BT 101 MODERN BIOLOGY

SEMESTER II

3 1 0 8

Pre-requisite: Nil

Before Midsem:

Dr. Ranjan Tamuli: Diversity in biological systems; cell biology and cell structure; biological membranes

Dr. Pranjal Chandra: Fundamental concepts. Why biology for engineers, Genetics: DNA as genetic material; structure of DNA; gene expression and regulation; recombinant DNA technology.

After Midsem:

Dr. Priyadarshi Satpati: bioenergetics; DNA replication; transcription; translation; genes to proteins and to protein function

Dr. Dr. Souptick Chanda: Human physiology: biological axons and neurons, neuromuscular and synaptic junctions; sensory systems - hearing, taste, smell and visual receptors.

http://shilloi.iitg.ernet.in/~biotech/BT%20Syllabus/BT%20101.htm

Study Materials

Texts:

1. J. L. Tymoczko, J. M. Berg and L. Stryer, Biochemistry, 5th Ed, W. H. Freeman & Co, 2002.
2. D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, Macmillan Worth, 2000.

References:

1. N. Hopkins, J. W. Roberts, J. A. Steitz, J. Watson and A. M. Weiner, Molecular Biology of the Gene, 4th Ed, Benjamin Cummings, 1987.
2. C. R. Cantor and P. R. Schimmel, Biophysical Chemistry (Parts I, II and III), W.H. Freeman & Co., 1980.
3. C. C. Chatterjee, Human Physiology, Vol 1 & 2, 11th Ed, Medical Allied Agency, 1987.

Grading Pattern

- Mid semester: 40 marks
- Surprise (unannounced) quiz test before mid semester: 10 marks
- Final semester : 40 marks
- Surprise (unannounced) quiz test after mid semester: 10 marks

- No re-examination
- Attendance guideline will be strictly followed

About Myself

DR. PRANJAL CHANDRA

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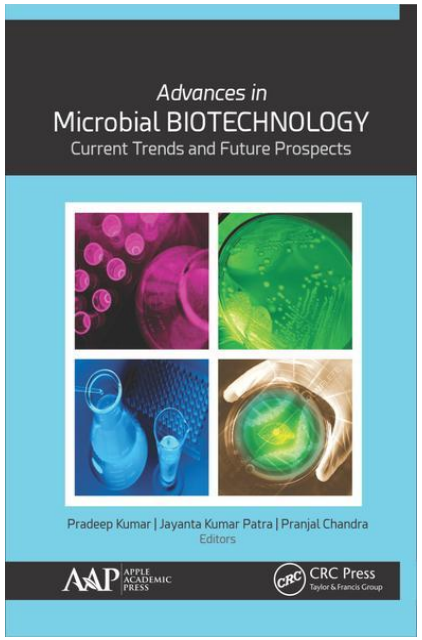
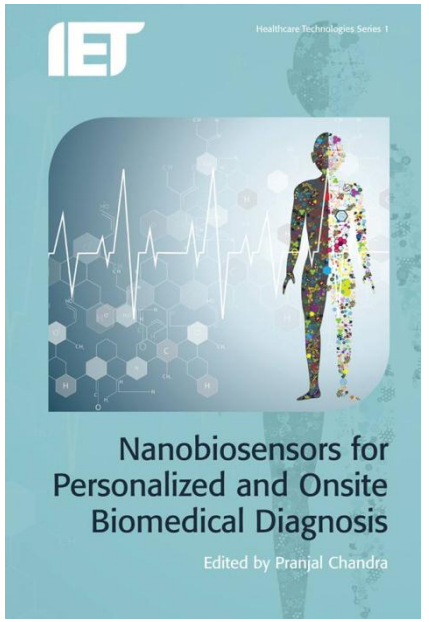
Education and Experience

- ✓ **PhD :** Pusan National University South Korea
- ✓ **Postdoc:** Technion, Israel
- ✓ Visiting Scientist: PNU South Korea
- ✓ Visiting Professor : IBST, South Korea
- ✓ **Asst. Professor:** Since July 2015 – till date

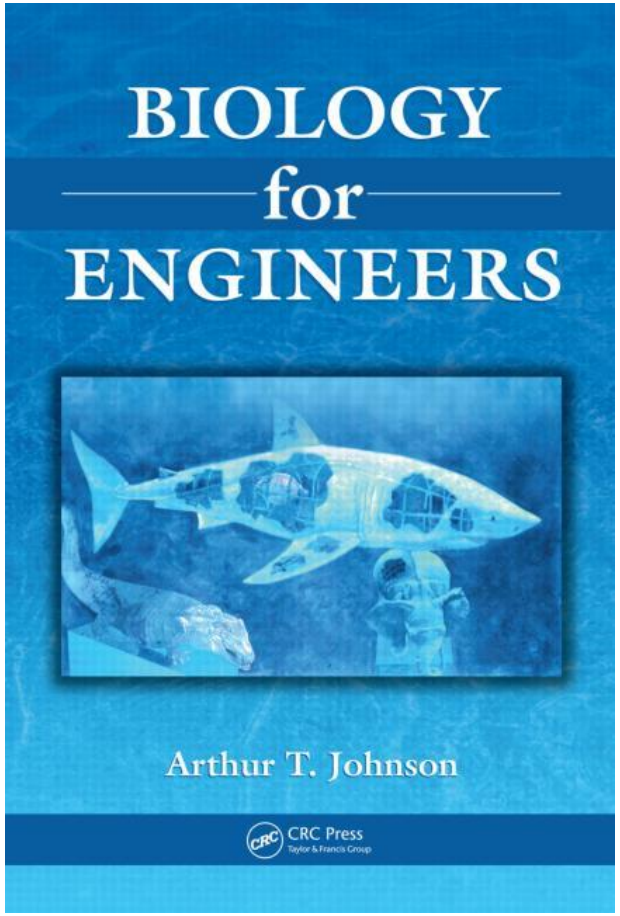


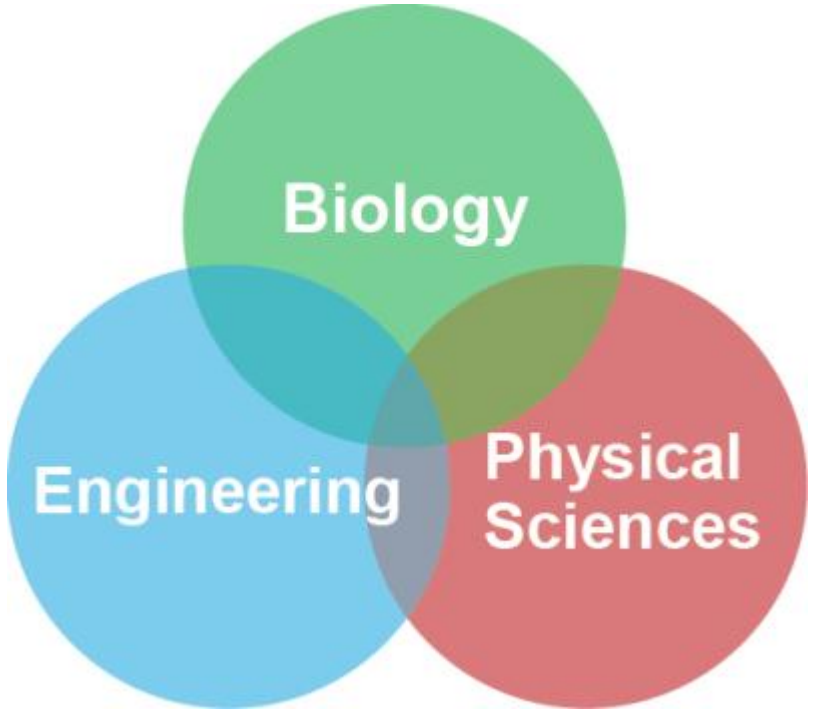


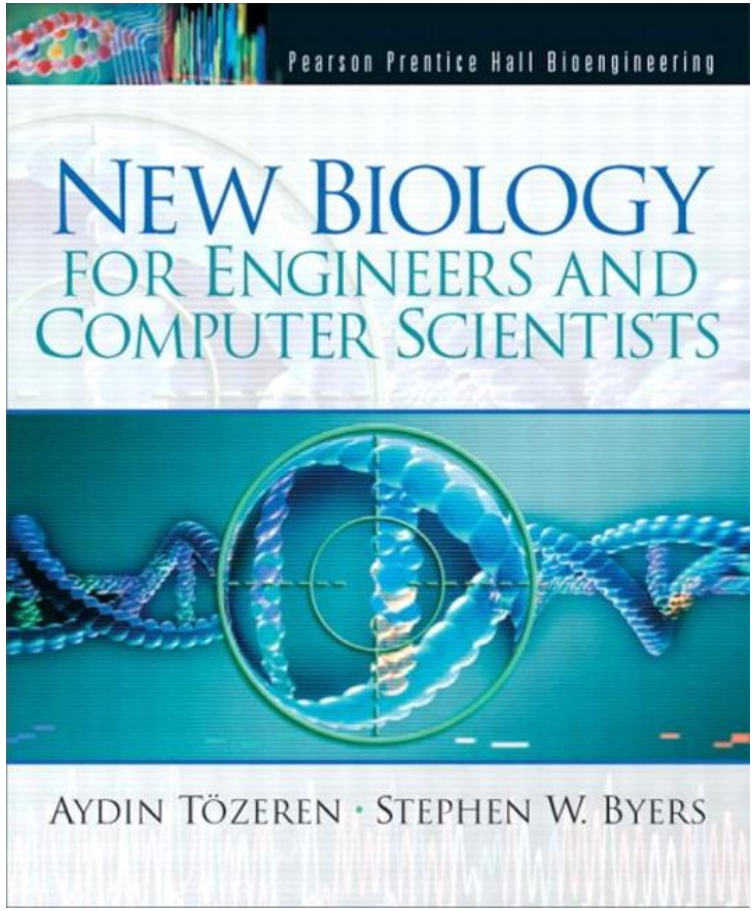
Research Interest

Biomedical Device, Biosensors, Bioelectronics, Microfluidics, Nanomedicine.

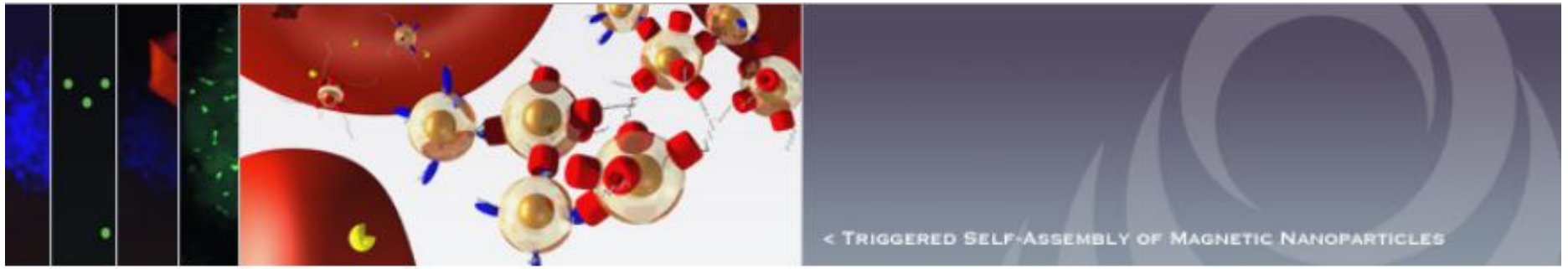
The idea of this course ????



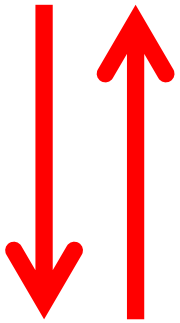




To understand the interfacing discipline



Biology



Engineering

Laboratory for Multiscale Regenerative Technologies

PRINCIPAL INVESTIGATOR
GROUP MEMBERS
OUR COLLABORATORS
RECENT ALUMNI

Sangeeta Bhatia, M.D., Ph.D.

