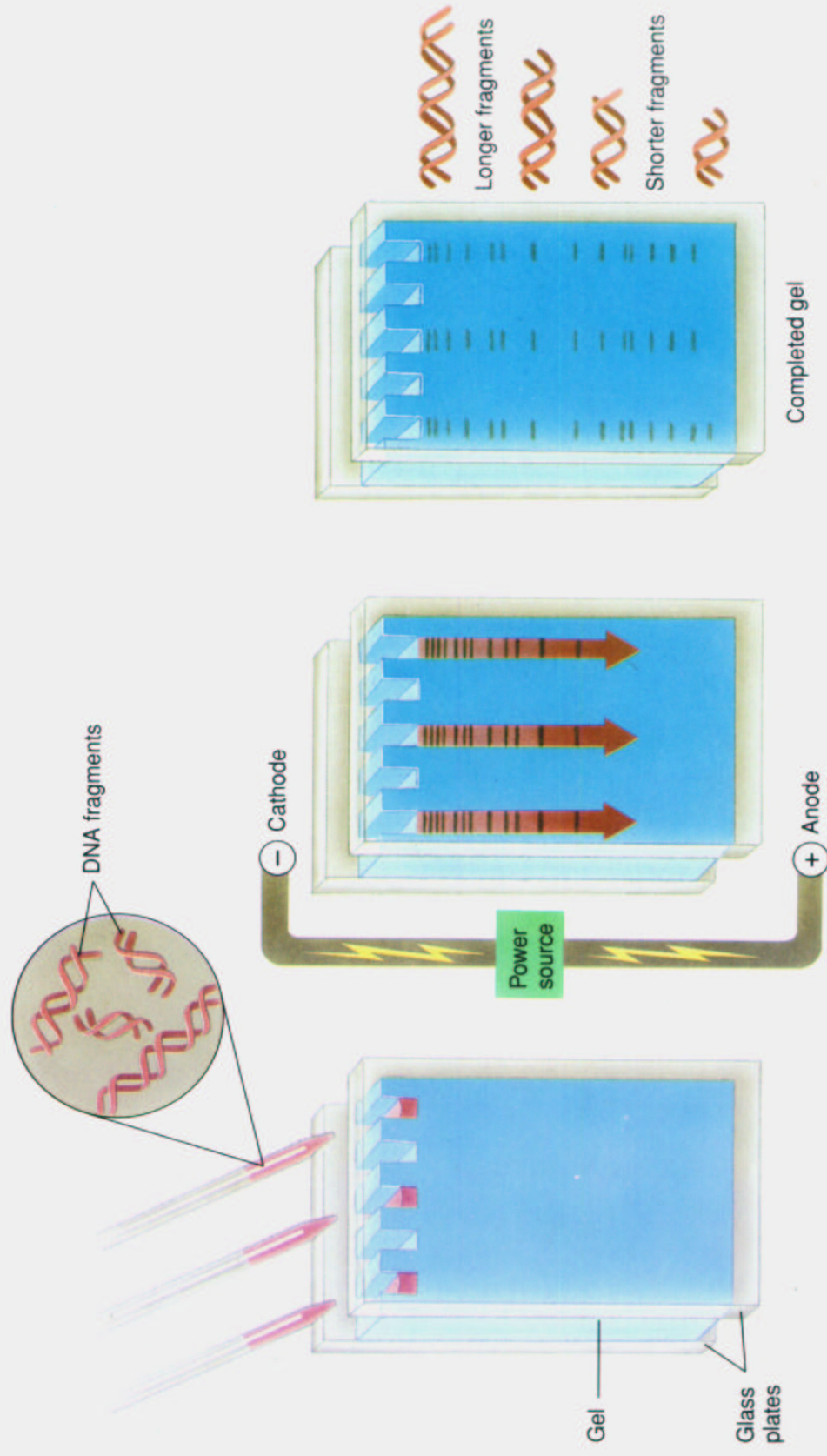


Outils de la BM

- Objectifs
 1. Manipuler
 2. Synthétiser / exprimer
 3. Détecter
 4. Séparer
- Applications
 1. Thérapeutiques
 2. Biophysique

