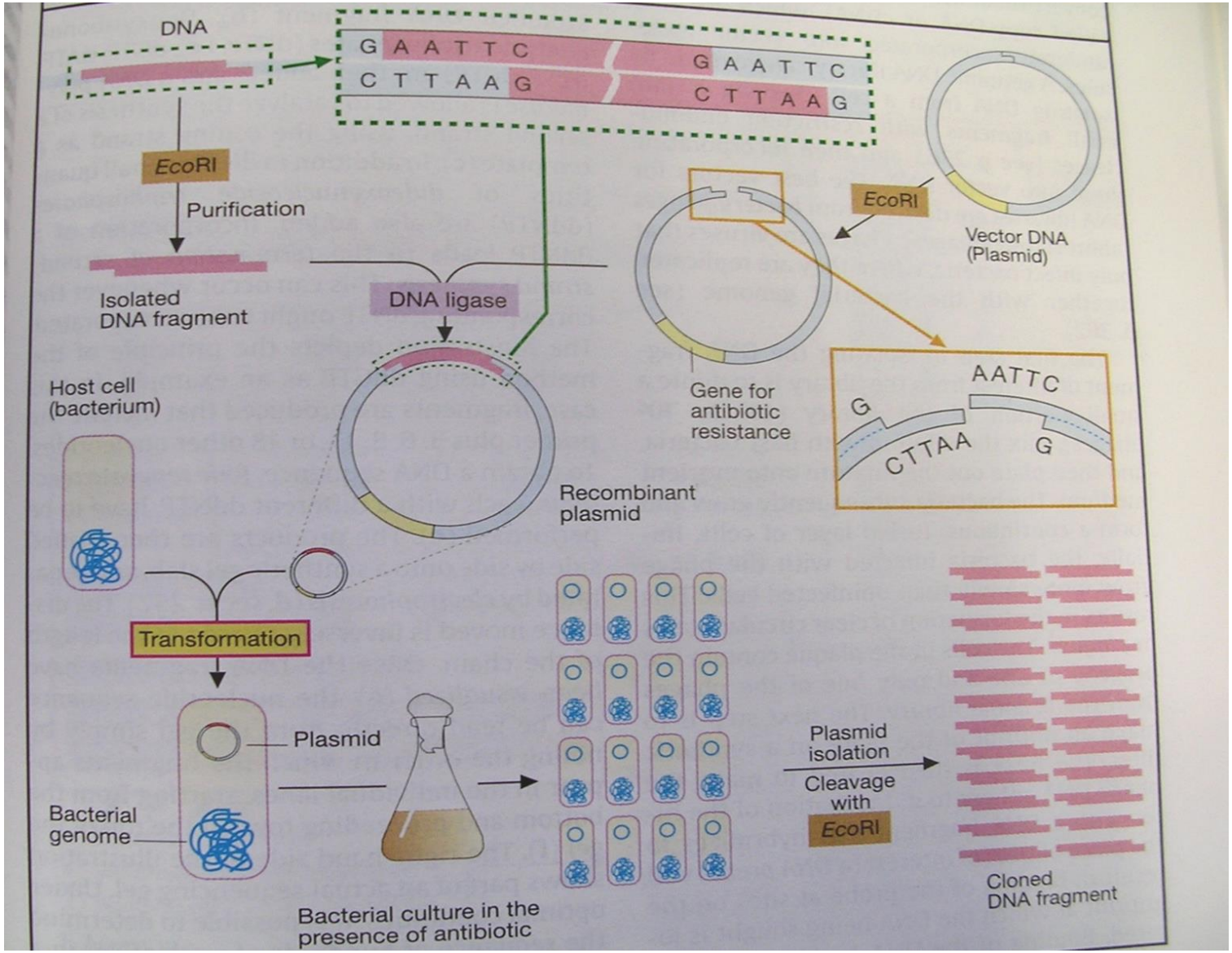
CISC 636 Computational Biology &Bioinformatics (Fall 2016)

Molecular Biology Tools

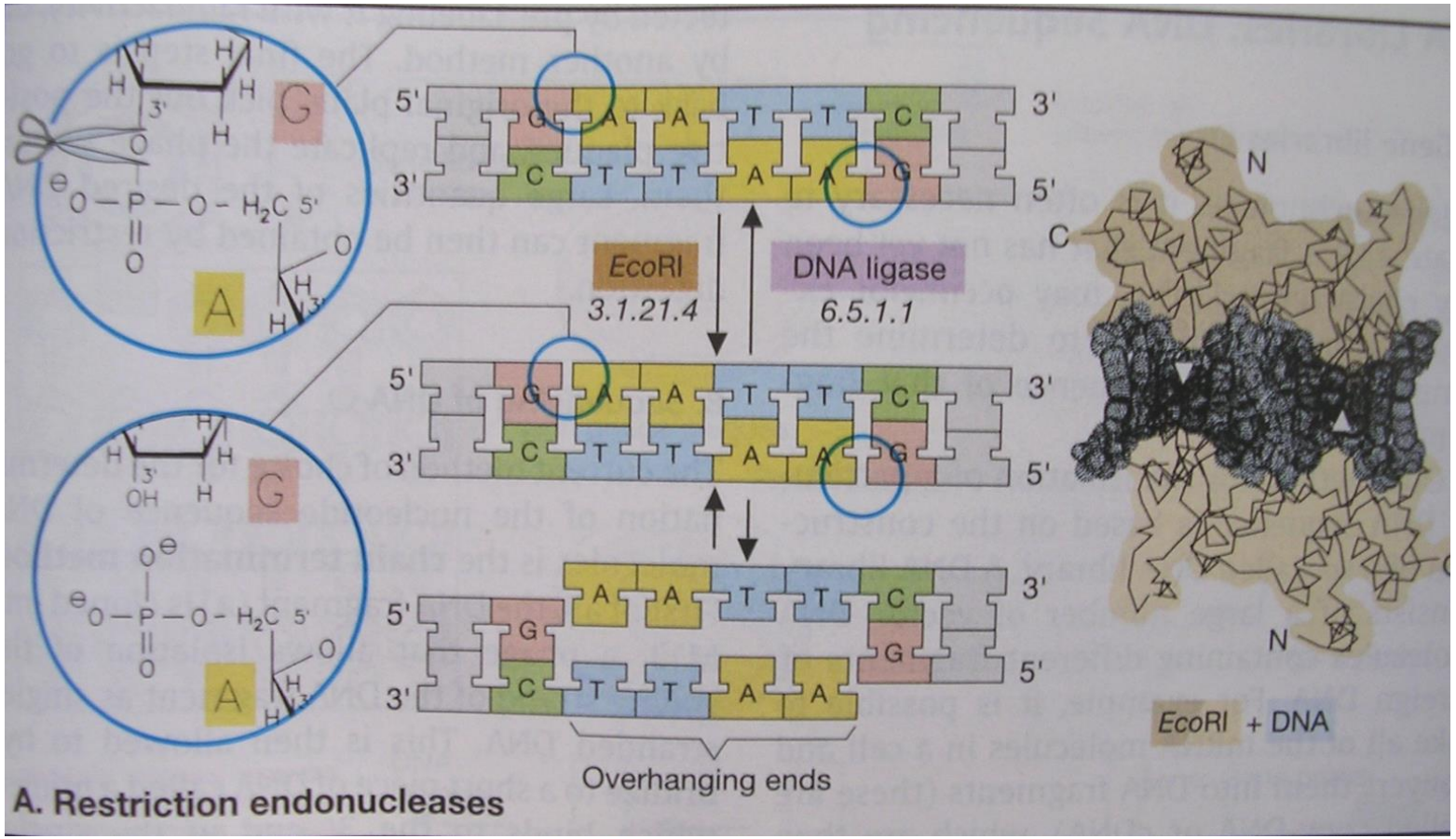
Gel electrophoresis
Cloning
PCR
DNA Sequencing

