RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

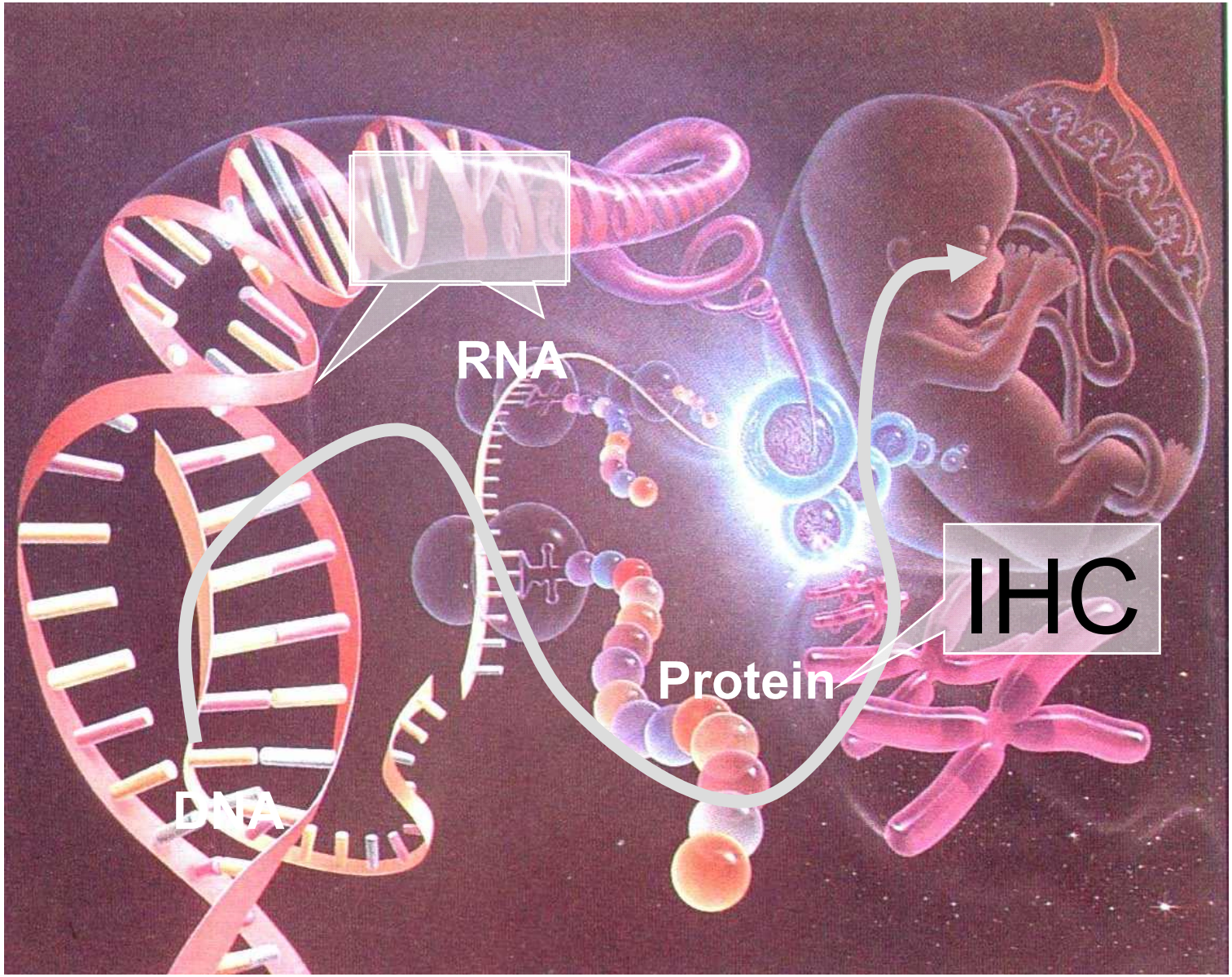
In situ Hybridization

To identify a specific genes (DNA or RNA) in intact cells, tissues or even whole animals.

