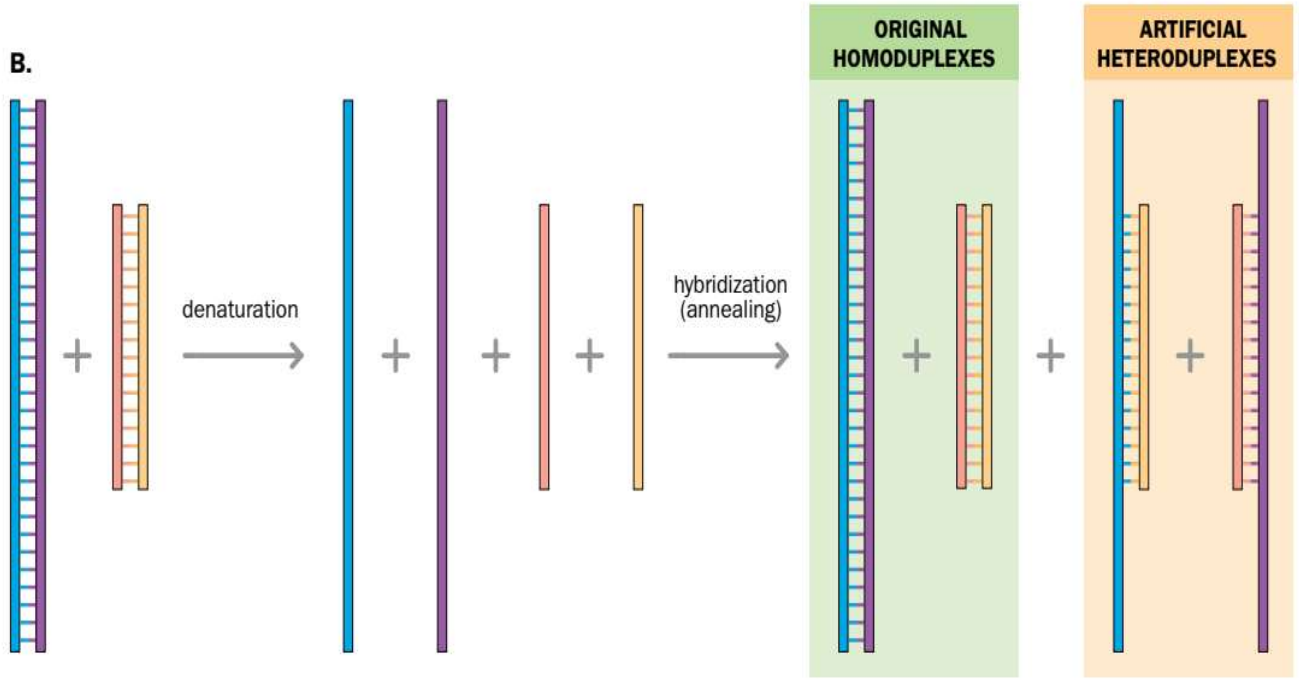
Chapter III:

Molecular hybridization, probes and DNA labeling

Molecular hybridization

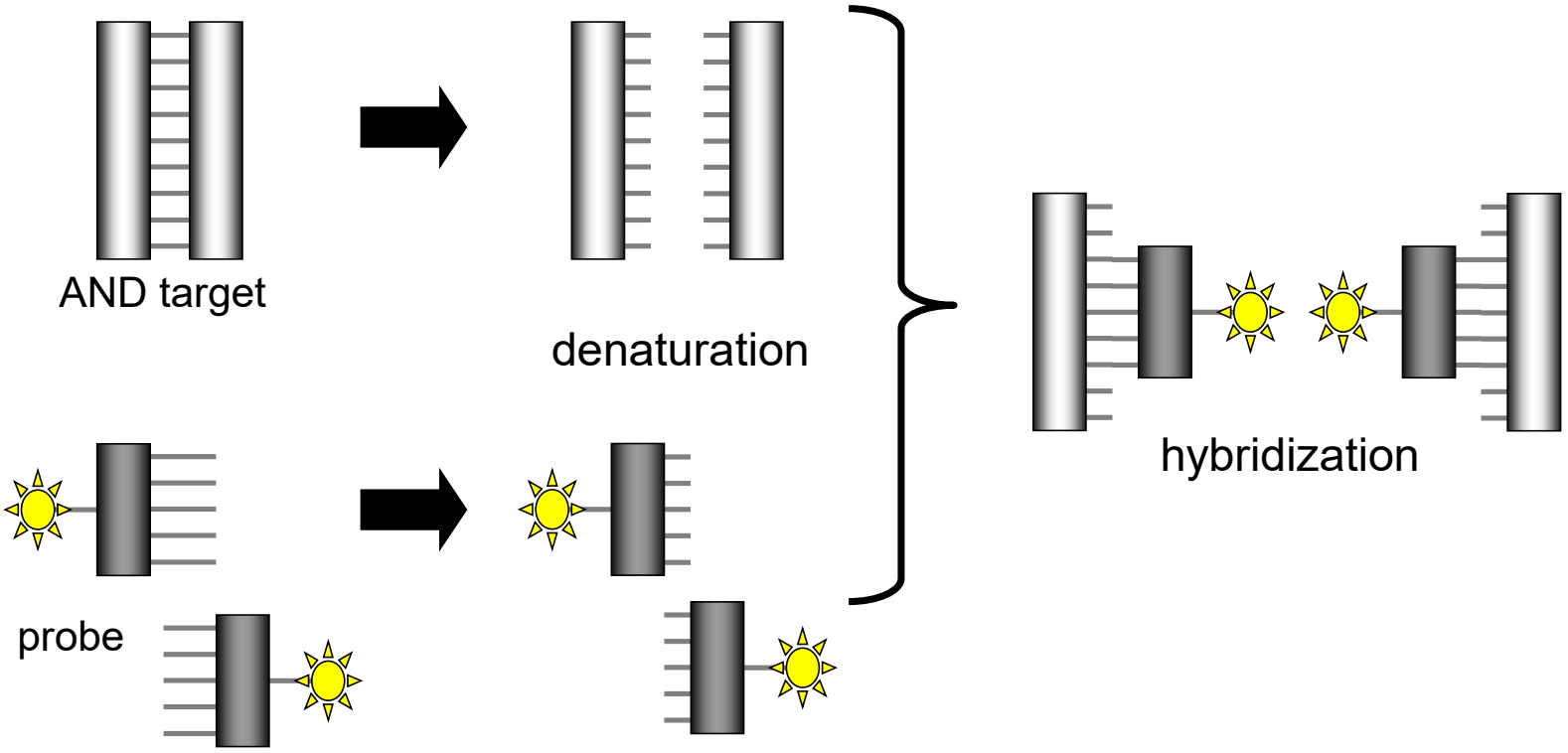
- Definition:
  - Molecular hybridization refers to all applications that use the specific recognition of a nucleic acid sequence (DNA and RNA) by another sequence that is complementary to it.
  - When the second sequence is labeled, it is called a ' **molecular probe** '.
  - The duplex formed is detectable by a signal that provides qualitative and quantitative information about the detected target sequence.

