In Situ Hybridization

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Outline

- ISH
- Probe
- Labeling Techniques
- FISH
- CISH

Introduction

In Situ Hybridization

natural or original position

pairing of complementary RNA or DNA to produce a double stranded nucleic acid

Introduction

- In situ hybridization (ISH) detection of specific DNA or RNA sequences in tissue sections or cell preparations using a labeled probe under appropriate conditions.

- This probe will hybridize the target DNA or RNA which will be visualized by radioactive or non-radioactive labels incorporated into the probe .

Introduction

- The first ever ISH protocol to visualize nucleic acids was described by Gall and Pradue (1969), and it was based on radiolabeled RNA probes to detect DNA sequences.

- In 1977, in situ applications used RNA probes marked with a fluorophore, called fluorescent ISH (FISH), to detect chromosomal targets.

- Few years later, chromogenic-based approaches were used with biotin and digoxigenin.

Probe

- A probe (a labeled complementary single strand) is incorporated with the DNA/RNA strands of interest.
- Strands will anneal with complementary nucleotides bonding back together with their homologous partners when cooled.
- Chances of a probe finding a homologous sequence other than the target sequence decrease as the number of nucleotides in the probe increases

Probe Types and their Advantages and Disadvantages

Probe Types	Advantages	Disadvantages
Double-stranded DNA (dsDNA) probes	Stable, available, easier to obtain	Self-hybridize, less sensitive, need denaturation before hybridization
Single-stranded DNA (ssDNA) probes	Stable, easier to work with, more specific, resistant to RNases, better tissue penetration, without self-hybridize	Time consuming, expensive
RNA probes (riboprobes)	Higher thermal stability, better tissue penetration, more specific, low background noise by RNase	Sensitive to RNases
Synthetic oligonucleotides	Economical, stable, available, easier to work with, more specific, resistant to RNases, better tissue penetration, better reproducibility	Know the information of nucleotide sequence Probes

