FAST and PARTIAL FLIGHT OVER GENETICS

First part
Peculiarity of the genetic approach
Relationship between genotype and phenotype

Second part
DNA as the genetic material
DNA structure and function

Third part
Genetic markers and their use
Genetic maps and their use

SOME IMPORTANT DEFINITIONS - 1

Genetics: the study of biological inheritance

Genotype: the genetic constitution of a CELL or ORGANISM. It can refer to single or multiple genes

Phenotype: the observable characteristics of a CELL or ORGANISM including the result of tests and/or measurements

Gene:
1. an hereditary unit
2. a functional DNA unit
3. a factor that controls a phenotype and segregates in pedigrees

Alleles: Alternative forms of the same gene

Genome: Athe whole DNA content, i.e. the genetic information

SOME IMPORTANT DEFINITIONS – 2

Mutation: a HERITABLE alteration of a gene or chromosome or the genome

Mutant: 1. a biological entity with a change due to a mutation
2. a "changed" gene

Segregation: the distribution of allelic sequences between daughter cells at meiosis

Recombinant: a gamete that contains a combination of allelles that is different from the combination inherited by parents

GENOTYPE- PHENOTYPE RELATIONSHIP: STILL THE MAIN QUESTION

To understand the nature of genetic variation, which is at the basis of evolution

DIFFERENT SCHOOLS

1. DARWINIAN SCHOOL

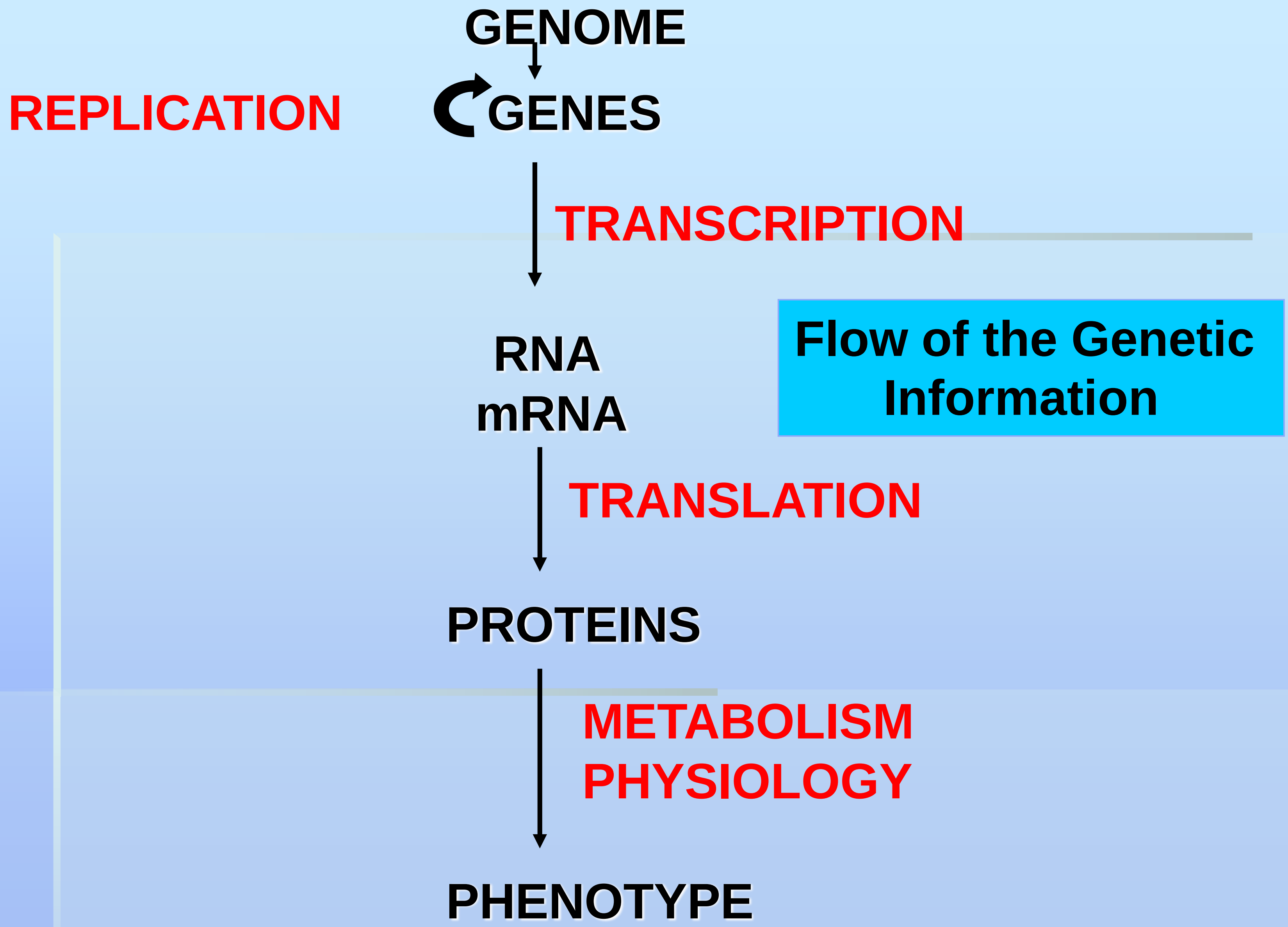
Approach: Olistic

Methods: Biometry and Quantitative Genetics

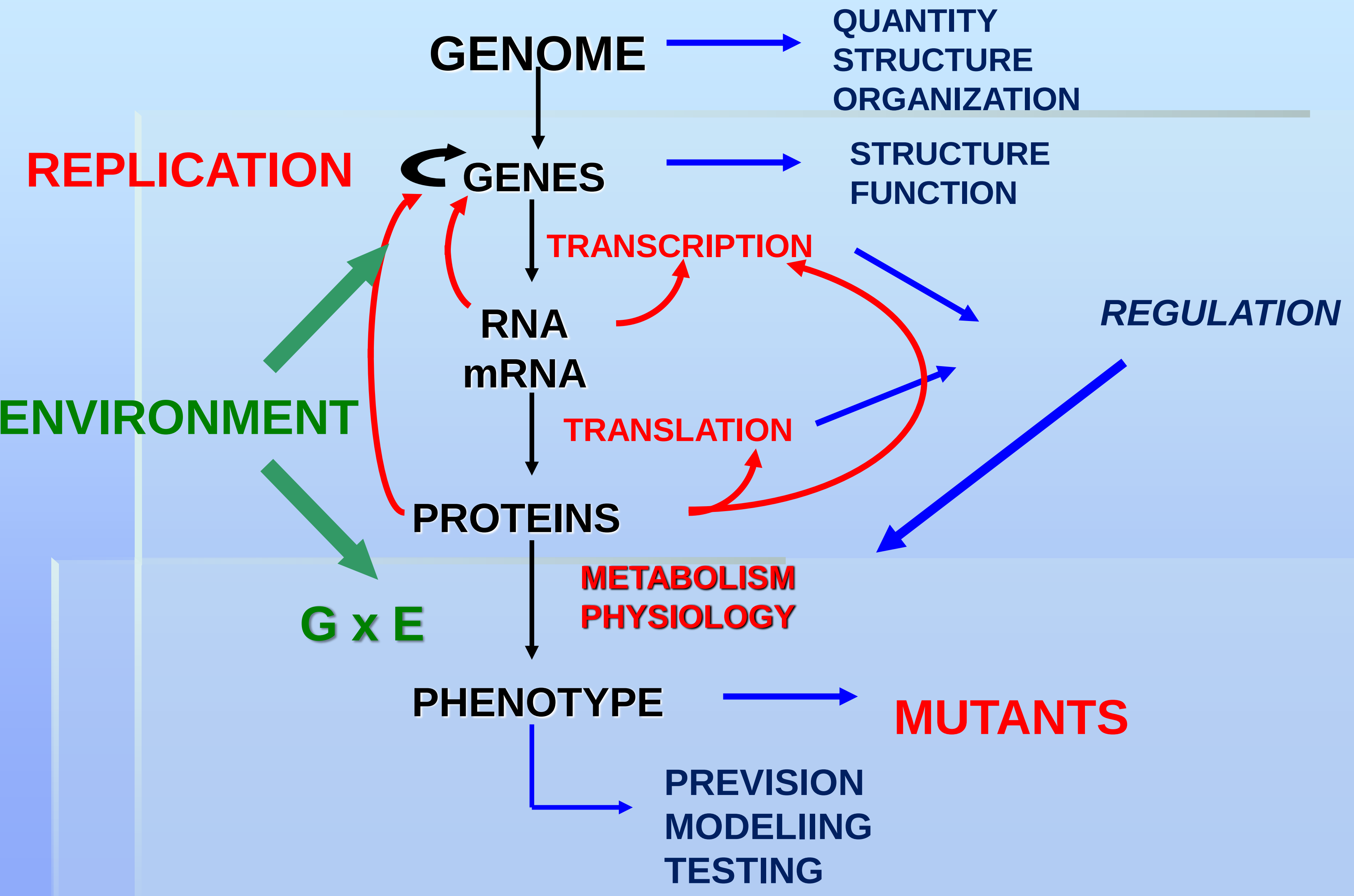
2. MENDELIAN SCHOOL

Approach: Reductionist

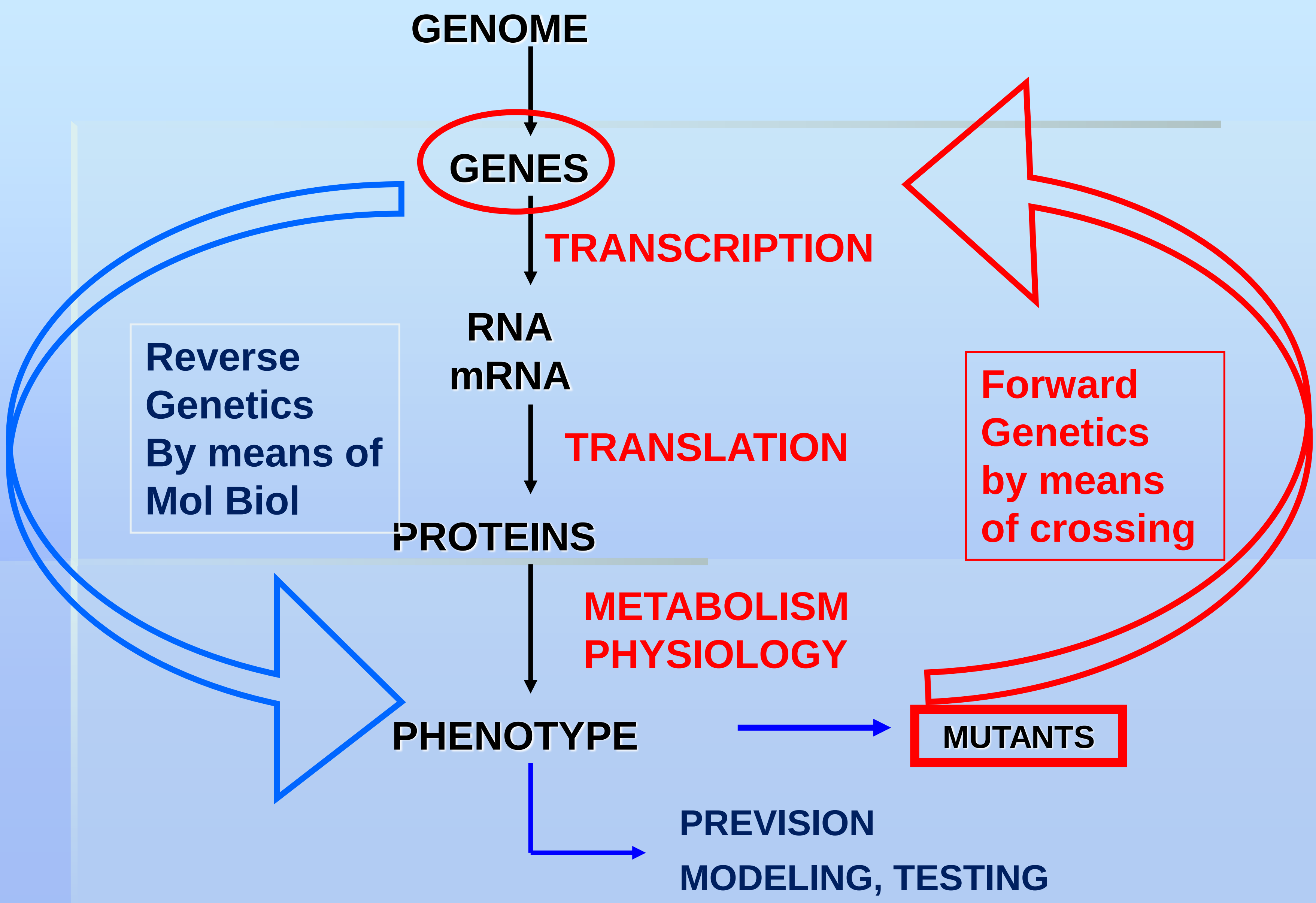
Methods: Mendelian Genetics, Molecular Genetics


GENOME
REPLICATION
GENES
TRANSCRIPTION
RNA
mRNA
Flow of the Genetic Information
TRANSLATION
PROTEINS
METABOLISM
PHYSIOLOGY
PHENOTYPE

The Flow of the Genetic Information



Genetic Approaches



Genetic Variability: NO genetic analysis without it

- Genetics addresses those differences among individuals of a species (Sturtevant & Beadle 1939)
- Variability among individuals is crucial for a species to adapt to the environment and evolve, either by selection and / or by random drift
- Selection (natural and artificial) works on PHENOTYPIC differences

MUTATIONS

1. Source of new genetic variability
2. Cornerstone of genetic analysis
3. Studying aberrant phenotypes may lead to the discovery of wild type function of a gene

MUTATIONS: the souce of genetic variation

Mutations can be classified in three main types

1. **Genome mutations**

    Changes in chromosome number

2. **Chromosome mutations**

    Changes in chromosome structure

3. **Single-gene mutations**

    Relatively small changes in DNA structure that occur within a particular gene

Mutations, Alleles and the Concept of Polymorphisms

HOW MANY POSSIBLE ALLELES FOR A GENE?

- **Mutations in the same gene can produce different alleles**
- **Several alleles for a single gene**
- **Polymorphism derives from mutations which spread in the population**
- **By definition a polymorphic allele > 1% in the population**

Multi-allelism is a population genetics concept

Types of Mutations

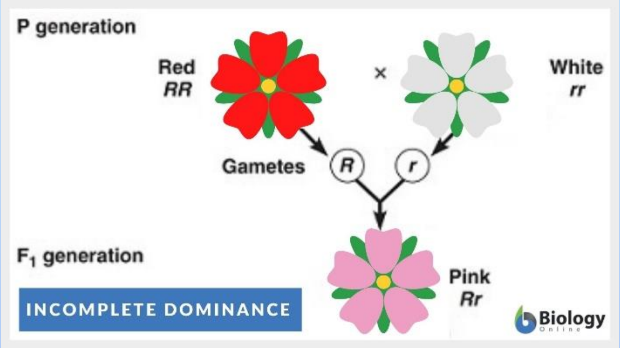
les1 Lesion Mimic

RECESSIVE

m1/m1 display a mutant phenotype BUT M1/m1 are wildtype

DOMINANT

M2/m1 display a mutant phenotype BUT m2/m2 are wildtype



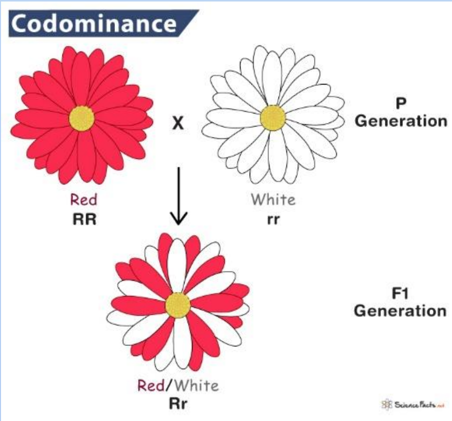
SEMIDOMINANT

Based on severity of the phenotypic change

M3/M3 > M3/m3 > m3/m3

CO-DOMINANT

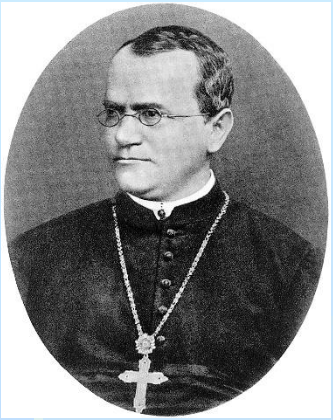
The both alleles deternine the phenotype /bllod types). Very useful in genetic analysis



VERY SIMPLE CLASSIFICATION BUT NOT ALWAYS ADEQUATE

Mutations and Phenotypic Effects

- **Not all mutations lead to phenotypic changes**
  the majority are SILENT but still provide genetic variation
- **Only those which cause phenotypic effects provide phenotypic variability on which selection can act**
- **Most mutations produce negative phenotypic effects**
- **Linking mutation, i.e. genetic variation, to phenotypic effects IS a major problem**



1822 - 1884
Metereologist, mathematician and botanist

- THE USE OF MUTATIONS WITH PHENOTYPIC EFFECTS

Gregor Mendel: Augustinian friar

- Lived in what was the Austro-Hungarian Empire (now Czech Rep.)
- Adopted a mathematical approach to the study of hybridization
- Defined the concept of “factors” transmitted from one generation to the next according to specific laws
- STILL OPEN THE QUESTION OF THE PHYSICAL ASPECTS OF HEREDITY

Physical basis of Inheritance: The Birth of the Chromosome Theory of Inheritance

Walter Sutton
1877 – 1916
American
Geneticist and
Physician

Theodor Boveri
1862 – 1915
German
Zoologist

- **Around 1900, cytologists and biologists began to see parallels between the behavior of chromosomes and the behavior of Mendel's factors.**
  - **Chromosomes and genes (genetic factors) are both present in pairs in diploid cells**
  - **Homologous chromosomes (diploidy) separate and alleles segregate during meiosis**
  - **Fertilization restores the paired condition for both chromosomes and genes**

