Advance Genetic Engineering

Prof.Dr.Abdul Hussein M.AlFaisal
Ph.D. in Cancer Molecular Genetics
Wales University- UK.

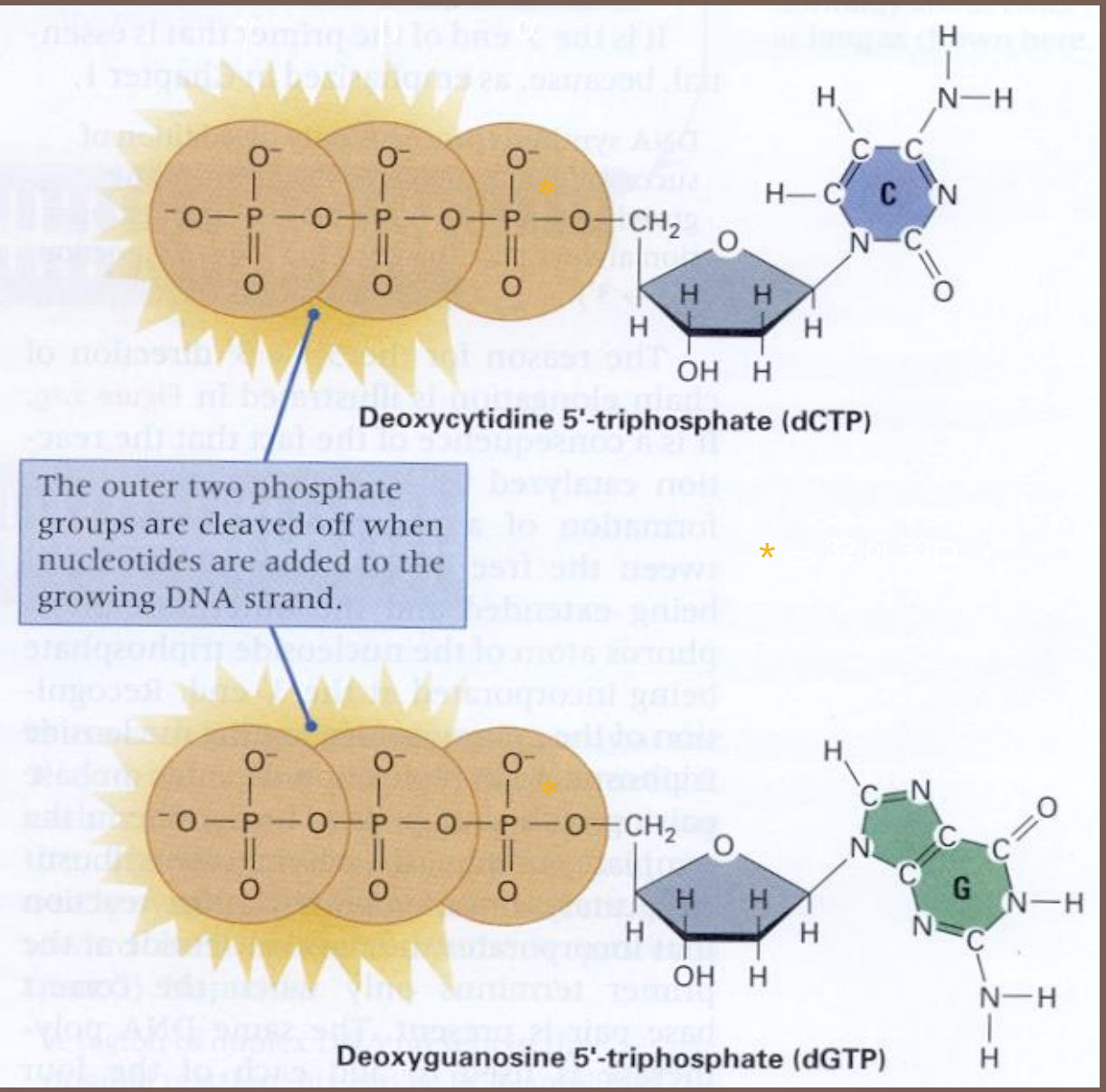
PROBES LABELING

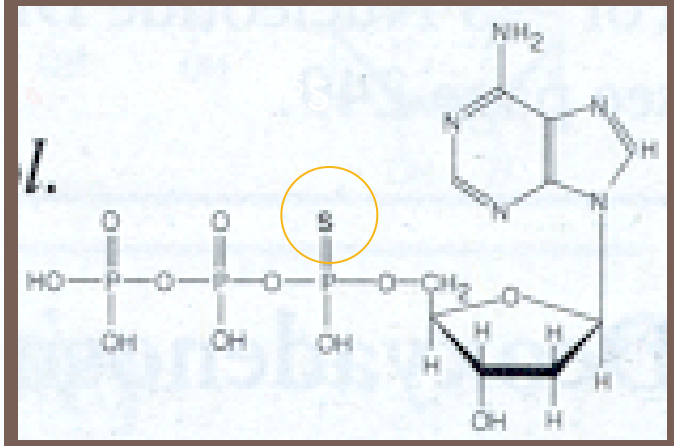
Introduction

1. Normal atoms replacement
2. H^3 P^{32} N^{14}, N^{15}, C^{14}
3. Fluorescents.
4. Applications: detection of

- Compounds movements
- Enzymatic reaction
- Secondary metabolites
- Diseases diagnosis
- DNA replication
- Proteins studies

Radioactively labeled dNTPs





- Isotopes Safety procedure

General use of the probes in genetics

1. Enzymatic monitoring
2. Detection and isolation of genes
3. Analysis of the structure of genes
4. Detection of gene expression
5. Many other purposes

1. Enzymatic monitoring

e.g. monitoring the synthesis of the 1st and 2nd cDNA strands., DNA synthesis in cells.

2. Detection and isolation of genes

e.g. gene detection on DNA fragments., detection of genes among chromosomes.

