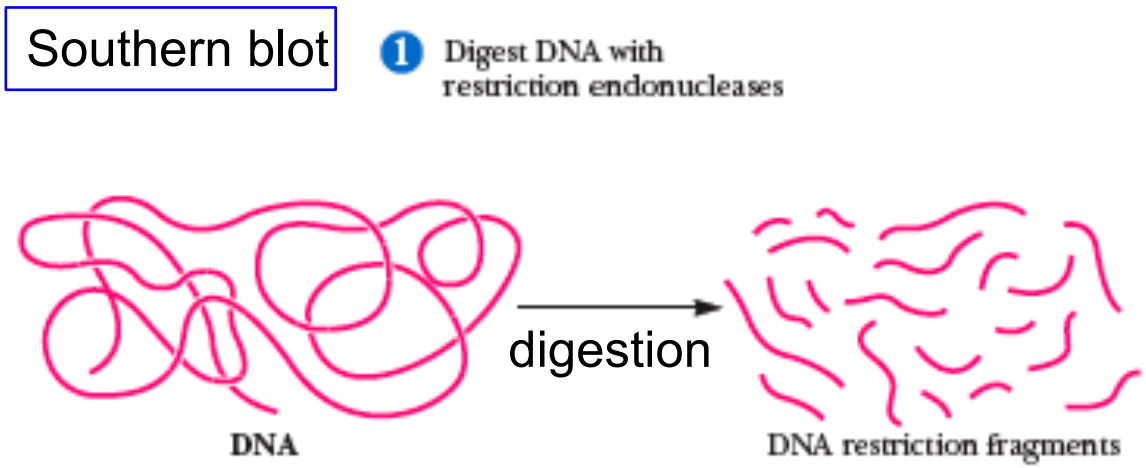
Southern blot



ADN génomique

$3 \cdot 10^9$ pb

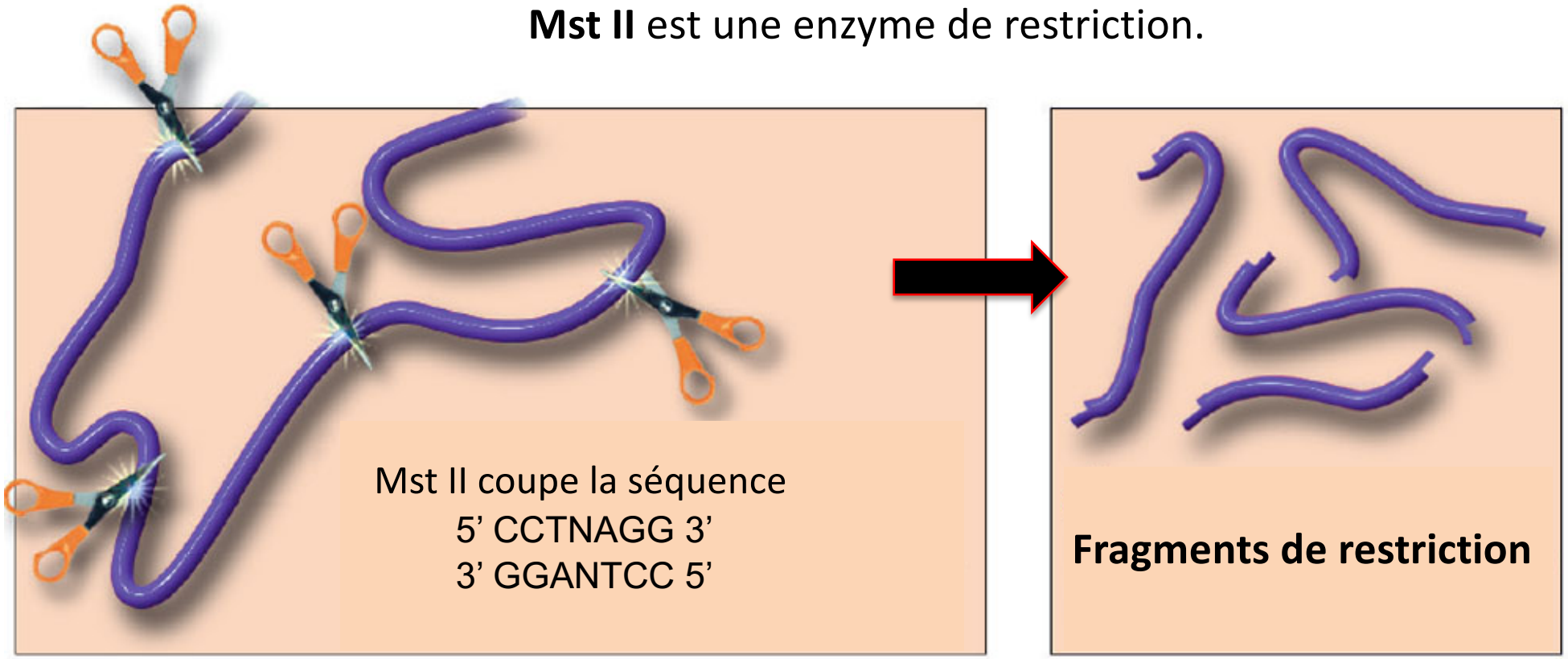
~1 million de fragments de toutes les tailles

Digestion de l'ADN génomique avec une enzyme de restriction.

(p.ex. Mst II)

G & G

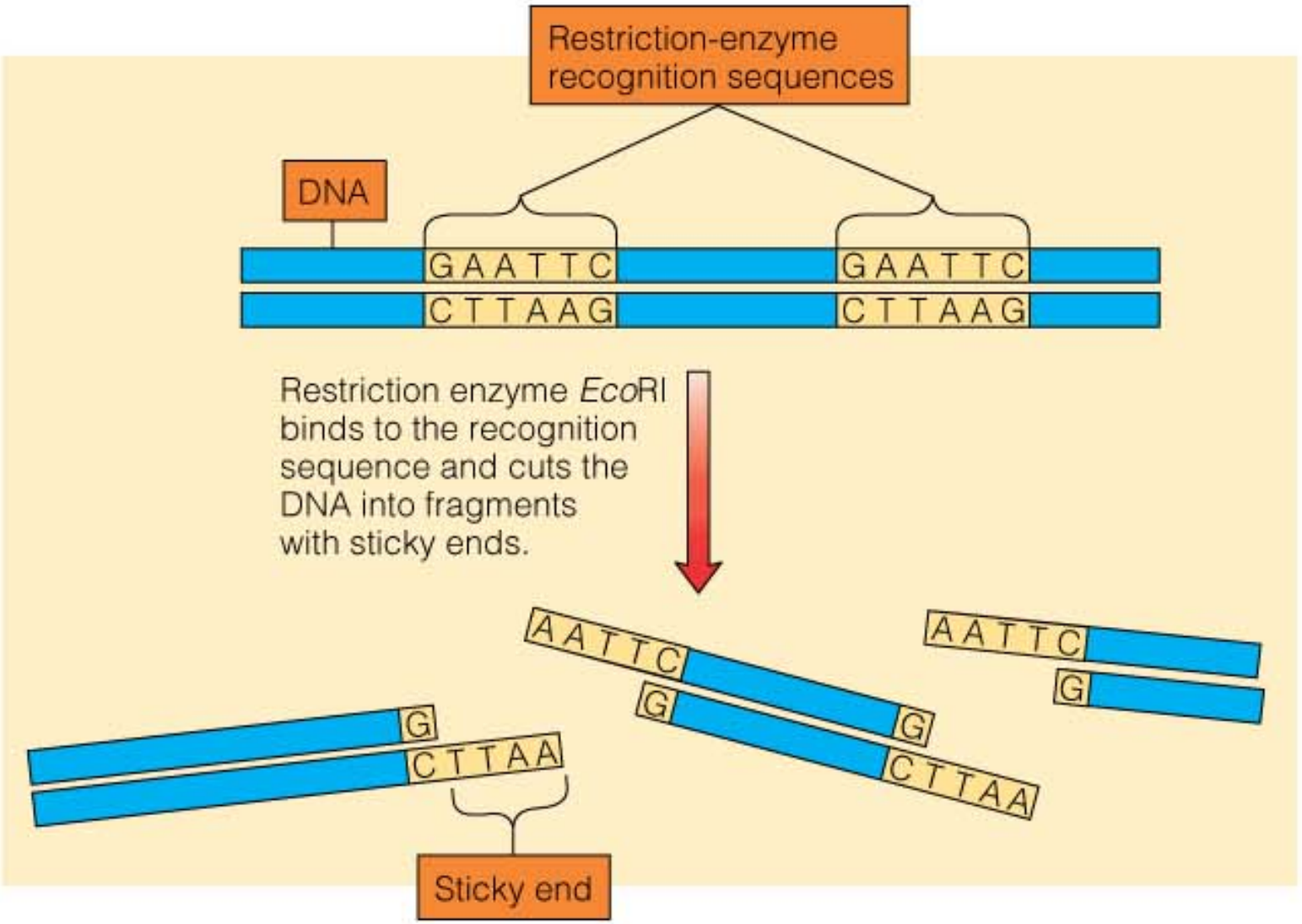
Mst II est une enzyme de restriction.



Notez les bouts collants

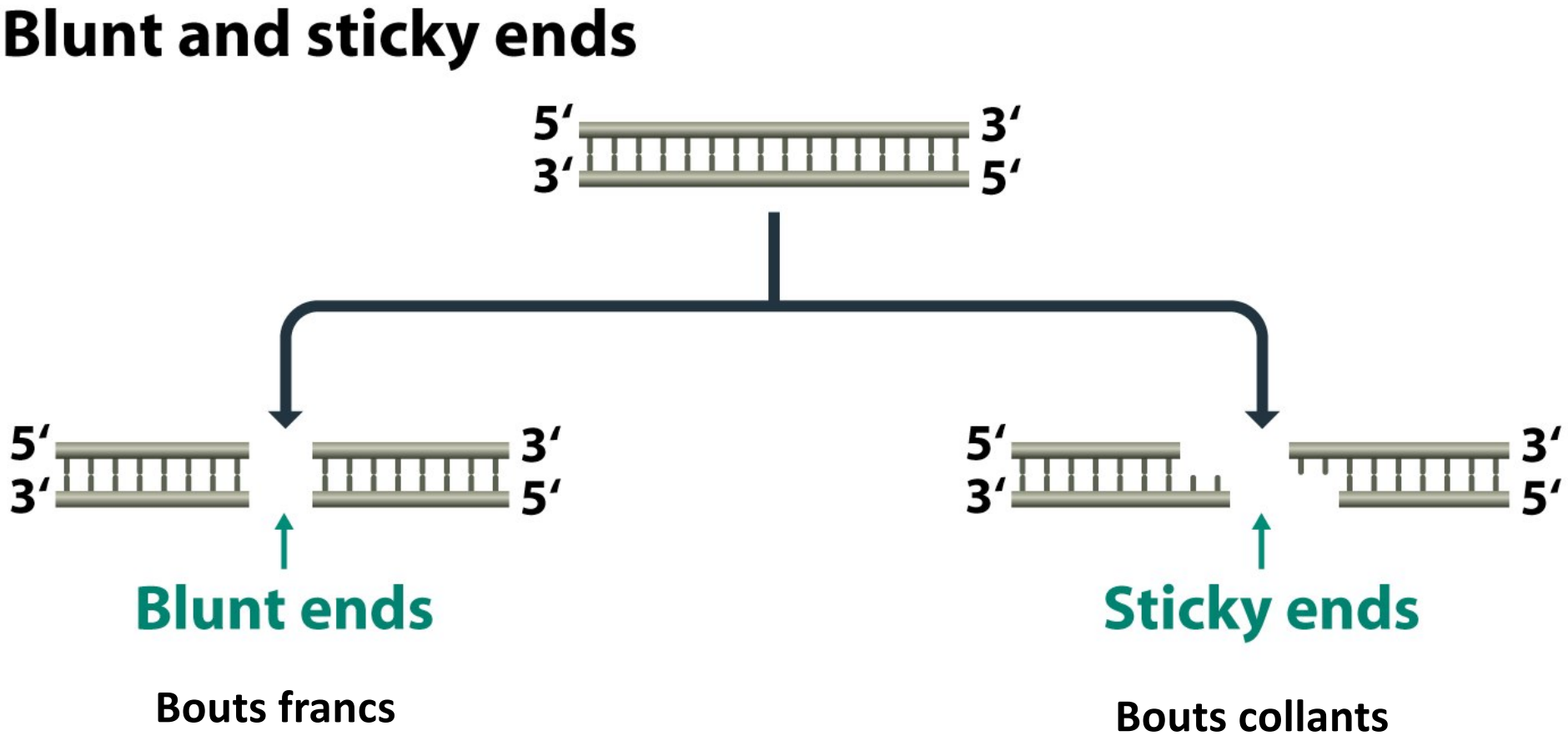
Digestion de l’ADN d’une cellule
(ADN génomique)
par une **enzyme de restriction**.

Les enzymes de restriction reconnaissent des ***séquence spécifiques*** : sites de restriction.



Bouts collants

Enzymes de restriction :

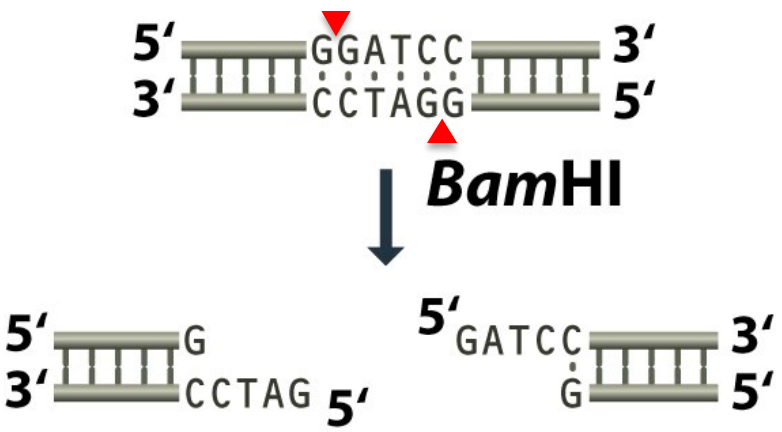


Bouts francs

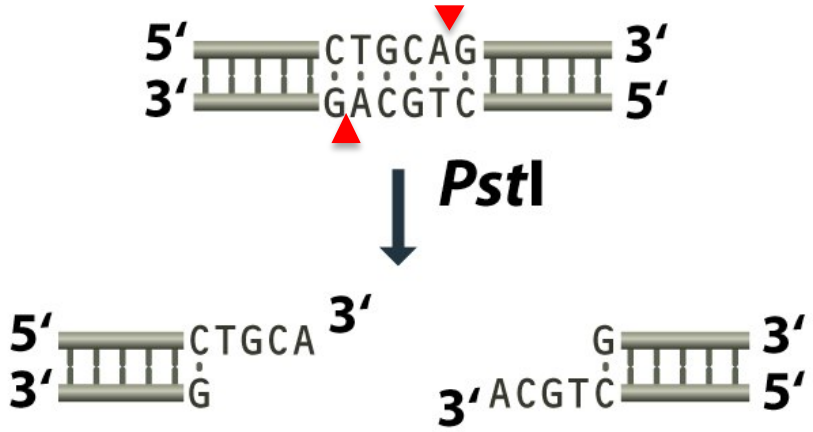
Bouts collants

Enzymes de restriction :

Deux sortes de bouts collants :

