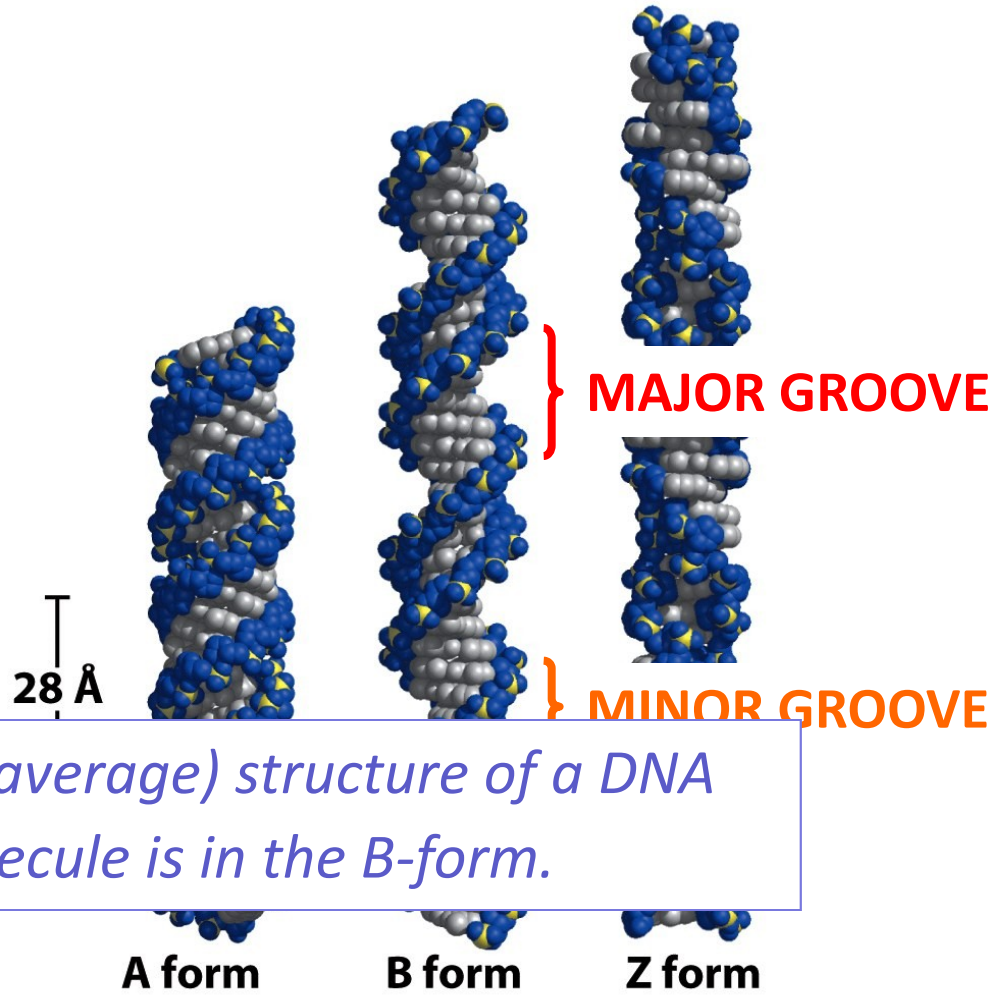
DNA is a double-stranded, helical molecule



Alternative DNA Helices

- The DNA helix may take different forms:

  A- and B-helices are *right-handed*; Z-DNA is *left-handed*.

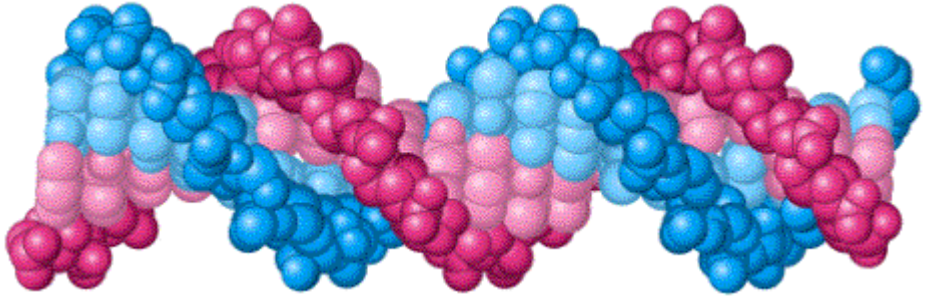


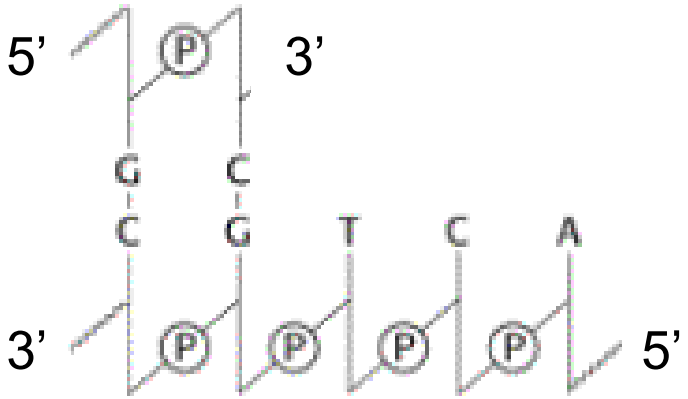
The typical (average) structure of a DNA molecule is in the B-form.