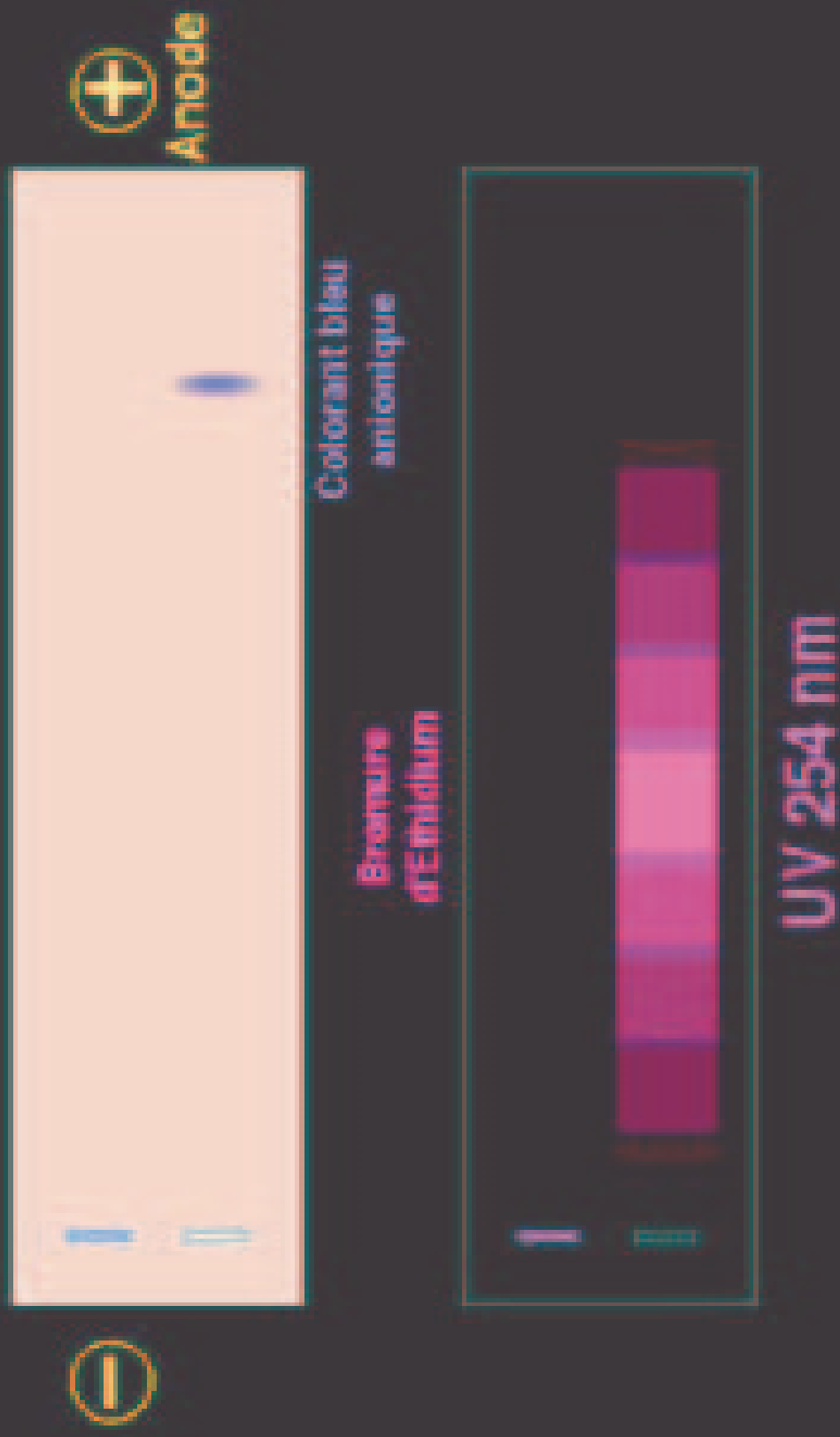


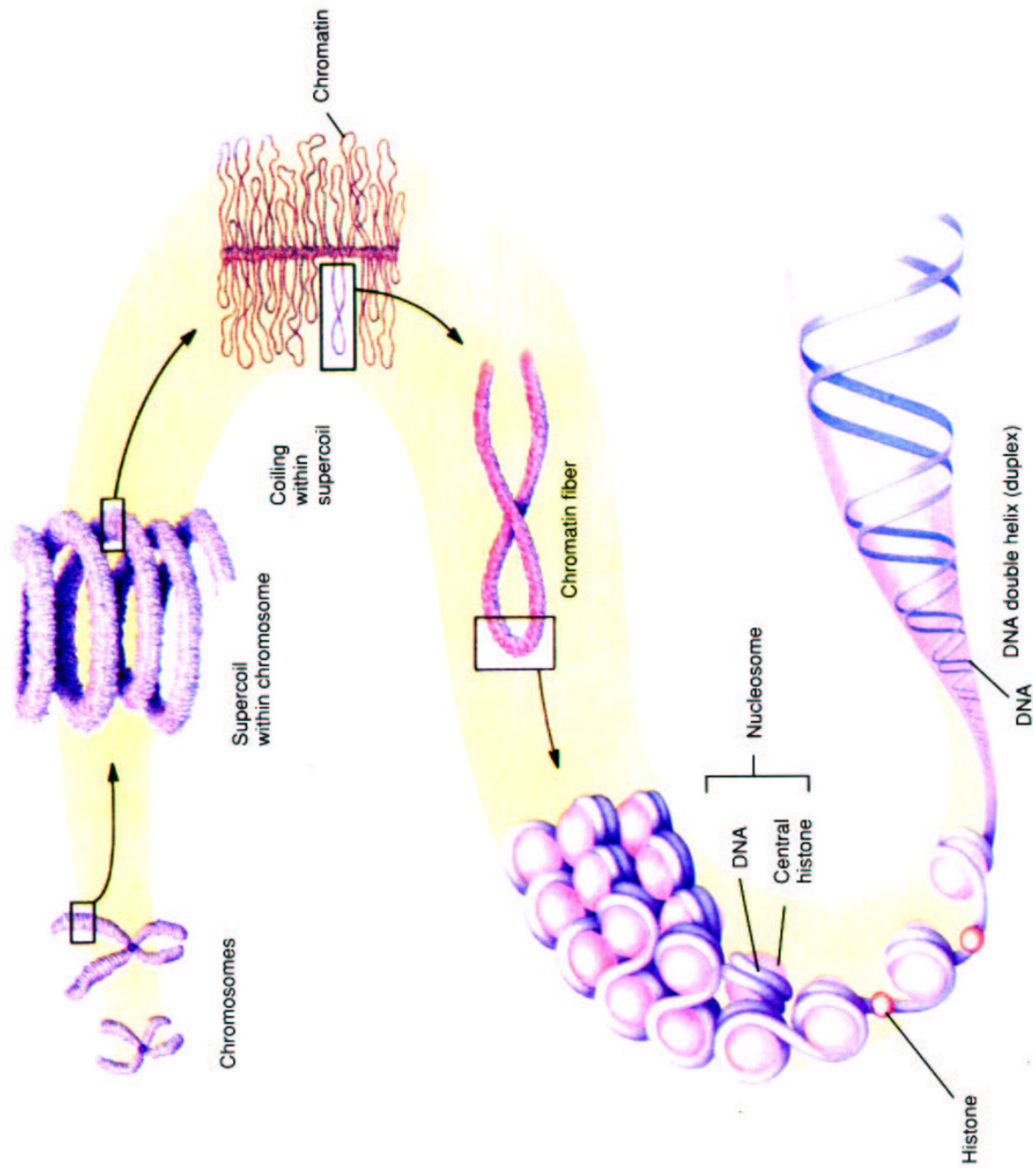
Mixture of DNA fragments of different sizes in solution placed at the top of "lanes" in the gel

Electric current applied, fragments migrate down the gel by size - smaller ones move faster (and therefore go farther) than larger ones

