Nucleic Acid Hybridization

Lab7

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Nucleic Acid Hybridization

Nucleic acid hybridization is a technique in which single-stranded nucleic acids, the DNA's and RNA's, are allowed to interact. This will result to occurrence of complexes called hybrids. These hybrids are .being formed by molecules with similar, complementary sequences Hybrids are detected by various means: visualization in the electron microscope; by radioactively labelling one component and removing non-complexed DNA; or by washing or digestion with an enzyme that attacks single-stranded nucleic acids and finally estimating the .radioactivity bound

Hybridizations are done in all combinations: DNA-DNA (DNA can be .rendered single-stranded by heat denaturation), DNA-RNA or RNA-RNA

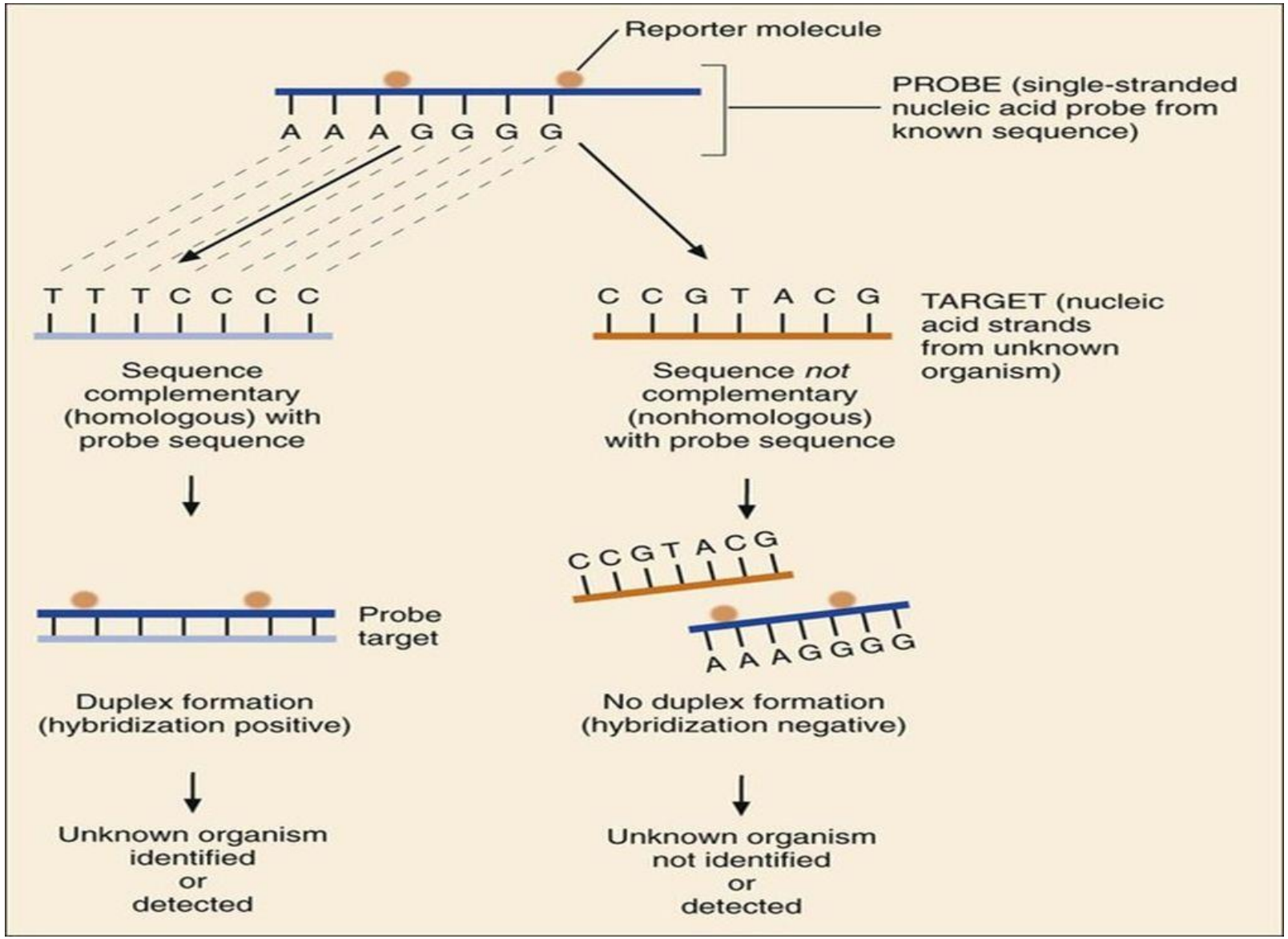
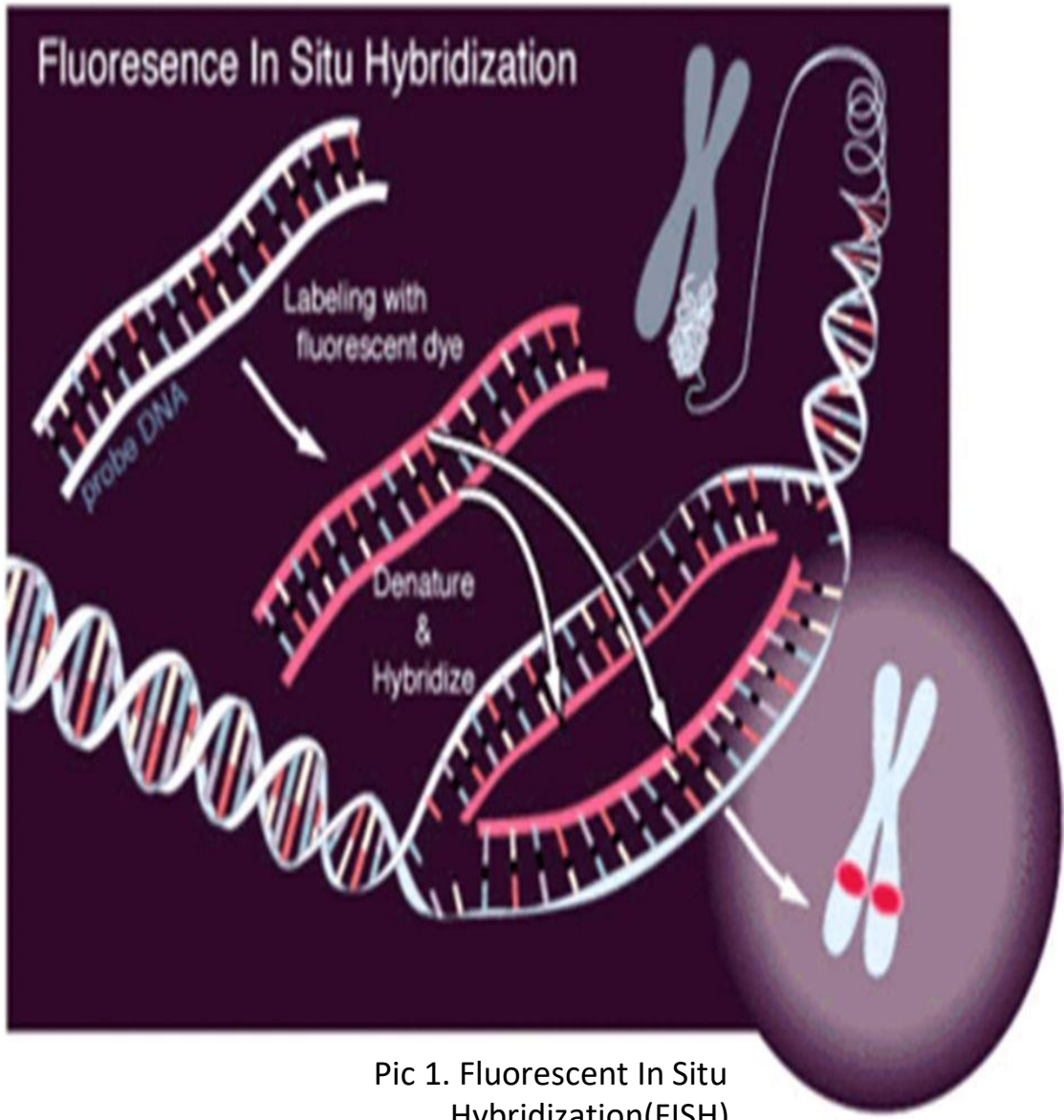


Figure 1-1 Principles of nucleic acid hybridization

Methods of Hybridization i. in situ hybridization

In situ meaning “in place” or “in position”) allows a pathogen to be identified from a specimen
using the patient’s cells or tissues as the solid support phase. Tissue specimens thought to be infected with a particular pathogen are processed in a manner that maintains the structural integrity of the tissue and cells, yet allows the nucleic acid of the pathogen to be accessed *in situ* and denatured toa single strand with the base sequence intact for hybridization with the pathogen-specifc probe.



