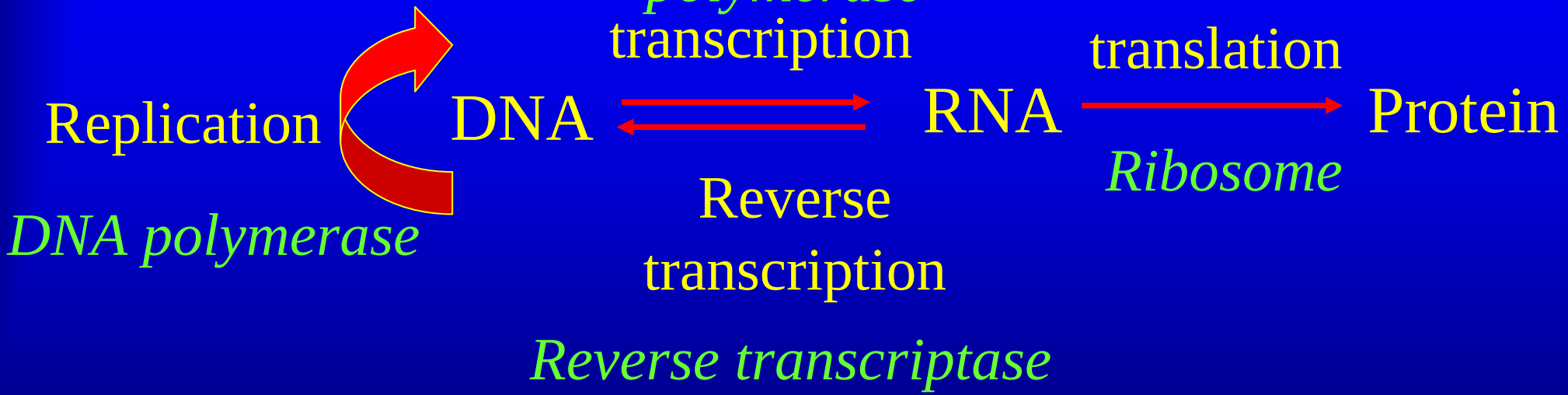
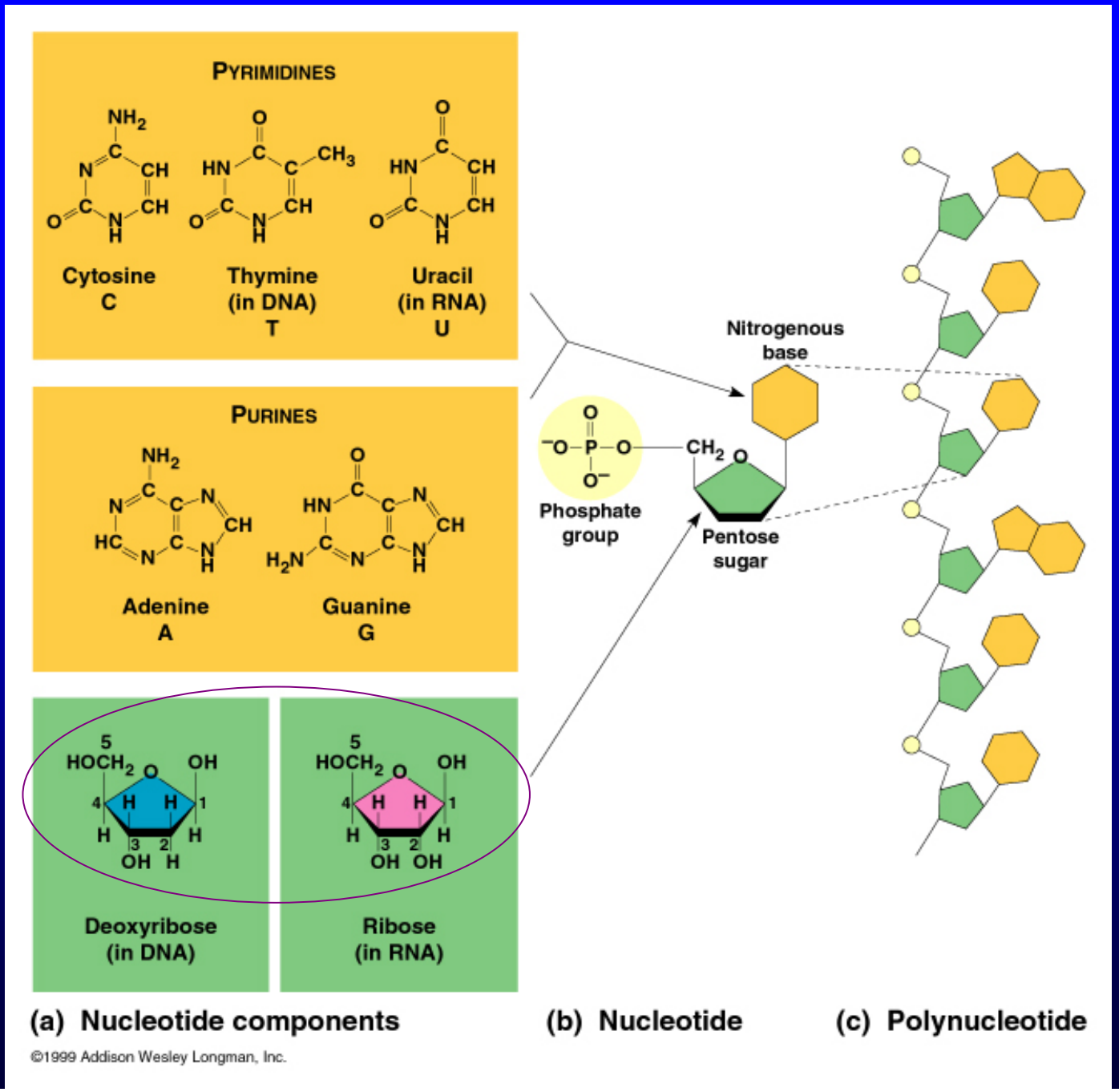
Molecular Techniques in Radiobiology

- DNA – RNA – Protein
- Gene Expression
- Gene Structure

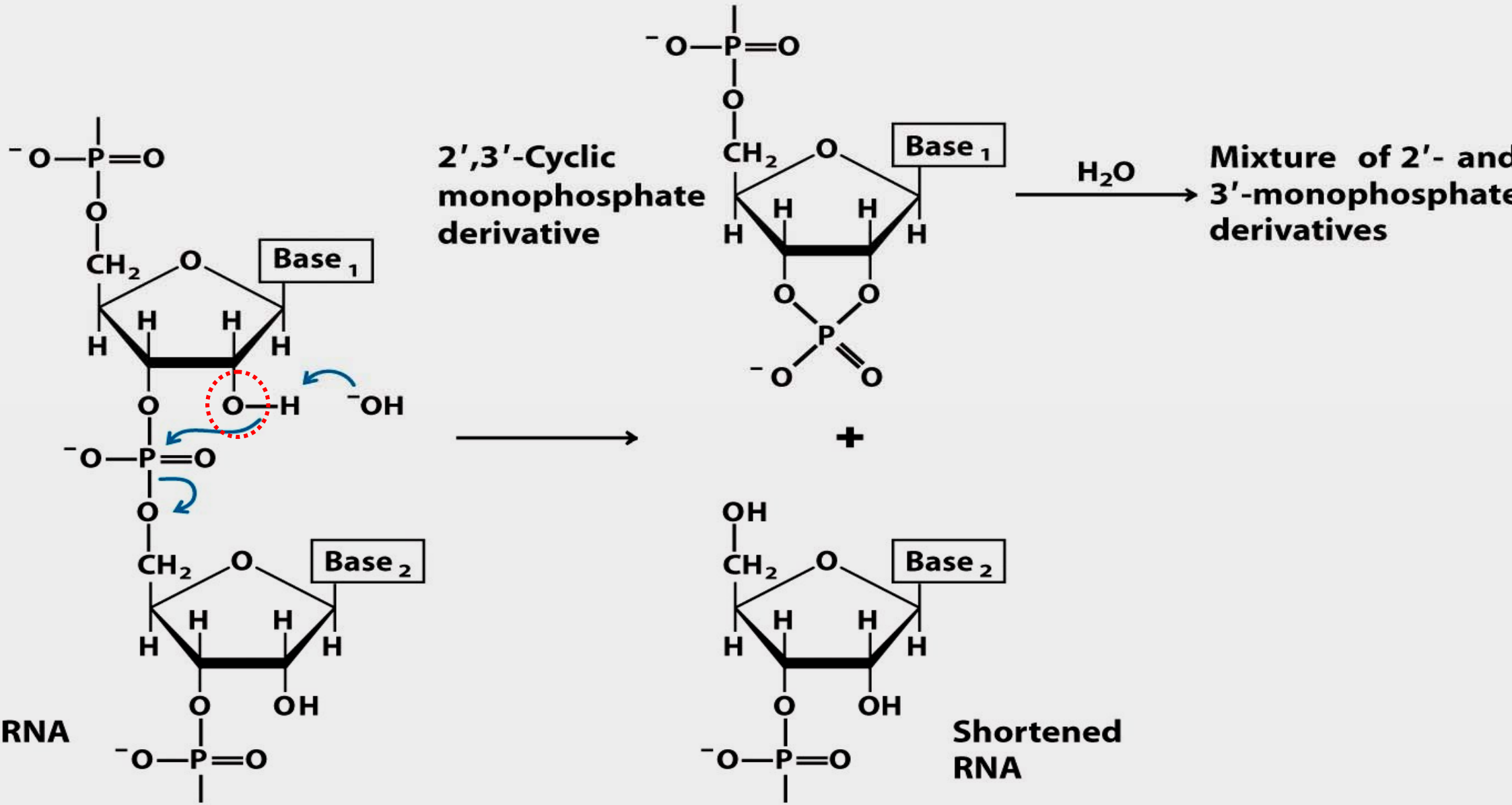
DNA – RNA - Protein



DNA and RNA



Hydrolysis of RNA under alkaline conditions



Techniques for the study of Gene Expression: RNA Level

- RNA Isolation
- Northern Blot Assay
- RNase Protection Assay (RPA)
- RT-PCR
- In Situ Hybridization
- Microarray (DNA Chips)

Techniques for the study of Gene Expression: Protein Level

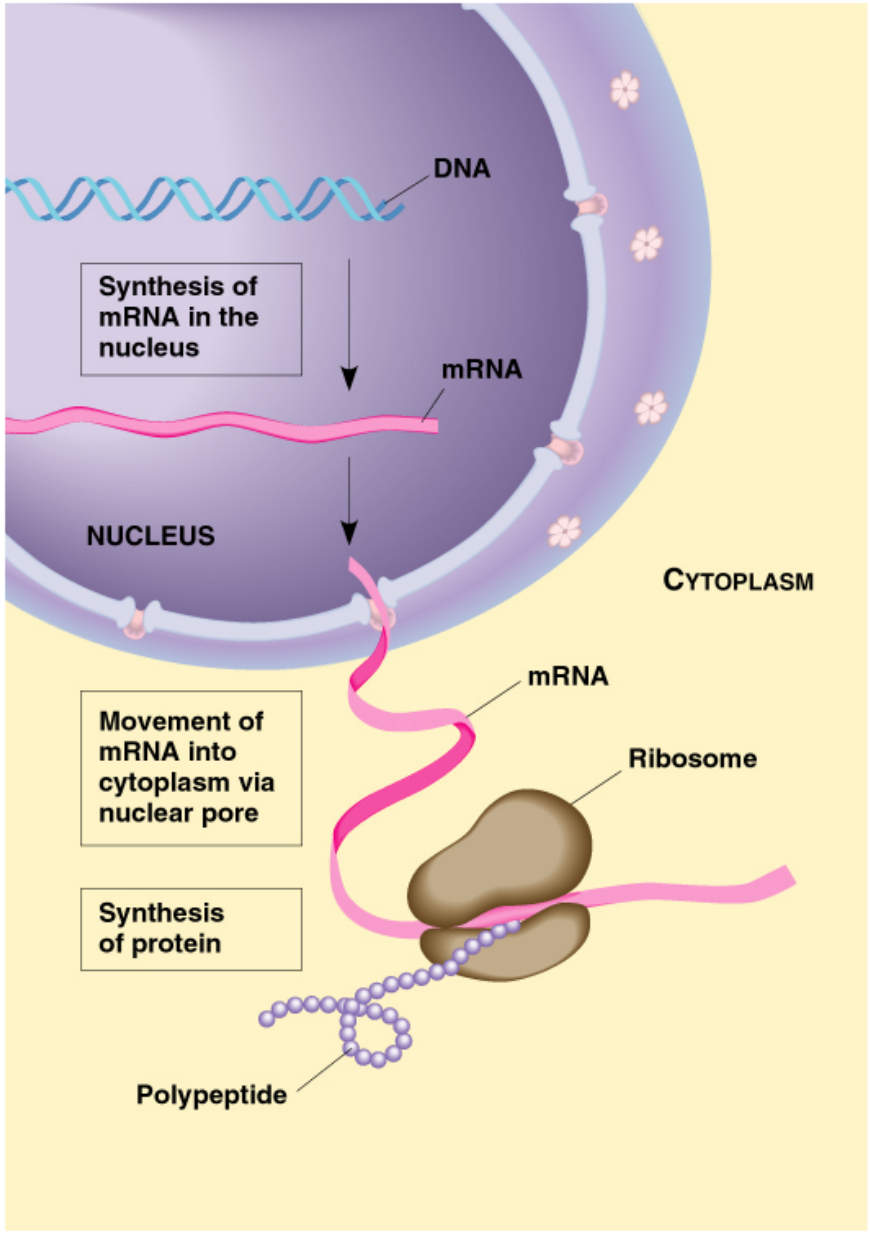
- **SDS PAGE (Gel Electrophoresis)**
- **Western Blot Assay**
- **ELISA Assay (RIA)**
- **Flow Cytometry (FACS)**
- **Immunostaining**
- **Protein Array (2-D Gel)**