How Gel Electrophoresis Works

Electrophorèse de l'ADN

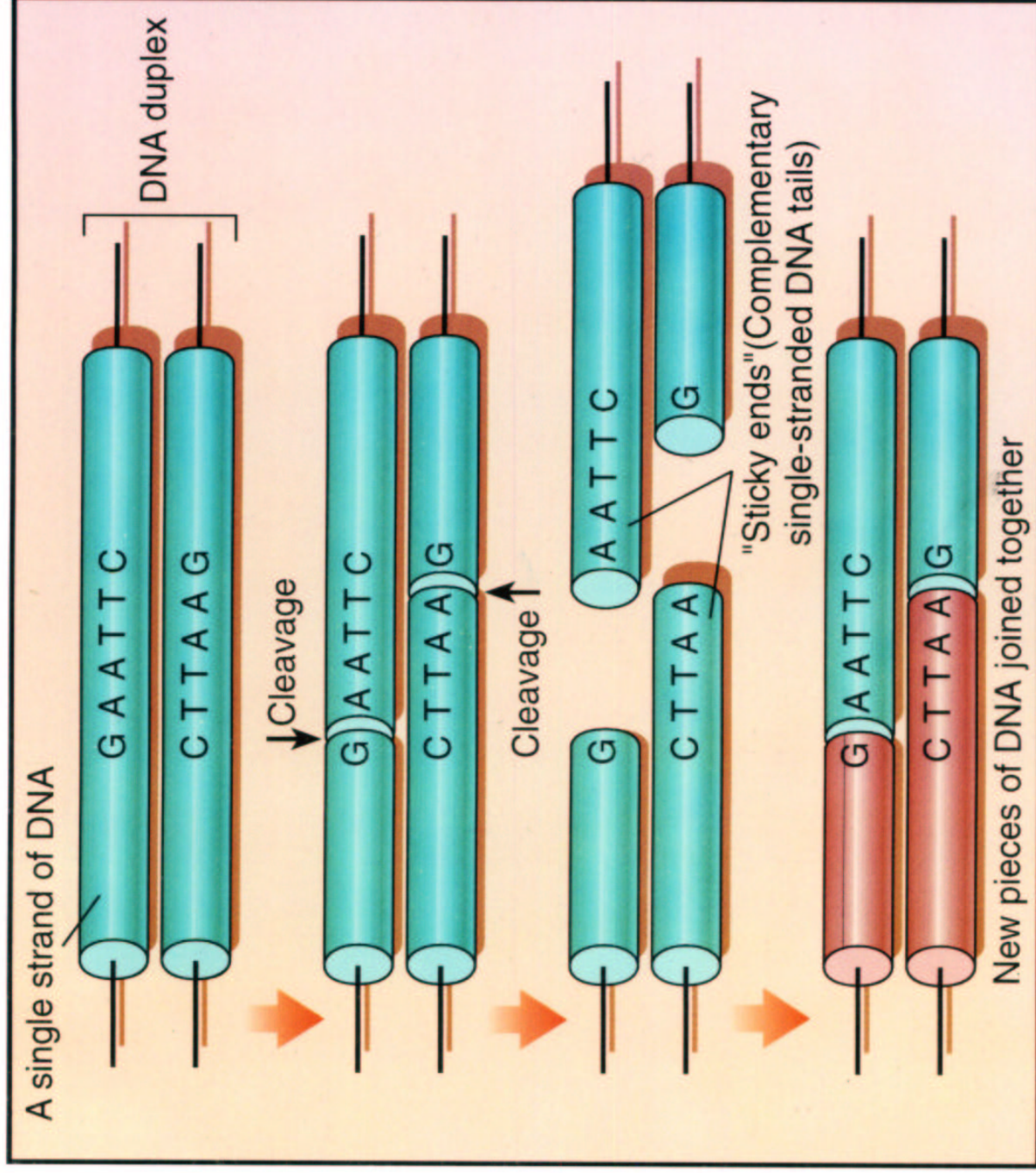




DNA is organized on chromosomes

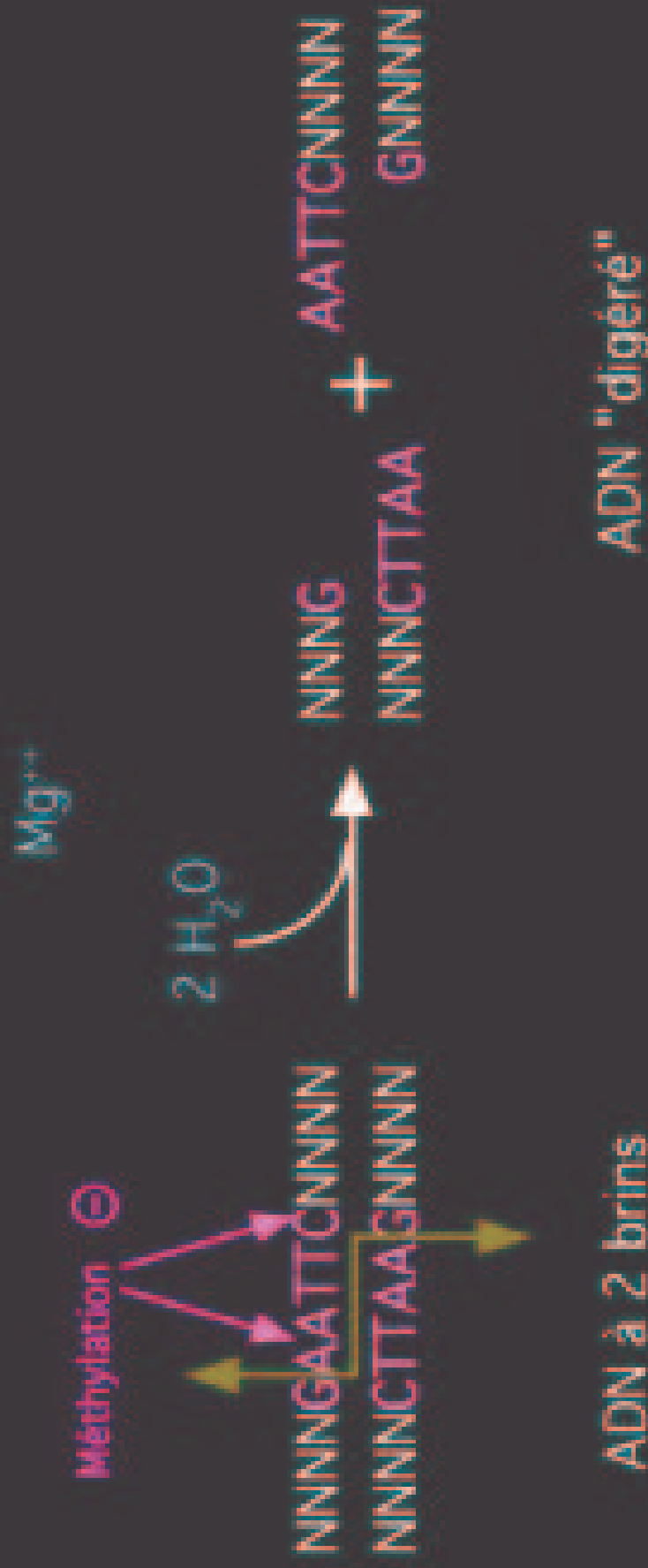
FRAGMENT DE RESTRICTION

- Produit de la digestion de l'acide désoxyribonucléique par une endonucléase spécifique d'une séquence de nucléotides.

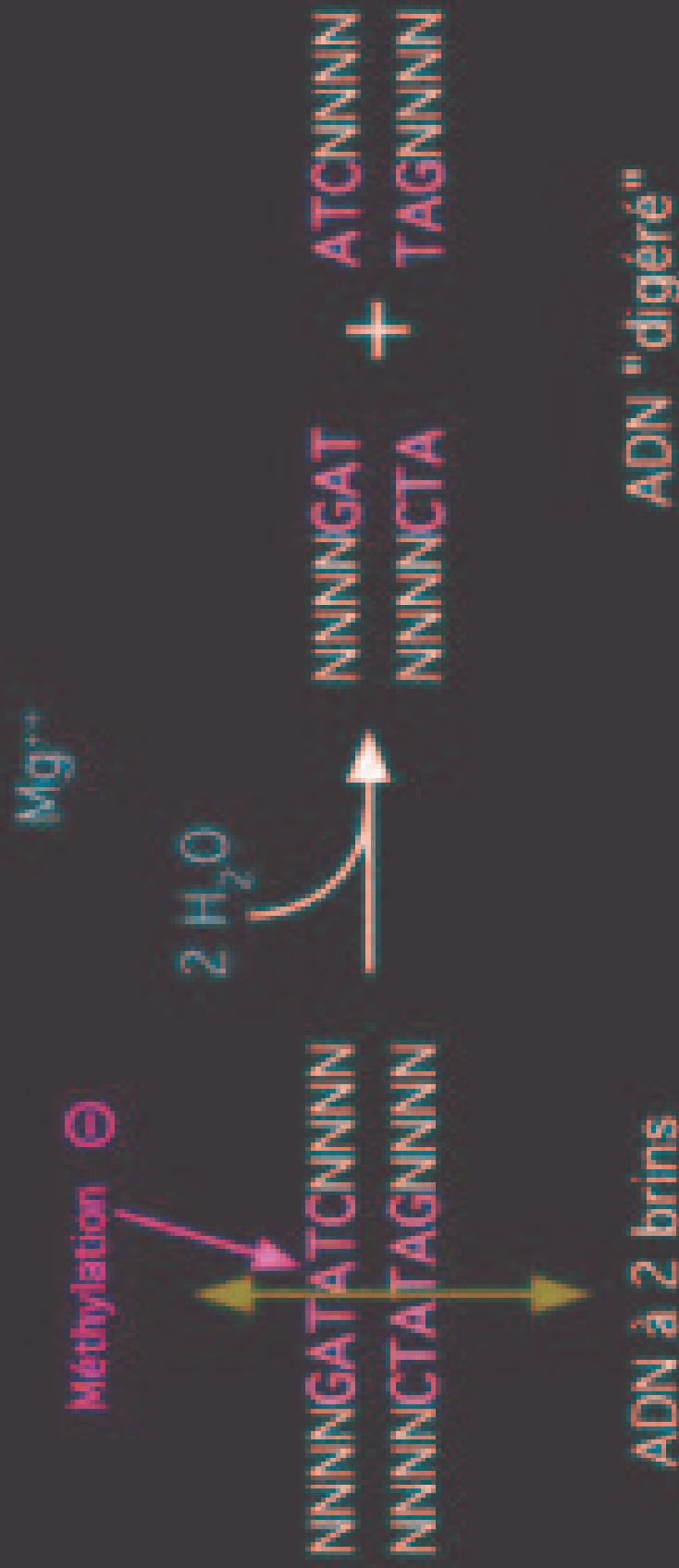


Restriction Enzymes/sticky ends

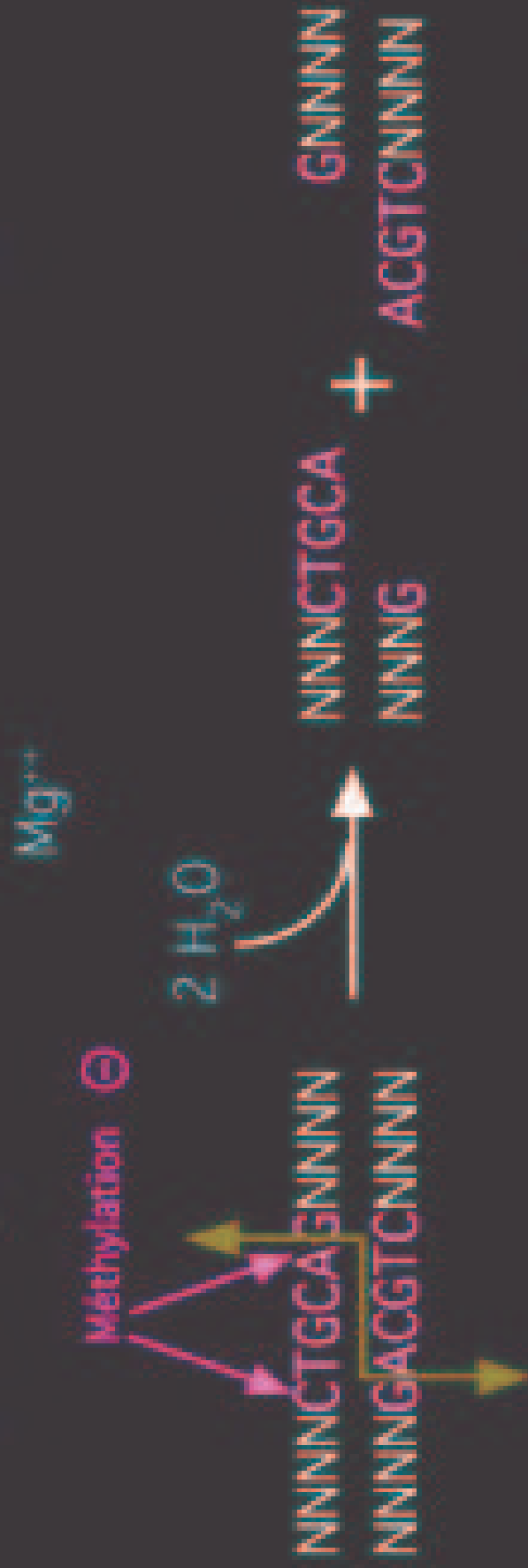
EcoR I (enzyme de restriction)



EcoR V (enzyme de restriction)

