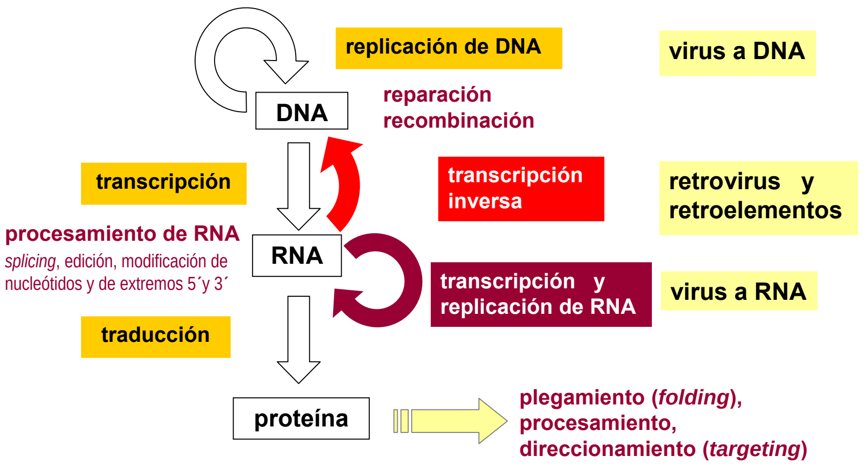
Genética Molecular

Email: genmol@gmail.com
Password: cromatina

“Dogma central de la biología molecular”



Víctor

ácidos nucleicos

estructura, estabilidad,
hibridación…

Alternativas de
tipos de ácidos nucleicos:

- DNA o RNA
- doble o simple cadena: ds, ss
- lineal o circular
- híbridos DNA:RNA

GENOMAS DE VIRUS: CLASIFICACIÓN

Considerando el tipo de organismo que parasitan

Los virus pueden clasificarse en:

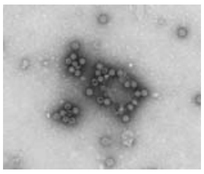
- Bacteriófagos o fagos: virus que parasitan a bacterias
- Virus animales
- Virus vegetales

Desde el punto de vista genético

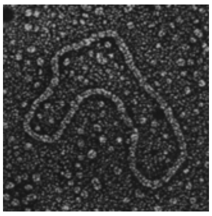
- Virus cuyo material hereditario es DNA (ss o ds).
- Virus cuyo material hereditario es RNA (ss o ds).
- Virus cuyo material hereditario es DNA-RNA

SV40 (*Papovavirus*)

tiene una cápside icosaédrica



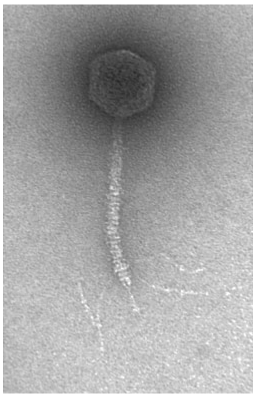
ADN circular doble hélice de
5.243 pares de nucleótidos.

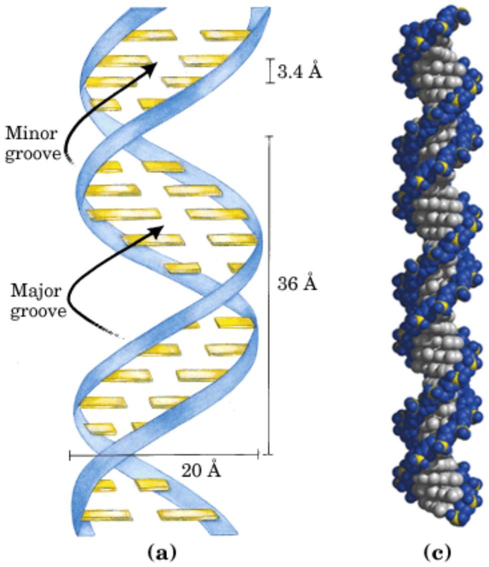


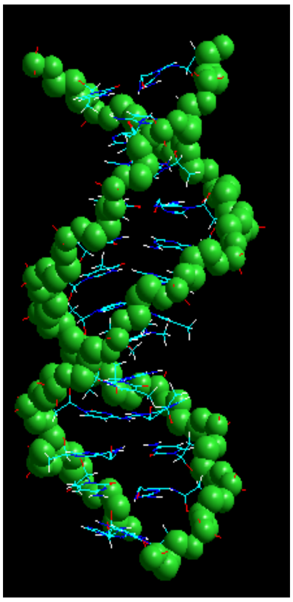
Su ADN se asocia con las
histonas de la célula huésped

fago λ

El fago λ posee una cápside icosaédrica con una cola.
Dentro de la cápside existe una molécula de ADN doble hélice lineal con 48.000 pares de bases con extremos cohesivos > circularización.

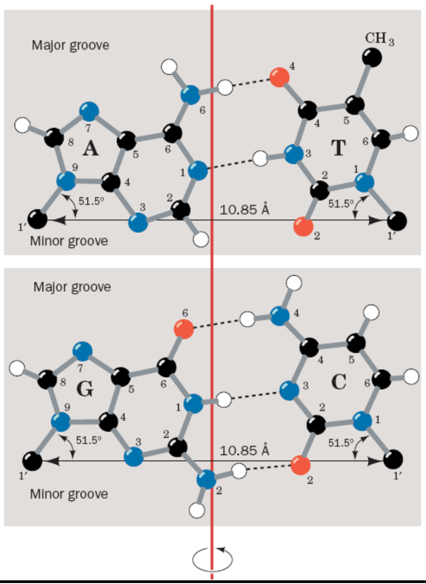


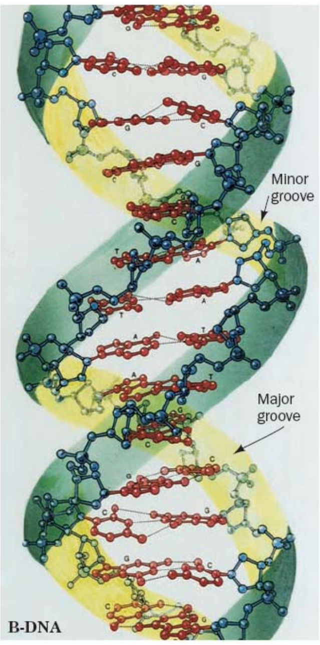




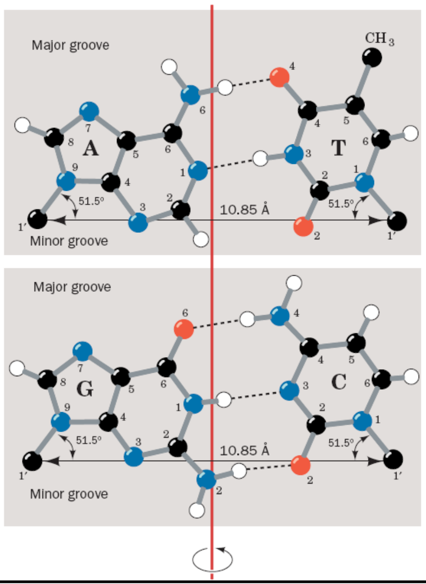
- El apareamiento de las dos cadenas genera un surco mayor y un surco menor en la superficie de la doble hèlice

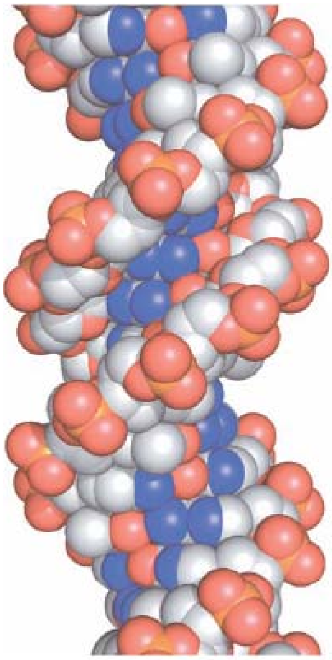
Doble hélice con un surco mayor y un surco menor





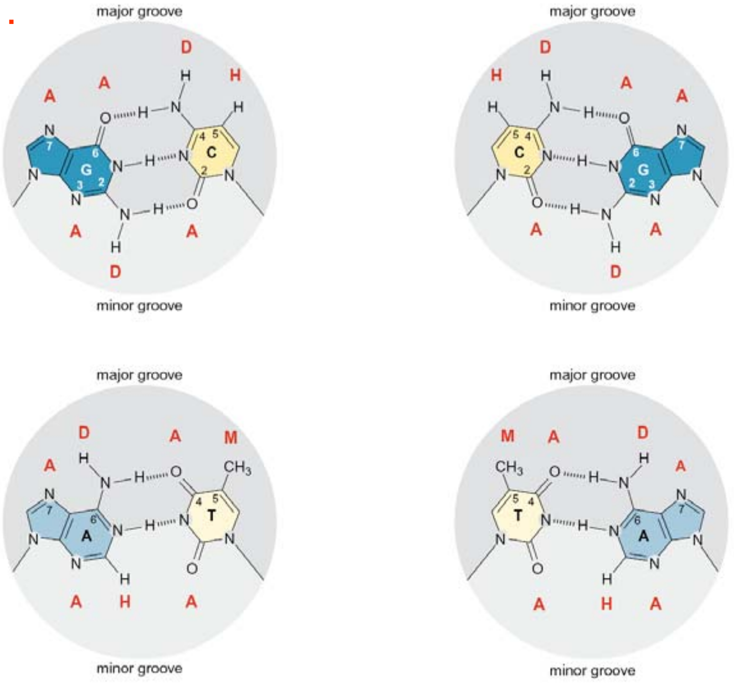
Doble hélice con un surco mayor y un surco menor