Techniques for the study of Gene

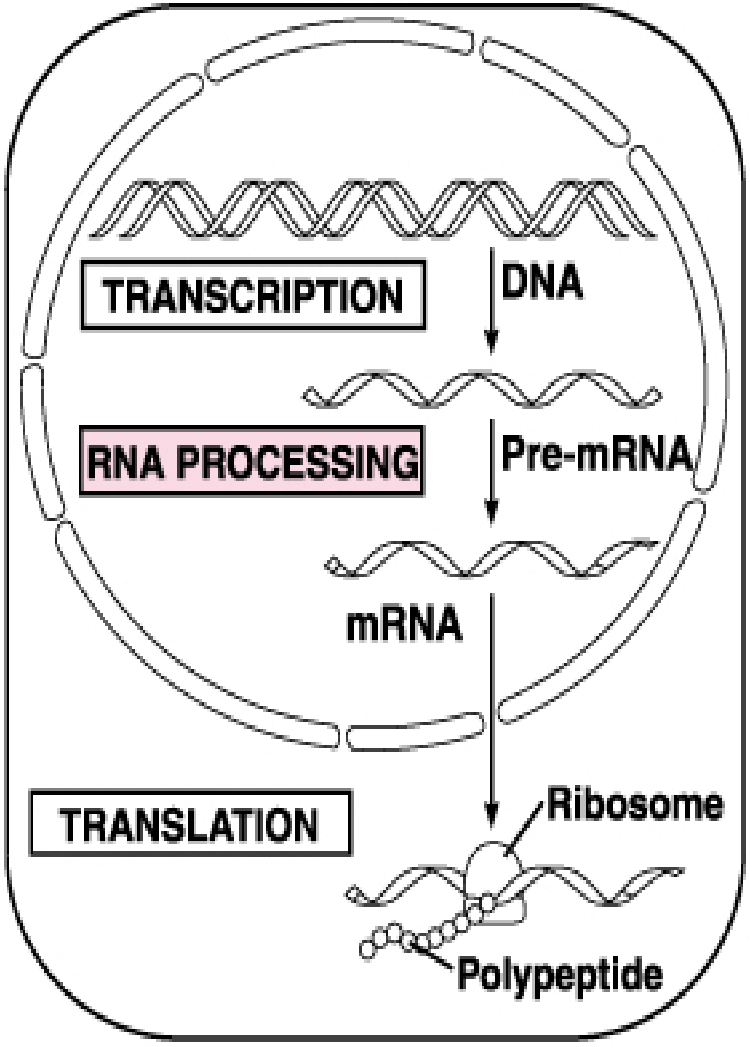
- Gene Cloning
- Gene Analysis

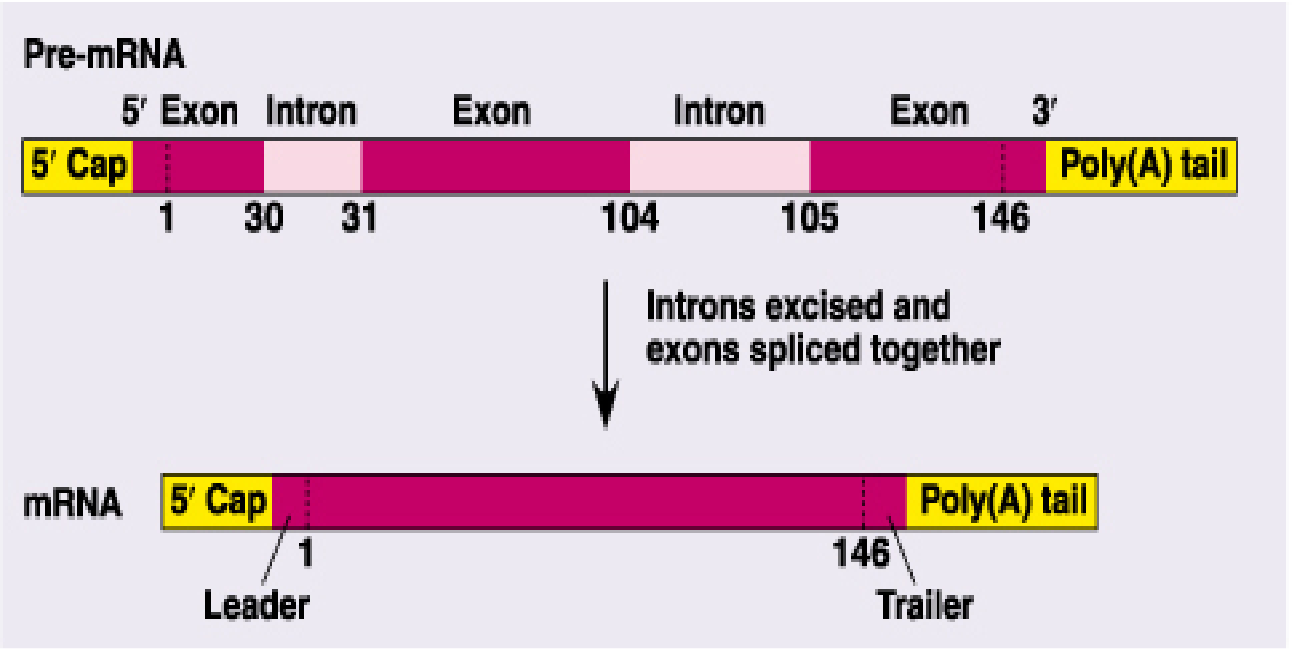

http://mx.nthu.edu.tw/~cschiang

Regulation of Gene Expression

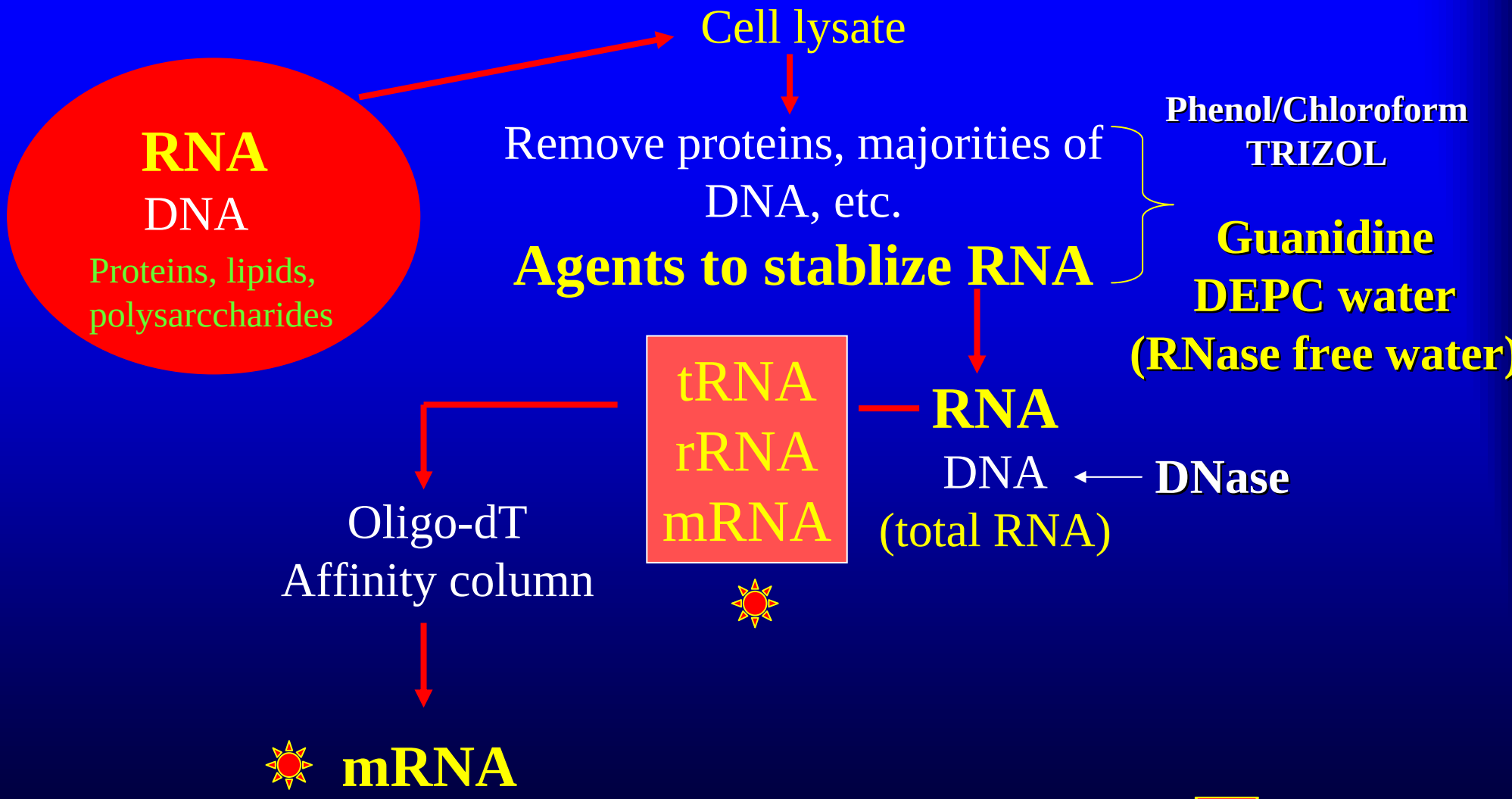


RNA Processing





Sample RNA isolation



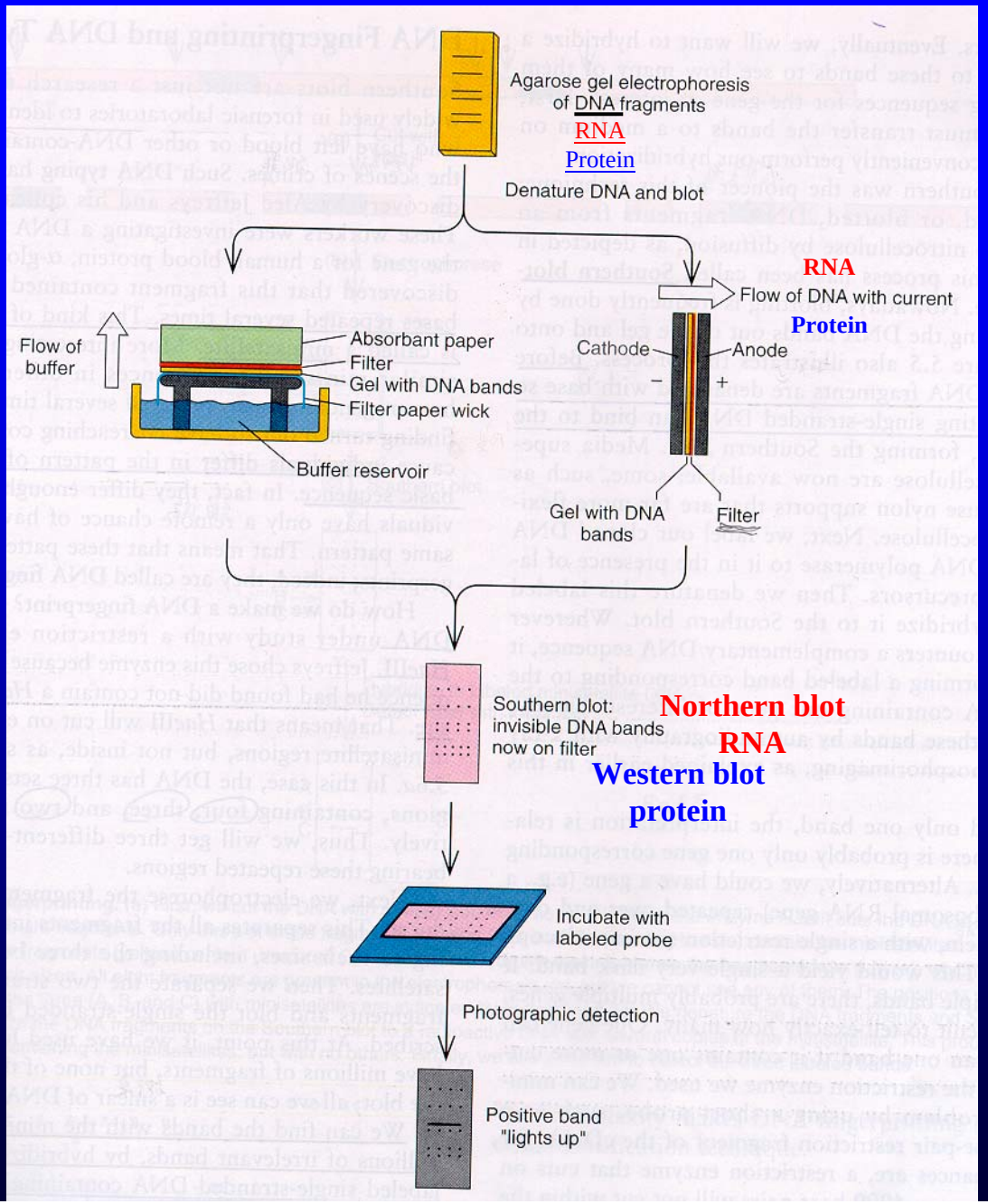
Northern Blot Assay 1

1. Electrophoresis

2. Transfer

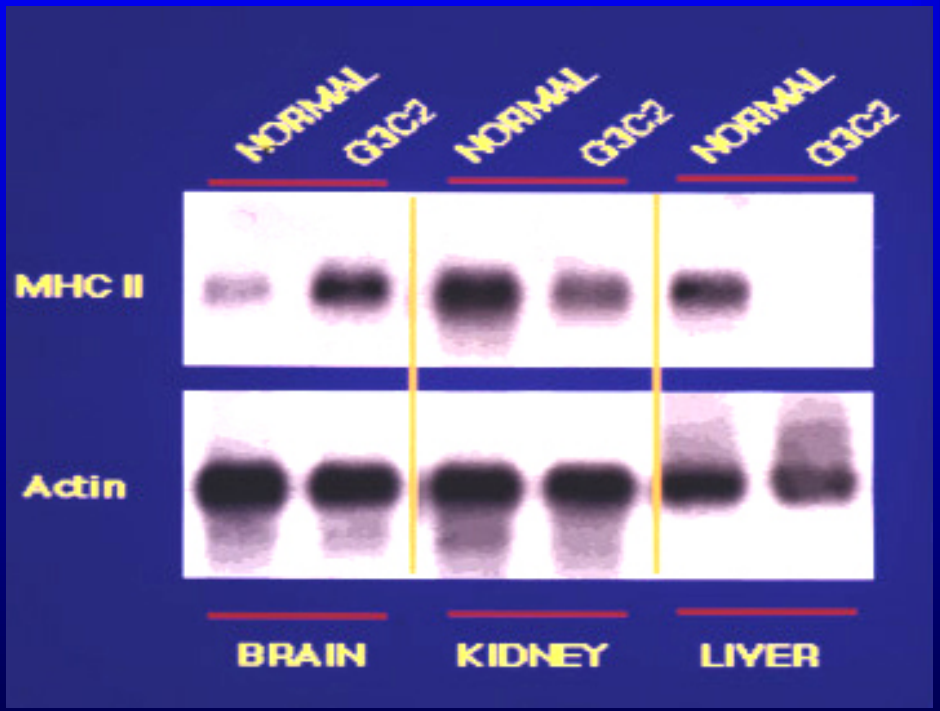
3. Hybridization (Probing)

4. Detection

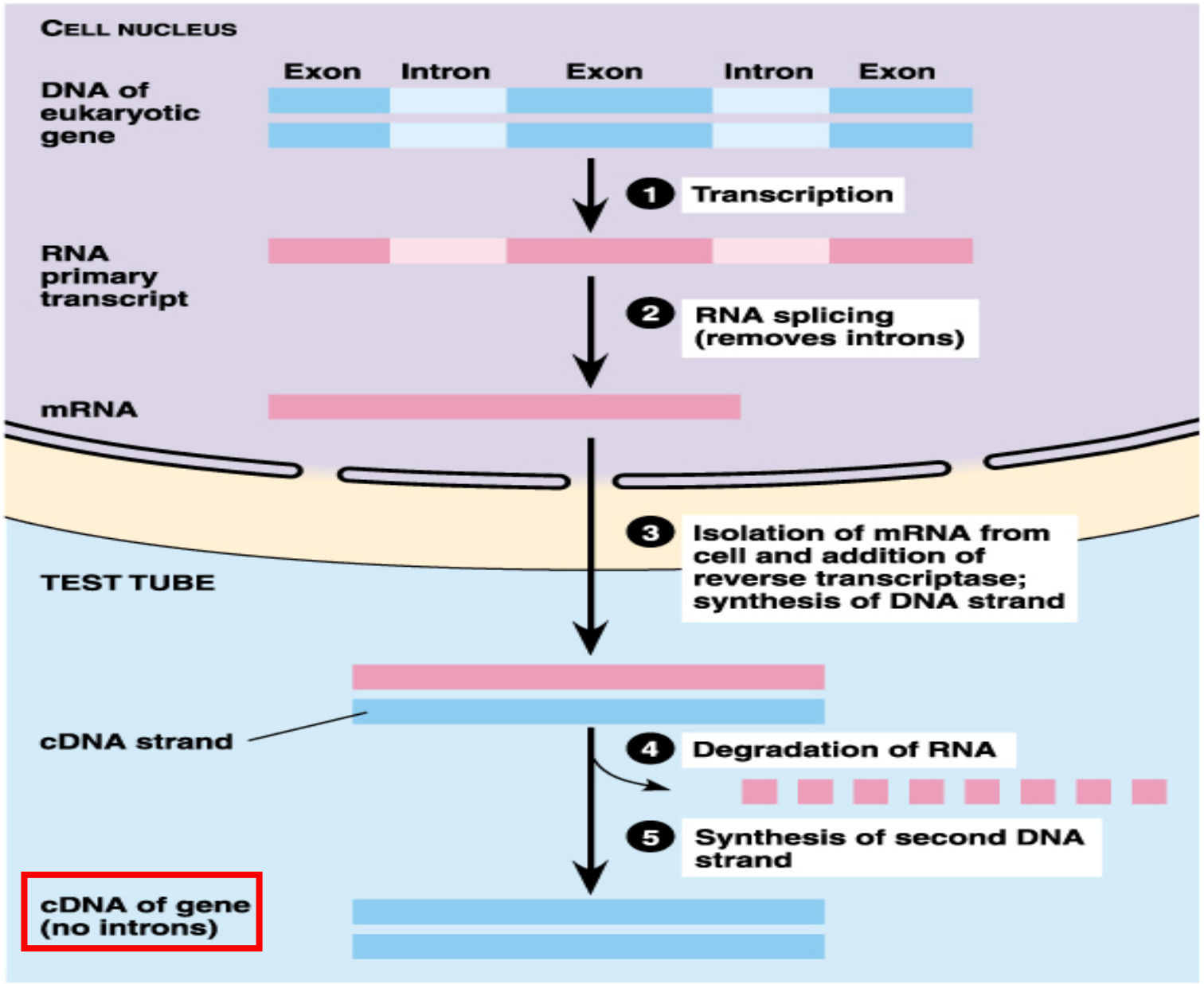


Northern Blot Assay 2

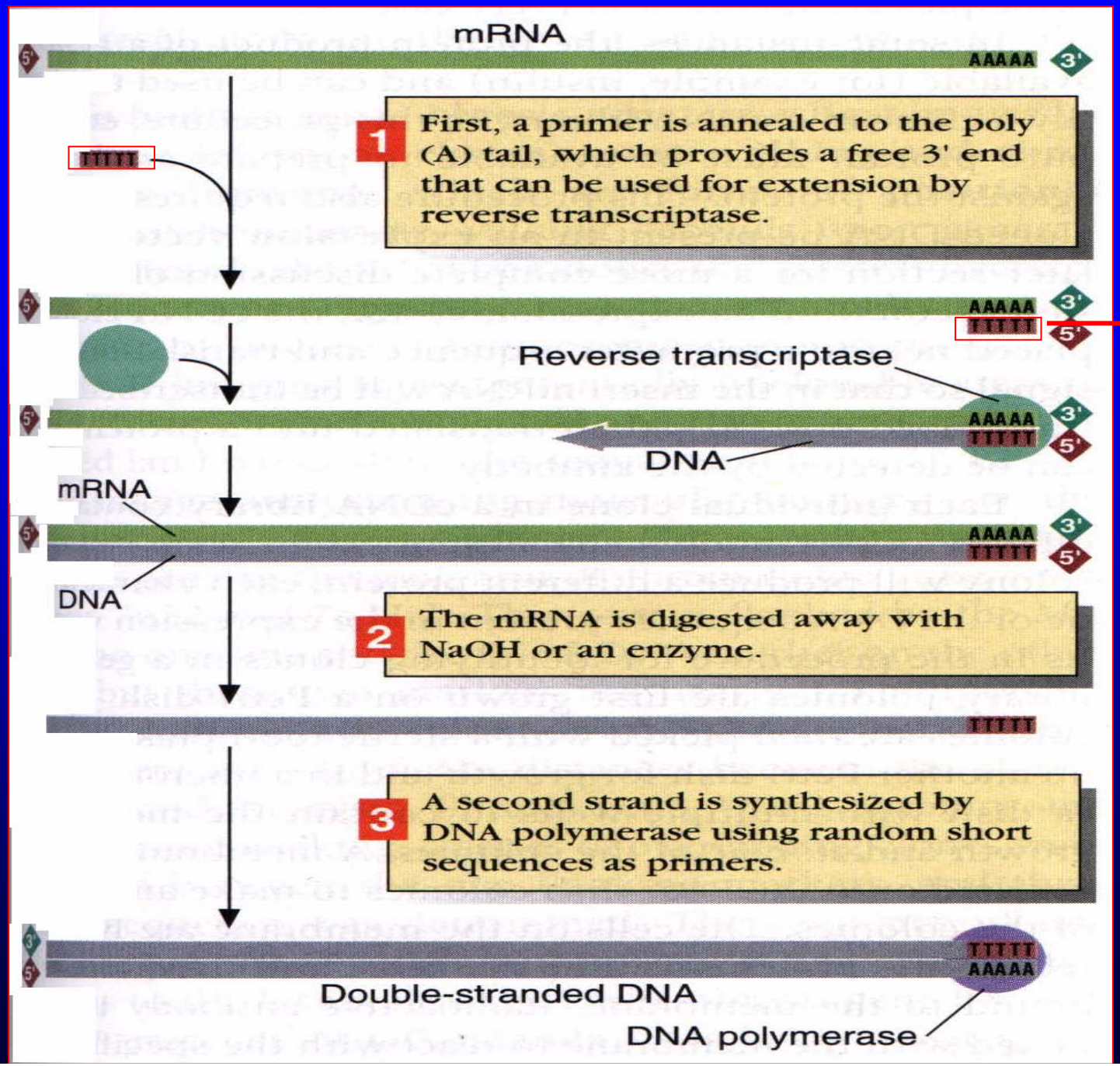
- Sample Target: total RNA or mRNA
- Detection:
  - Gel Electrophoresis
  - Probe Labeling
  - Detection



cDNA



Reverse Transcription (RT)



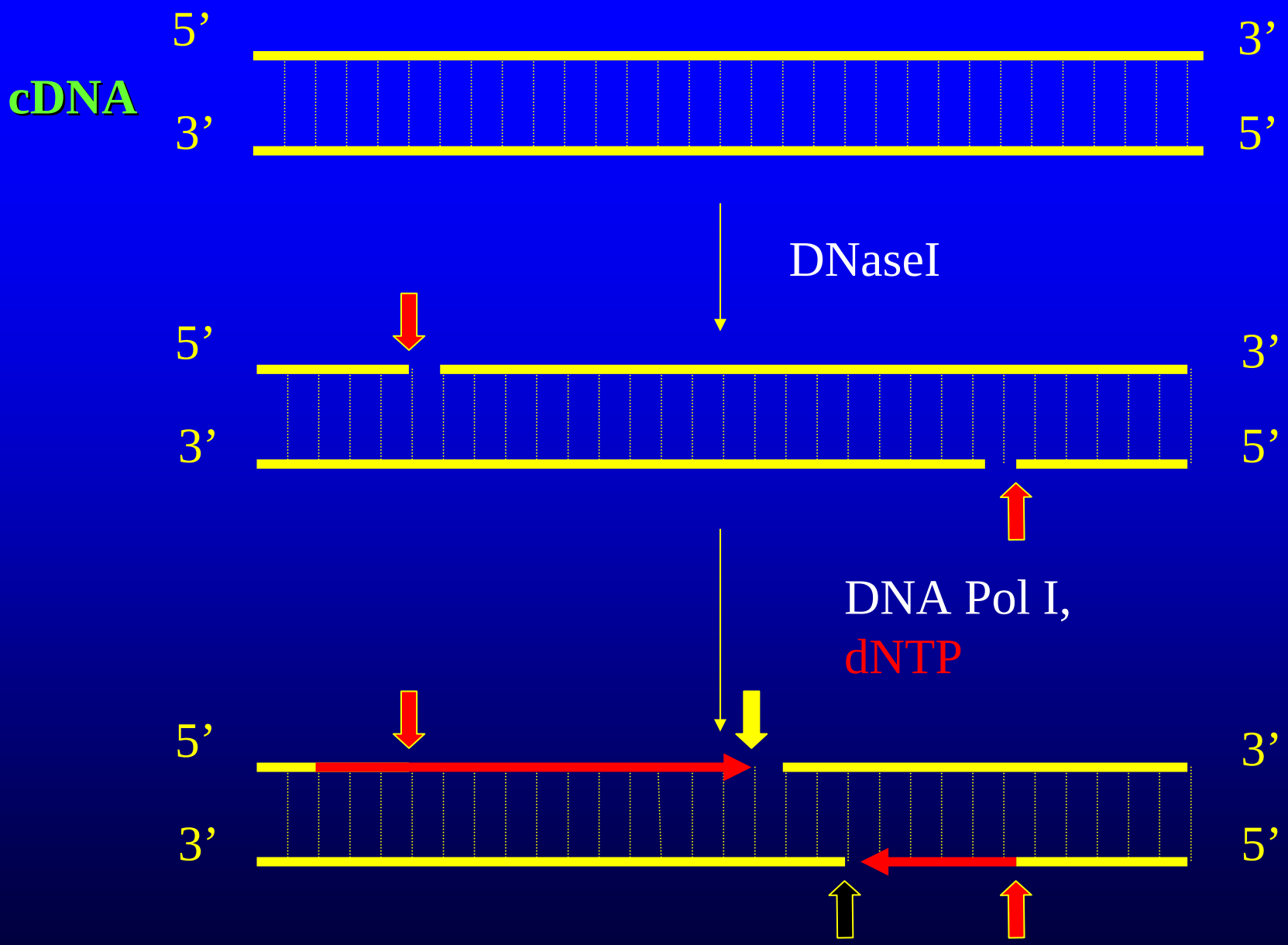
Oligo dT primer

cDNA

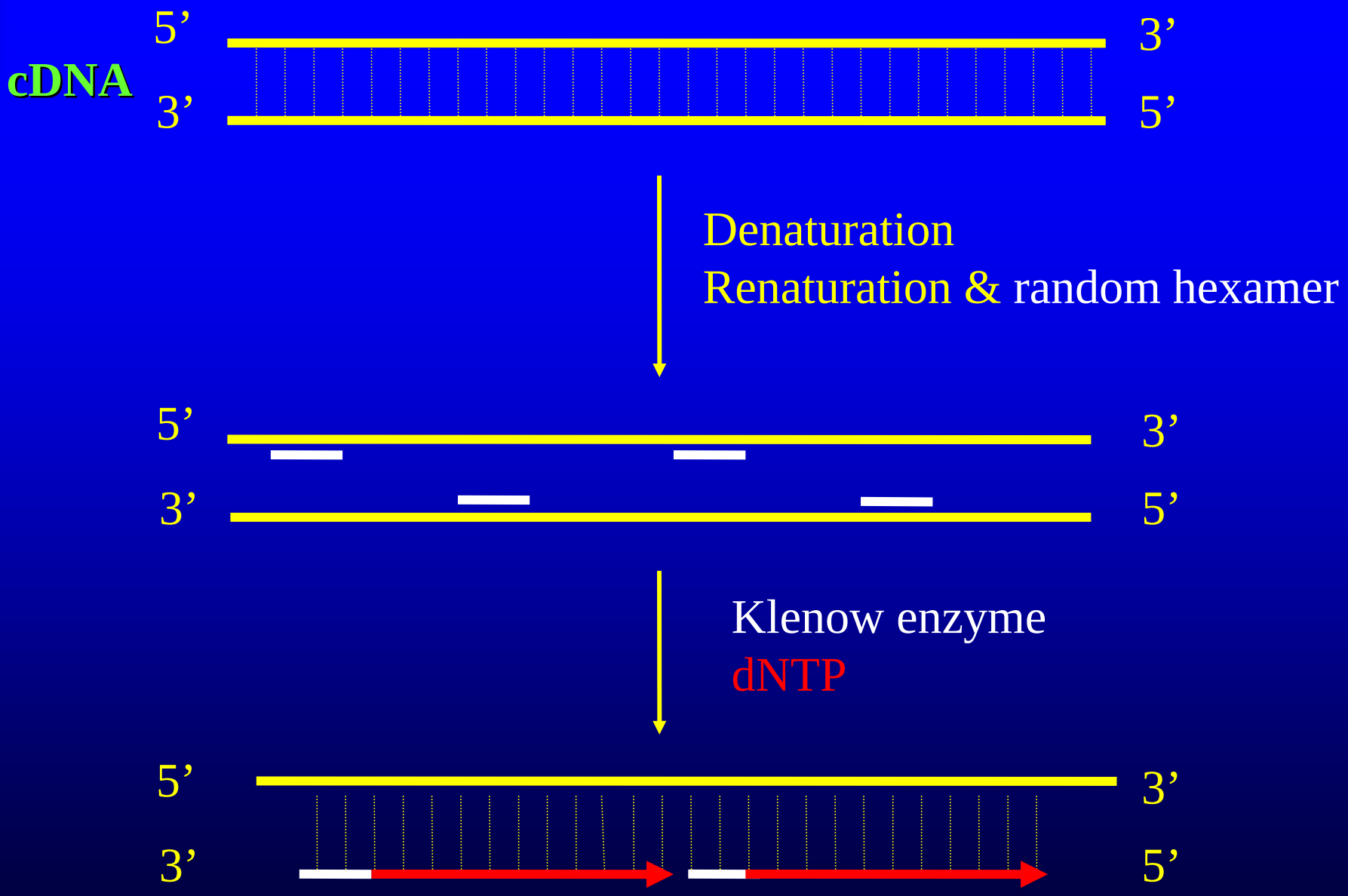
Probe Labeling

- Nick Translation
- Random Primed Labeling
- PCR Labeling
- 5’ End Labeling
- 3’ End Labeling
- Direct Labeling