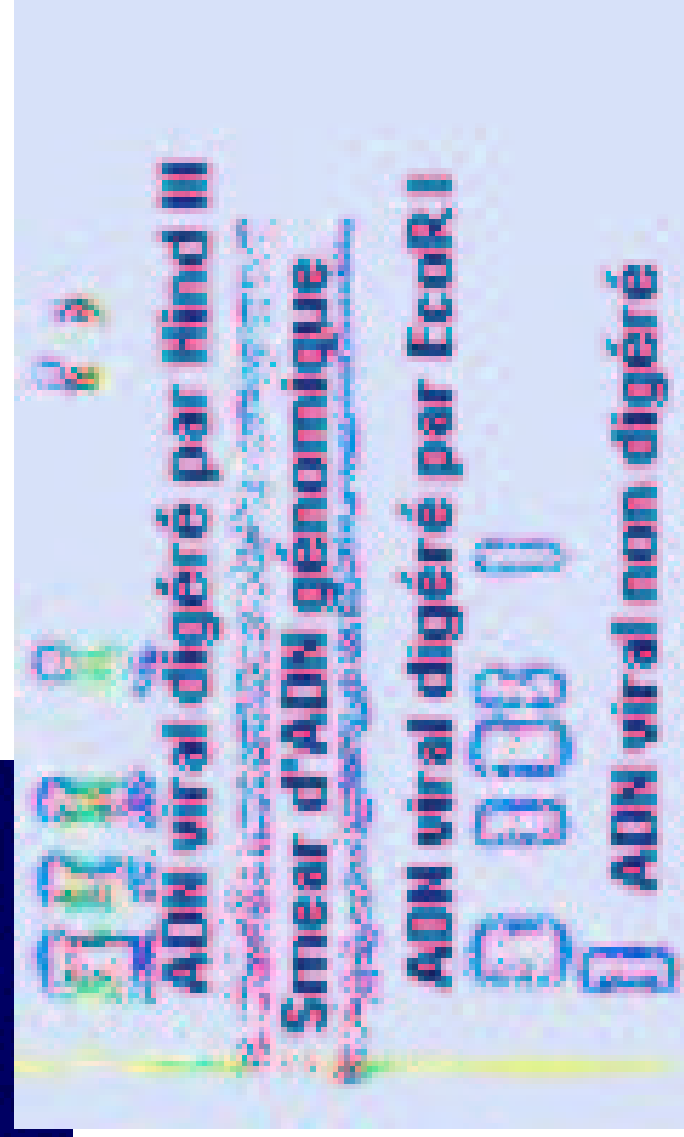
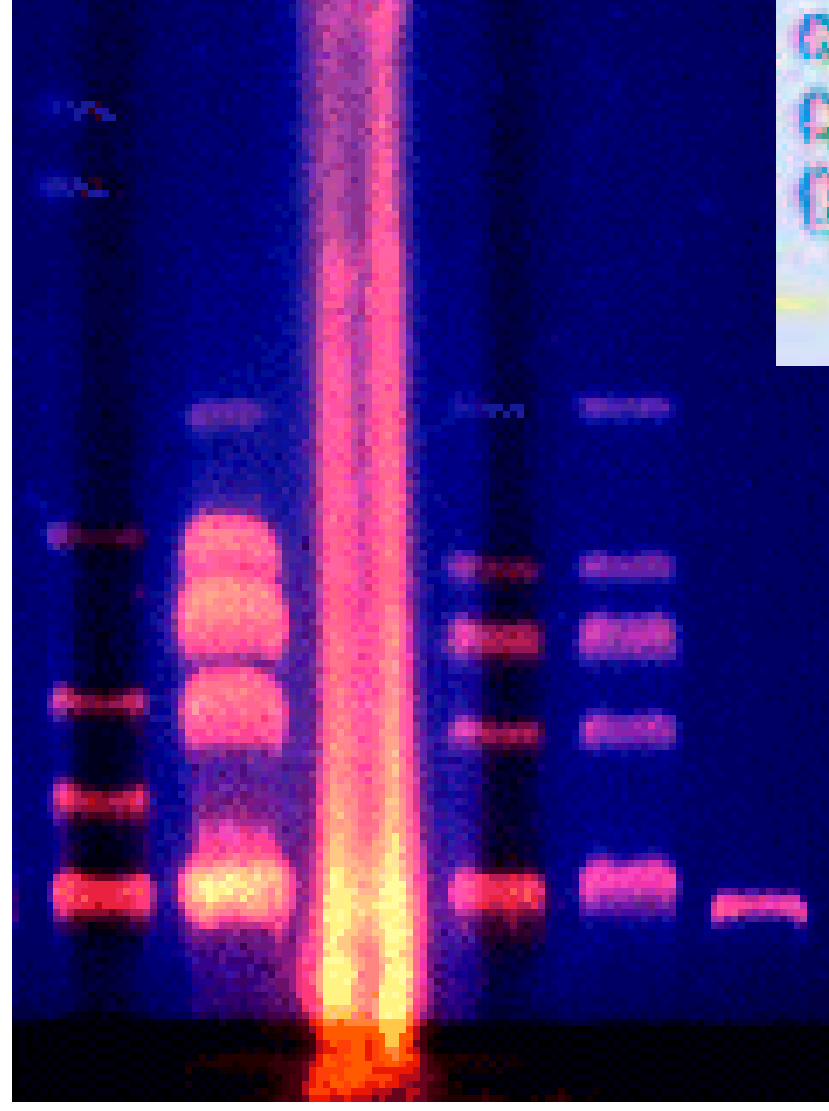


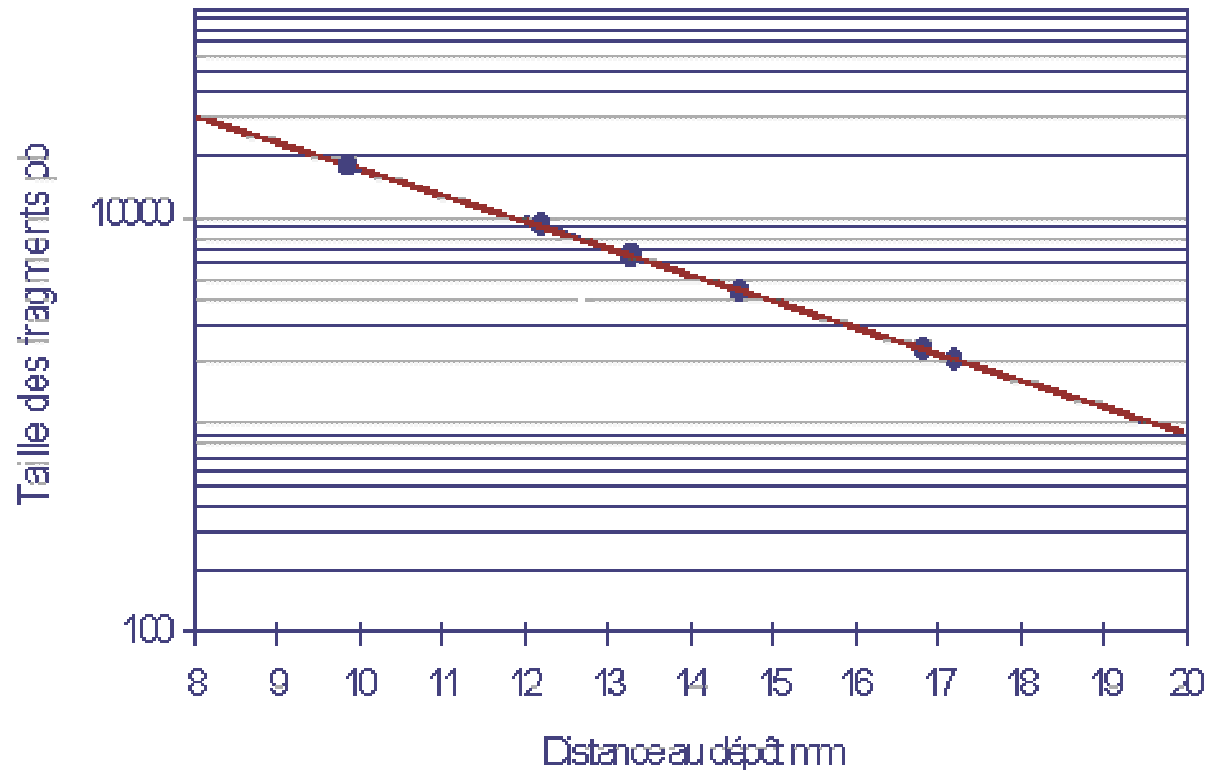
Pst I (enzyme de restriction)



ADN à 2 brins

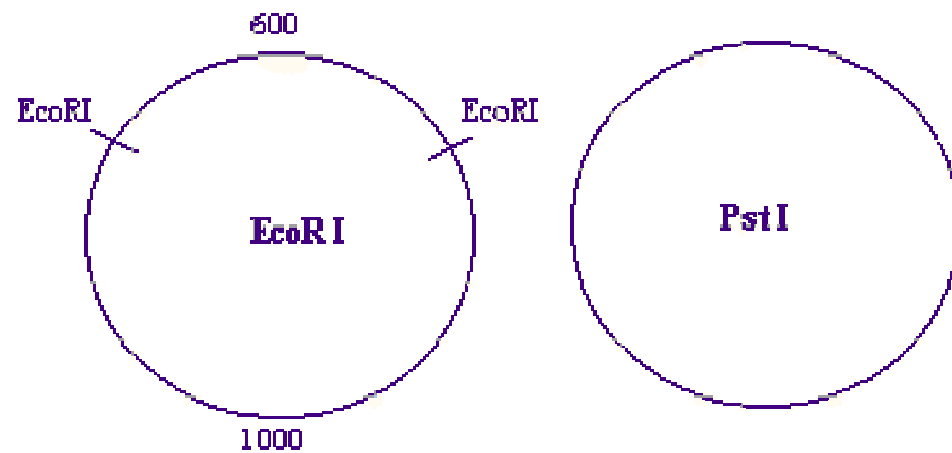
ADN "digéré"





