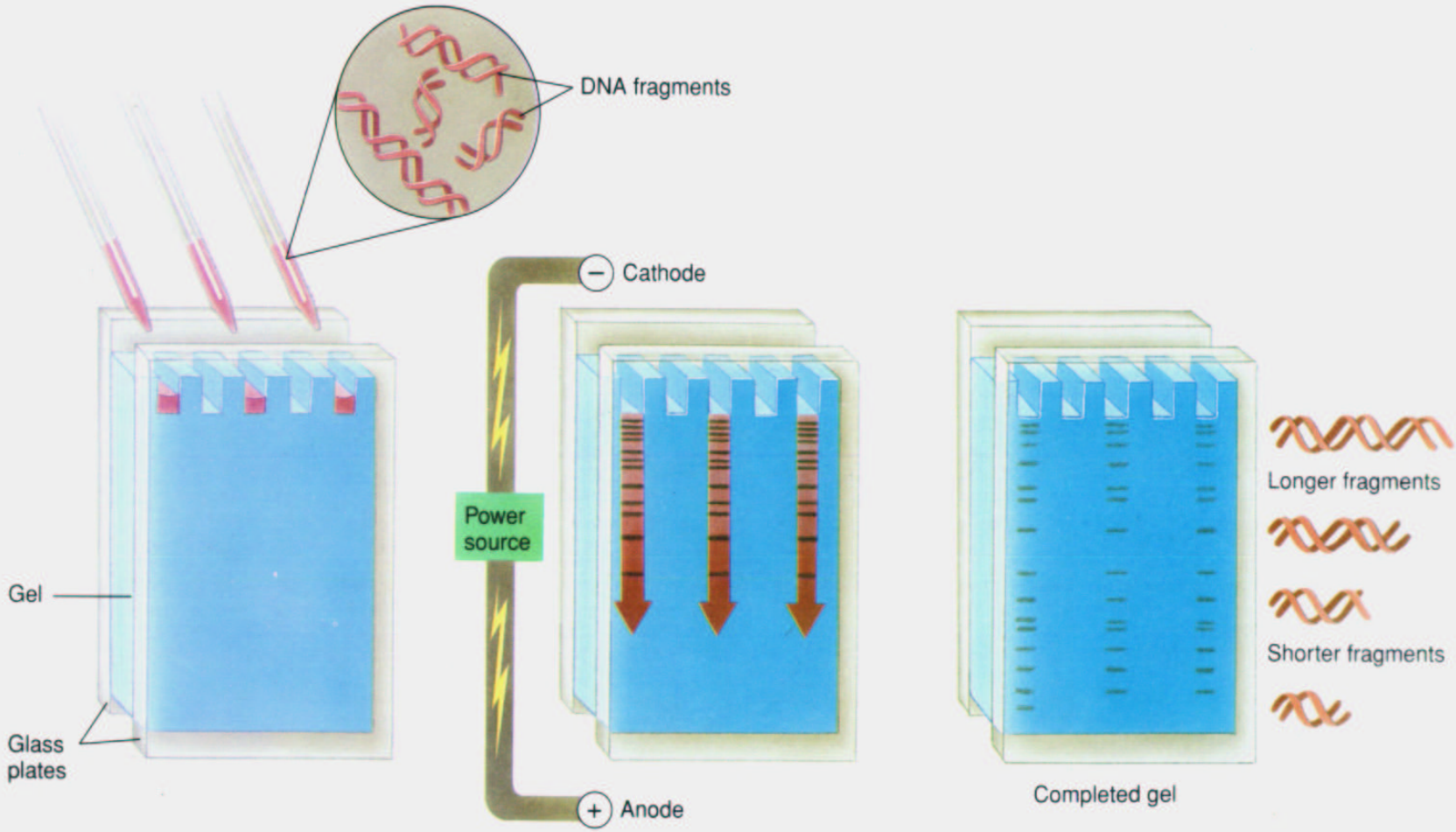
Outils de la BM

- Objectifs

1. Manipuler
2. Synthétiser / exprimer
3. Détecter
4. Séparer

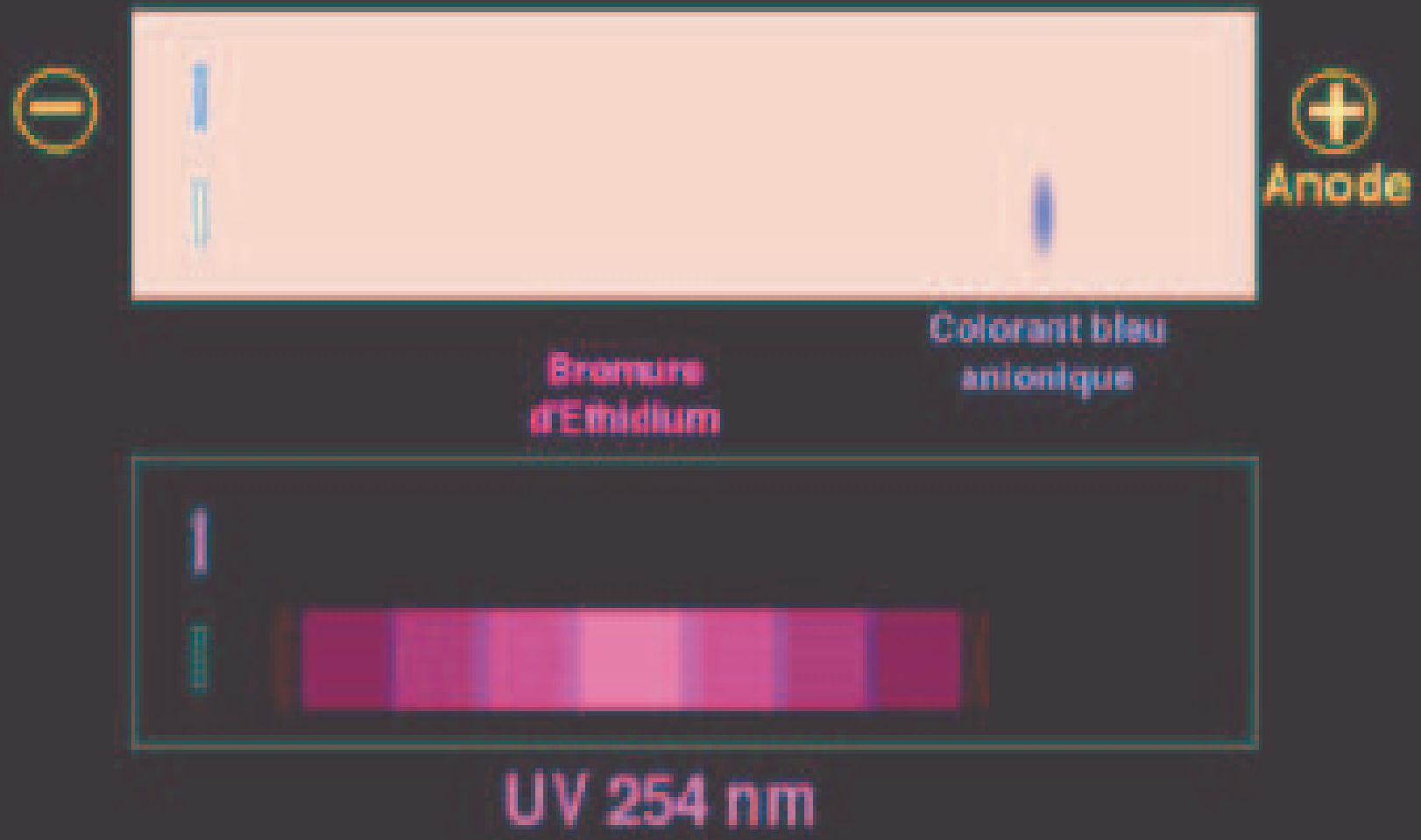
- Applications

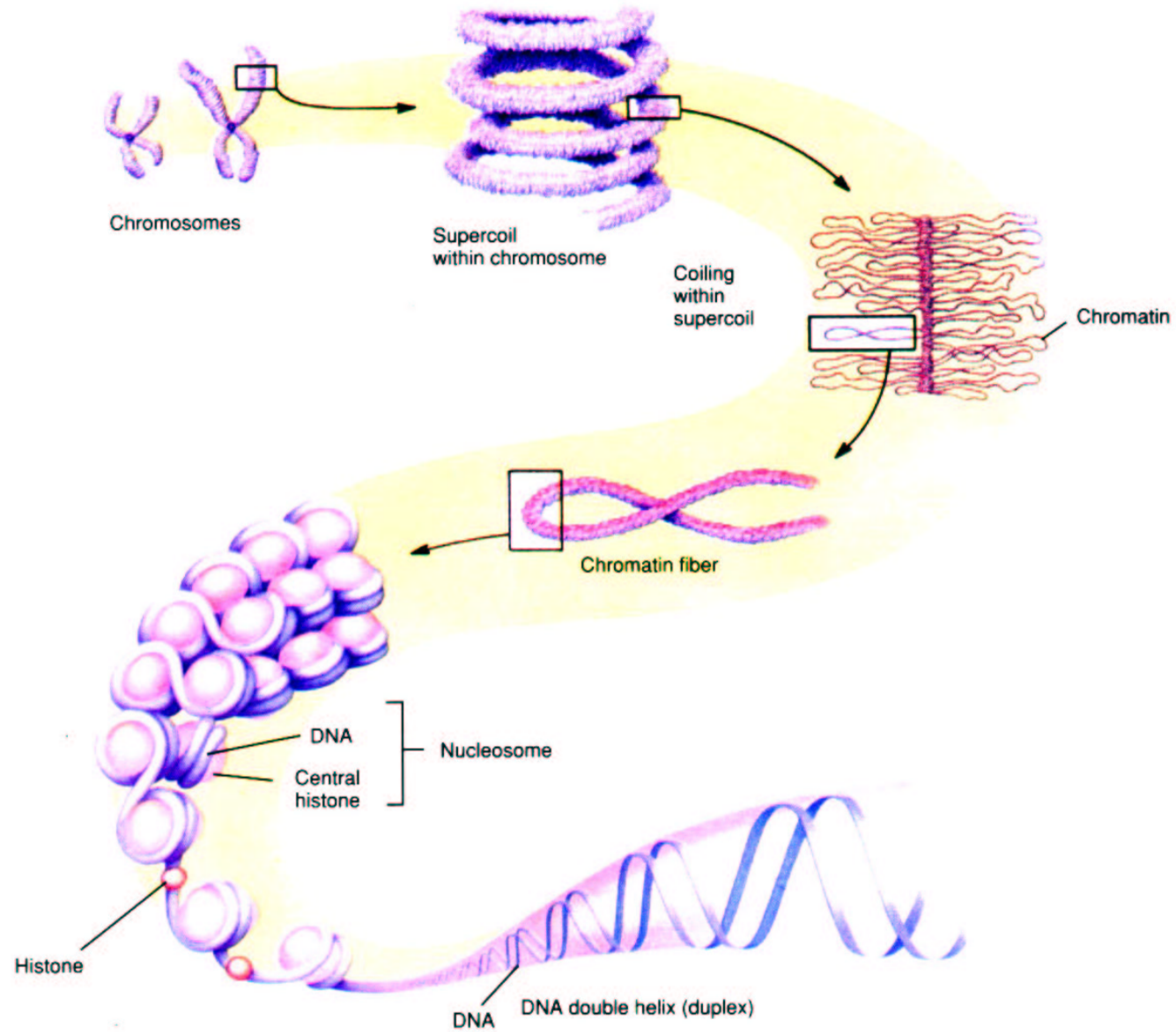
1. Thérapeutiques
2. Biophysique



How Gel Electrophoresis Works

Electrophorèse de l'ADN

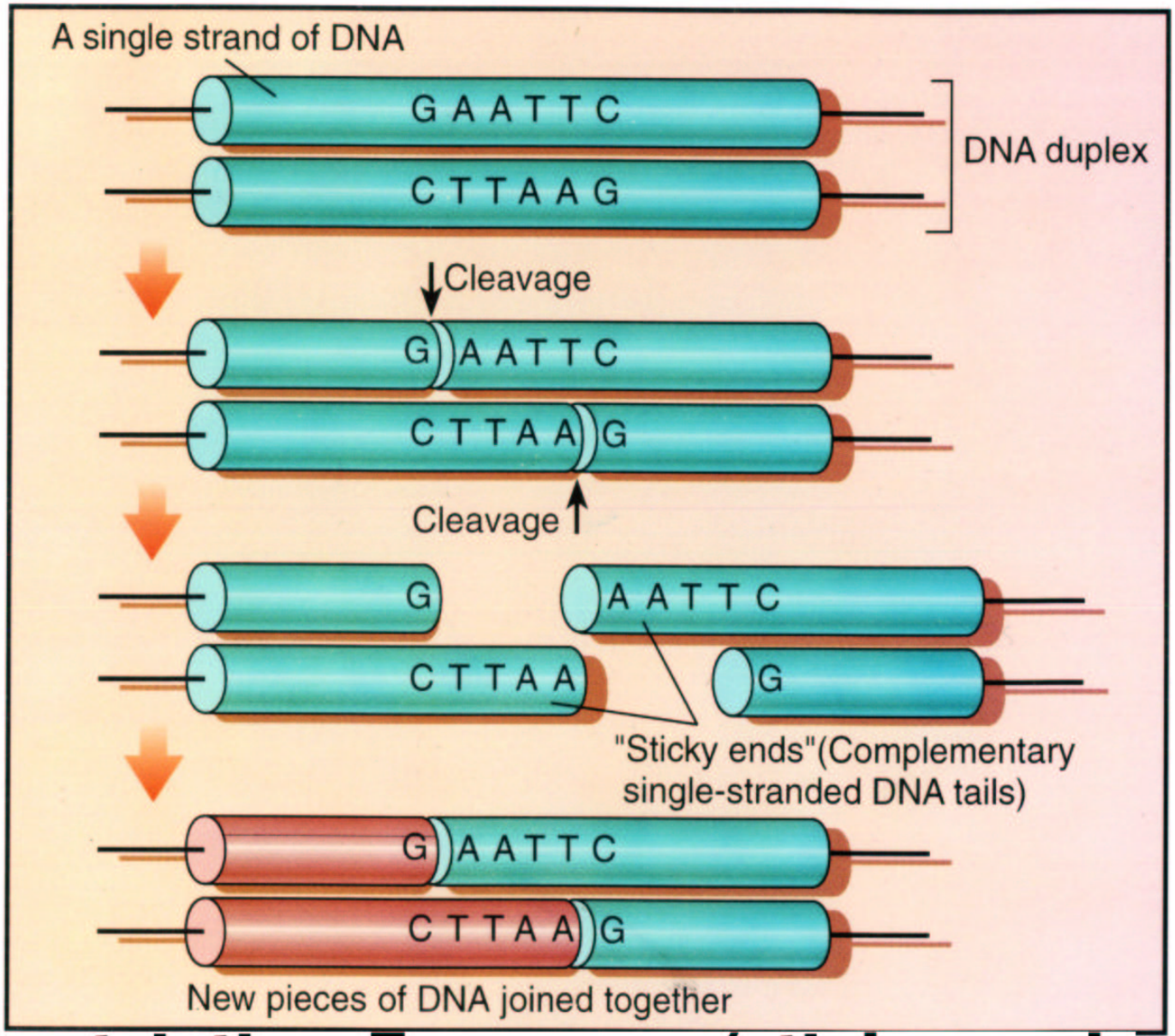




DNA is organized on chromosomes

FRAGMENT DE RESTRICTION

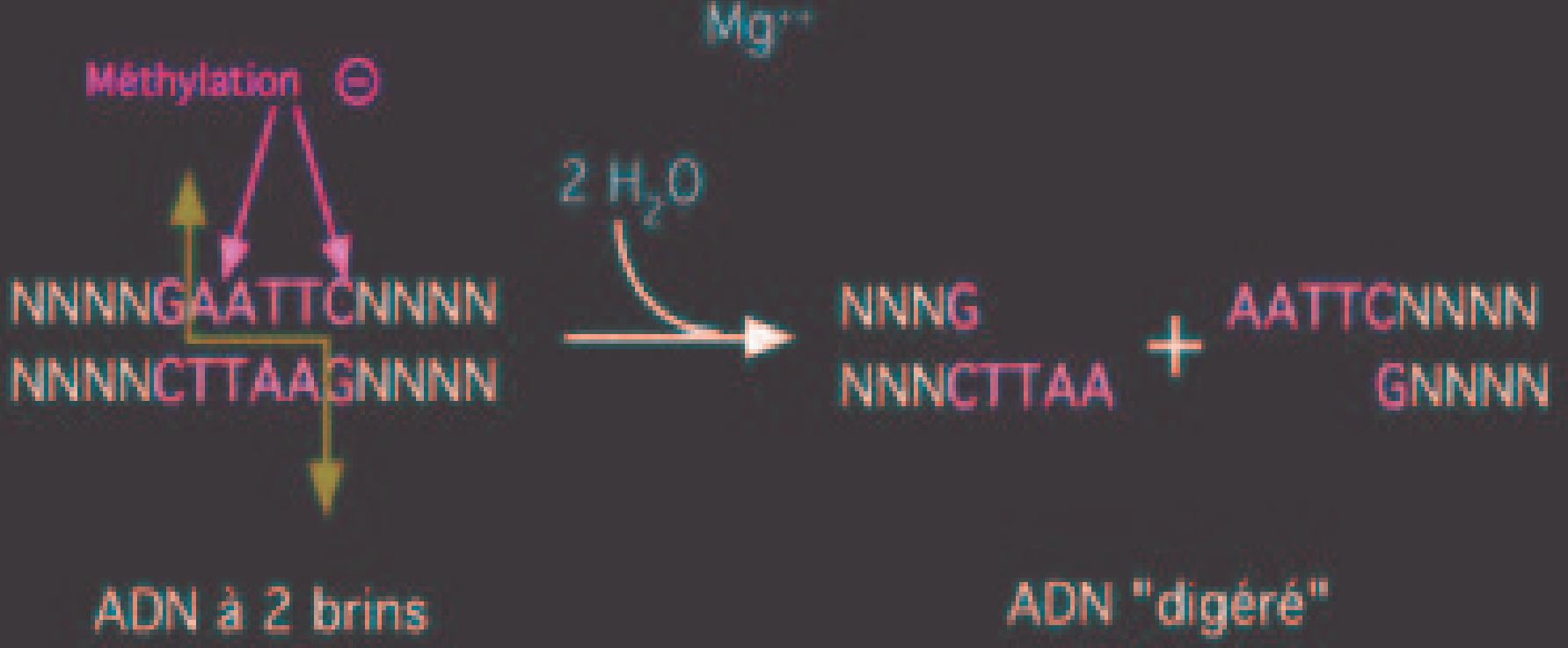
- Produit de la **digestion** de l'acide désoxyribonucléique par une **endonucléase** spécifique d'une séquence de nucléotides.



Restriction Enzymes/sticky ends

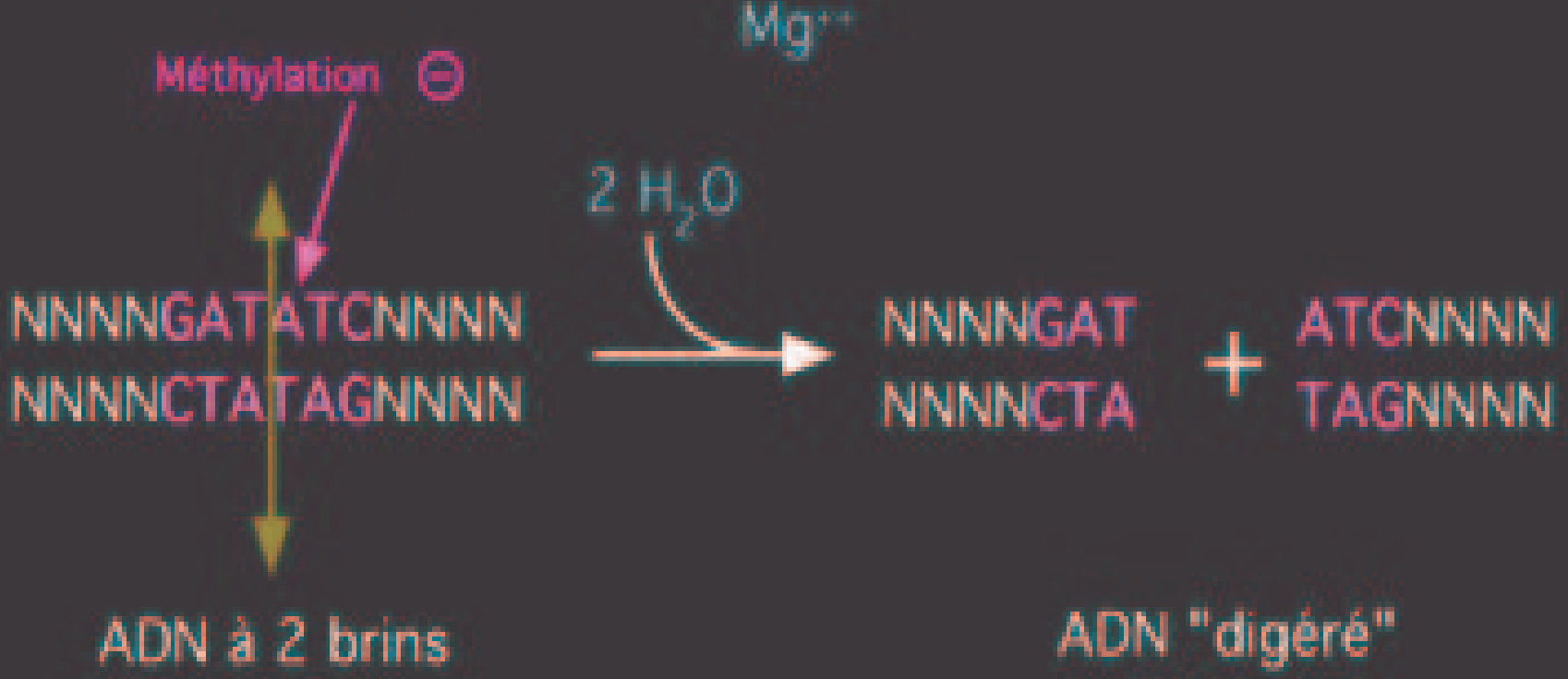
3.1.21.4

EcoR I
(enzyme de restriction)