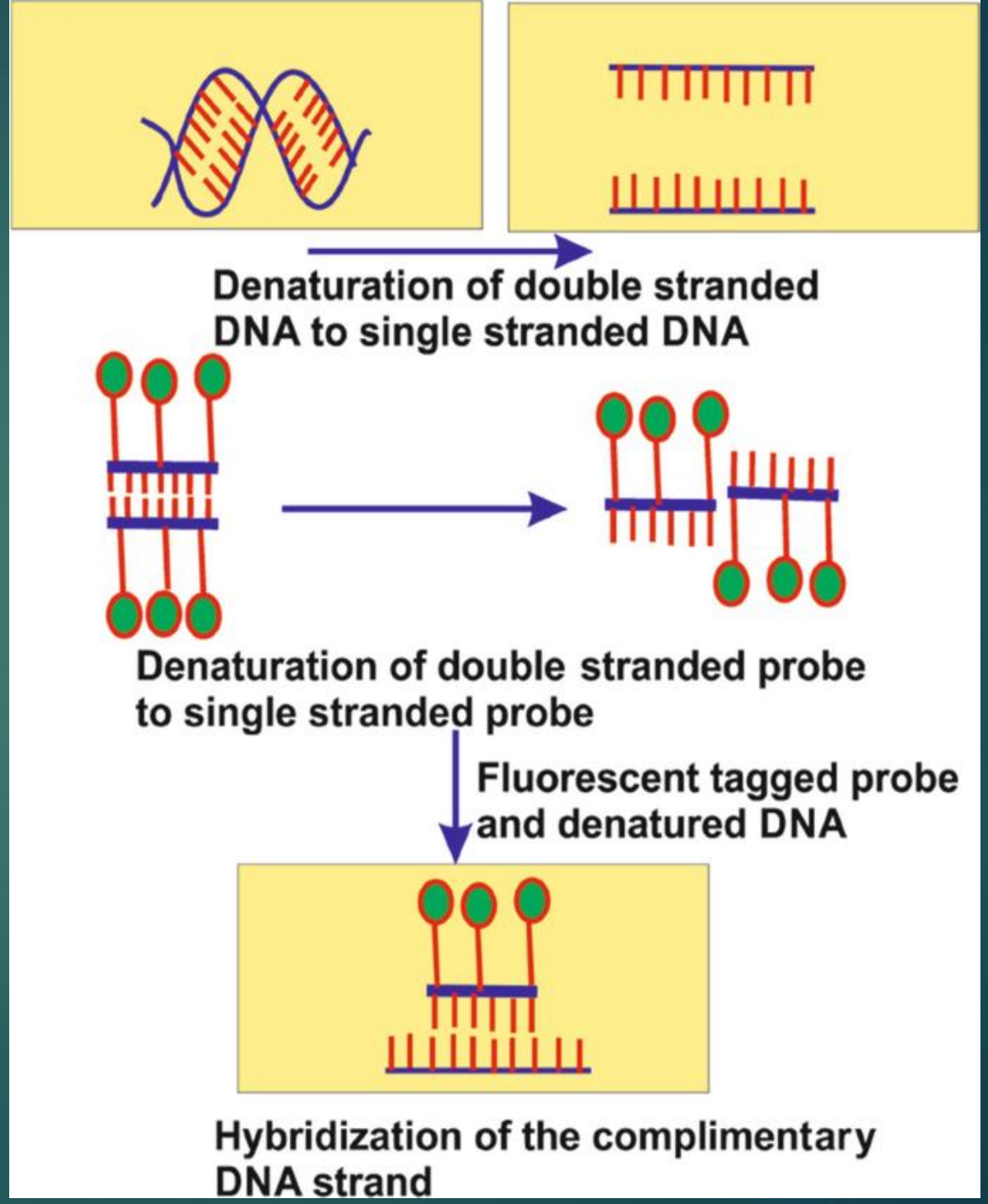
Labeling Techniques

- Radioactive probes are labelled with the radioactive isotopes of sulphur, phosphorus or nitrogen for detection.
- Nonradioactive probes are the ones that are labelled with chemical tags or fluorescent molecules such as biotin, fluorescein and digoxigenin.
- Non-radioactive detection methods offer several advantages over the usual radioactive methods.
- Non-radioactive detection methods eliminate the need to deal with the licensing, waste disposal, and safety concerns associated with the use radioactive material.

