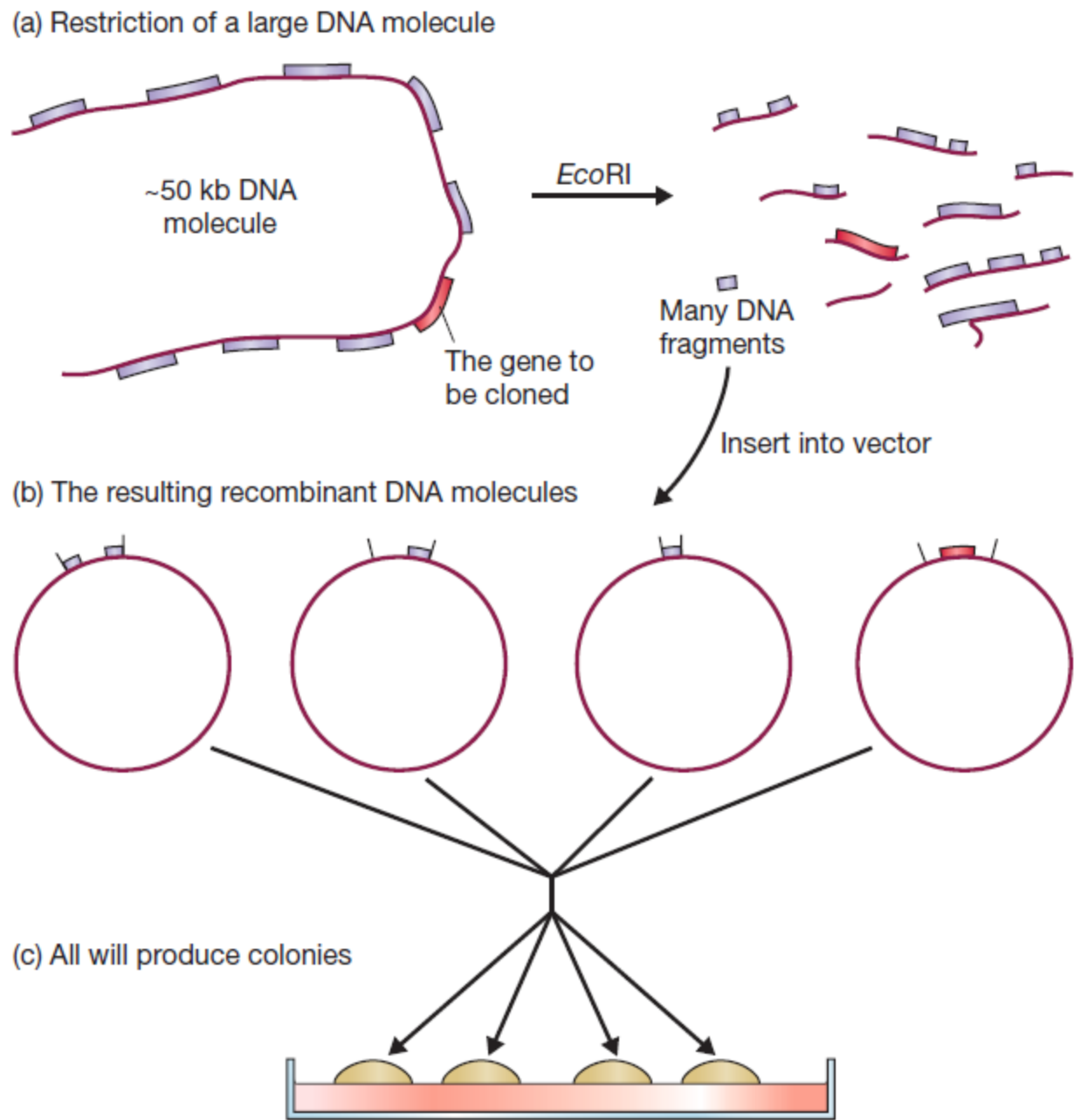


Screening of Cloned Gene



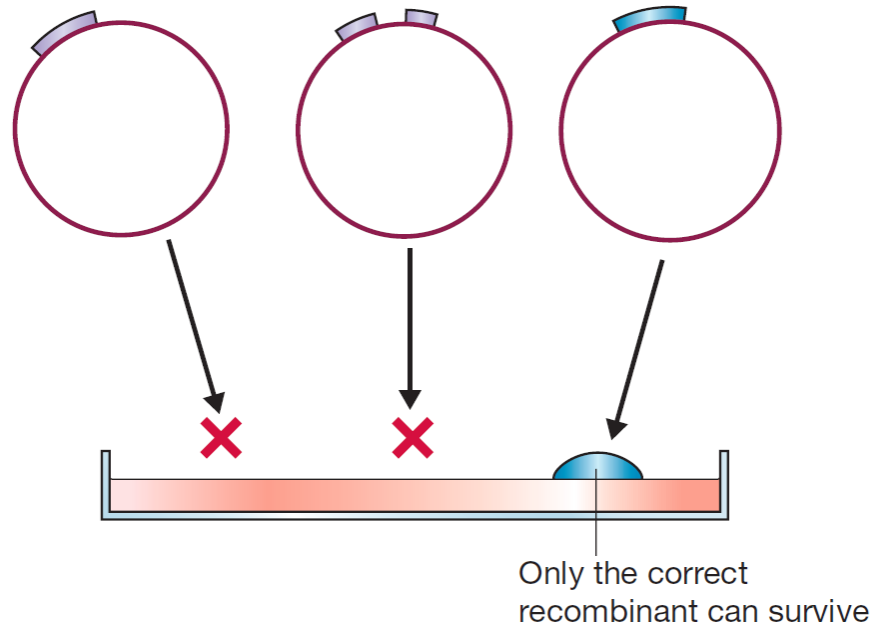
By
Dr. Jyotsnarani Pradhan
Assistant Professor
Department of Biotechnology
Utkal University

