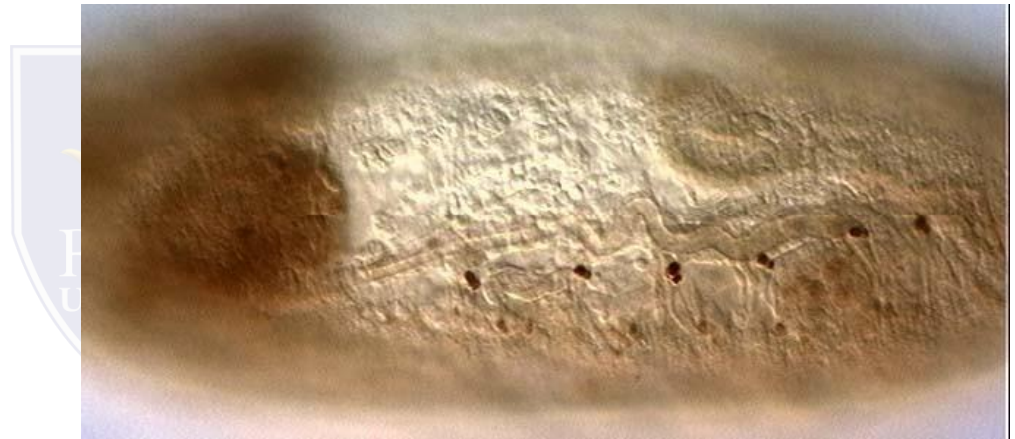




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DEPARTMENT OF BIOTECHNOLOGY

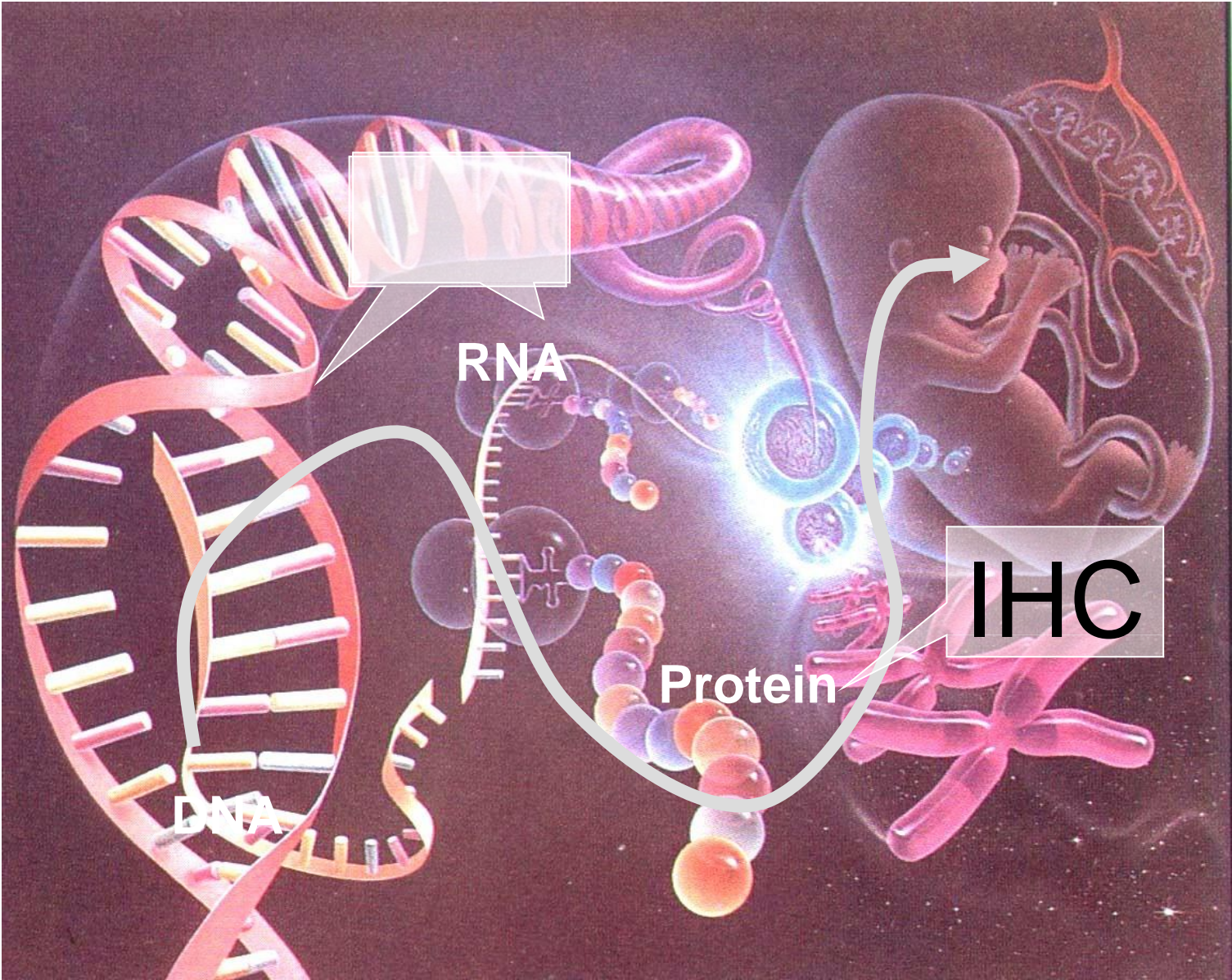
In situ Hybridization

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



In situ
Hybridization
Detection

= Inside (cell/tissue)
= Specific Binding of a Probe
= Visible Reaction



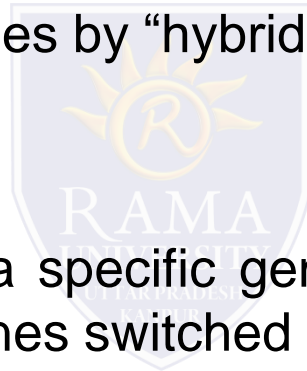
