

Genetics in Dentistry – Practice















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* 20. 7. 1822, Heizendorf

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- Founder of genetics
- Discoverer of basic principles of inheritance
 - Principle of F1 generation uniformity
 - Principle of random segregation of genes into gametes
 - Principle of independent assortment of alleles

	Flower Colour	Plant Height	Seed Color	Seed Shape	Pod Colour	Pod Shape	Flower Position
Dominant Trait	 Purple	 Tall	 Yellow	 Round	 Green	 Inflated (full)	 Axial
Recessive Trait	 White	 Short	 Green	 Wrinkled	 Yellow	 Constricted (flat)	 Terminal

Experiments on Plant Hybrids (1866)



Basic Concepts

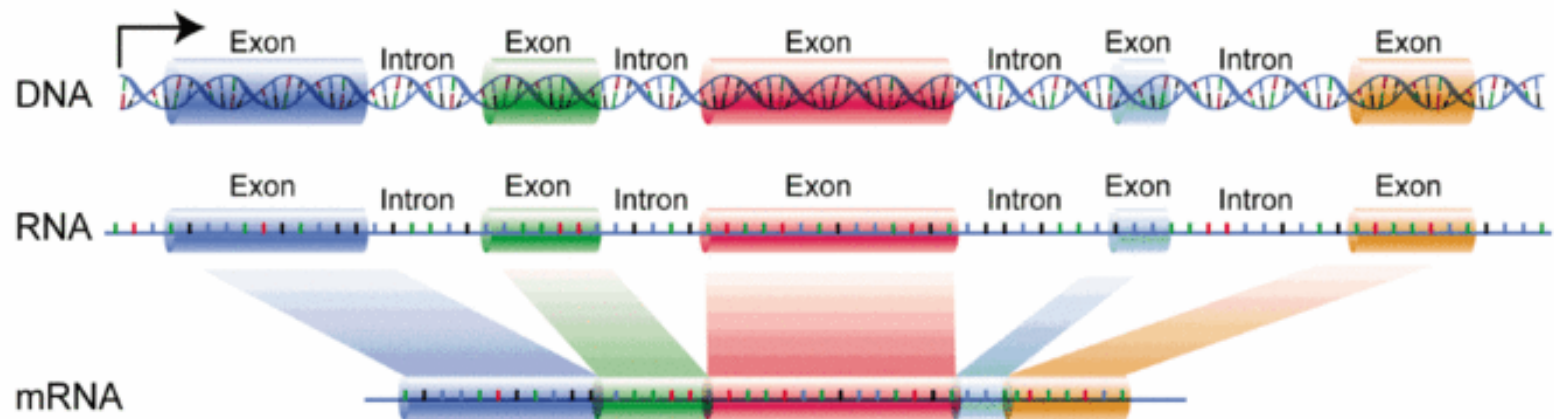
- **Genetics**

- Science field examining heredity and variability of quantitative and qualitative traits of all living organisms

- **Gene**

- Basic unit of heredity (genetic information)
- Sequence of DNA molecule carrying the information for production of protein or nucleic acid
- Consists of exons and introns

structural
functional



Basic Concepts

- **Chromosome**

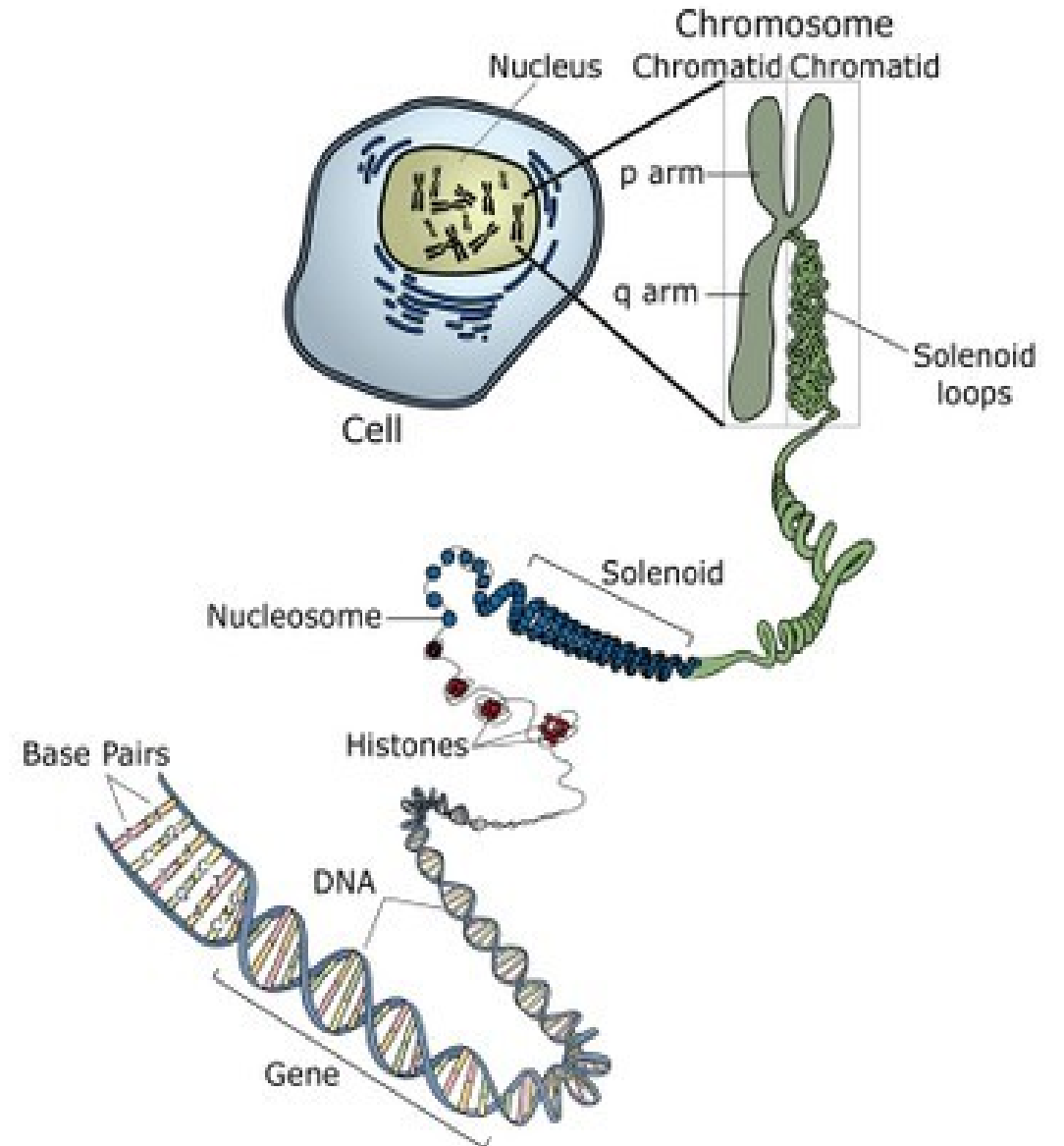
- Functional unit of hereditary record of genetic information in a cell
- Cell nucleus → 22 pairs of autosomes + 1 pair of gonosomes

- **Locus**

- Position of a gene on a particular location on a specific chromosome

- **Allele**

- Specific variant of the gene



Basic Concepts

- **Genomics**

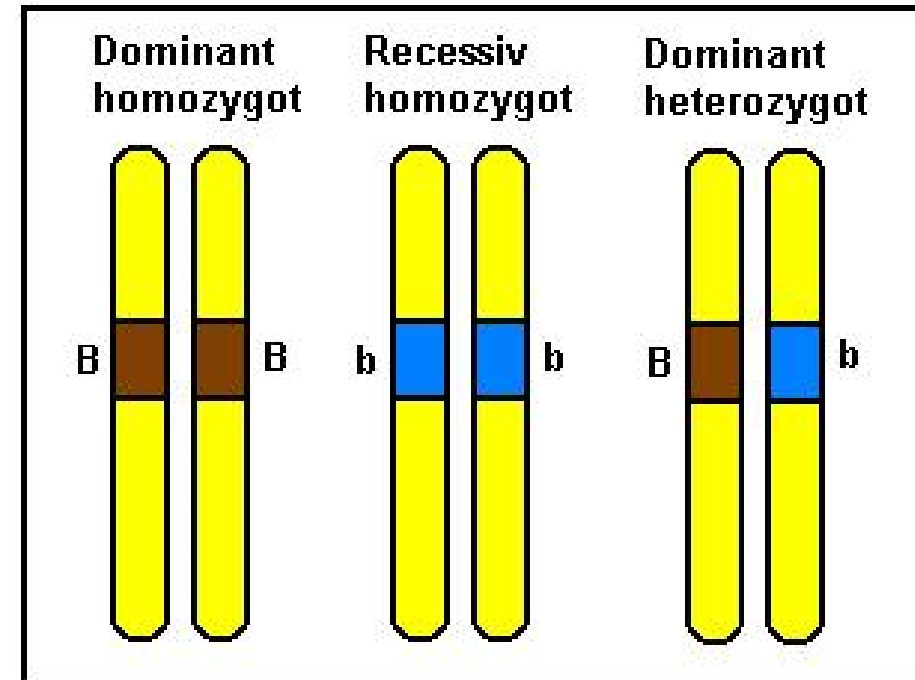
- Field of genetics trying to determine the whole genetic information of an organism and to interpret it in terms of life processes