Molecular hybridization

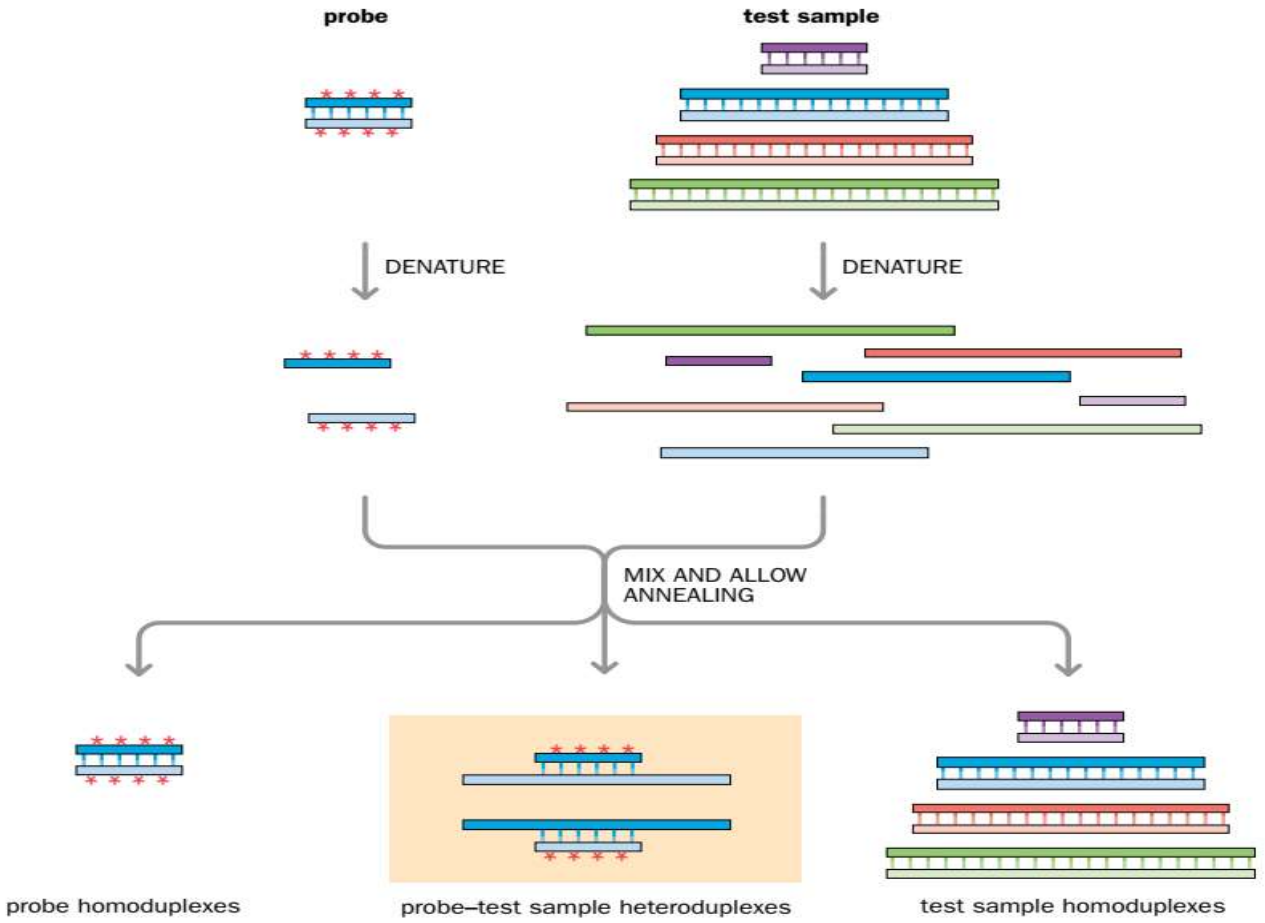


Denaturation and hybridization of homologous DNA molecules to form artificial heteroduplexes and natural homoduplexes.

Molecular hybridization



Molecular hybridization



Probe/target heteroduplex formation during a nucleic acid hybridization assay.

Molecular hybridization

The different types of hybridization:

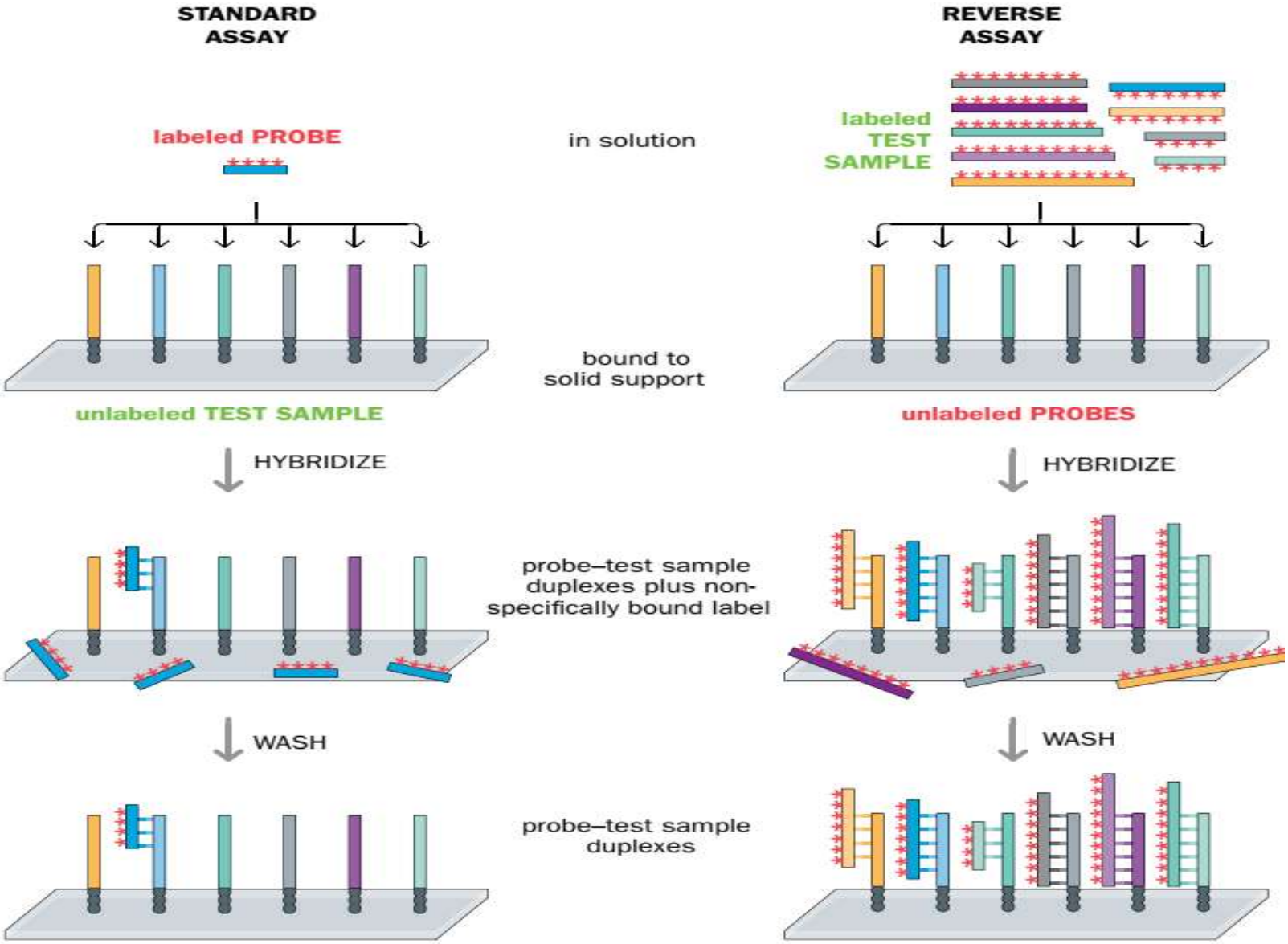
- Hybridization can occur
  - In solution
  - On solid supports: immobilization of the target on a membrane (nitrocellulose, nylon), on glass: bacterial colonies, chromosomes, tissue sections...

- **Objective** : to detect the presence of a nucleic acid of a given sequence using a complementary DNA fragment = probe

Molecular hybridization

The different types of hybridization:

- On a solid surface:

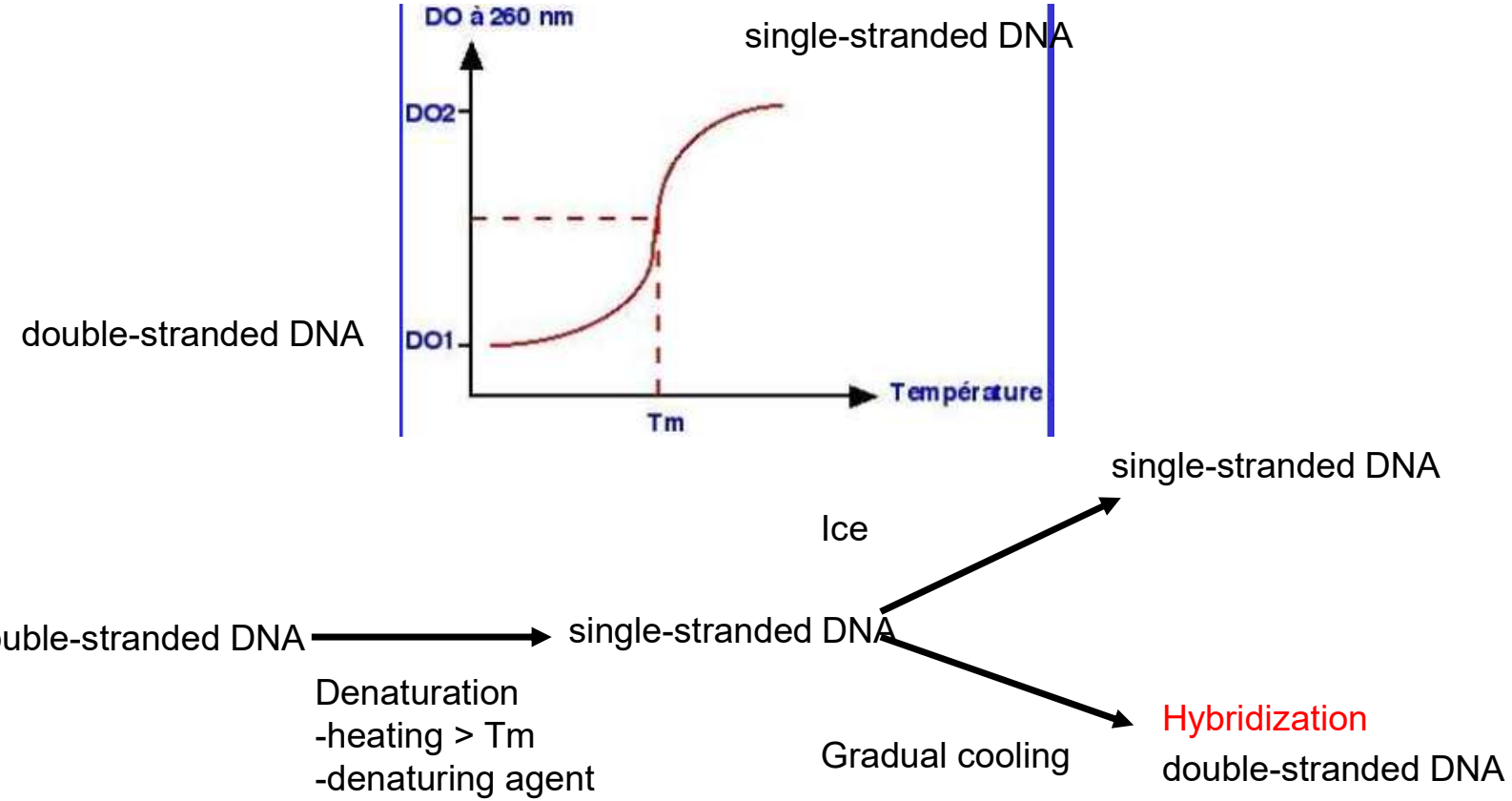

STANDARD ASSAY
REVERSE ASSAY
labeled PROBE
in solution
labeled TEST SAMPLE
bound to solid support
unlabeled TEST SAMPLE
unlabeled PROBES
HYBRIDIZE
HYBRIDIZE
probe–test sample duplexes plus non-specifically bound label
WASH
WASH
probe–test sample duplexes

Molecular hybridization

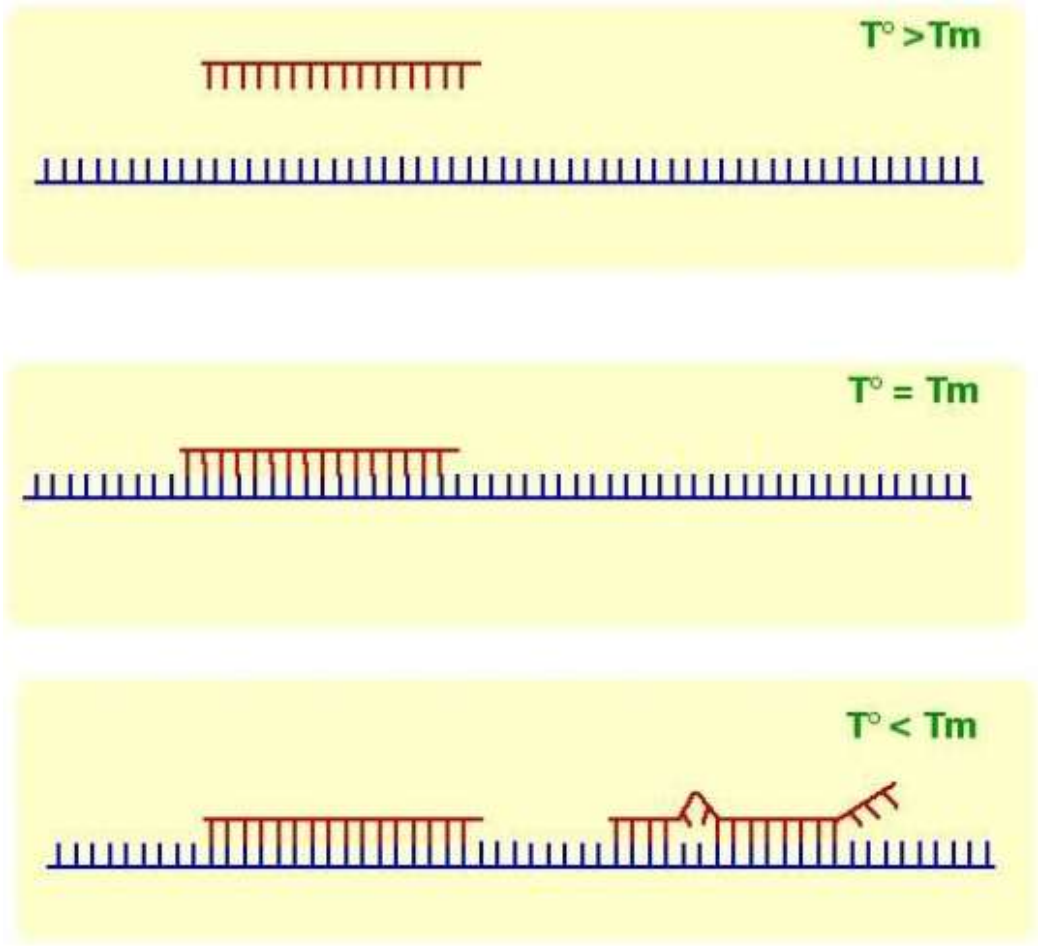
- Factors influencing hybridization:
  - The temperature
  - The percentage of DNA in CG and the time
  - The complexity of the sequences
  - Ionic strength

Molecular hybridization

Melting point and molecular hybridization:



Molecular hybridization



THE PROBES

- **Definition:**

  A probe is a labeled DNA (or RNA) sequence complementary to a target DNA sequence

PROBE LABELING

Labeling strategies:

- DNA labeling
  - Oligonucleotide end labeling
    - Via kinases (Oligosondes)
    - By end filling
  - Nick translation
  - Random primer
  - PCR labeling
- RNA labeling
  - *in vitro* transcription of cloned DNA

Different types of probe labeling:

- Radioactive Marking
- Non-radioactive marking

DNA Labbeling Strategies

End labeling of oligonucleotides by kinases

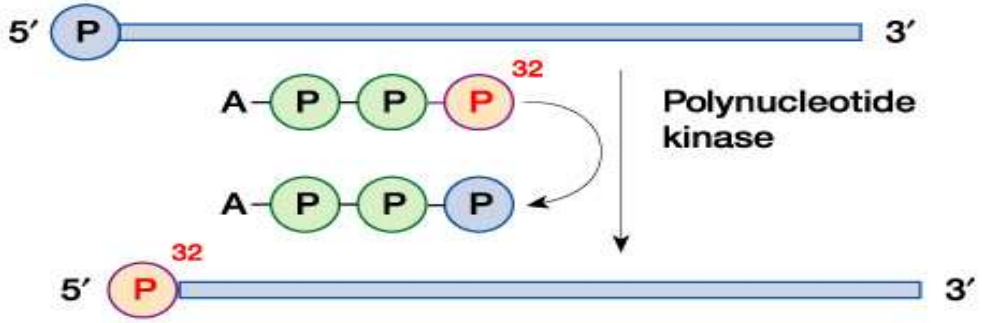


Figure 6-4 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

End-fill labeling

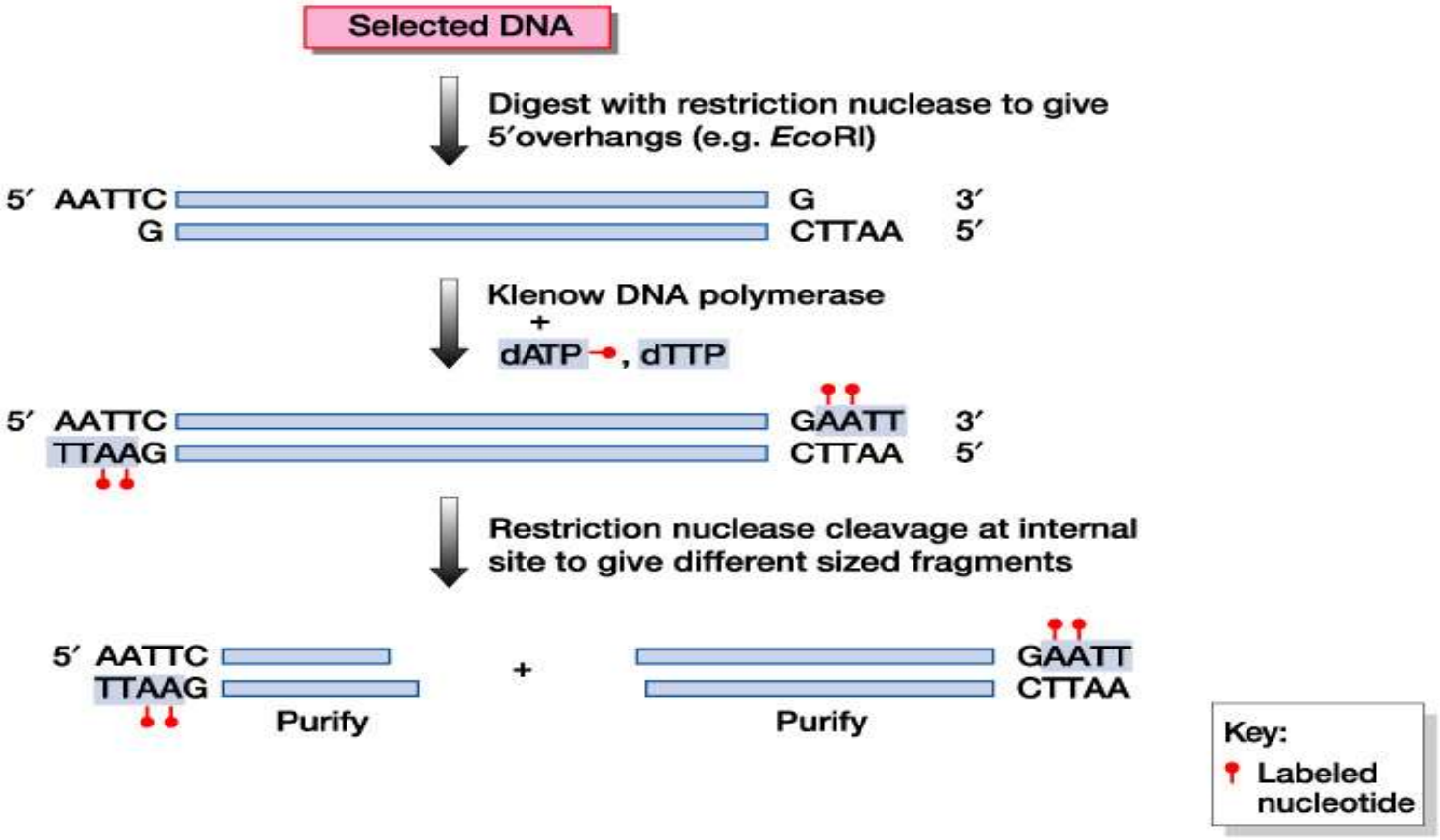


Figure 6-4 part 2 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Labeling by Nick translation

DNase generates some random single-strand breaks

At the breaks, DNA polymerase I destroys DNA by its exonuclease activity (5' 3') and synthesizes it by its polymerase activity in the presence of nucleotides, one of which is labeled

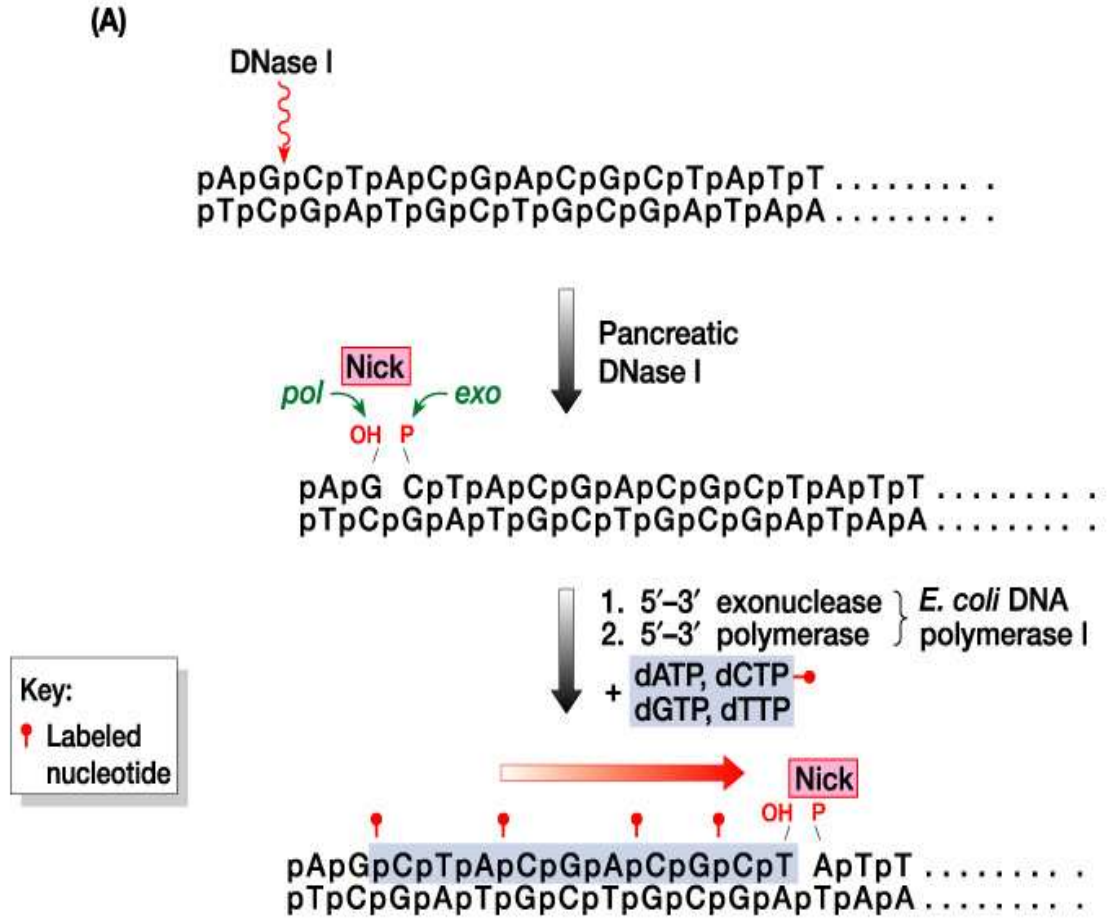


Figure 6-2 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

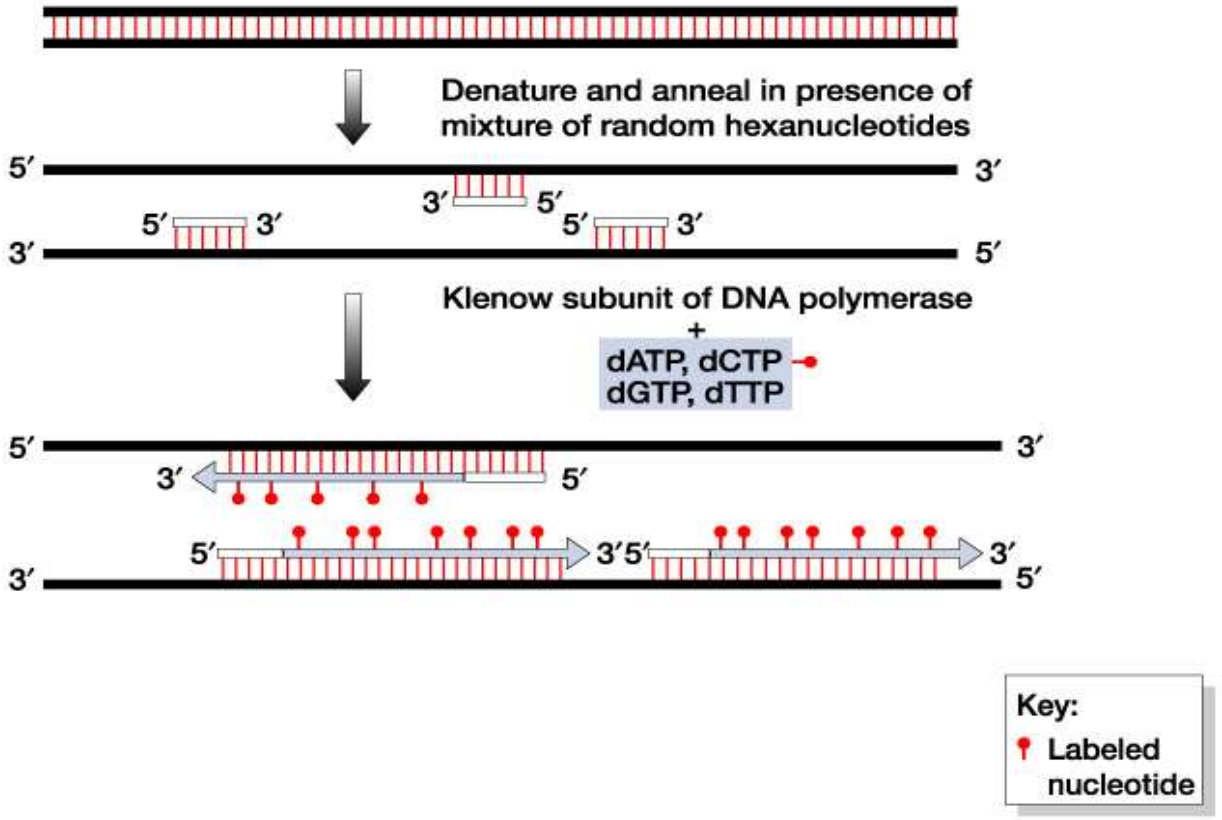
Random labeling

Use of hexanucleotides

-Addition of a cocktail of oligonucleotides (hexamers)