DNA Cloning



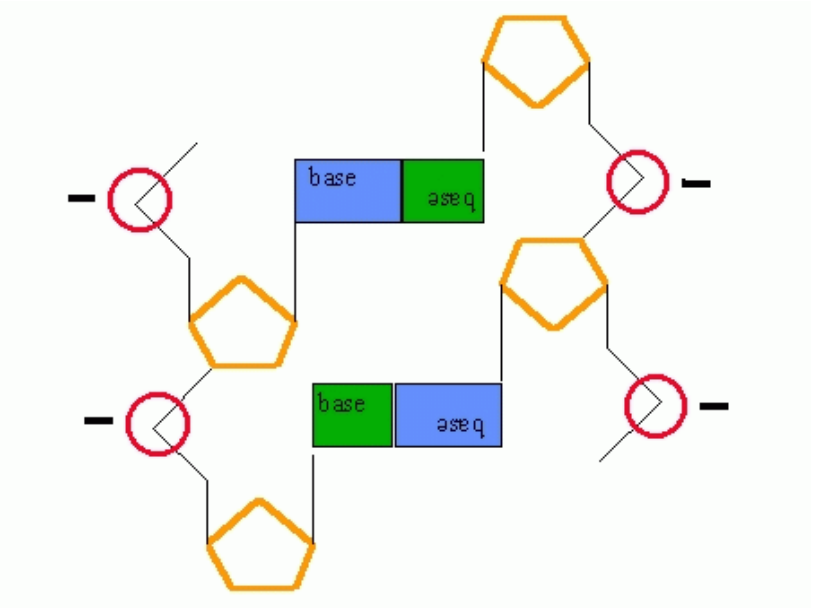
Courtesy of Color Atlas of Biochemistry

Restriction endonucleases

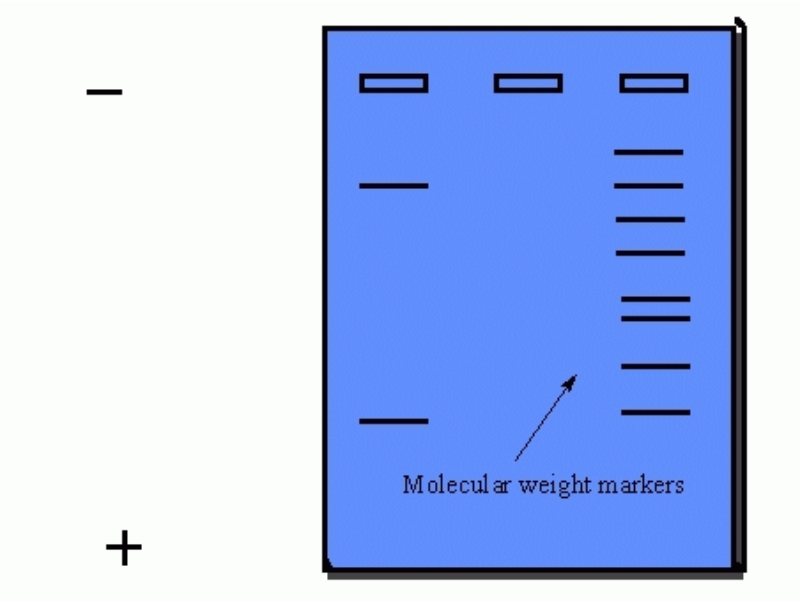


Gel electrophoresis

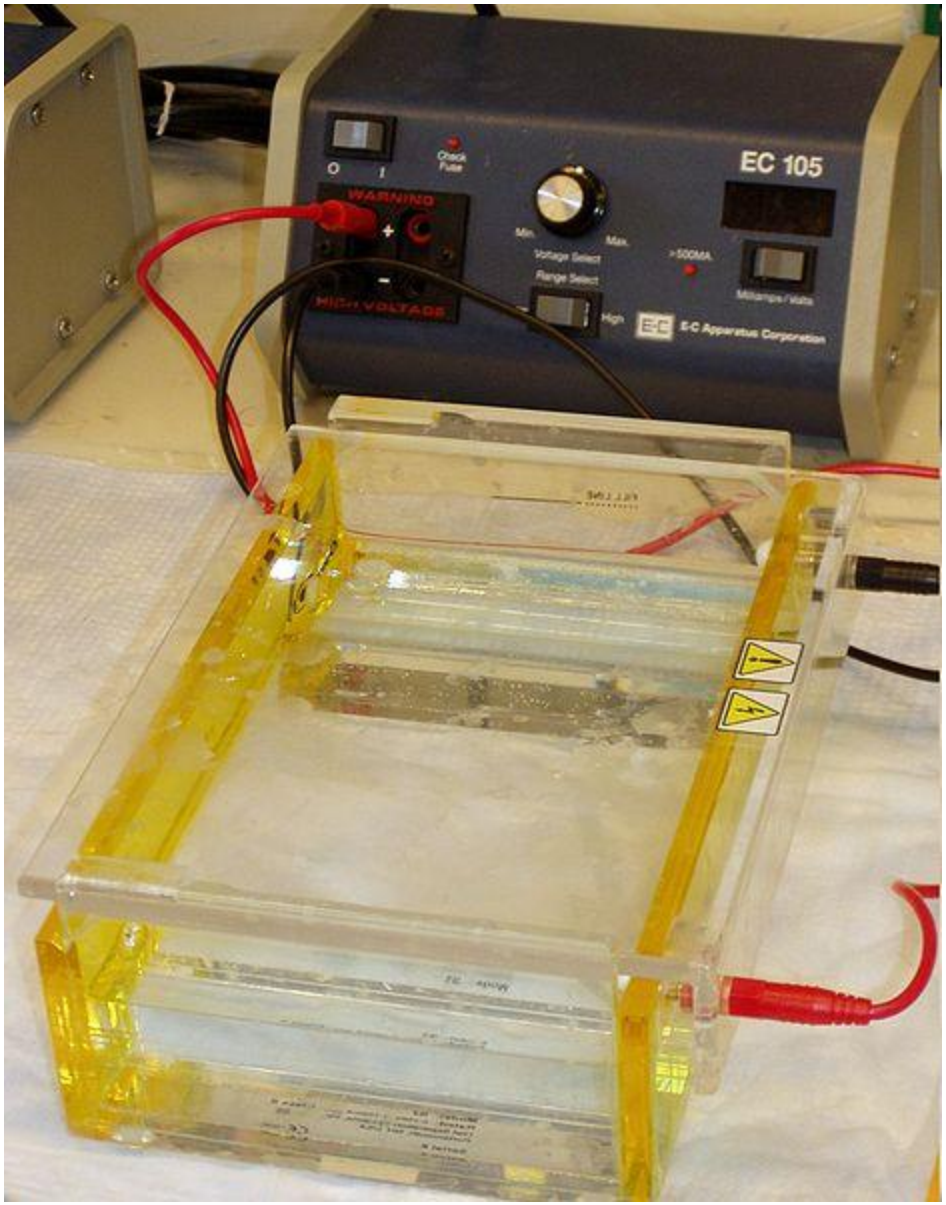
a procedure for separating a mixture of molecules through a stationary material (gel) in an electrical field.