3. Analysis of the structure of genes

e.g. DNA sequencing., gene mutations

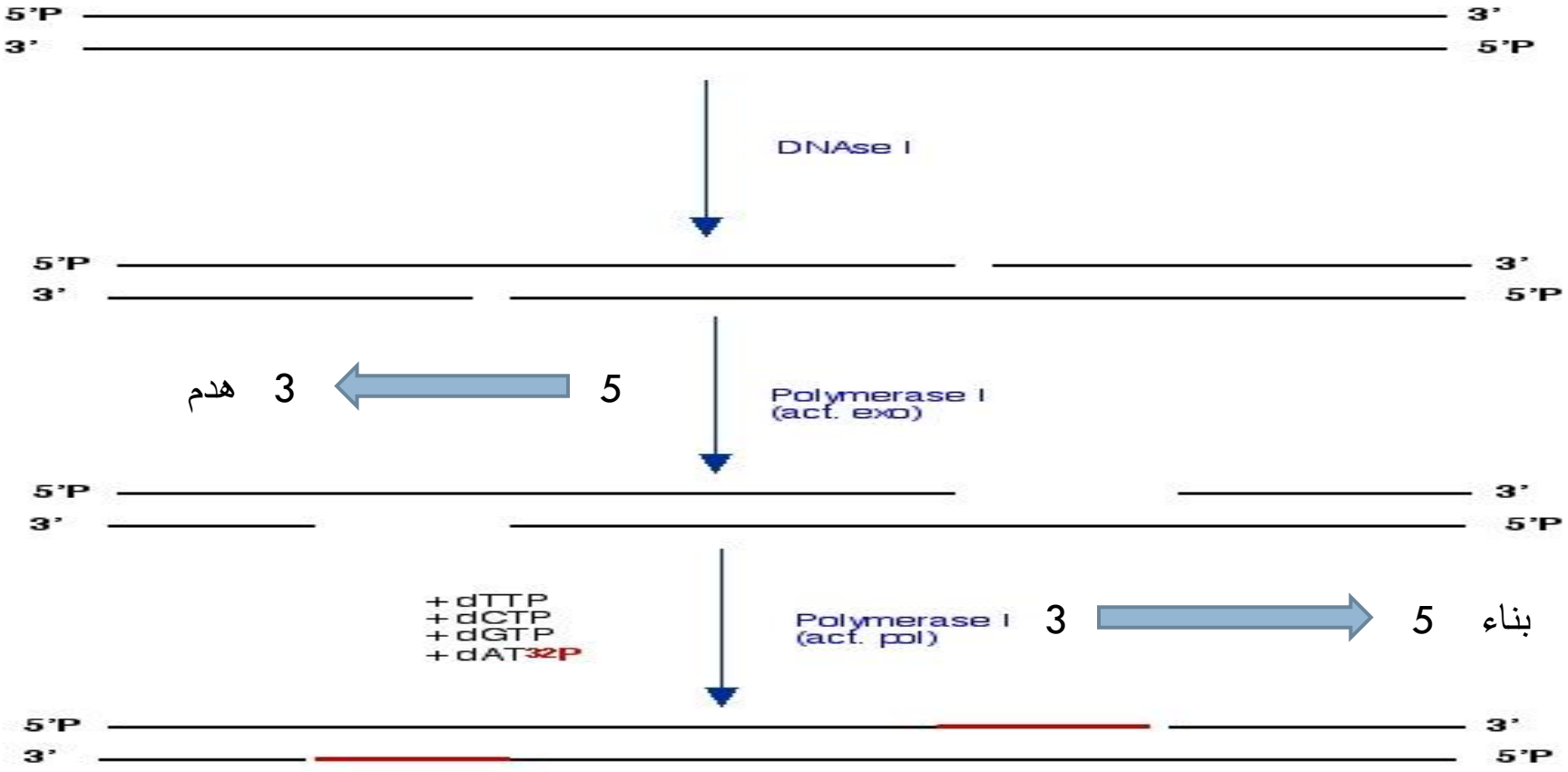
METHODS OF LABELING NUCLEIC ACID & PROBES

There are five basic methods for labeling nucleic acids. These are:

- **Nick translation**
- **Primer extension**
- **End labeling methods**
- **Methods based on RNA polymerase**
- **Direct labeling methods**

4. Nick translation based labeling

- Dnase 1 nicks the DNA (cuts phosphodiester bonds)
- DNA polymerase (with a 5' to 3' exonuc act) replaces nucleotides with new dNTPs, one or more of which is labeled.



Factors effect nick translation method:

1.Contamination

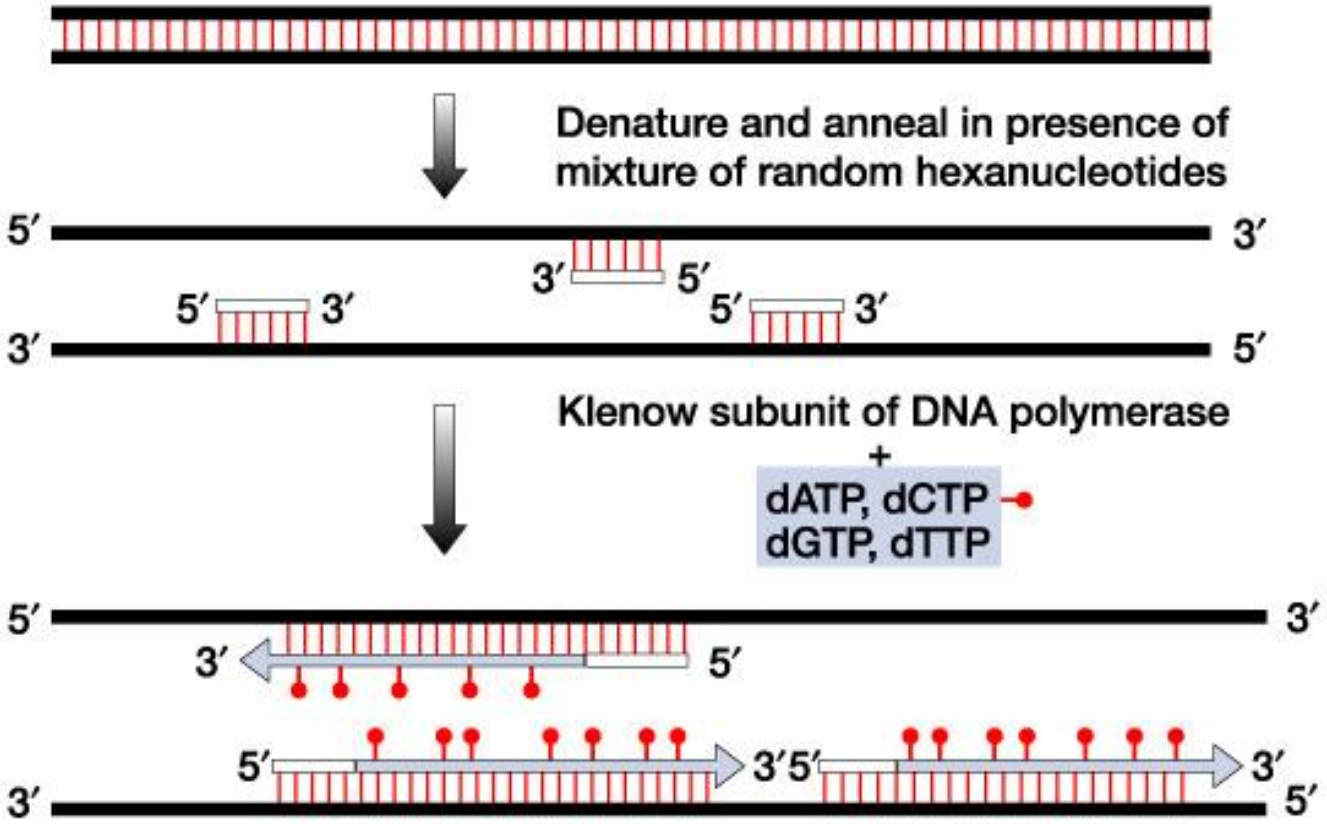
2. Enzyme concentration

3. Reaction temperature

Disadvantages

1. Need One ug DNA
2. Need to control reaction temperature.
3. Need to control enzymes concentration
4. The reaction is not suitable for single strand DNA