- **Heterozygote**

- Two different variants (alleles) of a particular gene or its part

- **Homozygote**

- Two same variants (alleles) of a particular gene or its part



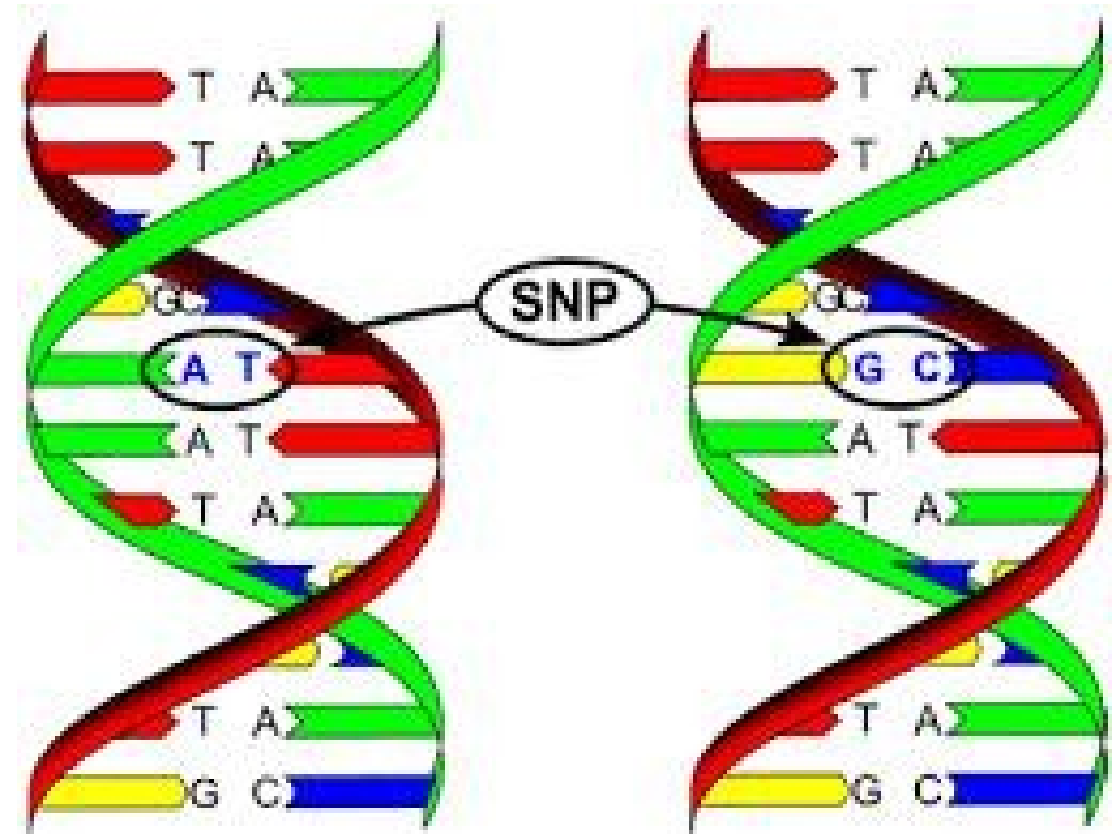
Basic Concepts

- **Polymorphism**

- Existence of several (at least two) alleles for a specific gene, of which the least common one has population frequency $\geq 1\%$

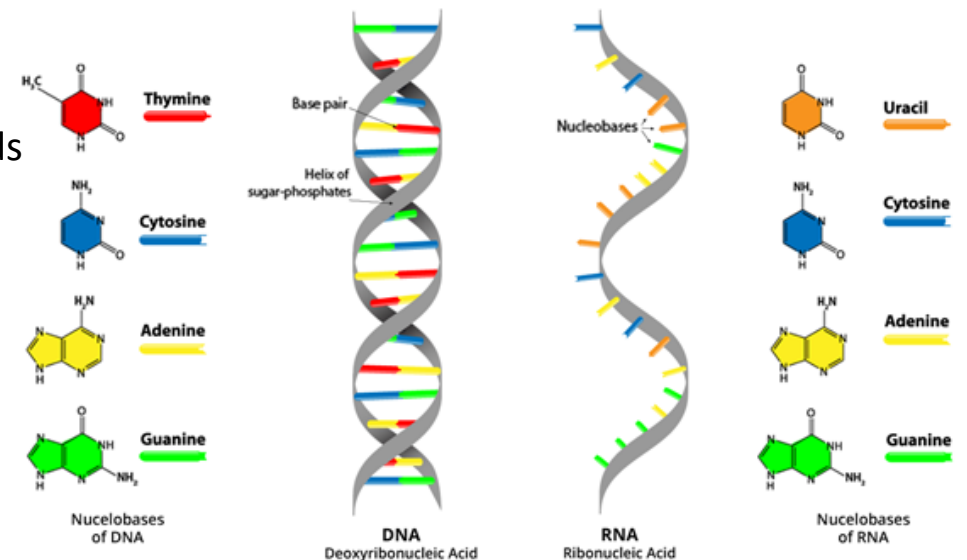
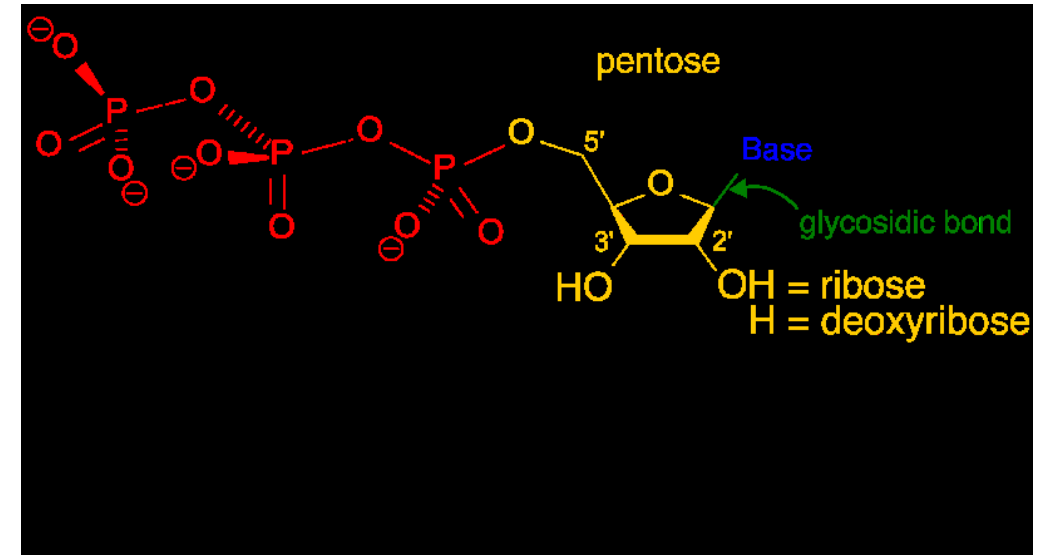
- **Mutation**

- Processes, during which changes in genotype occur due to different environmental factors
- Less common allele has population frequency $< 1\%$