Director, Laboratory for Multiscale Regenerative Technologies

Dr. Bhatia is a Howard Hughes Medical Institute Investigator and the John J. and Dorothy Wilson Professor at MIT's Institute for Medical Engineering and Science and Electrical Engineering and Computer Science (EECS). Bhatia is the Director of the Marble Center for Cancer Nanomedicine, and a member of the Ludwig Center for Molecular Oncology – both part of the Koch Institute for Integrative Cancer Research at MIT. She is also an Affiliated Faculty member of the Harvard Stem Cell Institute, an Institute Member of the Broad Institute, a Biomedical Engineer at the Brigham & Women's Hospital, and has been elected to Brown University's Board of Trustees. Trained as both a physician and engineer, Bhatia leads a laboratory dedicated to leveraging miniaturization tools from the world of semiconductor manufacturing to impact human health. She has pioneered technologies for interfacing living cells with synthetic systems, enabling new applications in tissue regeneration, stem cell differentiation, medical

Where Engineering and Biology Meet

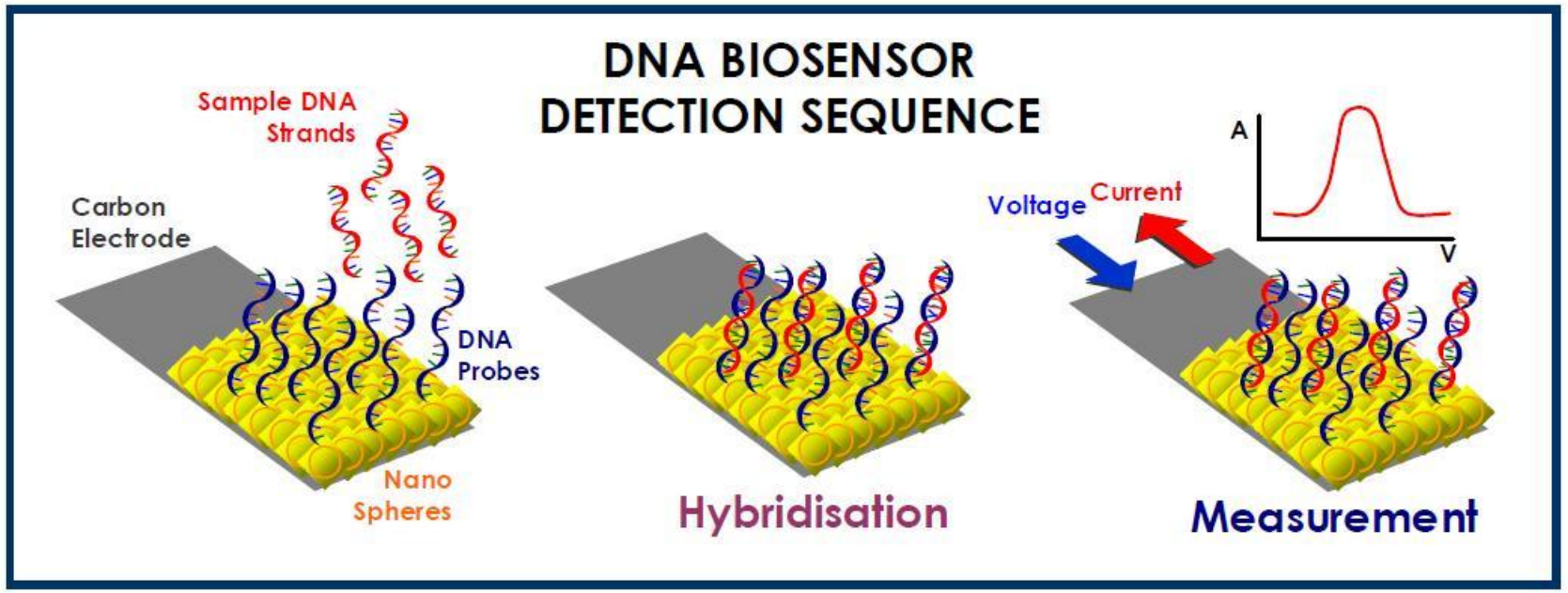
DNA EXTRACTION *via conventional methods*

→

DNA AMPLIFICATION *Not required if DNA amount > limit of detection* 2.6×10^{-17} *mol/liter*

→

DNA DETECTION *using DNA Biosensor via Hybridisation (see below)*



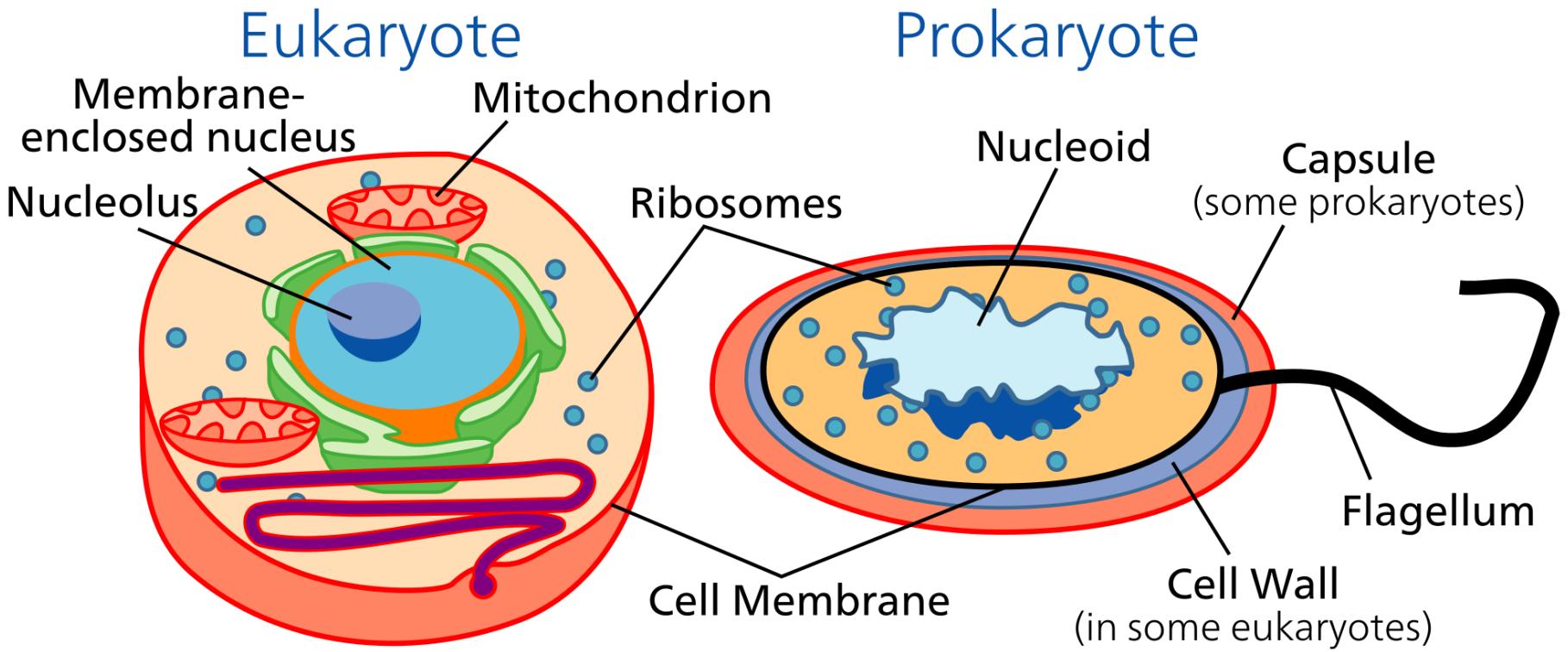
Lets understand the course structure

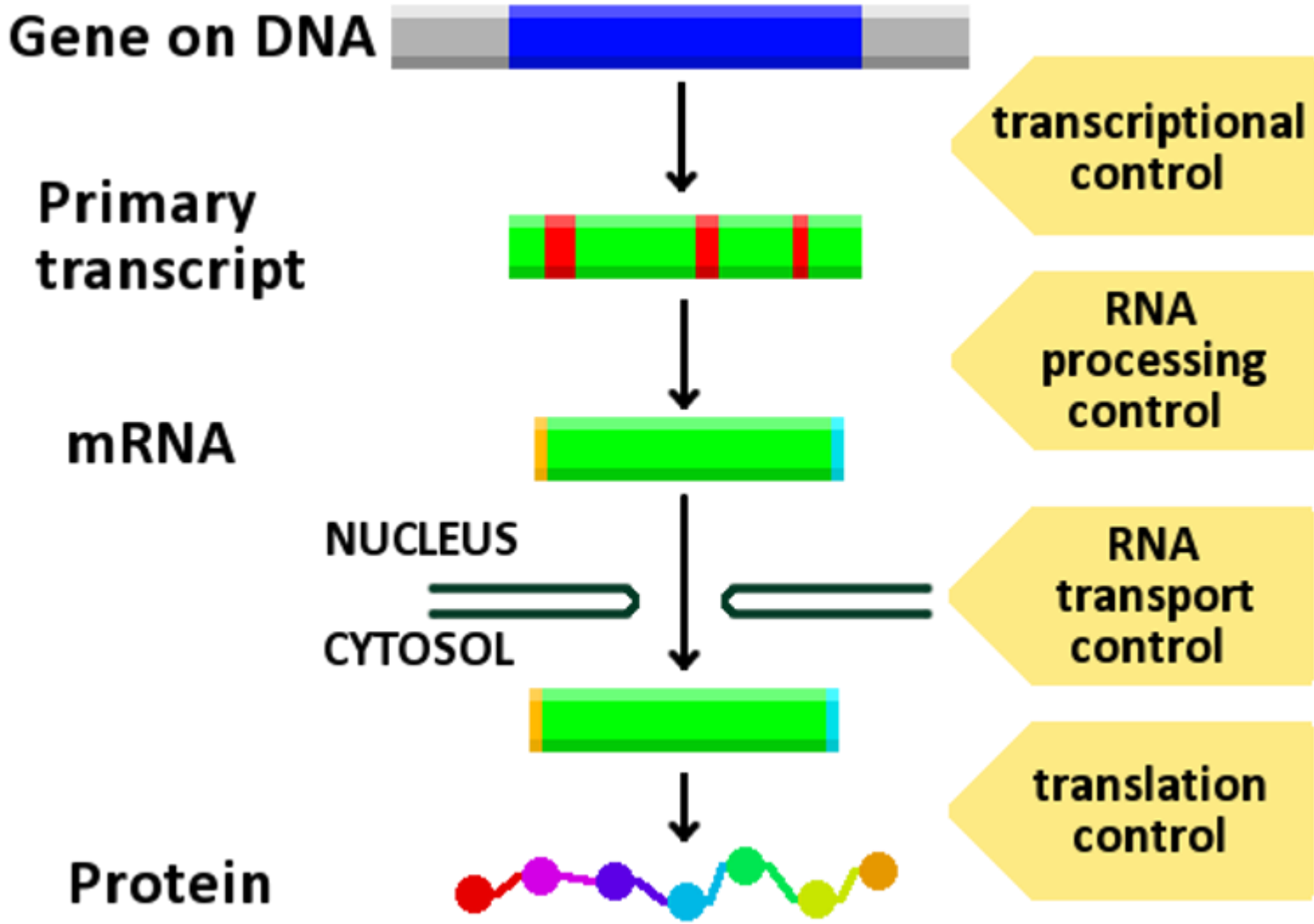
Fundamental concepts. Why biology for engineers, Genetics: DNA as genetic material; structure of DNA; gene expression and regulation; recombinant DNA technology.