In situ = Inside (cell/tissue)

Hybridization = Specific Binding of a Probe

Detection = Visible Reaction


RNA
DNA
Protein
IHC

In situ Hybridization

ISH -

Detection of specific nucleic acid sequences (signatures) within cells and tissues by “hybridizing” a complementary probe.

Uses -

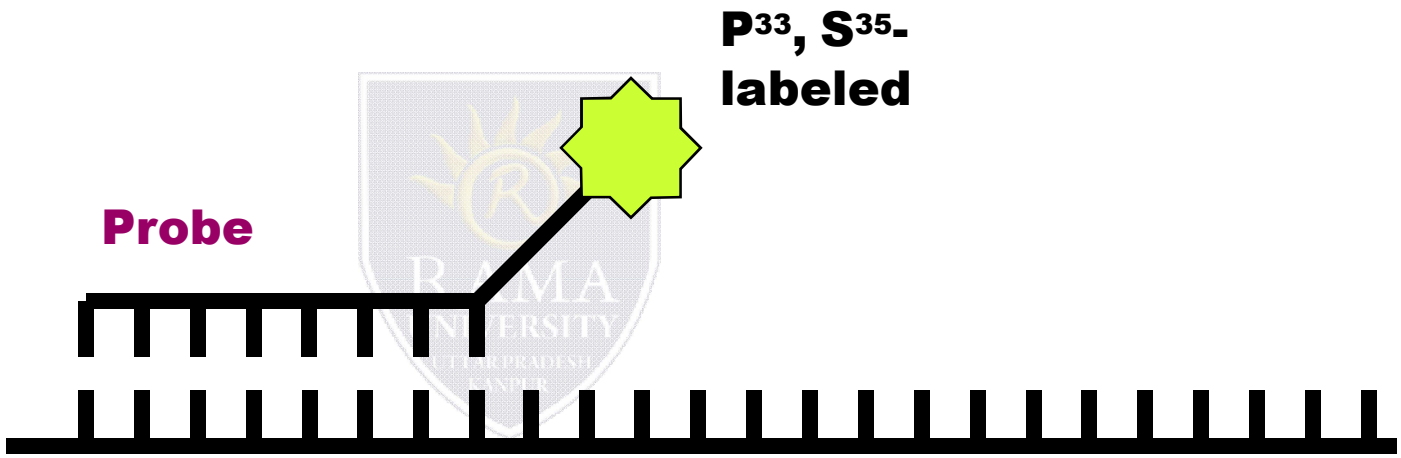
Finding pathogens, a specific gene, a mutant gene, cells that have certain genes switched on.

In situ Hybridization

- **Radioactive in situ hybridization**
  - (simple but time consuming and hazardous)
- **Fluorescent in situ hybridization**
  - (simple, quick but short-lived results)
- **Colorimetric in situ hybridization**
  - (simple, quick and long-lived results)