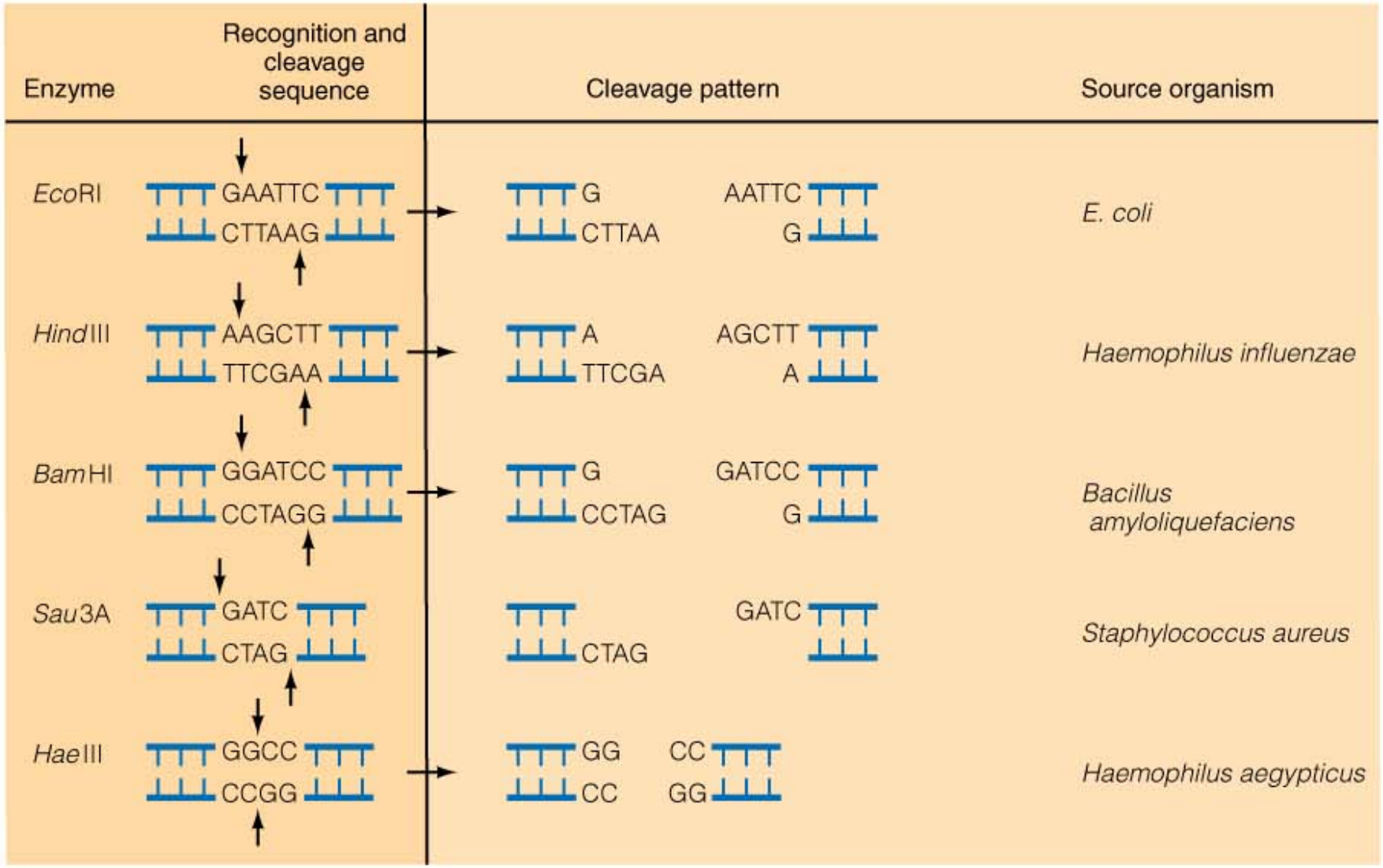


5' overhang



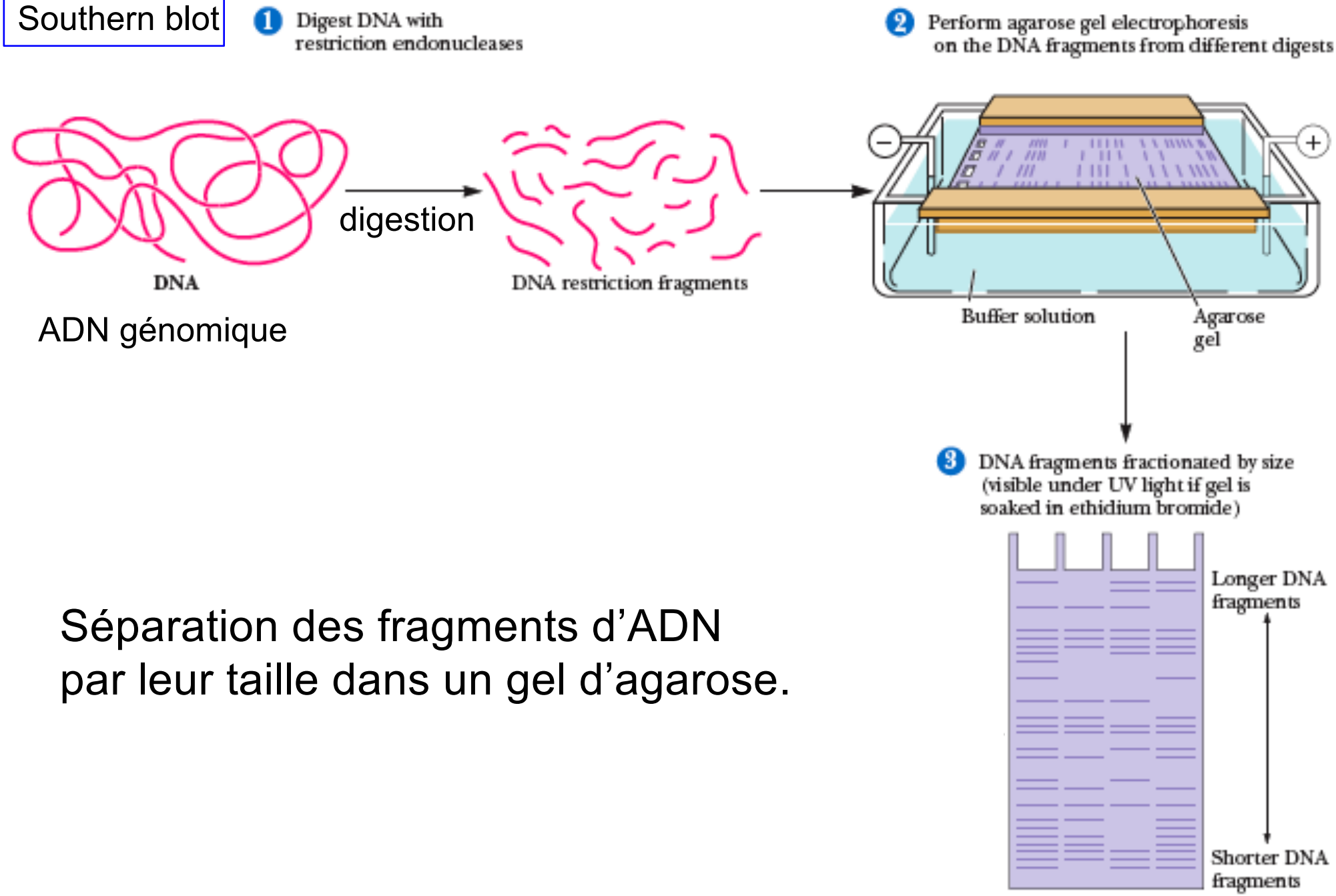
3' overhang

Exemples d'enzymes de restriction :



bouts francs (blunt ends)

Southern blot



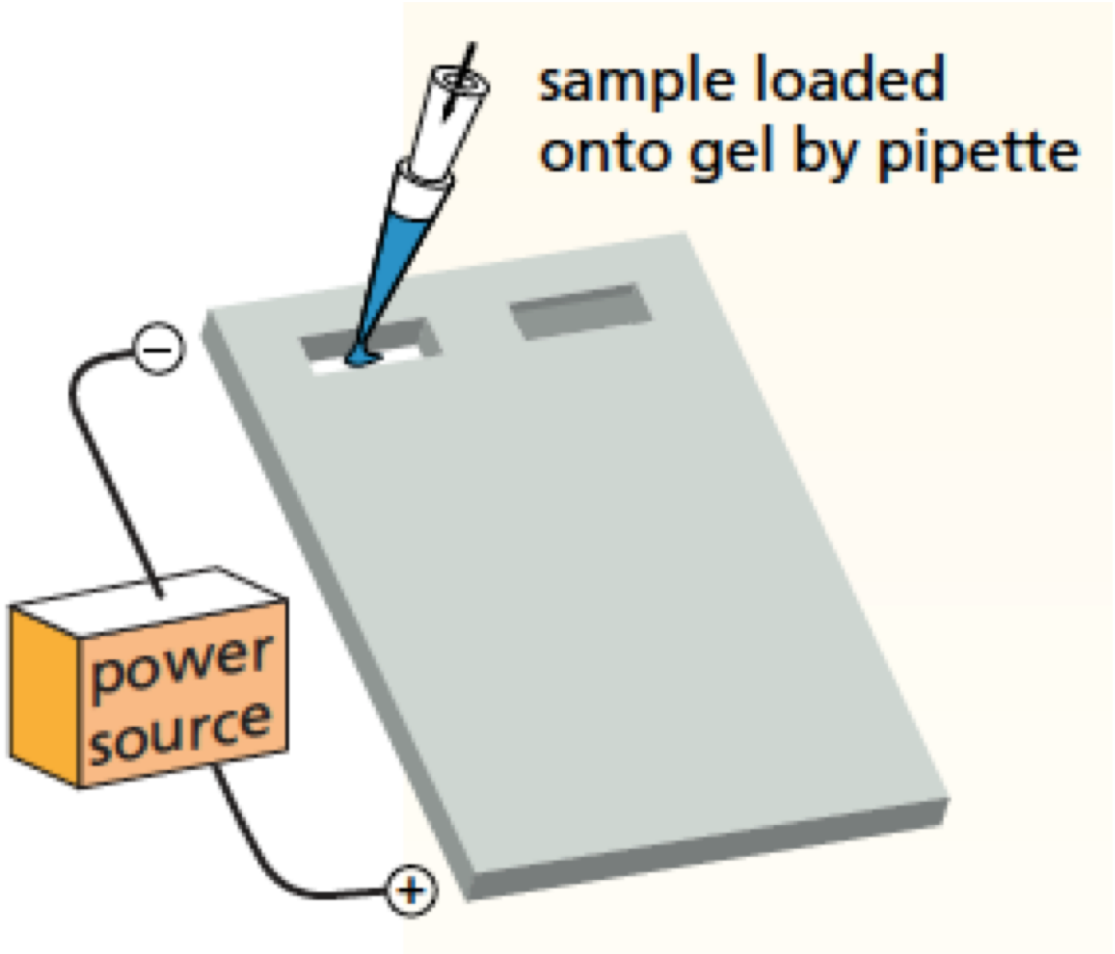
ADN génomique

Séparation des fragments d'ADN par leur taille dans un gel d'agarose.

G & G

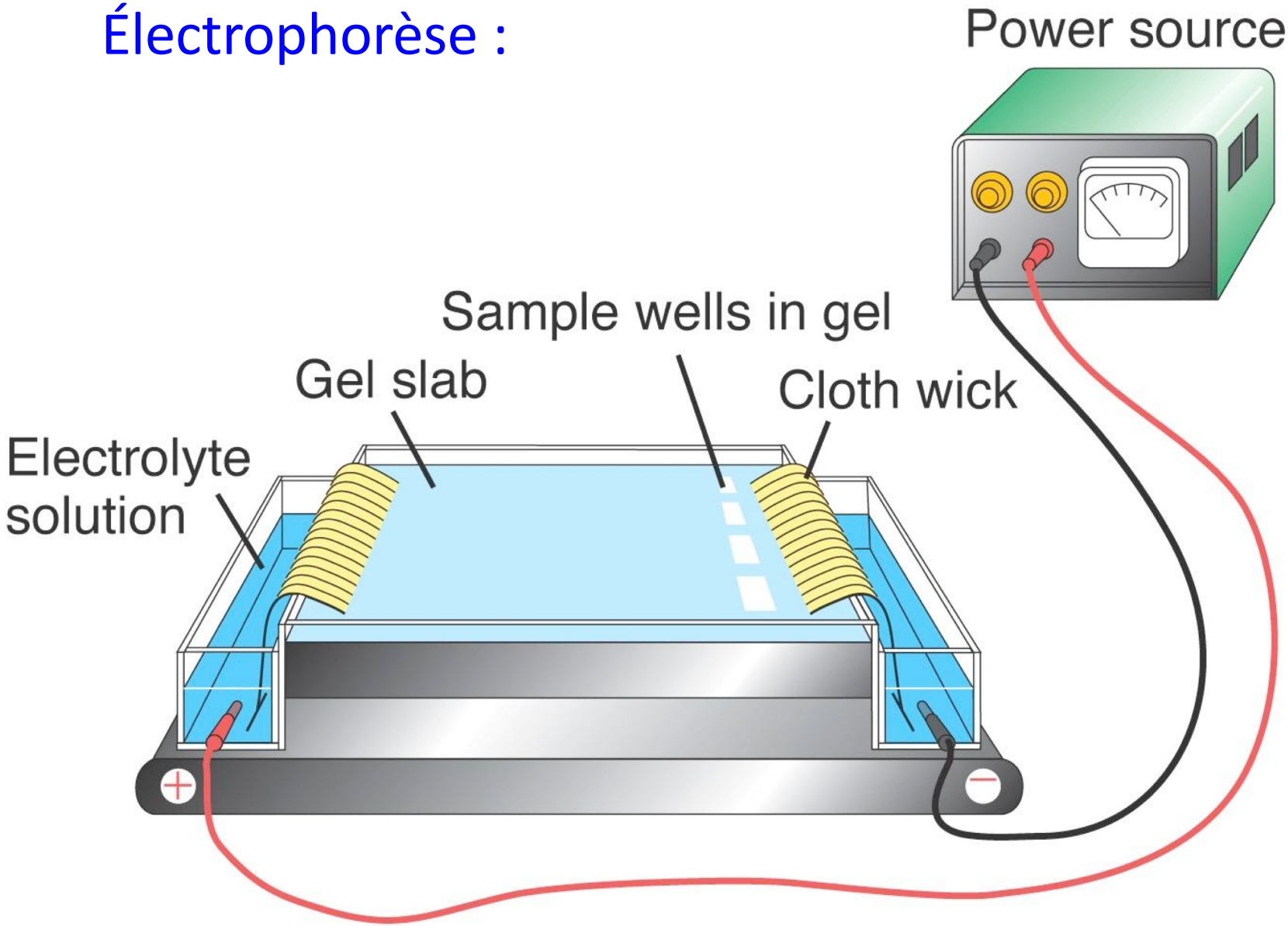
Électrophorèse :