Every thing is linked with these cells

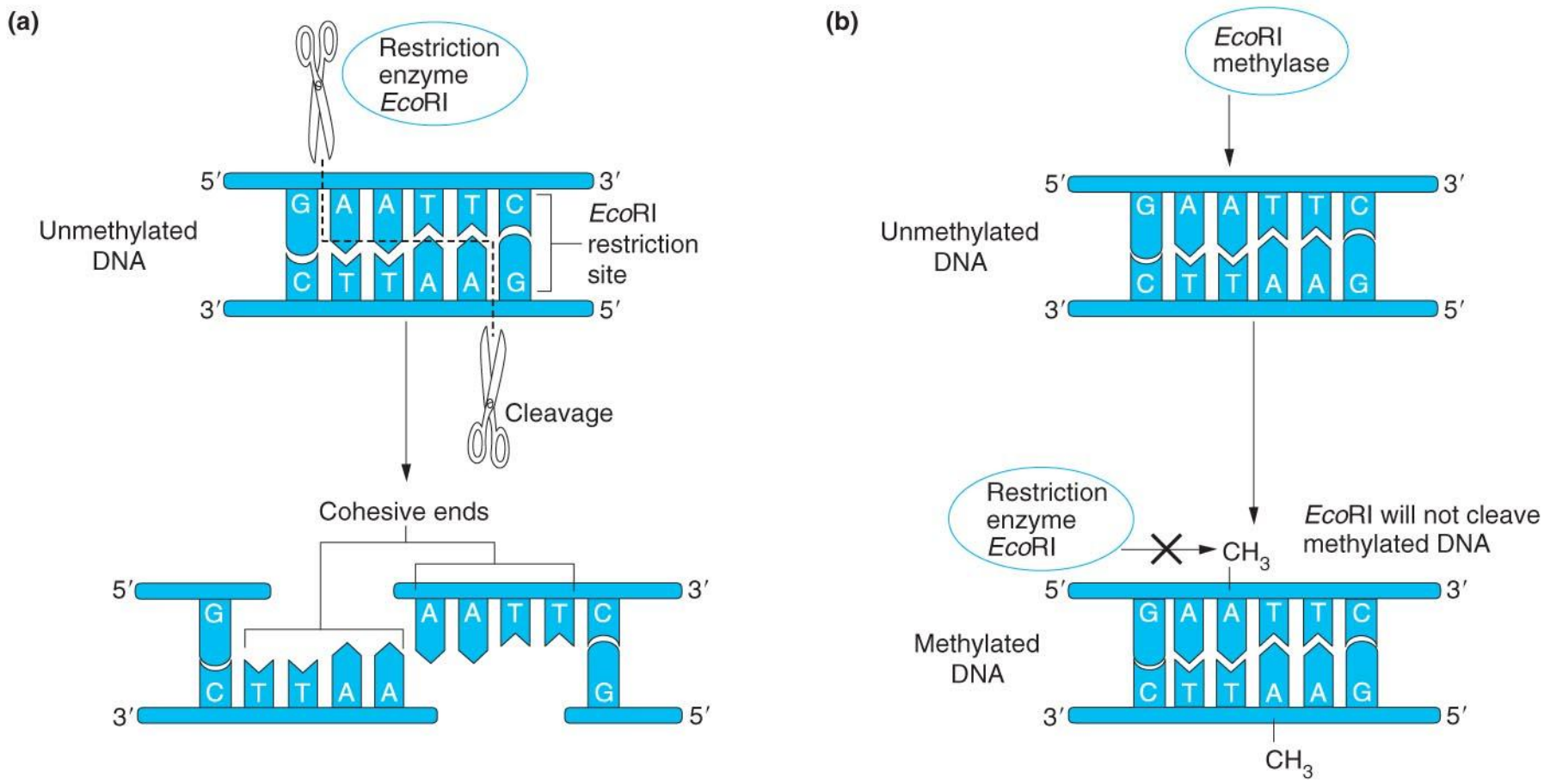
Knowledge of

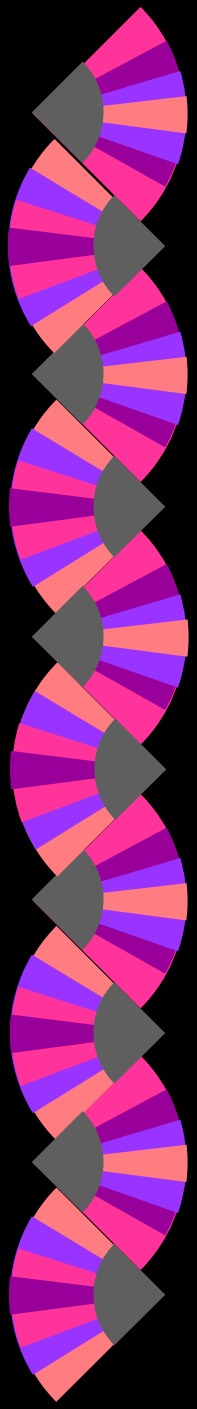
- **DNA sequence**
- **Proteins**
- **Metabolic products**
- **Excretory molecules**
- **Many more examples……**




Gene on DNA
Primary transcript
mRNA
NUCLEUS
CYTOSOL
Protein
transcriptional control
RNA processing control
RNA transport control
translation control

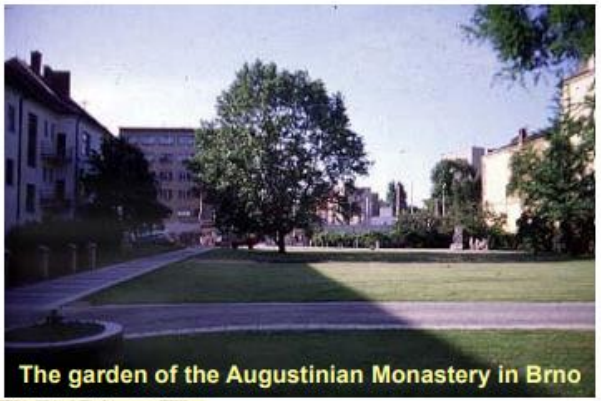
Recombinant DNA technology



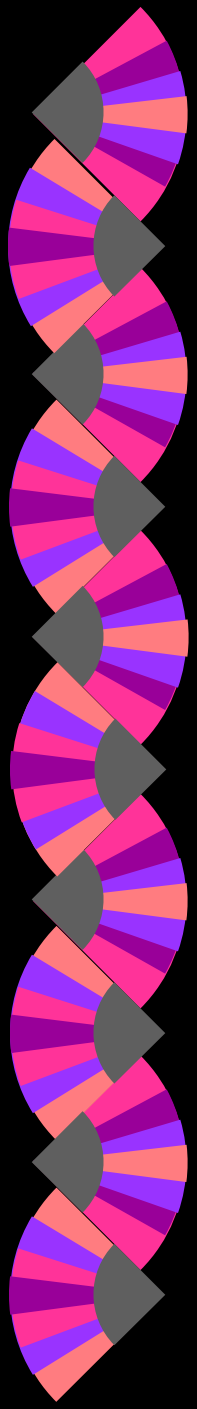


GENETICS

The History


The garden of the Augustinian Monastery in Brno


Friars of the Augustinian monastery in Brünn, in 1860-ies



- GENETICS - The study of the way animals & plants pass on to their offspring such as:

  eye color, hair color, height, body build, blood types, intelligence, gender, etc.

- HEREDITY - Characteristics that a child receives from both parents

What genetic principles account for the passing of traits from parents to offspring?

The "blending" hypothesis is the idea that genetic material from the two parents blends together (like blue and yellow paint blend to make green)

How about when one paint color is more in volume

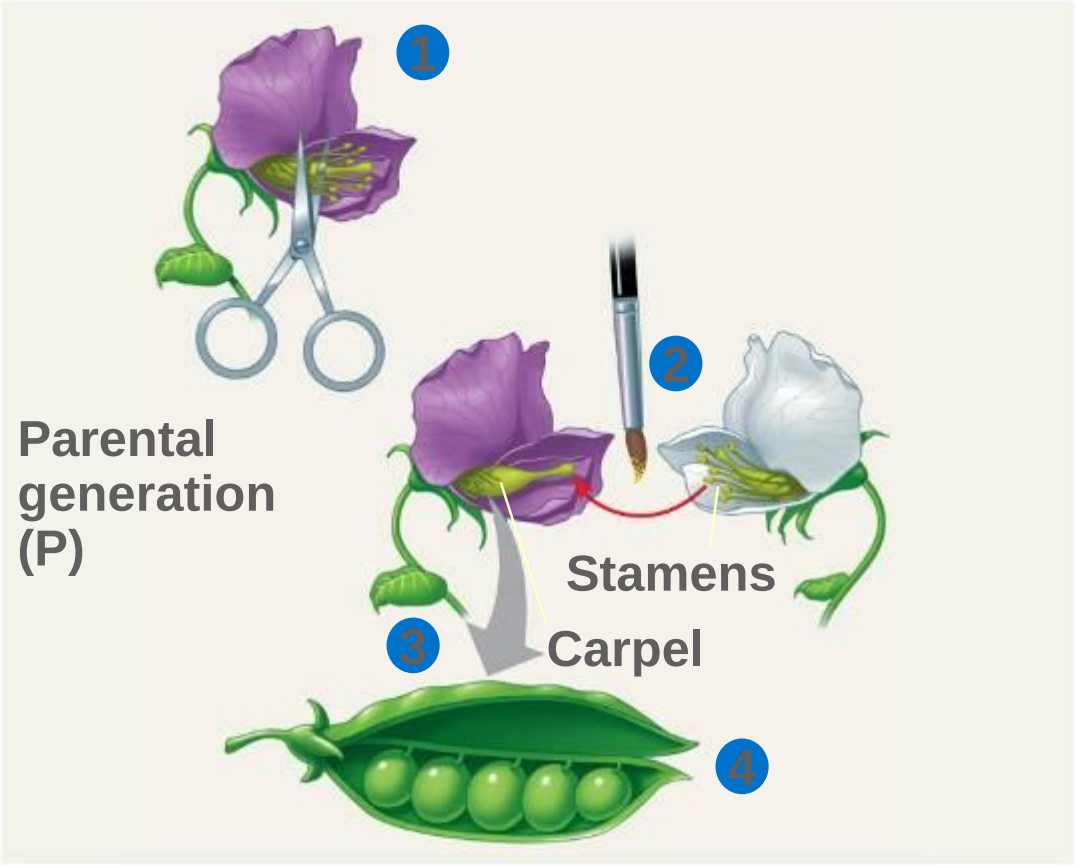
- The "particulate" hypothesis is the idea that parents pass on discrete heritable units (genes)
- This hypothesis can explain the reappearance of traits after several generations (Diabetes, eye colour)
- **Mendel** documented a particulate mechanism through his experiments with garden peas

Mendel did some experiments on garden pea: *Pisum sativum*

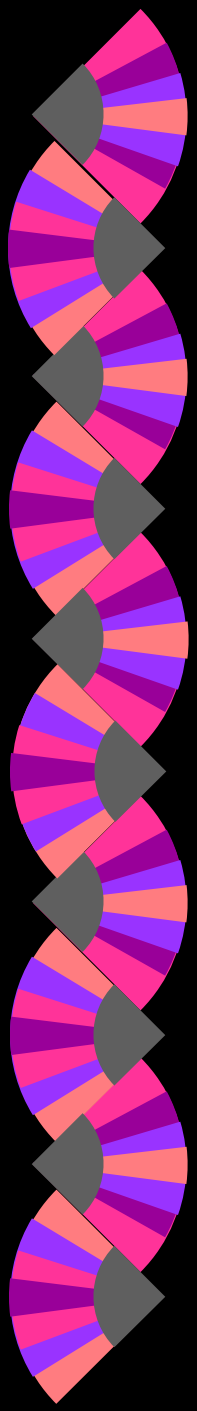
Advantages of pea plants for genetic study

- There are many varieties with distinct heritable features, or **characters** (such as flower color); character variants (such as purple or white flowers) are called **traits**
- Mating can be controlled to ensure the result's.
- Each flower has sperm-producing organs (stamens) and an egg-producing organ (carpel)
- Cross-pollination (fertilization between different plants) involves dusting one plant with pollen from another.

TECHNIQUE



RESULTS



EXPERIMENT

P Generation (true-breeding parents)

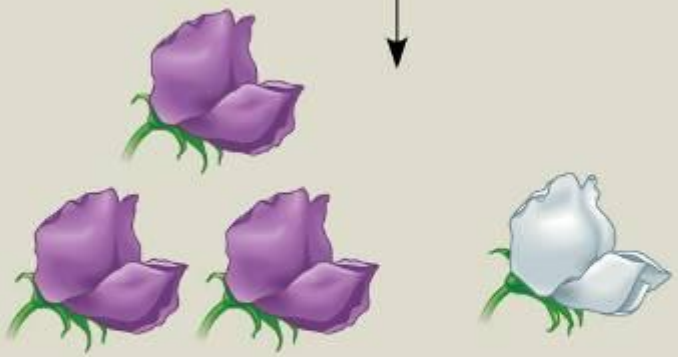
Purple flowers × White flowers

F_1 Generation (hybrids)

All plants had purple flowers

Self- or cross-pollination

F_2 Generation



705 purple-flowered plants

224 white flowered plants

The Law of Segregation

- When Mendel crossed contrasting, true-breeding white- and purple-flowered pea plants, all of the F_1 hybrids were purple
- When Mendel crossed the F_1 hybrids, many of the F_2 plants had purple flowers, but some had white
- Mendel discovered a ratio of about three to one, purple to white flowers, in the F_2 generation

Important

Why two colors in F2 generation ?

