Biomolecules II (nucleic acids)

- DNA structure
- DNA replication
- PCR
- RNA
- DNA separation

References:

- Bruce Alberts et al., Molecular Biology of the Cell (5th ed., 2007, Chs. 5-6)
- JC Giddings, *"Unified Separation Science"* (Wiley-Interscience, New York, 1991)
- *Electrophoresis* (Journal)

Chia-Fu Chou@2010

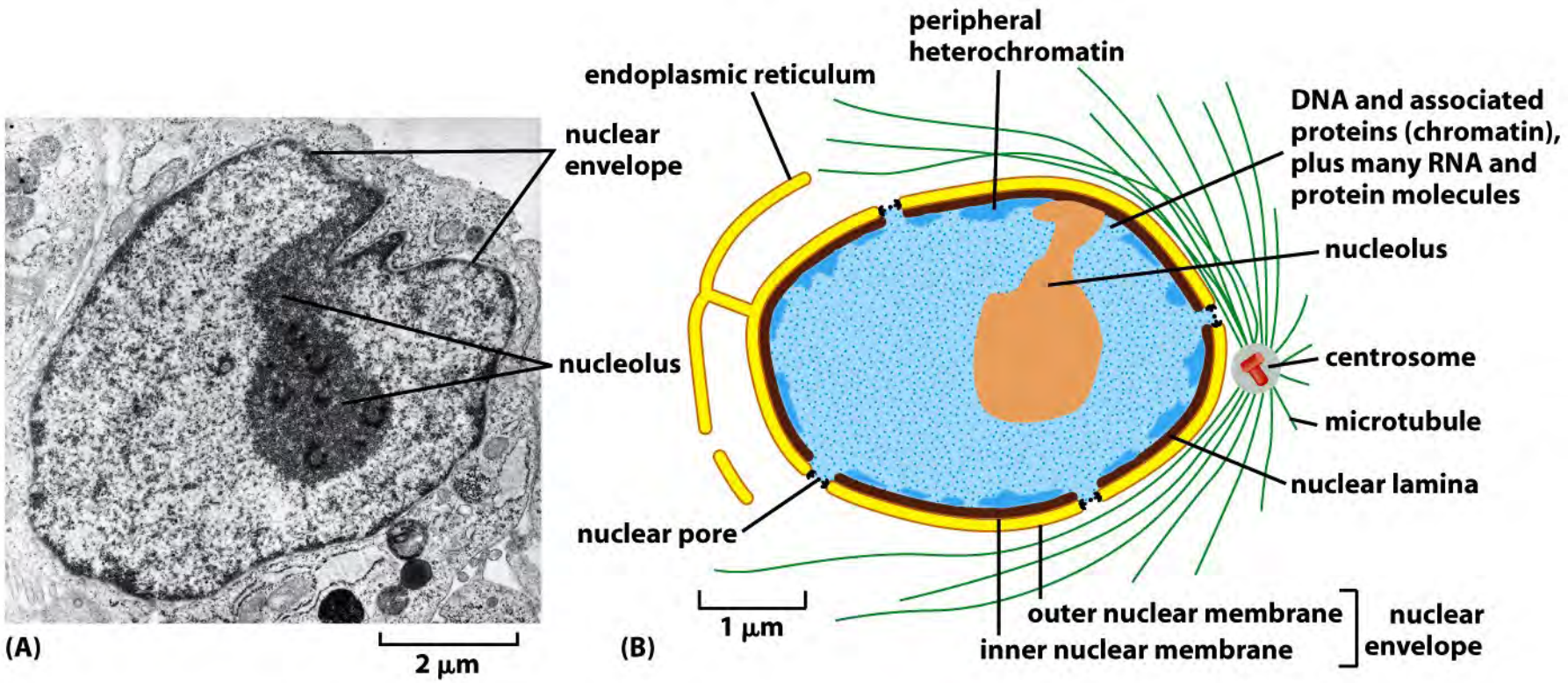


Figure 4-9 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

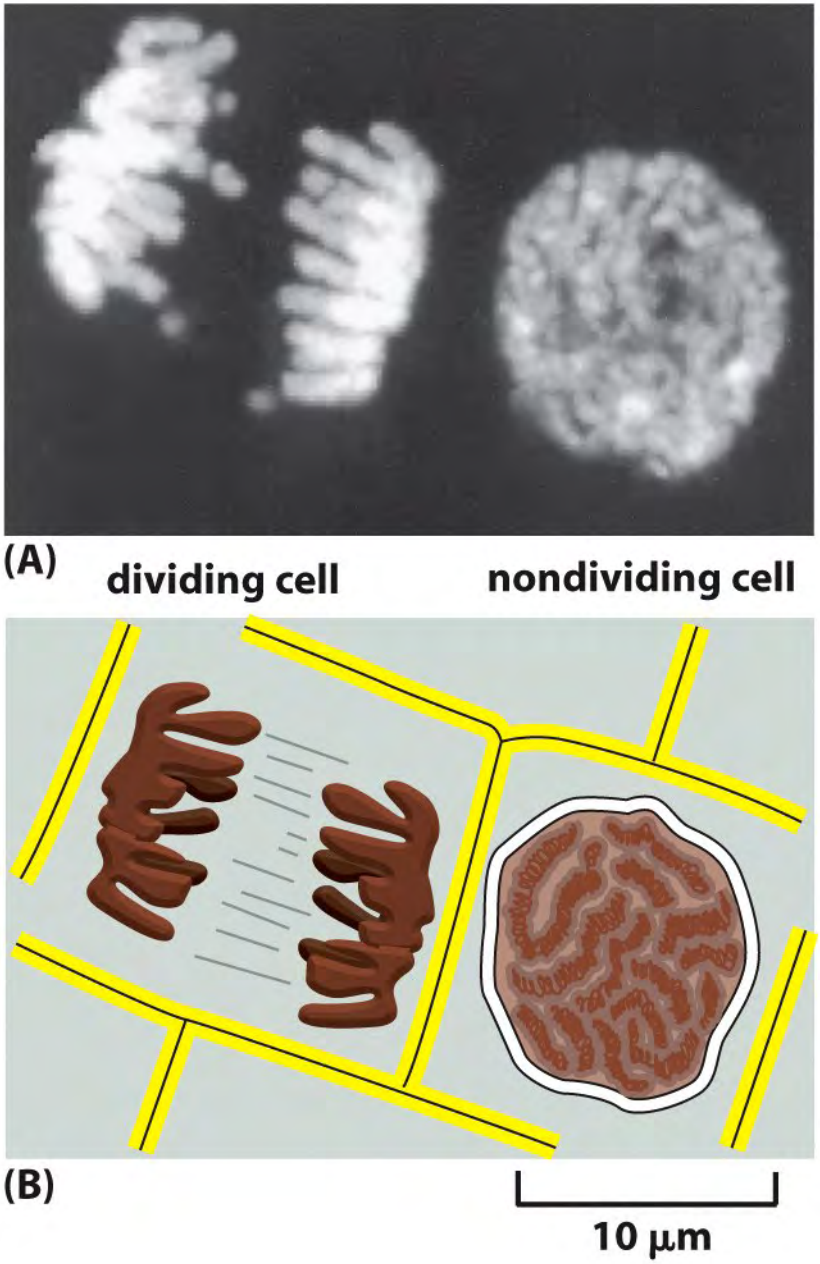


Figure 4-1 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

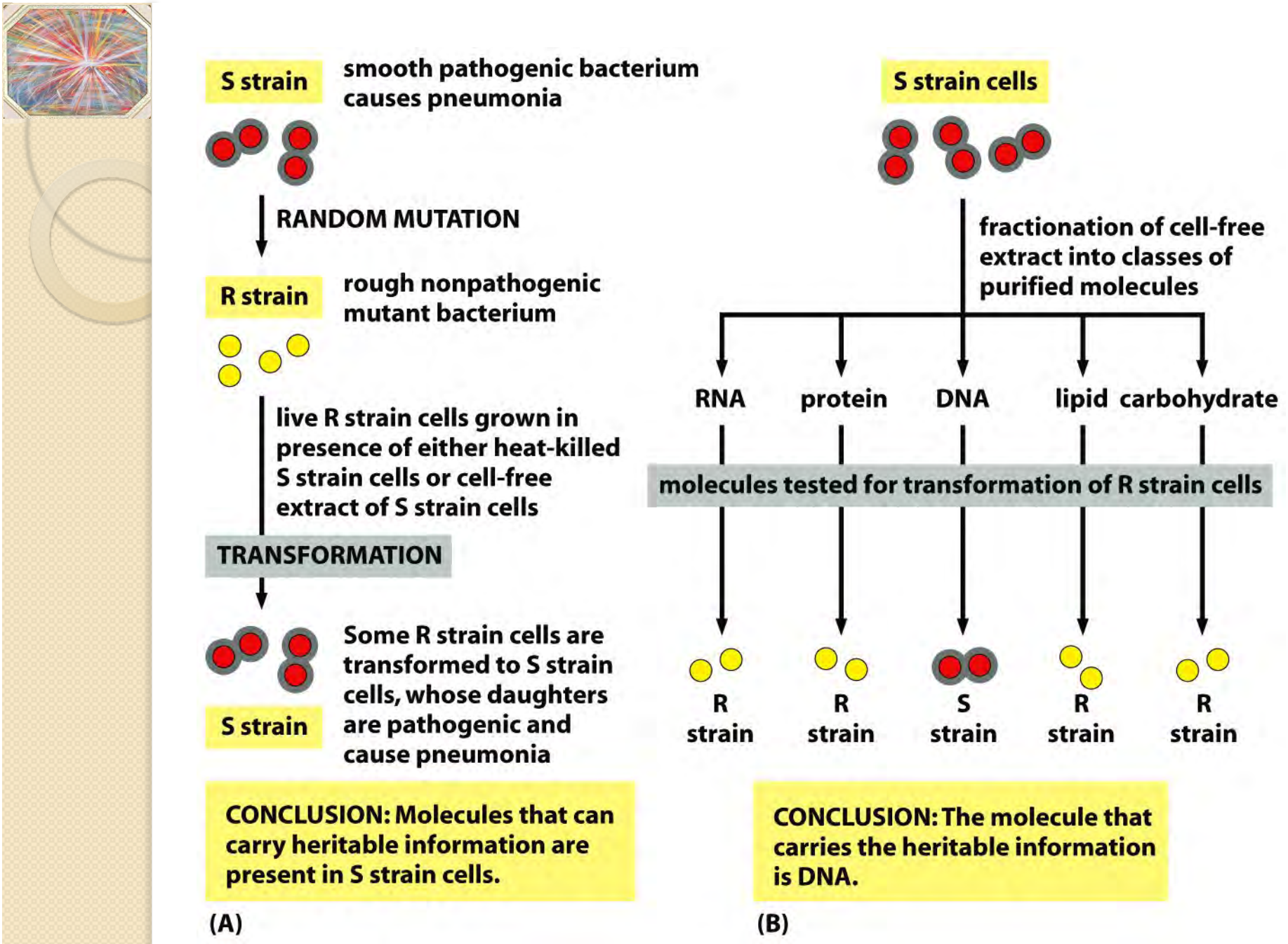


Figure 4-2 *Molecular Biology of the Cell* (© Garland Science 2008)

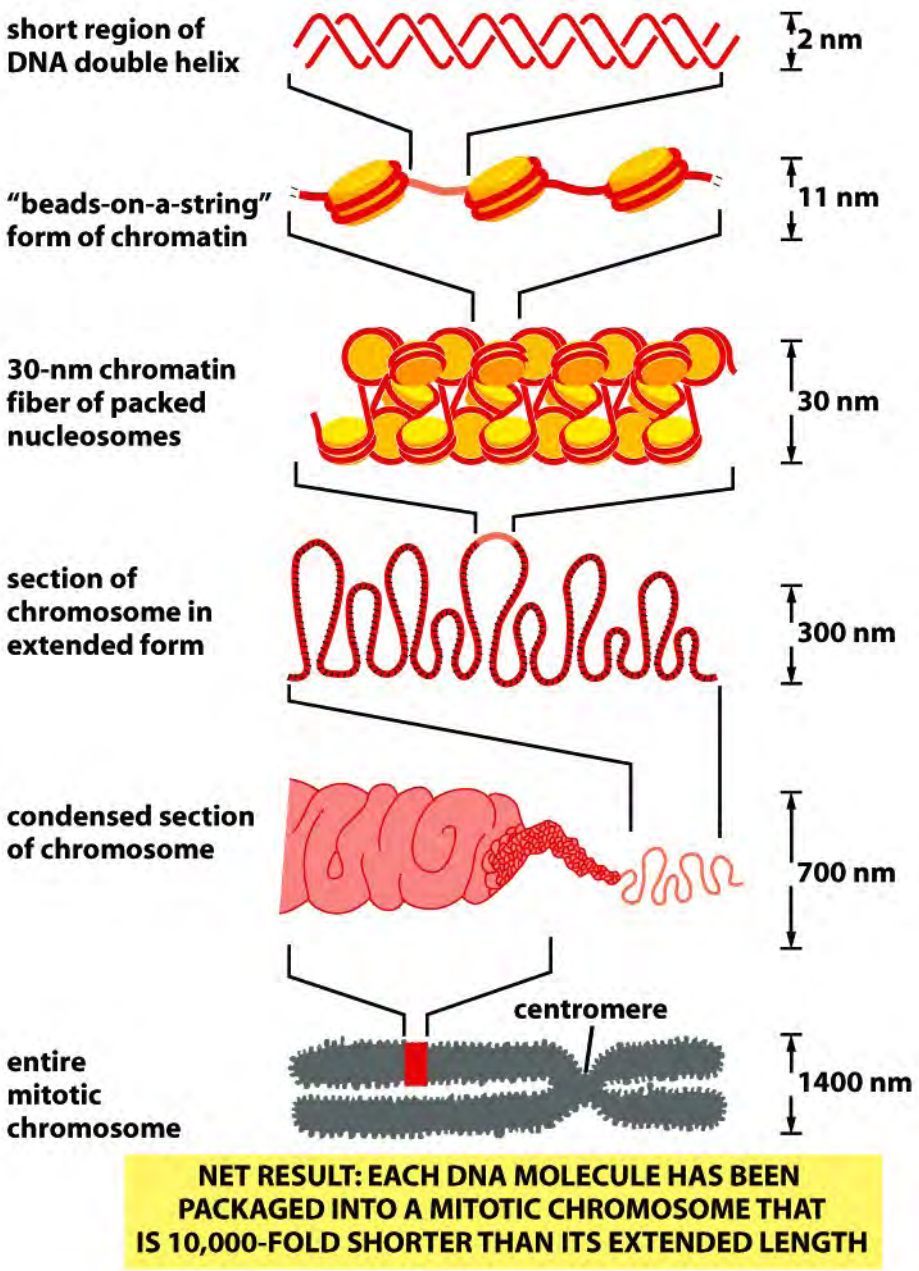


Figure 4-72 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

中央研究院講座

ACADEMIA SINICA LECTURE

Dr. James D. Watson

Nobel Laureate in Physiology or Medicine (1962)
1962 諾貝爾生理學或醫學獎得主

Chancellor Emeritus
Cold Spring Harbor Laboratory, USA

4/2 2010 Friday 10:00-11:30

From Discovery of Double Helix Structure of DNA to Developing a Research Career

Moderated by Dr. Ming-Ta Hsu
Director of the National Yang-Ming University
VYM Genome Research Center
國立陽明大學榮陽基因體研究中心徐明達主任主持

Convention Center
National Yang-Ming University
國立陽明大學活動中心

4/2 2010 Friday 15:30-17:00

Management of Science as an Enterprise

Moderated by Dr. Chi-Huey Wong
President of Academia Sinica
中央研究院翁啓惠院長主持

International Conference Hall (3F)
Building for Humanities and Social Sciences
Academia Sinica
中央研究院人文社會科學館國際會議廳

主辦單位：中央研究院
贊助單位：財團法人傑出人才發展基金會
財團法人溫世仁文教基金會
合作單位：國立陽明大學 (02) 2826-7373
連絡單位：中研院國際事務辦公室 (02) 2789-9895
網　　址：http://iao.sinica.edu.tw/ASL
線上報名：http://iao.sinica.edu.tw/ASL

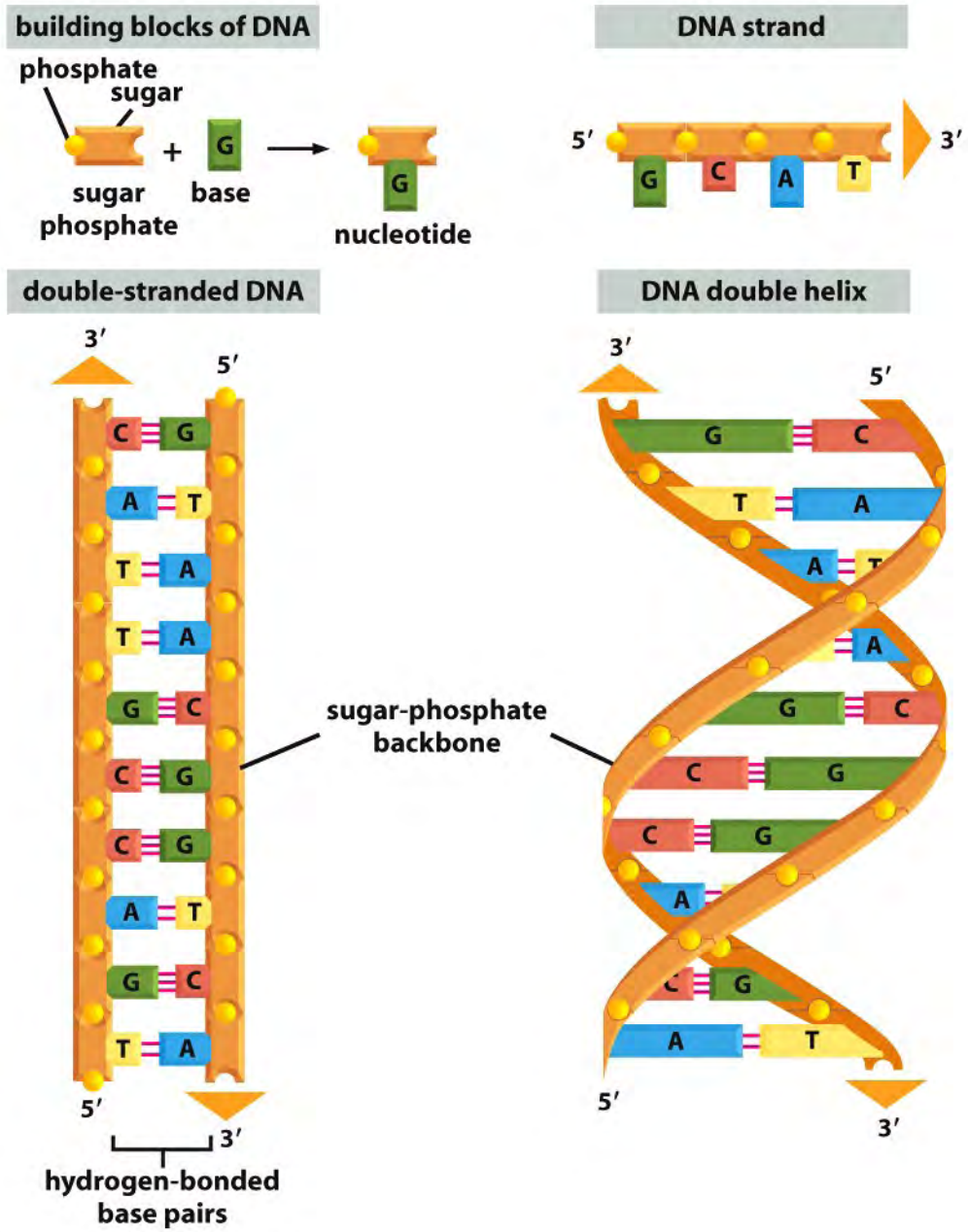


Figure 4-3 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

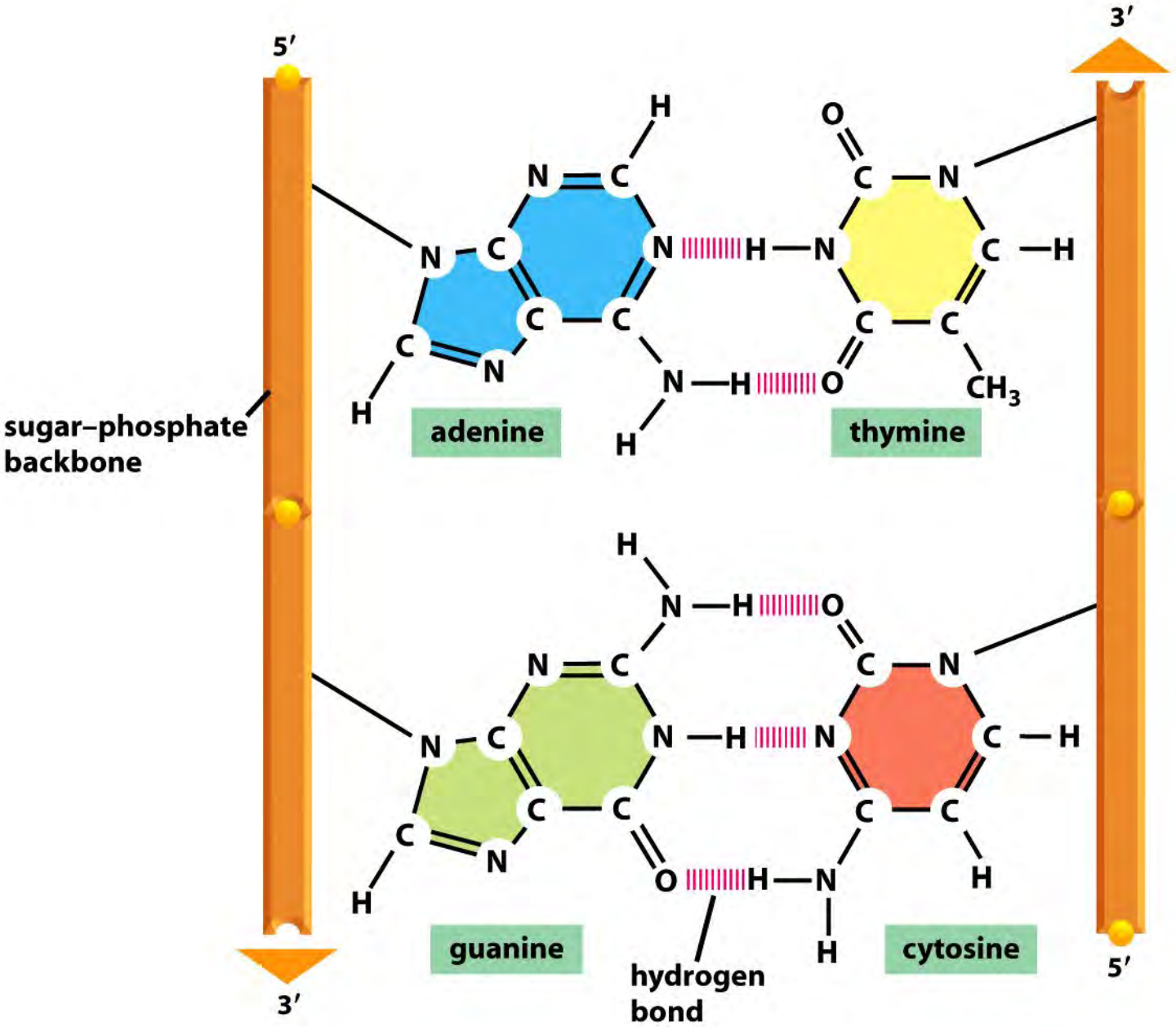


Figure 4-4 *Molecular Biology of the Cell* (© Garland Science 2008)