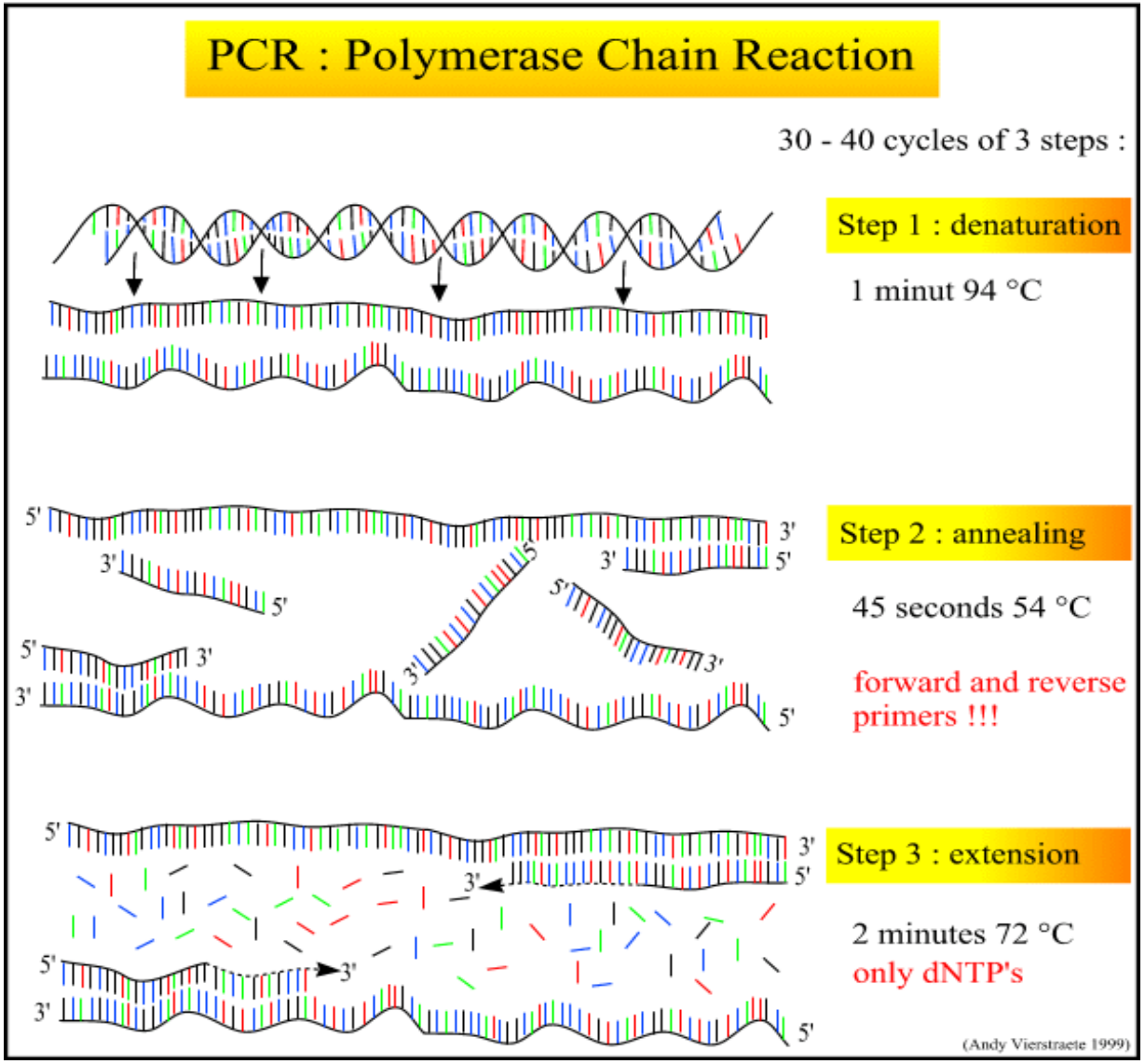


- **Organic molecules such as DNA are charged. DNA is negatively charged because the phosphates (red circles) that form the sugar-phosphate backbone of a DNA molecule have a negative charge.**
- **A gel is prepared which will act as a support for separation of the fragments of DNA. The gel is a jello-like material, usually agarose, a substance derived from seaweed.**
- **Holes are created in the gel. These will serve as a reservoir to hold the DNA solution.**
- **Large molecules have difficulty getting through the holes in the matrix. Small molecules move easily through the holes**
- **Because of this, the distance moved is inversely related to the weight, and therefore the length of DNAs.**
- **Molecular weight markers, usually a mixture of DNAs with known molecular weights, are often electrophoresed along with DNAs to estimate the sizes of DNA fragments in the sample.**