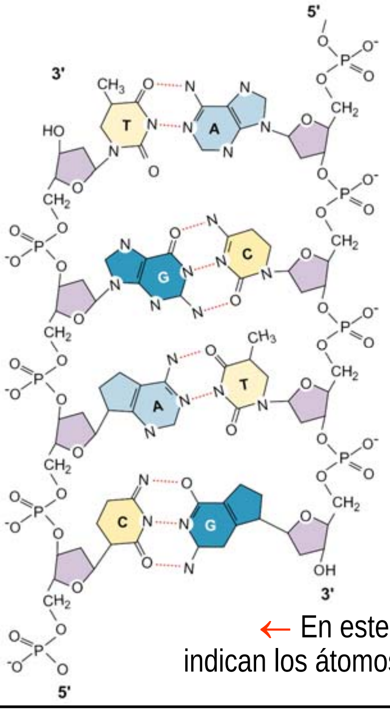


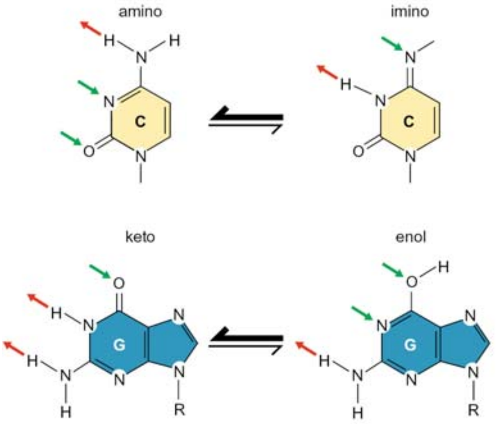


B-DNA

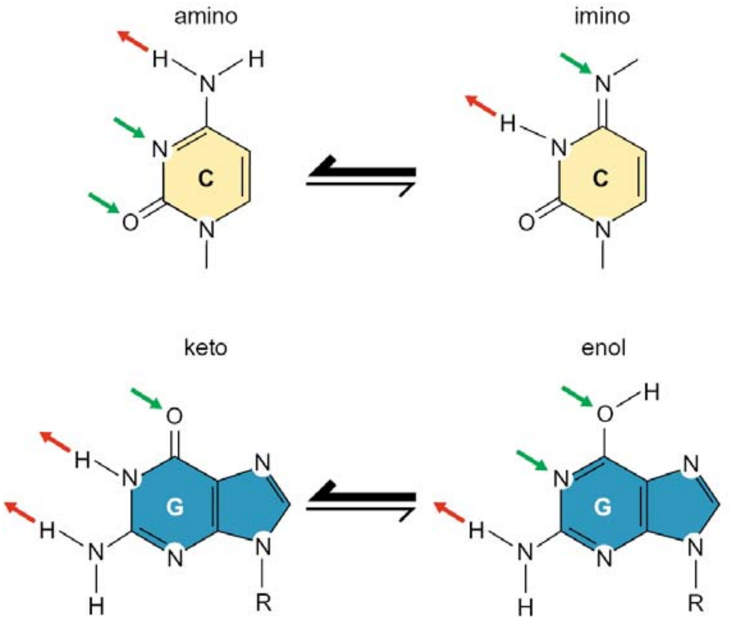
surcos…



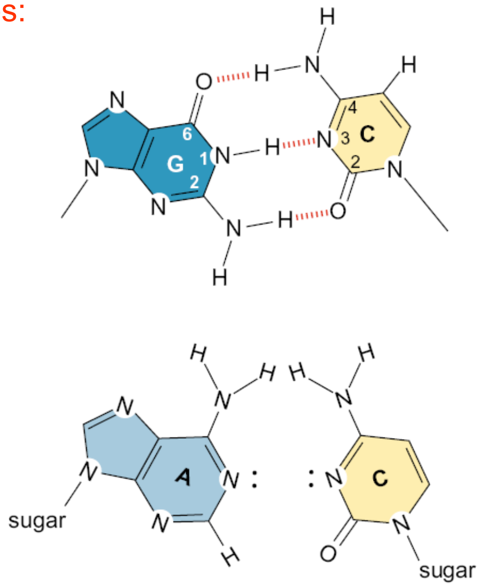




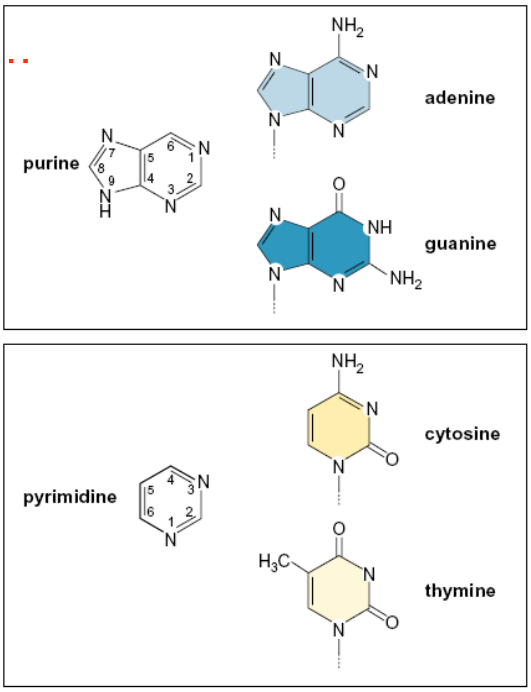
← En este esquema de la doble hebra de DNA no se indican los átomos de H que participan de los puentes de H



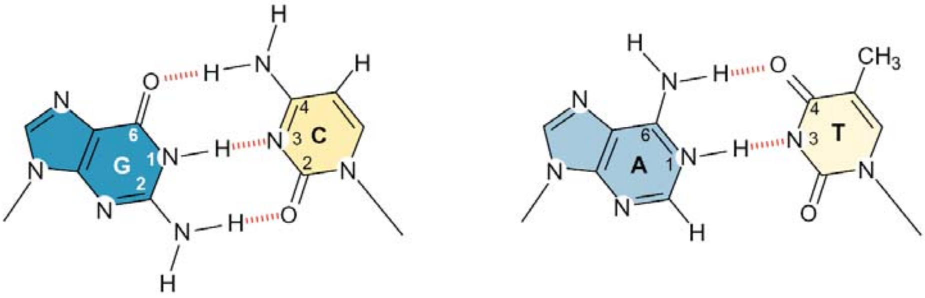
Distancia entre las bases: puentes de H

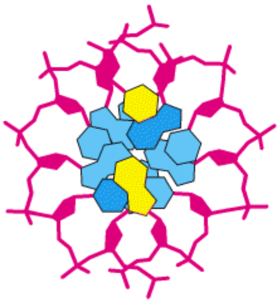


Descubra el error…

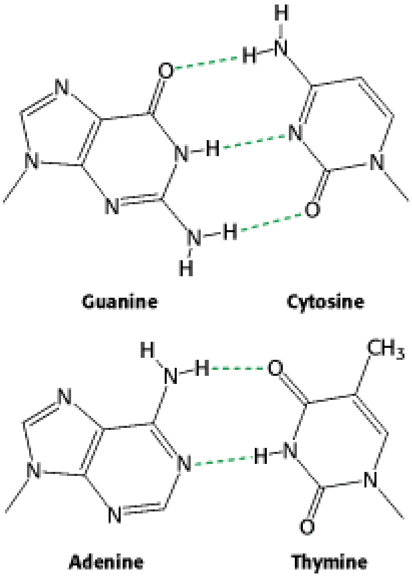


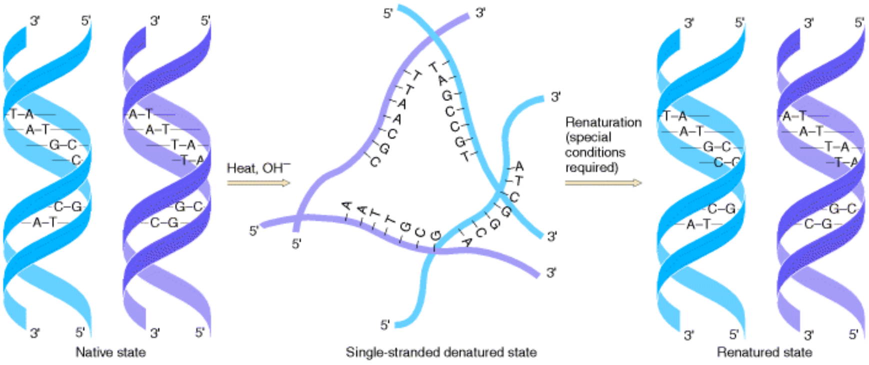
Descubra el error…



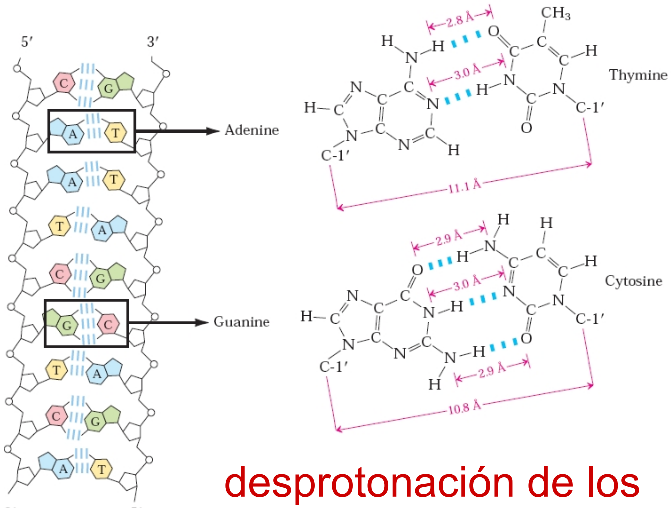


Axial view of DNA. Base pairs are stacked nearly one on top of another in the double helix.