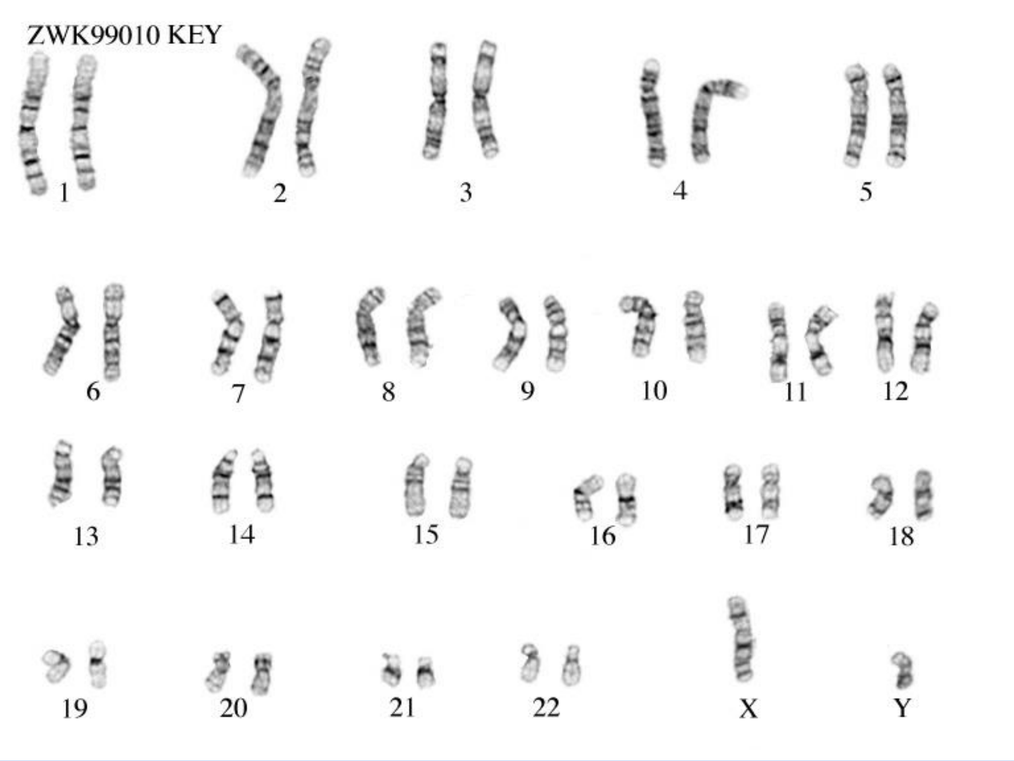
BIOLOGY REMINDERS BOX: chromosomes, kariotypes and meiosis

Karyotype of Eukaryotic Cells: the first description of genomes

In each diploid eukaryotic cell there are two HOMOLOGOUS chromosomes for each type of chromosome, *e.g.* in humans 23 pairs = 46 chromosome

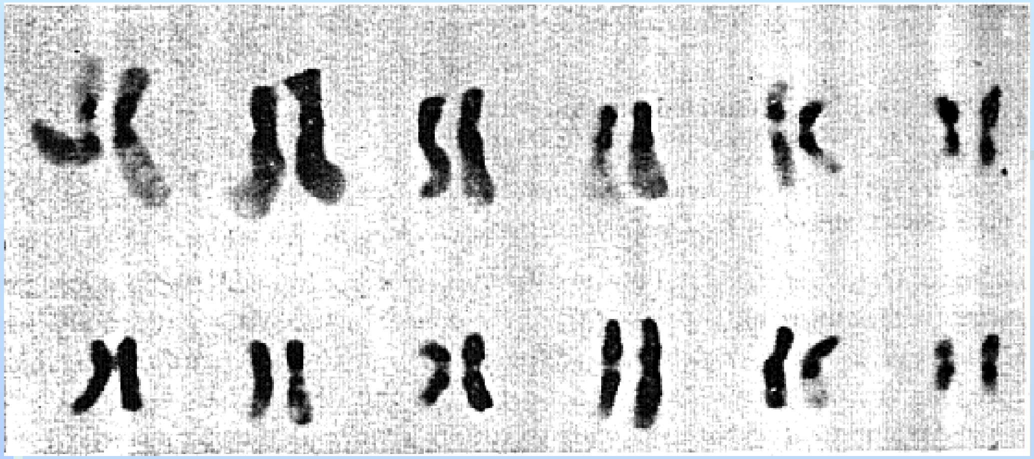
Chromosome content in a human diploid cell is 2n = 46

Also 2x, where x = number of pairs of homologue

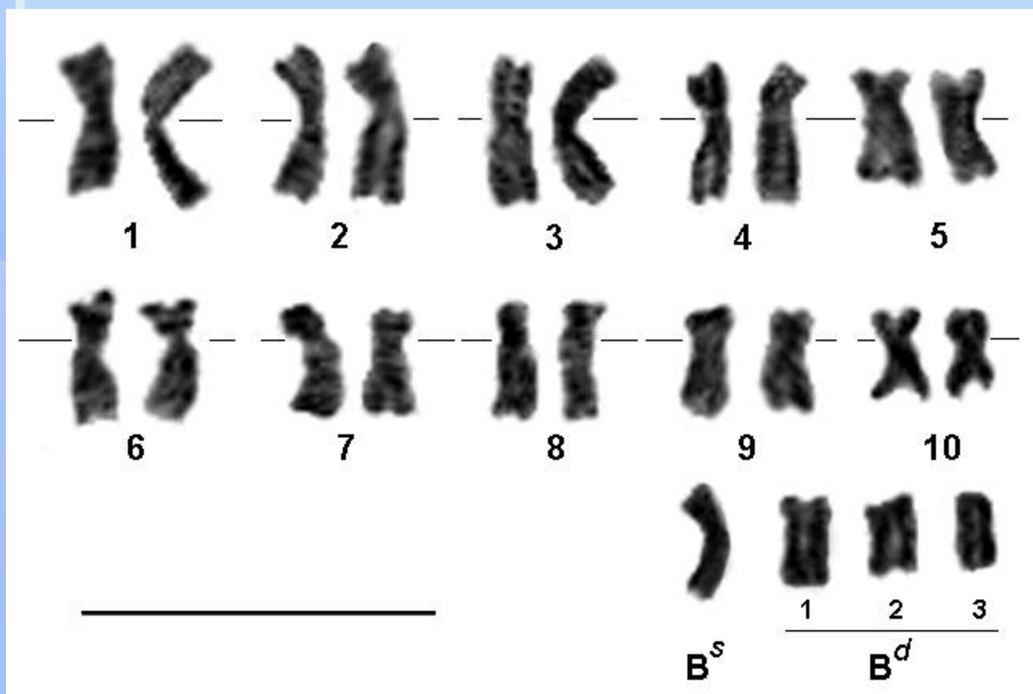


Human karyotype

Plant Karyotypes



Rice karyotype

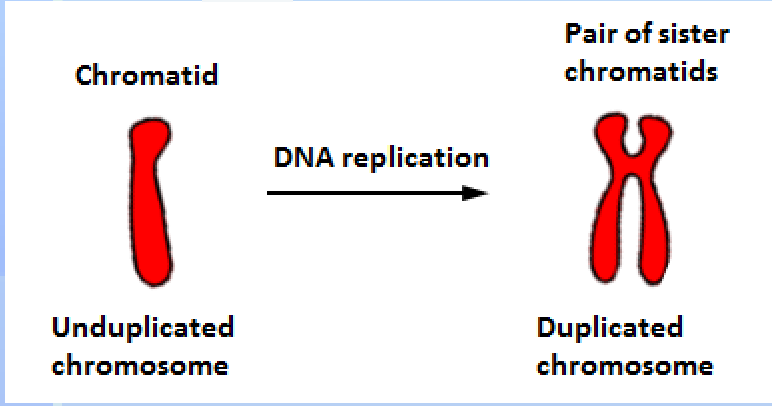


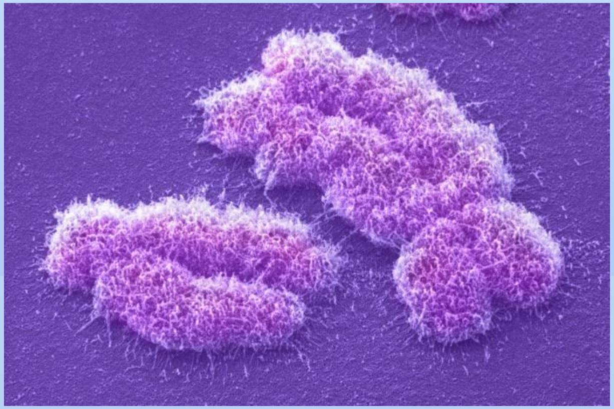
Maize karyotype

MITOSIS

Cellular Process by which from one single eukaryotic cell two daughter cells are produced that are equal among each other and equal to their mother cell

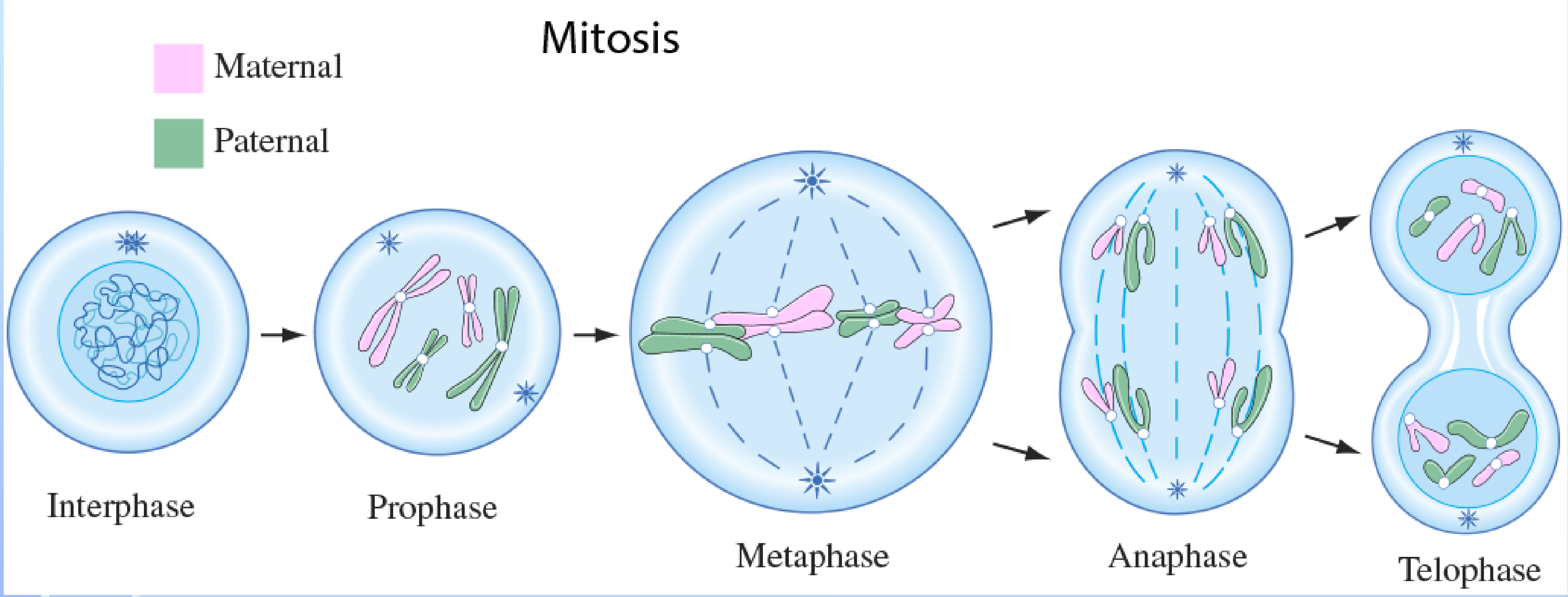
a. **One replication of cellular chromosomes → doubling of chromosomes = 4n**





b. Only one cell division, therefore from one 4n cell → two 2n cells

MITOSIS



Mitosis
Maternal
Paternal
Interphase
Prophase
Metaphase
Anaphase
Telophase

MEIOSIS

Cellular Process by which from one single eukaryotic cell four daughter cells are produced whose chromosome content is half that of the mother cell

IF the cell is diploid (2n) the results are four aploid (n) cells or gametes

a. One chrosomal replication → one 4n cell

b. Two cellular divisions → four n cells

c. The first is a reduction meiotic division
separation of homologous chromosomes

d. Second meiotic division is basically a mitosis

MEIOSIS IN THE PRESENCE OF GENETIC VARIATION

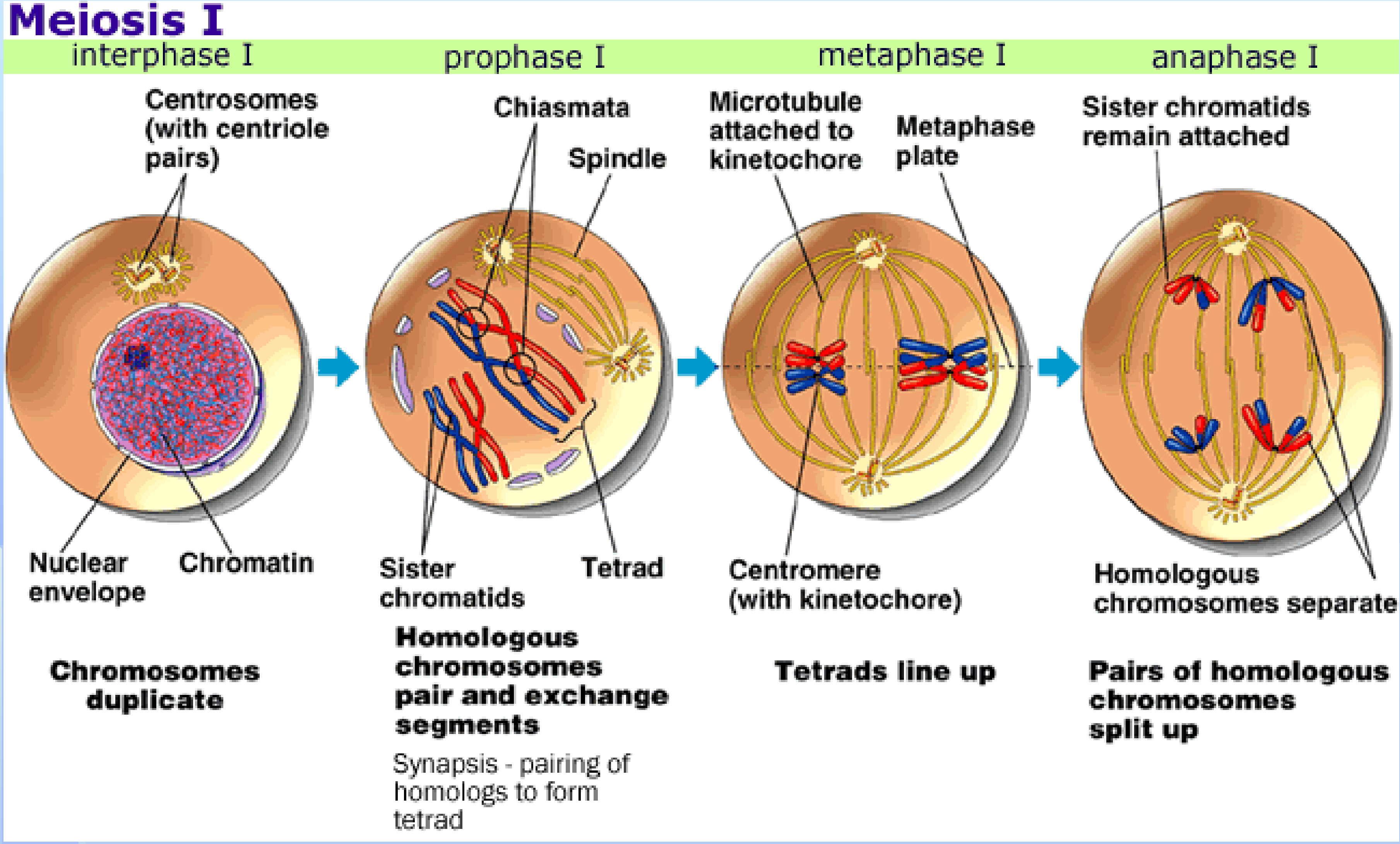
Segregation: the distribution of allelic sequences between daughter cells at meiosis

Crossing over: the exchange of genetic material between homologous crhomosome

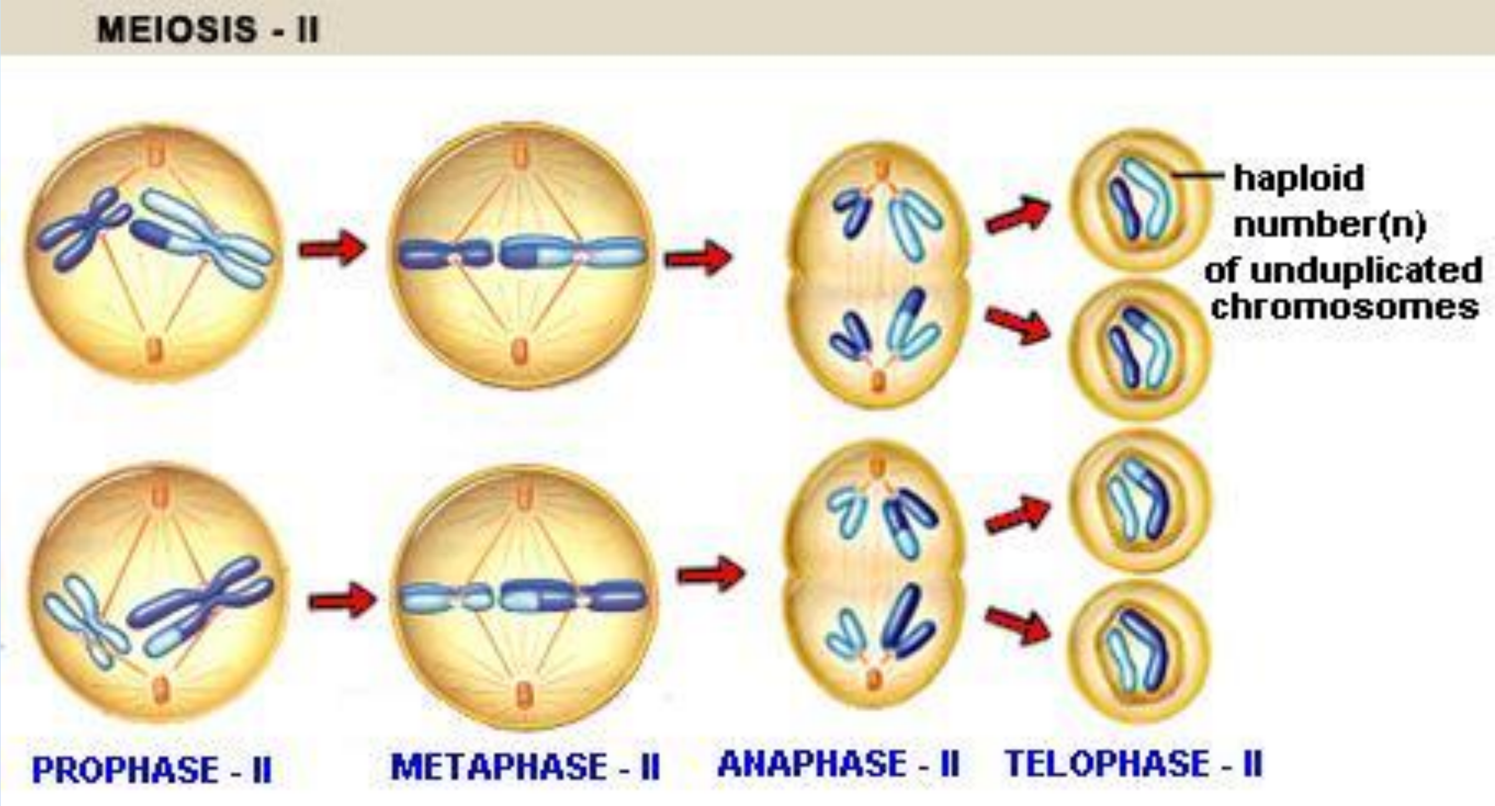
Recombinant: a gamete that contains a combination of allelles that is different from the combination inherited by parents