1. Déposition de l’échantillon dans un puits

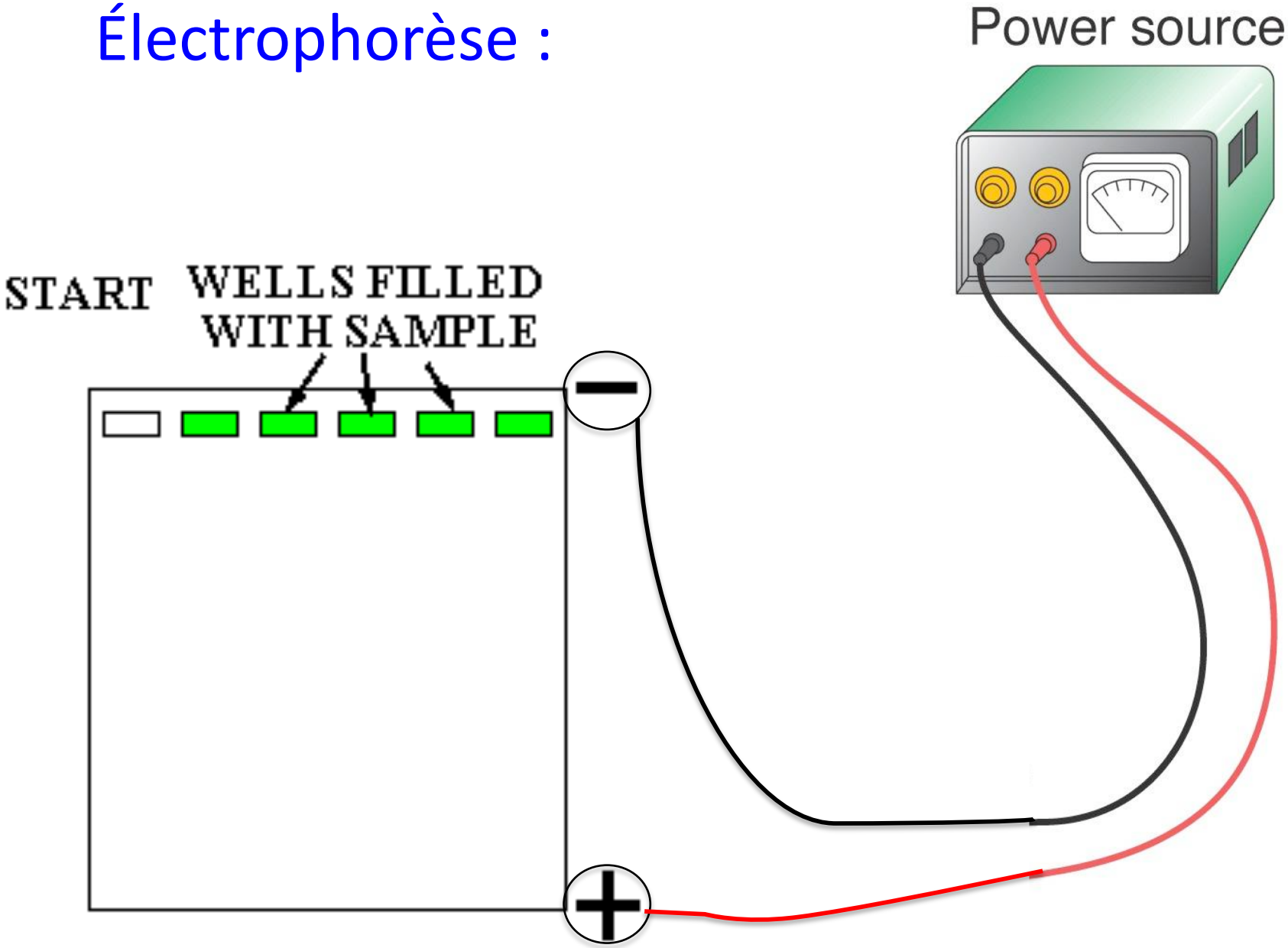


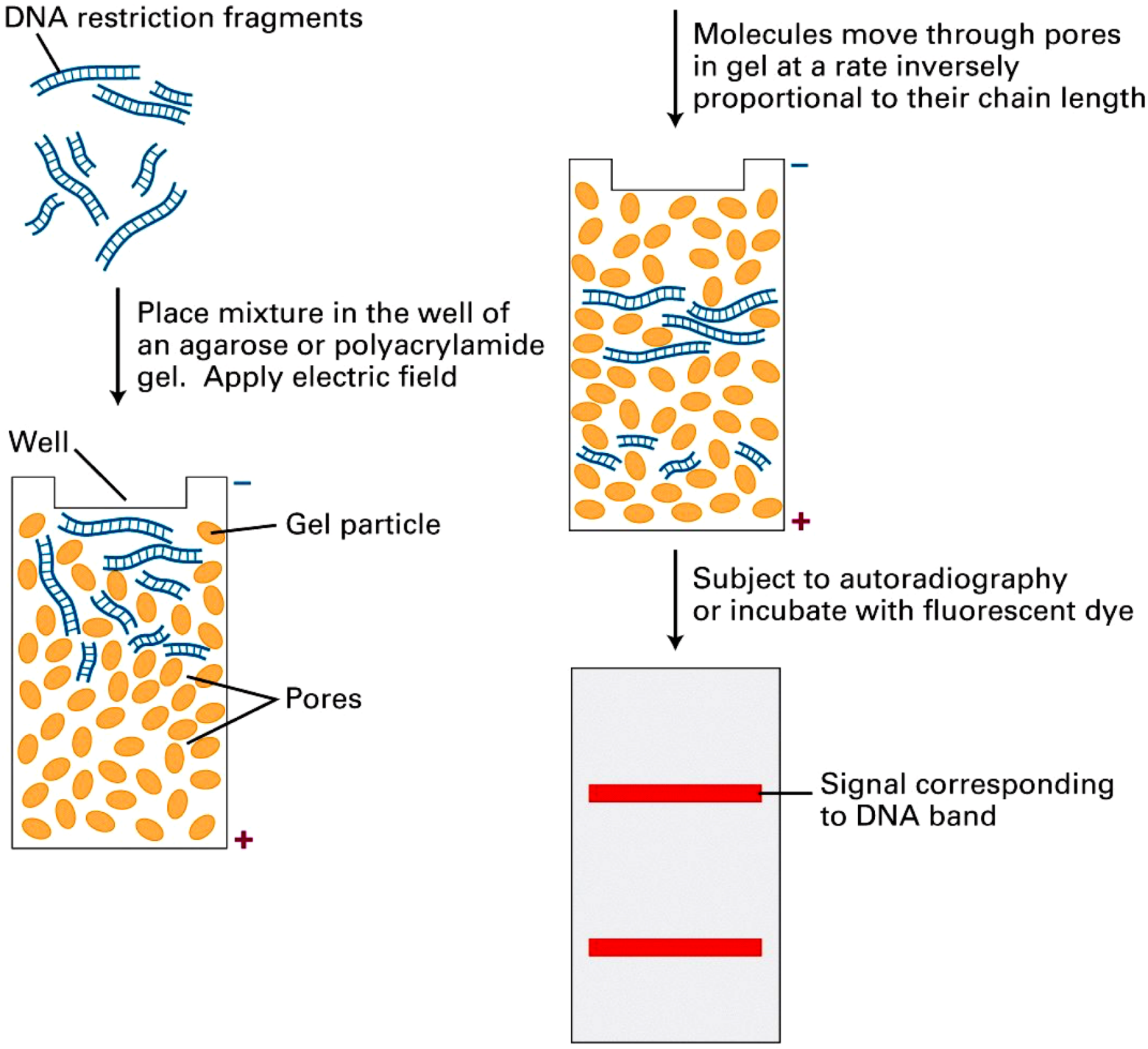
Les fragments d’ADN sont mélangés à un colorant bleu (pour voir l’échantillon) et du glycérol (pour alourdir l’échantillon).

Électrophorèse :

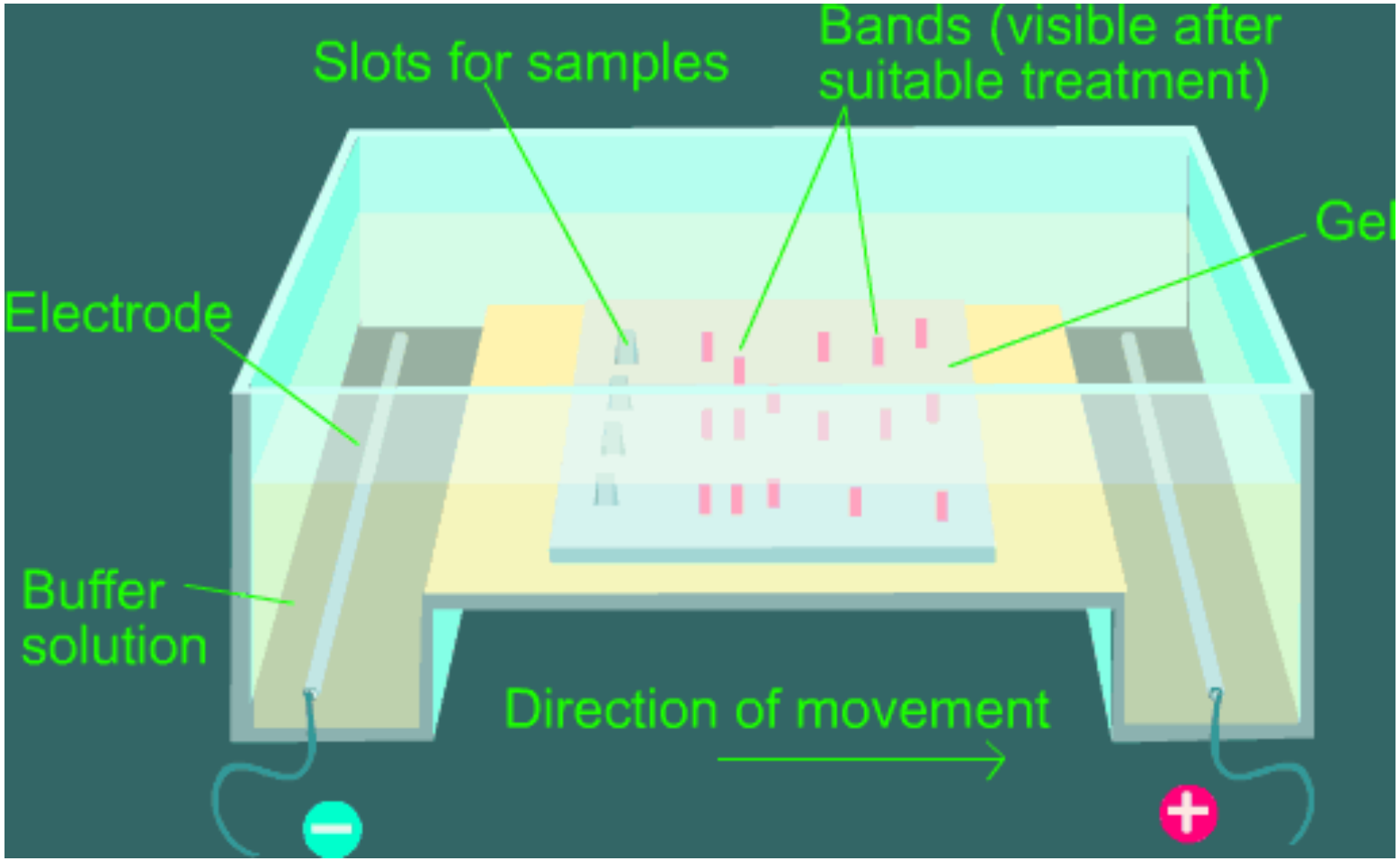


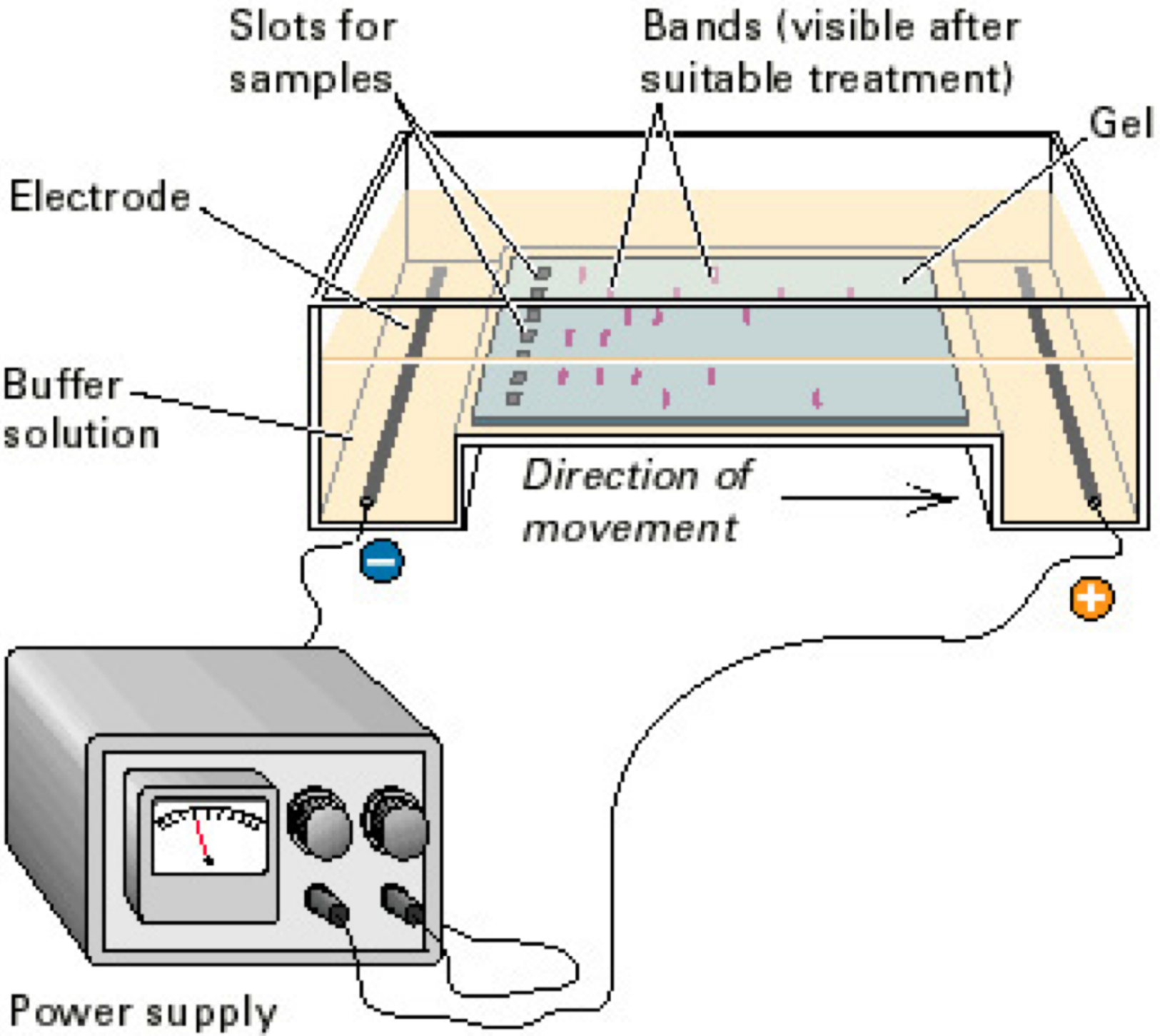
Électrophorèse :




DNA restriction fragments
Place mixture in the well of an agarose or polyacrylamide gel. Apply electric field
Well
Gel particle
Pores
Molecules move through pores in gel at a rate inversely proportional to their chain length
Subject to autoradiography or incubate with fluorescent dye
Signal corresponding to DNA band

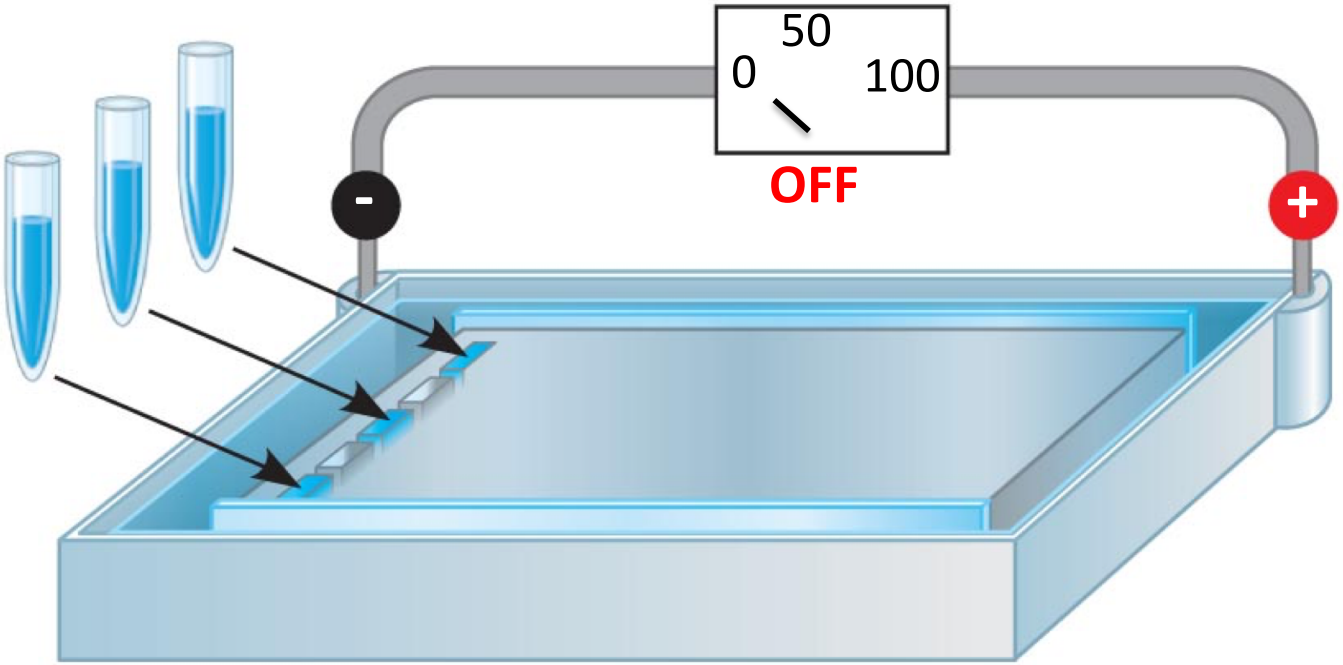
Électrophorèse de fragments d'ADN dans un gel d'agarose.



