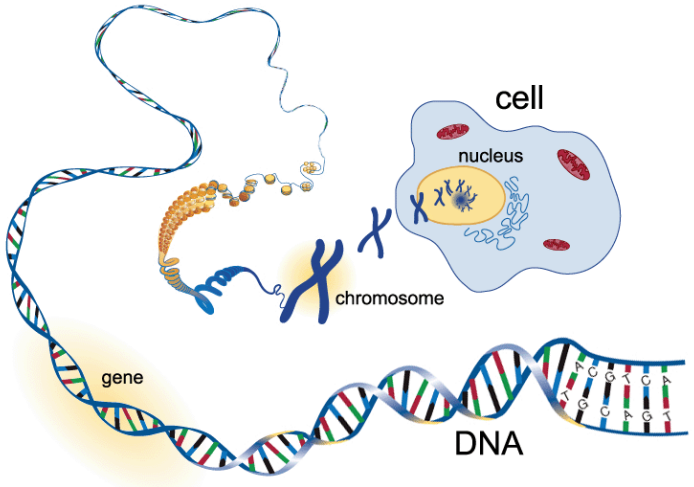
Cours Biochimie I

Leçon 5: Explorer les gènes et les génomes

Leçon	Subject	Page en Stryer
1	Introduction en biochimie / classes de biomolécules / aminoacides	4-17 (1.2, 1.3), 25-40 (2.1, 2.2)
2	Composition et structure des protéines	40-59 (2.3-2.6)
3	Exploration des protéines et des protéomes	65-90 (3.1-3.3), 93-101 (3.5, 3.6)
4	Structure de la DNA et RNA	107-119 (4.1-4.3)
5	Explorer les gènes et les génomes	134-144 (5.1, 5.2 premières 3 pages)
6	Synthèse des protéines / expression recombinante	117-119 (4.3), 119-127 (4.4), 142-148 (5.2)

Christian Heinis christian.heinis@epfl.ch, BCH 5305

Leçon 5



- Electrophorèse de DNA sur gel
- Enzymes de restriction (1970)
- Technologie du DNA recombinant (1970s)
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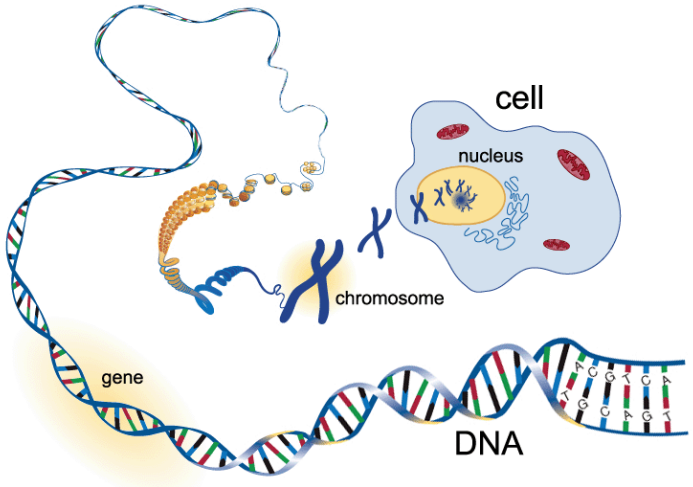

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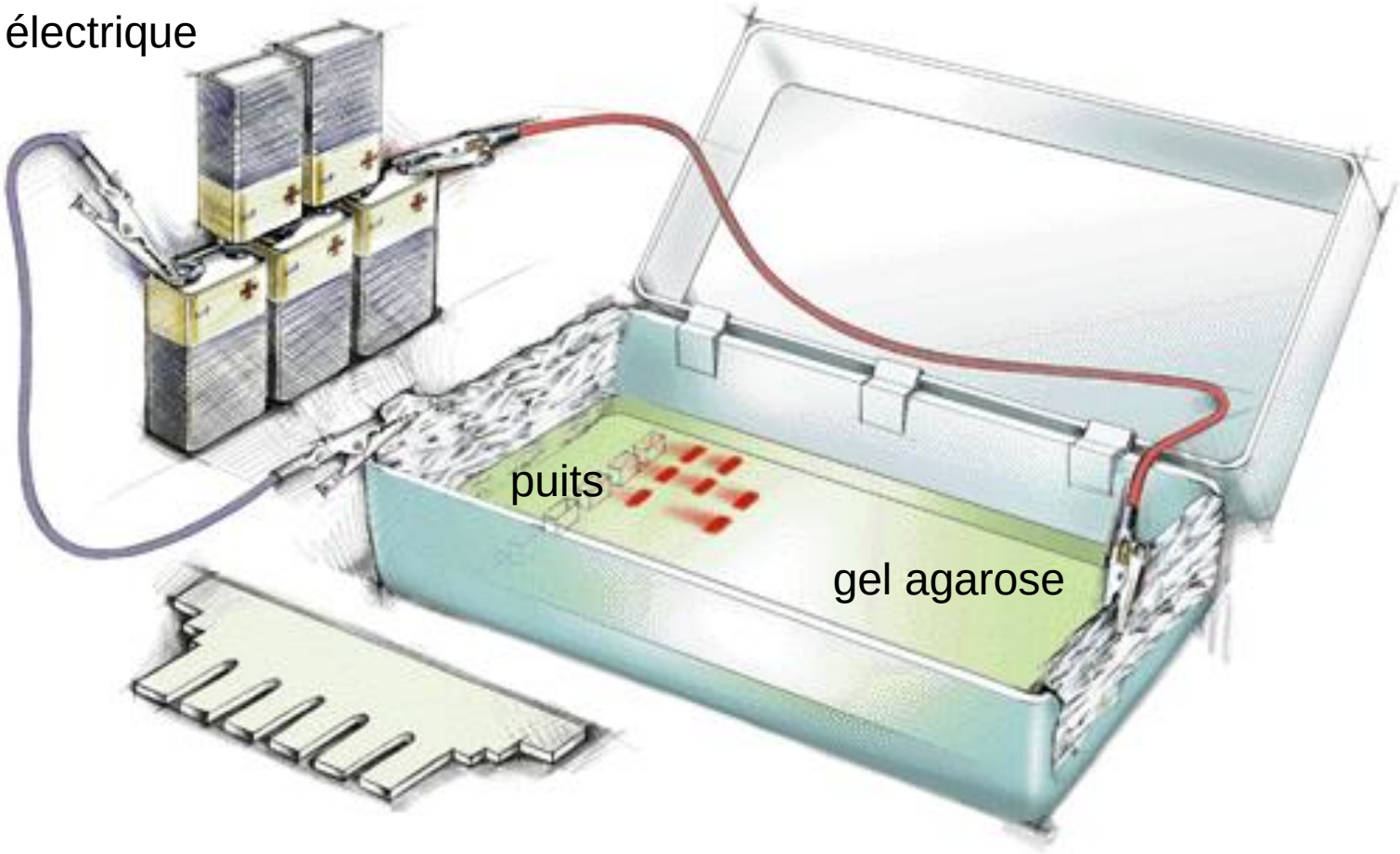
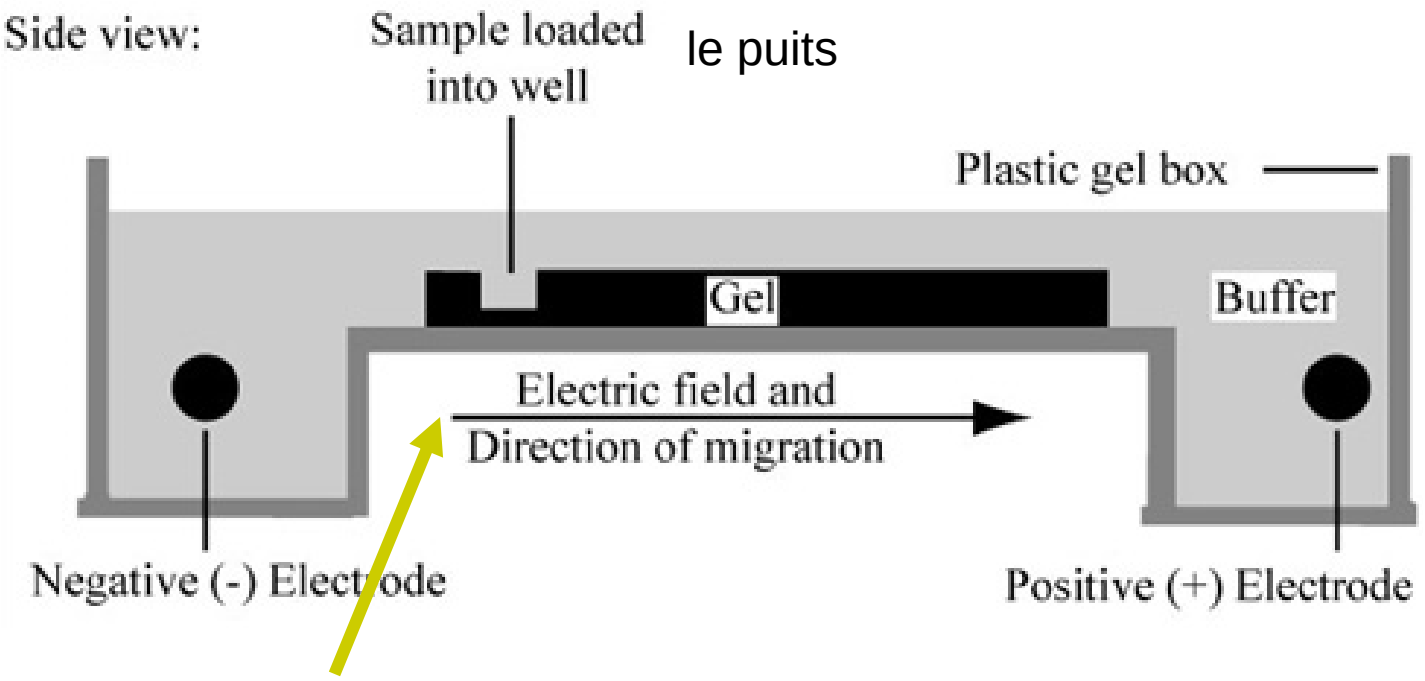
Electrophorèse de DNA sur gel

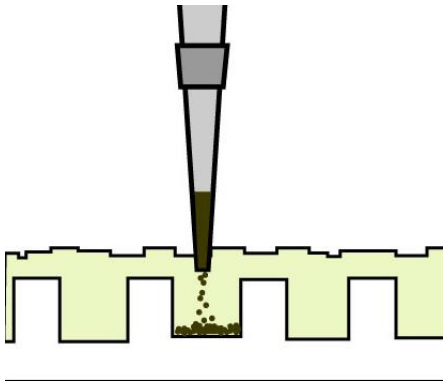
application d'un champ

électrique

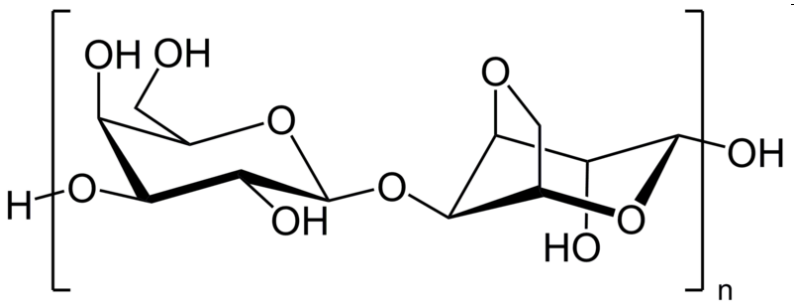


Electrophorèse de DNA sur gel





Gel d’agarose



Gel d’agarose

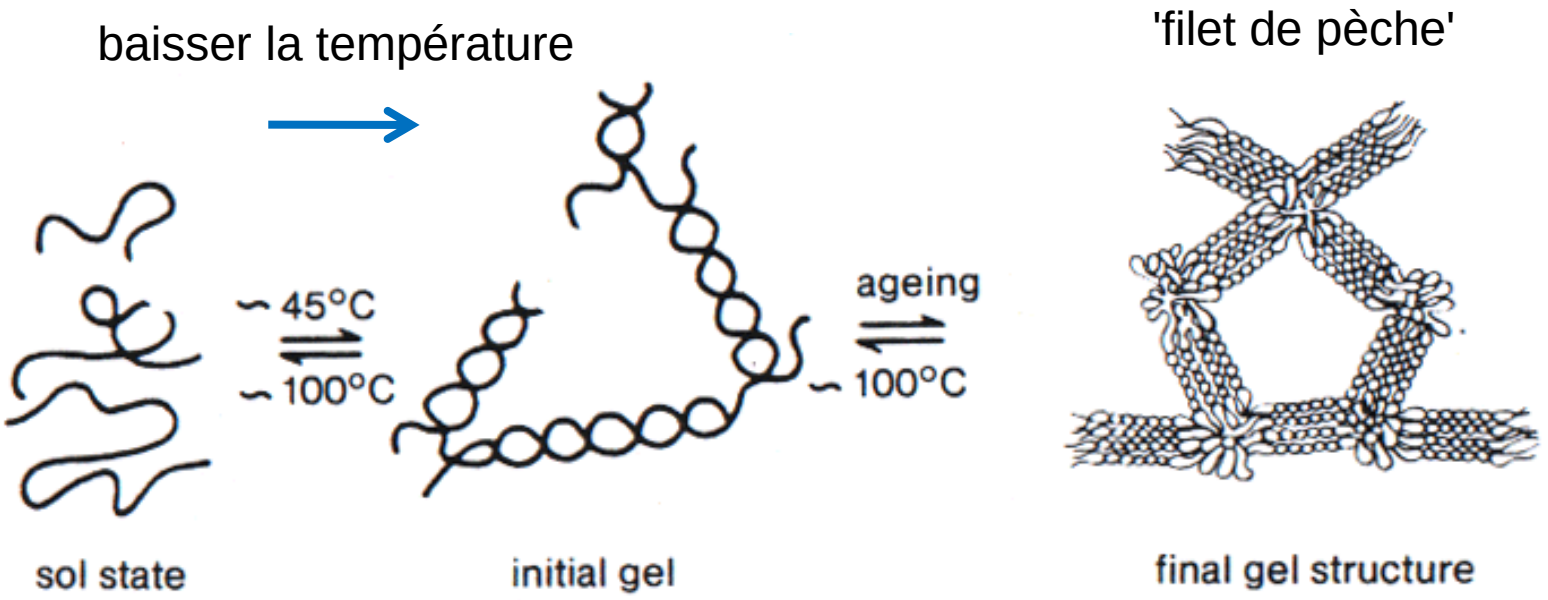
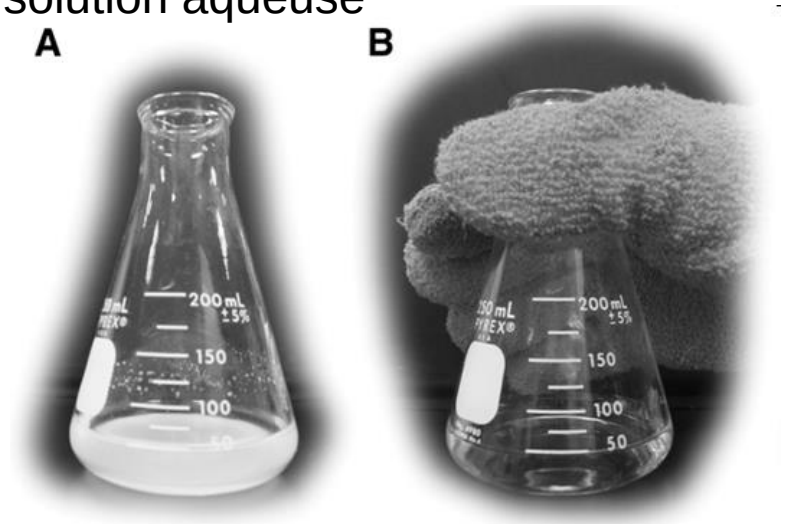
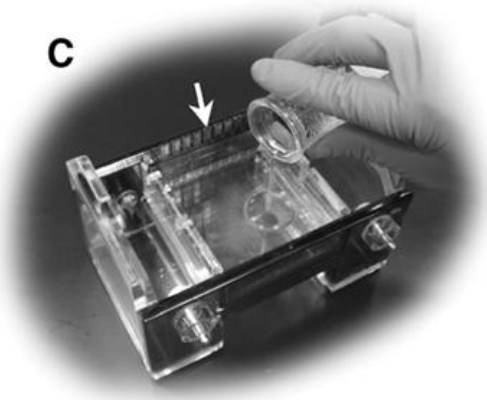


Fig. 25. Gel structure of agarose. (Låås, T. Doctoral thesis. Acta Universitatis Upsaliensis 1975. Reproduced by kind permission of the Author.)

Gel d’agarose

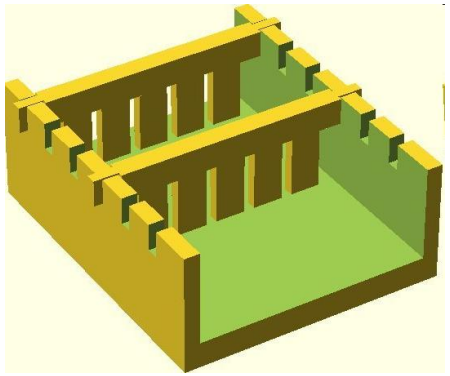
récipient avec agarose et solution aqueuse





verser l'agarose dans l'apparéil

un peigne



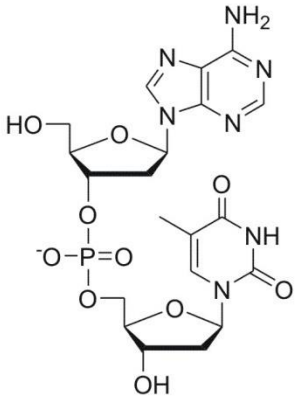
Electrophorèse de DNA sur gel

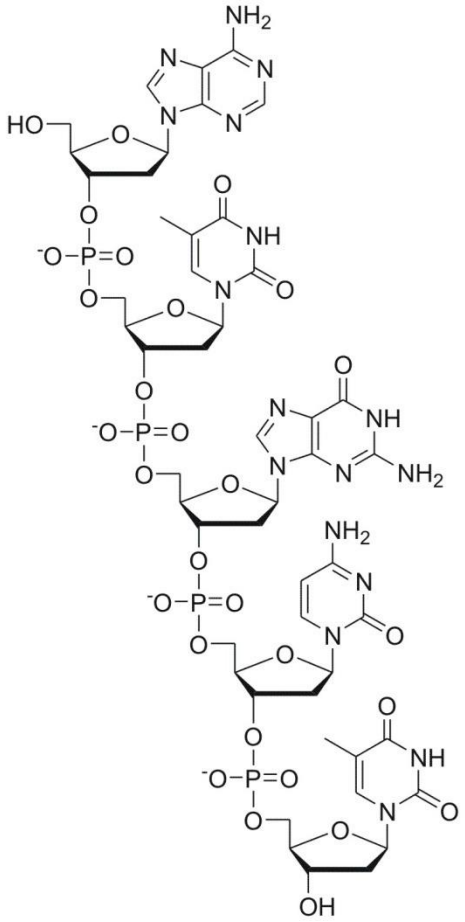
Dans quelle direction est-ce que le DNA va migrer?

(pôle négatif ou positif)

Charge des nucléotides

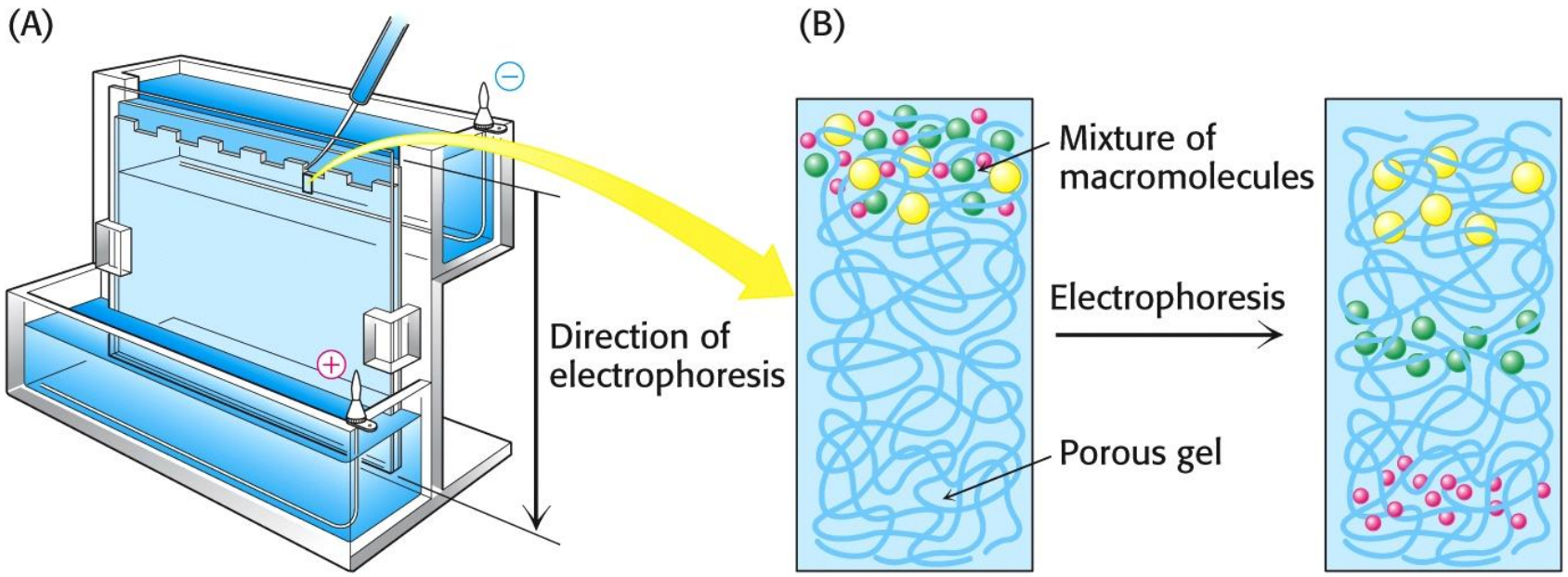
dinucleotide
5'-AT-3'





pentanucleotide
5'-ATGCT-3'

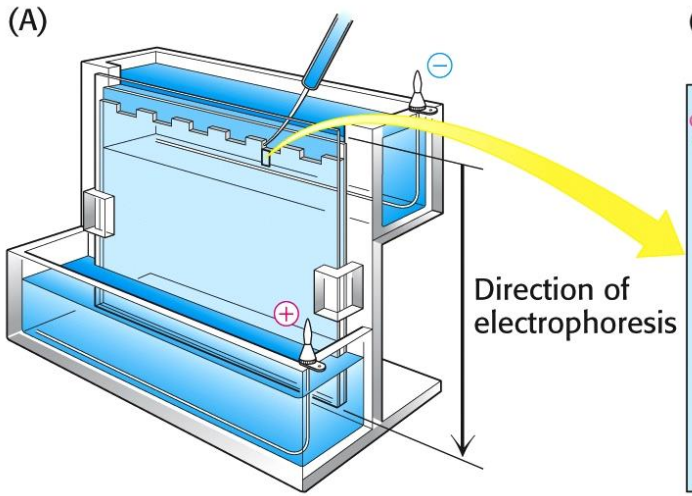
Électrophorèse sur gel des protéines (SDS PAGE)

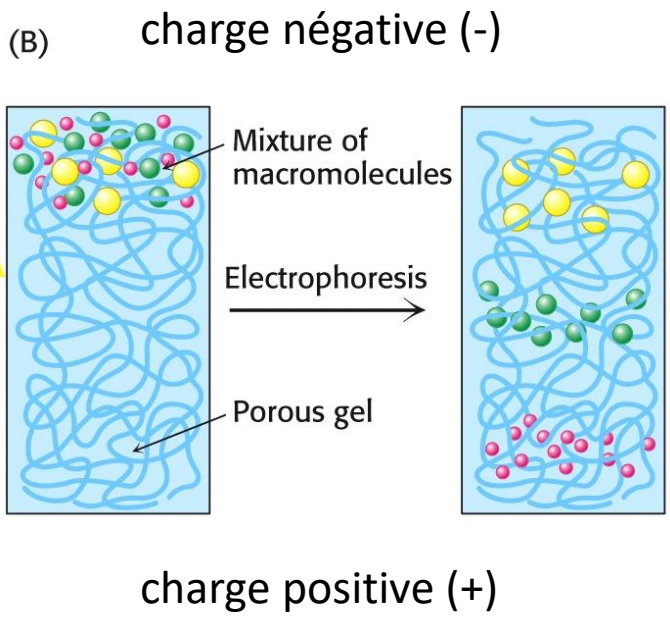


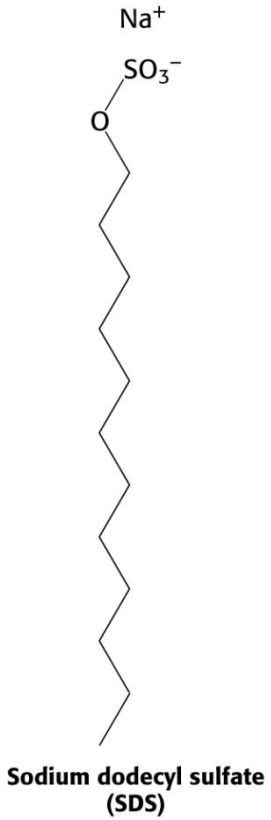
Dans quelle direction est-ce que les protéines se déplacent?