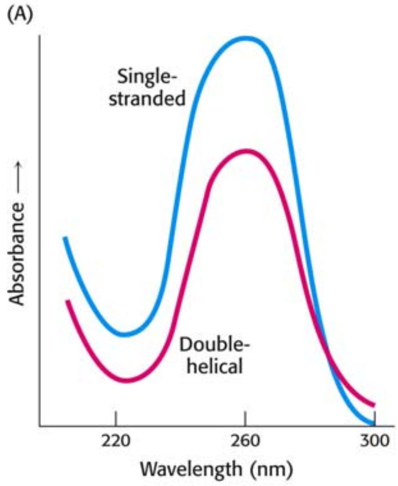


dsDNA en medio alcalino fuerte



desprotonación de los
>N-H de los heterociclos ➡ ¿…?

Efecto hipercrómico



ssDNA
(desnaturalizado a 90 ºC))

⇧

dsDNA
(nativo a 28 ºC)

The purine and pyrimidine bases in DNA absorb UV light maximally at a wavelength of approximately **260 nm.** In double-stranded DNA, however, the absorption is decreased due to base-stacking interactions. When DNA is denatured, these interactions are disrupted and an increase in absorbance is seen. This change is called the **hyperchromic effect**. The extent of the effect can be monitored as a function of temperature

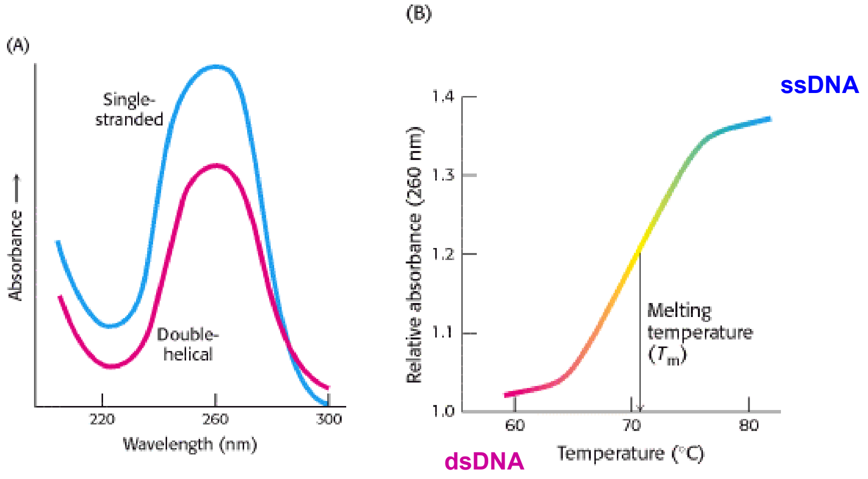
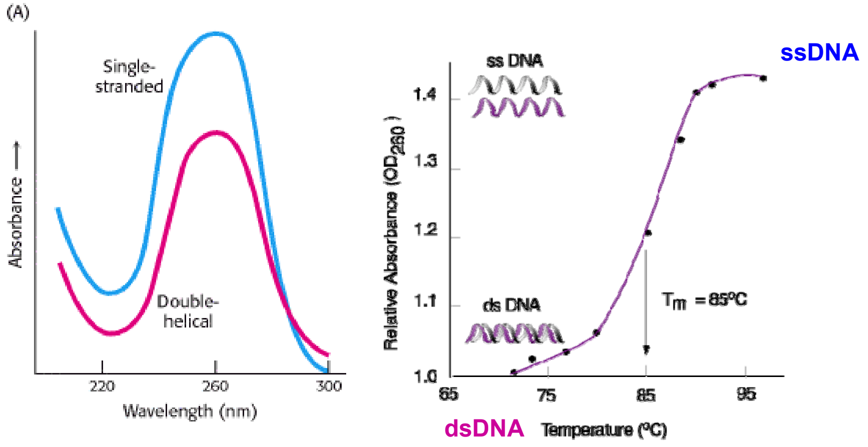


Figure 5.17. **Hyperchromic effect**. **(A) Single-stranded DNA absorbs light more effectively than does double-helical DNA. (B) The absorbance of a DNA solution at a wavelength of 260 nm increases when the double helix is melted into single strands.**



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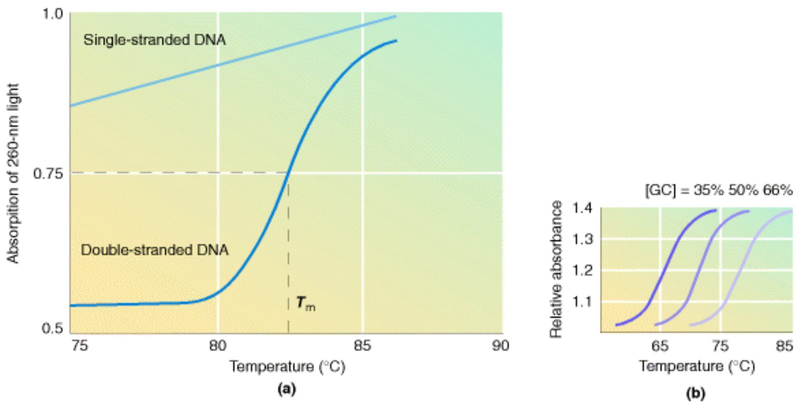


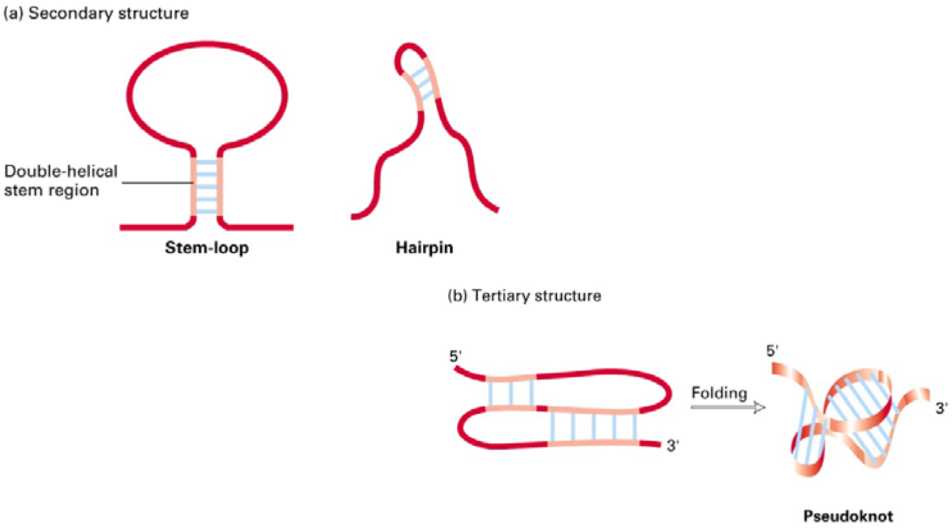
Figure 8-40. (a) The absorption of ultraviolet light of 260-nm wavelength by solutions of single-stranded and double-stranded DNA. As regions of double-stranded DNA unpair, the absorption of light by those regions increases almost twofold. The temperature at which half the bases in a double-stranded DNA sample have denatured is denoted *Tm*. (b) DNA melting curves. The absorbance relative to that at 25°C is plotted against temperature. (The wavelength of the incident light was 260 nm.) The *Tm* is 69°C for *E. coli* DNA (50 percent GC pairs) and 76°C for *Pseudomonas aeruginosa* DNA (68 percent GC pairs). (Part b from L. Stryer, *Biochemistry,* 4th ed. Copyright © 1995 by Lubert Stryer.)

Figure 5.19. Stem-Loop Structures. Stem-loop structures may be formed from single-stranded DNA and RNA molecules.

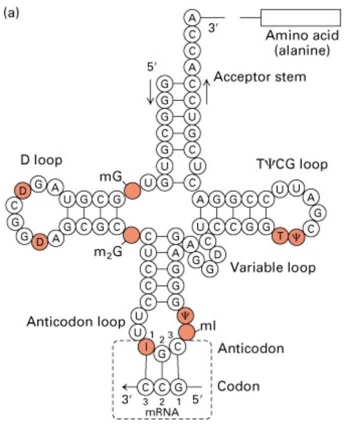
The simplest and most common structural motif formed is a *stem-loop*, created when two complementary sequences within a single strand come together to form double-helical structures (Figure 5.19). In many cases, these double helices are made up entirely of Watson-Crick base pairs. In other cases, however, the structures include mismatched or unmatched (bulged) bases. Such mismatches destabilize the local structure but introduce deviations from the standard double-helical structure that can be important for higher-order folding and for function (Figure 5.20).

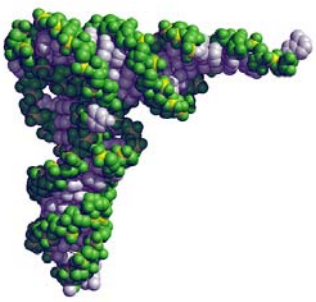
Single-stranded nucleic acids can adopt structures more complex than simple stem-loops through the interaction of more widely separated bases. Often, three or more bases may interact to stabilize these structures. In such cases, hydrogen-bond donors and acceptors that ordinarily participate in Watson-Crick base pairs may participate in hydrogen bonds of nonstandard pairings. Metal ions such as magnesium ion (Mg2+) often assist in the stabilization of these more elaborate structures.