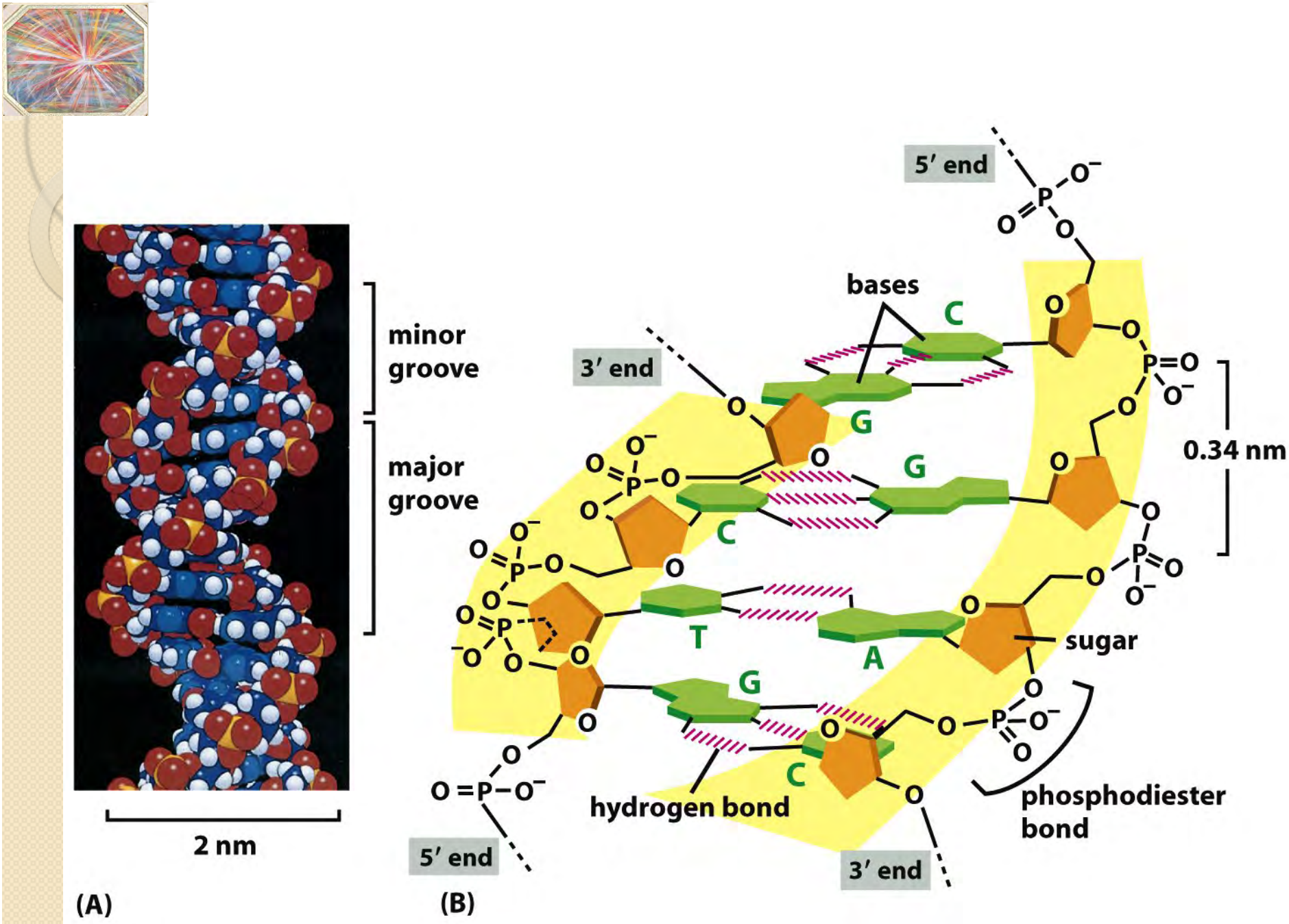
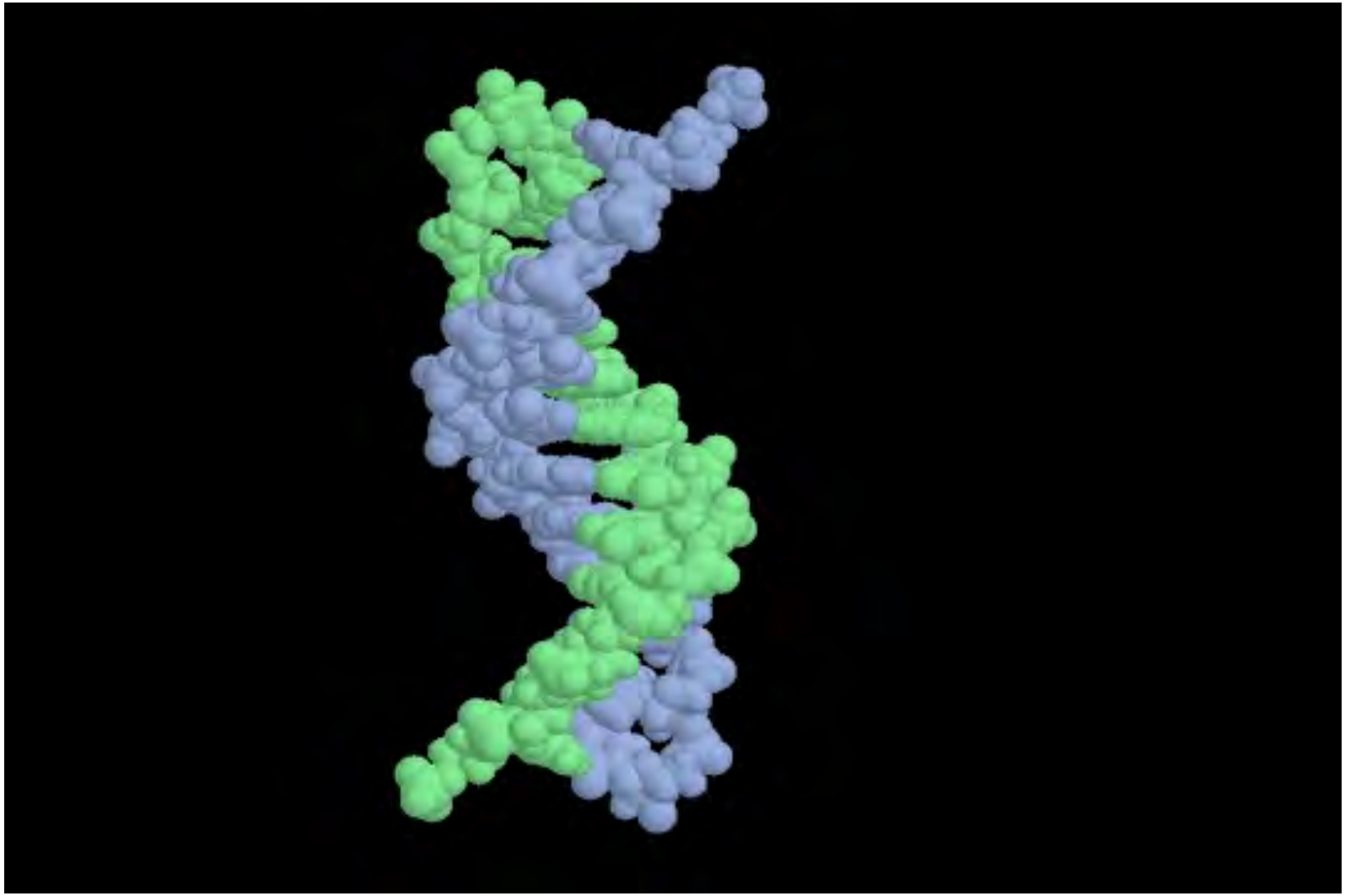


Figure 4-5 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA structure



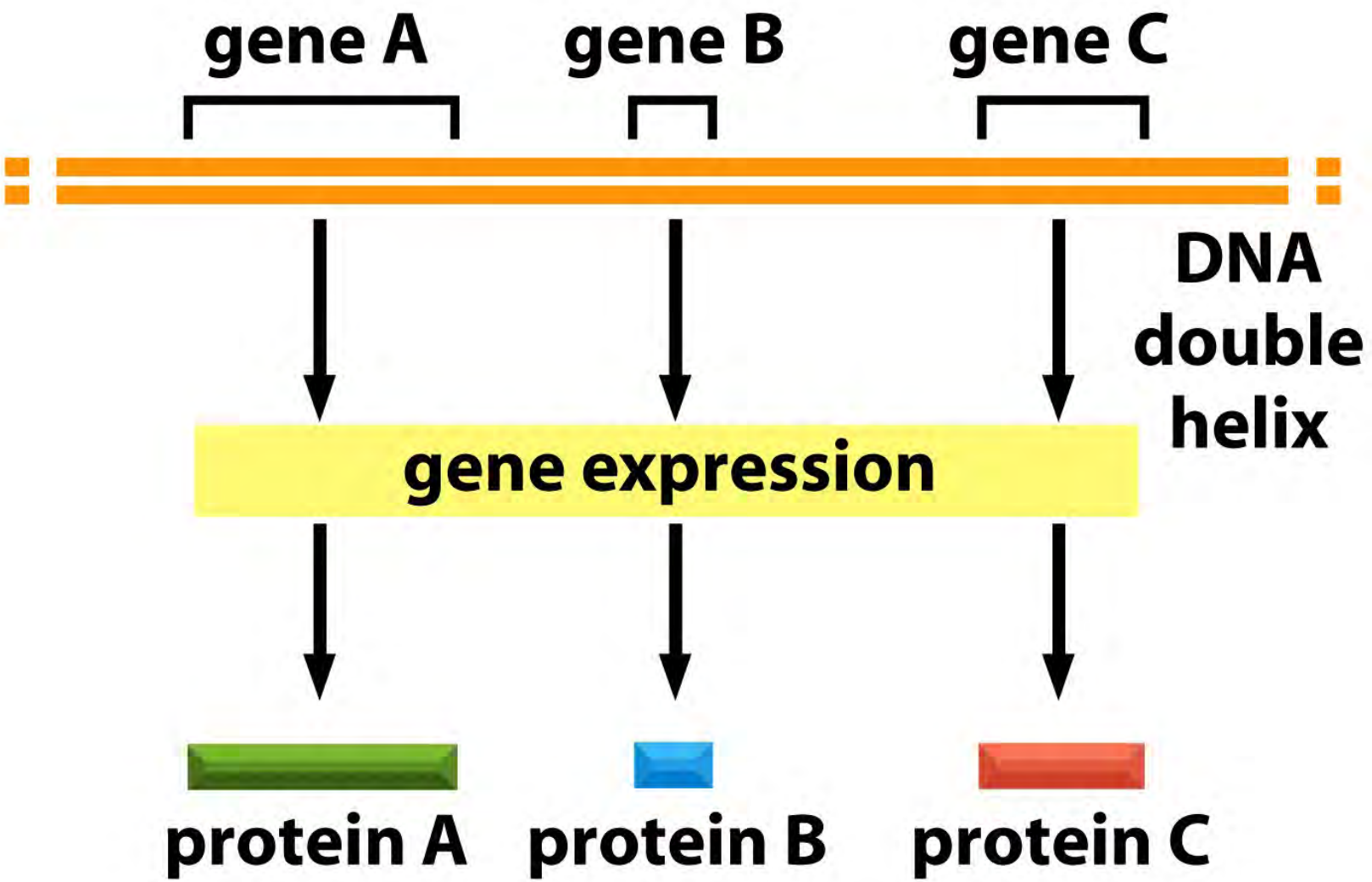


Figure 4-6 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

DNA replication

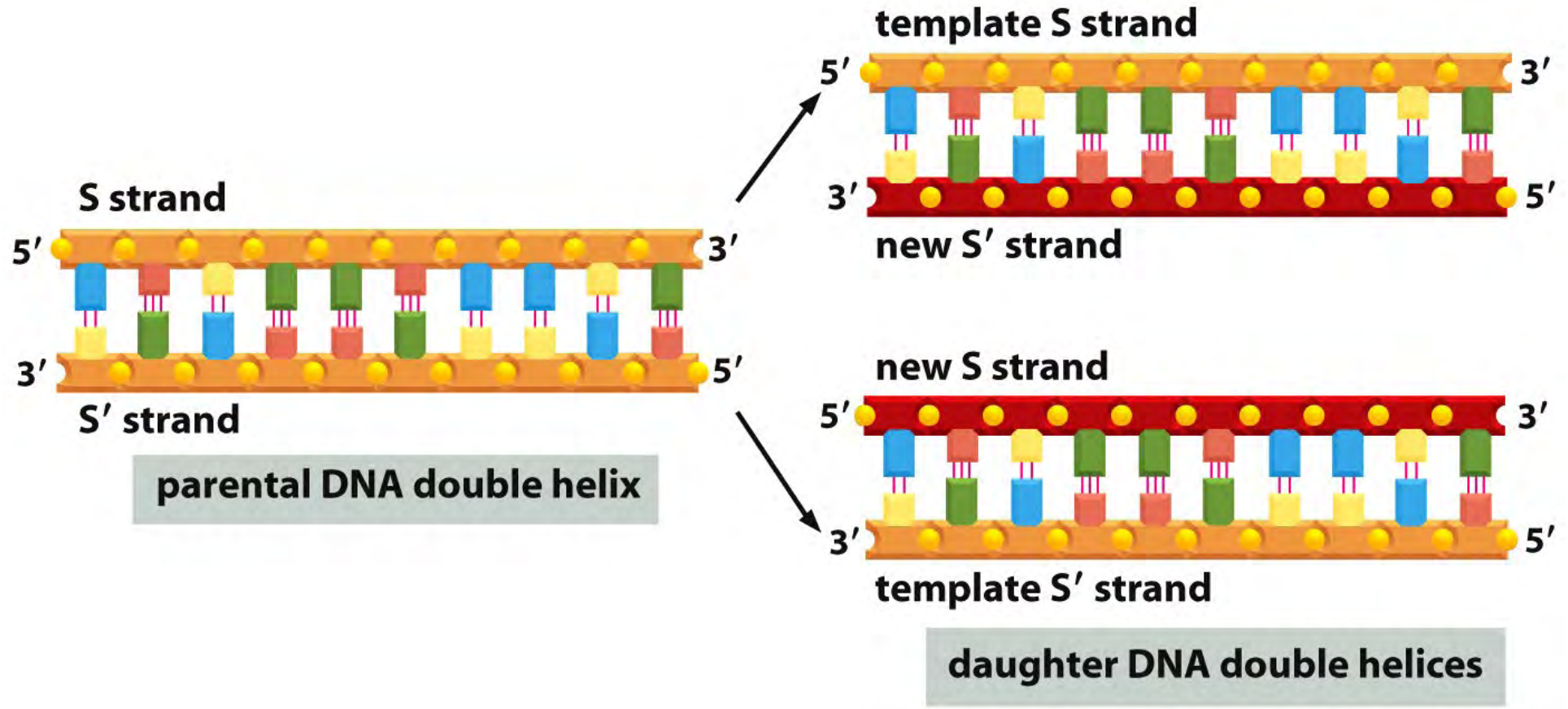
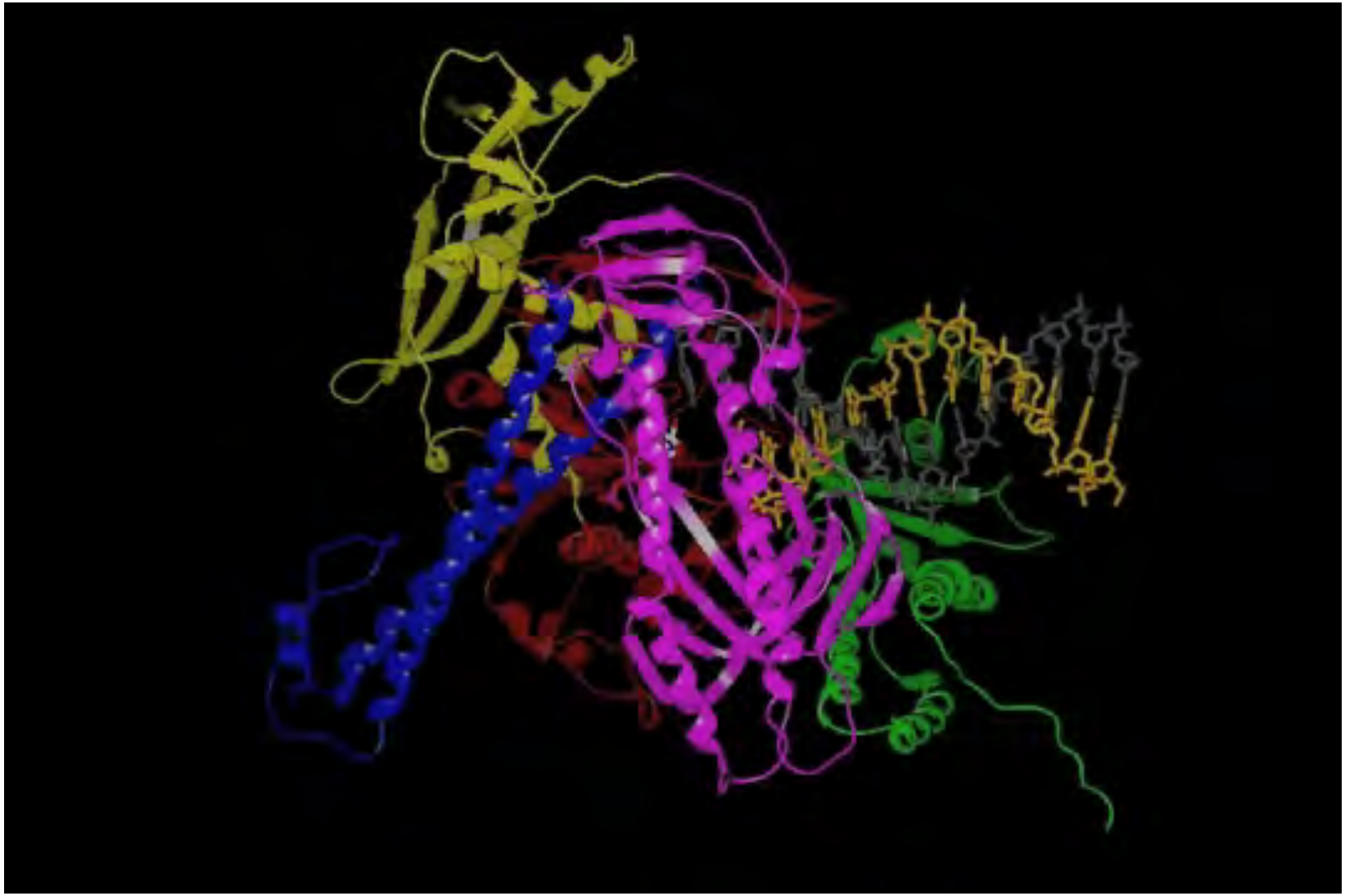
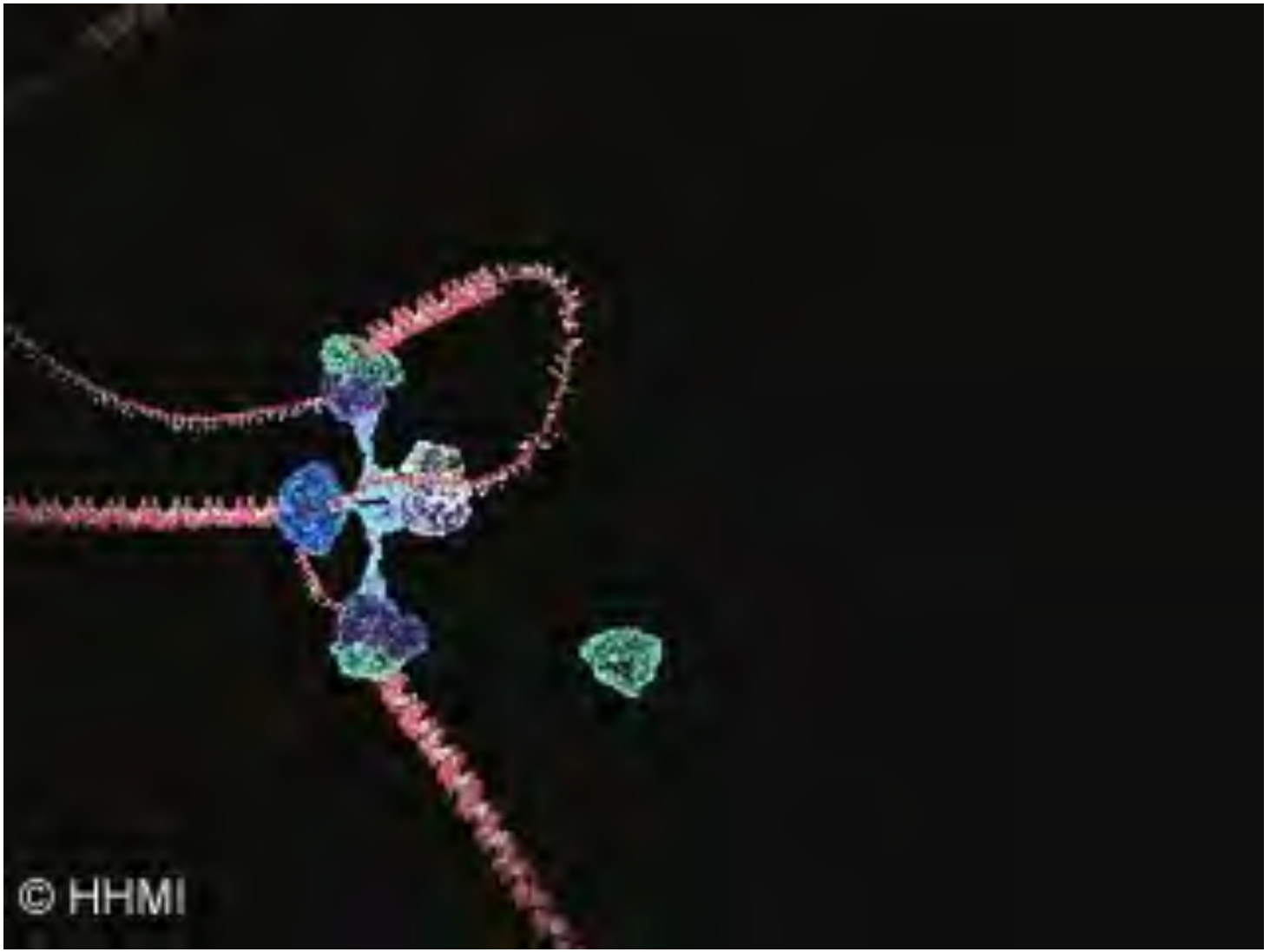