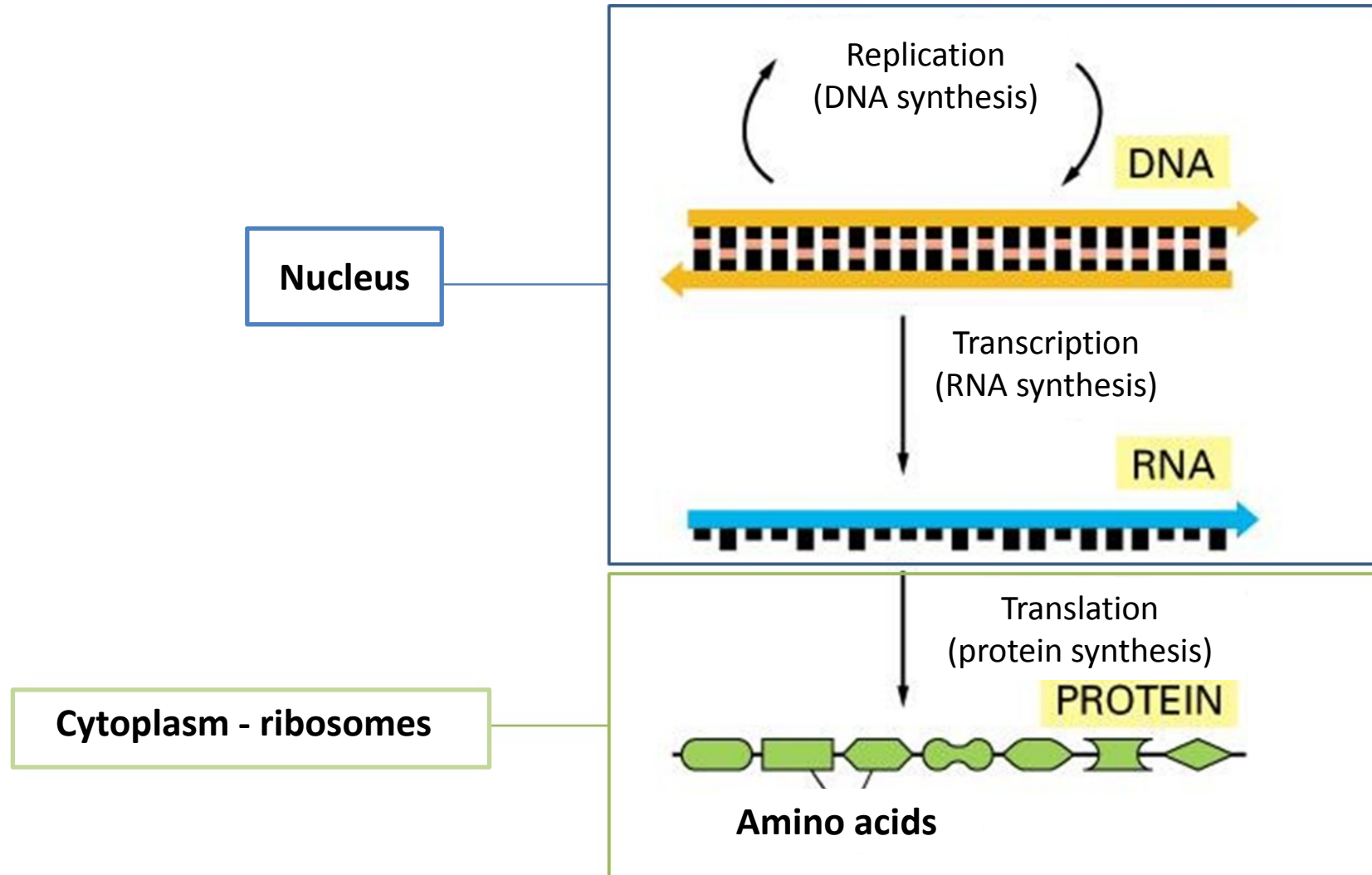


DNA vs. RNA

- DNA molecule = **deoxyribonucleic acid**
 - Double helix – 2 strands in opposing directions
 - Polynucleotide chain
 - Nitrogenous bases (T, A, C, G) connected with hydrogen bonds
 - Phosphate group
 - Sugar – deoxyribose
- RNA molecule = **ribonucleic acid**
 - One strand
 - Polynucleotide chain
 - Nitrogenous bases (U, A, C, G) connected with hydrogen bonds
 - Phosphate group
 - Sugar – ribose
 - Types – mRNA, tRNA, rRNA



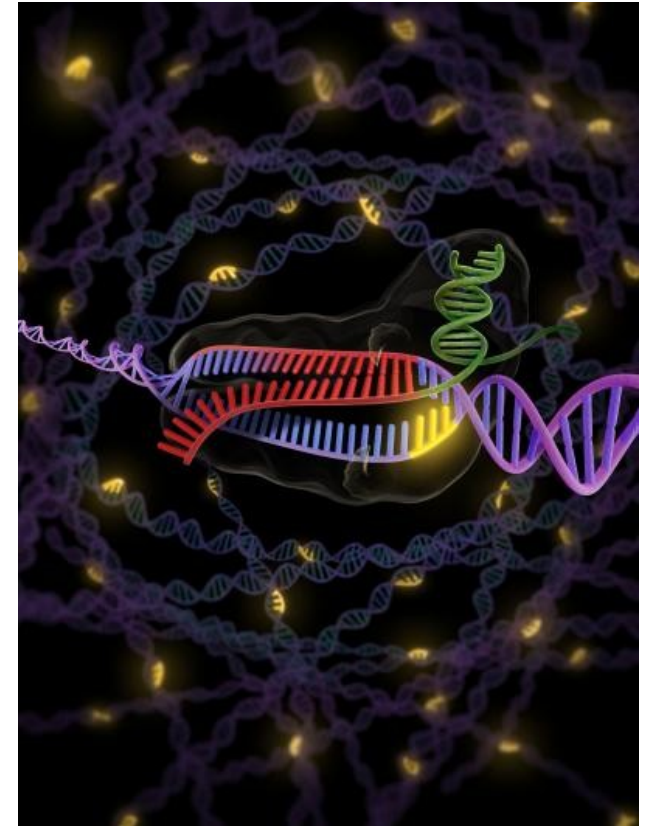
Central Dogma of Molecular Biology



DNA Replication

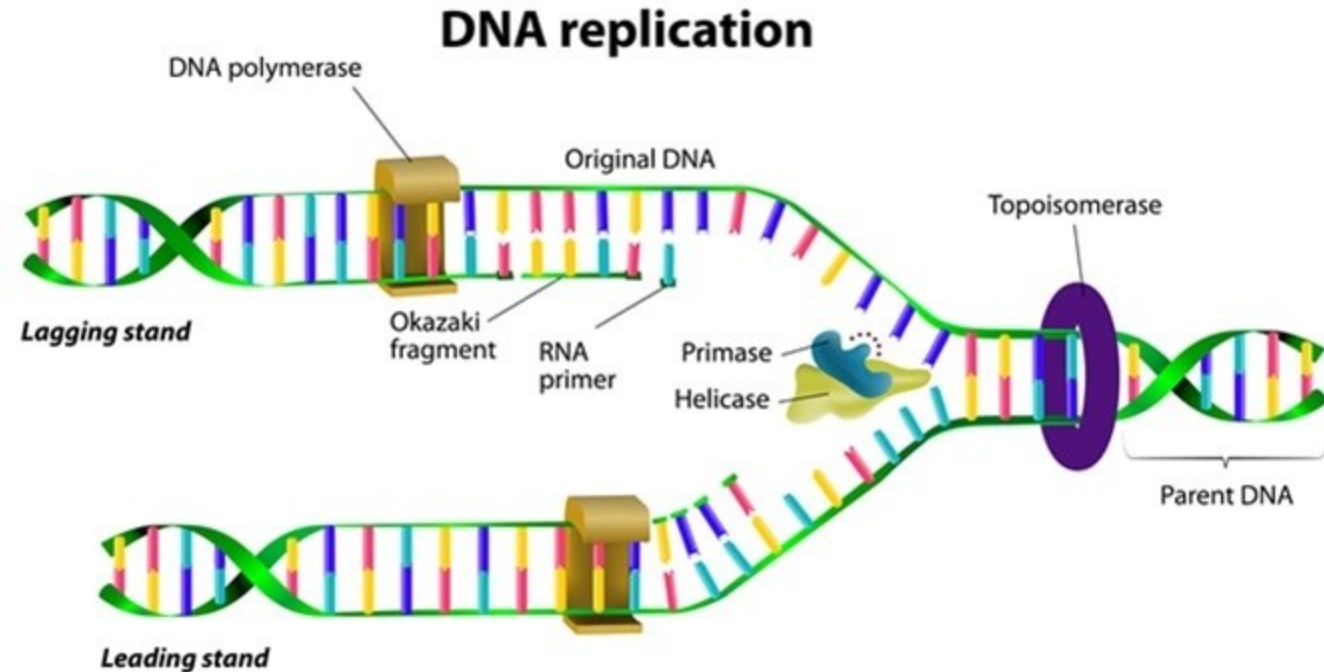
= **production of copies** of DNA molecules providing genetic information transmission from parental to daughter cell