Las moléculas de ssRNA pueden exhibir conformaciones variadas



Estructuras secundarias en RNA





tRNA

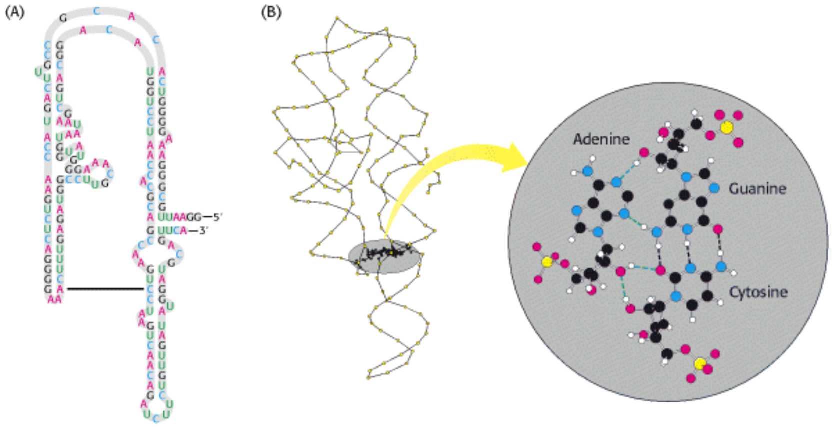
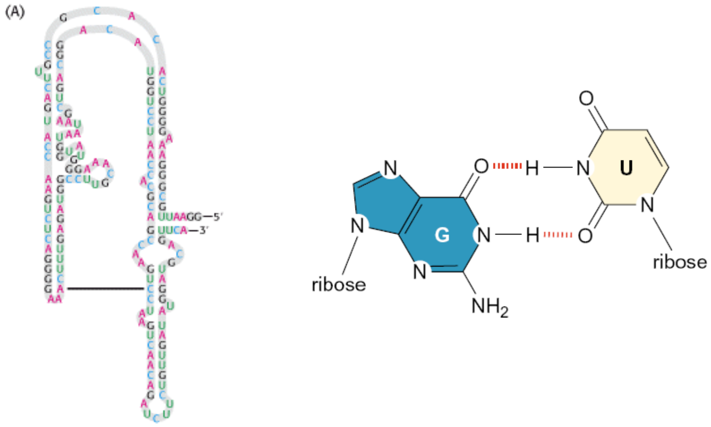


Figure 5.20. Complex Structure of an RNA Molecule. A single-stranded RNA molecule may fold back on itself to form a complex structure. (A) The nucleotide sequence showing Watson-Crick base pairs and other nonstandard base pairings in stem-loop structures. (B) The three-dimensional structure and one important long-range interaction between three bases. Hydrogen bonds within the Watson-Crick base pair are shown as dashed black lines; additional hydrogen bonds are shown as dashed green lines



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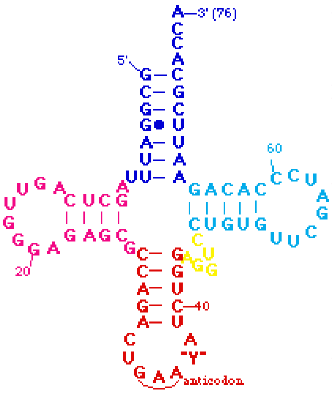
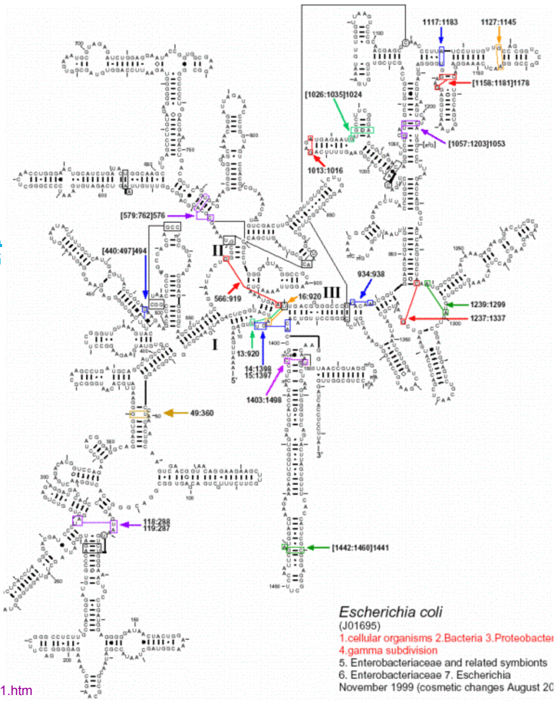
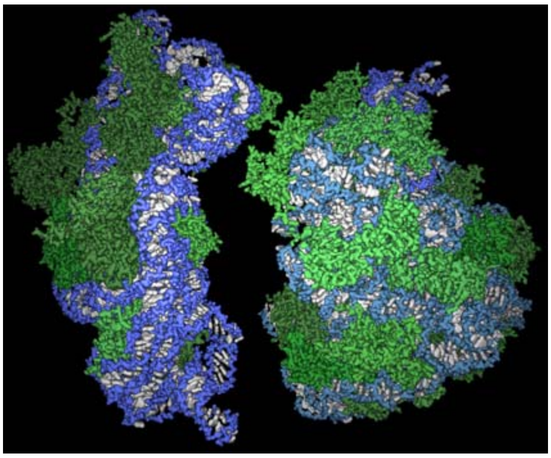


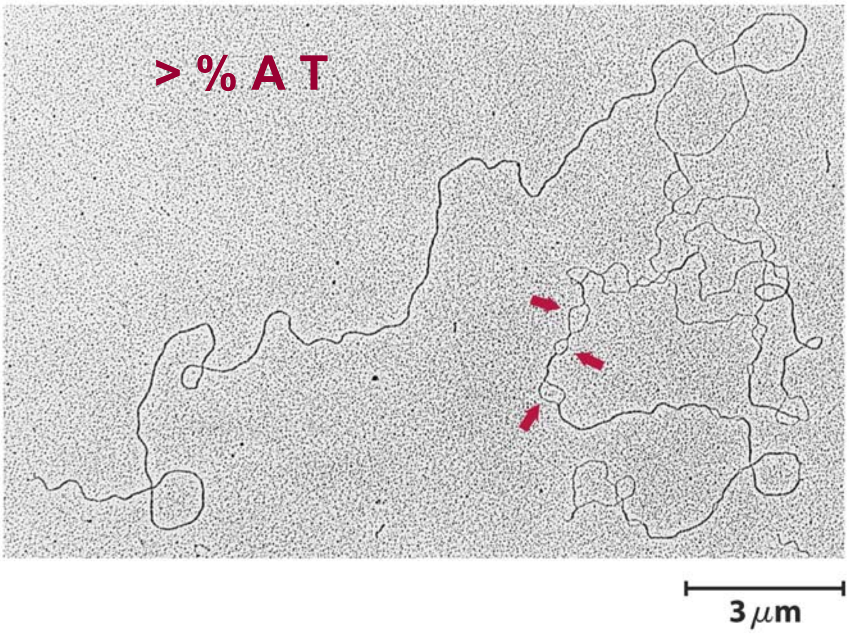


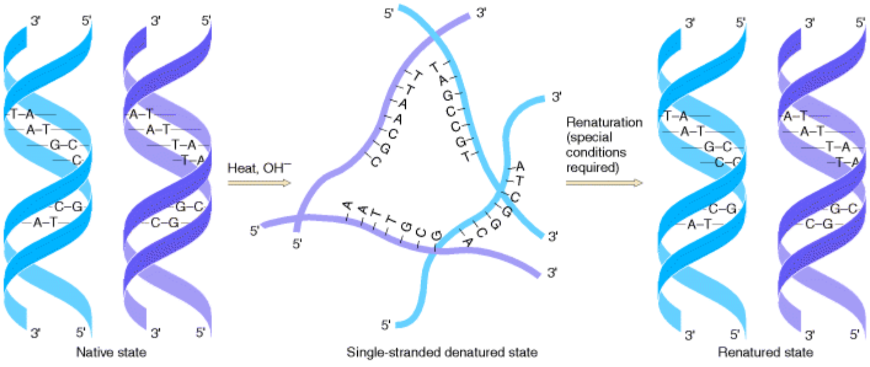
http://www.tulane.edu/~biochem/nolan/lectures/rna/RNAlec2001.htm



http://exploringorigins.org/ribozymes.html

The ribosome, a large molecular machine that drives protein synthesis, is a ribozyme.
Roll over to compare the ribosome structure with and without proteins.
Proteins are shown in green, and RNA is shown in blue and white.
(PDB #2HGR for the 30S subunit and #2HGU for the 50S subunit).