Problem in clone selection

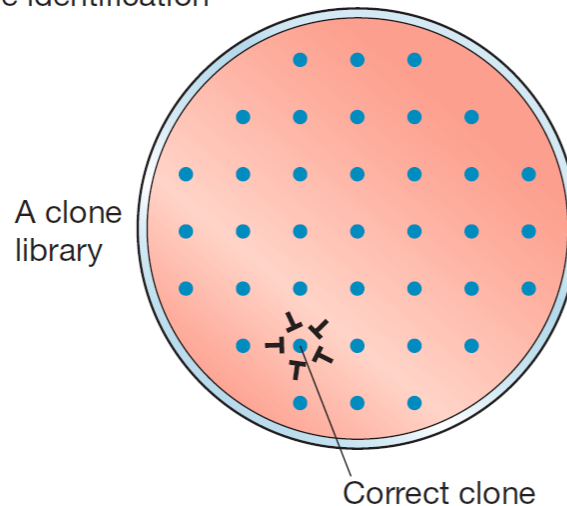


Strategy for clone selection

(a) Direct selection

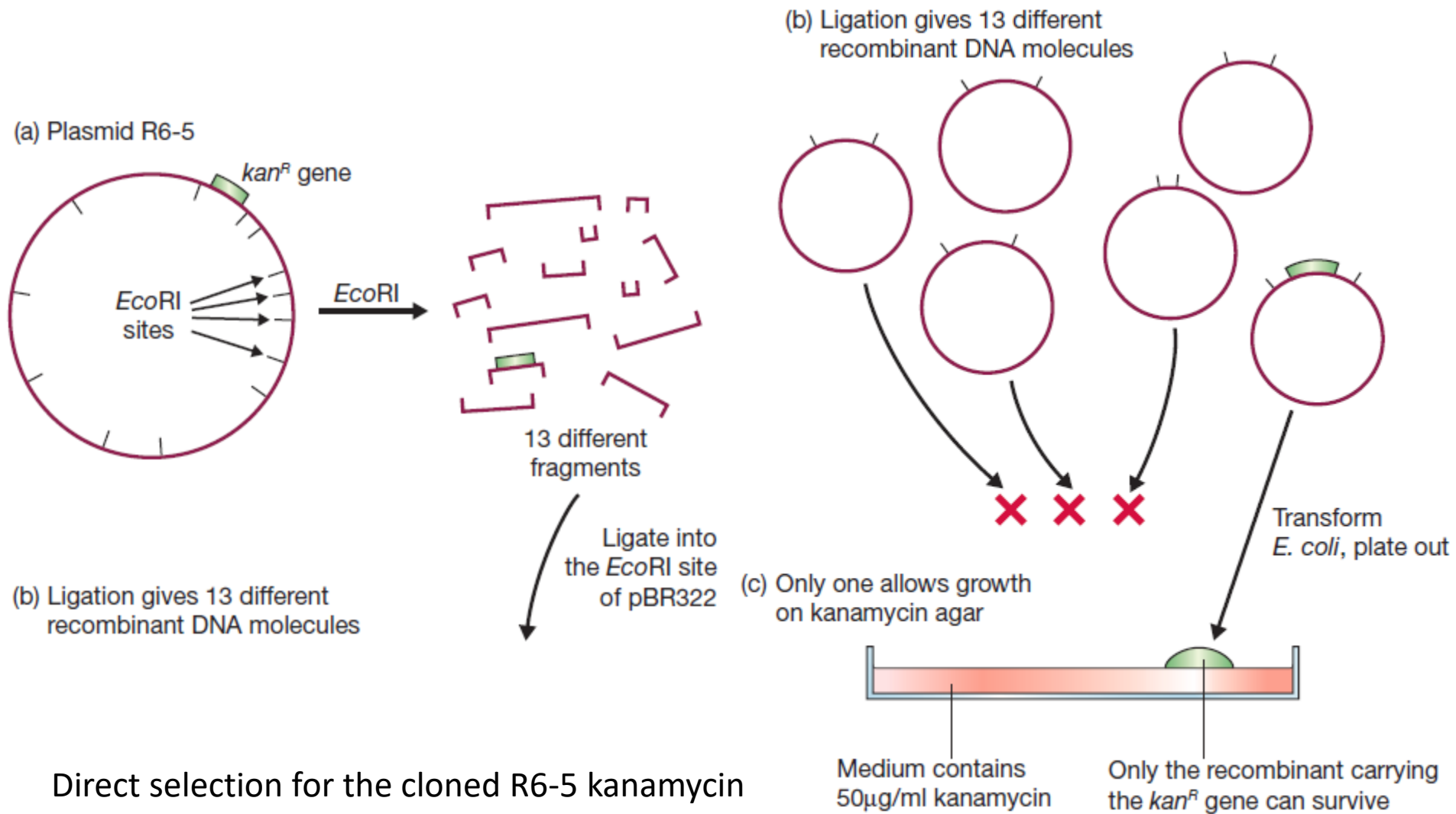


(b) Clone identification



The basic strategies that can be used to obtain a particular clone. (a) Direct selection. (b) Identification of the desired recombinant from a clone library.

Direct selection by Antibiotic resistance