- S-phase of cell cycle
- semiconservative process – 1 new + 1 old strand
- Components required for replication
 - template – parental strand
 - primer – short oligonucleotide with free 3'OH end
 - enzymes
 - nucleotides



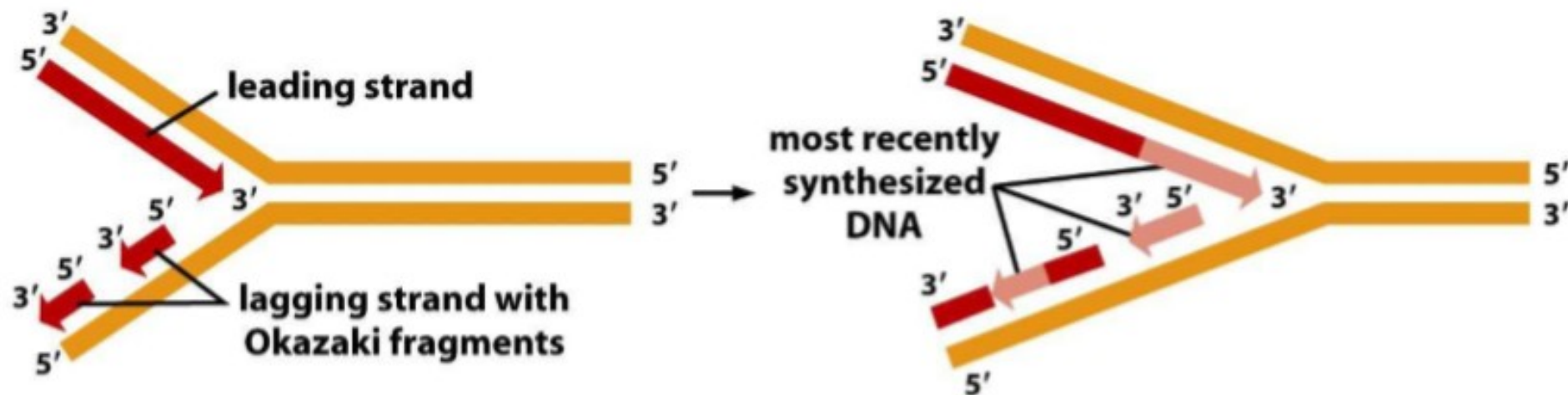
DNA Replication

- Forming of the replication fork
 - **helicase** – allows separation of both molecules of the double-helix
 - **SSB proteins** – helps keep strands separated
- **DNA primase** – production of RNA primers
- Replication is started in specific locations - **replication origins**
- **DNA polymerase** – catalyzes elongation of the strand
 - sequence of the new strand according to the principle of complementarity of bases - **adenine + thymine** (2 hydrogen bonds) and **cytosine + guanine** (3 hydrogen bonds)
 - synthesis from 5' end to 3' end



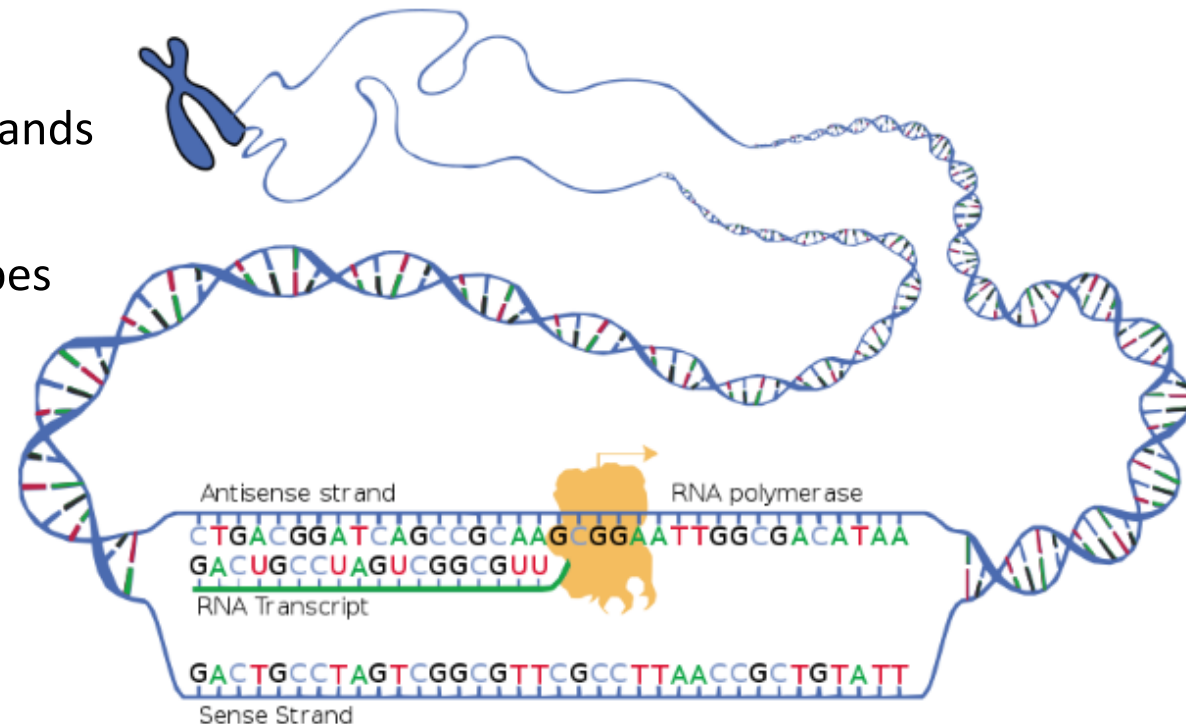
DNA Replication

- Template strands are **antiparallel** – one strand is lagging
 - Leading strand – one RNA primer at the beginning, replication without interruption
 - Lagging strand – in direction 5'-3' are discontinuously produced short **Okazaki fragments** (every from a new RNA primer), later connected by **DNA ligase**
- RNA primers are removed by 5'-3' exonuclease activity and replaced by 3'-5' polymerase activity



Transcription

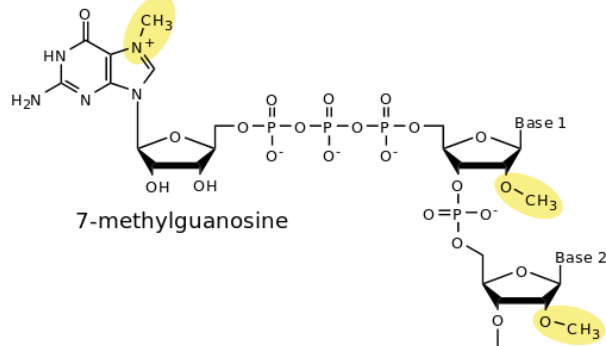
- **transcription** of the information in a form of DNA sequence to RNA sequence
- cell nucleus
- **template** - DNA strand
- transcripts are from the template released as single strands
- **DNA-dependent RNA polymerase**
 - 3 types (similar structure, transcribing different types of genes)
 - RNA pol. I (genes coding rRNA)
 - RNA pol. II (genes coding hnRNA)
 - RNA pol. III (genes coding tRNA)
 - requires presence of transcriptional factors (separating DNA strands, placement of RNA polymerase to promoter and releasing from promoter)
 - **Promoter** = starting point on DNA – TATA box, CAT box
 - **Terminator** = ending point - AAAA



