ANALYSIS OF GENE EXPRESSION

Objectives

- Determination of RNA level
  - Northern blot
  - Microarrays
- Analysis of proteins

  ELISA

  Western blot
- Gene Sequencing and application

ANALYSIS OF GENE EXPRESSION

Determination of RNA levels

Northern blots

Microarrays

Analysis of proteins

Enzyme-linked immunosorbent assays (ELISA)

Western blots

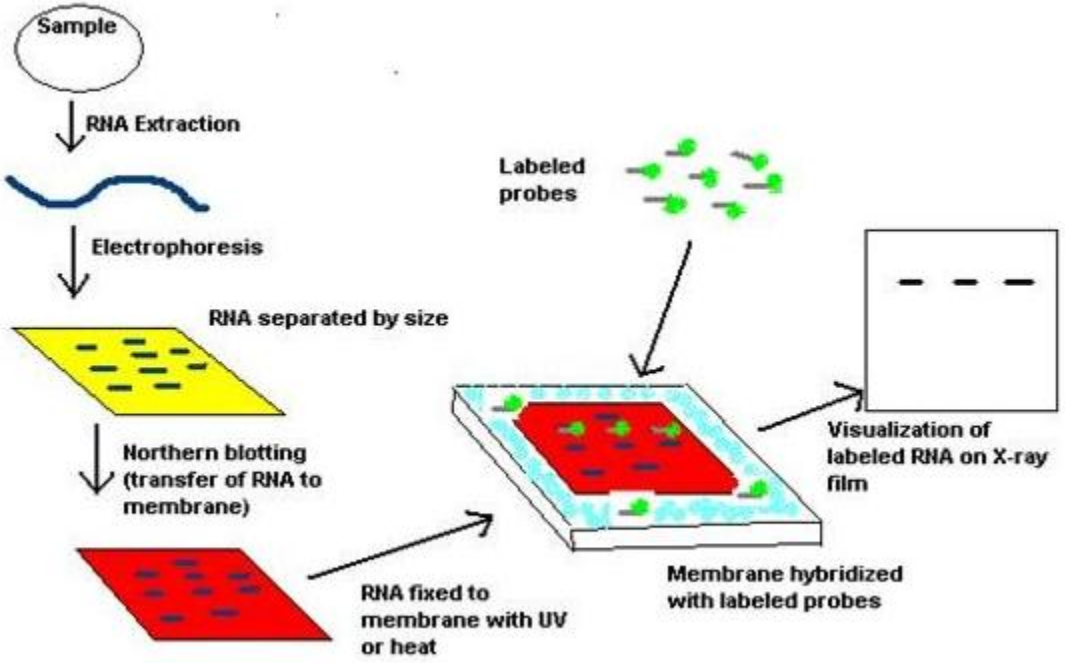
Proteomics: The study of all proteins expressed by a genome, including their relative abundance, distribution, posttranslational modifications, functions, and interactions with other macromolecules, is known as proteomics. Proteomics offer the potential of identifying new disease markers and drug targets.

Northern Blot

- The northern blot technique was developed in 1977 by James Alwine, David Kemp and George Stank at Stanford University

