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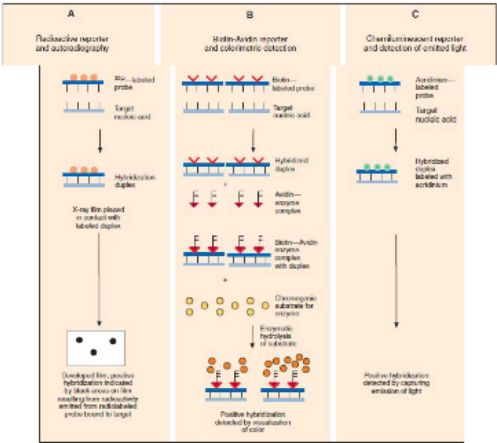
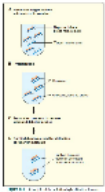


Figure 9-2 Reporter nucleic acid labeling of nucleic acid probes and principles of hybridization detection. One of probes labeled with a radioactive reporter, with hybridization detected by autoradiography (A); probes labeled with biotin or other reporter, with hybridization detected by a colorimetric assay (B); probes labeled with chemiluminescent reporter (e.g., acridinium), with hybridization detected by a luminometer to detect emitted light (C).

Detection of Hybridization:
The method of detecting hybridization depends on the reporter molecule used for labeling probe nucleic acid and on the hybridization format (see Figure 9-2). Detection of hybridization using radioactively labeled probes is done by exposing the reaction mixture to radiograph film (i.e., autoradiography). Hybridization with **nonradioactively** labeled probes is detected using colorimetry, fluorescence, or chemiluminescence, and detection can be somewhat automated using spectrophotometers, fluorimeters, or luminometers, respectively. The more commonly used nonradioactive detection systems (e.g., **digoxigenin, chemiluminescence**)



Hybridization probe and reporter:
The probe is a nucleic acid molecule that is complementary to the target sequence. The reporter is a molecule that is attached to the probe and can be detected by a specific method (e.g., autoradiography, colorimetry, fluorescence, or chemiluminescence).

Radioactive nucleic acid probes:
Radioactive probes are nucleic acid molecules that are labeled with a radioactive isotope (e.g., ³²P, ³⁵S, ¹²⁵I). The radioactivity is detected by autoradiography or scintillation counting.

ON-TARGET HYBRIDIZATION USING SENSITIVE DETECTION:
Usually to assess detecting the presence of specific sequences of nucleotides in a DNA molecule, or an RNA. First, specific for a particular sequence, in principle, the presence of a specific gene or a particular nucleic acid sequence is interpreted as a **relative identification of the organism.**

ORGANISM IDENTIFICATION USING GENOTYPIC CRITERIA

usually involves detecting the presence of **specific sequence of nucleotides in a DNA molecule**, or an **RNA** that is specific for a particular organism. In principle, the presence of a specific gene or a particular nucleic acid sequence is **interpreted as a definitive identification of the organism.**

NUCLEIC ACID HYBRIDIZATION METHODS

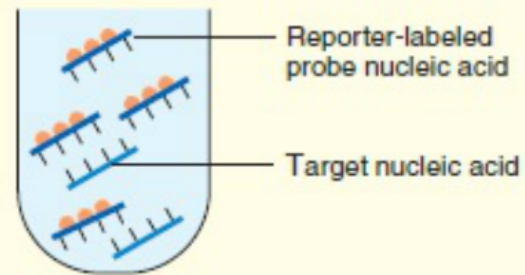
Hybridization methods are based on the ability of two nucleic acid strands that have complementary base sequences (i.e., are homologous) to specifically bond with each other and form a double-stranded molecule, or duplex or hybrid.

Hybridization Steps and Components

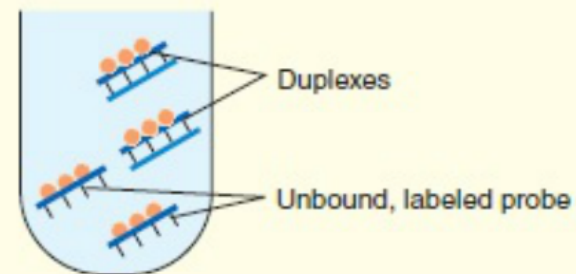
The basic steps in a hybridization assay include:

1. Production and labeling of single-stranded probe nucleic acid
2. Preparation of single-stranded target nucleic acid
3. Mixture and hybridization of target and probe nucleic acid
4. Detection of hybridization

A Probe and target nucleic acids mixed in solution



B Hybridization



C Separation process to remove unbound, labeled probes



D Purified duplexes read for detection of reporter molecule

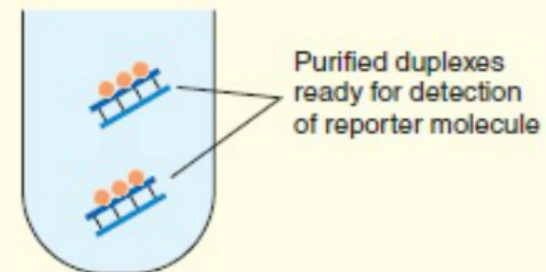


Figure 8-3 Principle of the solution hybridization format.