RECOMBINATION IS PRODUCES BY BOTH SEGREGATION AND CROSSING OVER

MEIOSIS I
Reduction Division



MEIOSIS II
Separation of Sister Chromatides



Thomas Hunt Morgan Demonstrated the Chromosome Theory of Inheritance

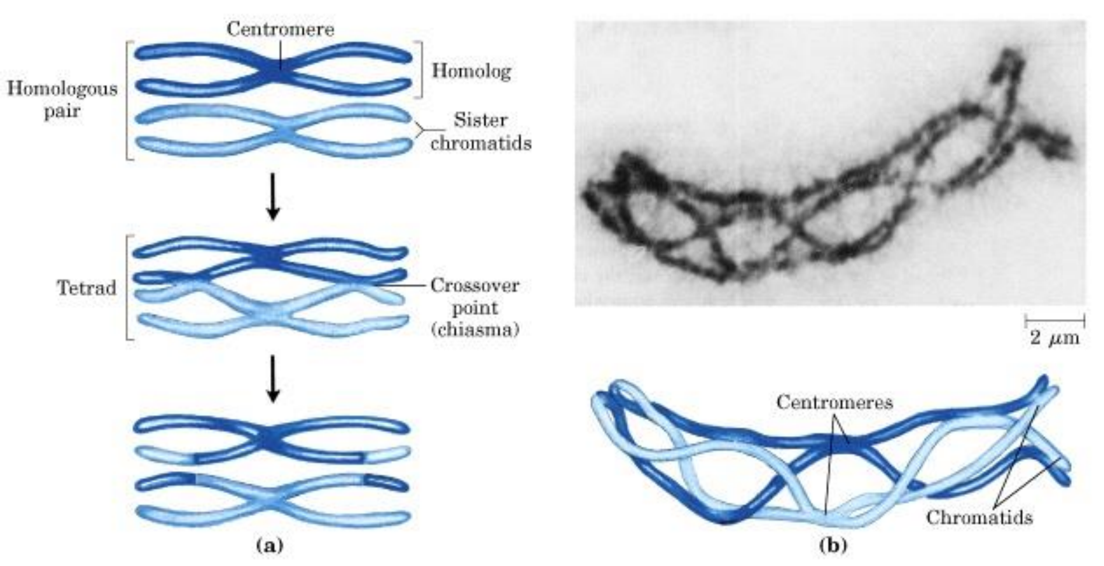
1866 – 1945
American biologist and geneticist
Winner of Nobel prize in Physiology in 1933

The first to associate a specific gene with a specific chromosome

- **Like Mendel, Morgan made an insightful choice of his experimental model, *Drosophila melanogaster***
- **Fruit flies are prolific breeders and have a generation time of two weeks**
- **Fruit flies have three pairs of autosomes and a pair of sex chromosomes n = 4**

PROBLEM: THERE ARE MORE GENES THAN CHROMOSOMES

GENES and CHROMOSOMES

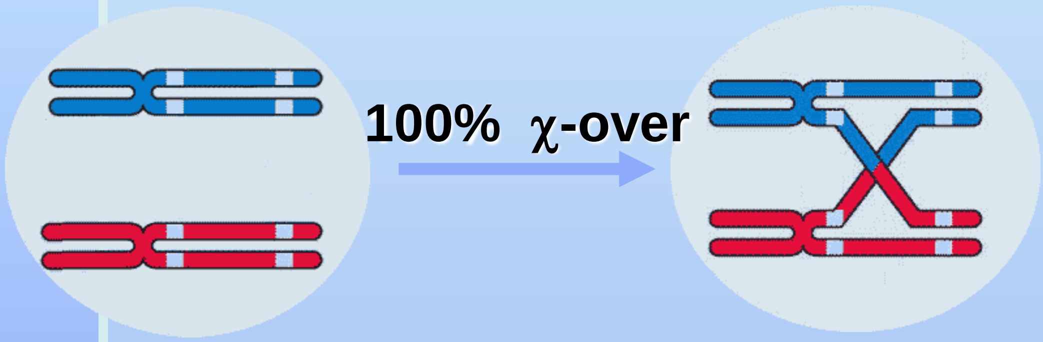


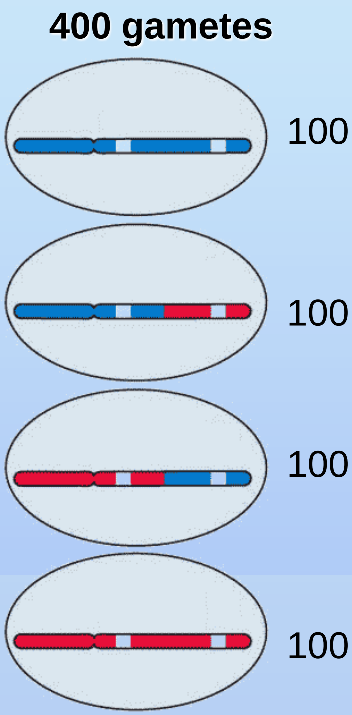
Ccrossing over occurs between two chromatides at a time. C.O. could involve multiple events involving all 4 chromatides

FREQUENCY of CROSS-OVER and FREQUENCY of RECOMBINATION

Maximum value of RECOMBINANTS is 50%

For instance: N° of Cells in meiosis = 100





freq of recombinant gametes = 50%

Alfred Sturtevant the FREQUENCY of RECOMBINATION to estimate genetic distances between genes

American geneticist
1891 – 1970 As a child
Built the pedigree of horses
PhD student of Thomas Morgan

Proposed his hypothesis in 1913
When he was still a PhD student

Alfred Sturtevant's Background knowledge

- **Maximum percentage of RECOMBINANTS due to segregation is 50% (Mendel's law)**

- **If two genes are on the same chromosome and there is always a c.o. between them, the maximum percentage of recombinant is 50%**

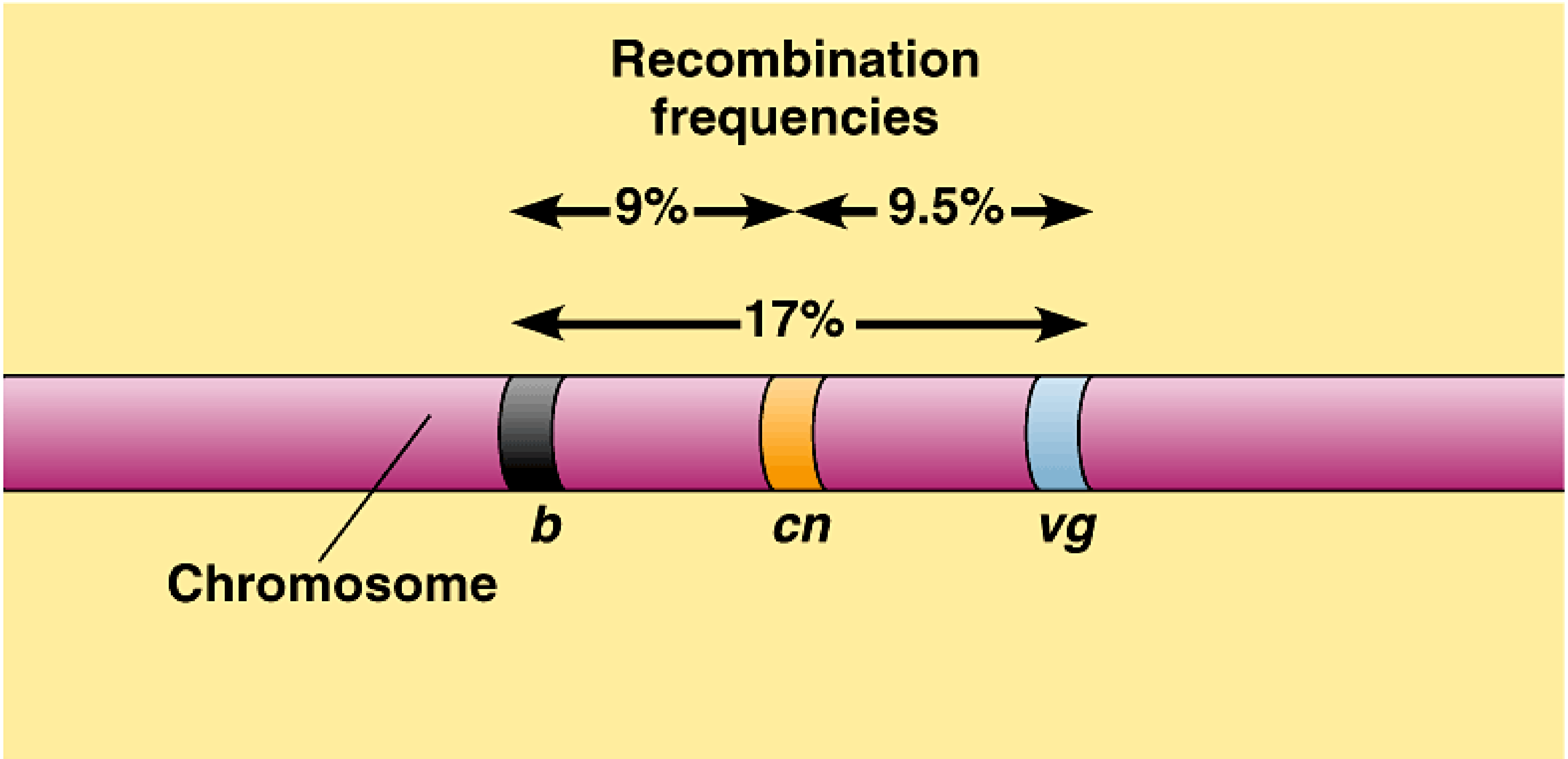
no diffrence from independent segregation

Alfred Sturtevant's Hypothesis of linkage between genes and estimate of genetic distance

Hypothesis: **If c.o. is a random event, its frquency would depend on the distance between genes. Therefore the Frequency of recombination can be an estimate of the genetic distance between the two genes**

NOTE THAT THE FREQUENCY OF RECOMBINATION CAN BE ESTIMATED ONLY IF THERE ARE 2 DIFFRENT ALLELES FOR EACH OF THE GENES CONSIDERED

Using Frequencies of Recombination to Construct Genetic Maps



How the discovery that DNA is the genetic material revolutionized biology

The discovery that DNA is the Genetic Material of all organisms changed the perspective and determined the birth of Molecular Biology

Only exceptions are a number of (both plant and animal) viruses whose genetic material is RNA

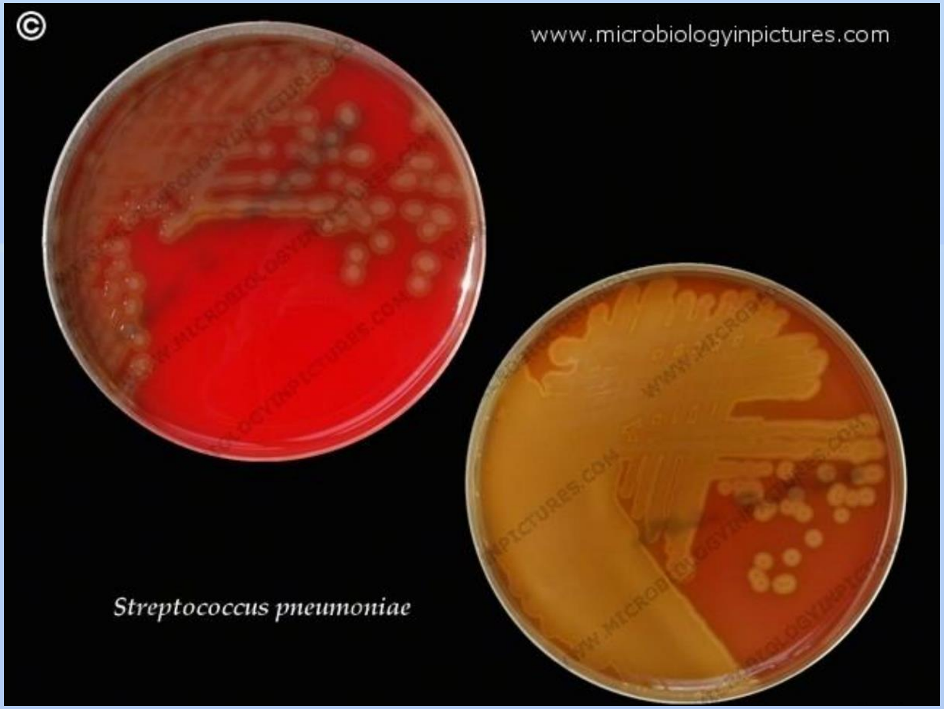
The Search for the Genetic Material: Griffith's Experiment 1928

- Frederick Griffith 1887 - 1941

British microbiologist

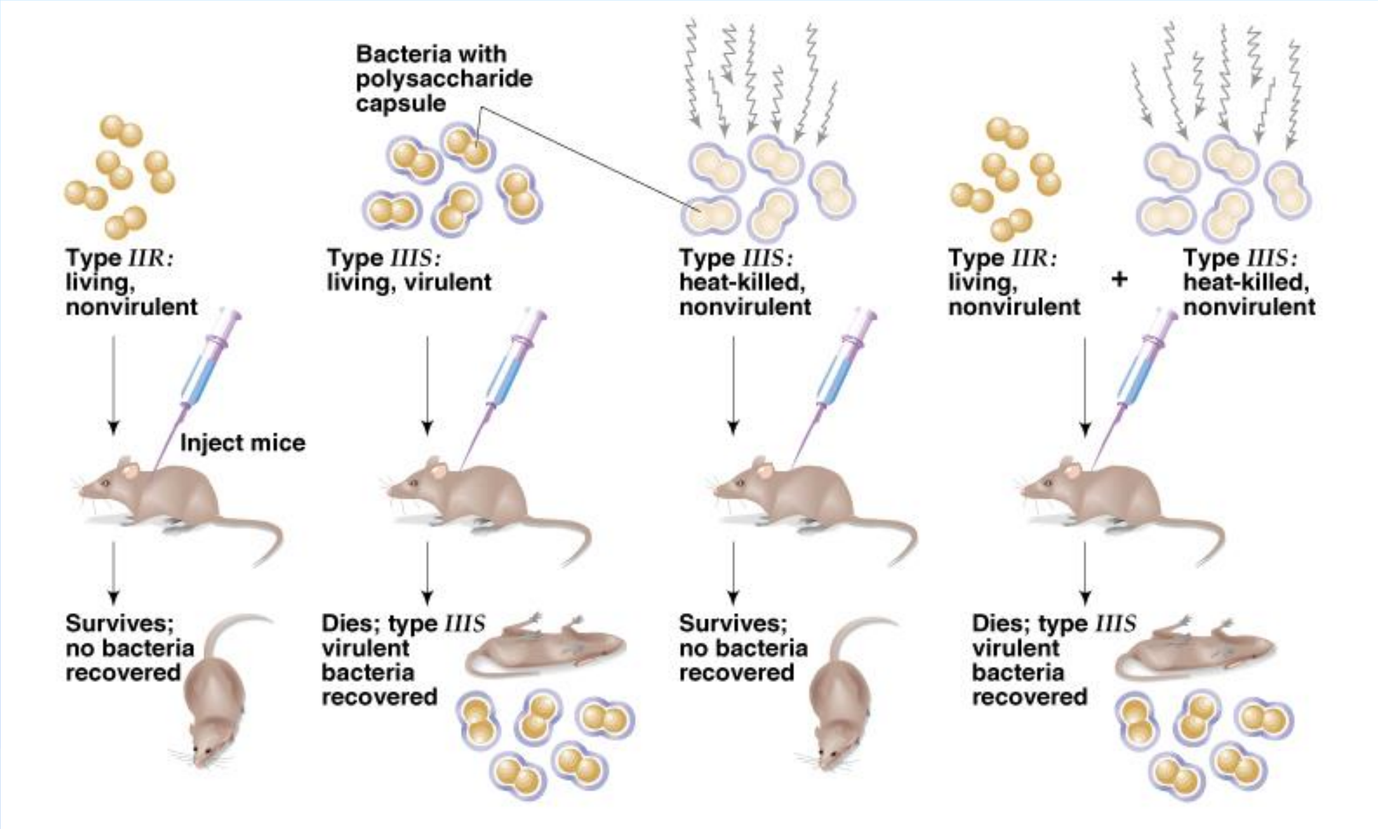
Worked on *Streptococcus pneumoniae* bacteria

Smooth strain (S): virulent



Rough strain (R)
avirulent
Lacks the capsule

TRANSFORMING PRINCIPLE



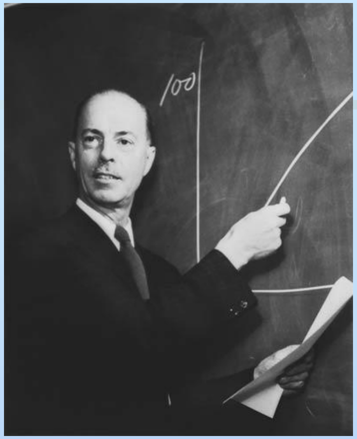
MAJOR FINDING

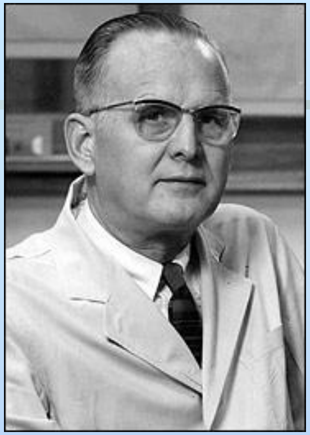
A substance that did not need a living cell was the transforming principle