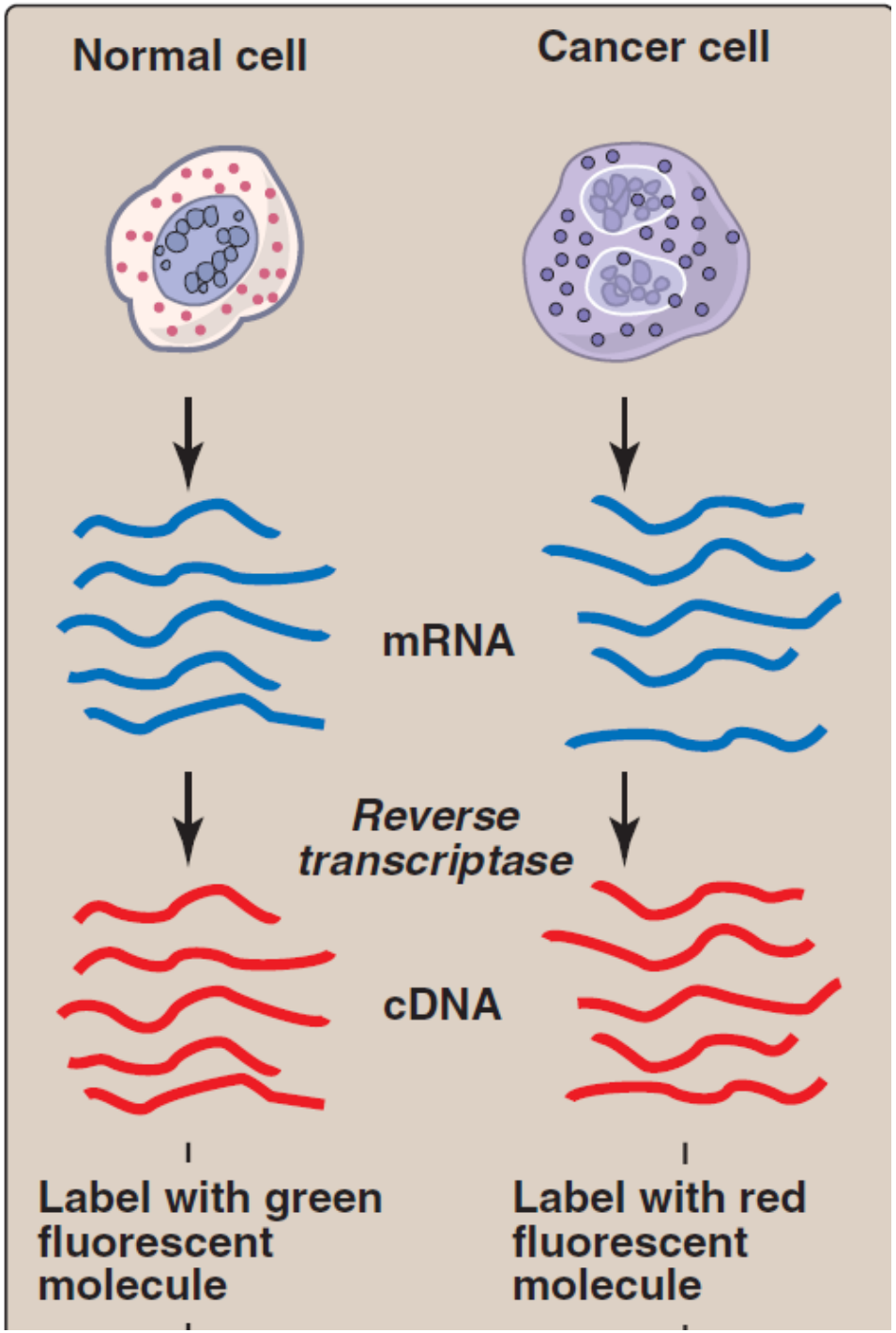
- Principle:
- Northern blotting involves the use of electrophoresis to separate RNA samples by size and detection with a hybridization probe complementary to part of or the entire target sequence.