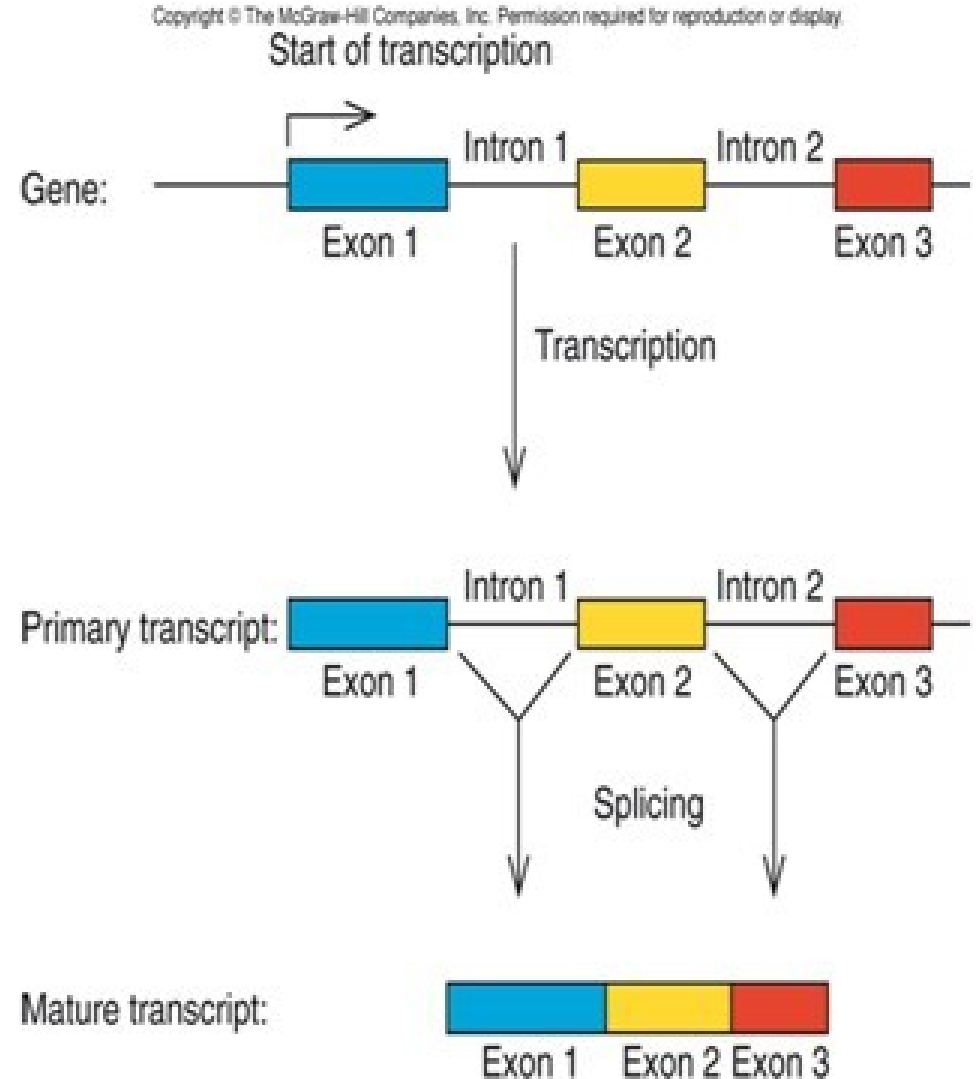
Post-transcriptional Modification

- Modification of primary transcripts:
 - addition of a **cap** to 5'end (helps controlling mRNA translation)

Obrázek 3 - Struktura tzv. RNA čepičky



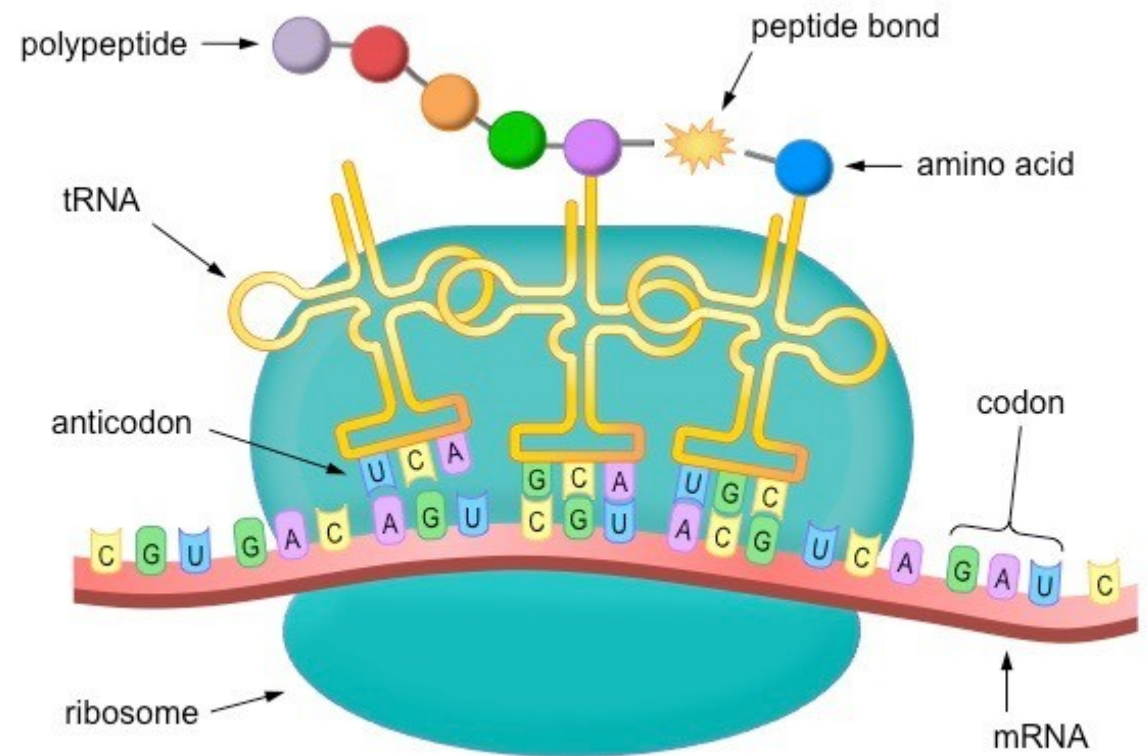
- connection of **polyadenylic chain** to 3'end
- **RNA splicing** – cutting out of introns to form mature mRNA



Translation

- **Translation** of genetic information from mRNA to AA sequence in polypeptide (using genetic code)
- Occurs on ribosomes in cell cytoplasm

- Phases – initiation, elongation, termination
- **Enzyme - Aminoacyl-tRNA synthetase**
- Initiation complex is formed at the 5'end of mRNA (cap), searching mRNA from 5'end, looking for **initiation codon AUG**
- Termination of translation: **UAA, UAG, UGA**
- **Post-translational modifications** – phosphorylation, glycosylation, methylation,



Genetic Code

- **System** for adding specific AAs to polypeptide chain according to mRNA sequence
- **Triplet** = codon – defines AA or terminates translation
- Every AA defined by one or several codons in mRNA
- **64 possible triplets:** 61 define AA, 3 terminate translation
- Codons are recognized by complementary sequences in tRNA (anticodons), which carry specific AAs on 3' end
- Insertion/deletion of one/two base pairs
changes reading frame
- **(almost) universal, degenerate**