(B) Primer extension: Random primer synthesis



Single strand DNA+ Klenow fragments

Key:
Labeled nucleotide

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

Random primer synthesis with reverse transcriptase E

mRNA + reverse transcriptase enzyme + dNTPA → labelled cDNA

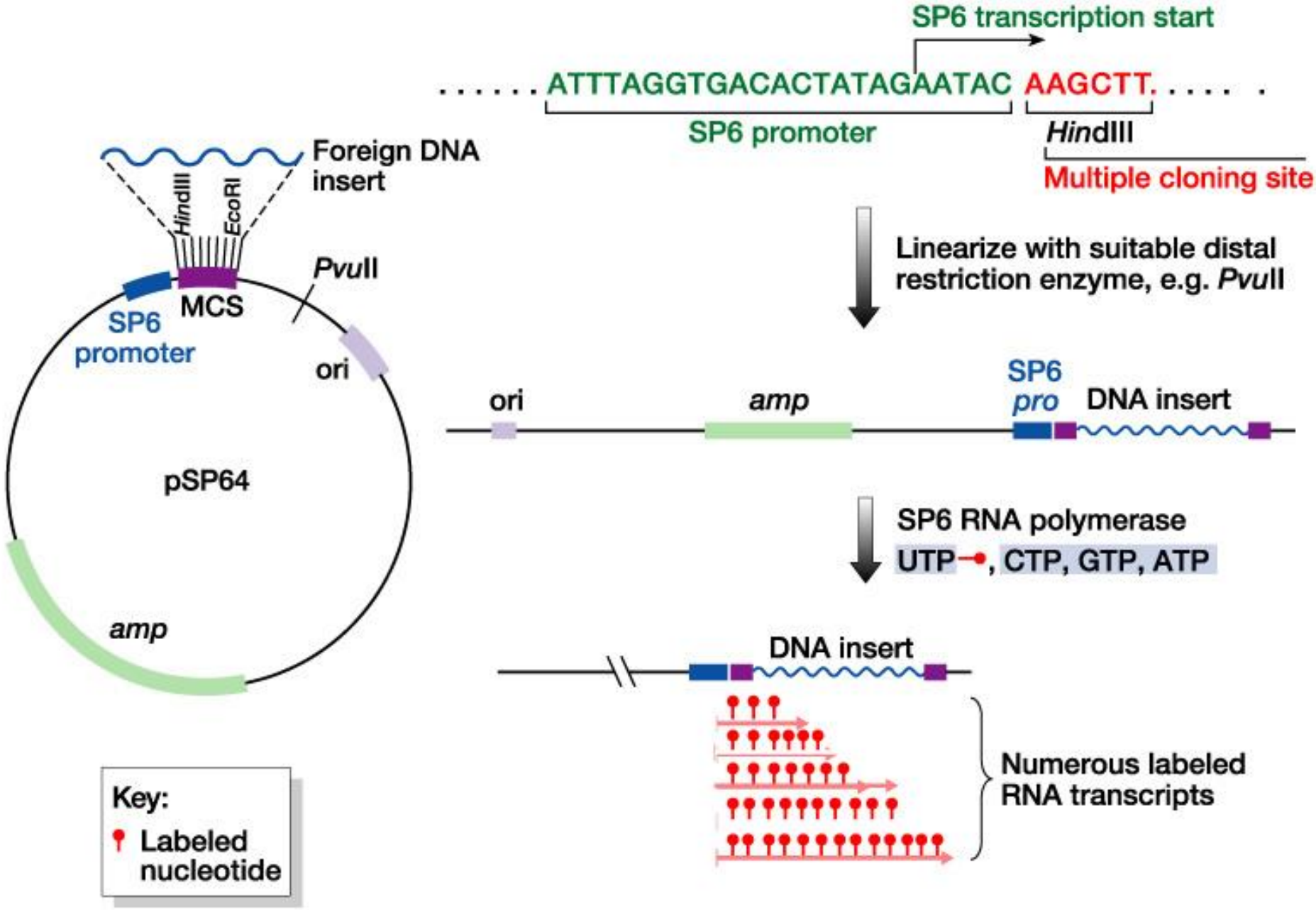


Figure 6-3 Human Molecular Genetics, 3/e. (© Garland Science 2004)