-Random hybridization

-Oligonucleotides serve as primers for Polymerase (Klenow fragment of Pol I) which uses free nucleotides, one of which is labeled



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RNA labeling strategies

RNA Probe Labeling

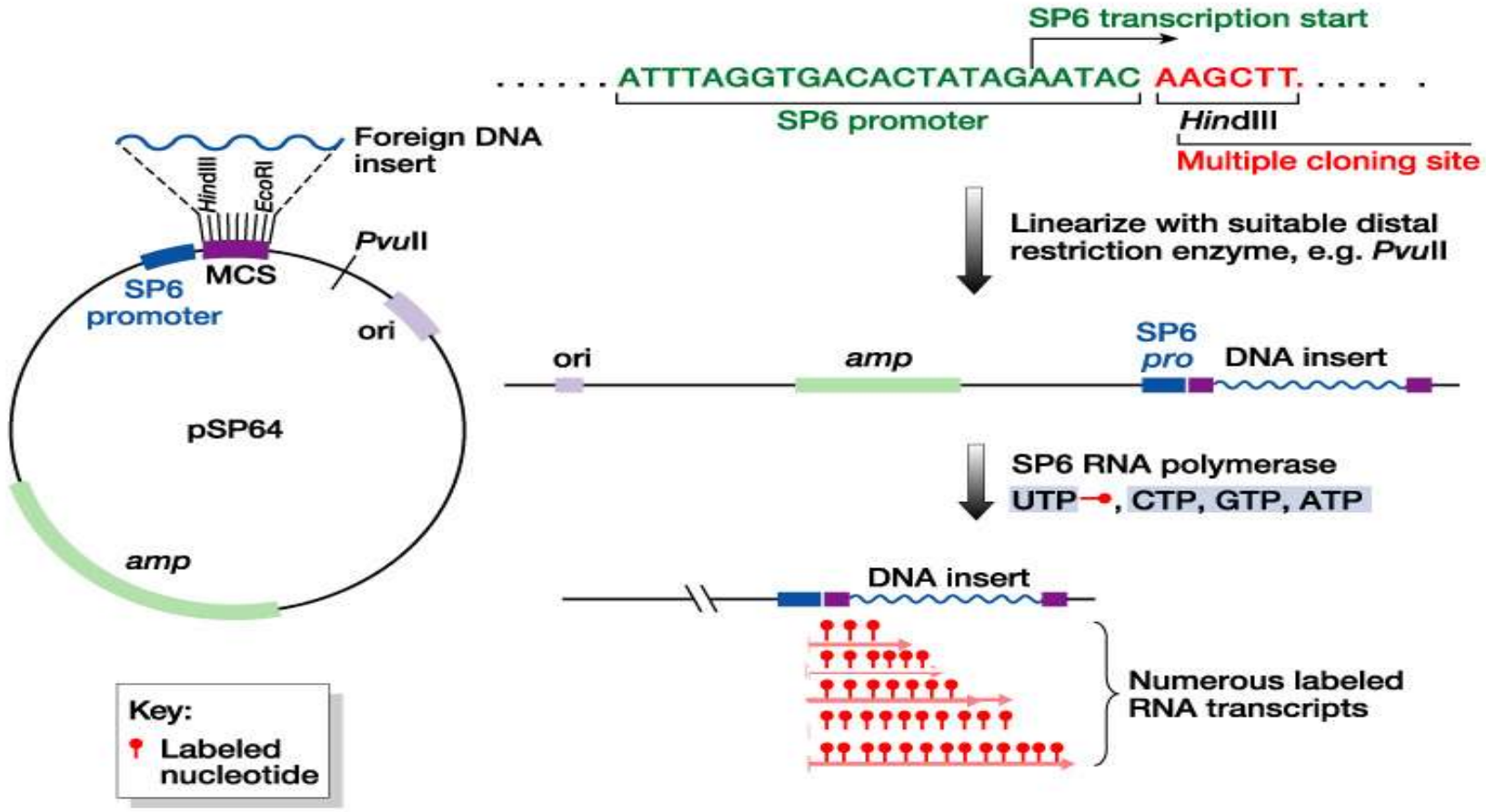


Figure 6-3 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Probe labeling

Different types of probe labeling :

- Radioactive labeling
- Non-radioactive labeling

THE PROBES

- **Radioactive marking of probes:**
  - ^{32}**P** , ^{35}S, ^{3}H, ^{14}C
  - **Warning** : radioactive probes have many drawbacks:
    - the need to protect oneself against the emitted radiation.
    - uncomfortable handling of the probes
    - rapid decay of radioactive P, hence the need to label the probes frequently
  - Advantage: high sensitivity

Characteristics of radioisotopes

Radioisotope	half-life
^{32}P	14.3 days
^{35}S	87.4 days
3H	12.4 years

THE PROBES

- **Non-radioactive labeling of probes:**

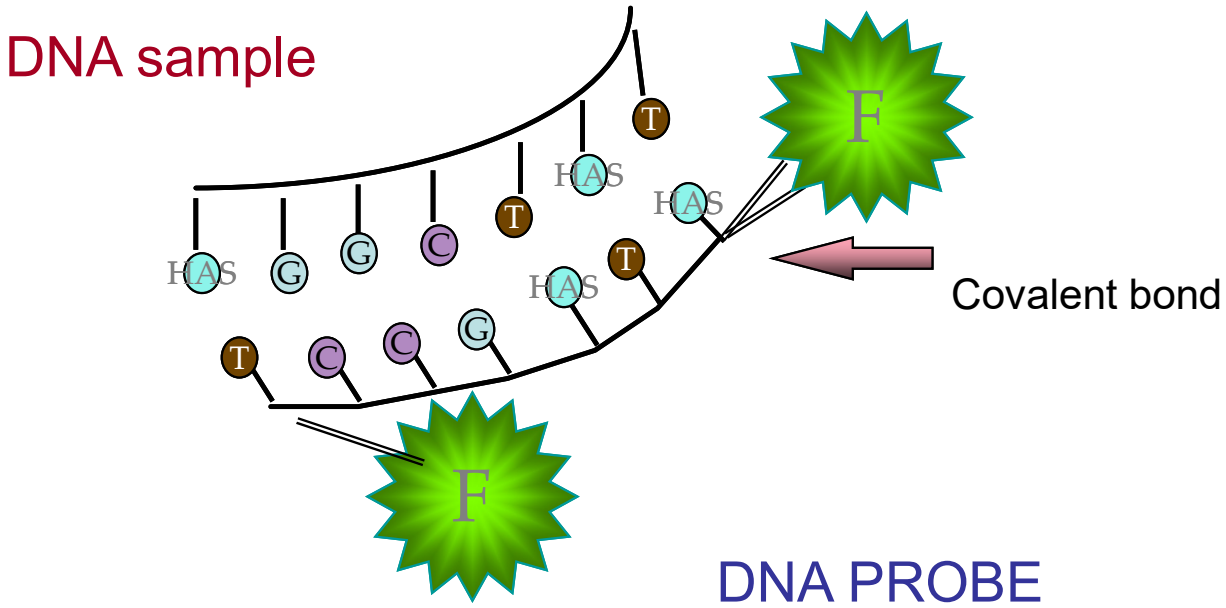
The use of non-radioactive markers offers many advantages:

  - security
  - greater stability of a probe
  - the effectiveness of the labeling reaction
  - in situ detection
  - less time taken to detect the signal

- The main types of non-radioactive labeling
  - direct labeling (e.g., labeled with a fluorophore)
  - indirect labeling (e.g., biotin, streptavidin system)

DIRECT labeling of PROBES by fluorophore:

Chemical group that fluoresces when exposed to a given wavelength: Fluorescein, Cy5, Cy3, Rhodamine, Texas Red, TAMRA, TET,