DNA IS the Genetic Material

1944 Avery, MacLeod and McCarty

Rockefeller Institute of Medical Research

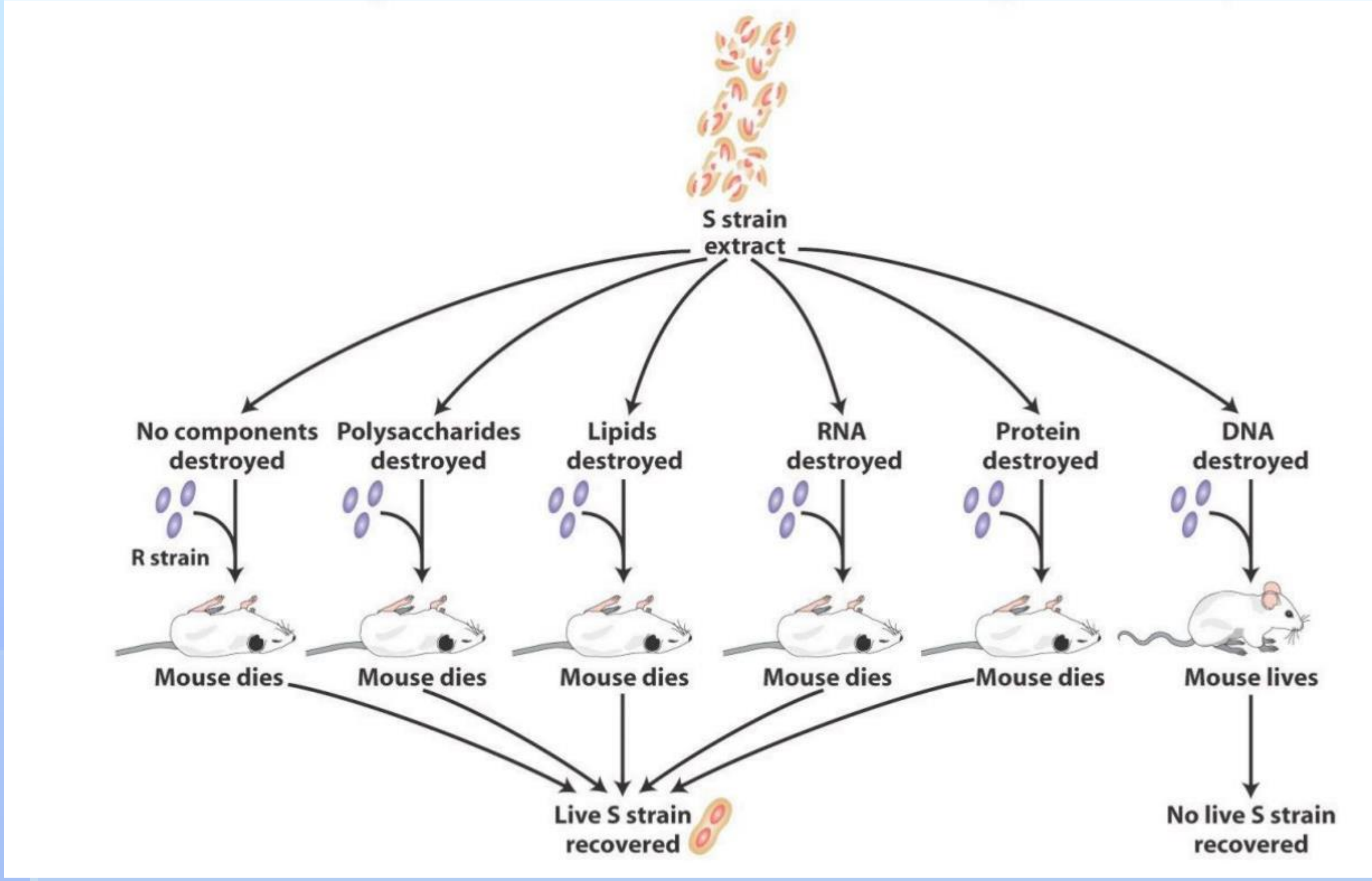




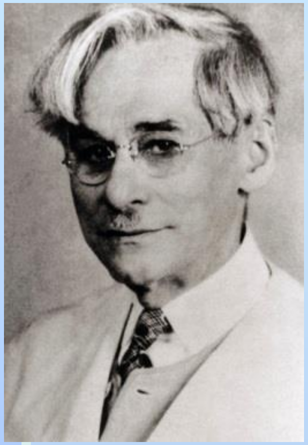
Canadian **Canadian** **American**

Identification of the transforming principle from *S. pneumoniae*.

Their approach was to break open dead cells, chemically separate the components (*e.g.*, protein, nucleic acids) and determine which was capable of transforming living *S. pneumoniae* cells


S strain extract
No components destroyed
Polysaccharides destroyed
Lipids destroyed
RNA destroyed
Protein destroyed
DNA destroyed
R strain
Mouse dies
Mouse dies
Mouse dies
Mouse dies
Mouse dies
Mouse lives
Live S strain recovered
No live S strain recovered

The Composition and Structure of DNA and RNA

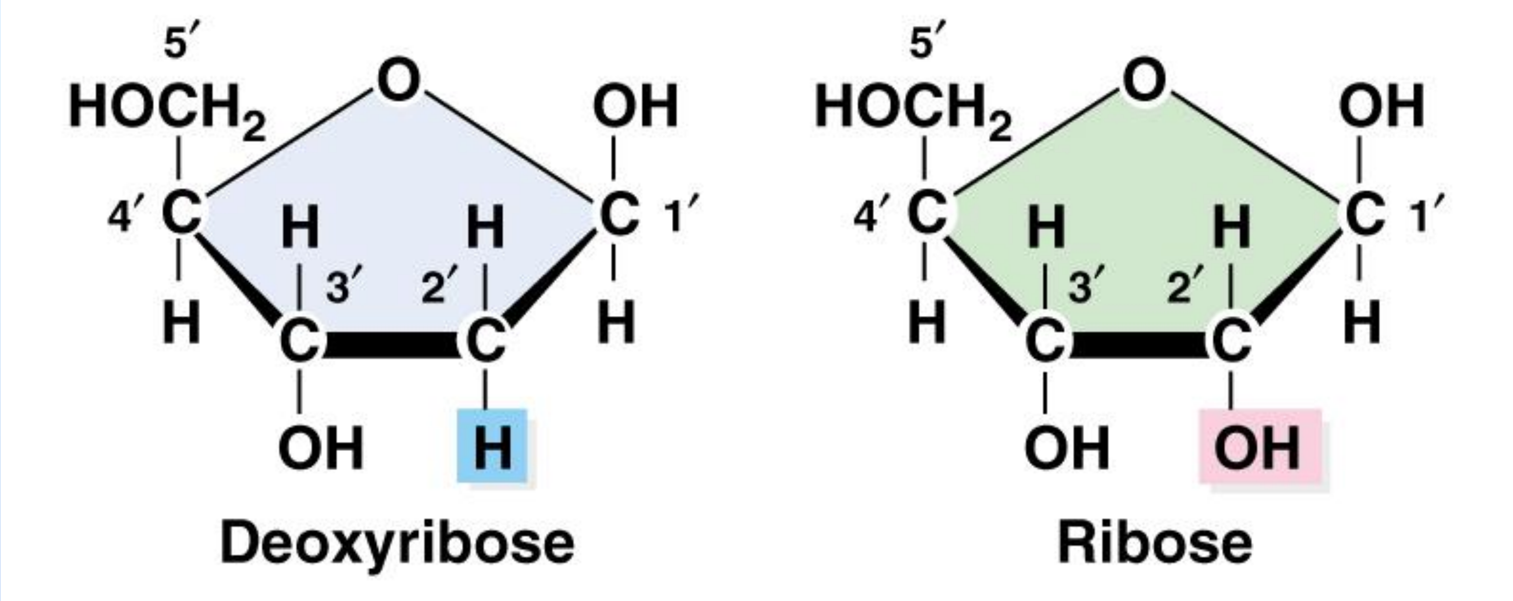


Poebus Levene (1863-1940) Biochemist Born in the Russian Empire emigrated to US, Worked at Rochefeller Institute NY

1. DNA and RNA are polymers composed of monomers called nucleotides
2. Each nucleotide has three parts:
   a. A pentose (5-carbon) sugar
   b. A nitrogenous base.
   c. A phosphate group

The Composition and Structure of DNA and RNA

SUGAR: A pentose (5-carbon) FLAT MOLECULE



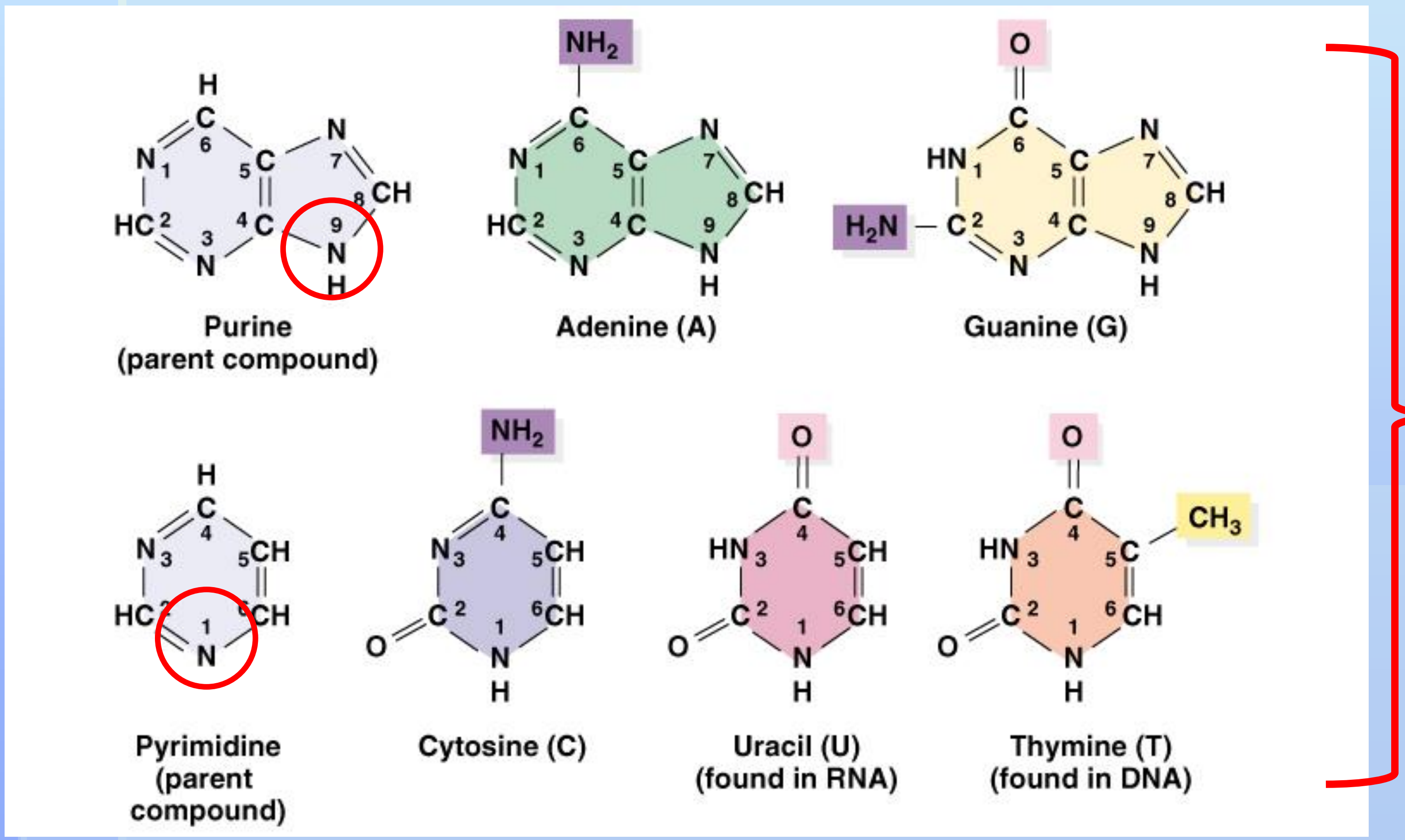
The Number of the C atoms is IMPORTANT

The Composition and Structure of DNA and RNA

There are two classes of nitrogenous bases (Nucleobases)

a. Purines (double-ring, nine-membered structures)

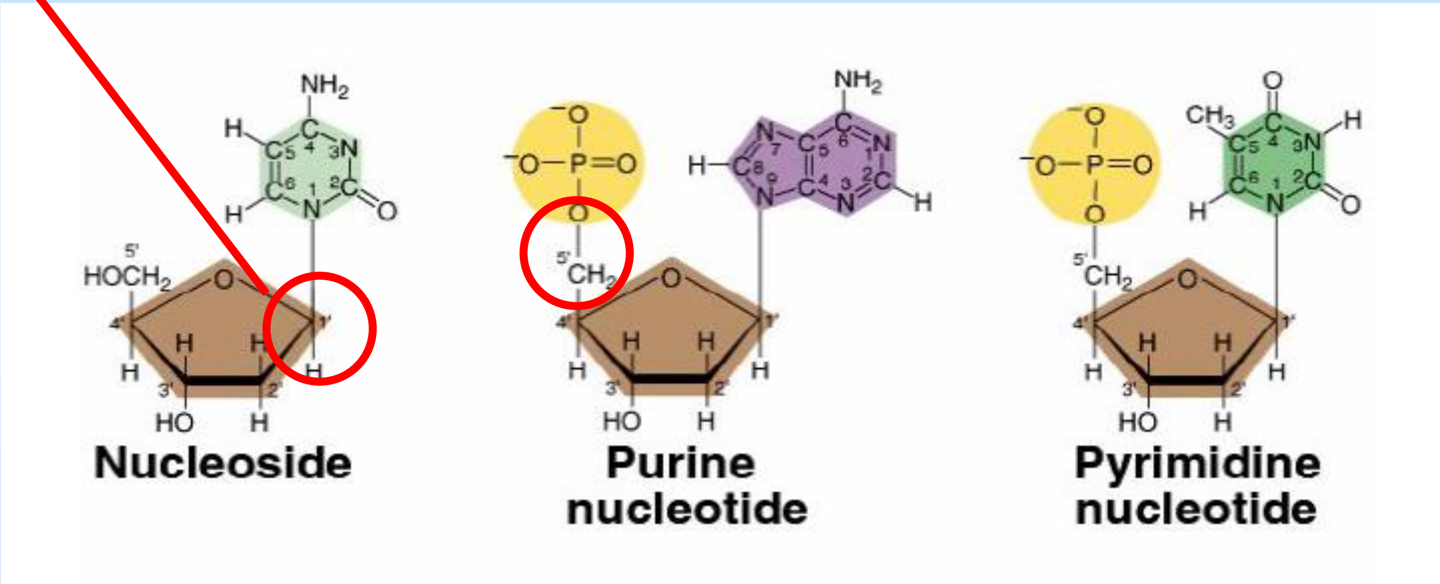
b. Pyrimidines (one-ring, six-membered structures)



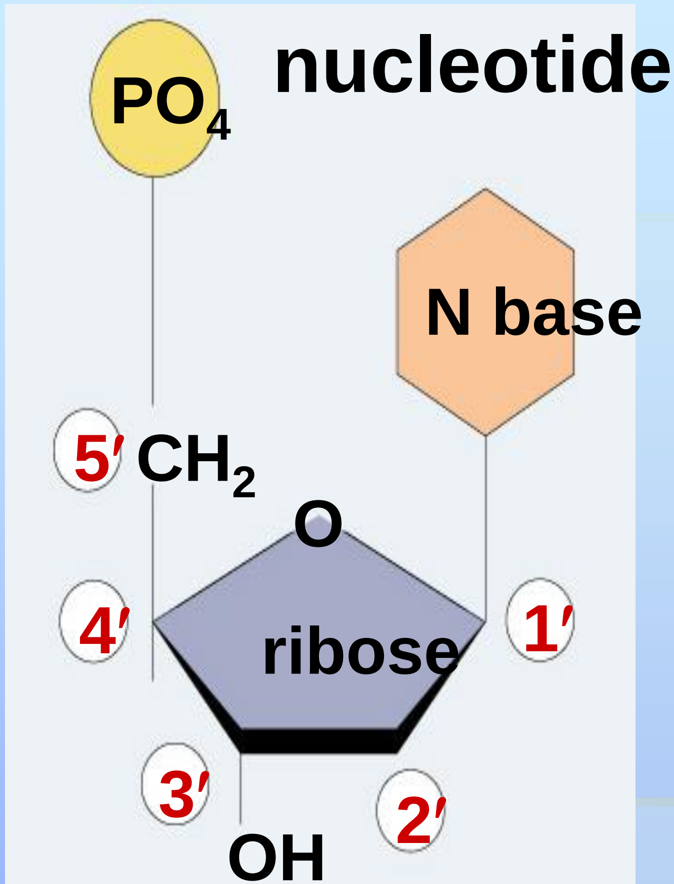
Natural Nucleo-bases

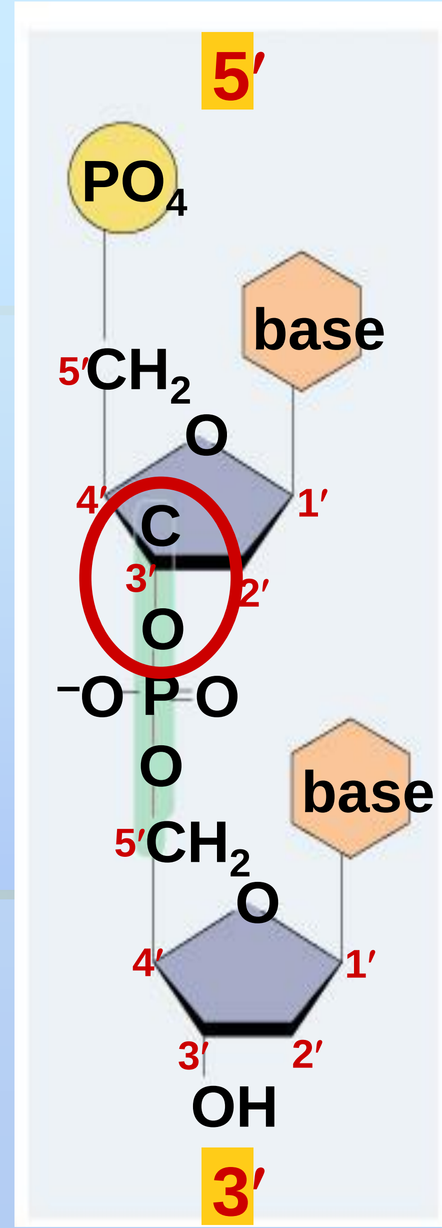
Nucleosides and Nucleotides

β– glycosidic bond



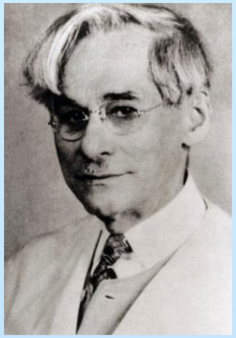
Carbon 1 linked to the nucleobase and Carbon 5 to the phosphate



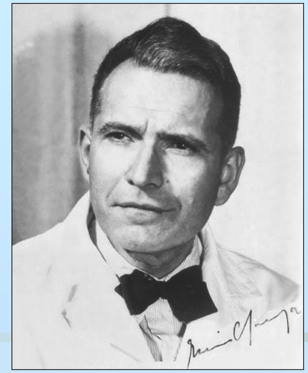


Polarity of the chain

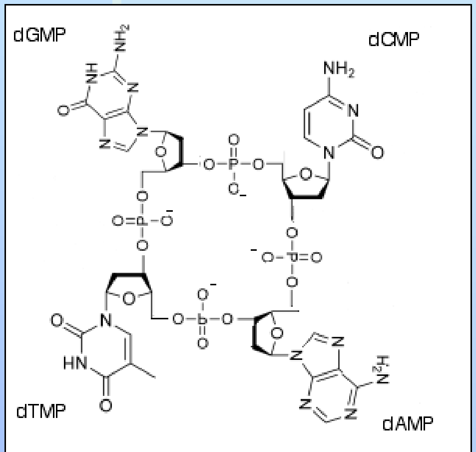
Sugar and P make up the backbone of the chain



Poebus Levene proposed the TETRANUCLEOTIDE HYPOTHESIS

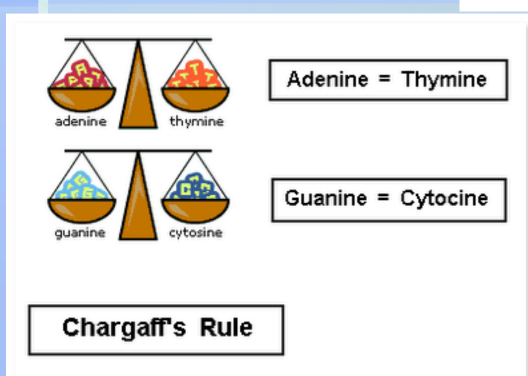


Erwin Chargaff
Austrian biochemist emigrated to US when Nazis annexed Austria



DNA source	Adenine	Thymine	Guanine	Cytosine
Calf Thymus	1.7	1.6	1.2	1.0
Beef Spleen	1.6	1.5	1.3	1.0
Yeast	1.8	1.9	1.0	1.0
Tubercle Bacillus	1.1	1.0	2.6	2.4

(From Vischer, Zamenhof and Chargaff, 1949, p. 433, and Chargaff *et al.*, 1949, p. 413).