Figure 8-17 part 1
Lehninger Principles of Biochemistry, Fifth Edition

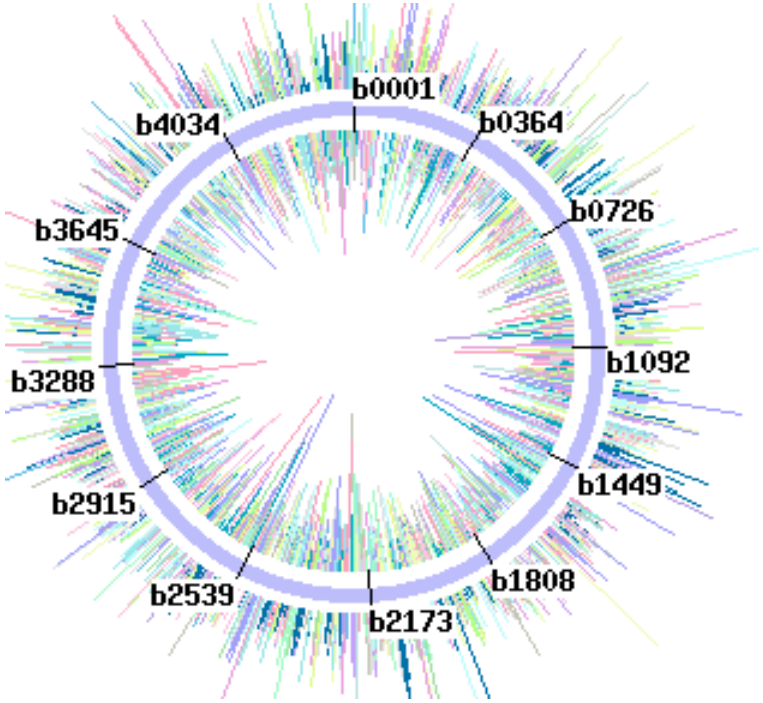
dsDNA has an Anti-Parallel Structure

The two strands of dsDNA have an anti-parallel polarity





Genomes

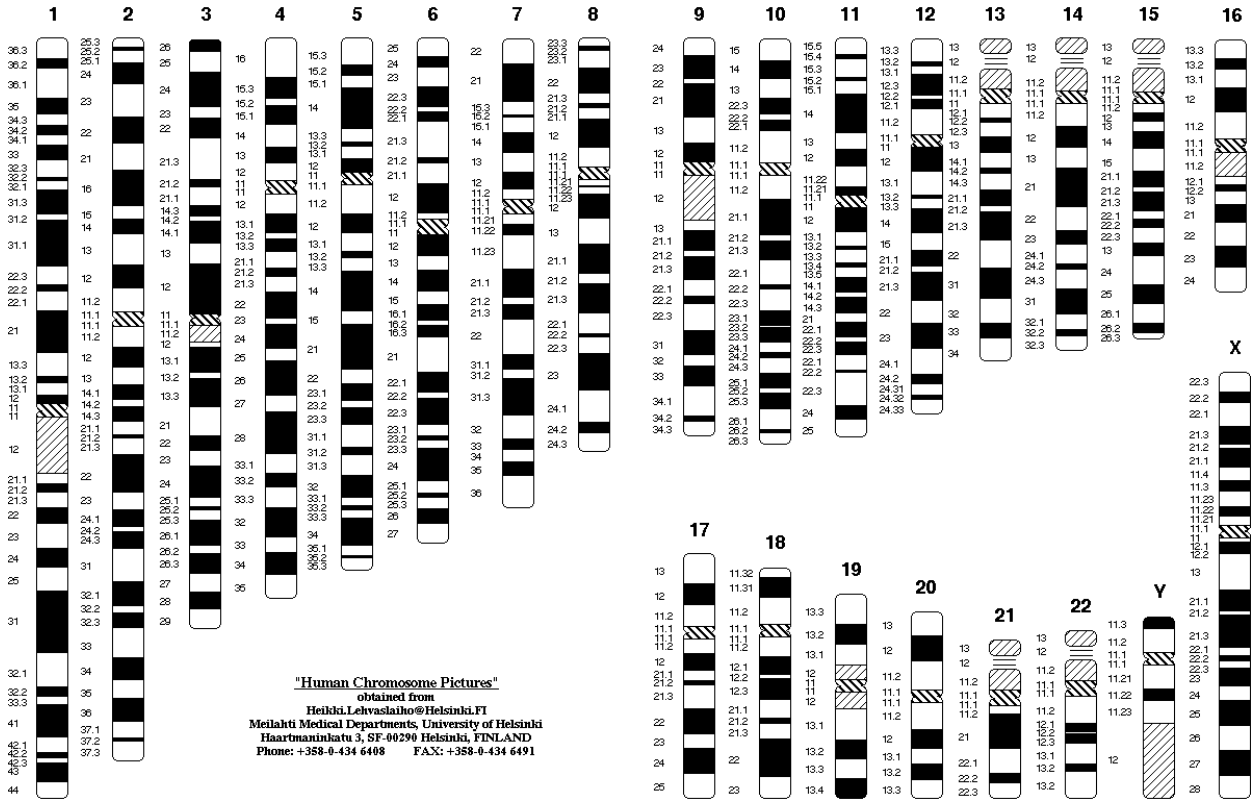


Genome of *E. coli* codes

for 4,500 genes

in 4.6 Mbp

Genomes



Human genome codes for ~30,000 genes in 3,000 Mbp

Cellular DNA

- "Coding" DNA is name given to genes that are transcribed & translated to make protein
- Eukaryotic genomes contain large amounts of *non-coding* DNA
- The length of DNA inside cells is extremely large relative to cell size

The Length of DNA in Cells is Very Large!

- length of DNA inside cells is <u>extremely large</u> relative to cell size

E. coli – lysed to show chromosomal DNA

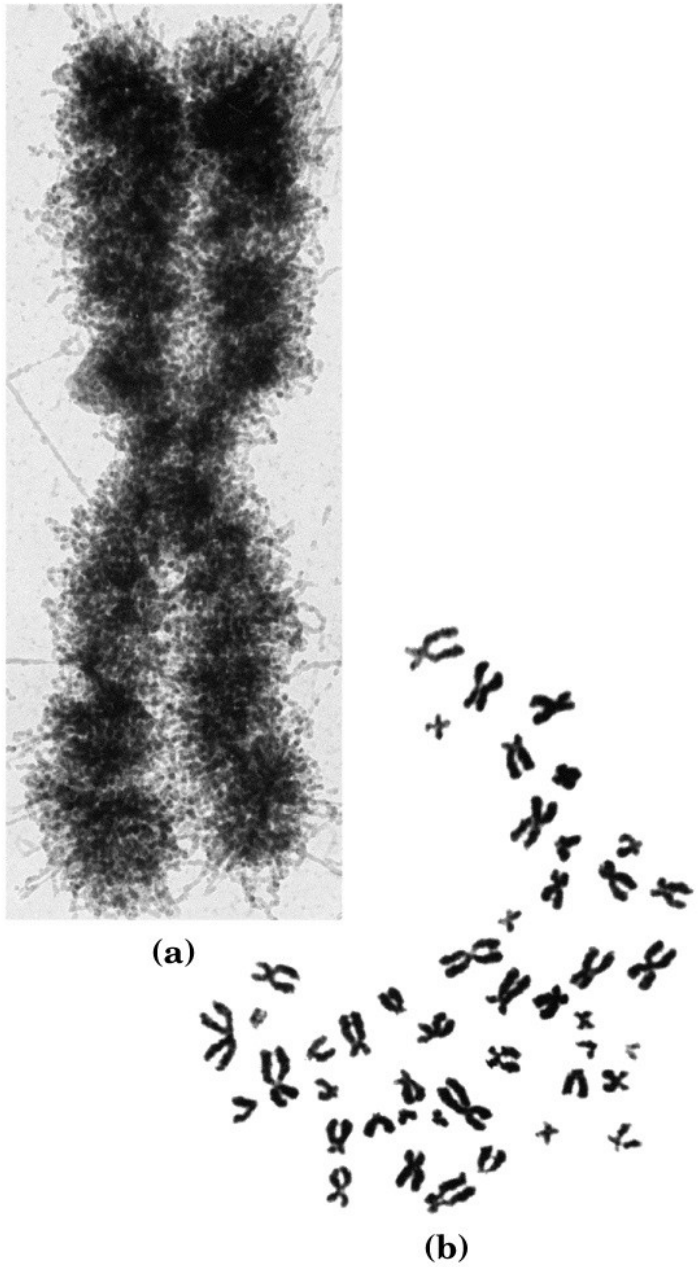
For related discussion, see also: Nelson & Cox, "Lehninger, Principles of Biochemistry", 5th edn, 2008, p. 950

Coding & Non-coding DNA

Organism	No. of genes	Total size of DNA (Mbp)	% of genome as coding DNA*
E. coli	4,500	4.6	98
Yeast (*S. cerevisiae*)	5,885	12	49
Human	30,000 (?)	3,000	1