Structure of fluorophores

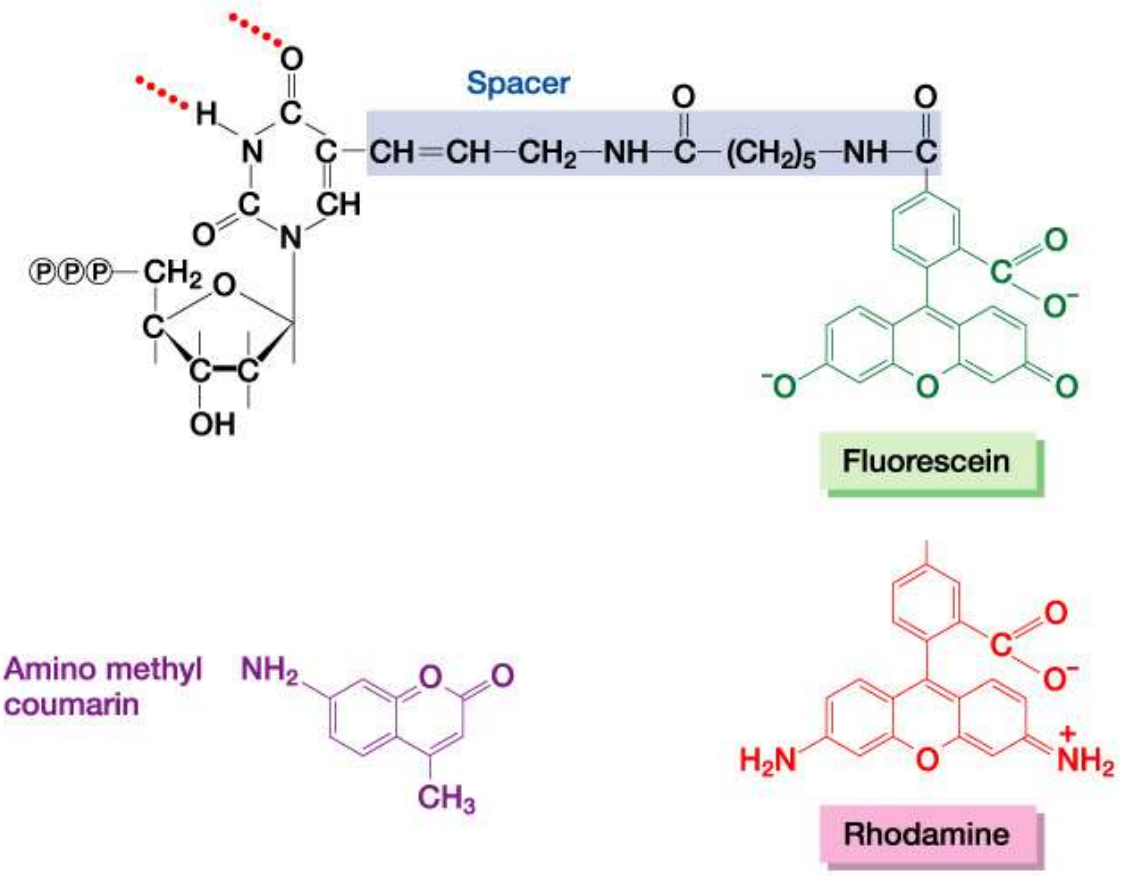
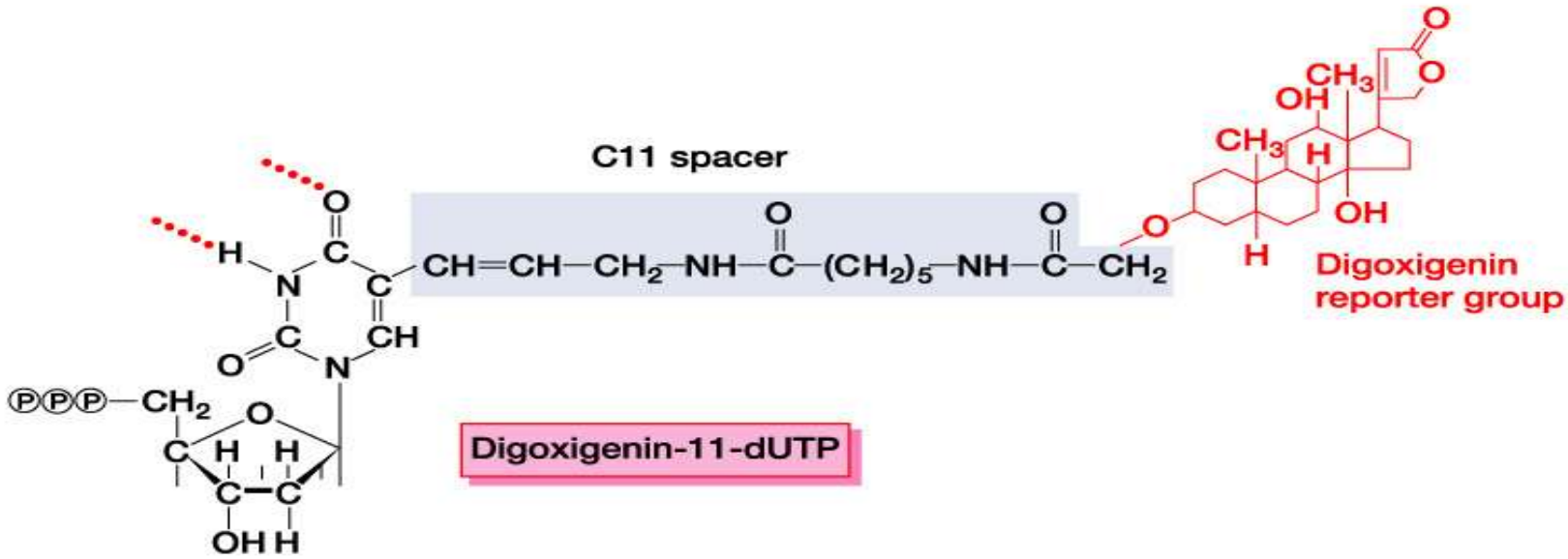


Figure 6-5 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Structure of digoxigenin-modified nucleotides



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Indirect non-radioactive labeling

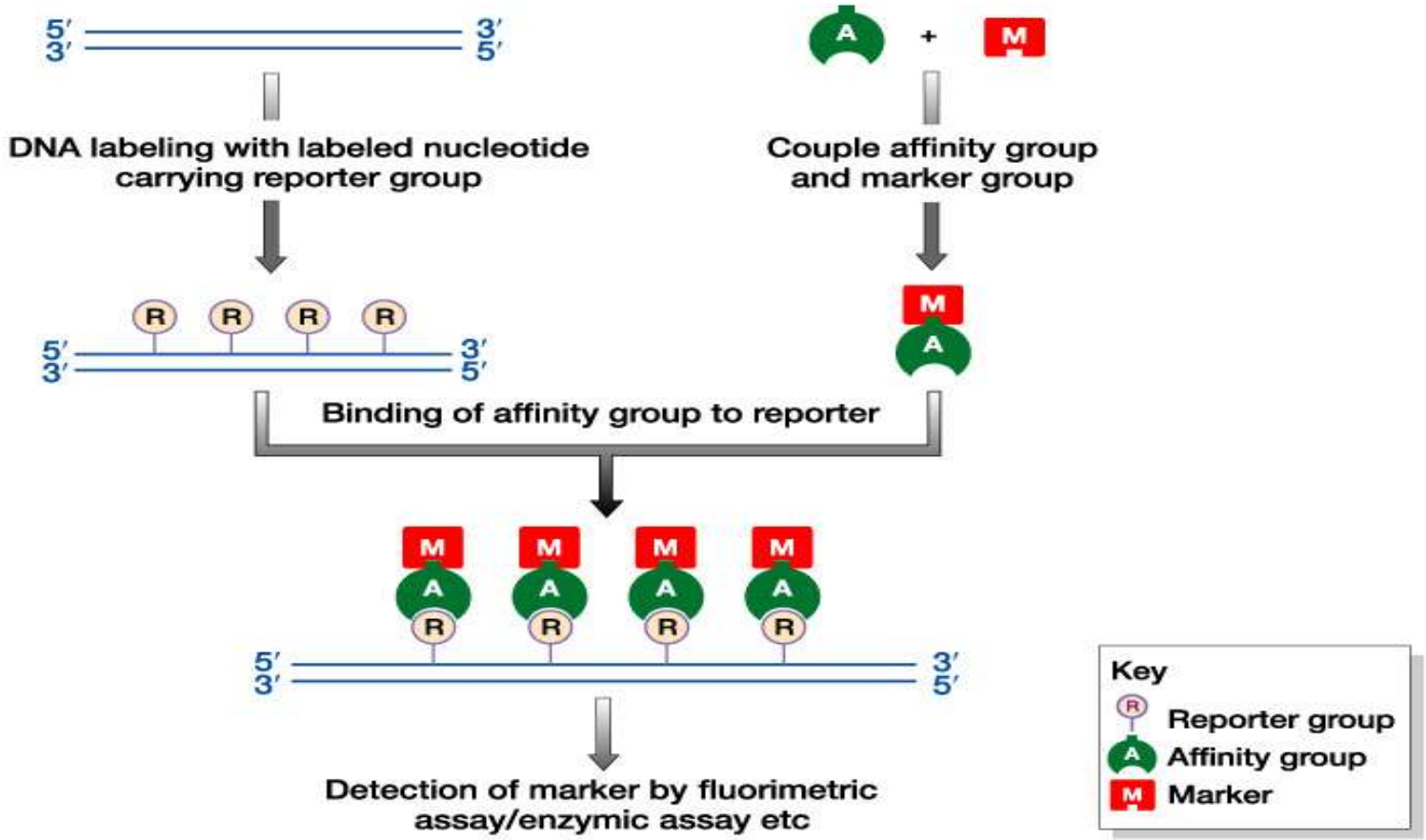


Figure 6-6 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Molecular Hybridization