Procedure of Northern Blotting

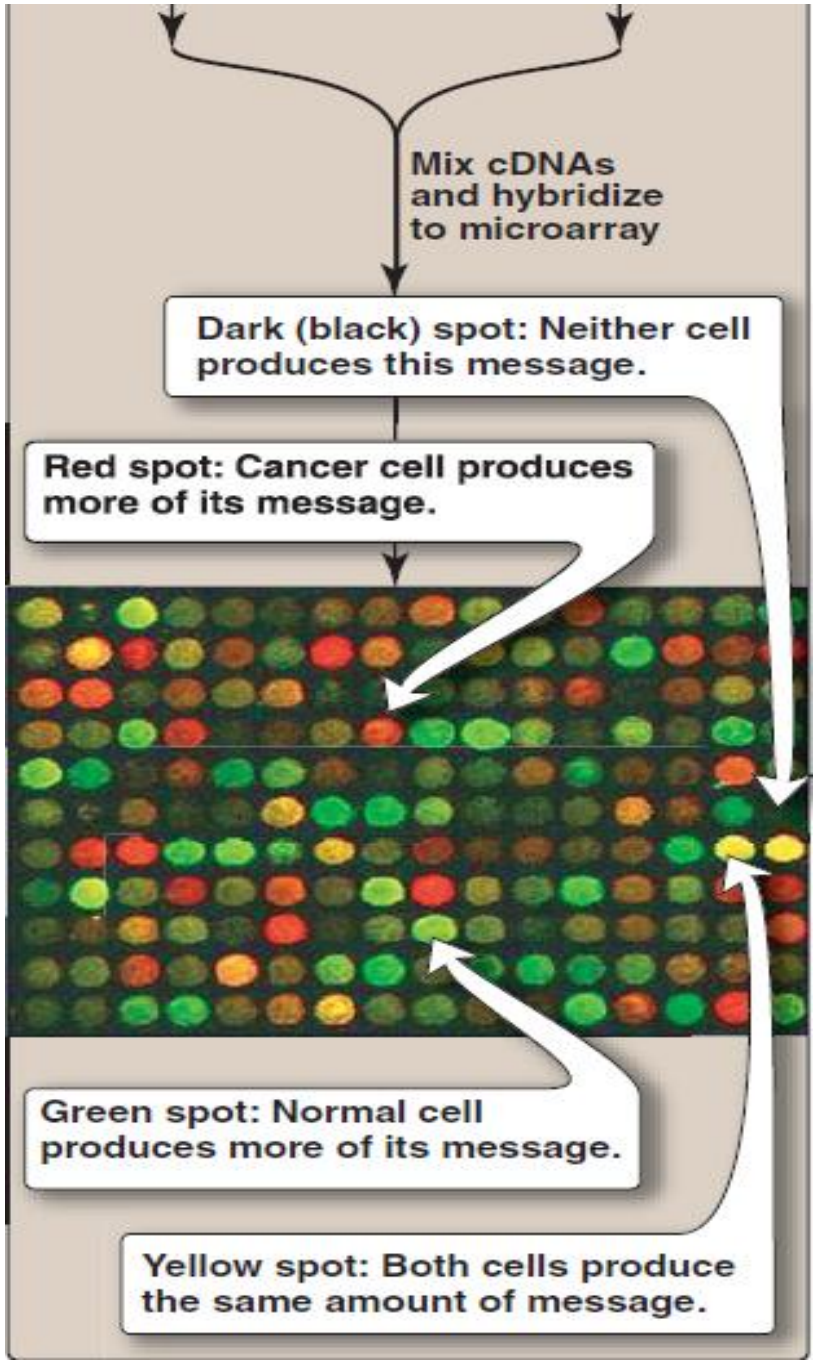


- Limitations:
- Northern Blotting using radioactive probes is very sensitive, but very **time-consuming.** Northern blotting is **not practical in large clinical studies** to detect the expression of hundreds of miRNAs and it also **requires large amounts (5–25 μg)** of total RNA from each sample

Microarray analysis of gene expression



Microarray analysis of gene expression



Gene Sequencing

"... [A] knowledge of sequences could contribute much to our understanding of living matter."

