Mutah University
Faculty of Medicine

First Year
Molecular Biology

Genetic testing

Lecture 7 (58 slides)

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L/O/G/O

What is Genetic Testing

- The **analysis of human DNA** in any of its forms or related products (chromosomes, RNA, proteins).
- The ultimate **goal** is to *recognize the potential for a genetic condition* at an early stage.

Definitions:

- Genotype vs. Phenotype
  - The genetic make-up, as distinguished from the physical appearance
- Mutation
  - A genetic change, usually one that is associated with a disease
- Karyotype
  - A visual presentation of chromosomes

Types of genetic tests

Diagnostic	Used to confirm a diagnosis based on physical signs
Predictive	Used to detect gene mutations associated with disorders that appear later in life
Carrier Identification	Used by people with a family history of recessive genetic disorders
Prenatal	Used to test a foetus when there is risk of bearing a child with metal or physical disabilities
Newborn Screening	Used as a preventative health measure once the baby is born
Forensic testing	Used to identify an individual for legal purposes
Research testing	Used for finding unknown genes and identifying the function of a gene

1. Diagnostic Testing

- used to **confirm** or **rule out** a known or suspected genetic disorder in a person *with disease symptoms*.
- Confirming a diagnosis may *alter medical management* for the individual (PKU).
- Diagnostic testing of an individual may have ***reproductive or psychosocial implications*** for other family members.

2. Predictive testing:

- offered to individuals *who do not have symptoms* at the time of testing but *have a family history* of a genetic disorder.
- can have psychological ramifications - may require patient assessment & **counseling.**

3. Carrier Testing

- Test to **identify individuals** who have a gene mutation for a disorder inherited in an ***autosomal recessive*** or ***X-linked recessive*** manner.
- **offered to individuals with:**

➢ family members who have a genetic condition.

➢ family members of an identified carrier.

➢ individuals in ethnic or racial groups known to have an increased risk for a specific condition.

- **If both parents are tested**, the test can provide information about a ***couple's risk*** of having a child with a genetic condition

4.1. Prenatal genetic testing

- Detect genetic disorders and birth defects. > 200 single gene disorders can be diagnosed. Testing done only when a family history or other risk.

a. Ultrasound: Noninvasive, uses reflected sound waves converted to an image. **Transducer** placed on abdomen

- See physical features of fetus, not chromosomes. May identify some chromosomal abnormalities by physical features.

b. Amniocentesis

• Diagnose > 100 disorders, cells analyzed for ***chromosomal and biochemical disorders.***

• Risk of **infection** and **spontaneous abortion.**

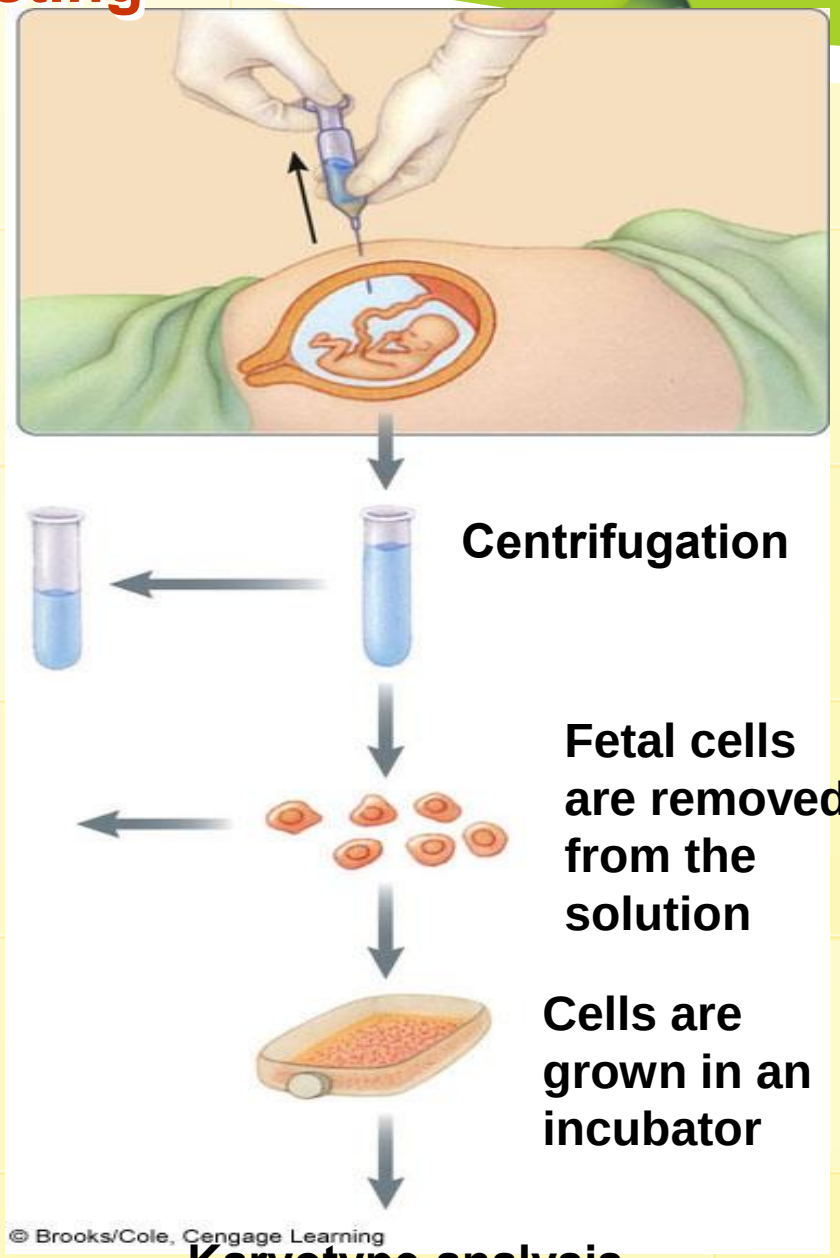
• Normally **only** used when: - Advanced maternal age. - History of chromosomal disorder. - Parent with chromosomal abnormality. - Mother carrier of X-linked disorder

4.1. Prenatal genetic testing
b. Amniocentesis

Removal of about 20 ml of amniotic fluid containing suspended cells that were sloughed off from the fetus

Biochemical analysis of the amniotic fluid after the fetal cells are separated out

Analysis of fetal cells to determine sex



4.2. Preimplantation Genetic Diagnosis

- Eggs collected, fertilized, allowed to develop.
- ~ 3rd day of fertilization, embryo has 6–8 cells.
- For PGD, one cell, **a blastomere**, is removed.
- ***DNA extracted*** and tested (DNA analysis, karyotyping, biochemical analysis for PKU).
- Embryo without genetic disorder are implanted into mother.

5. Newborn Screening

- *identifies individuals who have an increased chance of having a* ***specific*** *genetic disorder* so that **treatment can be started as soon as possible**.
- performed on a small blood sample, which is taken by pricking the baby's heel.
- a parent will usually only receive the result if it is positive. if the test result is positive, additional testing is needed to determine whether the baby has a genetic disorder.
- performed ***routinely at birth***

Types of genetic tests: Two main types of genetic tests

I. Constitutional

- Tests for mutations that **affect ALL CELLS** in the body, and have been there since conception

II. Acquired

- Tests for changes that **affect only certain cells** or **cell types** in the body, and that occurred later in life.

Genetic testing includes:

1. **Direct genetic testing (Molecular):** examination of DNA (or RNA) to determine if mutations are present.
2. **Cytogenetic testing:** examination of the chromosomes for visible alterations that indicate a genetic defect.
3. **Biochemical genetic testing:** assay for specific metabolites that indicate a genetic disease

I. Constitutional:

Genetic tests for constitutional mutations

1. **Molecular Tests**
2. **Cytogenetic Tests**
3. **Biochemical Tests**

.....

1. Molecular Test: Example

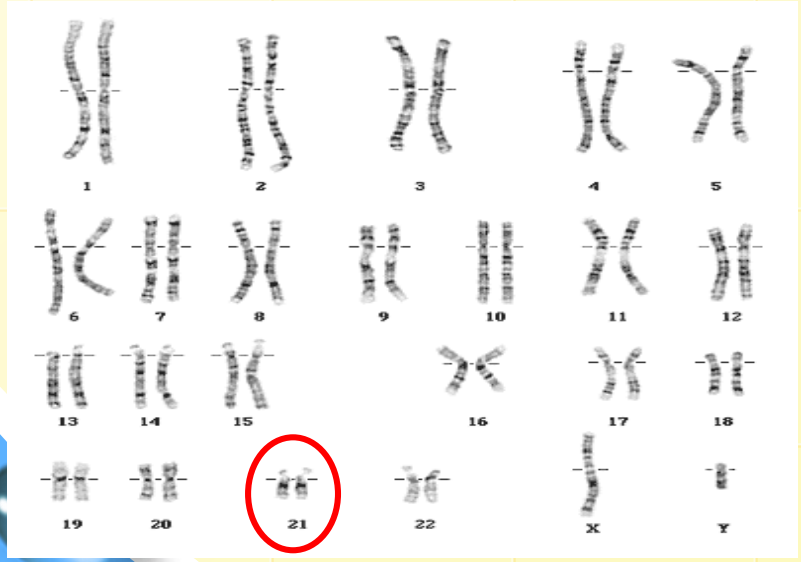
- Analysis of **DNA sequence** in patient with a rare inherited disease (Muscular Dystrophy).
- --Gene: DMD
  - Clinical Picture: - progressive muscle weakness starting in early childhood. wheelchair by age 12.
- Obtain blood sample from child
- Read the ***DNA sequence*** of the ***DMD gene.***
- Identify the mutation that caused the disease

I. Constitutional:

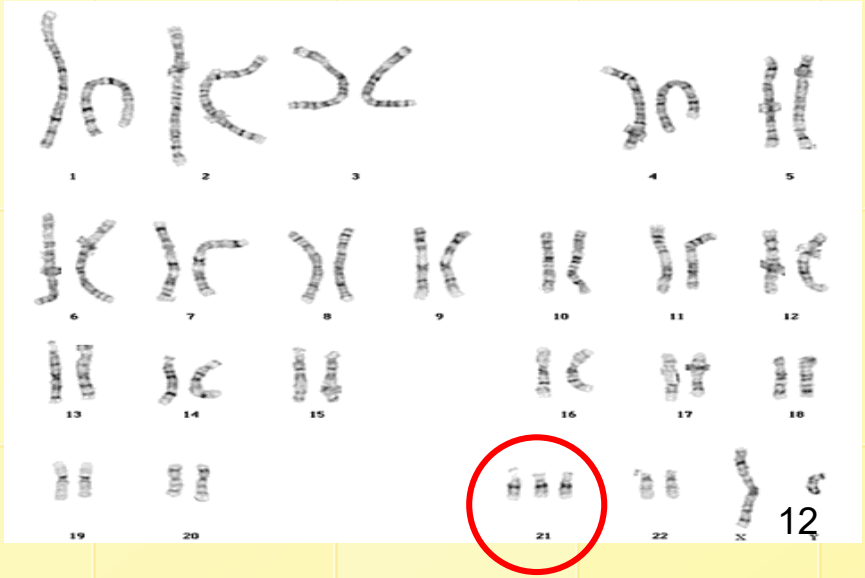
2. Cytogenetic Test: Example

- **Karyotype** – to examine the chromosomal complement of an individual including *number*, *form*, and *size* of the chromosomes.
- Frequently used for children who present with multiple anomalies, developmental delay, autism.