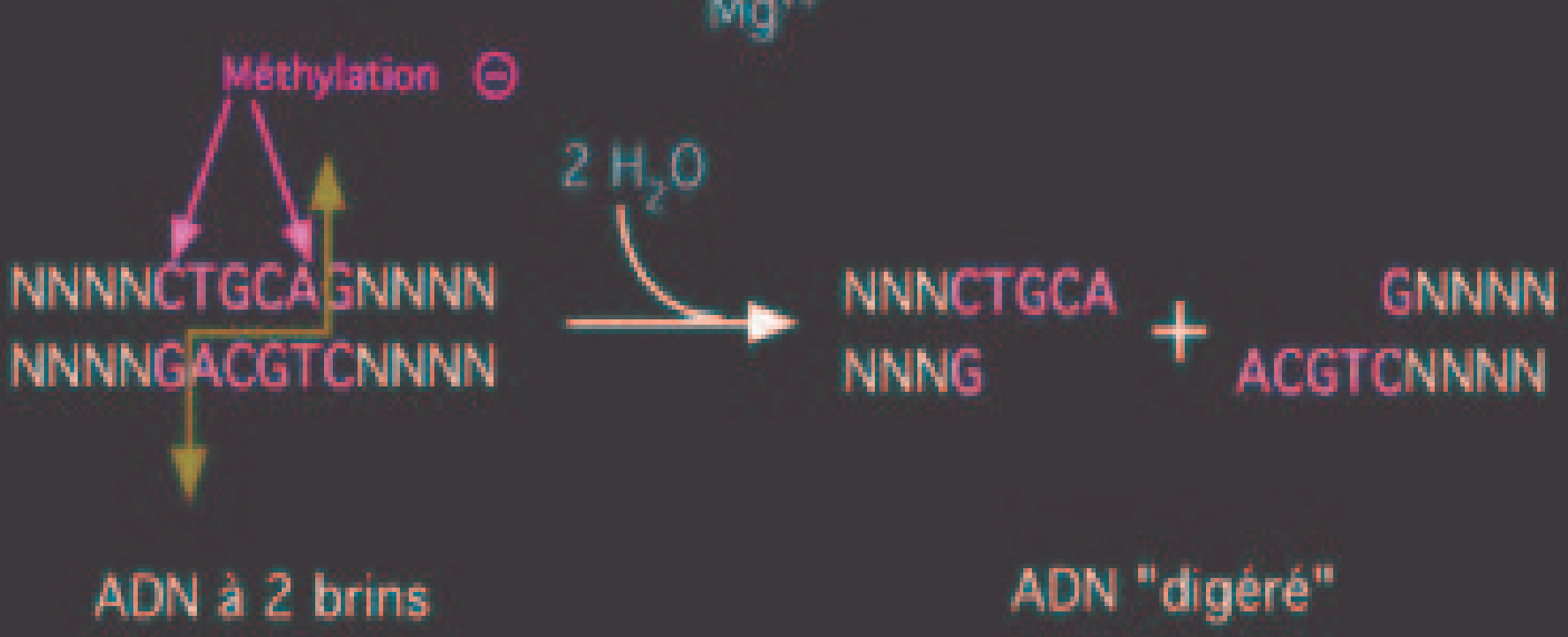
3.1.21.4

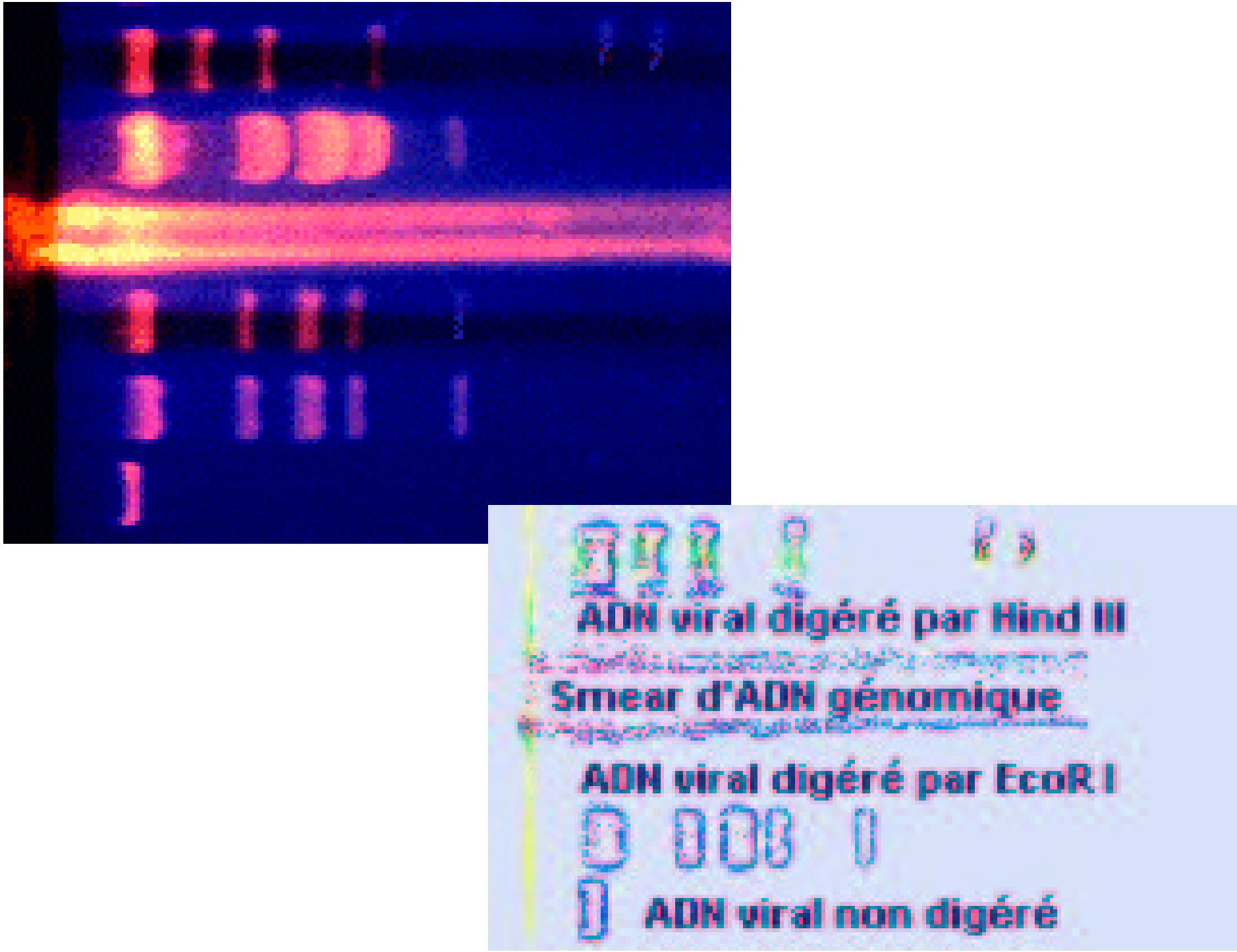
EcoR V
(enzyme de restriction)

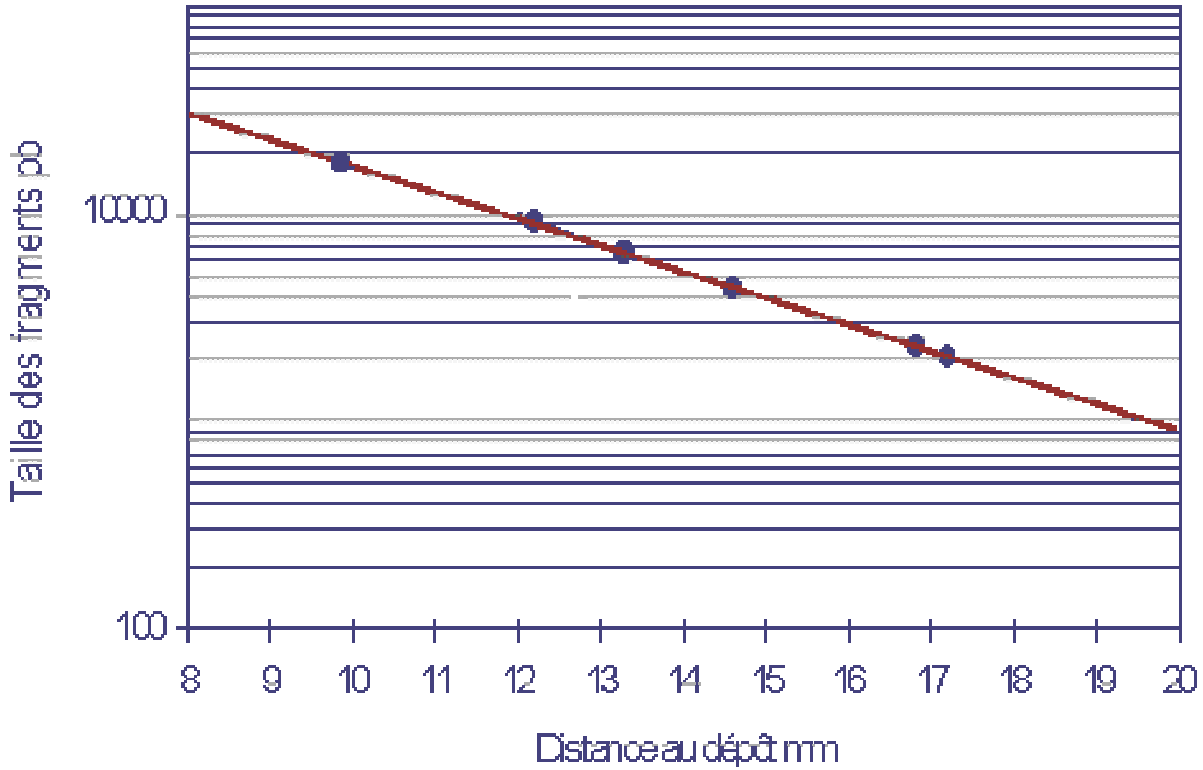


3.1.21.4

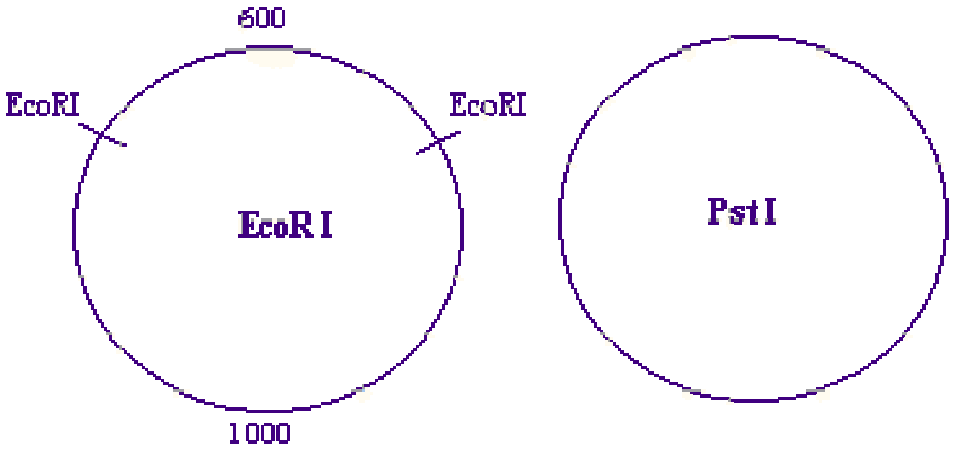
Pst I (enzyme de restriction)




ADN viral digéré par Hind III
Smear d'ADN génomique
ADN viral digéré par EcoR I
ADN viral non digéré



1-Détermination de la Taille des fragments d'ADN

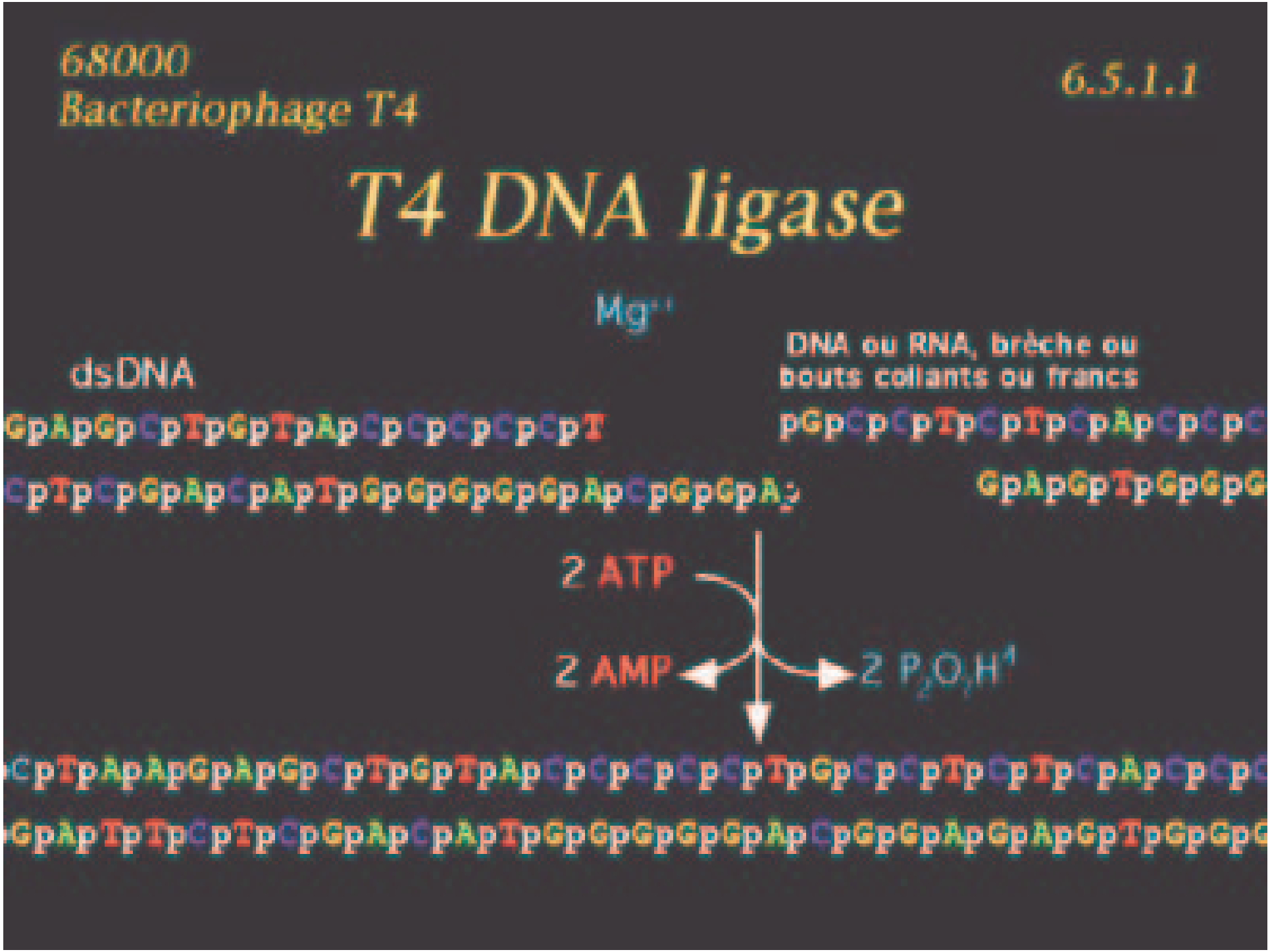


2- Etablissement de la carte de Restriction de l'ADN considéré


737 nt
152 nt
128 nt
98 nt
198 nt
195 nt
6 nt
196 nt

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.