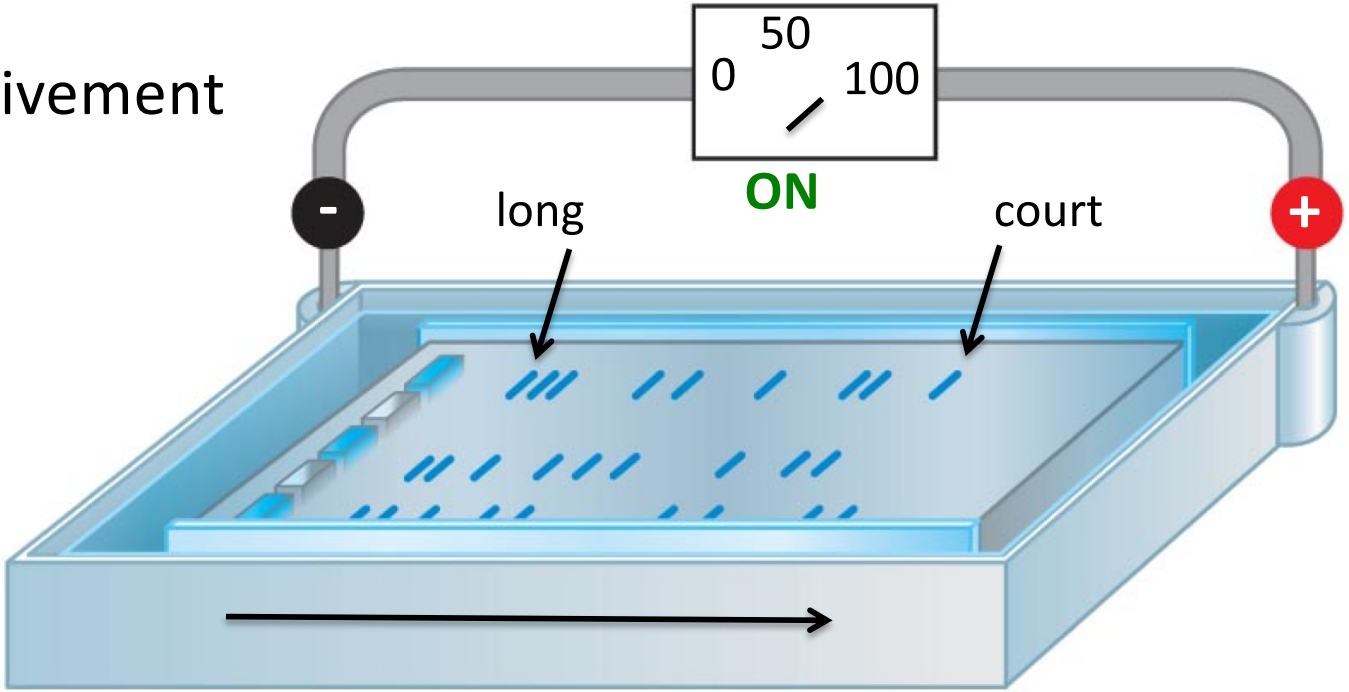

Slots for samples
Bands (visible after suitable treatment)
Gel
Electrode
Buffer solution
Direction of movement
Power supply

Déposition des échantillons :

fragments d'ADN
+ colorant bleu
+ glycérol



L'ADN chargé négativement migre vers l'anode.



Southern blot

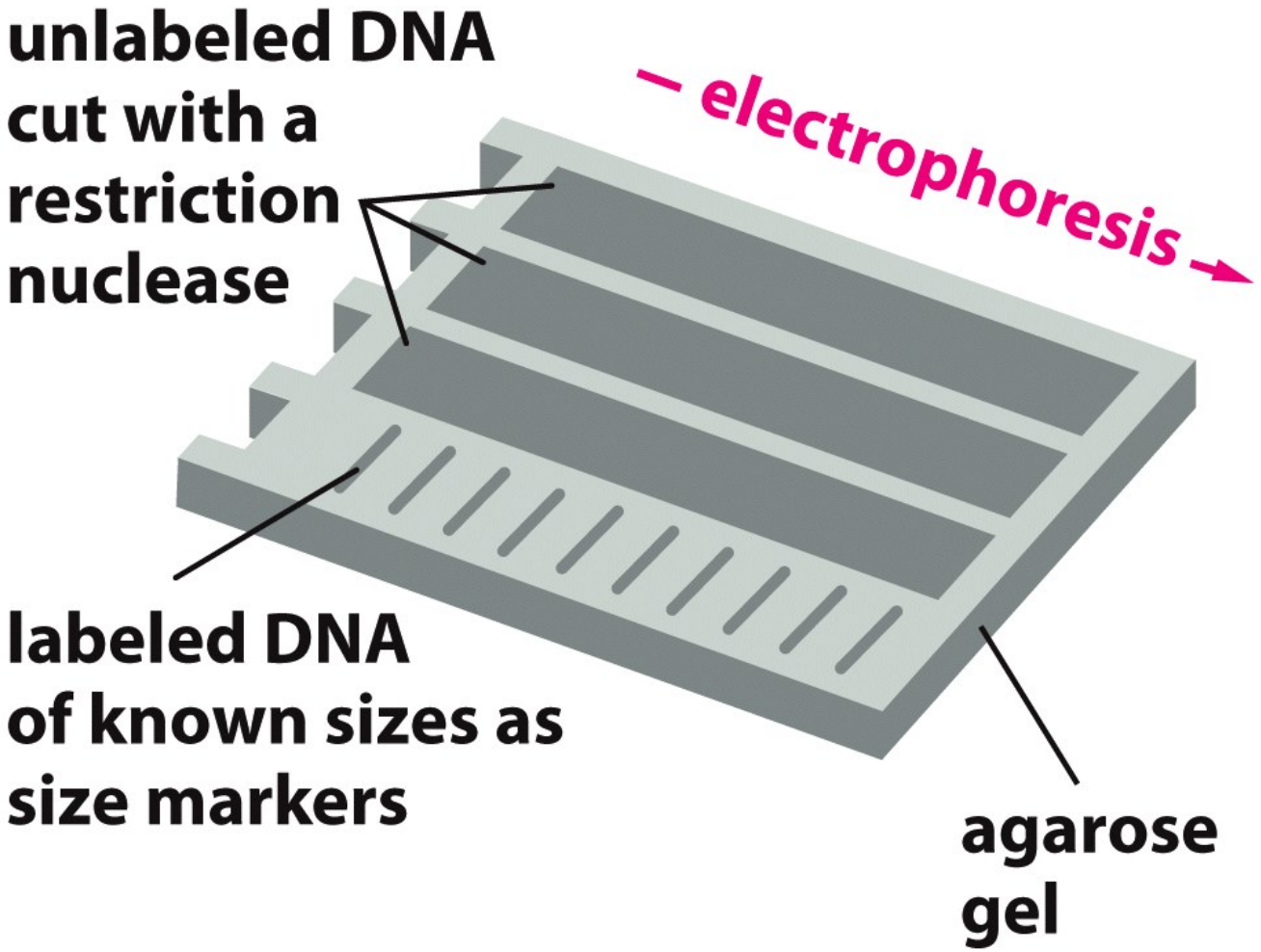


Figure 10-5 (part 1 of 5) *Essential Cell Biology* (© Garland Science 2010)

Southern blot

transfert

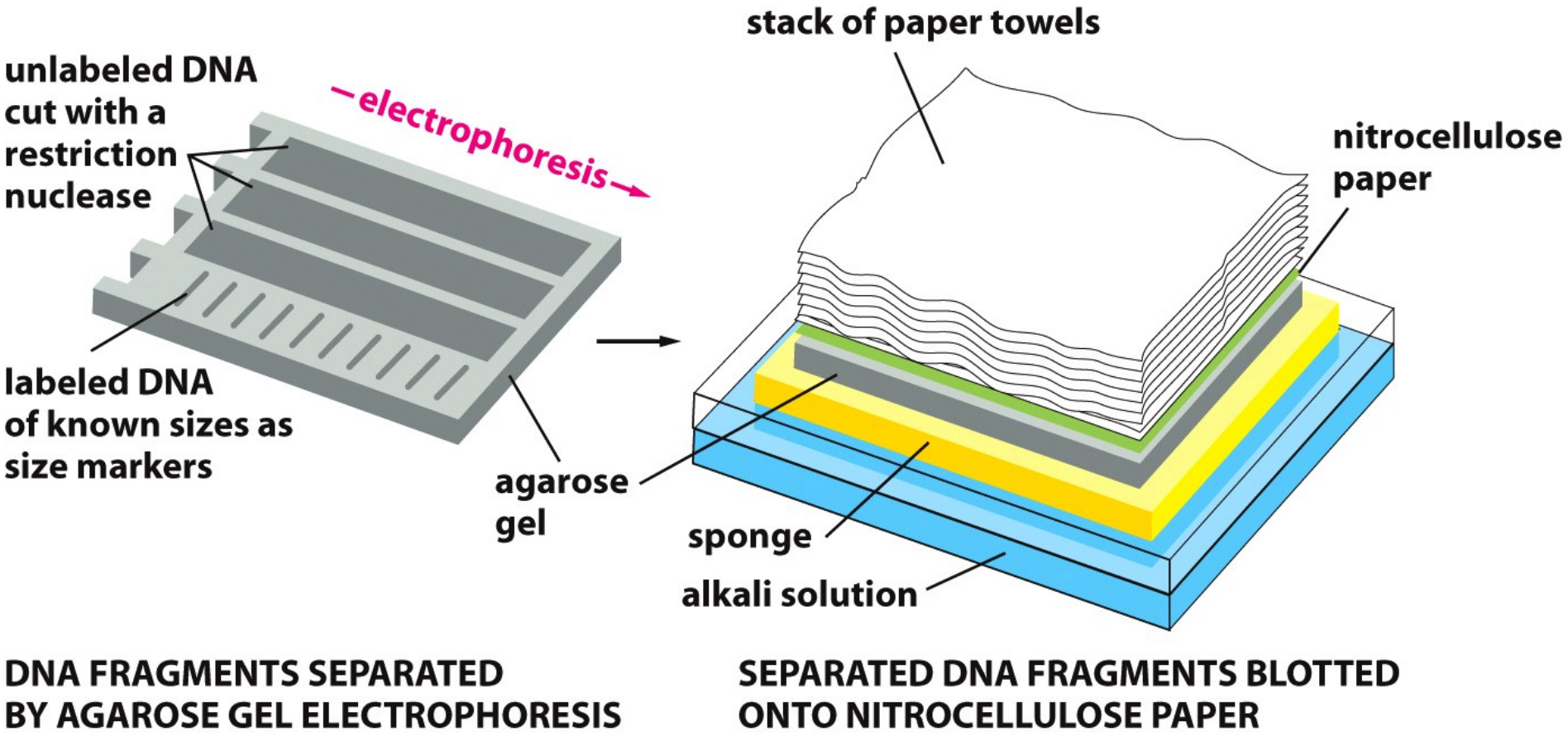
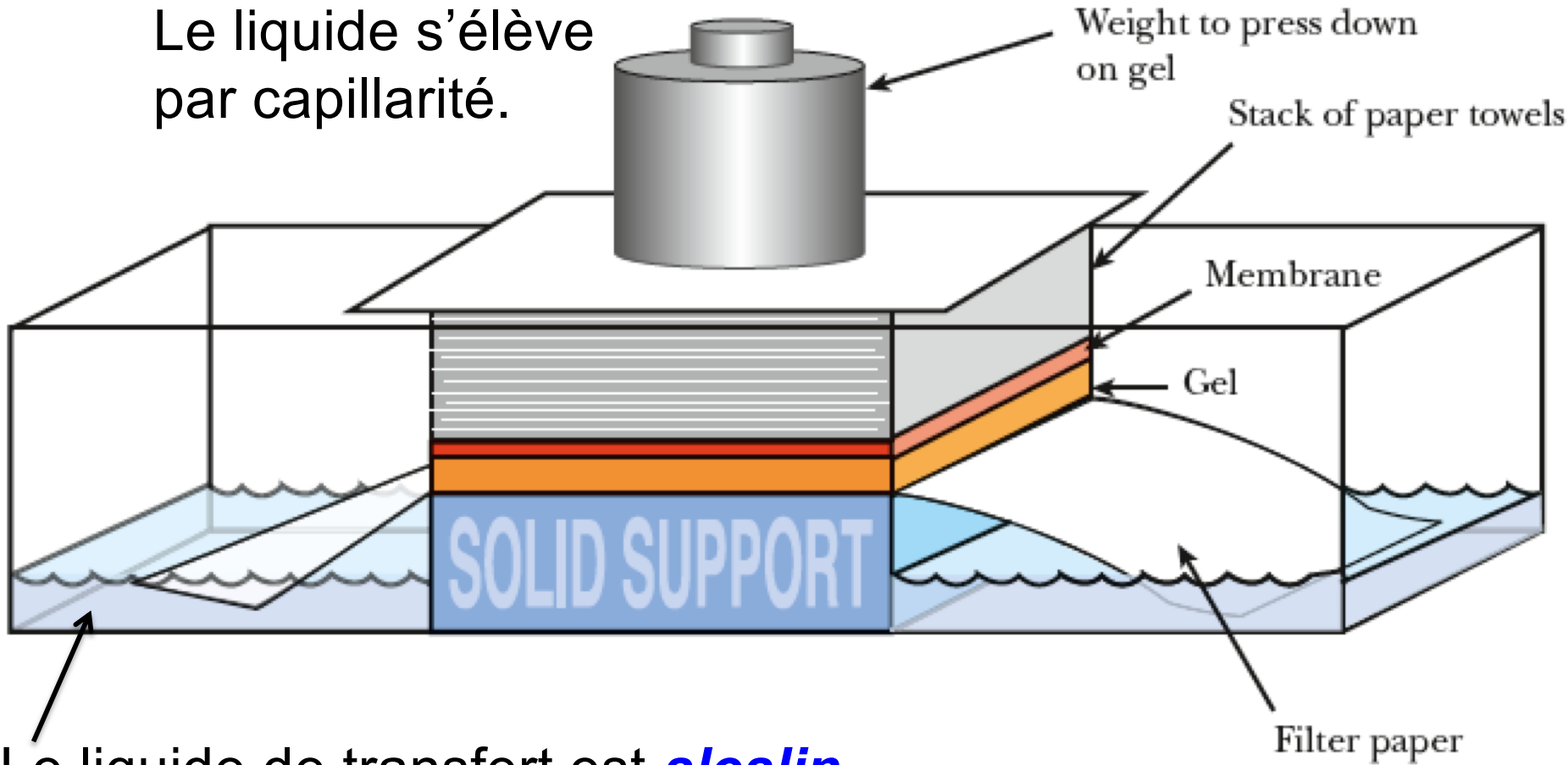


Figure 10-5 (part 2 of 5) *Essential Cell Biology* (© Garland Science 2010)

Le **transfert** de l'ADN d'un gel sur une membrane.

Le liquide s'élève par capillarité.



Le liquide de transfert est ***alcalin*** pour dénaturer l'ADN.

Dénaturation alcaline

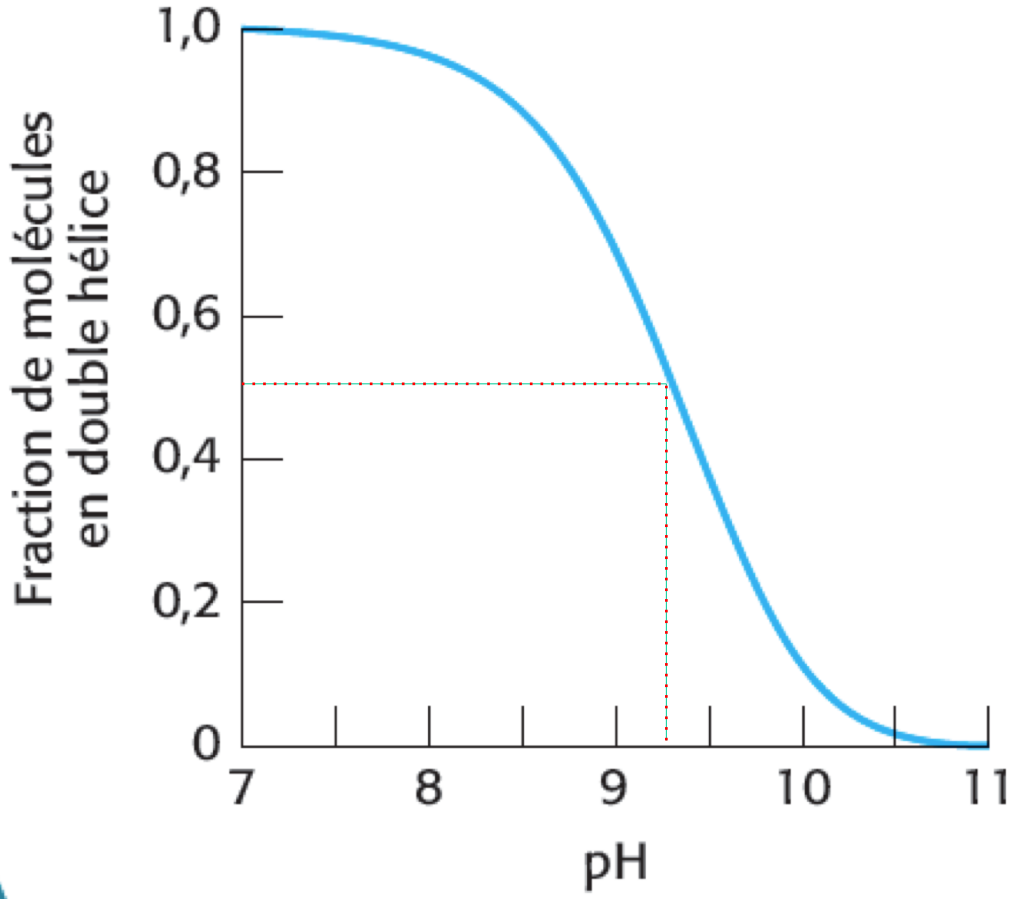


Figure 1.16 Dénaturation du DNA par l'addition d'une base. L'addition d'une base à une solution de DNA en double hélice initialement à pH 7 provoque la séparation de la double hélice en simples brins. Le processus est à moitié complet un peu au-dessus de pH 9.