- First: alternative versions of genes account for variations in inherited characters
- For example, the gene for flower color in pea plants exists in two versions, one for purple flowers and the other for white flowers
- These alternative versions of a gene are now called **alleles**
- Each gene resides at a specific locus on a specific chromosome

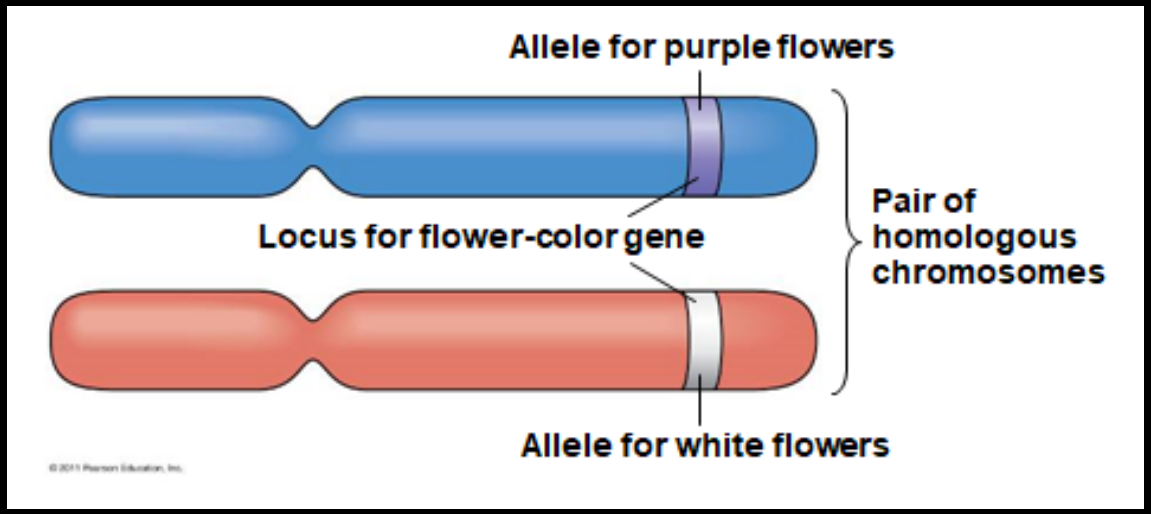
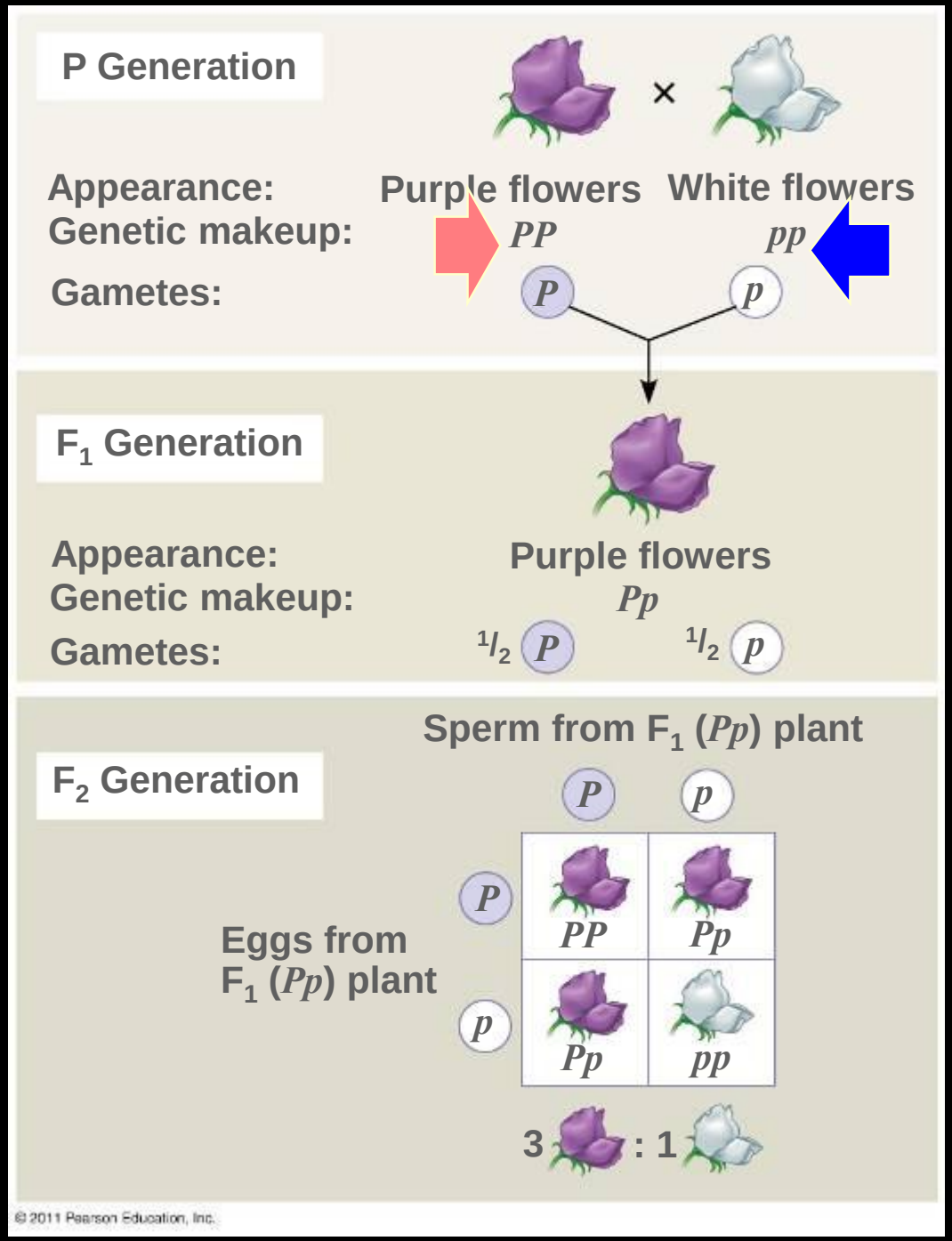


Figure 14.5-3

Lets understand again



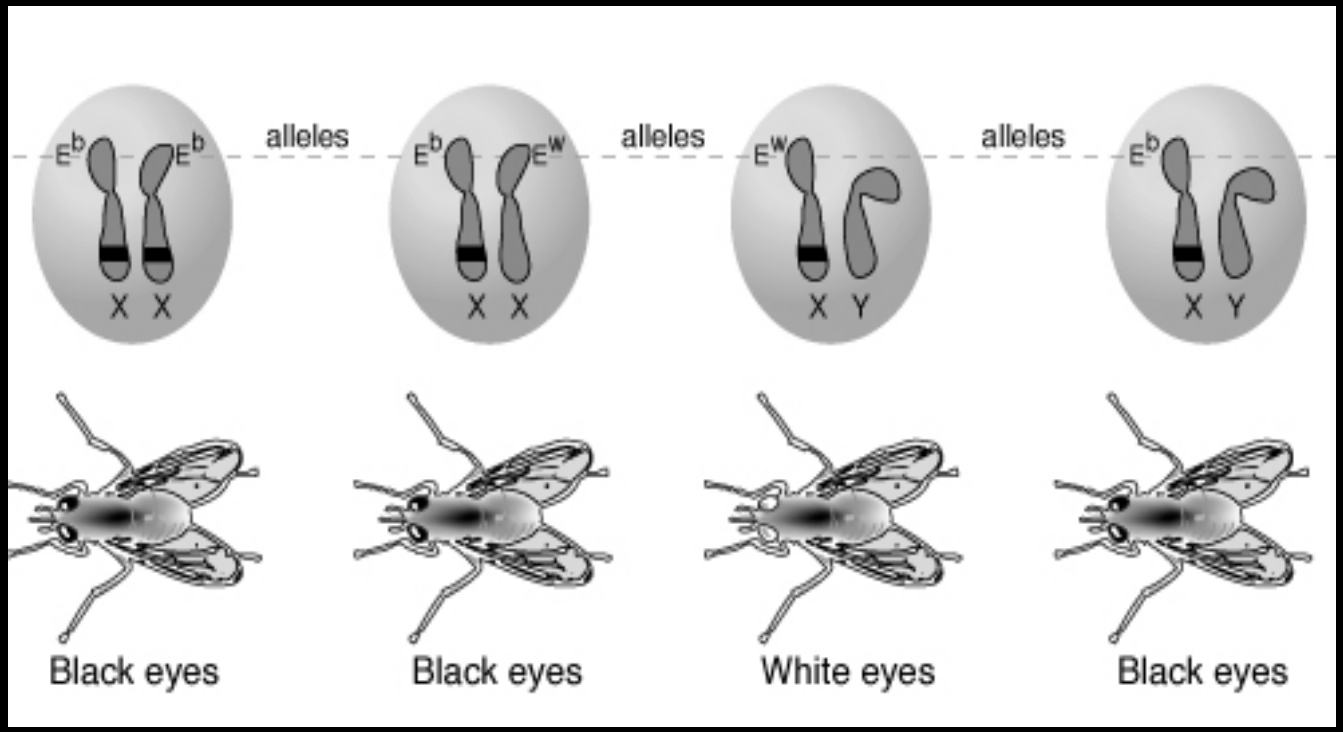
Useful Genetic Vocabulary

- An organism with two identical alleles for a character is said to be **homozygous** for the gene controlling that character
- An organism that has two different alleles for a gene is said to be **heterozygous** for the gene controlling that character
- Unlike homozygotes, heterozygotes are not true-breeding

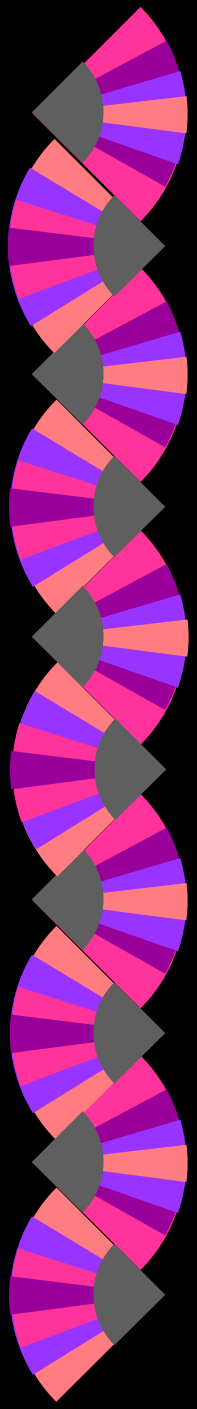
Figure 14.6

Phenotype		Genotype	
Purple		*PP* (homozygous)	1
Purple		*Pp* (heterozygous)	2
Purple		*Pp* (heterozygous)	
White		*pp* (homozygous)	1
3			
1			
Ratio 3:1		Ratio 1:2:1	

Genotypes ⟷ *Phenotypes (example)*

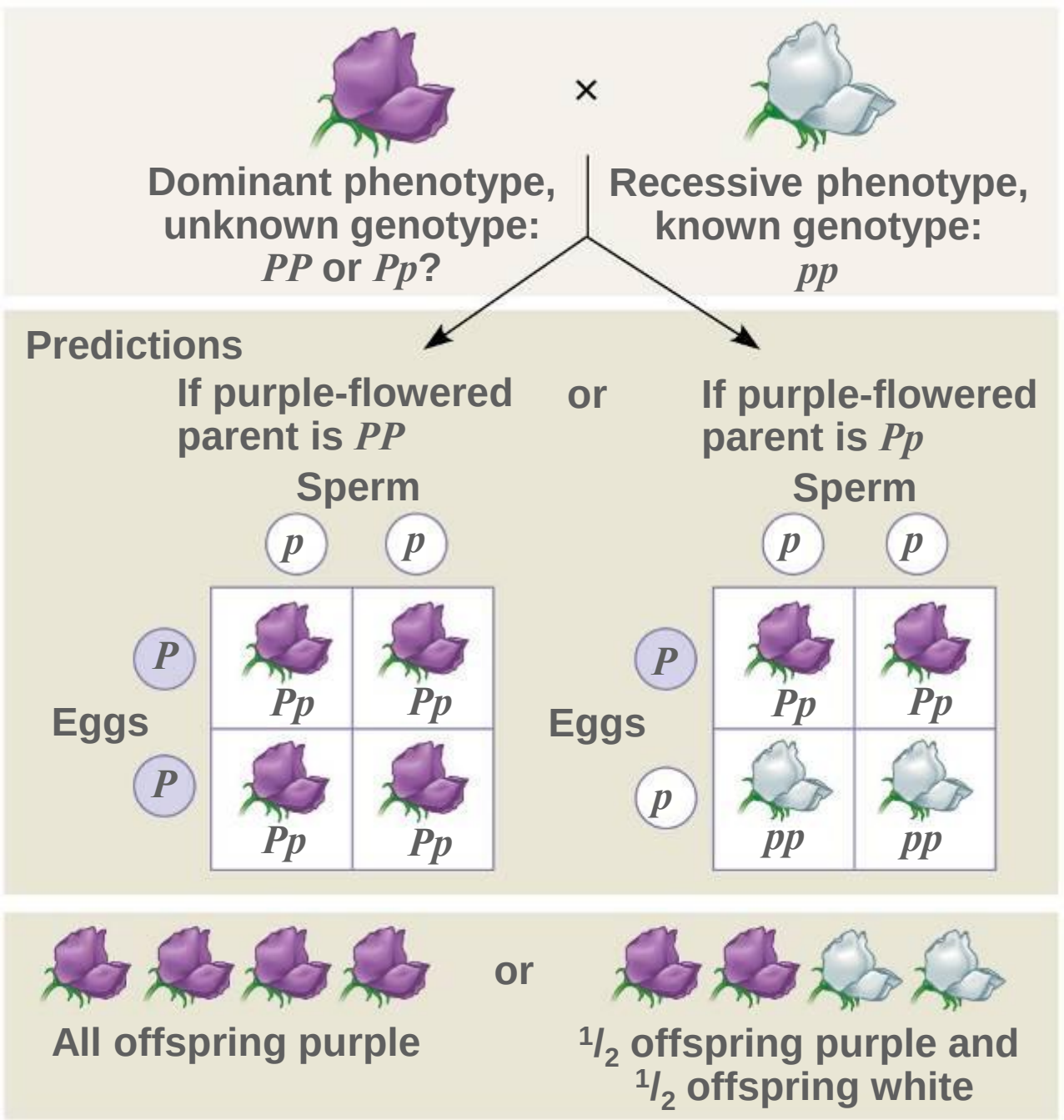


- E^b- *dominant* allele.
- E^w- *recessive* allele.