RNA codon table

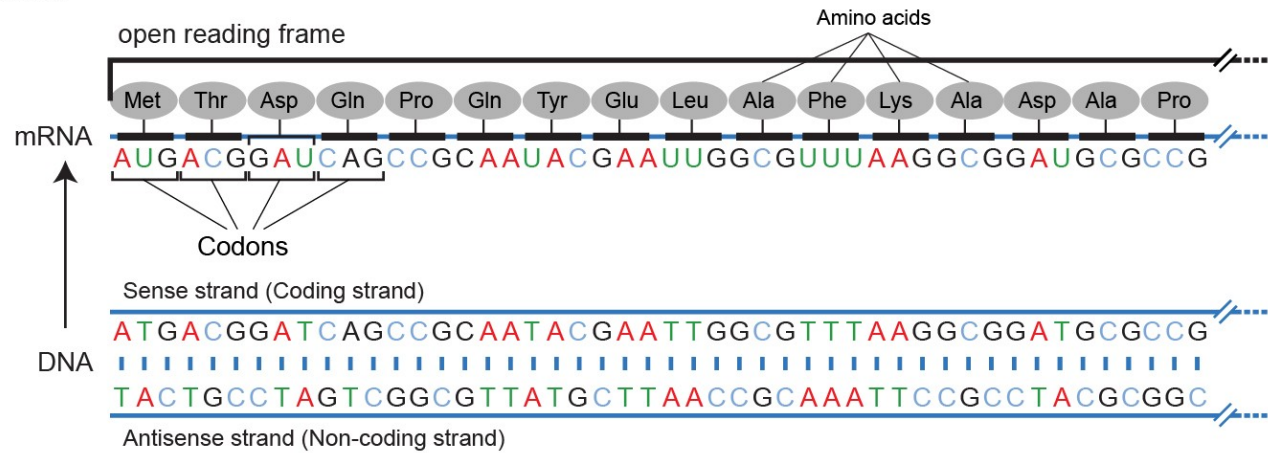
1st position	2nd position				3rd position
	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr stop stop	Cys Cys stop Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Amino Acids

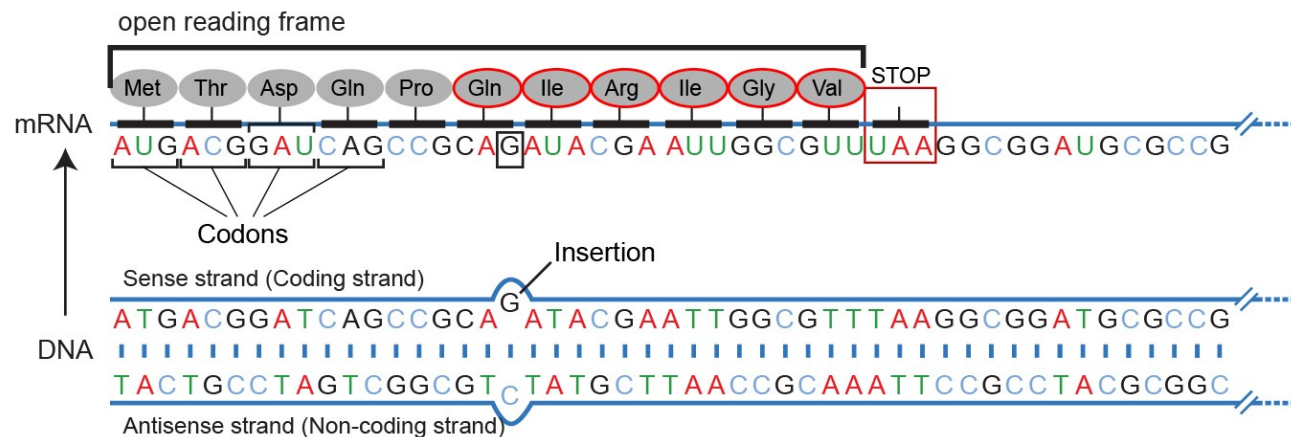
Ala: Alanine	Gln: Glutamine	Leu: Leucine	Ser: Serine
Arg: Arginine	Glu: Glutamic acid	Lys: Lysine	Thr: Threonine
Asn: Asparagine	Gly: Glycine	Met: Methionine	Trp: Tryptophane
Asp: Aspartic acid	His: Histidine	Phe: Phenylalanine	Tyr: Tyrosine
Cys: Cysteine	Ile: Isoleucine	Pro: Proline	Val: Valine

Genetic Code – Reading Frame Shift

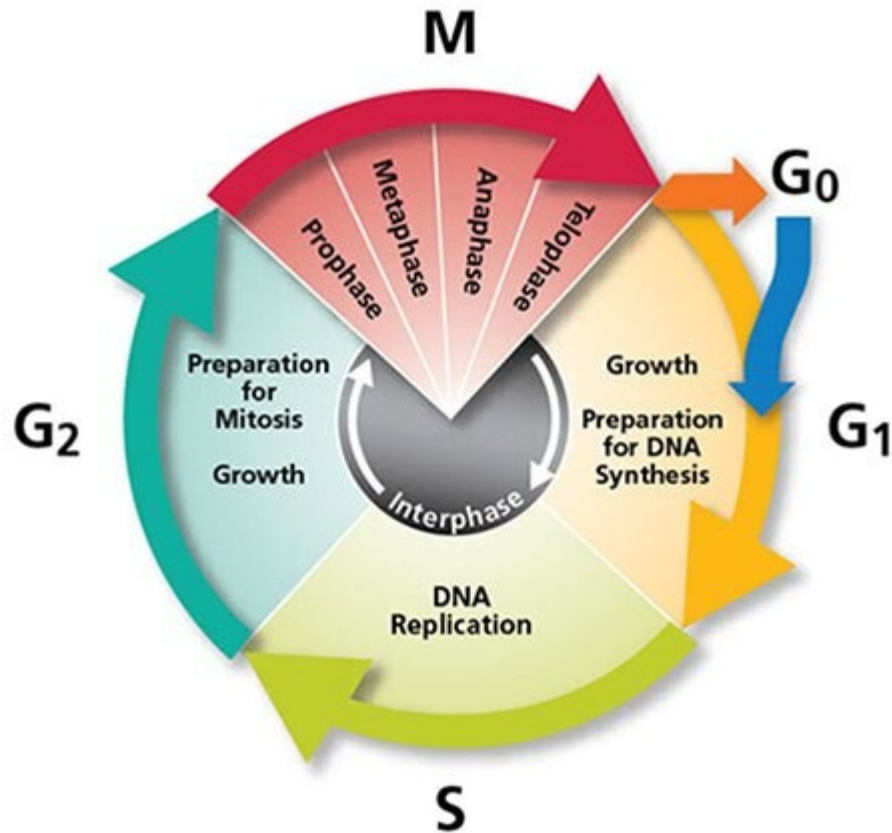
Normal



Frameshift mutation - single nucleotide insertion



Cell Cycle



= organized sequence of processes during which the cell doubles its content and subsequently divides into two daughter cells (both of them carry the same chromosomes)

Aim: Reproduction of genetic material for the next generation of cells

Cell Cycle

- **Single cell organisms**
 - coordinated with growth – parental cell must reach certain size to divide
- **Multicellular organisms**
 - coordination of DNA replication with the developmental program of the cell
 - coordination of replication and division of every cell with the development of respective tissues or organs
 - **maturity** – cells divide when necessary (replacement of dying cells, repair of damaged tissues)
 - loss of control over cell cycle -> **cancer**

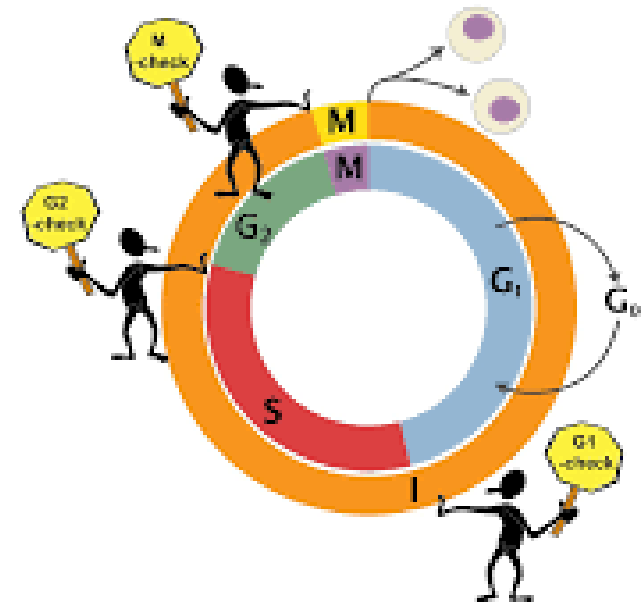
Cell Cycle

- **high accuracy requirements**

- flawless replication
- correct sequence of phases
 - mitosis before finished replication -> loss of genetic information in at least one cell
 - double replication before mitosis -> increased number of gene copies in a particular part of chromosome -> instability in gene expression, low viability
- precise segregation of chromosomes
- coordination with developmental programs