5 –end labelling

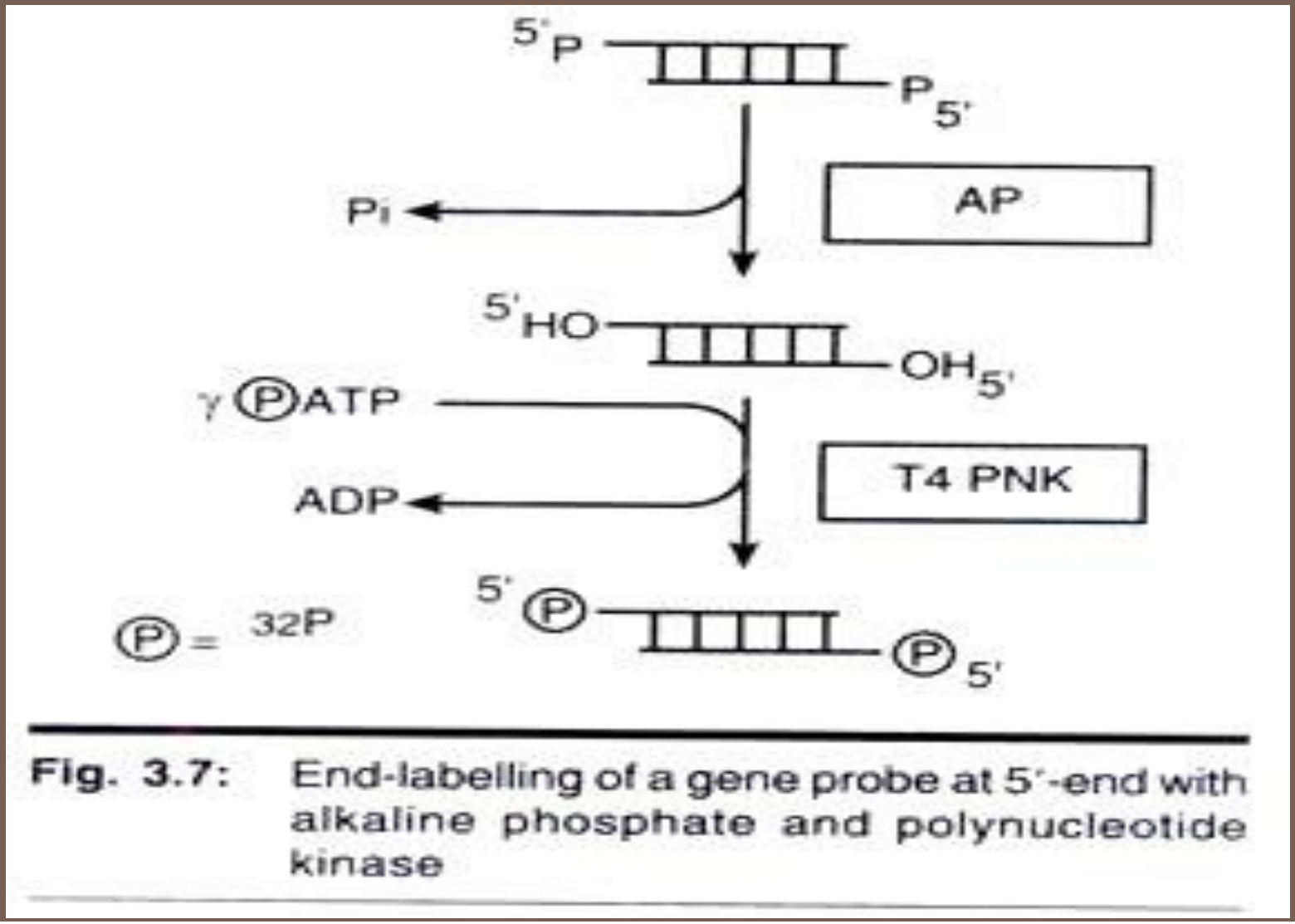
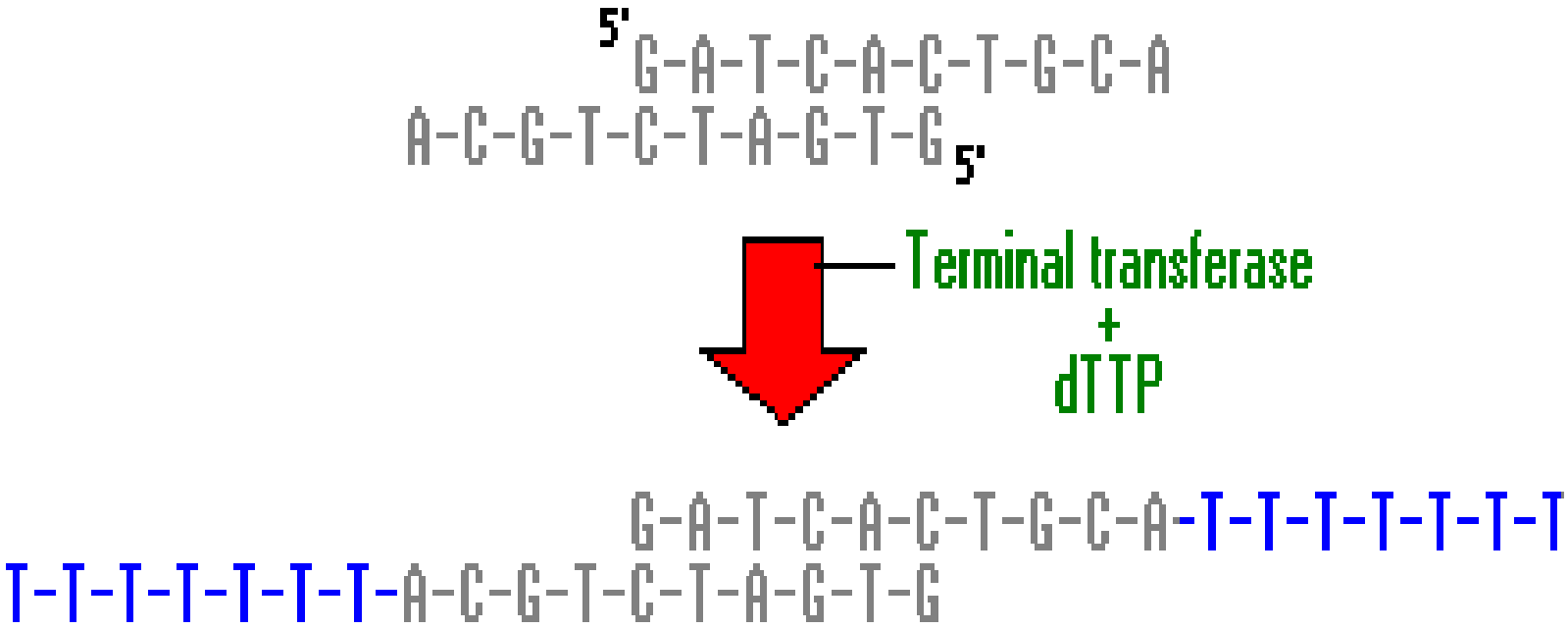
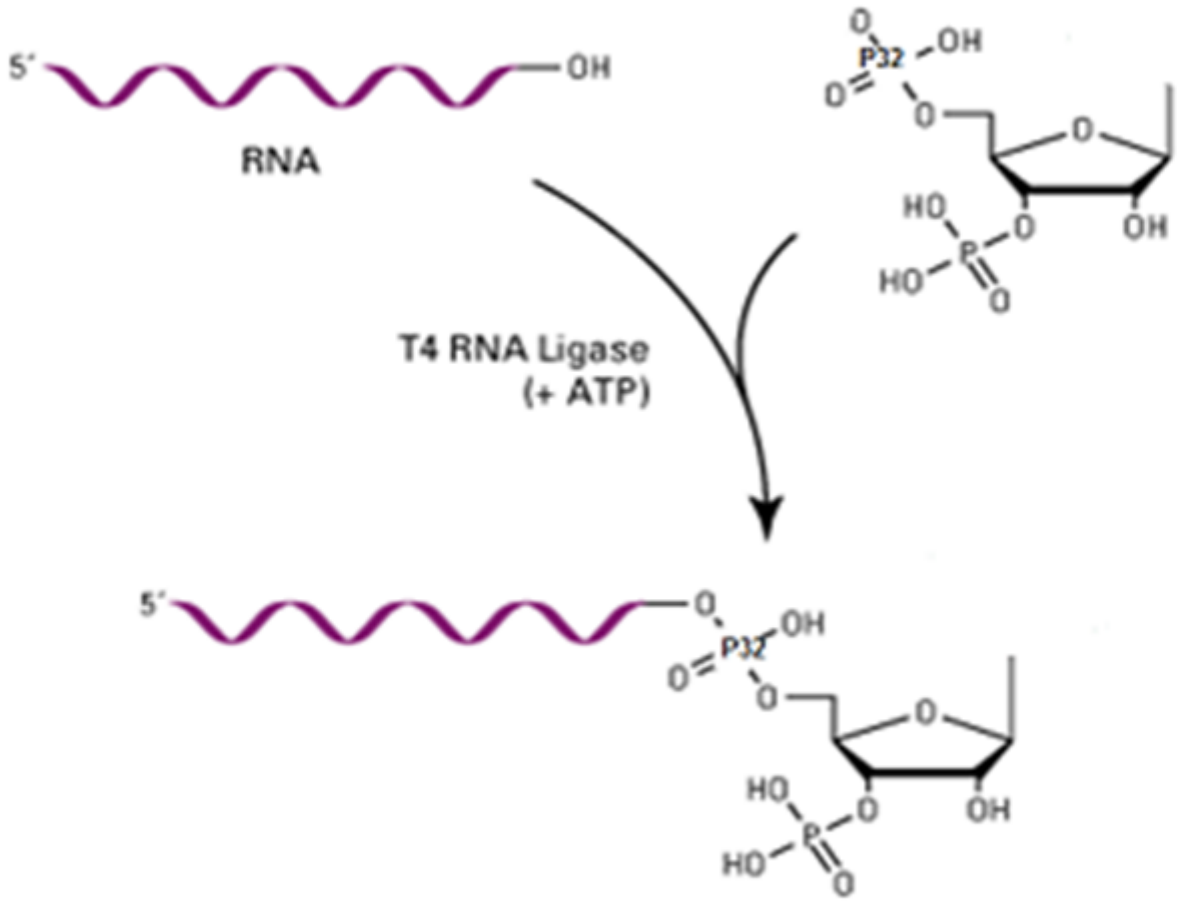


Fig. 3.7: End-labelling of a gene probe at 5'-end with alkaline phosphate and polynucleotide kinase

3’ end labeling

- **Use terminal deoxynucleotidyl transferase - TdT to add homopolymer extensions at the 3’OH of a probe. Works for ss and ds DNA**




5'
OH
RNA
T4 RNA Ligase
(+ ATP)
P32
OH
HO
HO
OH
5'
P32
OH
HO
HO
OH

Un-irradiated labeled probes

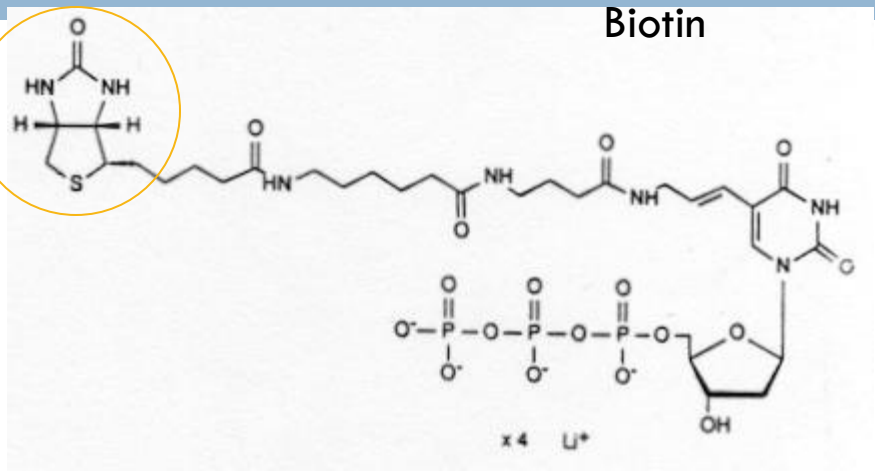
- Biotin
- Acetylamino fluorenyl
- Sulphonated cytidine