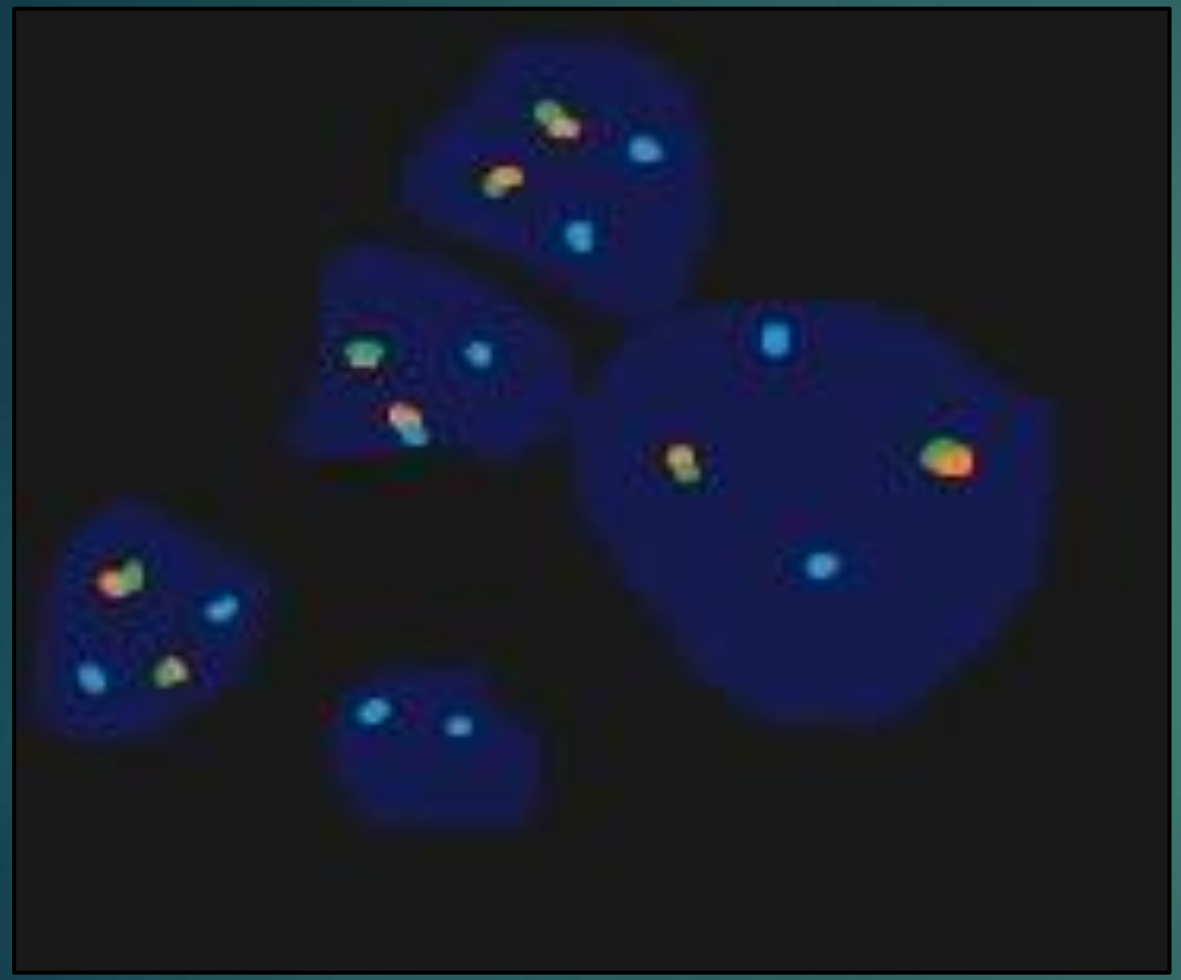
Types of ISH

- Today there are two basic ways to visualize RNA and DNA targets: fluorescence in situ hybridization (FISH) and chromogenic in situ hybridization (CISH).
- Characteristics inherent in each method of detection have made FISH and CISH useful for very distinct applications.
- While both use a labeled, target-specific probe that is hybridized with the sample, the instrumentation used to visualize the samples is different for each method.