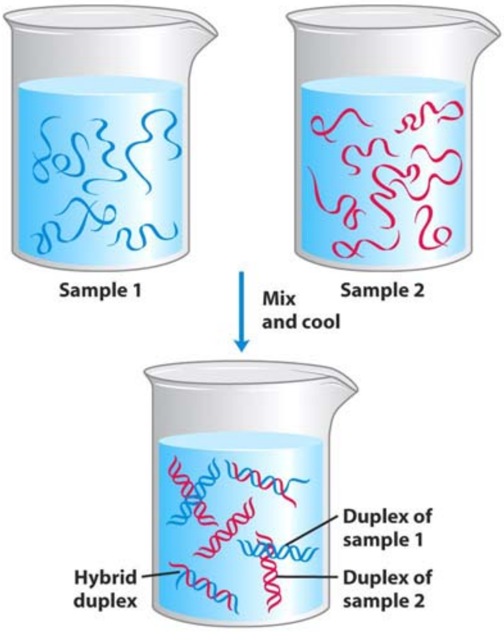


estudios termodinámicos

(temperatura de desnaturalización = Tm), relacionados con estructura (ss, ds) y composición (%GC)

estudios cinéticos

(velocidad de renaturalización), relacionados con la complejidad de la secuencia del dsDNA ($C_o t_{1/2}$).
Aplicaciones: hibridación de sondas…

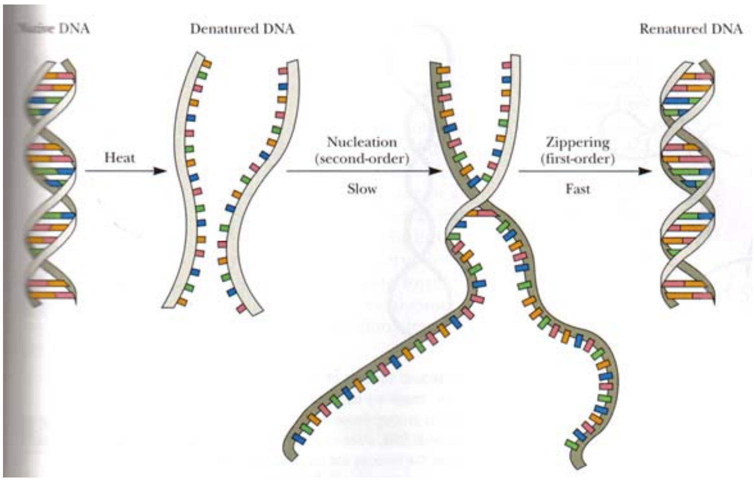


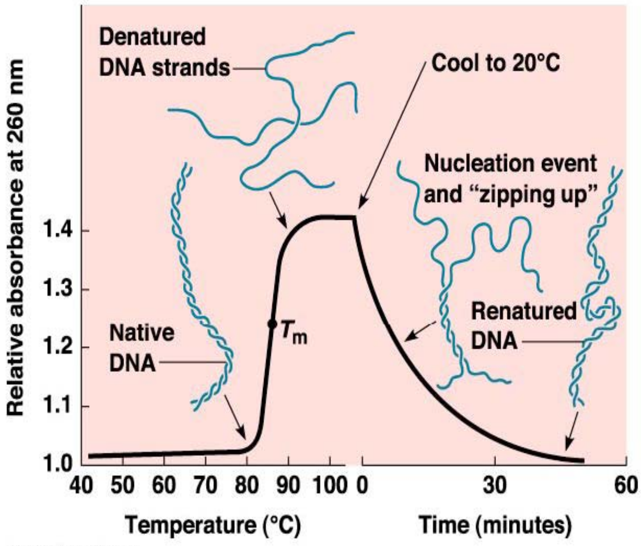
Parámetros que intervienenen en la **desnaturalización** del DNA y renaturalización o **reasociación** de hebras ssDNA con secuencias **complementarias** (*annealing*, hibridación)

Parámetro	**Efecto sobre T_m**	**Efecto sobre la velocidad de renaturalización**
Composición de bases	Incremento de T_m con el aumento del **%G-C**	No ejerce efecto
Longitud	**<150 bp**; incremento de T_m con el incremento de **longitud**; >500 bp no hay efecto	Incrementa la velocidad con la longitud
Fuerza iónica	Incremento de **T_m** con el aumento de **[Na^+]**	Optimo a 1.5 M Na^+
% bp *mismatch*	Disminuye T_m con el aumento de % *mismatch*	Disminuye la velocidad con el aumento de % *mismatch*
Concentración	No ejerce efecto	Aumenta la velocidad con el aumento de **[DNA]**
Agentes denaturalizantes	disminuye T_m con el aumento de [formamida], [urea]	Optimo a 50% formamida
Temperatura	-	Optima a **20°C por debajo de T_m**

Renaturalización

- La desnaturalización es un proceso reversible
- *Reannealing* – reasociación de las cadenas de DNA





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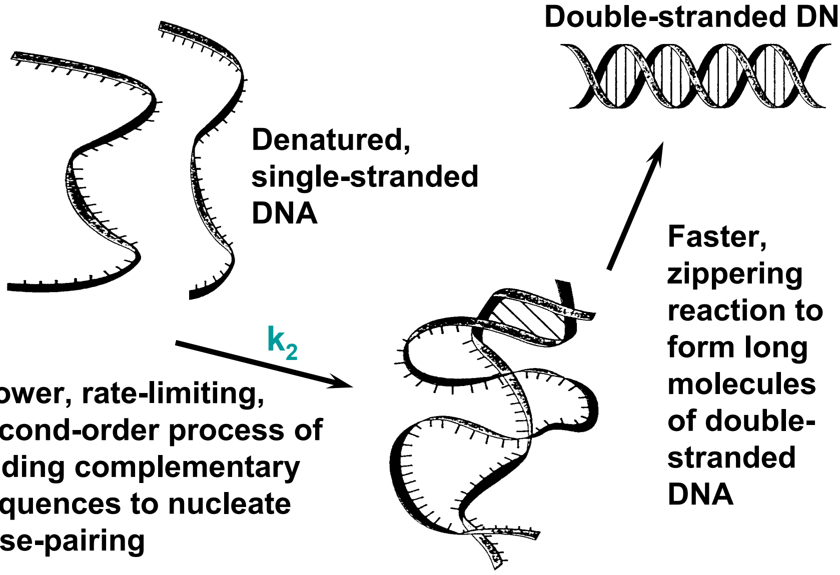
$$-\frac{dC}{dt} = k_2.C^2$$

Nucleación
2º orden

Cierre
1er orden

Complexity of chromosomal DNA

DNA reassociation (renaturation)



Slower, rate-limiting, second-order process of finding complementary sequences to nucleate base-pairing

Faster, zippering reaction to form long molecules of double-stranded DNA

Reasociación de hebras complementarias

Definición de Cot$_{1/2}$: función inversa de la constante de velocidad (k)

$$\frac{c}{c_0} = \frac{1}{1+kC_0t}$$

$$\frac{1}{2} = \frac{1}{1+kC_0t} \qquad \frac{1}{2}(1+kC_0t) = 1 \qquad (1+kC_0t) = 2$$

$$kC_0t = 2-1 = 1$$

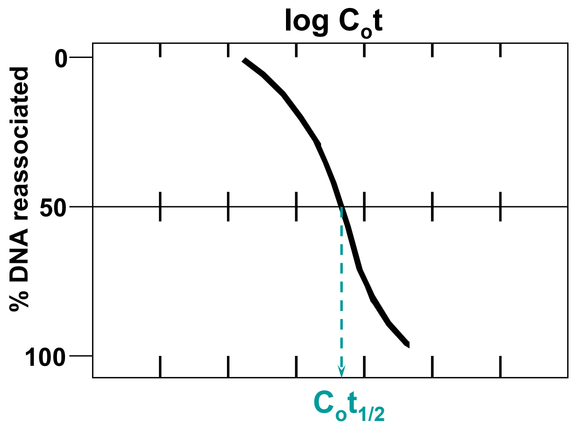
$$C_0t_{1/2} = \frac{1}{k}$$

$C_0t_{½}$: valor de C_0t cuando se reasoció un 50%

DNA reassociation kinetics (for a single DNA species)

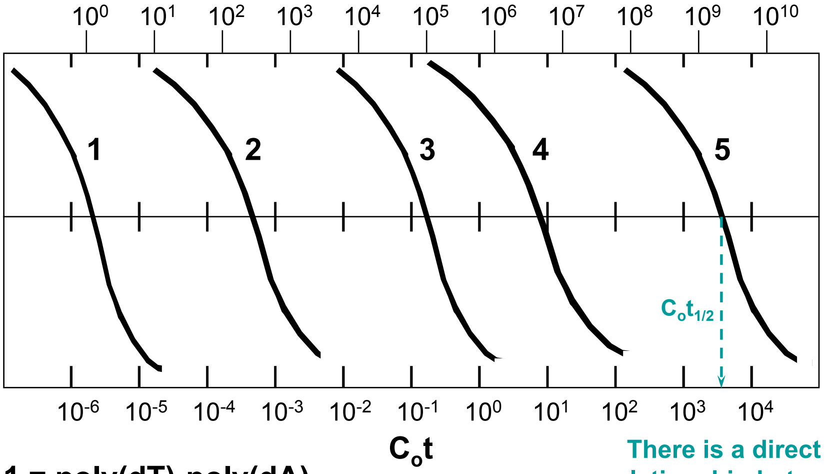
$C_o t_{1/2} = 1 / k_2$

k_2 = second-order rate constant
C_o = DNA concentration
$t_{1/2}$ = time for half reaction



Ideal second-order DNA reassociation curve (Cot curve)

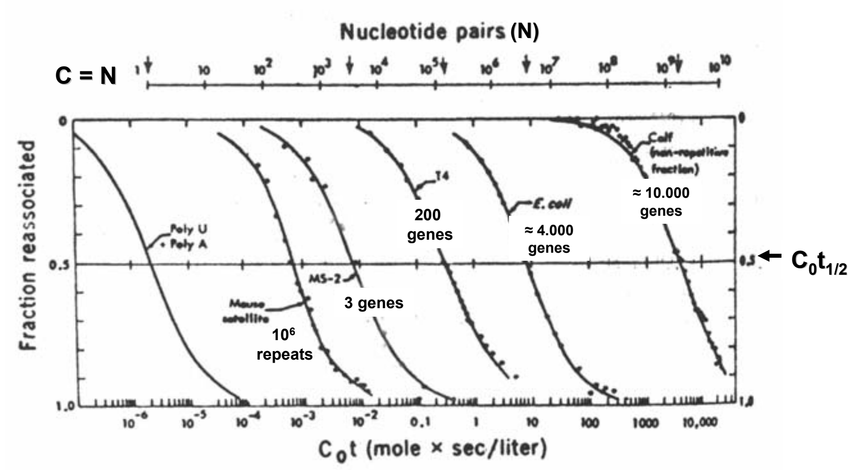
Complexity expressed as base-pairs (bp)