(pôle négatif ou positif)

Électrophorèse sur gel des protéines







→ En direction du pôle positif

sodium dodecyl sulfate (SDS)

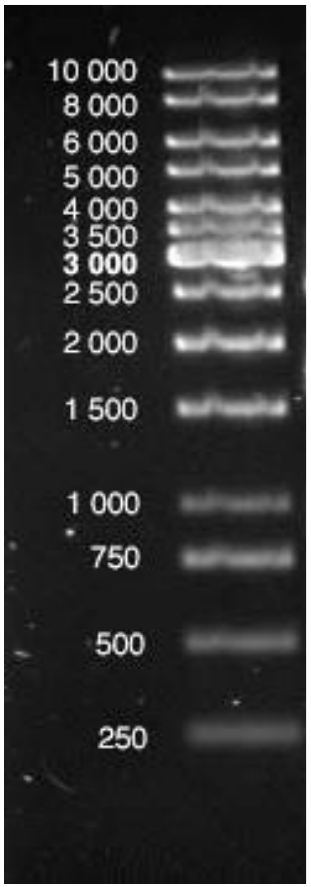
Electrophorèse sur gel du DNA

pôle -

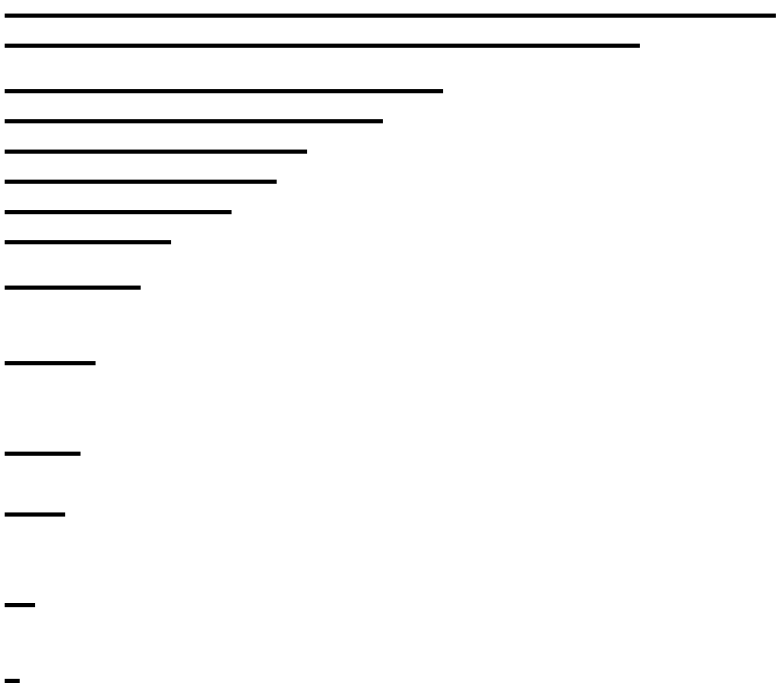
direction de migration

pôle +

marker



pièces de DNA séparées sur gel d'agarose

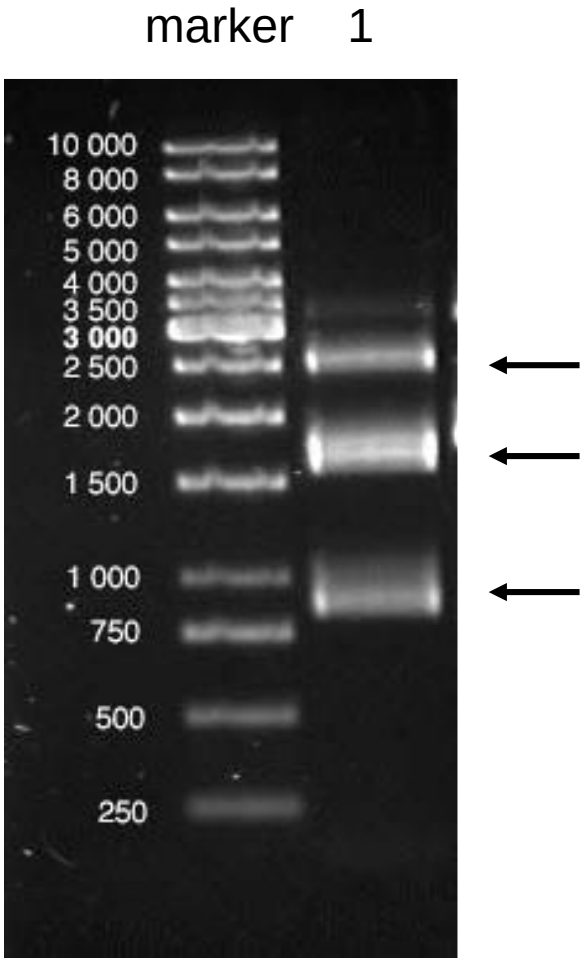


Electrophorèse sur gel du DNA

pôle -

direction de migration

pôle +



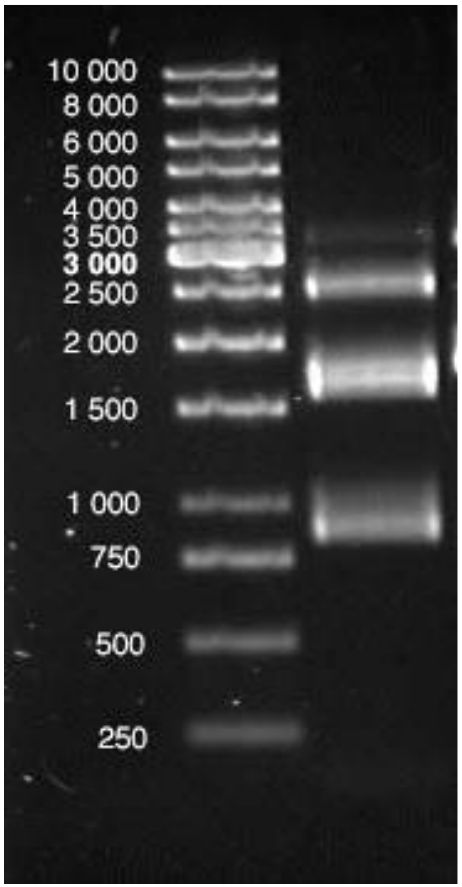
Quelles sont les tailles des fragments de DNA

Electrophorèse sur gel du DNA

pôle -

direction de migration

pôle +



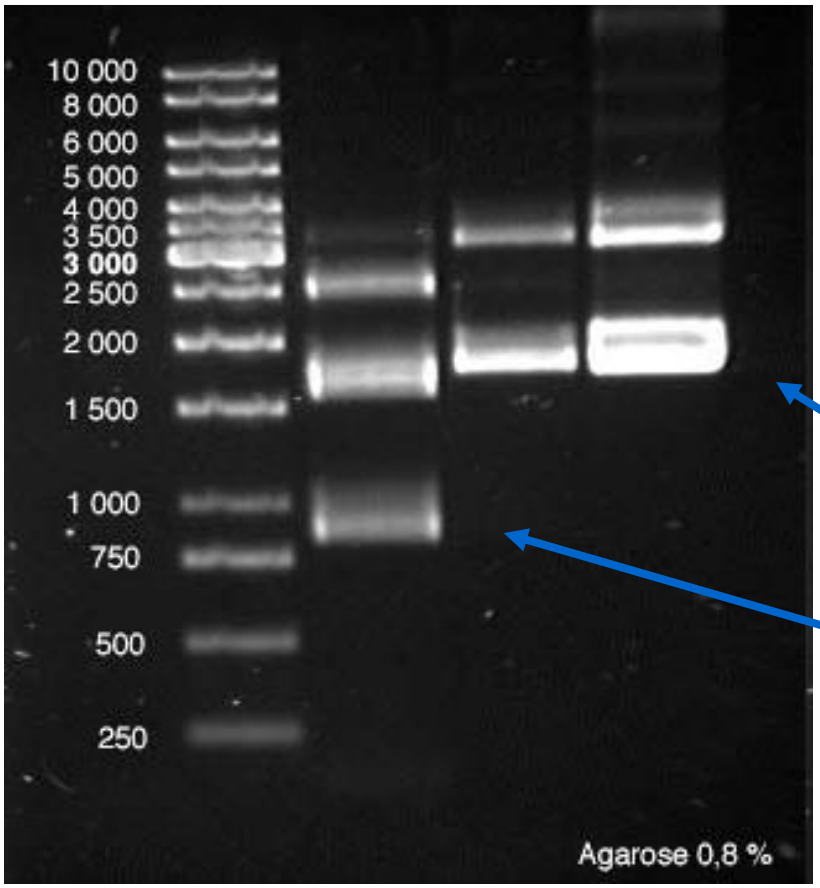
Quelles sont les tailles des fragments de DNA

← 2600 pairs de base

← 1700 pairs de base

← 850 pairs de base

Electrophorèse sur gel du DNA



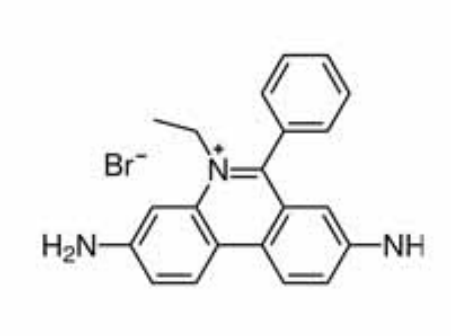
Le DNA peut aussi être quantifié avec l'électrophorese de gel agarose:

Marker: 100 ng de chaque fragment

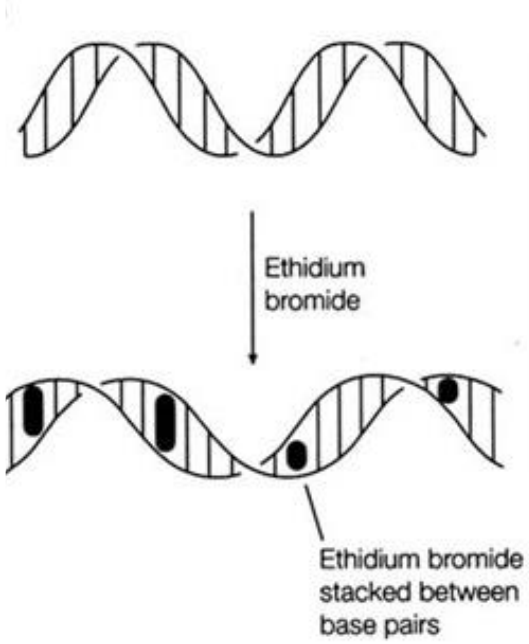
Electrophorèse sur gel du DNA

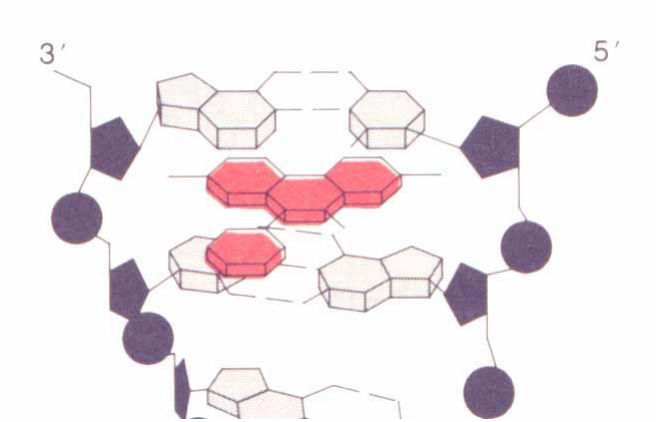
Comment peut-on visualiser le DNA dans un gel d'agarose?

Electrophorèse sur gel du DNA

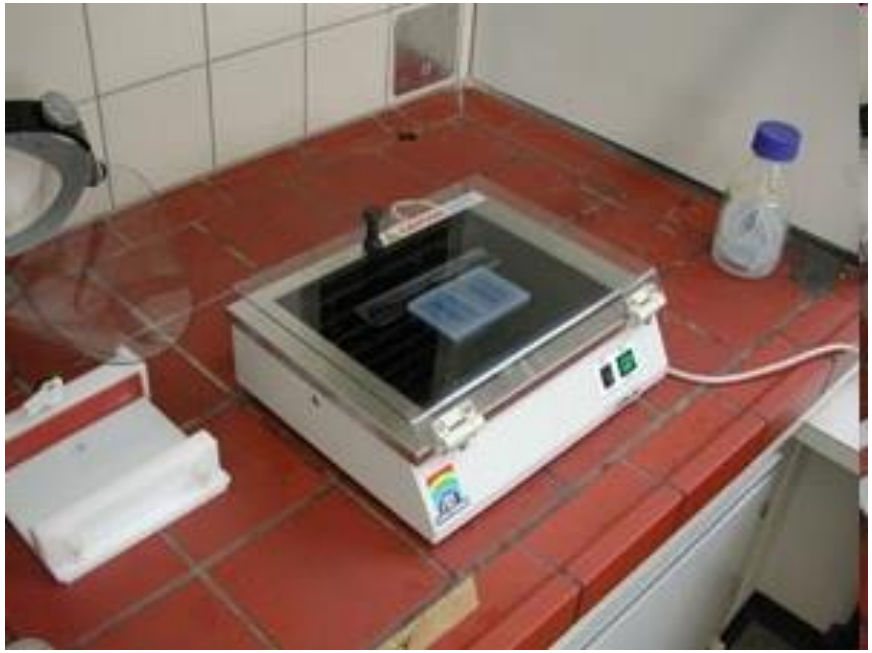


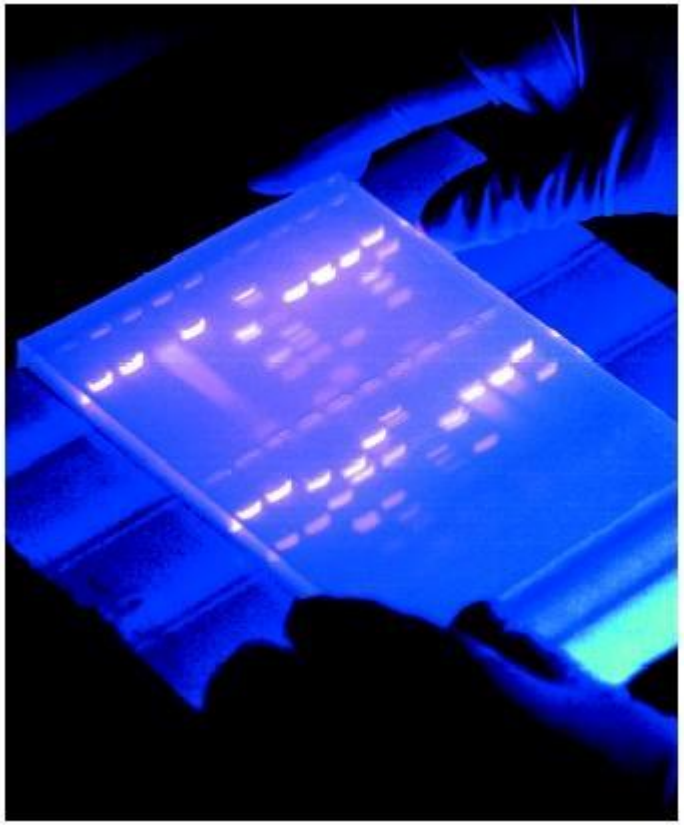
l'éthidium bromide



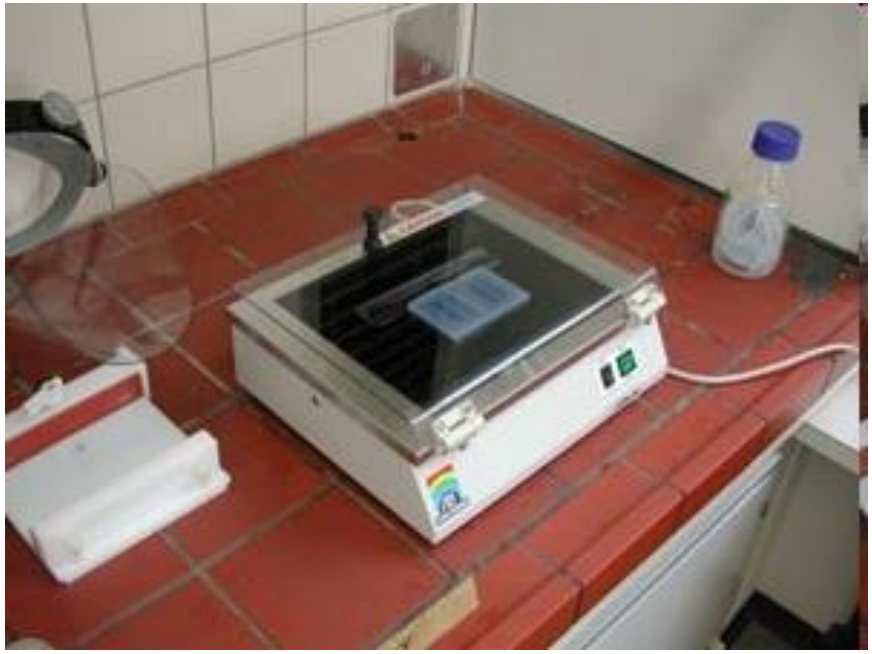


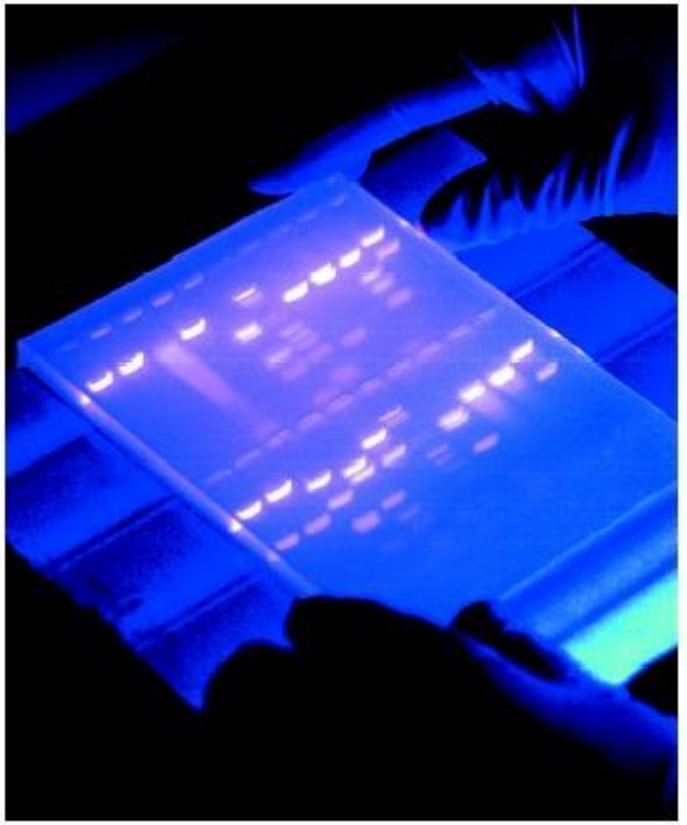
Electrophorèse sur gel du DNA





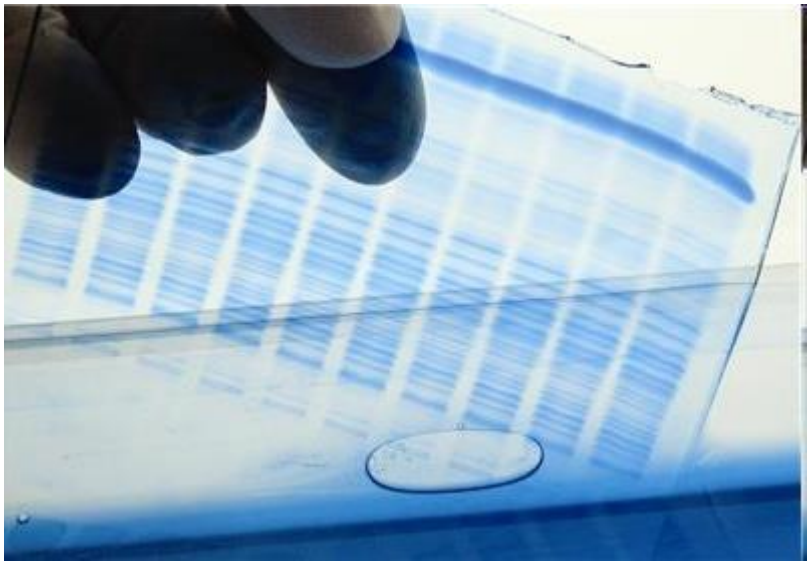
Electrophorèse sur gel du DNA





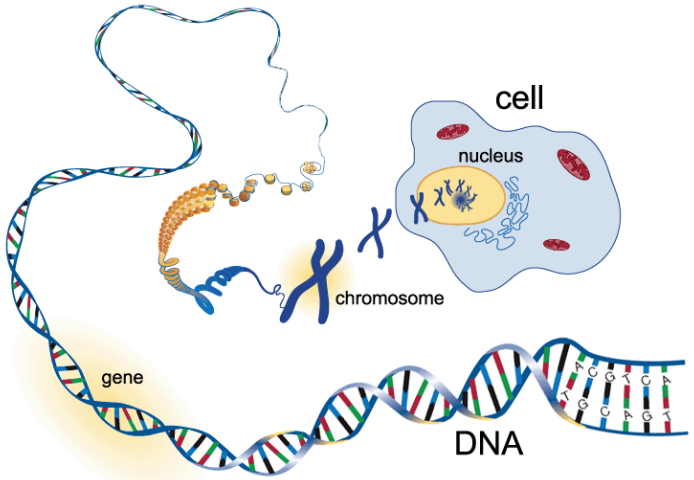
Comment peut-on visualiser les protéines sur des gels de polyacrylamide?