Normal



Down's syndrome (21 trisomy)



I. Constitutional:

3. Biochemical Test

- Analyzes the *quantity of a downstream product of a gene* (e.g. not looking directly at the gene, or the chromosome).
- Example: Newborn Screening.
- To determine if enzymes in the body are abnormal in some way.
- performed on a blood, urine, spinal fluid, or other tissue sample.
- the disease is usually the result of a mutation that causes an **enzyme** to be *absent*, *unstable*, or to have *altered activity*.
- diseases often called **"inborn errors of metabolism"** because they are present at birth and affect how the body's metabolism works

I. Constitutional: 3. Biochemical Test
Newborn Screening

- **Congenital Hypothyroidism:** Inadequate or absent production of ***thyroid hormone*** . Thyroid hormone replacement therapy begun by 1 month of age can prevent mental and growth retardation.

- **Galactosemia:** –***Galactose-1-Phosphate Uridyltransferase Deficiency*** - Failure to metabolize the milk sugar galactose. The classical form can lead to cataracts, liver cirrhosis, mental retardation and/or death. Treatment is elimination of galactose from the diet usually by substituting soy for milk products.

I. Constitutional: 3. Biochemical Test

Newborn Screening

- **Phenylketonuria (PKU)** - An enzyme defect that prevents metabolism of phenylalanine, an amino acid essential to brain development, is known as PKU.
  - Undetected and untreated with a special restricted protein diet, PKU leads to irreversible mental retardation.
  - Gene for PKU is ***Phenyl alanine hydroxylase.*** Cannot convert **phenylalanine** into **tyrosine.**

- **Sickle Cell anemia** – Sickle cell anemia is the most prevalent SCD and causes clogged blood vessels resulting in severe pain and other severe health problems.
  - Persons of ***African or Mediterranean descent*** are at an increased risk.
  - Early treatment with daily penicillin prevents death in the first few years of life.

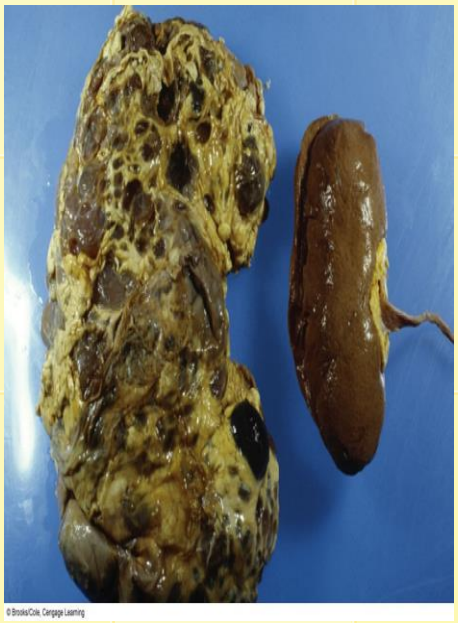
Testing adults for genetic conditions

Testing available for:

- Genetic predisposition to ***breast cancer***
- Polycystic kidney disease (***PCKD***).

Polycystic Kidney Disease (PCKD):

- **Dominant trait**, affects about 1/1,000
- Symptoms usually appear age **~35–50**
- Formation of **cysts** in one or both kidneys
- Cysts grow and gradually destroy the kidney.
- Treatment options are kidney dialysis or transplant.
- many affected individuals die



II. Acquired genetic diseases: Cancer

- Cancer is a heterogeneous disease. It is not a single disease.

Cancer is a genetic disease:

- All cancers involve genetic changes in somatic cells, the germ line, or both.
- Most gene mutations in cancer occur in **somatic cells** and are **acquired** (multifactorial etiology). (single tumors, late-onset, unilateral).
- However, some mutations do occur in the **germline** and may be **inherited** and passed on to future generations. (multiple tumors, early-onset, bilateral).

Features suggesting an inherited predisposition to cancer:

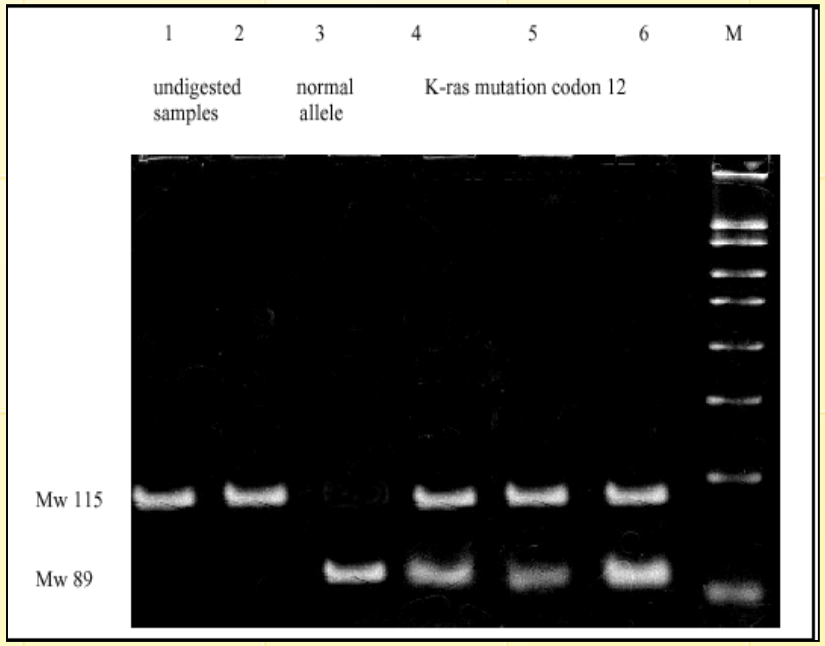
- Two or more close relatives affected.
- Early age of onset.
- Cancers of a specific type occurring together (***breast*** and ***ovary***).
- Multiple or bilateral cancers occurring in one person.

Genetic tests for acquired mutations

Tests for changes that **affect only certain cells** or cell types in the body, and that occurred later in life

1. Molecular test for acquired disease: e.g. K-ras gene test on tumor tissue from patients with *colorectal cancer*

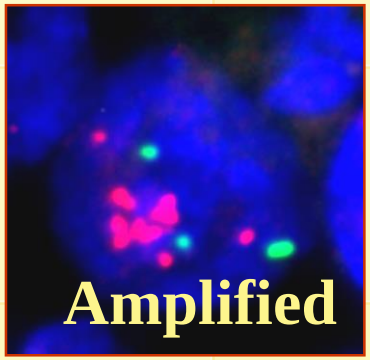
- Obtain tumor from patient.
- Extract DNA.
- treat with restriction enzyme that allows visualization of the mutation.

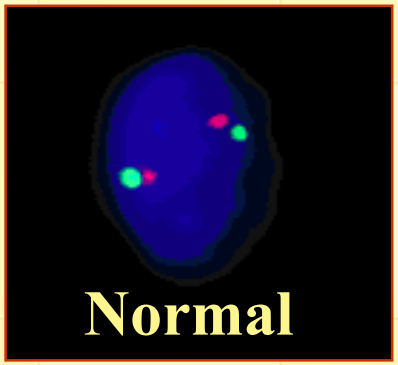


- Patient who has colon tumors do not have a K-ras mutation are much more likely to **respond** to certain therapy (as Cetuximab).
- Allows choice of alternative therapies (and saves time and money) for patients unlikely to respond.

2. Cytogenetic test for acquired disease: example

- **HER-2/neu** (Human Epidermal Growth Factor Receptor 2) **gene amplification** in ***Breast cancer***
  - Occurs early in oncogenesis
  - Associated with poor prognosis
  - Responds to ~~Herceptin~~ treatment. But Does not respond to ~~Tamoxifen~~ treatment.
  - **FISH** (Fluorescent In Situ Hybridization) for HER-2/neu is a gene-based test that allows one to count the number of HER-2 genes in a cell.





Red – *HER2;* **Green–Control Probe**

How is this information useful?

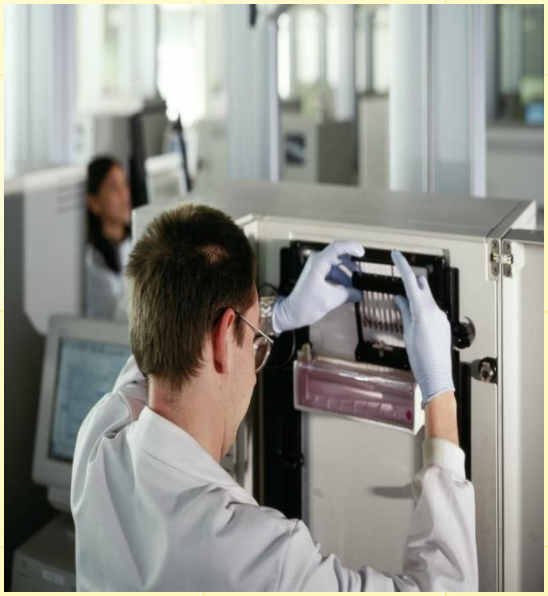
- Assists in selection of patients for chemotherapy, and which therapy to use
- Predicts response to adjuvant therapy
- Increases survival
- Allows choice of alternative therapies (and saves time and money) for patients unlikely to respond.

Summary:

- **Genetic testing** performed for both heritable and somatic gene mutations.
  - **Heritable (germline) mutations** may be predictive and performed in healthy women from ***blood***. Heritable mutations also predict risk for future disease in multiple organs.
  - **Somatic mutation** testing performed on the ***tumor*** to better assess risk and inform therapy.

Procedure:
Genetic testing & profiling

- Take a sample of cells (blood, hair root, amniotic fluid, mouth swab).
- ➢ Use staining of chromosomes to locate any chromosome abnormalities.
- ➢ Extract the DNA from cells
  - – Cut up the DNA
  - – Separate the DNA fragments
  - – Analyse the DNA fragments.