In situ Hybridization

ISH -

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

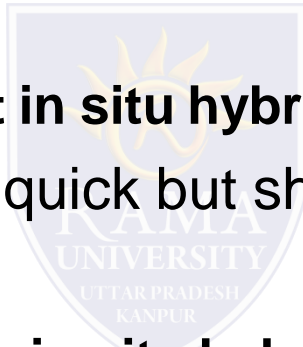
Uses -

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



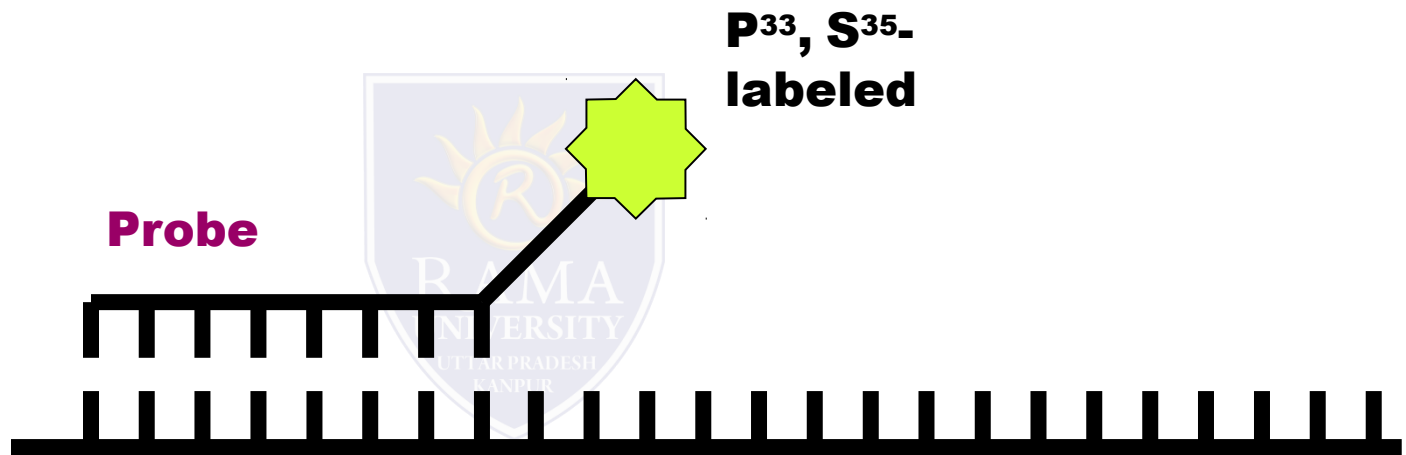
In situ Hybridization

- **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)

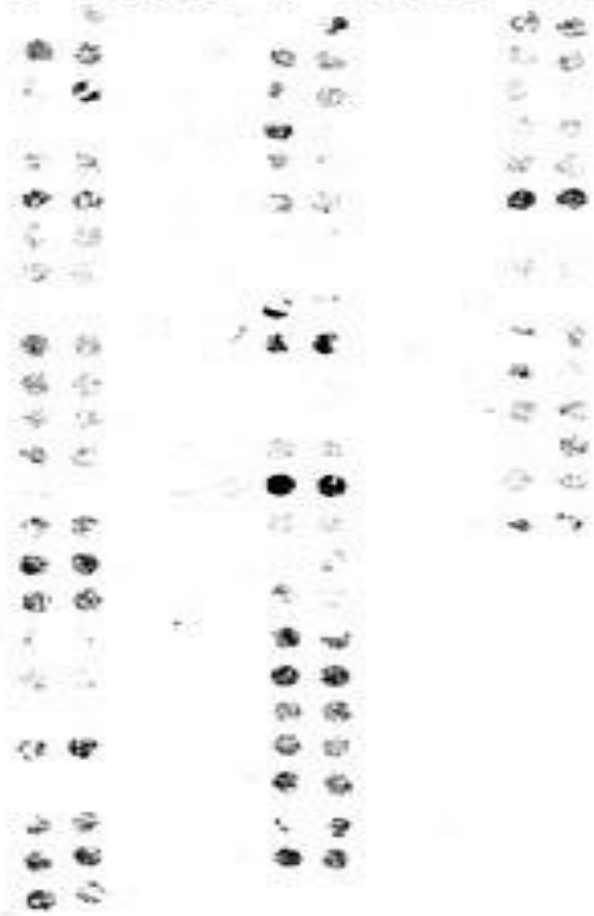