Frederick Sanger

History

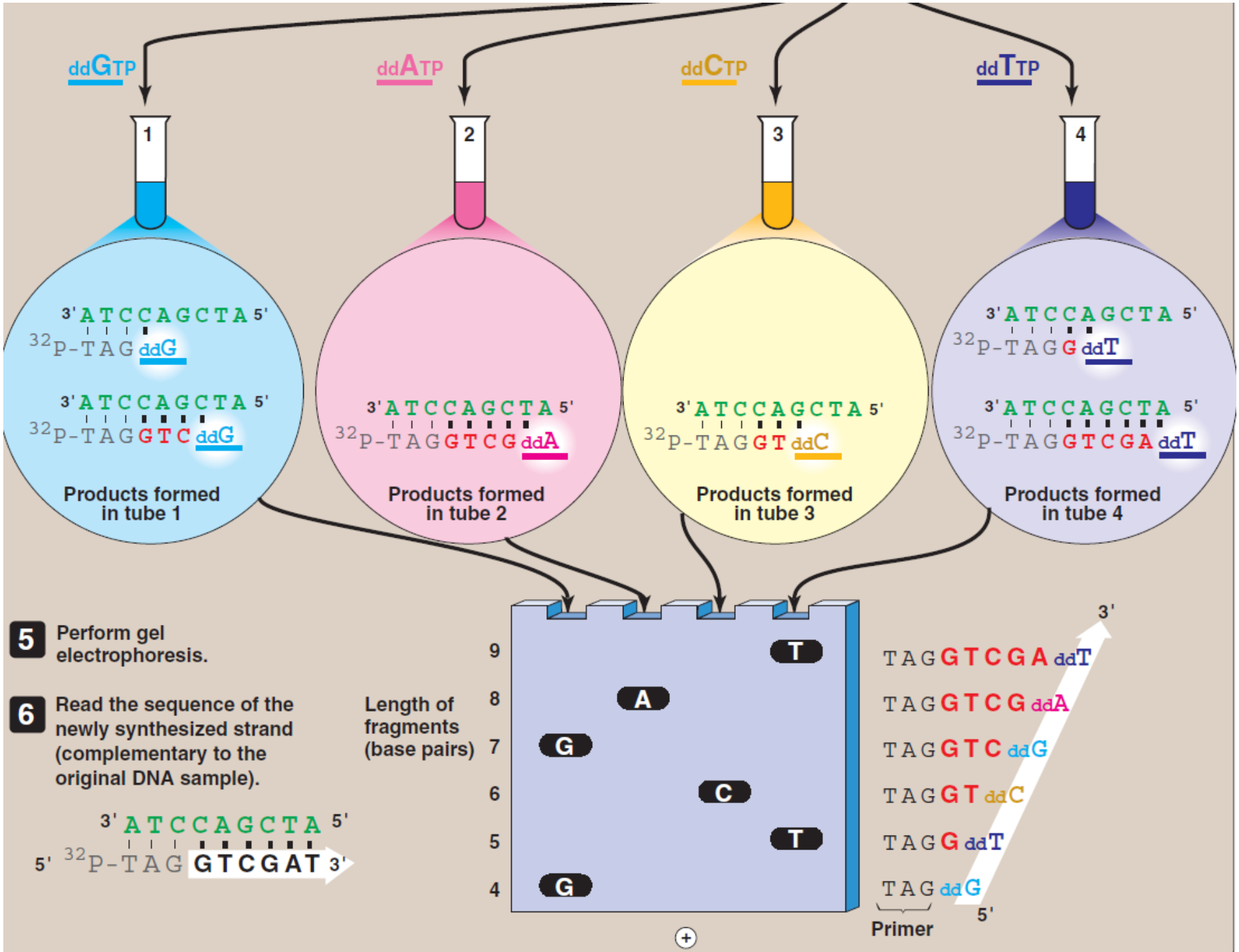
- The first method for determining DNA sequences involved a location-specific primer extension strategy established by Ray Wu in 1970
- The first DNA fragment to be sequenced belonged to T4 bacteriophage
- In the mid-1975, Frederick Sanger and Aln Coulson sequenced by using a **plus-minus system** for running a sequencing reaction.
- .