+ Avidin or strep avidin…… color light

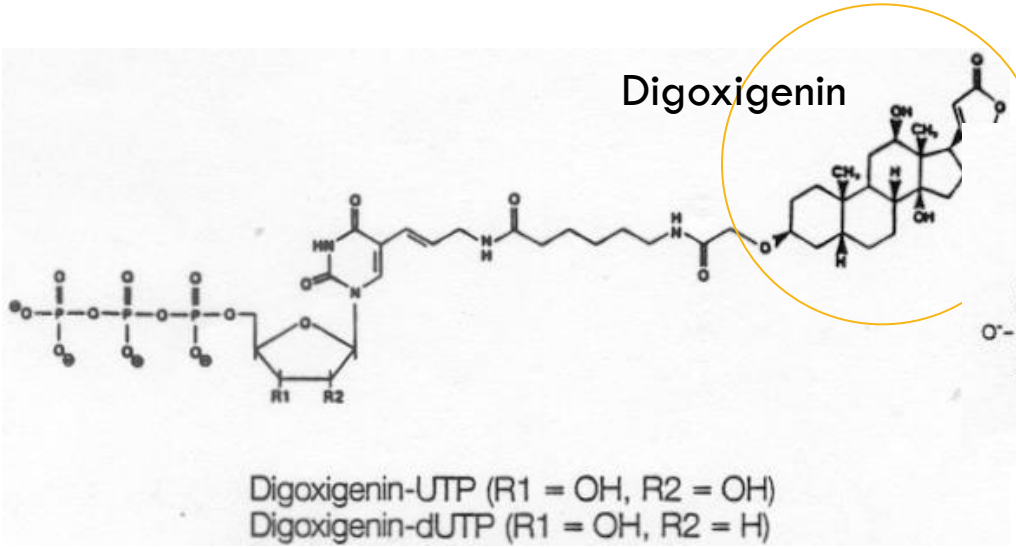
- Immuno labelling

Non-radioactively labeled (d)NTPs

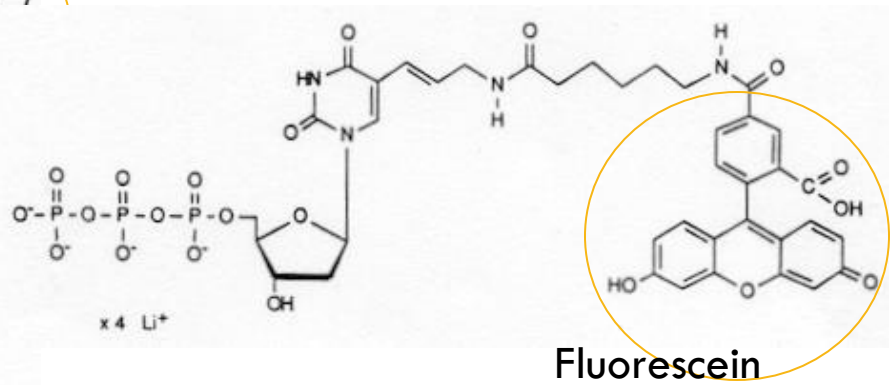
Biotin



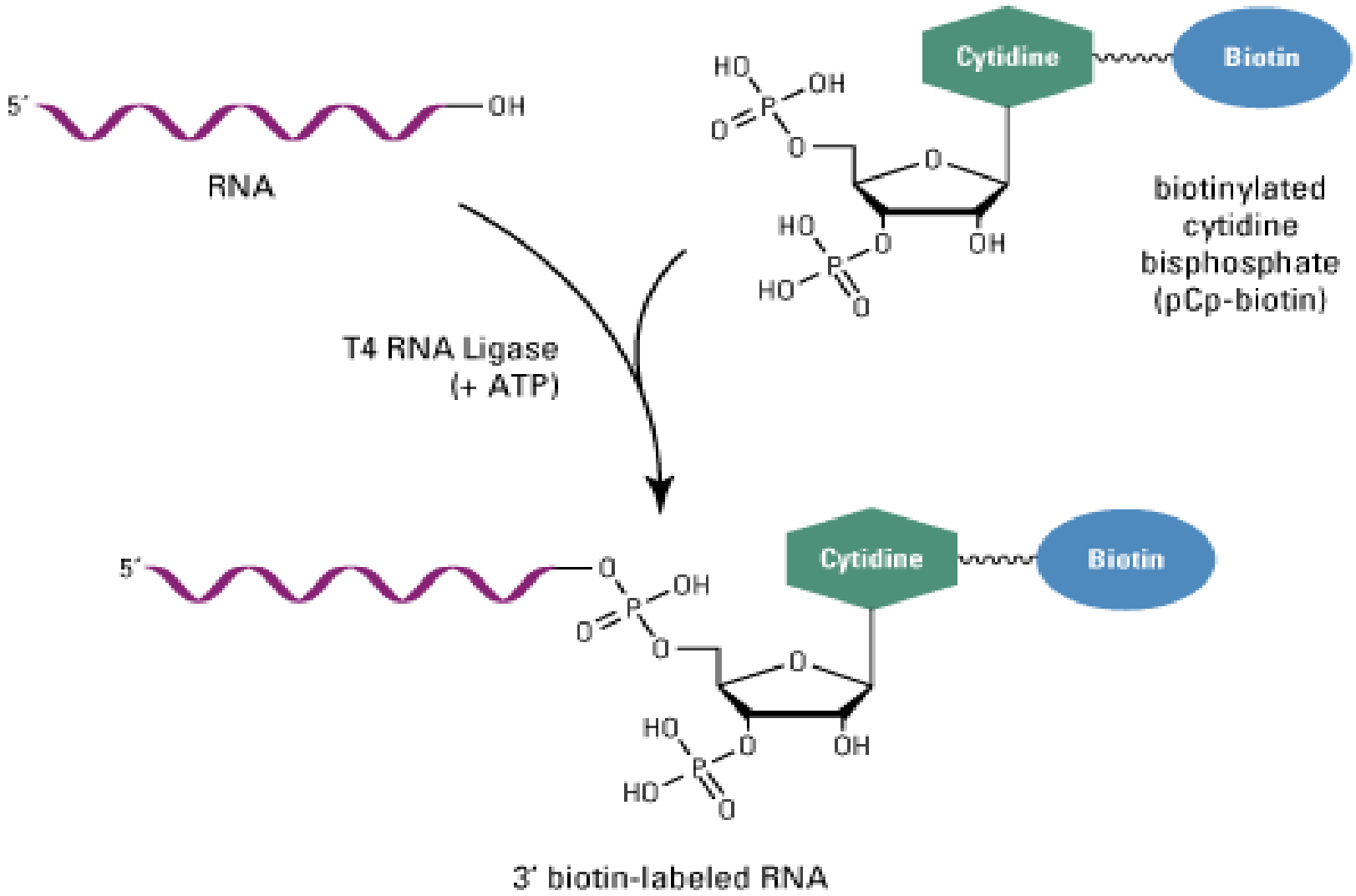
Digoxigenin



Digoxigenin-UTP (R1 = OH, R2 = OH)
Digoxigenin-dUTP (R1 = OH, R2 = H)



Fluorescein


5′
OH
RNA
Cytidine
Biotin
HO
OH
O
P
O
O
HO
O
OH
HO
P
HO
O
biotinylated
cytidine
bisphosphate
(pCp-biotin)
T4 RNA Ligase
(+ ATP)
5′
Cytidine
Biotin
3′ biotin-labeled RNA