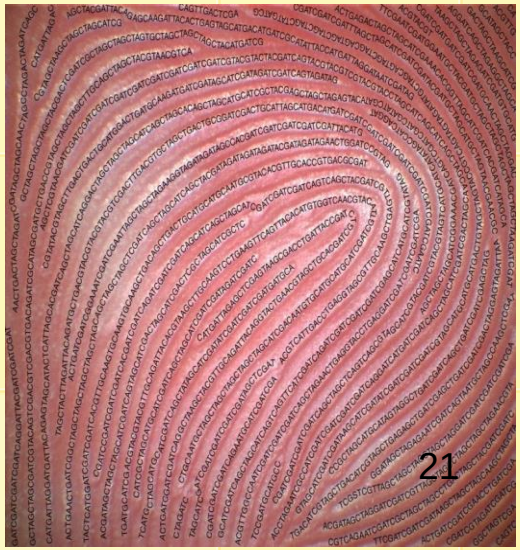


Molecular testing:

I. RFLP.

II. DNA sequencing.

III. Blotting techniques.



Restriction fragment length polymorphism (RFLP)

L/O/G/O

Molecular testing: I. RFLPs

Restriction Fragment Length Polymorphisms

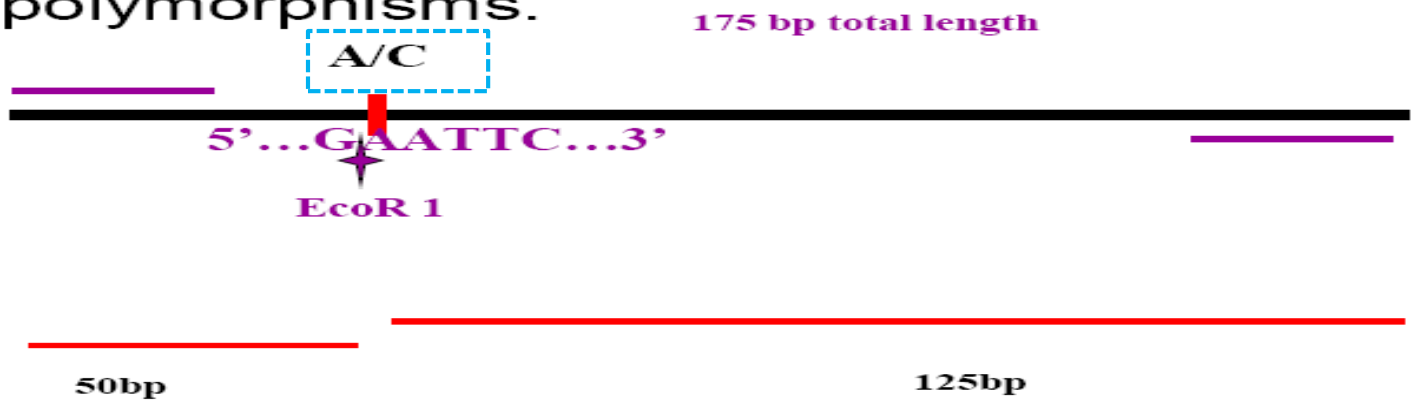
- polyms that alter the length of restriction fragments.
- Result from changes (e.g. SNPs) that ***introduce*** or ***delete*** a restriction enzyme site.
- Two alleles.
- Genotyping by Southern or PCR-RFLP.

Principle:

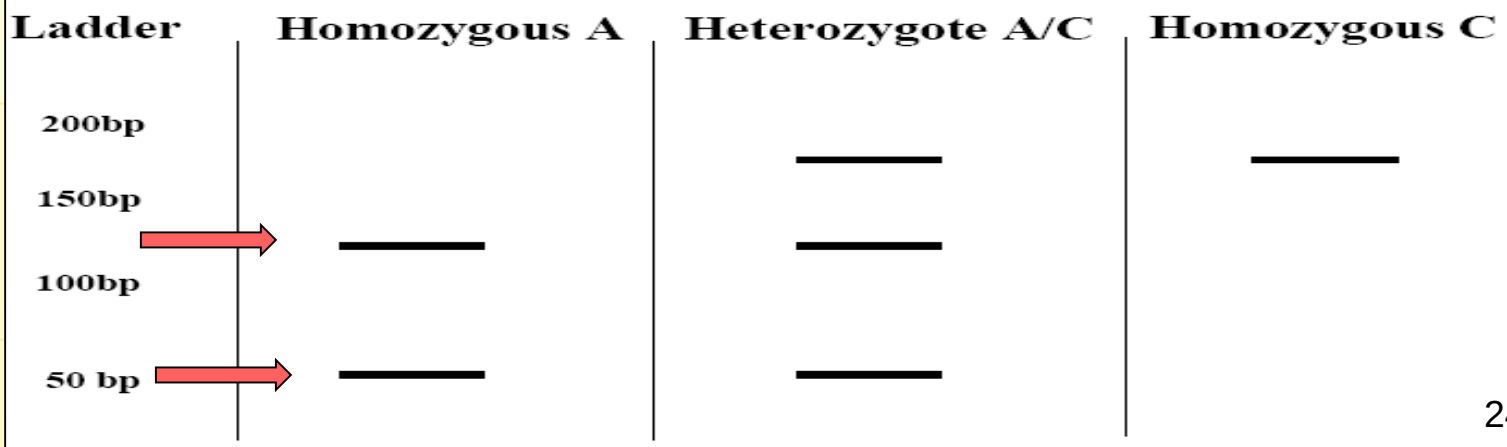
- Isolation of DNA.
- DNA is amplified by PCR.
- Amplified DNA is incubated with restriction endonucleases.
- Then electrophoresis.
- Visualization of different bands.
- Much faster than Southern analysis

RFLPs

- Restriction fragment length polymorphisms.



Run digested product on a Gel



RFLP

- Description of previous figure:
- eg., if A gene of length 175 bp. is cut by EcoR1 which specifically hydrolyses bond between G & A. so that 2 fragments are produced of length 50 & 125 bp.
- If a mutation occurs converting normal A to **C.** This R. endonuclease will not act. So, the DNA fragment will be only of 175 bp length.
- By electrophoresis, we can detect :

1. the normal subject which has 2 fragments (50 & 125), called homozygote for **A.**
2. The heterozygote containing one normal (**A**) and one mutated base (**C**).
3. The homozygote having mutation of both alleles of this gene (**C** & **C**).

Summary

- **Amplification of DNA:**

- PCR.

- **Analysis of DNA:**

- **Electrophoresis** only.
- R.E. & Electrophoresis …… (**RFLP**).
- R.E. & Electrophoresis & blot & probe……(**Southern blot**).
- ……..

RFLP – Applications:

- Forensics.
- Diagnosis of genetic diseases: Cystic fibrosis, Sickle cell anemia, ……

DNA sequencing

L/O/G/O

Eman Shaat

Genetic techniques
II. DNA sequencing: definition

- **DNA sequencing** is the process of determining the precise order of ***nucleotides*** within a ***DNA*** molecule.
- It is used to determine the order of the four bases in a strand of DNA isolated from cells of animals, plants, bacteria, or virtually any other source of genetic information.

Overall process:

- First, ***DNA has to be extracted*** from the cells of the organism being studied.
- The ***sequencing reaction*** is then performed on the DNA, and the sequenced DNA strands are sorted by size using capillary electrophoresis.
- Finally, ***the DNA code is read*** by a computer and analysed.

Uses of sequencing: Detect the presence of known genes **for medical purposes:** - genetic testing (ex. diagnostic). -Forensic identification. - Parental testing.

Genetic techniques. II. DNA sequencing:

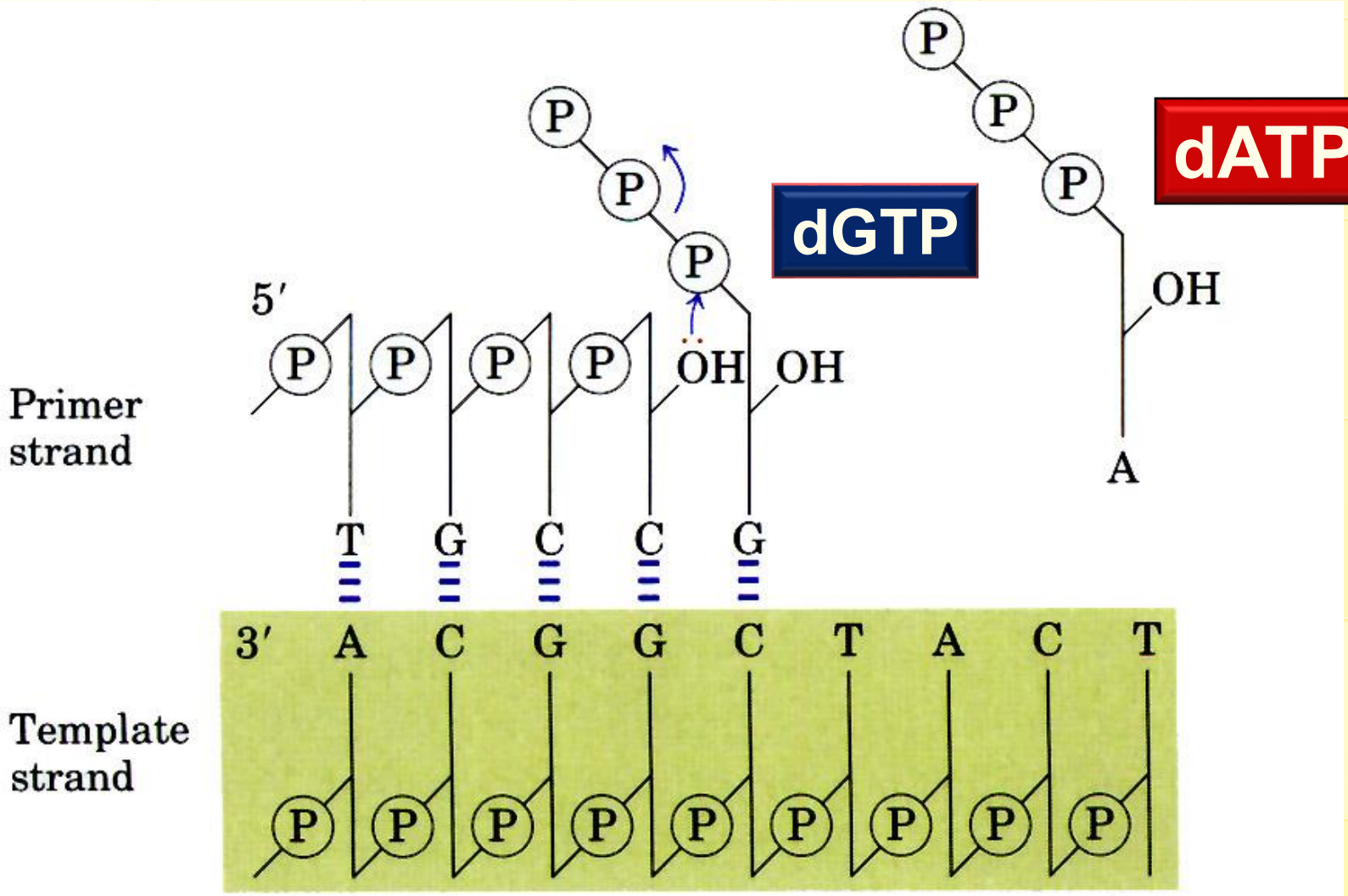
1. Chain termination (Sanger) sequencing