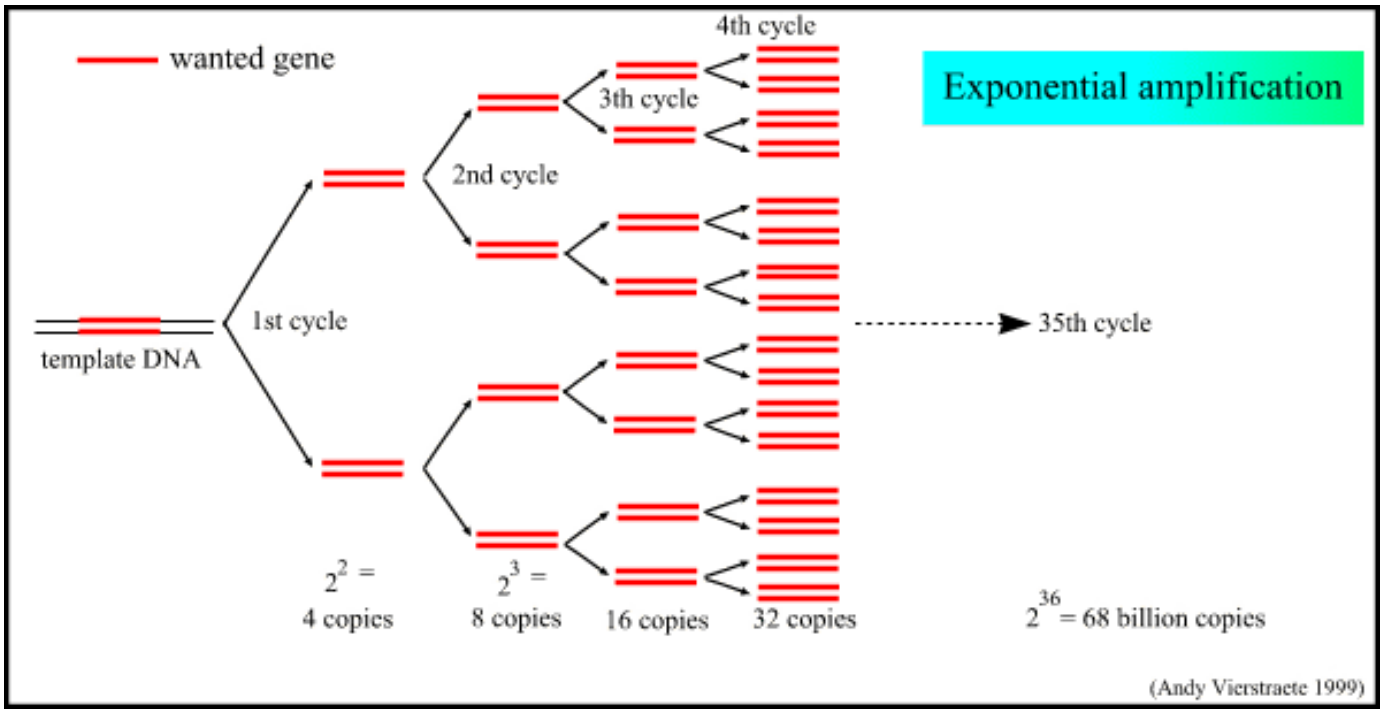


Gel electrophoresis apparatus - An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the camera.





The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template)

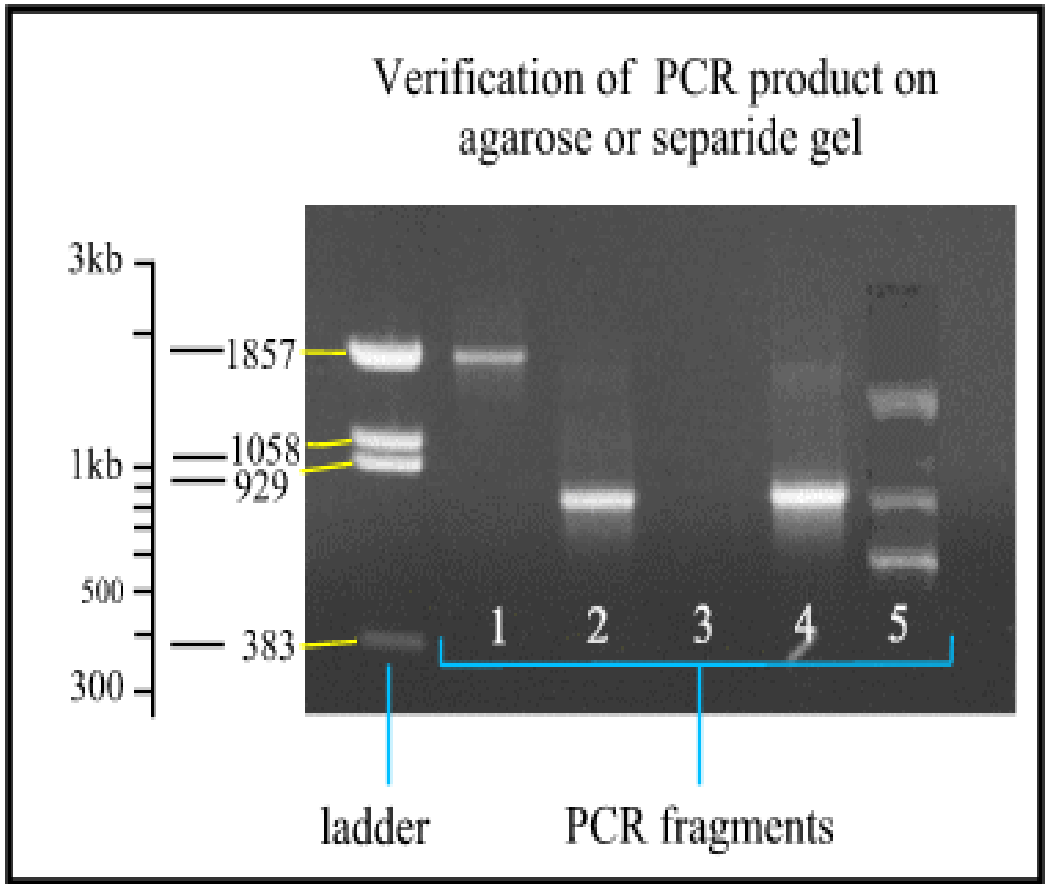


• No need for restriction enzymes, vectors, and host cells.

• Only need: two primers, sufficient amounts of the four deoxylribonulceoside triphosphates, and heat-tolerant DNA polymerase.

•Primer design is computationally aided.

- Is it the right size?
- Is there a product?
- Is it a mixture?