Coloration des protéines après électrophorèse en utilisant du bleu de Coomassie



- Une quantité de 0.1 μg de protéine peut être détectée
- Des protéines avec une différence de masse de 2% peuvent être séparées (e.g. 50 et 51 kDa; 10 aminoacides)

Leçon 5



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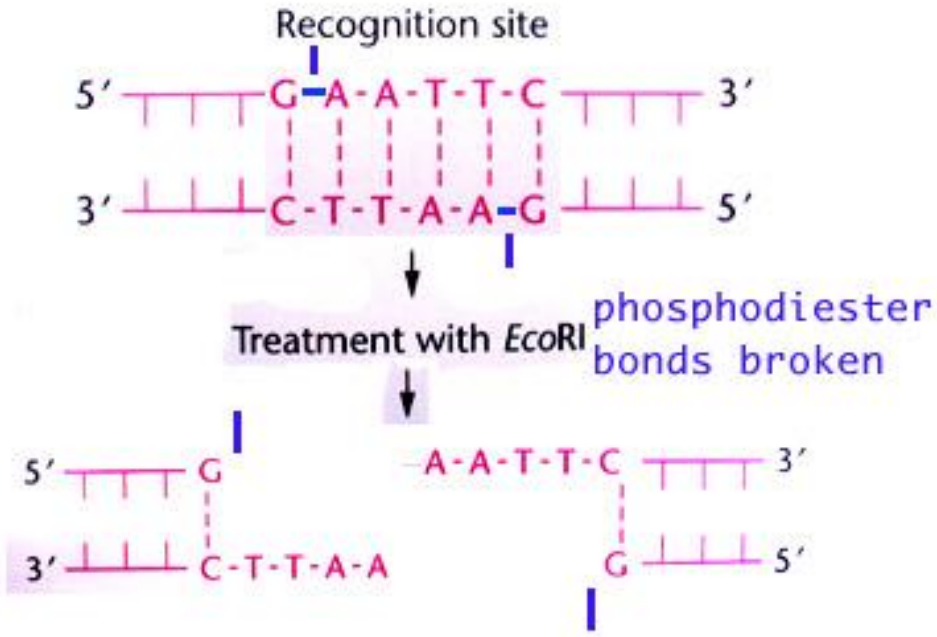
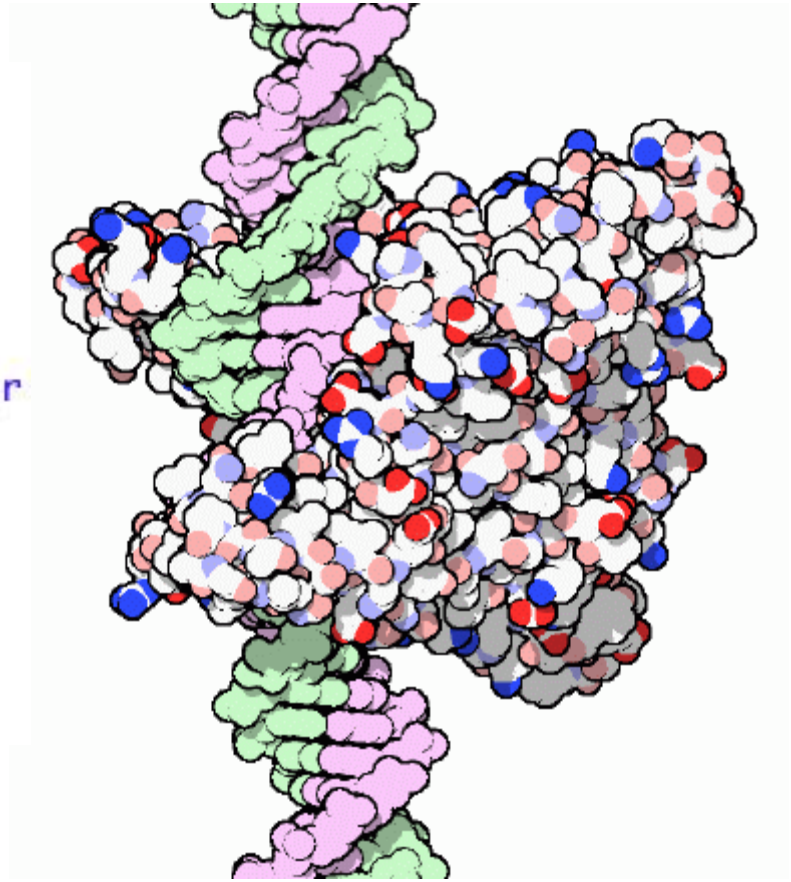
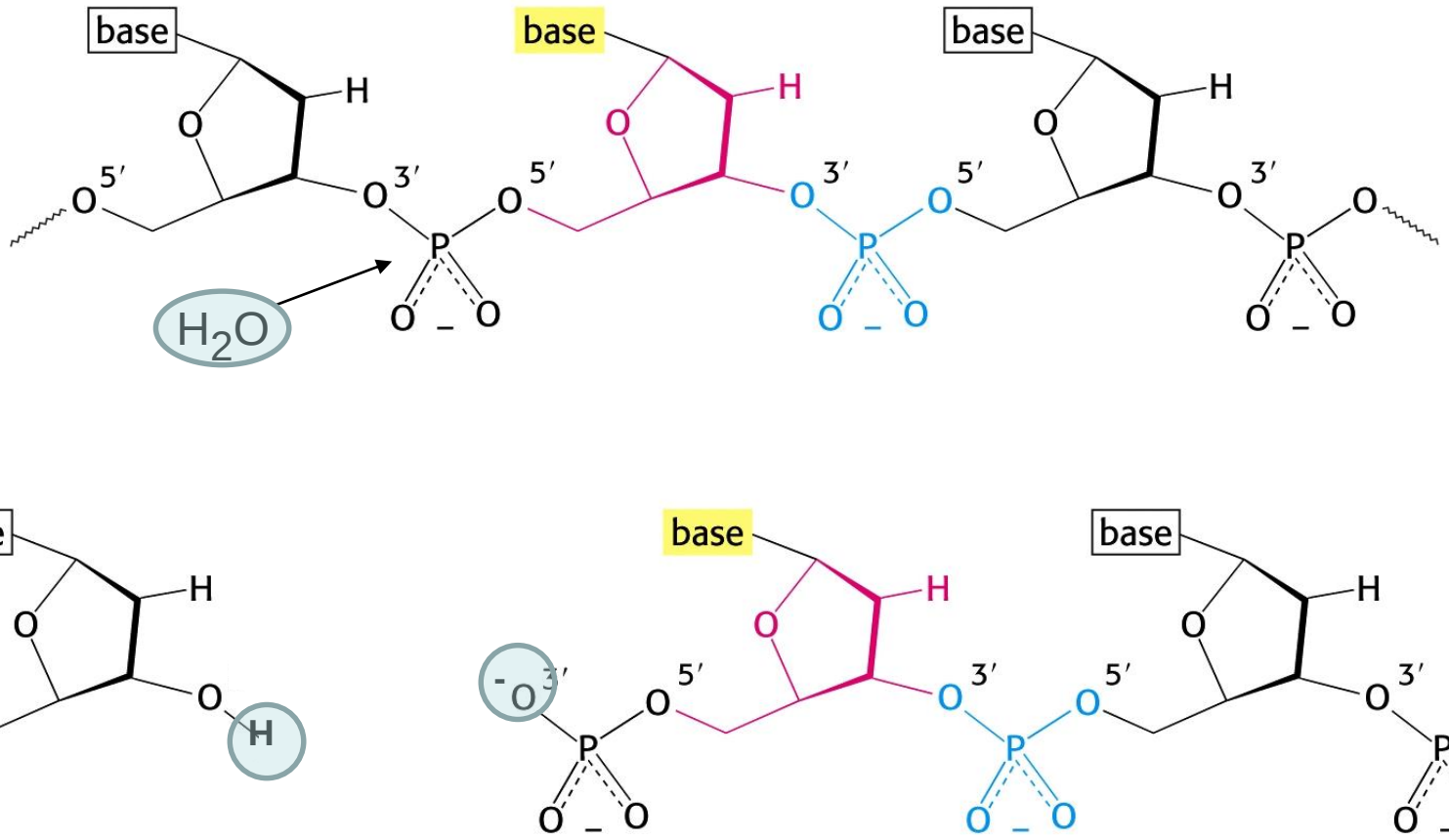
Enzymes de restriction





- Coupent les deux brins
- Réaction d'hydrolyse

Hydrolyse avec les enzymes de restriction



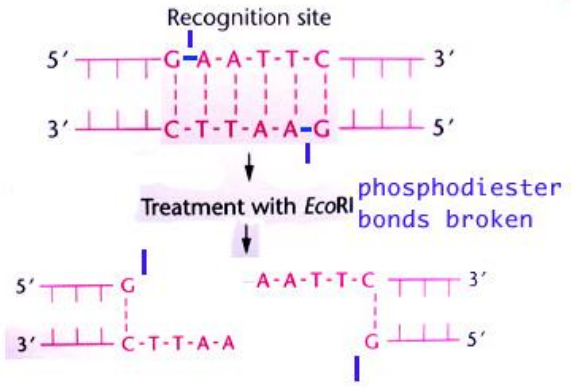
Exemple d'une réaction de restriction

5'-GTGACC**GAATTC**AAAGGTGCCTTTTATCATCACTTTAAAAATAAAAAACAATTACTCAGTGCCTGTTATA
3'-CACTGG**CTTAAG**TTTCCACGGAAAATAGTAGTGAAATTTTTATTTTTTGTTAATGAGTCACGGACAATAT

GCAATTAATTA**GAATTC**TGATTGATGCCTACATCACAACAAAAACTGATTTAACAAATGGTTGGTCTGCCTTA
CGTTAATTAAT**CTTAAG**ACTAACTACGGATGTAGTGTTGTTTTTGACTAAATTGTTTACCAACCAGACGGAAT

AAACCTTATCCCTATCCAAGAAGTGATG-3'
TTTGGAATAGGGATAGGTTCTTCACTAC-5'

*EcoR*I

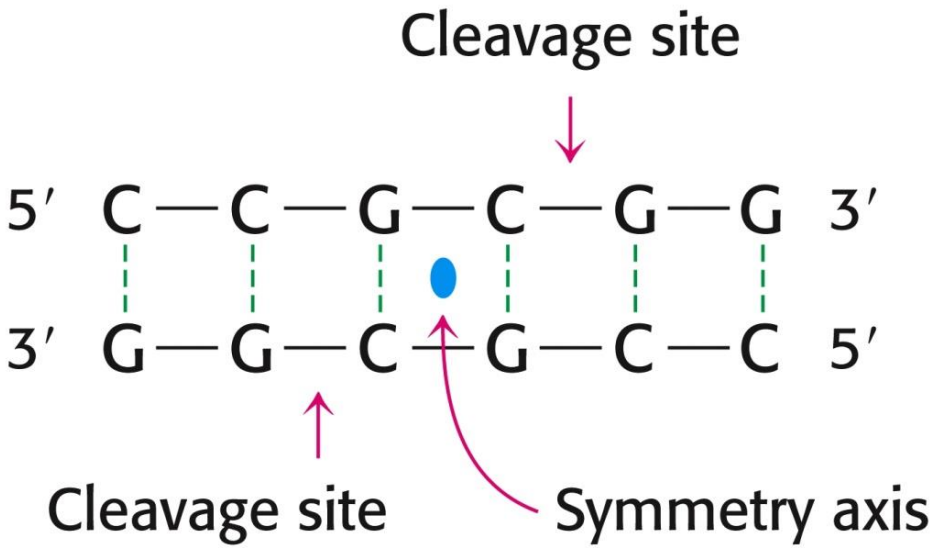


5'-GTGACC**G**-3'
3'-CACTGG**CTTAA**-5'

5'-**AATTC**AAAGGTGCCTTTTATCATCACTTTAAAAATAAAAAACAATTACTCAGTGCCTGTTATAAGCAGCAATTAATTA**G**-3'
3'-**G**TTTCCACGGAAAATAGTAGTGAAATTTTTATTTTTTGTTAATGAGTCACGGACAATATTCGTCGTTAATTAAT**CTTAA**-5'

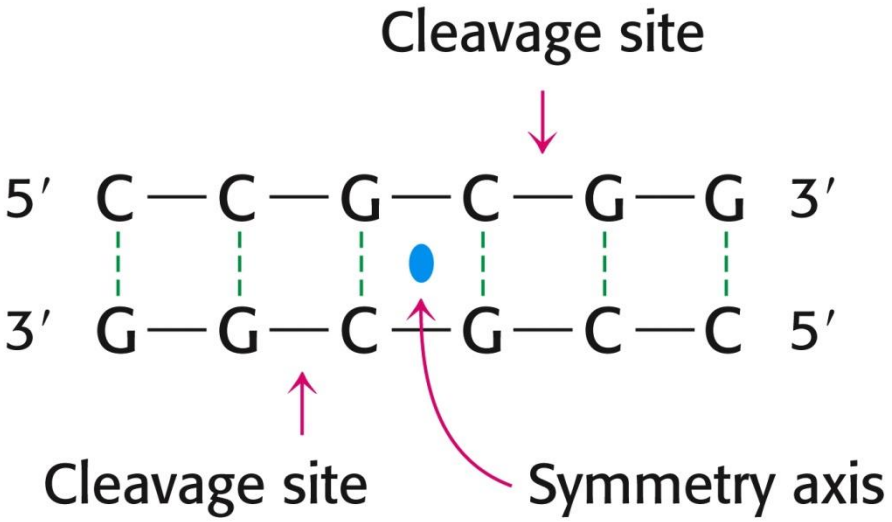
5'- **AATTC**TGATTGATGCCTACATCACAACAAAAACTGATTTAACAAATGGTTGGTCTATATTTGAACATTATCTTGATTATATTA-3'
3'- **G**ACTAACTACGGATGTAGTGTTGTTTTTGACTAAATTGTTTACCAACCAGATATAAACTTGTAATAGAACTAATATAAT-5'

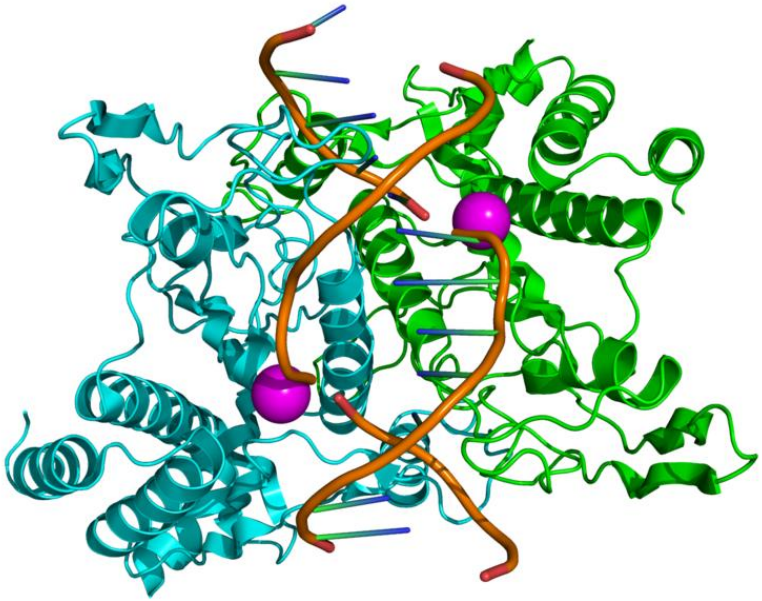
Les enzymes de restriction coupent toujours des séquences palindromiques



Pourquoi est-ce que les enzymes de restriction coupent des séquences palindromique?

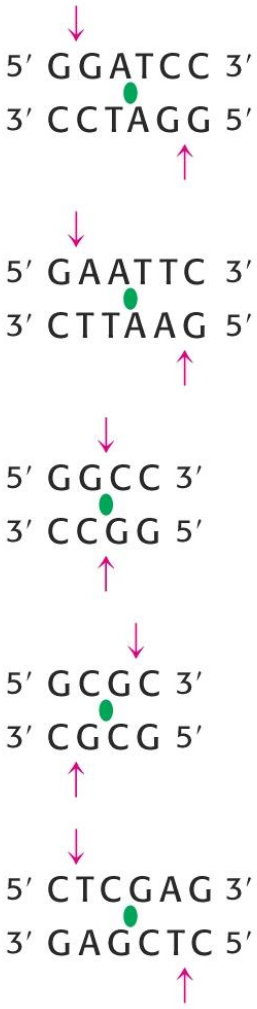
Les enzymes de restriction coupent toujours des séquences palindromiques

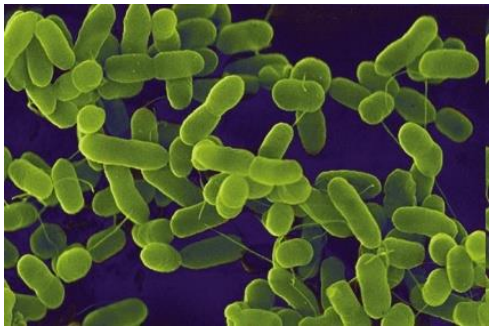




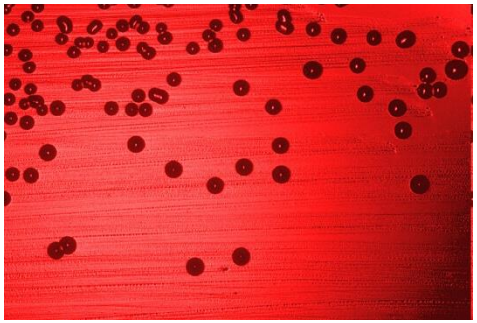
Les enzymes de restriction sont des homo-dimères: **unité 1** et **unité 2**

Plus de 1000 enzymes de restriction ont été isolées des bactéries



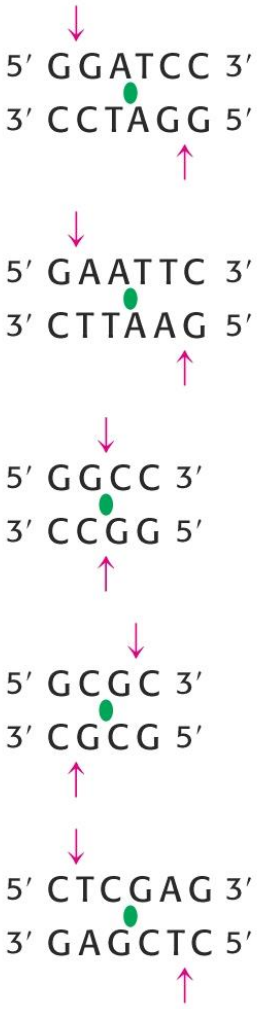


de *Escherichia Coli*



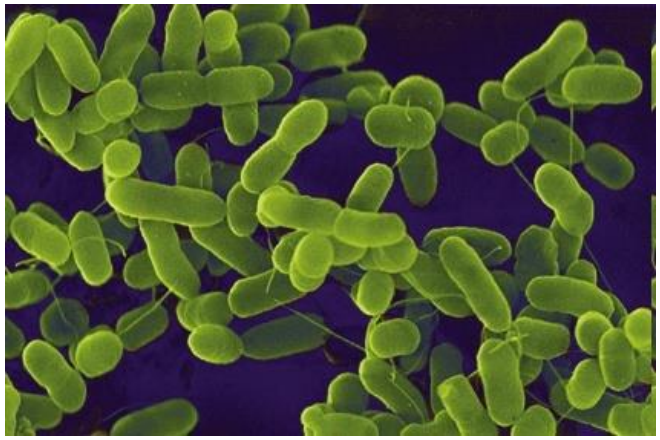
de *Haemophilius aegyptius*

Plus de 1000 enzymes de restriction ont été isolées des bactéries



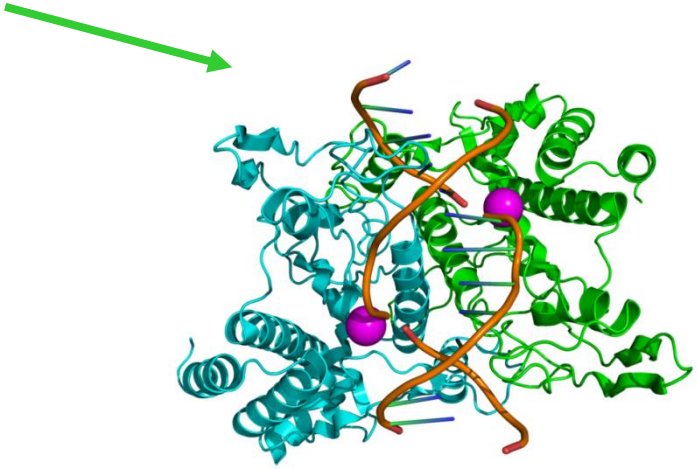
Pourquoi est-ce que les bactéries produisent des enzymes de restriction?

Mécanisme de protection contre les virus



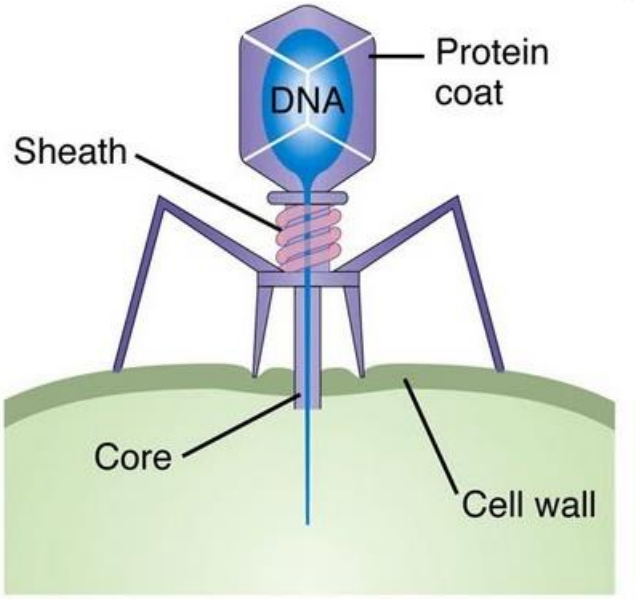
Escherichia Coli

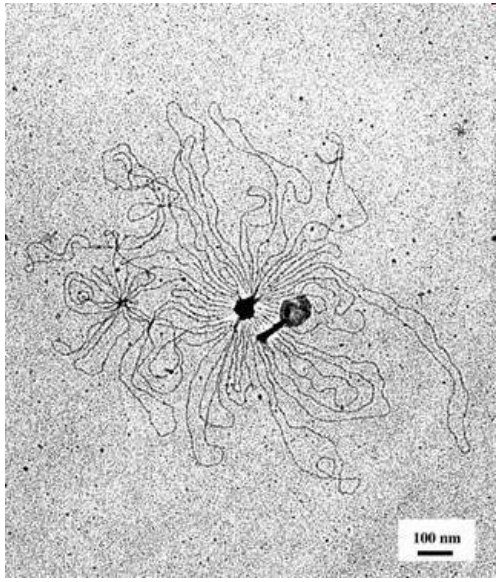
Les bactéries de *E.coli* produisent l'enzyme de restriction *EcoR*I



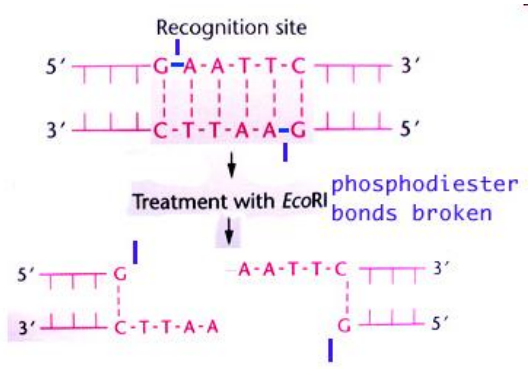
*EcoR*I

Mécanisme de protection contre les virus

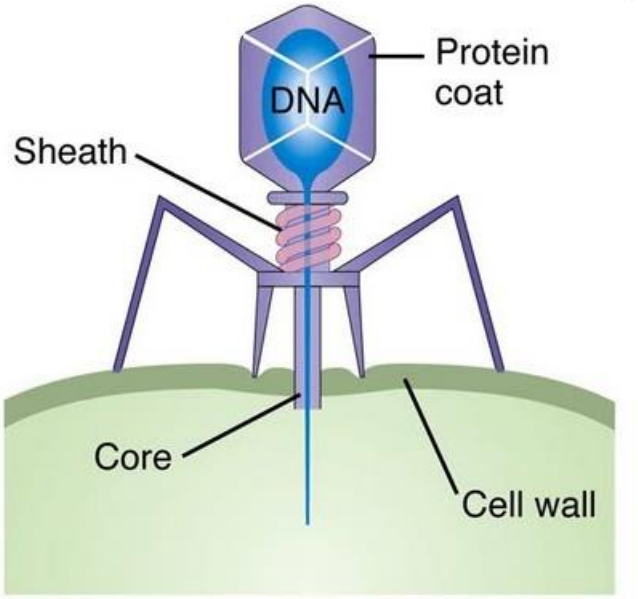


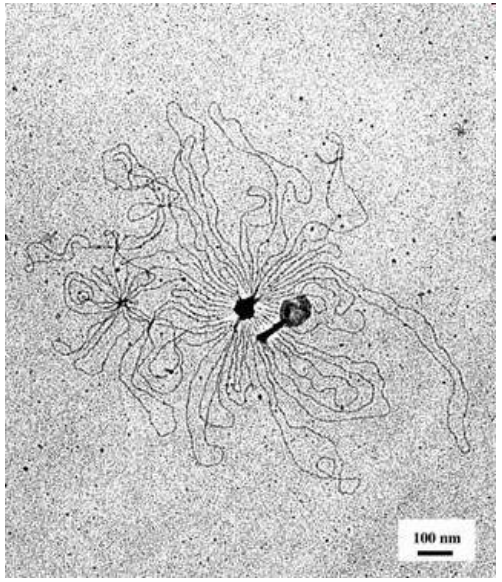


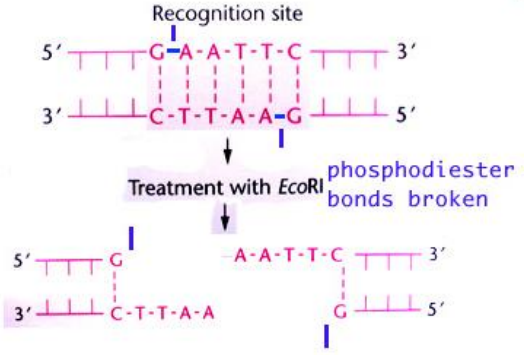
Infection d'une cellule de bactérie



Mécanisme de protection contre les virus



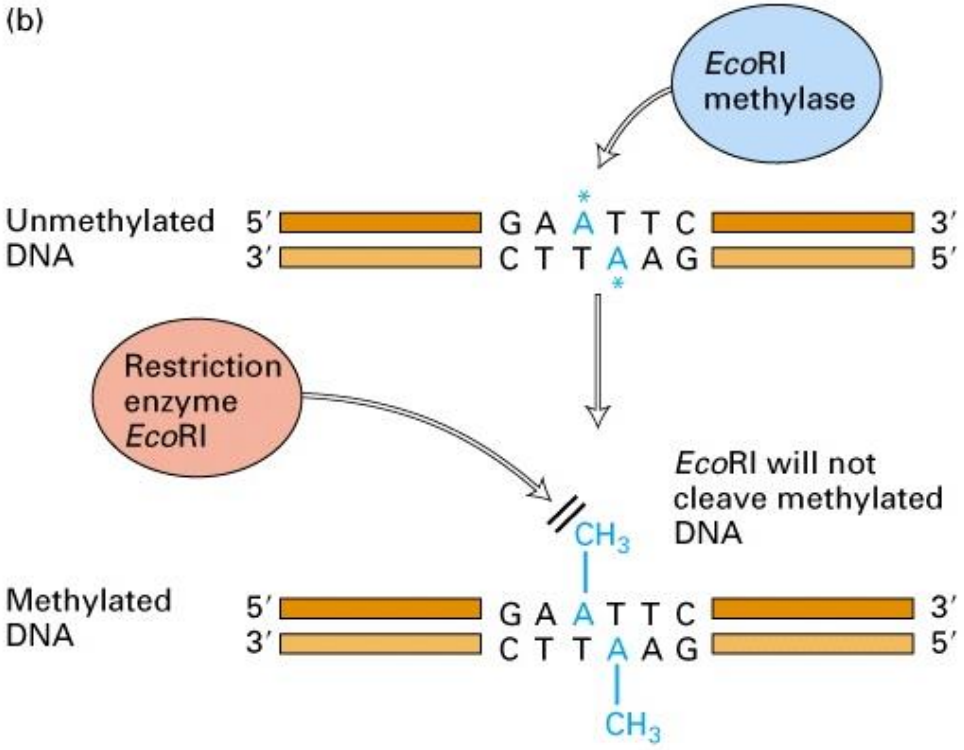


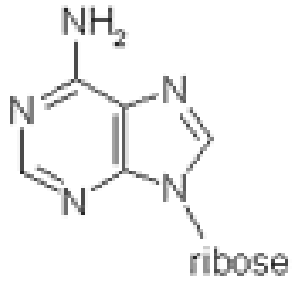


Infection d'une cellule de bactérie

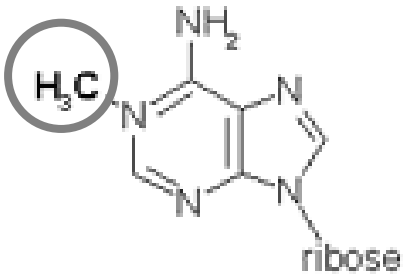
Pourquoi est-ce que le DNA des bactéries n'est pas clivé?