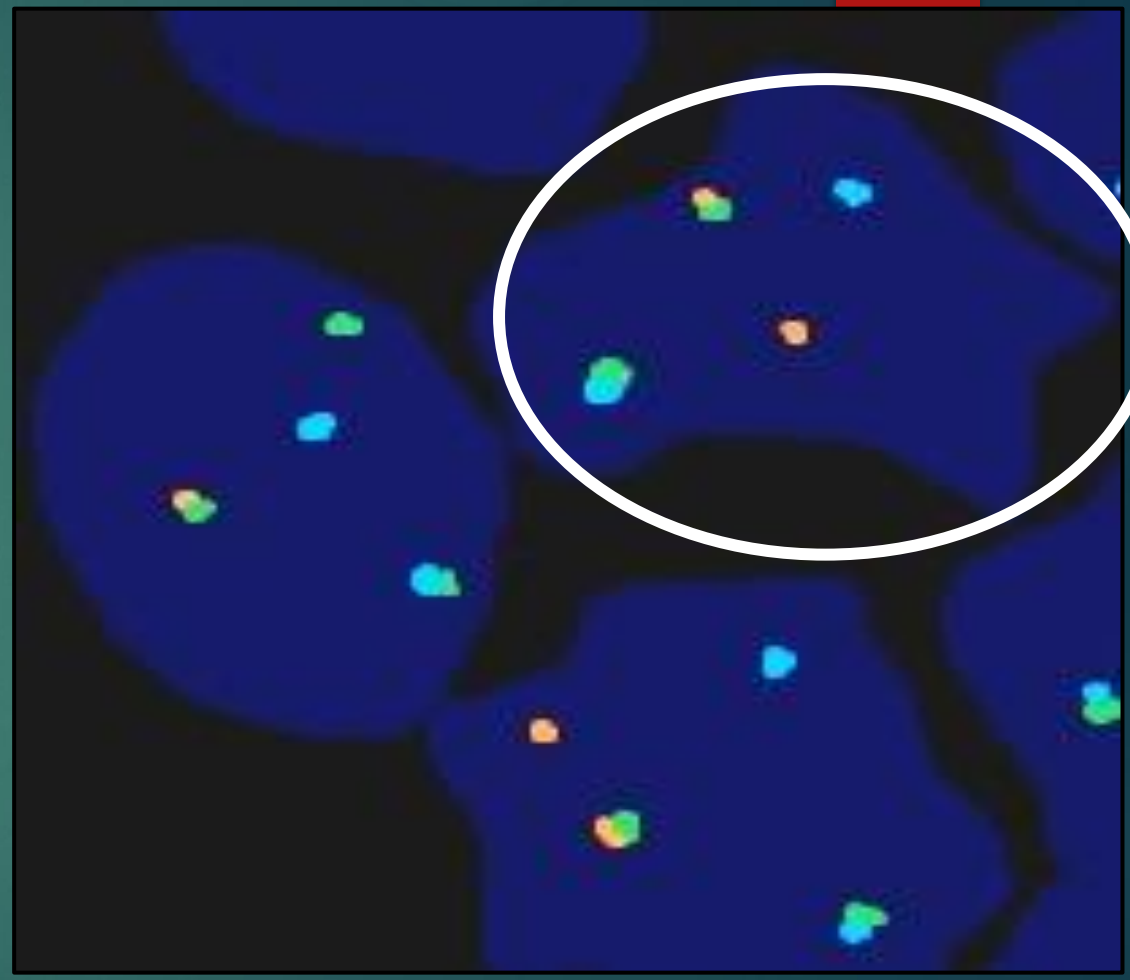
FISH steps


Denaturation of double stranded DNA to single stranded DNA
Denaturation of double stranded probe to single stranded probe
Fluorescent tagged probe and denatured DNA
Hybridization of the complimentary DNA strand