Stryer ch 1

Southern blot

À la fin du transfert :

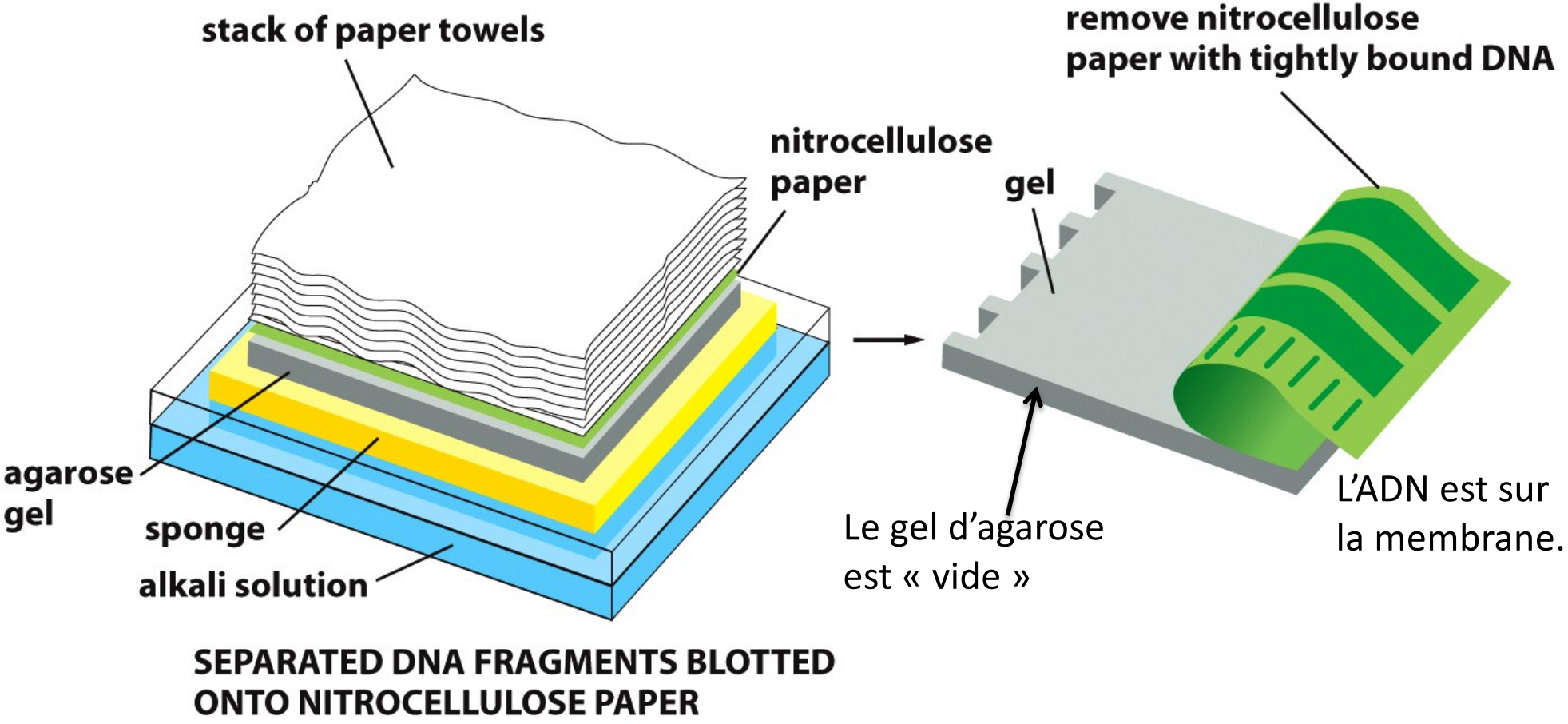
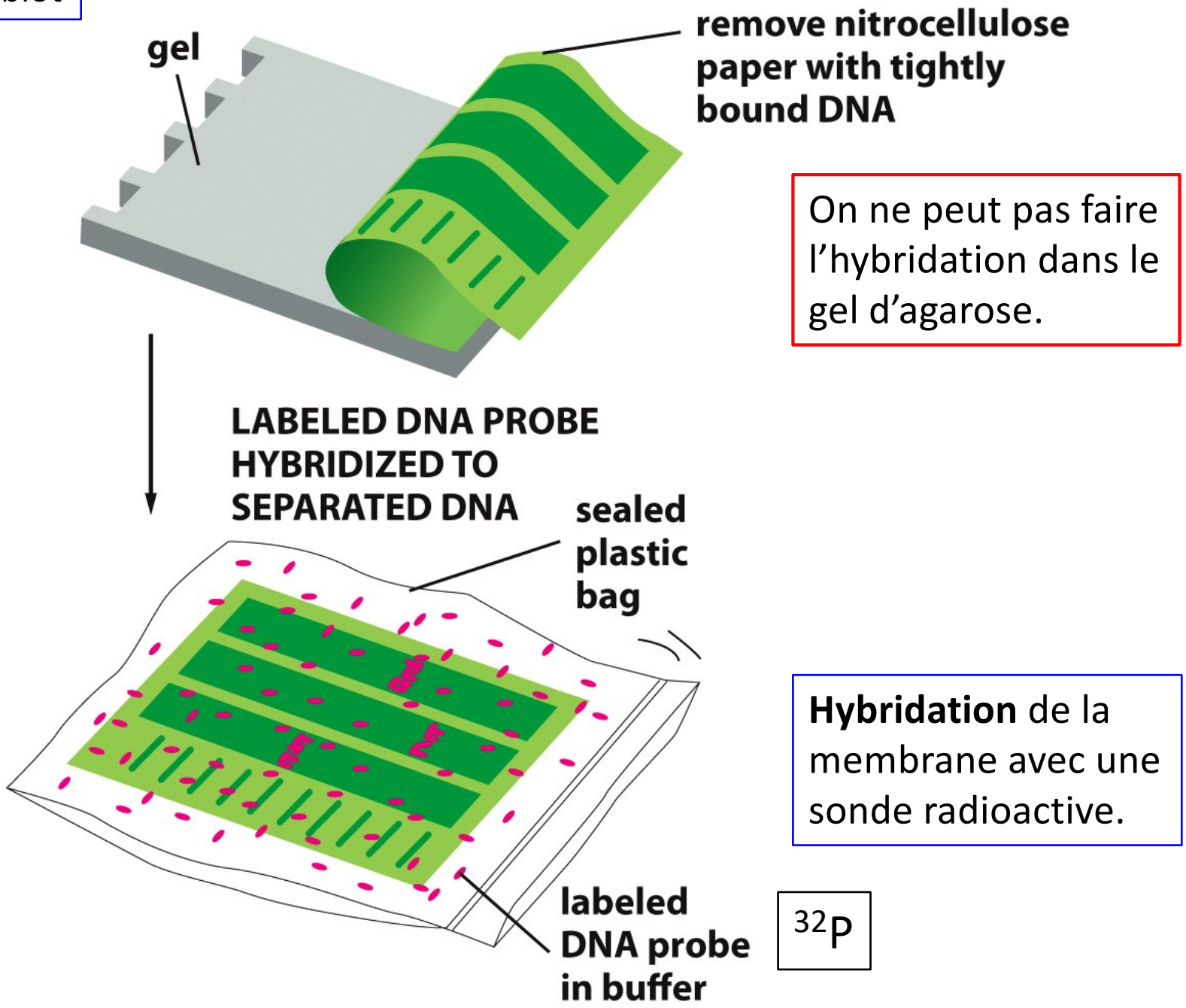


Figure 10-5 (part 3 of 5) *Essential Cell Biology* (© Garland Science 2010)

Southern blot



On ne peut pas faire l'hybridation dans le gel d'agarose.

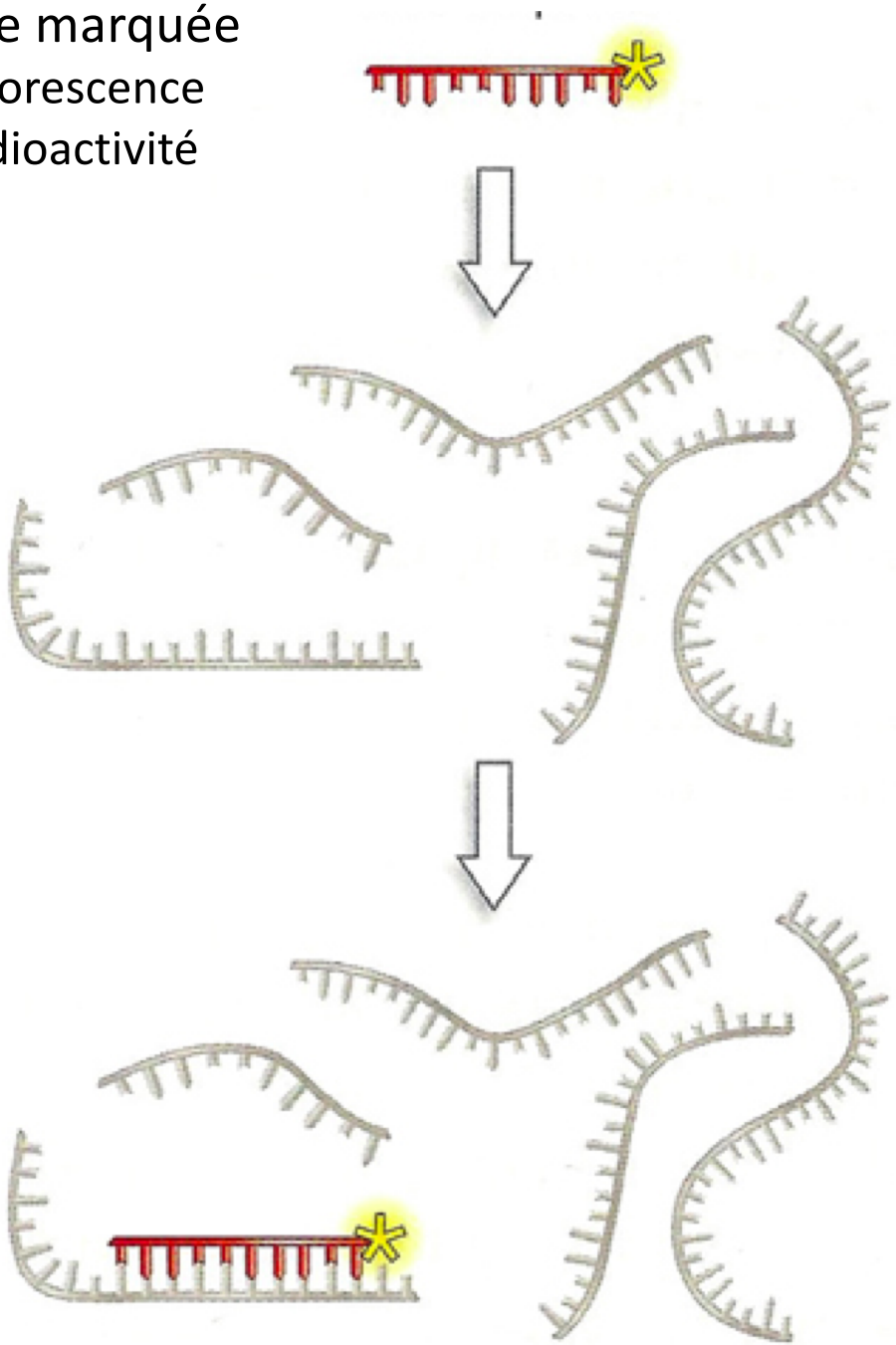
Hybridation de la membrane avec une sonde radioactive.

^{32}P

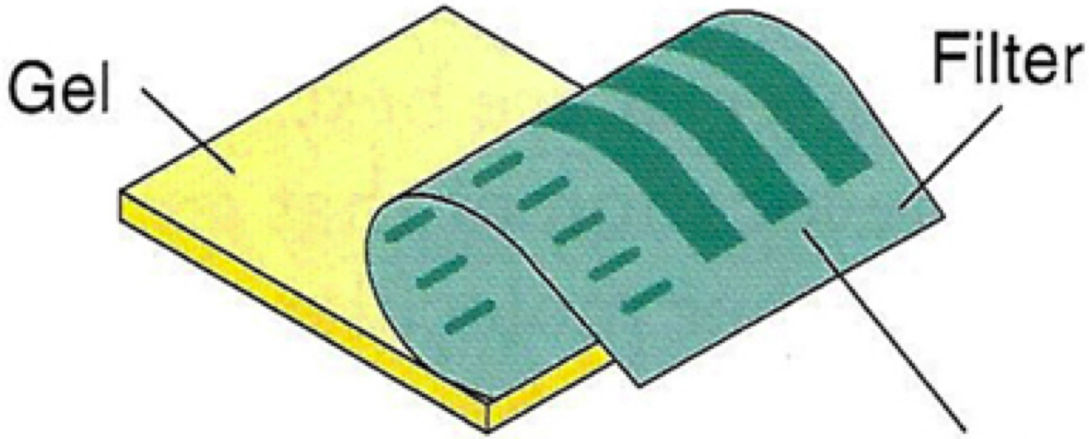
Figure 10-5 (part 4 of 5) *Essential Cell Biology* (© Garland Science 2010)