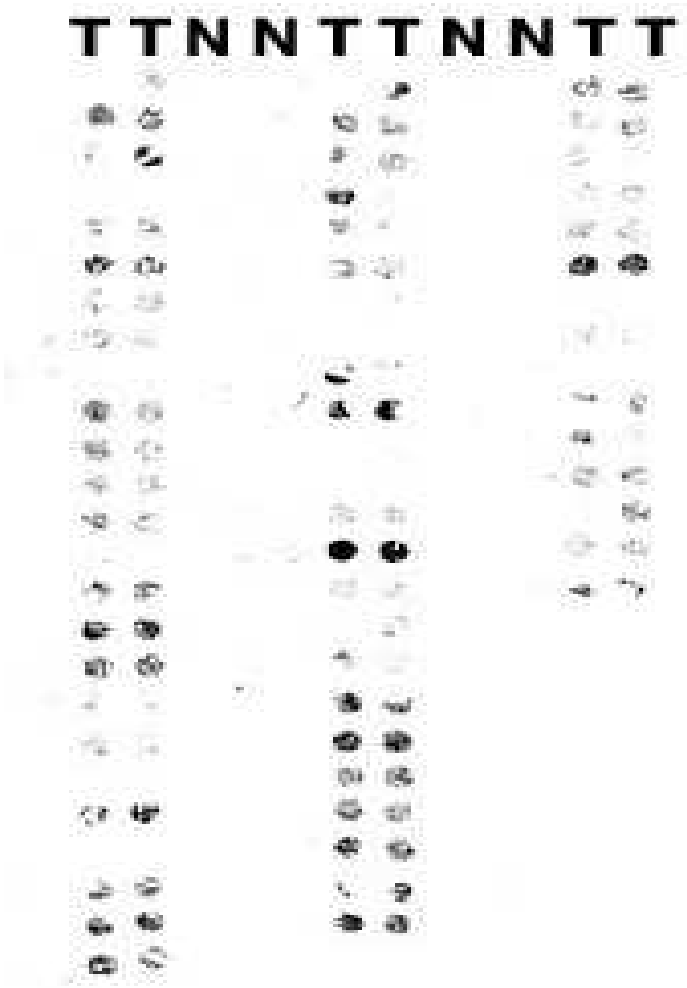
Radioactive ISH Protocol Summary

- Dewax Slides
- Permeabilize, target retrieve *& Post-fix*
- *Denature and Hybridize radiolabeled-Probe*
- *Post-Hybridization Washes*
- Counterstain
- Photographic emulsion
- Expose for days to weeks
- Develop
- Read



Target Gene

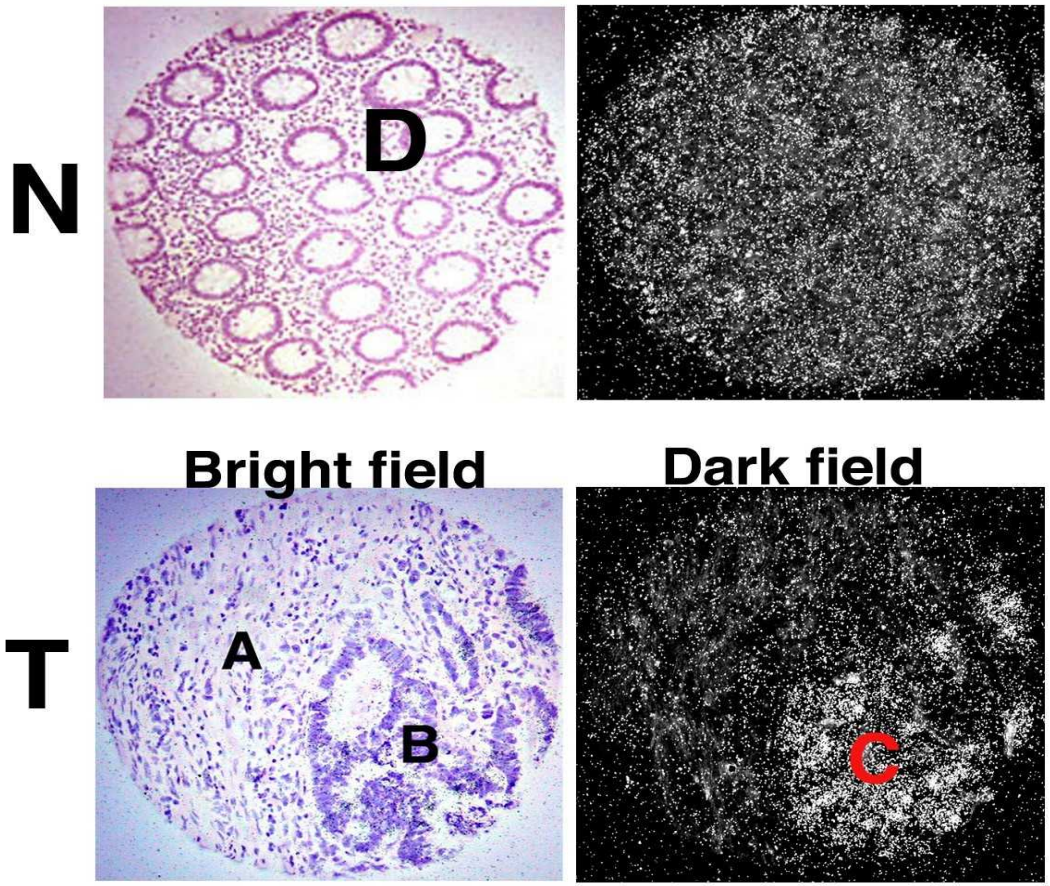
(DNA)