Molecular Application

- FISH and Multi-FISH
- Southern and northern blot
- CGH
- Microarray (AND chips)
- Real-Time PCR
- ……

In situ hybridization (FISH)

In situ hybridization on chromosome:

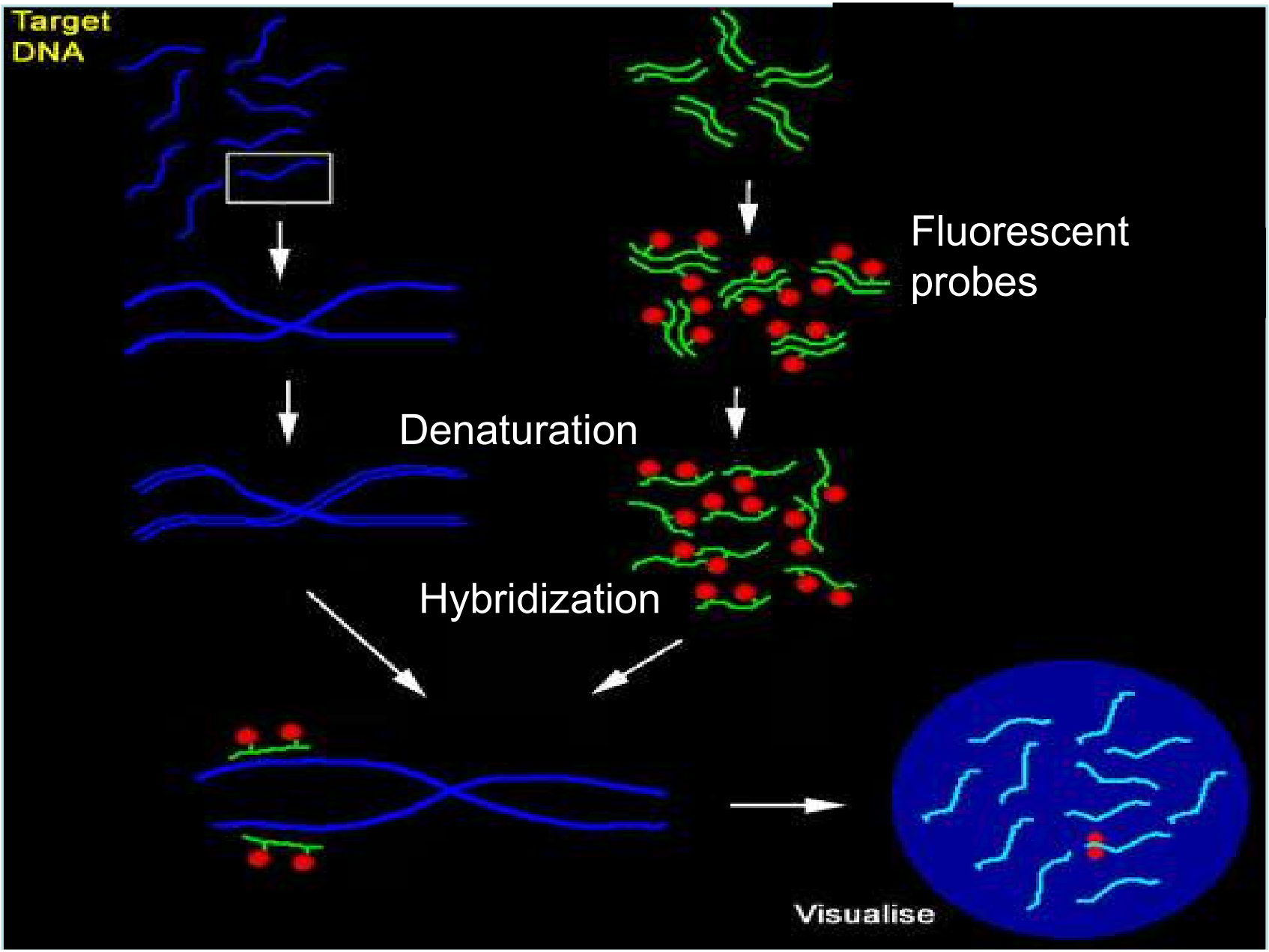
- **Objective** : Hybridization of fluorescent probes onto metaphase chromosomes