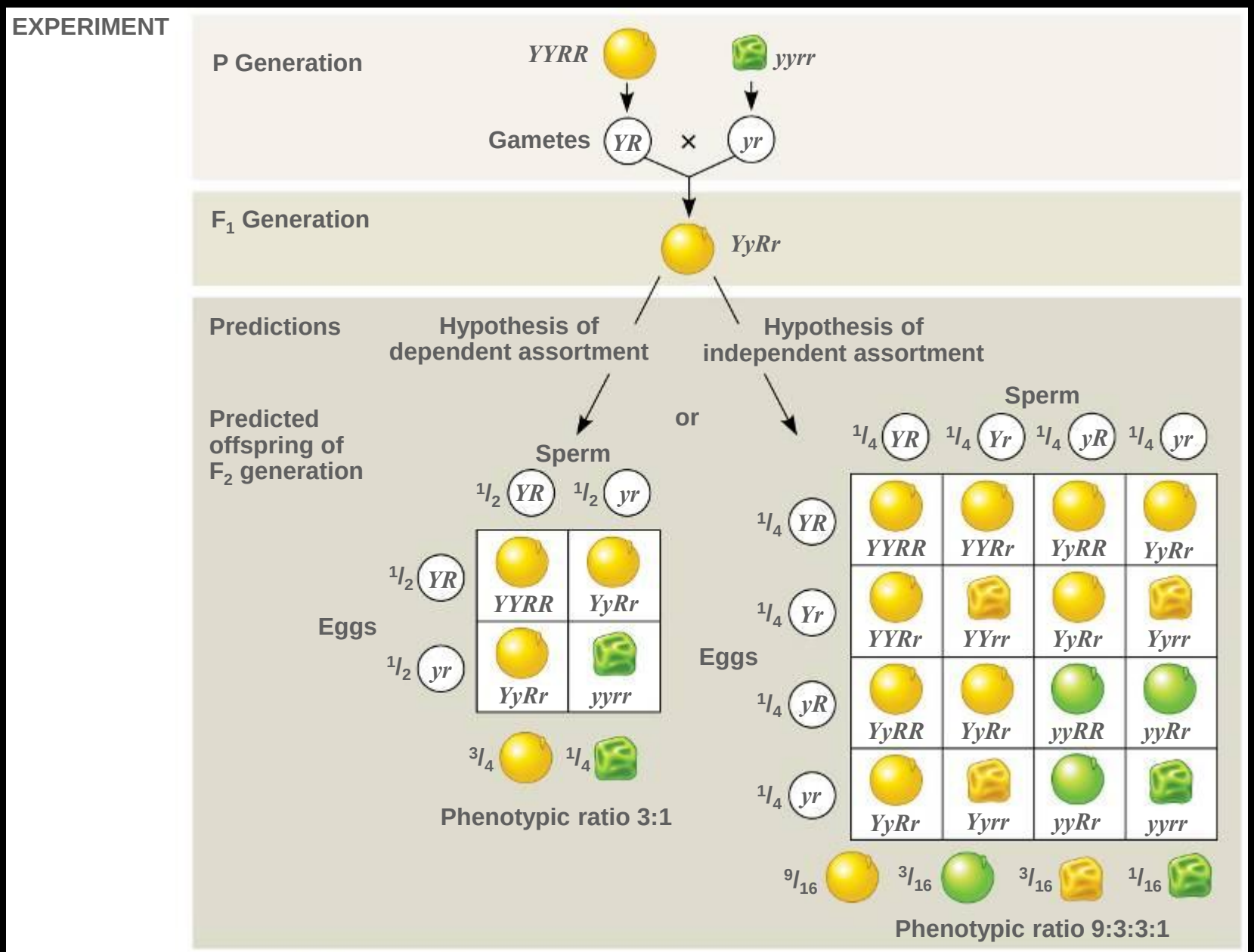


Another Experiment

TECHNIQUE



RESULTS



Important

Law	Definition
Law of segregation	During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene.
Law of independent assortment	Genes for different traits can segregate independently during the formation of gametes.
Law of dominance	Some alleles are dominant while others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele.

By that time people start knowing that there are something which carry genetic information.

What was that ????

Mendals time
1882-1894

Gene term coined
By Wilhelm Johannsen
In 1905

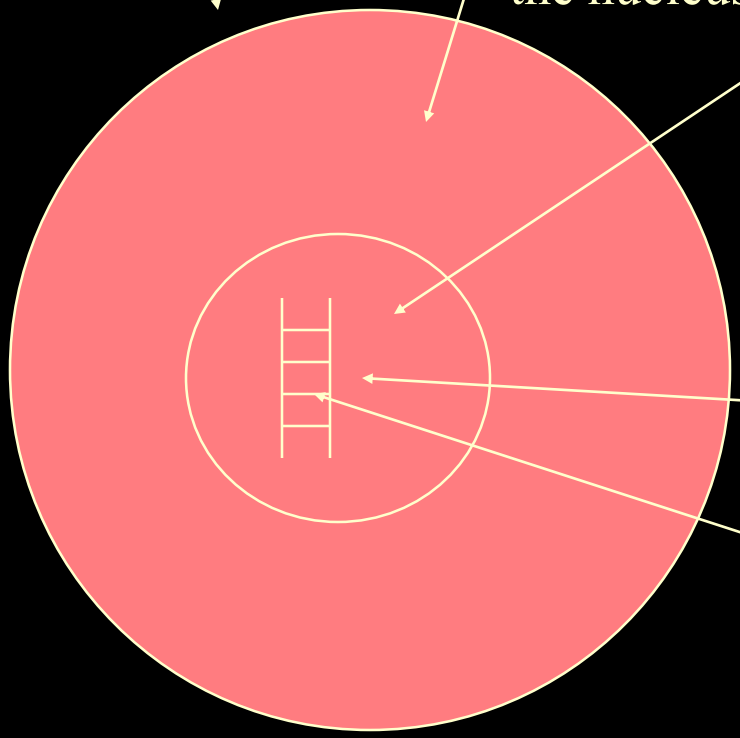
DNA molecular structure
1953

DNA was first isolated
by Friedrich Miescher in 1869

Any idea how Mendel's experiment is still in use to understand the a very important clinical condition

Where is the carrier molecule

- CELL: Basic unit of all living matter (Adult = over 10 trillion cells)



CYTOPLASM: Substance of a cell outside of the nucleus

NUCLEUS: Central point of cell / contains genetic coding for maintaining life systems and issuing commands for growth & reproduction

CHROMOSOMES:

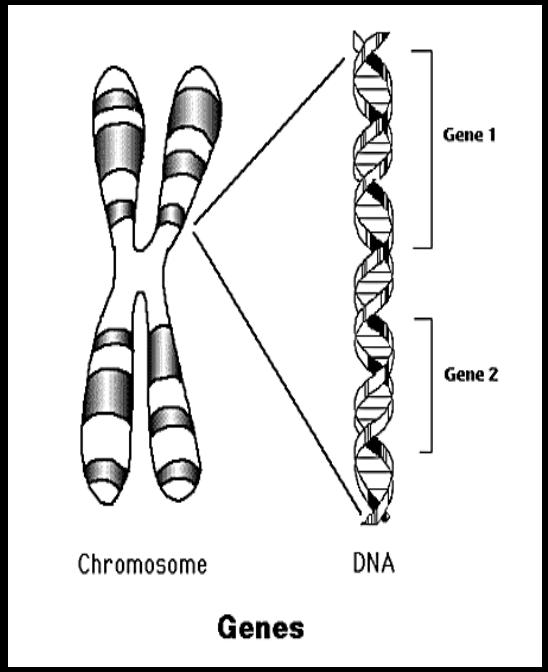
46 in each Nucleus (23 pairs)

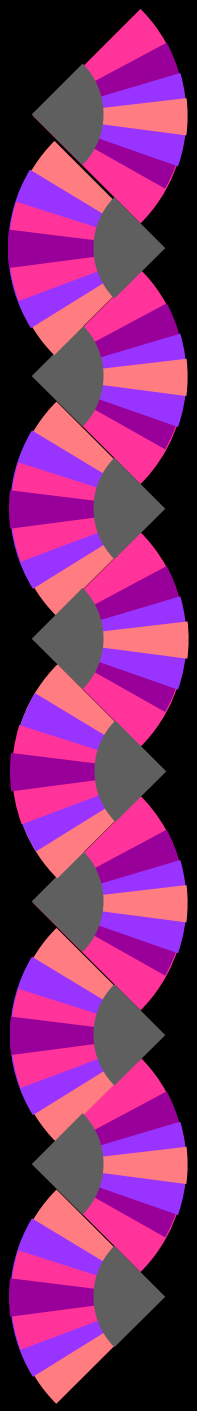
GENES: bands on chromosomes (thousands of genes)

DNA on genes (billions of DNA)

Genetic Information

- **Gene** – basic unit of genetic information. Genes determine the inherited characters.
- **Genome** – the collection of genetic information.
- **Chromosomes** – storage units of *genes*.
- **DNA** - is a nucleic acid that contains the genetic instructions specifying the biological development of all cellular forms of life

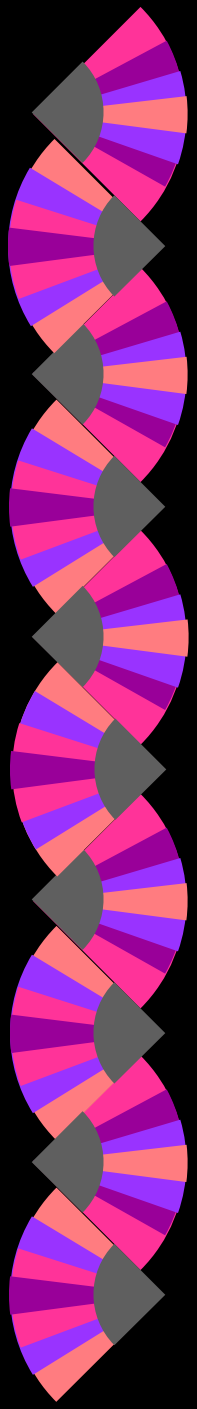




? ? ? ? ? ? ? ?

- How many chromosomes are there in each cell?
- 46 CHROMOSOMES or 23 PAIRS

- How many chromosomes are in Reproductive (egg & sperm) or Germ cells?
- 23 CHROMOSOMES
  - (combined = the 46 chromosomes)



CELL DIVISION

- MITOSIS: Cell divides by copying the DNA - cell splits - new cell with normal number of chromosomes (Cell growth & repair)

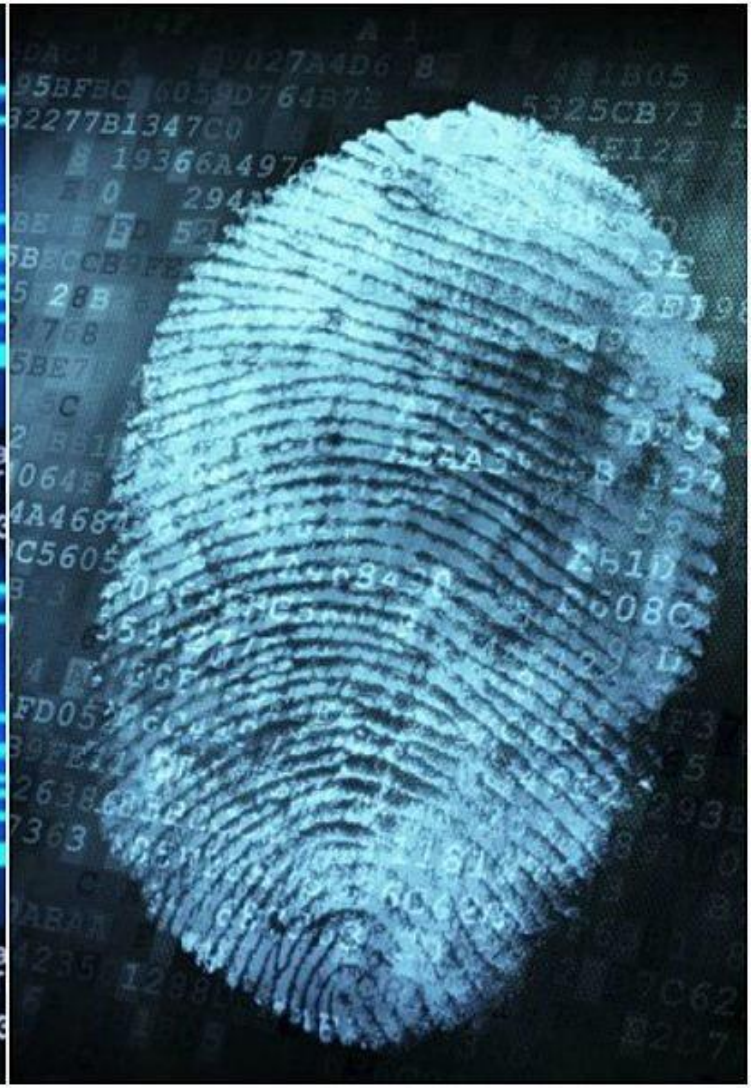
- MEIOSIS: Creates 1/2 sets of chromosomes
  - Women = 23 Men = 23 Combined = 46

- Female Sex Cells XX (Ovum or Egg)
- Male Sex Cells XY (Sperm)
- Baby Girl = XX Baby Boy = XY
  - Conception is the union of an OVUM and the SPERM

- DOMINATE Gene: More powerful - trait seen in person
- RECESSIVE Gene: Weaker and hides in the background. Trait can only determine when two of them are present - may show up in future generations.
- CARRIER: Has a recessive gene that is not visible
- SEX-LINKED: Mother passes the recessive X to son
  - Color-blind male receives the trait from his mother.
  - The mother is usually not color-blind herself.