Sonde marquée

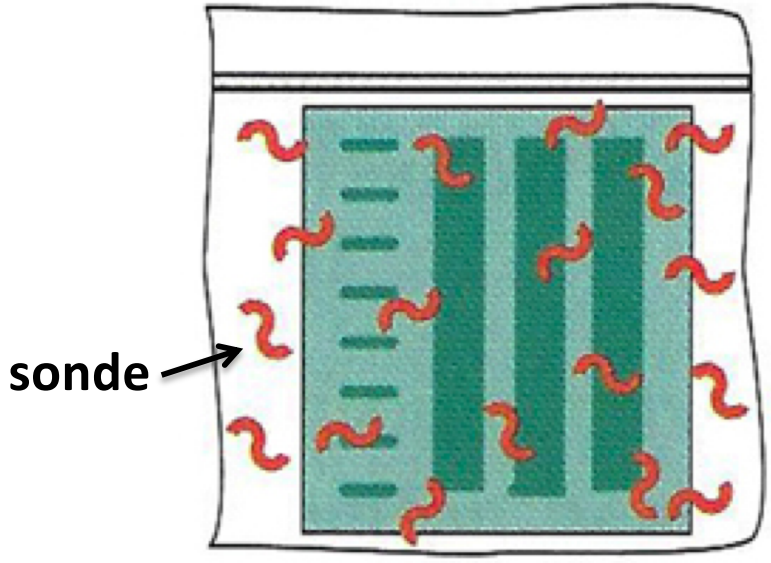
- fluorescence
- radioactivité

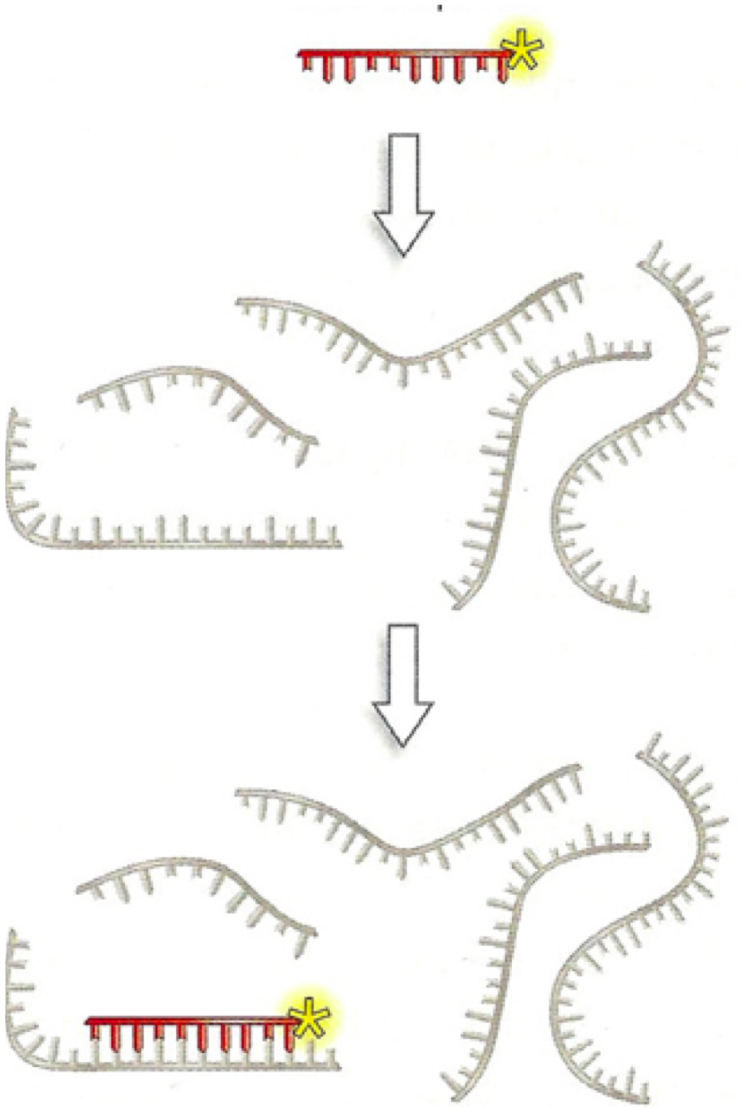


Fragments d'ADN dénaturés

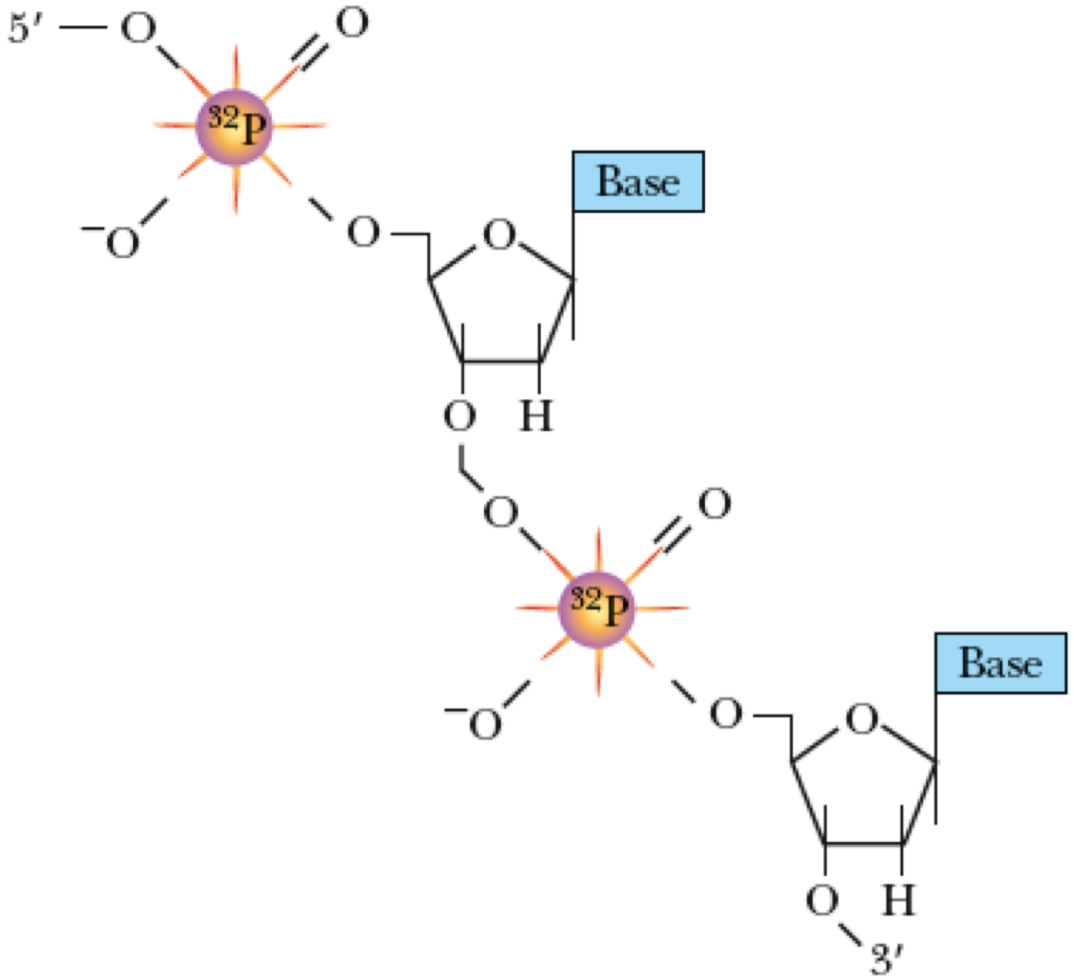


Hybridation



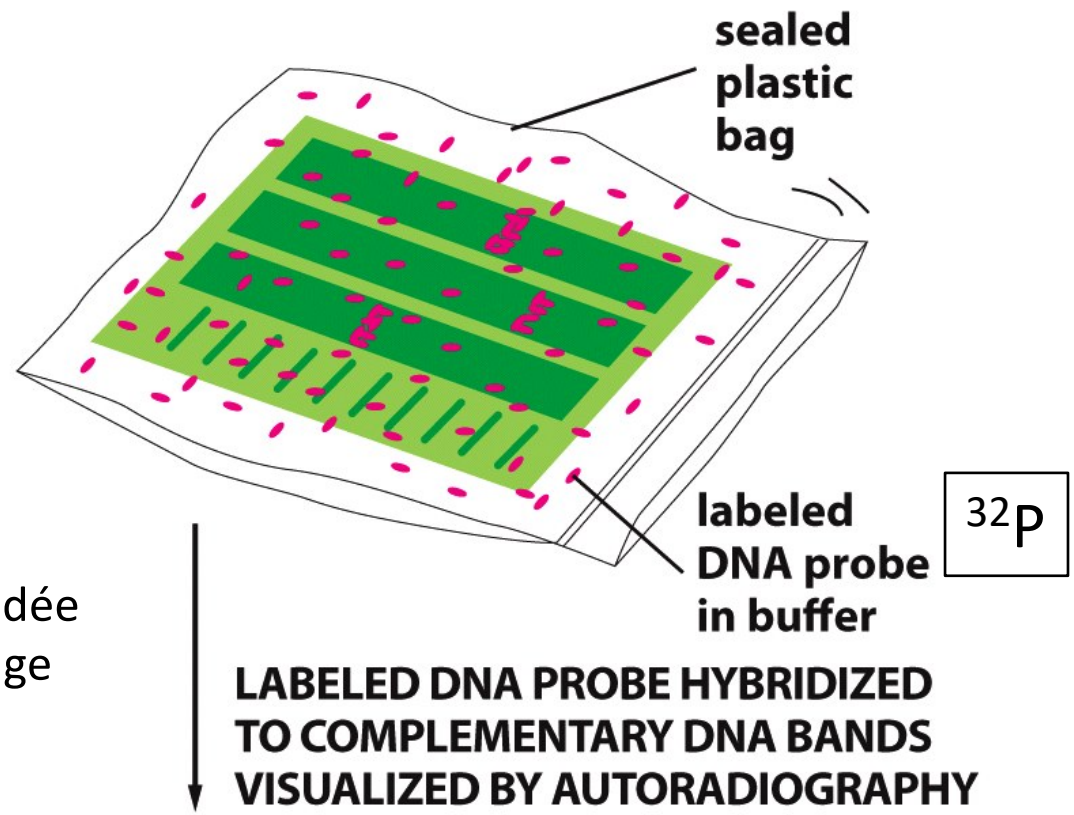


Une **sonde** : ADN simple brin radioactif.



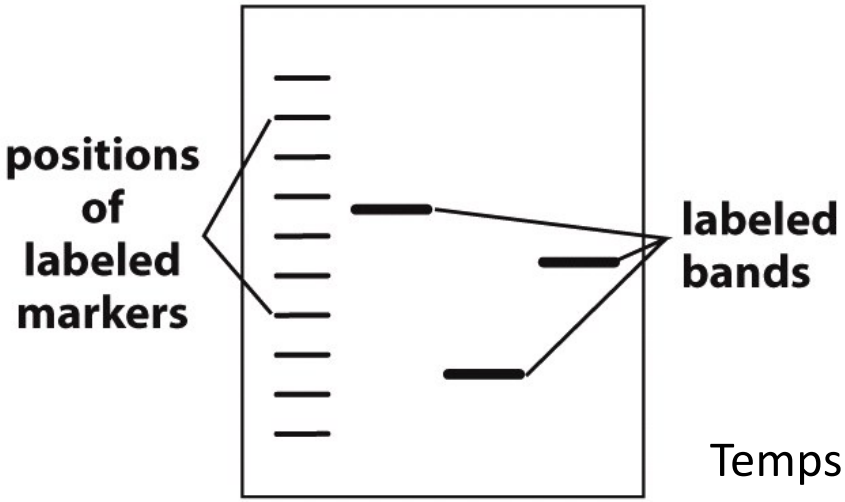
32P-LABELED DNA

Southern blot



Lavage :
la sonde hybridée
résiste au lavage

Autoradiographie



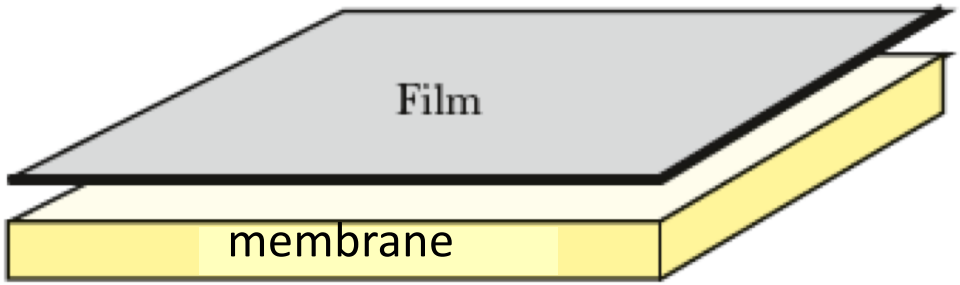
La **sonde** émet
de la radioactivité
qui noircit le film.

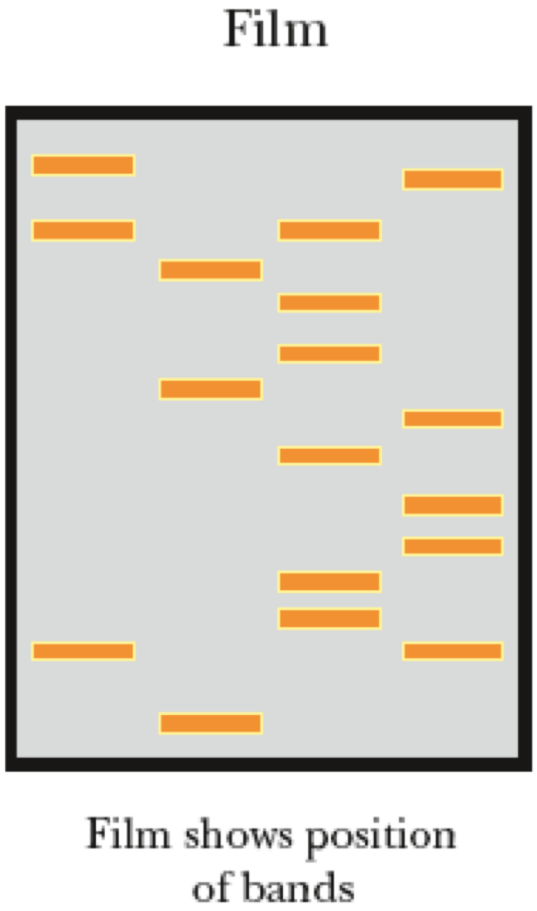
Temps d'exposition : ~24 heures

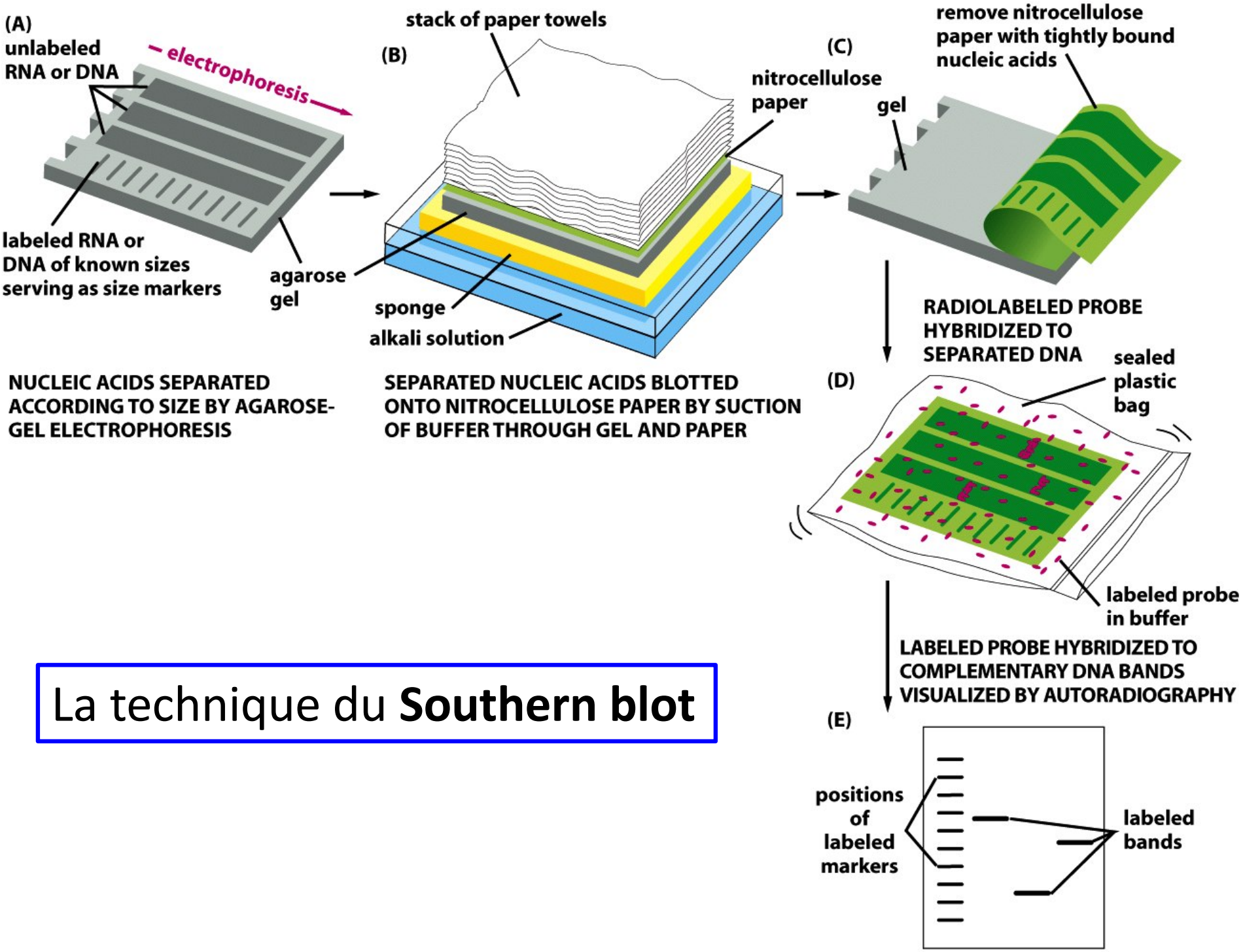
Figure 10-5 (part 5 of 5) *Essential Cell Biology* (© Garland Science 2010)

Autoradiographie

Un film est exposé sur la membrane dans l'obscurité.