History contd

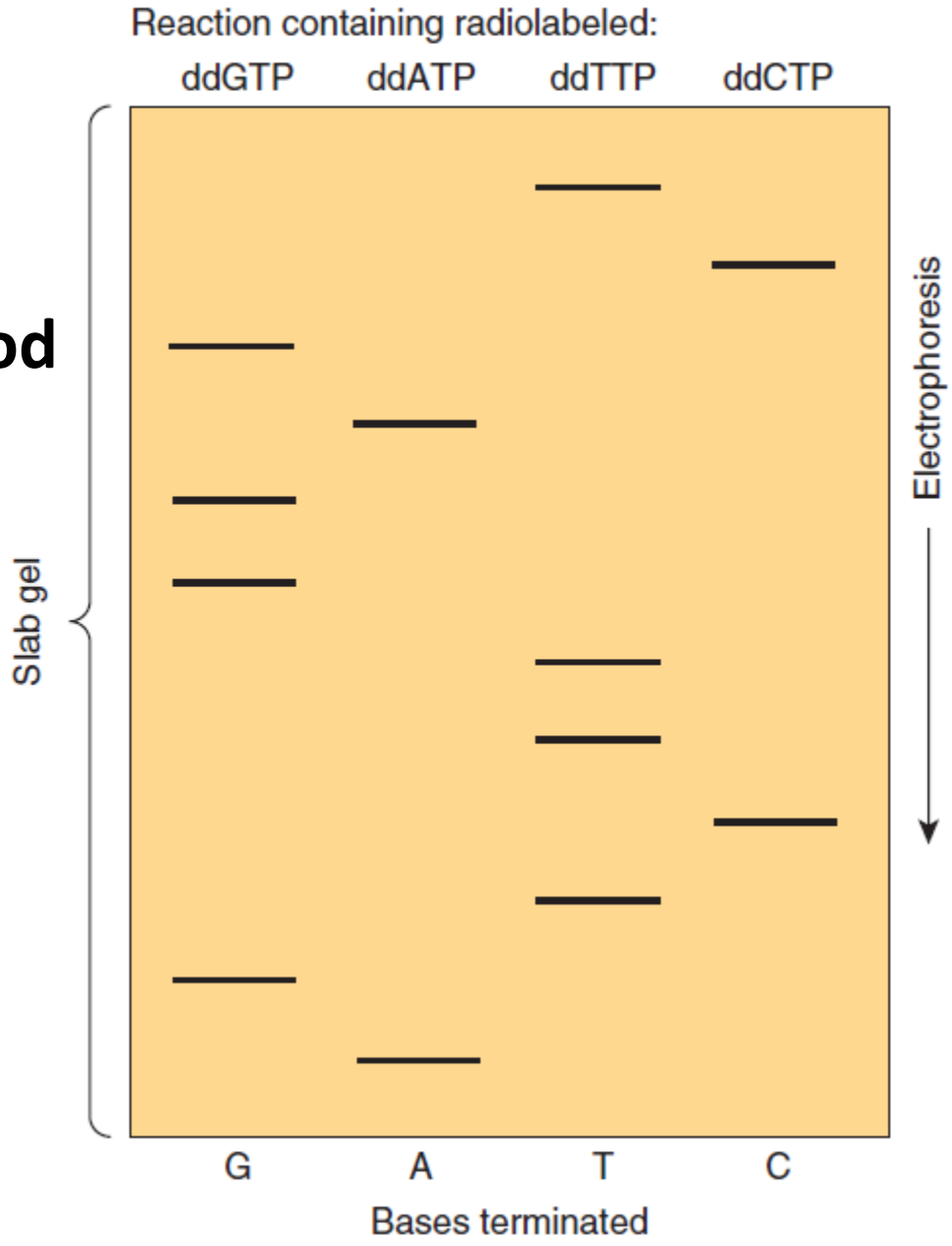
- Maxam and Gilbert further modified this method by using radiolabeled DNA and chemicals (such as hydrazine)
- One of the biggest breakthroughs in this field was the development of chain-termination technology using modified nucleotides by the Sanger lab in 1977
- In 1983, polymerase chain reaction (PCR) for amplifying stretches of DNA was discovered.