- **B = BROWN eyes (dominate)** **b = BLUE eyes (recessive)**
- BB = BROWN eyes
- bb = BLUE eyes
- Bb = BROWN eyes but carry the recessive BLUE eye gene

Genetics
DNA Finger printing



Sir Alec John Jeffreys

Father of DNA finger printing
Oxford
1950-
10 Sep 1984
EU and American Patent

Father of DNA finger printing in India
Born Jaunpur, UP.
Died Varanasi, UP.

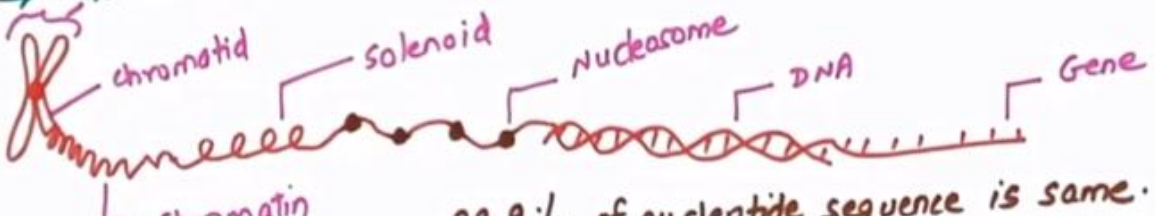
Conceptualized by
Prof. Lalji Singh

- DNA finger Printing:-

:- also called as DNA profiling or DNA Restriction Analysis.

:- Every individual has a unique genetic make up - DNA fingerprint.

→ Chromosome - chromatid - chromatin material - Solenoid fibre - Nucleosome - DNA - Gene.



DNA Fingerprinting

- technique is based on identification of Nucleotide sequence.

Nucleotide sequence
- 99.9% of nucleotide sequence is same.
- 0.1% of nucleotide sequence is different. → VNTR [variable number of Tandem Repeats]

- VNTR's → repeated several times [20-100 bp]
  - first studied by A. Wyman & R. White (1980)
  - university of utah.

* Probability of 2 individuals having same set of VNTR is 1in 300 million.

- This (VNTR) is our unique identity. → This VNTR is used in DNA fingerprinting.

STEPS →
1. DNA ISOLATION
2. DNA AMPLIFICATION
3. DNA FRAGMENTATION
4. GEL ELECTROPHORESIS
5. SOUTHERN BLOTTING
6. HYBRIDIZATION
7. PHOTOGRAPHY & DOCUMENTATION.


# of Repeats = VNTR Weight ÷ Sequence Weight
GTCAACGTAGCT
079200 DALTONS

STEPS OF DNA FINGERPRINTING

STEP:-1 DNA ISOLATION

CRIME SPOT

search sample from which DNA can be isolated

- Hair (Root Hair)
- Blood (WBC)
- Tissue
- Semen

Any one of the sample is obtained.
→ DNA is isolated.
→ DNA amount will be very less.

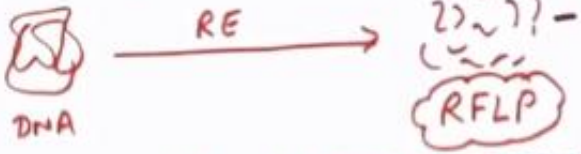
STEP:- 2 DNA AMPLIFICATION

DNA sample obtained is amplified. → In vitro (Lab.) → PCR Technology.
[Polymerase chain Reaction]

- multiple copies are generated in short time.

STEP:- 3 DNA FRAGMENTATION.

DNA sample is subjected to RE [Restriction Endonuclease] ∴ Double stranded DNA is broken in many fragments.

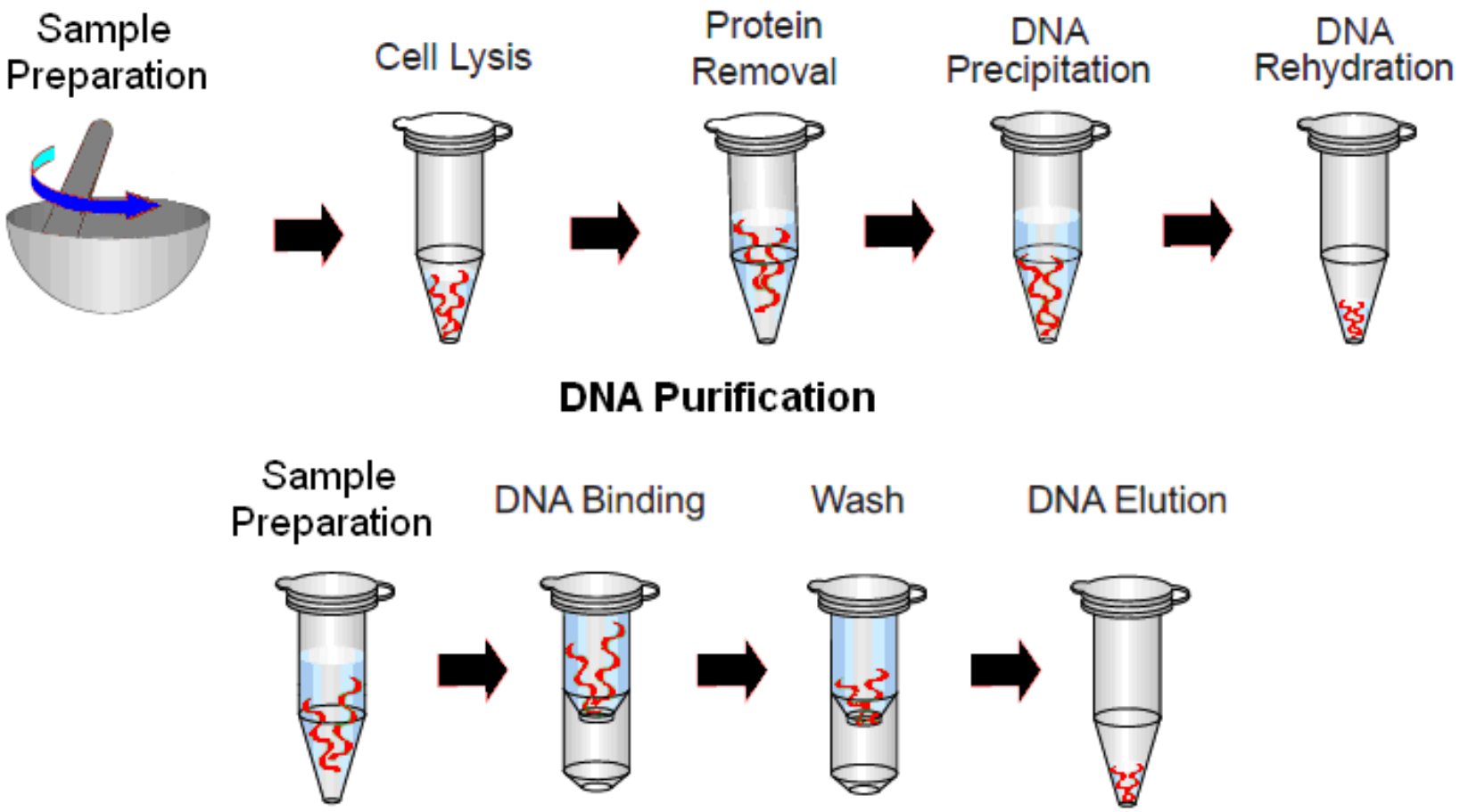


- Restriction fragment length polymorphism.
- DNA will be of different length.

STEP:- 4 GEL ELECTROPHORESIS [Electro - current, Phoresis - movement]

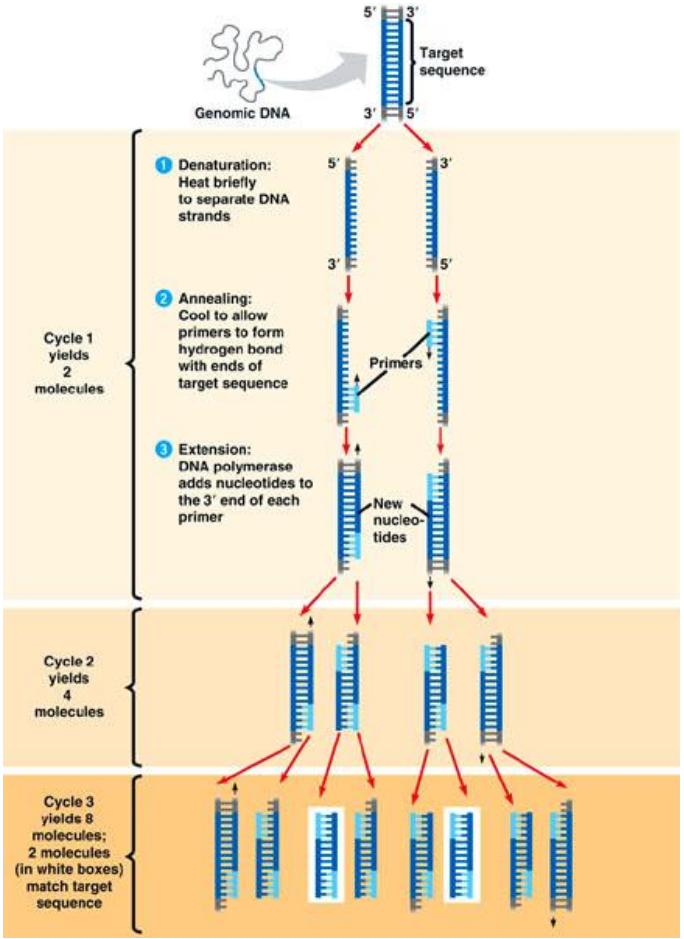
- DNA fragments are seperated based on their size using Agarose Gel.
- Charge of DNA :- -ve charge [PO_4^{3-}] ∴ DNA is added at -ve end. current flows from -ve to +ve.

DNA Isolation

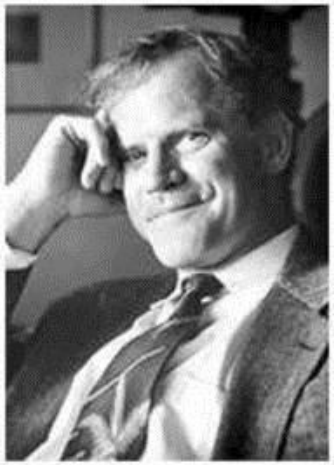


PCR

What's the need ?????