Radioactive ISH Protocol Summary

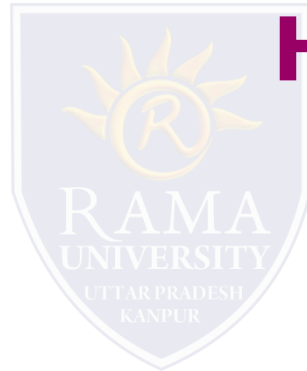
- Dewax Slides
- Permeabilize, target retrieve & *Post-fix*
- *Denature and Hybridize radiolabeled-Probe*
- *Post-Hybridization Washes*
- Counterstain
- Photographic emulsion
- Expose for days to weeks
- Develop
- Read



TTNNTTNTT

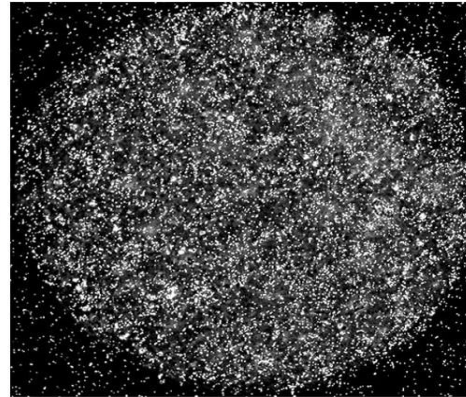
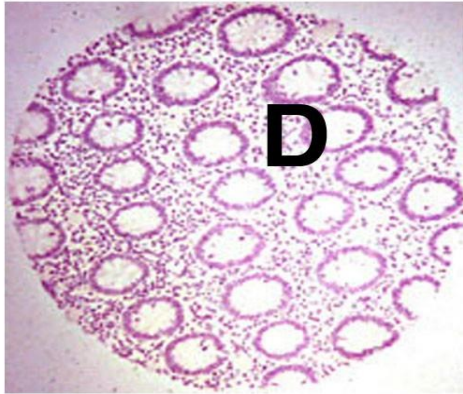