1 = poly(dT)-poly(dA)
2 = purified human satellite DNA
3 = T4 bacteriophage DNA
4 = E. coli genomic DNA
5 = purified human single-copy DNA

There is a direct relationship between $C_o t_{1/2}$ and complexity

Curvas de reasociación de DNA no repetitivo (fragmentos de 500 nt)



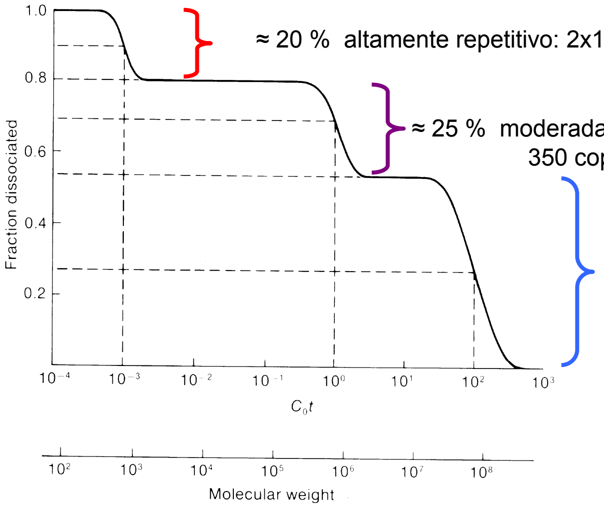
If no repeated sequences: C (complexity) = genome size (bp)
N (genome size) is determined directly from $C_0t_{1/2}$

¿Complejidad del Genoma ?

¿Qué es?

AAAAAAAAAAAAAAAA	C= 1; L=16
ATATATATATATATATA	C= 2; L=16
ATCGATCGATCGATCG	C= 4; L=16
ATCGCTAGAACGTCTG	C= 16; L=16

Reasociación de DNA de eucariotes



≈ 20 % altamente repetitivo: $2x10^6$ copias

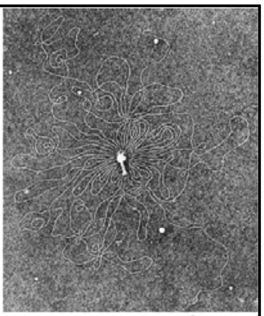
≈ 25 % moderadamente repetitivo
350 copias

≈ 55% copia única

Tamaños de genomas

Tamaños de genomas

Virus a DNA



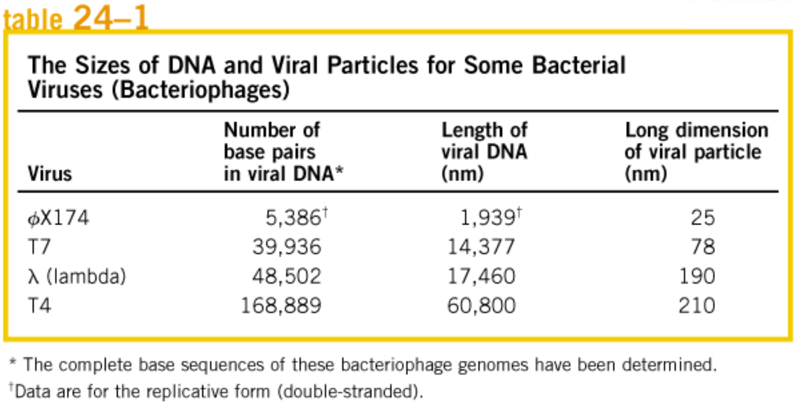


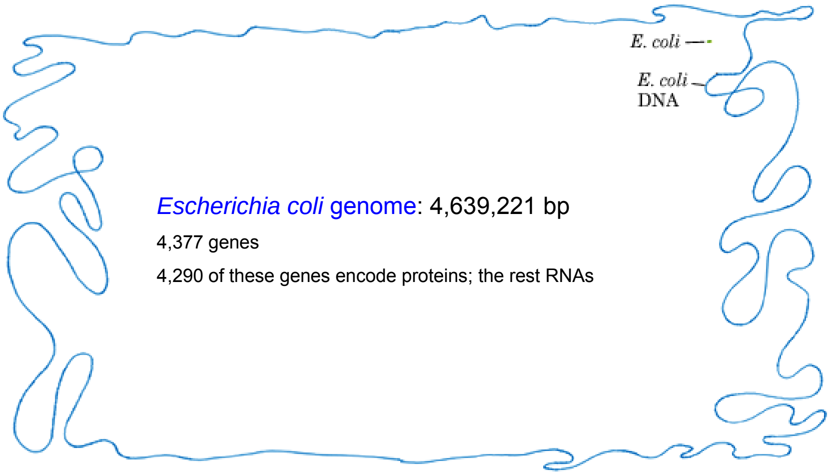
table 24–1

The Sizes of DNA and Viral Particles for Some Bacterial Viruses (Bacteriophages)

Virus	Number of base pairs in viral DNA*	Length of viral DNA (nm)	Long dimension of viral particle (nm)
ϕX174	5,386[†]	1,939[†]	25
T7	39,936	14,377	78
λ (lambda)	48,502	17,460	190
T4	168,889	60,800	210

* The complete base sequences of these bacteriophage genomes have been determined.

[†] Data are for the replicative form (double-stranded).



Escherichia coli genome: 4,639,221 bp

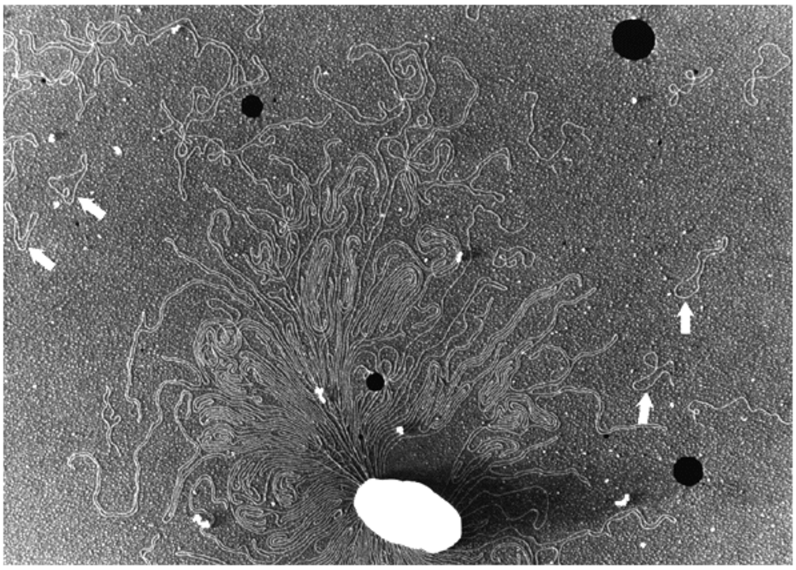
4,377 genes

4,290 of these genes encode proteins; the rest RNAs

1 Cell length 2 µm or 2x10^{-6} m

2 Cell diameter 0.8 µm or 0.8x10^{-6} m

3 Cell total volume 1x10^{-15} L



Tamaños de genomas

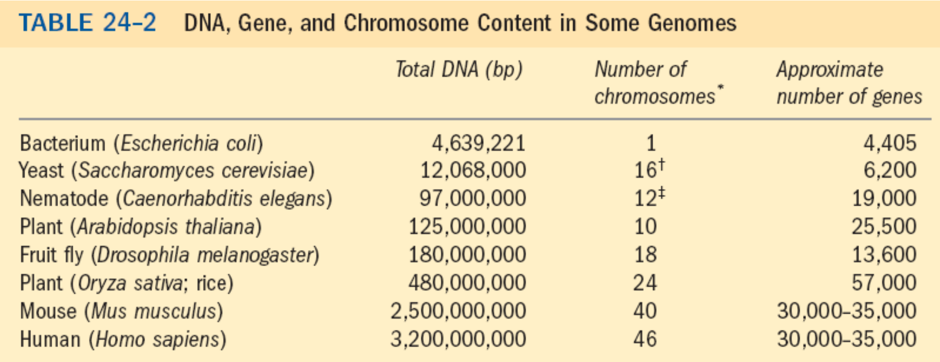
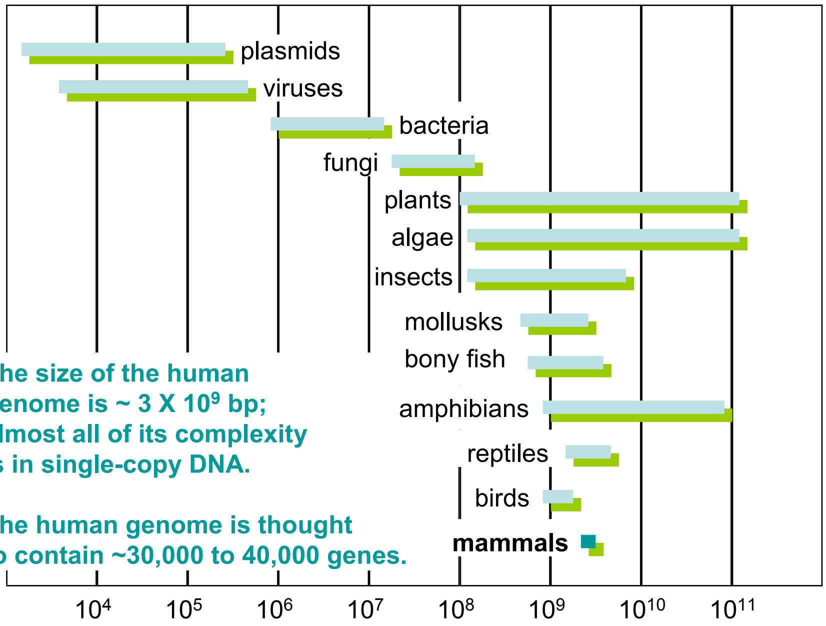