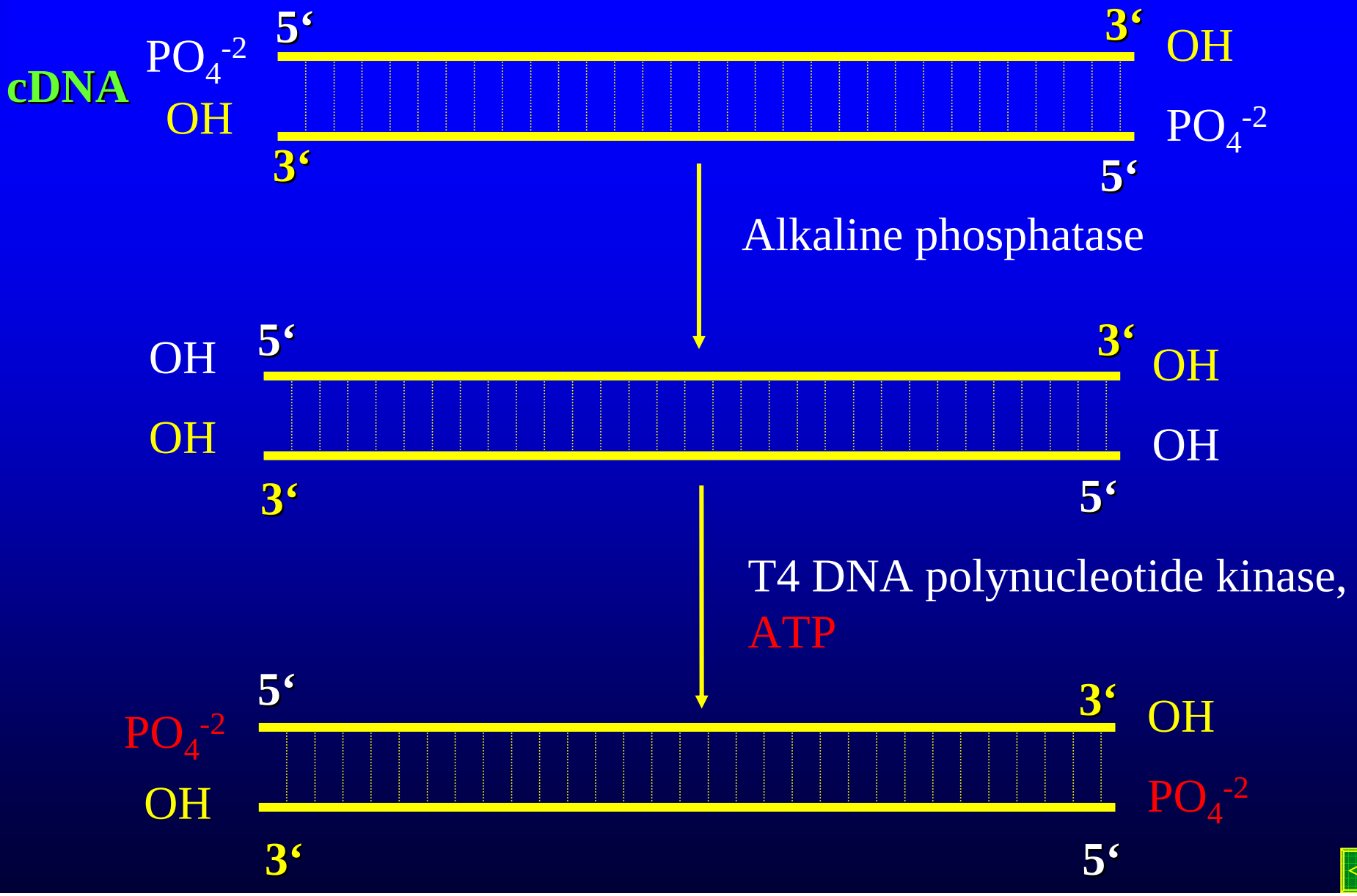
Nick Translation



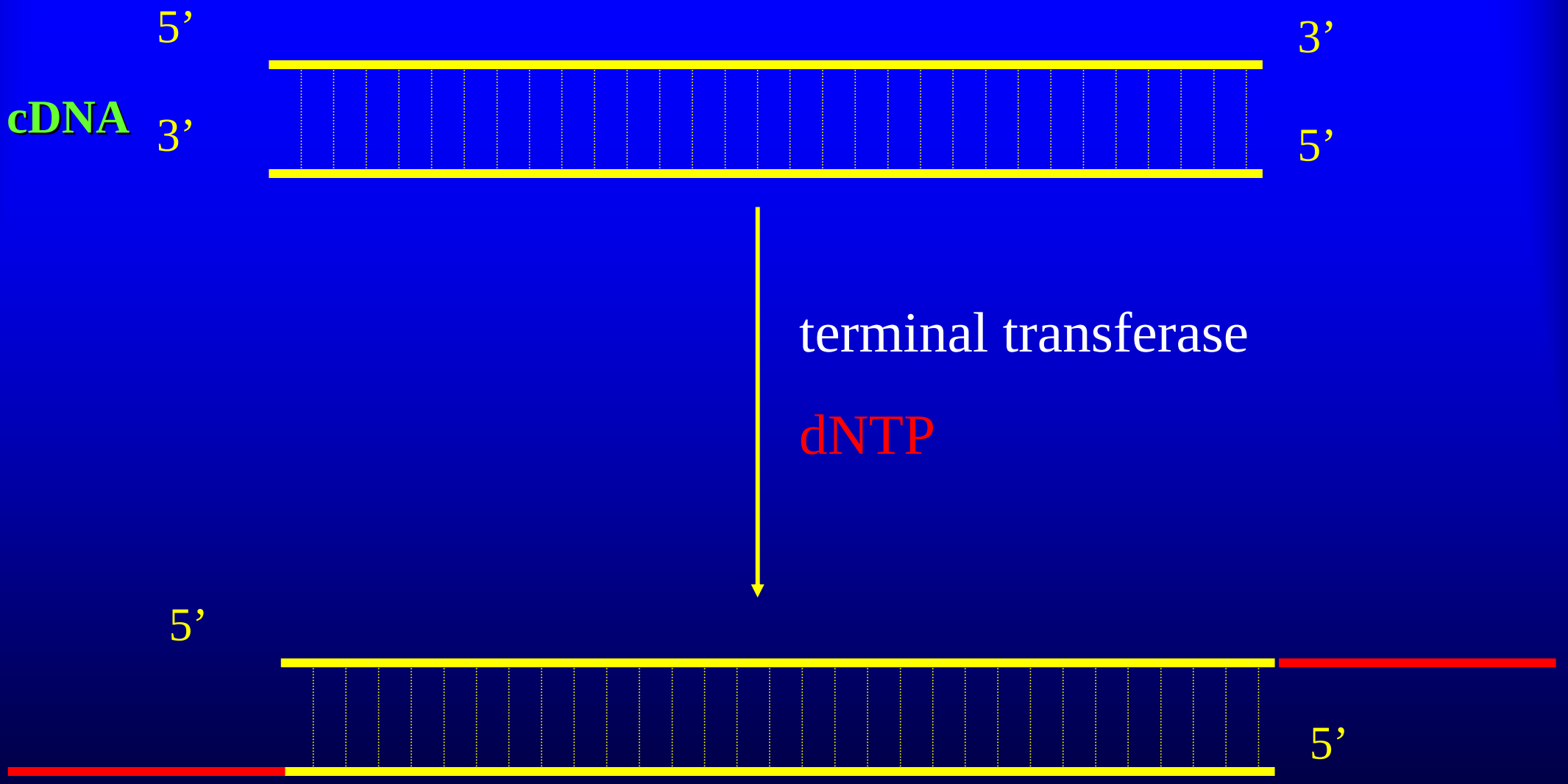
Random Primed Labeling



5’ end labeling with polynuclotide kinase



3’ end labeling with terminal transferase

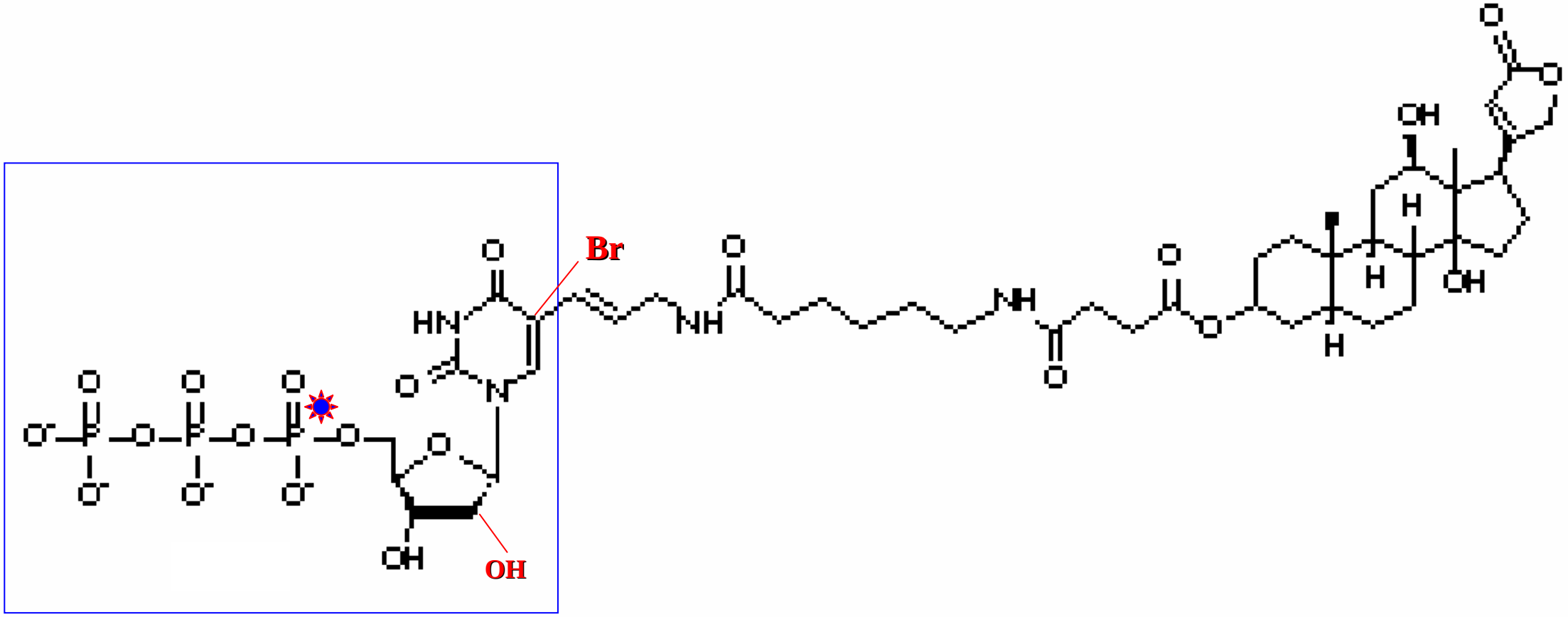


Choice of Labeling Nucleotides

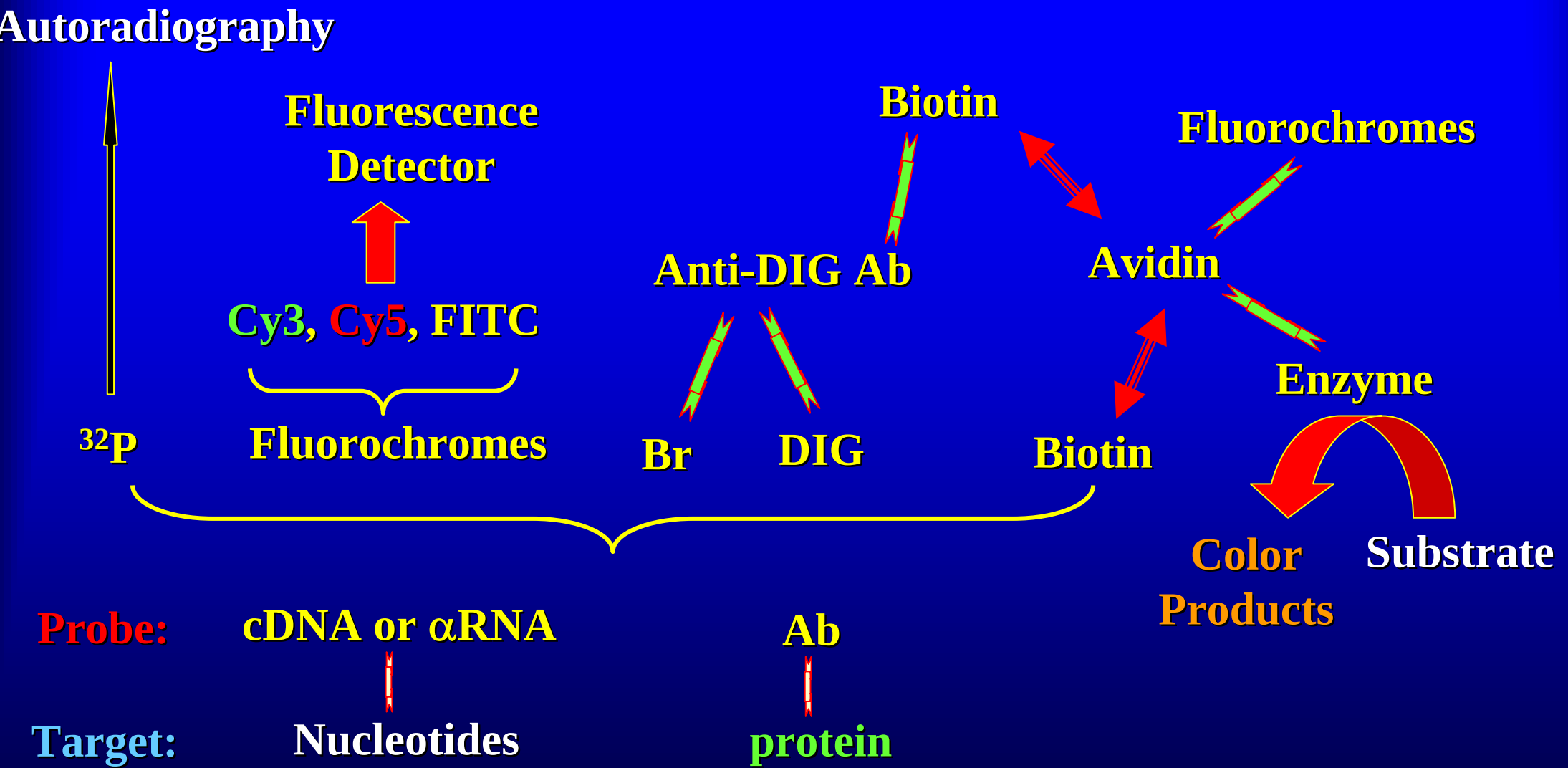
Amplified signals

- Fluorescent dyes (Cy3, Cy5)
- Color detection dyes (Biotin, DIG)
- Radioisotope: ^{32}P-dATP, ^{32}P-rUTP

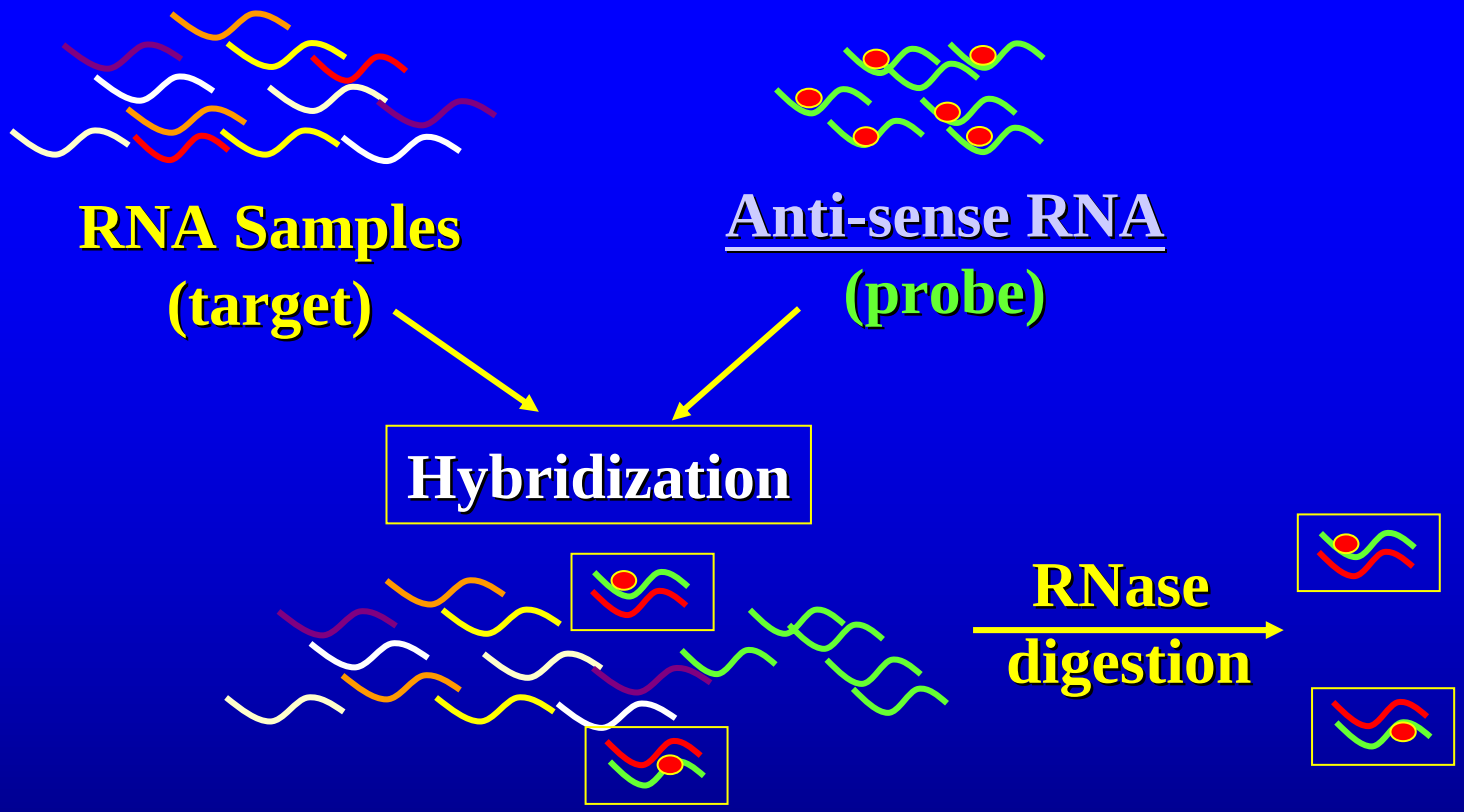
Structure of Digoxidenin (DIG)-dUTP

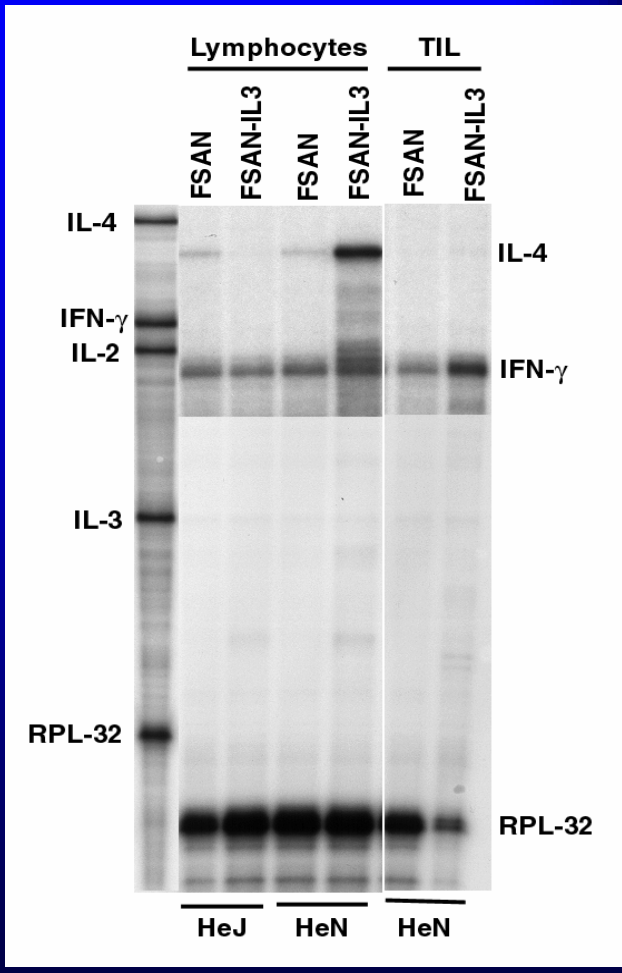


Detection

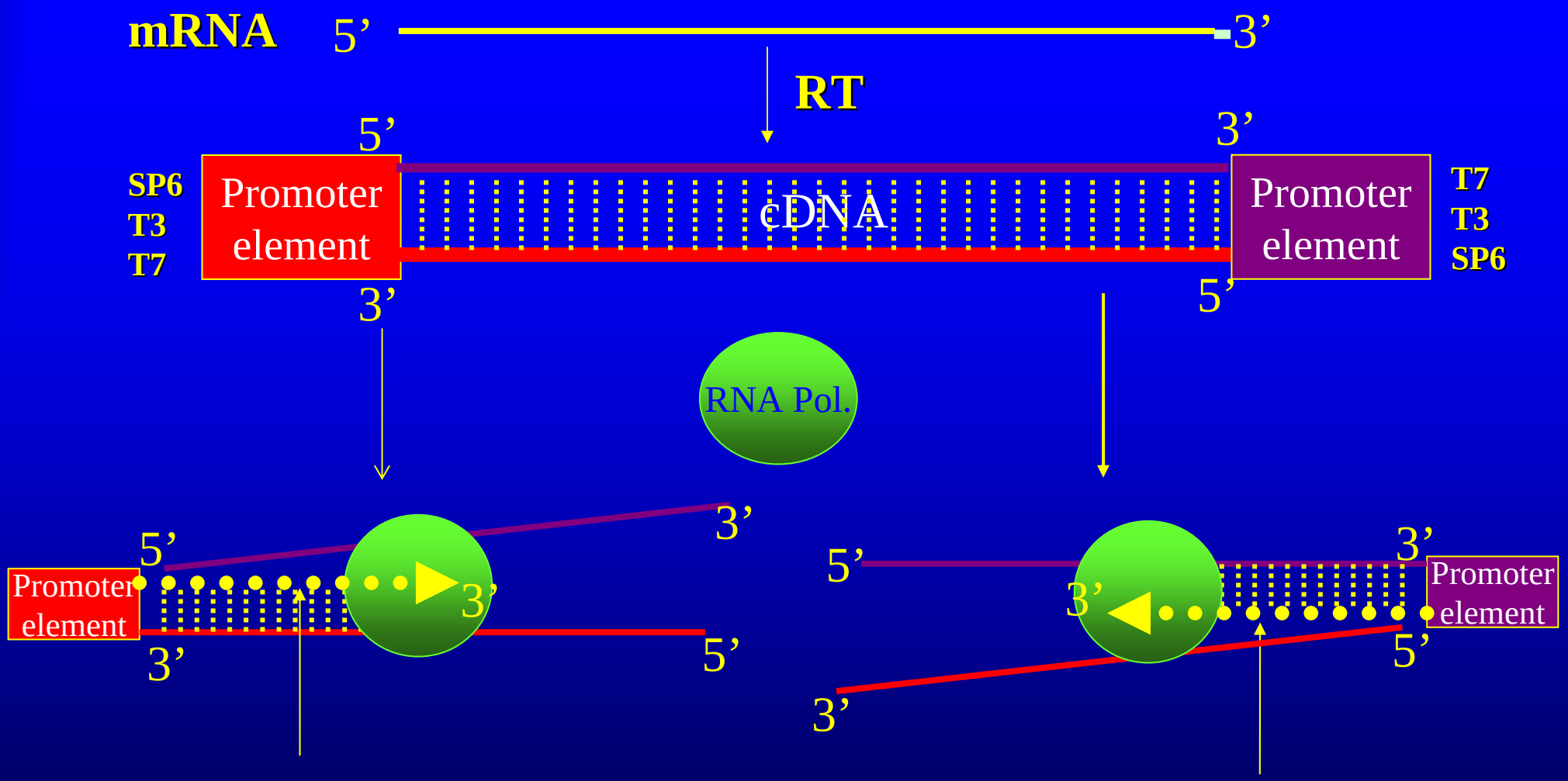

Autoradiography
Fluorescence Detector
Cy3, Cy5, FITC
Fluorochromes
32P
Biotin
Anti-DIG Ab
Br
DIG
Fluorochromes
Avidin
Enzyme
Biotin
Color Products
Substrate
Probe:
cDNA or αRNA
Ab
Target:
Nucleotides
protein

RNase Protection Assay (RPA)