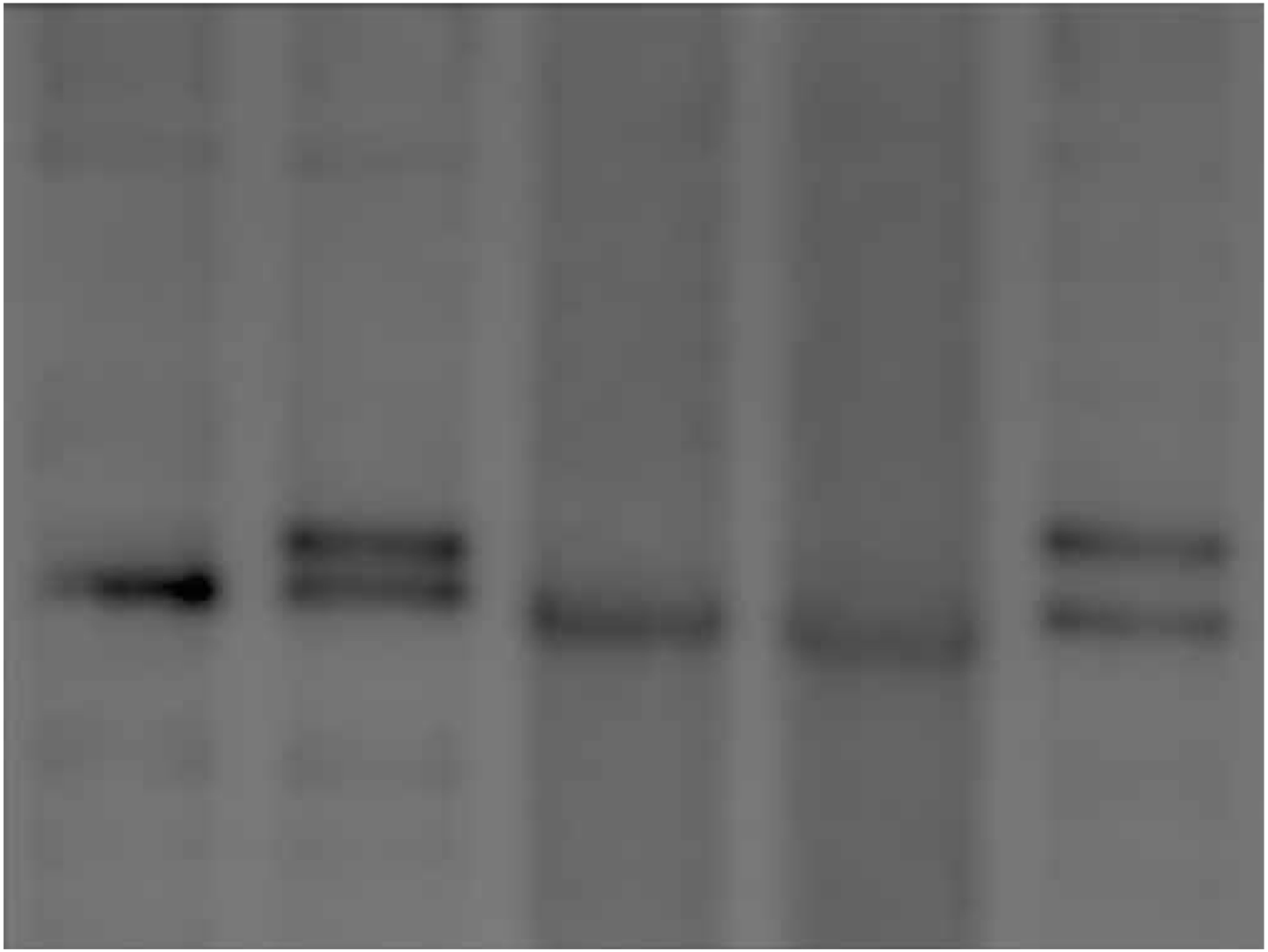


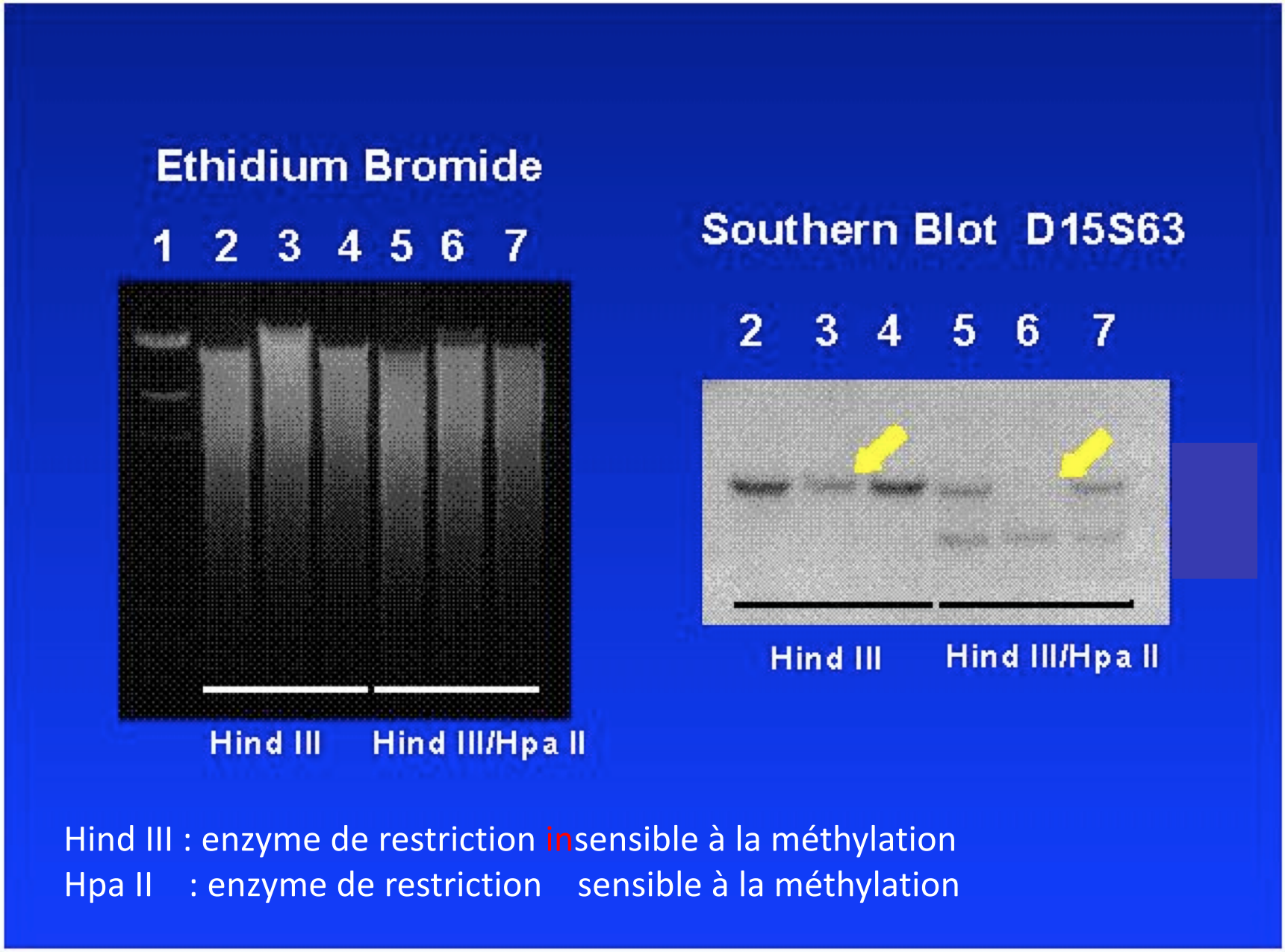




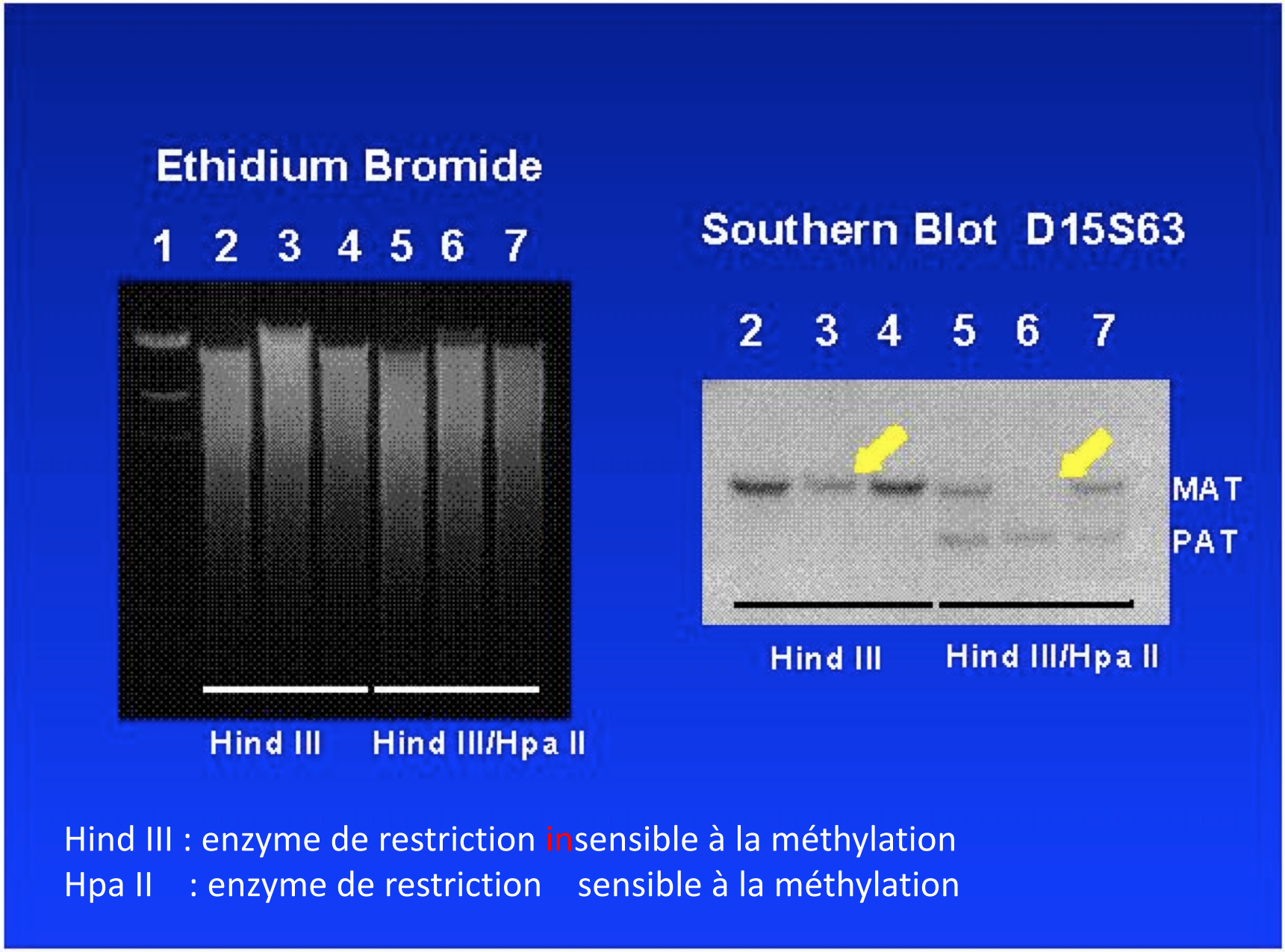
La technique du **Southern blot**

Un exemple de Southern blot :



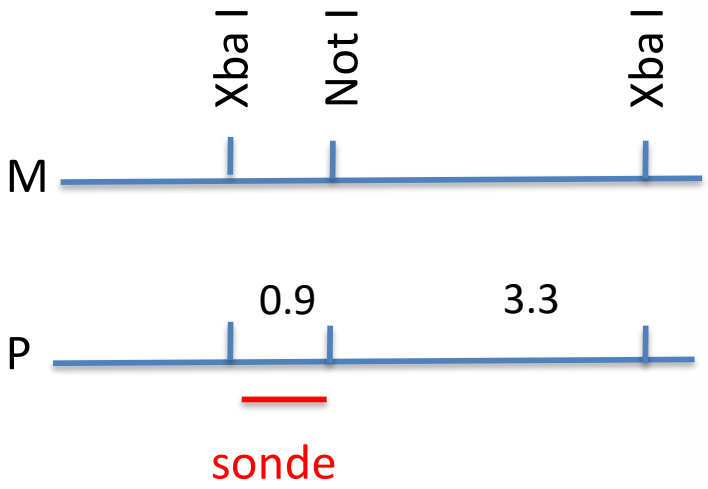


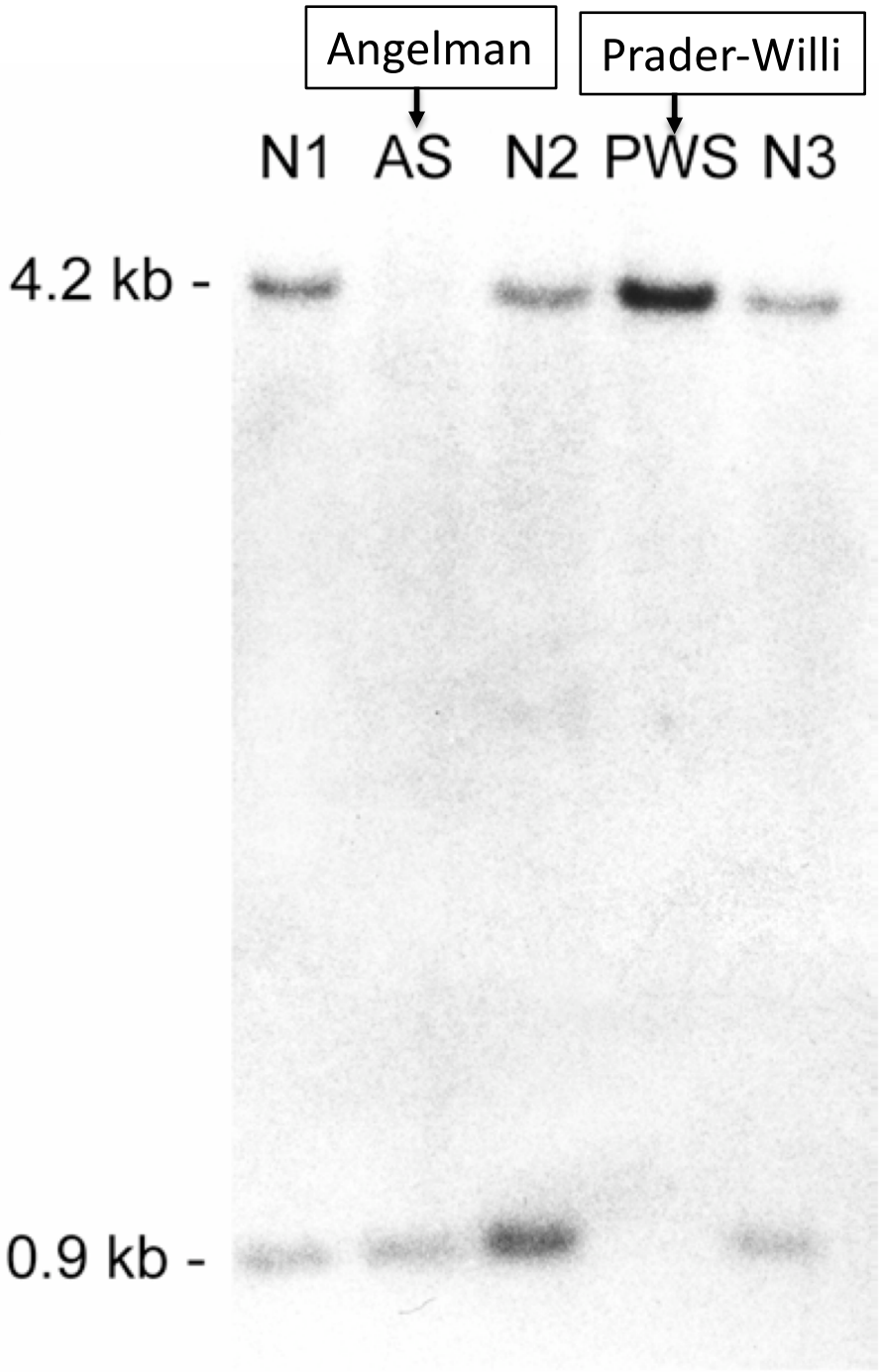
Hind III : enzyme de restriction insensible à la méthylation
Hpa II : enzyme de restriction sensible à la méthylation



Hind III : enzyme de restriction insensible à la méthylation
Hpa II : enzyme de restriction sensible à la méthylation

Southern blot :





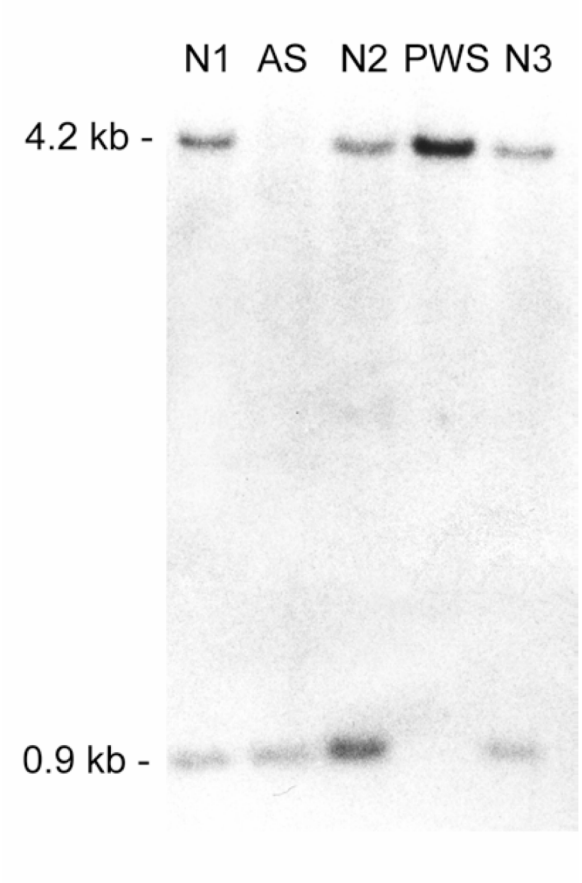
N = personne normale

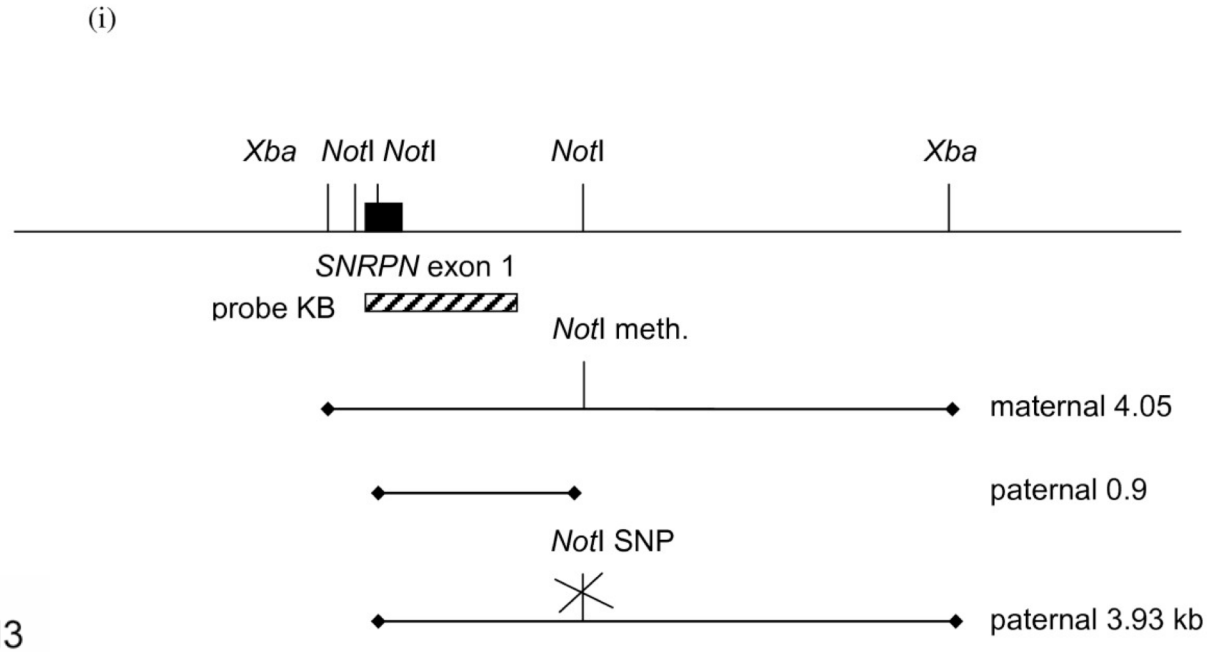
MAT (méthylé)