Figure 4-8 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA polymerase



DNA replication



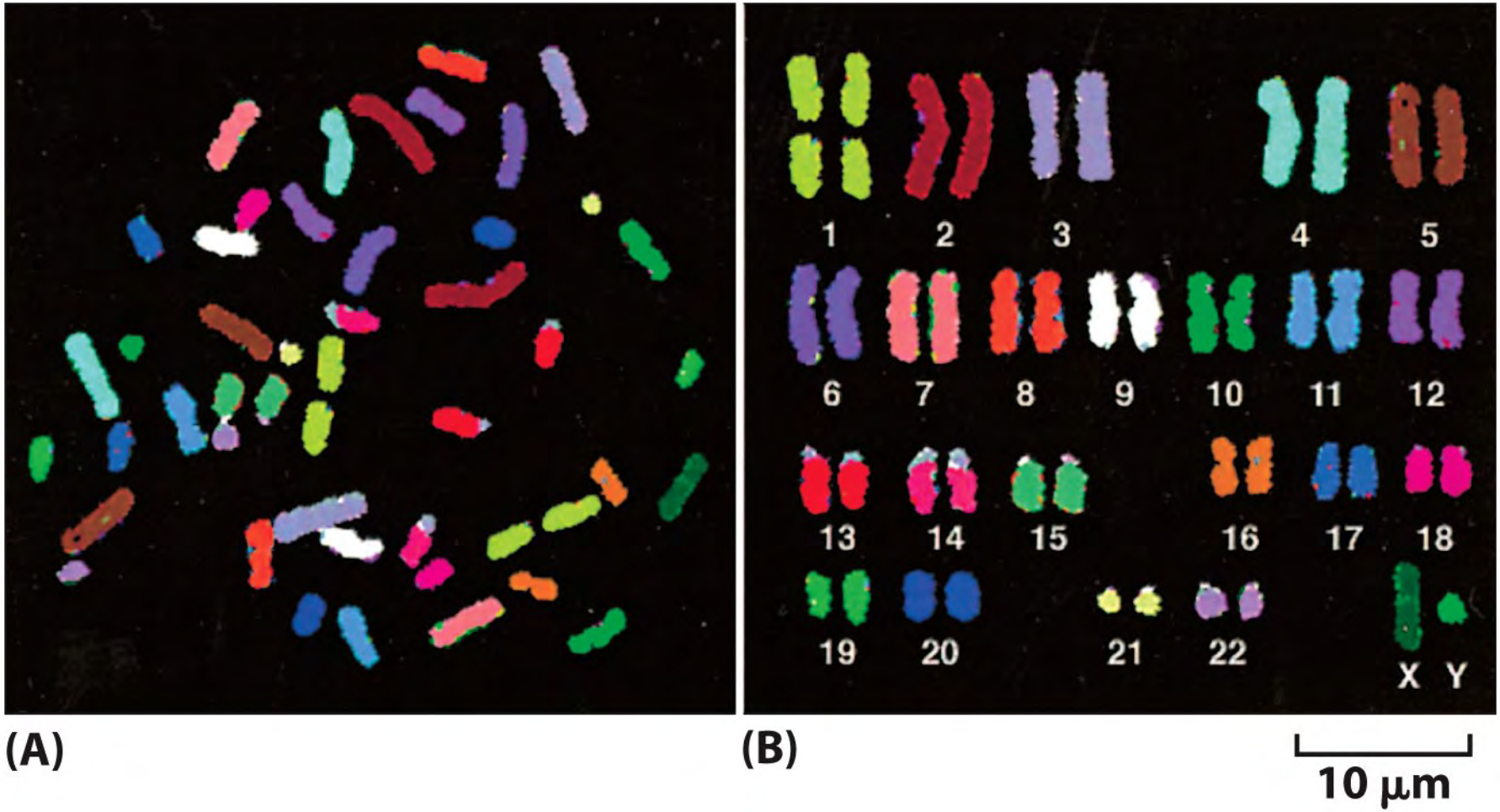


Figure 4-10 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

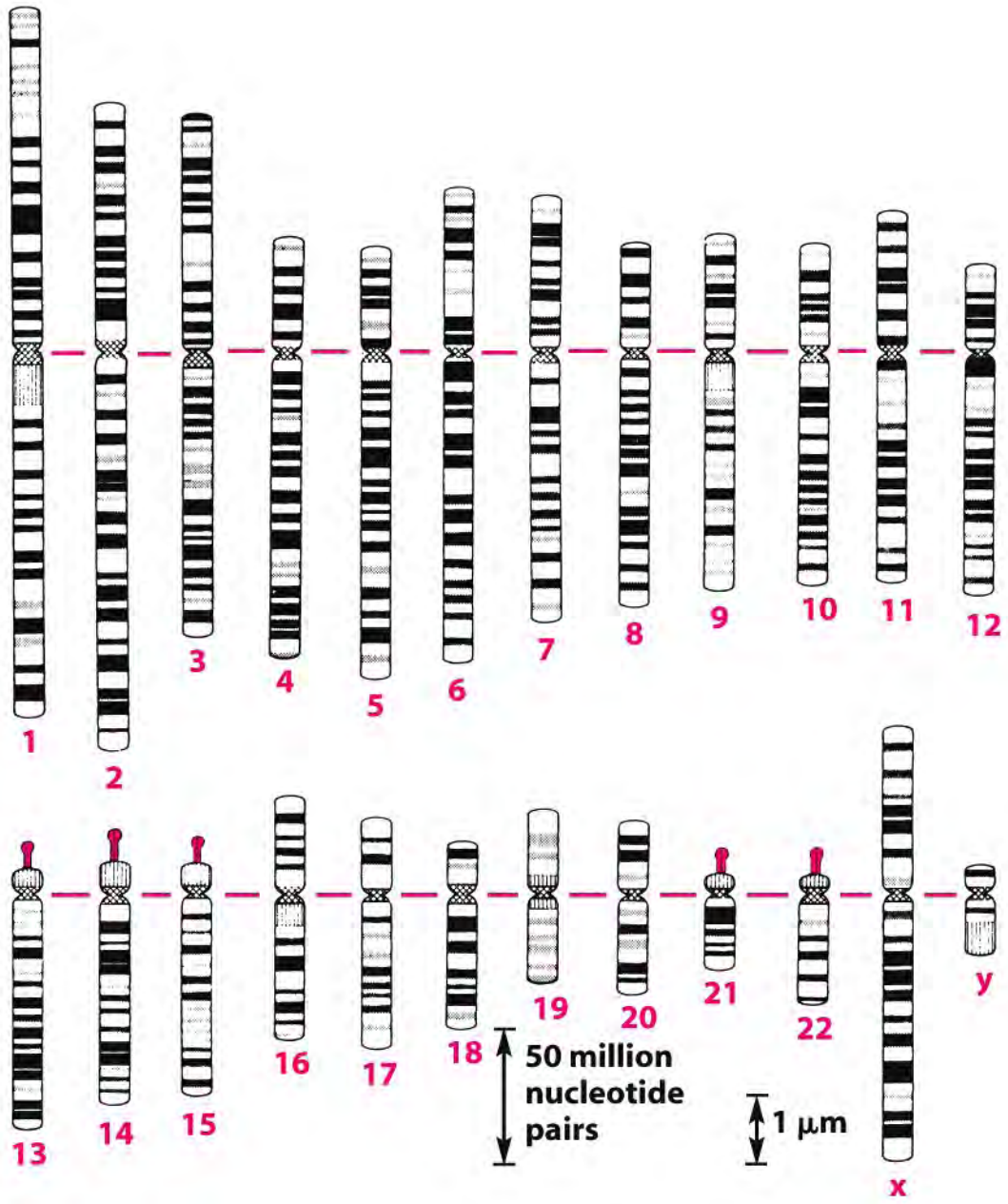
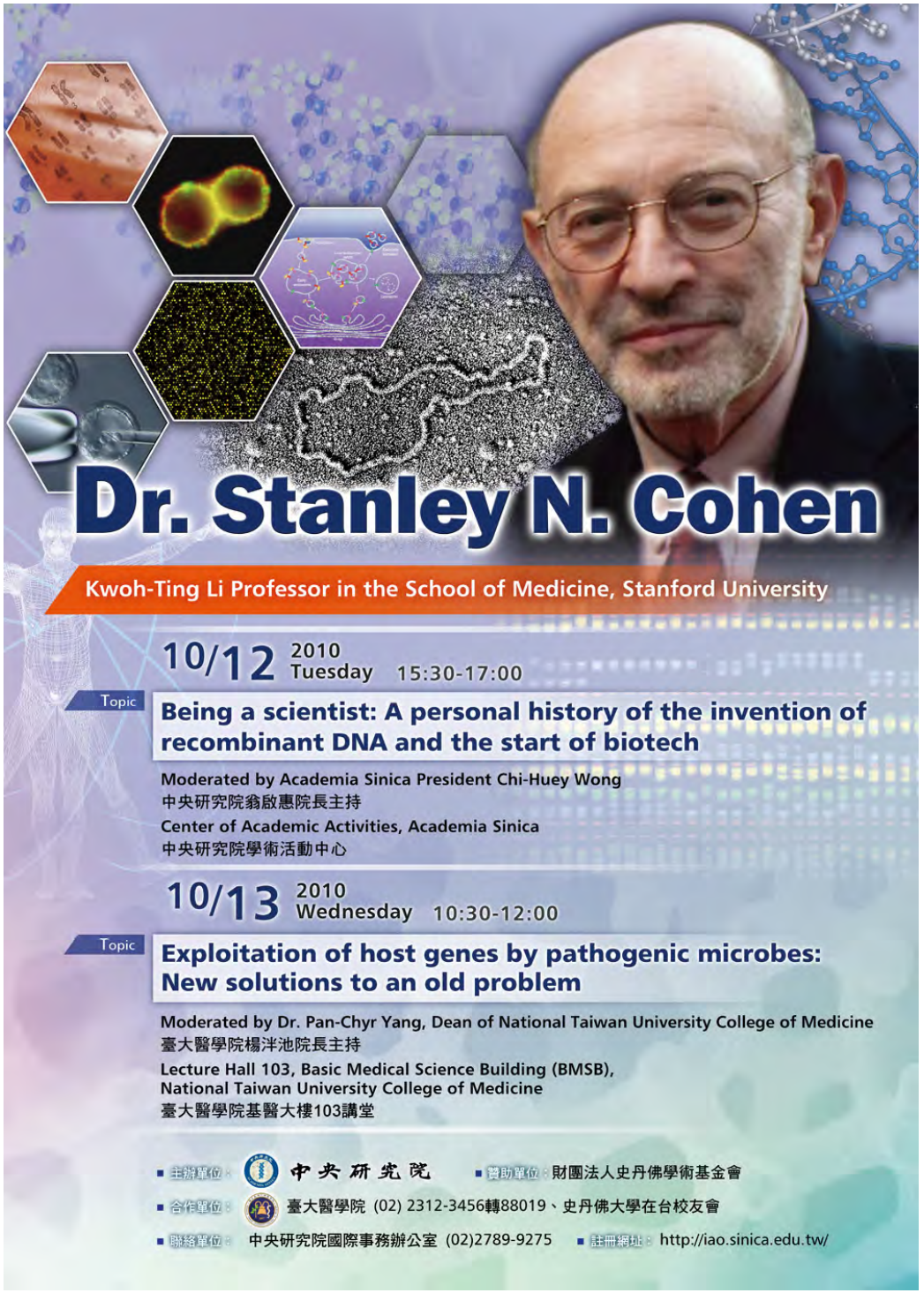


Figure 4-11 ***Molecular Biology of the Cell*** **(© Garland Science 2008)**


Dr. Stanley N. Cohen
Kwoh-Ting Li Professor in the School of Medicine, Stanford University
10/12 2010 Tuesday 15:30-17:00
Topic
Being a scientist: A personal history of the invention of recombinant DNA and the start of biotech
Moderated by Academia Sinica President Chi-Huey Wong
中央研究院翁啟惠院長主持
Center of Academic Activities, Academia Sinica
中央研究院學術活動中心
10/13 2010 Wednesday 10:30-12:00
Topic
Exploitation of host genes by pathogenic microbes: New solutions to an old problem
Moderated by Dr. Pan-Chyr Yang, Dean of National Taiwan University College of Medicine
臺大醫學院楊泮池院長主持
Lecture Hall 103, Basic Medical Science Building (BMSB), National Taiwan University College of Medicine
臺大醫學院基醫大樓103講堂
主辦單位：中央研究院
贊助單位：財團法人史丹佛學術基金會
合作單位：臺大醫學院 (02) 2312-3456轉88019、史丹佛大學在台校友會
聯絡單位：中央研究院國際事務辦公室 (02)2789-9275
註冊網址：http://iao.sinica.edu.tw/

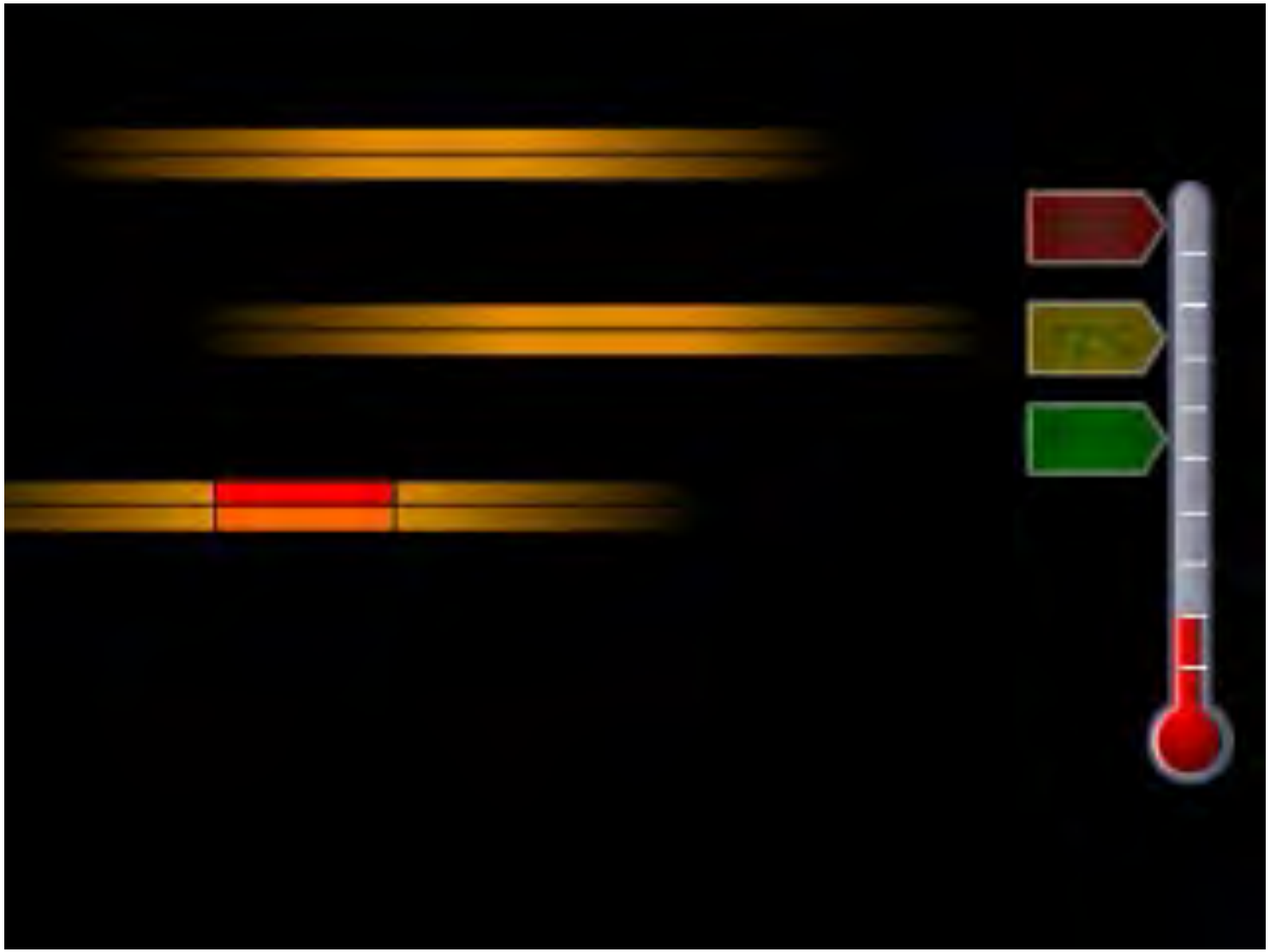
1968

Polymerase Chain Reaction (PCR)

The Nobel Prize in Chemistry 1993: Kary B. Mullis, Michael Smith

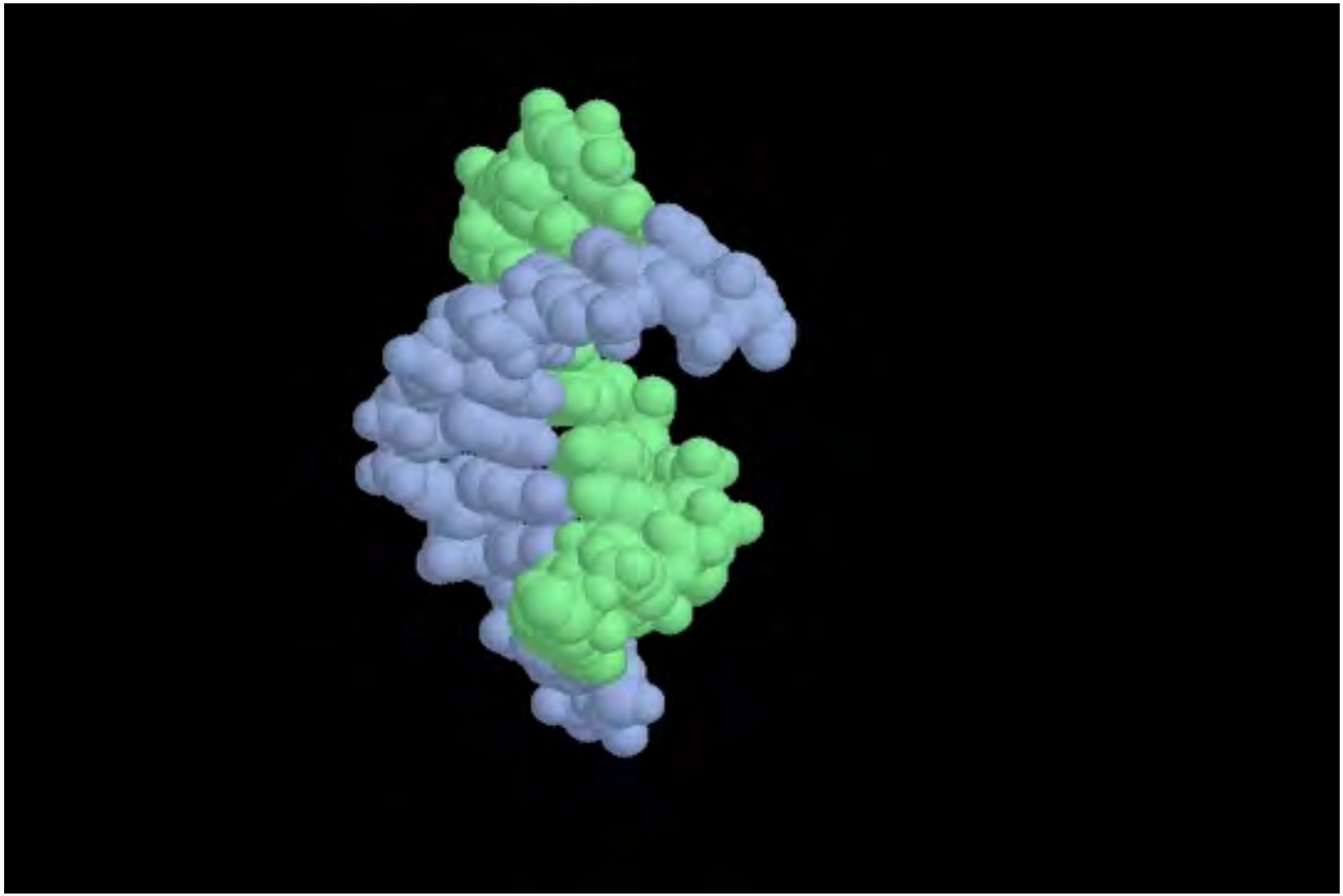
95°C—DNA melting
55°C—primer annealing
72°C—extension

Polymerase Chain Reaction (PCR)-2

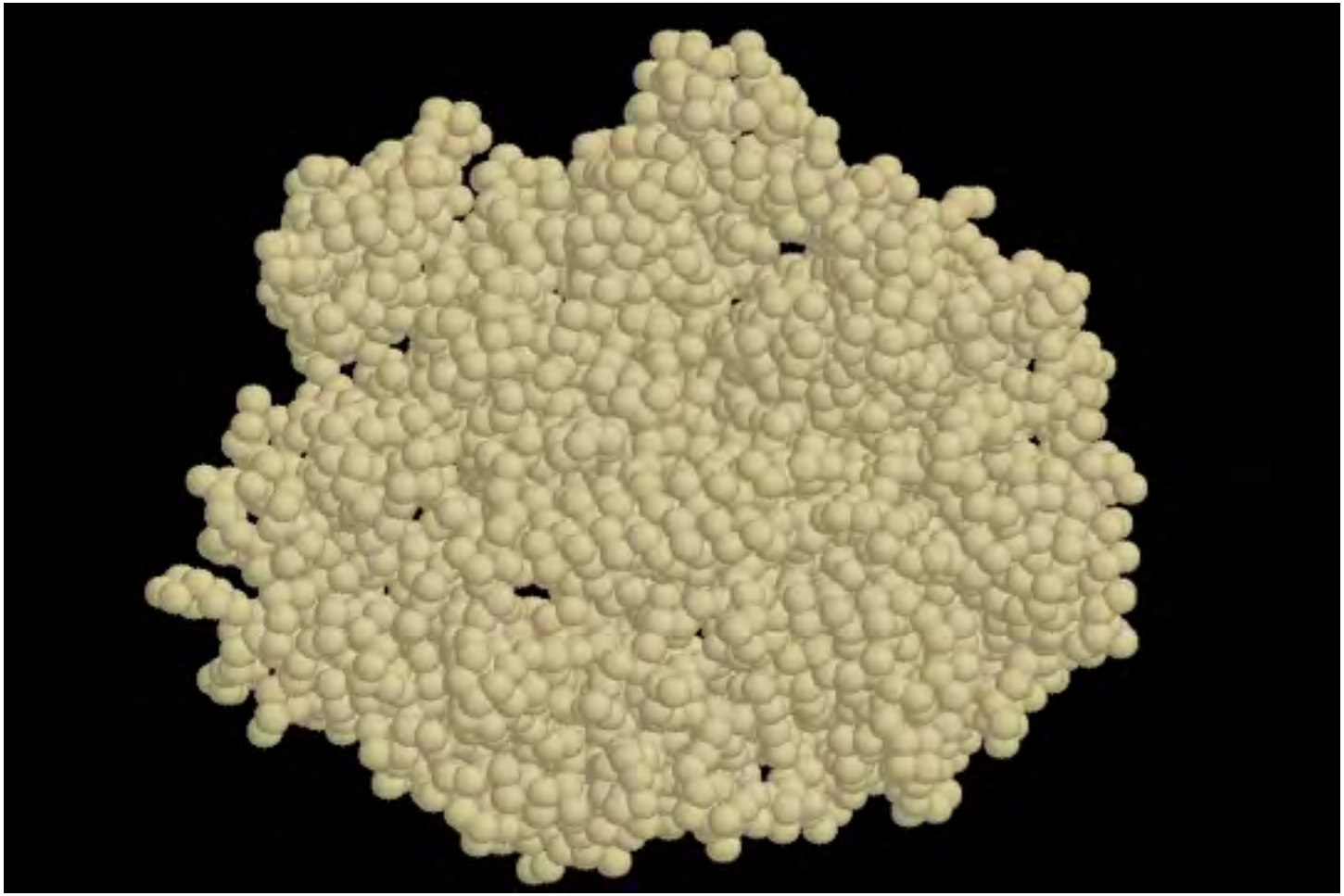


http://www.youtube.com/watch?v=eEcy9k_KsDI

RNA structure



RNA polymerase II



DNA separation

- Introduction to separation science
- Conventional gel electrophoresis
- Conventional pulsed-field gel electrophoresis
- Capillary electrophoresis
- Advanced DNA separation techniques

(On-chip electrophoresis and pulsed-field electrophoresis, DNA prism, etc.)

References:

- JC Giddings, *"Unified Separation Science"* (Wiley-Interscience, New York, 1991)
- *Electrophoresis* (Journal)

The big column for separation…

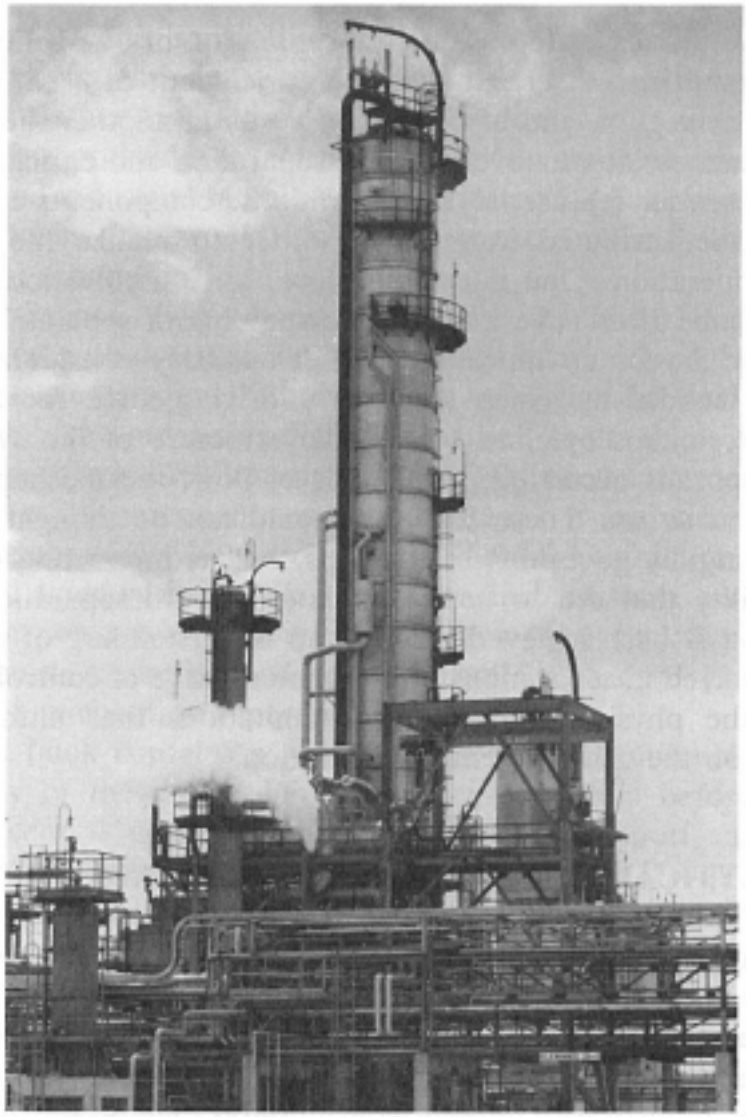
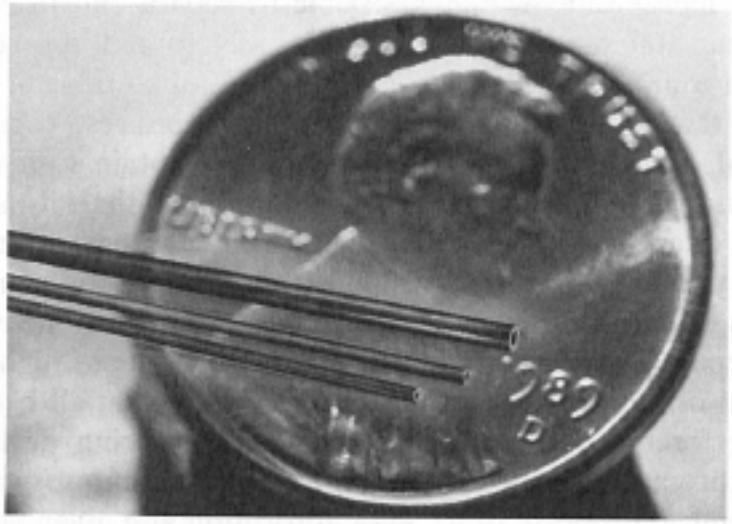


Figure 1.1. Crude fractionation tower at the Phillips Oil Refinery in Woods Cross, Utah. In this crude fractionator, 25,000 barrels per day of incoming crude oil is divided into six fractions by distillation. (Photo by Alexis Kelner.)

Figure 1.2. Preparative scale liquid chromatography unit with 6 ft (1.8 m) long column (in center) having an inner diameter of 6 in (15 cm). Up to 2 L of sample solution can be injected and processed in a single 90-min run. (Courtesy Thomas J. Filipi and Whatman Chemical Separation Division.)