Radioactive in situ Hybridization in

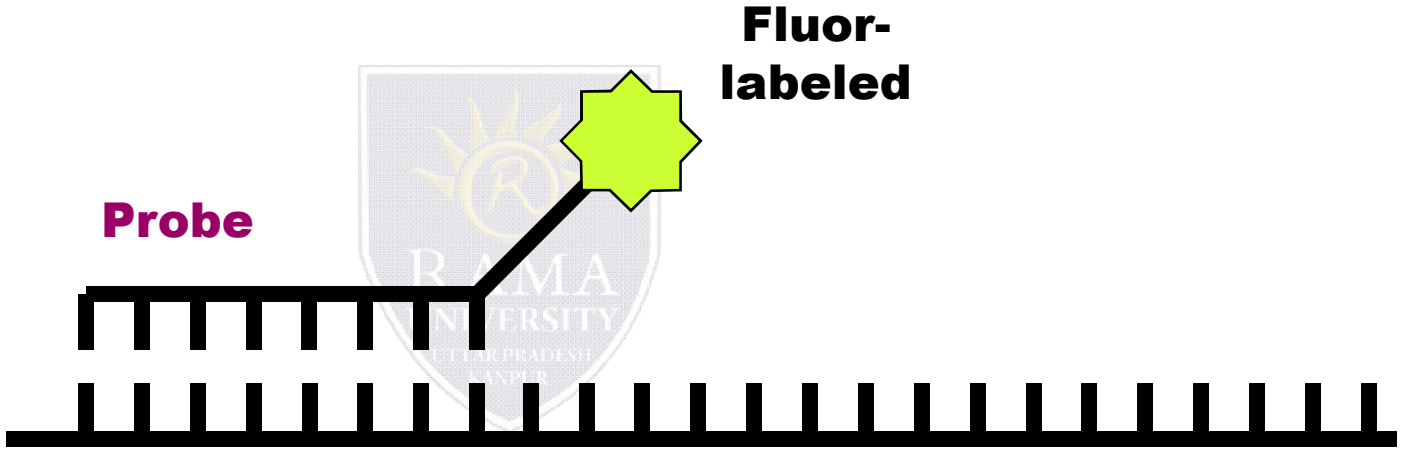
Normal and Tumor cells

Radioactive ISH



Fluorescent ISH Protocol Summary

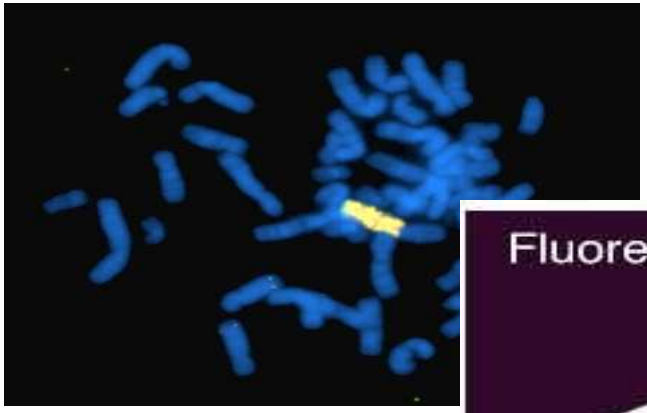
- **Dewax Slides**
- **Permeabilize, target retrieve *& Post-fix***
- ***Denature and Hybridize fluorescent labeled-Probe***
- ***Post-Hybridization Washes***
- **Counterstain**
- **Fluorescence microscopy**