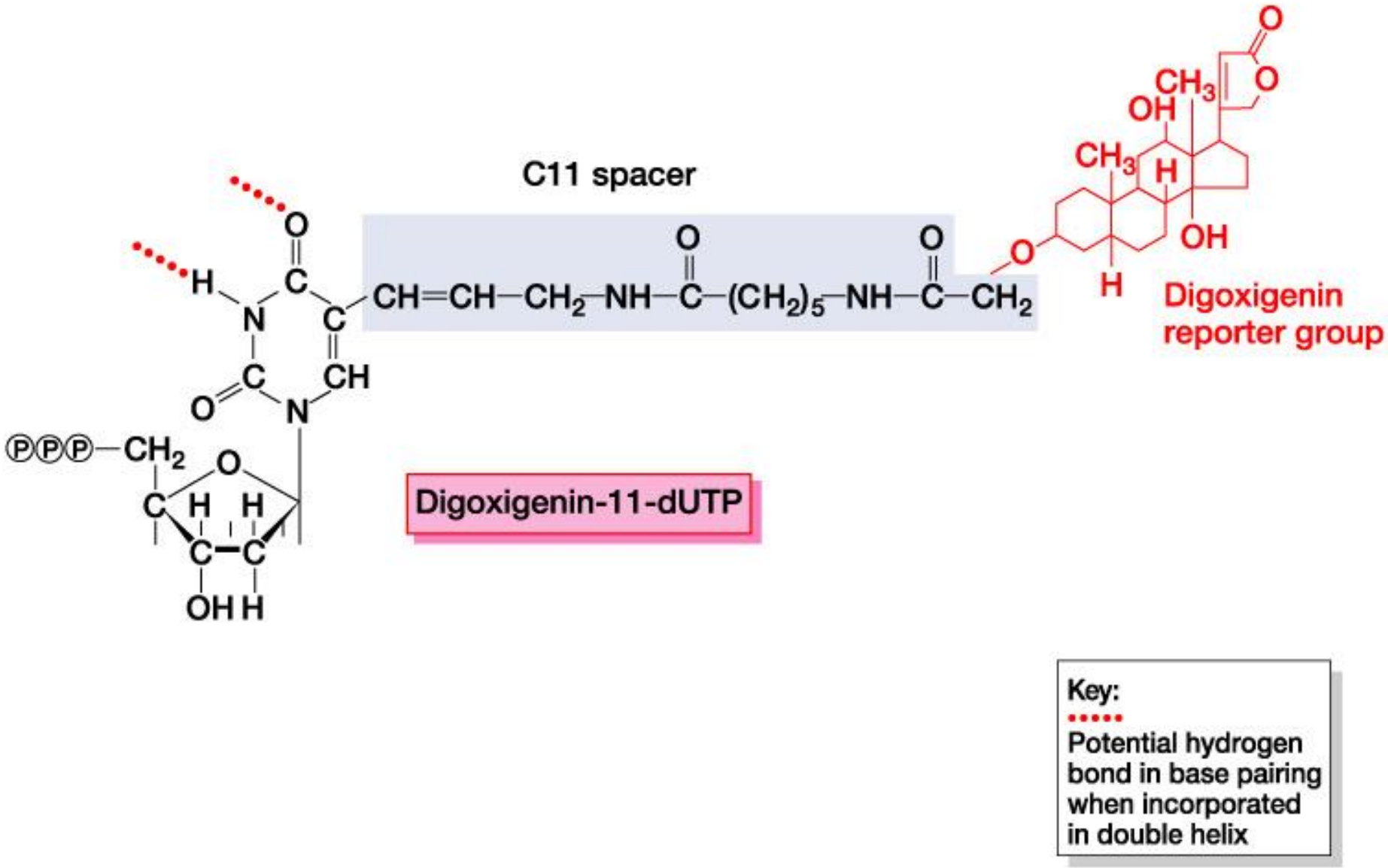
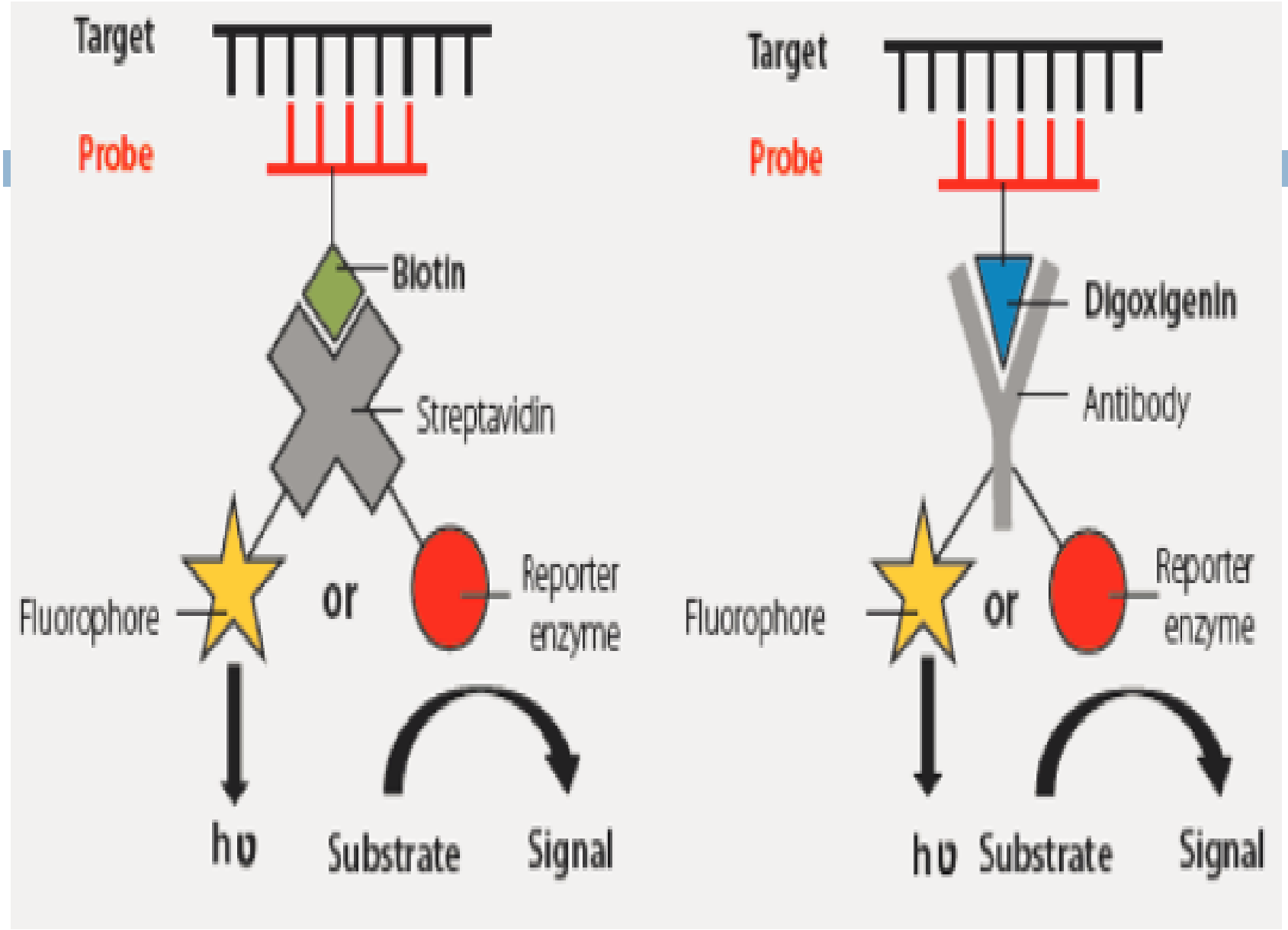


Figure 6-7 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)


Target
Probe
Biotin
Streptavidin
Fluorophore
or
Reporter enzyme
hυ
Substrate
Signal
Target
Probe
Digoxigenin
Antibody
Fluorophore
or
Reporter enzyme
hυ
Substrate
Signal