Direct selection for the cloned R6-5 kanamycin resistance (*kan^R*) gene

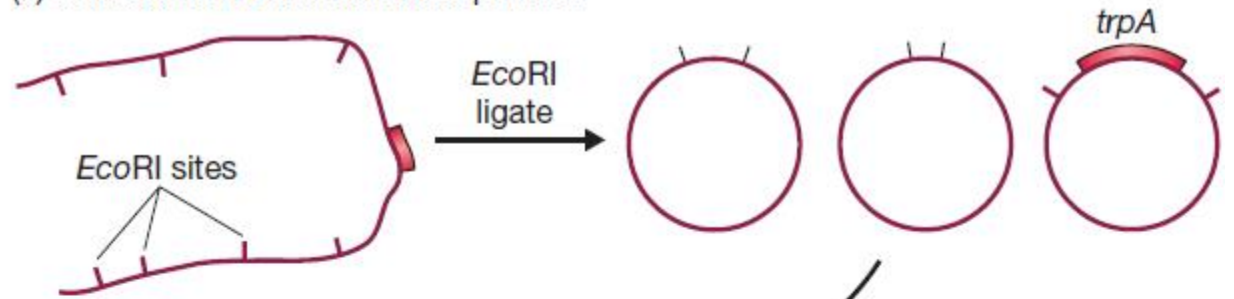
Direct selection by marker rescue

Direct selection for the *trpA* gene cloned in a *trpA*⁻ strain of *E. coli*

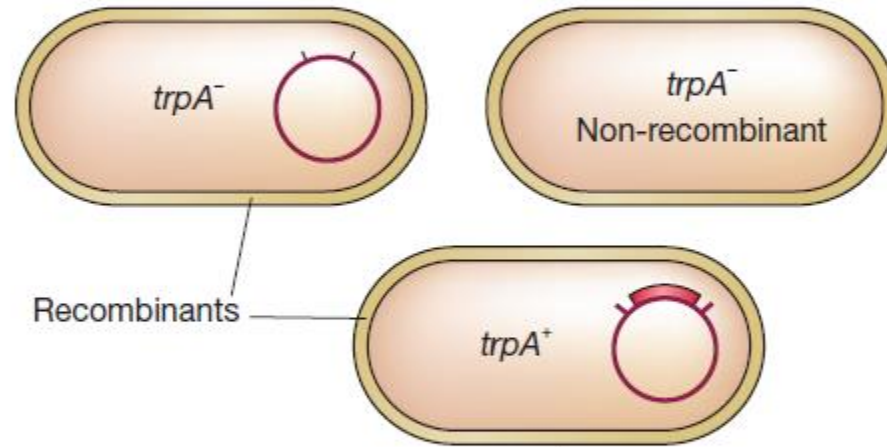
Limitations

- A mutant strain must be available for the gene in question.
- A medium on which only the wild-type can survive is needed.