- A modified DNA replication reaction.
- Growing chains are terminated by dideoxynucleotides.
- **Limitations:** The dideoxy method is good only for ***500-1000 bp*** reactions. ***Expensive & Takes time.***

Brief Bio Background as regards DNA Polymerase

- DNA polymerase can add free nucleotides and forms phosphodiester bonds.
- No known DNA polymerase is able to begin a new chain, so needs ***primer***.
- It requires DNA ***template*** (ss DNA).
- It requires presence of ***dNTPs*** (dATP, dGTP, dCTP, TTP)

DNA Polymerase Action

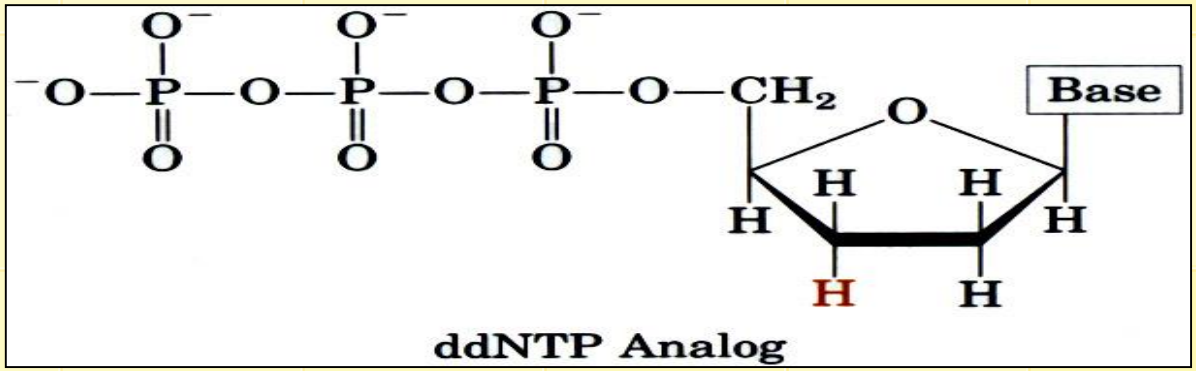


Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

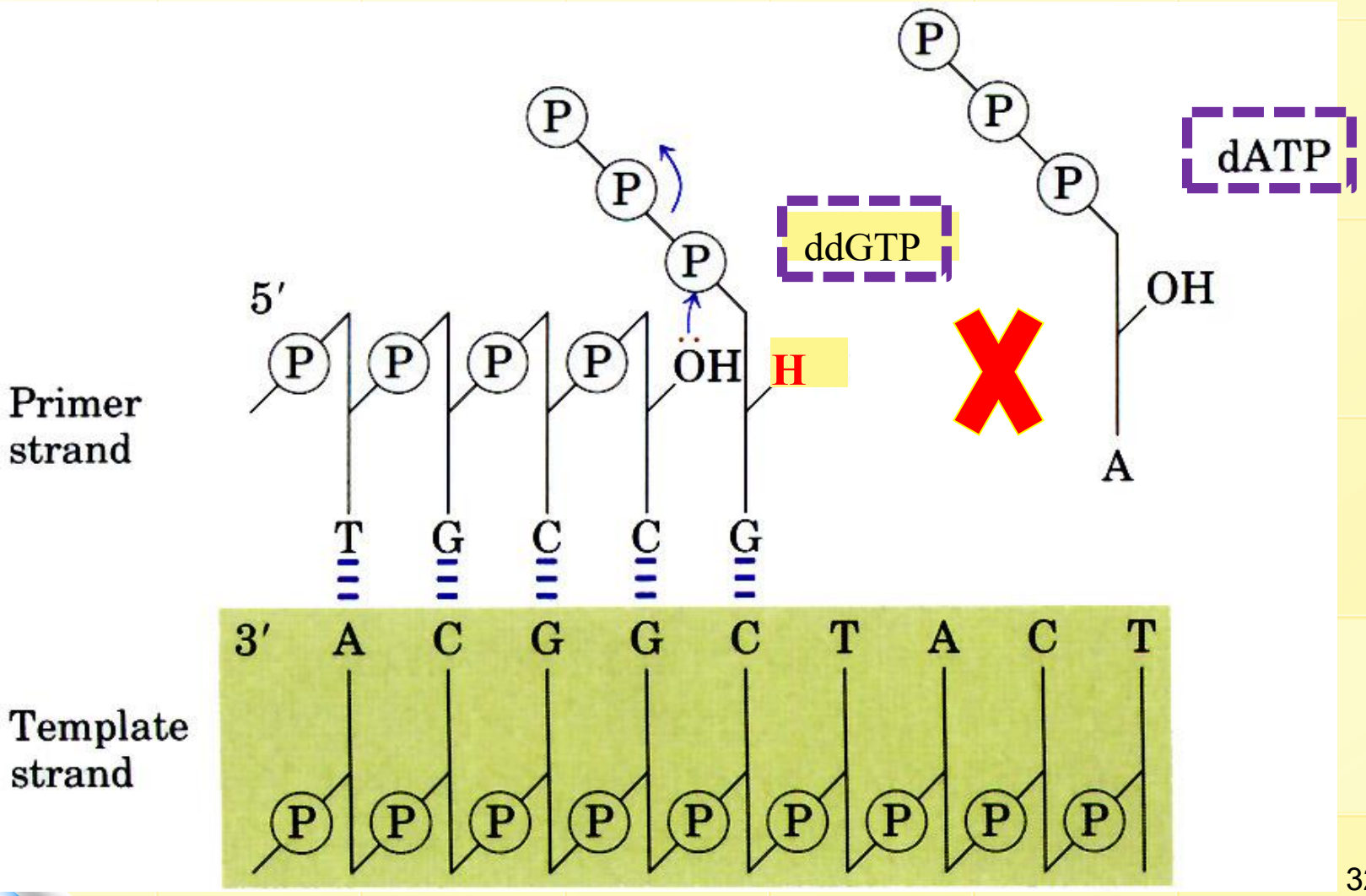
Principle:

- Uses DNA polymerase to synthesize a second DNA strand that is labeled.
- DNA polymerase always adds new bases to the 3’ end of a primer that is base-paired to the template DNA.
- Also uses chain terminator nucleotides: **dideoxy nucleotides *(ddNTPs)***, which lack the -OH group on the 3' carbon of the deoxyribose. When DNA polymerase inserts one of these ddNTPs into the growing DNA chain, the chain terminates, as nothing can be added to its 3' end.



ddNTP Analog

Dideoxies block elongation



Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

Steps:

1. Strand separation. *(denaturation; heating)*
2. Primer annealing. *(primer)*
3. Primer extension. *(DNA polymerase, dNTPs)*
4. Chain termination. *(ddNTPs)*
5. Electrophoresis. *(capillary)*
6. Detection & analysis . *(computer; electropherogram).*

……

Steps 1 (strand separation)

- Double-stranded DNA needs to be **denatured**, or separated into single strands, before it can be sequenced.
- This process is accomplished by **heating** the DNA.

Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

Steps 2 (primer annealing)

- Next, a small single-stranded DNA piece of about ***20 bases***, called an **oligonucleotide**, is annealed to the denatured template strand.
- In addition, a **large excess of primers** is used to again ensure that the primers will out-compete the complementary DNA strand for annealing to the template.
- The oligonucleotide primer must be of **complementary** sequence to the template strand in order to bind by base-pair interactions.
- **Primers: -** Oligonucleotides. - Complementary to template strand. - In excess.

Steps 3 (primer extension): During the extension phase, a ***bacterial DNA polymerase*** enzyme begins assembling a new DNA chain from the **dNTPs**, provided in the reaction mixture.The nucleotides are added in the order specified by the complementary bases in the template strand.

Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

Steps 4 (chain termination)

- The reaction mixture also contains small amounts of each of the 4 dideoxynucleotides, or "**ddNTPs**," which lack the 3'-hydroxyl group necessary for chain extension.
- Whenever a ddNTPs is incorporated into a growing DNA chain, it ***terminates*** chain growth.
- When DNA polymerase reaches a base for which some ddNTP is present, the chain will either:
  - terminate if a ddNTP is added, or:
  - continue if the corresponding dNTP is added.
  - which one happens is random, based on ratio of dNTP to ddNTP in the tube.

dNTPs >>> ddNTPs

Template 3\ T C A G A 5\

ddA

A ddG

A G ddT

A G T ddC

A G T C ddT

Four different labels: ***Each*** of the four nucleotide chains has a ***different dye***.

For example:

Red for ddATP.

Yellow for ddGTP.

Blue for ddTTP.

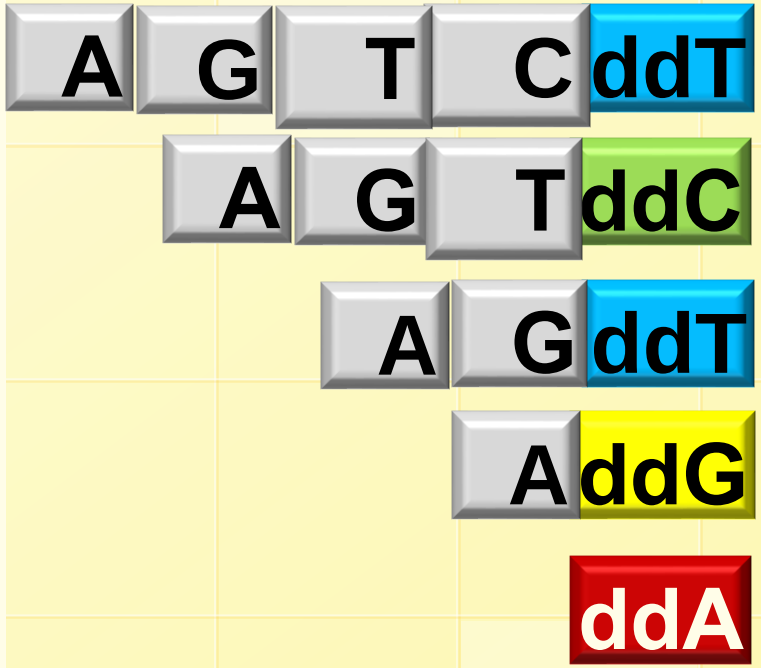
Green for ddCTP.