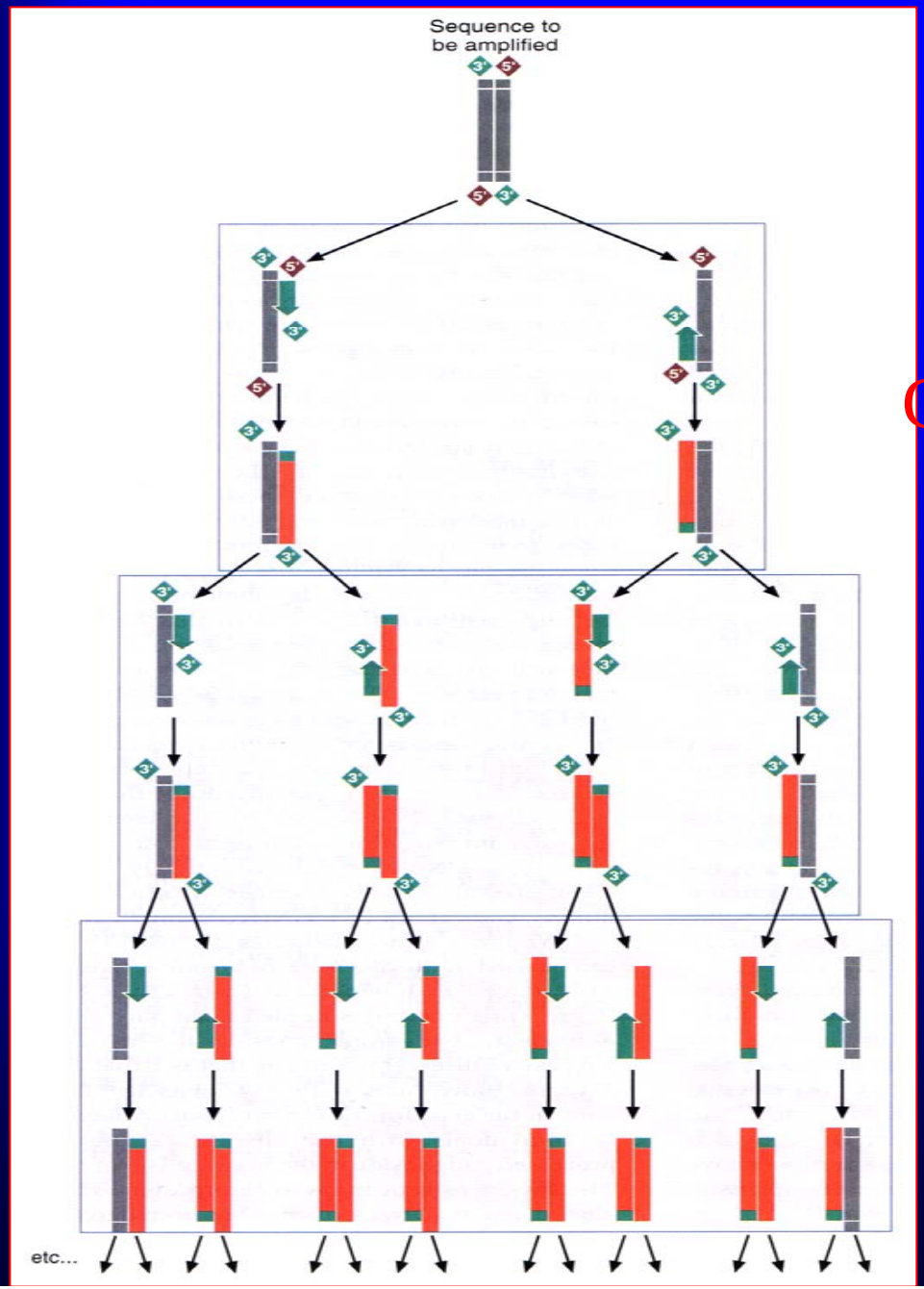
In Vitro Transcription



cRNA (complementatry RNA)
sense RNA

anti-sense RNA

Polymerase Chain Reaction (PCR)



Template (cDNA or DNA)
Primers
NTPs (ATP, GTP, CTP, TTP)
Tag polymerase

- denature DNA (95°C)
- annealing step (55-60°C)
- extension step (74°C)

After 30 cycles
2^{30} molecules
1 pg = 1 µg

1 pg template yields
200 to 300 ng

RT -PCR

mRNA

1 pg

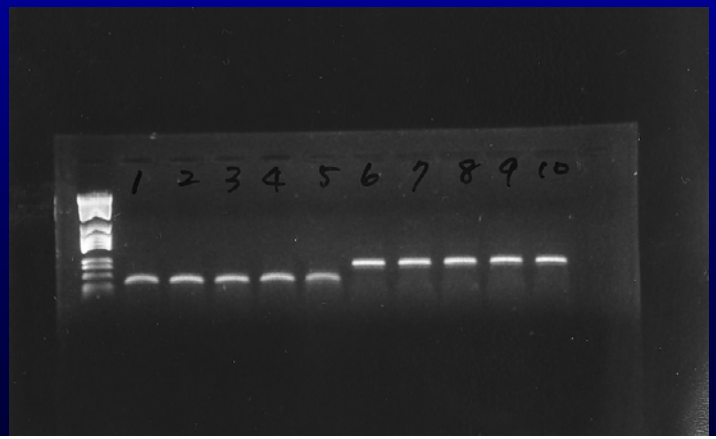
Reverse Transcription

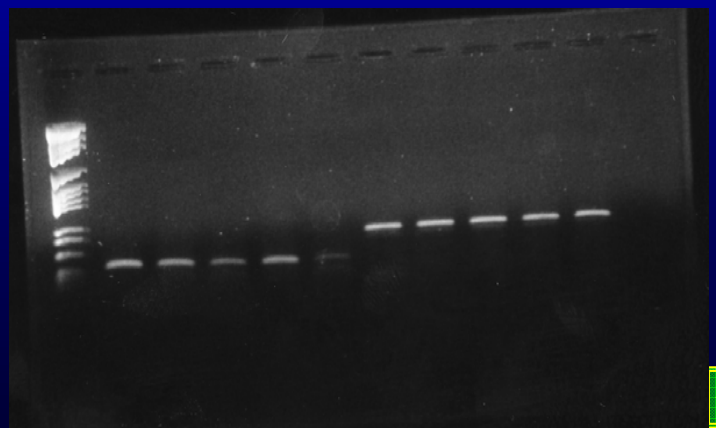
cDNA

Primer pairs

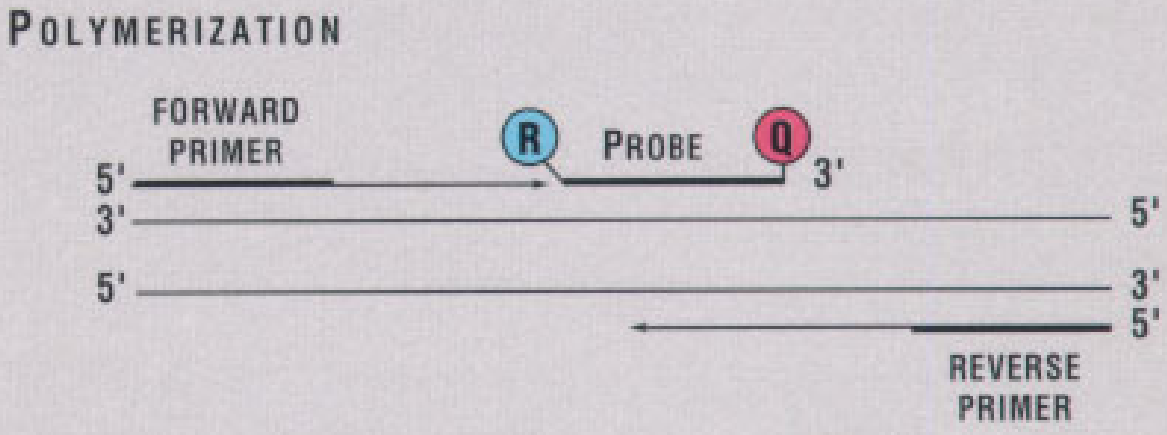
PCR

1 μg

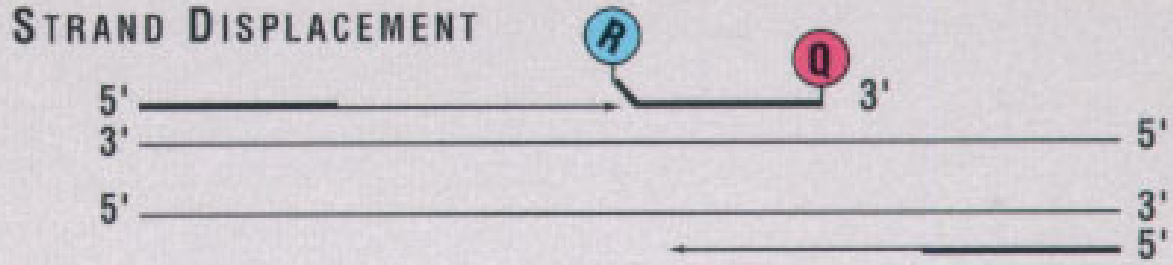




Real Time PCR

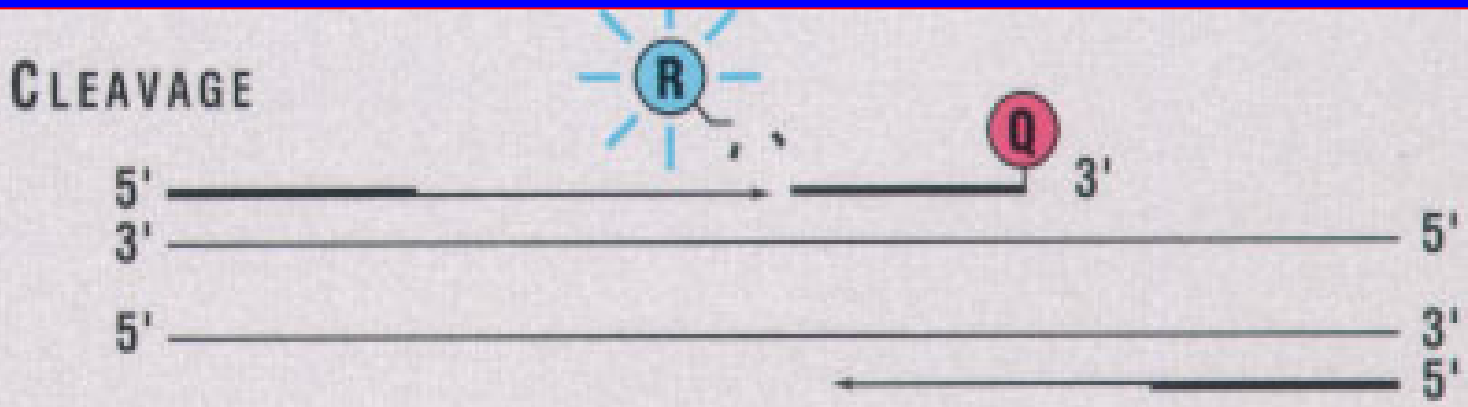


The reporter (R) and the quencher (Q) dyes are attached to the probe.

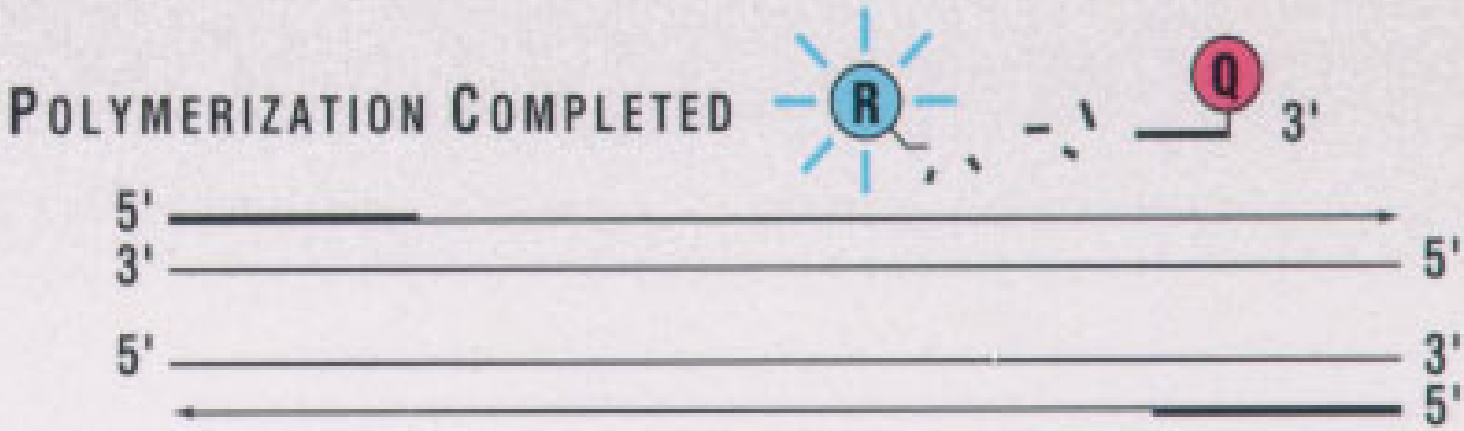


When both dyes are attached to the probe, reporter dye emission is quenched.

Real Time PCR

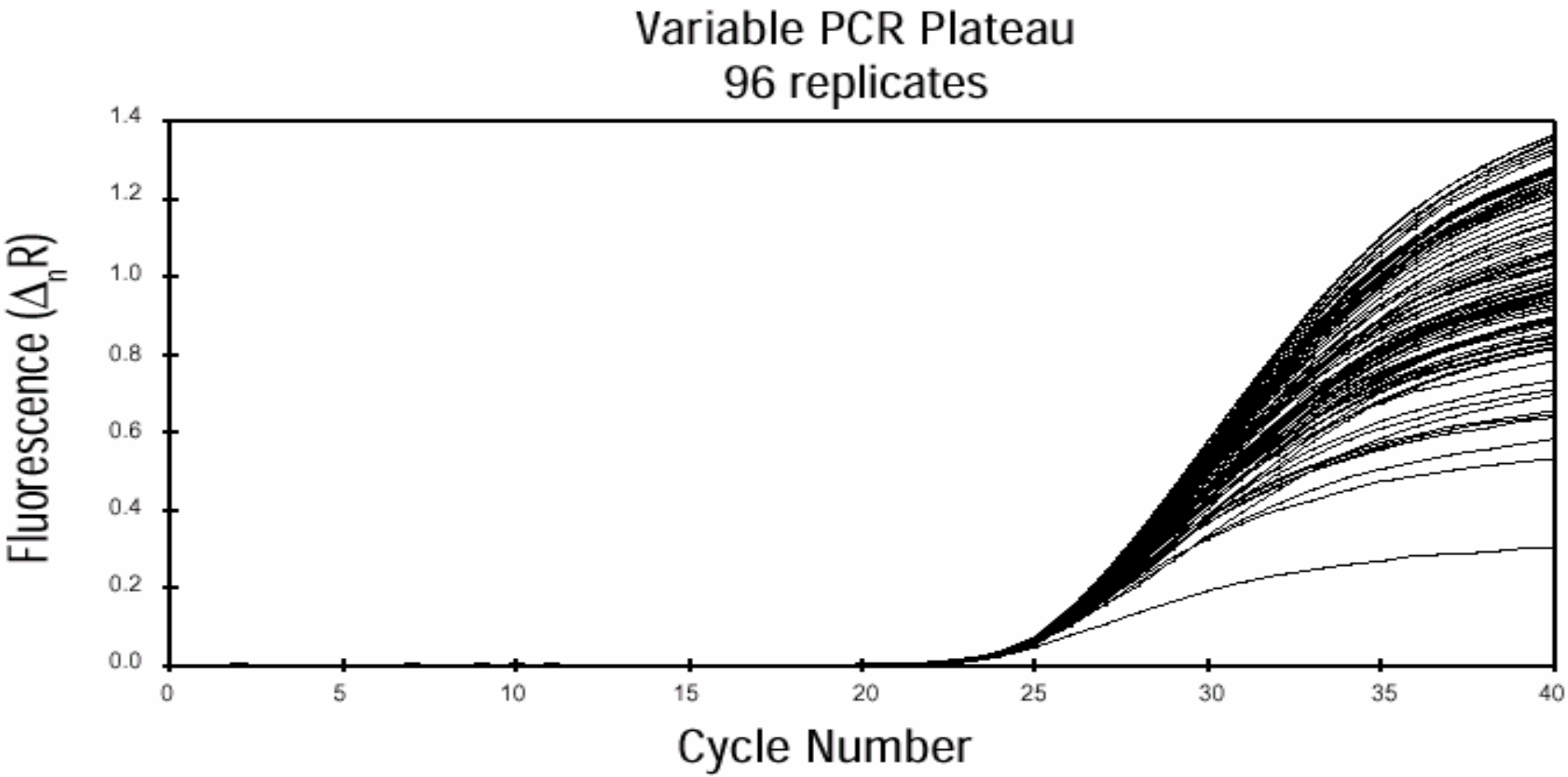


During extension, DNA polymerase cleaves the reporter dye from the probe.

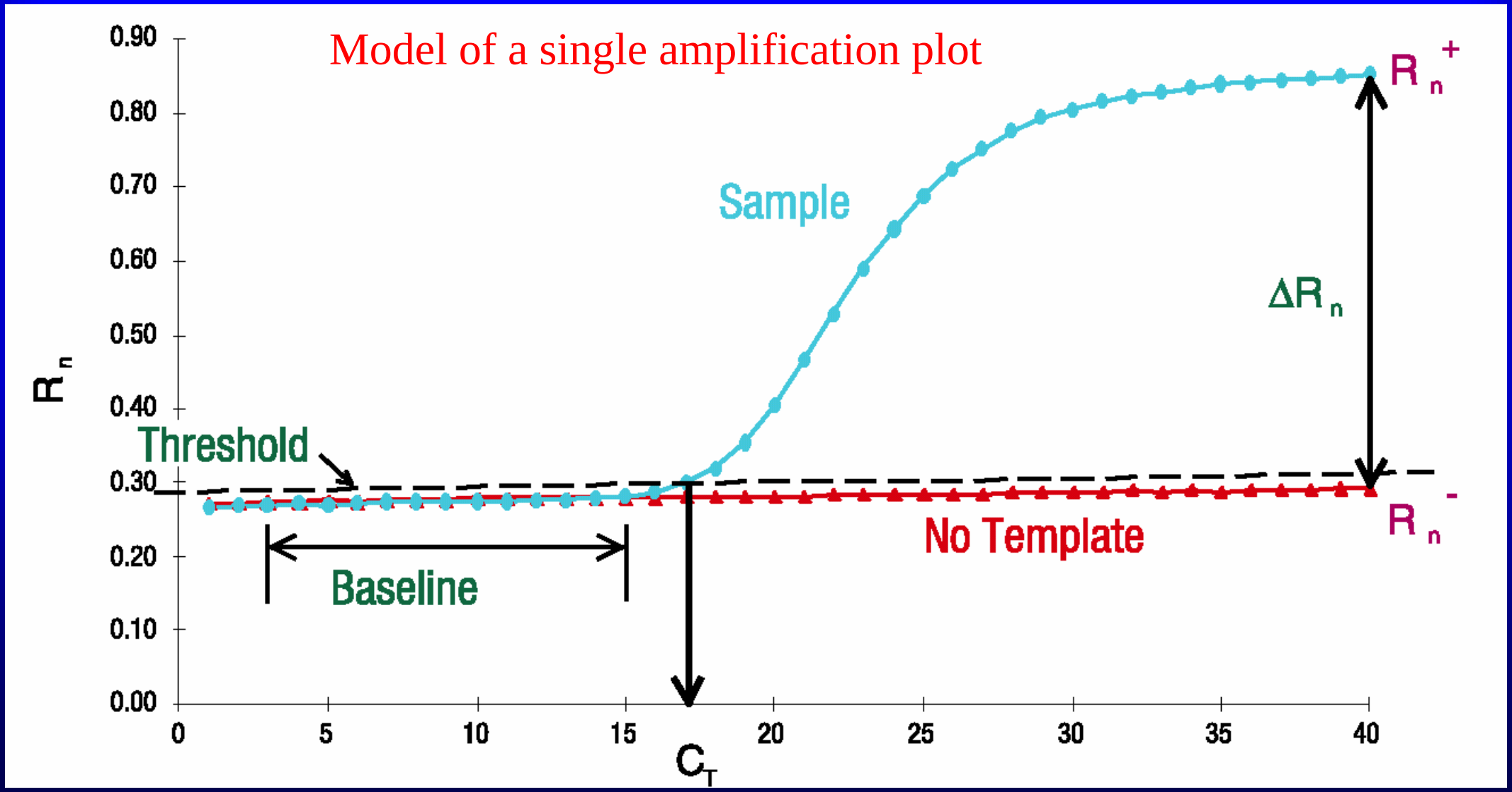


Real Time PCR

4a)

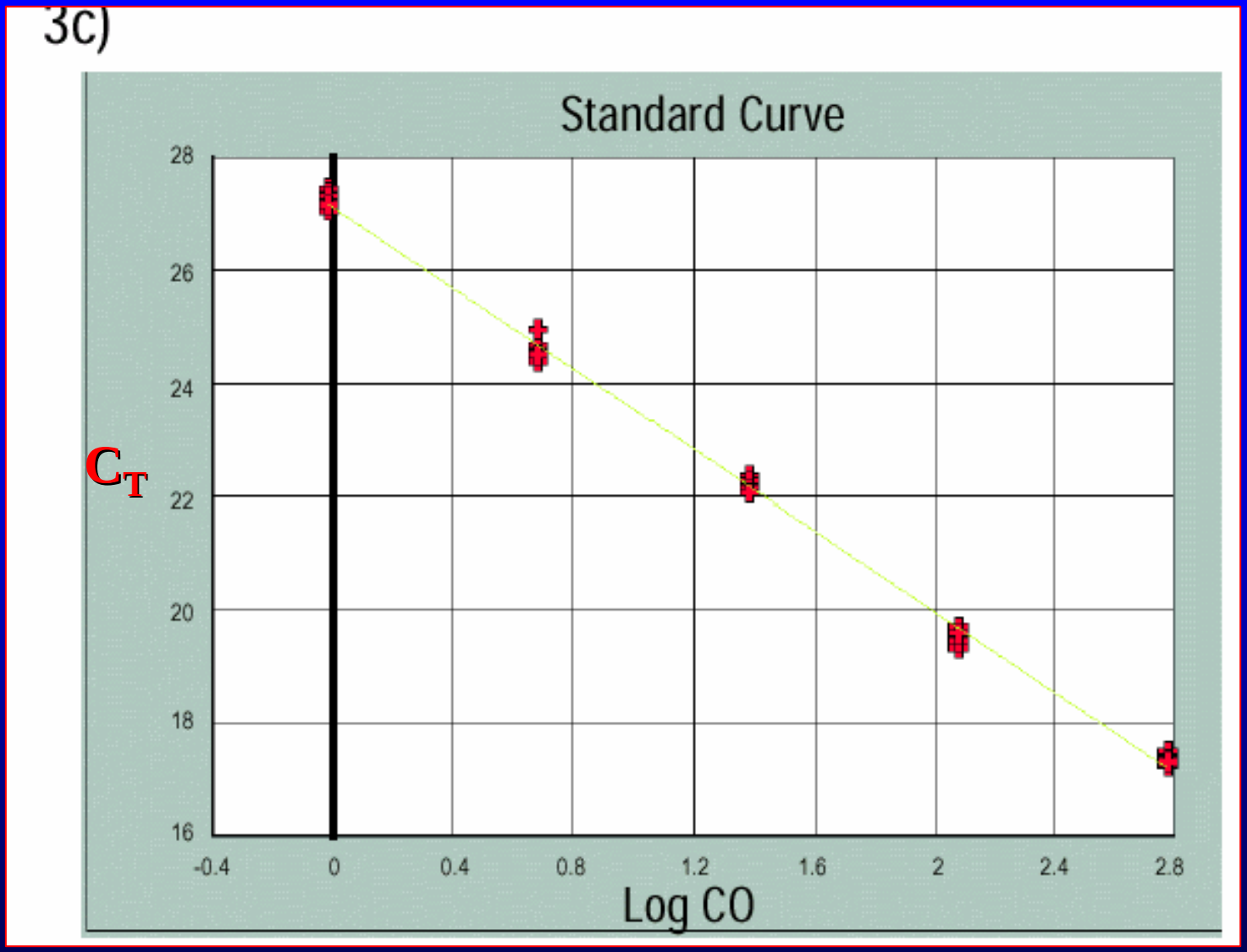


Real Time PCR



C_T : threshold cycle

Real Time PCR



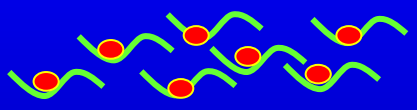
Standard curve showing C_T values plotted versus the log of the initial amount of genomic DNA.