Mécanisme de protection





Adenosine



1-methyladenosine

Enzymes de restriction

Werner Arber
Biozentrum Basel
Nobel prize in 1978

Hamilton O. Smith
Johns Hopkins University
Nobel prize in 1978

Daniel Nathans
Johns Hopkins University
Nobel prize in 1978

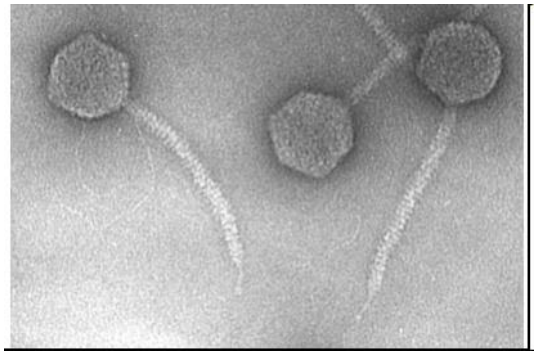
Ont découvert les enzymes de restriction

Première personne à avoir utilisé les enzymes de restriction

Application des enzymes de restriction

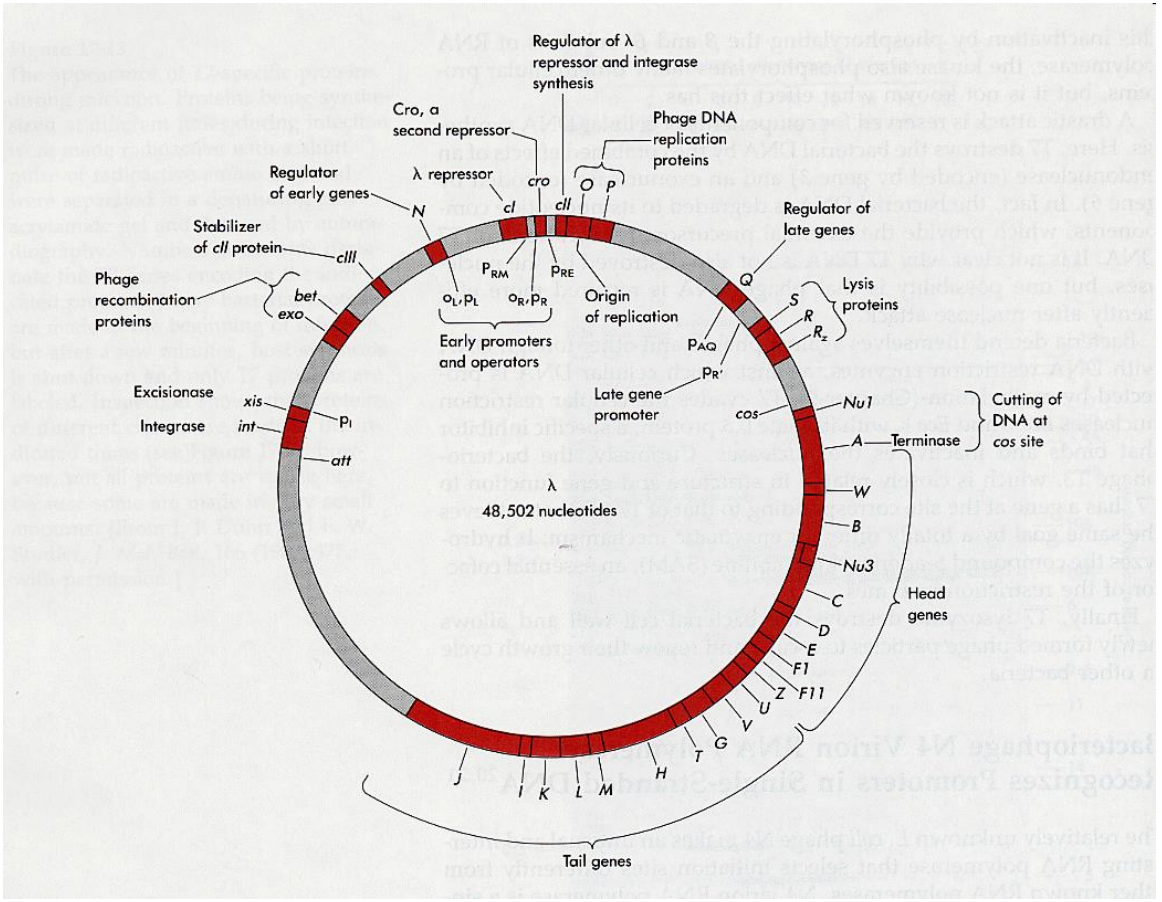
Pour quel application est-ce qu'on pourrait utiliser les enzymes de restriction?

L’analyse des longues molécules de DNA

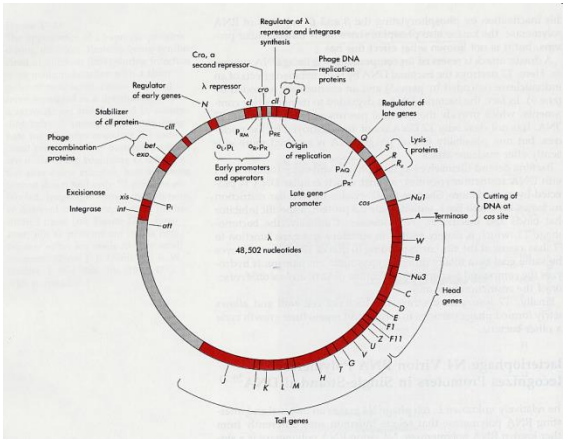


Lambda phage
(un virus)

cyclic DNA, **48’500** pairs de bases

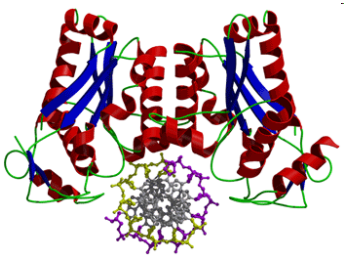


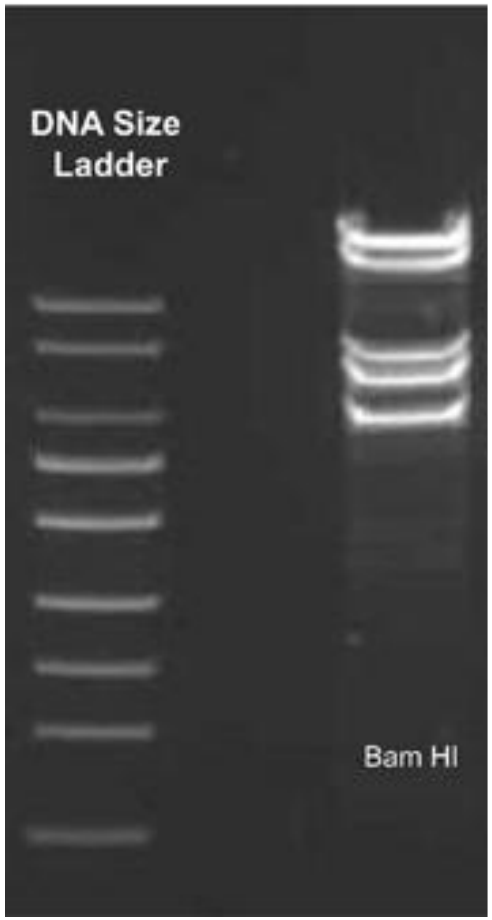
L’analyse des longues molécules de DNA

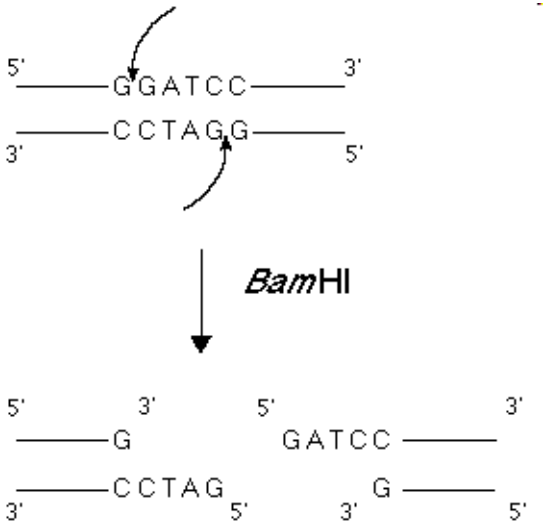


+

enzyme de restriction
(p.ex. *BamHI*)

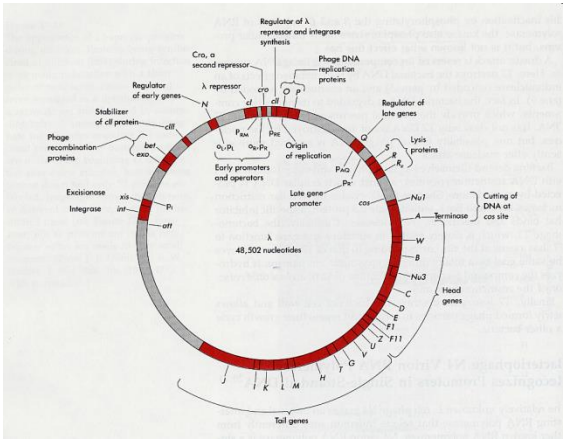






Combien de fois est-ce que l’enzyme BamHI a coupé le DNA du lambda phage ?

L’analyse des longues molécules de DNA



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enzyme de restriction
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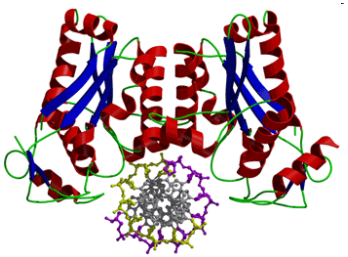
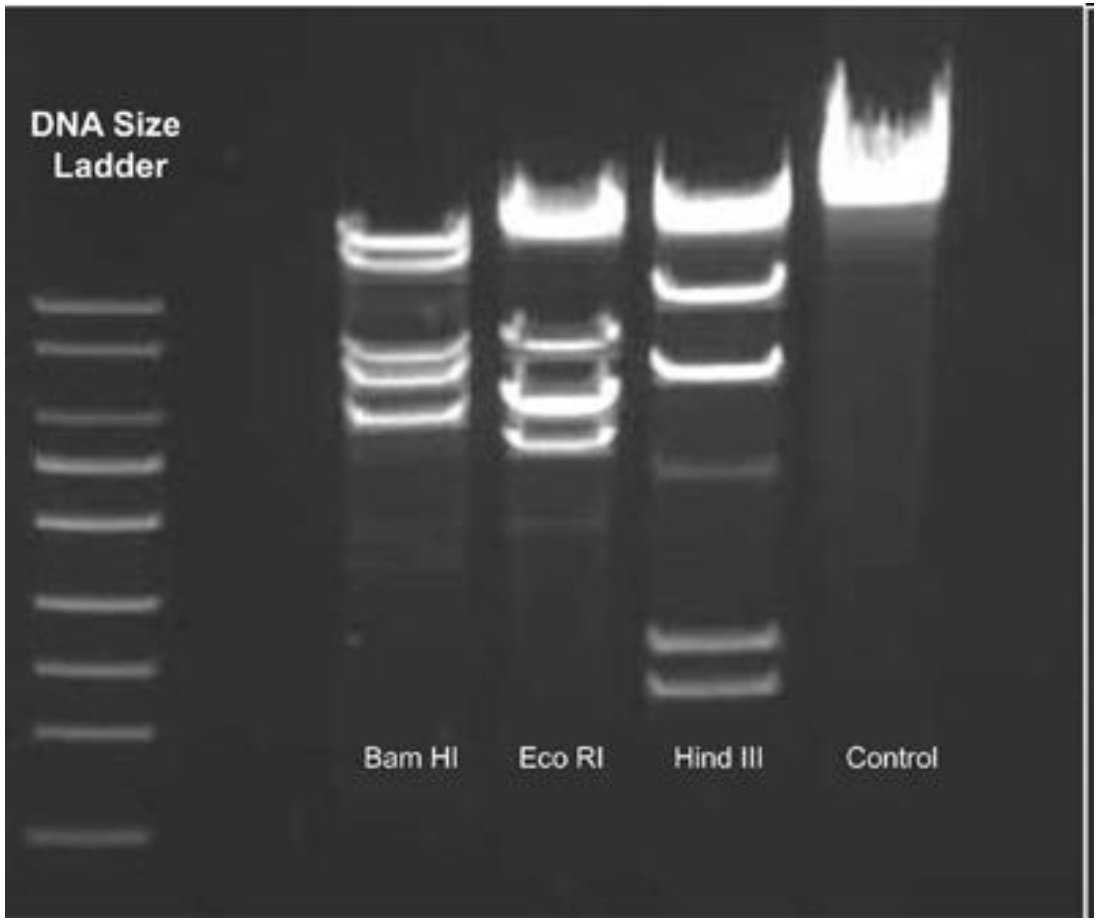
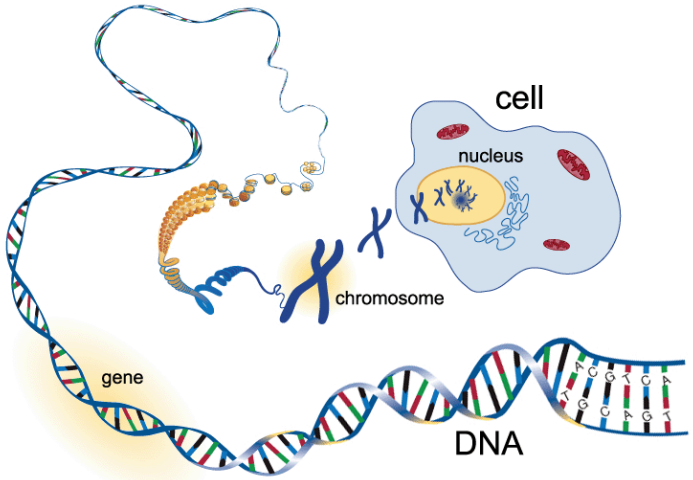




Leçon 5



- Electrophorèse de DNA sur gel
- Enzymes de restriction (1970)
- Technologie du DNA recombinant
- Séquençage de DNA (1975)
- Réaction en chaîne par polymérase (PCR) (1985)

Technologie du DNA recombinant

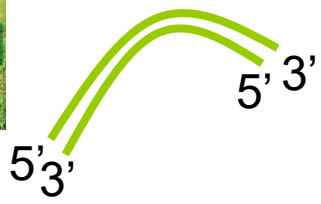
organisme 1

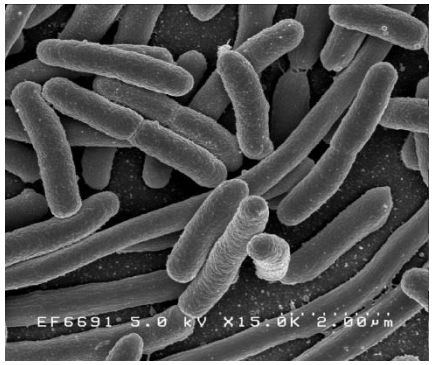
(p.ex. une vache)

transférer

un gène

spécifique





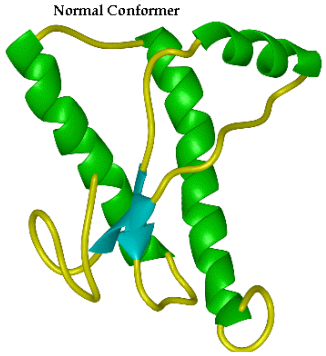
organisme 2

(p.ex. bactérie)

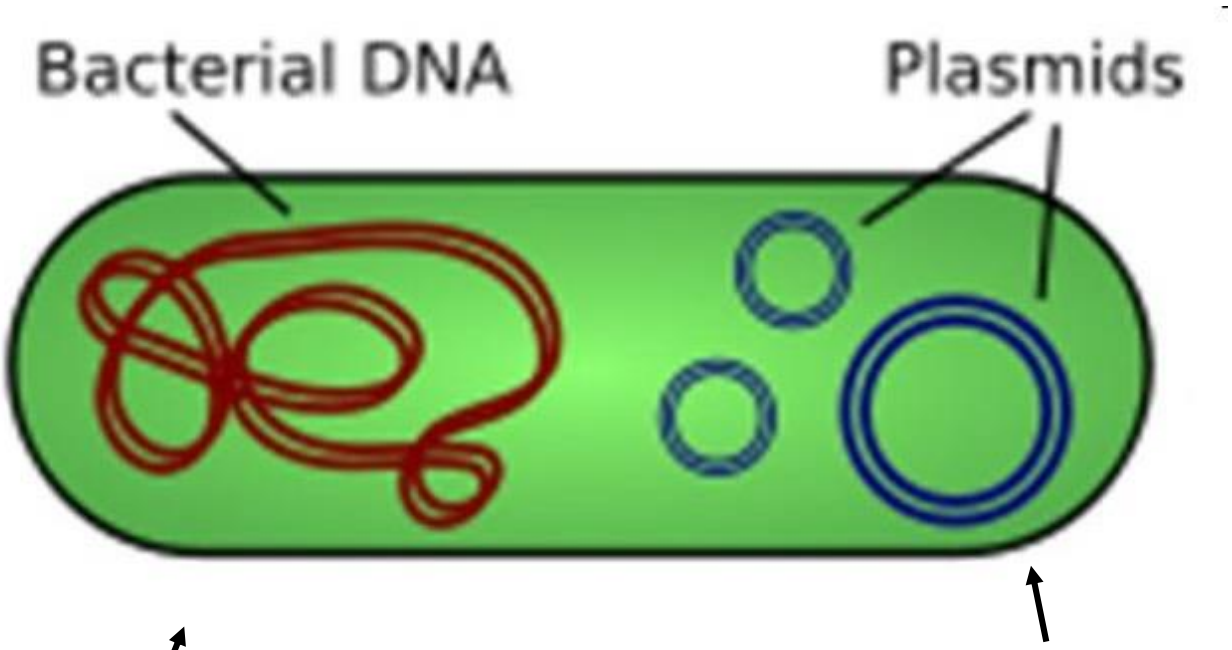
expression

d’une protéine

spécifique



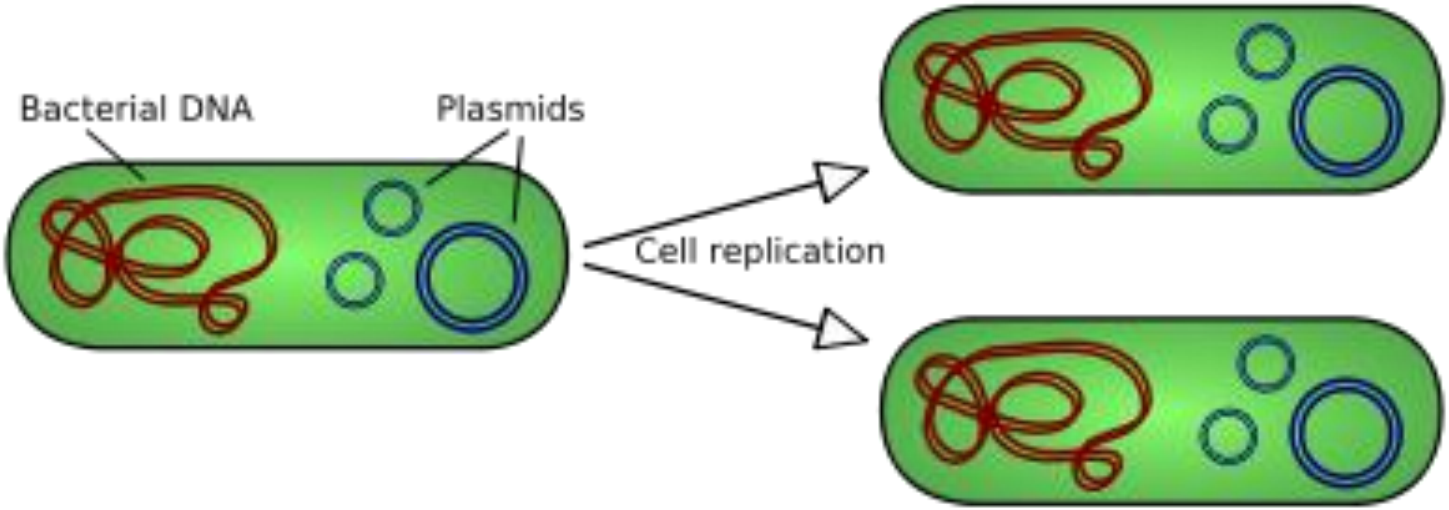
‘Vecteur’ de DNA



DNA génomique de bactérie
(En *E.coli,* 4.6 million de nucléotides,
contient tous les gènes du bactérie)

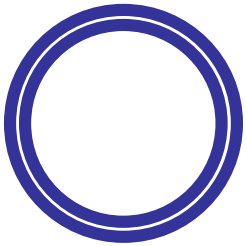
Plasmide = DNA circulaire
(environ 4000 nucléotides, peut se
répliquer de façon autonome
dans la bactérie)

Vecteur de DNA

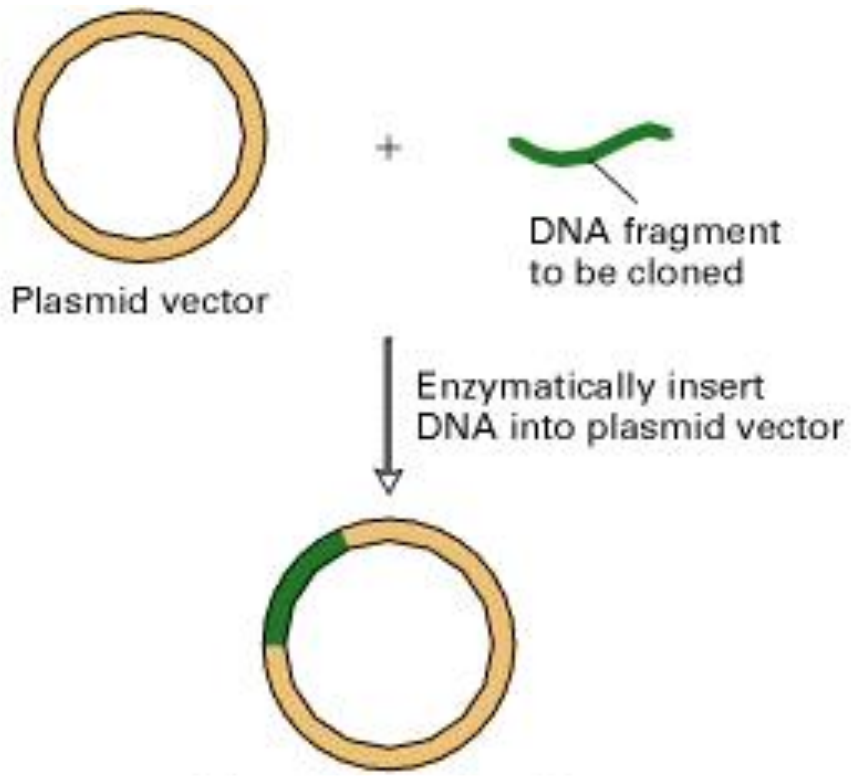


ouverture des cellules,
isolation des plasmides

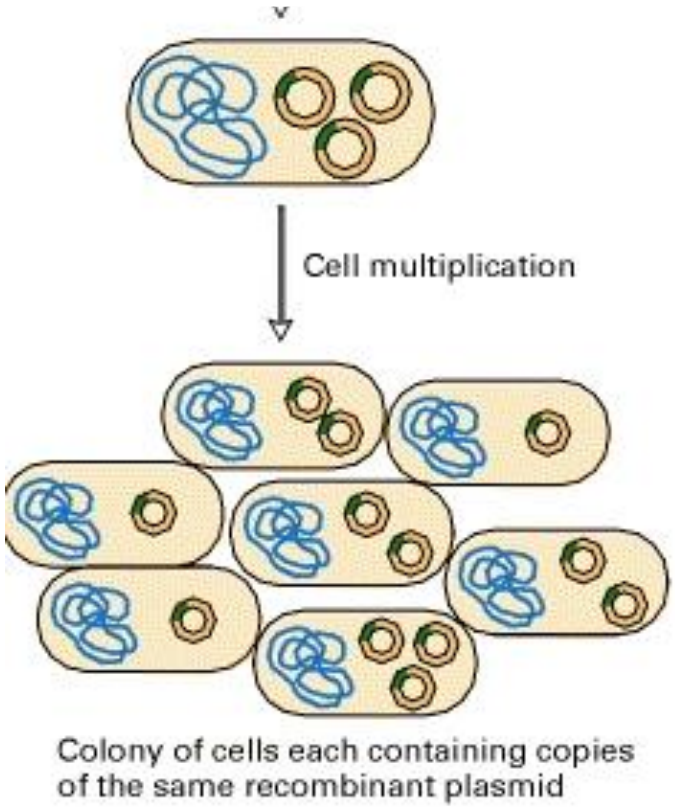
Plasmide isolé
(deux brins)