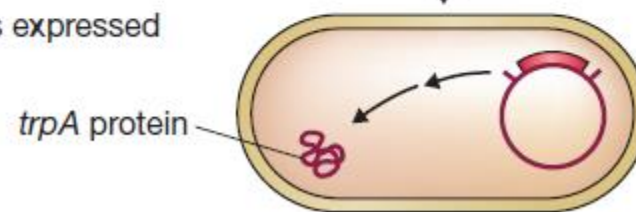
(a) Construction of recombinant pBR322



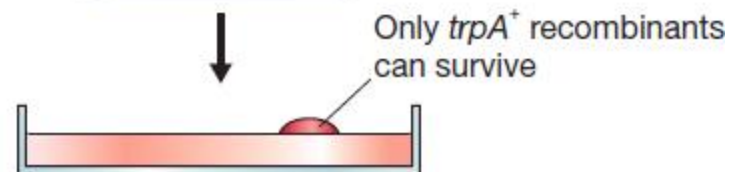
(b) Transform *E. coli trpA*⁻



(c) Plasmid gene is expressed



(d) Plate onto minimal medium

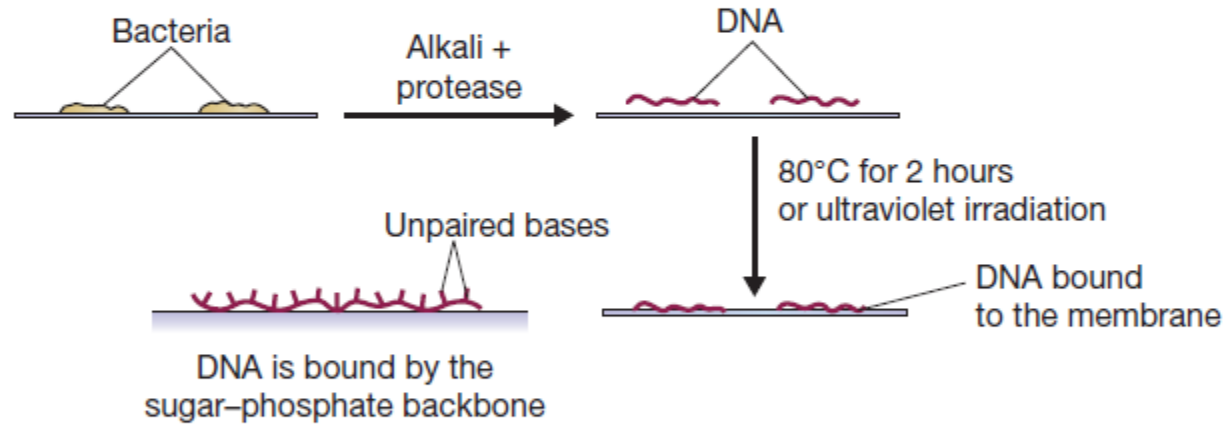


Hybridization probing

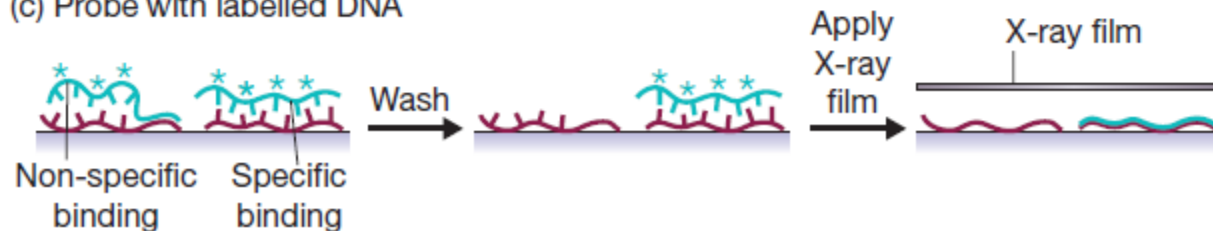
(a) Transfer colonies to nitrocellulose or nylon