Genetic techniques. II. DNA sequencing:
1. Chain termination (Sanger) sequencing

Steps 5 (capillary electrophoresis)

- The newly synthesized DNA strands, ***each labeled with one of four dyes, are now sorted by length*** using capillary electrophoresis.
- An electrical current pulls the negatively charged DNA strands through the capillary. This tube is used t***o separate strands that differ in length by only one base***.
- ***Shorter DNA*** strands migrate through the gel material more quickly, and ***come out the bottom of the capillary first.***
- The fragments are separated by

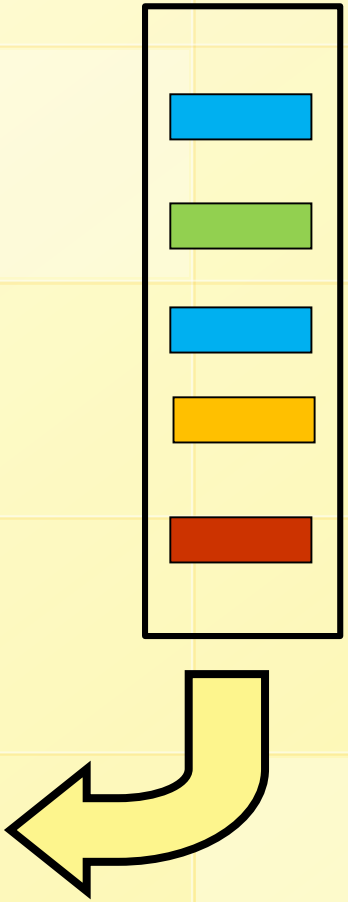
Size & color



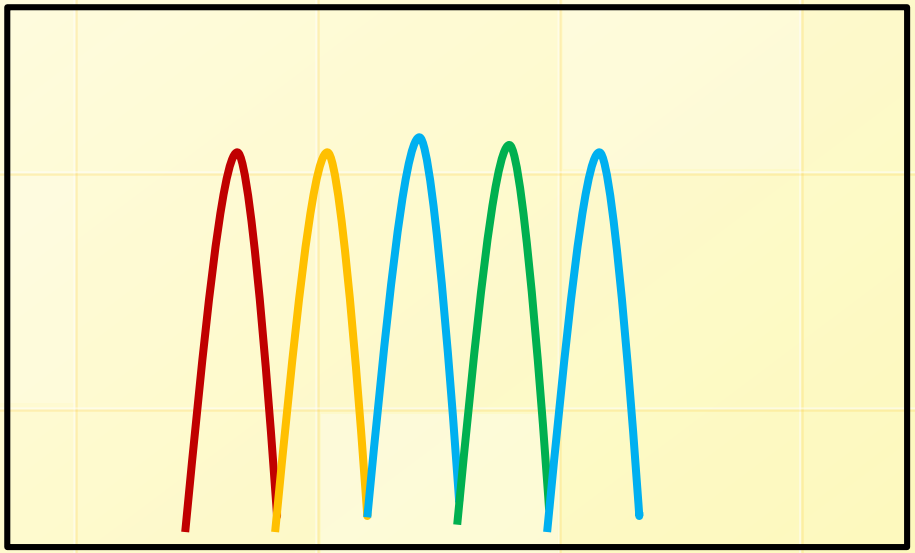
Genetic techniques. II. DNA sequencing:

1. Chain termination sequencing

Capillary electrophoresis



Electropherogram



5\- A G T C T -3

Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

Steps 6 (detection)

- **Fluorescent dyes** can absorb and emit fluorescent light at specific wavelengths.
- As the strands emerge out the bottom of the capillary they pass through a ***laser beam*** that ***excites the fluorescent dye attached to the ddNTPs*** at the end of each strand.
- This causes the ***dye to fluoresce***, or glow, at a ***specific wavelength, or color***.
- This color is then ***detected by a photocell***, which feeds the information to the **computer.**

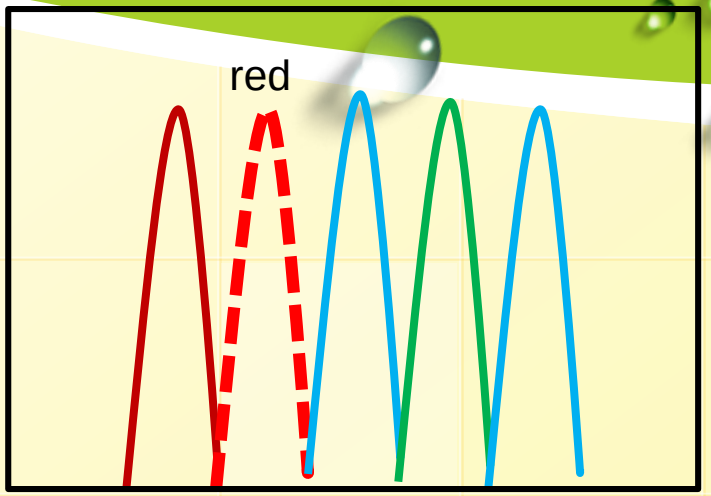
Reading the sequence

- The sequenced strand is: **5\- A G T C T -3**
- The template strand sequence is: **3\- T C A G A -5**

Automated Sequencing: detecting a mutation

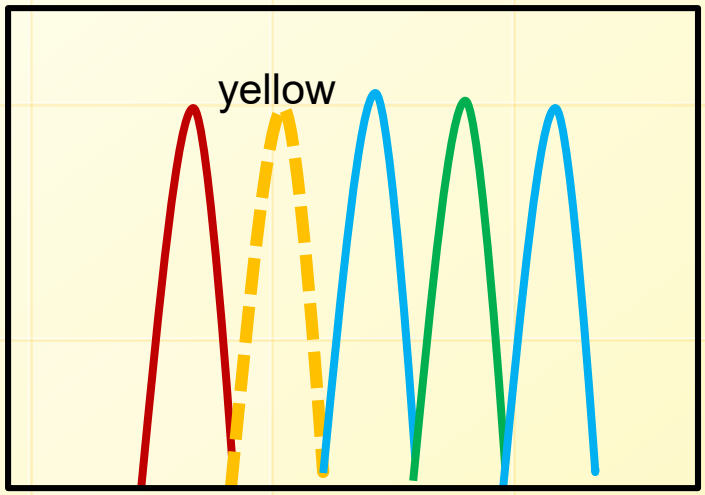
A/A

Homozygos mutation



A A T C T

G/G

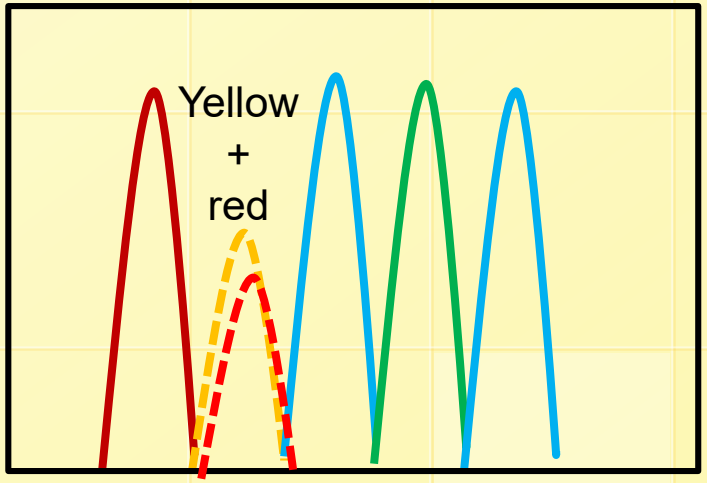


A G T C T

Normal

G/A

Heterozygos mutation



A G/A T C T

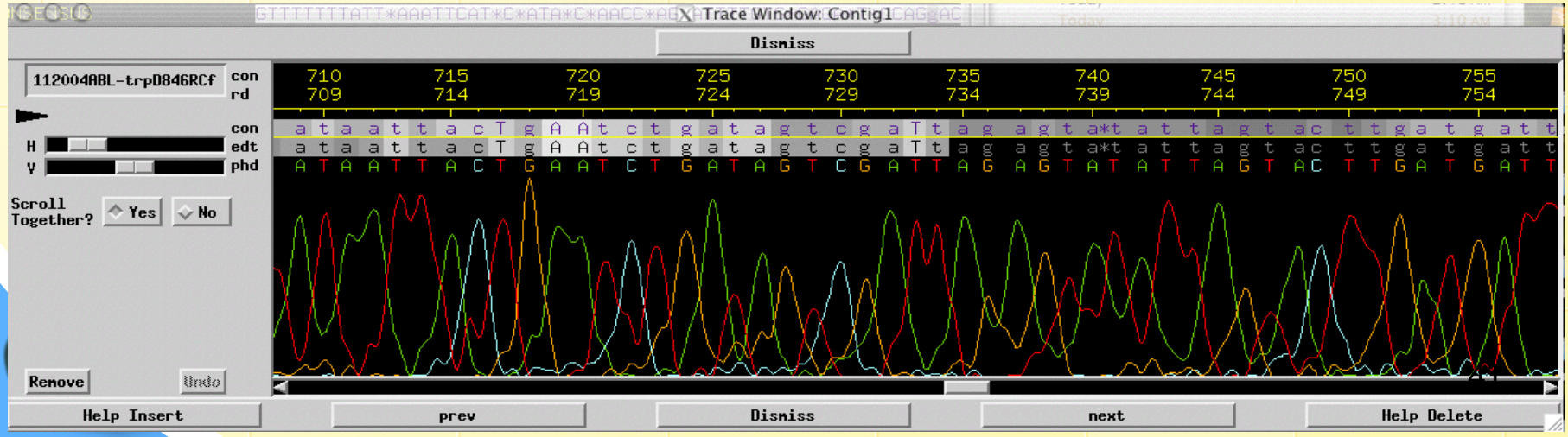
Genetic techniques. II. DNA sequencing:

1. Chain termination (Sanger) sequencing

Steps 6 (detection)

Computer analysis:

- The computer displays the information received from the photocell as an **electropherogram; Chromatogram.**
- It also ***prints*** the letter of the appropriate base below each of the signal peaks.
- successive peaks correspond to DNA segments differing in length by one nucleotide.



Blotting techniques

L/O/G/O

Genetic techniques. **III. Blotting technique:**

Definition: Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis.

- This method Involves:
  - separation.
  - Transfer.
  - Hybridization.

History: Professor **Sir Edwin Southern** ,**Professor of Biochemistry** developed this method in **1975**. The technique is known as DNA transfer or 'Southern blottin.