* Assuming average gene size ~ 1,000 bp

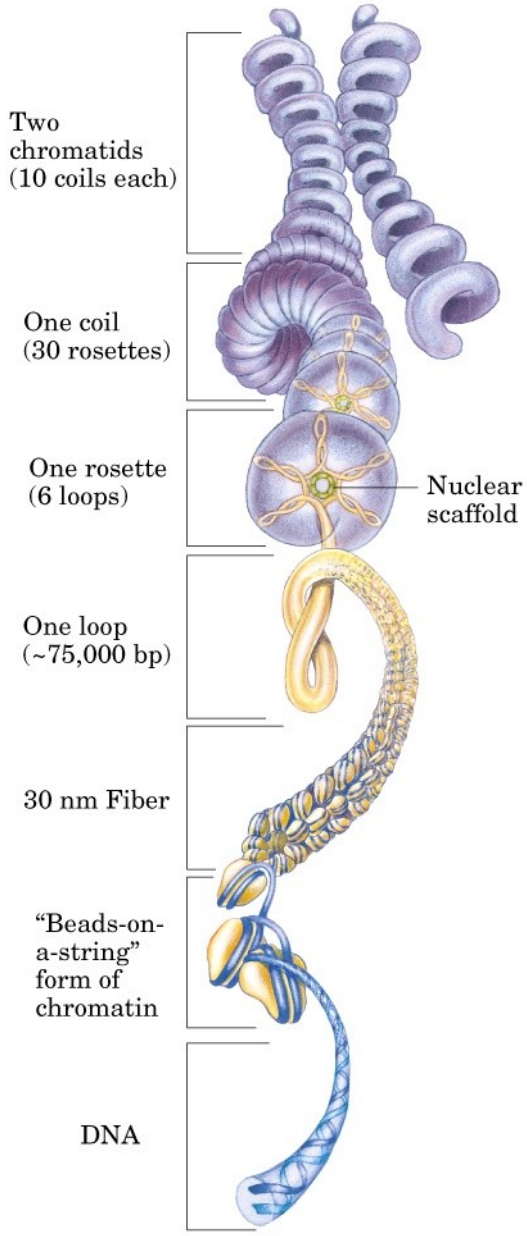
Chromosomal DNA



- *chromosomes* are complexes of protein & DNA

Nelson & Cox, "Lehninger, Principles of Biochemistry", 5th edn, 2008, p. 951

DNA Compaction within Cells



Nelson & Cox, "Lehninger, Principles of Biochemistry", 5th edn, 2008, p. 968

DNA Structure: Overview

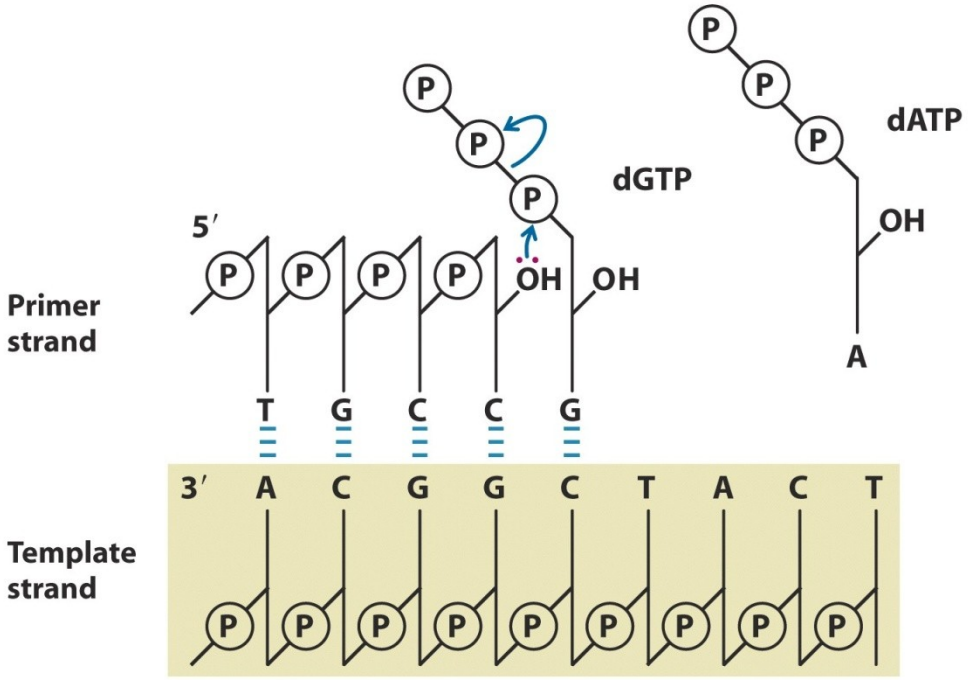
- Inside cells, the structure of DNA is *dynamic*: usually in B-form helix, but can exist in different structures/conformations
- DNA molecules can be linear or circular
- Most genomes have significant amounts of non-coding DNA
- DNA molecules in cells are *very long* – therefore the helix has further levels of well-organised structure that allow it to be contained and used in cells

Genomics & Technology

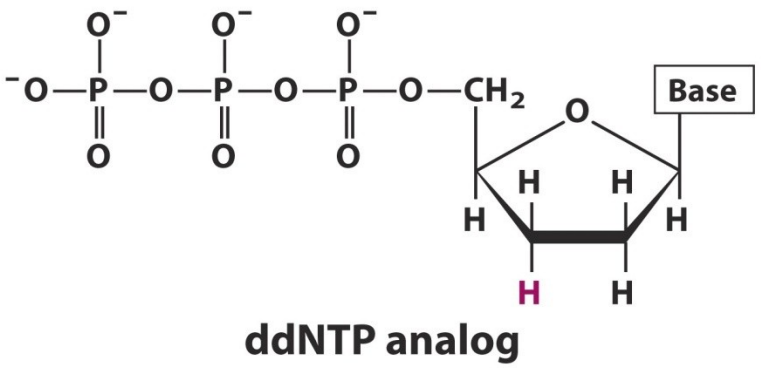
- Molecular biology: major scientific discipline for past ~50 years
- Genomics: became important science during 1990's
- Transcriptomics/Proteomics: developed during past 5 years
- Bioinformatics: has developed as major branch of science - enables efficient analysis of data from "omics" experiments

A Primer About DNA Sequencing

- Major advance in DNA sequencing occurred with use of DNA polymerases



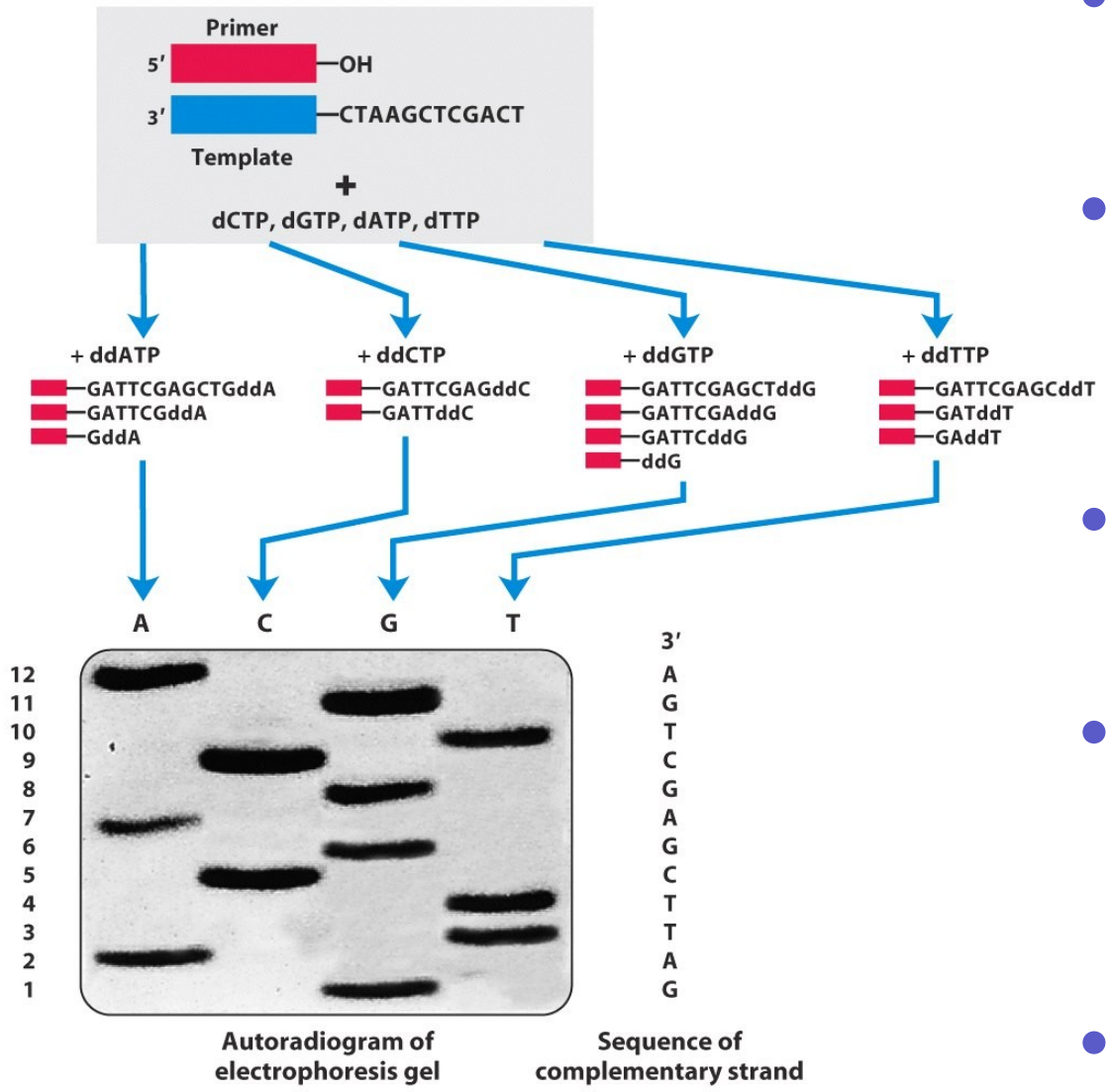
Synthesis requires template strand, "primer" & dNTPs