Radioactive in situ Hybridization in Normal and Tumor cells



Radioactive ISH

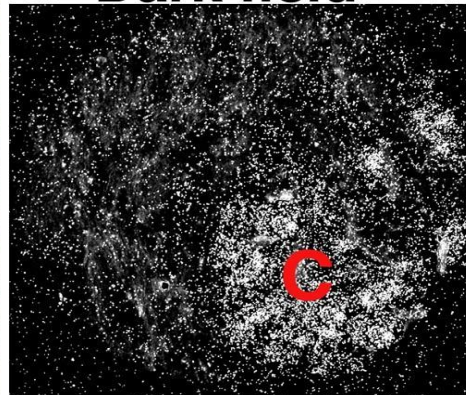
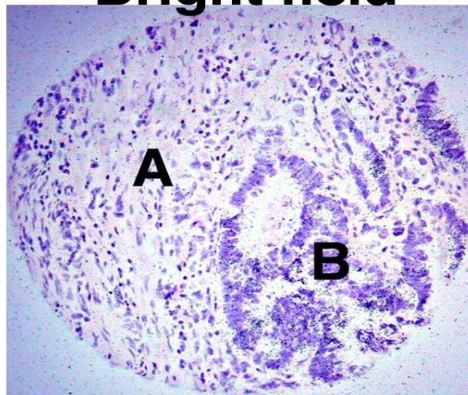
N