How PCR is used in forensic science

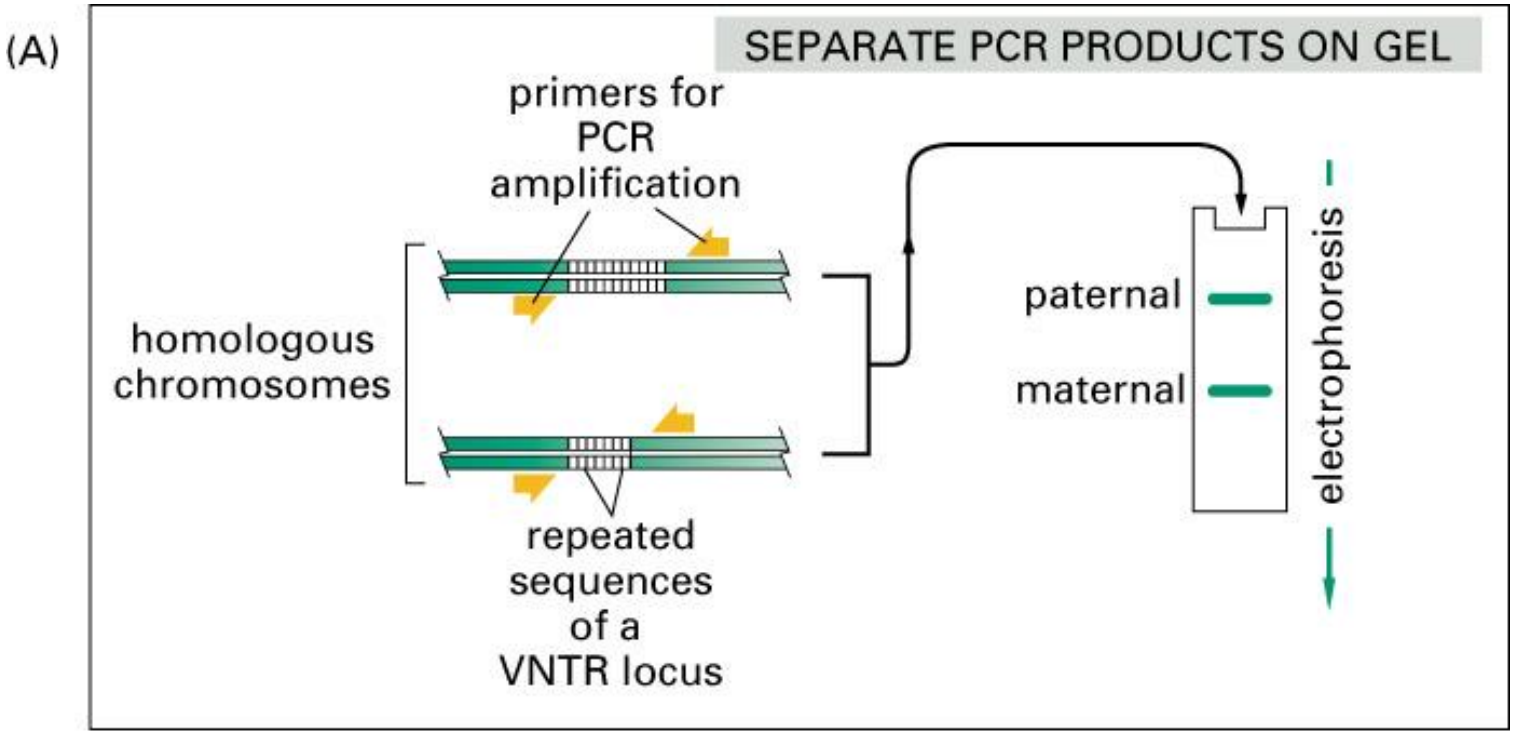
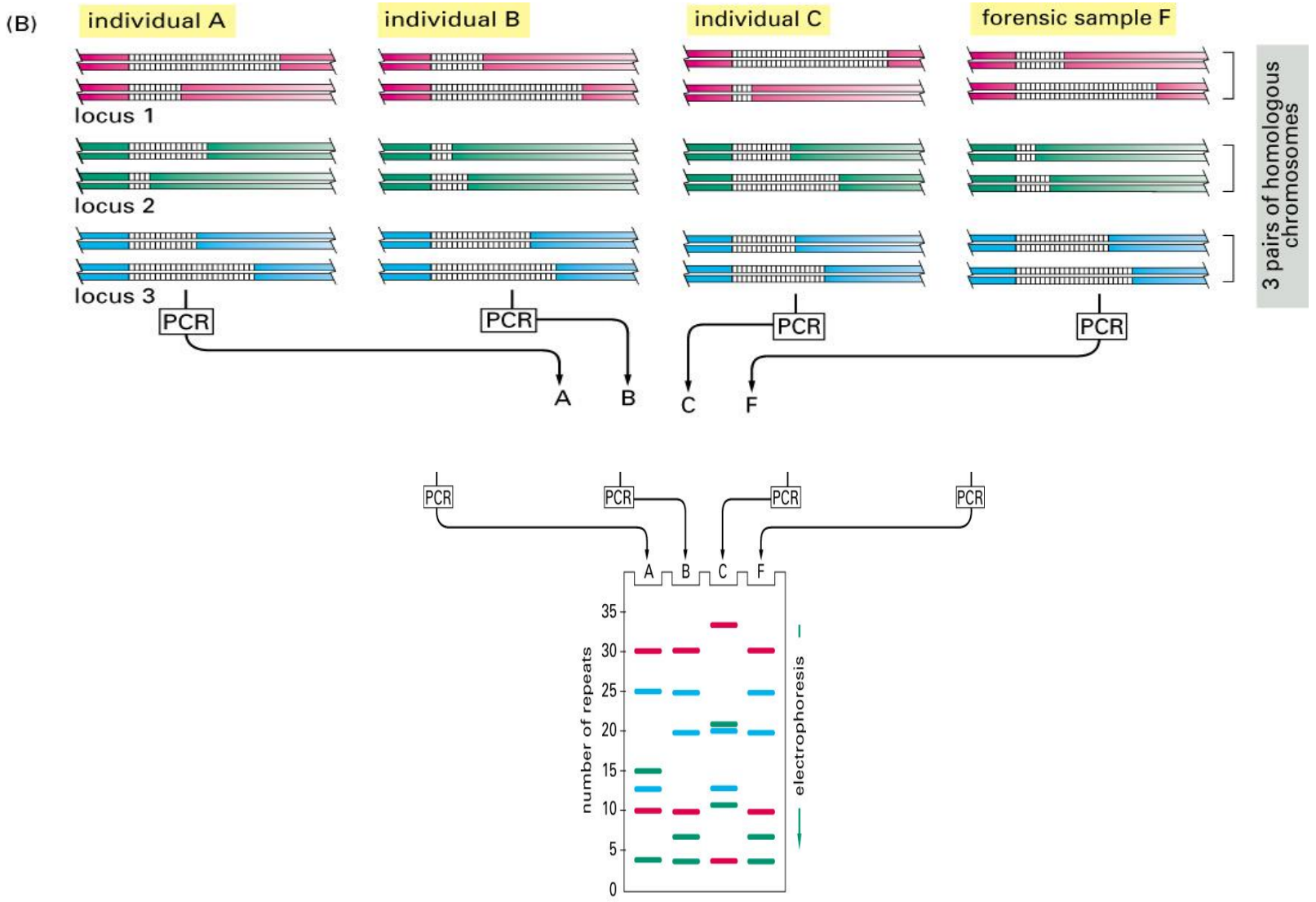