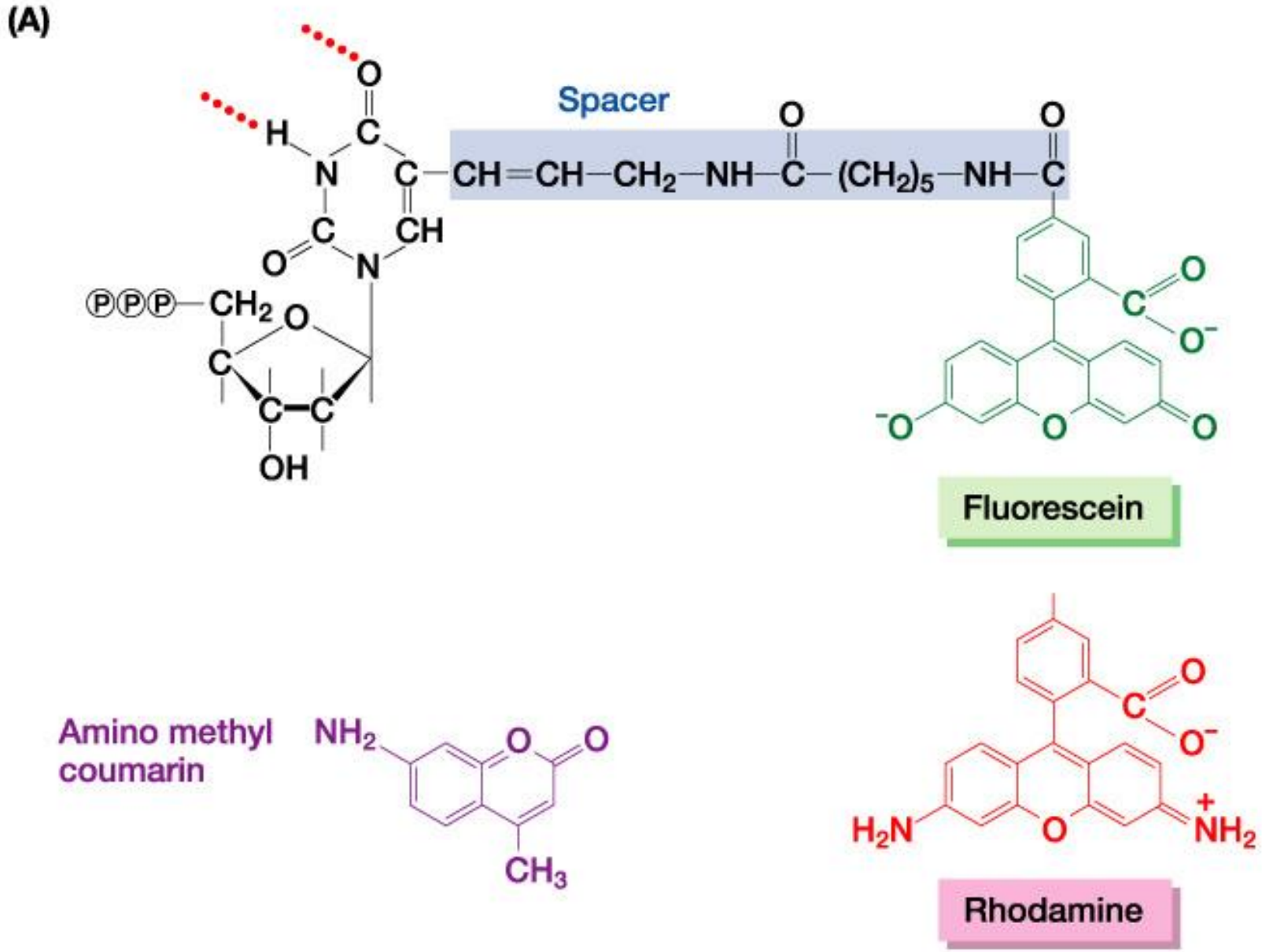
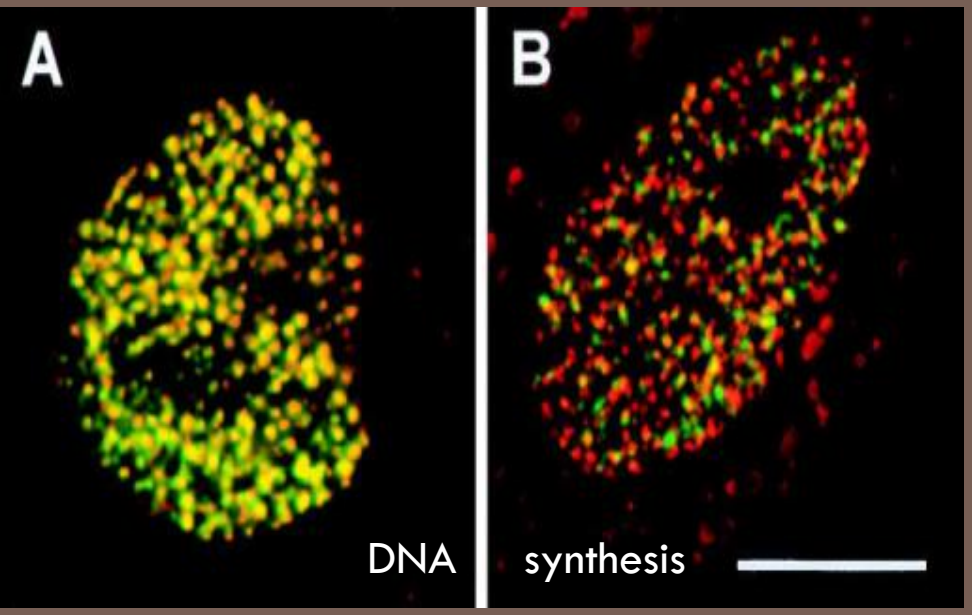


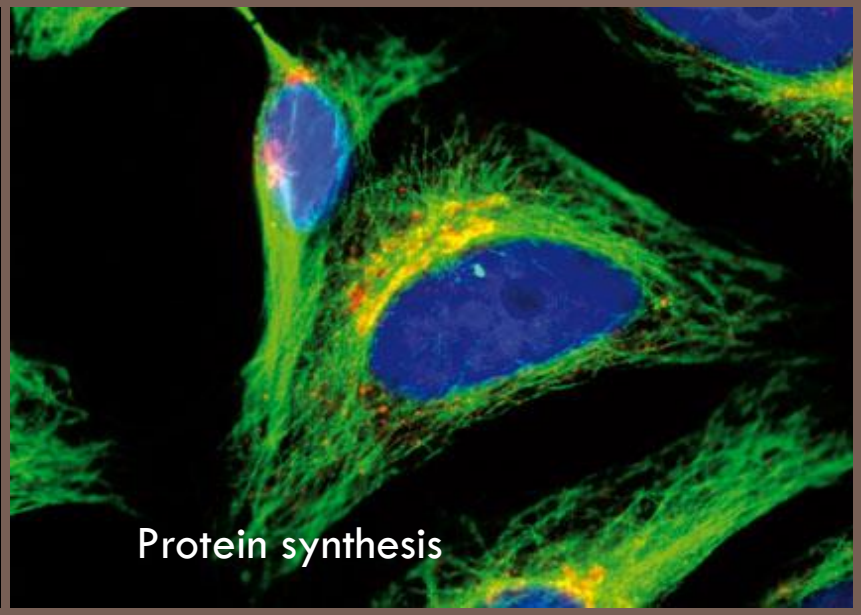
Figure 6-5 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