Bright field

Dark field

T



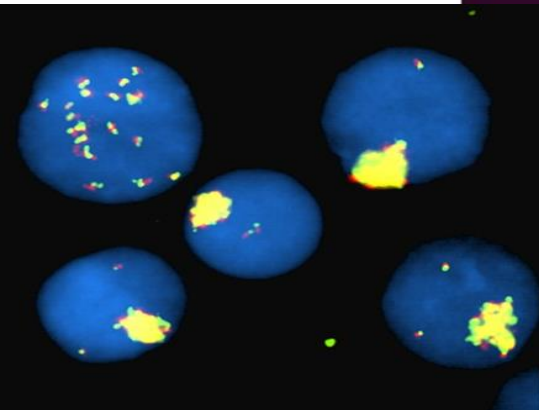
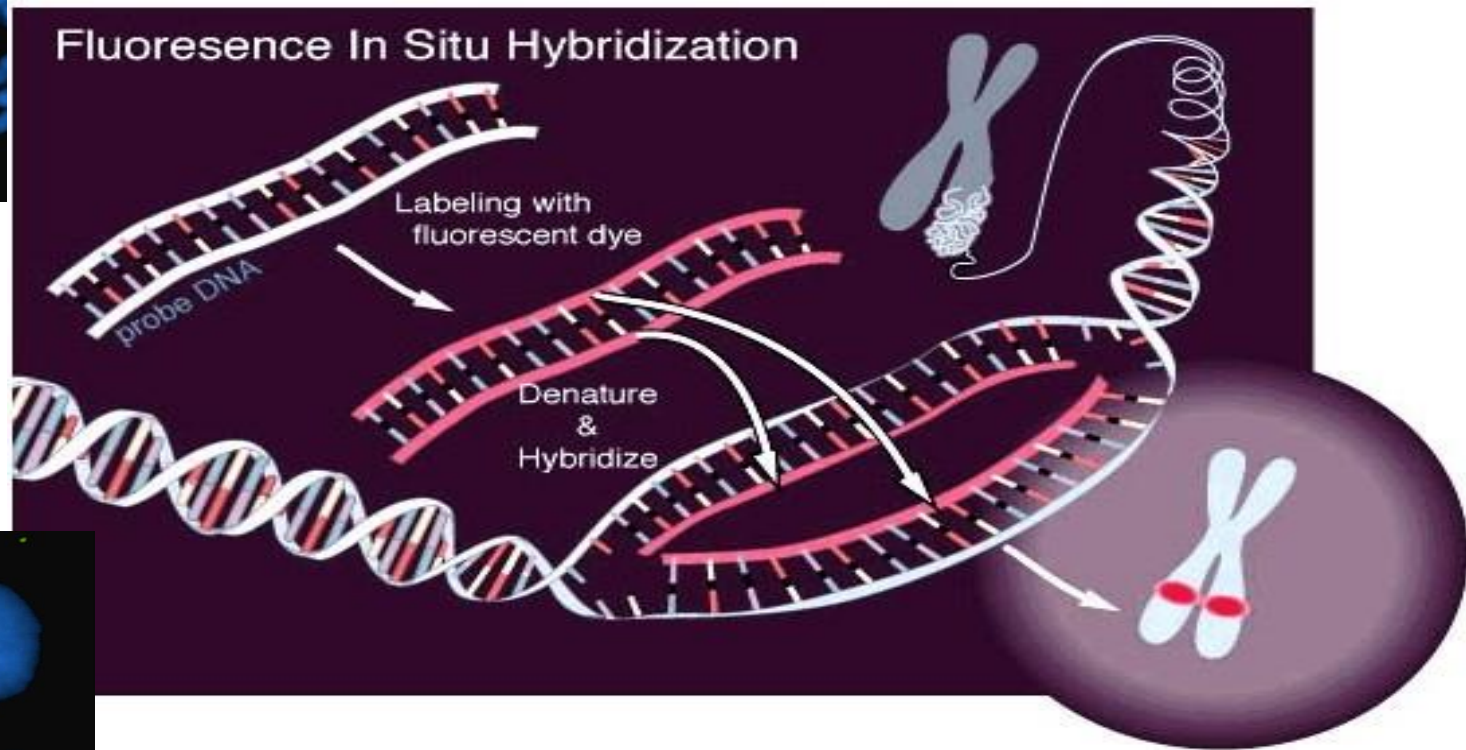
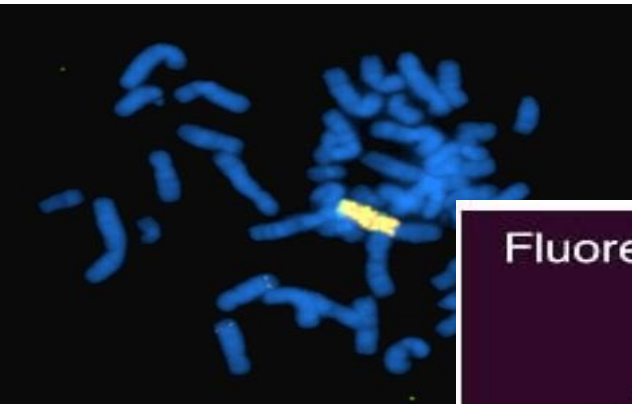
Fluorescent ISH Protocol Summary