Common types:

- Southern blot: it is used to detect DNA using DNA probe.
- Northern blot : it is used to detect RNA using DNA probe.
- Western blot : it is used to detect protein using antibody as a probe.

Genetic techniques. **III. Blotting technique:**

Definitions: Hybridization

•The binding between ***ss labeled probe*** to a ***complementary*** nucleotide sequence on the target DNA.

Making a probe:

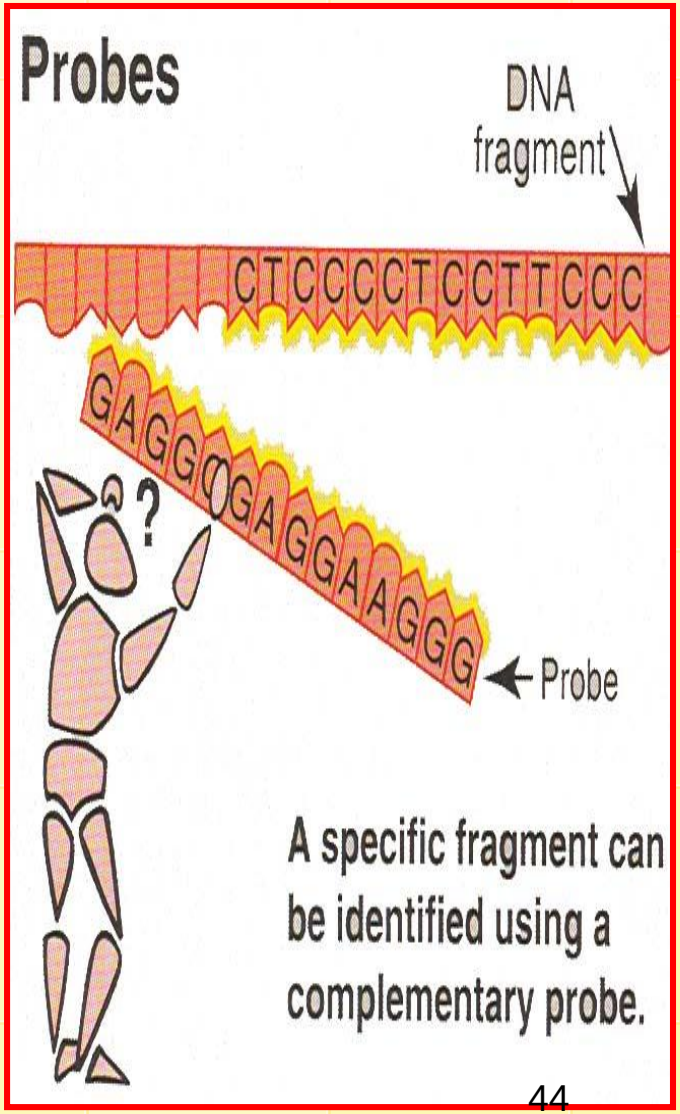
•A probe is a ***small*** length of ***DNA (20-30 nucleotides)*** or RNA.

•**Complementary** to the sequence (gene) of interest.

•**Labeled** for subsequent detection procedures by:

- ➢Radioactive e.g. ^{32}P (sensitive, cheap, hazardous)

• The probe used to visualize the restriction fragments.

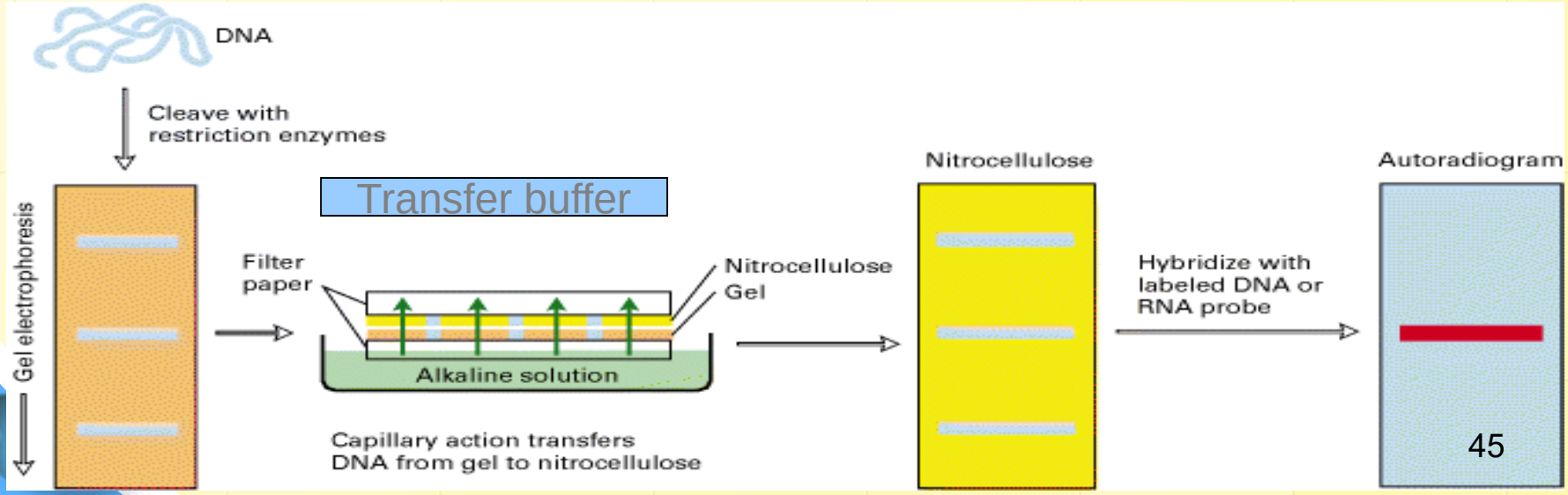


Genetic techniques. **III. Blotting technique:**

1. Southern hybridization

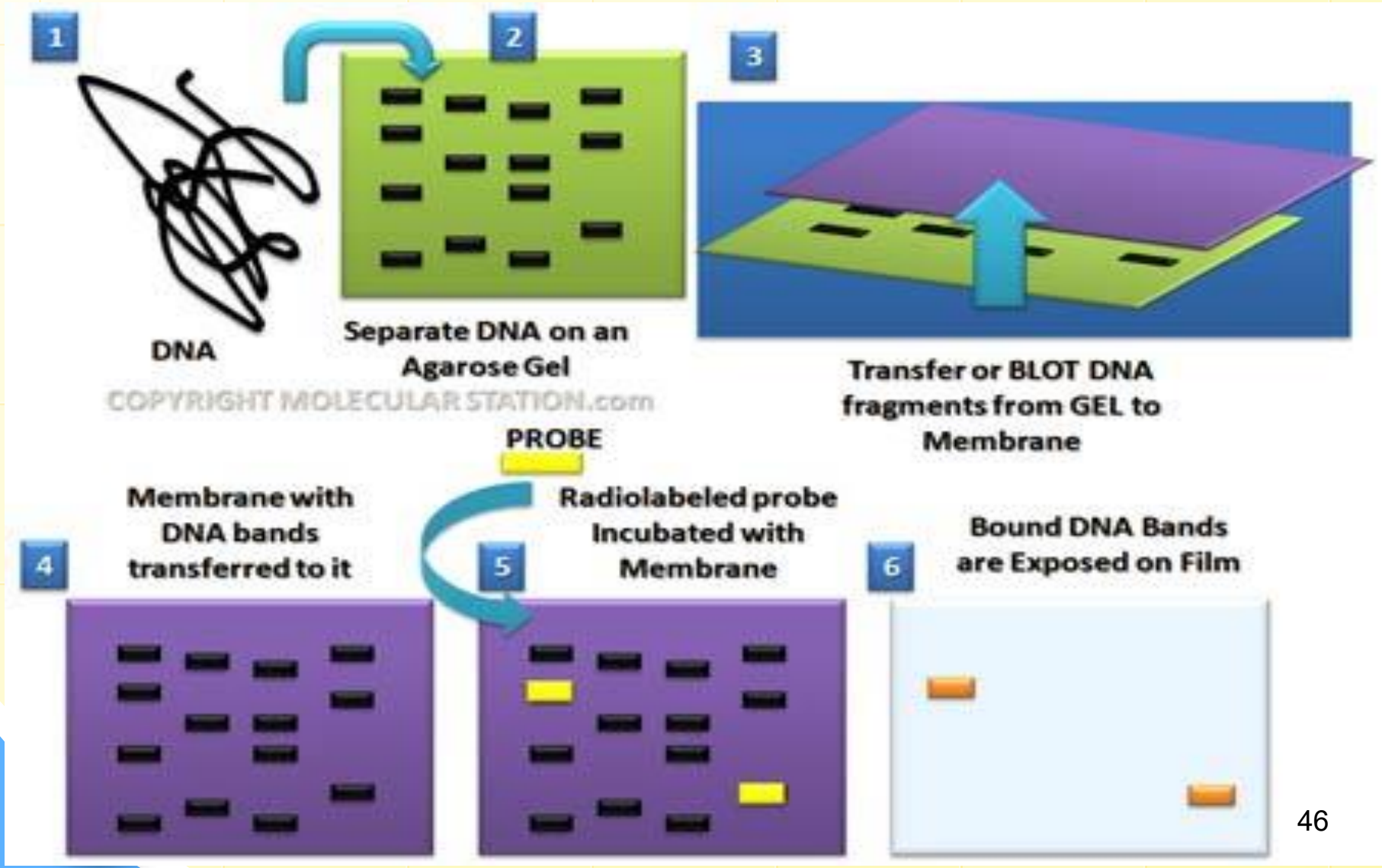
Steps:

1. ***DNA extraction*** & digestion.
2. Separation (***gel electrophoresis***).
3. ***Denaturation*** (alkali).
4. Transfer to nitrocellulose membrane (***blotted***).
5. ***Hybridize*** with ***labeled probe.***
6. ***Band visualization***. The labeled probes detect specific DNA sequences.