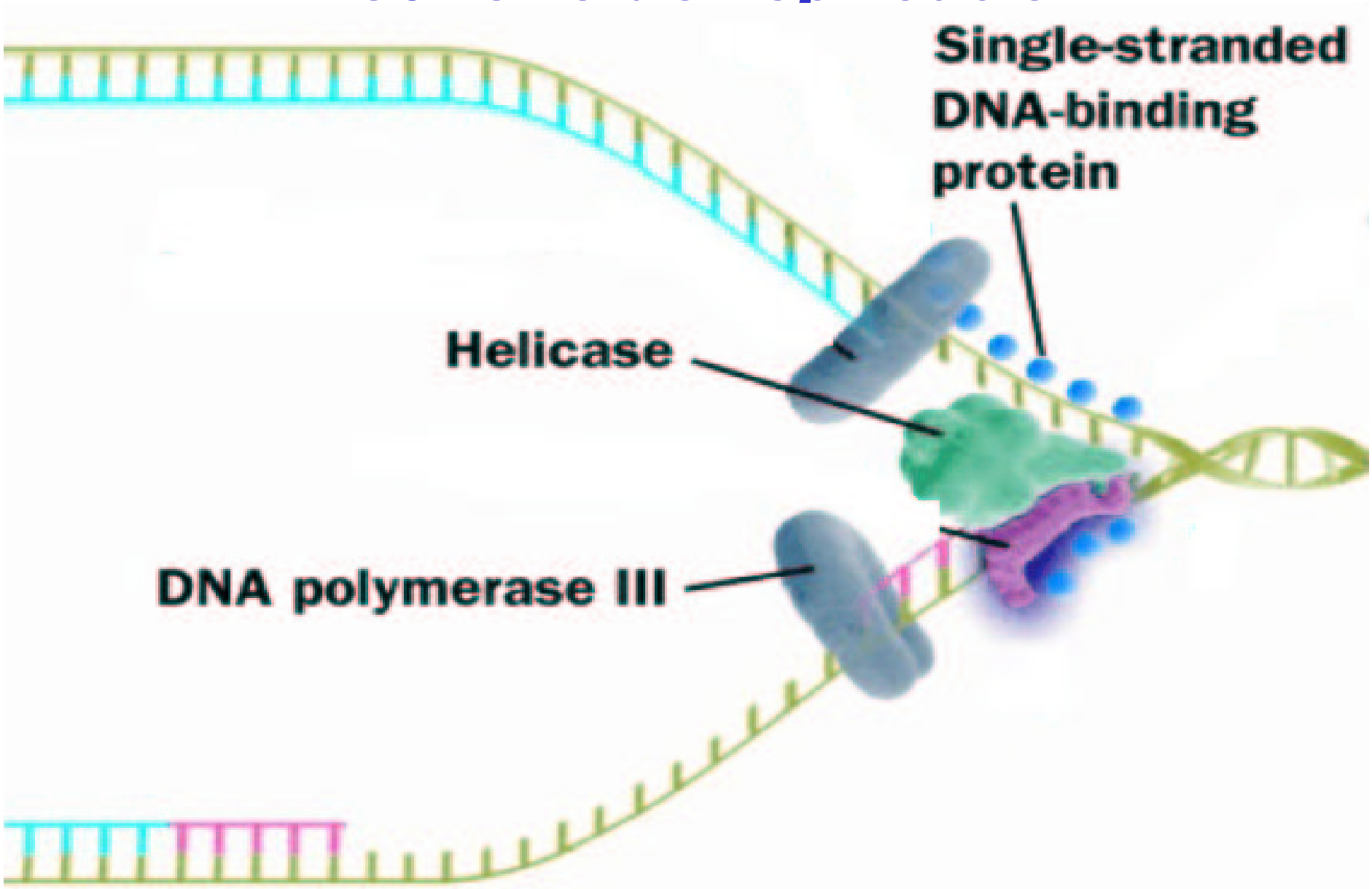

68000
Bacteriophage T4
6.5.1.1
T4 DNA ligase
Mg++
dsDNA
DNA ou RNA, brèche ou
bouts collants ou francs
2 ATP
2 AMP
2 P2O7H4