ddNTPs interrupt DNA synthesis

Nelson & Cox, "Lehninger, Principles of Biochemistry", 4th edn, 2004, p. 297

A Primer About DNA Sequencing

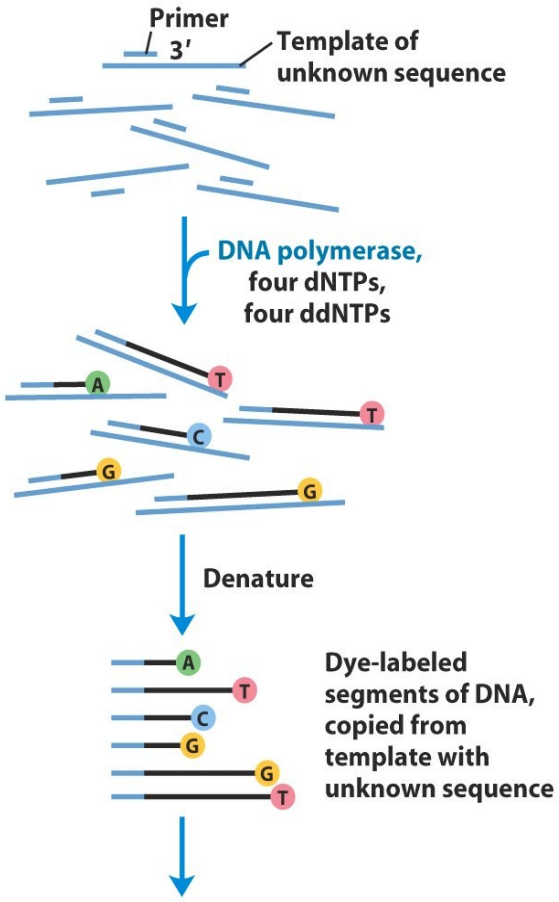


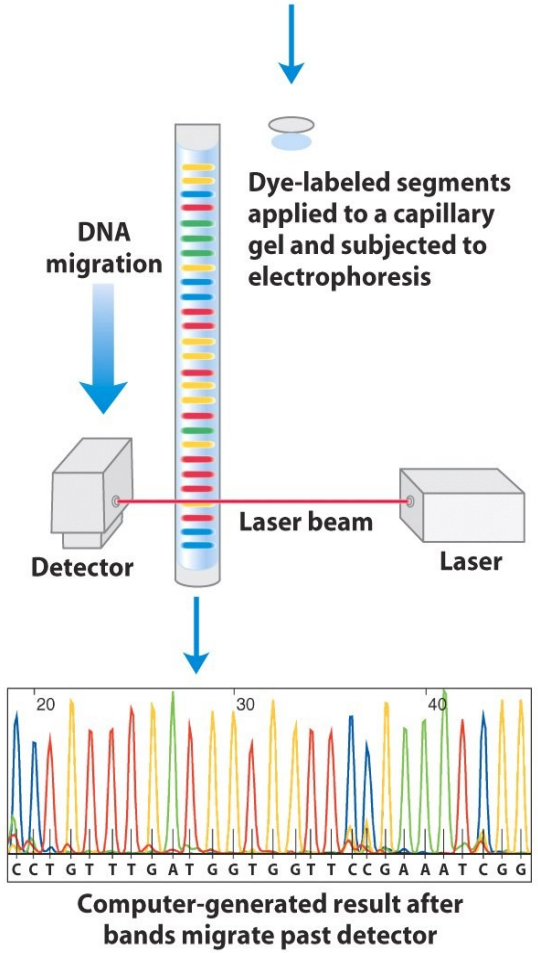
- DNA to be sequenced acts as template
- Oligonucleotide allows sequencing to start at any point
- Small amounts of "labelled" ddNTP
- Identification of specific bases after electrophoresis
- *<500 bases per day*

Nelson & Cox, "Lehninger, Principles of Biochemistry", 4th edn, 2004, p. 297

Improved DNA Sequencing

- "Labelled" DNAs separated by capillary electrophoresis
- DNA sequence read as series of colours
- Computer deciphers sequence
- *>2,000 bases per day*

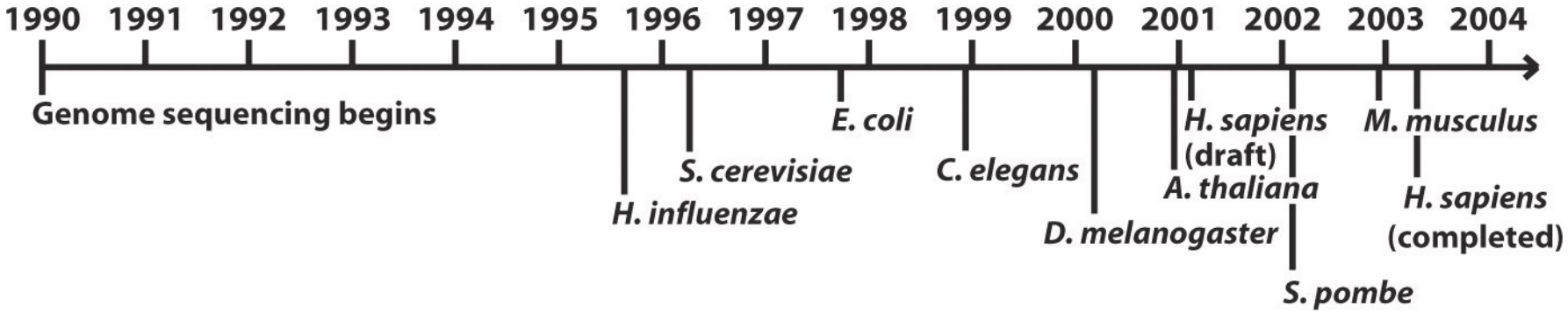




Nelson & Cox, "Lehninger, Principles of Biochemistry", 4th edn, 2004, p. 298

Genomic Sequencing

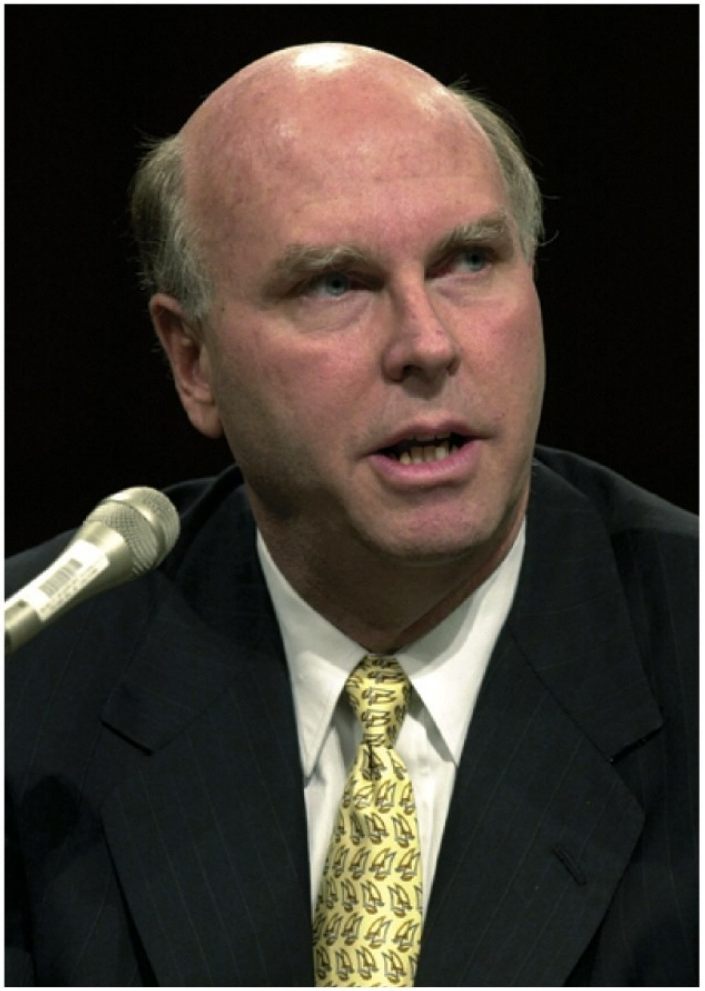
- Sequencing centres have hundreds of machines working continuously
- Each can generate equivalent of human genome sequence each month