Genetic techniques. **III. Blotting technique:**

1. Southern hybridization

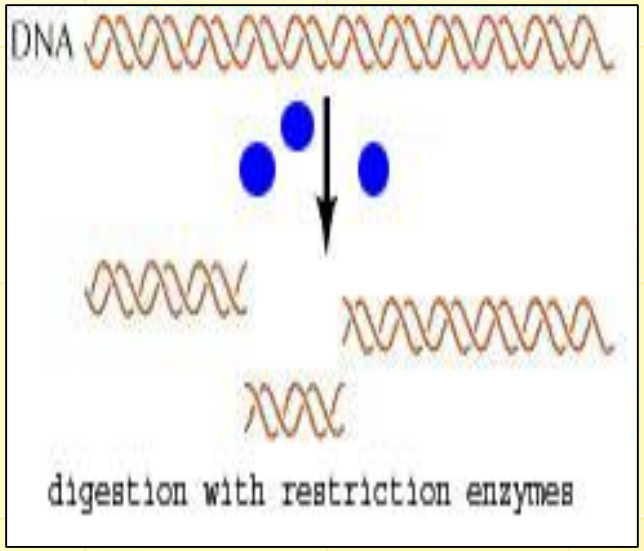


Genetic techniques. **III. Blotting technique:**

1. Southern hybridization

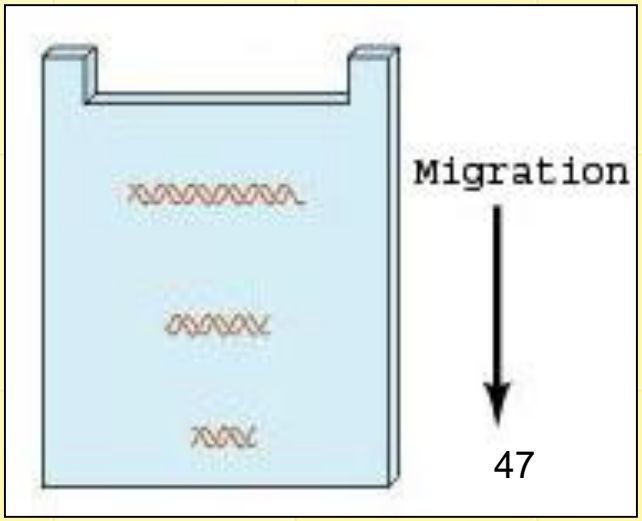
Steps 1:DNA separation & digestion:

- DNA is extracted from cells (ex. leukocytes).
- Digest the DNA with an appropriate ***restriction enzyme.***



Steps 2: electrophoresis:

- The complex mixture of fragments is subjected to gel electrophoresis to **separate** the fragments according to ***size.***
- The lengths of the fragments are compared with band of relative standard fragments of known size (Marker).

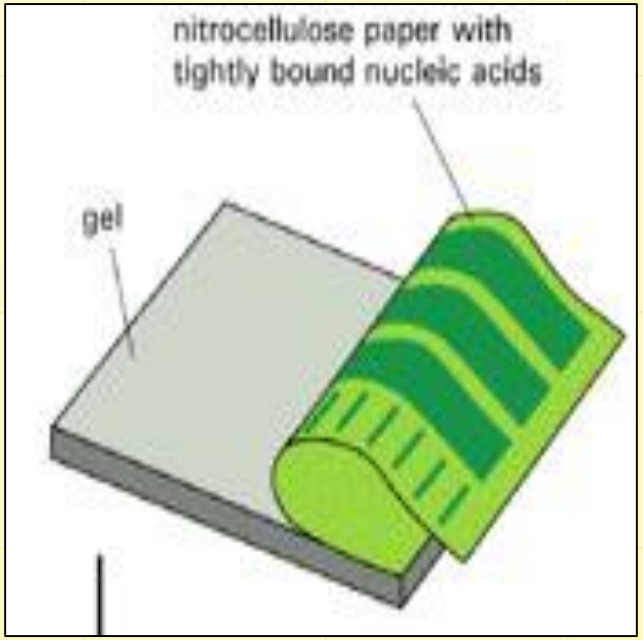


Genetic techniques. **III. Blotting technique:**

1. Southern hybridization

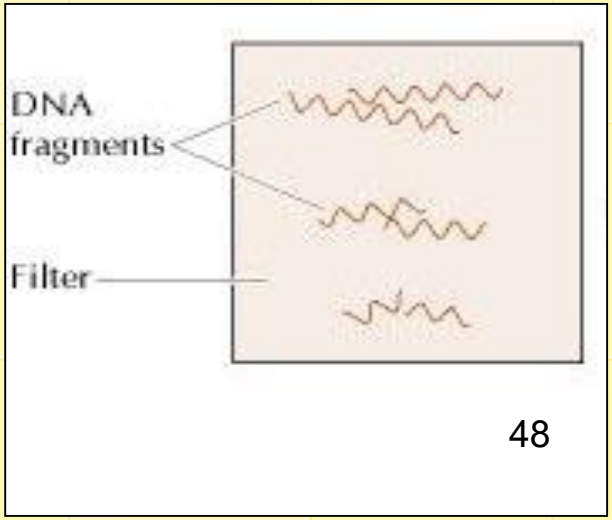
Steps3: denaturation & blot:

- The restriction fragments present in the gel are denatured with **alkali**.
- The denatured DNA fragments are transferred to nitrocellulose membrane for analysis.
- The gene of interest is on **only** one of these pieces of DNA.



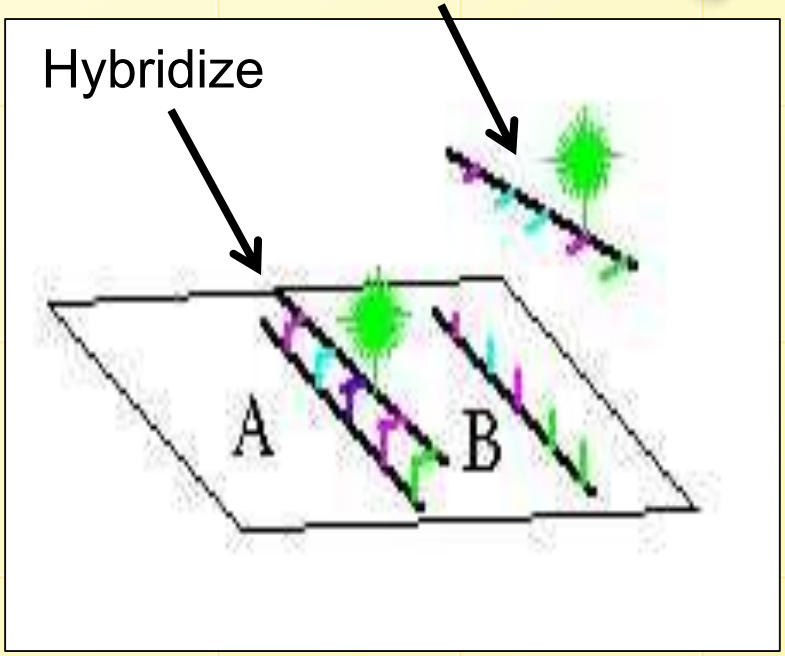
Blot

- This procedure preserves the distribution of the fragments in the gel, creating a replica of the gel on the filter.



Genetic techniques. **III. Blotting technique:**

1. Southern hybridization

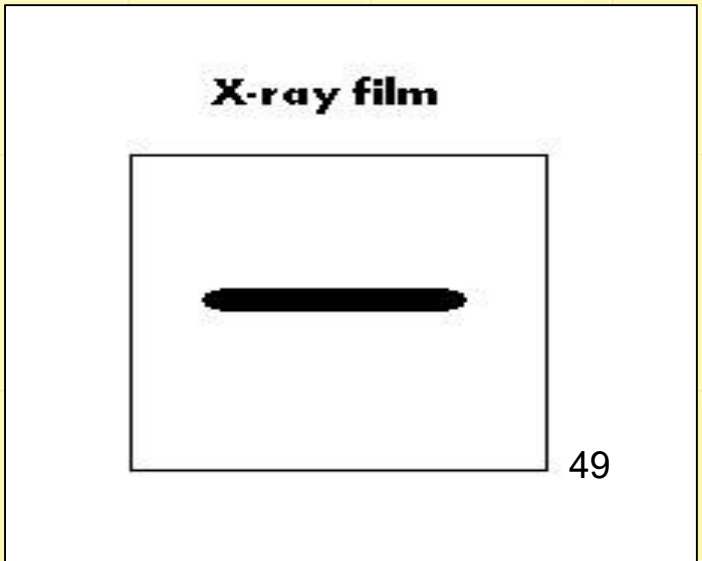


Steps4: Hybridization:

- The filter is incubated with a ***specific labeled DNA probe.***
- The probe hybridizes to the complementary DNA restriction fragment.

Steps5: Detection:

- Excess probe is washed away and the probe bound to the filter is detected by **autoradiography,** which reveals the DNA fragment to which the probe hybridized.



Genetic techniques. **III. Blotting technique:**
1. Southern hybridization

Application: Southern blots allow investigators to:

- determine the ***molecular weight*** of a restriction fragment
- ***measure relative amounts*** in different samples.

Southern blots are used in:

- ***gene discovery.***
- ***Mapping.***
- ***Diagnostics***
- ***forensics (It is used for DNA fingerprinting, …)***

Genetic techniques. III. Blotting technique:
2. Northern Blot

Definition:

- Northern blotting is a technique for ***detection of specific RNA sequences***.
- RNA is isolated from several biological samples (e.g. various tissues, various developmental stages of same tissue etc.)
- RNA is more susceptible to degradation than DNA.

Application:

- Study of **gene expression** **at the level of mRNA** in eukaryotic cells.
- To ***measure the amount*** & ***size*** of RNAs transcribed from eukaryotic genes.

Genetic techniques. III. Blotting technique:

3. Western Blot

Definition:

- Western blotting is an **Immunoblotting technique** which rely on the specificity of binding between **a protein** of interest and a probe (antibody) raised against that particular protein).

Applications:

- To determine ***the molecular weight of a protein (identification).***
- To measure relative amounts **(quantitation)** of the protein present in complex mixtures of proteins. It is used as confirmatory test for **HIV** .