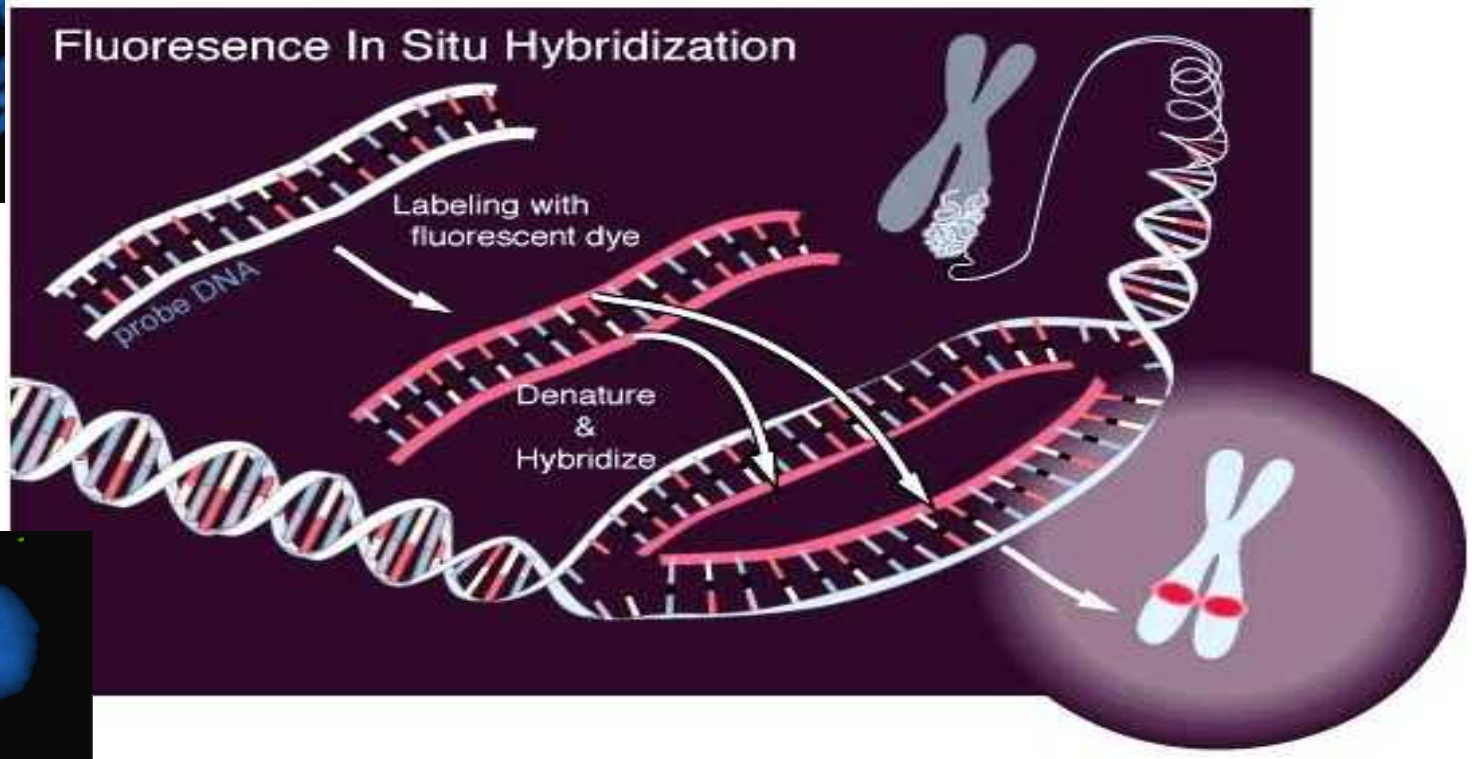


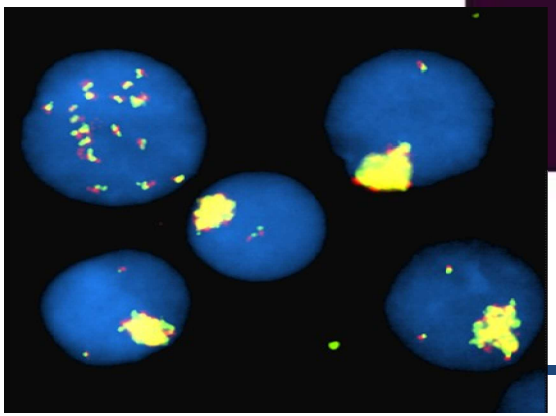
Target Gene

(DNA)