1-Détermination de la Taille des fragments d'ADN

2- Etablissement de la carte de Restriction de l'ADN considéré



CTAAGAGCTGTACCCCTGCTCTGACCCCTACCAATGAGTCTTCCAGGTGCTG
TCCTGCTGCCCCATTCTCTCCAGCTTGCTTATGCTTCAGCATAAACCTGACCCCACT = 737 nt

GG

CCCATTCCATAGTCCCTGTCACCTGACAGGGGTGGGTAAACAGACAGGTATATAG
CCCTTCTCTGTCAGGCAGGGCAGGCACAGACACAGAGACAGGACACAGGCTAG
GTAGATAGGGAGGCAGATGTGTGAGCAGCATCCAAAGAGG

= 152 nt

CCCTGGGCTTCAGTT

GTGGAGAGGGAGAGACAGGTTTGGAAATGGCAGCAGGTAGGGAGATCCCTGGGA
GAGCTGAGGCCATTGTGGCTTCAGGTGTCGCCCAAGCCCCAGCCTCTTCCGA
GG

= 128 nt

CCCCCTGCCCCAGTGTTCACAGACAGAGGCTGCTGCGAGCACTGGGCTACTGC
TCACCATGTGCAACCTTTGAAAGGTGGGTGTGATATGGAGAGGGGGG

= 98 nt

CCAAAGAGGAGAA

ATGTGCTGGTGGTTATAGCTACCTATCTGGCTGTGCTCTTATATGCCAGGTCTGGCA
ACCAAGAGGCTCAAAAGGAGAGTCAATGCTCTCTTAAGGCCAGAAAGCCCAATCCA = 198 nt
AAGATCTGGCTGGATACCTTTTGGGGAGGGGGCAGAGAGCTGGGGTTGGGTACTGG
CAAGGCTATGGCAGGAGG

CCAGAGATTCACTGCTGTGGACCCAGCTGAAAGAGGTGT

GTGTGTGGTGTGTGTGTGTGTGGCGAGGCTTGGGTTCGGAGACAGGC = 195 nt
AAGGAGGCCATGTGTGGAGAGCCTAGTTTCTCAGTACTTCCAGAGCCGTGACTGACT
ATGGCAAGGACCTGATGGAGAGGCTCAAGAGGCCAGAGCTTCAAGG

CCGAGG = 6 nt

CCAA

GTAGCTCTCAGGGCAGGGGGTTCAAGGGCTGTGGAGCTGTGGAGAGAGAGGGA = 196 nt
AGATGAGAGGTCCTCCACAGAGTCTGAGCCAGGGGTGGGATTAAGGCAAGTTAGG
CTTAAATGCGAGAGAAAGTATTTCATCAAGCCAGAGAGAGGCGAGGCTCTCTAGA

RESTRICTION-MODIFICATION :

- Système de défense des Bactéries vis-à-vis des bactériophages, incluant des enzymes de restriction pour digérer l'ADN parasite et des enzymes de méthylation pour protéger l'ADN de la bactérie.

68000

Bacteriophage T4

6.5.1.1

T4 DNA ligase

Mg²⁺

dsDNA

DNA ou RNA, brèche ou
bouts collants ou francs

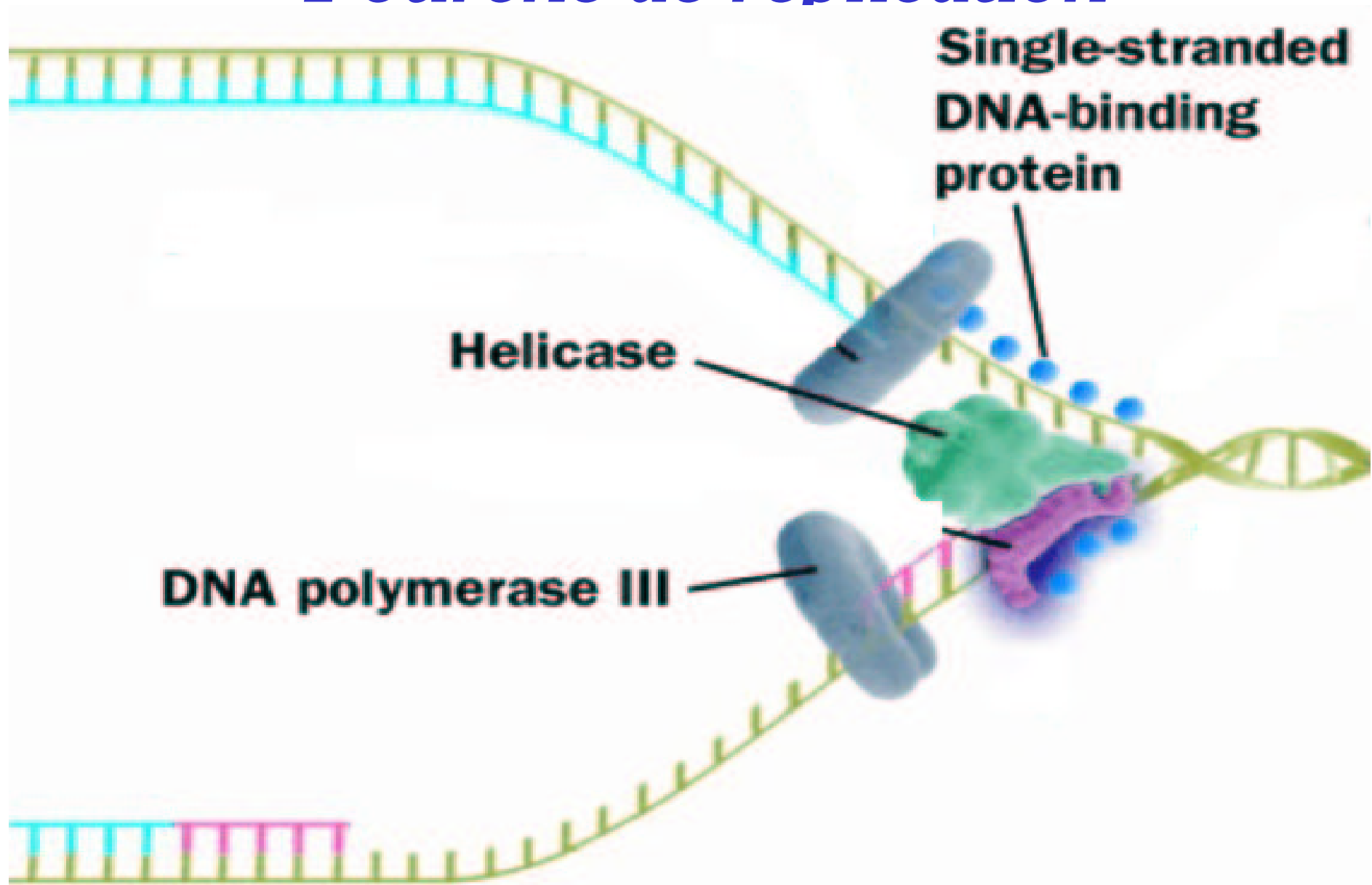


DNA polymérase

DNA-primase, DNA-ligase, Mg^{2+}



Fourche de réplication



94000

Thermus aquaticus

2.7.7.7

Taq DNA pol (*AmpliTaq*)

Mg²⁺

Amorce

pGpApGpCpTpG

pCpTpCpGpApCpApTpGpGpGpGpApGpApGpTpGpGpGpTpA

DNA (modèle)