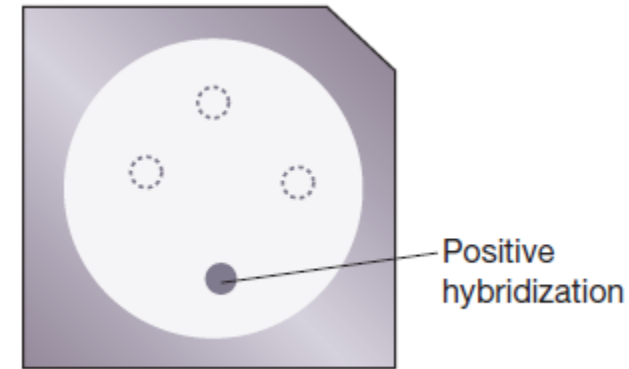
(b) Degrade cells, purify DNA



(c) Probe with labelled DNA



(d) The resulting autoradiograph

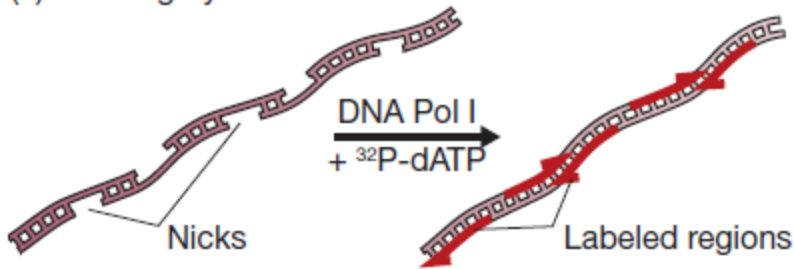


Colony hybridization probing. In this example, the probe is labelled with a radioactive marker and hybridization detected by autoradiography, but other types of label and detection system can also be used