FISH result



Normal ERG and TMPRSS2 genes



TMPRSS2-ERG GEN FUSION

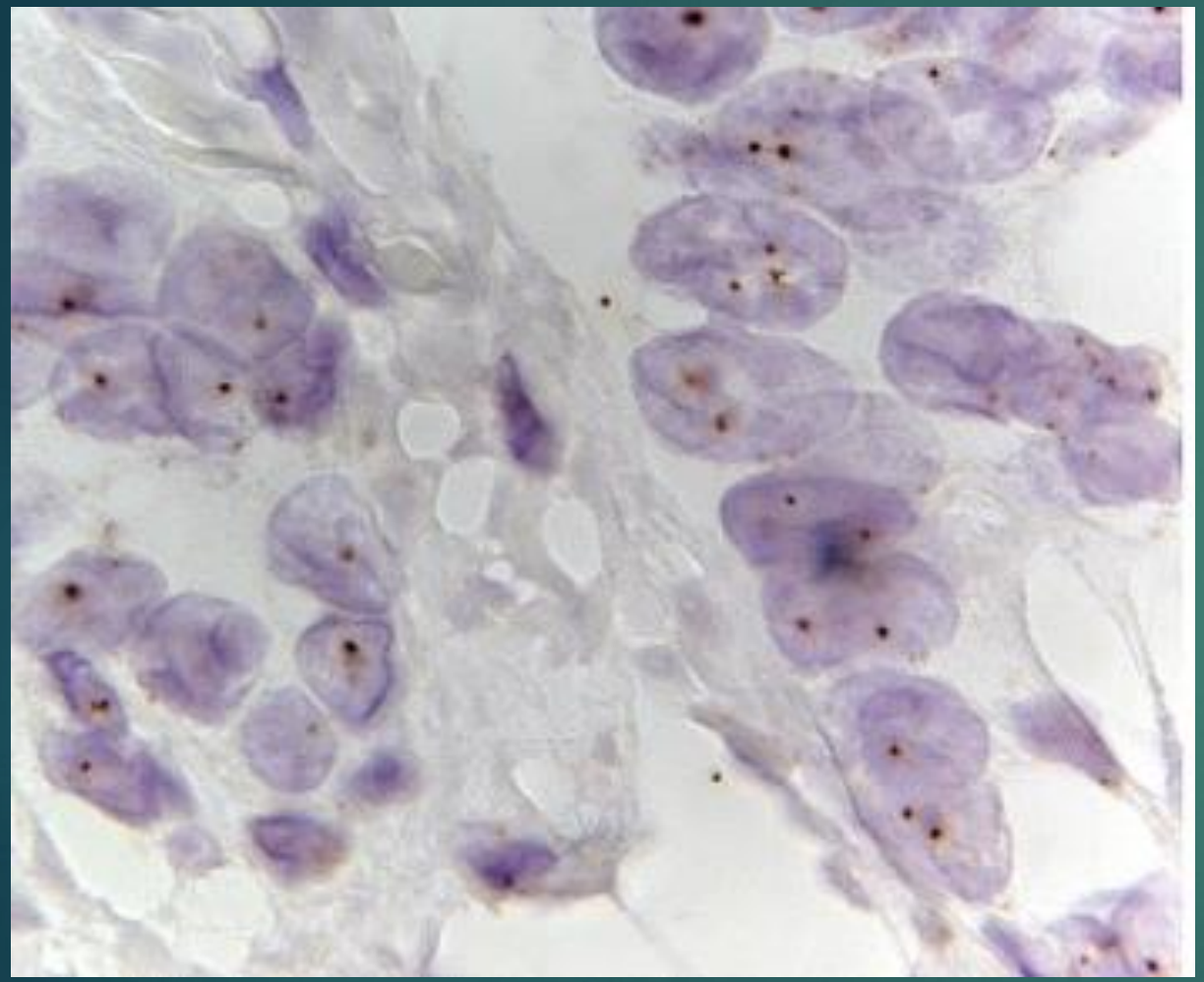
CISH

- Chromogenic in situ hybridization (CISH) is a cytogenetic technique that combines the chromogenic signal detection method of immunohistochemistry (IHC) techniques with in situ hybridization.