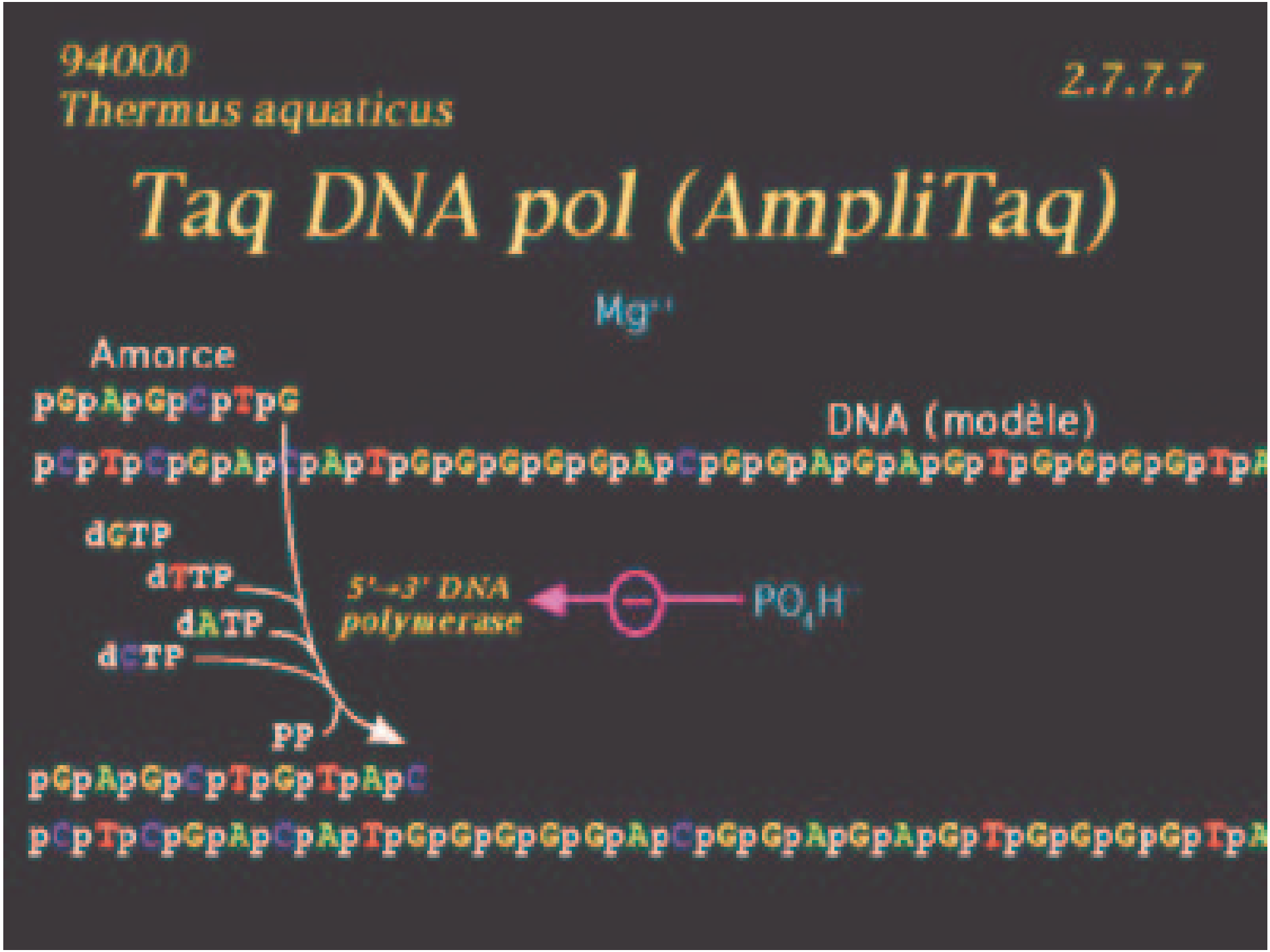
2.7.7.7

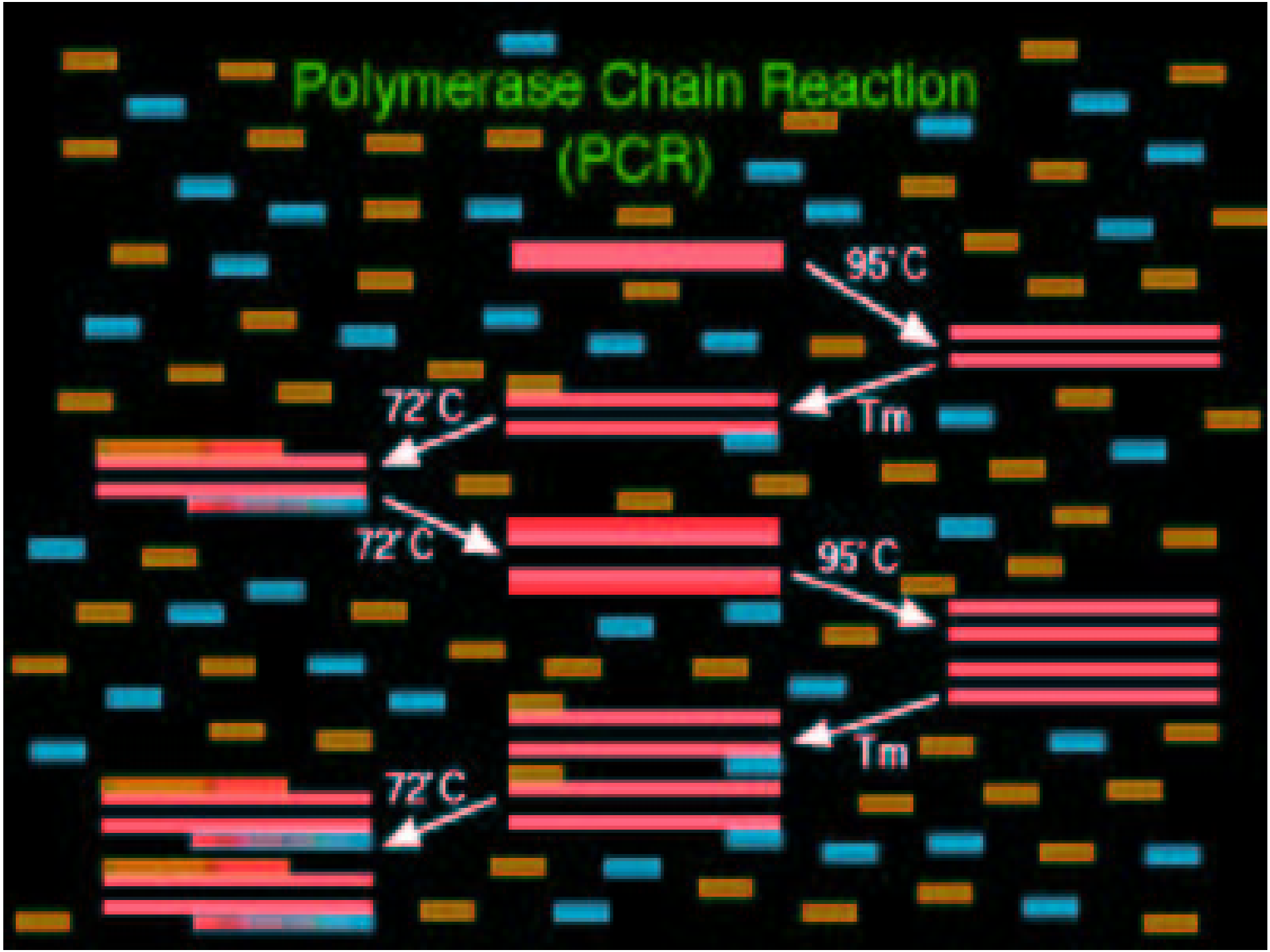
DNA polymérase

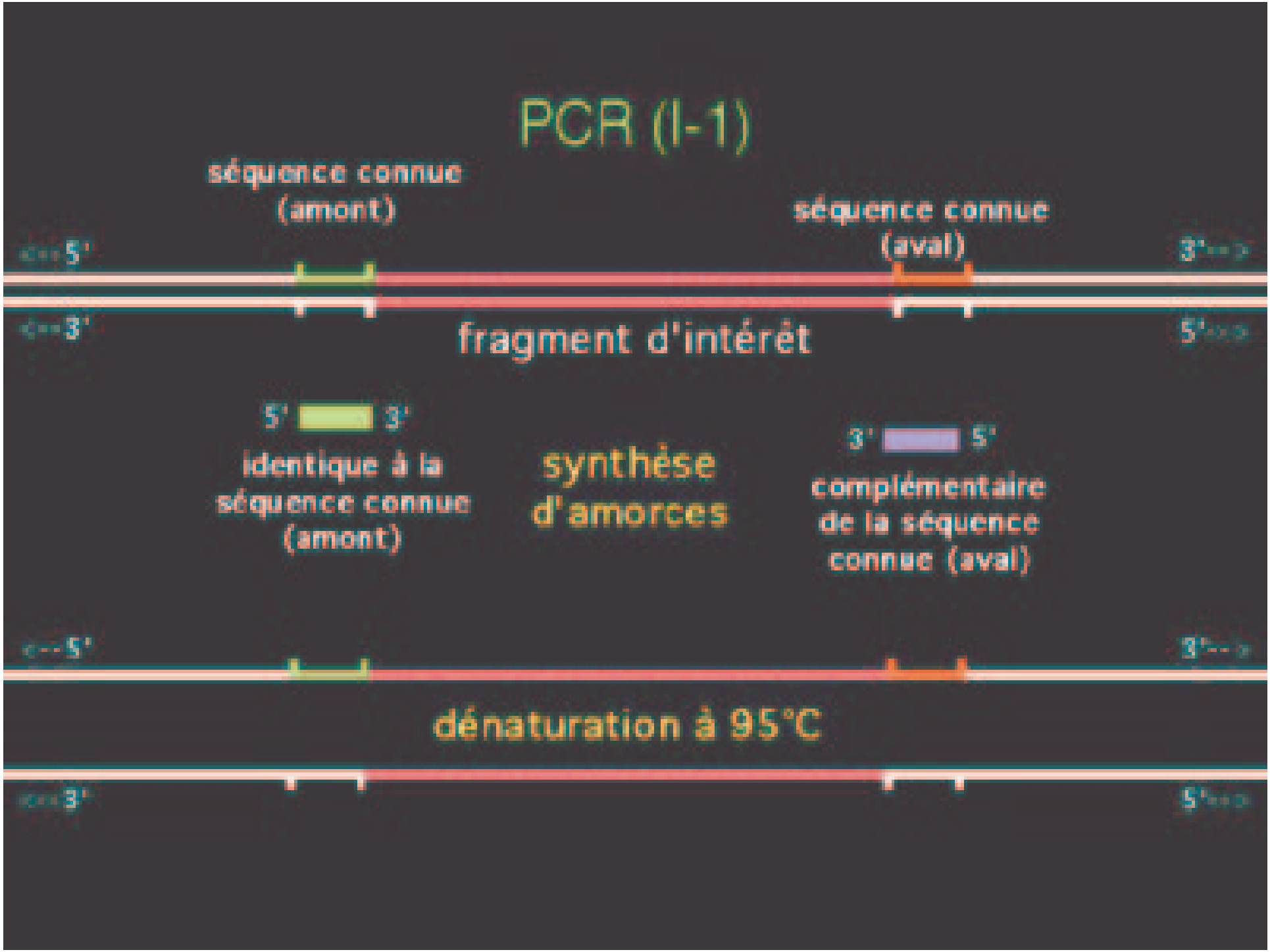
DNA-primase, DNA-ligase, Mg^{++}

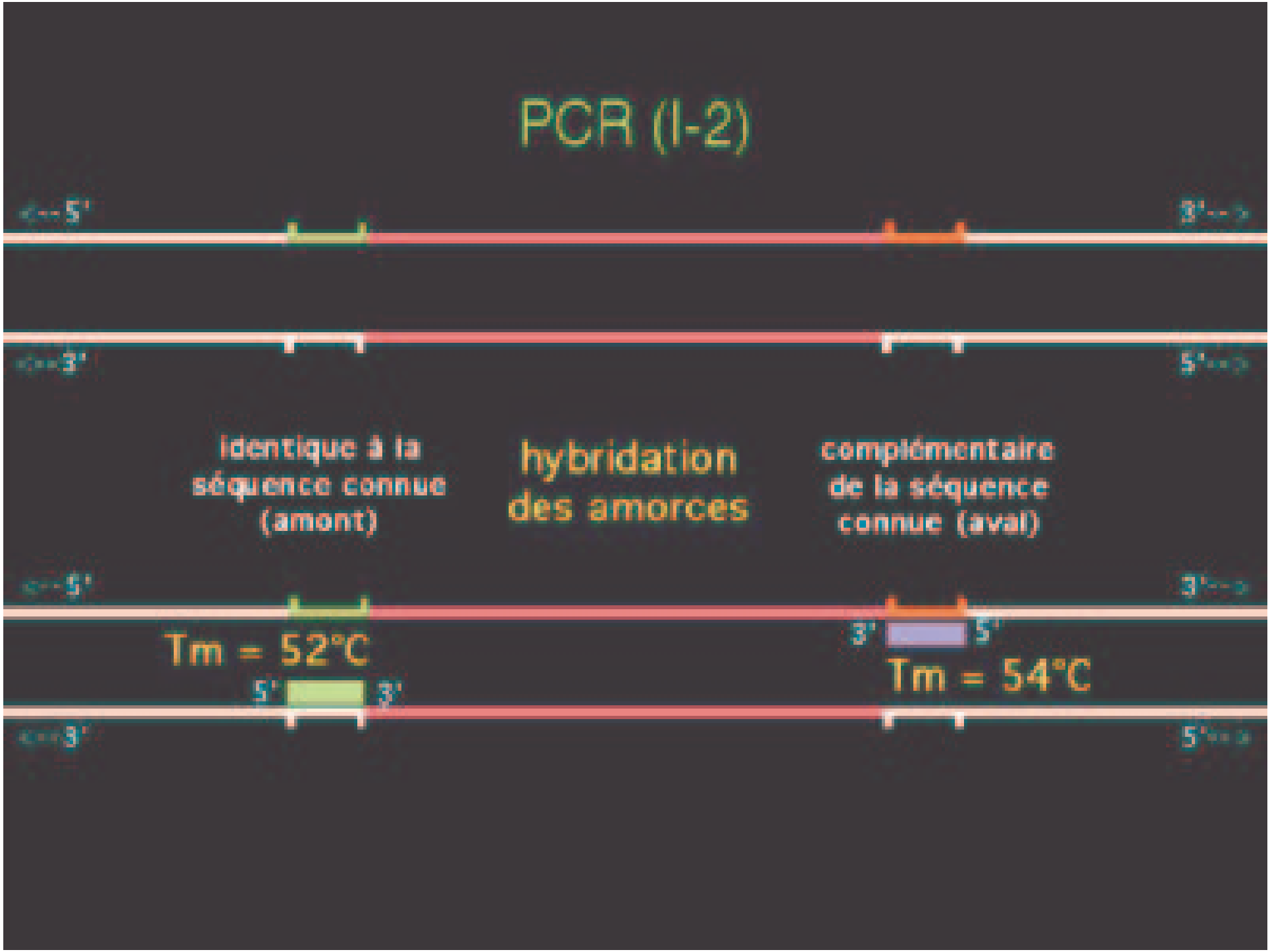
Fourche de réplication

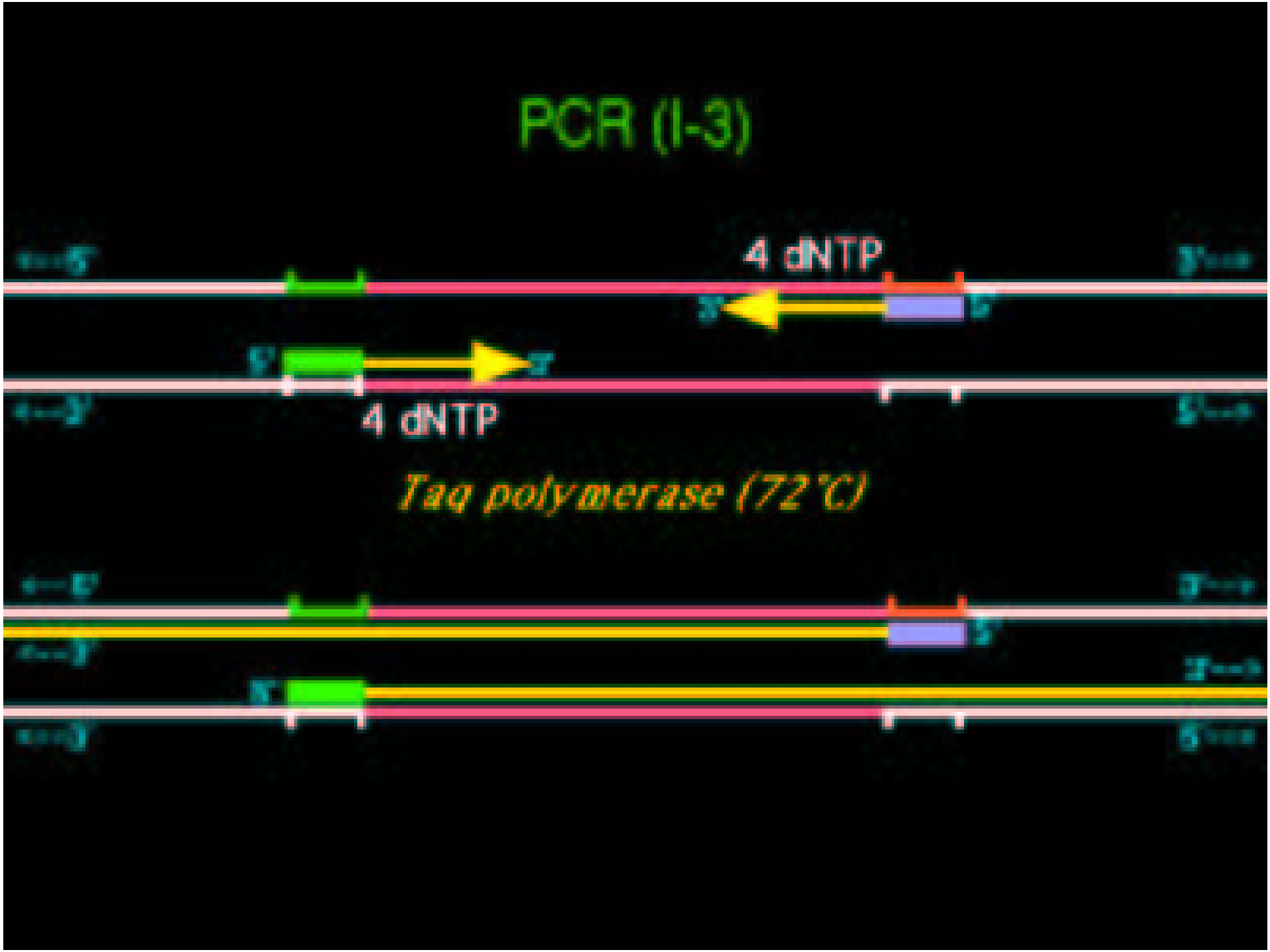