Nelson & Cox, "Lehninger, Principles of Biochemistry", 4th edn, 2004, p. 324

Genomic Sequencing

Francis S. Collins



J. Craig Venter

Unnumbered 9 p322
Lehninger Principles of Biochemistry, Fifth Edition

The Human Genome Project

- Sequencing of the human genome allows for:
  - Identification and categorization of different haplotypes
  - Understanding the differences between humans and chimpanzees
    - Based on phylogenetic trees and comparison of differences
    - Especially in regulatory sequences, which may be more important to evolution than protein changes
  - Identification of genes involved in disease
  - Track the path of human migration

Human genome contains many different sequence types

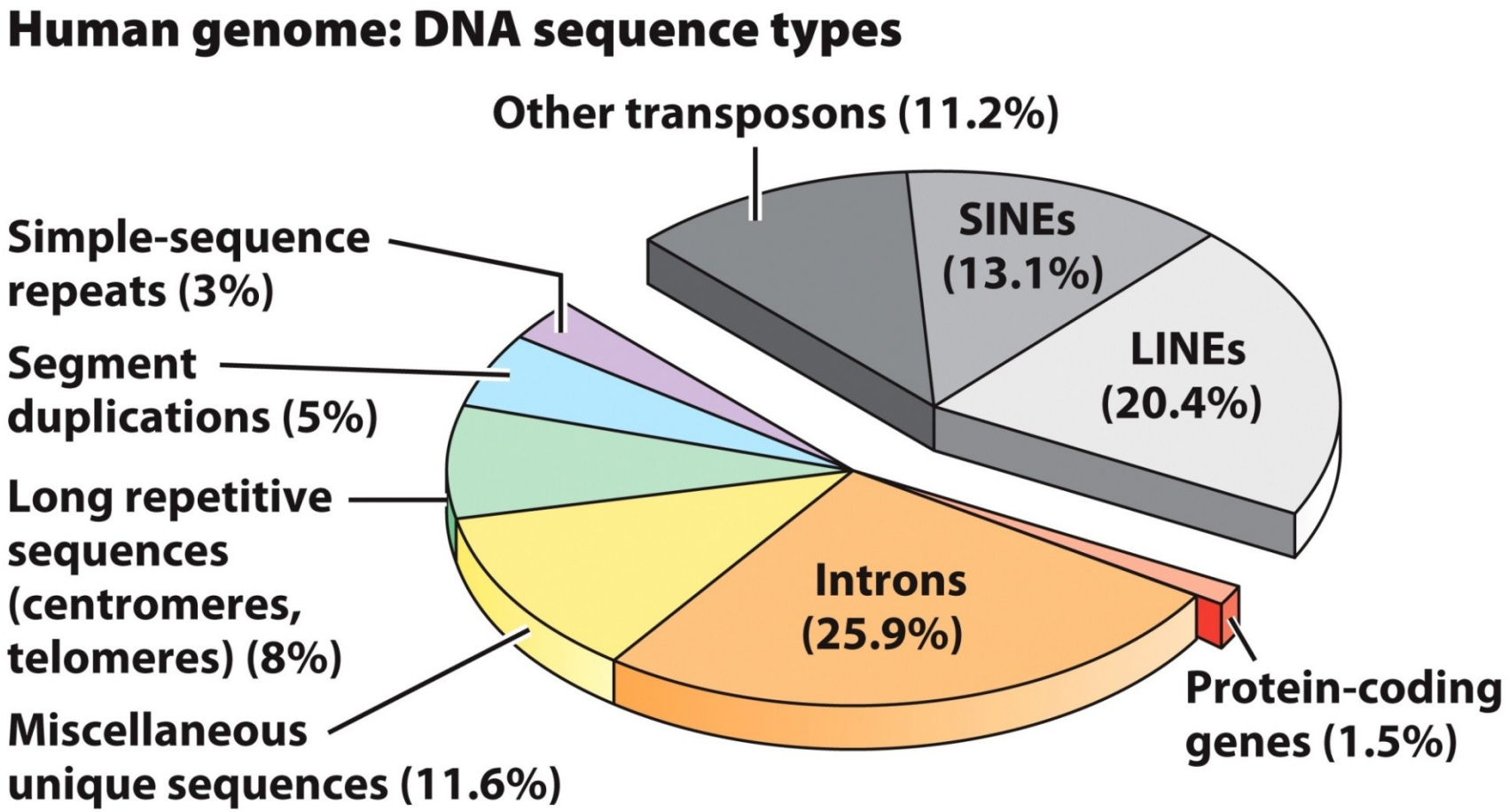


Figure 9-29a
Lehninger Principles of Biochemistry, Sixth Edition