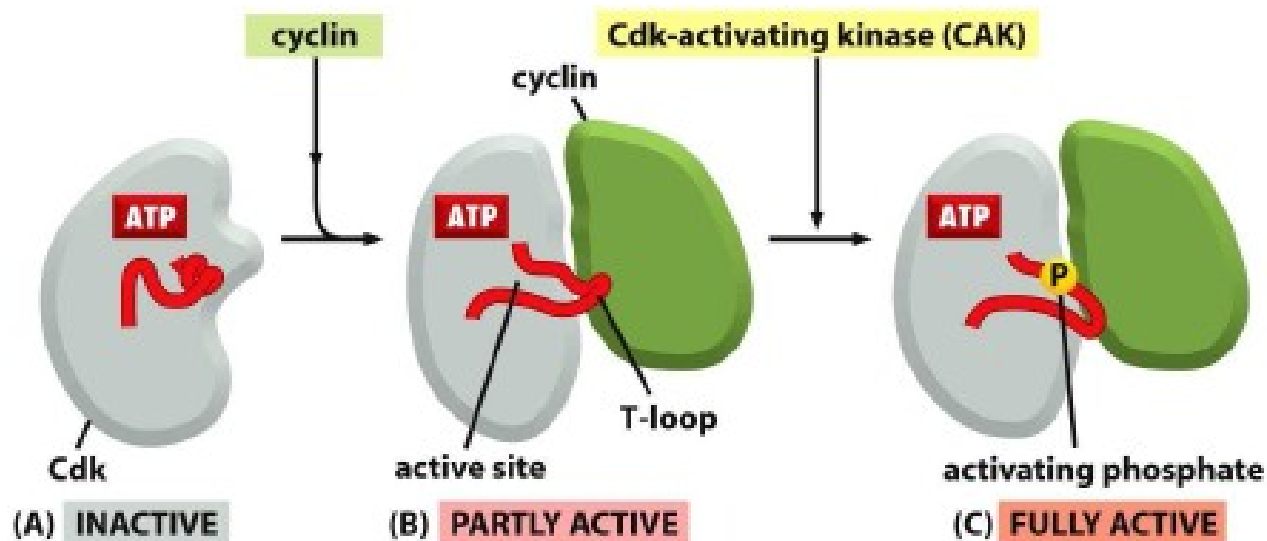


checkpoints



Cell Cycle

- controlling elements – **cyclin-dependent kinases**
 - control the activity** of many proteins connected to DNA replication and mitosis *via phosphorylation* in specific sites (activation/inactivation)
- Cyclin + CDK -> complex connects to protein -> protein phosphorylation -> after phosphorylation, complex breaks down and the activity of the protein changes



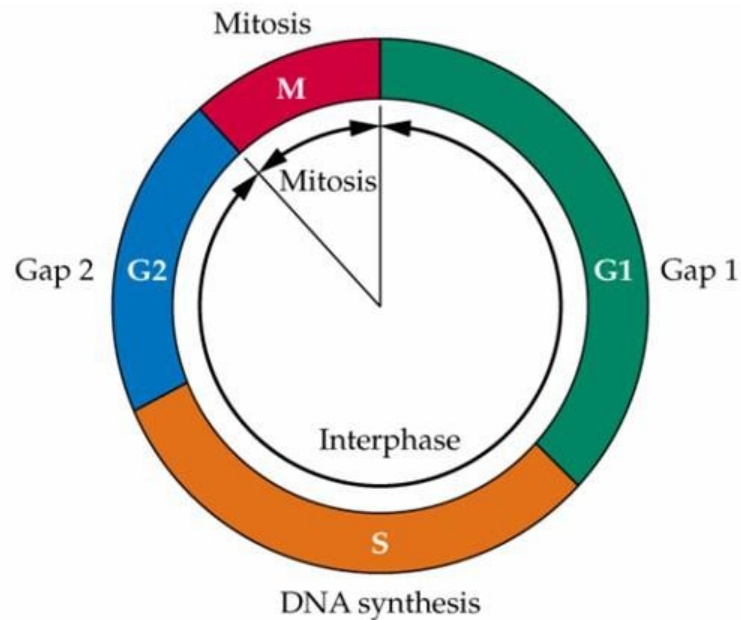
Cell Cycle - Interphase

Interphase – G1, G2, S

- **preparation** for cell division, outer nuclear membrane connected to ER
- **unfavorable conditions**
 - stopping in G1/entering G0; cells do not grow, may stay suspended for several months/years

- **G2-phase**
 - double the amount of DNA (compared to G1)
 - synthesis of proteins necessary to enter mitosis

- **S-phase**
 - DNA replication
 - synthesis of proteins associated with DNA



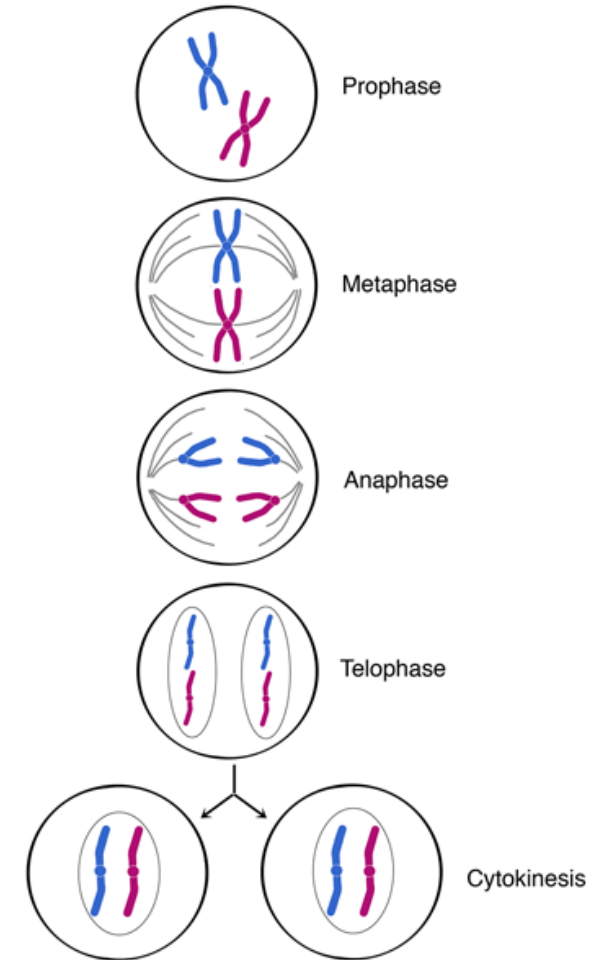
- **G0-phase**
 - most of cells in multicellular organisms (differentiated and specialized cells performing one function, do not divide)
 - after receiving pro-growth factor may enter back into the cell cycle
- **G1-phase**
 - the longest and the most variable
 - the cell **grows and doubles its organelles**
 - checkpoint at the end of this phase:
 - restriction point**
 - the cell has abundant nutrients and growth factors, shows high metabolic activity -> passes the restriction point and enters the next phase
 - nutrient deficiency, anti-proliferative signals -> slowing down of the phase progression/exiting of the cell cycle (entering G0)