Tetranucleotide hypothesis DEMOLISHED because the Amount of the 4 nucleotide Is NOT equal, BUT what is its structure?

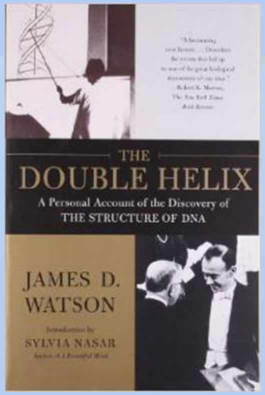
The Discovery of the DNA Structure: Drama, Intrigue and Brilliance.

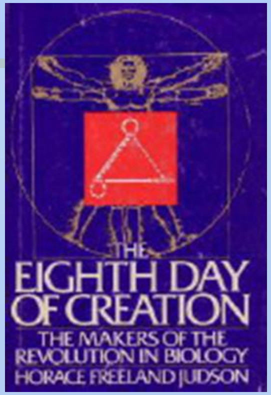
LIST OF PLAYERS

Cal Tech Institute: Linus Pauling

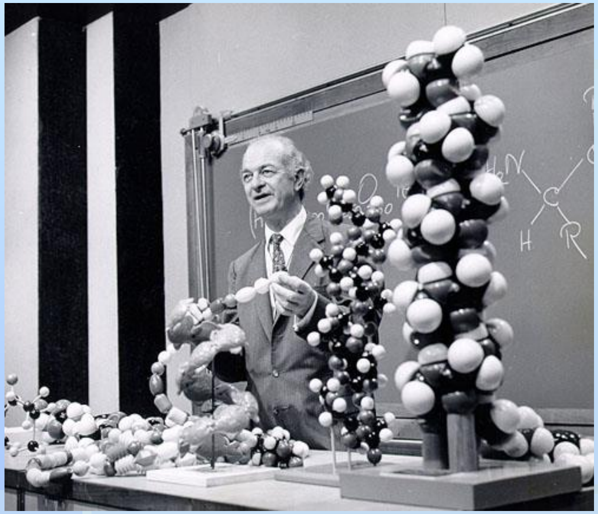
King's College London: Maurice Wilkins
Rosalind Franklin

Cavendish Lab Cambridge: Francis Crick
James Watson





The Cal Tech Institute1953: Pauling's Incorrect Triple-Helical Structure



Linus Pauling Chemist 1901 – 1994

The most important American Scientist at the time
Nobel Prize in Chemistry in 1954
- nature of the chemical bonds
- helical pattern of hemoglobin
Nobel Peace Prize in 1963
- Activism against nuclear weapons

FATAL WEAKNESS
He could not produce DNA crystals

The London Group

STRONG POINTS

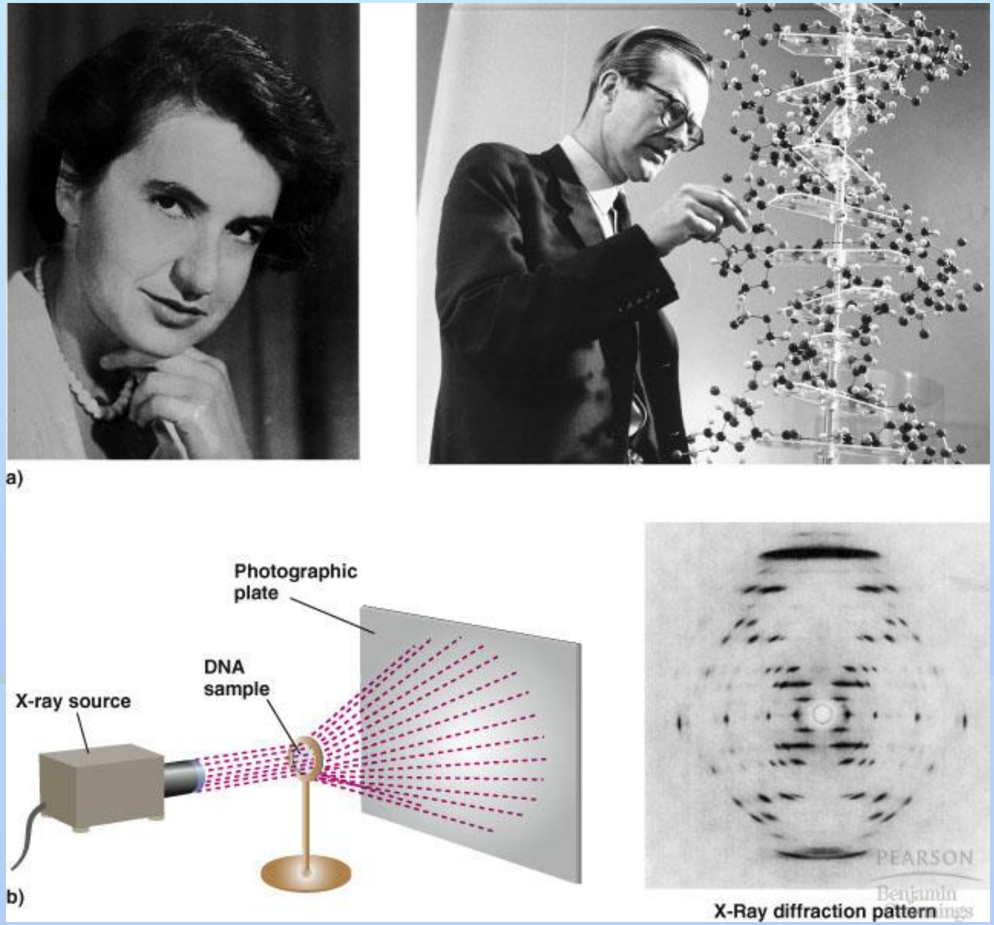
- ❖ Experimentally very advanced
- ❖ Able to produce crystalized DNA
- ❖ X-Ray analysis

WEAKNESS

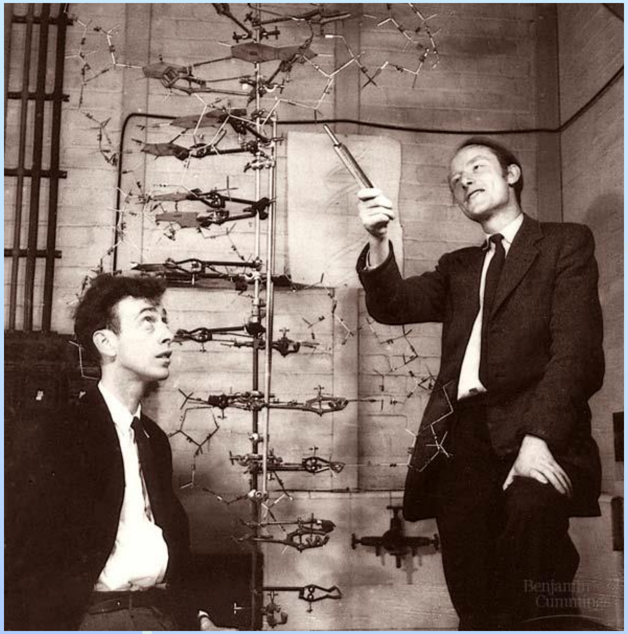
- ❖ Disagreement between Wilkins and Franklin

Lack of communication

X-Ray analysis suggests that DNA might be a double helix



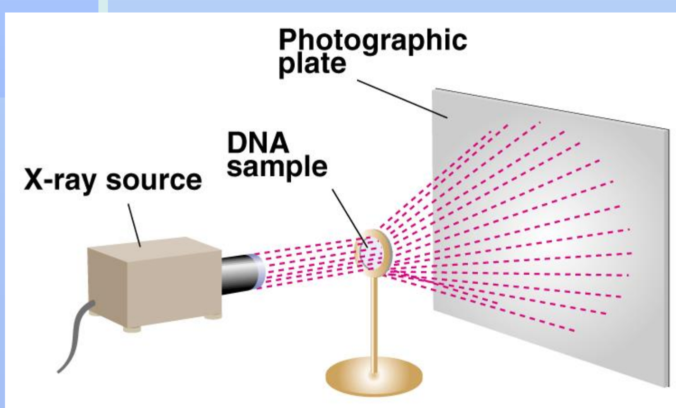
The Cambridge Group



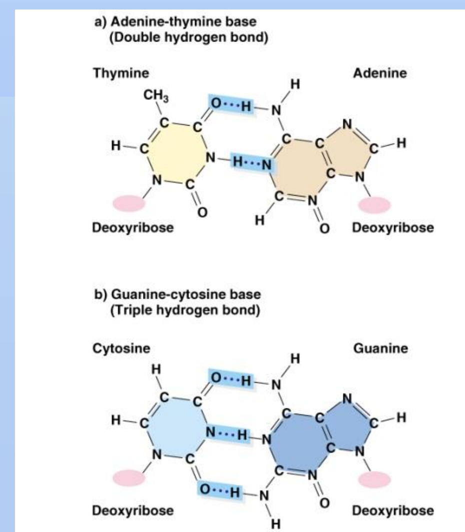
Francis Crick British Physiscs (1916 – 2004)
the genius of the team
PhD student working on protein structure

James Watson Americam Biologist (1928 -)
the driving force
With the ambition to discover the structure of the genetic material

WINNING STRATEGY
To build tridimentional models



+ Chargaff's rule =
COMPLEMENTARY BASE PAIRING



The Discovery of the DNA Double Helix

No. 4356 April 25, 1953 NATURE 737

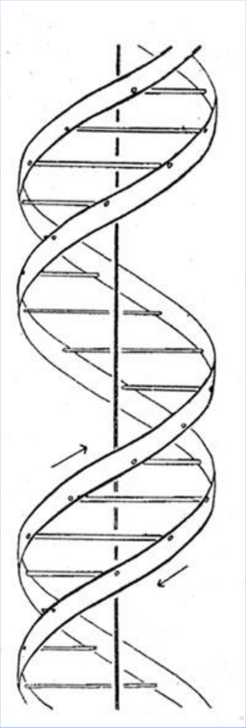
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

Watson and Crick's Three-dimensional Model

a. **It implies two polynucleotide chains wound around each other in a right-handed helix**
b. **The two chains are antiparallel**
c. **The sugar-phosphate backbones are on the outside of the helix, and the bases are on the inside, stacked perpendicularly to the long axis**



Why was the double helix such a Revolutionary Discovery?

FROM THE SECONDARY STRUCTURE TO HYPOTHESIS ON HOW IT FUNCTIONS

> It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

1. **Each strand contains the information necessary to generate a companion strand through complementary base pairing**

   → **REPLICATION**

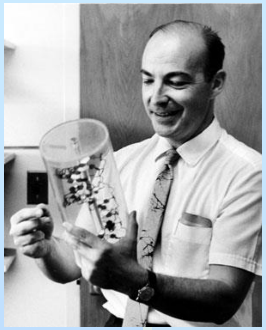
2. **Specificity depends on nucleotide sequence**

   → **GENETIC CODE**

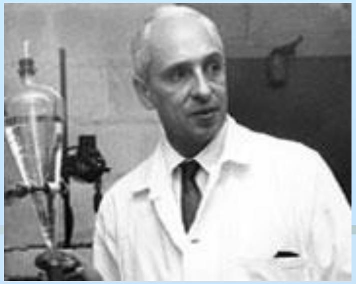
3. **Modification of the nucleotide sequence**

   → **GENETIC VARIATION**

The Mechanism of DNA Replication



Arthur Kornberg (1918 - 2007)
American Biochemist
NYU
DNA synthesis
Nobel Prize in 1959 with
Severo Ochoa



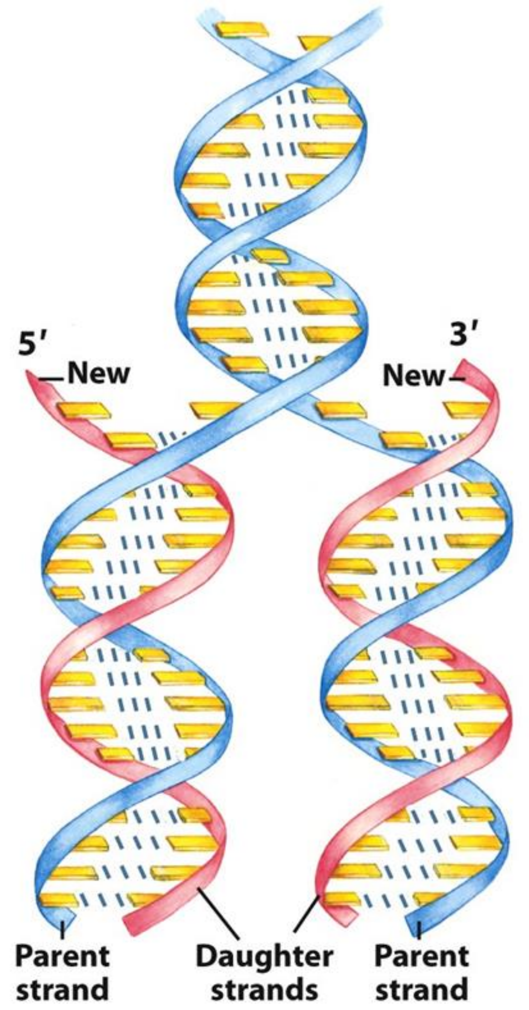
Severo Ochoa (1905 – 1993)
Spanish physician

Four components are required

1. dNTPs: dATP, dTTP, dGTP, dCTP (deoxyribonucleotide 5’-triphosphates) (sugar-base + 3 phosphates)
2. DNA template
3. DNA polymerase
4. Mg^{2+} (optimizes DNA polymerase activity)

DNA Replication

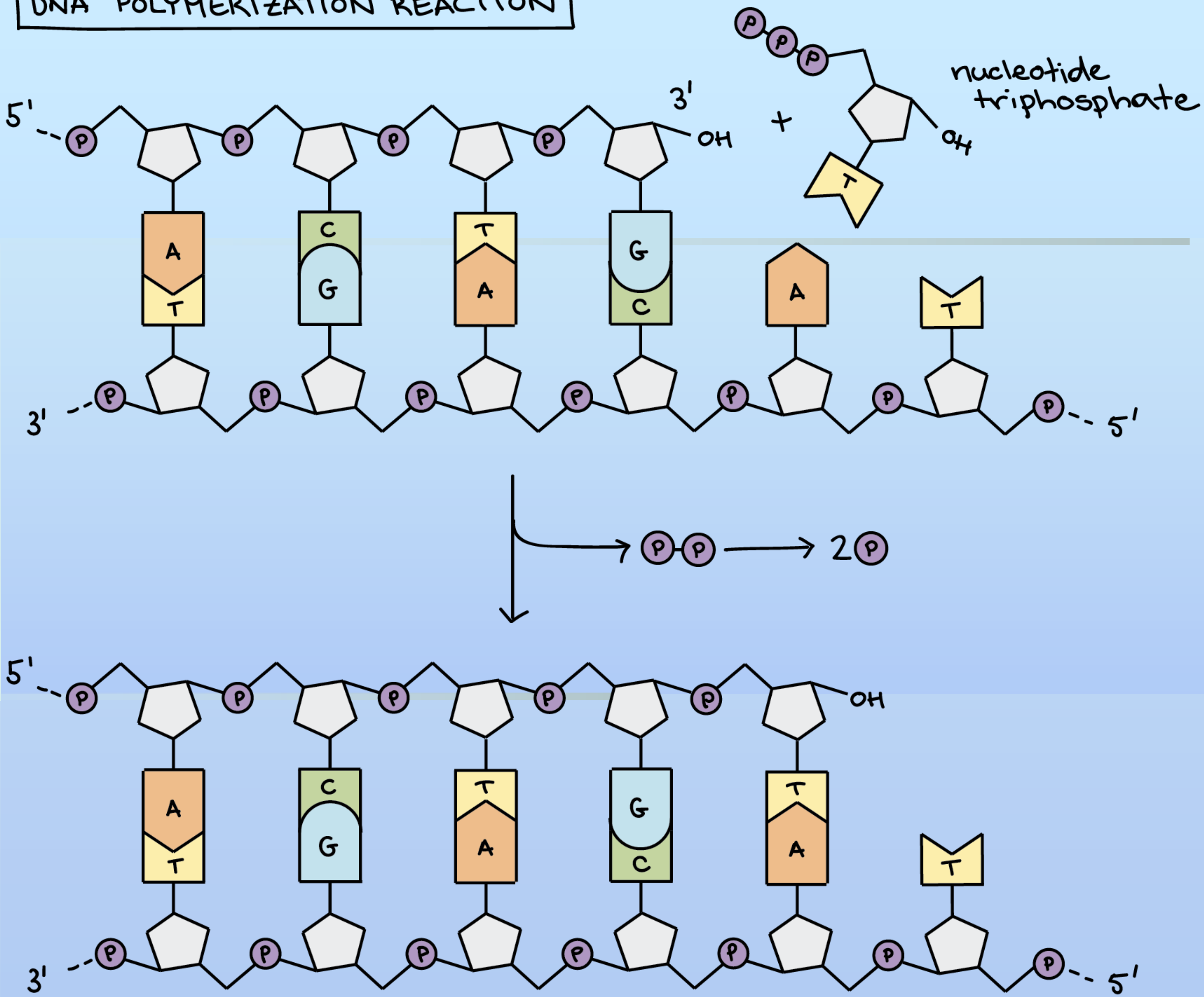
EACH STRAND IS USED AS TEMPLATE TO SYNTHESIZE A NEW COMPLEMENTARY STRAND



Phases of DNA Replication

- **Initiation**
  - **Proteins bind to DNA and open up the double helix**
  - **DNA is prepared for complementary base pairing**
- **Elongation**
  - **Proteins connect the correct sequences of nucleotides into a continuous new strand of DNA**
  - **POLYMERIZATION**
- **Termination**
  - **Proteins refine the product of replication and release the replication complex**