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

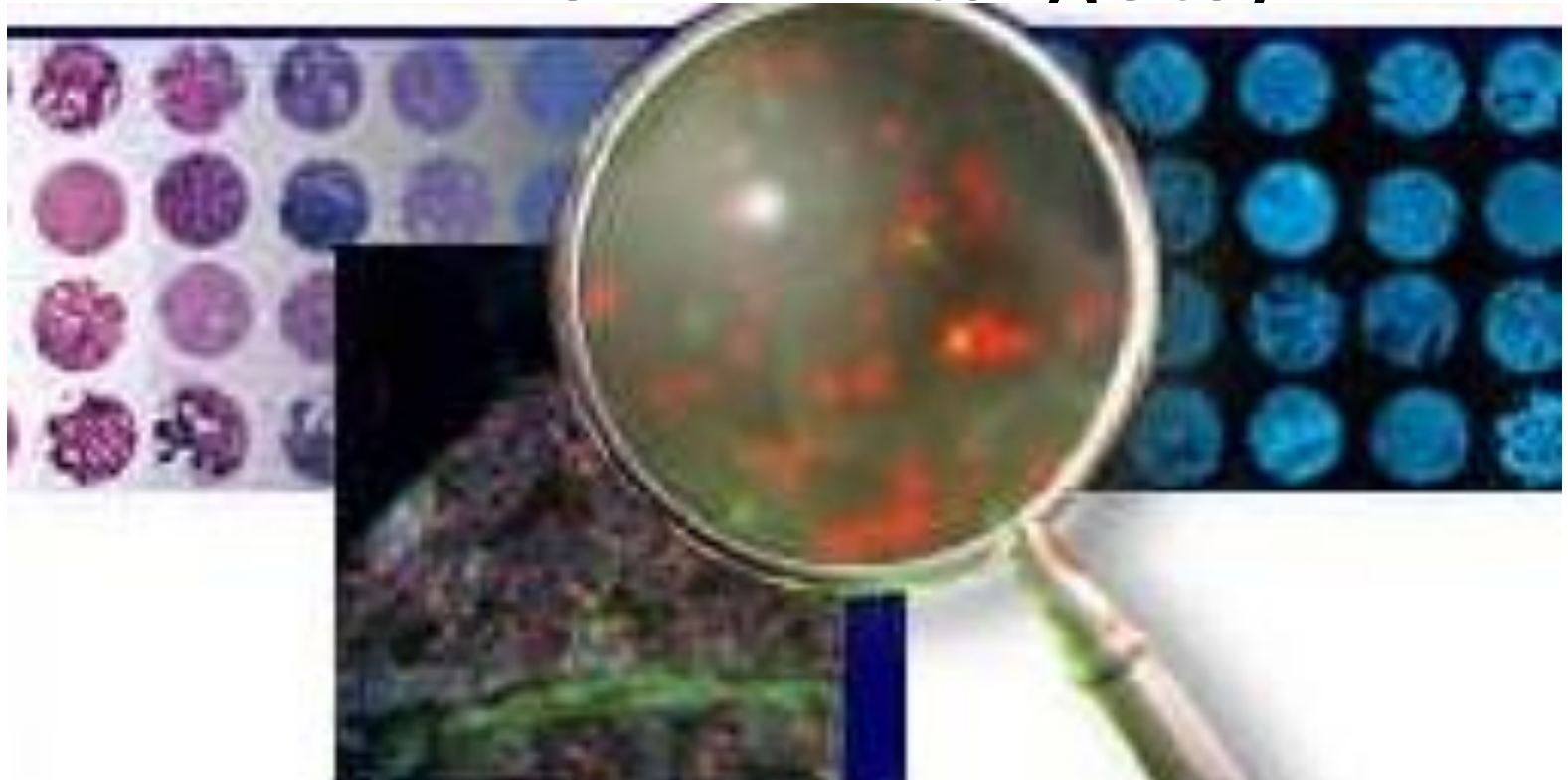


**Target Gene
(DNA)**

FISH



Fluorescent ISH (usually for DNA targets)