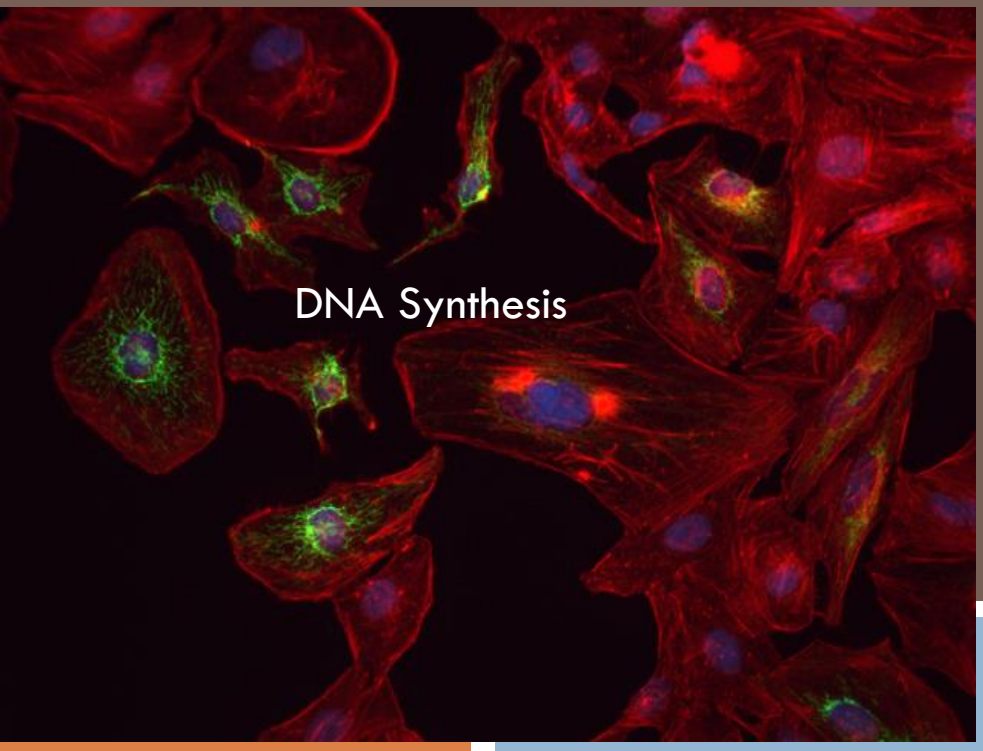

A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
B
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

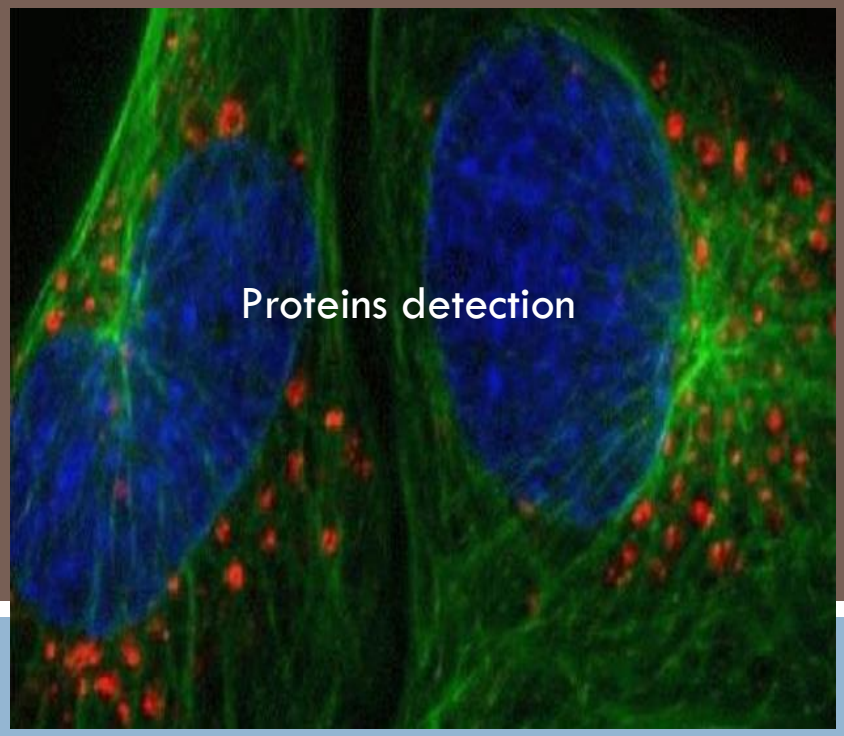
Living cells labeling

-- Use S^{35} – $N^{14, 15}$ for protein monitoring
or P^{32} for DNA-RNA monitoring
-- Thymidin or cytidin or Amonium chloride
-- Semi conservative replication Experiments
-- DNA replication experiments

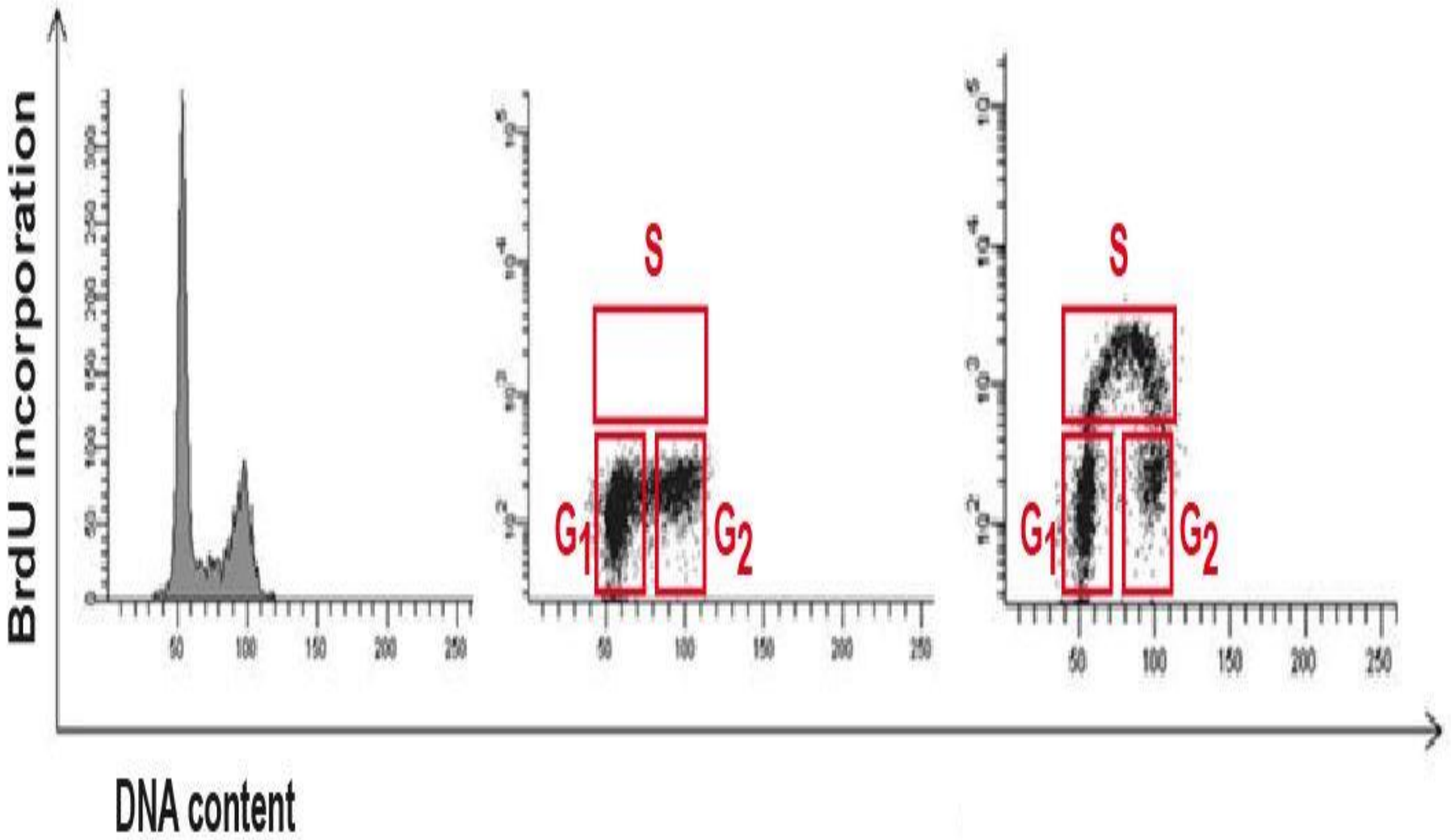

A
B
DNA
synthesis


Protein synthesis


DNA Synthesis


Proteins detection

(A) Flow cytometry



Thank you