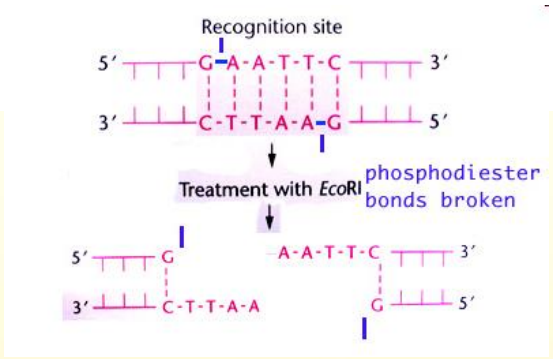
Insertion d'un gène dans un plasmide de DNA



Insertion d'un gène dans un plasmide de DNA



Clivage du plasmide et du DNA extérieur

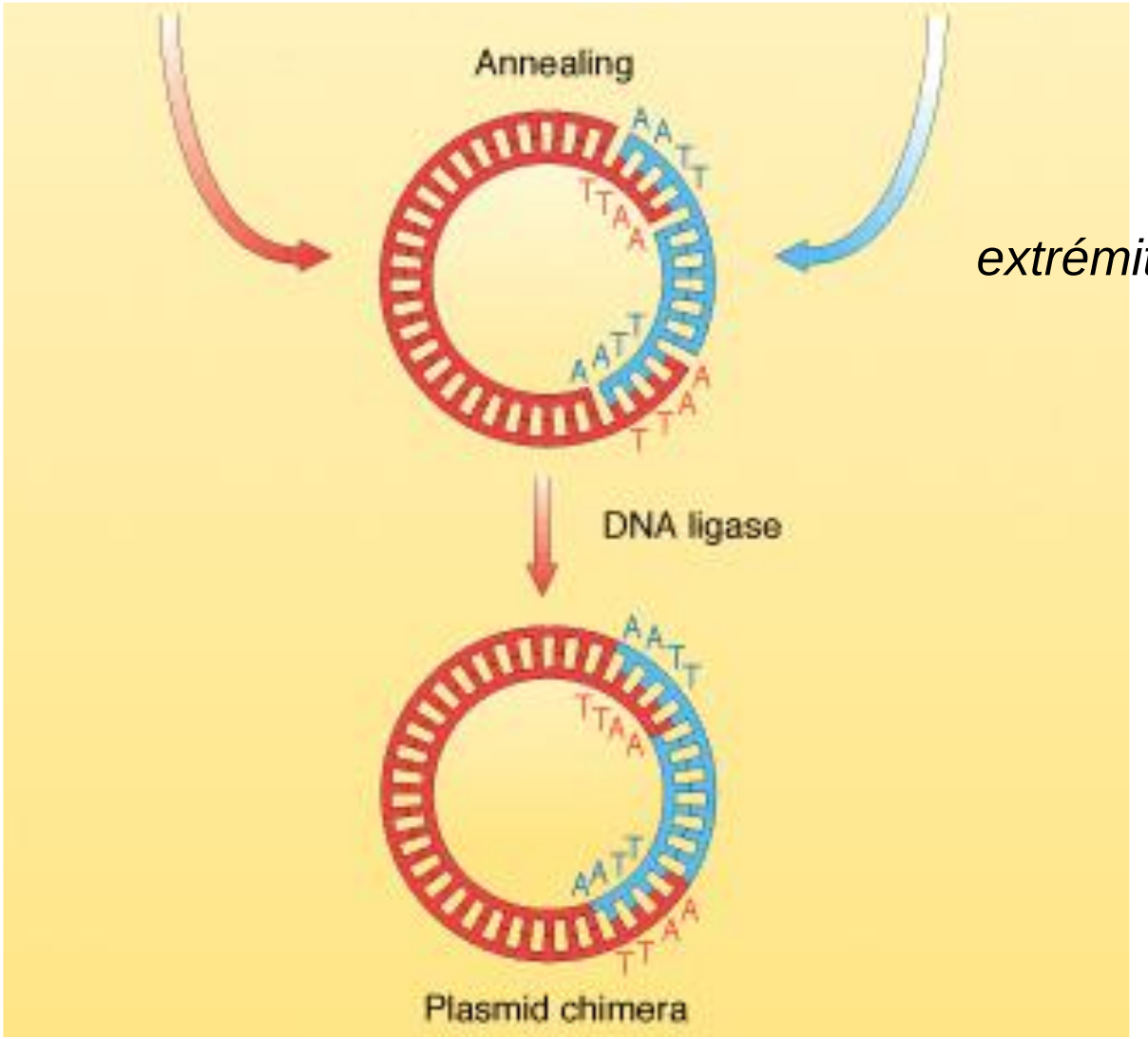


*Eco*RI

extrémités cohésives

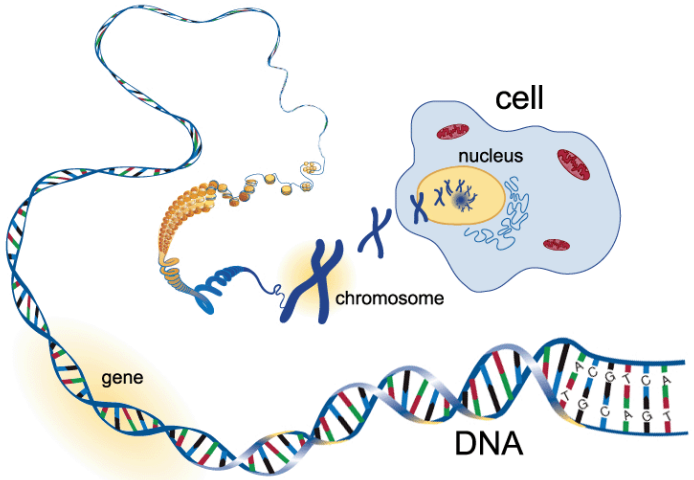
extrémités cohésives

Fusion du plasmide et du DNA extérieur



extrémités cohésives

Leçon 5



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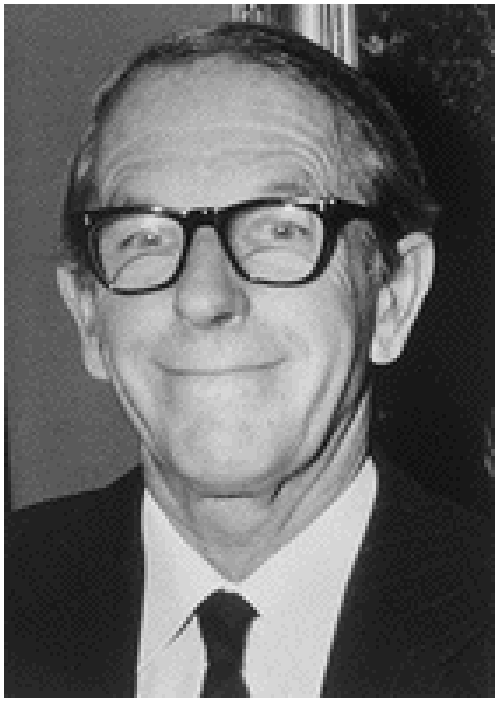
Séquençage du DNA



Frederick Sanger

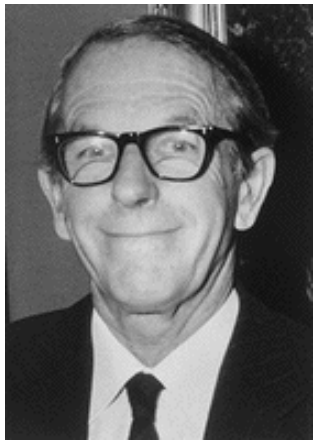
Prix Nobel en 1958 et 1980

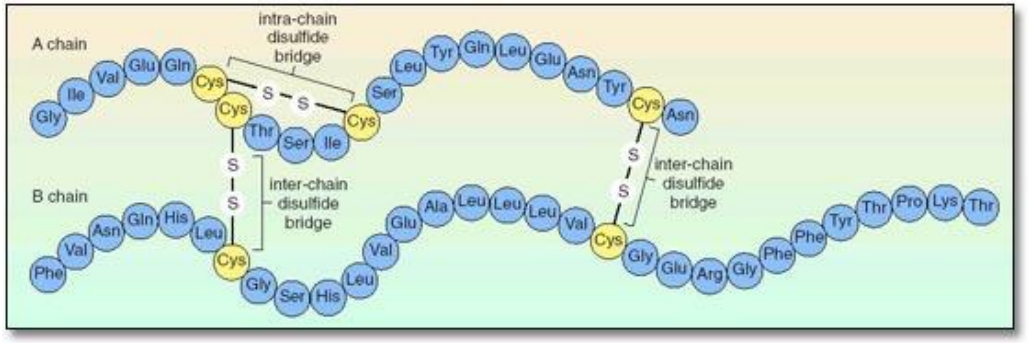
Cambridge University

Pour quelle contribution

a-t-il reçu le premier Prix Nobel?

Frederick Sanger





Séquence primaire de l'insuline

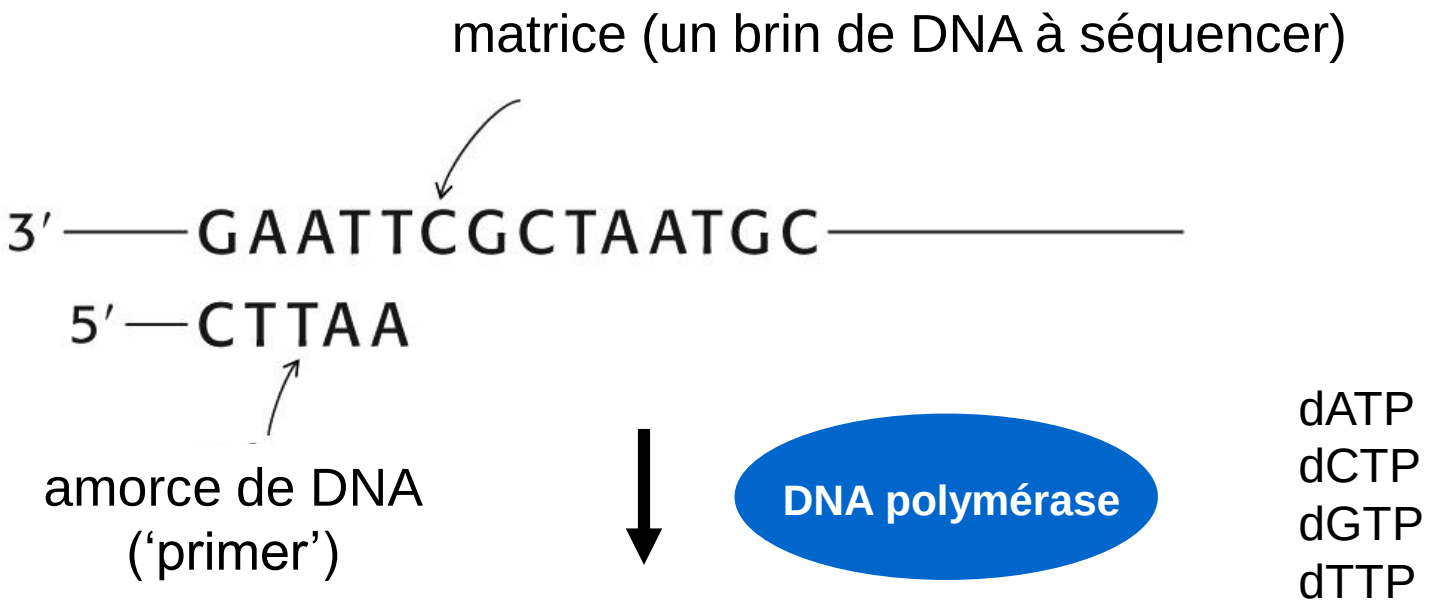
- 1953: Détermination de la séquence des aminoacides de l'insuline
- 1958: Prix Nobel en chimie
- 1970ies: Développement d'une méthode pour séquencer le DNA
- 1980: Prix Nobel en chimie

Séquençage du DNA

Comment pourrait-on séquencer un gène dans un morceau de DNA?

5‘-GTGACCAAAGGTGCCTTTTATCATCACTTTAAAAATAAAAAACAATTACTCAGTGCCTGTTA
TAAGCAGCAATTAATTATGATTGATGCCT**ACATCACAACAAAAACTGATTTAACAAATGGTTGGT**
CTGCCTTAGAAAGTATATTTGAACATTATCTTGATTATATTATTGATAATAATAAAAACCTTATCCC
TATCCAAGAAGTGATGCCTATCATTGGTTGGAATGAACTTGAAAAAATTAGCCTTGAATACATT
ACTGGTAAGGTAAACGCCATTGTCAGCAAATTGATCCAAGAGAACCAACTTAAAGCTTATGAT
AGTGATGTGCTTAAAAACTTACTCAATGGCTGGTTTATGCATATCGCAATACATGCGAAAAACC
TAAAAGAGCTTGCCGATAAAAAAGGCCAATTTATTGCTATTTACCGCGGCTTTTTATTGAGCTT
GAAAGATAAATAAAATAGATAGGTTTTATTTGAAGCTAAATCTTCTTTATCGTAAAAAATGCCCTC
TTGGGTTATCAAGAGGGTCATTATATTTCGCGGAATAACATCATTTGGTGACGAAATAACTAAGCA
CTTGTCTCCTGTTTACTCCCCTGAGCTTGAGGGGTTAACATGAAGGTCATCGATAGCAGGATAA
TAATACAGTAAAACGCTAAACCAATAATCCAAATCCAGCCATCCCAAATTGGTAGTGAATGATTAT
AAATAACAGTAAACAGTAATGGGCCAATAACACCGGTTGCATTGGTAAGGCTCACCAATAATCCC
TGTAAAGCACCTTGCTCATGACTCTTTGTTTGGATAGACATCACTCCCTGTAATGCAGGTAAAG-
3’

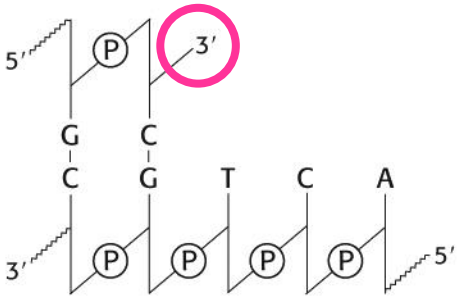
Synthèse du brin complémentaire du gène à séquencer



5’-**CTTAA**GCGATTACG-3’

Synthèse du brin complémentaire du gène à séquencer

amorce de DNA
(‘primer’)

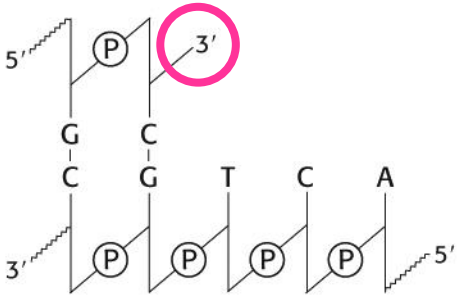


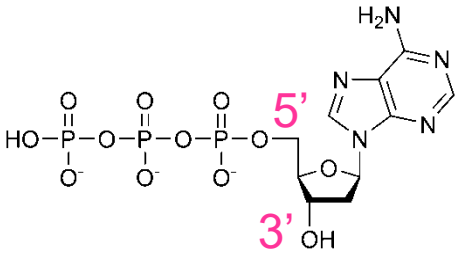
matrice (un brin de DNA à séquencer)

Synthèse du brin complémentaire du gène à séquencer

DNA polymérase

amorce de DNA
(‘primer’)

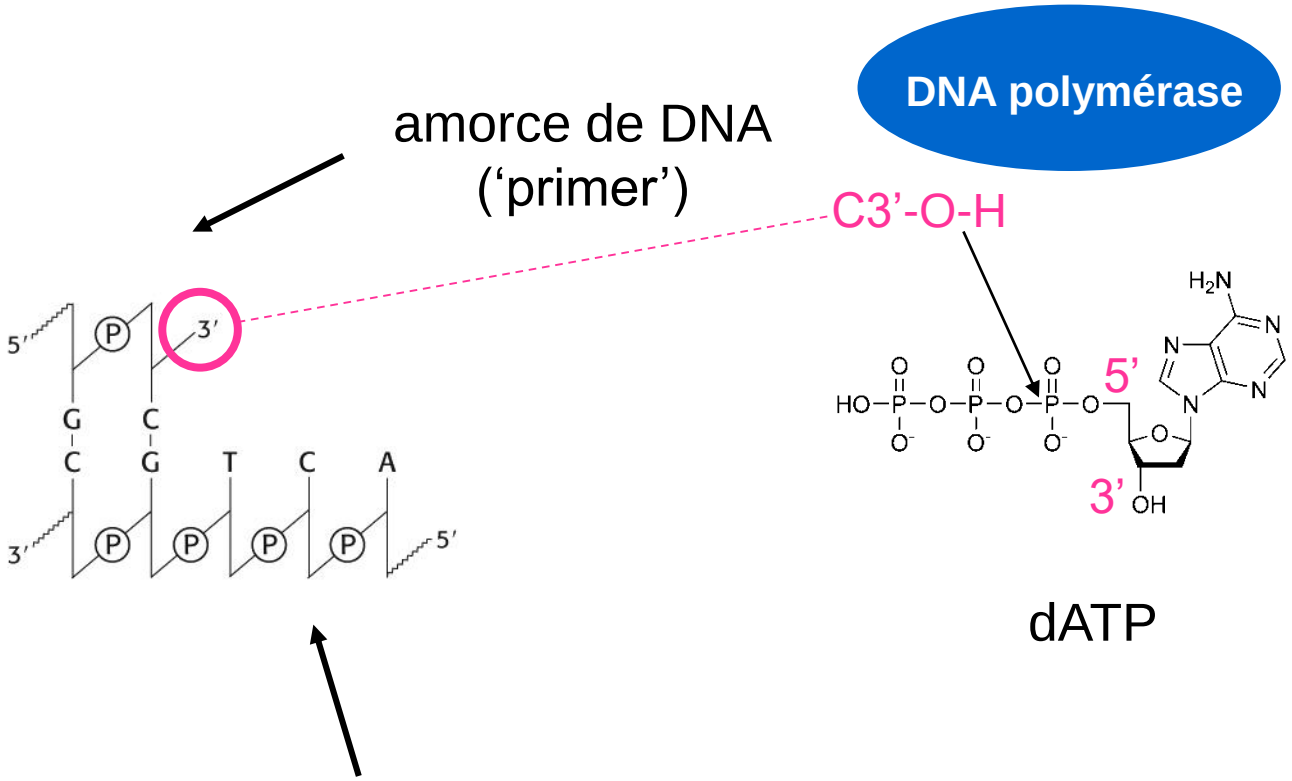




dATP

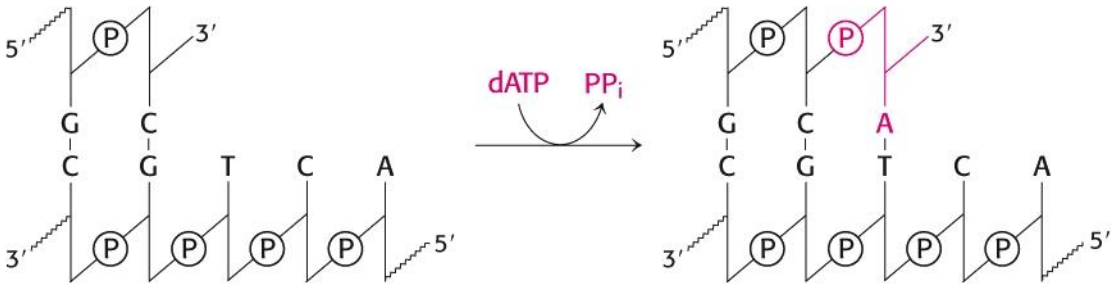
matrice (un brin de DNA à séquencer)

Synthèse du brin complémentaire du gène à séquencer

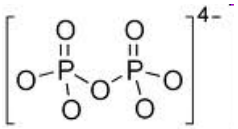


Synthèse du brin complémentaire du gène à séquencer

DNA polymérase

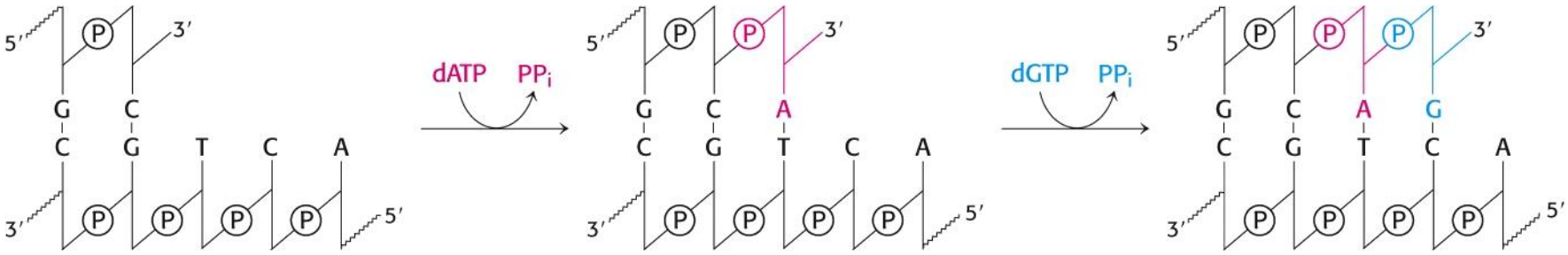


PP_i =



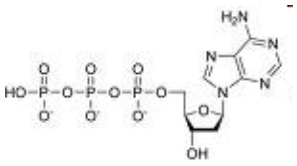
Synthèse du brin complémentaire du gène à séquencer

DNA polymérase

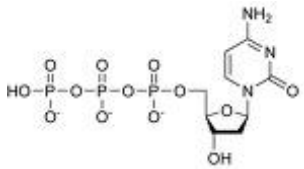


- DNA polymérase catalyse la ligation des nucleotides
- Élongation dans la direction 5’ → 3’

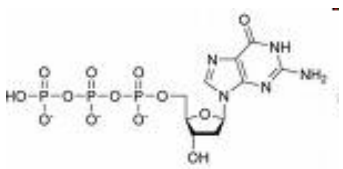
Synthèse du brin complémentaire du gène à séquencer