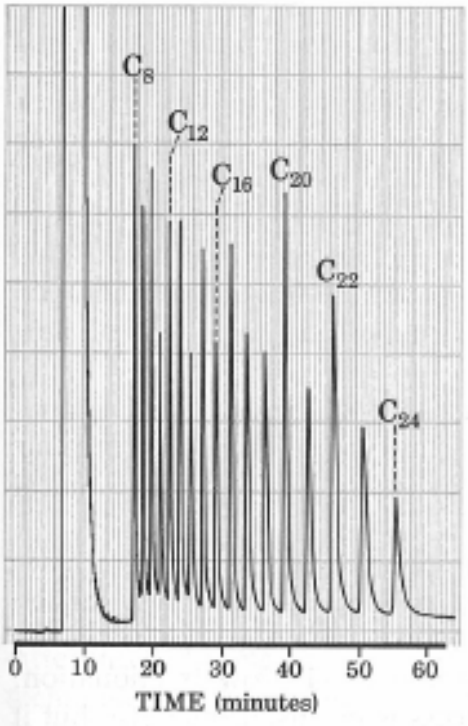
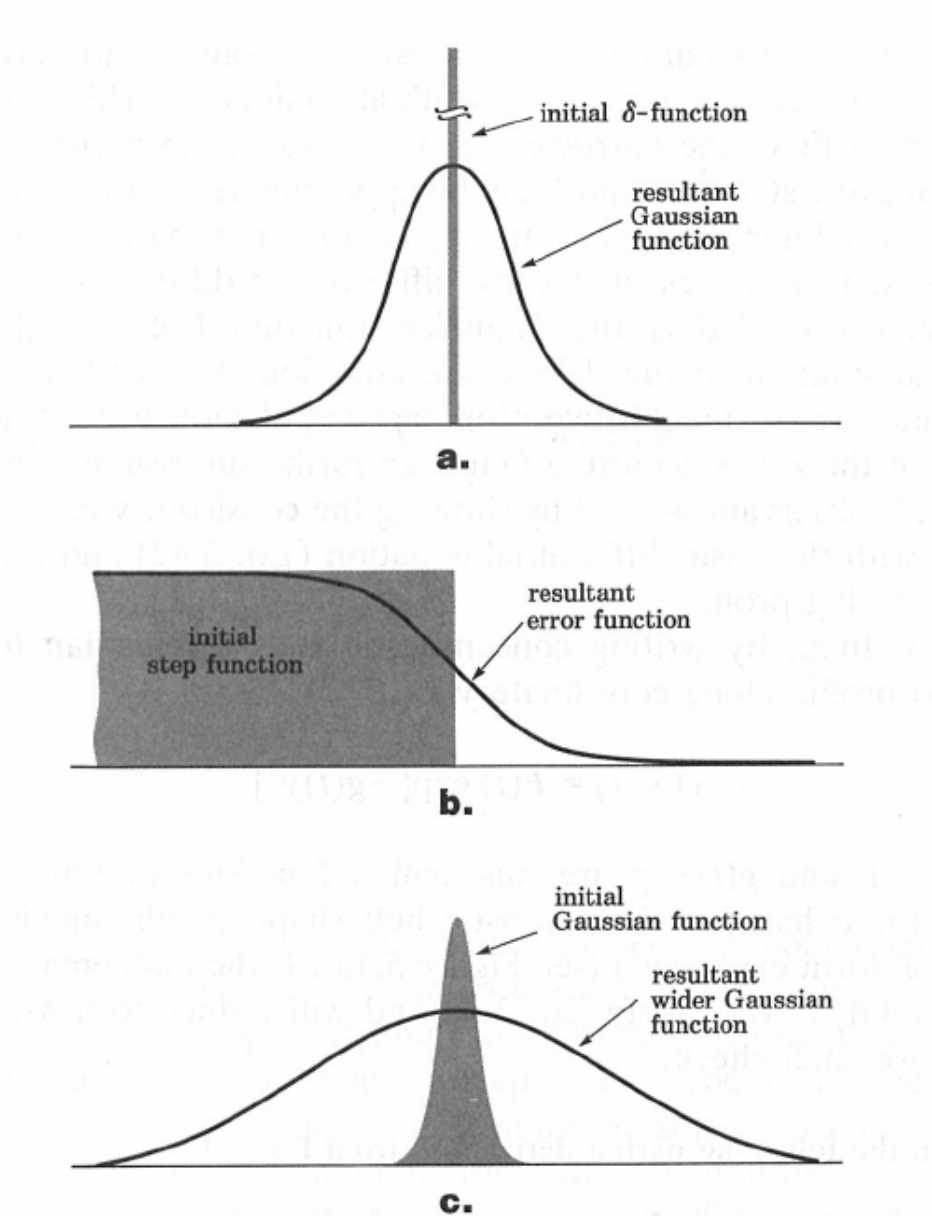


Figure 1.4. High-resolution separation of free fatty acids of indicated carbon numbers by supercritical fluid chromatography using a compressed CO_2 mobile phase at 170°C. Column is 50 cm long fused silica capillary tube of 250 μm internal diameter. (Courtesy of Frank J. Yang.)

Concentration (band) broadening by diffusion



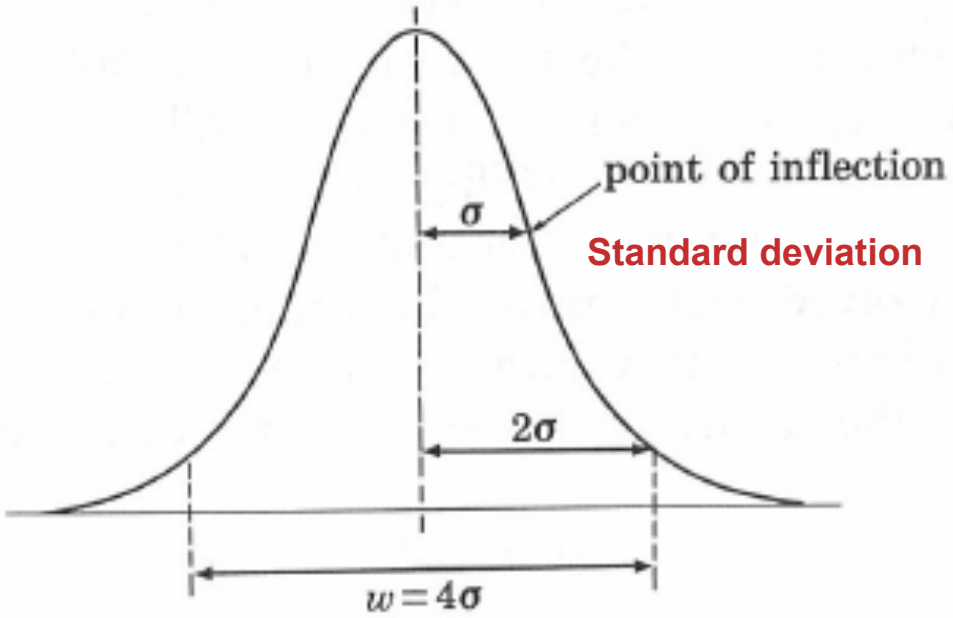
Normalized Gaussian Profile

$$c = \frac{1}{(4\pi Dt)^{1/2}} \exp\left(\frac{-y^2}{4Dt}\right)$$

$$\sigma^2 = 2Dt$$

$$\sigma = (2Dt)^{1/2}$$

$$c = \frac{n}{(4\pi Dt)^{1/2}} \exp\left(-\frac{y^2}{4Dt}\right)$$



Effective zone width

$$X = v\,t$$

$$\sigma^2 = (2D/v)\,X$$

Plate height: H

$$\sigma^2 = H\,X$$

$$H = \sigma^2 / X$$

Theoretical plates: N = X / H

Resolution

$$R_s = \frac{\Delta X}{4\sigma} \qquad \sigma = (HX)^{1/2}$$

$$R_s = \frac{\Delta v}{v}\left(\frac{X}{16H}\right)^{1/2} = \frac{\Delta v}{v}\left(\frac{N}{16}\right)^{1/2}$$

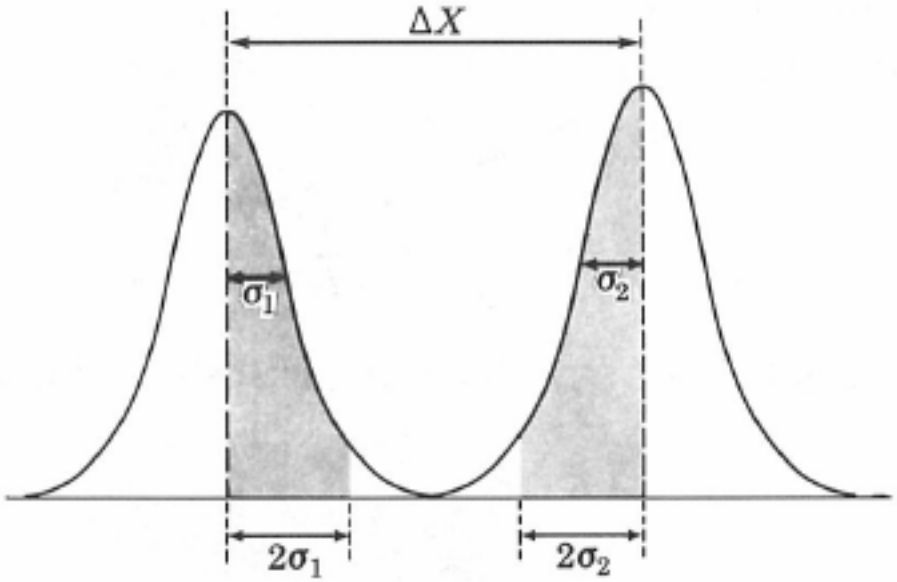


Figure 5.7. The resolution of two peaks, defined as $R_s = \Delta X/(2\sigma_1 + 2\sigma_2)$, measures peak separation ΔX relative to the zone dispersion ($2\sigma_1$ and $2\sigma_2$) leading to overlap and contamination.

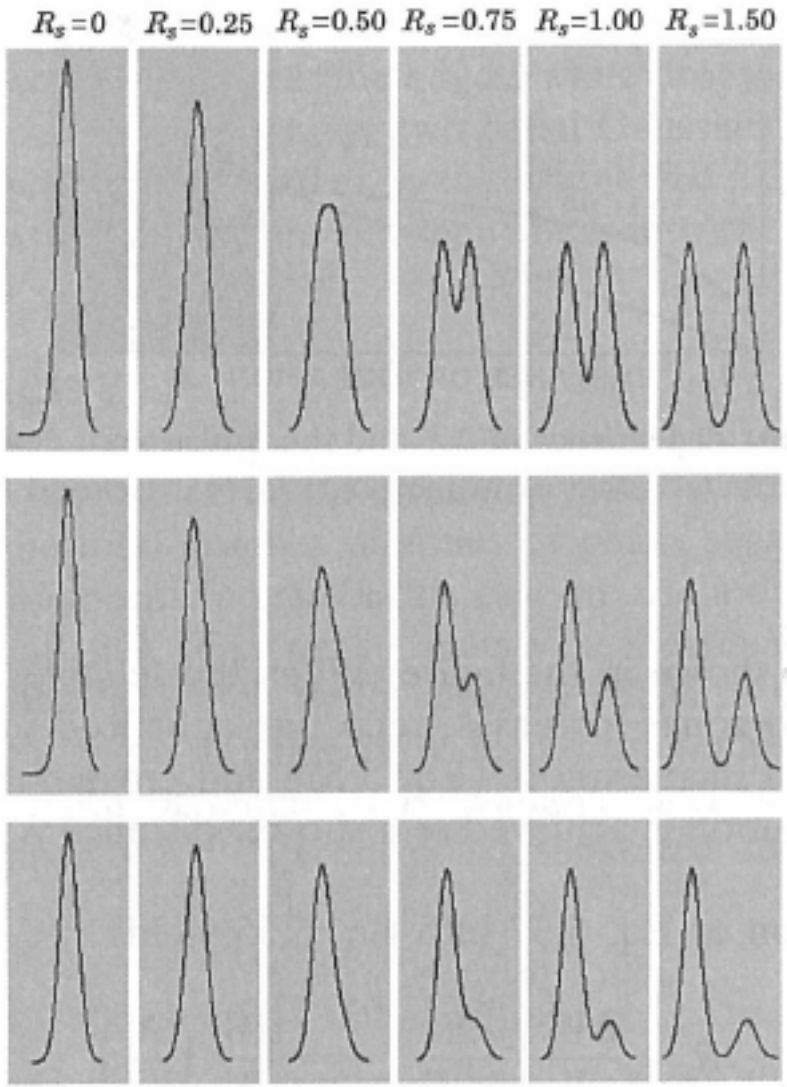
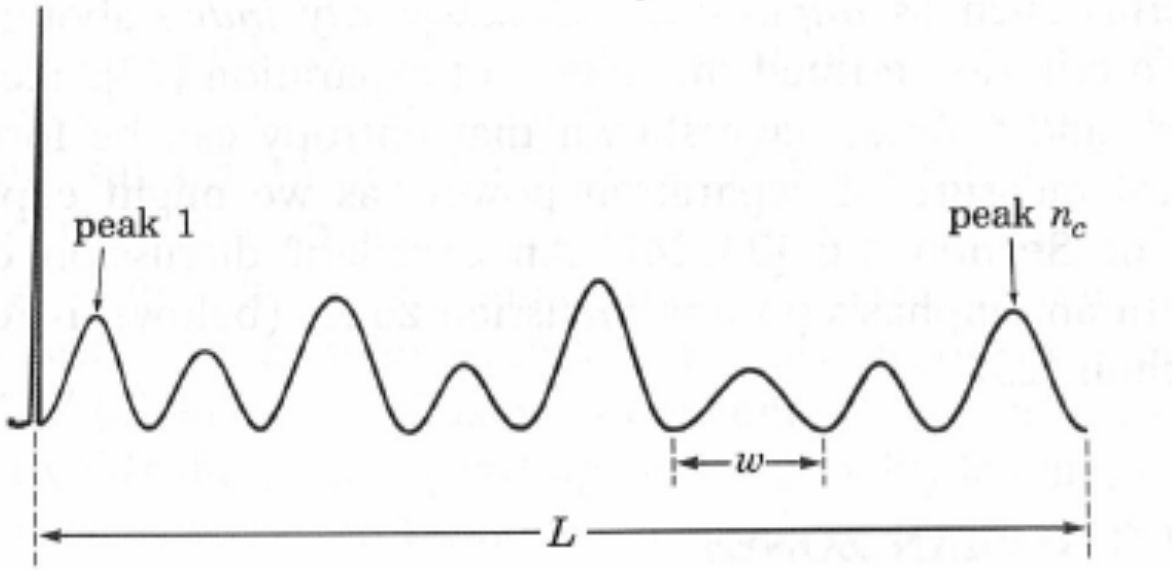


Figure 5.8. Left to right sequence gives the profile of a pair of Gaussian zones of equal σ at increasing levels of resolution, as shown. Top row shows this sequence for two zones of equal peak height; middle and bottom rows show the sequence for 2:1 and 5:1 peak height ratios (profiles courtesy of Joe M. Davis).

Peak capacity: $n_c = L/w = L/4\sigma$ (at Rs = 1)

the max number of separated peaks that can be fit (with adjacent peaks at some specified Rs value) into the path length.

$$n_c = \frac{L}{4\sigma R_s}$$



Theoretical plates: N = L / H

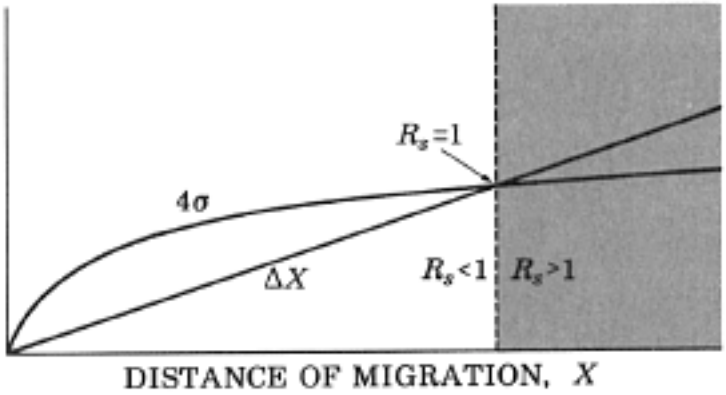


Figure 5.9. The linear dependence of ΔX and the square root dependence of 4σ on X lead to their inevitable crossing, at which point $R_s = 1$. Beyond the crossing (shaded area), $R_s > 1$.

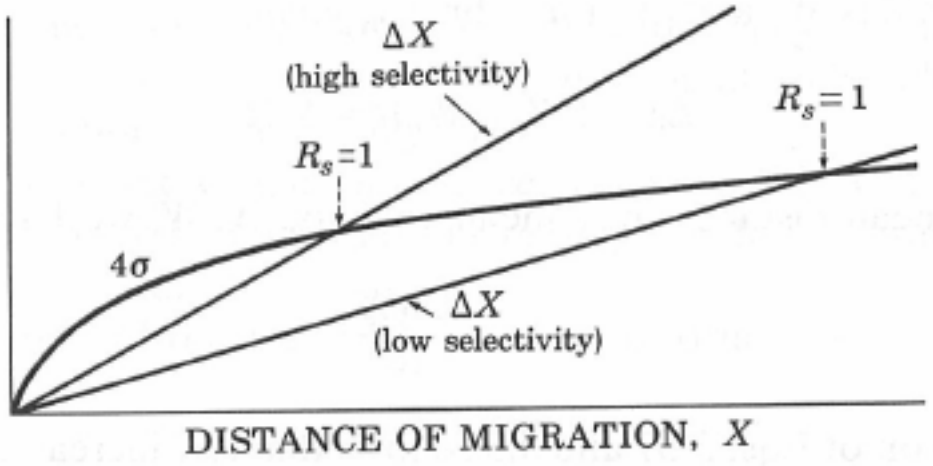
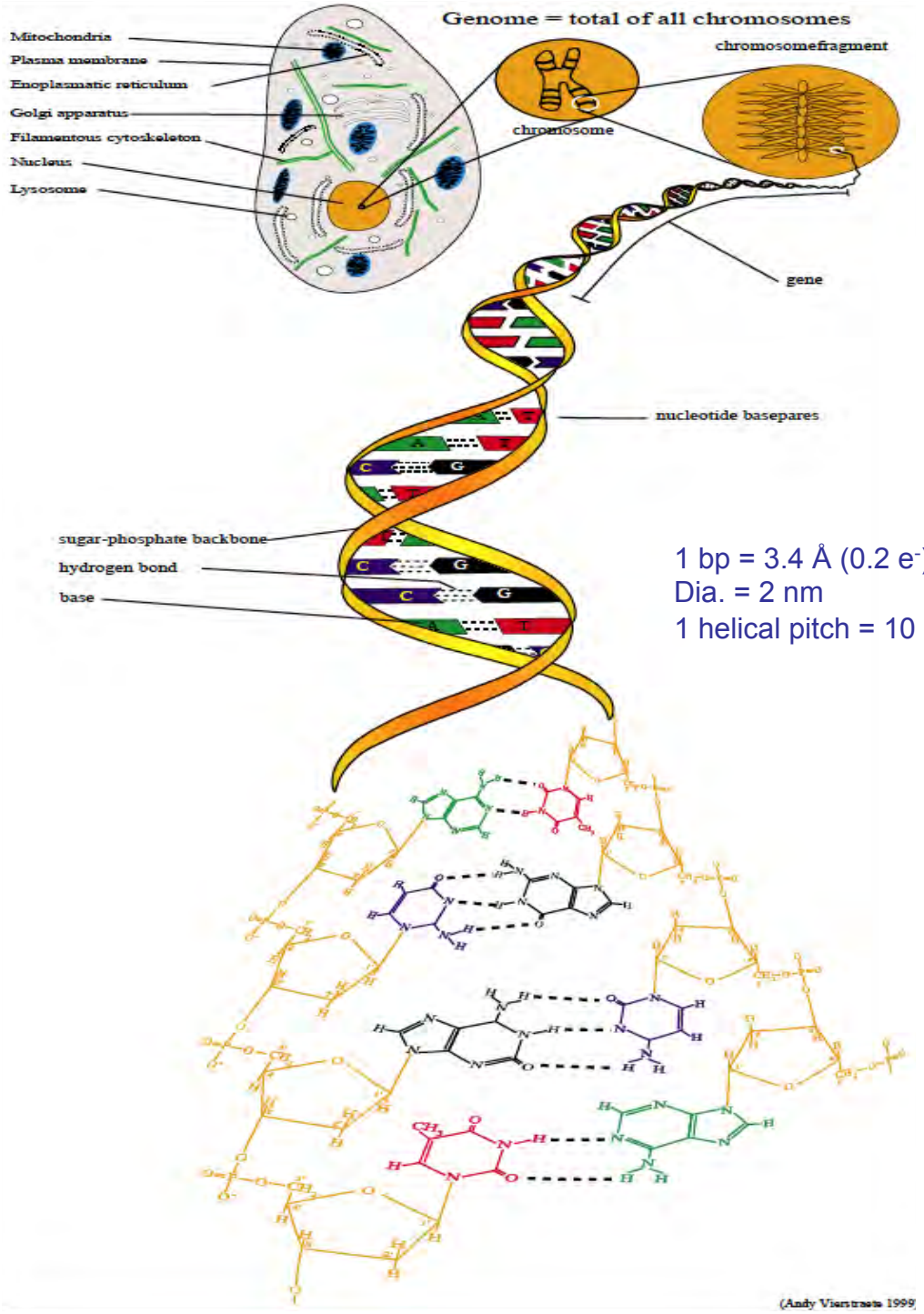


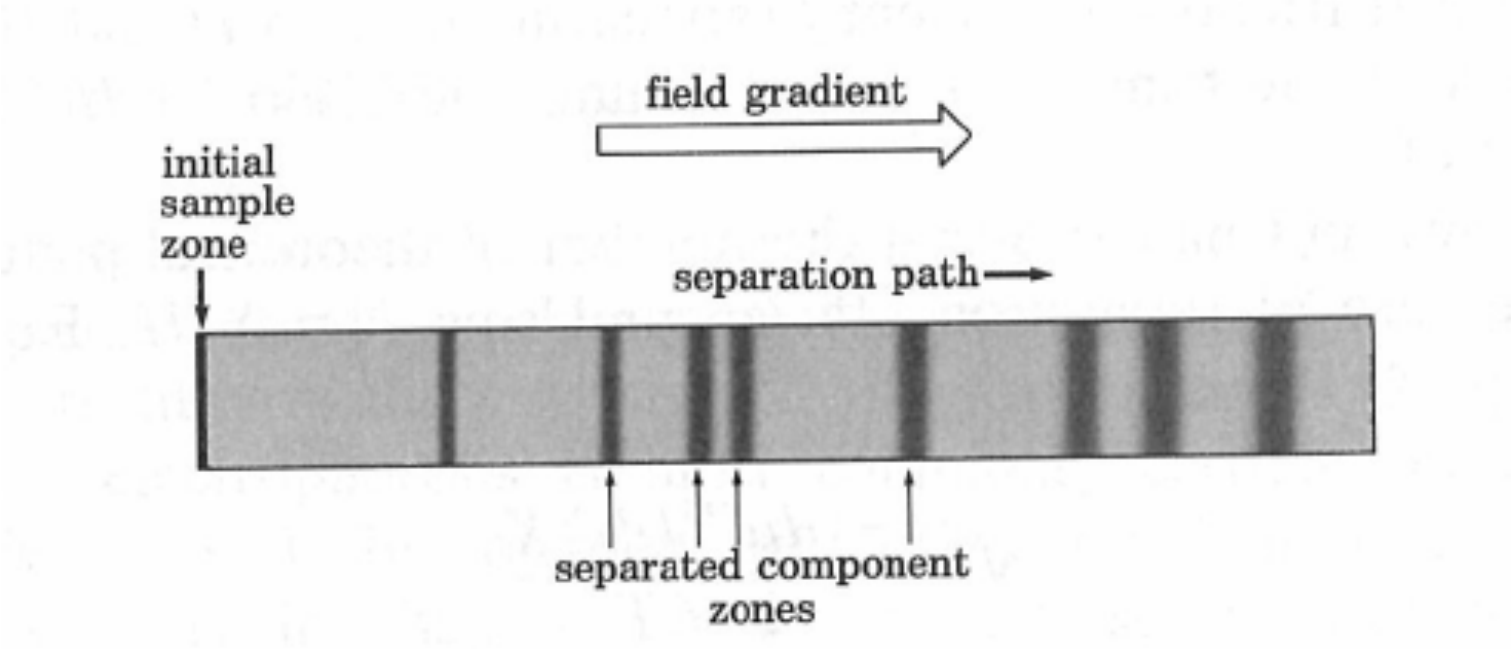
TABLE 8.1 Types of "Fields" that Cause Relative Displacement and thus (potentially at least) Some Degree of Relative Separation[a]

Field or Gradient	Static (*Sc*) Separation Methods or Phenomena	Other (non-*Sc*) Separation Methods
1. Electrical	Electrophoresis, isoelectric focusing, isotachophoresis	Electrodialysis, electrodeposition, electrostatic precipitation, countercurrent electrophoresis, electrical FFF[b]
2. Sedimentation	Rate-zonal sedimentation, isopycnic sedimentation	Equilibrium sedimentation, centrifugal elutriation, sedimentation FFF[b]
3. Temperature gradient	Thermophoresis	Thermogravitational methods, thermal FFF[b]
4. Electrical (nonuniform)	Dielectrophoresis	
5. Magnetic (nonuniform)	Magnetophoresis	Mass spectroscopy[c], magnetic separations, magnetic FFF[a]
6. em radiation	Photophoresis	
7. Concentration gradient	Diffusophoresis	