DNA POLYMERIZATION REACTION



Main features of the DNA Synthesis

1. DNA polymerases a multi-protein enzymes that catalyzes formation of phosphodiester bonds
2. Energy for this reaction is derived from the release of pyrophosphate of the three phosphates of the dNTP
3. DNA polymerase "finds" the correct complementary dNTP at each step in the lengthening process
4. Higly processing enzyme

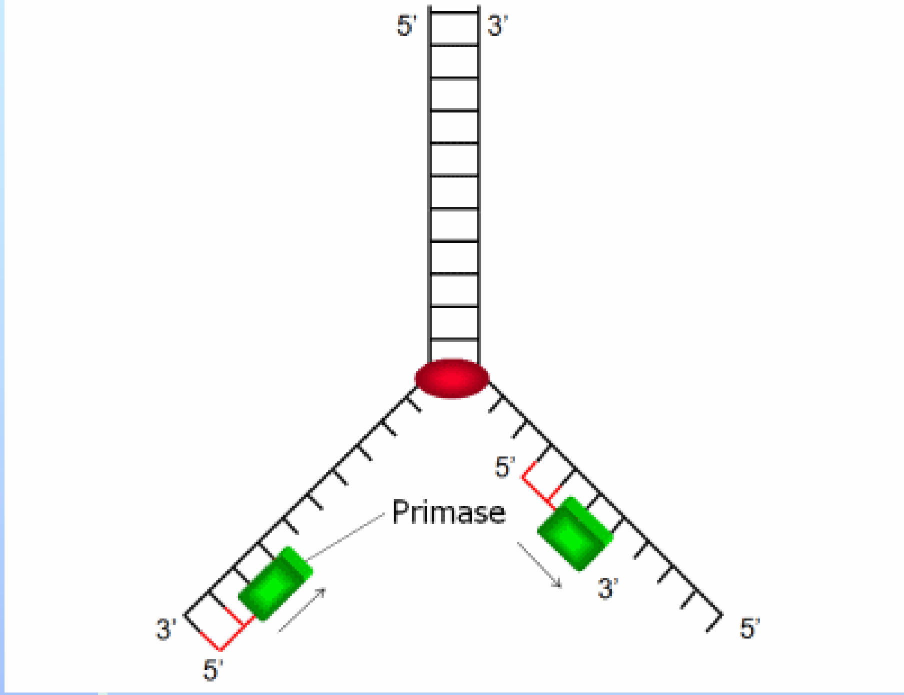
- rate ≤ 800 dNTPs/second
- low error rate because of PROOF READING PROPERTY due to 3' – 5' DNAse activity

5. Direction of synthesis ONLY 5' to 3'

ONE MAJOR DRAWBACK

It CANNOT START polymerization

Priming: DNA Primase



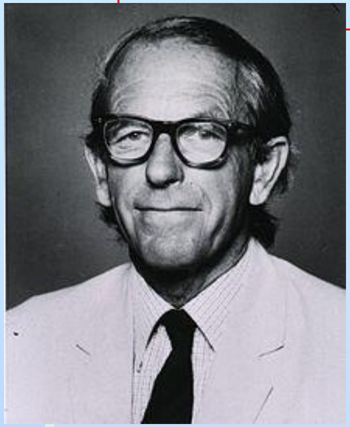
- It is DNA-dependent RNA polymerase
- Synthetizes short (11 nt) RNA fragments on both strands called PRIMERS
- DNA Polymerase works by elongating the RNA primers

Priming is only the last step of Initiation

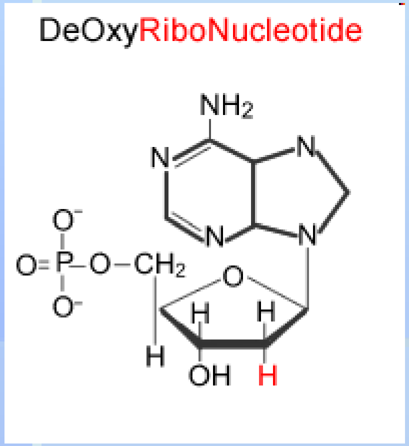
1. Open the double strand
2. Unwind the double helix and form the replication bubble
3. Keep the bubble open

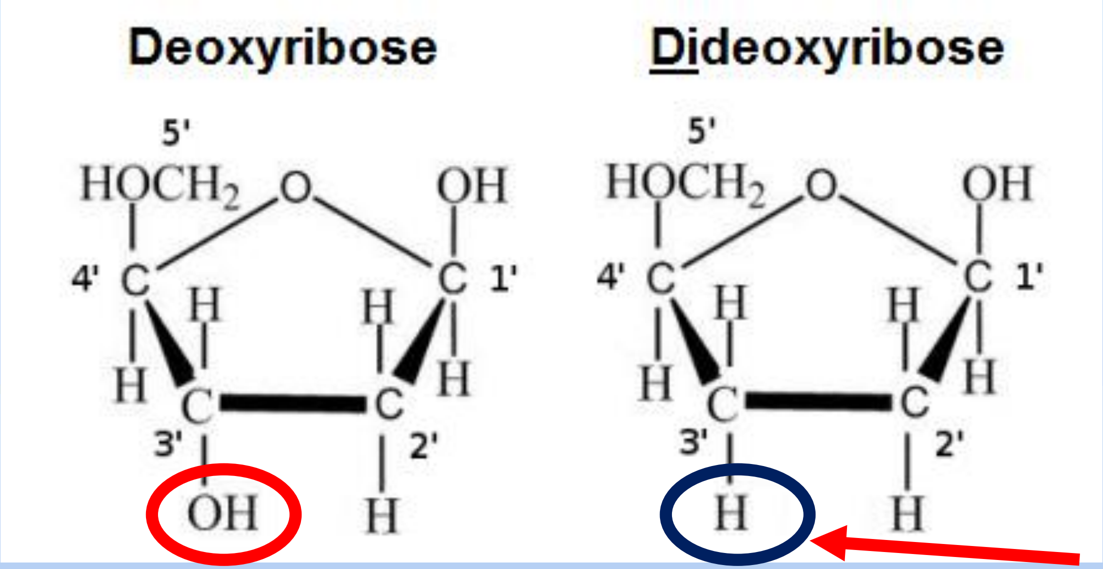
METHODOLOGICAL BOX

DNA sequencing: the SANGER' S CHAIN TERMINATION METHOD



Sir Federick Sanger (1918- 2013)
British biochemist
Cambridge University UK
Two Nobel Prizes in Chemistry
- 1958 structure of insulin
- 1980 DNA sequencing





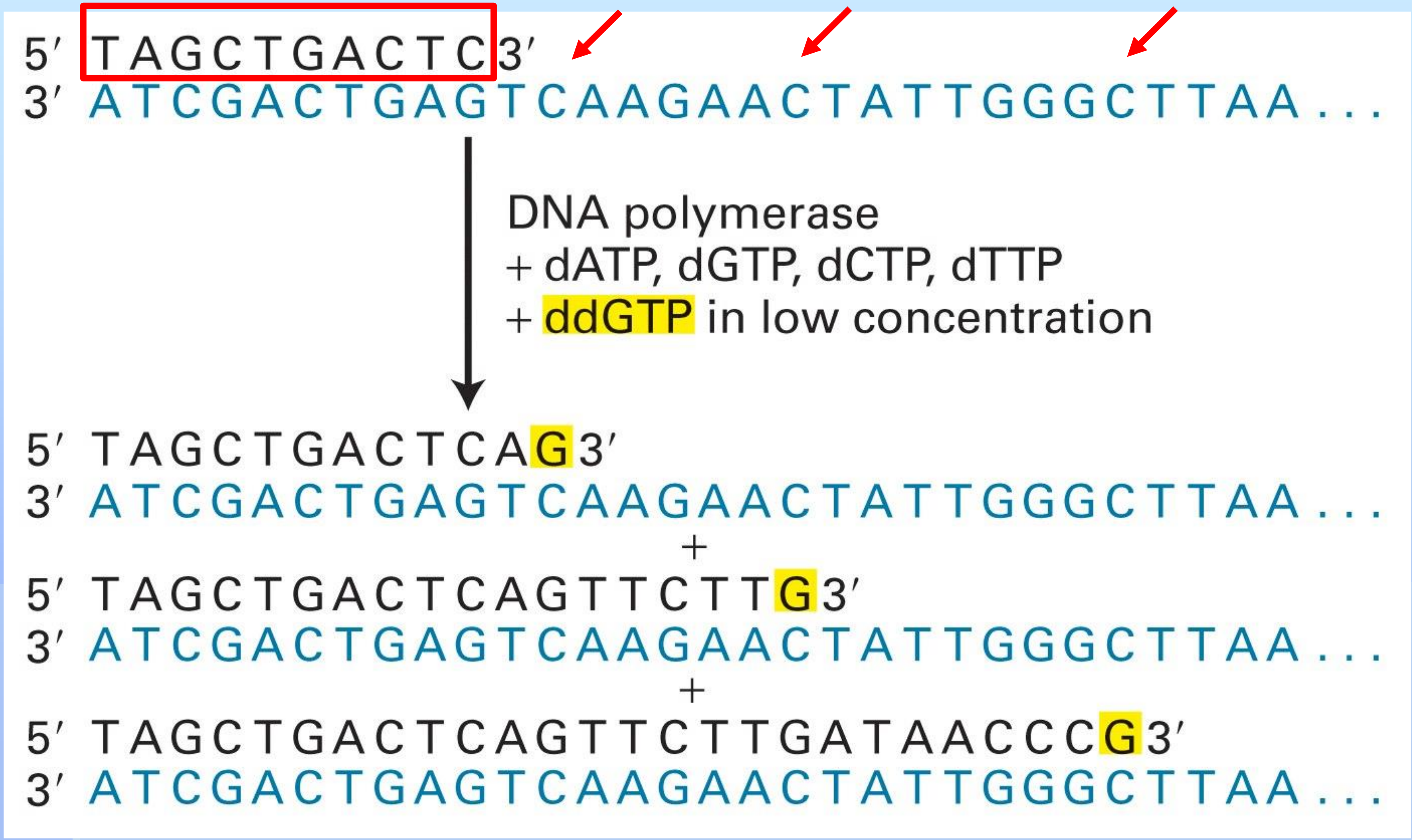
Developed in the first version in 1968!!

SANGER' S CHAIN TERMINATION METHOD

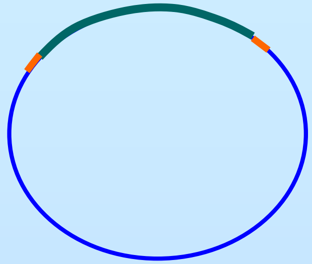
5′ TAGCTGACTC 3′
3′ ATCGACTGAGTCAAGAACTATTGGGCTTAA . . .

DNA polymerase
+ dATP, dGTP, dCTP, dTTP
+ ddGTP in low concentration

SANGER' S CHAIN TERMINATION METHOD

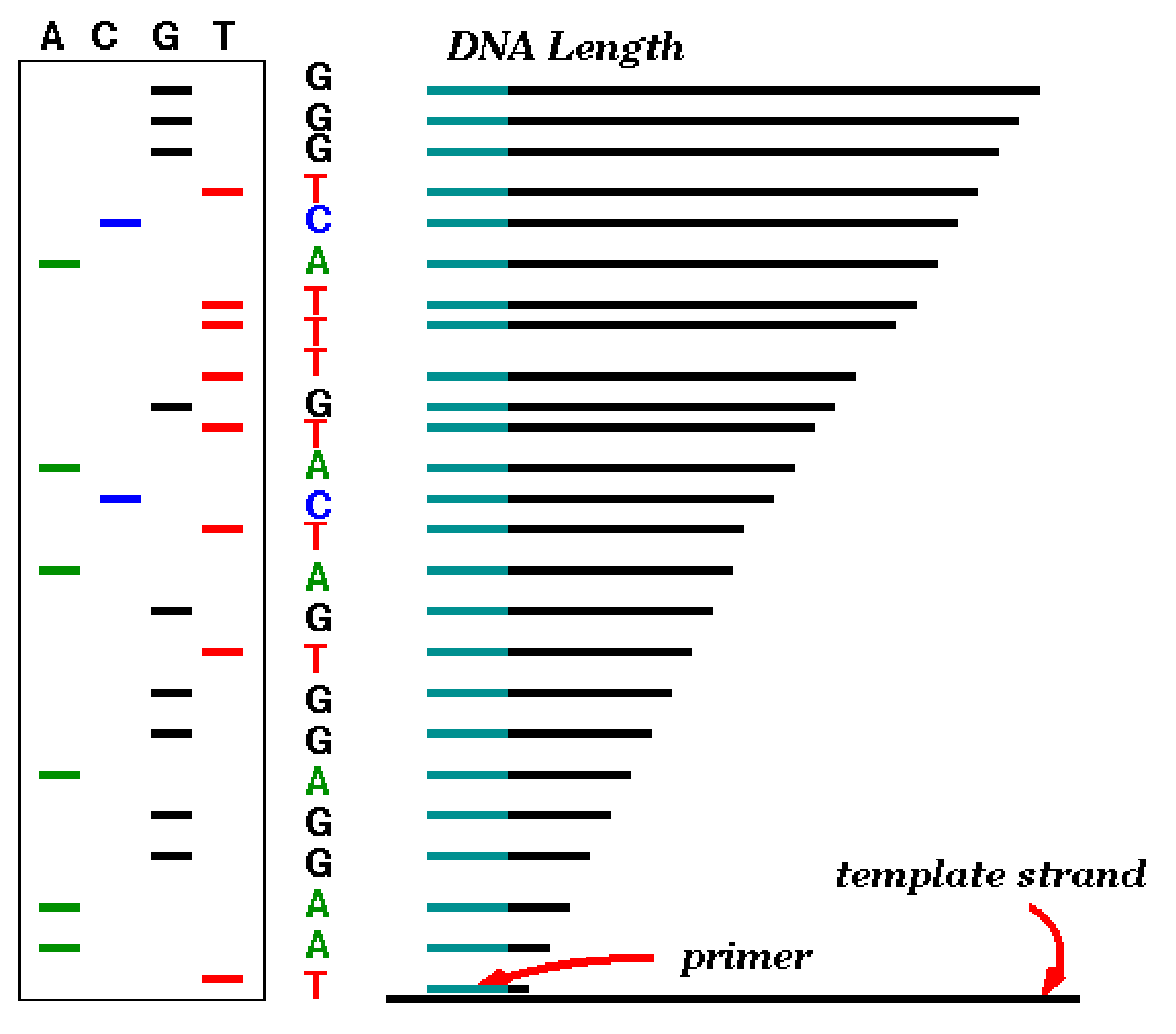


Sanger Method: Generating Read

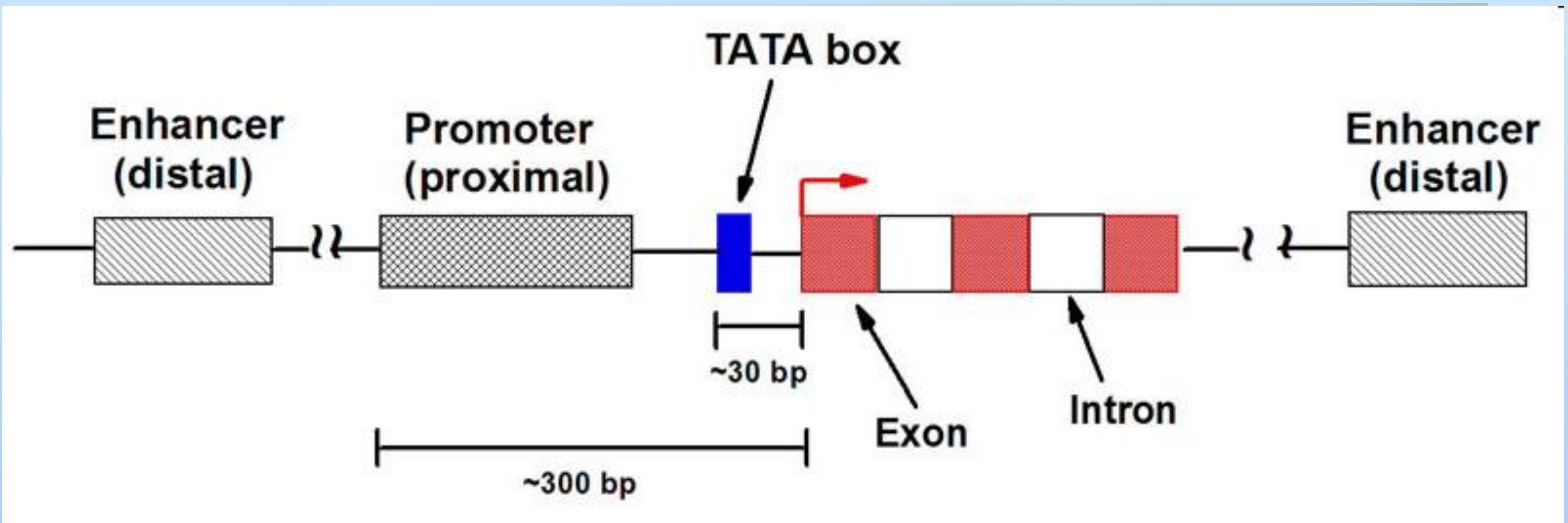


Fragment cloned into a plasmid

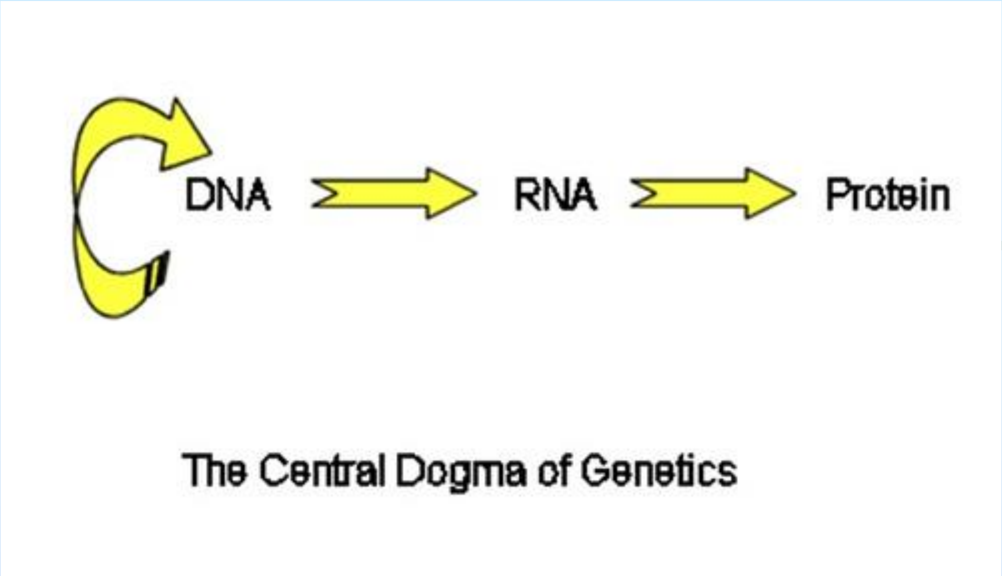
1. Start at primer (restriction site)
2. Included dNTPs
3. Grow DNA chain
4. Stops reaction at all possible points
5. Separate products by length, using gel electrophoresis