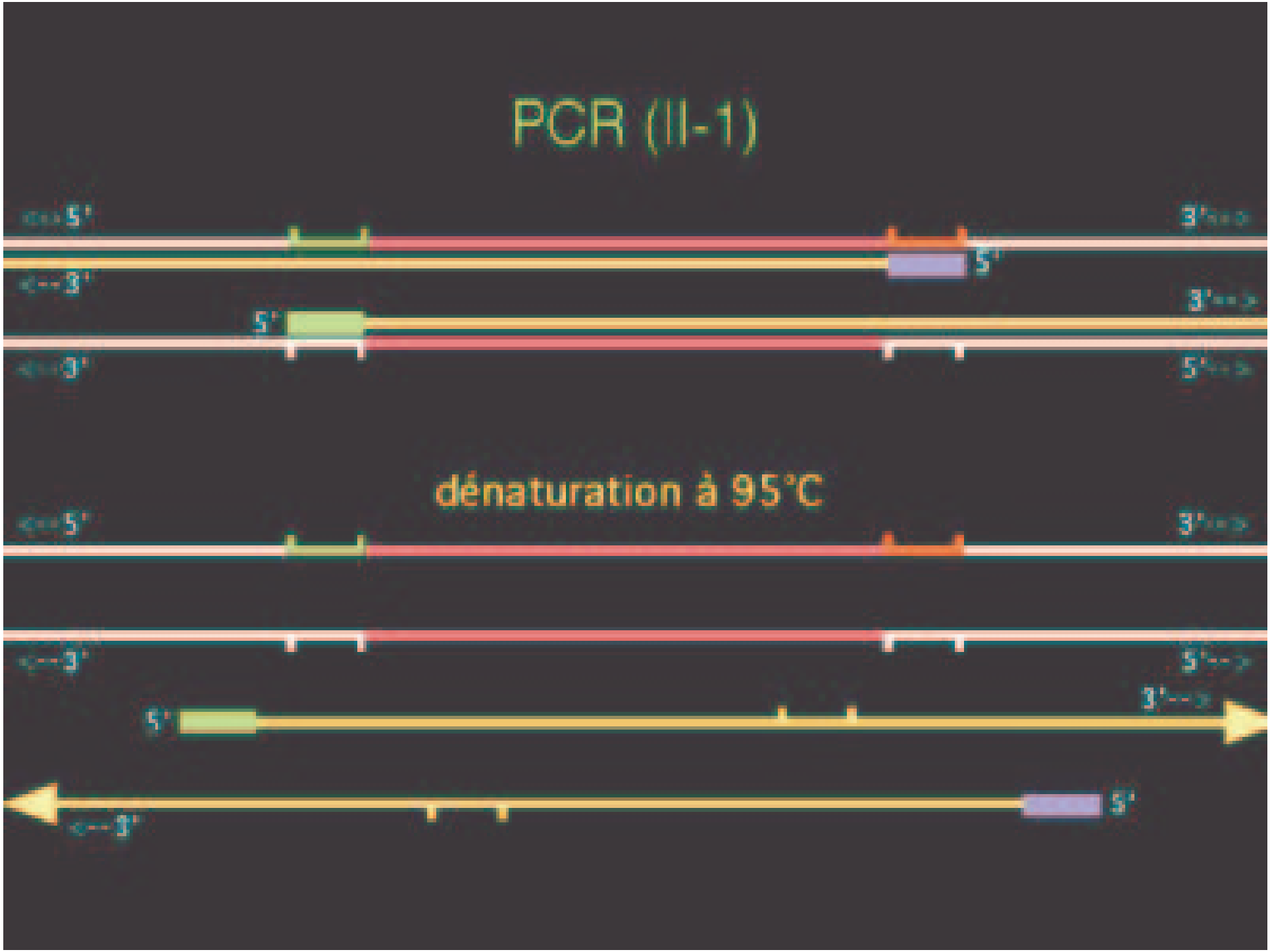

94000
Thermus aquaticus
2.7.7.7
Taq DNA pol (AmpliTaq)
Mg++
Amorce
pGpApGpCpTpG
DNA (modèle)
pCpTpCpGpApCpApTpGpGpGpGpGpApCpGpGpApGpApGpTpGpGpGpGpTpA
dGTP
dTTP
dATP
dCTP
5'→3' DNA polymerase
PO4H-
PP
pGpApGpCpTpGpTpApC
pCpTpCpGpApCpApTpGpGpGpGpGpApCpGpGpApGpApGpTpGpGpGpGpTpA