Human genome contains many different protein types

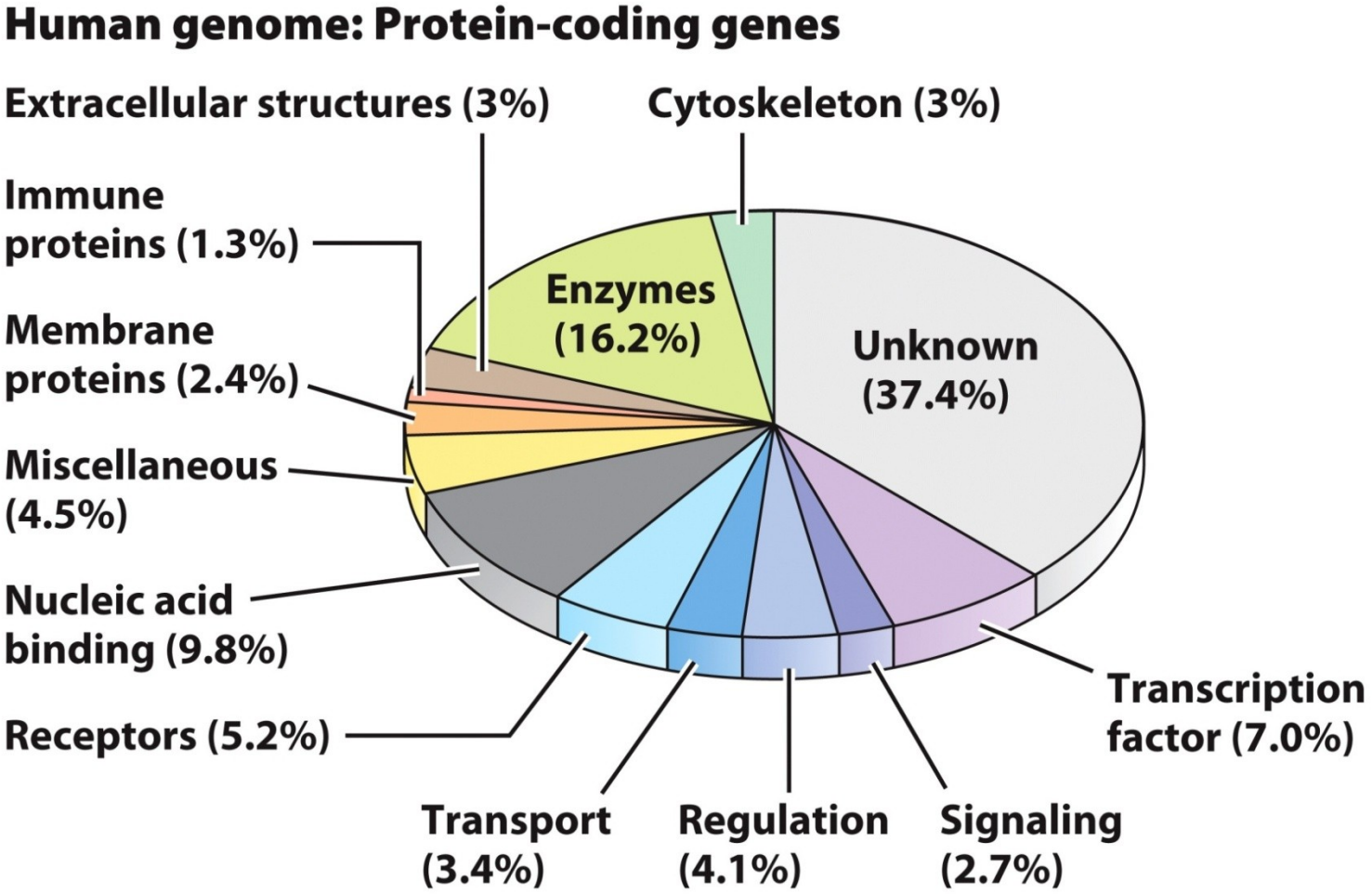
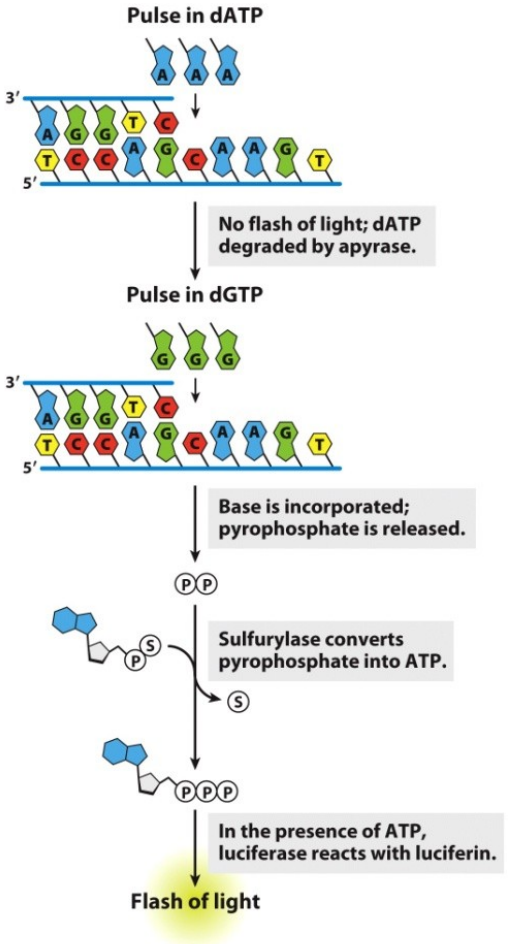


Figure 9-29b
Lehninger Principles of Biochemistry, Sixth Edition

New Generation of DNA Sequence Analysis

- Full genome is immobilized on a chip in fragments a few hundred bases long
  - All sequenced at once, allowing for faster detection
- Pyrosequencing
  - DNA synthesized from the template a single nucleotide at a time, each generating a pulse of light
  - Can read 400–500 nucleotides in the sequence
- Reversible terminator sequencing
  - Fluorescently labeled terminal nucleotide is added to the sequence and detected
  - Terminal nucleotide is removed, sequence extended, and next nucleotide is detected

Pyrosequencing



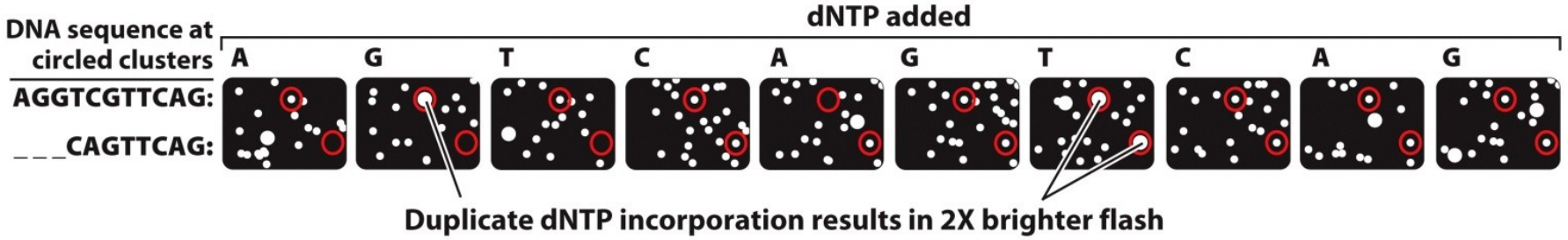
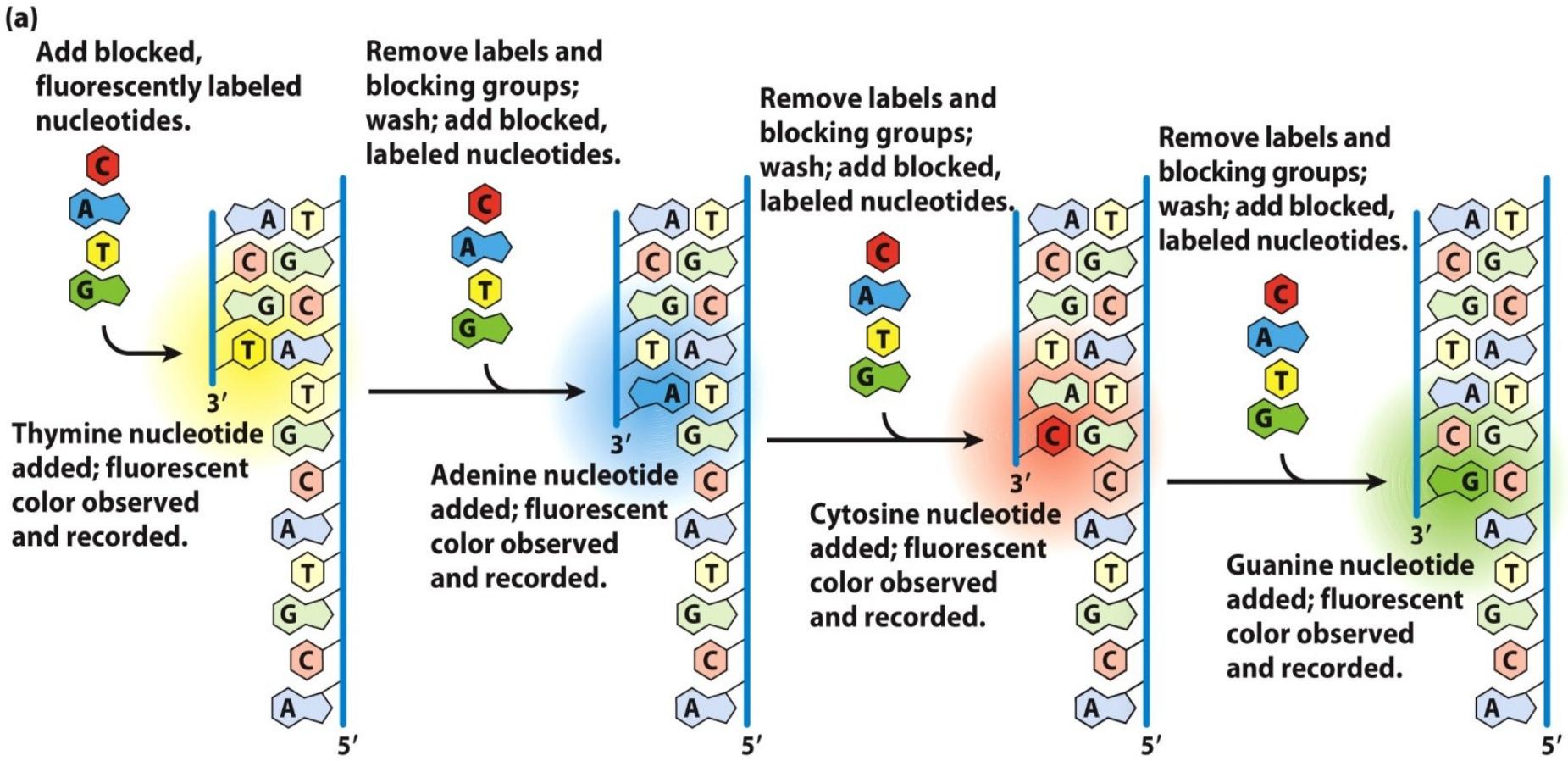