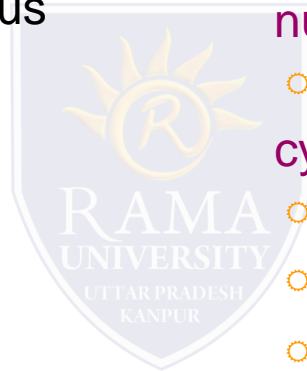
C-ISH v/s FISH

- **FISH**

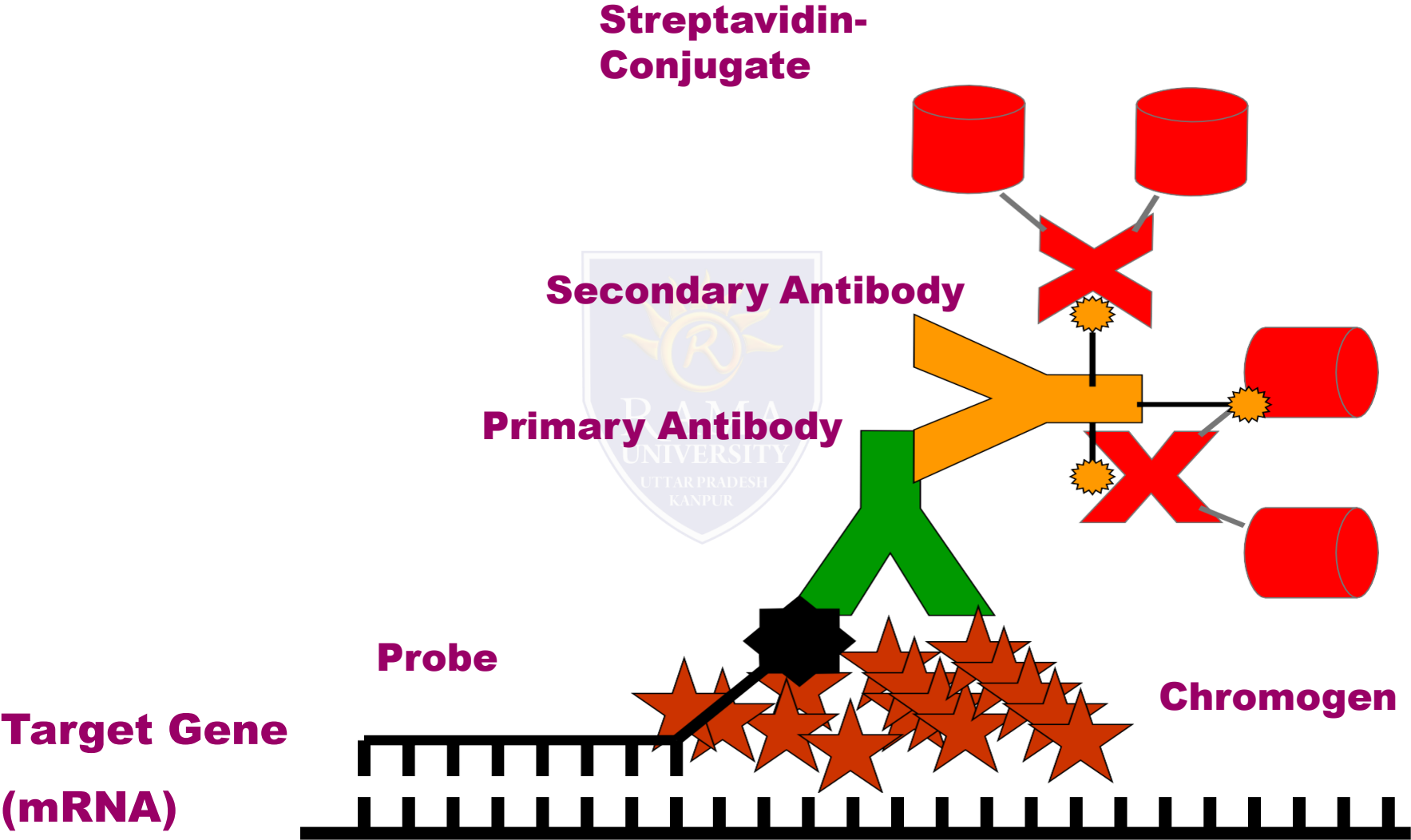
- ○ Mostly DNA detection
- ○ DNA located in the nucleus
- ○ Fluorescent end-point
- ○ Cannot be archived
- ○ Hard to read morphology
- ○ Can detect multiple genes simultaneously
- ○ DNA does not degrade

- **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