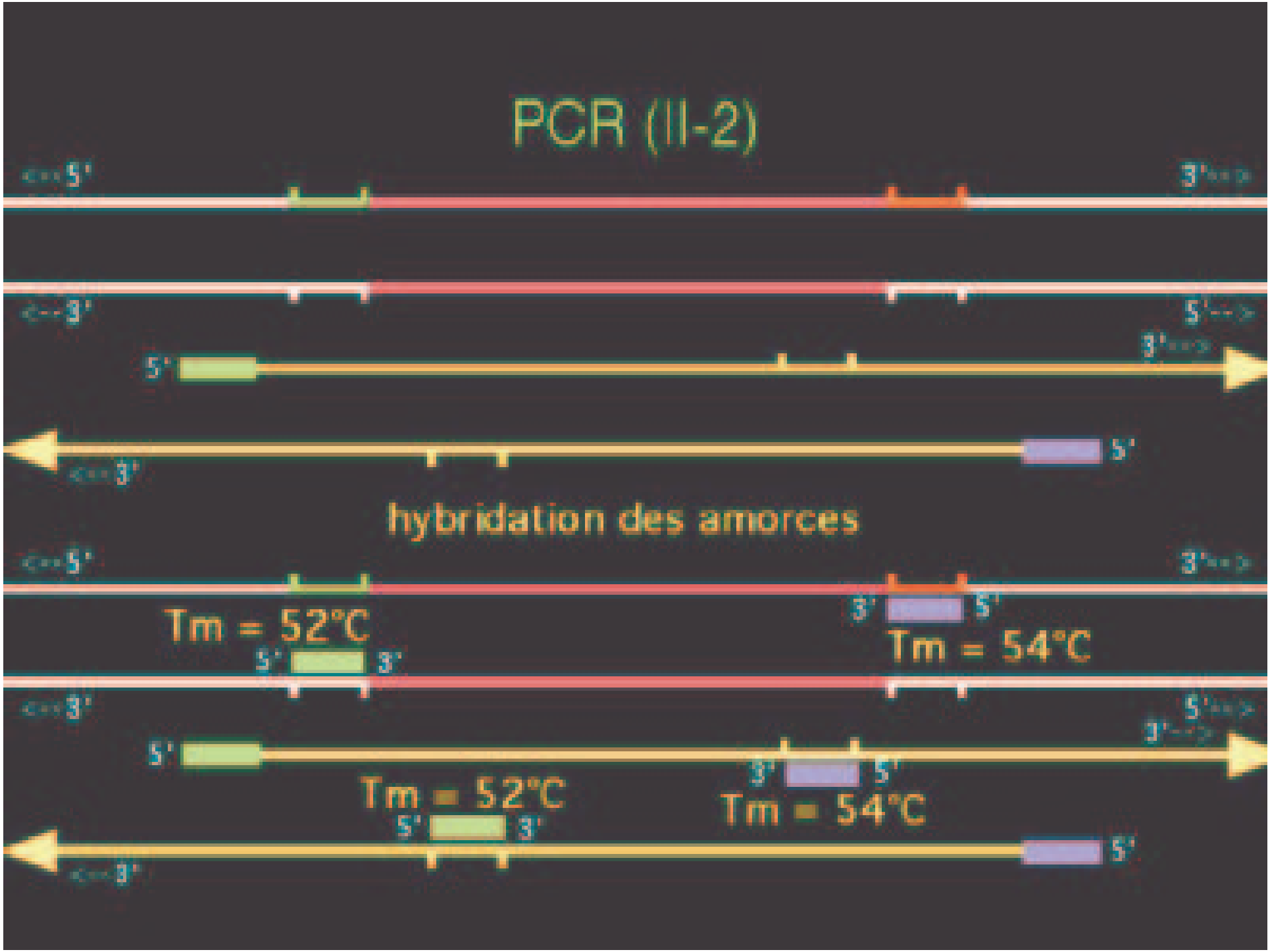

Polymerase Chain Reaction
(PCR)
95°C
72°C
Tm
72°C
95°C
Tm
72°C

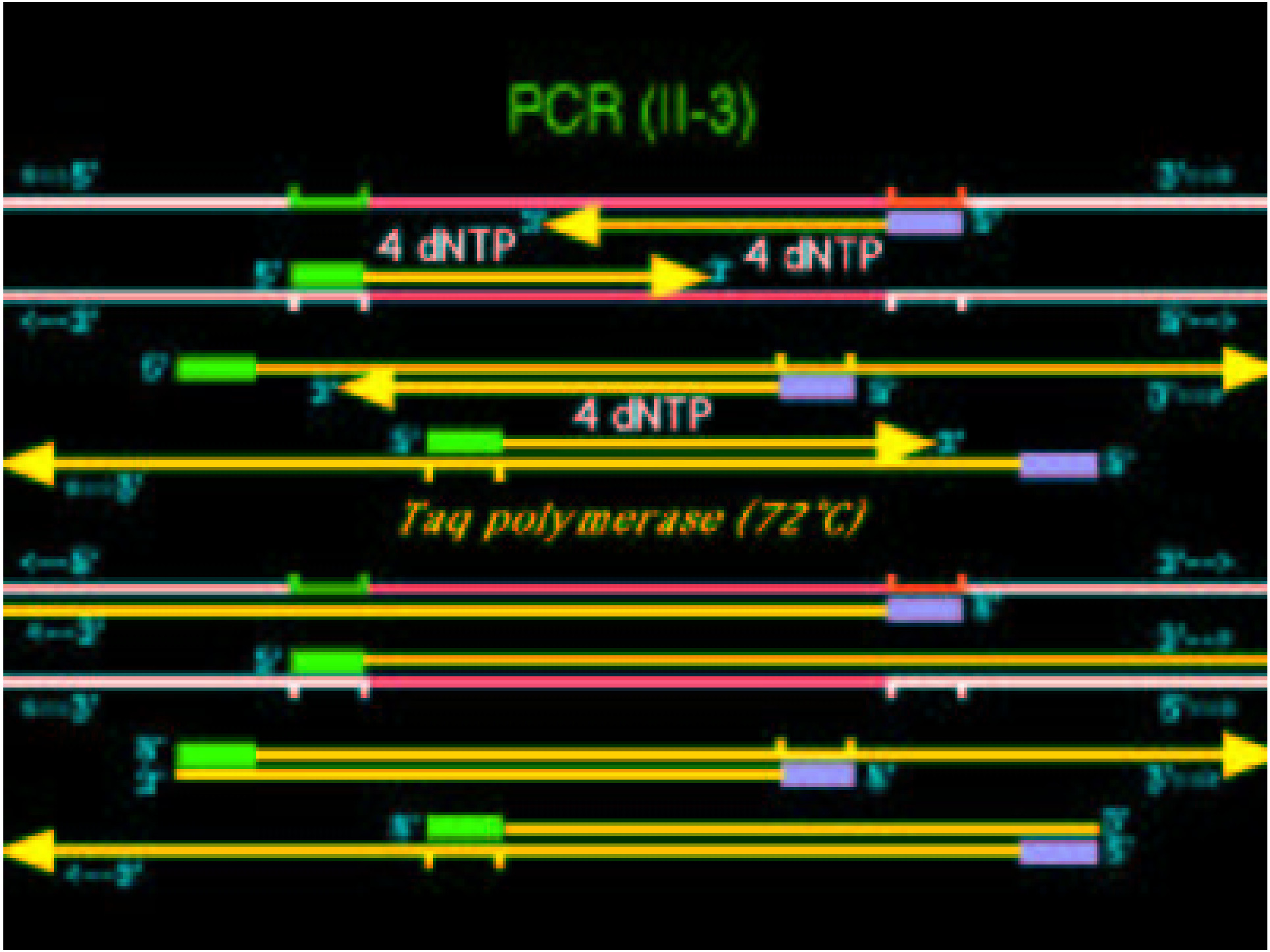

PCR (I-1)
séquence connue (amont)
séquence connue (aval)
<--5'
3'-->
<--3'
5'-->
fragment d'intérêt
5' 3'
identique à la séquence connue (amont)
synthèse d'amorces
3' 5'
complémentaire de la séquence connue (aval)
<--5'
3'-->
dénaturation à 95°C
<--3'
5'-->

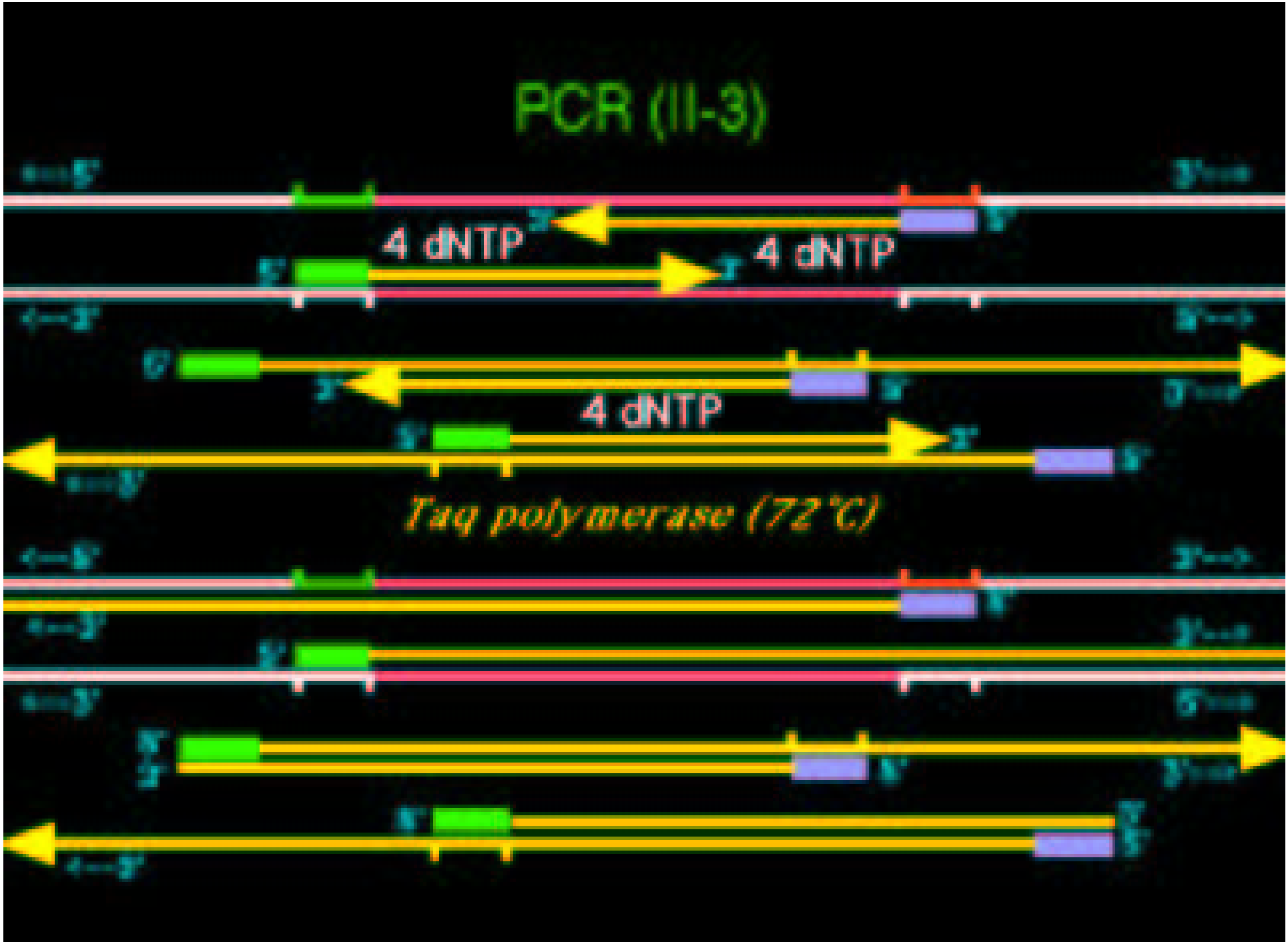

PCR (I-2)
<--5'
3'-->
<--3'
5'-->
identique à la
séquence connue
(amont)
hybridation
des amorces
complémentaire
de la séquence
connue (aval)
<--5'
3'-->
Tm = 52°C
5'
3'
3'
5'
Tm = 54°C
<--3'
5'-->