FISH




Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize



Fluorescent ISH (usually for DNA targets)

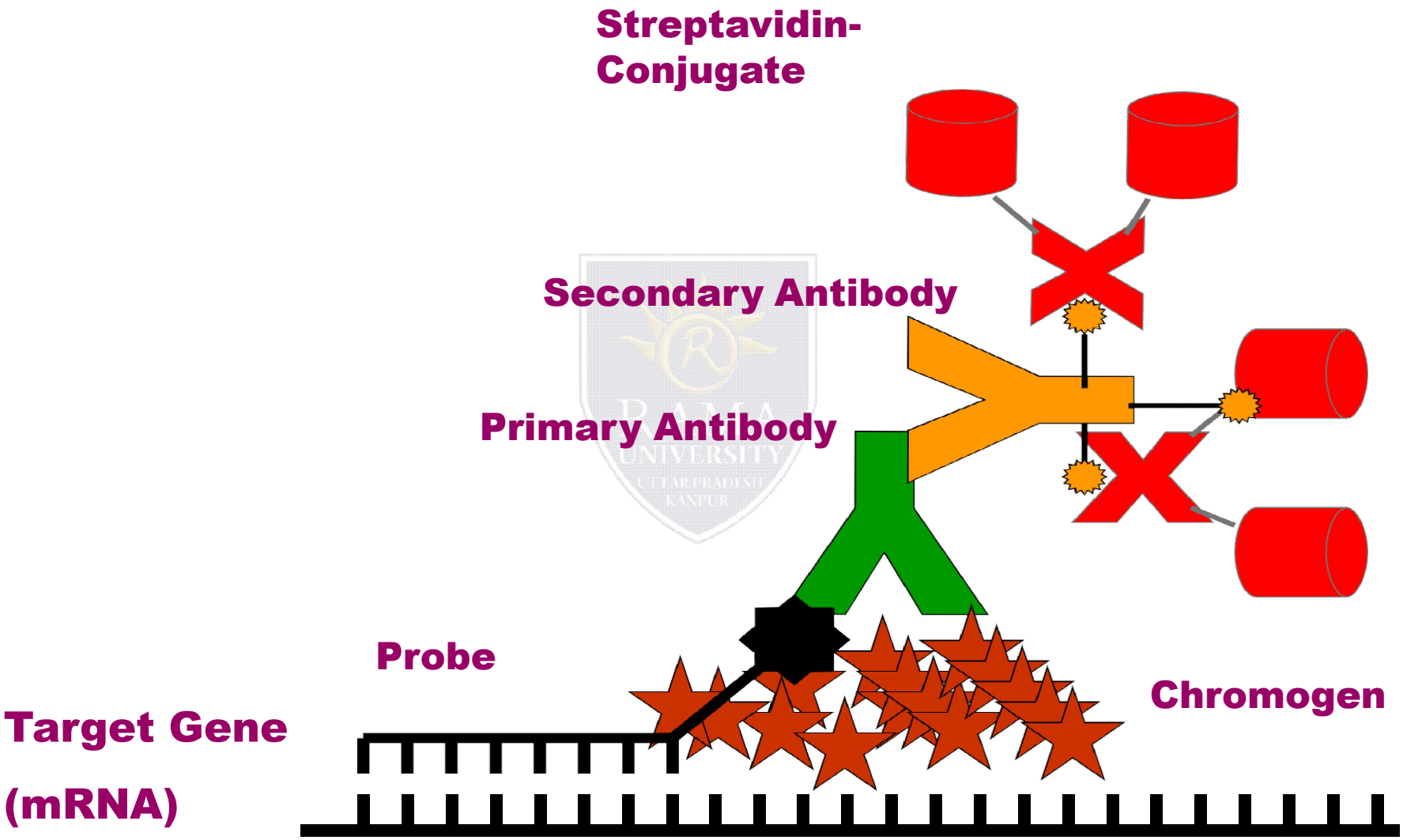
C-ISH v/s FISH

- FISH
  - Mostly DNA detection
  - DNA located in the nucleus
  - Fluorescent end-point
  - Cannot be archived
  - Hard to read morphology
  - Can detect multiple genes simultaneously
  - DNA does not degrade