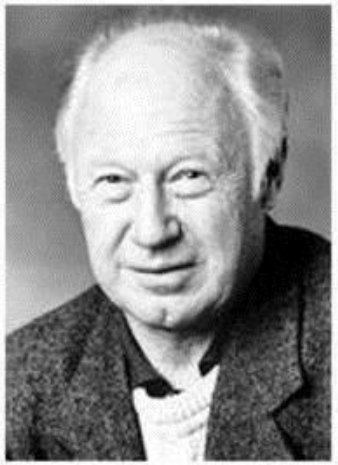


The Nobel Prize in Chemistry 1993



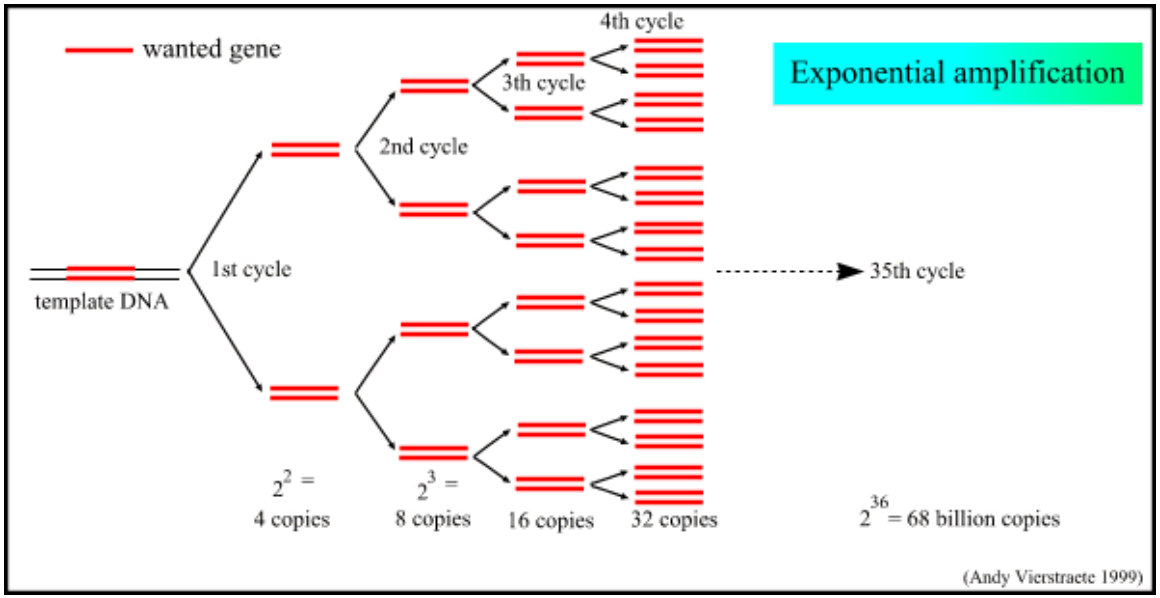
Kary B. Mullis

Prize share: 1/2

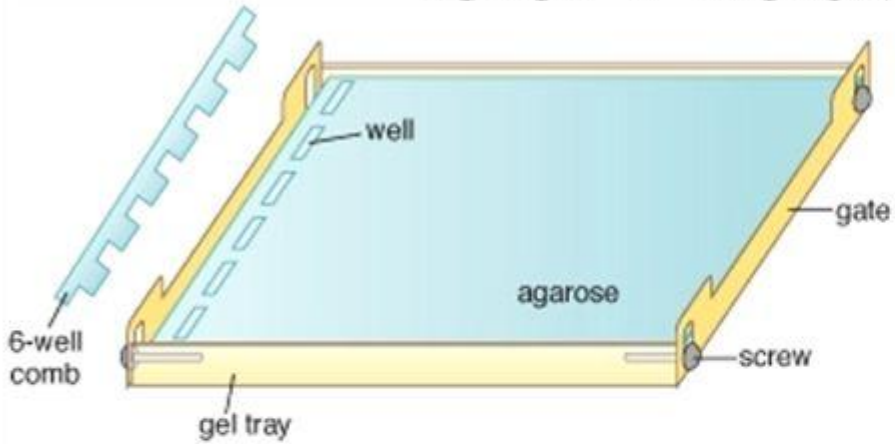


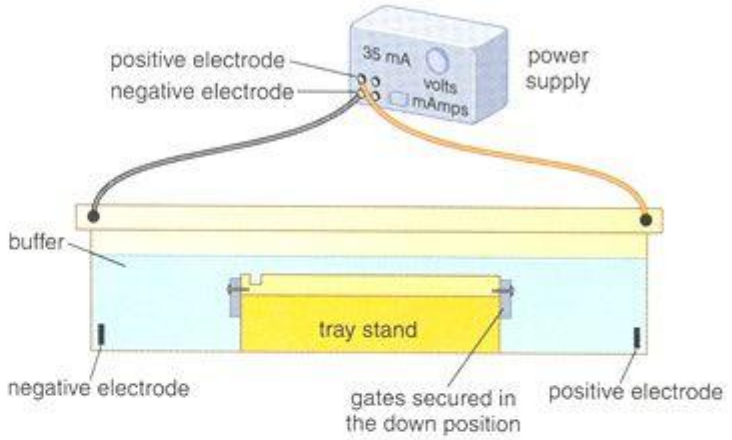
Michael Smith

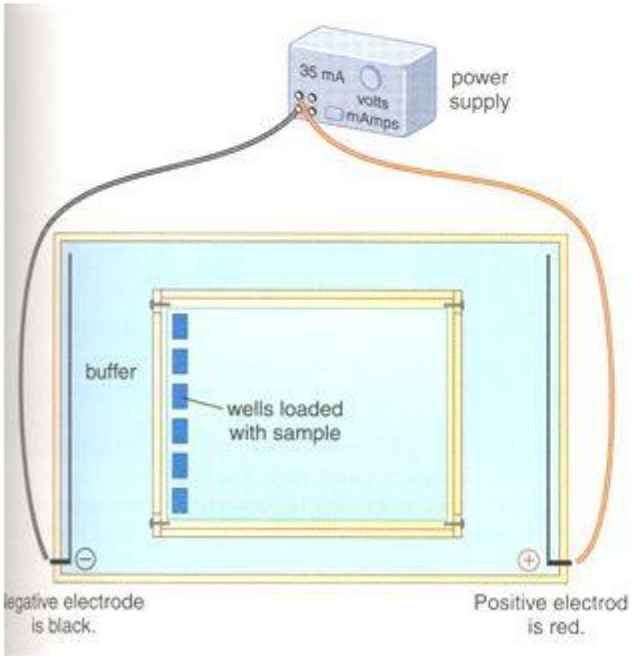
Prize share: 1/2

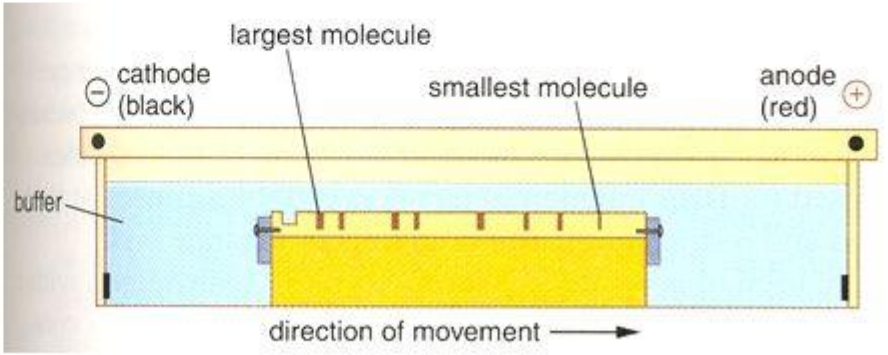


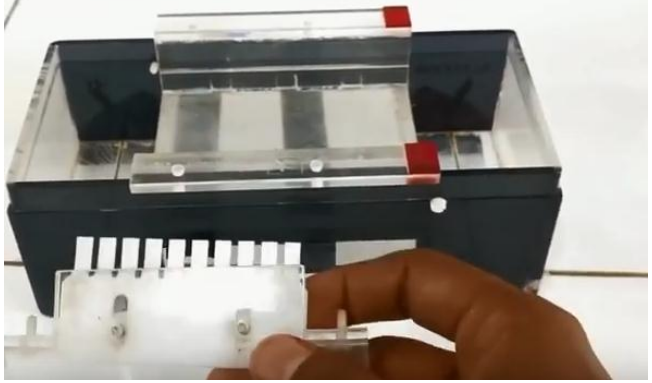
Gel Electrophoresis

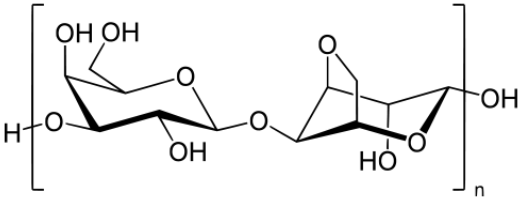


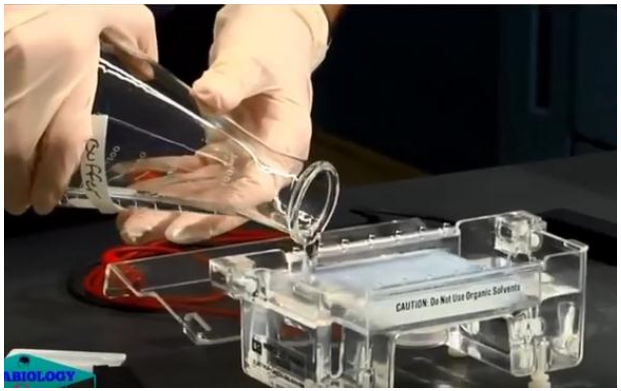






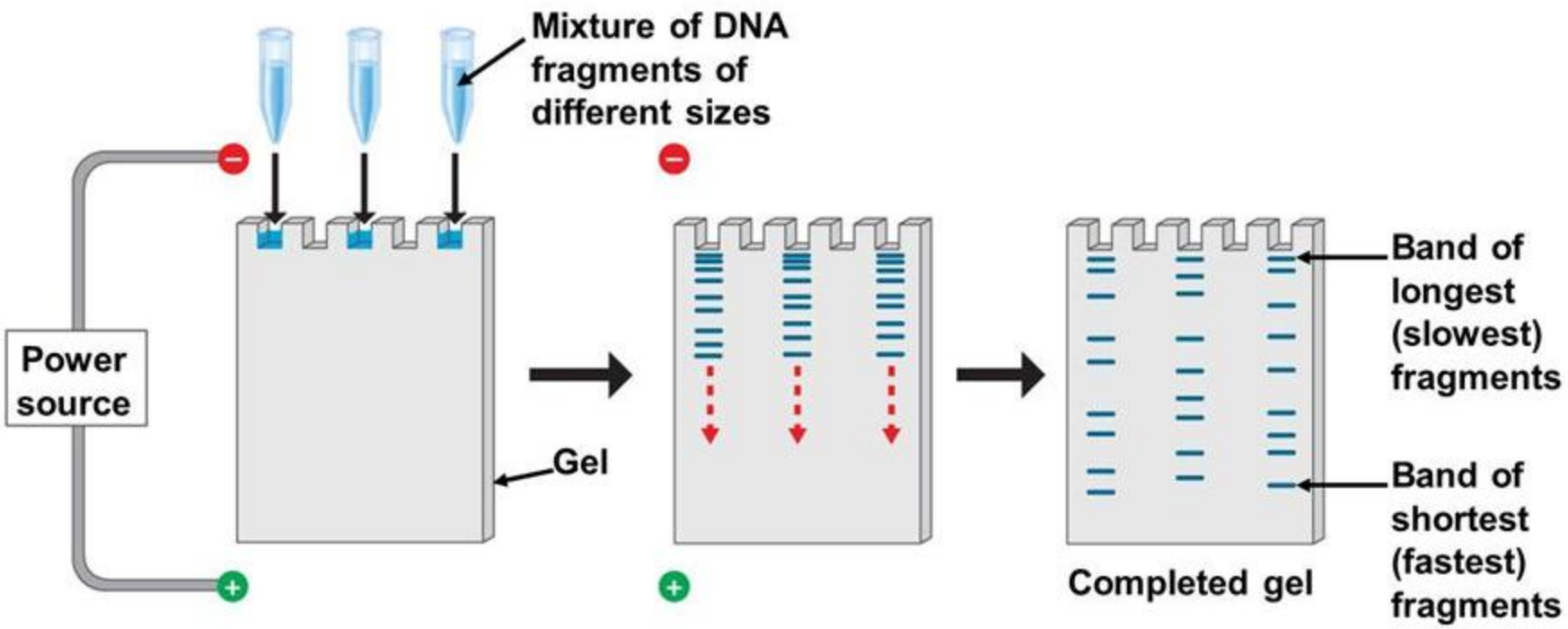






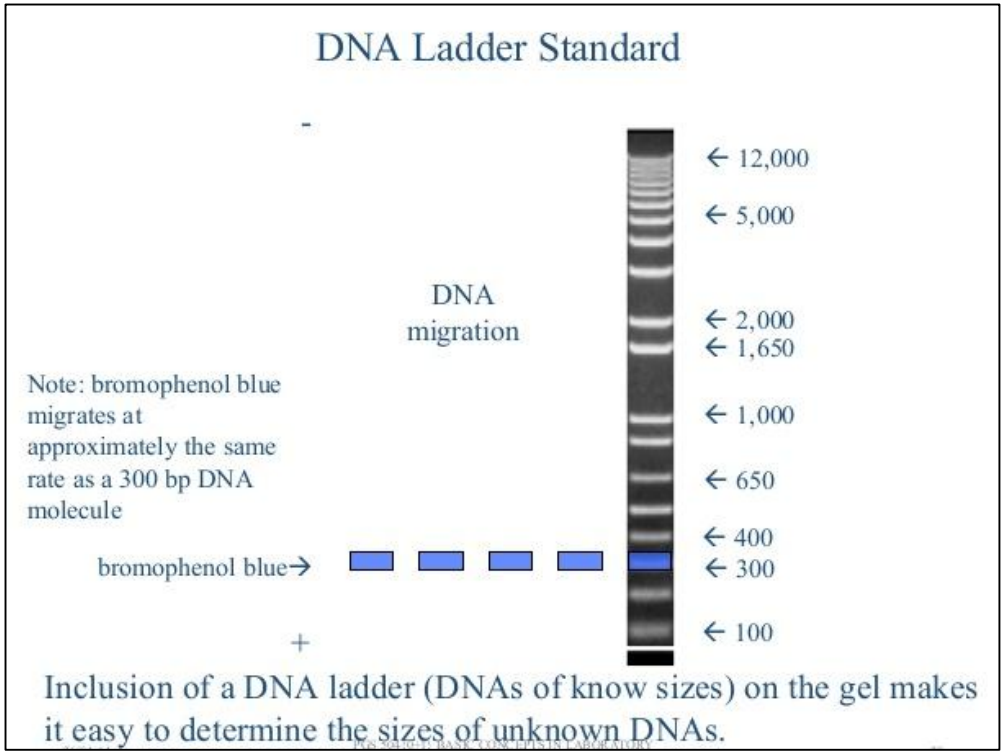
What's the importance of GEL and how it controls the movement of DNA molecules ??