Type of gene sequencing

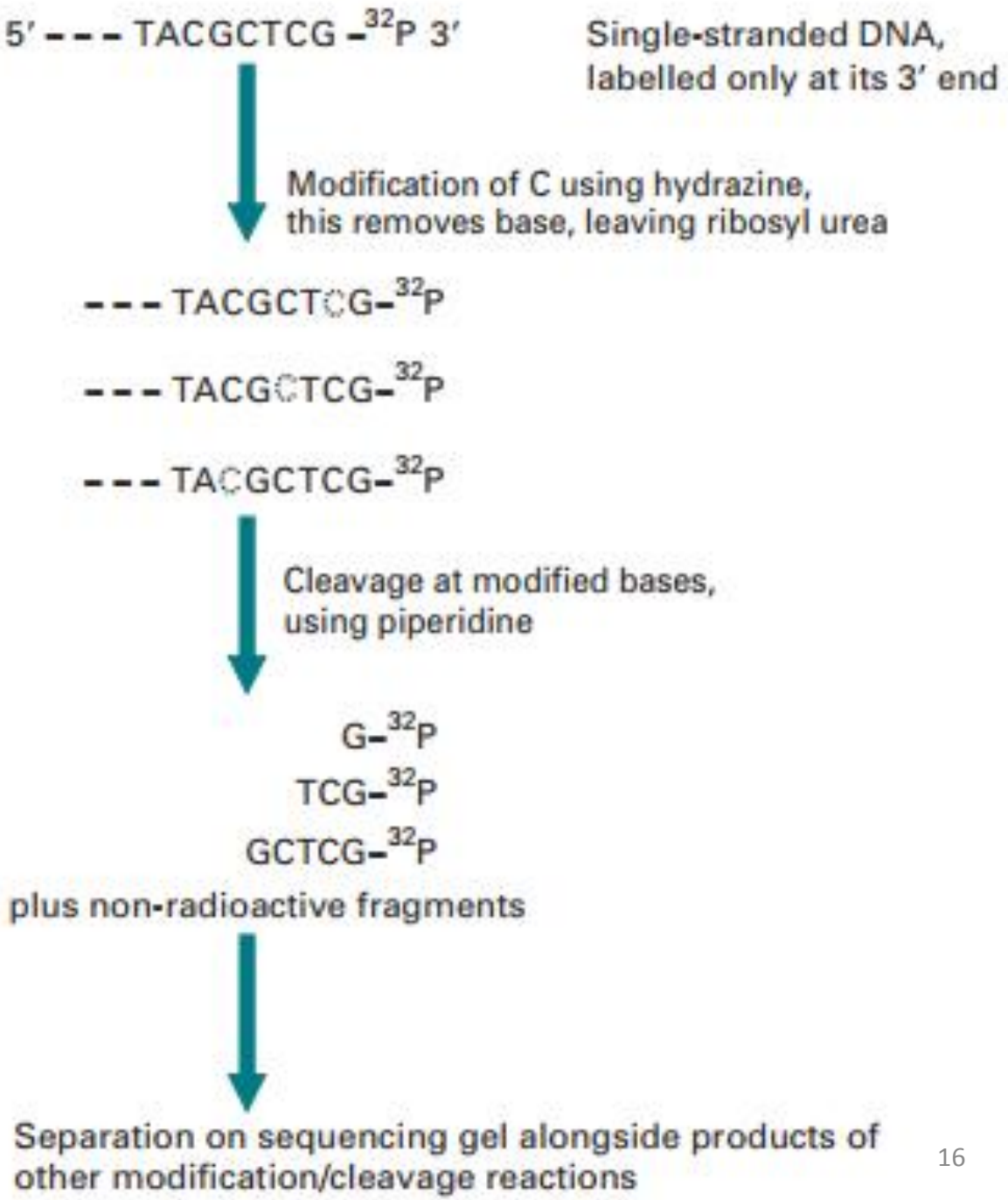
- **First generation DNA sequencing**
  - Sanger sequencing
  - Maxam Gilbert sequencing
  - Automated DNA sequencing
  - Shot Gun sequencing