TABLE 24–2 DNA, Gene, and Chromosome Content in Some Genomes

	Total DNA (bp)	*Number of chromosomes**	*Approximate number of genes*
Bacterium (*Escherichia coli*)	4,639,221	1	4,405
Yeast (*Saccharomyces cerevisiae*)	12,068,000	16[†]	6,200
Nematode (*Caenorhabditis elegans*)	97,000,000	12[‡]	19,000
Plant (*Arabidopsis thaliana*)	125,000,000	10	25,500
Fruit fly (*Drosophila melanogaster*)	180,000,000	18	13,600
Plant (*Oryza sativa*; rice)	480,000,000	24	57,000
Mouse (*Mus musculus*)	2,500,000,000	40	30,000–35,000
Human (*Homo sapiens*)	3,200,000,000	46	30,000–35,000

Genome sizes in nucleotide pairs (base-pairs)



The size of the human genome is ~ 3 X 10^9 bp; almost all of its complexity is in single-copy DNA.

The human genome is thought to contain ~30,000 to 40,000 genes.

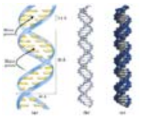
- ***Escherichia coli* *1,5 µm***

1 par de bases (pb) → 0,34 nm = 0.34 x 10^{-9} m

1 molécula de DNA genómico: 4,6 10^6 pb / célula

DNA: 1,5 mm !!!

Longitud del DNA
de una célula humana



1 par de bases (bp) → 0,34 nm = 0,34 x 10^{-9} m

2 juegos of 3,0 10^9 pb / célula

2 metros !!!

Contenido de DNA (longitud total)
en un humano adulto

2 m / célula x 10^{14} células **2 x 10^{11} kilómetros**

200.000 millones de km

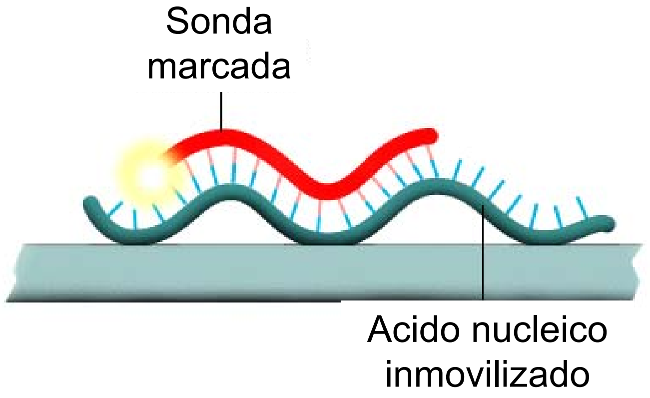
perímetro: 4 x 10^4 km

distancia de la Tierra al Sol: 1,5 x 10^8 km

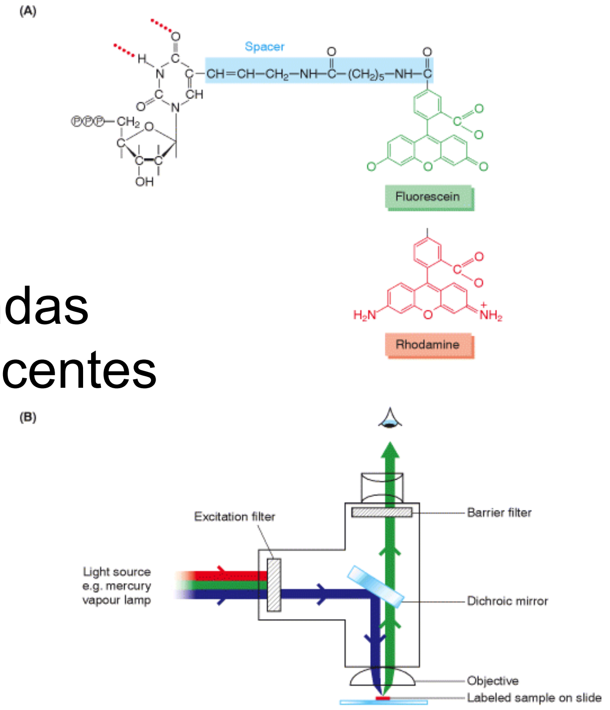
Hibridación

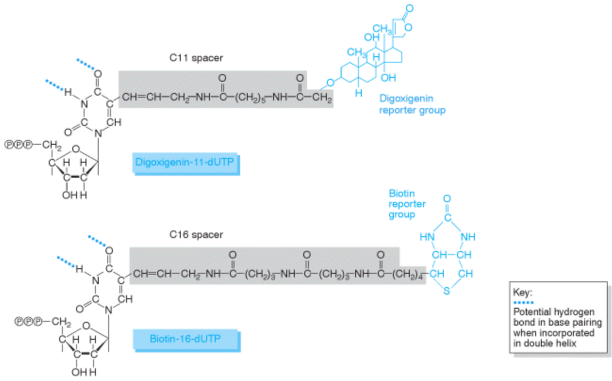
reasociación de secuencias complementarias

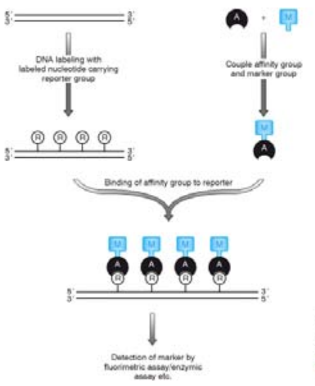
secuencia blanco + ***sonda*** ★



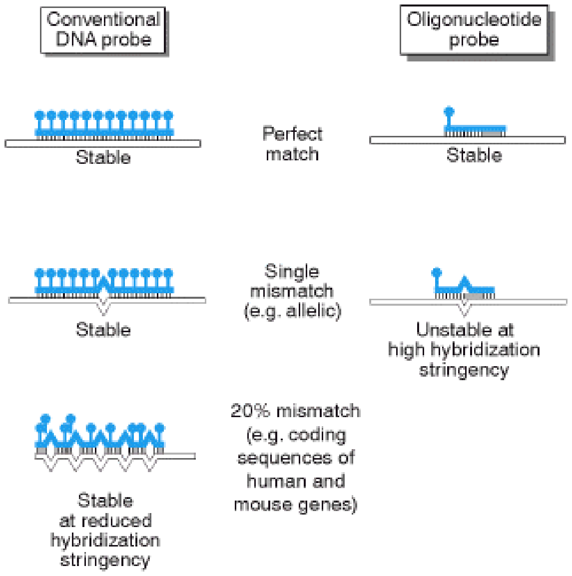
Sondas fluorescentes







Sondas con grupos detectables por afinidad con anticuerpos (DIG) y otras proteínas (avidina o estreptavidina)

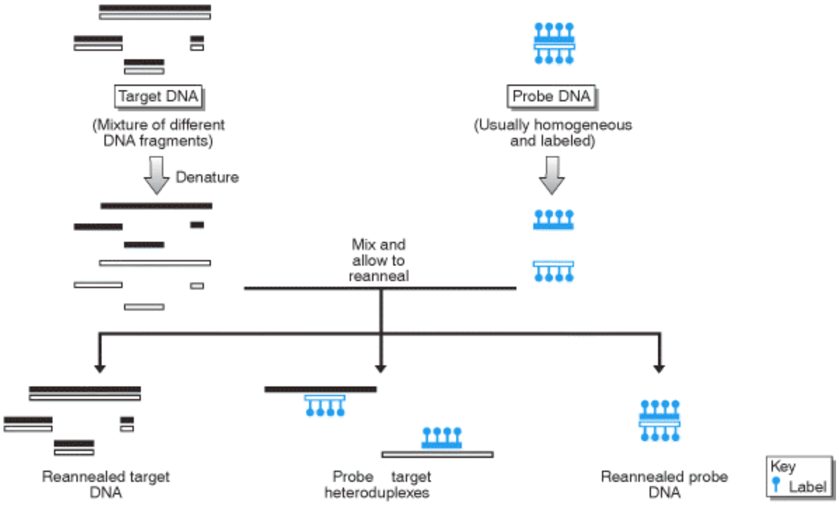


High stringency: condiciones estrictas de hibridación y lavado de la sonda, tales que solamente se mantienen unidas las secuencias blanco y sonda que son 100% complementarias

Low stringency: condiciones de hibridación y lavado de la sonda, tales que se mantienen unidas las secuencias blanco y sonda entre las que no hay complementariedad perfecta (con “mismatches”)