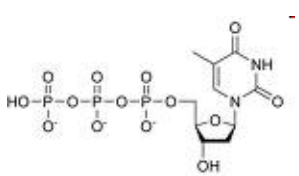
dATP



dCTP

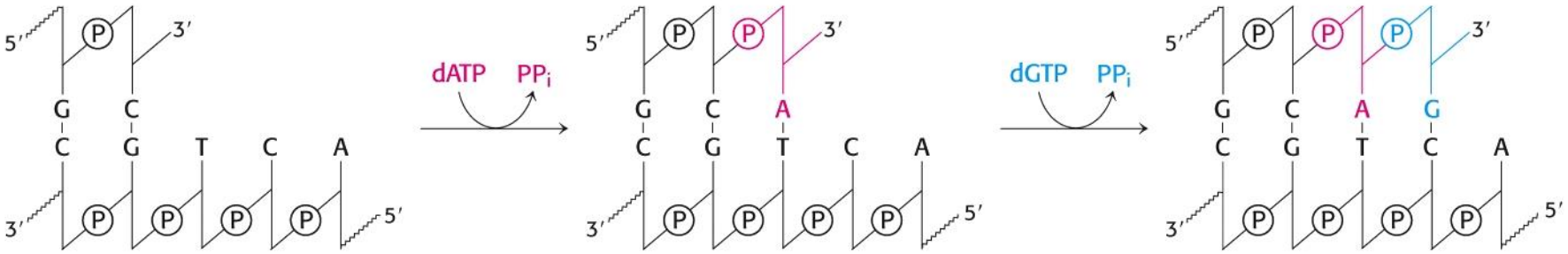


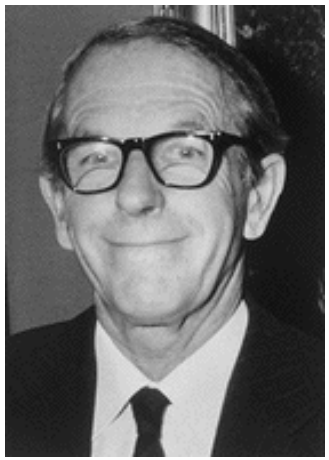
dGTP

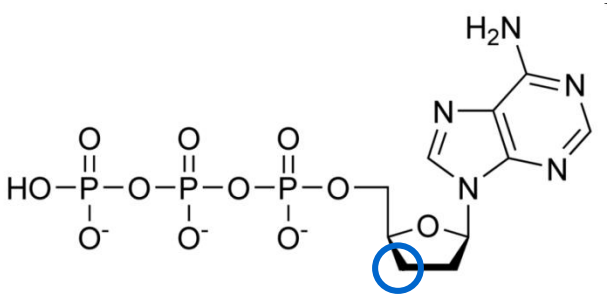


dTTP

DNA polymérase



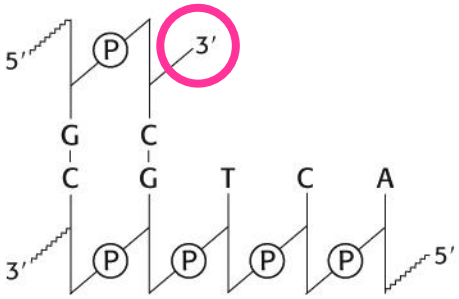




di-deoxy-adenosine-triphosphate

Synthèse du brin complémentaire du gène à séquencer

amorce de DNA
(‘primer’)

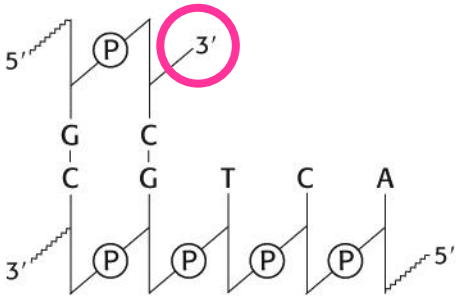


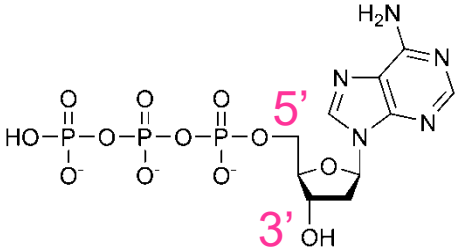
matrice (un brin de DNA à séquencer)

Synthèse du brin complémentaire du gène à séquencer

DNA polymérase

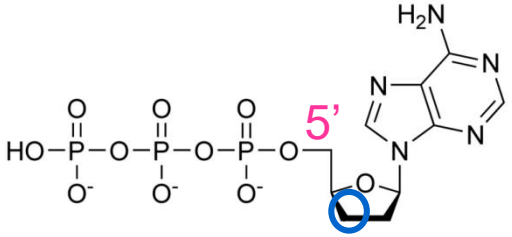
amorce de DNA
(‘primer’)





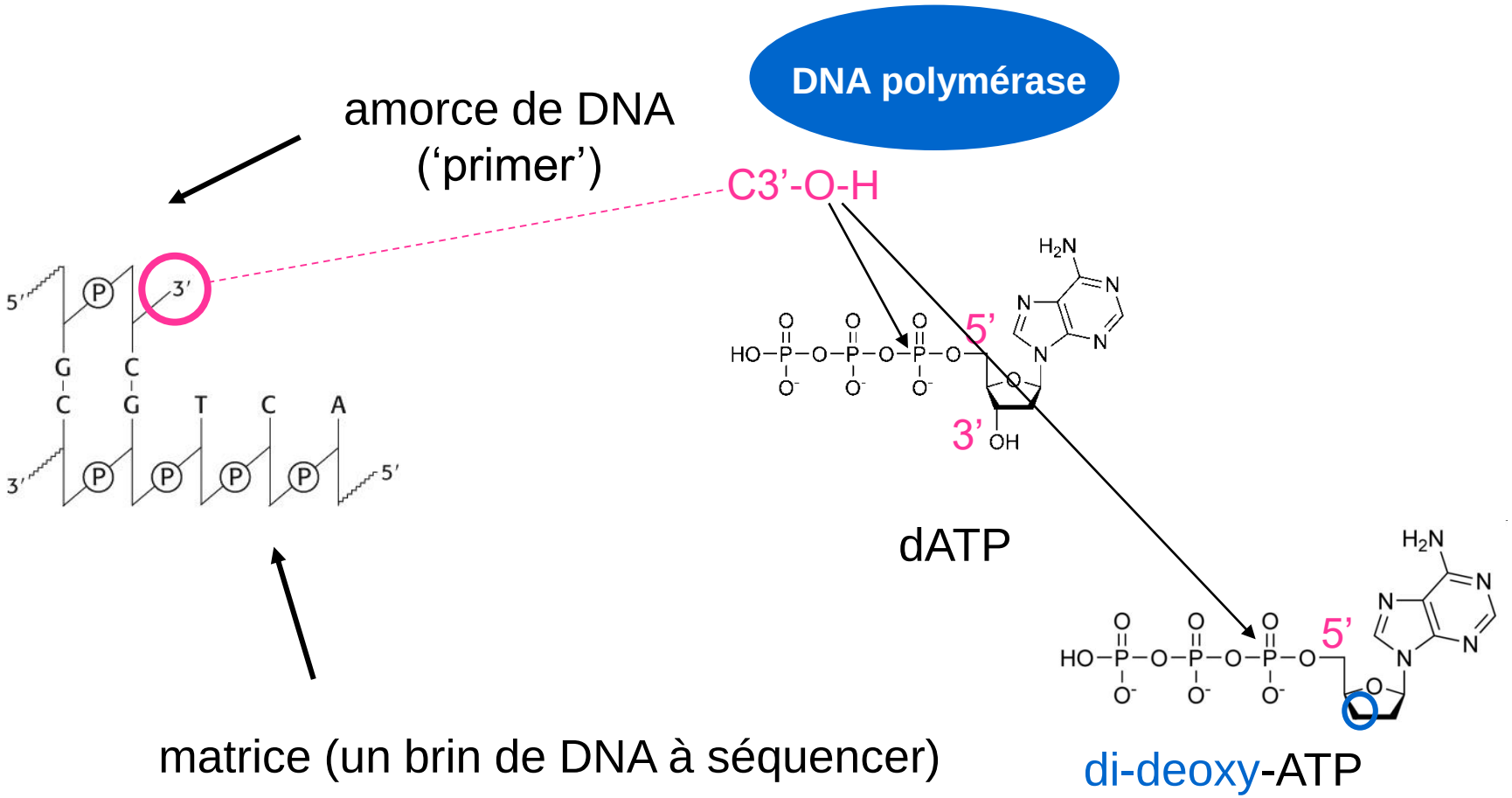
dATP

matrice (un brin de DNA à séquencer)

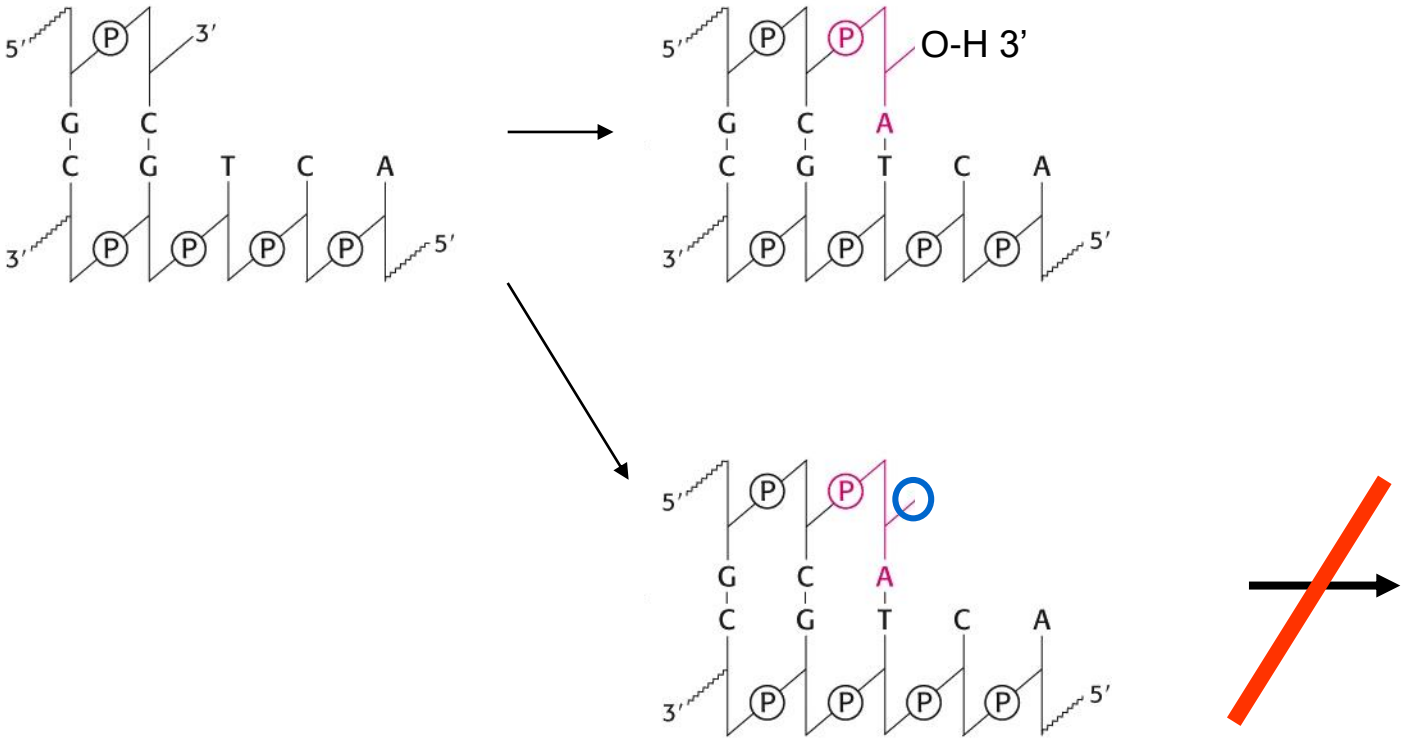


di-deoxy-ATP

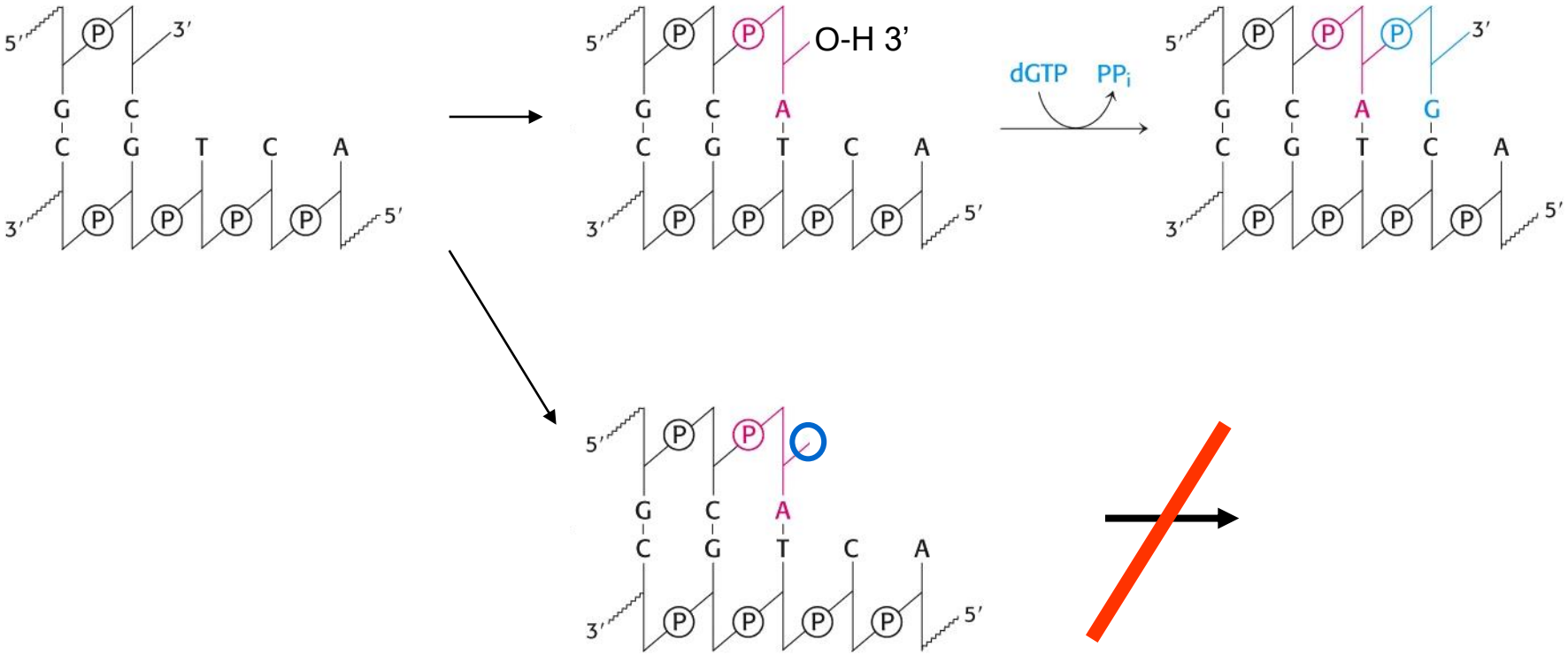
Synthèse du brin complémentaire du gène à séquencer



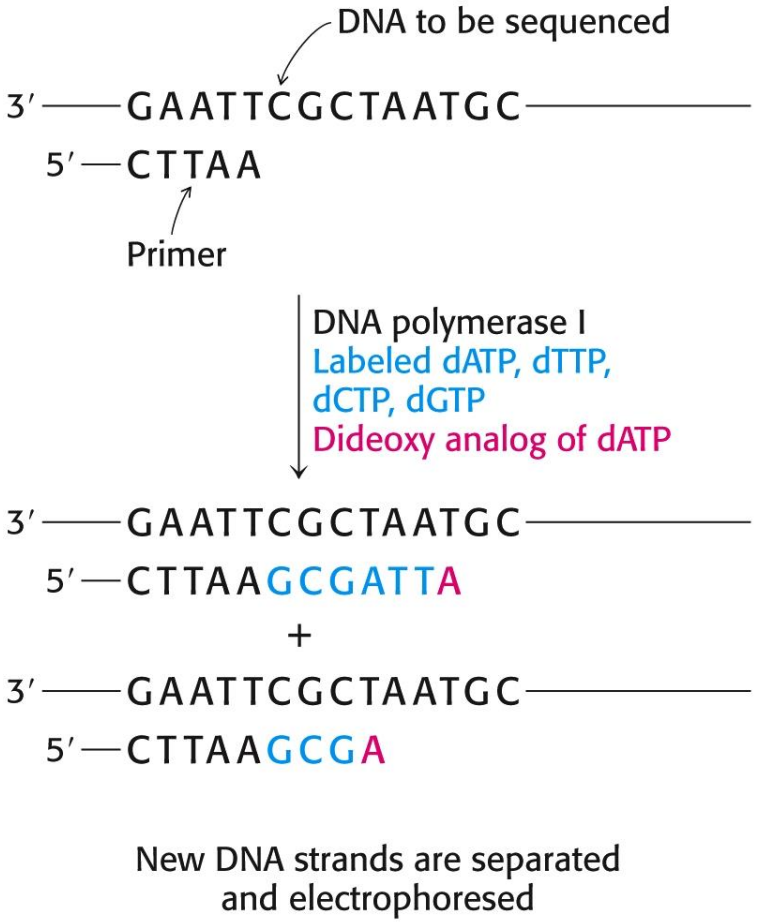
Synthèse du brin complémentaire du gène à séquencer

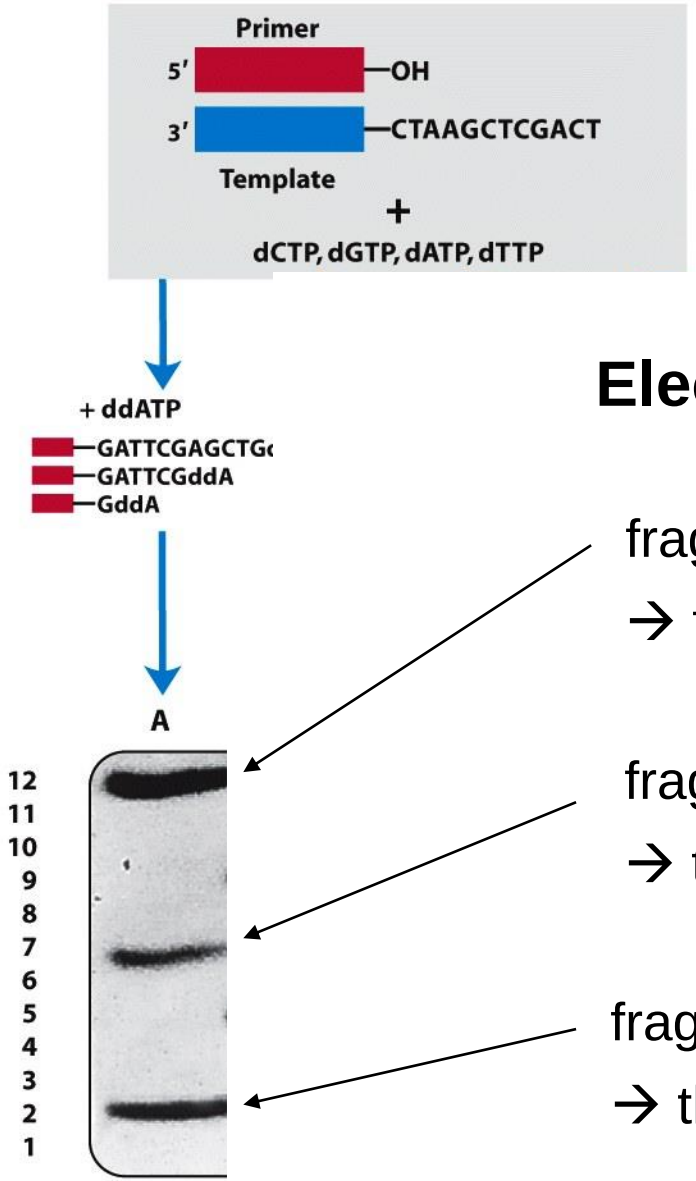


Synthèse du brin complémentaire du gène à séquencer



Séquençage du DNA d'après Sanger





Electrophorèse sur gel:

fragment de 12 nucléotides

→ thymidine en position 12

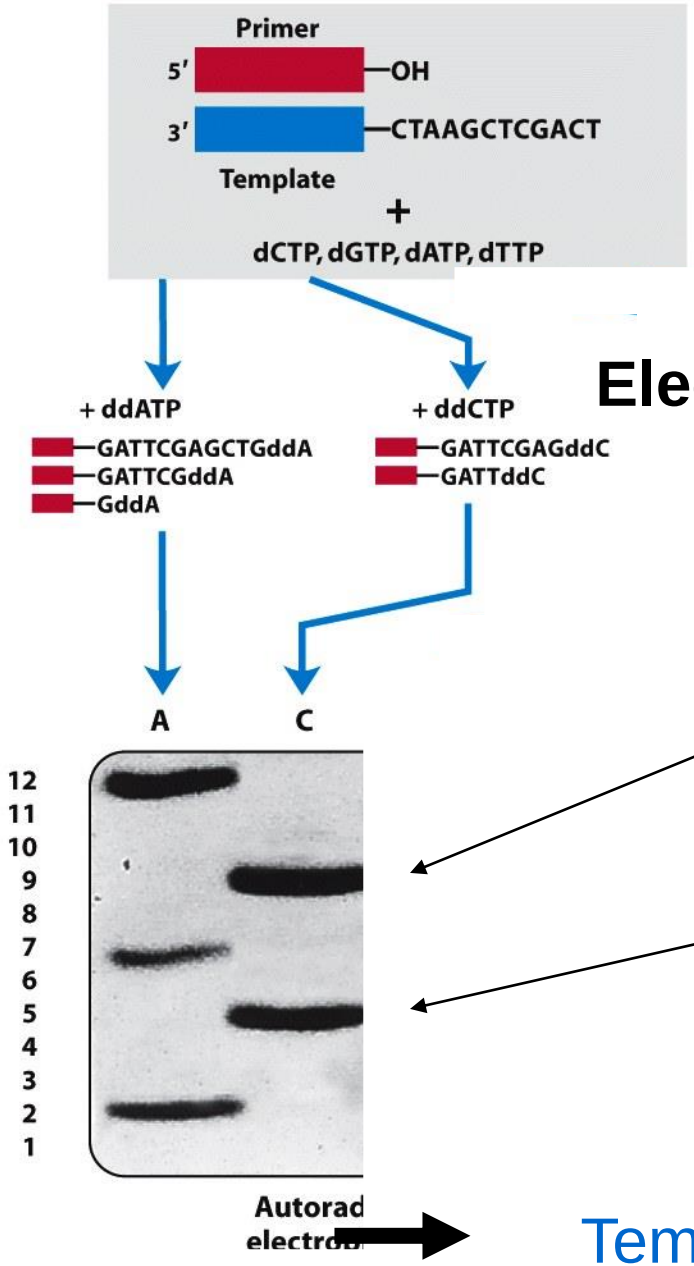
fragment de 7 nucléotides

→ thymidine en position 7

fragment de 2 nucléotides

→ thymidine en position 2

→ Template-X**T**XXXX**T**XXXX**T**



Electrophorèse sur gel:

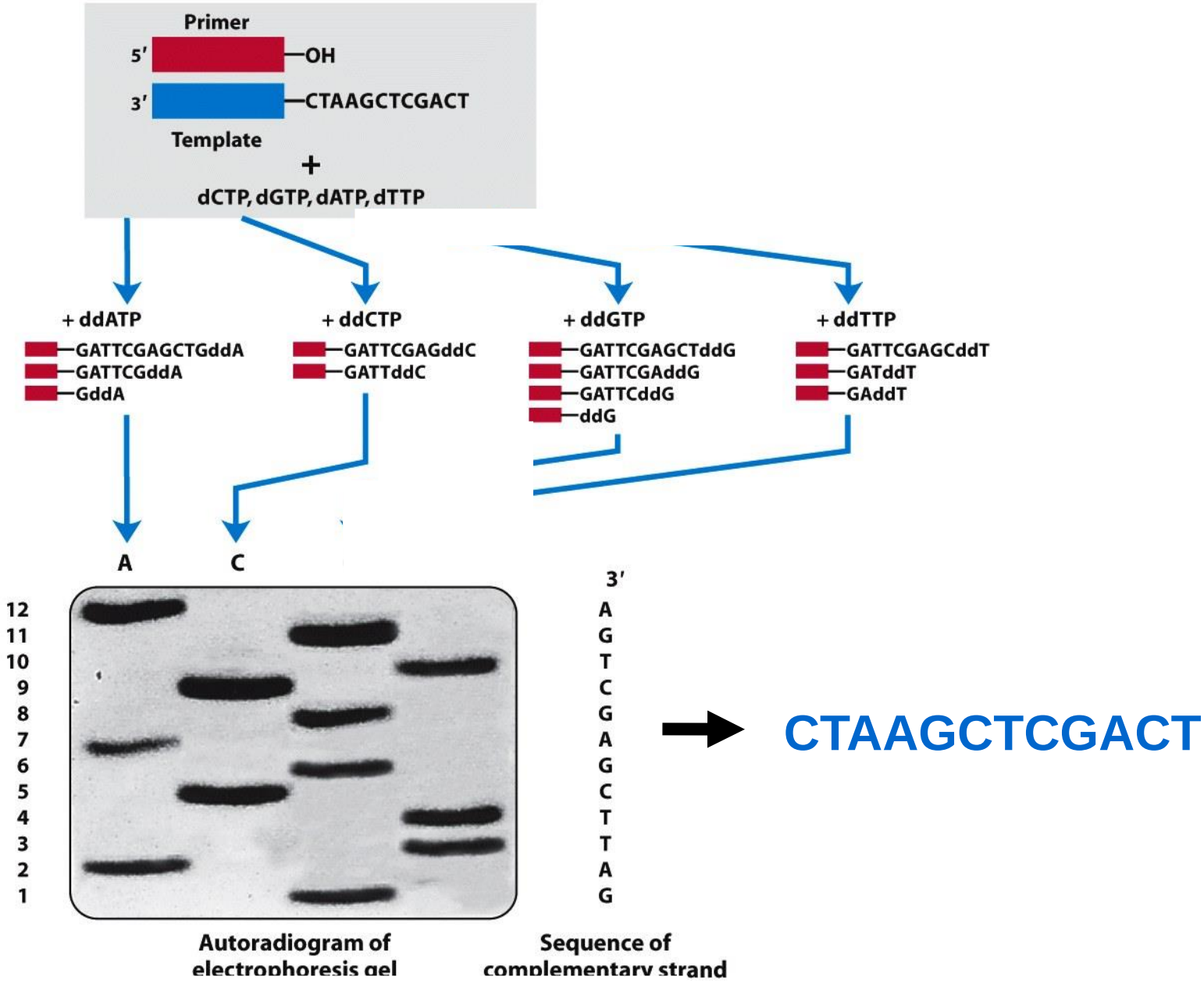
fragment de 9 nucléotides

→ guaninosine en position 9

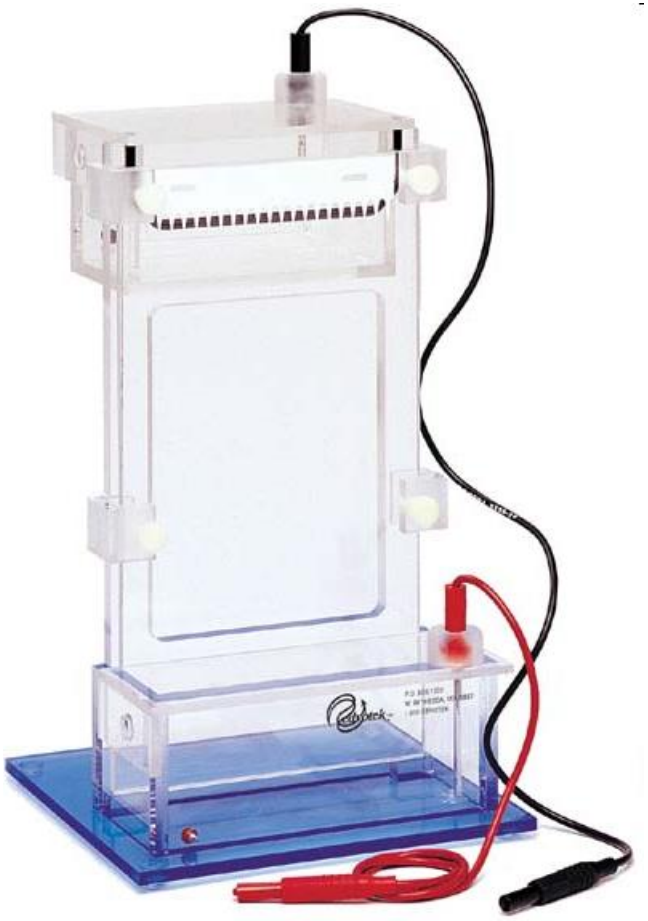
fragment de 5 nucléotides

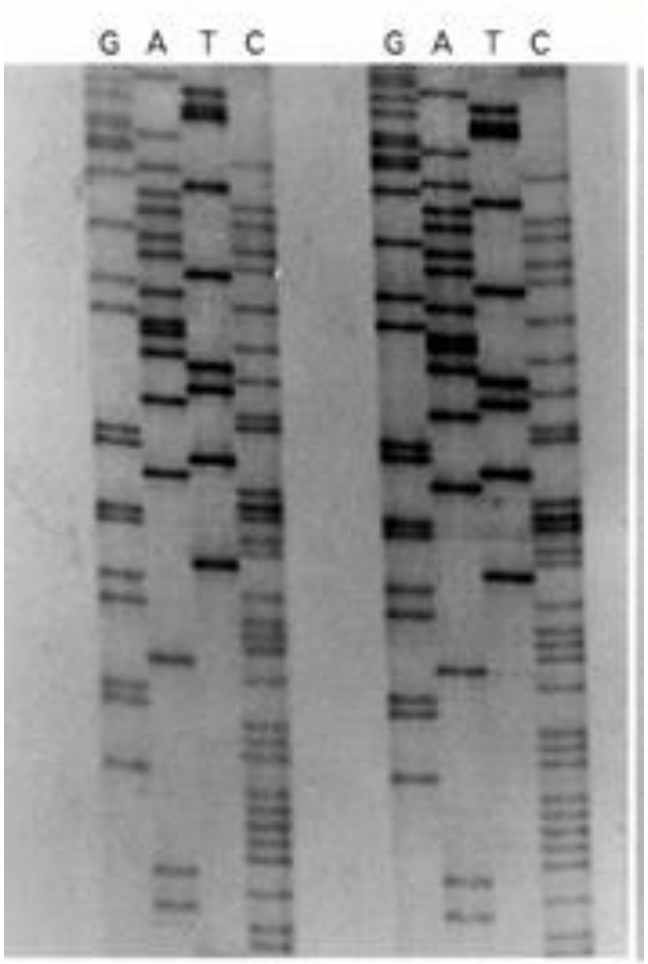
→ guanosine en position 5

Template-X**T**XX**G**X**T**X**G**XX**T**

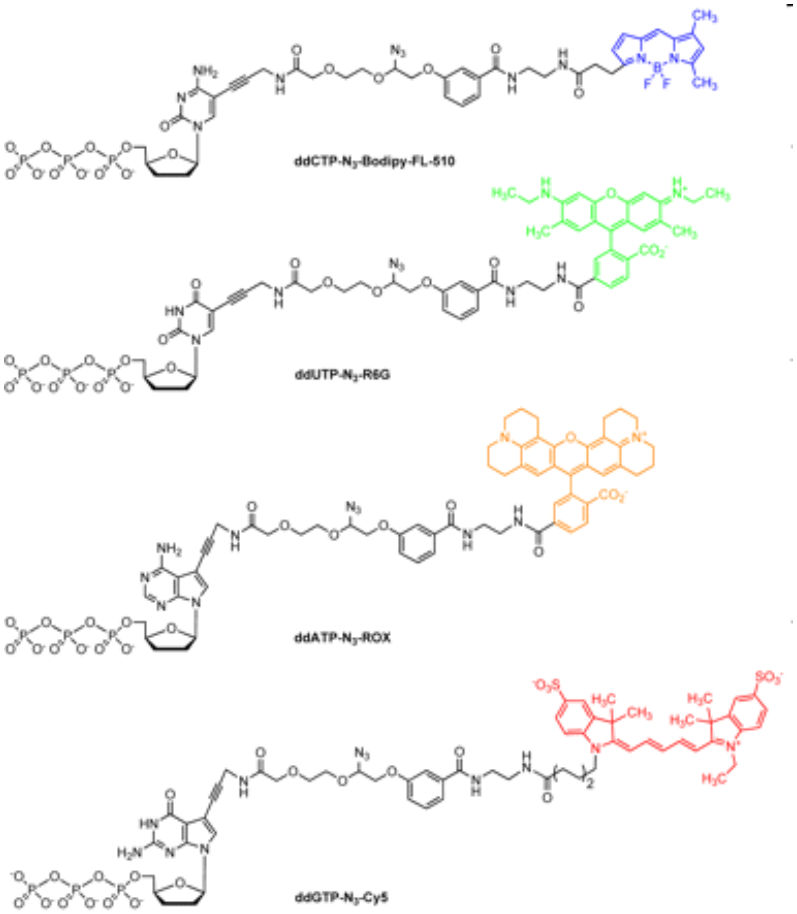

Primer
5′
OH
3′
CTAAGCTCGACT
Template
+
dCTP, dGTP, dATP, dTTP
+ ddATP
GATTCGAGCTGddA
GATTCGddA
GddA
+ ddCTP
GATTCGAGddC
GATTddC
+ ddGTP
GATTCGAGCTddG
GATTCGAddG
GATTCddG
ddG
+ ddTTP
GATTCGAGCddT
GATddT
GAddT
A
C
12
11
10
9
8
7
6
5
4
3
2
1
3′
A
G
T
C
G
A
G
C
T
T
A
G
CTAAGCTCGACT
Autoradiogram of electrophoresis gel
Sequence of complementary strand

Di-deoxy-nucléotides dans les année 1990s



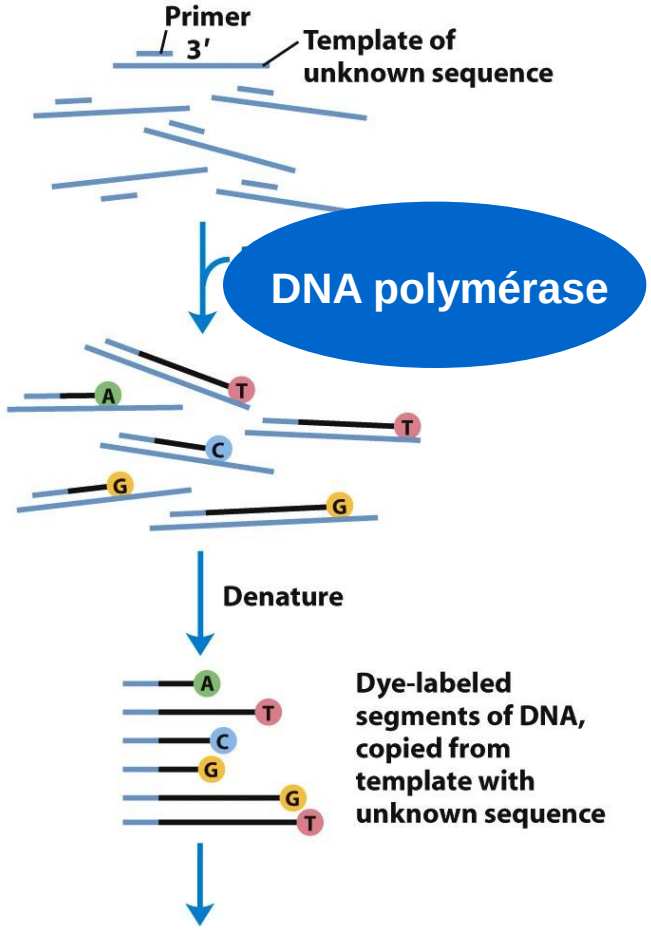


Di-deoxy-nucléotides fluorescents



Quel est l'avantage des di-deoxy-nucléotides fluorescents?

Séquençage du DNA d'après Sanger avec des nucléotides fluorescents



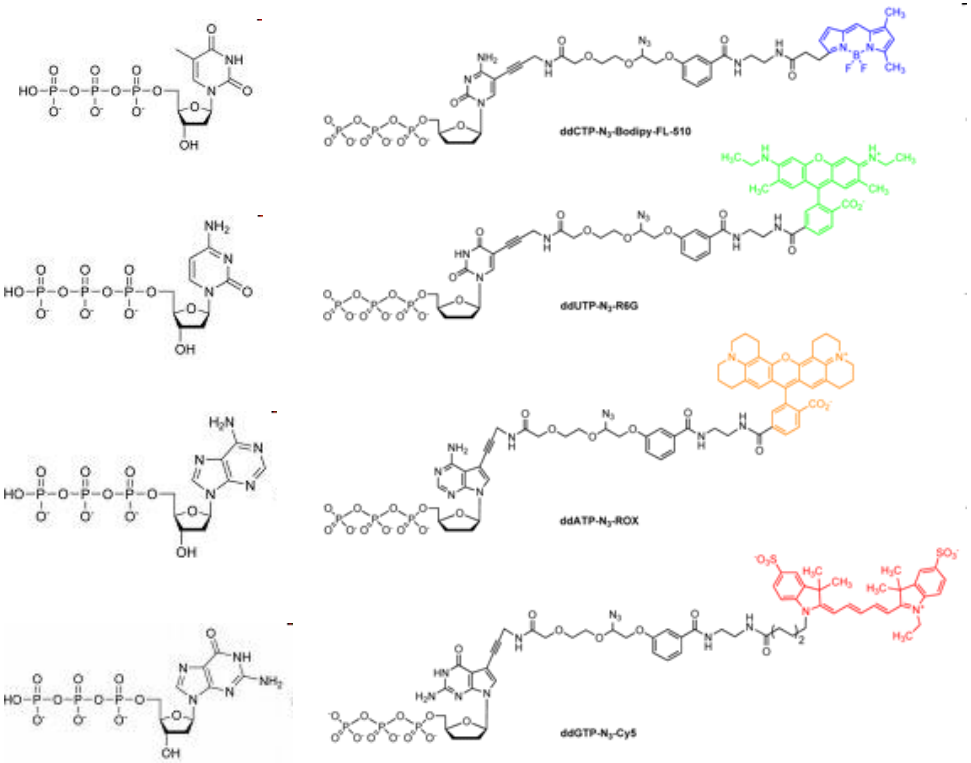


Figure 8-34 part 1
Lehninger Principles of Biochemistry, Fifth Edition

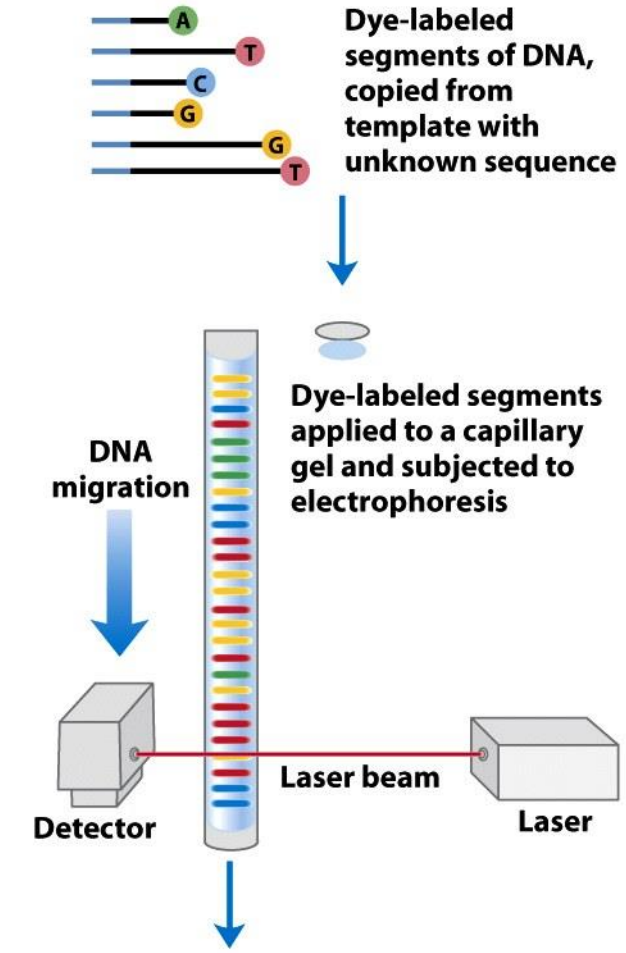
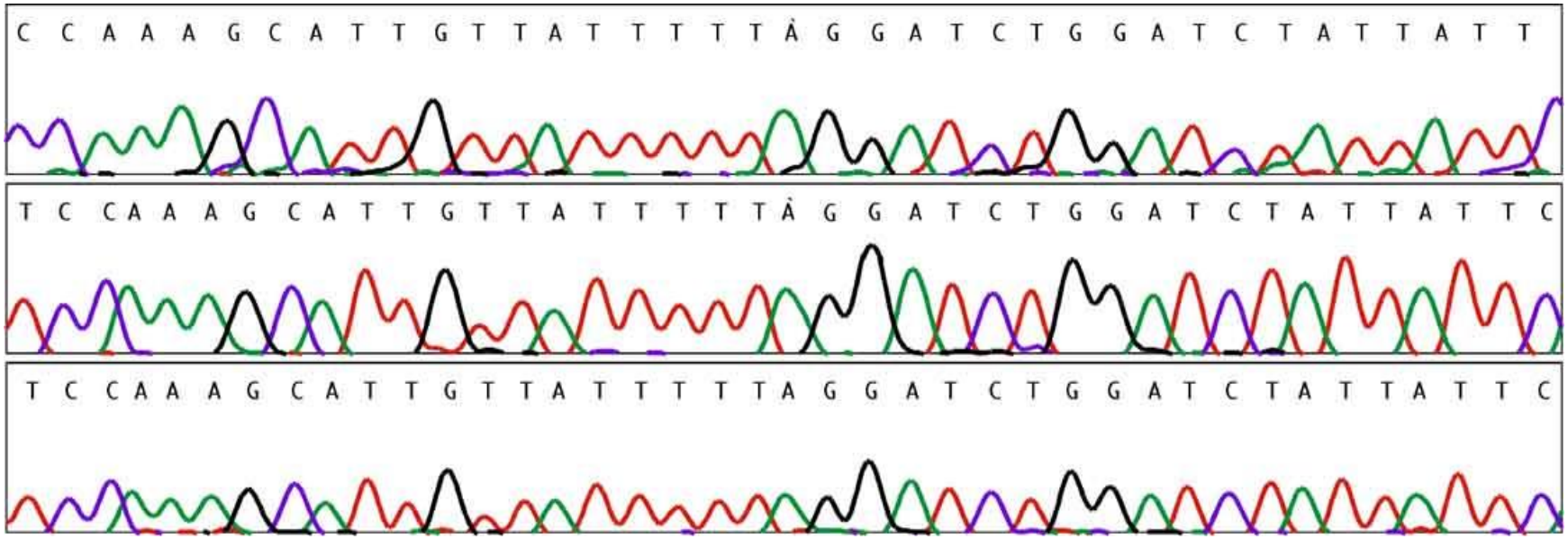
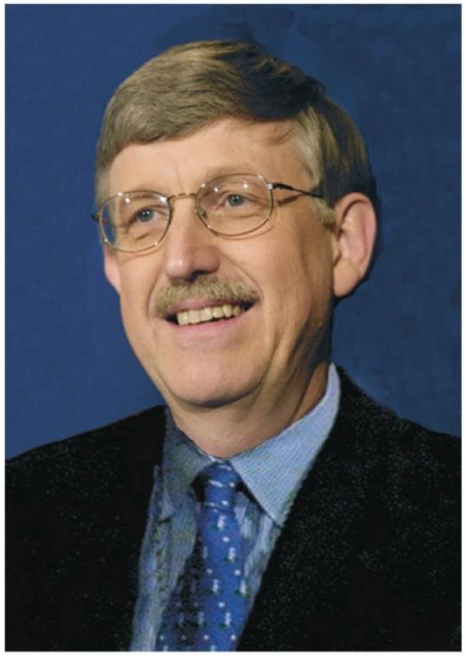


Figure 8-34 part 2

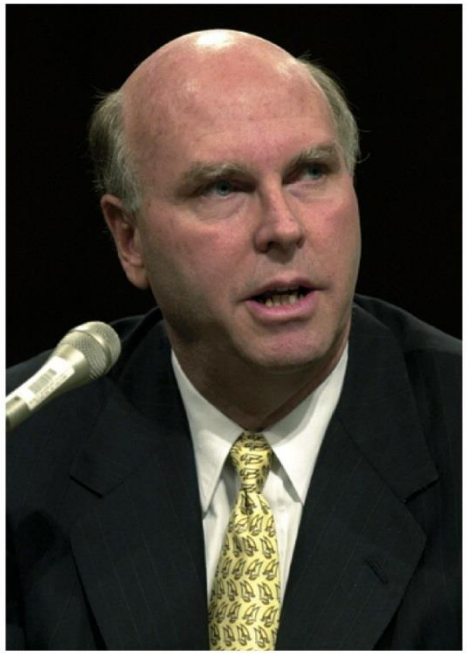
Lehninger Principles of Biochemistry, Fifth Edition


C C A A A G C A T T G T T A T T T T T Å G G A T C T G G A T C T A T T A T T
T C C A A A G C A T T G T T A T T T T T Å G G A T C T G G A T C T A T T A T T C
T C C A A A G C A T T G T T A T T T T T A G G A T C T G G A T C T A T T A T T C

Séquençage de génomes entiers



Francis S. Collins



J. Craig Venter

Chef du ‘Human Genome Project’

Fondateur de Celera Genomics

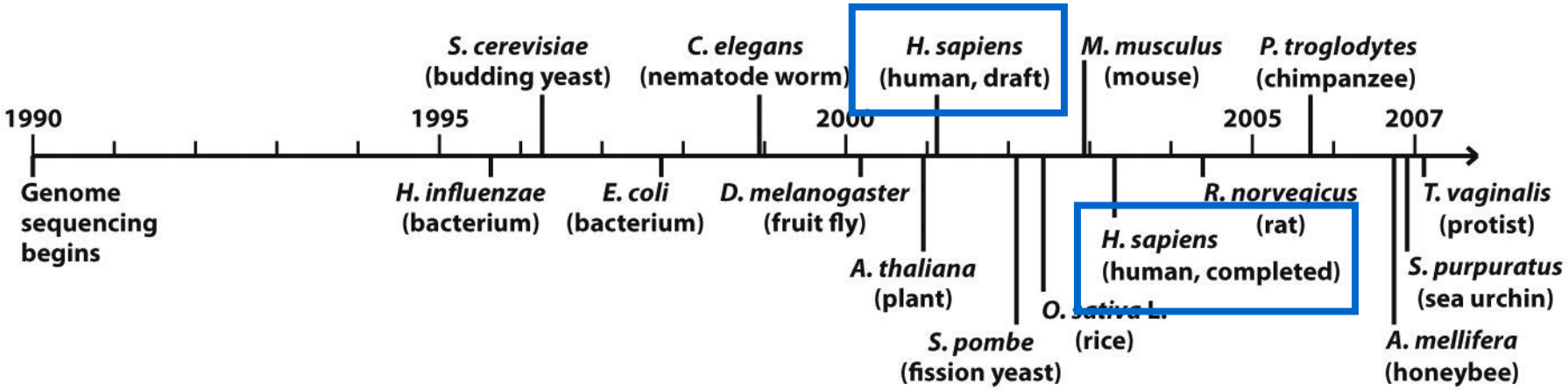
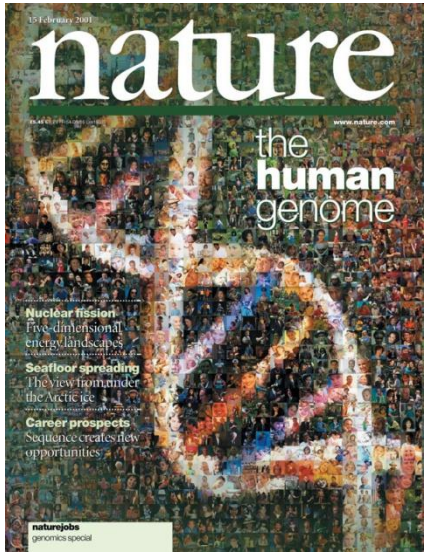


Figure 9-18
Lehninger Principles of Biochemistry, Fifth Edition

Human Genome Project



Février 15, 2001

Février 16, 2001

Celera Genomics

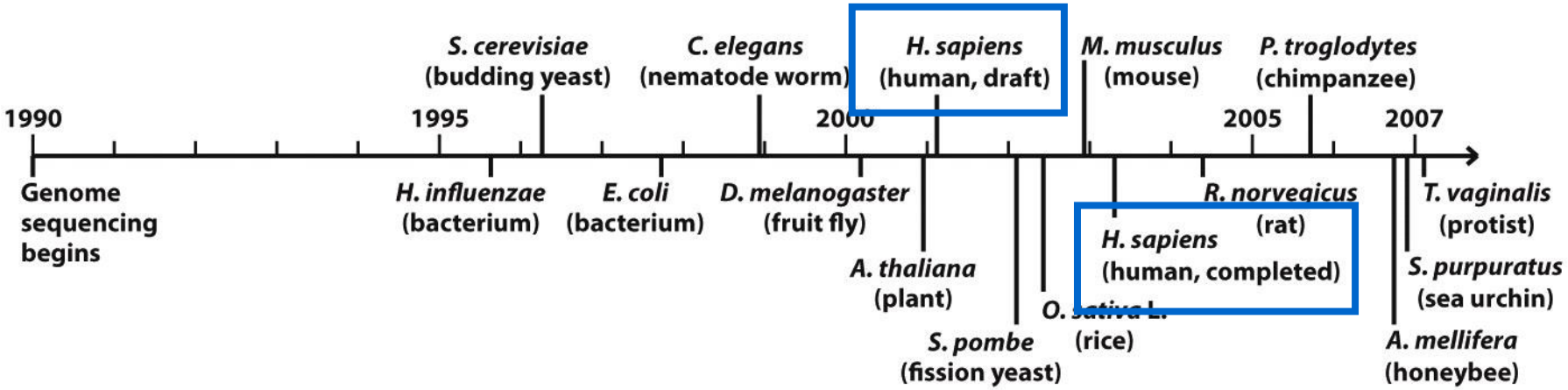


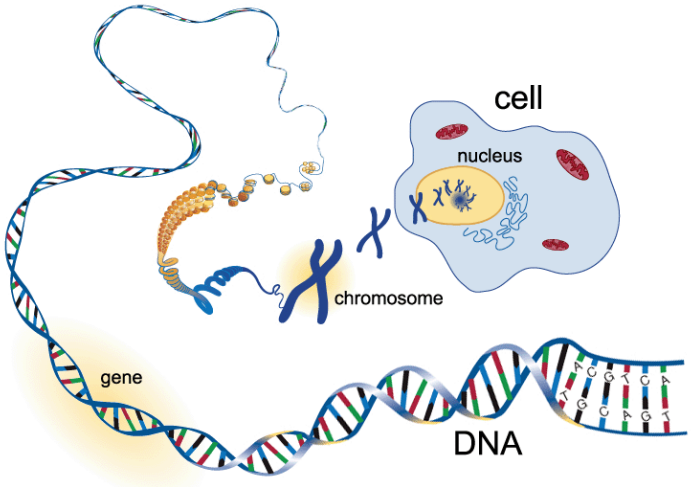
Figure 9-18
Lehninger Principles of Biochemistry, Fifth Edition