ddGTP
ddATP
ddCTP
ddTTP
1
2
3
4
3' A T C C A G C T A 5'
32P-TAG ddG
3' A T C C A G C T A 5'
32P-TAG G T C ddG
Products formed in tube 1
3' A T C C A G C T A 5'
32P-TAG G T C G ddA
Products formed in tube 2
3' A T C C A G C T A 5'
32P-TAG G T ddC
Products formed in tube 3
3' A T C C A G C T A 5'
32P-TAG G ddT
3' A T C C A G C T A 5'
32P-TAG G T C G A ddT
Products formed in tube 4
5 Perform gel electrophoresis.
6 Read the sequence of the newly synthesized strand (complementary to the original DNA sample).
3' A T C C A G C T A 5'
5' 32P-TAG G T C G A T 3'
Length of fragments (base pairs)
9
8
7
6
5
4
T
A
G
C
T
G
TAG G T C G A ddT
TAG G T C G ddA
TAG G T C ddG
TAG G T ddC
TAG G ddT
TAG ddG
Primer
3'
5'
+

Sequencing of DNA by the chain termination method devised by Sanger



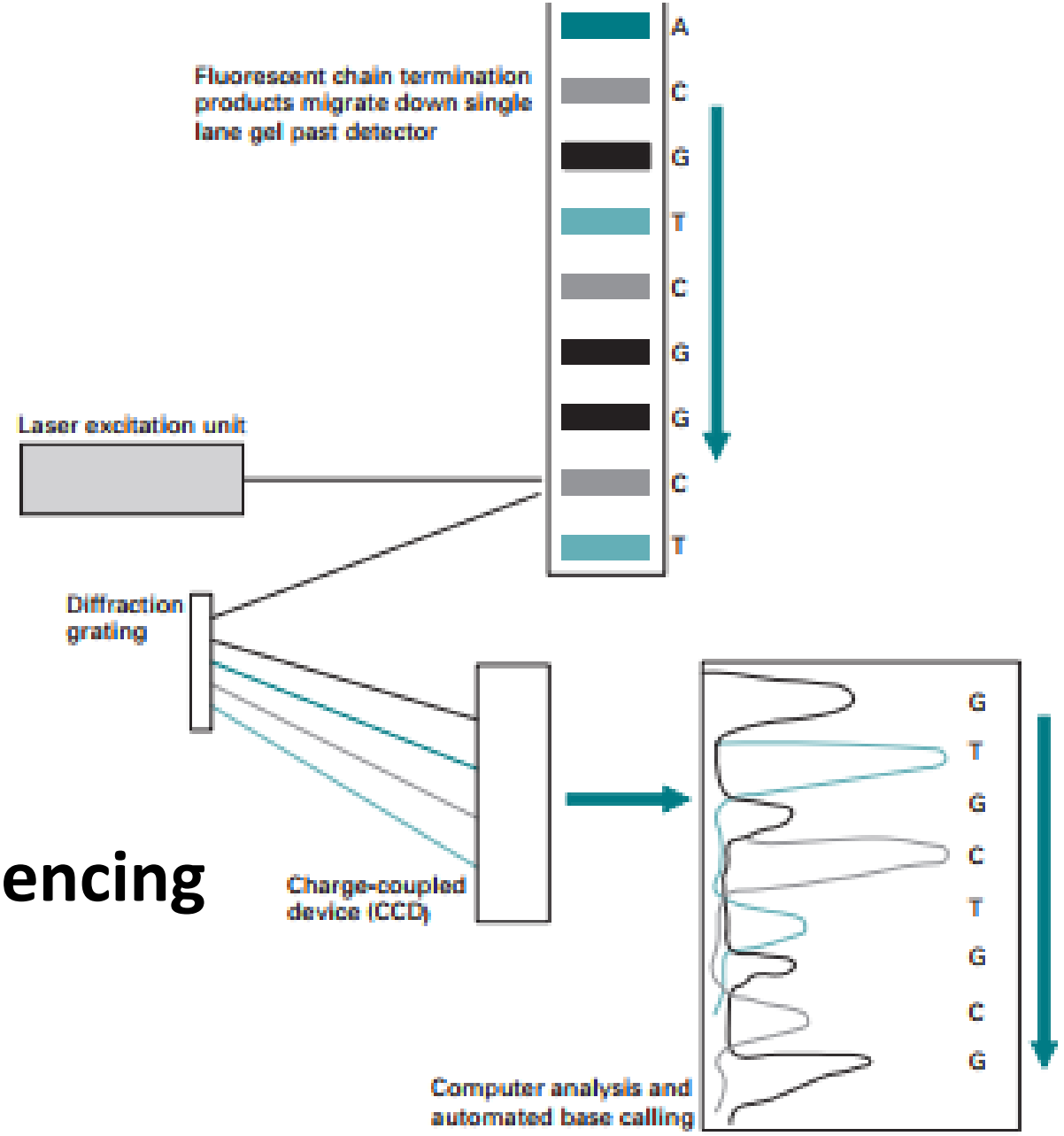
Maxam and Gilbert sequencing of DNA



Automated DNA sequencing

- PCR used for making sequencing templates
- Fluorescently labelled ddNTPs are used
- Capillary electrophoresis

Automated fluorescent sequencing detection



- **Second-generation DNA sequencing**

: (Next generation sequencing)

  - Pyrosequencing
  - Sequencing by synthesis
  - Sequencing by ligation
  - Ion semiconductor sequencing

- **Third-generation DNA sequencing**
  - **Real-time, single-molecule sequencing**