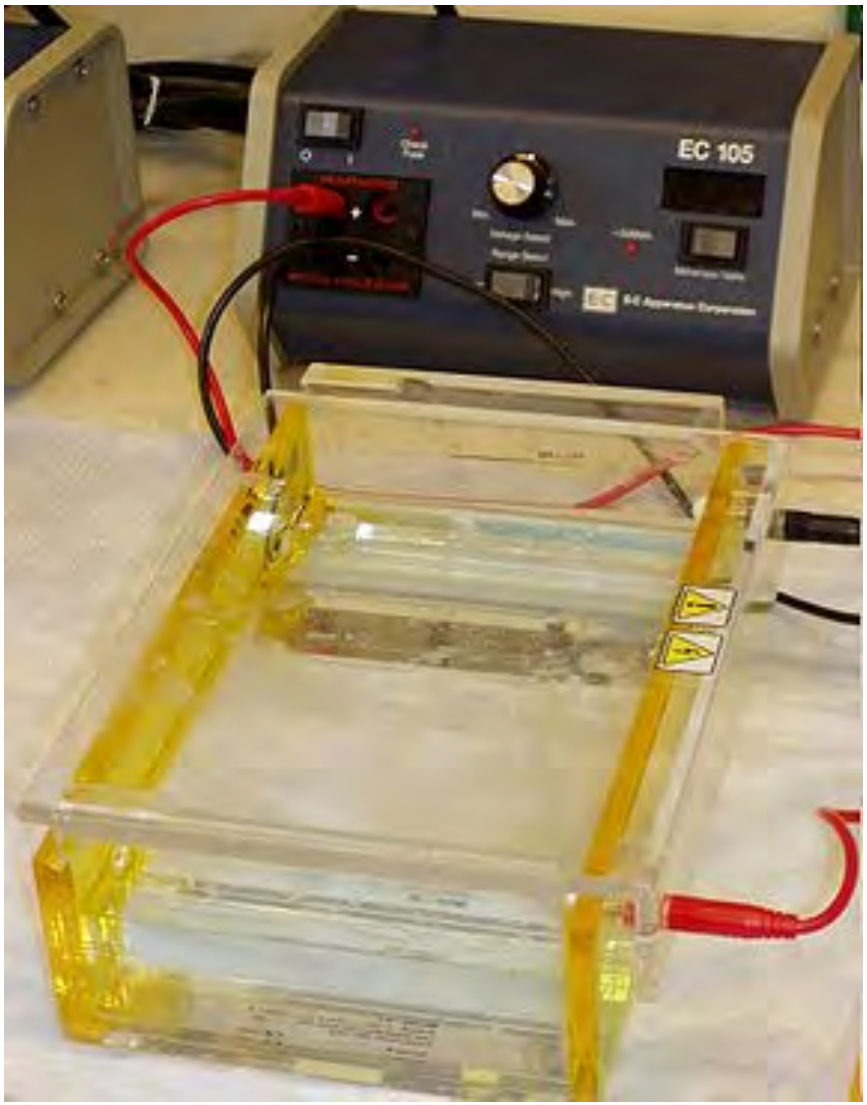
[a]Principal separation techniques, if they exist, are listed for each field.
[b]FFF = field-flow fractionation
[c]Inertial transport term important


Genome = total of all chromosomes
Mitochondria
Plasma membrane
Enoplasmatic reticulum
Golgi apparatus
Filamentous cytoskeleton
Nucleus
Lysosome
chromosomefragment
chromosome
gene
nucleotide basepares
sugar-phosphate backbone
hydrogen bond
base
1 bp = 3.4 Å (0.2 e-)
Dia. = 2 nm
1 helical pitch = 10 bp
(Andy Vierstraete 1999)

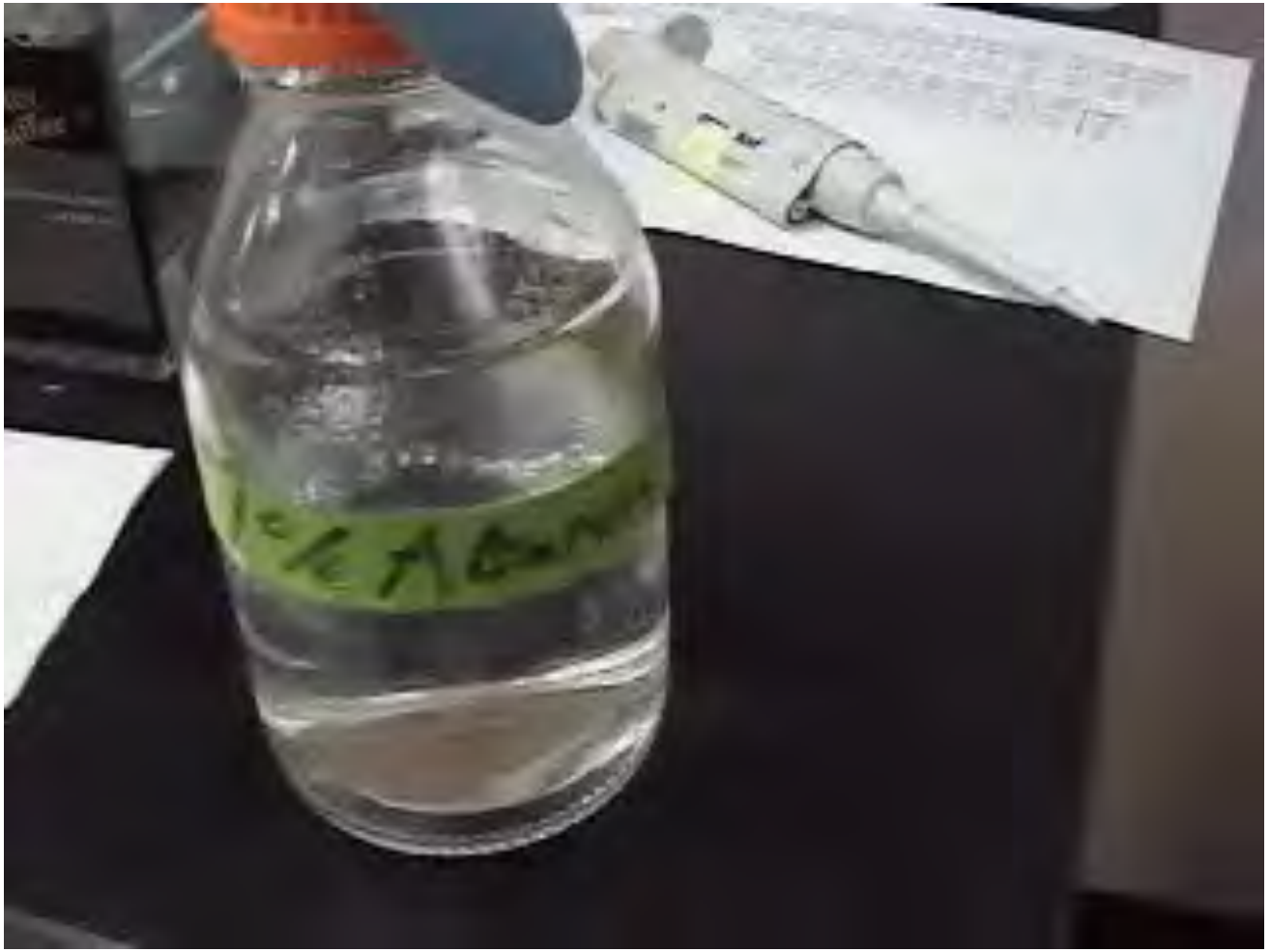
Electrophoresis



Gel electrophoresis apparatus

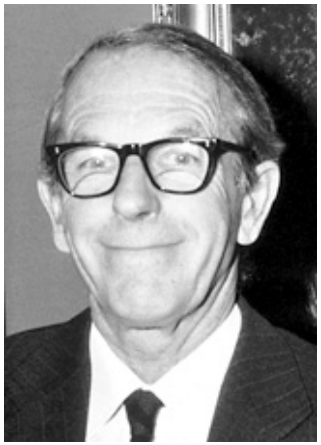


Gel Electrophoresis (movie)



History

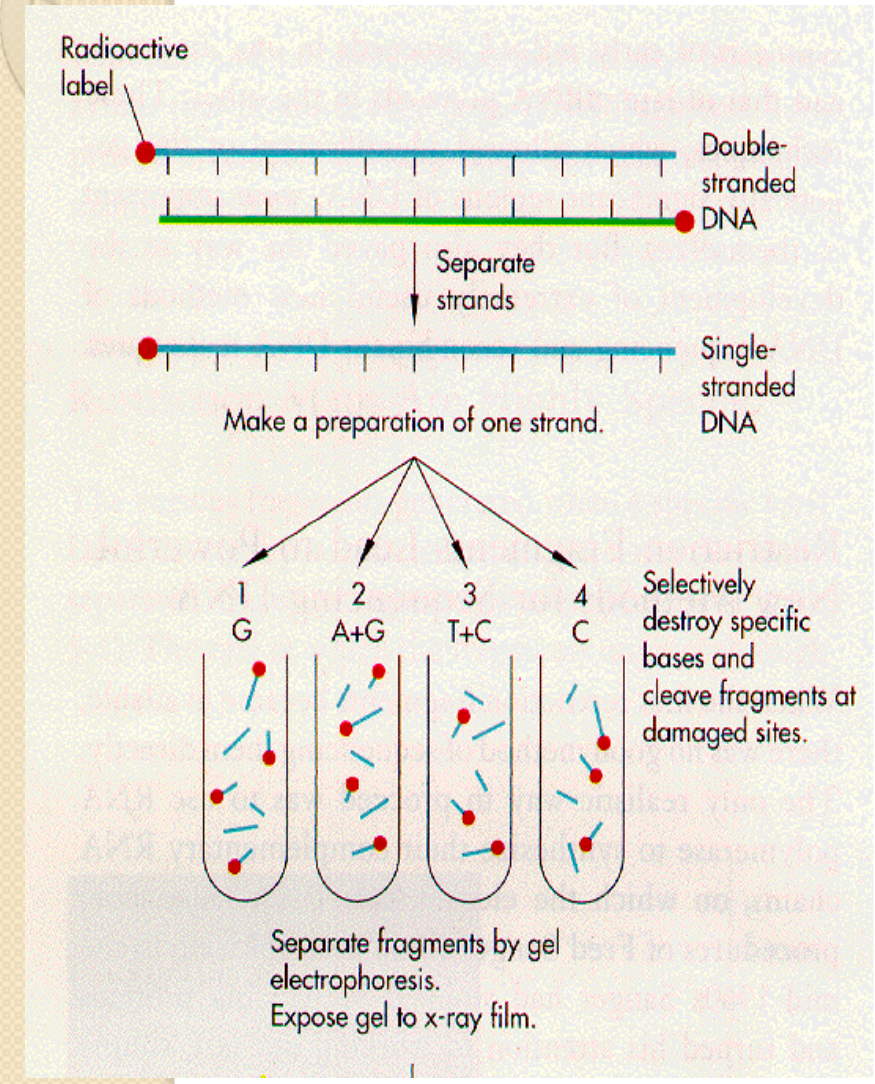
Walter Gilbert

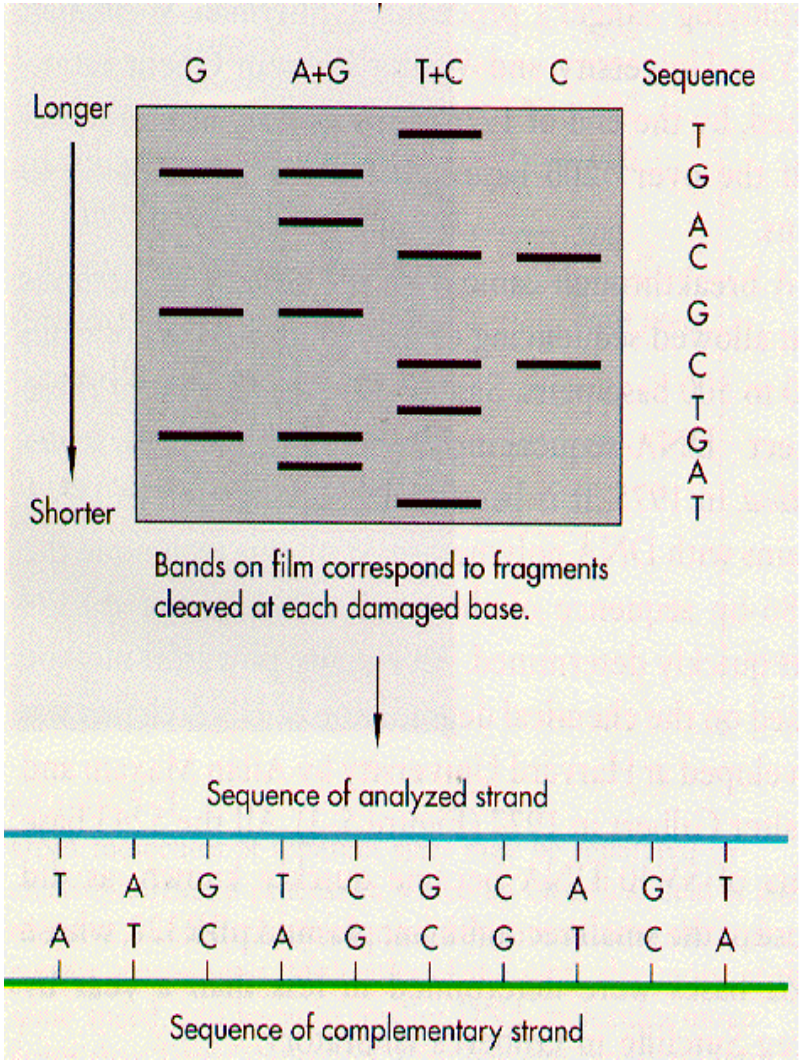


Frederick Sanger

- Year 1977
- Maxem and Gilbert – “Chemical Cleavage Protocol”
- Sanger – Dideoxy sequencing or chain termination method.
- Gilbert and Sanger shared 1/2 Nobel Prize in 1980.
- Sanger method is used widely because of its practicality.

Maxem-Gilbert Sequencing





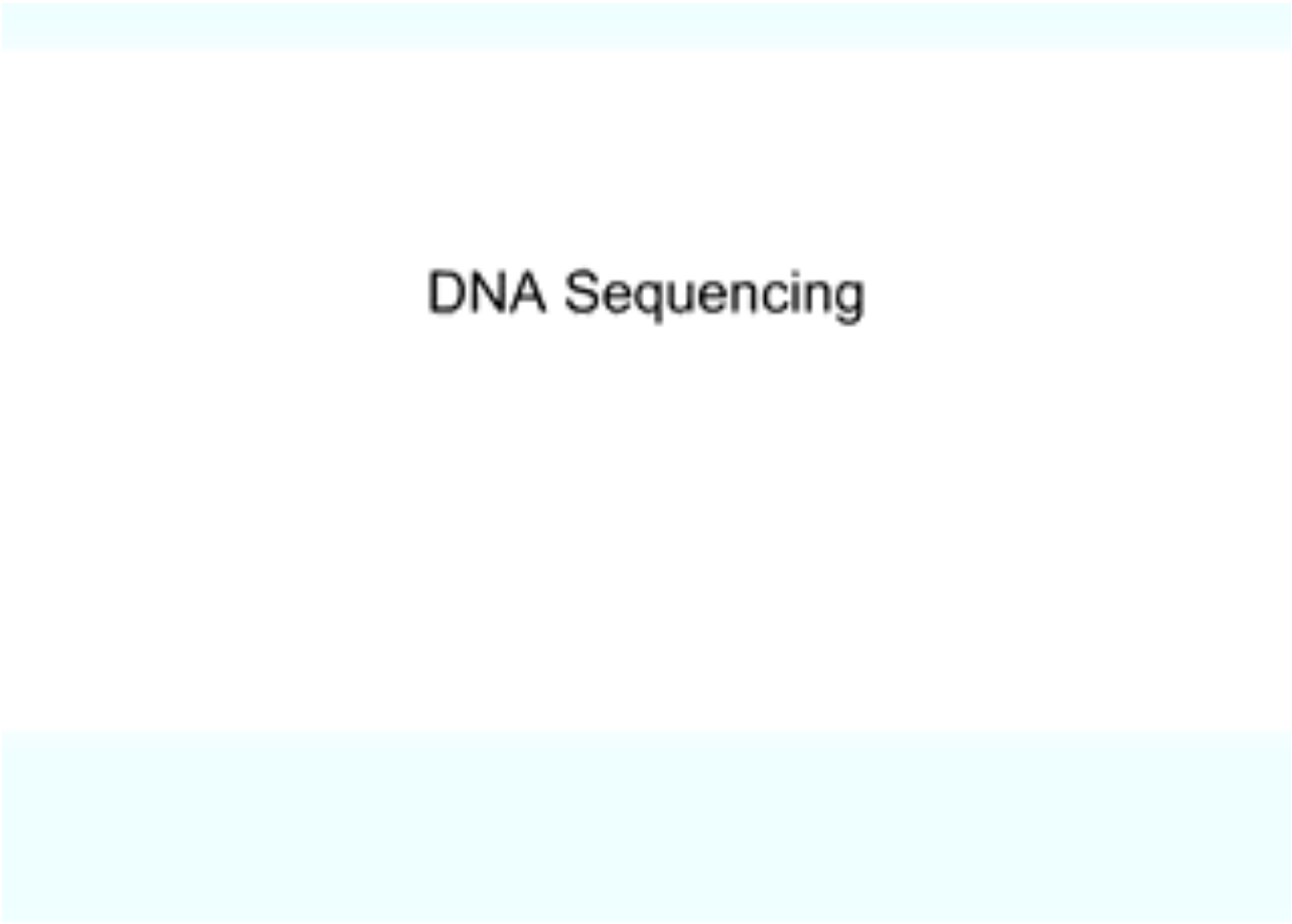
Sanger Method

- Based on the use of ddNTP's in addition to the normal nucleotides found in DNA.
- ddNTP's are same as NTP's except they contain a Hydrogen group on the 3' carbon instead of –OH group.
- ddNTP's when integrated into a sequence, prevent the addition of further nucleotides.

Sanger Method (Cont.)

- This is because a Phosphodiester bond can't form between ddNTP and the next incoming nucleotide.
- Thus, DNA chain is terminated.
- Automated Sequencer Method.
- Human Genome Project (1990) by US Dept. of Energy and National Institute of Health.

DNA sequencing–Sanger Method



Sequencing Methods

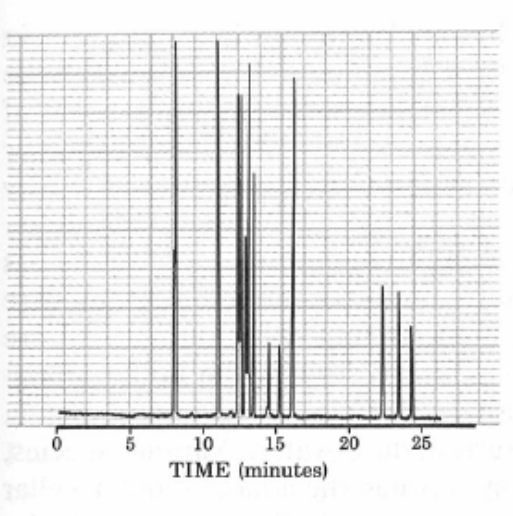
Electrophoretic Methods:

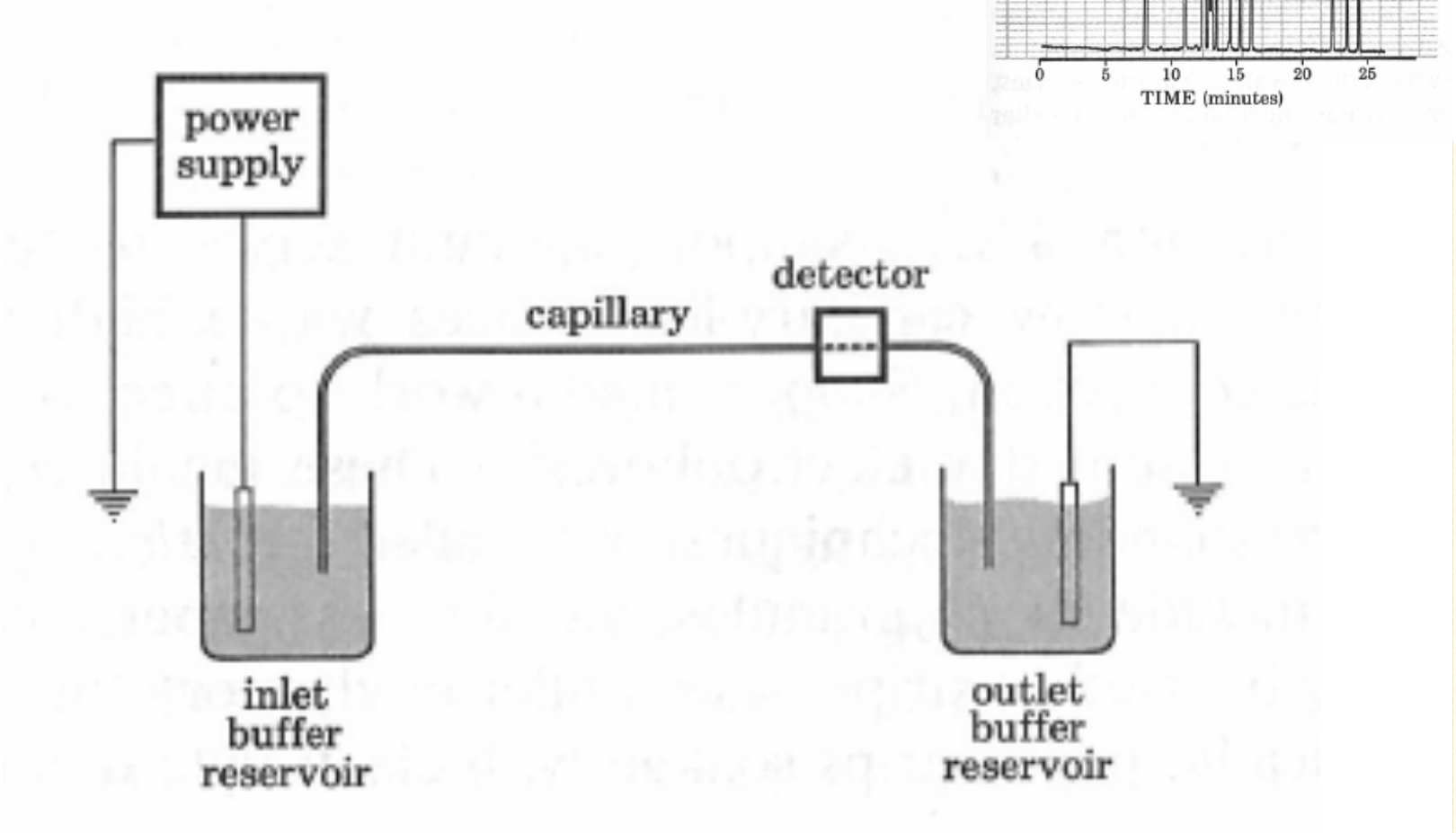
- Slab Gel Electrophoresis
- Capillary (Array) Electrophoresis
- Microfabricated Capillary Arrays
- Free Solution Electrophoresis

Non-Electrophoretic Methods:

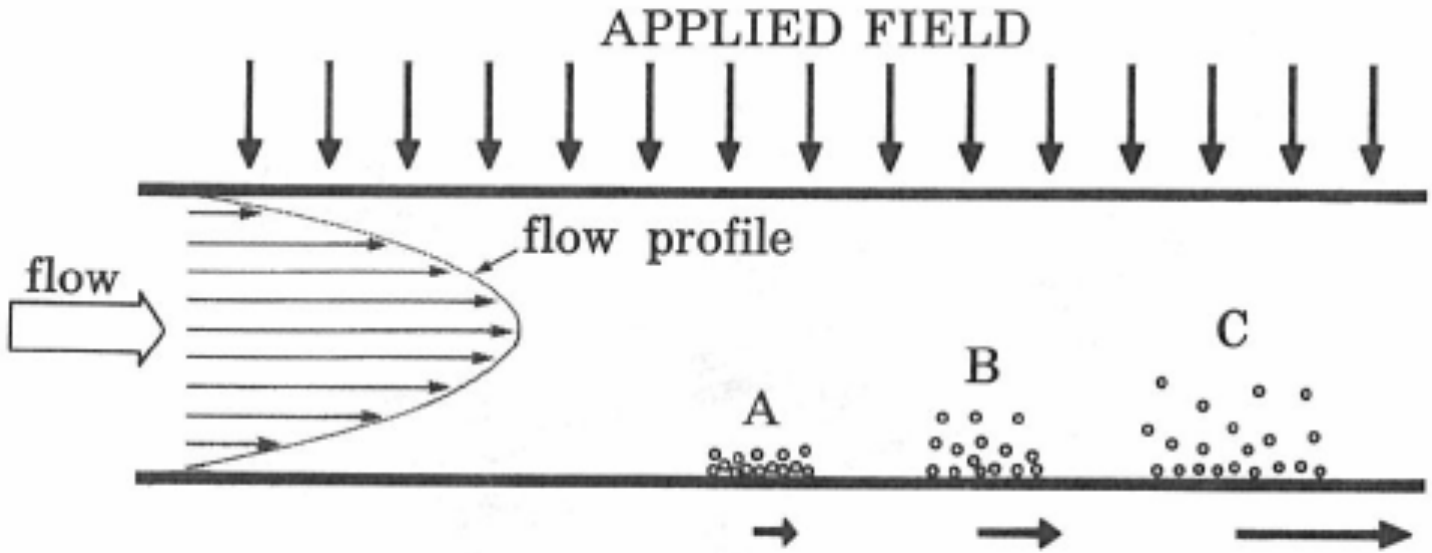
- Pyrosequencing
- Sequencing by Hybridization
- Massively Parallel Signature Sequencing
- MALDI-TOF Mass Spectrometry
- Single Molecule Methods

Capillary electrophoresis

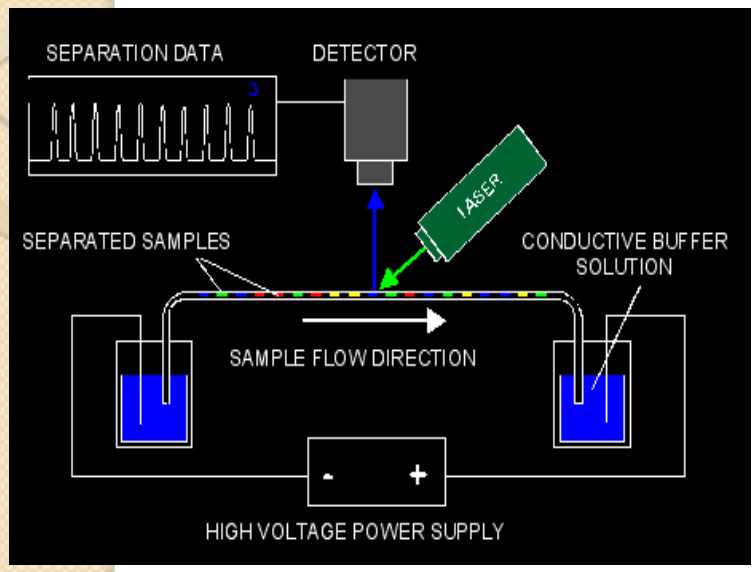


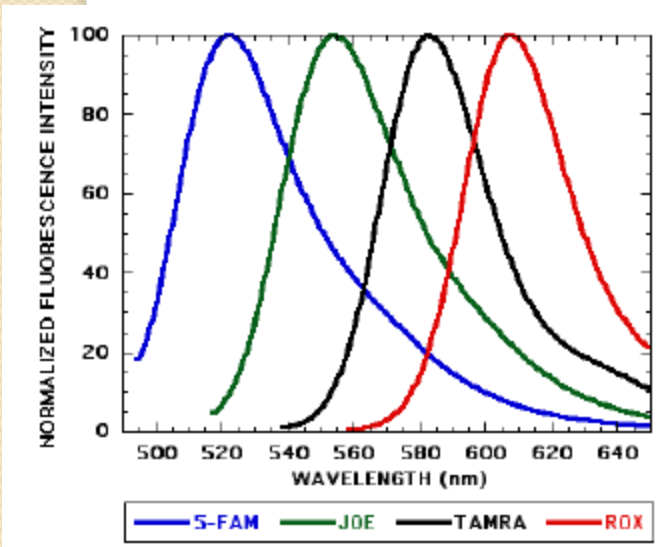


Field flow fractionation (FFF)



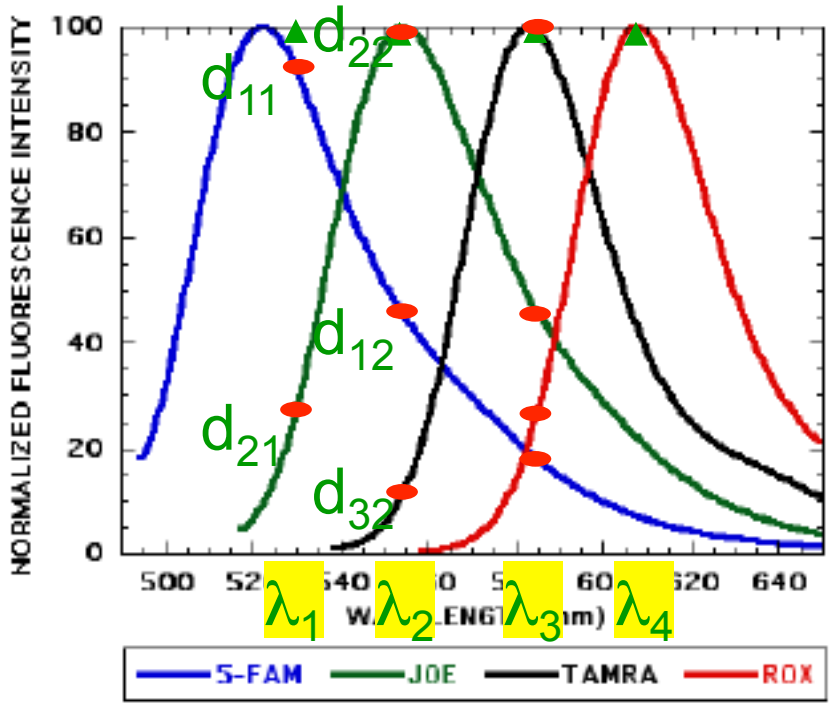
Capillary (Array) Electrophoresis





CE DNA Readout→Least-square fitting

What is needed: optical spectrum of 4 channels vs. time



Calibrated Coefficient:
d_{11}, d_{21}, d_{31}, d_{41}.....