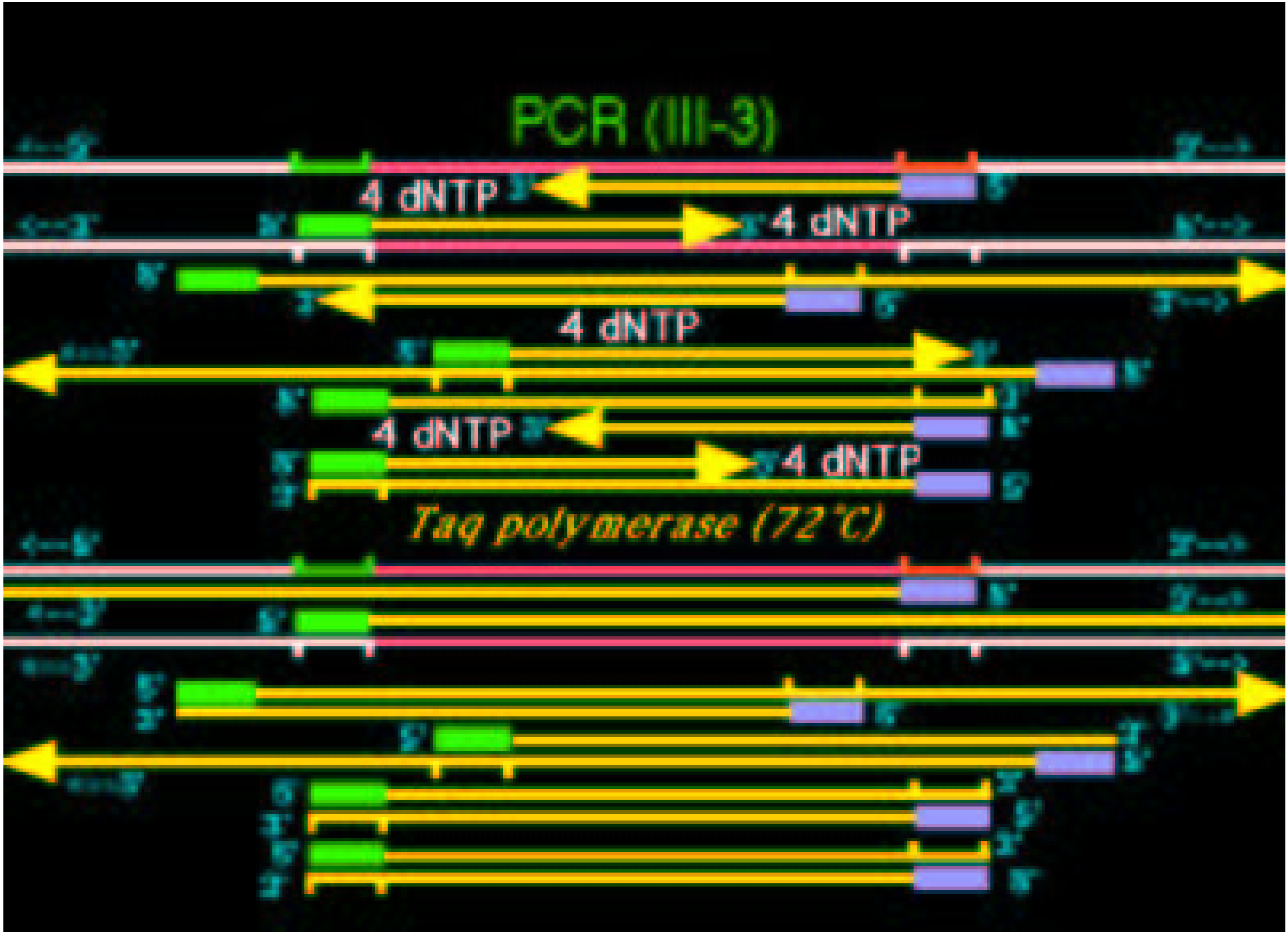

PCR (I-3)
4 dNTP
4 dNTP
Taq polymerase (72°C)


PCR (II-1)
5'
3'
5'
3'
5'
3'
5'
3'
dénaturation à 95°C
5'
3'
3'
5'
5'
3'
5'
3'

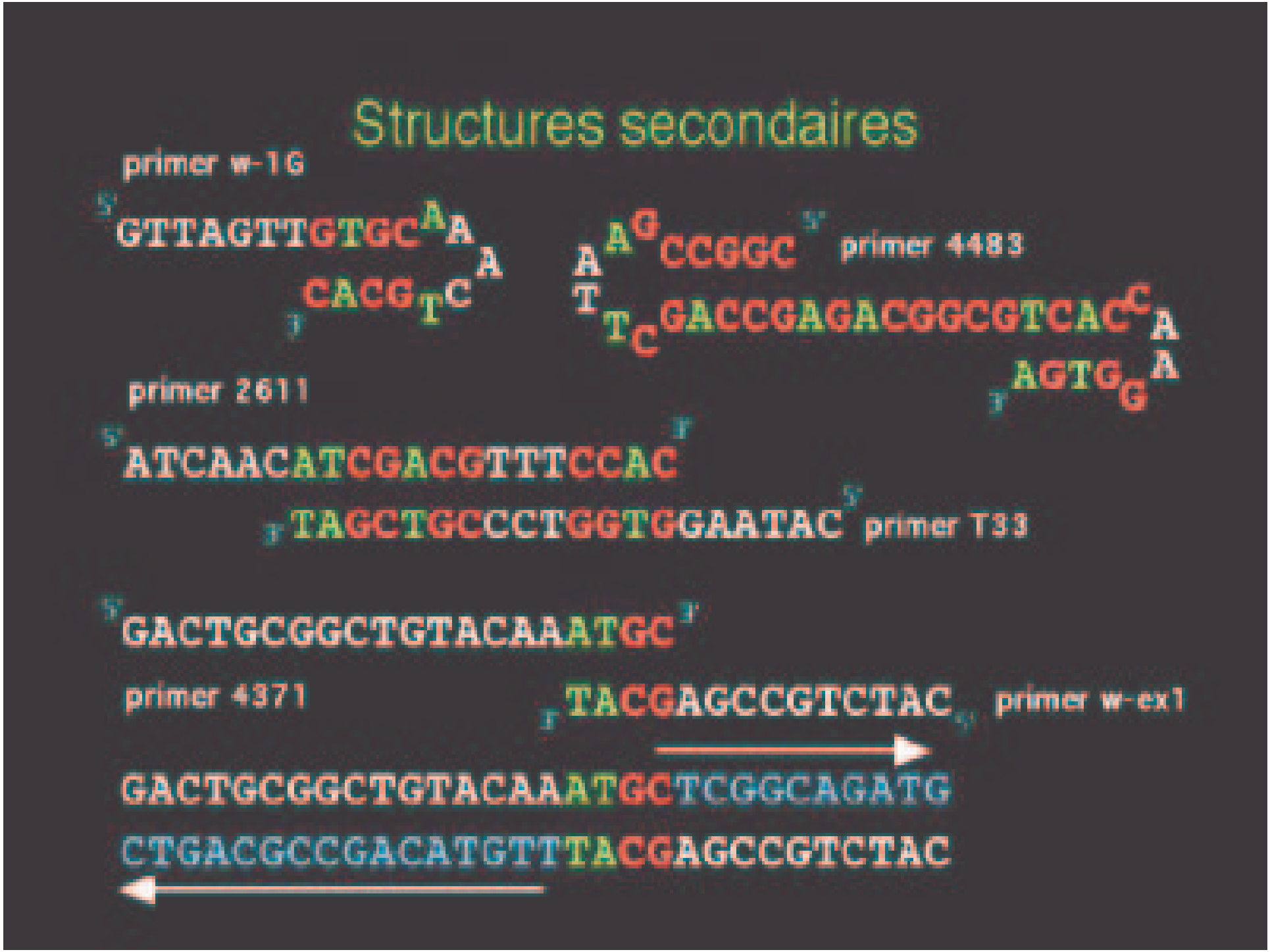

PCR (II-2)
<--5'
3'-->
<--3'
5'-->
5'
3'-->
5'
<--3'
hybridation des amorces
<--5'
3'-->
Tm = 52°C
5'
3'
3'
5'
Tm = 54°C
<--3'
5'-->
3'-->
5'
Tm = 52°C
5'
3'
3'
5'
Tm = 54°C
5'
<--3'


PCR (II-3)
4 dNTP
4 dNTP
4 dNTP
Taq polymerase (72℃)


PCR (II-3)
4 dNTP
4 dNTP
4 dNTP
Taq polymerase (72℃)


PCR (III-3)
4 dNTP
4 dNTP
4 dNTP
4 dNTP
4 dNTP
Taq polymerase (72℃)

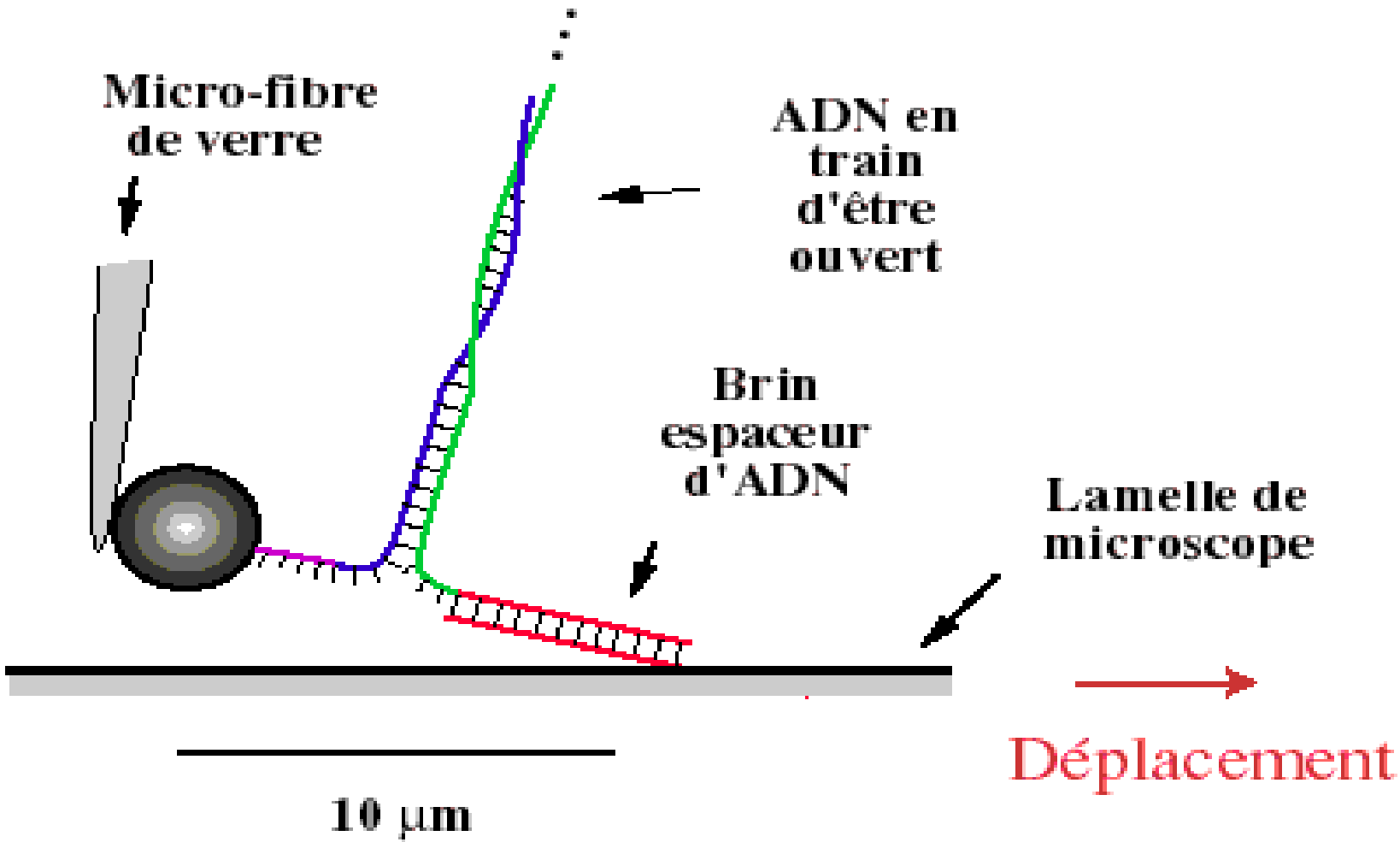
Nested PCR?