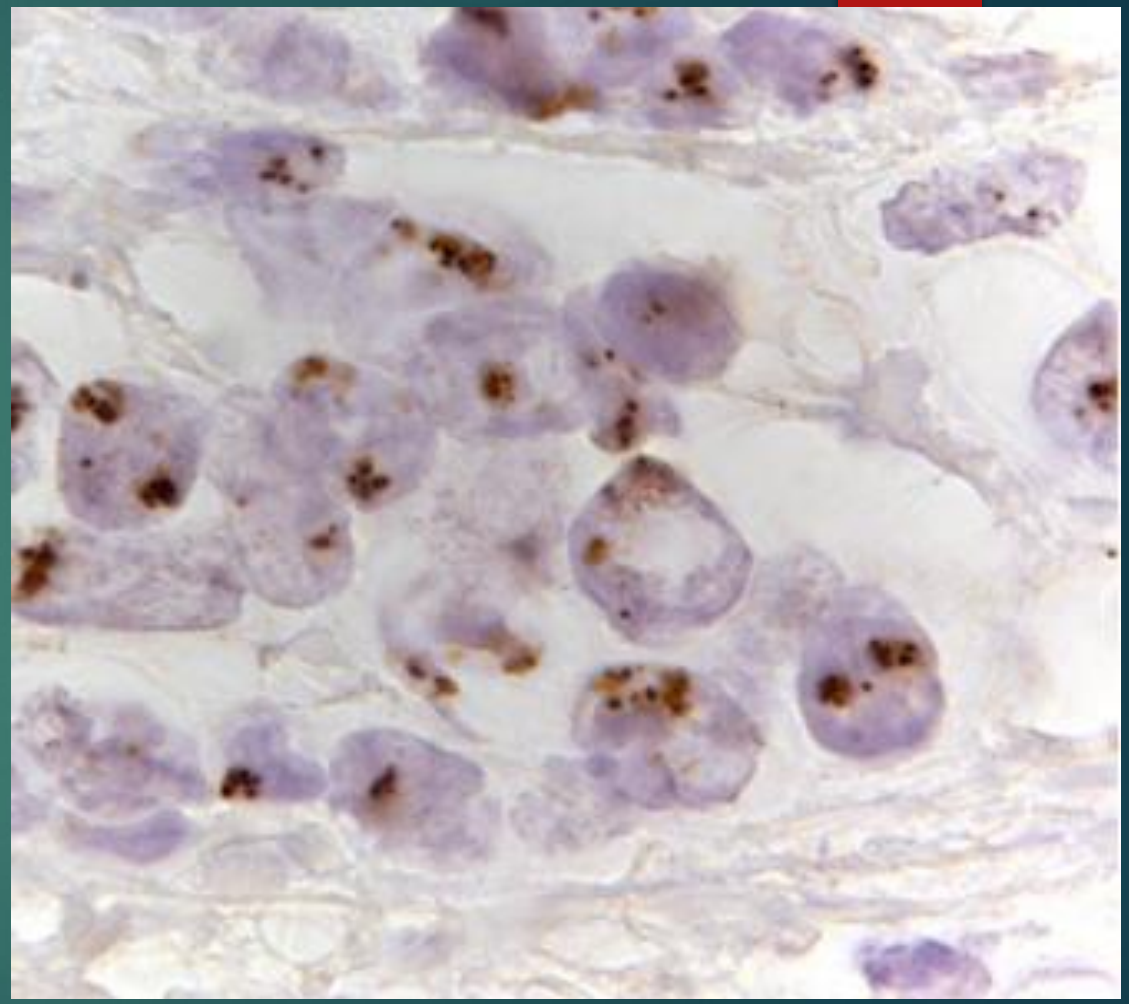
- CISH is similar to FISH in that they are both in situ hybridization techniques used to detect the presence or absence of specific regions of DNA.

- However, CISH is much more practical in diagnostic laboratories because it uses bright-field microscopes rather than the more expensive and complicated fluorescence microscopes used in FISH.