$I(\lambda_1)=d_{11}c_1+d_{21}c_2+d_{31}c_3+d_{41}c_4$

$I(\lambda_2)=d_{12}c_1+d_{22}c_2+d_{32}c_3+d_{42}c_4$

$I(\lambda_3)=d_{13}c_1+d_{23}c_2+d_{33}c_3+d_{43}c_4$

$I(\lambda_4)=d_{14}c_1+d_{24}c_2+d_{34}c_3+d_{44}c_4$

$I(\lambda_5)=d_{15}c_1+d_{25}c_2+d_{35}c_3+d_{45}c_4$

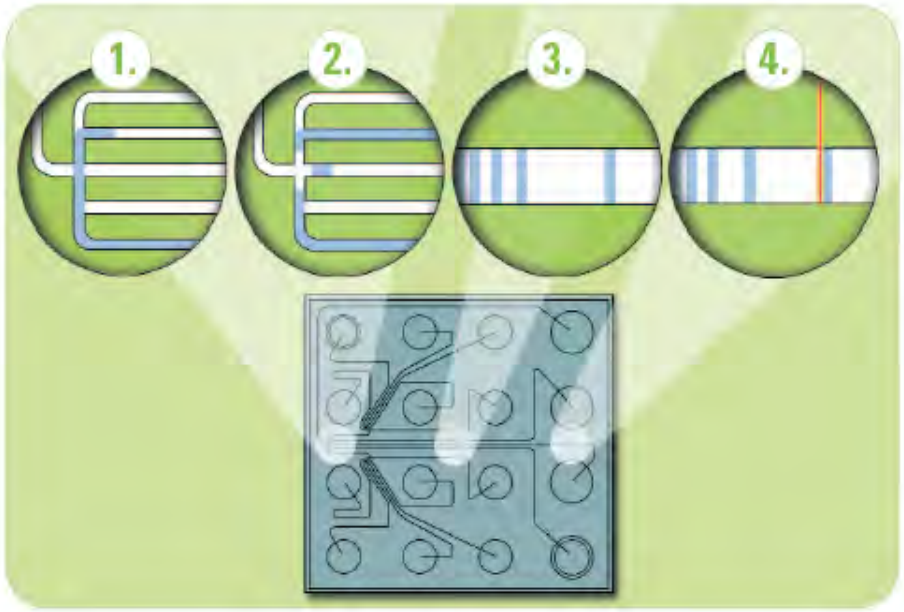
---

c_1, c_2, c_3, c_4 are the dye (DNA) concentration

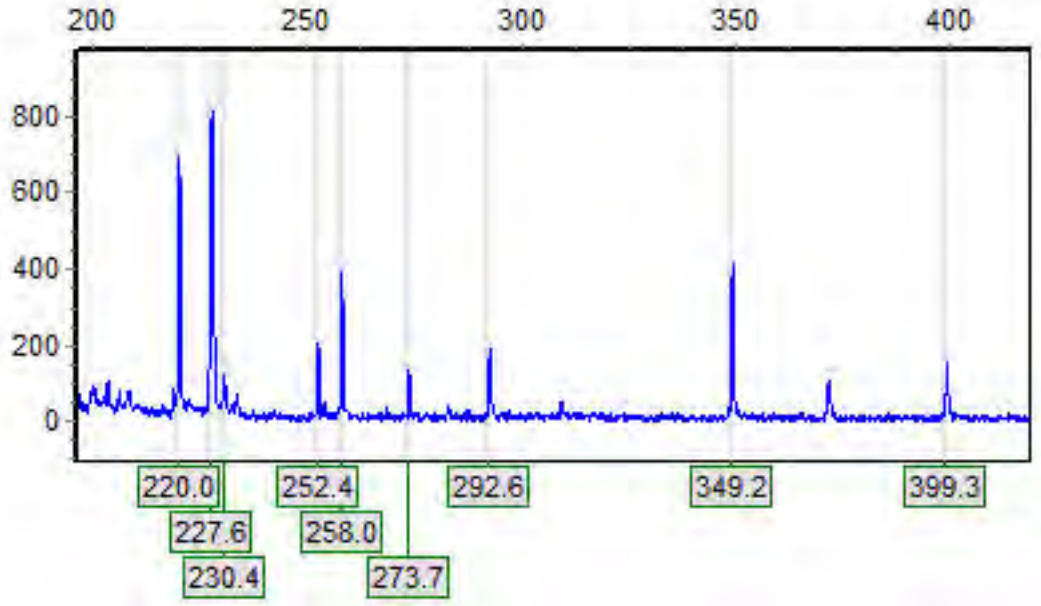
Agilent 2100 Bioanalyzer

Applied microfluidic lab-on-a-chip technology

Principle of lab-on-a-chip operation



1. The sample moves through the microchannels from the sample well.
2. The sample is injected into the separation channel.
3. Sample components are electrophoretically separated.
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms (peaks).



Organism	Genome size	Size relative to human	Status
HIV-1	9,750	0.0003%	complete 1985, Wain-Hobson,et.al.
Mycoplasma genitalium	580,070	0.0171%	complete 1995, TIGR
Mycoplasma pneumoniae	816,394	0.0240%	complete 1995, Univ. of Heidelberg
Lyme disease spirochete	946,000	0.0278%	complete 1997, TIGR
Methanococcus jannaschii	1,664,974	0.0490%	complete 1996, TIGR
H. influenzae	1,830,137	0.0538%	complete 1995, TIGR
Mycobacterium tuberculosis	4,397,000	0.1293%	complete 1998, Sanger Centre
Escherichia coli	4,639,221	0.1364%	complete 1997,Univ. of Wisc. + others
Yeast, Baker's	12,067,280	0.3549%	complete, 1996, Stanford Genome Center
C. elegans	100,000,000	3%	complete, 1999 Washington Univ. and Sanger Ctr.
Fruit fly	180,000,000	5%	complete, 2000 European and US groups
Pufferfish, Japanese (Takifugu rubripes)	400,000,000	12%	90% complete, 2005
Rice	400,000,000	12%	complete, 2002 International Rice Genome Seq. Proj.
Pig	2,700,000,000	79%	Mapping complete - sequencing mid stages
Rat	2,900,000,000	85%	Draft assembly
Cattle	3,000,000,000	88%	Begun in April, 2003, draft assembly
Mouse	3,000,000,000	88%	Complete, 2002
Rabbit	3,000,000,000	88%	
Human	3,400,000,000	1.0	Complete, 2001,2002,2003
Onion	18,000,000,000	5.3	
Marbled lungfish	139,000,000,000	40.9	
Fern	160,000,000,000	47.1	
Amoeba proteus	290,000,000,000	85.3	

Table 4–1 Some Vital Statistics for the Human Genome

	HUMAN GENOME
DNA length	3.2×10^9 nucleotide pairs*
Number of genes	approximately 25,000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes**	more than 20,000
Percentage of DNA sequence in exons (protein coding sequences)	1.5%
Percentage of DNA in other highly conserved sequences***	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%

* The sequence of 2.85 billion nucleotides is known precisely (error rate of only about one in 100,000 nucleotides). The remaining DNA primarily consists of short highly repeated sequences that are tandemly repeated, with repeat numbers differing from one individual to the next.

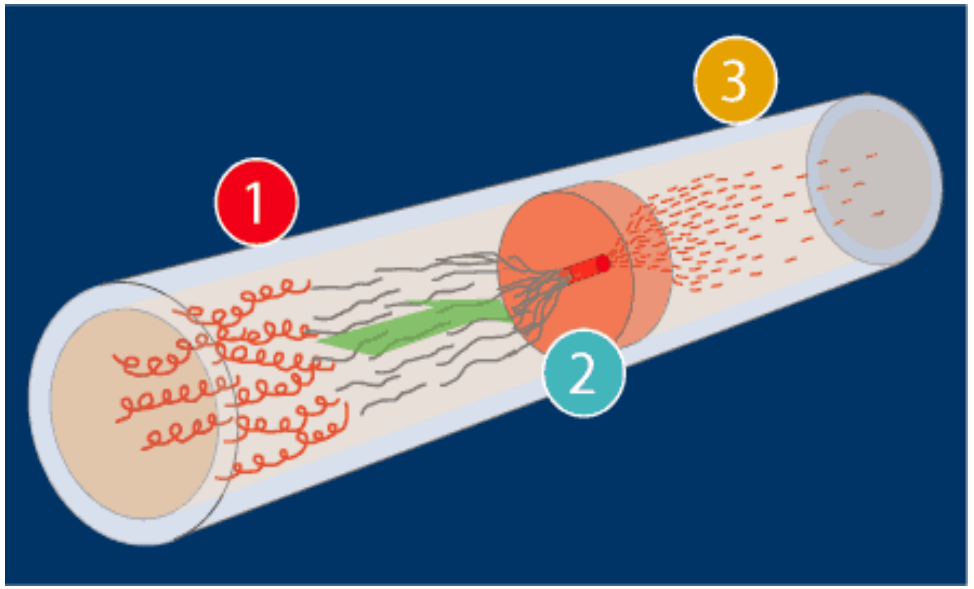
** A pseudogene is a nucleotide sequence of DNA closely resembling that of a functional gene, but containing numerous mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene followed by the accumulation of damaging mutations in one copy.

*** Preserved functional regions; these include DNA encoding 5′ and 3′ UTRs (untranslated regions), structural and functional RNAs, and conserved protein-binding sites on the DNA.

Table 4-1 *Molecular Biology of the Cell* (© Garland Science 2008)

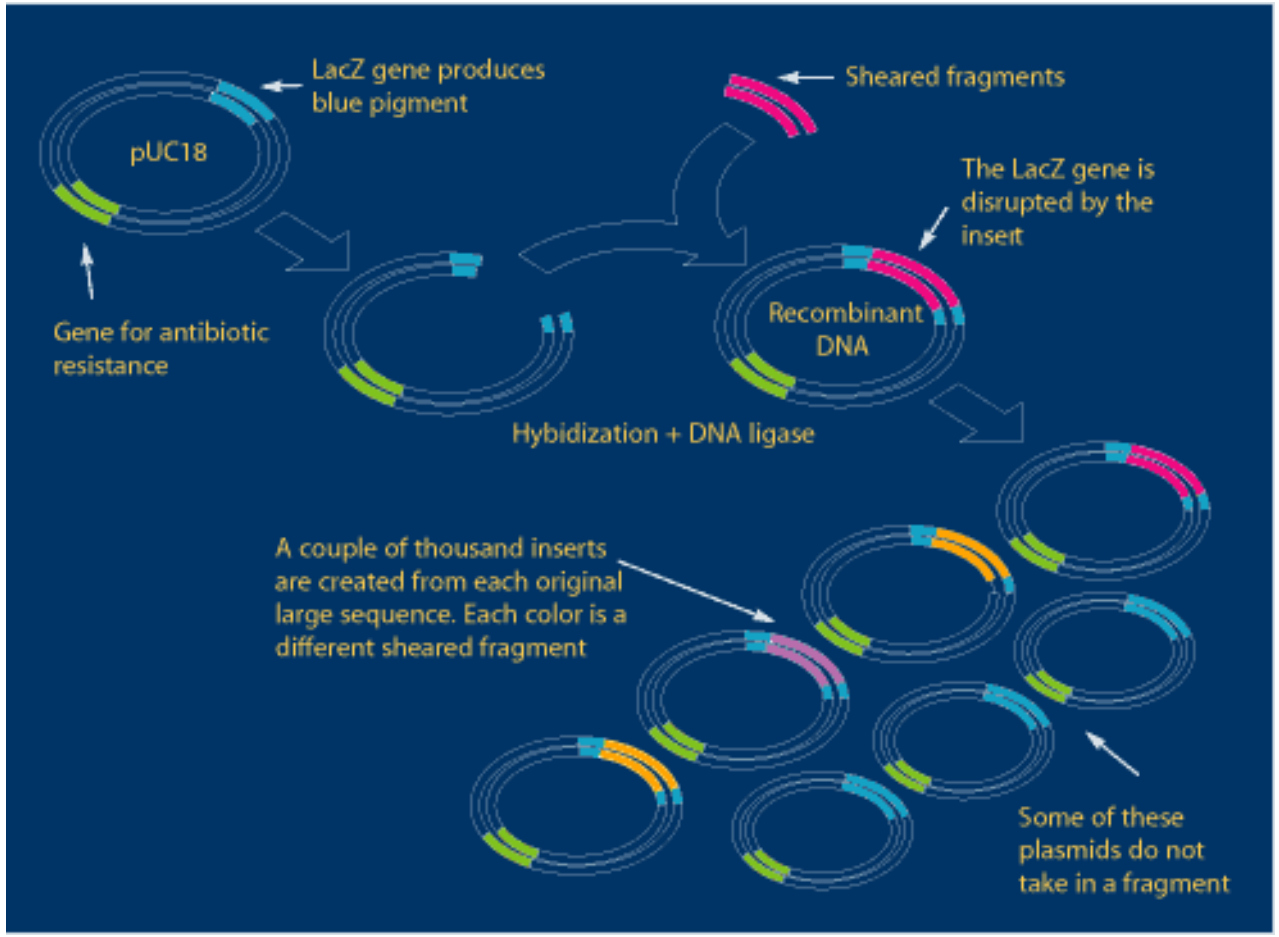
Human genome sequencing with CAE

Step 1: Shearing of DNA



1. The HydroShear takes up the sample containing long strands of source DNA.
2. The sample passes through a narrow tube where it is forced through a small aperture in a ruby, and fragments are randomly snapped (sheared) as the DNA is stretched.
3. Short sheared fragments (2-4 kb) pass through and are collected for the next step.

Step 2: Insertion of Fragments into a Plasmid

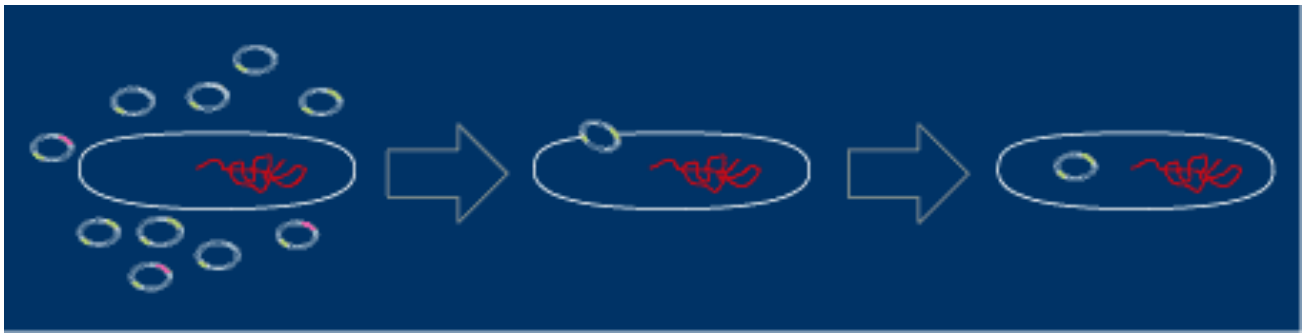


Step 3: Transformation

A cuvette for use in the electroporation machine

An electroporation machine: induces bacteria to open their pores with an electric shock

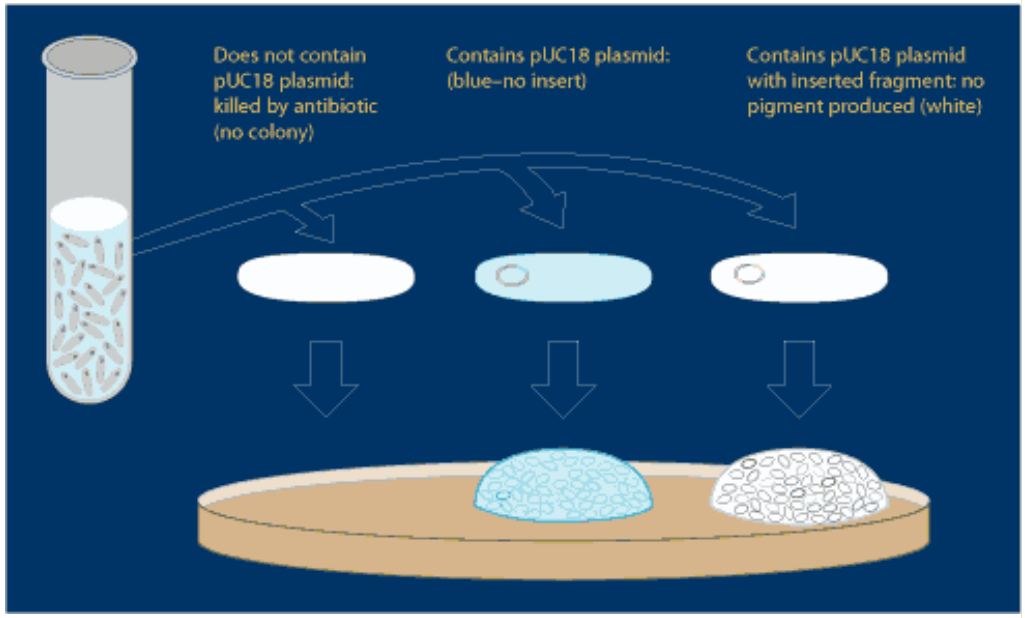
In this machine, the plasmids are encouraged (not very gently!) to enter the bacteria cells. Think of it as a bacterial torture chamber ;^)



Just one?

Step 4: Sub-Cloning the Sheared Fragment

An agar plate with many transformed colonies



Some of the colonies are blue and some are white, depending on whether they were successfully transformed (white) or not (blue)

All the plates containing fragments of the original 150~200-kb piece of DNA now are referred to as a "library."

Step 5: Colony Picking

A 384-well culture stock plate.

Believe it or not, millions of copies of the DNA fragments are just not enough; we need billions!
So each colony needs to be cultured again. This entails picking the good colonies from the agar plates and transferring each one to a well on a 384-well plate.

A technician loads plates into the colony picking machine.

Instead of taking a chance that a technician might go blind picking colonies all day, we use a robot called the Genetix Q-Pix. JGI has four pickers that operate 24 hours a day. The robots' imaging system can discriminate between white and blue colonies. Each colony is put into a separate well that contains 70 μl of a nutrient-rich liquid medium.

All together, JGI can produce 500 384-well plates in a day (but we average 240). That's 192,000 colonies! The plates are incubated for 18-20 hours at 37°C. After incubating, they can be stored at -80°C until they are ready for production sequencing.

Step 6: Lysing the Cell

At this point, our plasmids are still trapped inside the bacterial cell. To get to our original fragment, we need to isolate the plasmid from the rest of the cell. This starts with the PlateMate Plus robot (shown below). The robot takes 2 μl from the 384-well culture stock plate and puts it onto a new 384-well plate. Each well on the new plate also contains 8 μl of Amersham denature buffer.

The PlateMate Plus robot transfers colonies from 384-well stock plates to new 384-well plates for lysing.

The 384-well plates containing the denature buffer and bacteria from the culture stock plate are placed in a thermal cycler at 95°C for five minutes. At this point, the cells "lyse," meaning that their plasma membrane breaks and the cellular contents spill out into solution.

Thermal cyclers heat up bacterial cells to break them open.

Step 7: Rolling-Circle Amplification

Now that the plasmid is free of the cell, we need to amplify (make many, many copies of) just the fragment-containing pUC18 plasmid. This is achieved by a reaction called rolling-circle amplification, or RCA. If you are familiar with PCR (the polymerase chain reaction), then it may help you to know that RCA is very similar. The end result of RCA will be millions of snowflake-like structures, each containing several copies of the plasmid DNA.

New strands of DNA matching the DNA in the plasmid are formed in connected arcs as polymerase "rolls" around the circular plasmid (center ring).

To prepare the reaction, 10 μl of Amersham TempliPhi reagent is added to each of the 384 wells containing only 2 μl of the plasmid. The TemliPhi reagent consists of

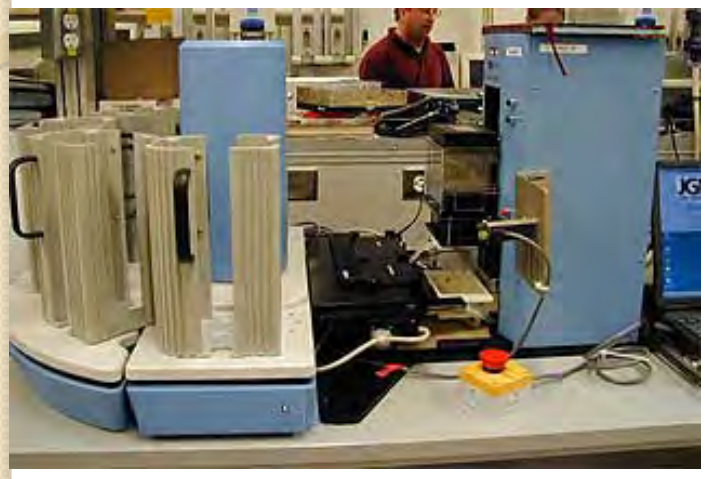
- dNTPs (the four nucleotides, A, T, G, and C)
- phi29 polymerase (an enzyme that adds nucleotides to a strand of DNA)
- random primers (which give polymerase a place to start building)

The reaction is incubated for 18 hours at a constant 30°C.

An incubator is used for the RCA reaction.

Step 8: Sequencing Chemistry

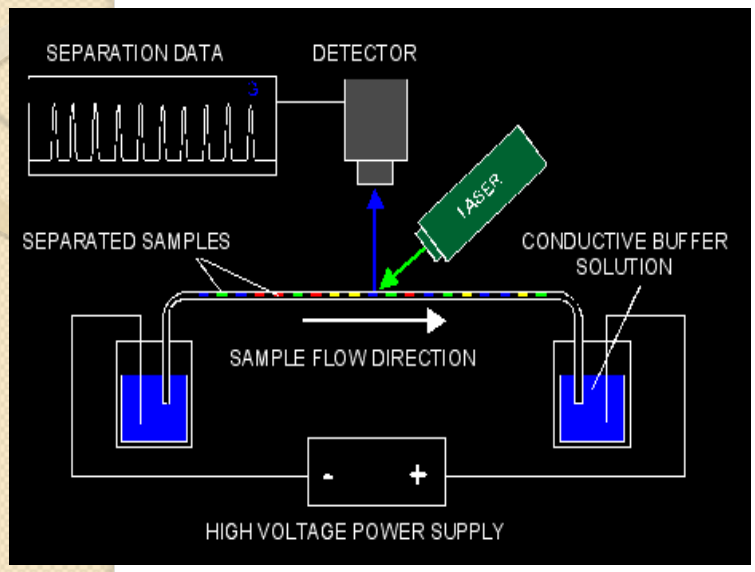
Only 1 μl from the RCA reaction plate is transferred to a new 384-well plate using the Robbins Twister robot.