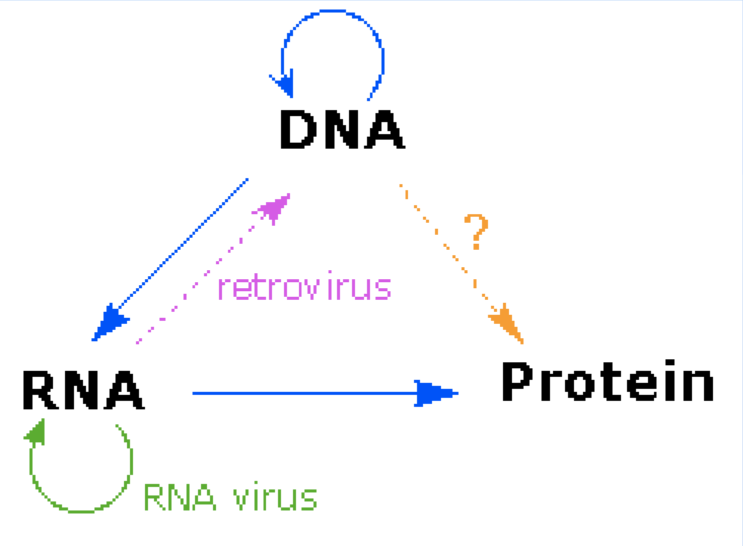


Eukaryotic Gene Structure



CENTRAL DOGMA of BIOLOGY





Transcription

The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA

DNA —Transcription→ **RNA**

tRNA
rRNA
snRNA
mRNA —Translation→ **Proteins**
ncRNA

EACH STEP REPRESENTS AN AMPLIFICATION OF THE GENETIC INFORMATION

METHODOLOGICAL BOX

DNA —Transcription→ RNA

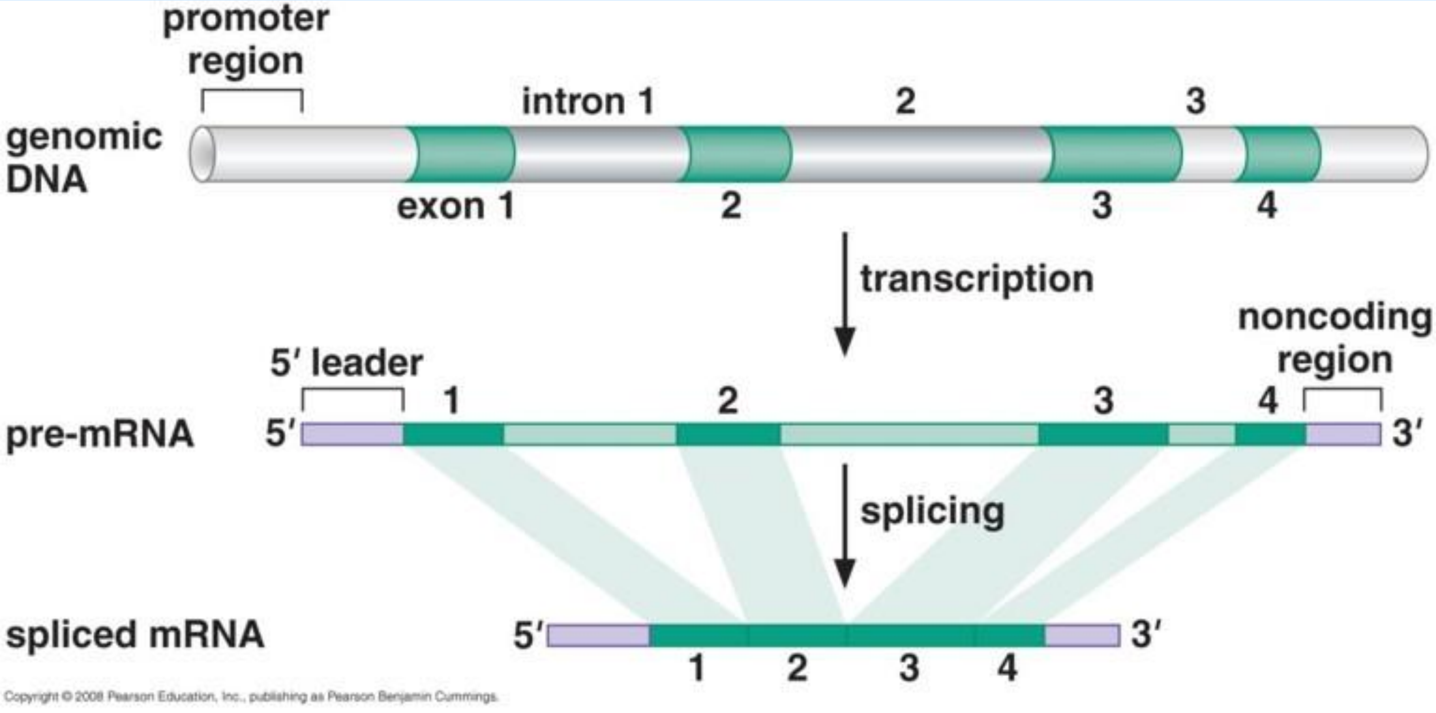
rRNA
mRNA
ncRNA

RNA is a highly unstable molecule
Molecular biology is mostly done on DNA

The discovery of retrovirus (RNA genome) brought to the discovery of a key enzyme called REVERSE TRANSCRIPTASE that can copy an RNA molecule into a copy DNA – cDNA

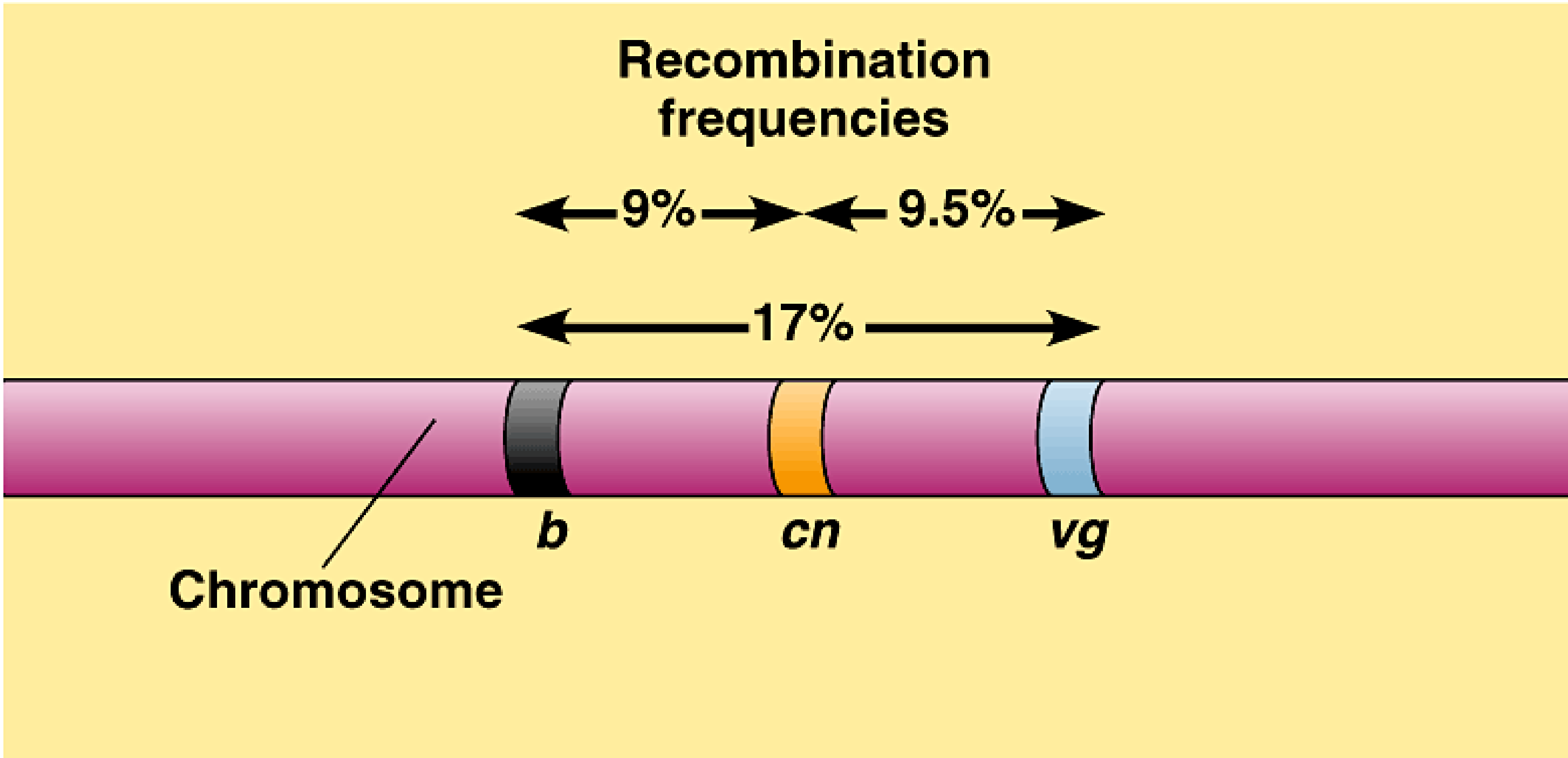
Extracting total mRNAs with Reverse Transcription a population of cDNAs can be produced and studied opening the path to TRANSCRIPTOMICS

Eukaryotic genes contain introns which are spliced to form mature mRNA

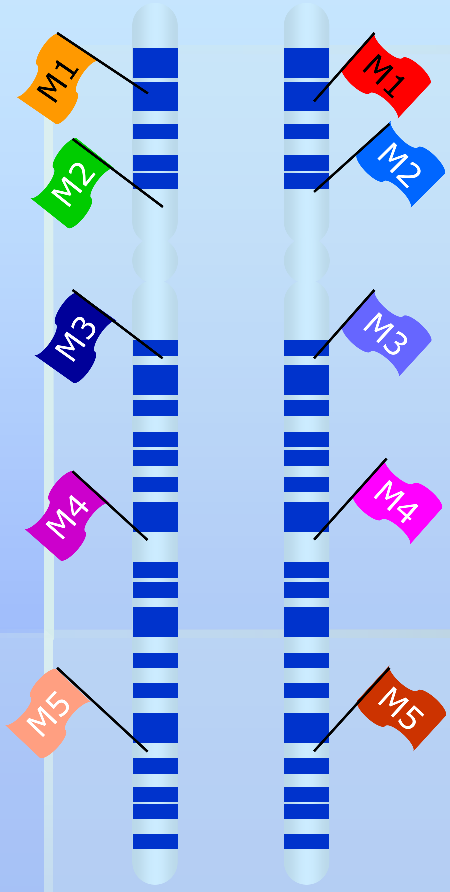


Integrating molecular biology into genetic mapping

Using Frequencies of Recombination to Construct Genetic Maps



Genetic Markers



Genetic Marker

A *Locus* which identifies un-ambigously a specific chromosomla region

- **Tool** → **not the objective**
- **Marker** → **indirect approach**

Genetic markers

TOOLS FOR GENETIC ANALYSIS

morphological

- Classical Mendelian traits

biochemicals

- isoenzymes
- structural proteine (endosperm)

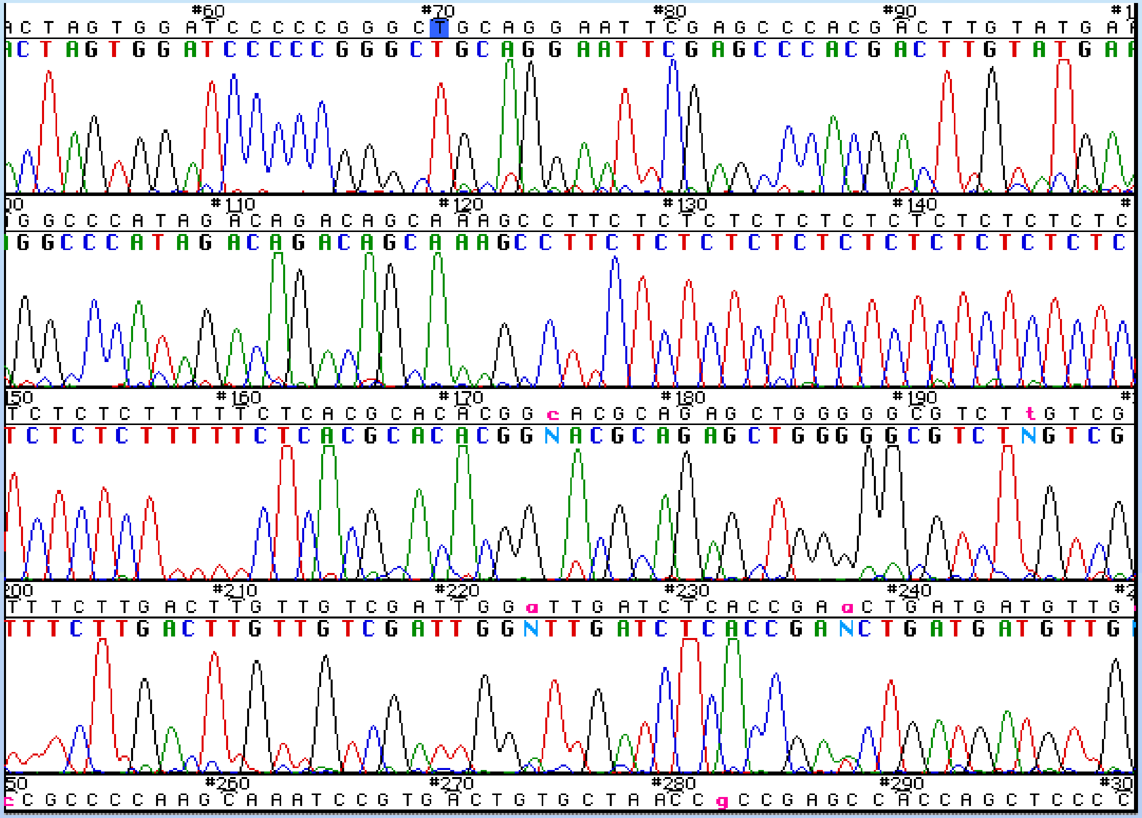
molecular

- based on DNA (RFLP, VNTR, RAPD, **microsatellites**, SSCP, AFLP, **Single Nucleotide Polymorphisms**

Ideal GENETIC Marker: Characteristics

- Mendelian behaviour
- Not influenced by the environment
- Highly polymorphic
- Easy to detect and analyze
- Robust
- Not expensive
- Automation

Simple Sequence Repeat - SSR – (Microsatellites)



METHODOLOGICAL BOX

POLYMERASE CHAIN REACTION – PCR –

Kary Mullis (1944 -)
American Biochemist
Cetus Corp.
Nobel prize in Chemistry in 1993

Polymerase: DNA polymerase

- DNA polymerase REPLICATES DNA

Chain Reaction: DNA polymerase

- The product of a reaction is used to amplify the products of each reaction

It is the PERFECT MEHODOLOGY to amplify regions containing SSRs and identify GENETIC VARIATION AT THE DNA LEVEL