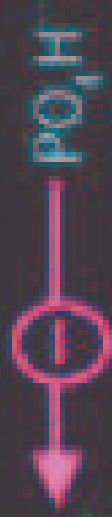
dCTP

dTTP

dATP

dGTP

5'→3' DNA
polymerase

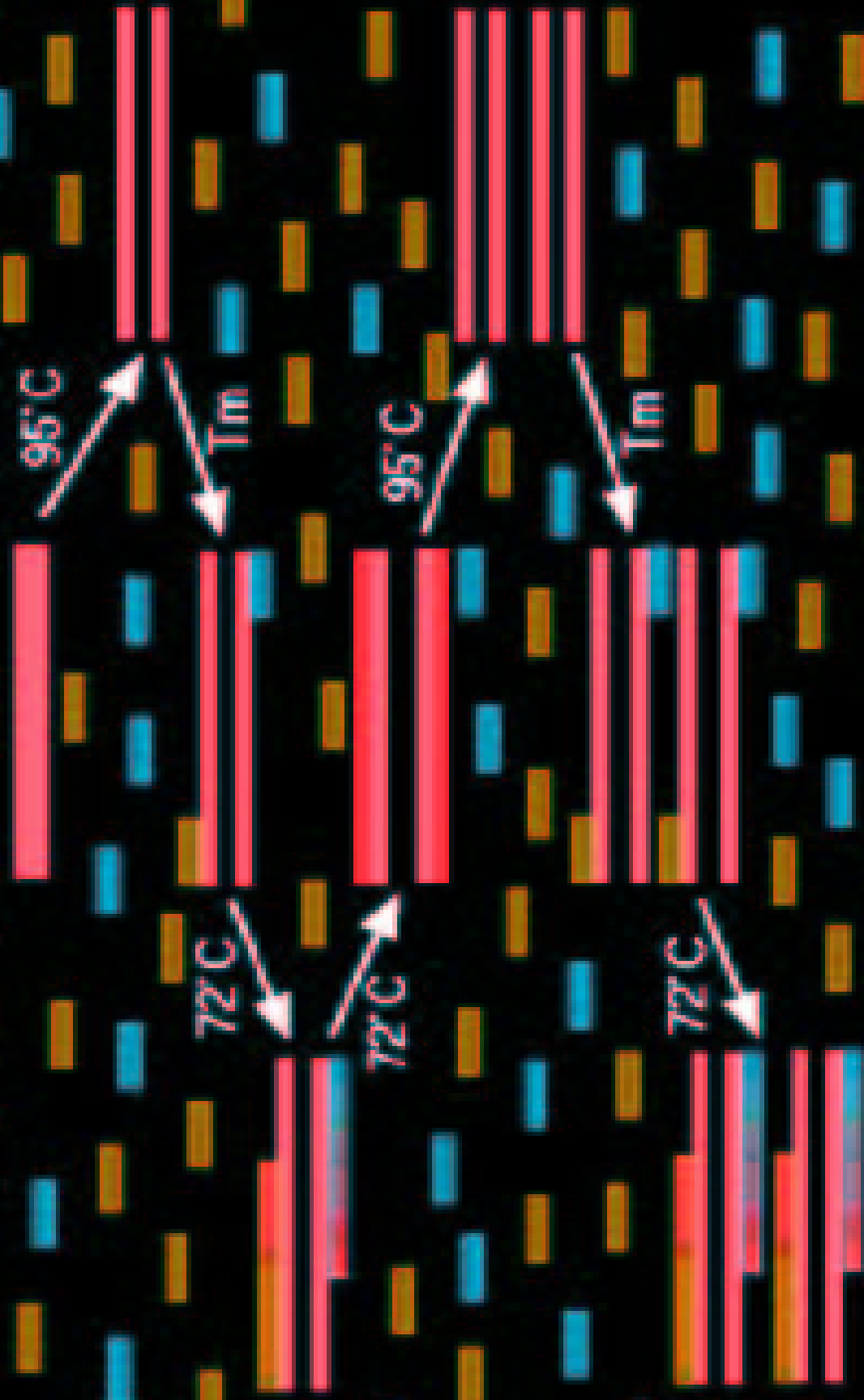


pp

pGpApGpCpTpGpTpApC

pCpTpCpGpApCpApTpGpGpGpGpApGpApGpTpGpGpGpTpA

Polymerase Chain Reaction (PCR)



PCR (I-1)

séquence connue
(amont)

séquence connue
(aval)

fragment d'intérêt

5' 3'

identique à la
séquence connue
(amont)

3' 5'

synthèse
d'amorces

complémentaire
de la séquence
connue (aval)

dénaturation à 95°C

PCR (1-2)



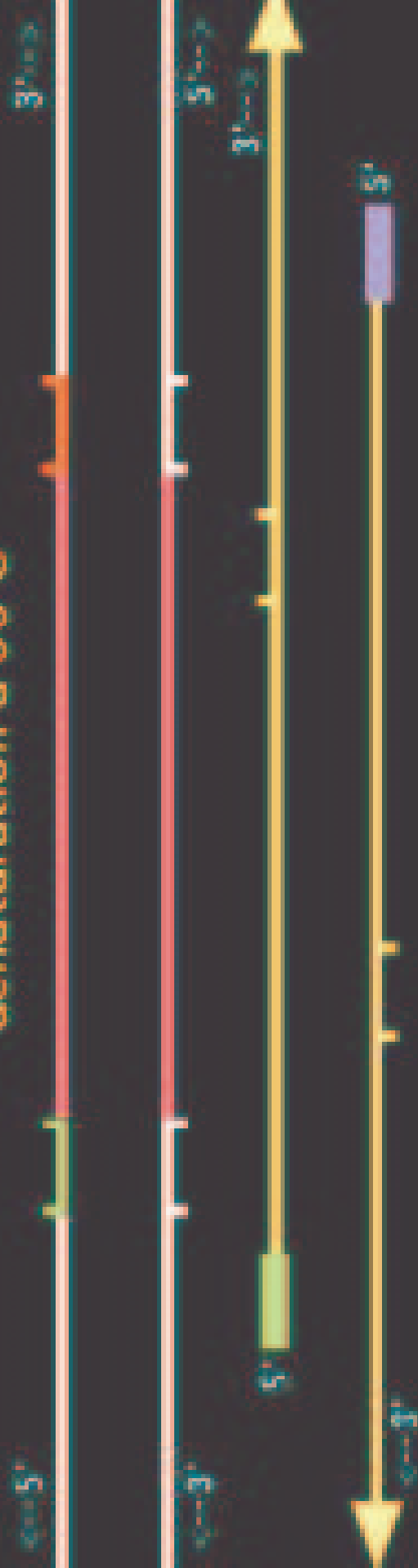
PCR (1-3)



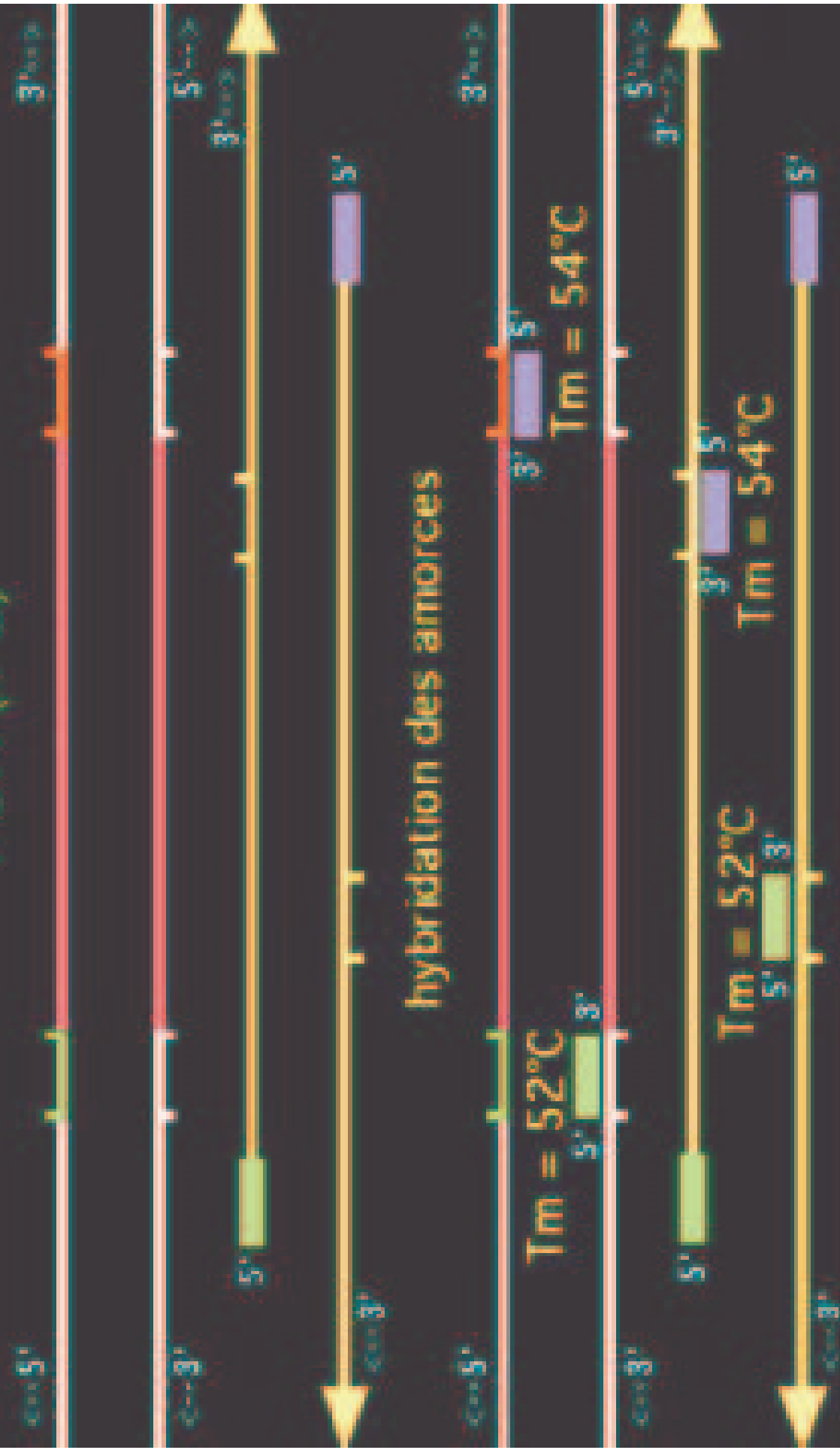
PCR (11-1)