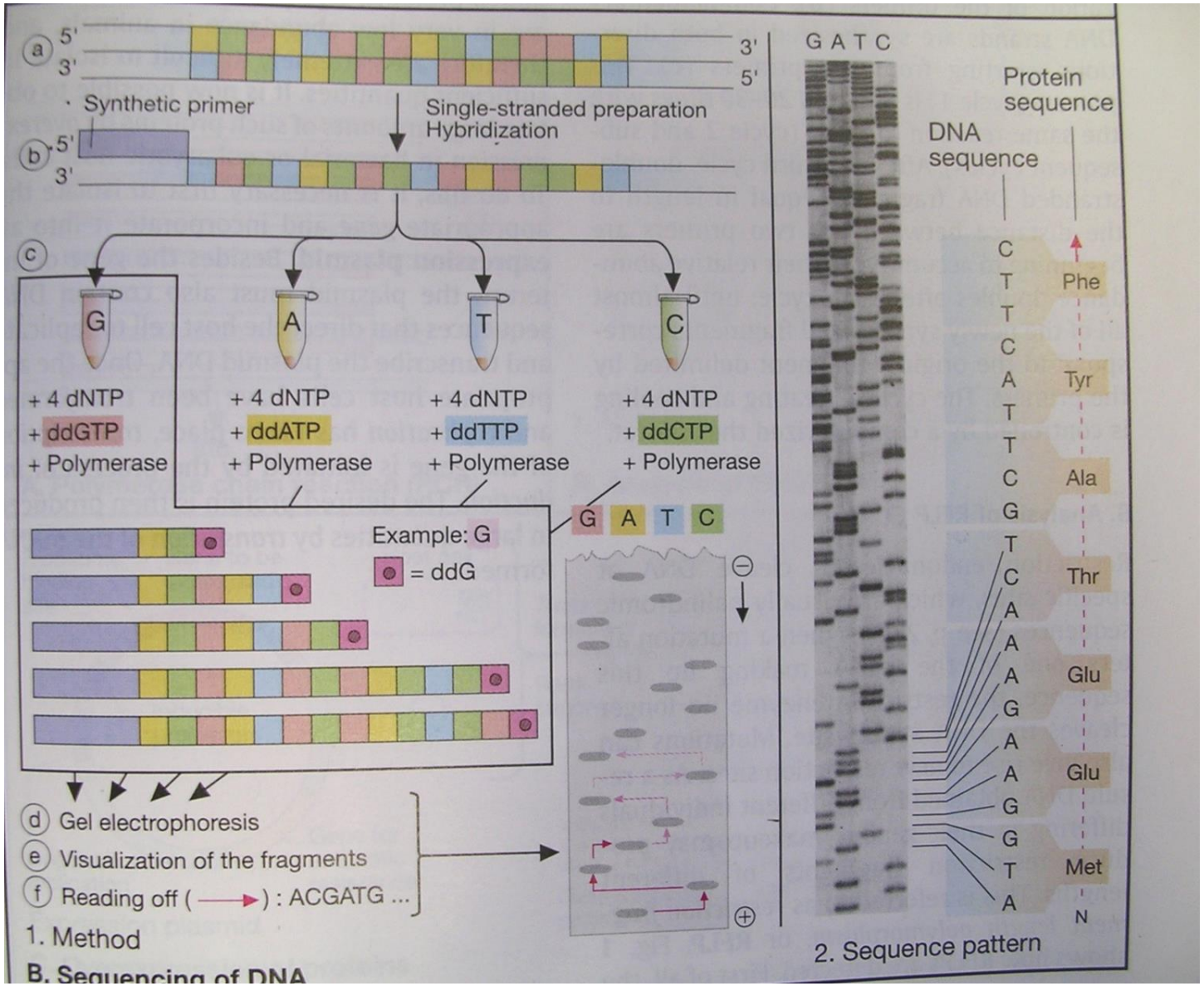
Figure 8–41 part 1 of 4. Molecular Biology of the Cell, 4th Edition.


(B)
individual A
individual B
individual C
forensic sample F
locus 1
locus 2
locus 3
3 pairs of homologous chromosomes
PCR
PCR
PCR
PCR
A
B
C
F
PCR
PCR
PCR
PCR
A
B
C
F
35
30
25
20
15
10
5
0
number of repeats
electrophoresis

Evolution of Sequencing Technology

- ABI Platform
- 1988
  - 16 samples per/day ~250 bp/sample
- 1992
  - 48 samples 3 times/day ~450 bp/sample
- 1996-8
  - 96 samples 4 times/day ~600 bp/sample
- 2006
  - 96 samples 4 times/day ~700-800 bp/sample
- 454
  - 300,000 – 400,000 samples 2 times per day 100 bp/sample
- Solexa
  - 2,000,000 – 3,000,000 samples once every 3 days 35-50 bp/sample
  - 40,000,000 samples once every 3 days 50 bp/sample

Sequencing DNA (Sanger's method)