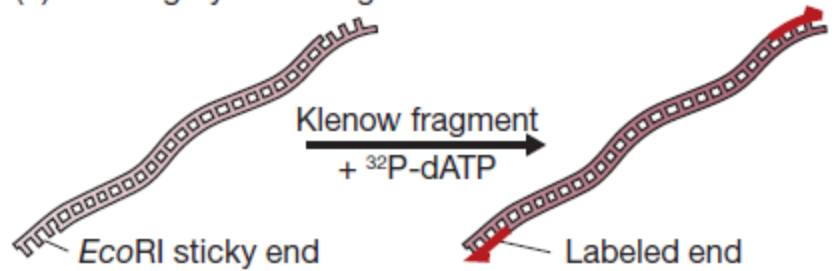
Methods for labeling of DNA

1. Nick Translation
2. End labeling
3. Random Priming

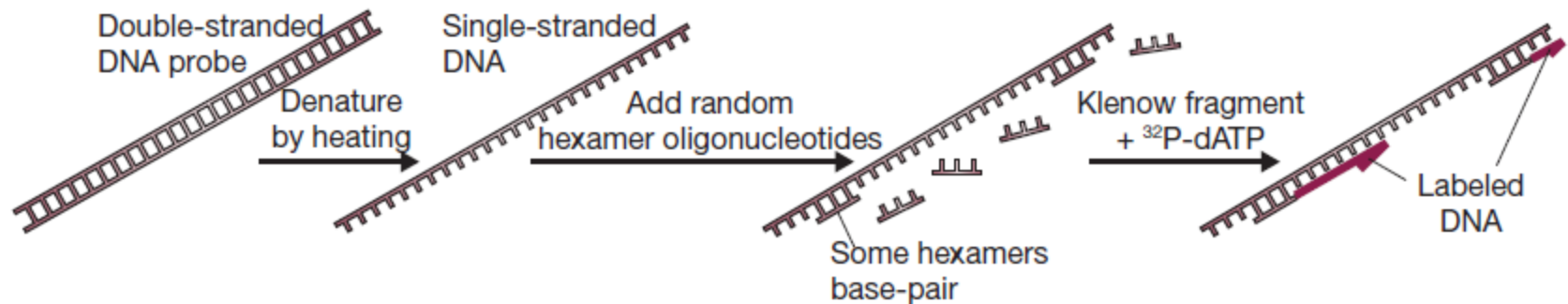
(a) Labeling by nick translation



(b) Labeling by end-filling

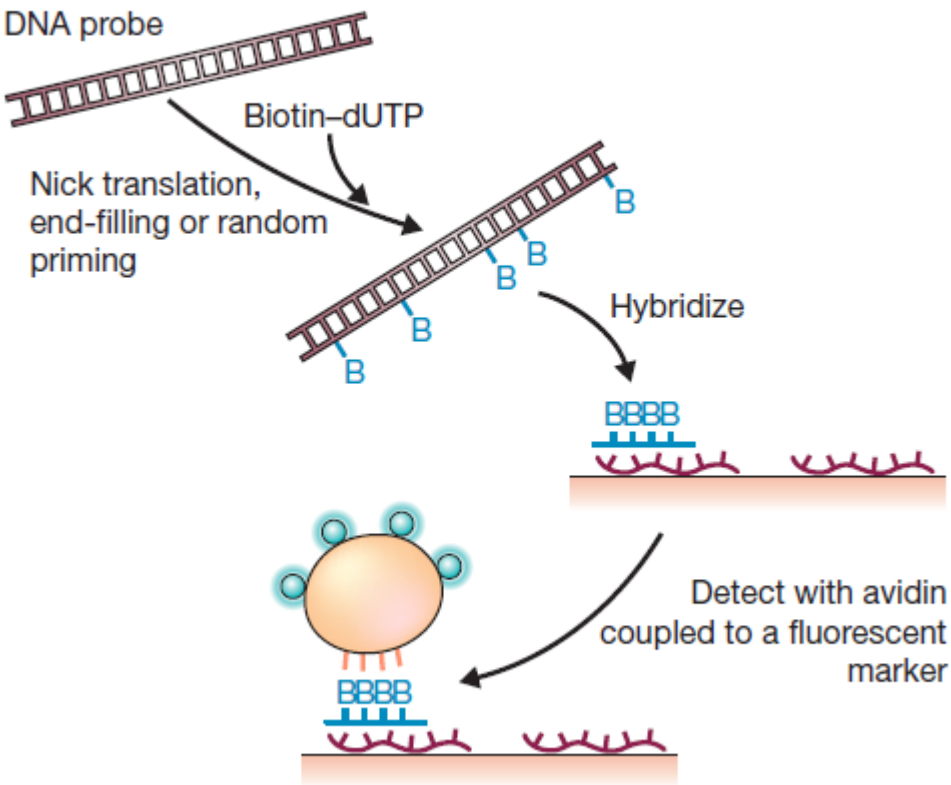


(c) Labeling by random priming

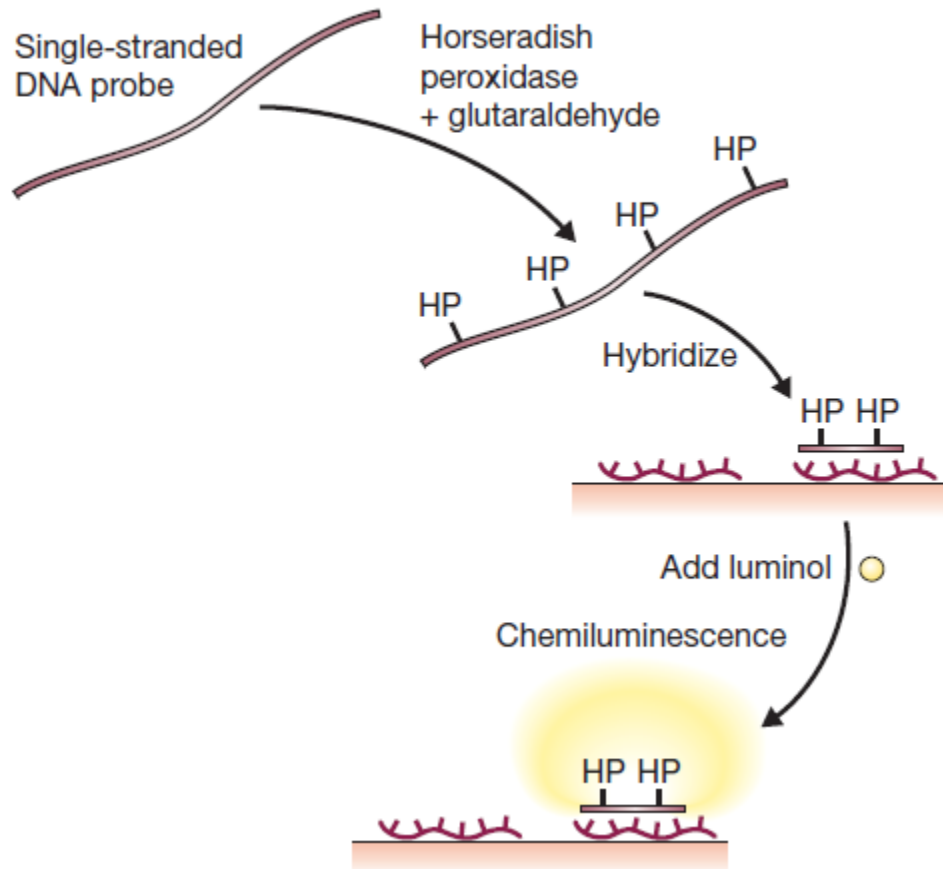