Advantages:

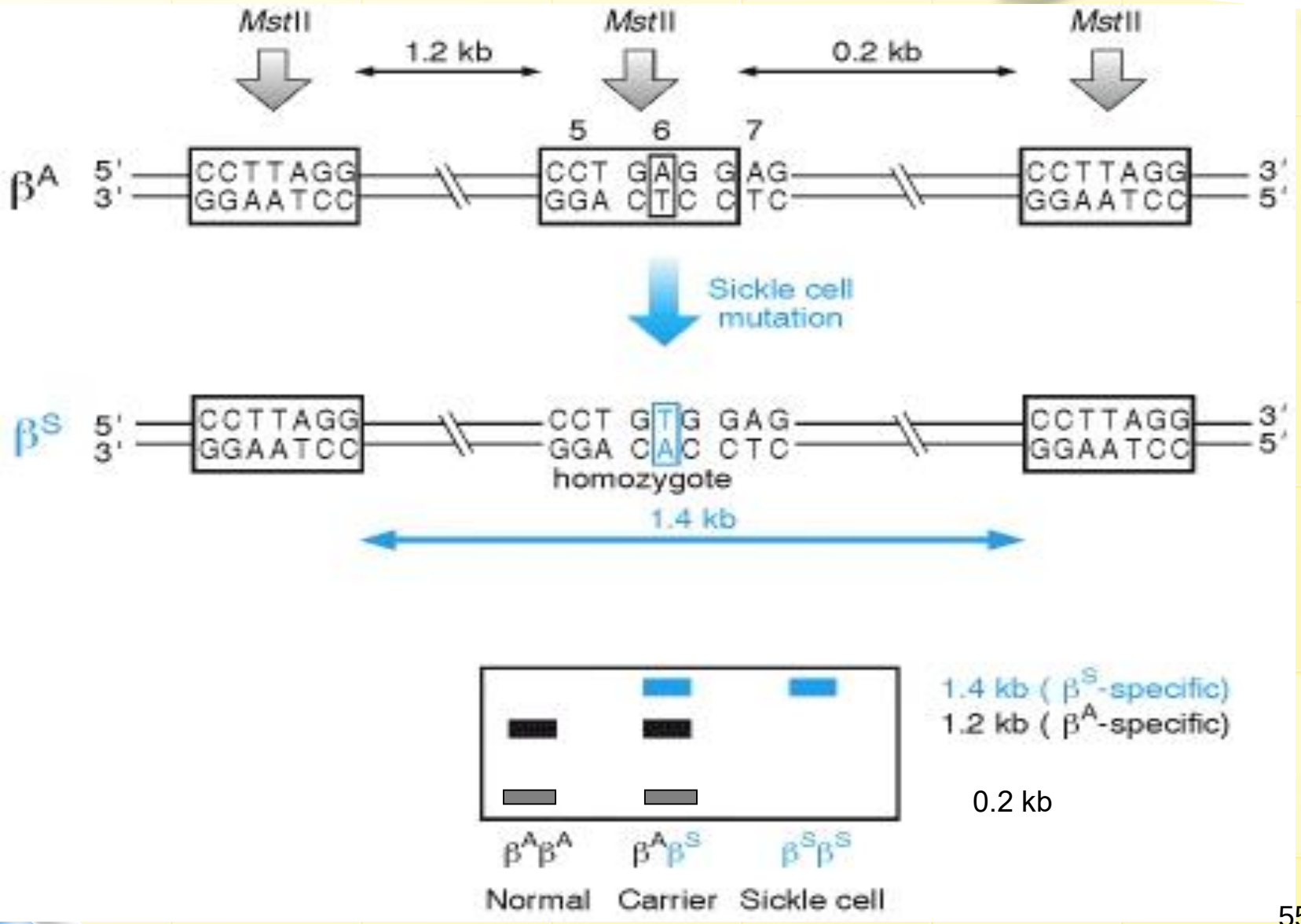
- WB is highly sensitive technique.
- **Detection and interpretation: A prestained MW standard** is included during electrophoresis to allow the identification of the MW of the target protein.

Applications of some genetic tests

L/O/G/O

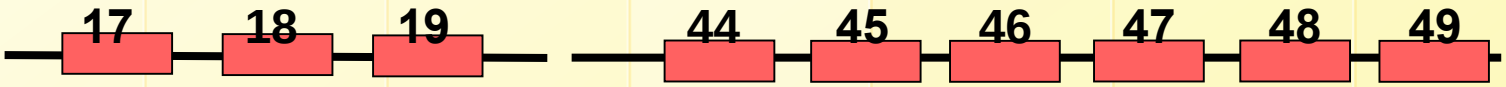
Applications of some genetic tests: **Sickle Cell Disease

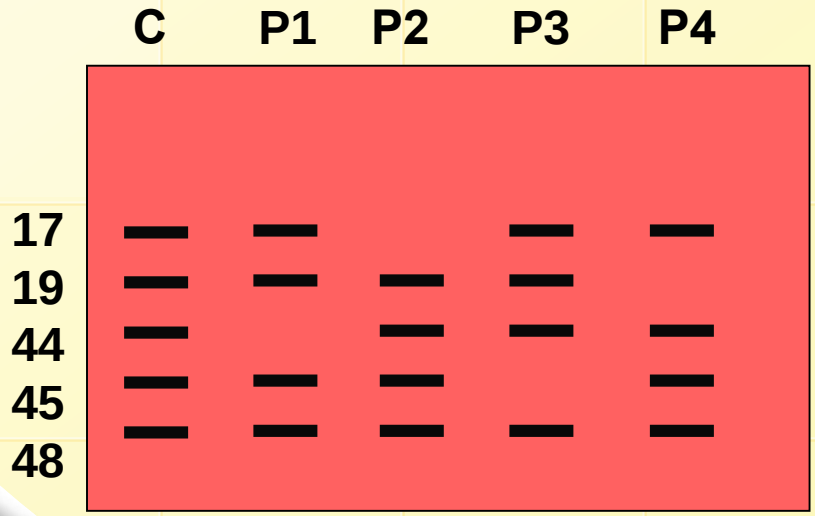
- Sickle cell anemia is a disease of red blood cells. It is caused by a mutation in the hemoglobin gene. A single base change results in a single amino acid substitution.
- Sickle cell anemia is considered a **recessive trait**, since both chromosomes have to carry the mutation in order for the full blown disease symptoms to appear.
- The sickle cell mutation **eliminates** a restriction enzyme site - the recognition site for the restriction enzyme **MstII**.
- To detect the sickle cell mutation, a patient's DNA is digested with MstII and a **Southern blot** is performed using a **probe** corresponding to this region of the hemoglobin gene.
- The presence or absence of the sickle cell mutation can be determined based on the **size** of the fragment identified by the probe.


MstII
1.2 kb
MstII
0.2 kb
MstII
5 6 7
βA
5'
3'
CCTTAGG
GGAATCC
CCT GAG GAG
GGA CTC CTC
CCTTAGG
GGAATCC
3'
5'
Sickle cell mutation
βS
5'
3'
CCTTAGG
GGAATCC
CCT GTG GAG
GGA CAC CTC
homozygote
CCTTAGG
GGAATCC
3'
5'
1.4 kb
1.4 kb (βS-specific)
1.2 kb (βA-specific)
0.2 kb
βAβA
βAβS
βSβS
Normal
Carrier
Sickle cell

Applications of some genetic tests:
** Duchenne muscular dystrophy

- Xp21; gene is 2.3 Mb long; **79 exons**
- 2/3 of cases due to ***deletions of some exons***
- Diagnosis: -Southern analysis. - PCR of exons





Patient1: exon 44 deleted

Patient2: exon 17deleted

Patient3: exon 45 deleted

Patient4: exon 19 deleted

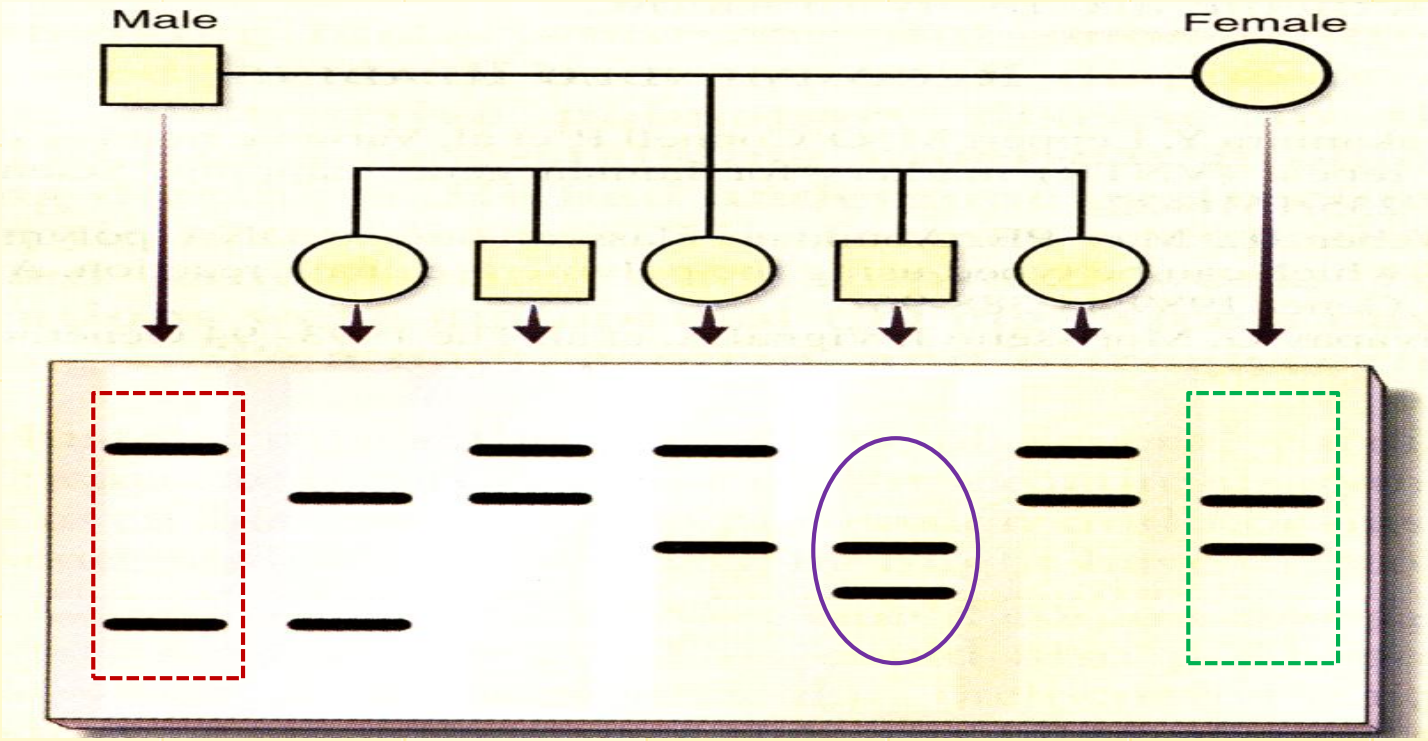
DNA profiling (DNA fingerprinting)

Every cell in the body contains the same set of DNA (except sperm/eggs).

- Although **99.9%** of human DNA sequences are the **same** in every person, enough of the DNA is different that it is possible to distinguish one individual from another, unless they are **monozygotic ("identical") twins.** Most variation exists in non-coding regions.
- DNA profiling uses repetitive ("repeat") sequences that are highly variable, called **variable number tandem repeats** (VNTRs), also known as **microsatellites**, and **minisatellites**.
- **VNTR loci** are very ***similar*** between closely related individuals, but are so ***variable*** that unrelated individuals are extremely unlikely to have the same VNTRs.
- DNA fingerprinting is a lab. technique used to establish a link between ***biological evidence*** and a ***suspect*** in a criminal investigation.
- DNA fingerprinting is also used to establish ***paternity***.

Applications of some genetic tests:
** Forensic (Paternity testing)

To determine the genetic father of a specific child



Best wishes

Dr. Eman Shaat