In situ Hybridization

Tissue slide

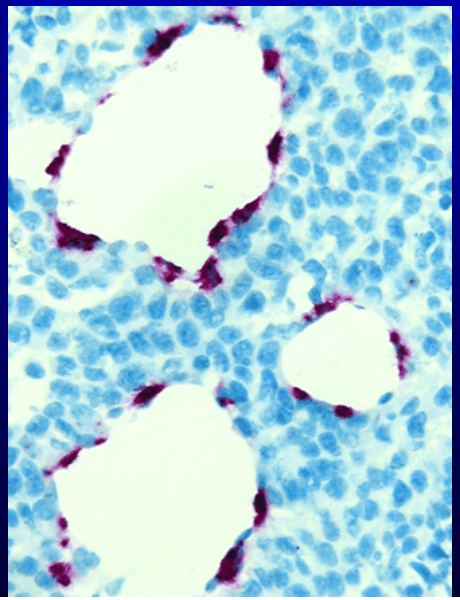
+

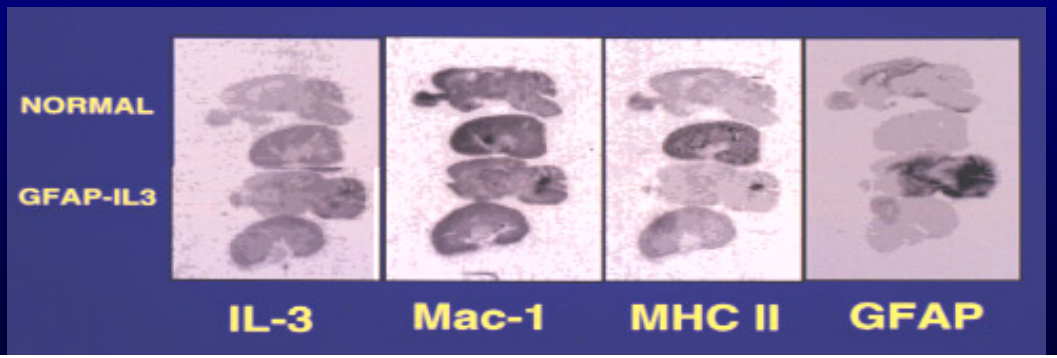
Anti-sense RNA
(probe)

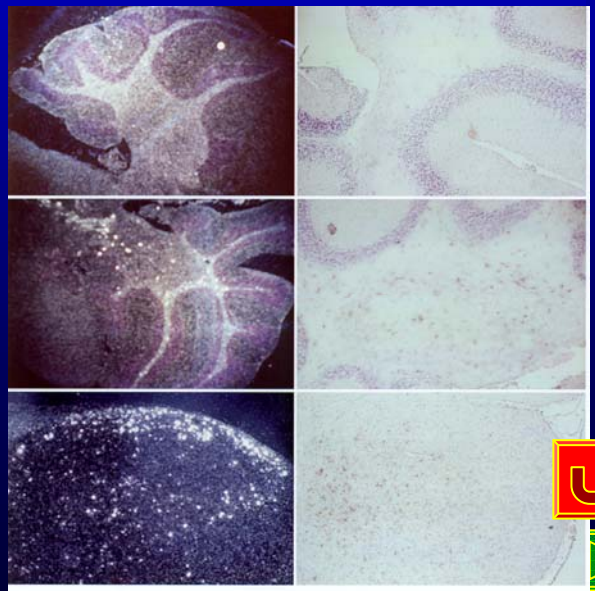


Hybridization

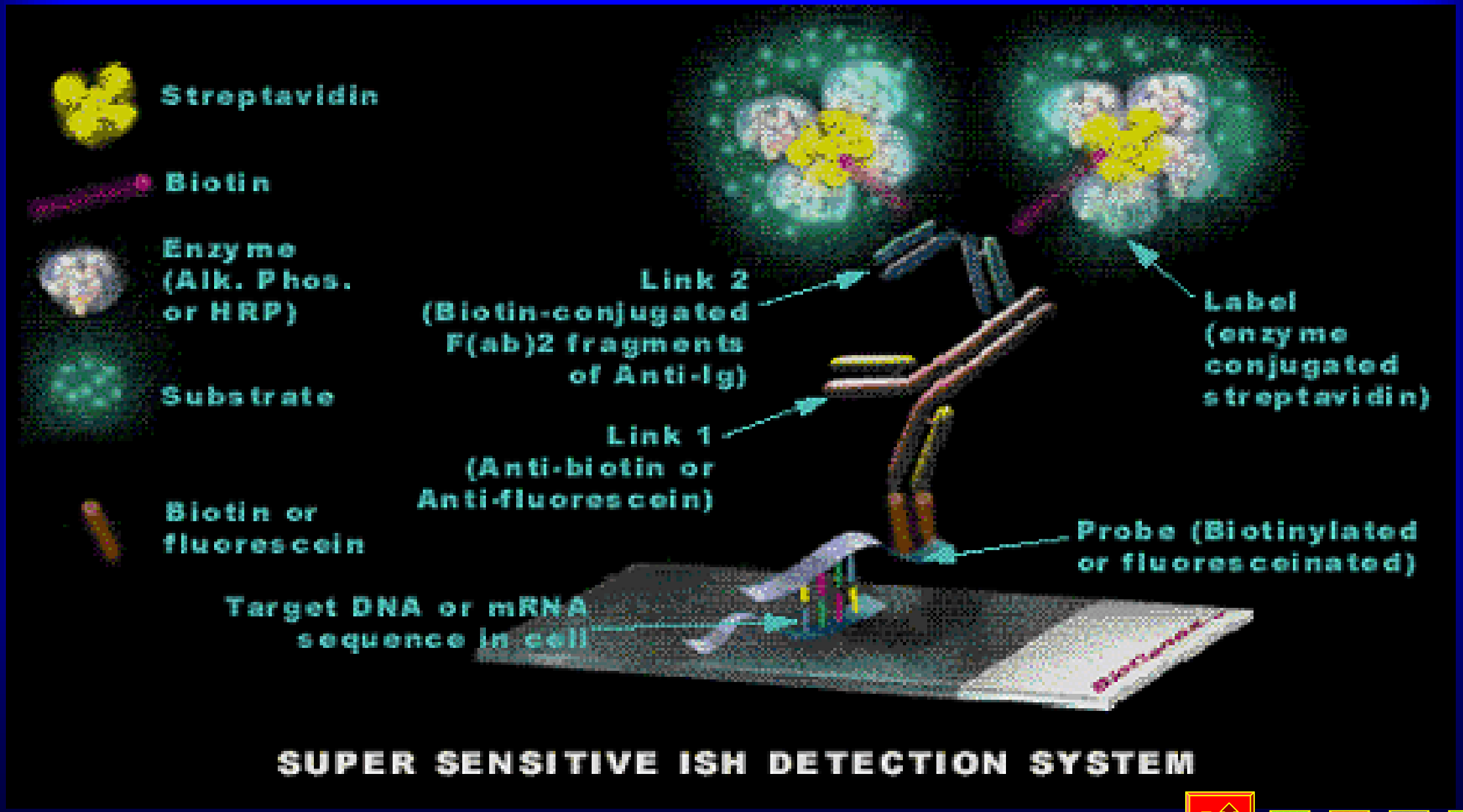
RNase Digestion



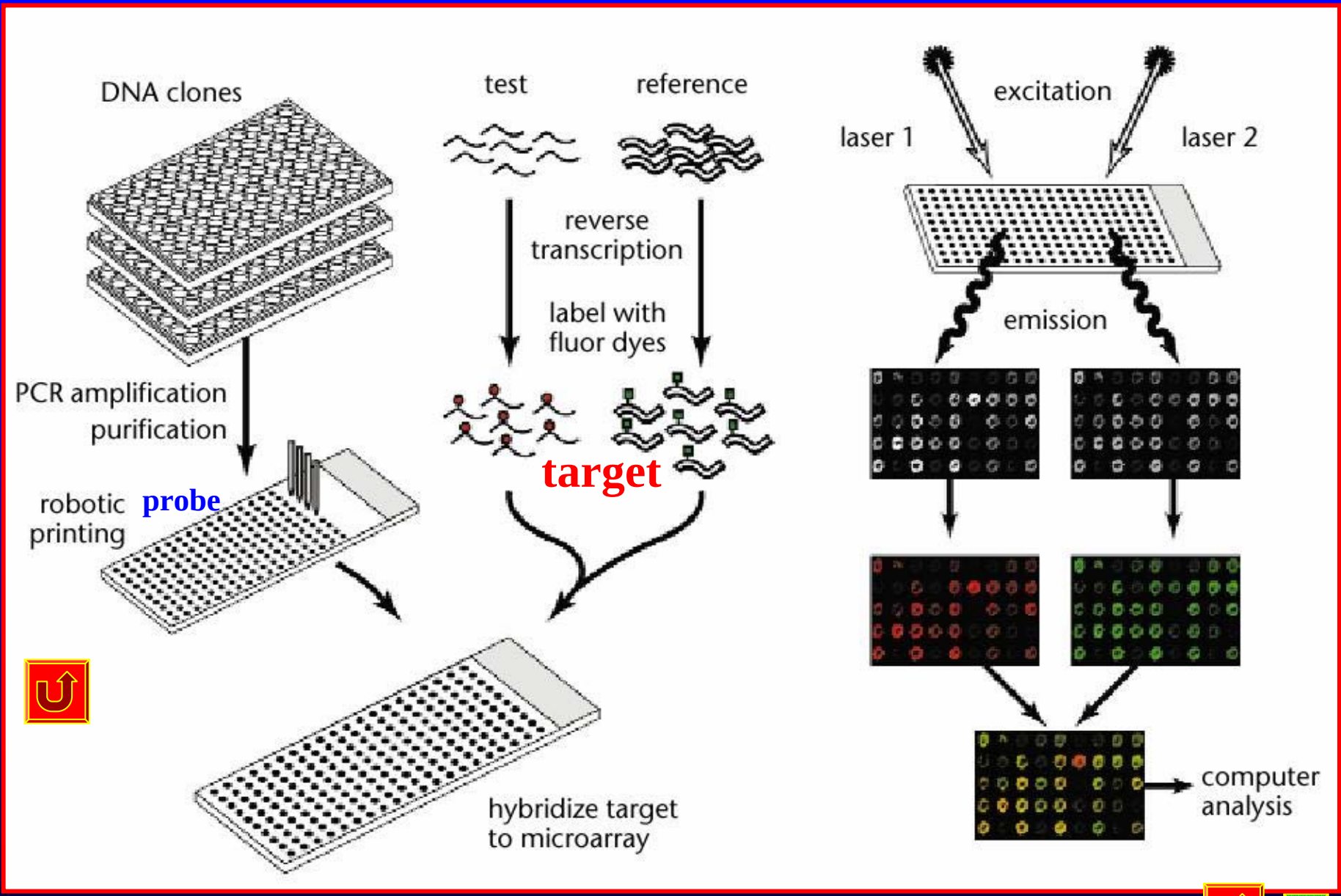




In situ Hybridization 2



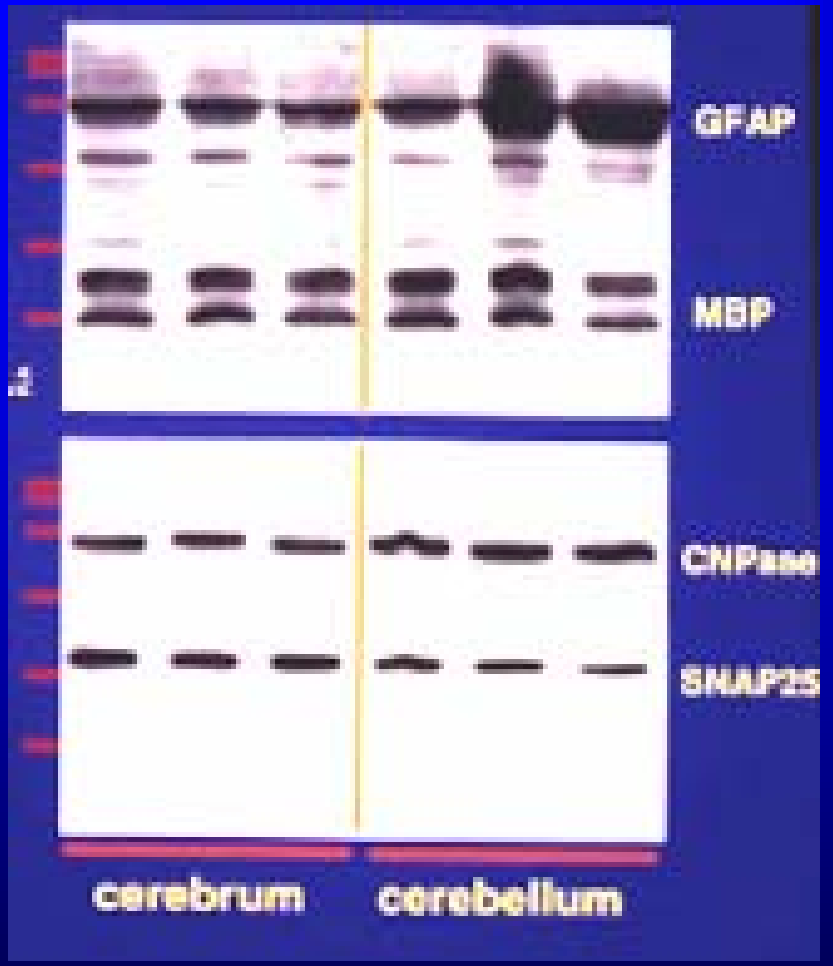
DNA Microarray



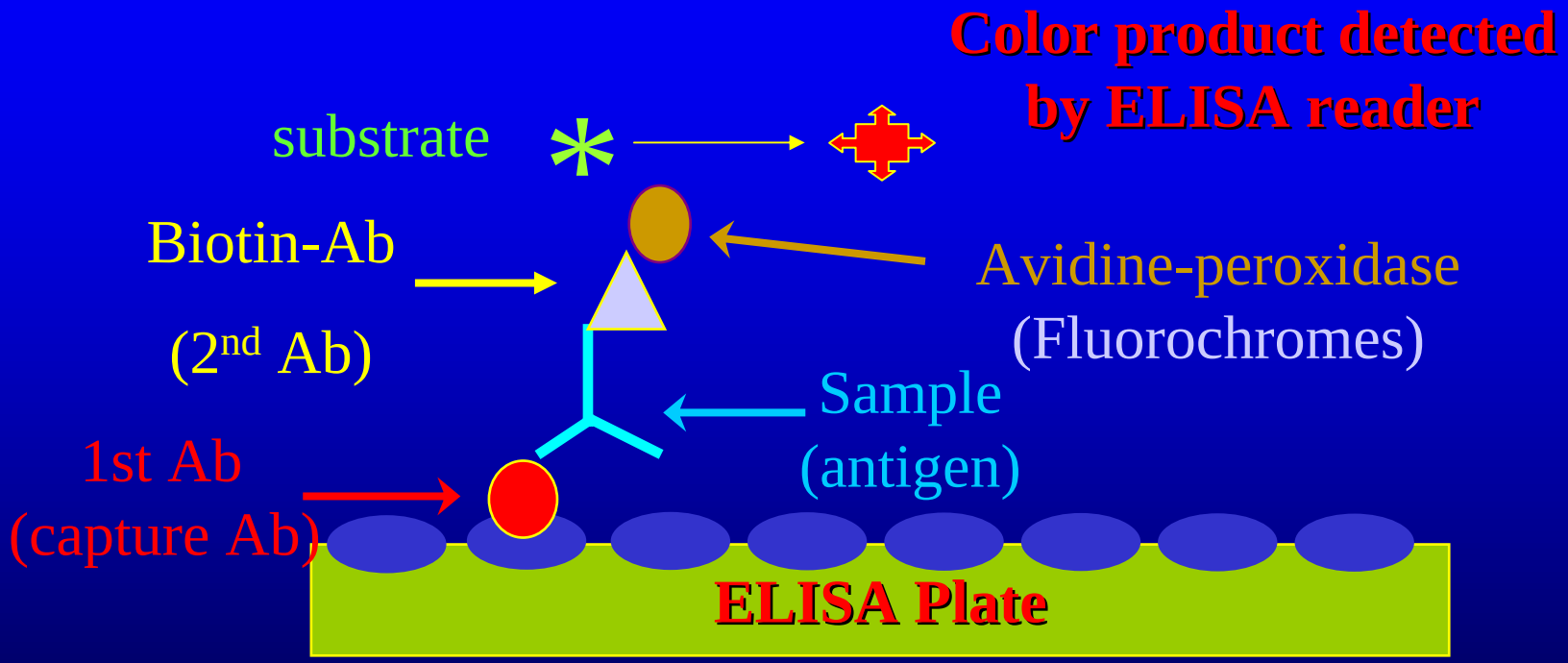
Duggan, D. *et al.*, *Nature Genet.* **21**, 10–14 (1999)

Western Blot

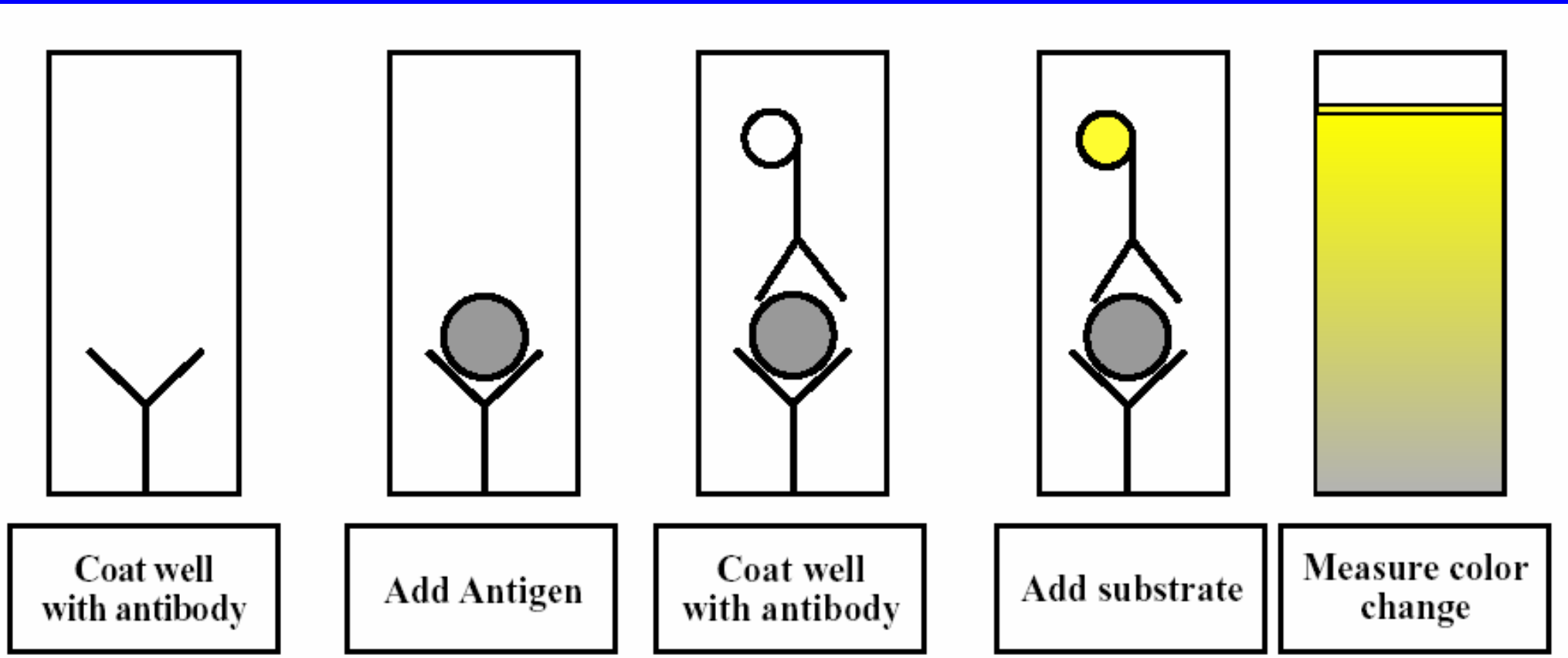
- Sample Target: total proteins
- Probe : Antibody
- Detection:
  - Gel Electrophoresis
  - Detection



ELISA
(Enzyme Link ImmunoSorbent Assay)



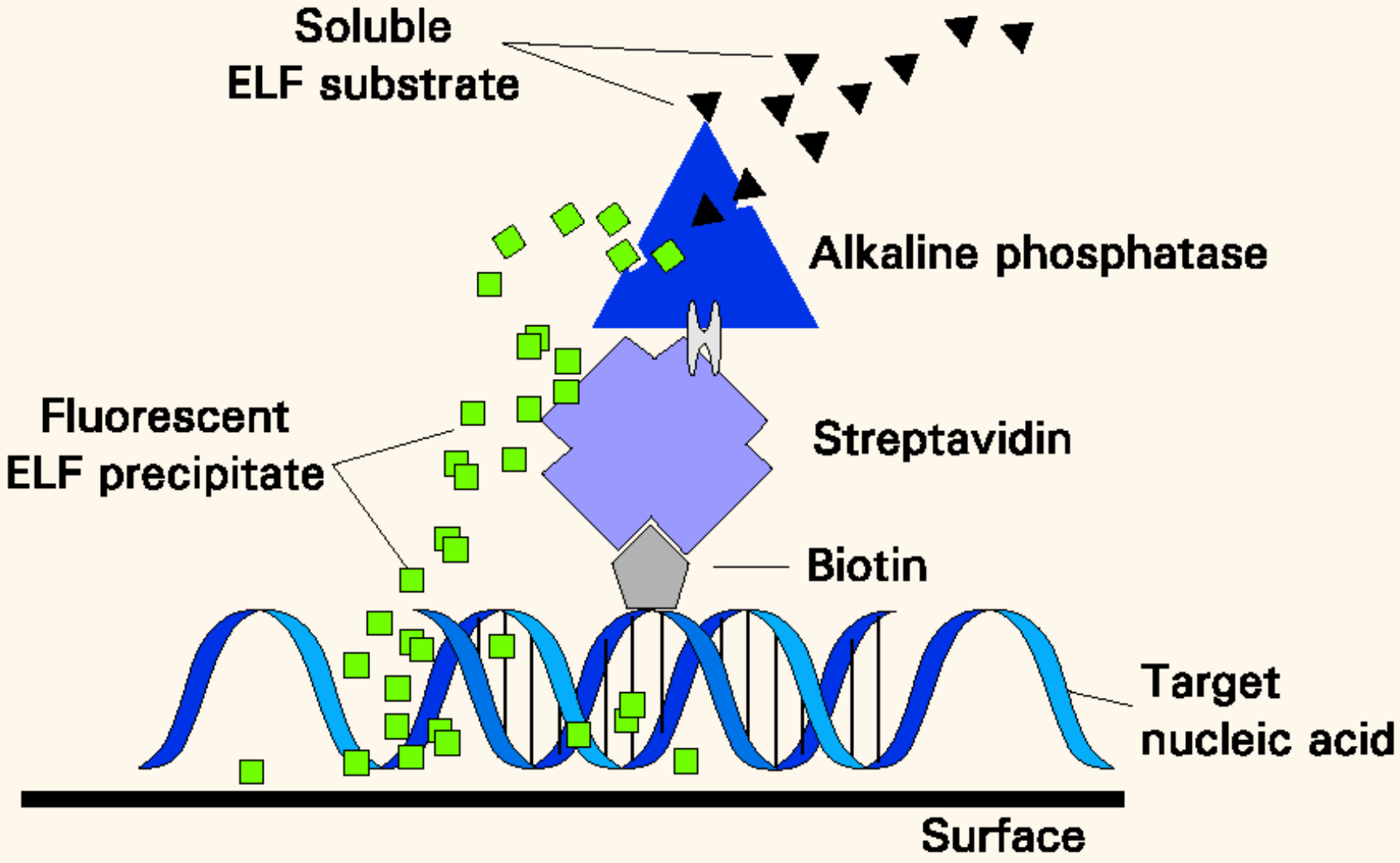
ELISA (Enzyme Link ImmunoSorbent Assay)