Non-radioactive labeling

(a) Labelling with a biotinylated nucleotide

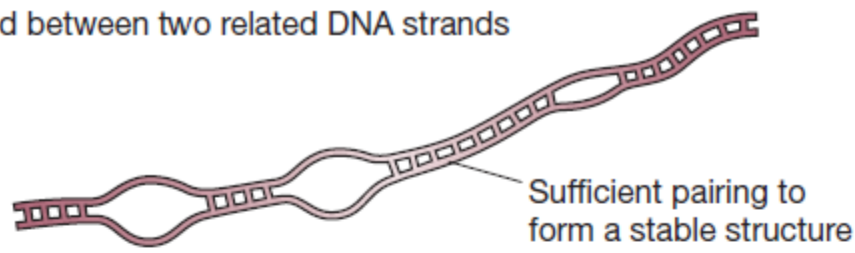


(b) Labelling with horseradish peroxidase

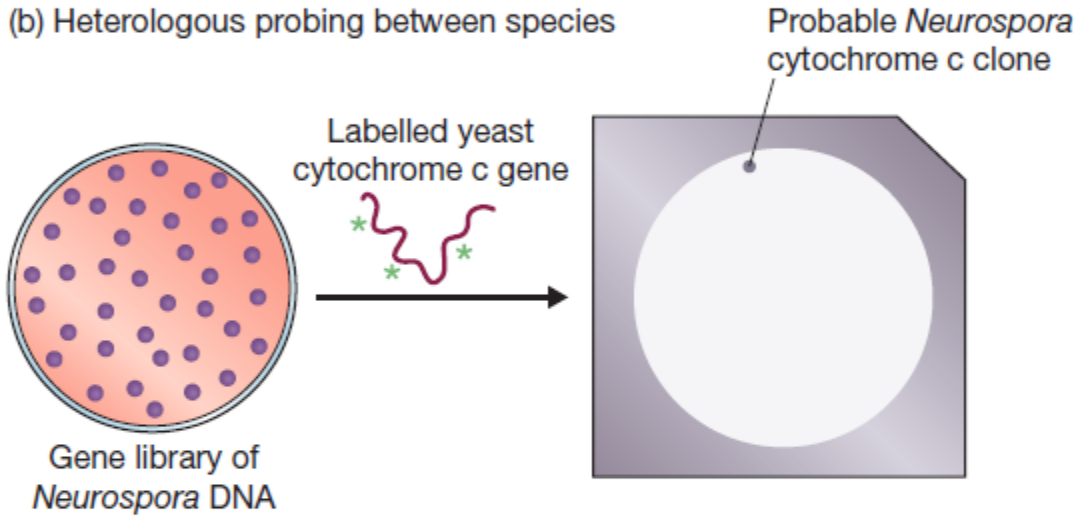


Heterologous Probing

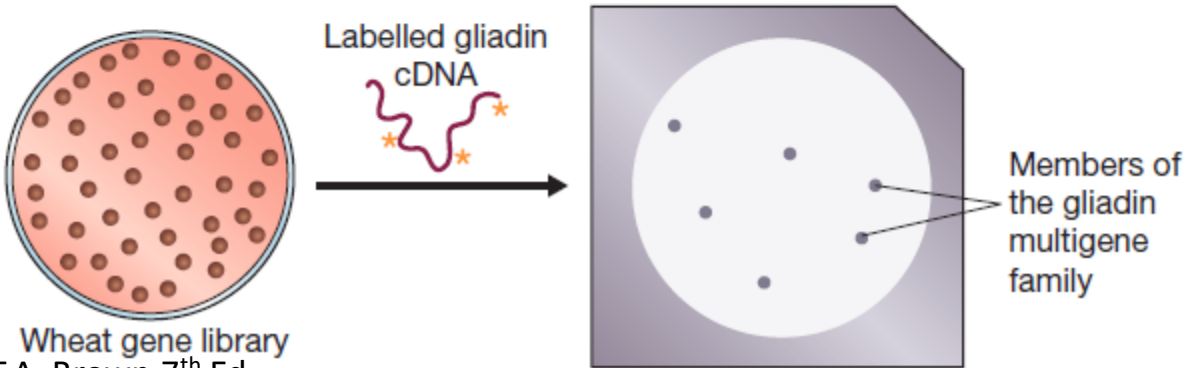
(a) A hybrid between two related DNA strands