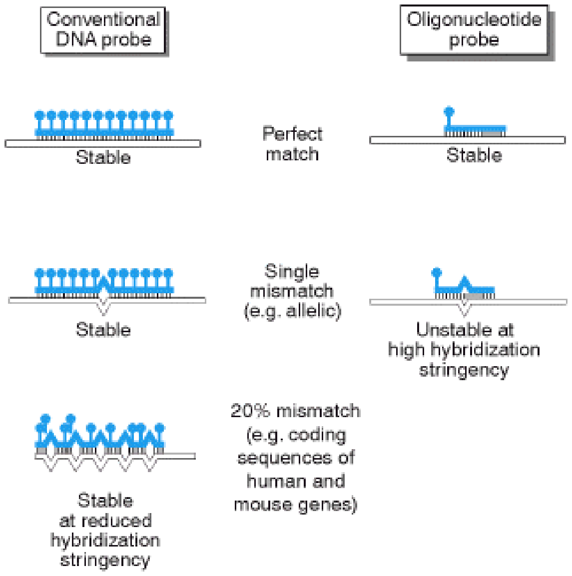
Sondas

de ácidos nucleicos



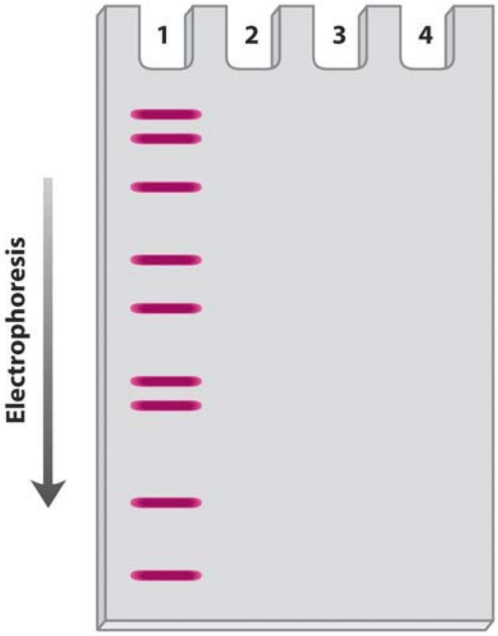
La hibridación de ácidos nucleicos posee múltiples usos

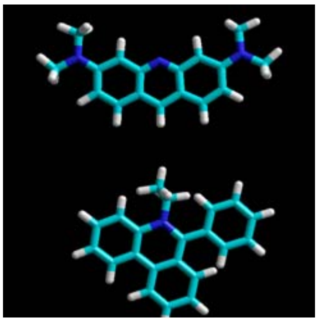
- Southern blot / Northern blot
- Colony blot
- PCR
- Purificación
- Microarrays
- FISH



High stringency: condiciones estrictas de hibridación y lavado de la sonda, tales que solamente se mantienen unidas las secuencias blanco y sonda que son 100% complementarias

Low stringency: condiciones de hibridación y lavado de la sonda, tales que se mantienen unidas las secuencias blanco y sonda entre las que no hay complementariedad perfecta (con “mismatches”)





Agentes intercalantes

Naranja de acridina

Bromuro de etidio

- Moléculas aromáticas que se interaccionan en el ADN entre bases apiladas
- Fluorescentes
- Detección de DNA y RNA
- Agentes mutagénicos

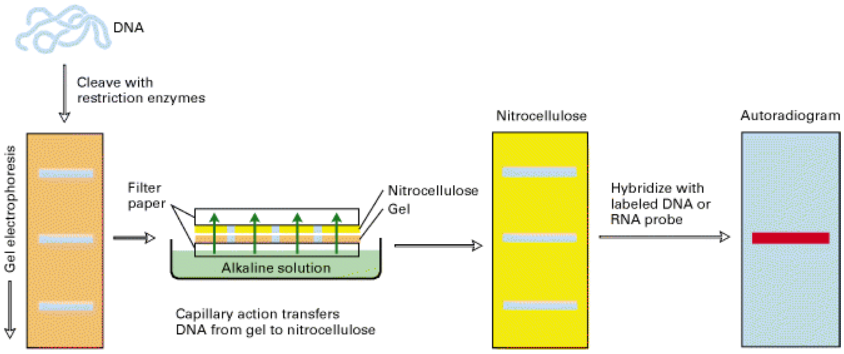


Figure 7-32. The **Southern blot** technique for detecting the presence of specific DNA sequences following gel electrophoresis of a complex mixture of restriction fragments. The diagram depicts three restriction fragments in the gel, but the procedure can be applied to a mixture of millions of DNA fragments. A similar procedure, called *Northern blotting*, is used to detect specific RNA sequences. [See E. M. Southern, 1975, *J. Mol. Biol.* **98**:508.] Lodish

Northern blotting detects specific mRNAs

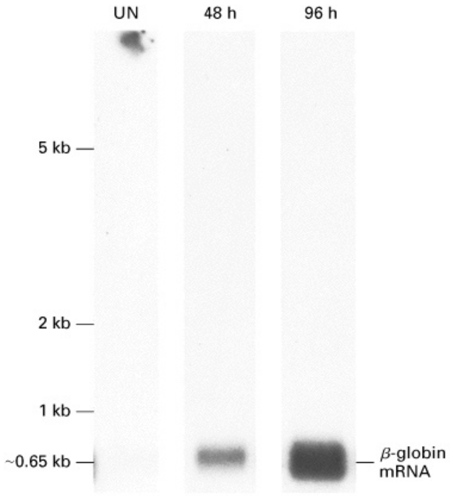


Figure 7-33

PCR: the polymerase chain reaction

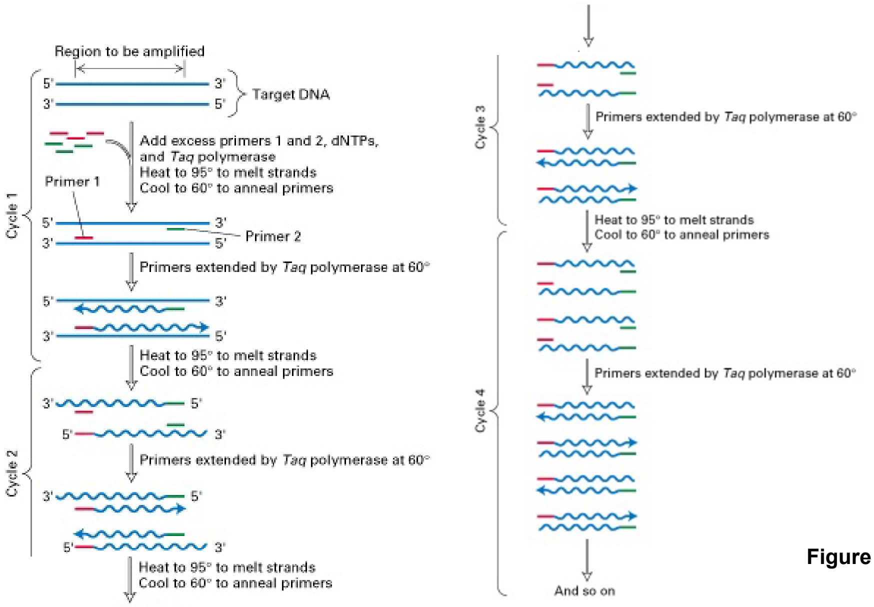
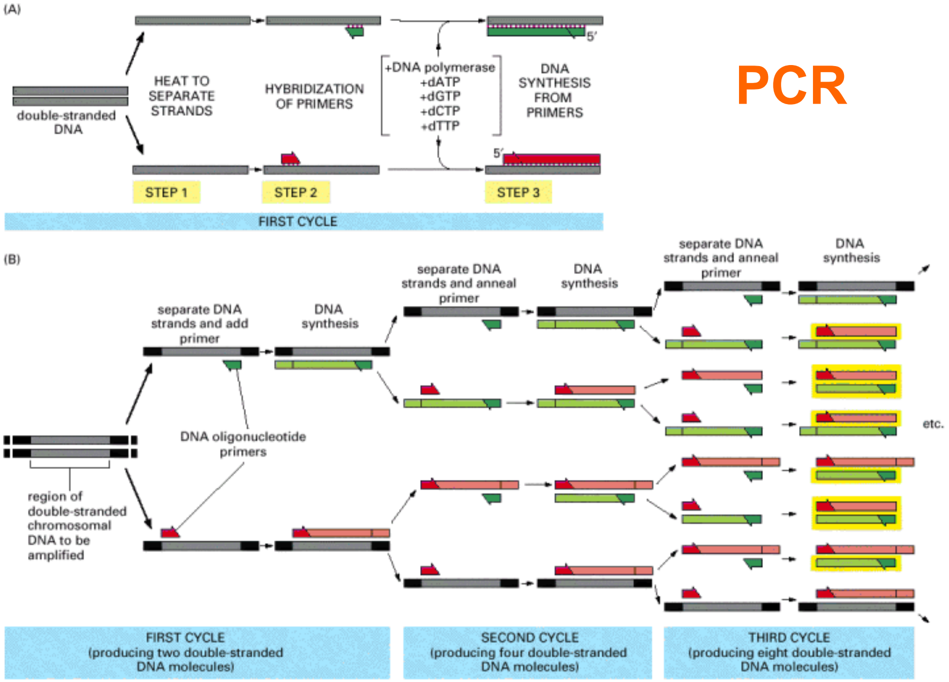


Figure 7-38

PCR


(A)
double-stranded DNA
HEAT TO SEPARATE STRANDS
HYBRIDIZATION OF PRIMERS
+DNA polymerase
+dATP
+dGTP
+dCTP
+dTTP
DNA SYNTHESIS FROM PRIMERS
5′
5′
STEP 1
STEP 2
STEP 3
FIRST CYCLE
(B)
separate DNA strands and add primer
DNA synthesis
separate DNA strands and anneal primer
DNA synthesis
separate DNA strands and anneal primer
DNA synthesis
DNA oligonucleotide primers
region of double-stranded chromosomal DNA to be amplified
etc.
FIRST CYCLE (producing two double-stranded DNA molecules)
SECOND CYCLE (producing four double-stranded DNA molecules)
THIRD CYCLE (producing eight double-stranded DNA molecules)