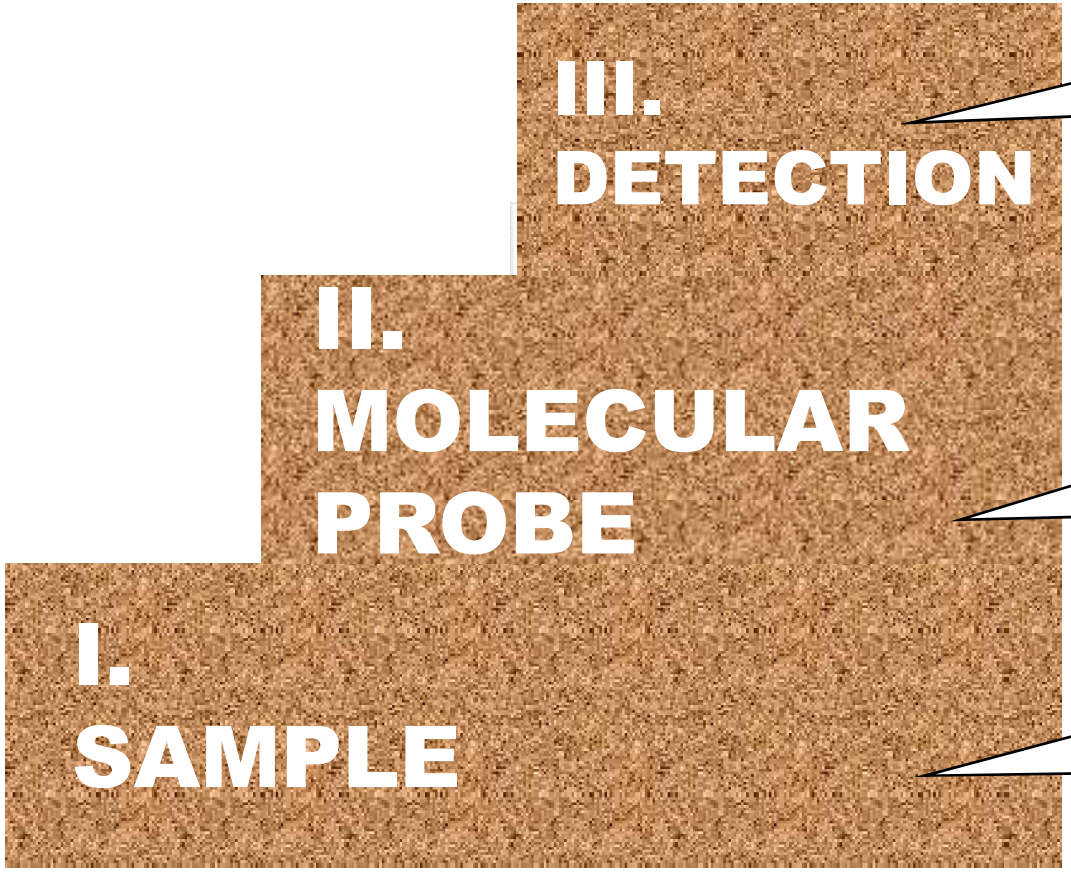
CISH

- Detect mRNA and DNA
- DNA located in the nucleus
- RNA located in the cytoplasm
- Colored end-point
- Can be archived
- Greater comfort level for pathologists
- Cannot detect more than 2 genes
- RNA degrades easily

Colorimetric In situ hybridization



In situ assays:
Three main variables



Least important

Medium importance

Most important