- Génome humaine:

  3'000'000'000 nuclèotides

- Sequencer avec la méthode de Sanger:

  500-1000 nucléotides



milliards de nucléotides

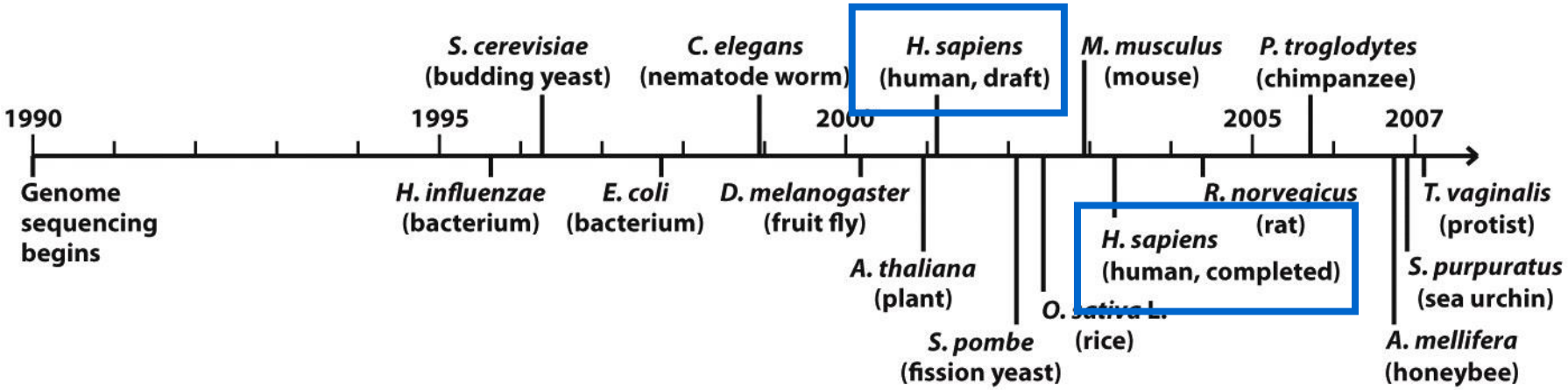
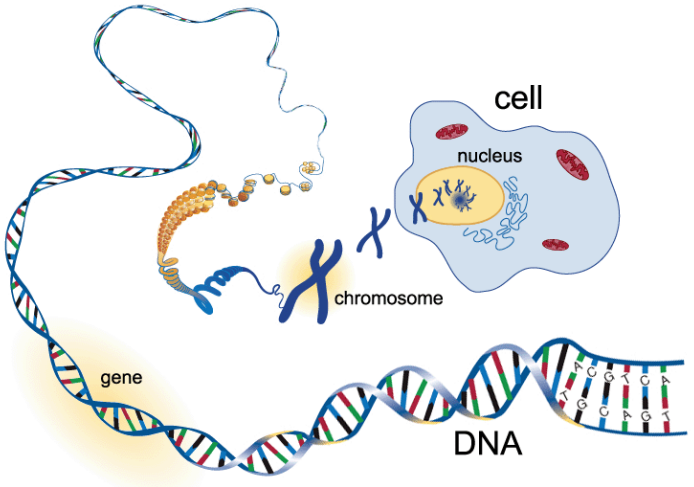


Figure 9-18
Lehninger Principles of Biochemistry, Fifth Edition

- Génome humaine:
  3'000'000'000 nuclèotides
- Sequancer avec la méthode de Sanger:
  500-1000 nucléotides

Comment serait-il possible
de séquencer un génome humain entier
avec la méthode de Sanger?



milliards de nucléotides

Séquençage du génome

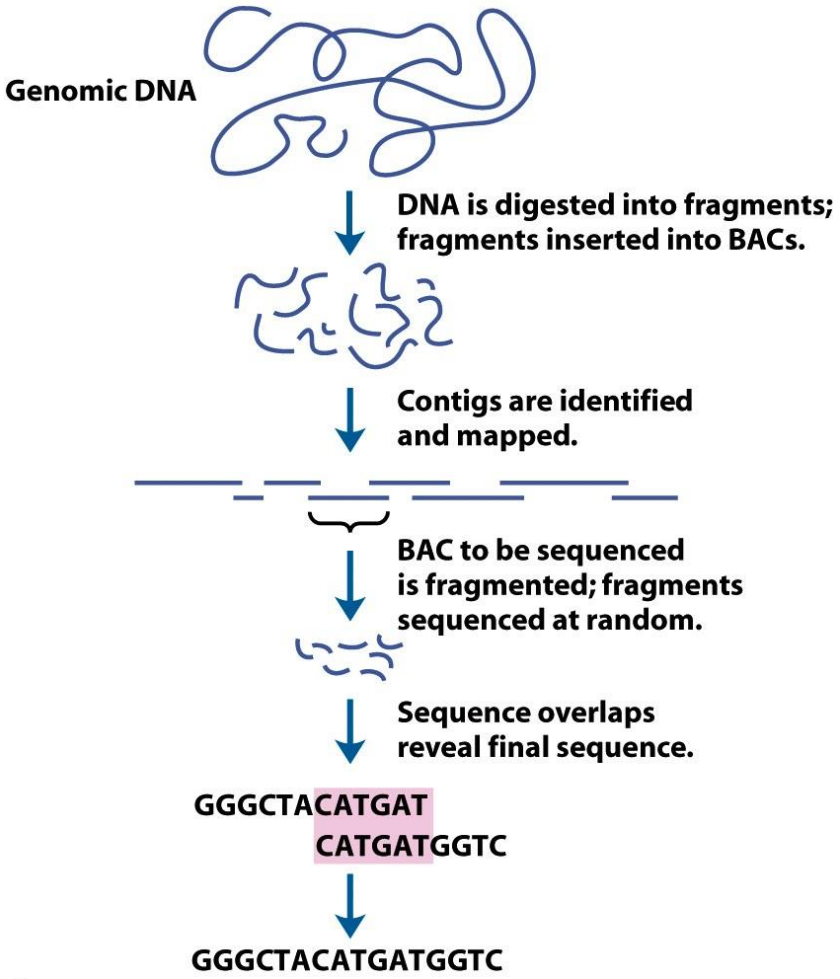
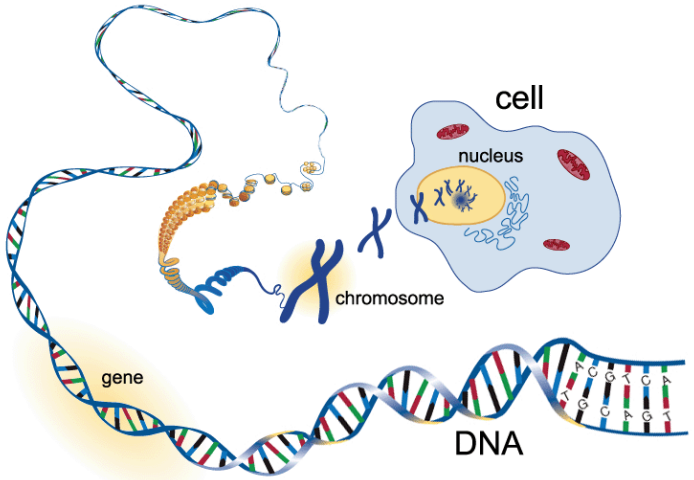


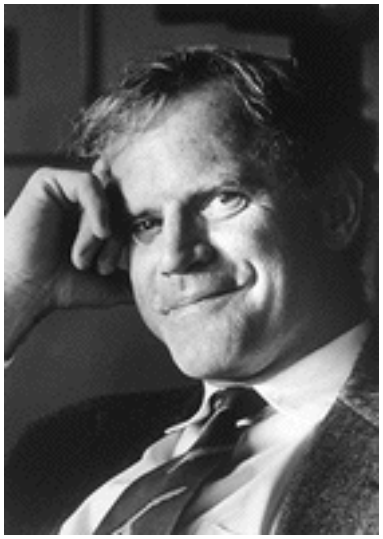
Figure 9-17
Lehninger Principles of Biochemistry, Fifth Edition

Leçon 5



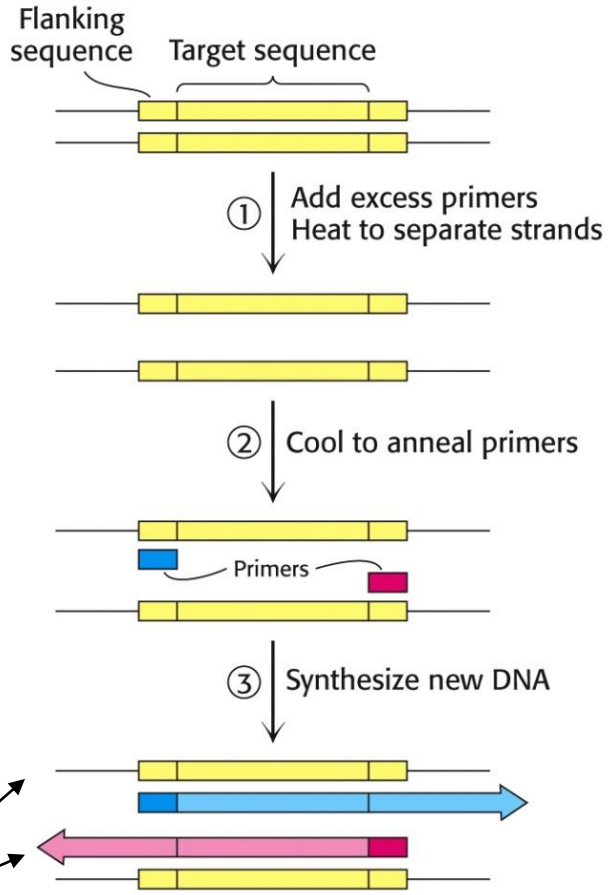
- Electrophorèse de DNA sur gel
- Enzymes de restriction (1970)
- Technologie du DNA recombinant
- Séquençage de DNA (1975)
- Réaction en chaîne par polymérase (PCR) (1985)

Amplification des séquences de DNA par la « polymerase chain reaction » (PCR)



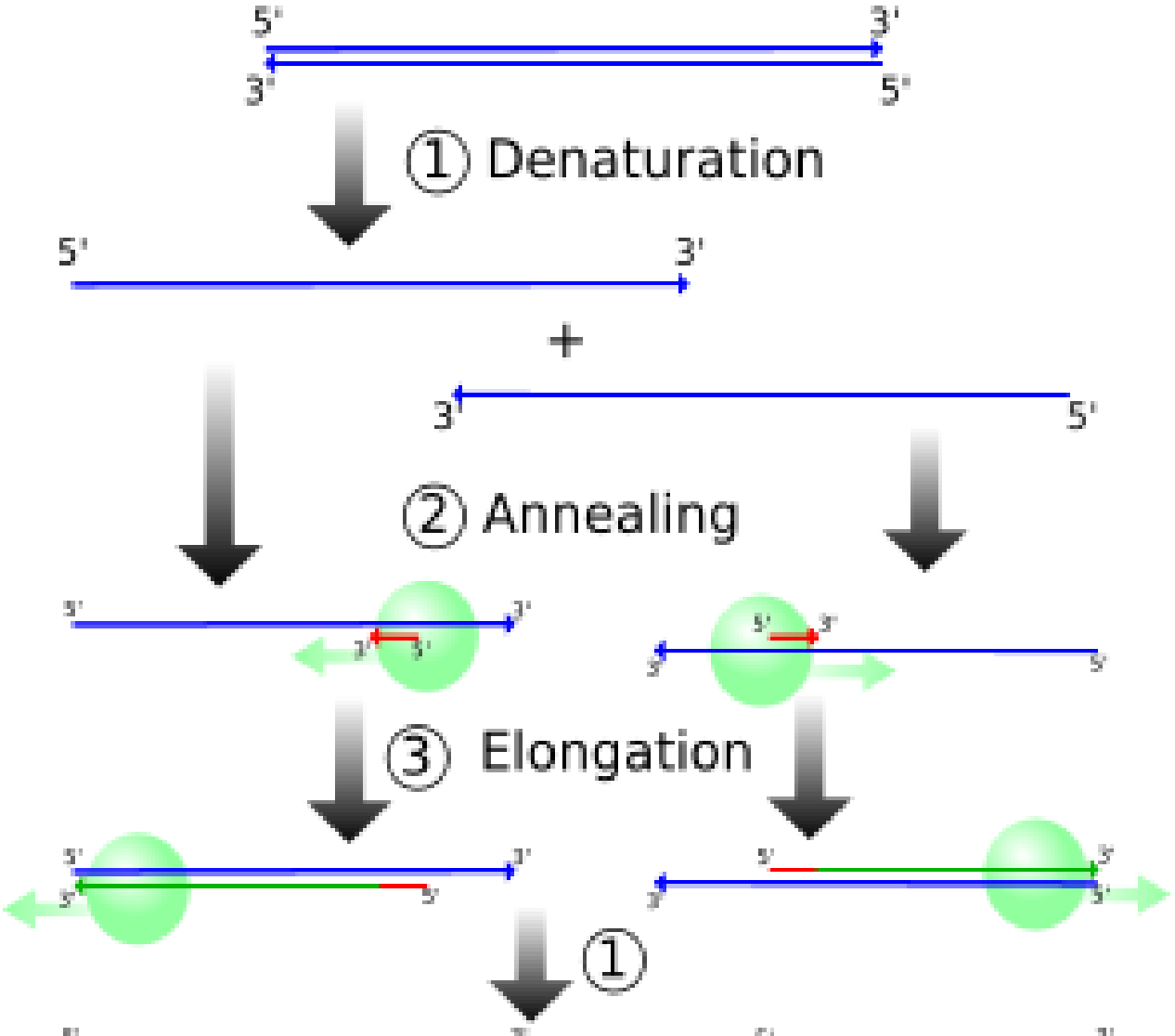
Kary Mullis

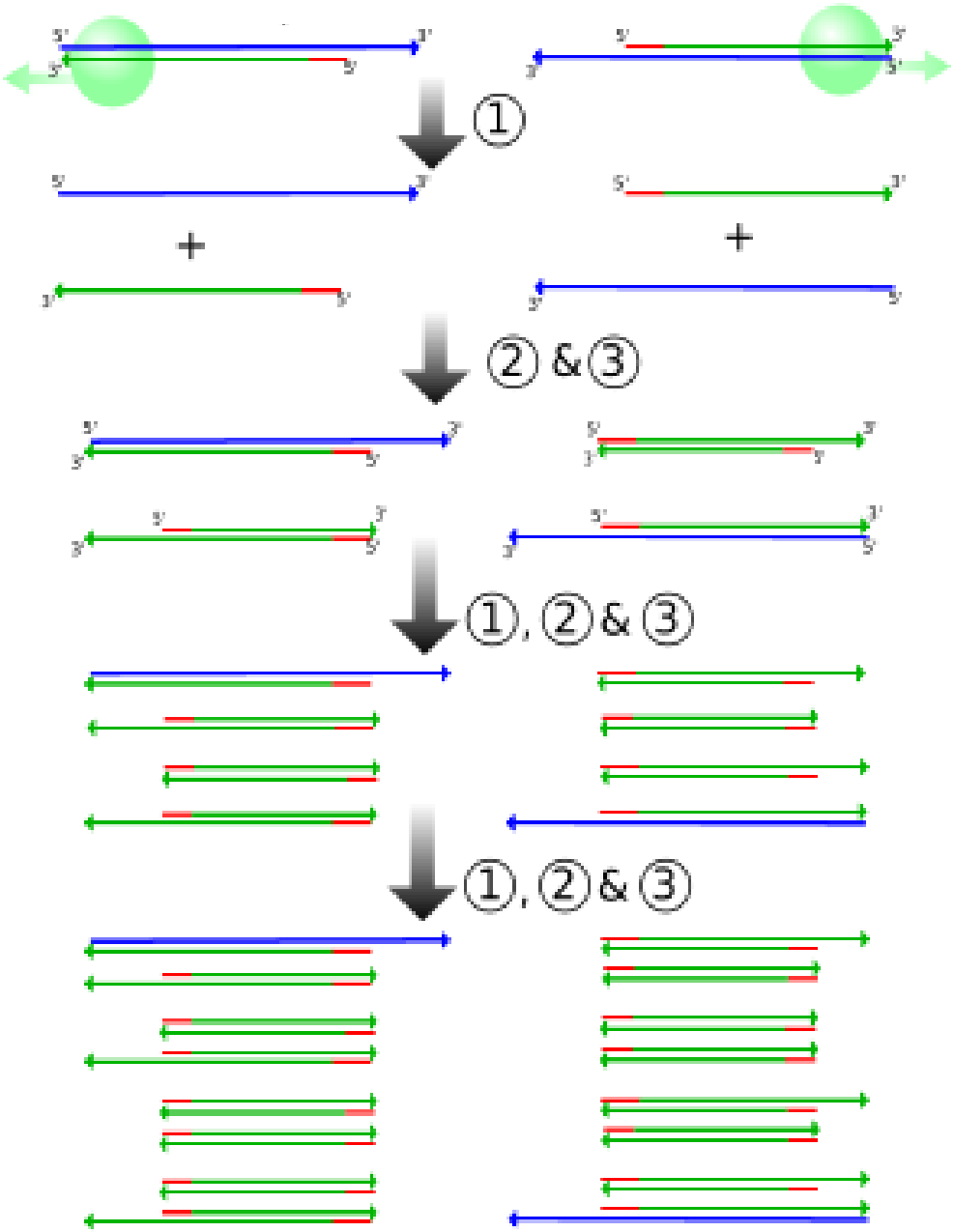
Prix Nobel en 1993



deux matrices!

Amplification des séquences de DNA par la « polymerase chain reaction » (PCR)




①
②&③
①,②&③
①,②&③

PCR vs réplication normale

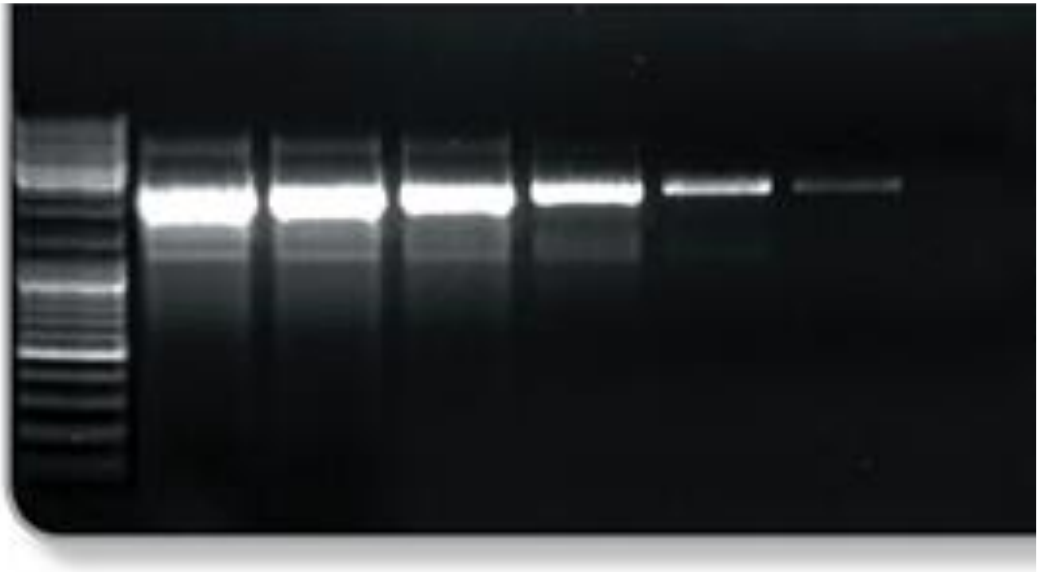
Copies de DNA:

Cycles	PCR	Réplication normale
0	1	1
1	2	2
2	4	3
3	8	4
4	16	5
5	32	6
6	64	7
7	128	8
8	256	9
9	512	10
10	1024	11
....	....	....

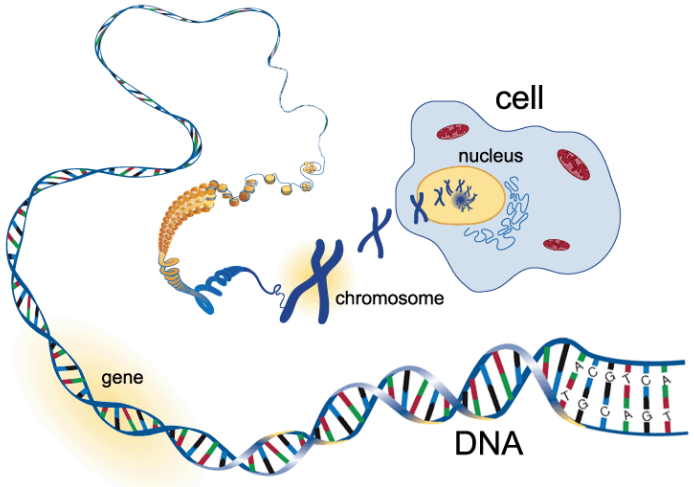
DNA produit par une réaction PCR

nombre de cycles de PCR

marker 30 25 20 15 10 5 0



Leçon 5



- Electrophorèse de DNA sur gel
- Enzymes de restriction (1970)
- Technologie du DNA recombinant
- Séquençage de DNA (1975)
- Réaction en chaîne par polymérase (PCR) (1985)

Série 4

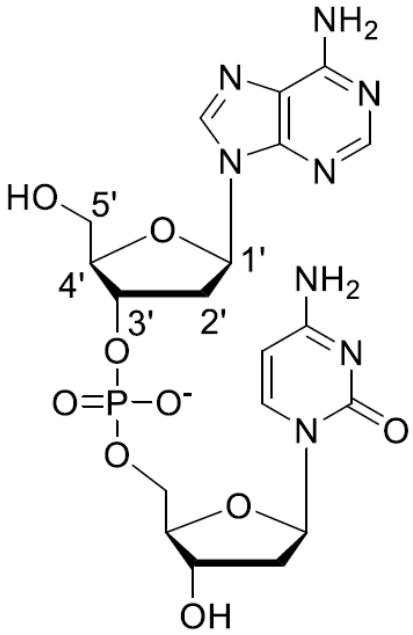
Question 1

(a) 5'-AGTAC-3’

(b) 5’-TACGGGCAGG-3’

Série 4

Question 2



Série 4

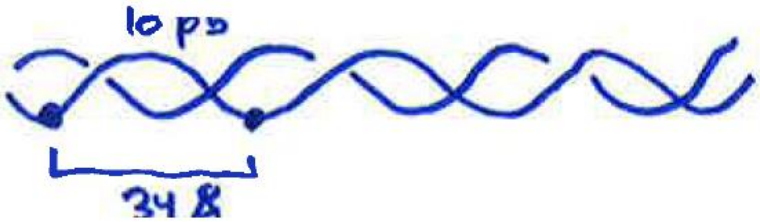
Question 3

(a) $[T] + [C] = 0.5$

(b) $[A] + [G] = 0.5$, $[T] = 0.2$, $[C] = 0.3$

Série 4

Question 4

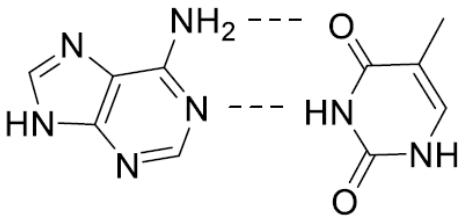


$$\frac{800\,Å}{3.4} = 235 \text{ p. d. b.}$$

Environ 235 pairs de bases

Série 4

Question 5



adenine thymine

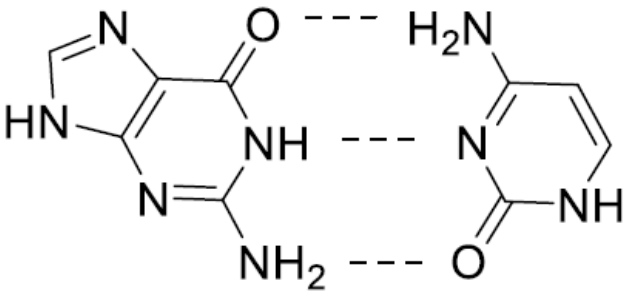
Série 4

Question 6

Liaisons hydrogènes, Van der Waals, π-stacking

Série 4

Question 7



guanine cytosine