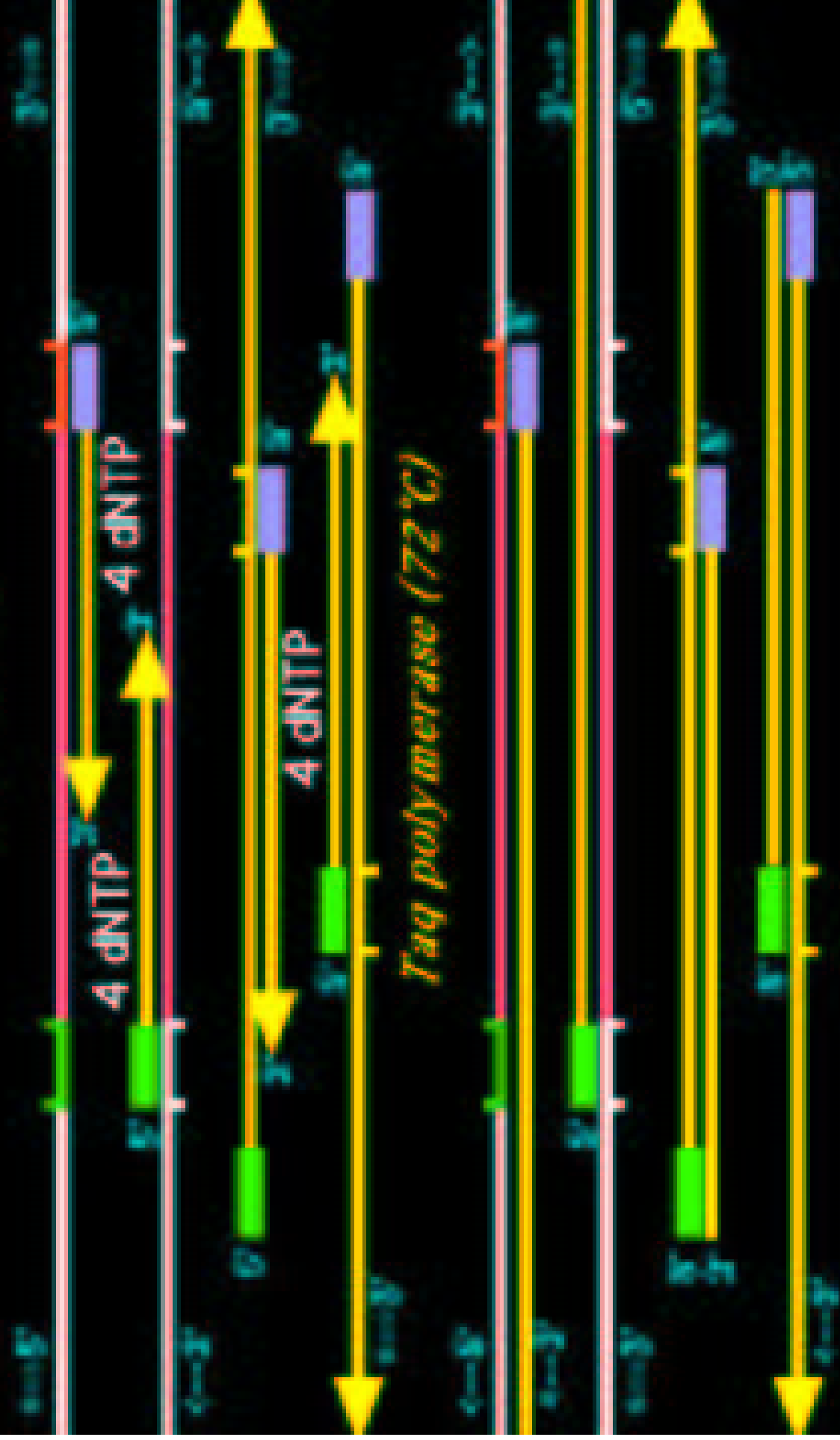
dénaturation à 95°C



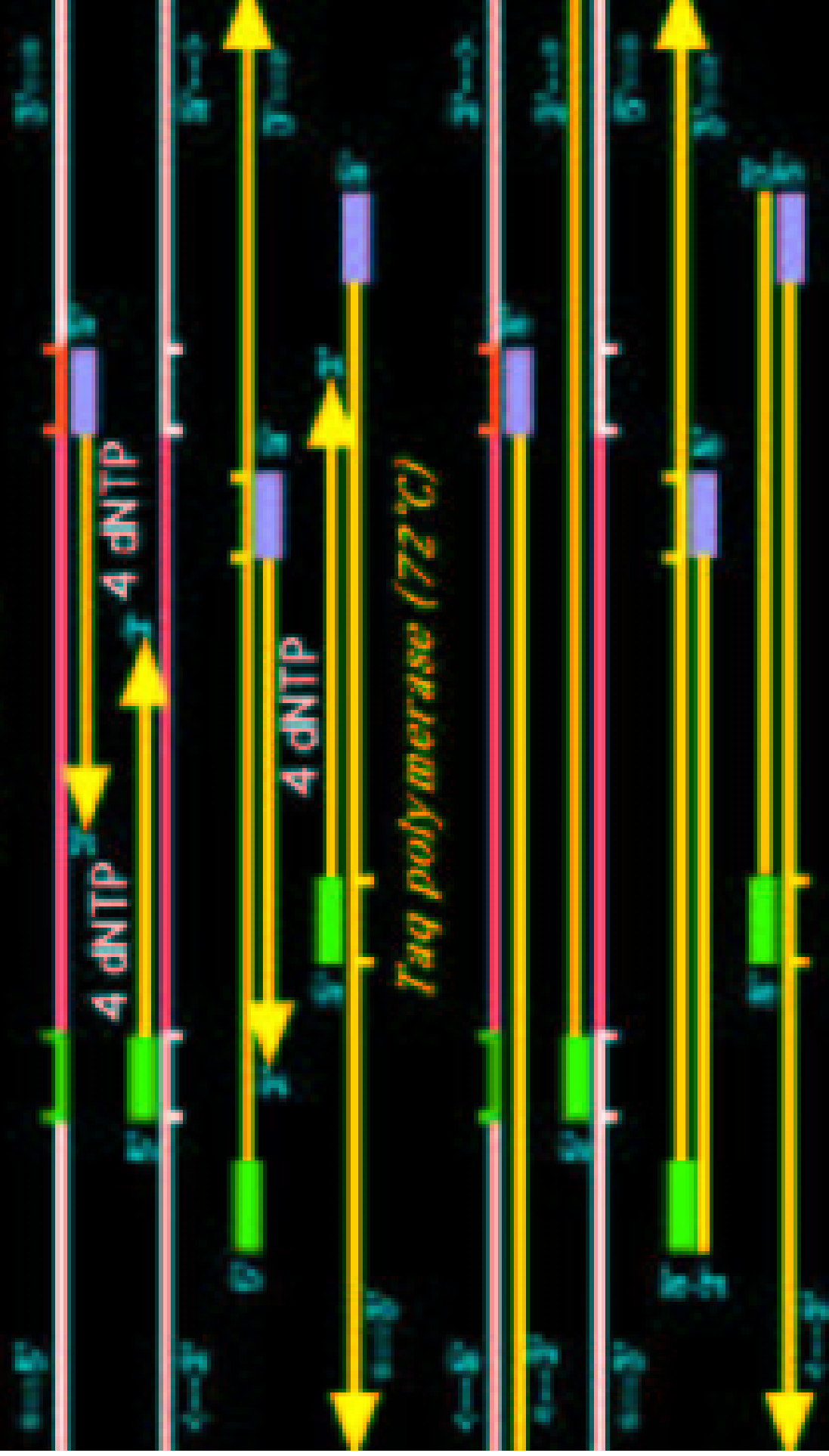
PCR (11-2)



PCR (11-3)



PCR (11-3)



PCR (III-3)



Nested PCR?