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The more commonly used nonradioactive detection systems (e.g., **digoxigenin, chemiluminescence**)

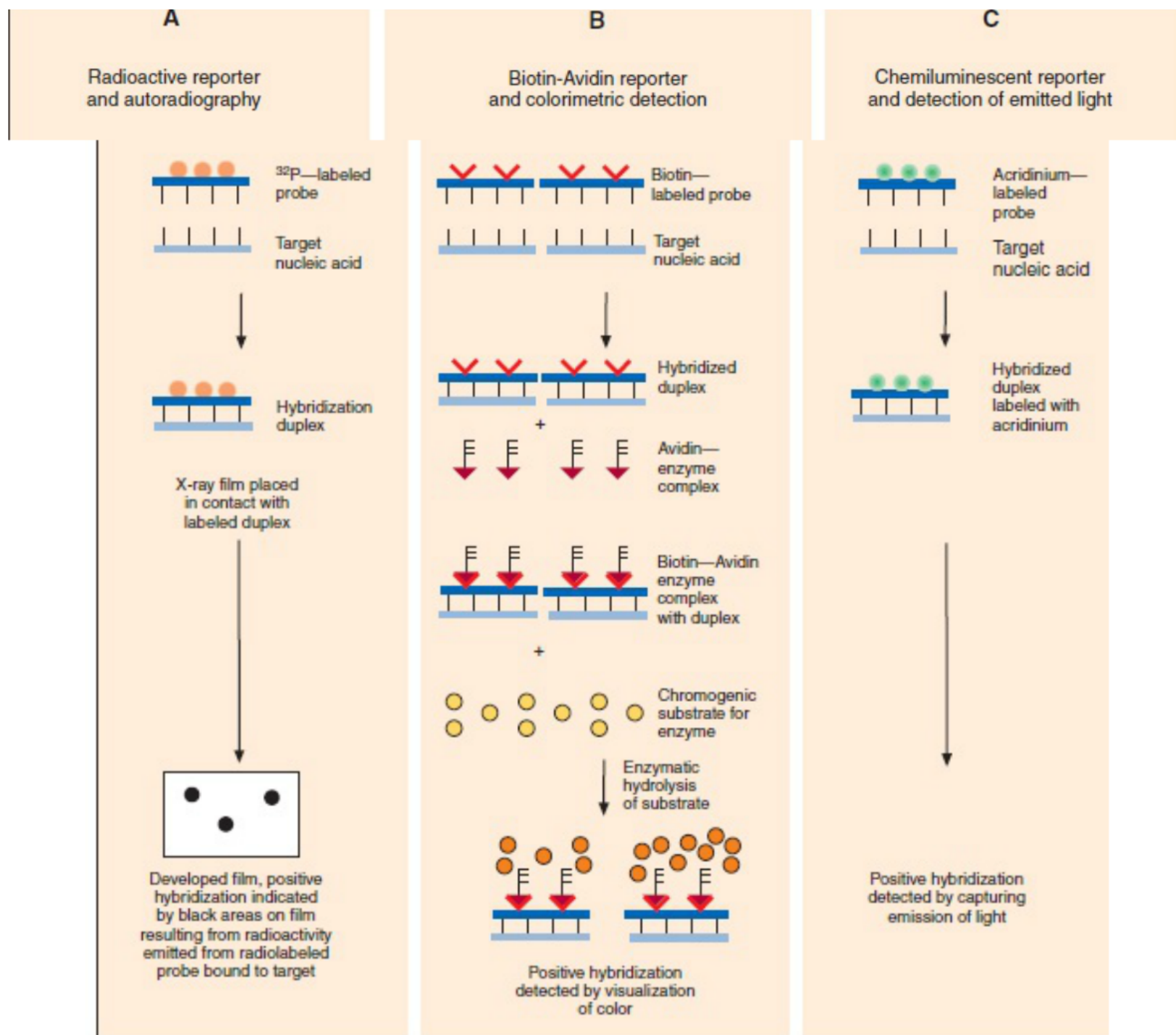


Figure 8-2 Reporter molecule labeling of nucleic acid probes and principles of hybridization detection. Use of probes labeled with a radioactive reporter, with hybridization detected by autoradiography (A); probes labeled with biotin-avidin reporter, with hybridization detected by a colorimetric assay (B); probes labeled with chemiluminescent reporter (i.e., acridinium), with hybridization detected by a luminometer to detect emitted light (C).

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