Structures secondaires
primer w-1G
5'GTTAGTTGTGCAA
CACGTC A
AAGCCGGC 5' primer 4483
ATTCGACCGAGACGGCGTCACCA
AGTGGA
primer 2611
5'ATCAACATCGACGTTCCAC 3'
TAGCTGCCCTGGTGGAATAC 5' primer T33
5'GACTGCGGCTGTACAAATGC 3'
primer 4371
TACGAGCCGTCTAC 3' primer w-ex1
GACTGCGGCTGTACAAATGCTCGGCAGATG
CTGACGCCGACATGTTTACGAGCCGTCTAC

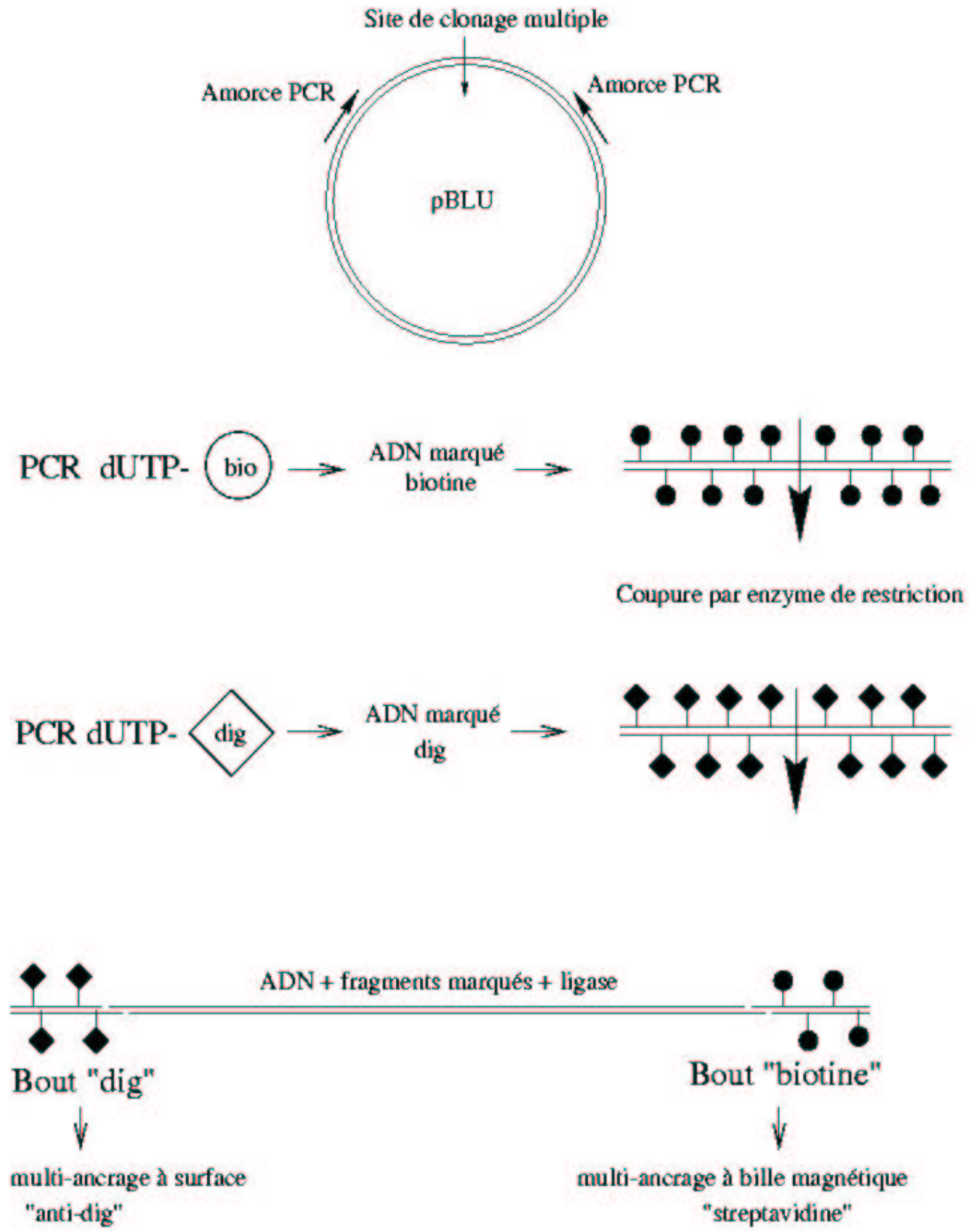
Oligonucléotides

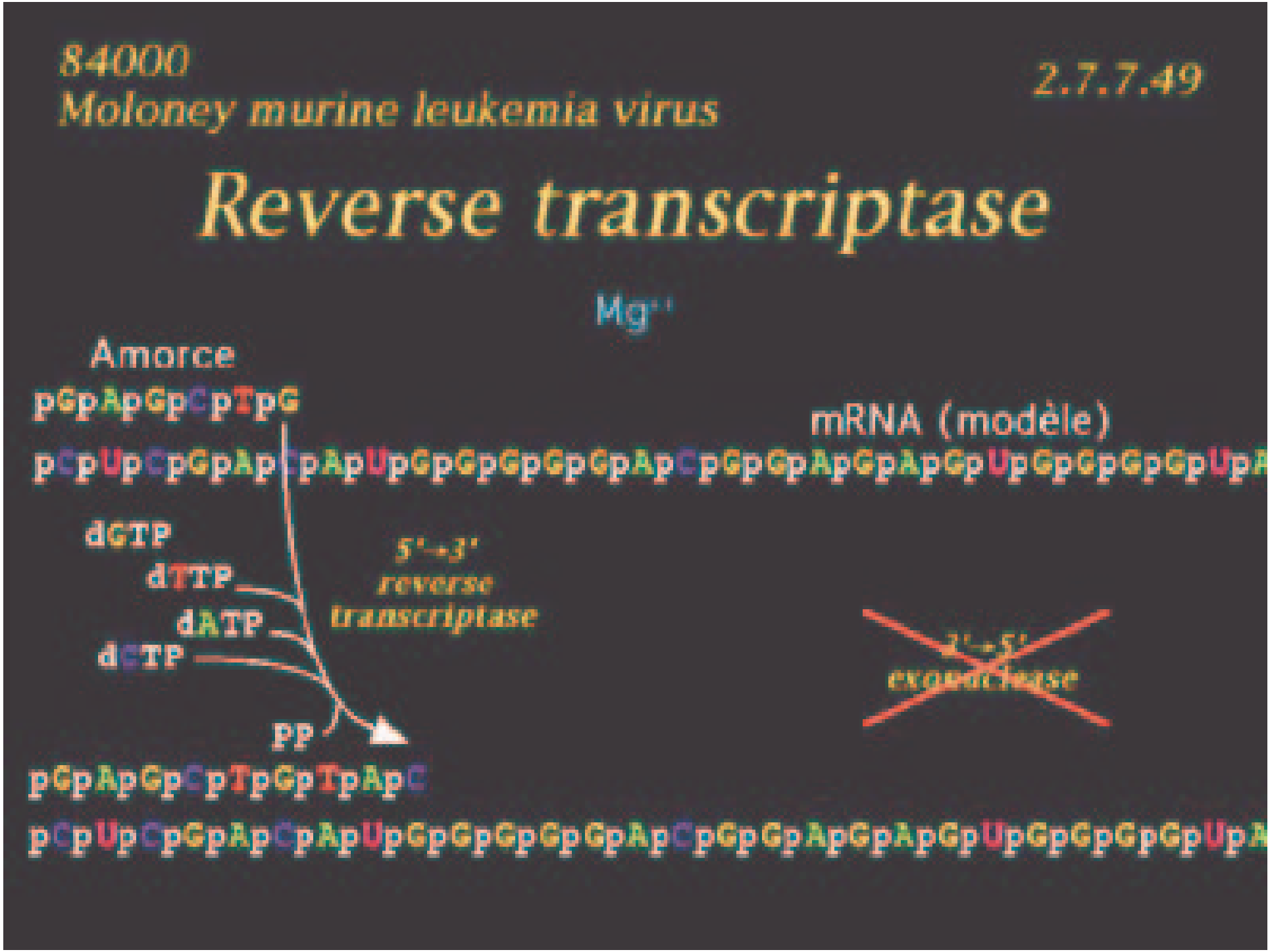
- Amorces
  - aléatoires — 6 pb — 4096 possibles
  - PCR — 20 à 30 pb — Hybridation en 3'
  - modifiées — nouveau site de restriction, mutagénèse dirigée
- Sondes
  - Southern — 30 à 5000 pb — Hybridation des exons

Exemple 1 (F. Heslot et al)

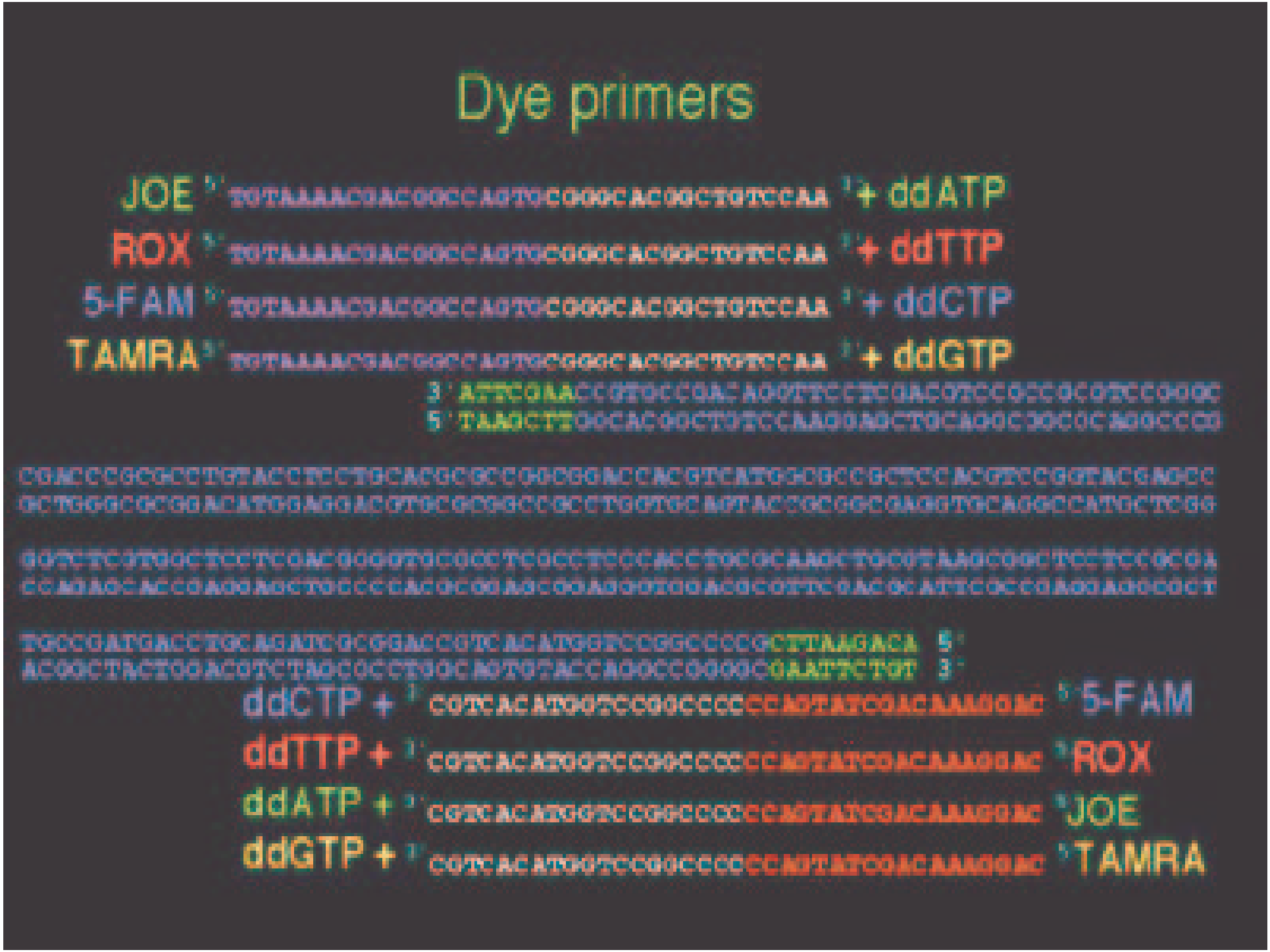


Exemple 2 (V.Croquette et al)




84000
Moloney murine leukemia virus
2.7.7.49
Reverse transcriptase
Mg++
Amorce
pGpApGpCpTpG
mRNA (modèle)
pCpUpCpGpApCpApUpGpGpGpGpGpApCpGpGpApGpApGpUpGpGpGpGpUpA
dGTP
dTTP
dATP
dCTP
5'→3'
reverse
transcriptase
3'→5'
exonuclease
PP
pGpApGpCpTpGpTpApC
pCpUpCpGpApCpApUpGpGpGpGpGpApCpGpGpApGpApGpUpGpGpGpGpUpA

Dye primers


JOE
ROX
5-FAM
TAMRA
+ ddATP
+ ddTTP
+ ddCTP
+ ddGTP
ddCTP +
ddTTP +
ddATP +
ddGTP +
5-FAM
ROX
JOE
TAMRA