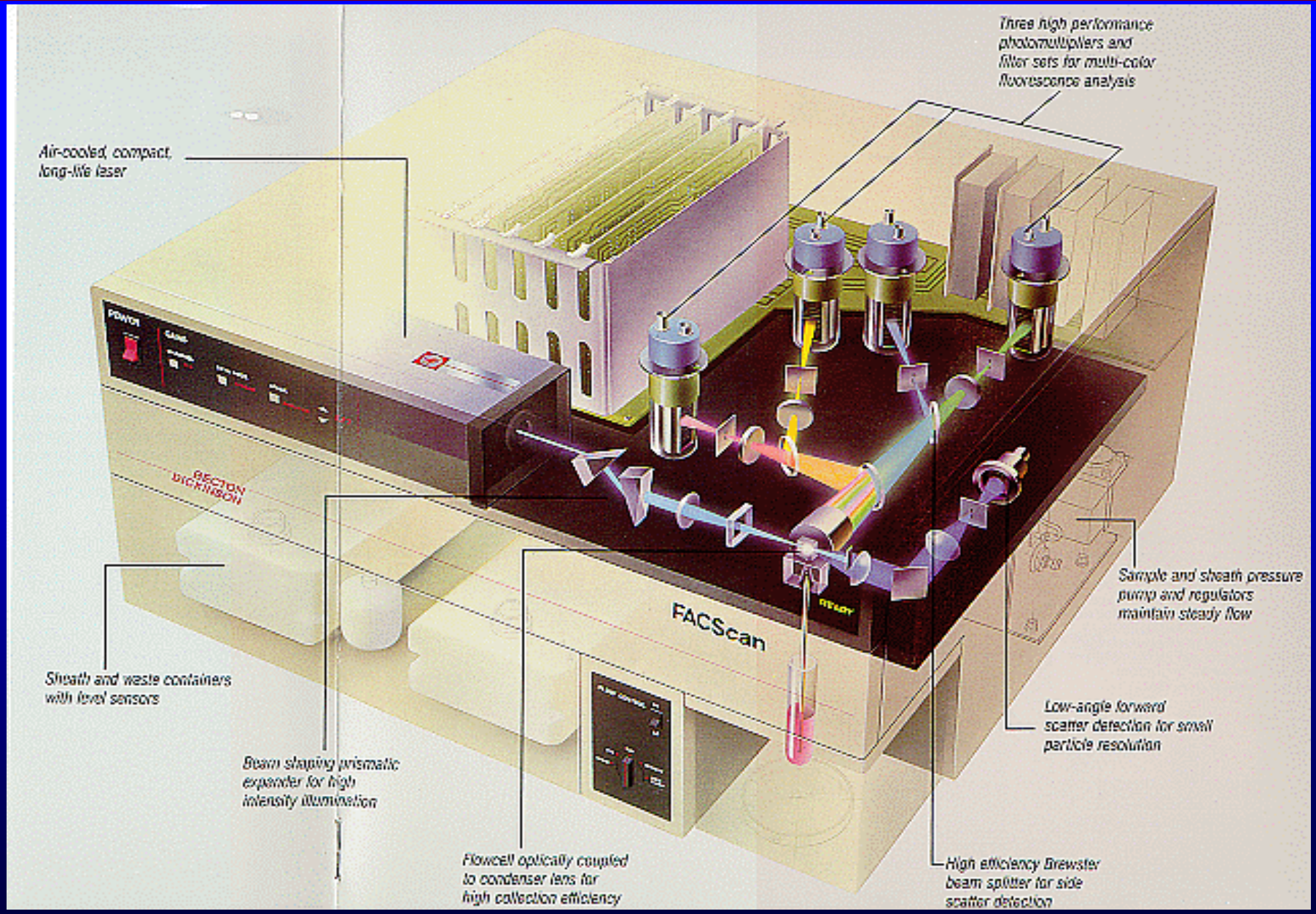
ELISA-IL3 standard curve

Capture antibody 4ug/ml
Detection antibody 1ug/ml

Hybridization of Nucleic Acids

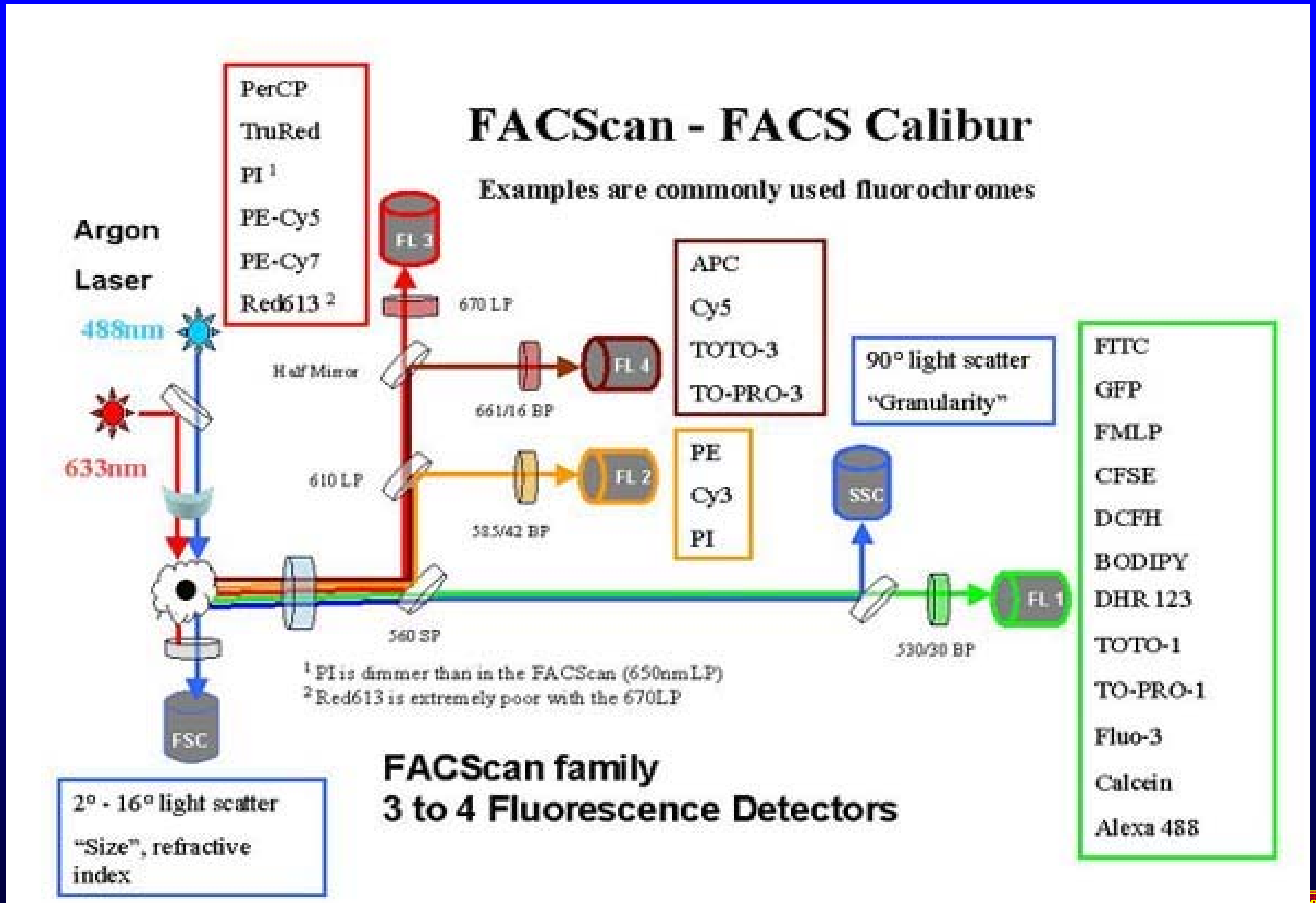


Flow Cytometry (FACS)



Flow Cytometry (FACS)

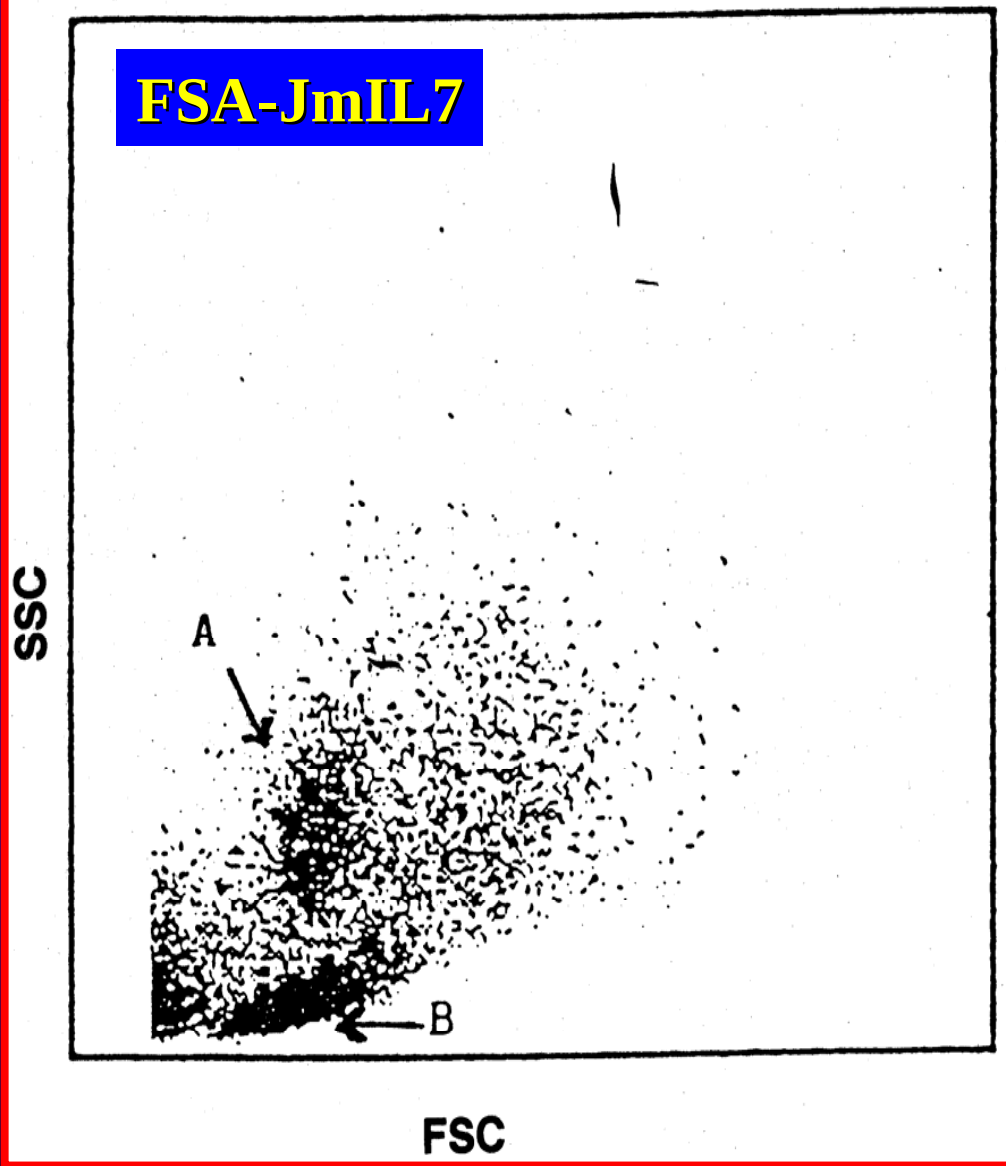
Fluorescence Activated Cell Sorting

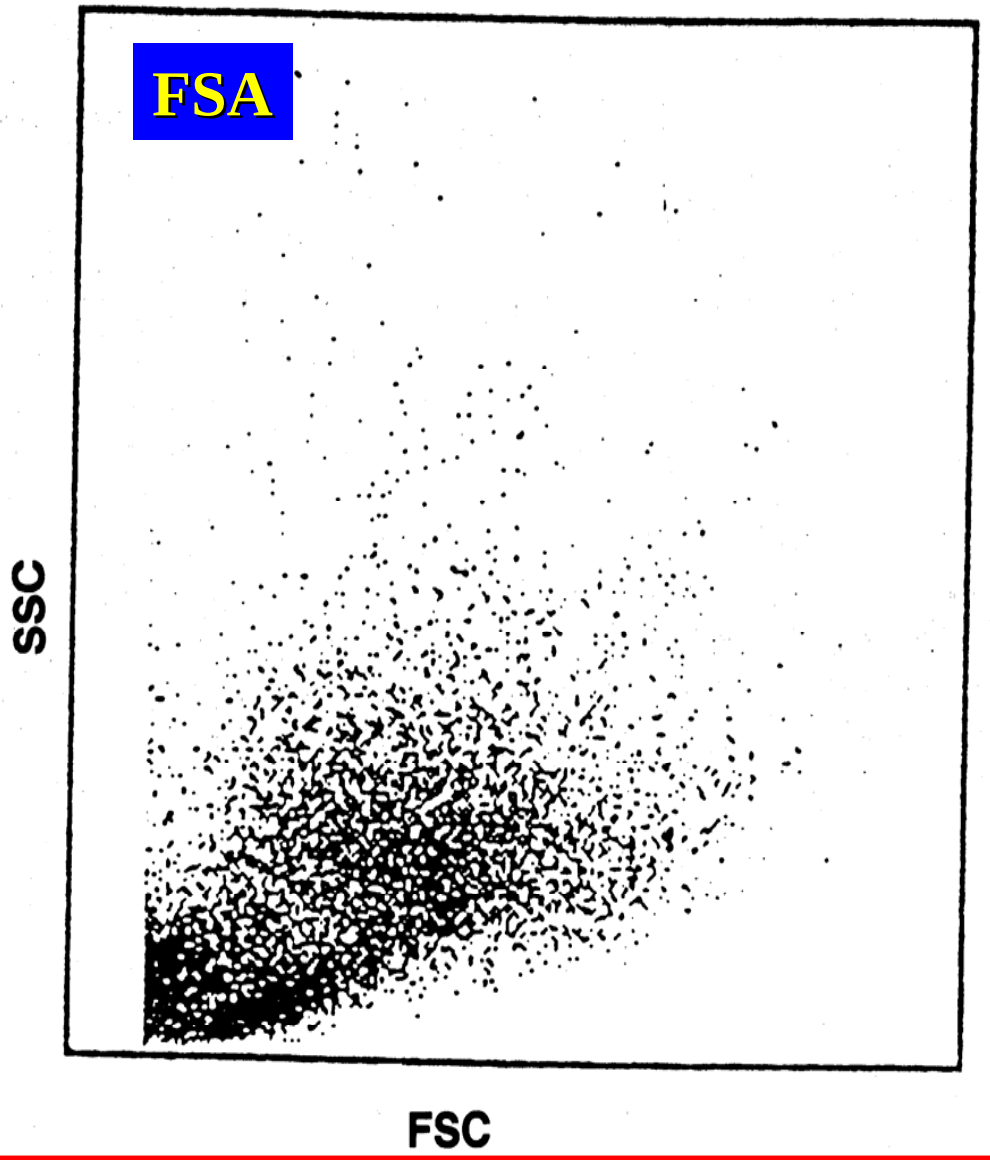


Principles of Using FACS

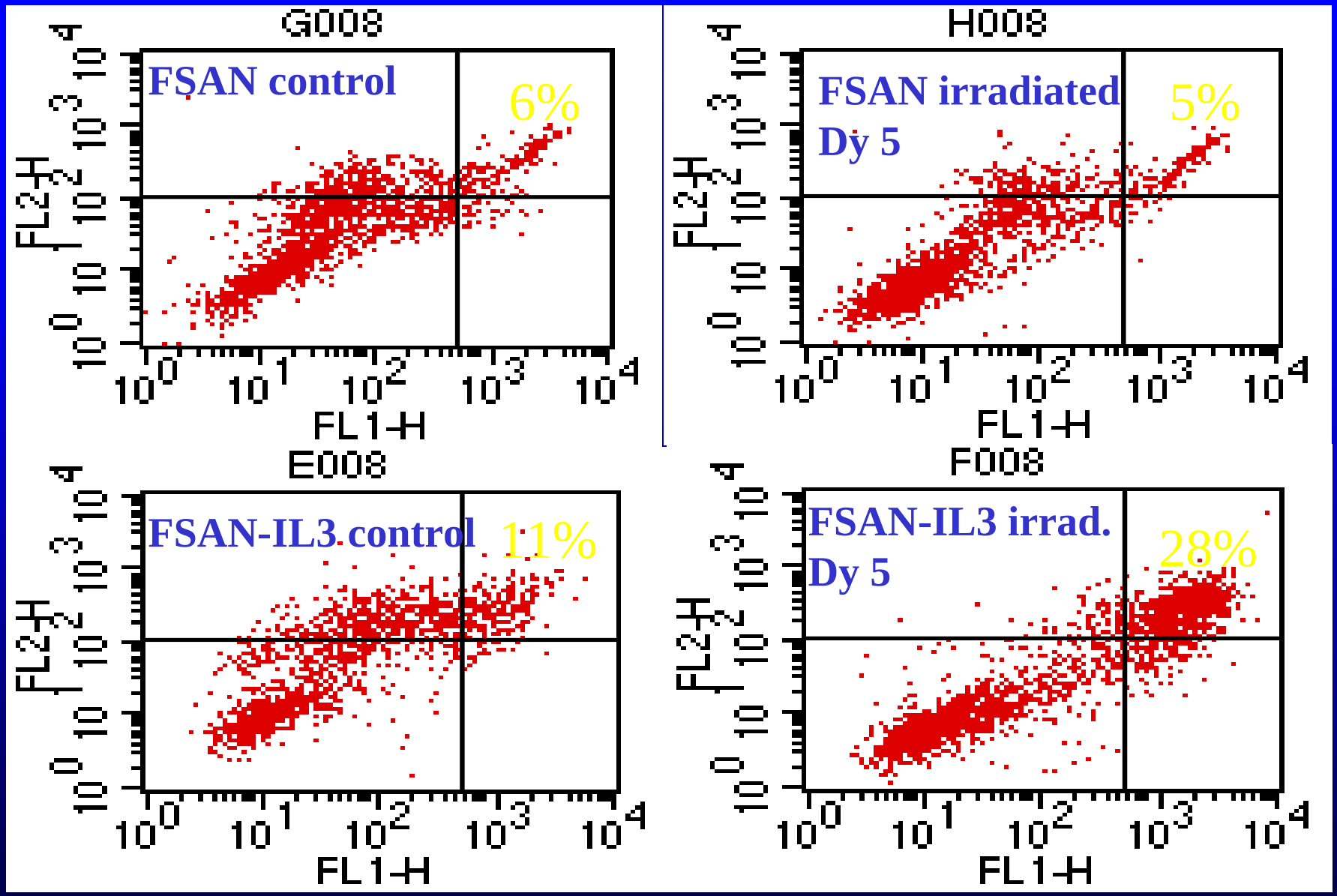
- Size difference (FSC)
- Granularity difference (SSC)
- Binding ability to fluorochromes
  - FL1
  - FL2
  - FL3
  - FL4

Example 1 of FACS





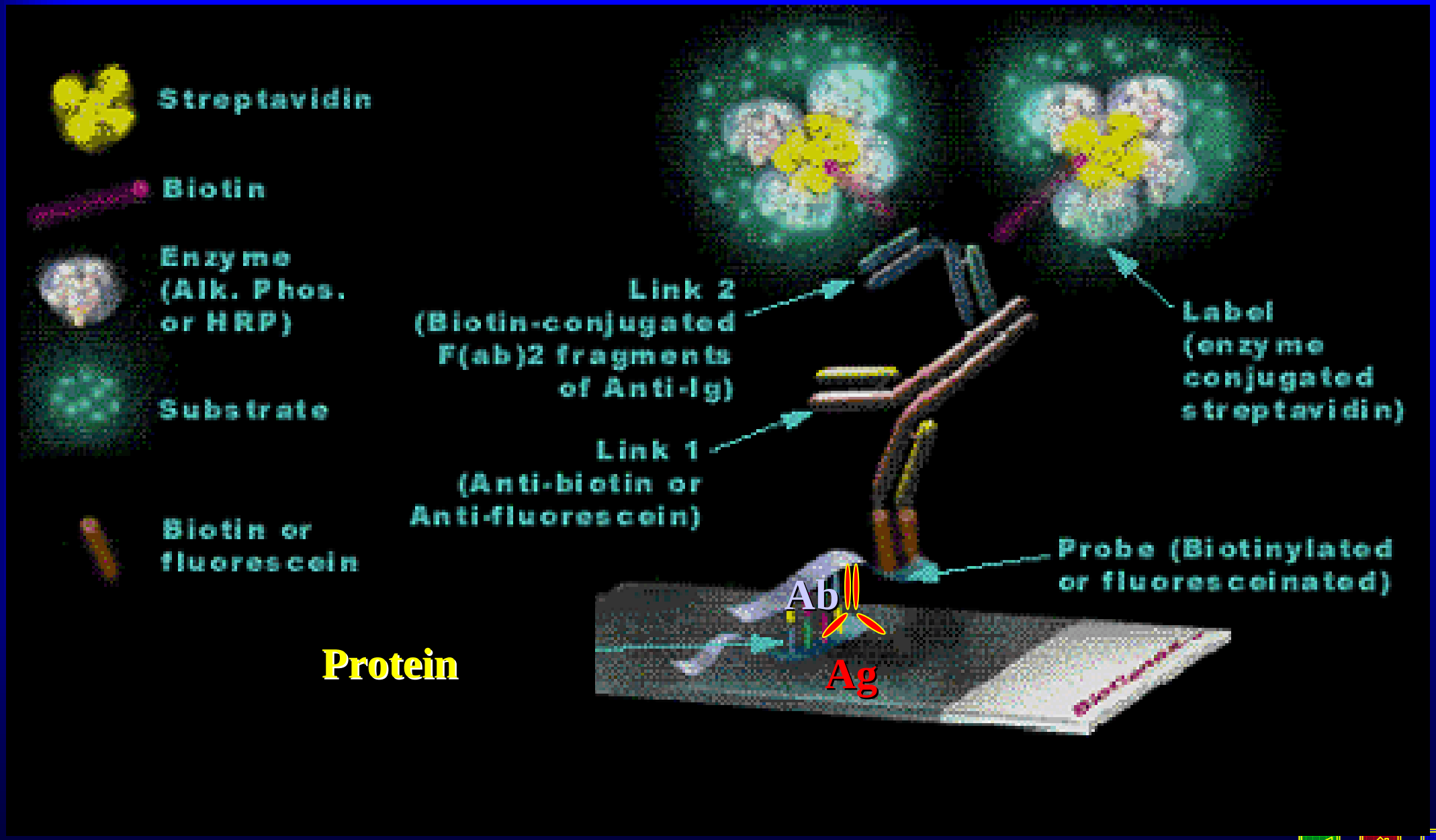
Example 2 of FACS



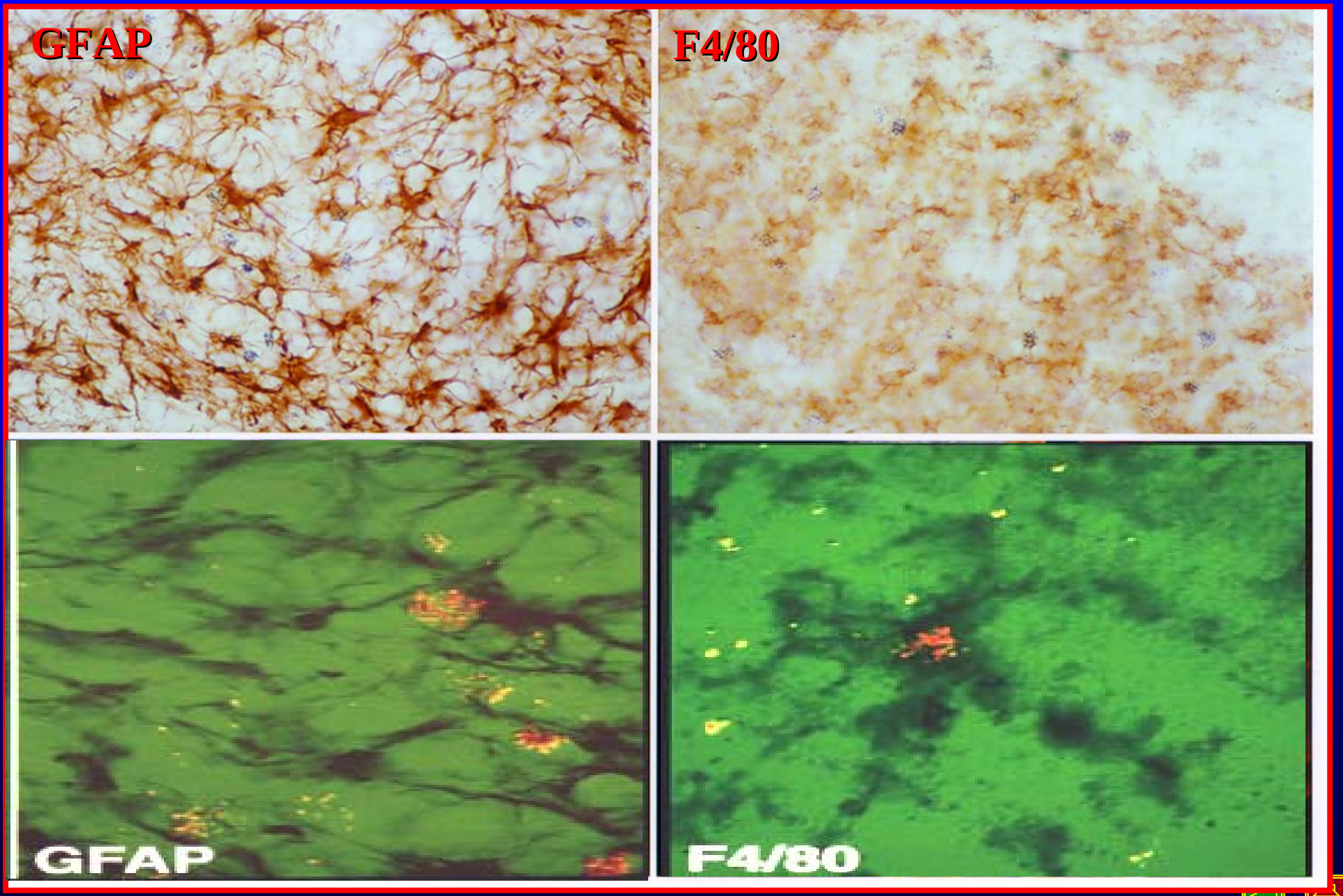
Applications of FACS

- Cell cycle analysis
- Chromosome analysis (DNA analysis)
- Cell Sorting
- Multicolor Analysis: Cell Phenotyping
- Functional studies
- Apoptosis