(b) Heterologous probing between species

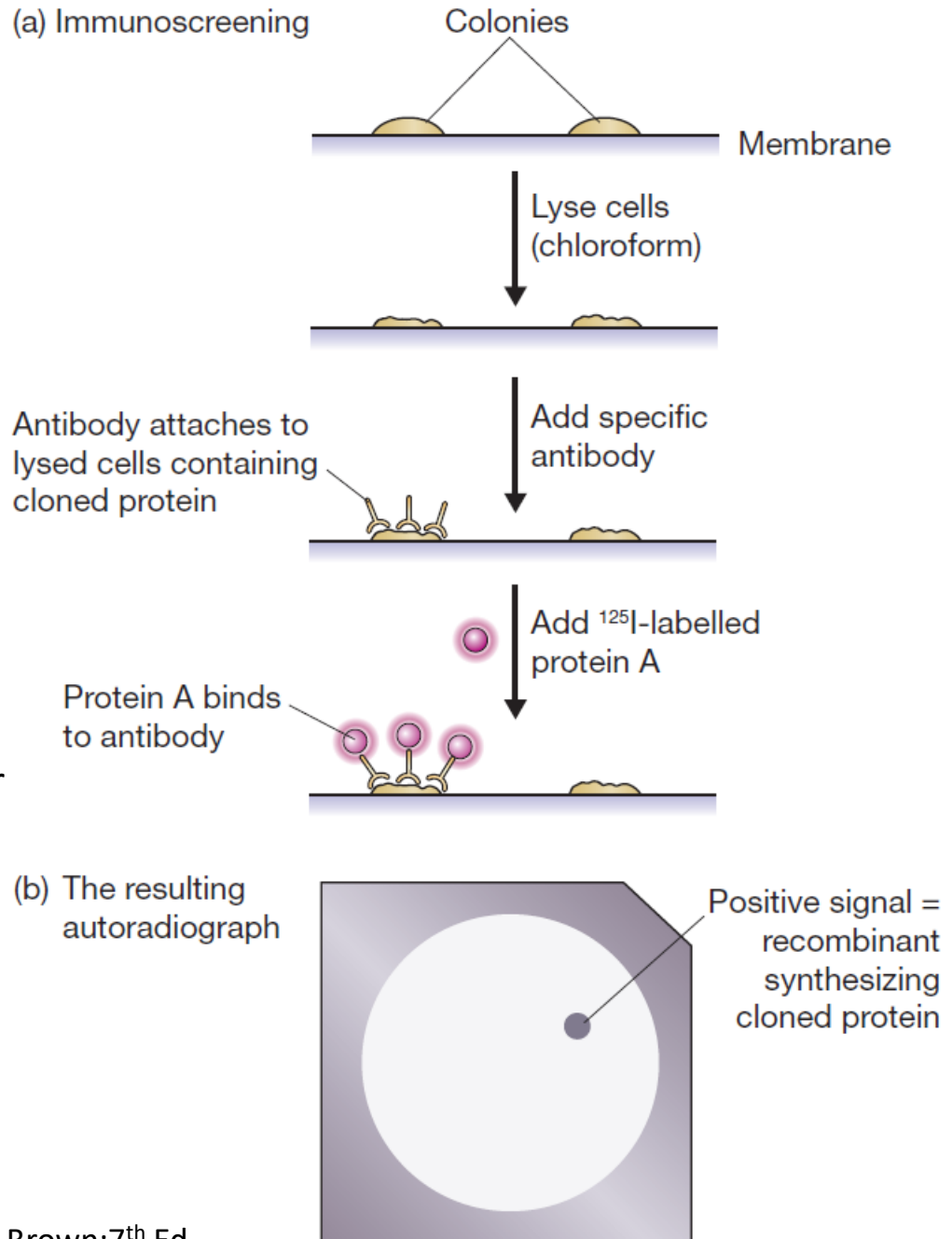


(c) Heterologous probing within a species



Immunological detection method

Using a purified antibody to detect protein in recombinant colonies. Instead of using a secondary antibody, the primary antibody itself can be labelled, or the antibody can be detected with labelled protein A.



Thank You