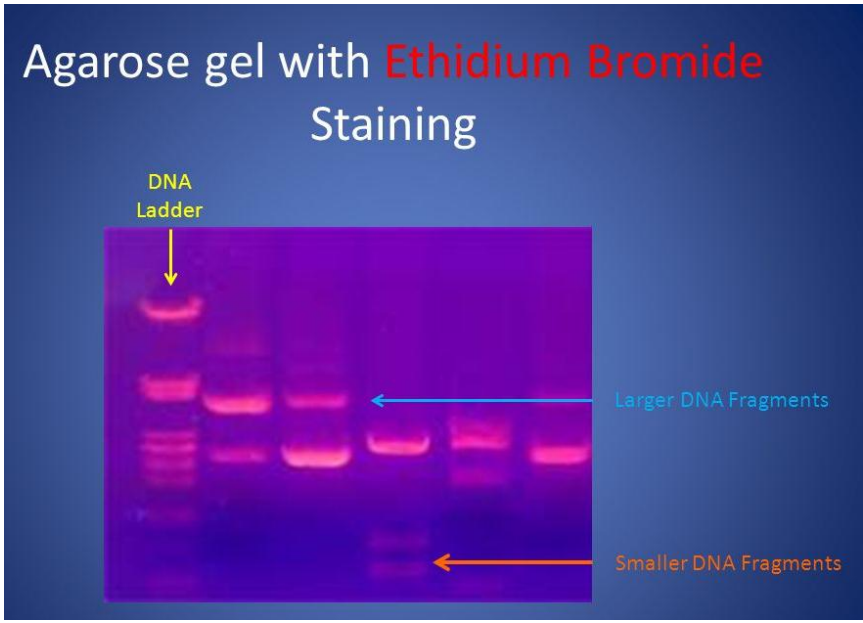
Gel Electrophoresis of DNA



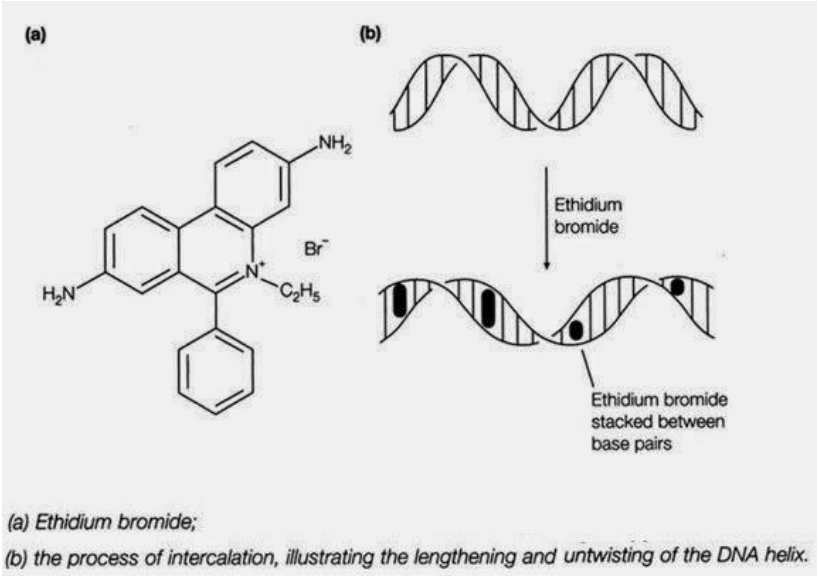
How to know the migration ???

How to visualized DNA ???





Fluorescent tag (nucleic acid *stain*)



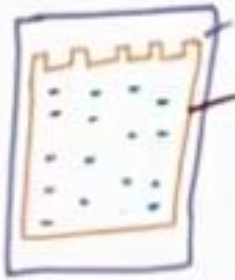
(a) Ethidium bromide;
(b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

Things to remember:

- Gel is made up of Agarose,
- Chemically it is agarobiose, which is a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose
- The current used in the separation process is DC.
- What is the importance of GEL ?

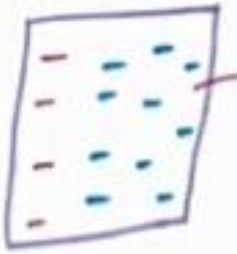
STEP 5 :- SOUTHERN BLOTTING

- DNA fragments obtained on gel is blotted on NYLON MEMBRANE (OR) NITROCELLULOSE PAPER.
- All gel are fragile & weak.
  ∴ Southern blotting is done.
  DNA has more affinity for Nylon or Nitrocellulose paper.



→ Some weight & paper is kept on top

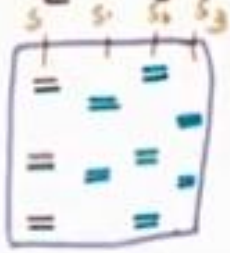
Southern Blotting →



STEP 6 :- HYBRIDIZATION.

- For Hybridization - Radioactive DNA probe is prepared.
- Band is flooded with Probe [single stranded DNA ⟶ ds DNA]
- Excess probe is washed off.

⟶



→ sample matches with Suspect 3.

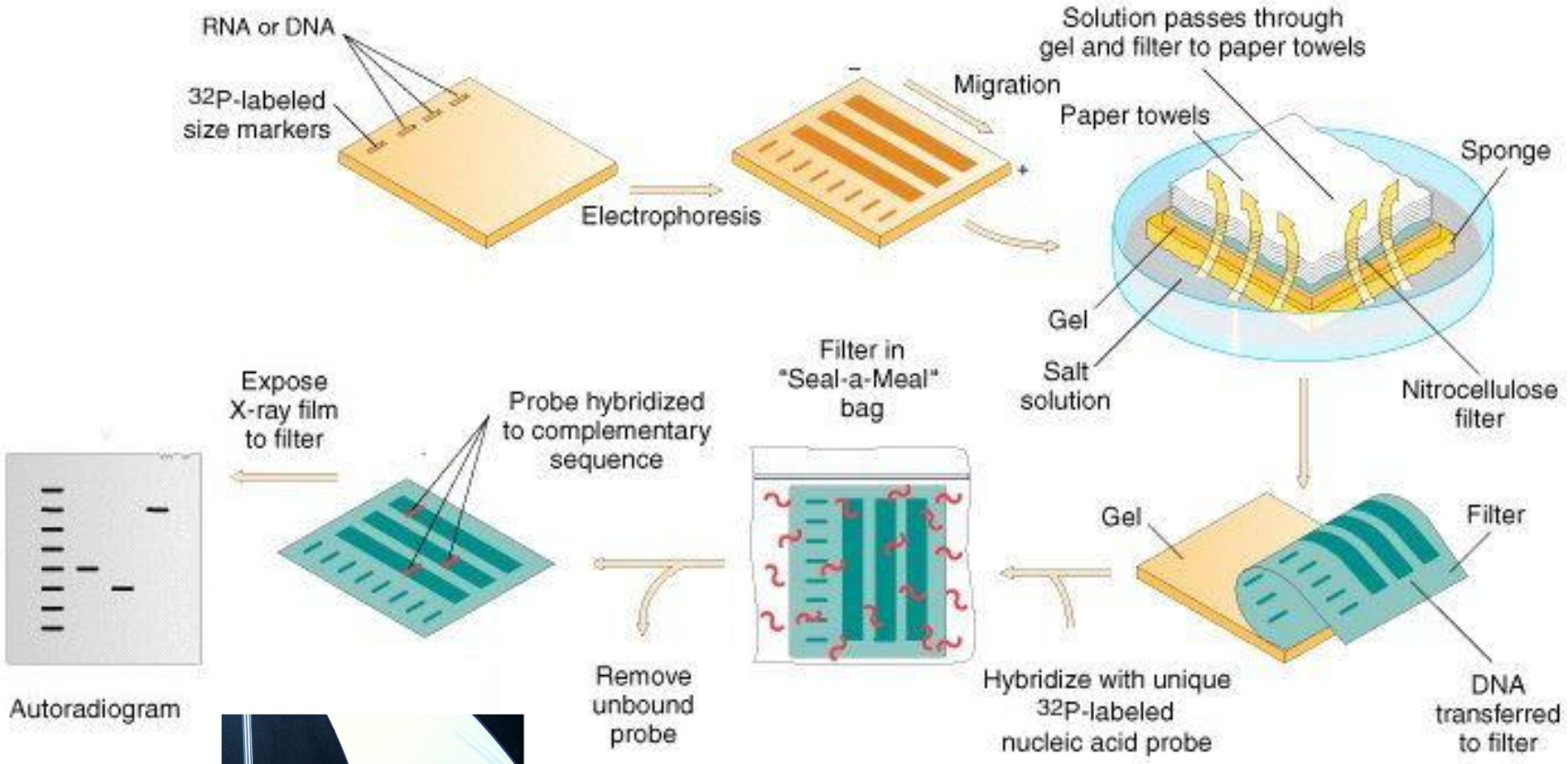
STEP 7 : PHOTOGRAPHY

- Nylon membrane is kept on X-ray film.

→ DNA band due to radioactive probe gives photographic image on X-ray film.

* Documentation is done.

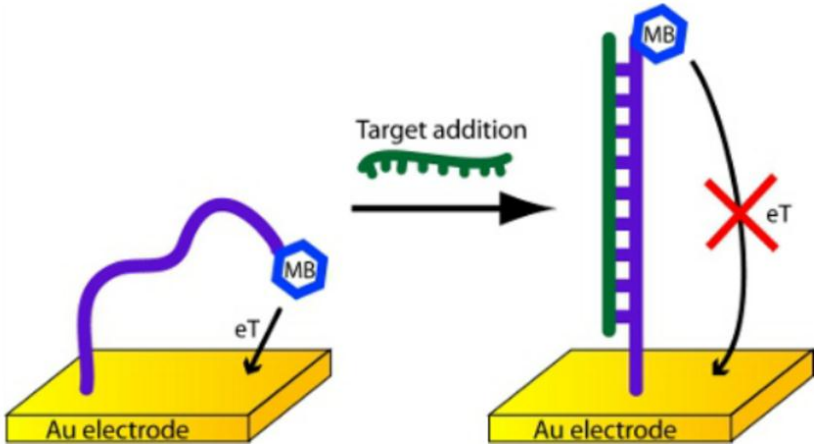
Sum-up the procedure

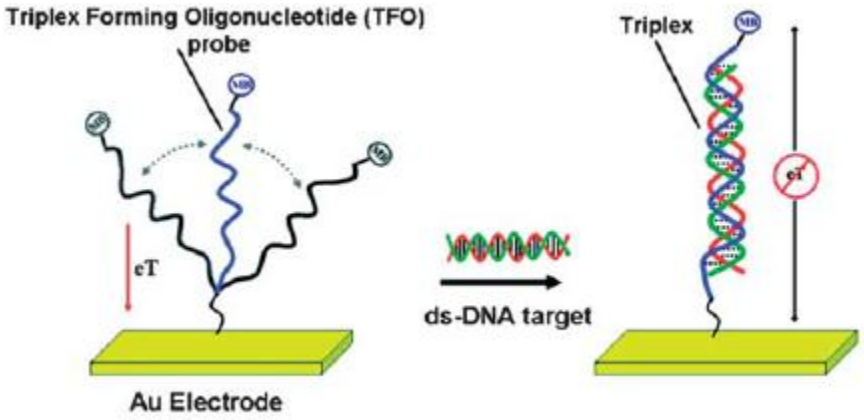


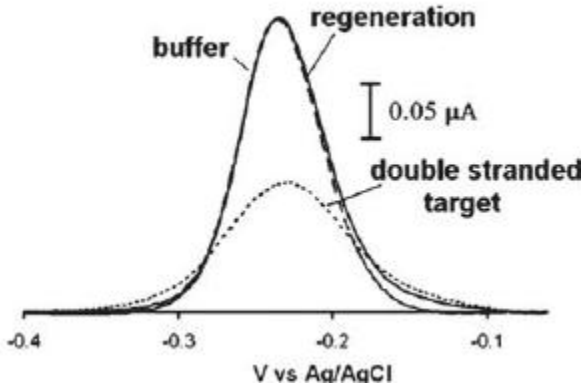
Ed Southern, Oxford University

E. Southern, "Detection of specific sequences among DNA fragments separated by gel-electrophoresis *J Mol Biol*, 98:503, 1975.

The most recent technology based for Gene detection







Where are in the course.

Genetics, **DNA as genetic material**; structure of DNA; gene expression and regulation; recombinant DNA technology.

For Quiz 1: Lecture 1 to 5 course content.