Oligonucléotides

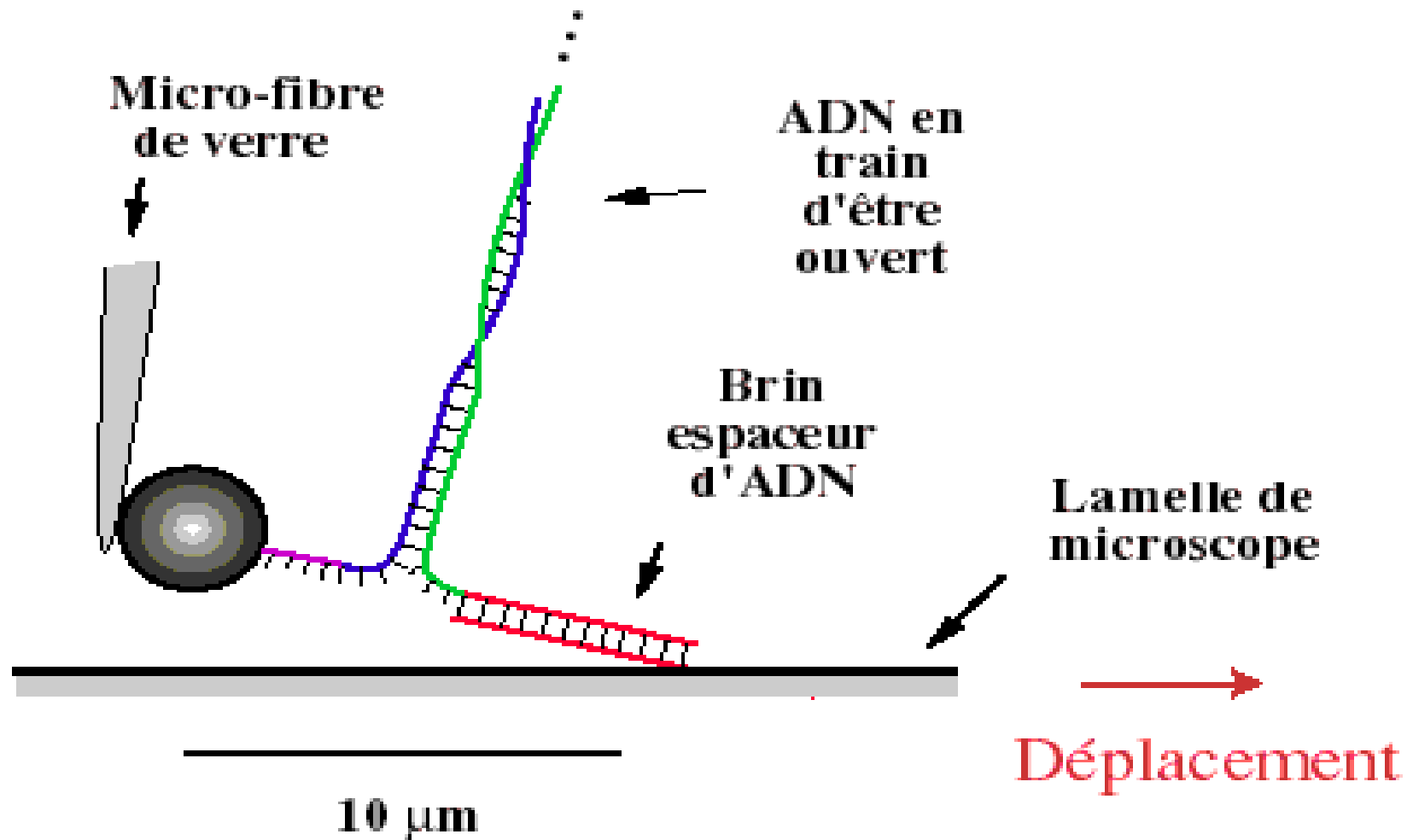
• Amorces

- aléatoires 6 pb 4096 possibles
- PCR 20 à 30 pb Hybridation en 3'
- modifiées nouveau site de restriction mutagenèse dirigée

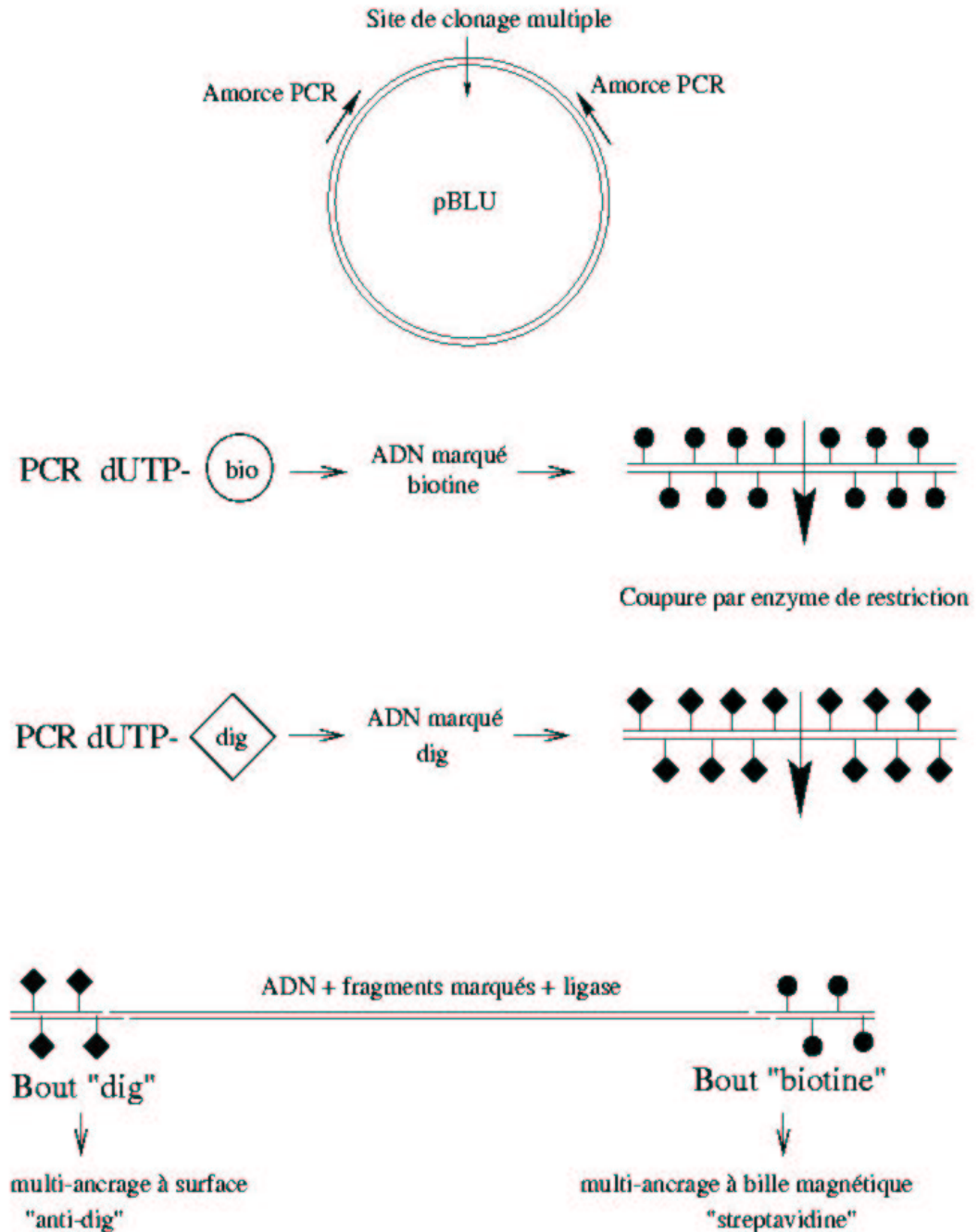
• Sondes

- Southern 30 à 5000 pb Hybridation des exons

Exemple 1 (F. Heslot et al)



Exemple 2 (V.Croquette et al)



84000

Moloney murine leukemia virus

2.7.7.49

Reverse transcriptase

Mg⁺⁺

Amorce

pGpApGpCpTpG

pCpUpCpGpApCpApUpGpGpGpGpApCpGpGpApGpUpGpGpGpUpA

mRNA (modèle)

dGTP

dTTP

dATP

dCTP

5'→3'

reverse

transcriptase

pp

pGpApGpCpTpGpTpApC

pCpUpCpGpApCpApUpGpGpGpGpApCpGpGpApGpUpGpGpGpUpA

~~3'→5'~~
~~exonuclease~~

Dye primers

JOE: 5' TGT TAA A A C G A C G G C C G A G T G C G G C C A C C G G C T G C C A A 3' + ddATP

BOX 1 <https://doi.org/10.1016/j.jhealeco.2020.100206> + ddTTP

5-FAM + ddCTP

TAMRA + ddGTP

3. **Atypical Aortic Dissection** Aneurysms that disintegrate or rupture before the aortic arch.

[illegible]

to the extent that the model is able to capture the underlying structure of the data, it is more likely to be able to generalize to new data. This is why it is important to have a good understanding of the data and the problem at hand, and to choose a model that is appropriate for the data and the problem. In this paper, we will discuss the importance of understanding the data and the problem, and how this can be used to choose a model that is appropriate for the data and the problem.

[illegible]

ddCTP +
5-FAM
5-FAM

ddTTP + 1. csrc.nist.gov/encryption/SP800/SP800-56A.html BOX

ddATP + CCGTCACAGGGGTCGGCCGCCGCCTTGTCTCTCAAGGAGC
JOE

ddGTP + T