Immunostaining



Examples of Immunostain



Gene Analyses

- Screening a gene
  - Southern Blotting
  - DNA Sequence Analyses
  - PCR
- Gene Structure
  - Promoter
  - intron

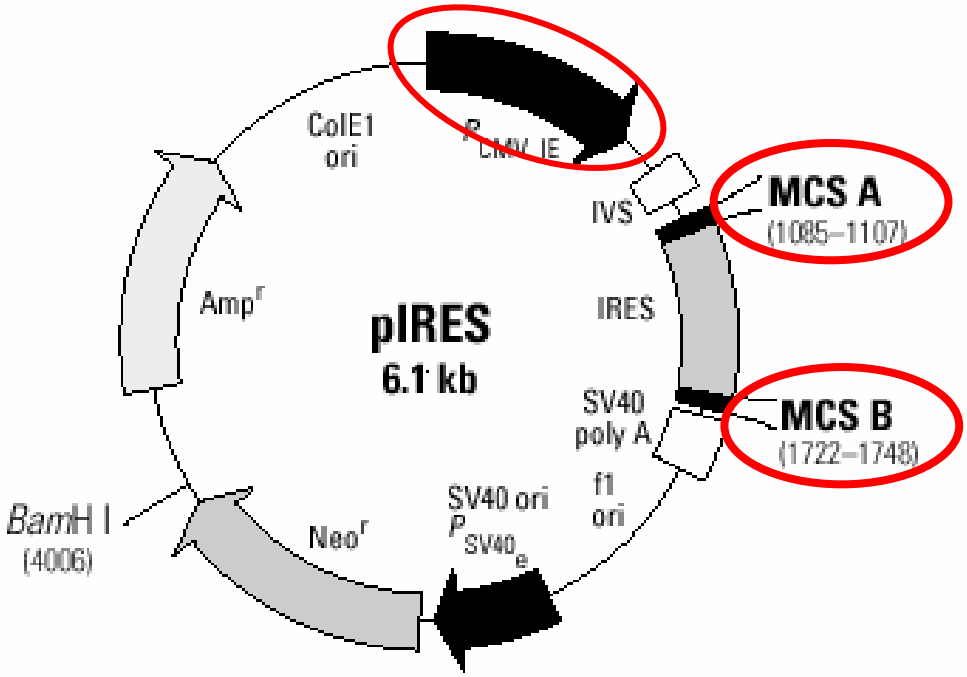
Gene Cloning

- **Restriction Endonuclease**
- **Vectors**
- **DNA-mediated Gene Transfer**
- **Hosts**
- **Libraries**

Vectors

- Plasmids (naked DNA)
- Virus
  - Retrovirus
  - Adenovirus
  - AAV
  - Lentivirus
  - Phage
  - …..
- Inducible vectors
- Tissue Specific vectors

Plasmid



MCS A

T7 Promoter

1070 1080 1090 1100 1110

TAATACGACTCACTATAGGCTAGCCTCGAGAATTCACGCGTCGAGCA IRES sequence...

***Nhe* I** ***Xho* I** ***EcoR* I** ***Mlu* I** *Kpn* I / *Asp718* I

MCS B

T3 Promoter

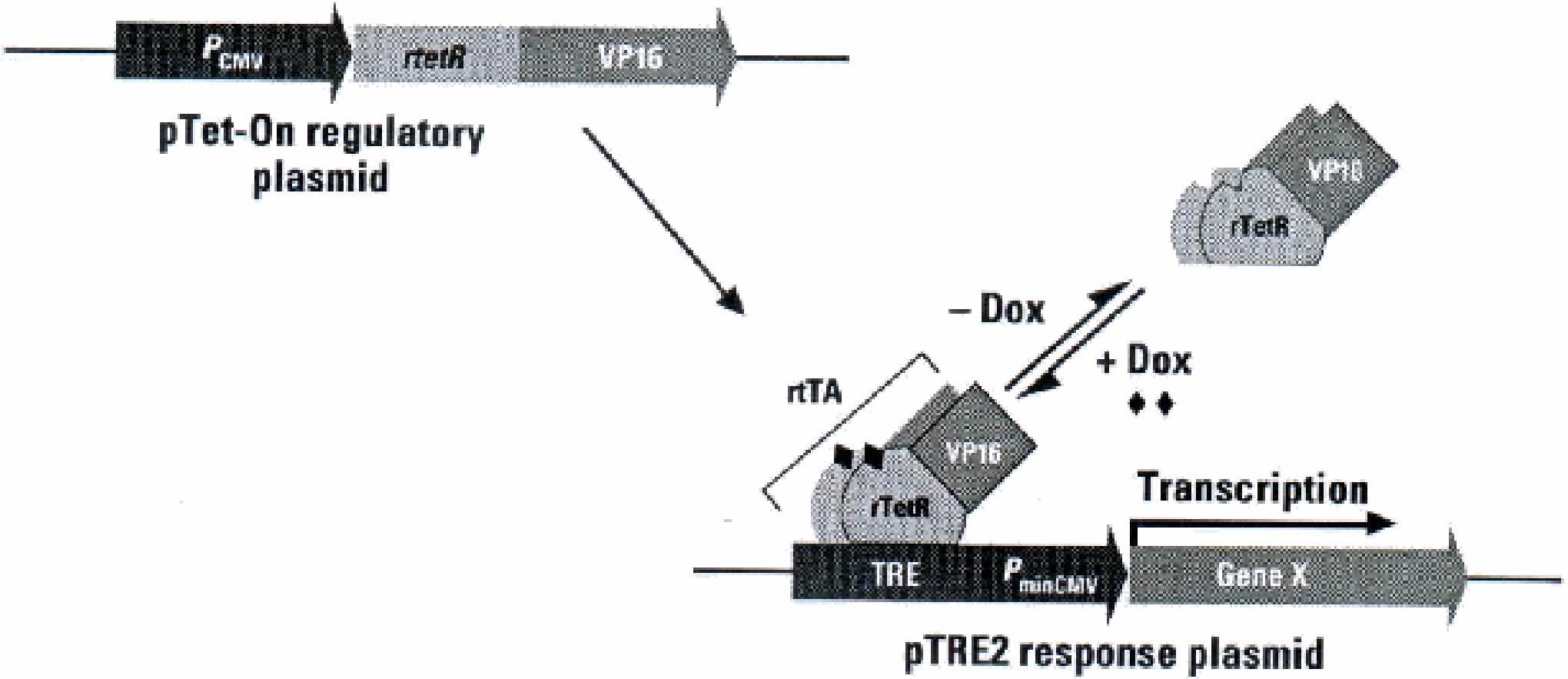
1720 1730 1740 1750 1760 1770

...IRES sequence CAACCCGGGATCCTCTAGAGTCGACCCGGGCGGCCGCTTCCCTTTAGTGAGGGTTAATG

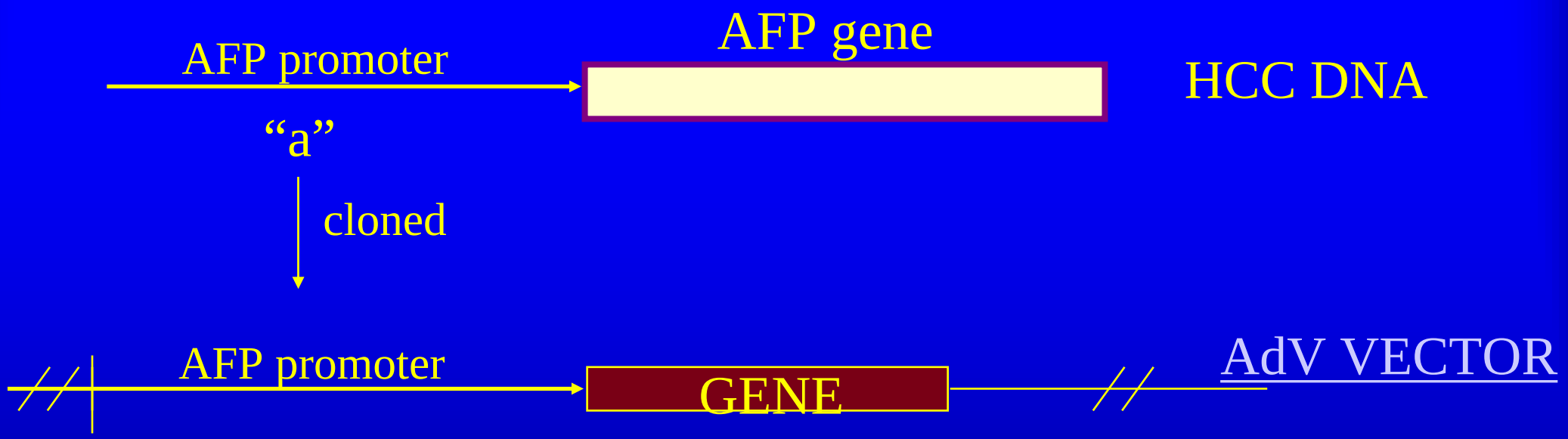
Xma I / *Sma* I * ***Xba* I** *BamH* I ***Sal* I** *Xma* I / *Sma* I * *Eag* I ***Not* I**

Inducible vectors

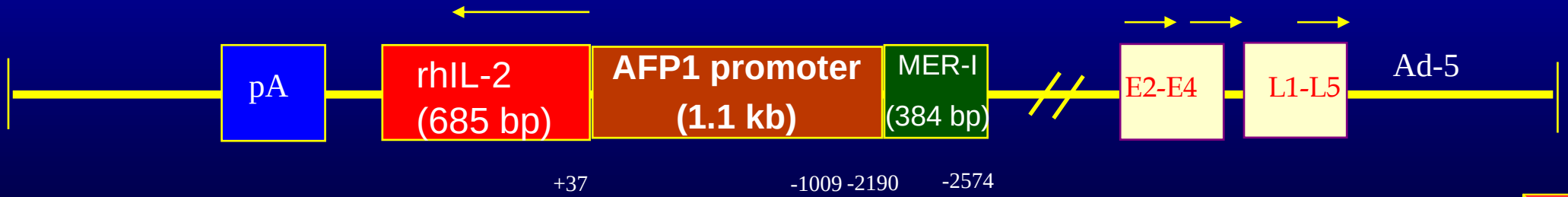
Tet-On



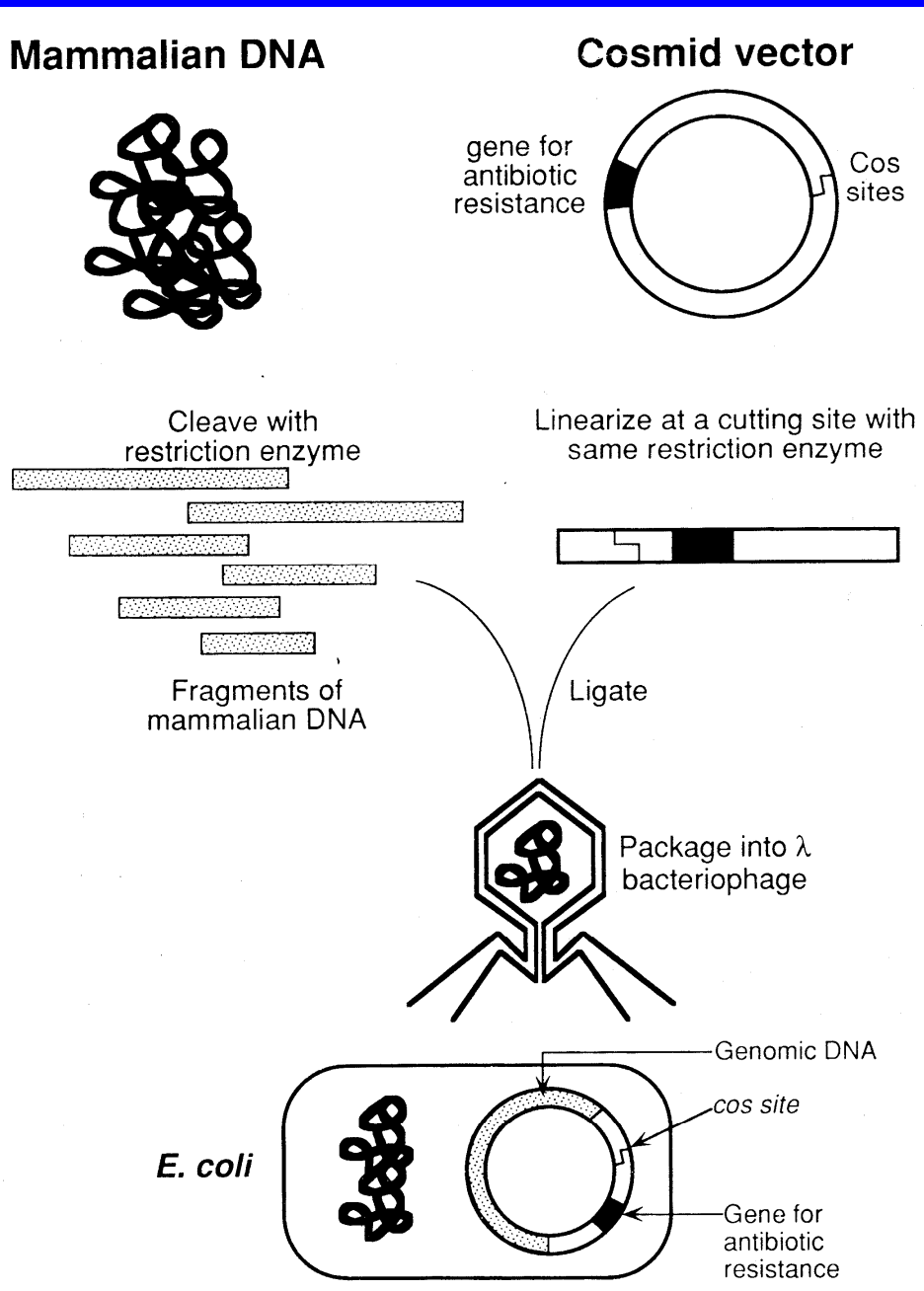
ADENOVIRAL AFP CONSTRUCTS



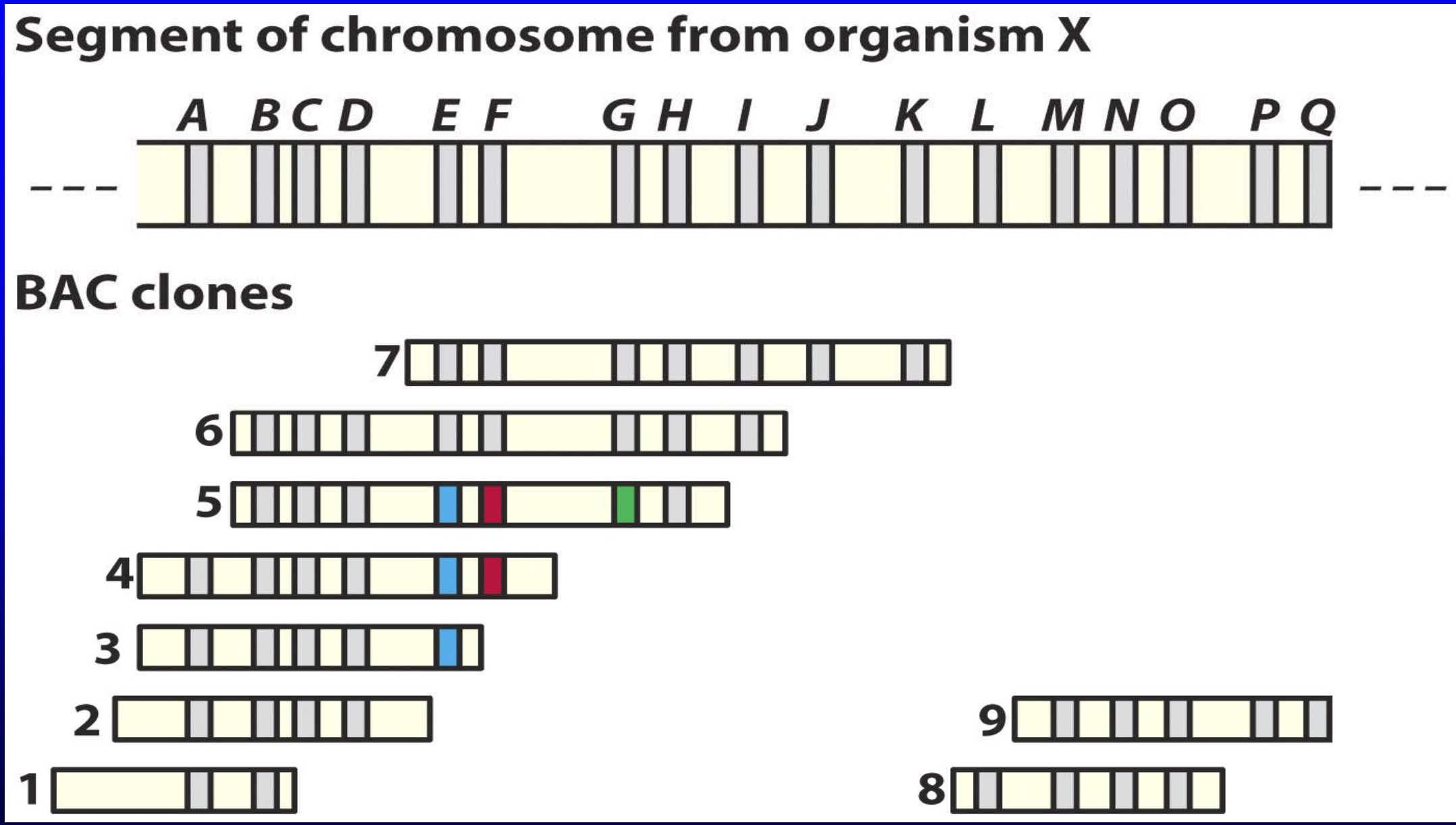
AdVAFP1-IL2



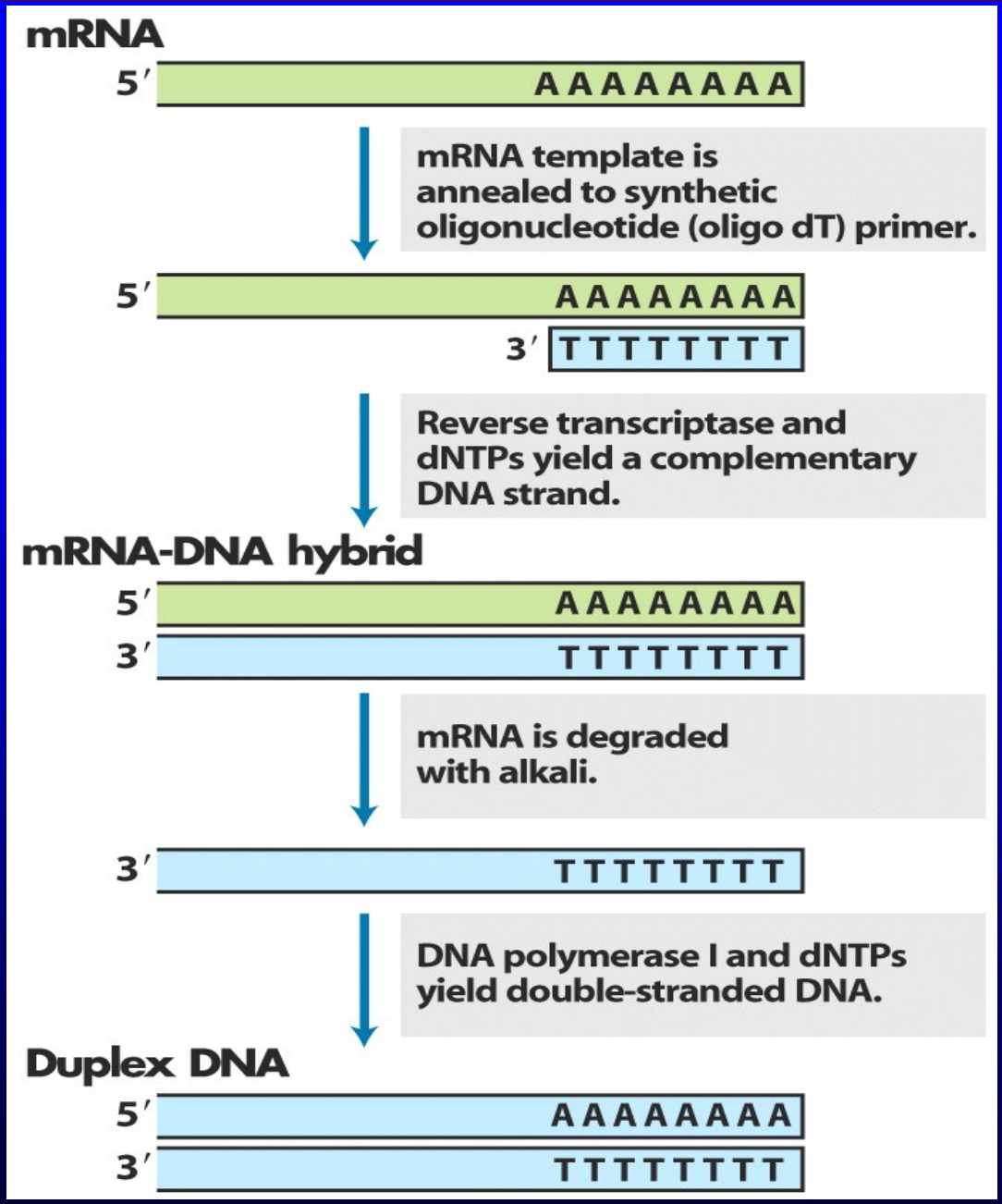
Genomic Library



Ordering of the clones in a DNA library (genomic library)