Courtesy of Color Atlas of Biochemistry

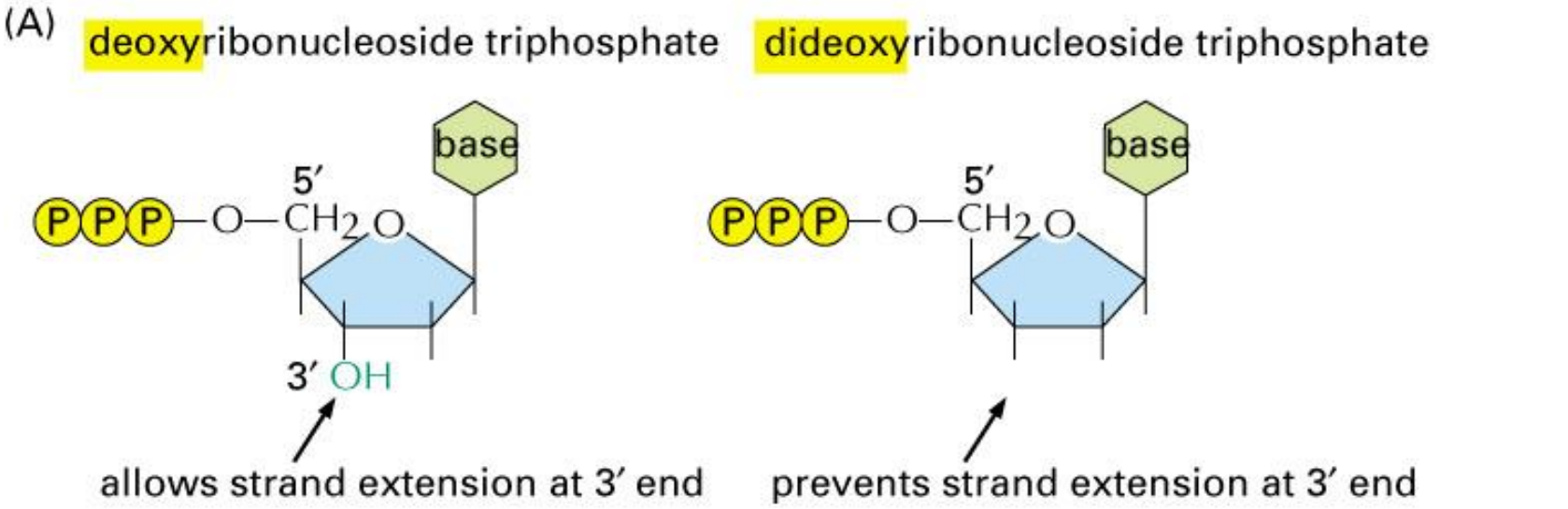
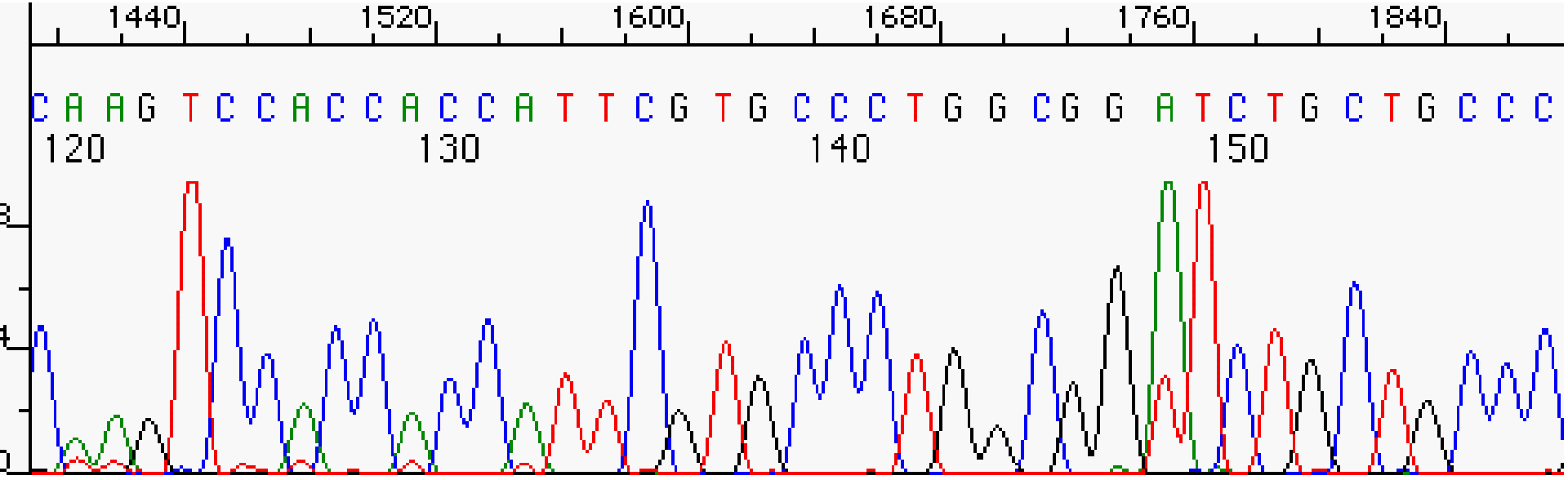
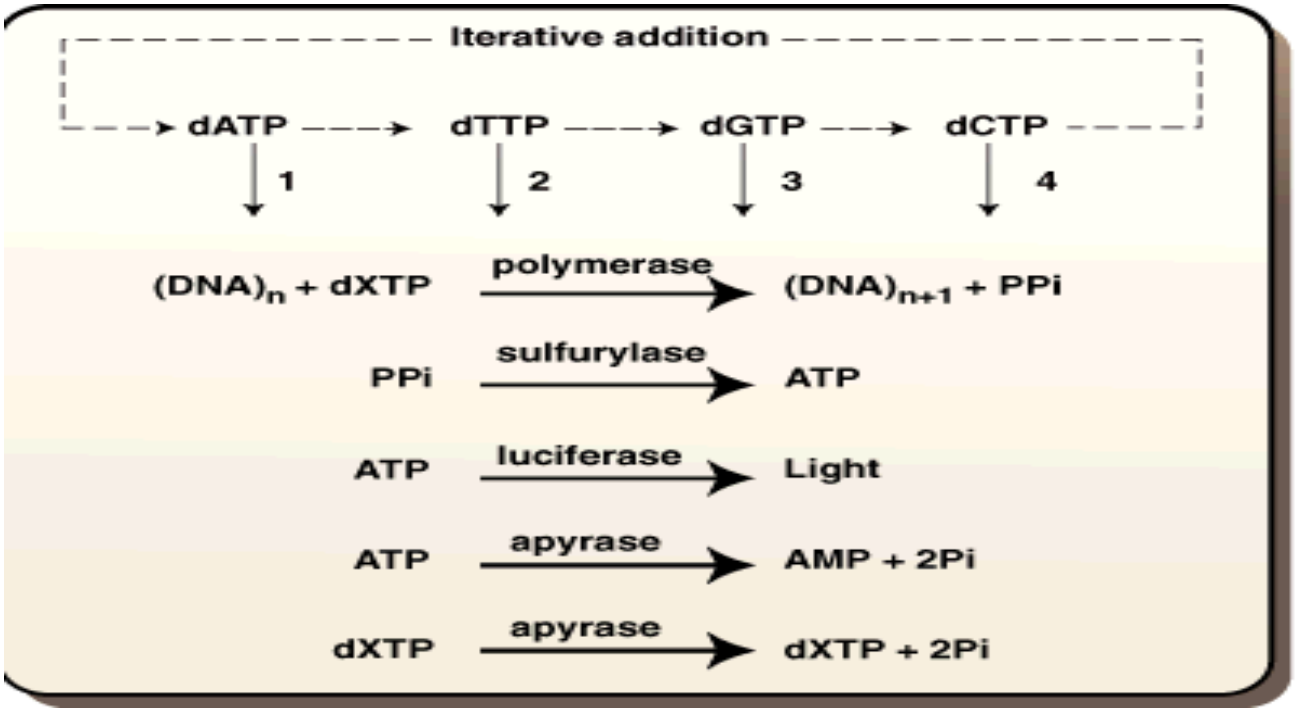


Figure 8–36 part 1 of 4. Molecular Biology of the Cell, 4th Edition.