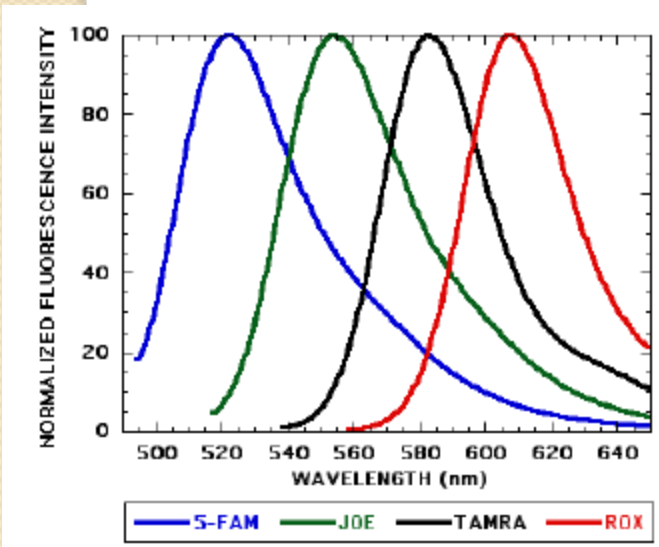


The Robbins Twister robot transfers samples from RCA reaction plates.

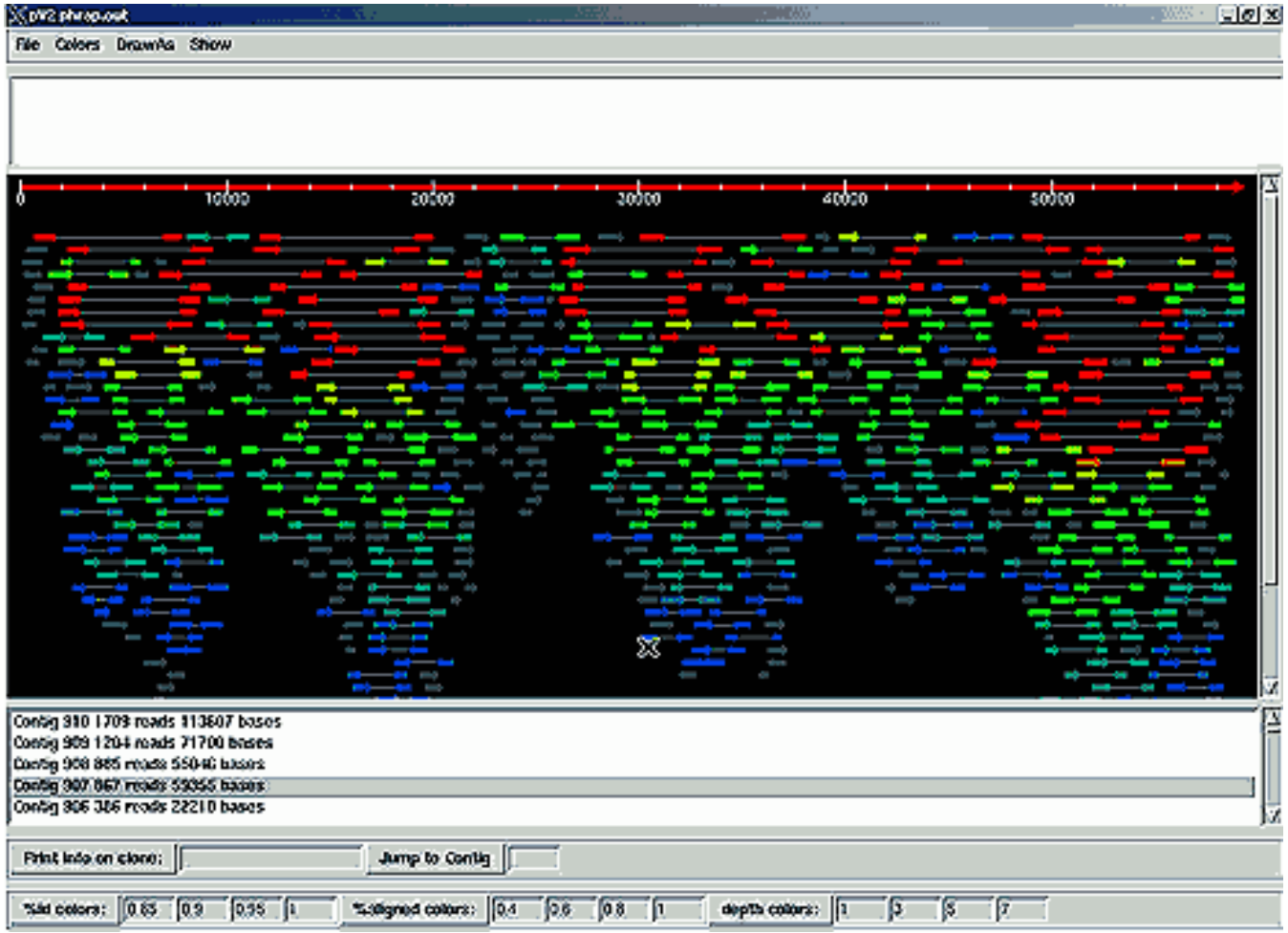
Cavro Dispensing Robot

Step 9: Capillary Array Electrophoresis





Step 10: Assembly



Assembling sequenced reads in Phrap.

Reads are aligned to form a "consensus read." Phrap scores for each base in the consensus are assigned by using Phred scores for bases at that position sequencing chemistry read orientation depth of read

A Farewell to Sanger

After a decade of service, the Sanger sequencing line was shut down at the DOE JGI the week of October 11, 2010. This video was produced in tribute to the people and the process who made sequencing projects such as the Human Genome Project, the poplar tree and the frog possible.

Cost: $Millions/genome
Time: months

goal

Cost: $1,000/genome
Time: hours

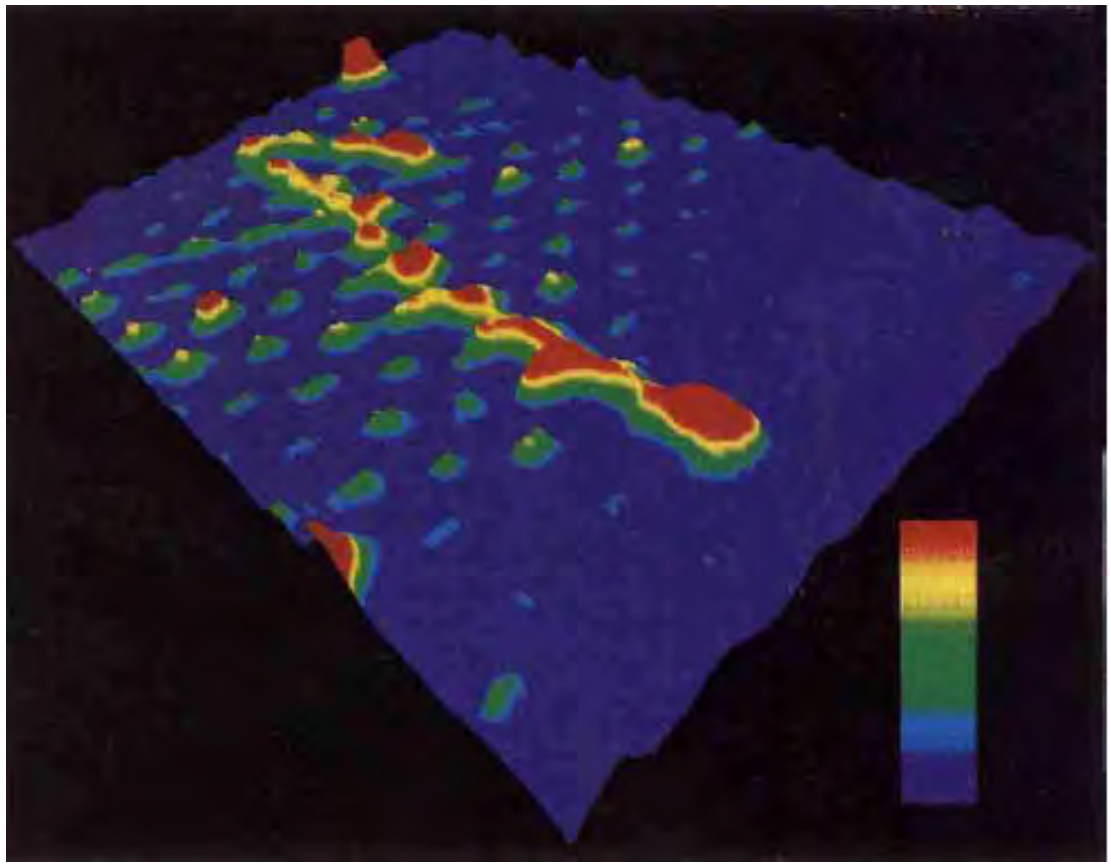
LETTERS TO NATURE

Nature 358, 600 (1992)

DNA electrophoresis in microlithographic arrays

W. D. Volkmuth & R. H. Austin

Department of Physics, Princeton University, Princeton, New Jersey 08544, USA



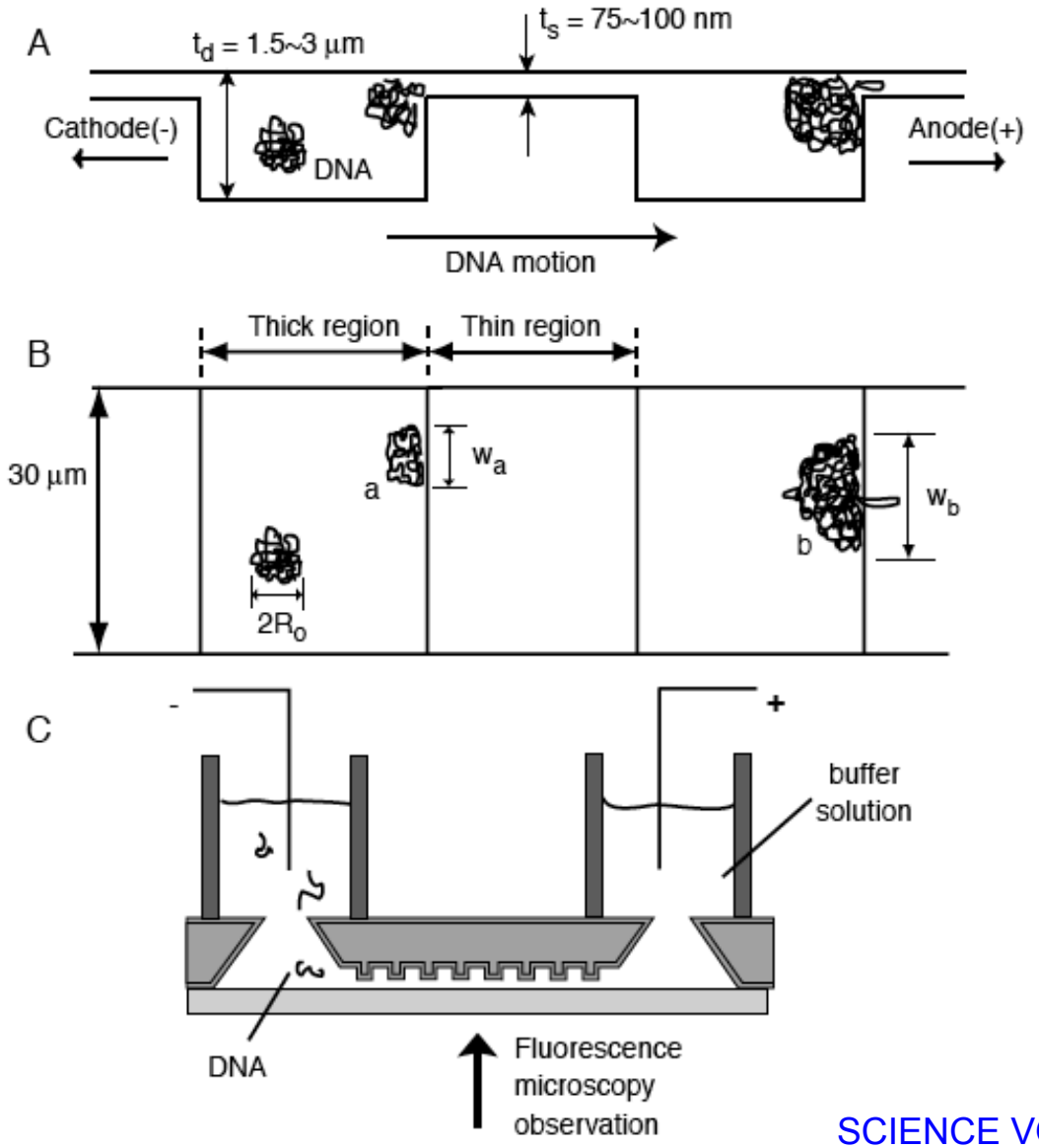
SCIENCE VOL 288 12 MAY 2000 1026-1029

Separation of Long DNA Molecules in a Microfabricated Entropic Trap Array

J. Han and H. G. Craighead*

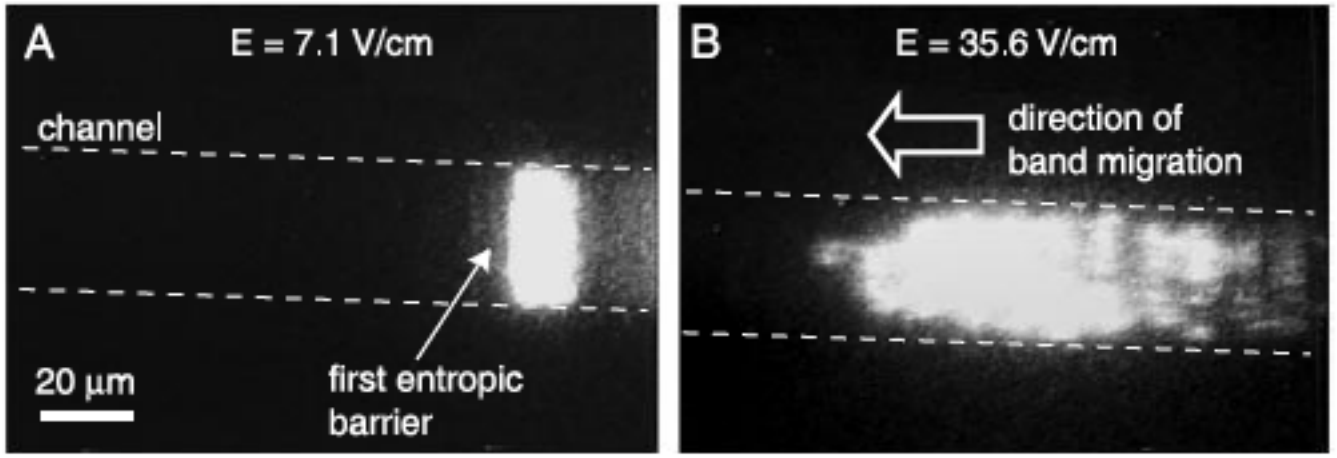
A nanofluidic channel device, consisting of many entropic traps, was designed and fabricated for the separation of long DNA molecules. The channel comprises narrow constrictions and wider regions that cause size-dependent trapping of DNA at the onset of a constriction. This process creates electrophoretic mobility differences, thus enabling efficient separation without the use of a gel matrix or pulsed electric fields. Samples of long DNA molecules (5000 to ~160,000 base pairs) were efficiently separated into bands in 15-millimeter-long channels. Multiple-channel devices operating in parallel were demonstrated. The efficiency, compactness, and ease of fabrication of the device suggest the possibility of more practical integrated DNA analysis systems.

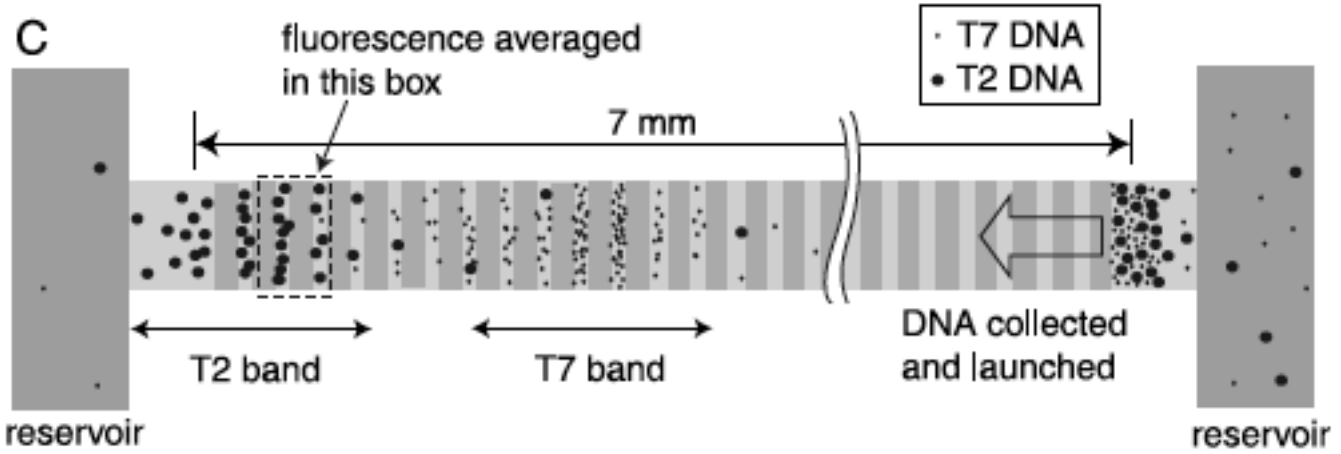
Nanofluidic DNA separation device with entropic trapping array



SCIENCE VOL 288 (2000) 1026

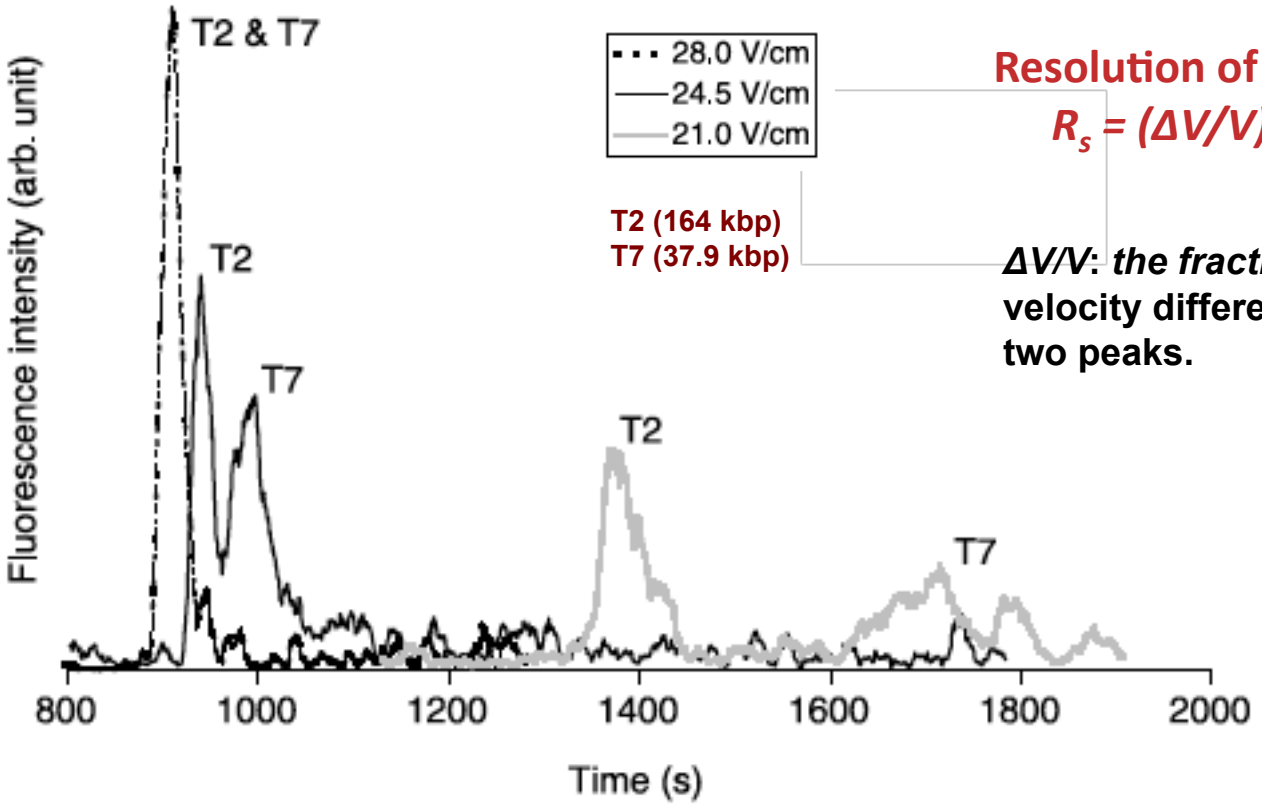
Band launching and analysis of bands





SCIENCE VOL 288 (2000) 1026

Electric field dependence of the separation of T2-T7 DNA mixture



Resolution of separation:

$R_s = (\Delta V/V)\sqrt{(N/16)}$

***ΔV/V*: *the fractional band* velocity difference between two peaks.**

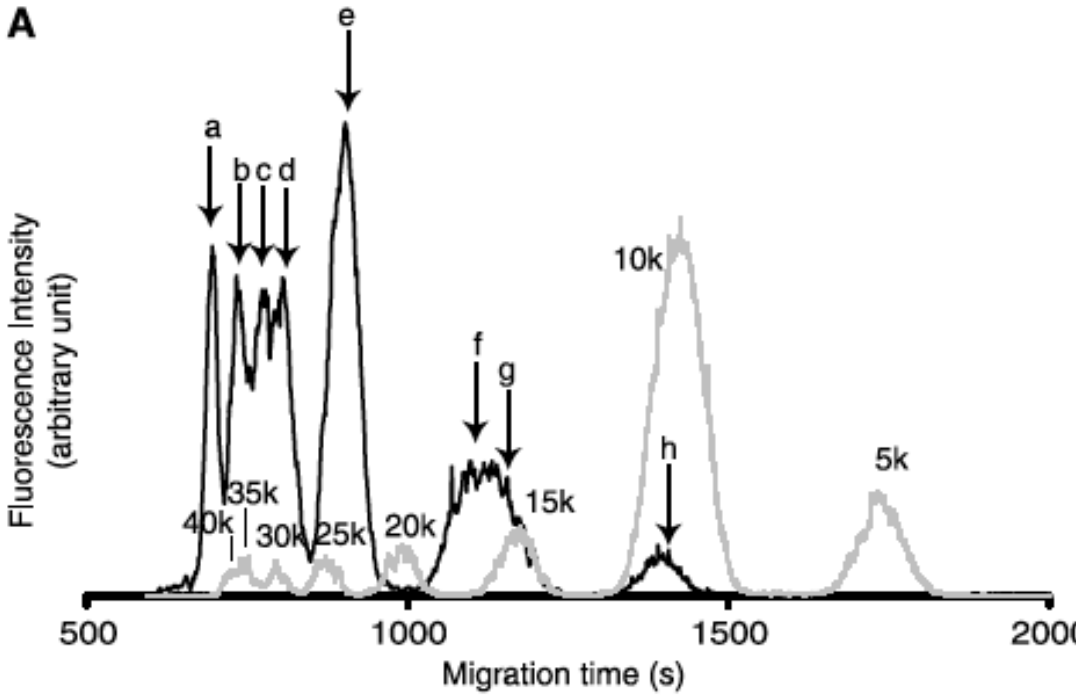
At 21.0 V/cm (gray line), the theoretical plate number (*N*) *was* 4900 for the T2 peak and 970 for the T7 peak, and the resolution was 1.95.

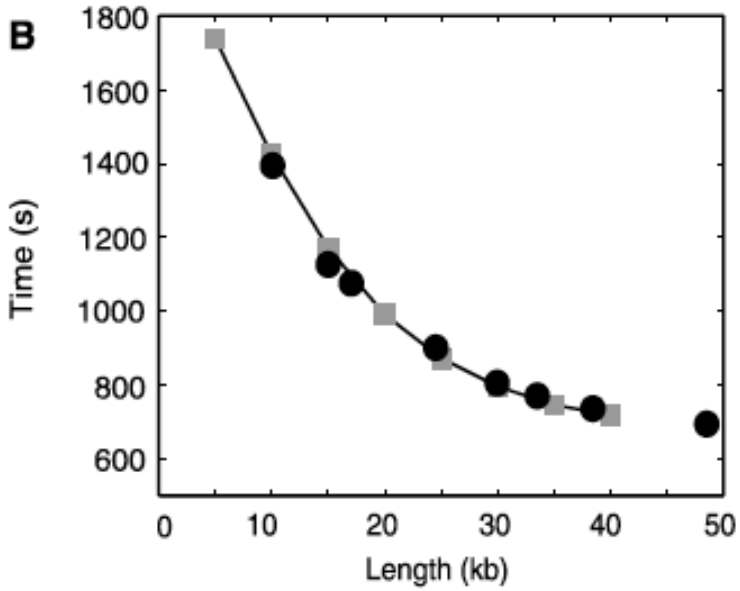
At 24.5 V/cm (black line), *N = 8500 for the T2 peak* and 3400 for the T7 peak, and the resolution was 0.89.