Construction of a cDNA library from mRNA



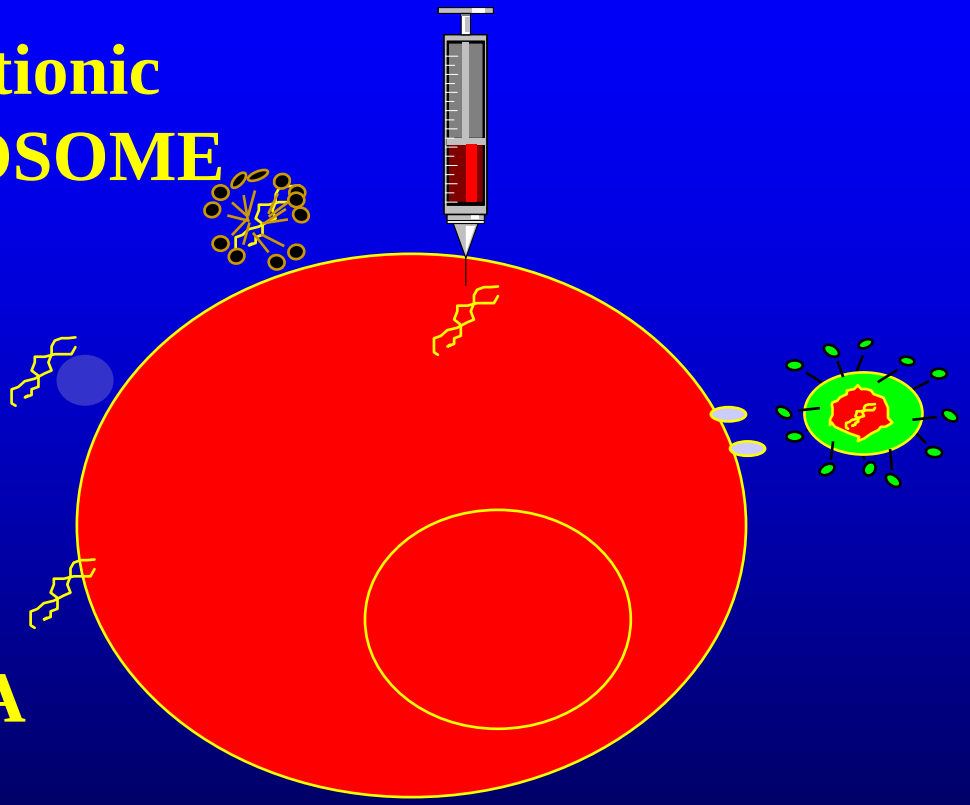
DNA-Mediated Gene Transfer

Microinjection/
Gene Gun

Cationic
LIPOSOME

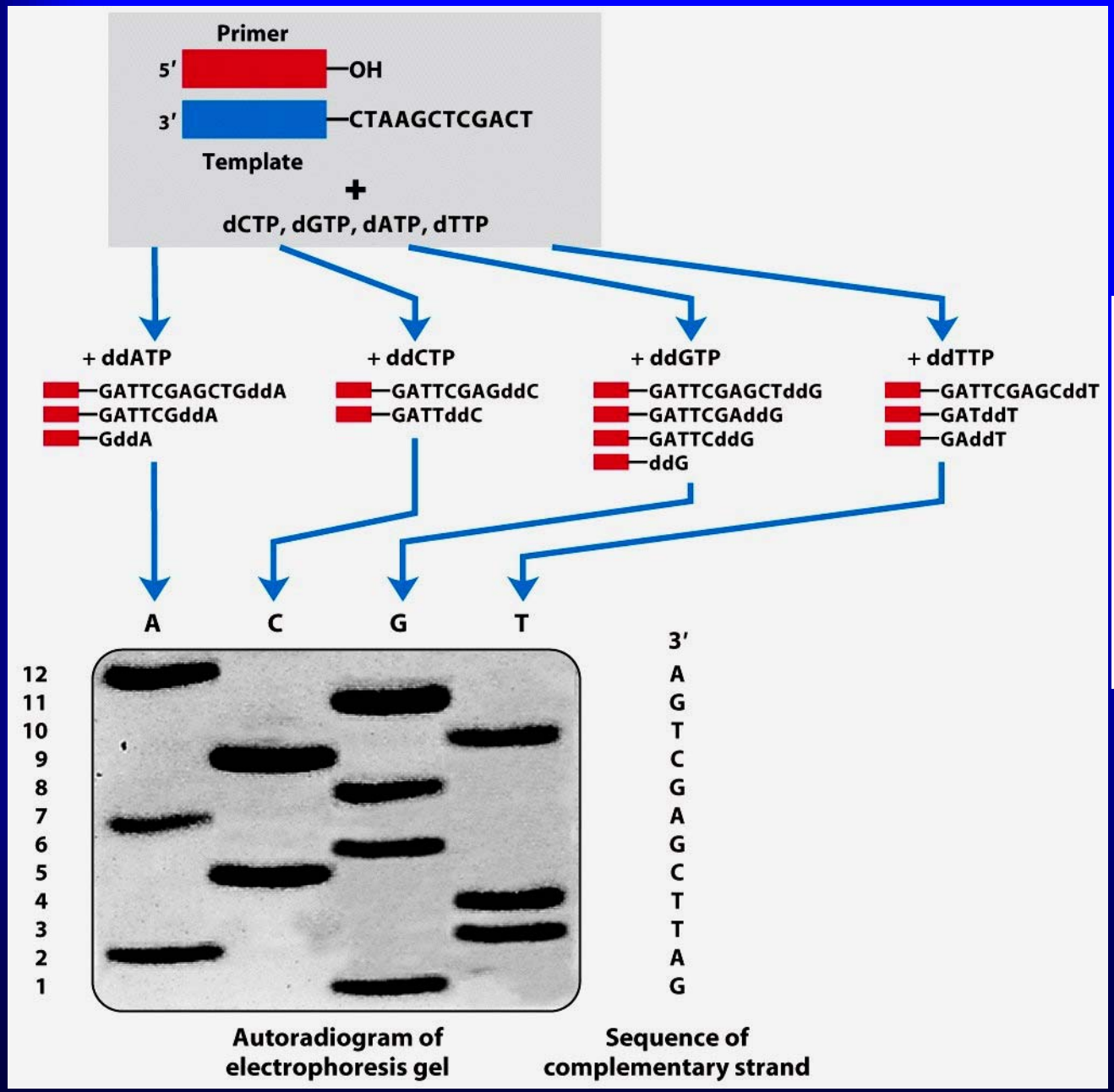
Calcium
Phosphate

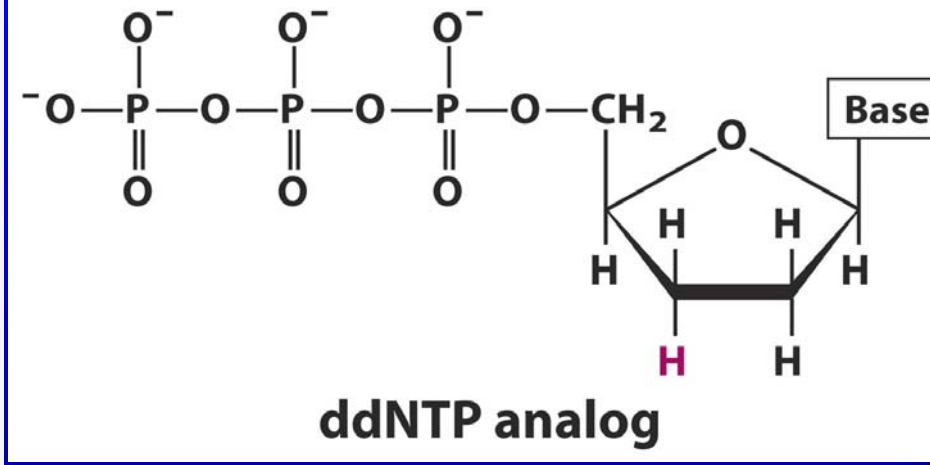
Naked DNA



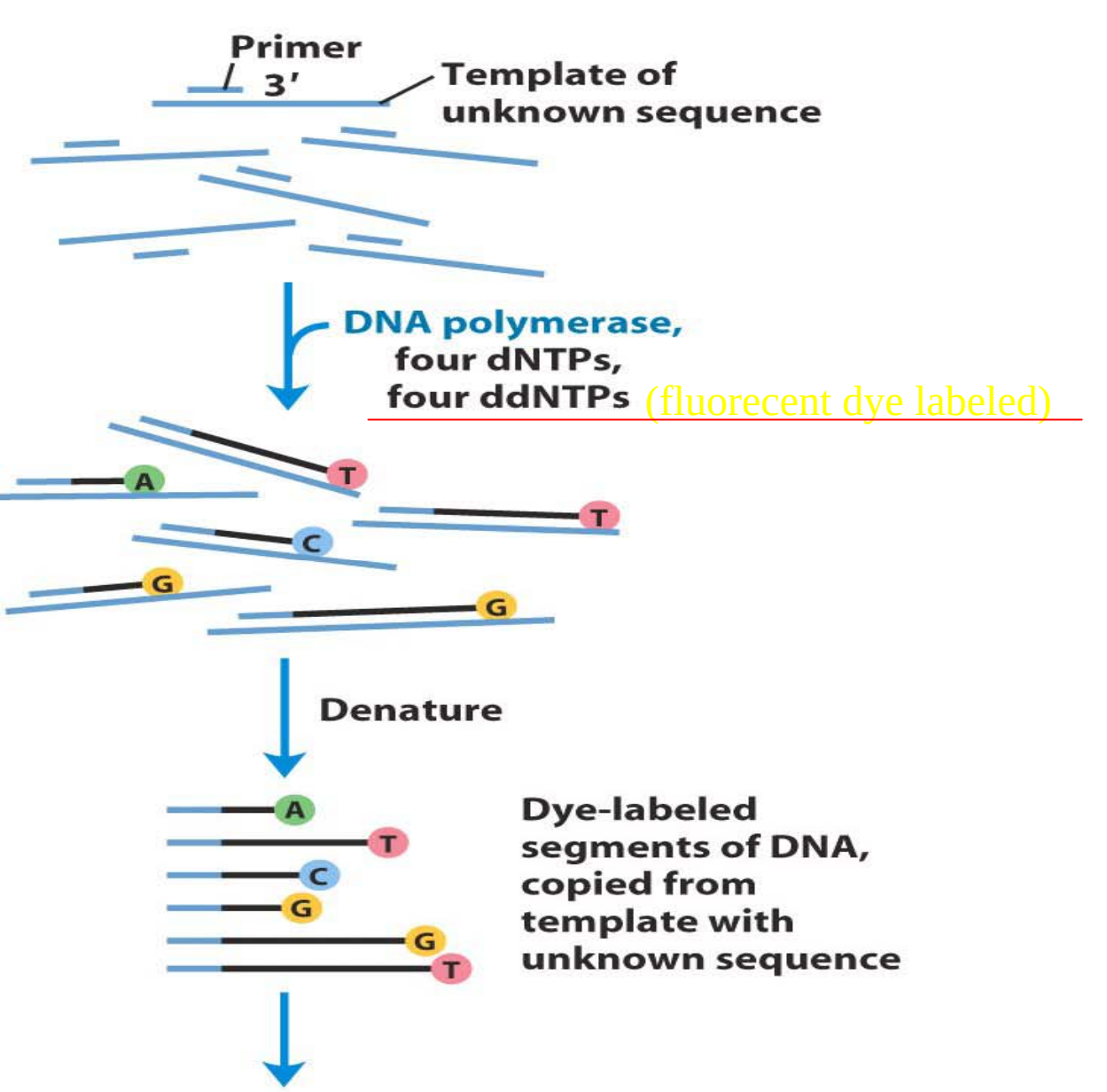
Retrovirus
Adenovirus
AAV
HSV
etc.

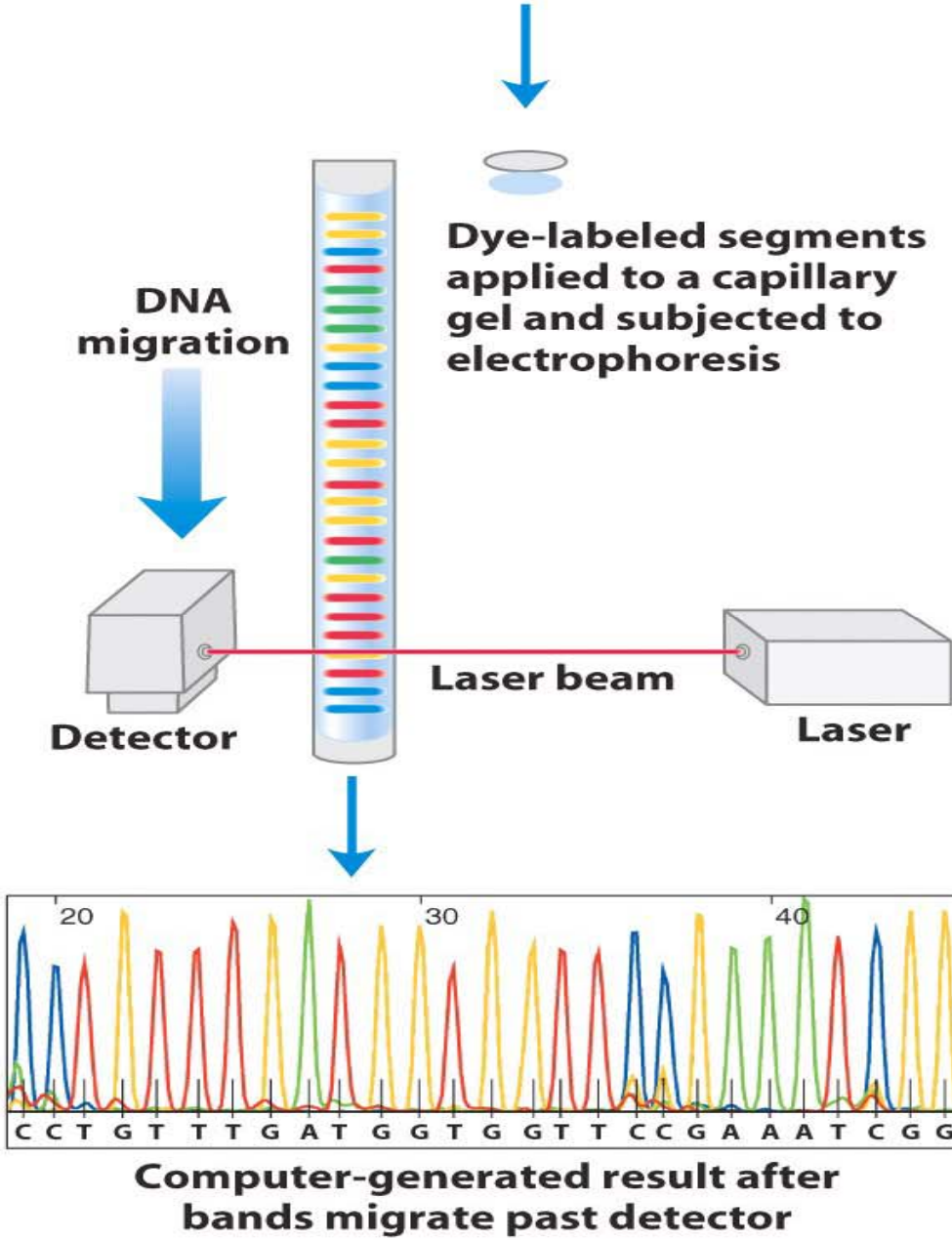
DNA Sequencing by Sanger Method





Strategy for automating DNA sequencing reactions

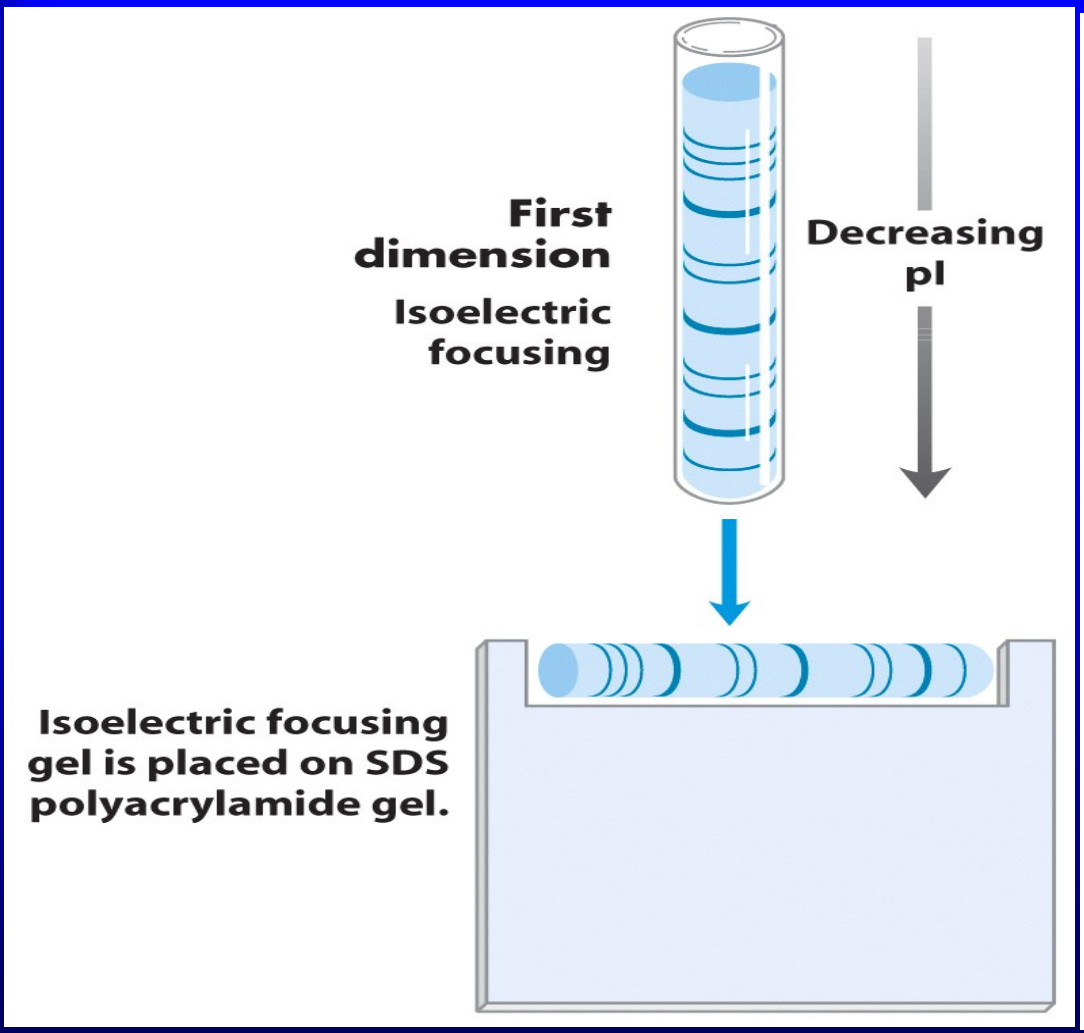


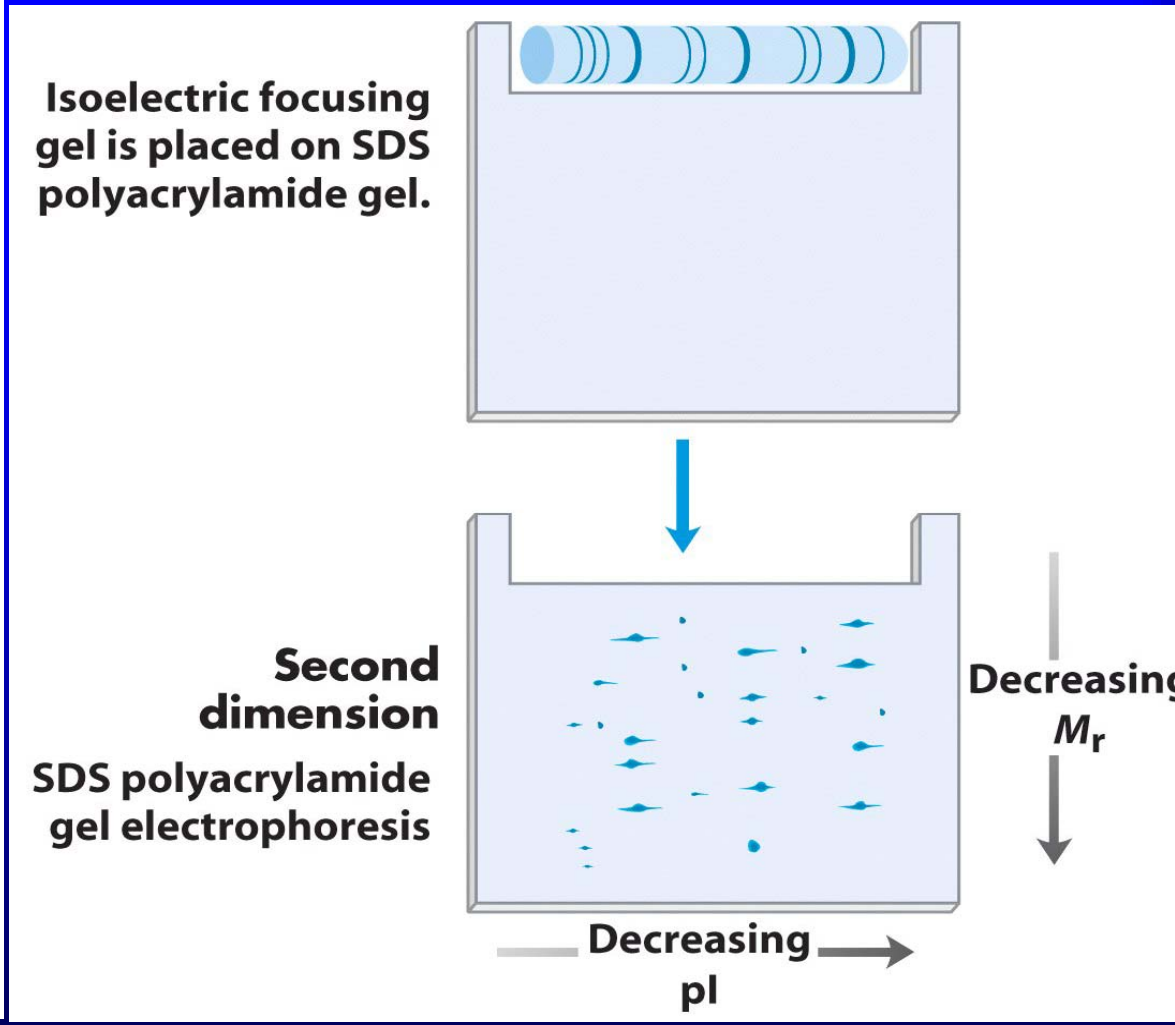


DNA libraries

A DNA library is a collection of DNA clones, gathered together as a source of DNA for sequencing, gene discovery or gene function studies.

Two dimensional electrophoresis



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