Chromatograph of Sequence data



Sequencing by synthesis

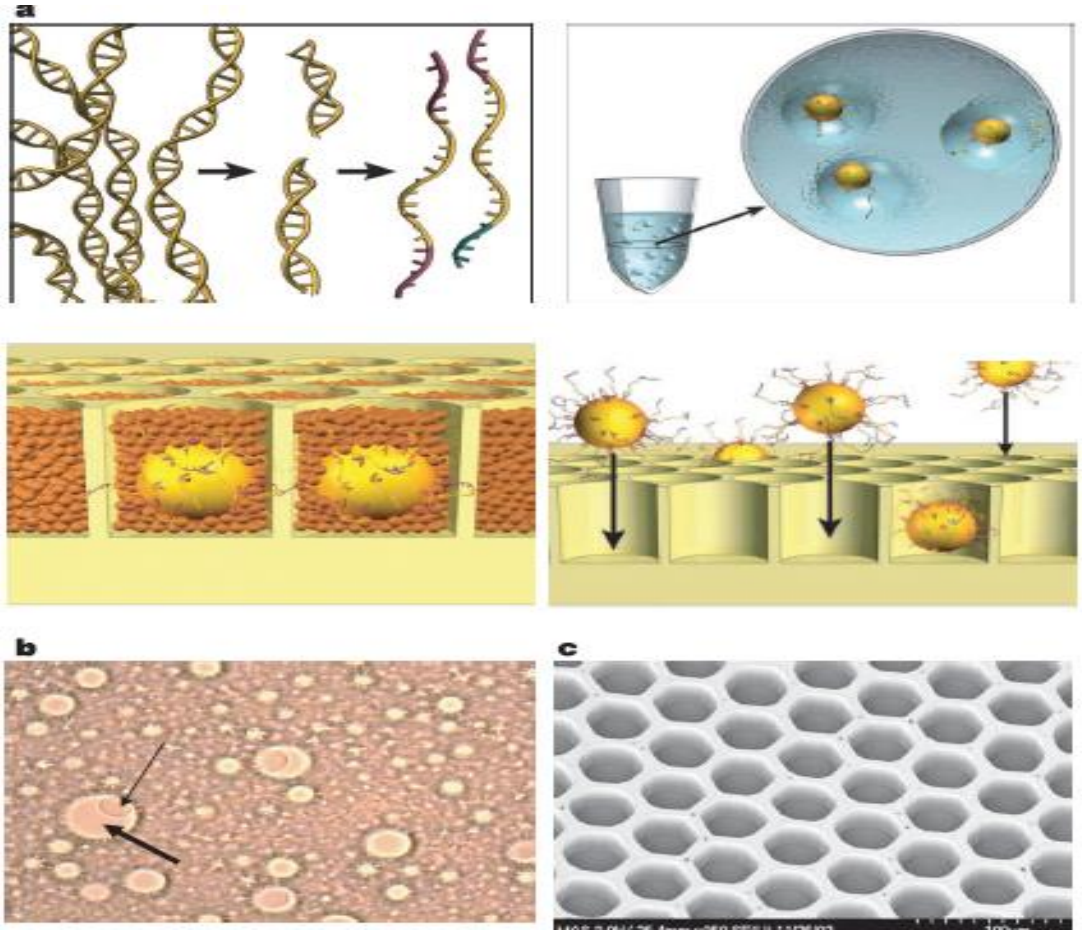


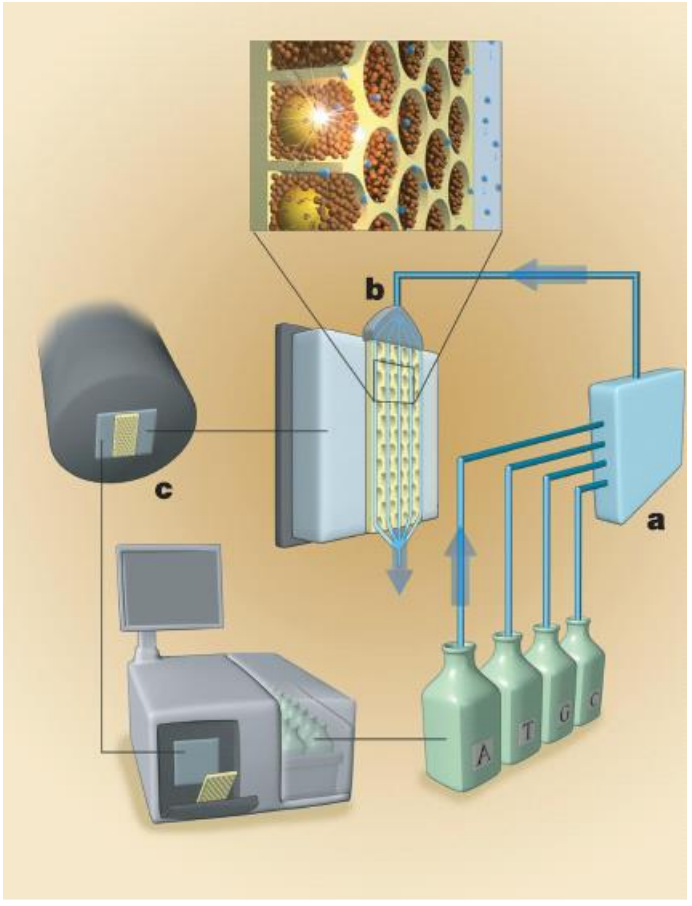
• Four nucleotides are added stepwise to the template hybridized to a primer.

• Incorporation of a deoxynucleotide, determined by complementing with the template, will release PPi and light, which can be detected by a CCD (charge-coupled device) camera

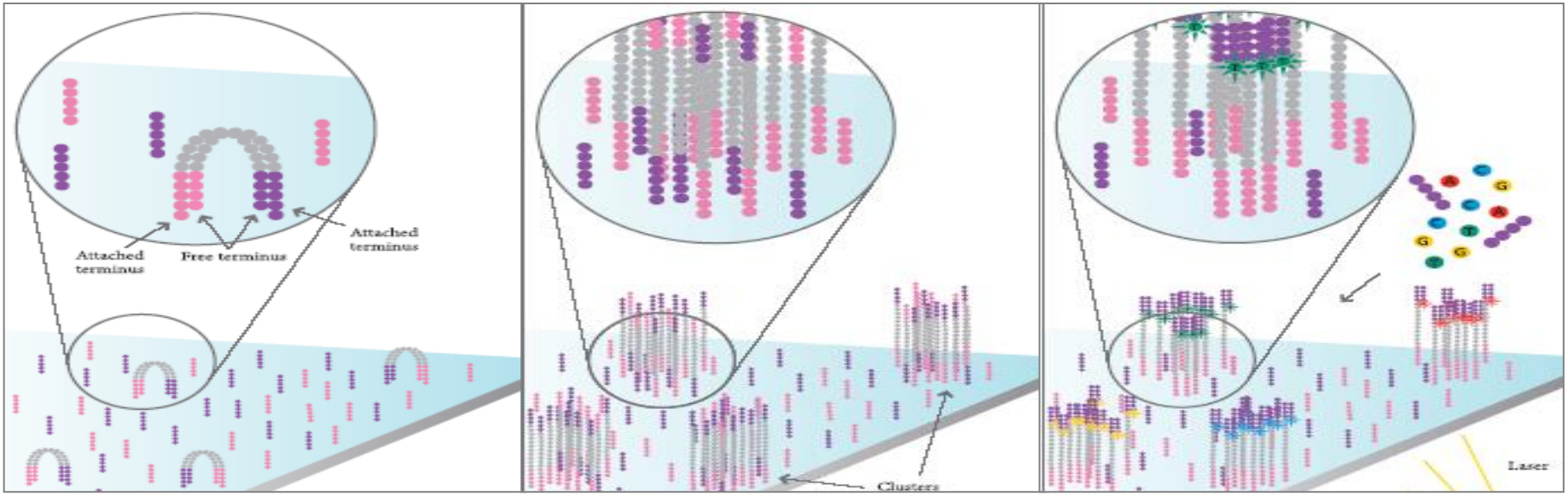
• Unincorporated deoxynucleotides and the produced ATP are degraded between each cycle by the nucleotide-degrading enzyme.

454 Sequencing





SBS Sequencing



SBS Sequencing**.** cDNA or genomic template (nebulized DNA) is prepared by the addition of 5' and 3' adapters. This is diluted and applied to the surface of a chip pre-coated with a dense lawn of primers **(A)**; "bridge-PCR" with unlabelled nucleotides is used to create a PCR colony covalently linked to the surface. This creates millions of dense clusters of dsDNA template **(B)**. Sequencing is performed by addition of four dye-labeled *reversible* nucleotide terminators along with the same primers and a custom polymerase. Laser excitation and image capture is used to determine the first nucleotide for all clusters in parallel **(C)**. The 3' blocked terminus is removed along with the fluorophore (dye), and the second bases are determined the same way. The process continues for 25-35 nucleotides. (Figure Modified from www.solexa.com).

The New York Times
nytimes.com

July 18, 2006

The Quest for the $1,000 Human Genome

By NICHOLAS WADE

As part of an intensive effort to develop a new generation of machines that will sequence DNA at a vastly reduced cost, scientists are decoding a new human genome — that of James D. Watson, the co-discoverer of the structure of DNA and the first director of the National Institutes of Health's human genome project.

The New York Times
nytimes.com

October 5, 2006

$10 Million Prize Set Up for Speedy DNA Decoding

By NICHOLAS WADE

A $10 million prize for cheap and rapid sequencing of the human genome was announced today by the X Prize Foundation of Santa Monica, Calif.