Pic 1. Fluorescent In Situ Hybridization(FISH)

Methods of Hybridization

ii. Fluorescent in situ hybridization (FISH)

A genetic mapping technique using fluorescent tags for analysis of chromosomal aberrations and genetic abnormalities. Called also chromosome painting. The DNA is labeled with a fluorescent dye and hybridized to a cytological preparation of chromosomes that has been denatured to allow nucleic acid hybridization between chromosomal .DNA and the probe

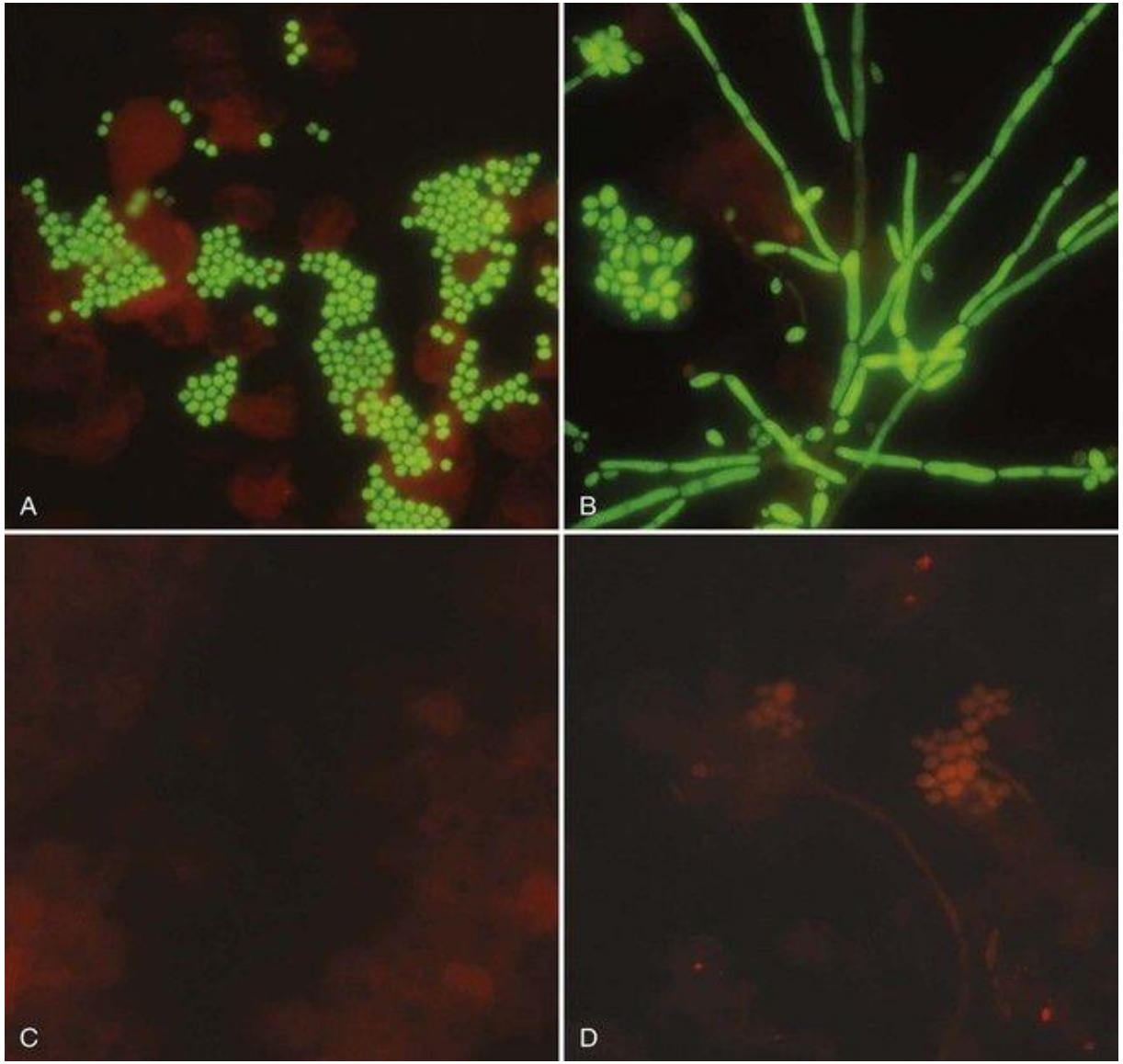


Figure 1-2 Using a fluorescent-tagged peptide nucleic acid (PNA) probe in conjunction with fluorescent in situ hybridization (FISH), Staphylococcus aureus (A) or Candida albicans (B) can be directly identified in .blood cultures
Blood cultures negative for either S. aureus (C) or C. albicans (D) by PNA
FISH technology are shown for comparison. (Courtesy of AdvanDx, Woburn, MA.)