Figure 9-25b
Lehninger Principles of Biochemistry, Sixth Edition

Reversible Terminator Sequencing

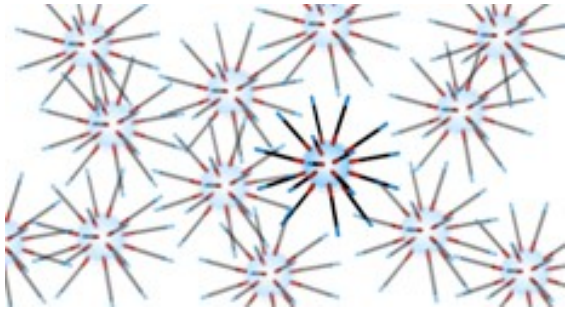


Nelson & Cox, "Lehninger, Principles of Biochemistry", 6th edn, Fig. 9.26

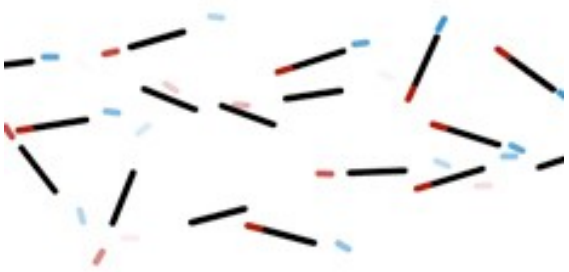
High-throughput Sequencing Technologies

- Recently, several new technologies have increased the throughput and reduced the cost for genome sequencing
- Examples are:

454 Sequencing



Illumina method



- Animations illustrating these methods available at:
- www.wellcome.ac.uk/Education-resources/Teaching-and-education/Animations/DNA/index.htm

DNA Sequencing: Overview

- Production of pure DNA polymerases made it feasible to consider sequencing of genomes
- Sequencing of large genomes became possible with:
  - Increased sensitivity of nucleic acid detection
  - Automated robotic technology
  - Improved computer power
- Further advances are increasing speed, reducing size and cost – suggesting it will soon be possible to sequence individual human genomes for $1,000

Polymerase Chain Reaction (PCR)

- Used to amplify DNA in the test tube
  - Can amplify regions of interest (genes) within DNA
  - Can amplify complete circular plasmids
- Mix together
  - Target DNA
  - Primers (oligonucleotides complementary to target)
  - Nucleotides: dATP, dCTP, dGTP, dTTP
  - Thermostable DNA polymerase
- Place the mixture into thermocycler
  - Melt DNA at ~95°C
  - Cool to ~ 50–60°C, primers anneal to target
  - Polymerase extends primers in 5'→3' direction
  - After a round of elongation is done, repeat steps

General Steps of PCR

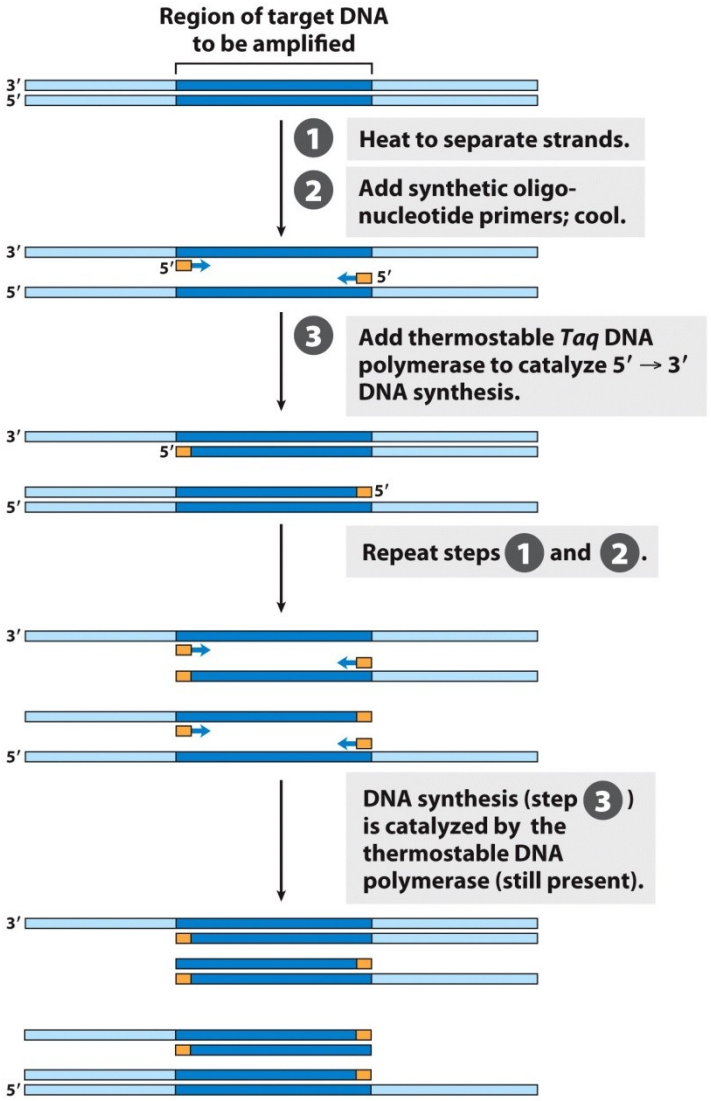


Figure 9-12a part 1
Lehninger Principles of Biochemistry, Sixth Edition

General Steps of PCR

- Repeat steps 1–3 many times:

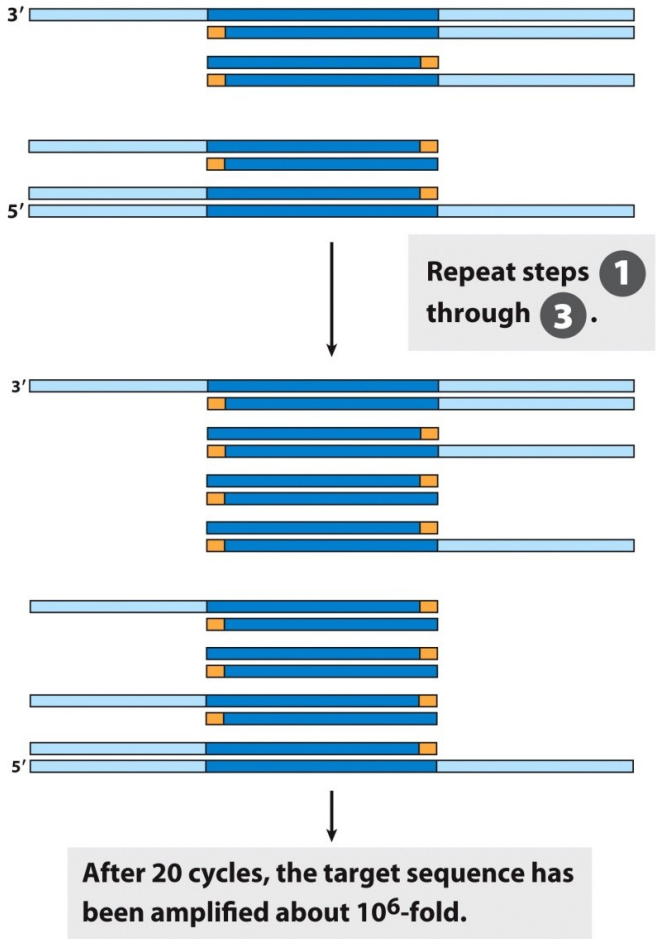
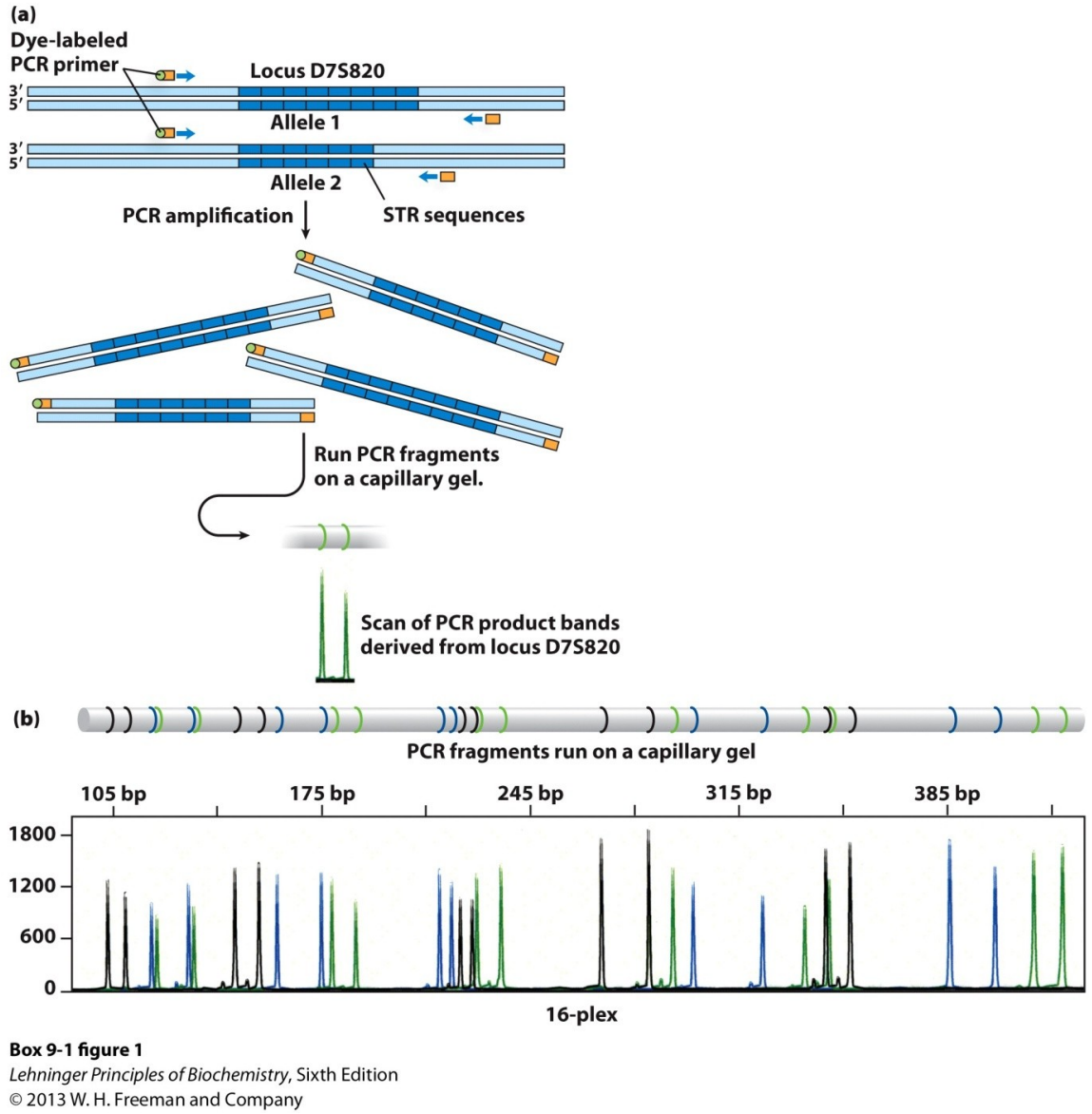


Figure 9-12a part 2
Lehninger Principles of Biochemistry, Sixth Edition

DNA Fingerprinting

- Humans have short sequences that repeat next to each other (Short tandem repeats (STR))
- Differences in the number of repeats cause varying fragment lengths when sample subjected to PCR using a primer specific for that region
- Fragment sizes determined by using a capillary gel
- Multiple STR locations exist in the human genome
- Allows matching of “suspect” samples to known individuals
- 13 well-studied locations are used in identifications
  - Based on number of alleles at each location misidentification is <1 in 10^{18} (with good data)

DNA Genotyping



Box 9-1 figure 1
Lehninger Principles of Biochemistry, Sixth Edition

Adaptations to PCR

- Reverse Transcriptase PCR (RT-PCR)
  - Used to amplify RNA sequences
  - First step uses reverse transcriptase to convert RNA to DNA
- Quantitative PCR (Q-PCR)
  - Used to show quantitative differences in gene levels

qPCR

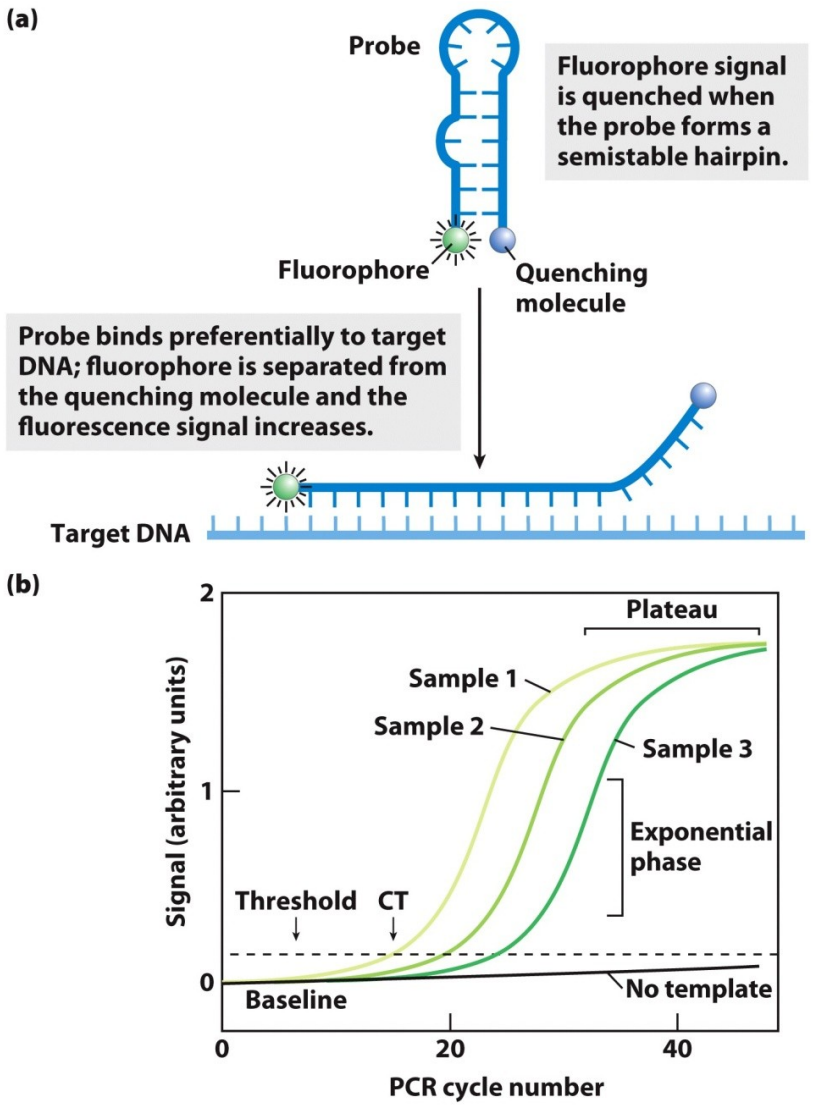


Figure 9-13
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