At 28.0 V/cm, no separation was achieved (broken line).

SCIENCE VOL 288 (2000) 1026

Simultaneous separation of the Mono Cut Mix sample (black line) and 5-kbp ladder sample (gray line) by the entropic trap array, run at 80 V/cm.





Large molecules move faster!!

SCIENCE VOL 288 (2000) 1026

On-chip pulsed-field electrophoresis

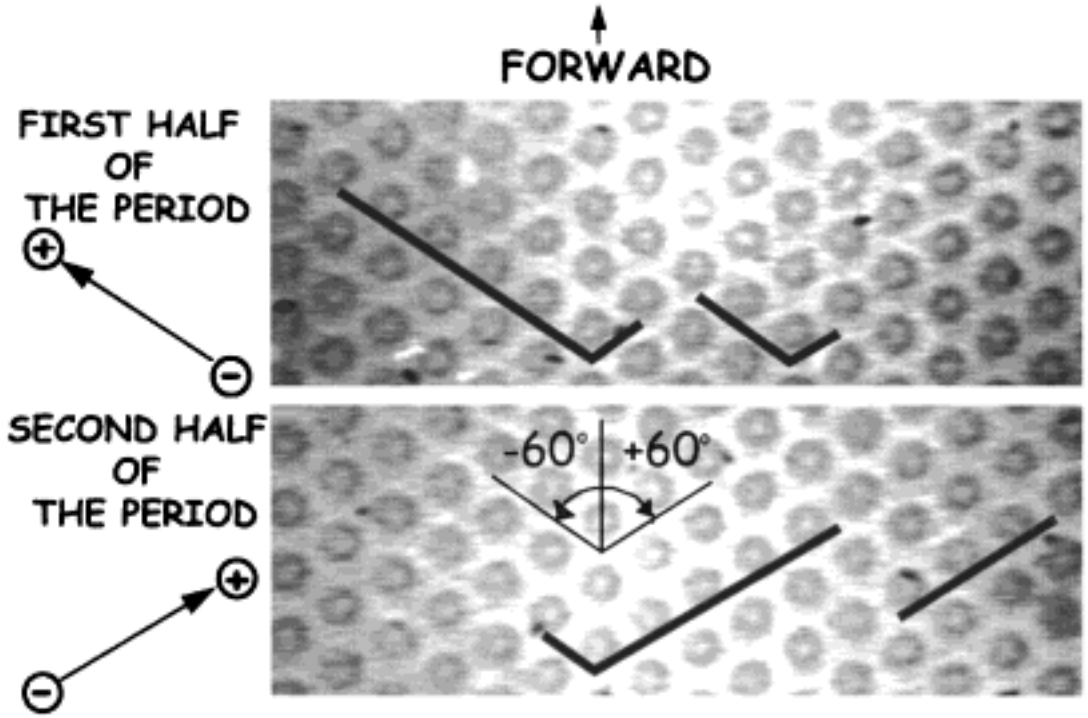
Separation of 100-Kilobase DNA Molecules in 10 Seconds

Anal. Chem. 2001, 73, 6053-6056

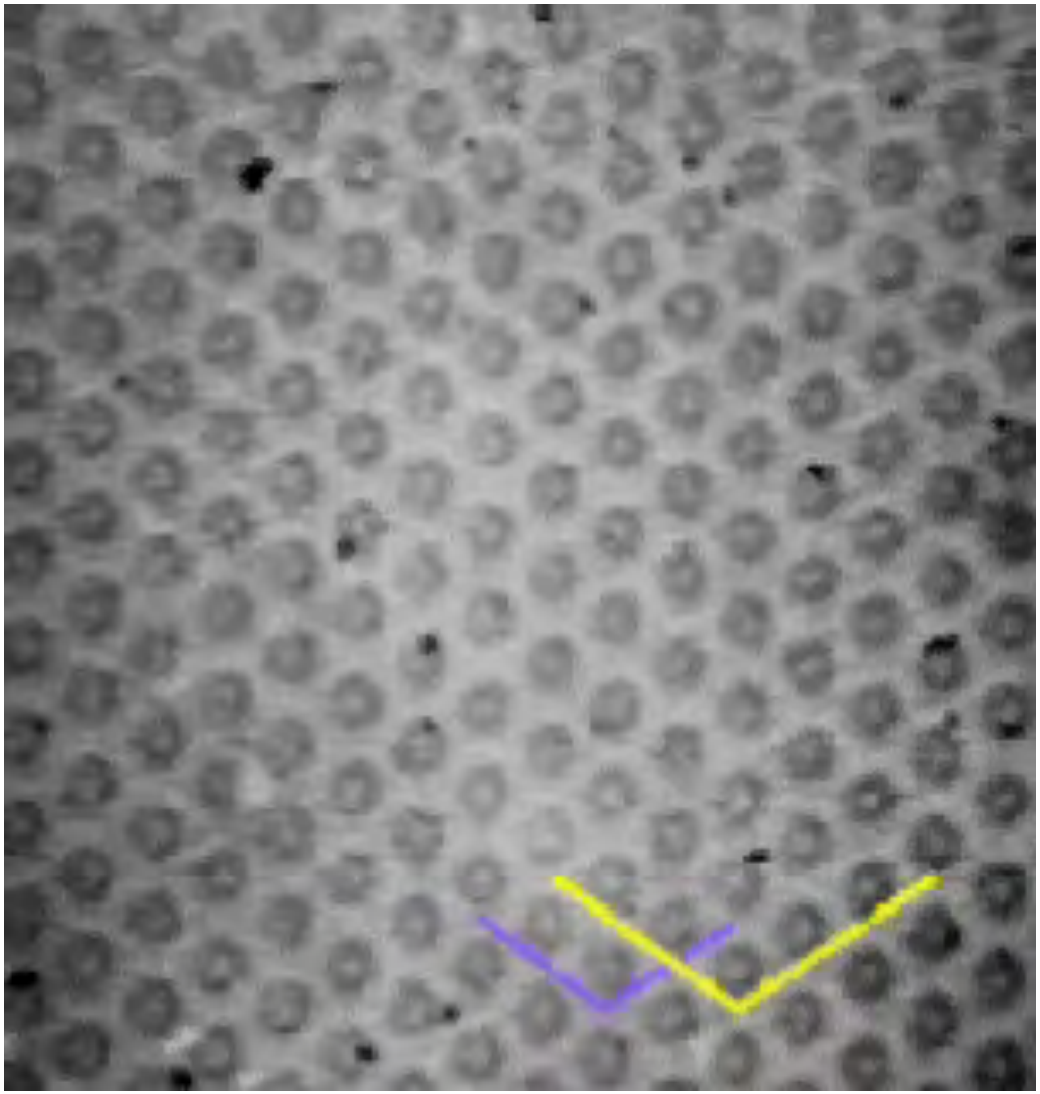
Olgica Bakajin,[†,||] Thomas A. J. Duke,[‡] Jonas Tegenfeldt,[†] Chia-Fu Chou,[†] Shirley S. Chan,[†] Robert H. Austin,[*,†] and Edward C. Cox[§]

Physics Department, Princeton University, Princeton, New Jersey 08544, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, U.K., and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

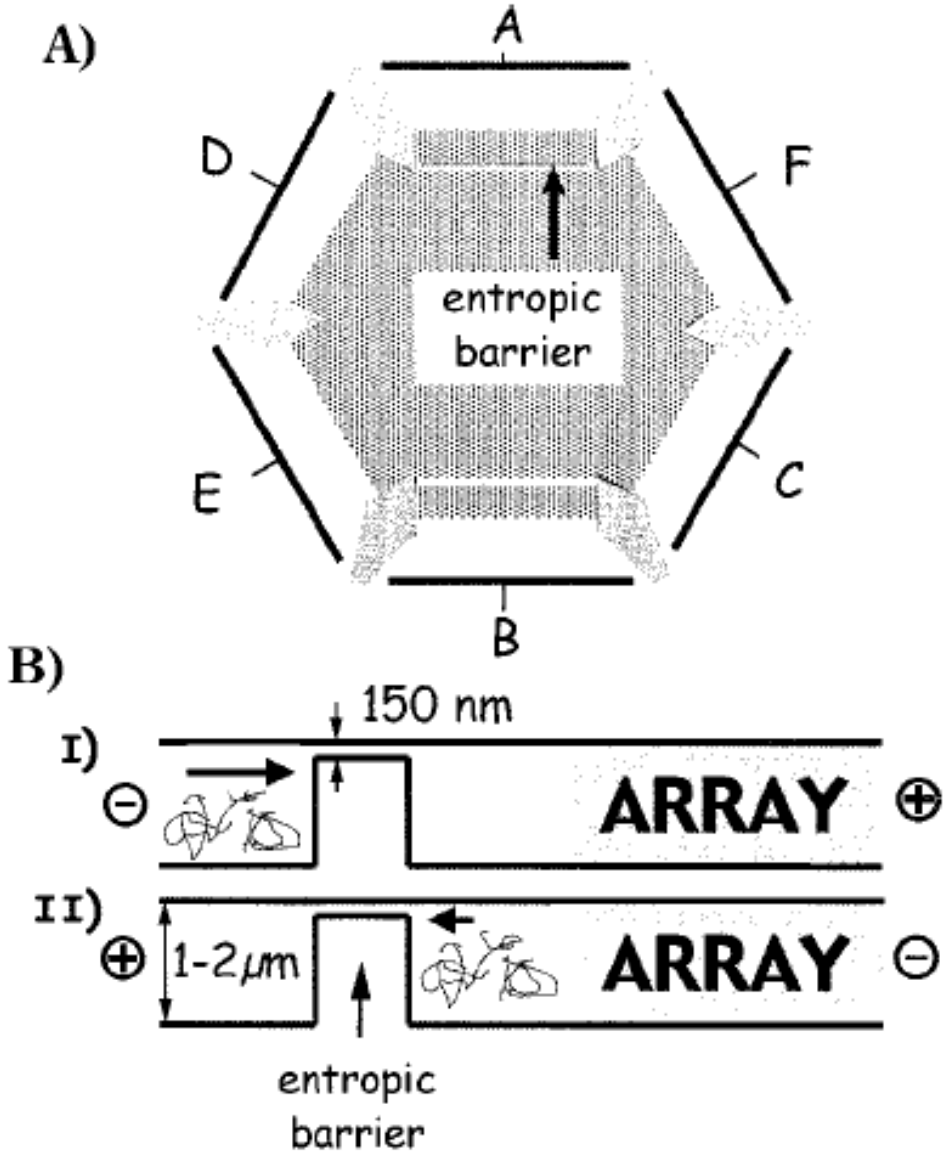
Microscopic view of the pulsed hexagonal array



On-chip pulsed-field electrophoresis (animation)

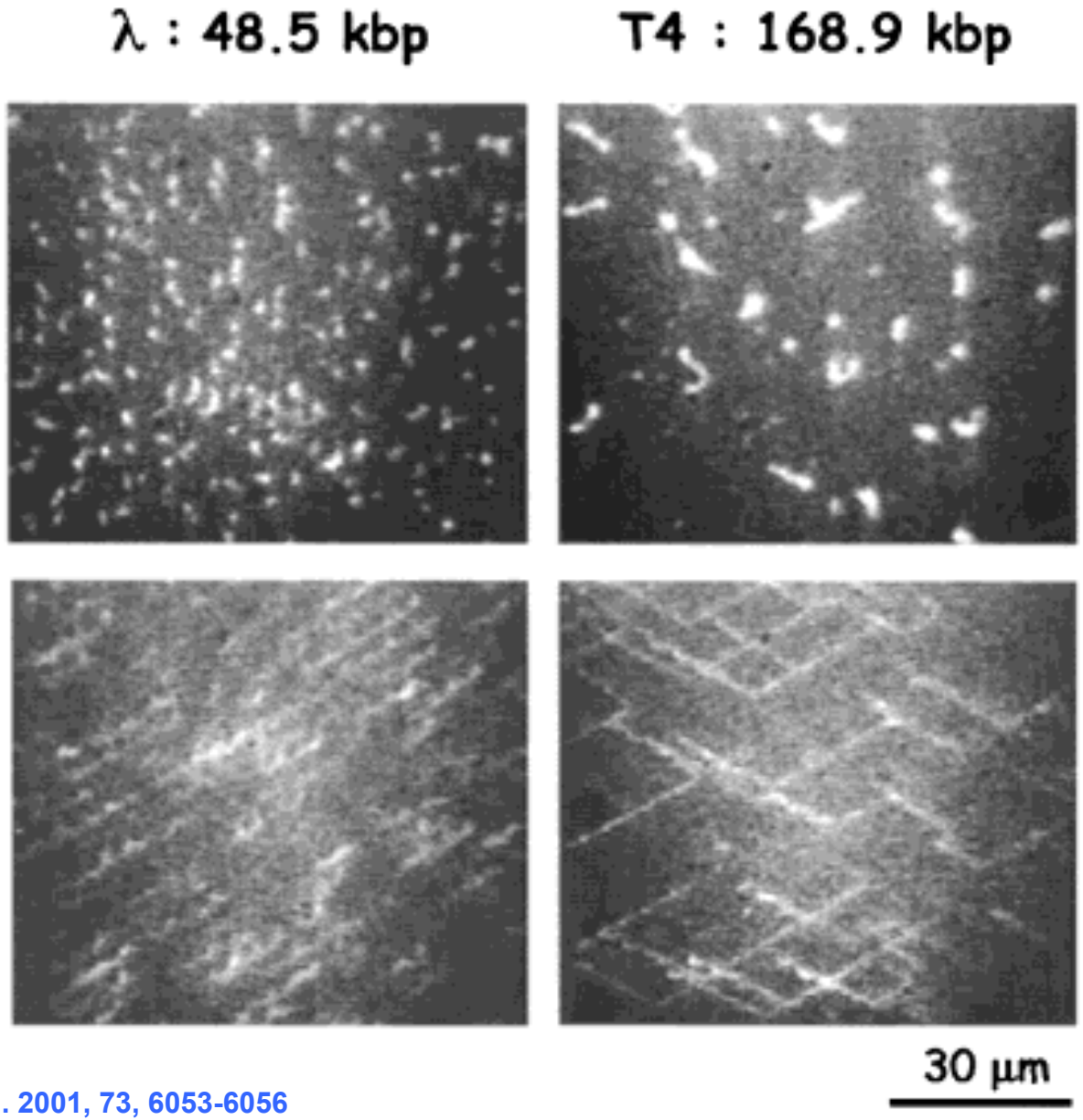


Device configuration of a pulsed hexagonal array



Anal. Chem. 2001, 73, 6053-6056

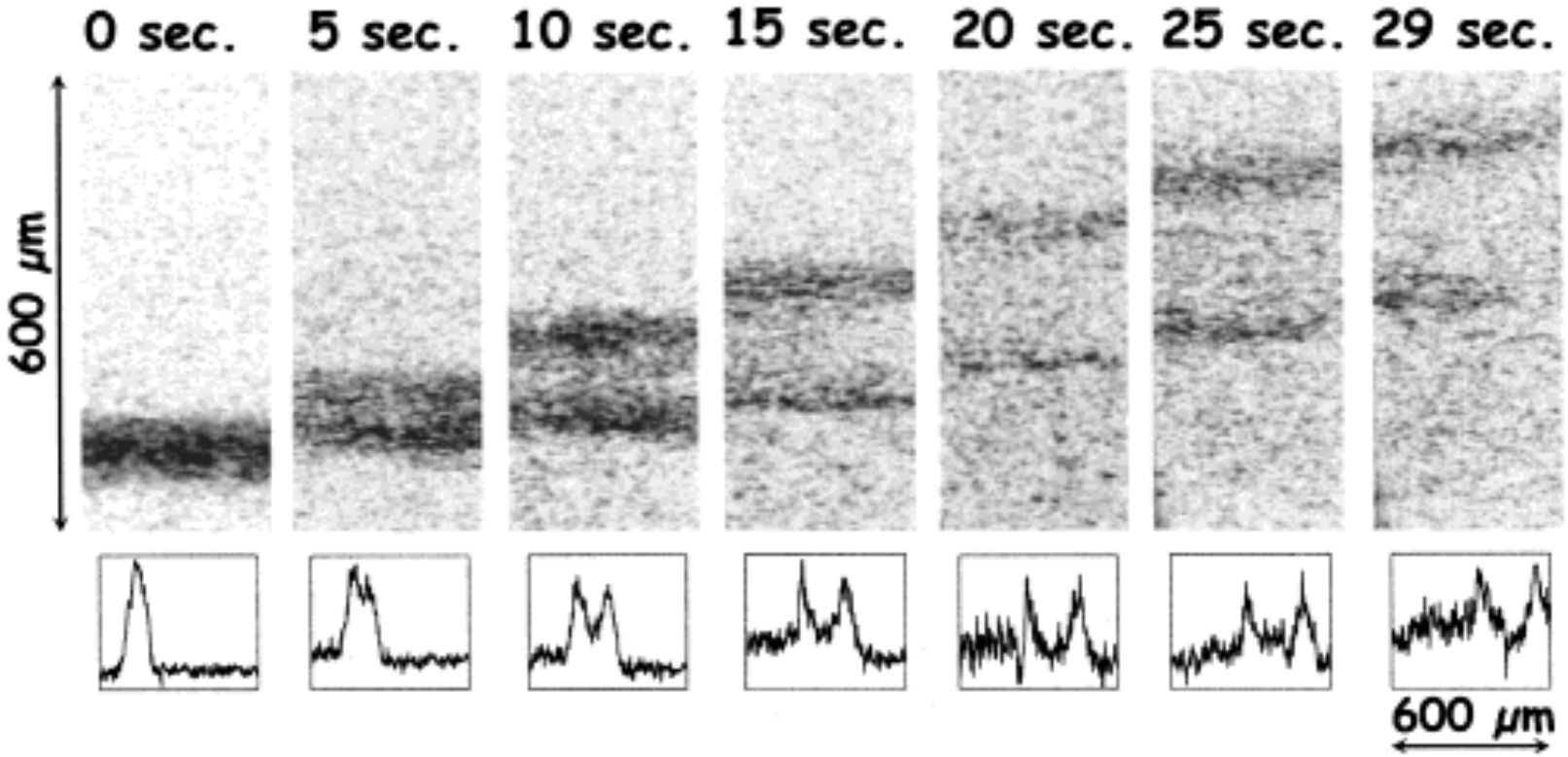
Single-molecule images



Anal. Chem. 2001, 73, 6053-6056

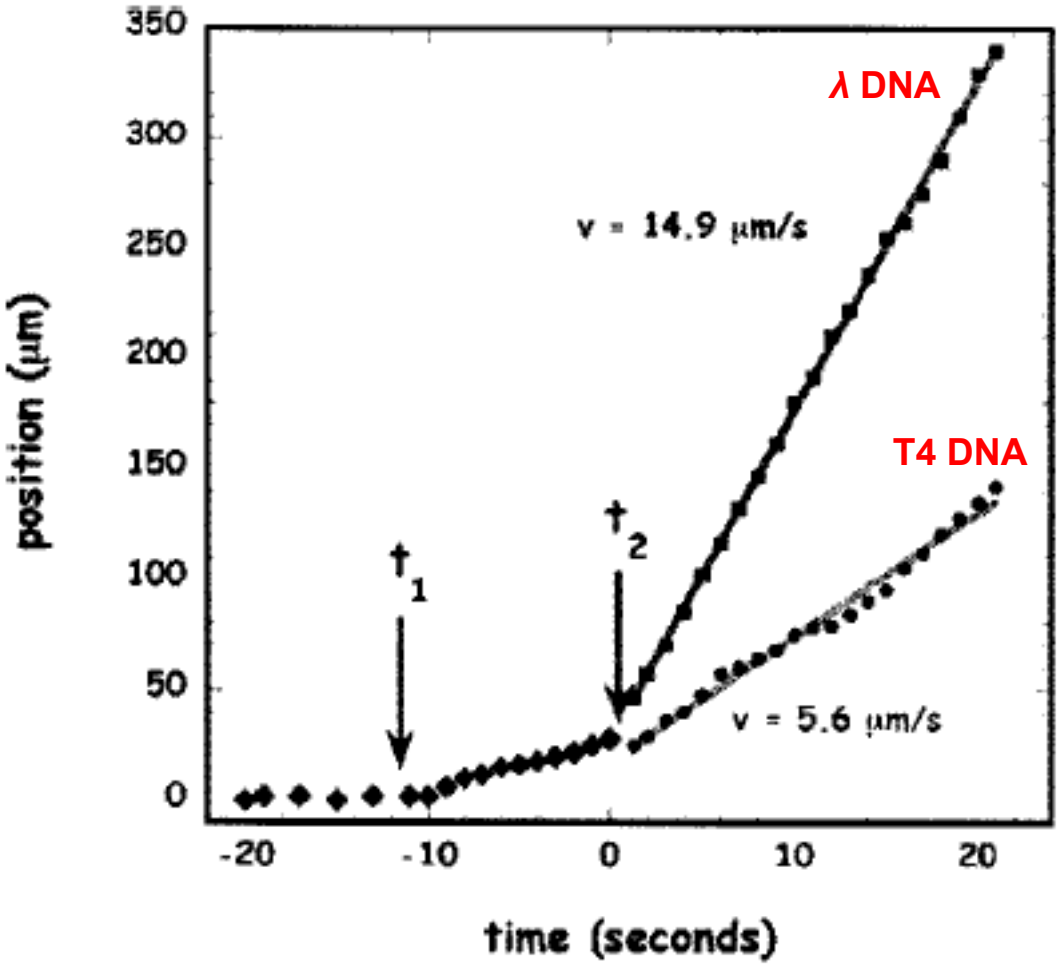
λ and T4 DNA separation

***λ and* T4 DNA pulsed at 244 V and with period T = 1 s after release from the entropic trap**



Anal. Chem. 2001, 73, 6053-6056

DNA position as a function of time

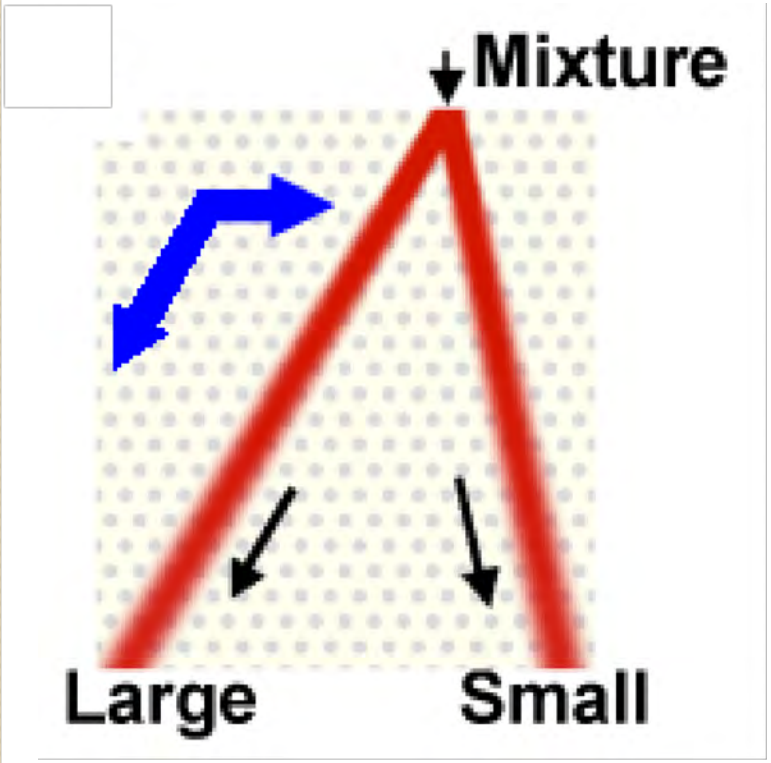


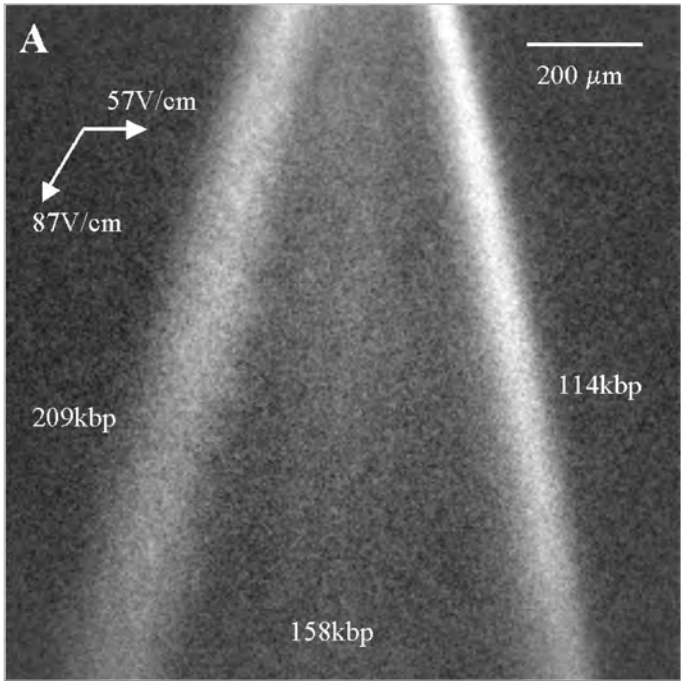
Anal. Chem. 2001, 73, 6053-6056

DNA Prism

Asymmetric pulsed fields:

The direction of the net flow motion of the DNA is angle-dependent.





Continuous separation of BAC/PAC mixture of 114, 158, and 209 kbp fragments (Huang *et al.*, Nat. Biotech. 2001)