Hybridization Steps and Components

- Production and labeling of single-stranded nucleic acid probe
- Preparation of single-stranded target nucleic acid
- Mixture and hybridization of target and probe nucleic acid
- Detection of hybridization

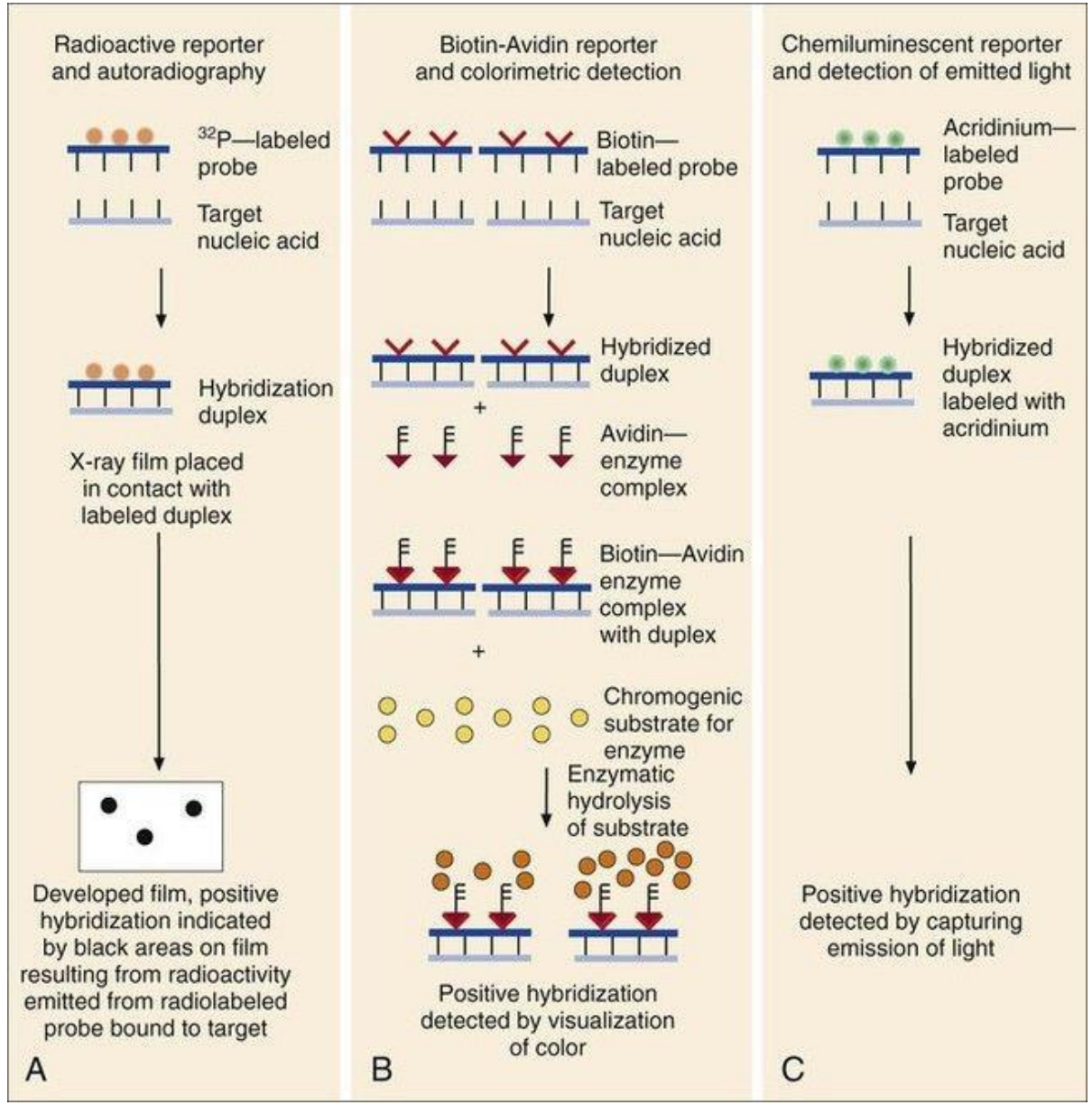
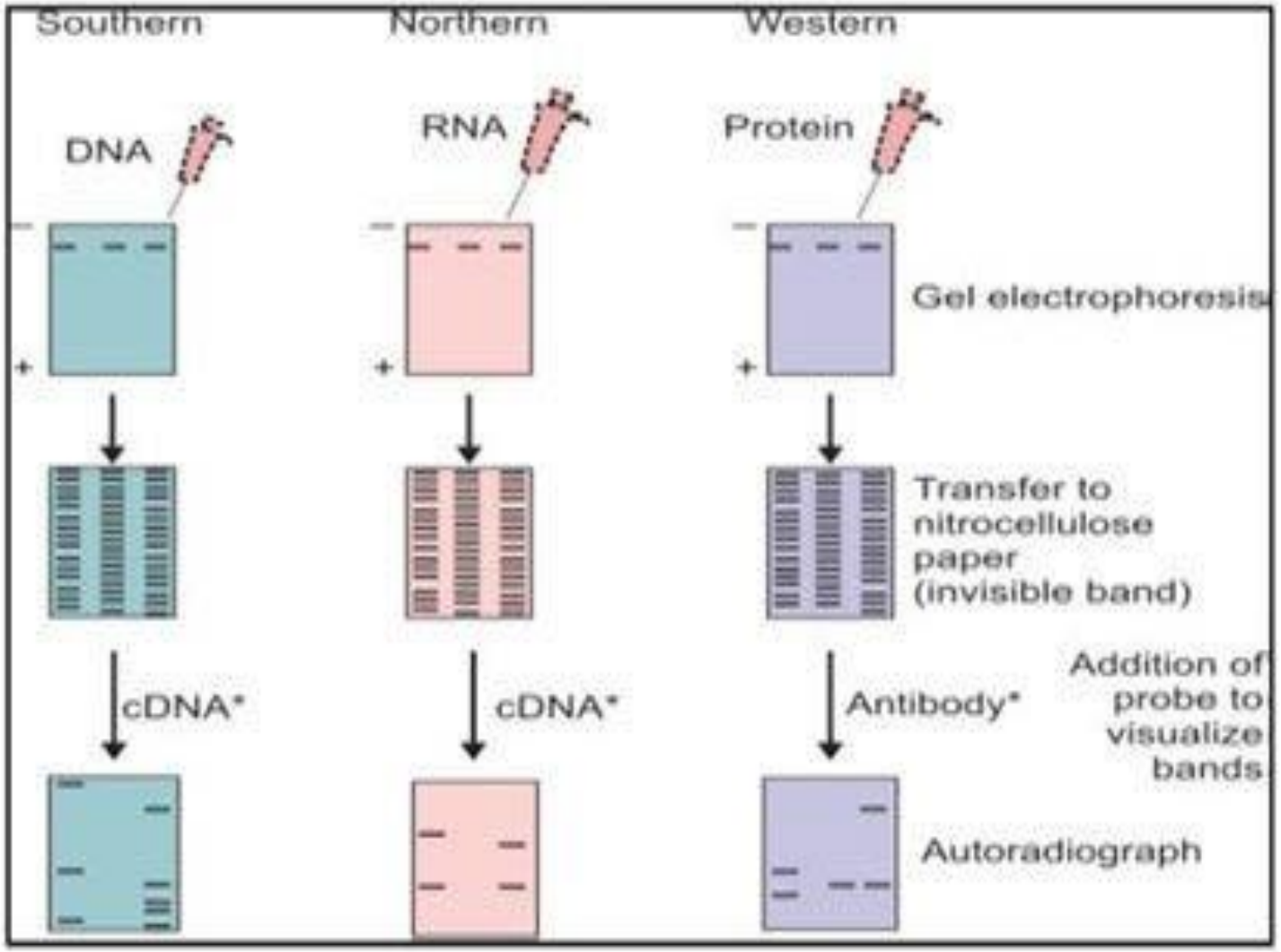


Figure 8-2 A, Reporter molecule labeling of nucleic acid probes and principles of hybridization detection. Use of probes labeled with a radioactive reporter, with hybridization detected by autoradiography. B, Probes labeled with a biotin-avidin reporter, with hybridization detected by a colorimetric assay. C, Probes labeled with a chemiluminescent

reporter (i.e., acridinium), with hybridization detected by a luminometer to detect emitted light

Types of blotting techniques

Southern blotting •
Northern blotting •
Western blotting •
Colony blotting •
Dot blotting •



Applications

a) Isolation and quantification of specific nucleic acid sequences

b) Intracellular localization: presence and absence of a particular gene and its copy number in the genome of an organism

c) Degree of similarity between chromosomal gene and the probe .sequence

d) Presence and absence of recognition sites for particular restriction .endonucleases in the gene

.e) Expression and regulation of a particular gene

.f) Diagnosis of infectious and inherited diseases