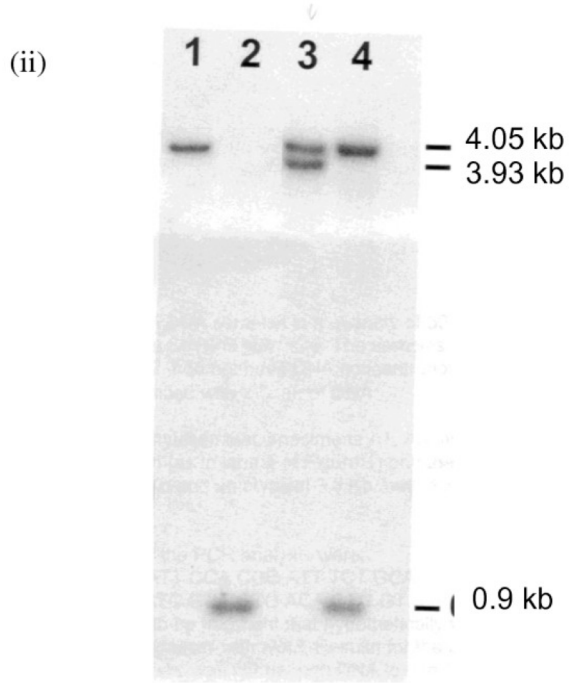
PAT (pas méthylé)

Southern blot :

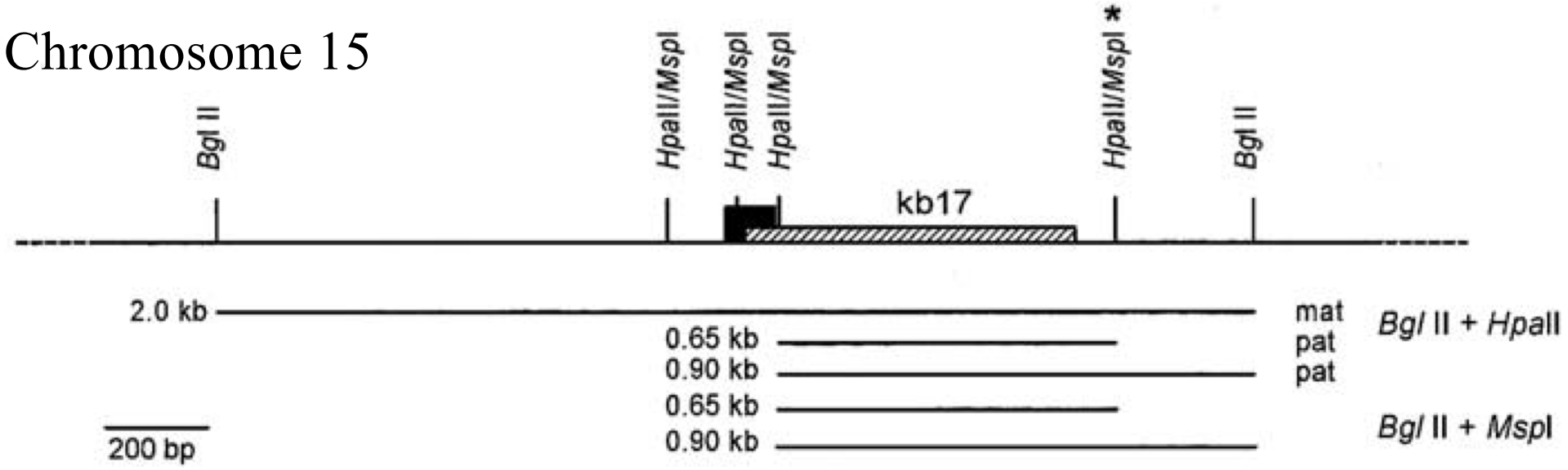
Bgl II / Hpa II







Analyse de la méthylation de IC par Southern blot :



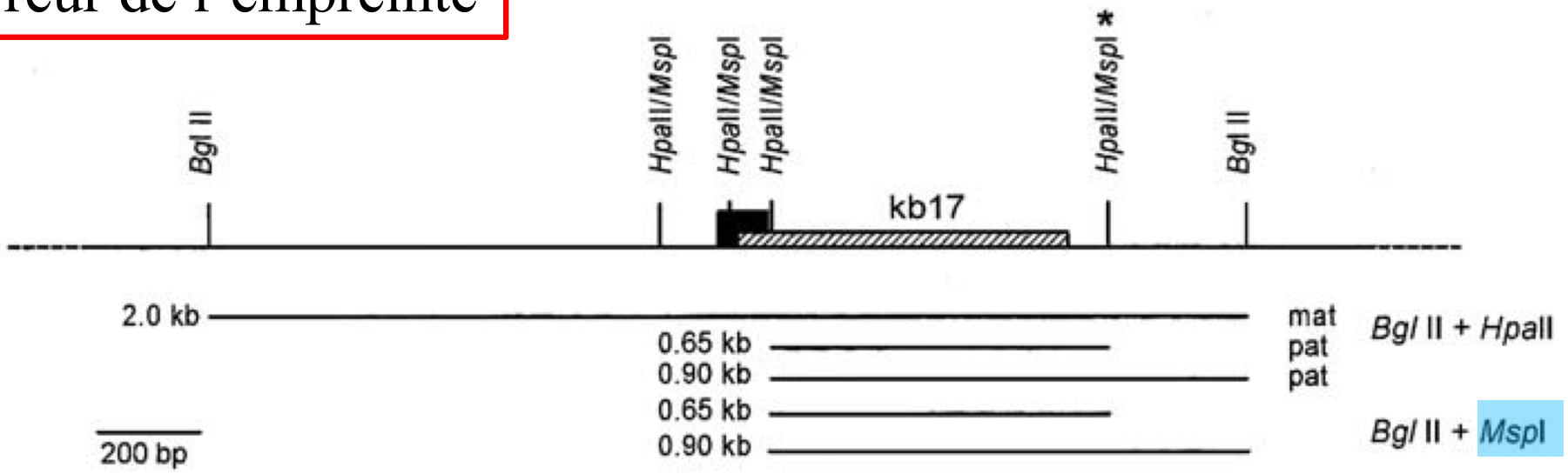
La digestion avec Bgl II donne un fragment de **2 kb**.

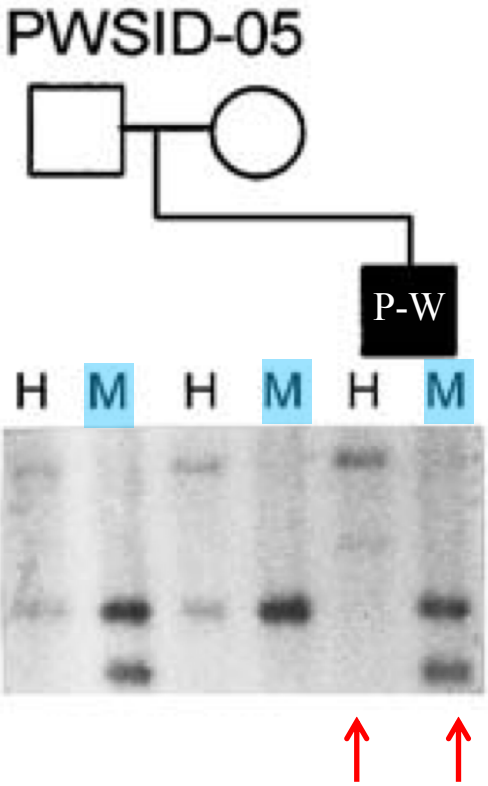
Ce fragment possède toujours 3 sites CCGG coupé par HpaII / Msp I. Un 4ème CCGG est polymorphe.

Empreinte maternelle : tous les CCGG sont méthylés → $C^{m}CGG$

Empreinte paternelle : aucun CCGG n'est méthylé.

Erreur de l'empreinte



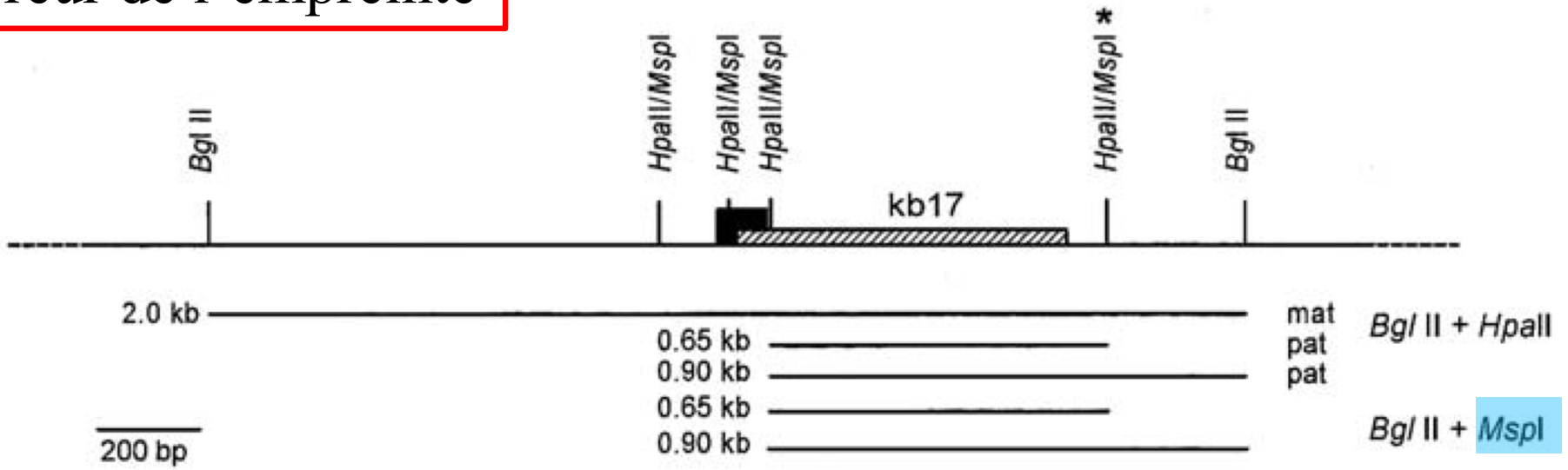


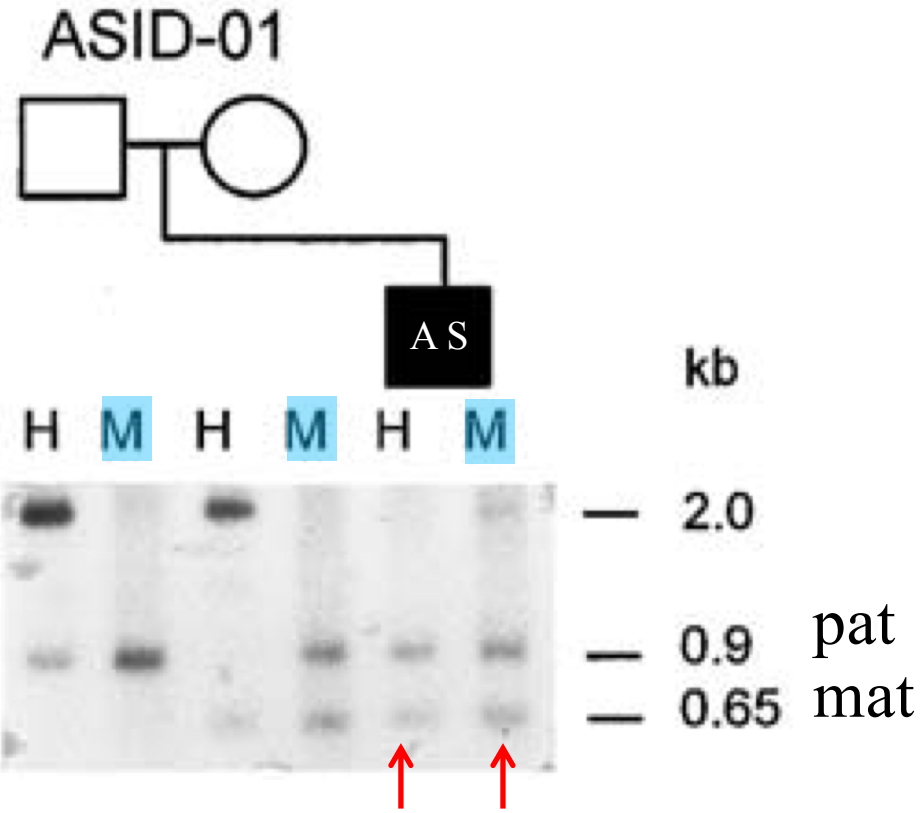
Southern blot :

Chez le patient :
les 2 chromosomes 15 sont méthylés.

Les 2 chromosomes portent une empreinte maternelle.
1 des chromosomes vient du père mais porte une empreinte mat.

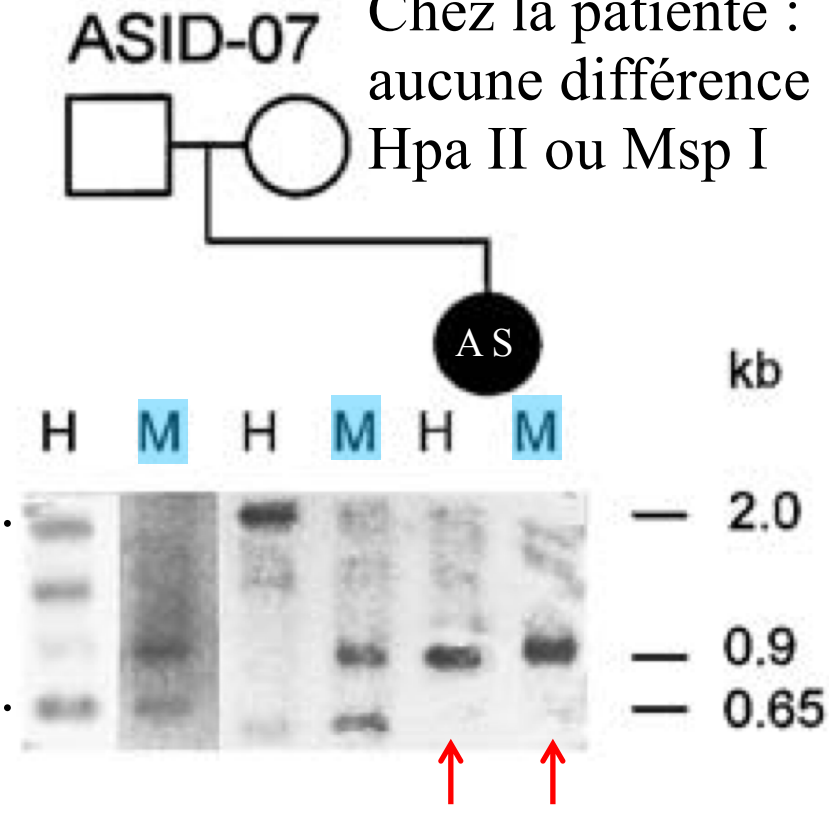
Erreur de l'empreinte





pat

mat



Chez la patiente : aucune différence Hpa II ou Msp I