- Applications

– Search for genome rearrangements

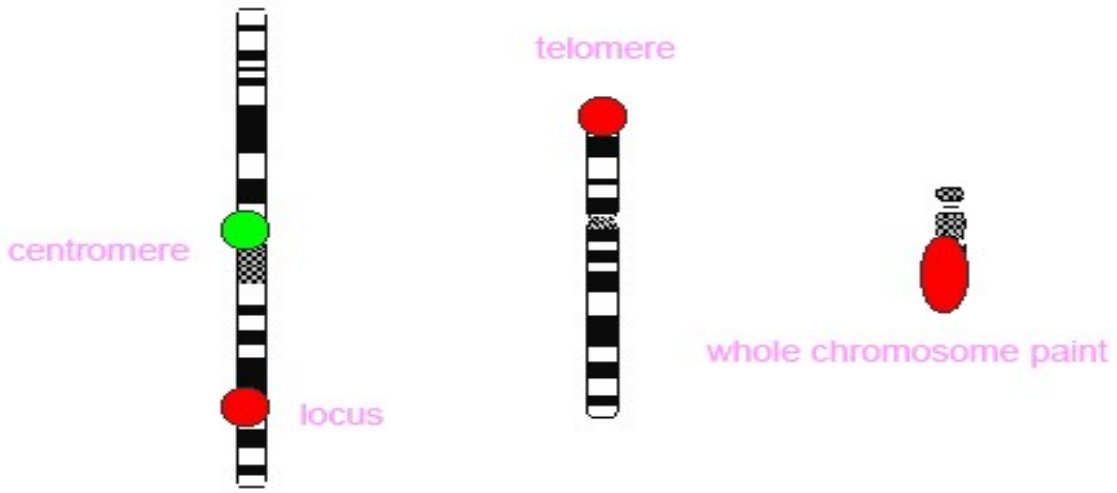
– Gene localization

– …

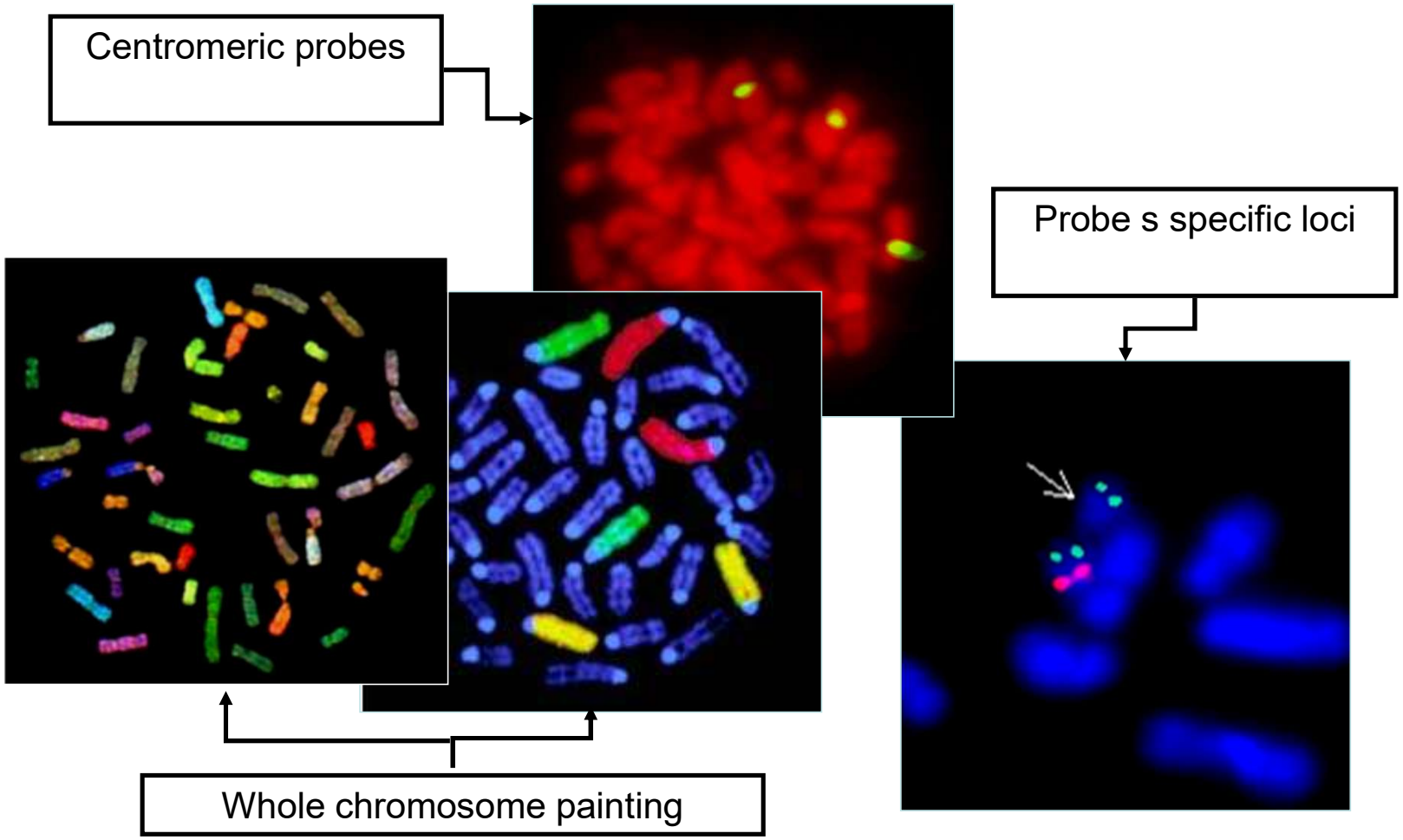

Target
DNA
Fluorescent
probes
Denaturation
Hybridization
Visualise

Types of probes for FISH

- Probes for centromere detection
- Probes for telomere detection
- Probes for “Whole chromosome paint”
- Probes for locus detection



Types of probes

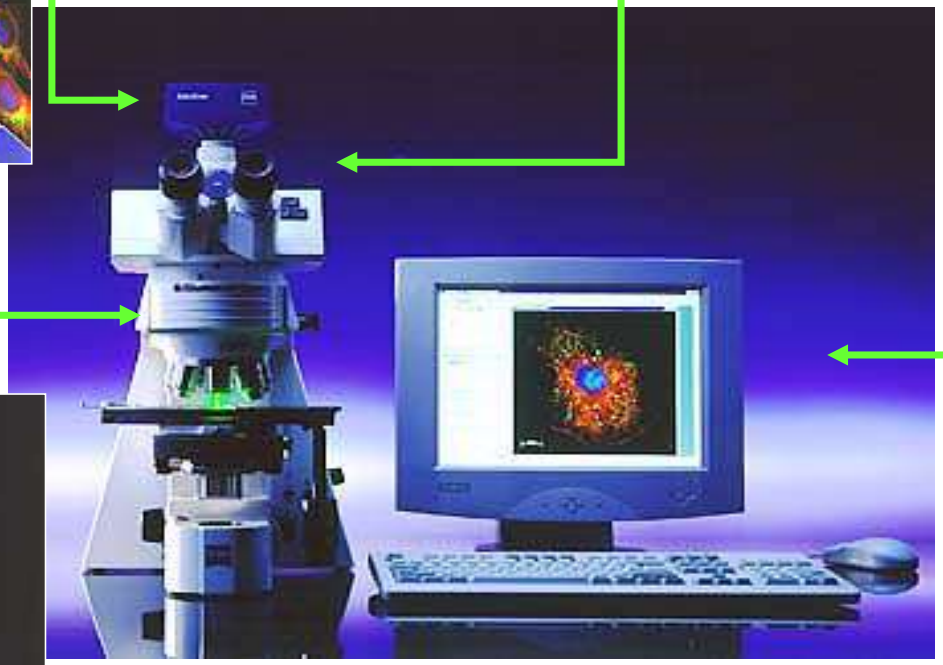


Visualizing hybridization

- The DNA probe is labeled with a colored fluorescent molecule.
- This fluorescent molecule remains attached to the DNA during the hybridization process.
- The molecule emits a particular color when viewed through a fluorescence microscope equipped with the appropriate filters.

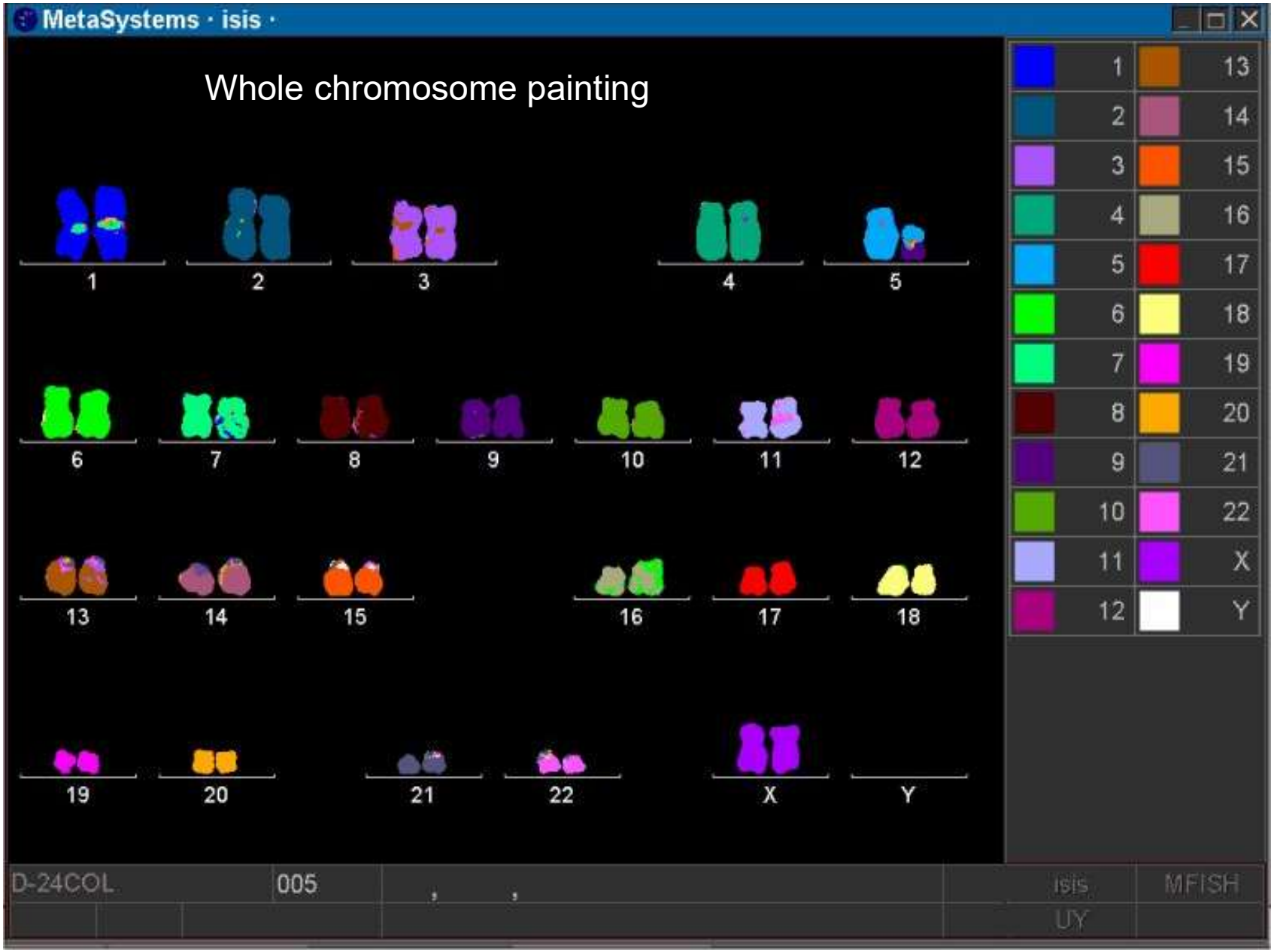
CCD Camera

Fluorescence Microscope



Filters

Analysis Software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