CISH result

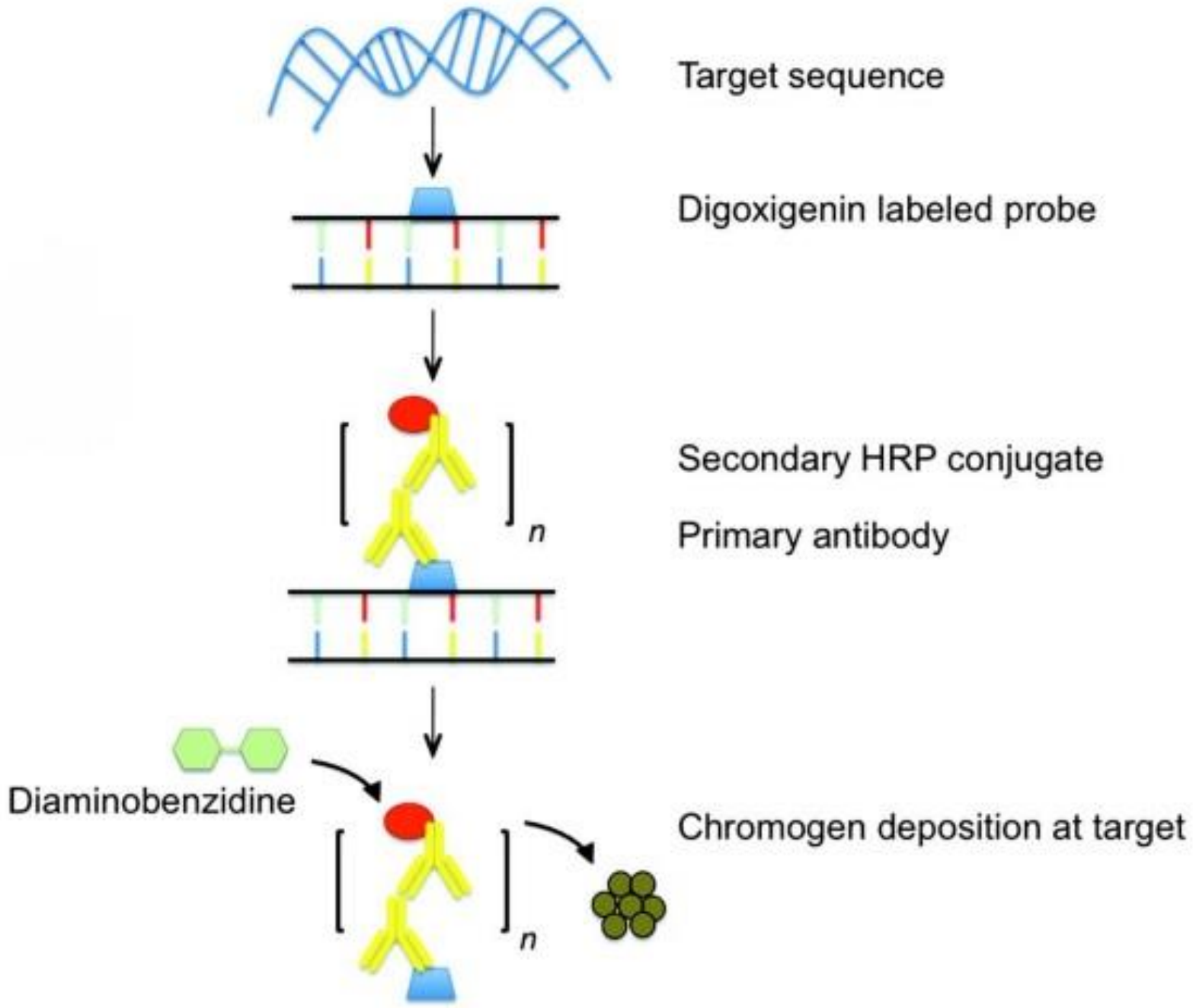


normal HER2 copy number



high level HER2 gene amplification

CISH steps


Target sequence
Digoxigenin labeled probe
n
Secondary HRP conjugate
Primary antibody
Diaminobenzidine
n
Chromogen deposition at target

General Procedure

Tissue Prep

- Fix tissue to a glass slide using paraffin
- Wash and heat slide several times to remove paraffin from surface
- Pepsin digestion to ensure access to target DNA sequence

Hybridi-zation

- Add 10-20 µL probe to tissue, cover slide, and seal coverslip
- Heat slide to 97℃ for 5-10 min to denature DNA
- Place slide in 37℃ oven overnight for probe to hybridize

Blocking

- Add a blocker to bind nonspecific binding sites
- Add hydrogen peroxide to suppress endogenous peroxidase activity

Probe Detectio-n

- If digoxigenin is the label: add anti-digoxigenin fluorescein primary antibody followed by a HRP-conjugated anti-fluorescein secondary antibody
- Add DAB which is converted to a brown precipitate by HRP Probe Detection

FISH versus CISH

- FISH is considered to be the gold standard for the detection of chromosomal abnormalities because it is very sensitive and has high resolution
- Other techniques that are developed to detect chromosomal abnormalities are usually compared to the sensitivity and specificity of FISH to see how they measure up.
- CISH is much cheaper and is easier to use because it uses bright-field microscopes instead of fluorescence microscopes.

FISH versus CISH

- In addition, the CISH reagents are more stable than the FISH reagents so it is possible to store the samples and examine the same sample multiple times.
- FISH reagents fade over time due to photobleaching so a sample can only be examined once.
- Apart from the expensive fluorescence microscope, FISH also requires a high-resolution digital camera to capture micrographs of the sample before the fluorescence fades.

FISH versus CISH

- The tissue or cell sample as a whole can be visualized through CISH whereas cell morphology is difficult to assess using fluorescence microscopy in FISH.


Applications of
in situ hybridization
Antibody validation
Gene expression analysis
Disease mechanism
Target distribution
Exogenous nucleic acid distribution
Xenotransplant localization
Mechanism of toxicity
Infectious agent localization
Patient selection
Tissue biomarker