    - capable of sequencing single molecules, negating the requirement for DNA amplification shared by all previous technologies.

Application of DNA sequencing

- Forensics:
  - To identify the individual

- Medicines
  - To detect genes associated with some hereditary or acquired diseases
  - E,g. Huntingtons disease
    - CAG codon in exon 1
  - Fragile X syndrome
    - CGG >200
  - Myotonic Dystrophy
    - CUG >100

- Agriculture
  - Mapping and sequencing of whole genome of microorganisms helps in making them useful for foods or crops

MCQ 1

- Which of the following is not an exclusively DNA sequencing method?
- 1. Sangers
- 2. Maxam Gillbert
- 3. Edman
- 4. LMPCR (Ligation mediated PCR)

LMPCR (Ligation mediated PCR)

- (1) primary DNA nucleotide sequences
- (2) cytosine methylation patterns
- (3) DNA lesion formation and repair, and
- (4) in vivo protein-DNA footprints

MCQ2

- The sample in Sangers method after reaction separated in
- 1. AGE
- 2. PAGE
- 3.PFGE (Pulse field gel electrophoresis)
- 4. 2-D gel electrophoresis

MCQ3

- If a hypothetical peptide has the sequence Phe-Tyr-Met-Pro-His.

- Calculate number of possible nucleotide sequences.
- A. 11
- B. 8
- C. 22
- D. 32

References

- Principle and techniques of Biochemistry and Molecular Biology : Edited by K Wilson and J Walker: 7th Edition
- Harpers illustrated Biochemistry 30th Edition
- Lippincott's illustrated review: 5th edition