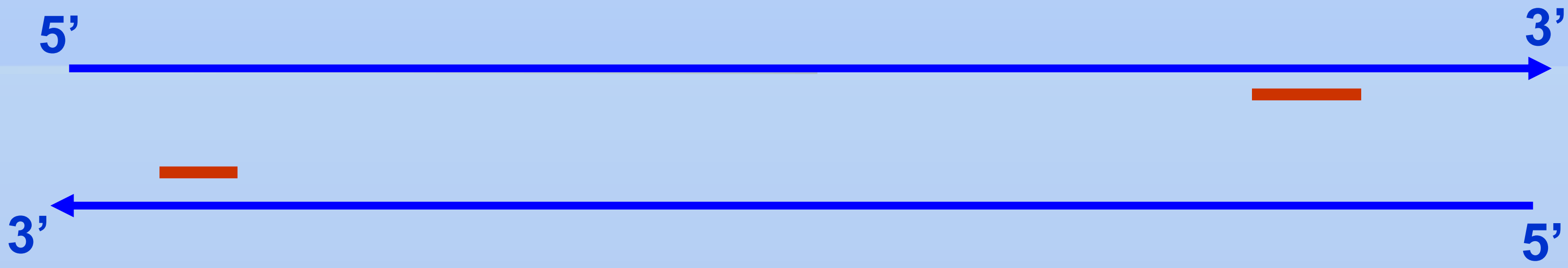
Properties of DNA polymearse

It needs a pre-existing DNA to duplicate

- Cannot assemble a new strand from components
- Called template DNA

It can only extend an existing piece of DNA

- Called primers

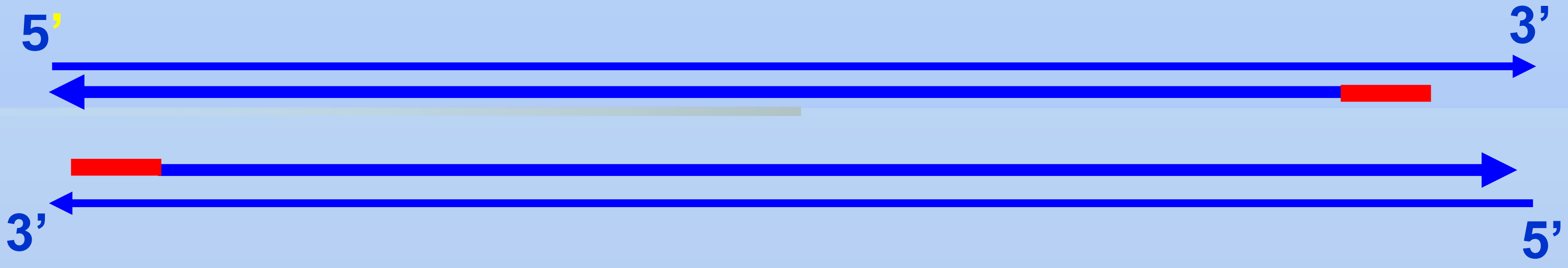


Properties of DNA polymearse

DNA strands are anti-parallel

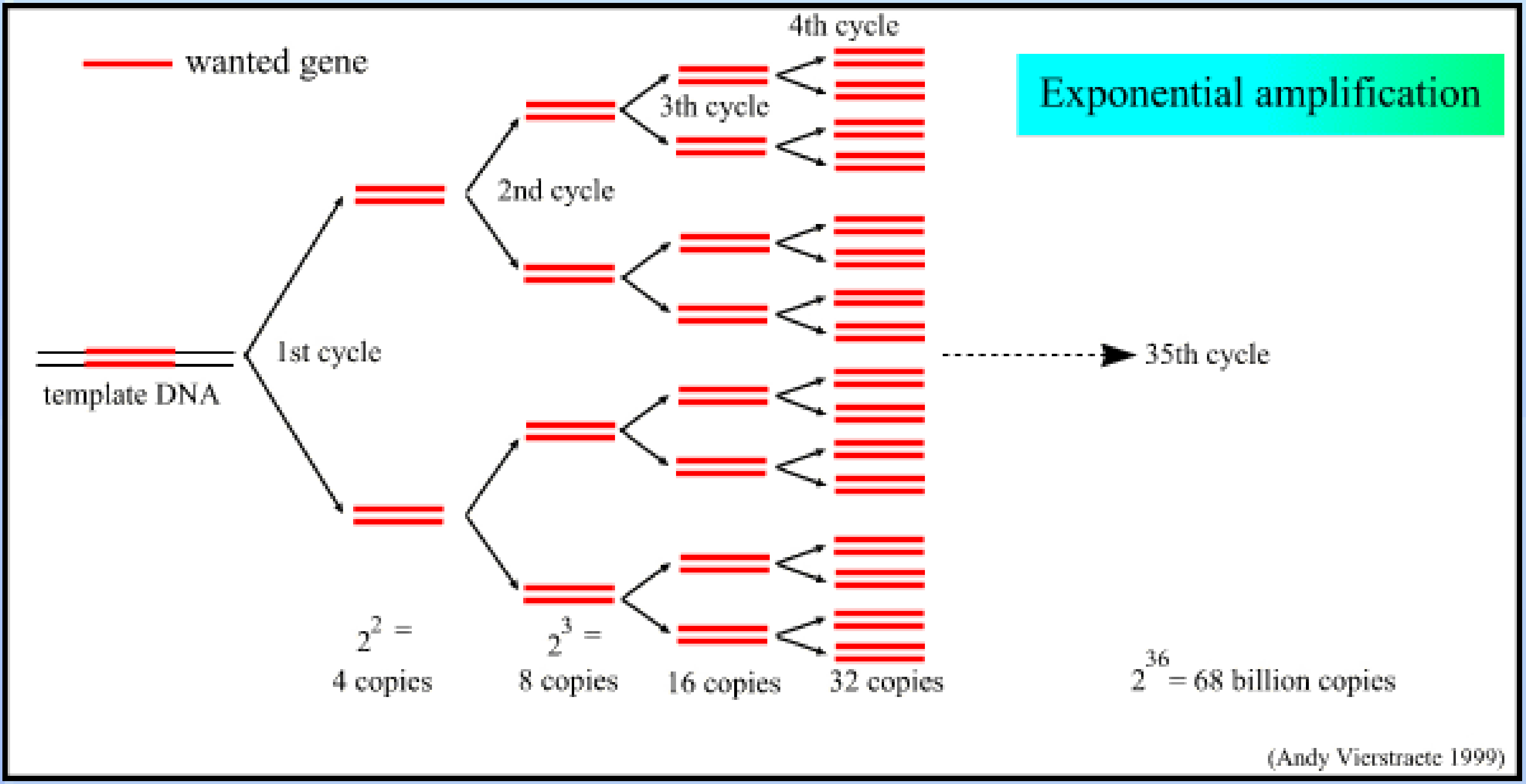
- One strand goes in 5' → 3'
- The complementary strand is opposite

DNA polymerase always moves in one direction (from 5' → 3')



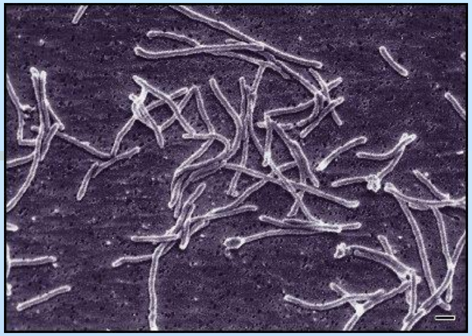
Properties of DNA polymearse

- The newly generated DNA strands serve as template DNA for the next cycle
- PCR is very sensitive
- Widely used



Taq DNA polymerase

- Derived from *Thermus aquaticus* thermophylus bacterium
- Heat stable DNA polymerase
- 1000 nt /sec at 72°C
- No proof reading activity



Lower Geiser at Yellowstone

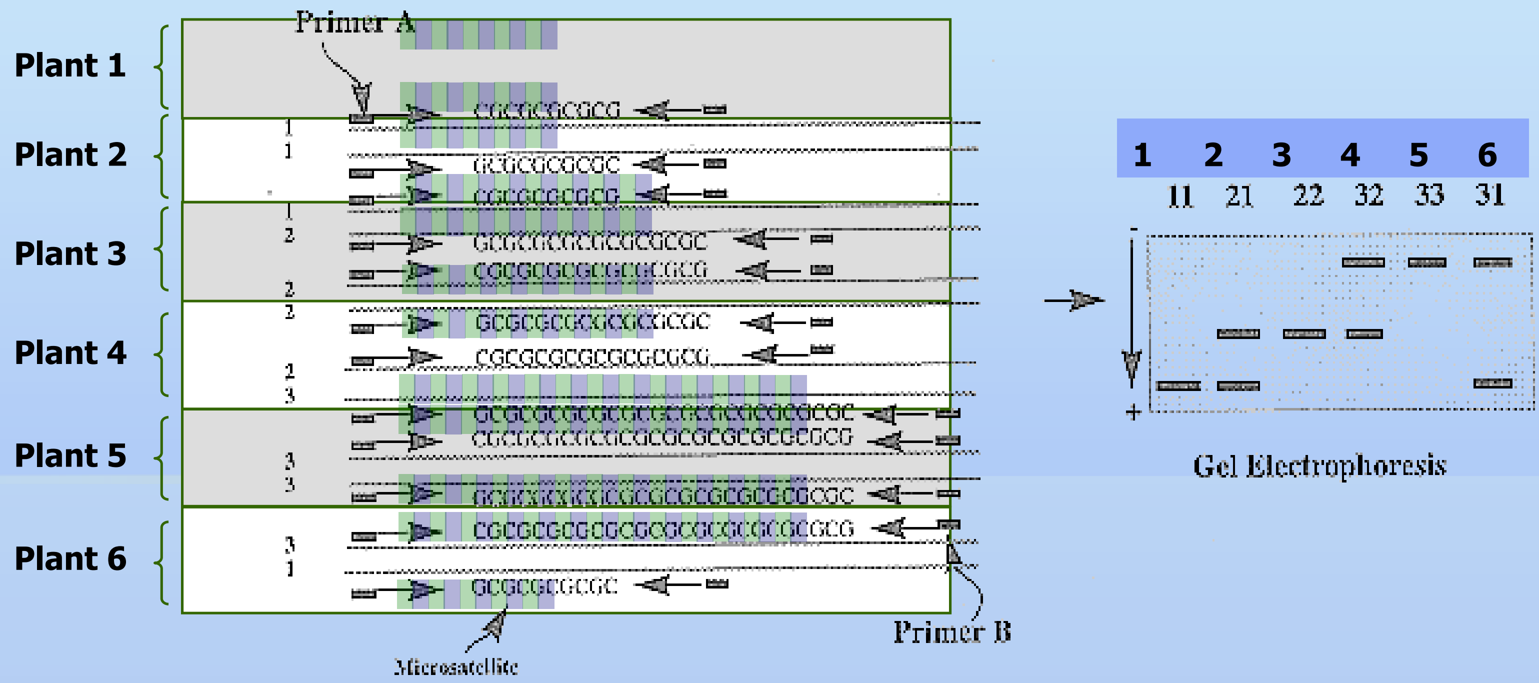
Thermal Cycling

A PCR machine controls temperature

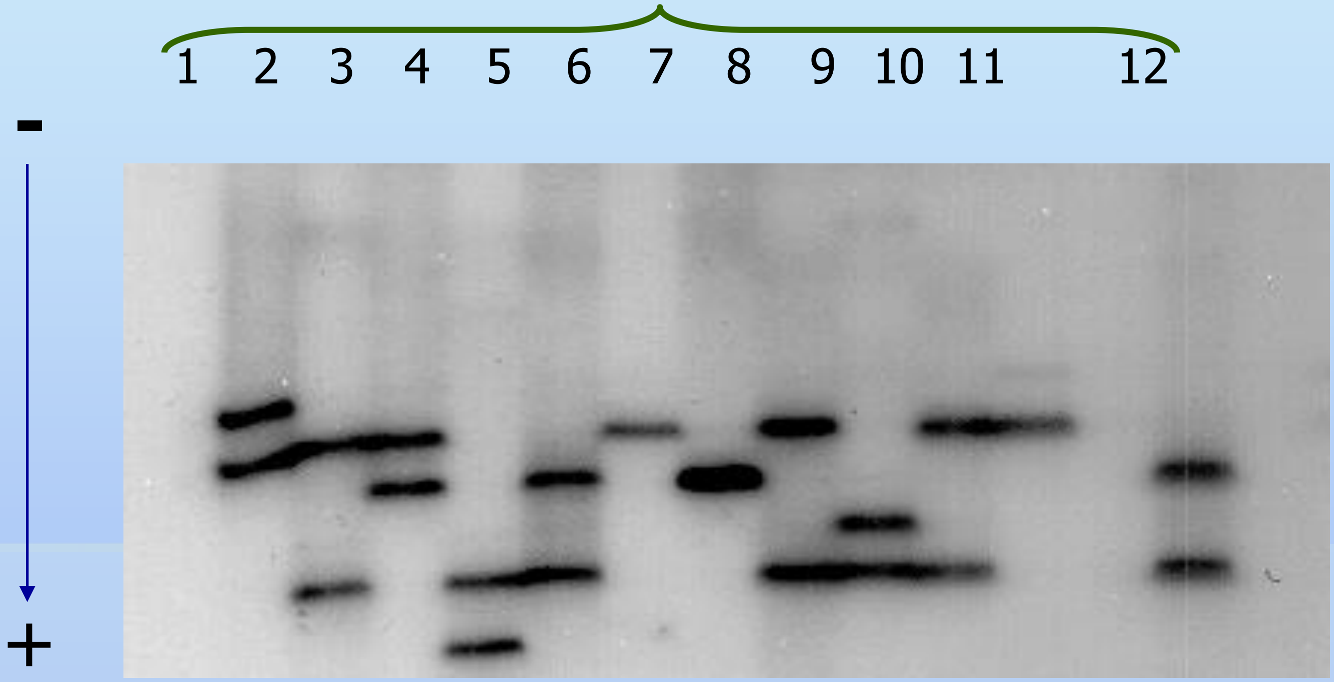
Typical PCR go through three steps

- **Denaturation**
- **Annealing**
- **Extension**

Simple Sequence Repeats - SSR – (Microsatellites)



SSR Analysis of different individuals from a natural population



Separation of different alleles by PAGE

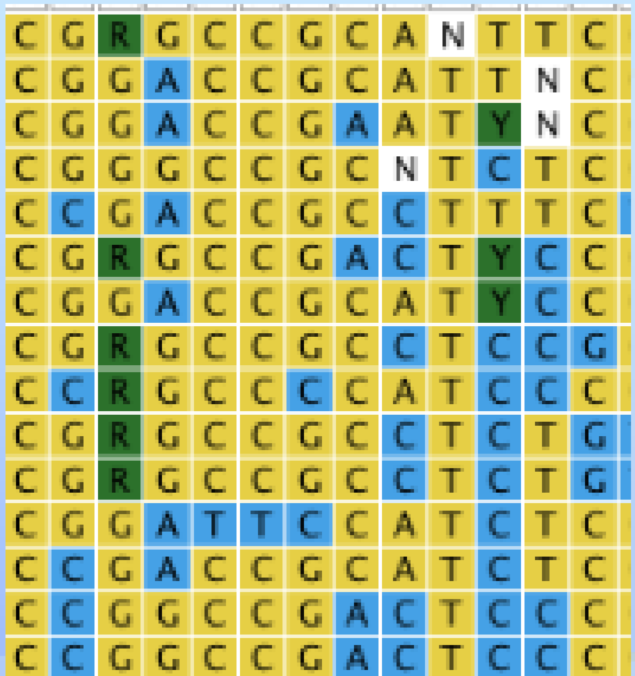
Concept of Polymorphisms

- Several alleles for a single gene
- Polymorphism derives from mutations which spread in the population
- By definition a polymorphic allele > 1% in the population

HOW MANY POSSIBLE ALLELES FOR A GENE?

Multi-allelism is a population genetics concept

Single Nucleotide Polymorphism - SNP -

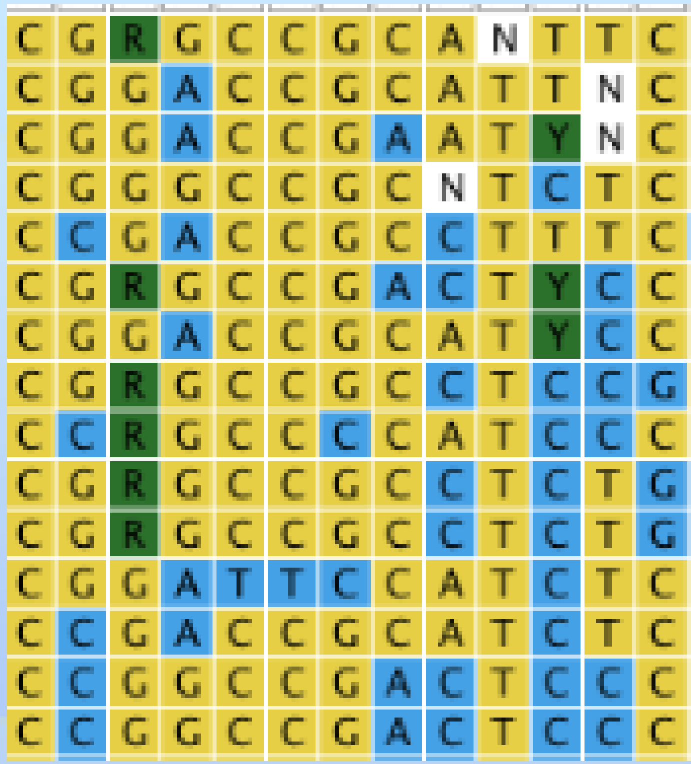


...C C **A** T T G A C...
...G G **T** A A C T G...

...C C **G** T T G A C...
...G G **C** A A C T G...

- **Identified by sequencing**
- **Their frequency depends on several factors**

Allele Identification is NOT the Bottleneck Anymore



In the post genomics era the challenge is to assign these sequences a meaning

Molecular Markers Applications

- **Population Analysis**
  - Population Genetics
  - Taxonomy and Evolution
- **Mapping**
  - Linkage maps
  - Mapping of single genes
  - Mapping of complex traits (QTL)
  - Dignostics
  - Marker assisted breeding
  - Gene Isolation
- **Fingerprinting**
  - Cultivar Identification
  - Forensic
  - Germplasm characterization

GENETIC MAP

Map

- **It defines linear relationships among *loci***
- **Produced by genetic analyses**
- **Distance between genes is measured as frequency of recombination**

IT IS THE RESULT OF GENETIC EXPERIMENTS

1 centi-Morgan (cM) = 1 recombinant / 100 meiotic prodoucts

Polymorphism

- **Allelic Variants for a specific gene**

Molecular Polymorphism

- **Differences at the DNA level**

Molecular Marker

- **Genetic *locus* that identifies a position on the genetic map**

Relevance of a Genetic Map

- Integration with known mutants
  - ⇨ FUNCTION
- Quantitative Traits
  - ⇨ IMAPPING PHENOTYPES
- Genomic Organization
  - ⇨ COMPARATIVE STUDIES

Genetic Maps are an application of FORWARD GENETICS

STEPS

1. Identification of polymorphism
   → Molecular markers generation
2. Selection of parental genotypes and breeding
3. Production of a segregating population
   → F2 – Backcross – etc.
4. Genotyping of single individuals in the population
   → Alleles at polymorphic *loci*
5. Analysis of segregation data
   → Genetic Map

Types of Mapping Populations

F_2

- All heterozigous *loci* segregate in a single meiosis

Back-cross = F_1 x P_x

- Only the alleles of the NON recurrent parent segregate

Ricombinant Inbred Lines (RIL)

- All heterozigous *loci* segregate in multiple meiosis

Pseudo back-cross

- F1 x C where C is NOT homozygous

Recombinant Inbred Lines (RIL)