Cell Cycle - Mitosis

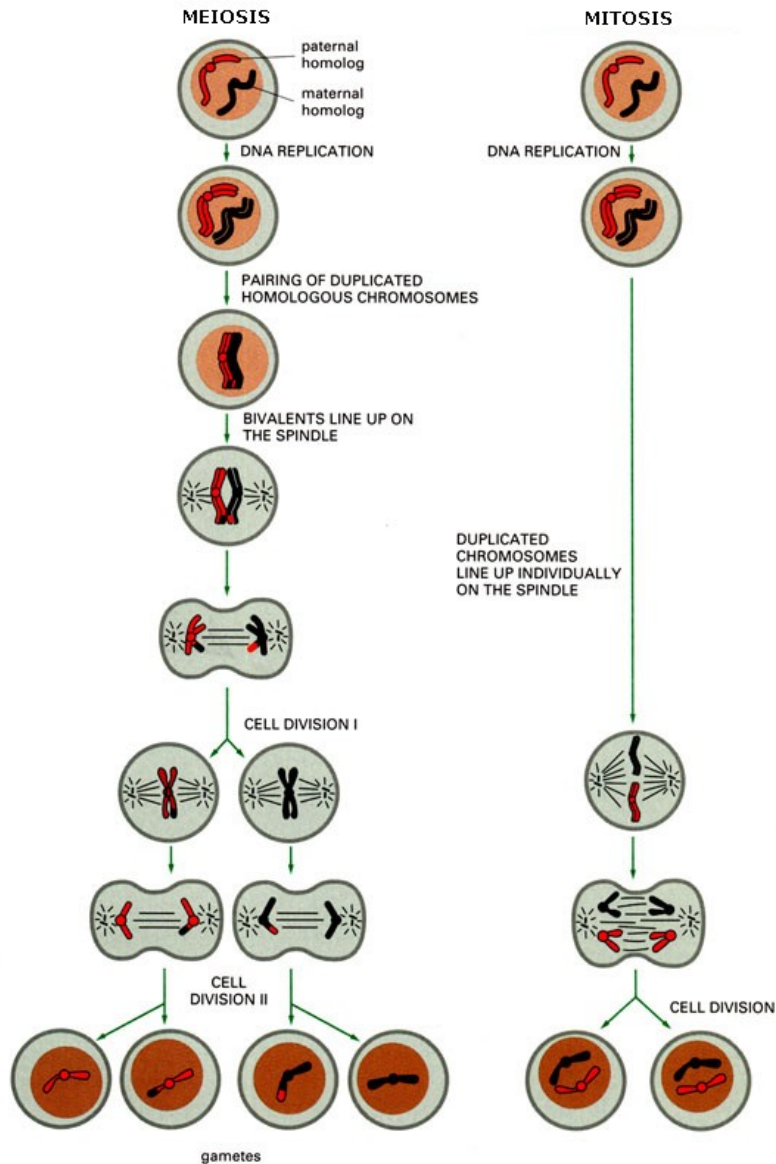
- nuclear division (mitosis) + subsequent cytoplasm division = cytokinesis

Mitosis

- division of somatic cells
- product - **two diploid** cells with identical genetic information
- **prophase** – spiralization of DNA strands, formation of the mitotic spindle
- prometaphase – breakdown of the nuclear membrane
- **metaphase** – formation of kinetochore on every centromere, connection of chromatids to spindle (equatorial plane)
- **anaphase** – separation of chromatids to the opposing poles of the cell
- **telophase** – finalization of chromatid separation, breakdown of spindle, recovery of the nuclear membrane



Mitosis vs. Meiosis



Mitosis = 2 daughter cells with diploid number of chromosomes; 1 cycle of DNA replication, followed by separation of chromosomes and nuclear division (prophase → prometaphase → metaphase → anaphase → telophase) and subsequently the whole cell division (cytokinesis)

Meiosis = 1 cycle of replication followed by 2 cycles of chromosome segregation and cell division, formation of haploid gametes

first meiotic (reductional) division – separation of homologous chromosomes; meiotic crossing-over occurs here (gene recombination) – no two gametes are identical!
separation disorders – e.g. trisomy.

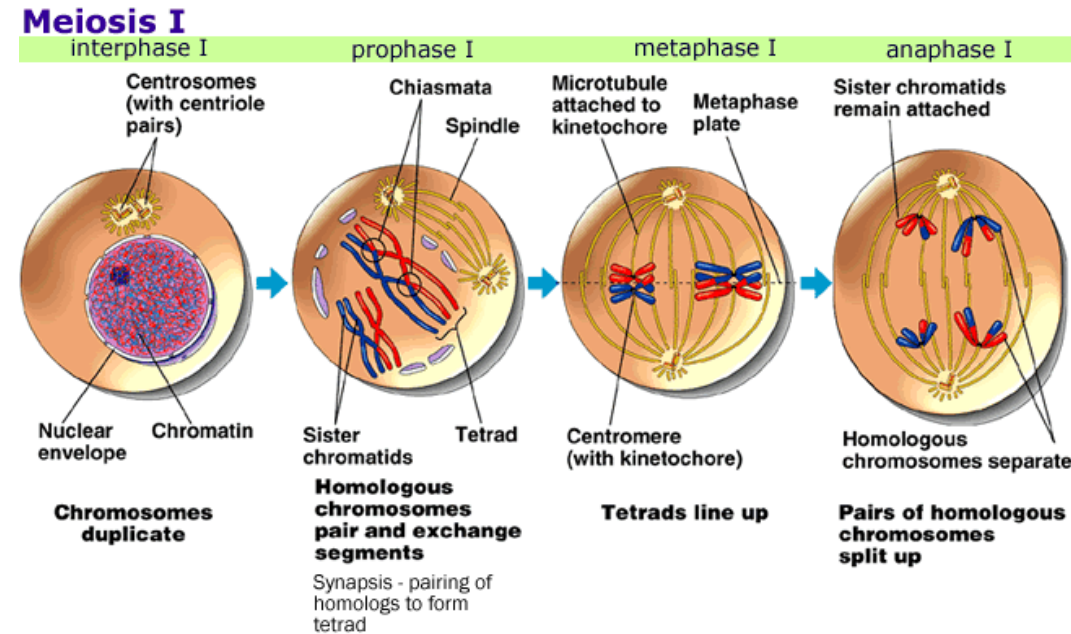
second meiotic division – separation of daughter chromatids -> 2 daughter cells with haploid number of chromosomes, formation of germ cells (sperm, egg), further swapping of genetic material by crossing-over

Meiosis

- formation of 4 haploid gametes (germ cells)
- genetic variability

Heterotypic division

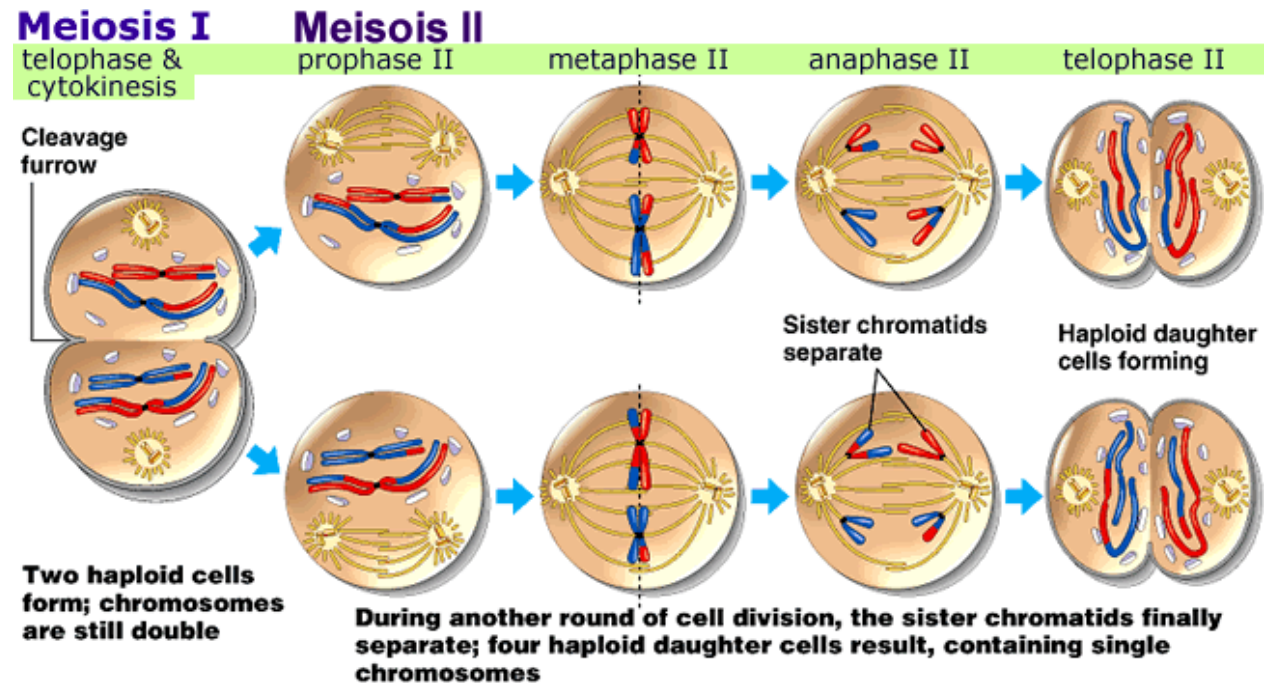
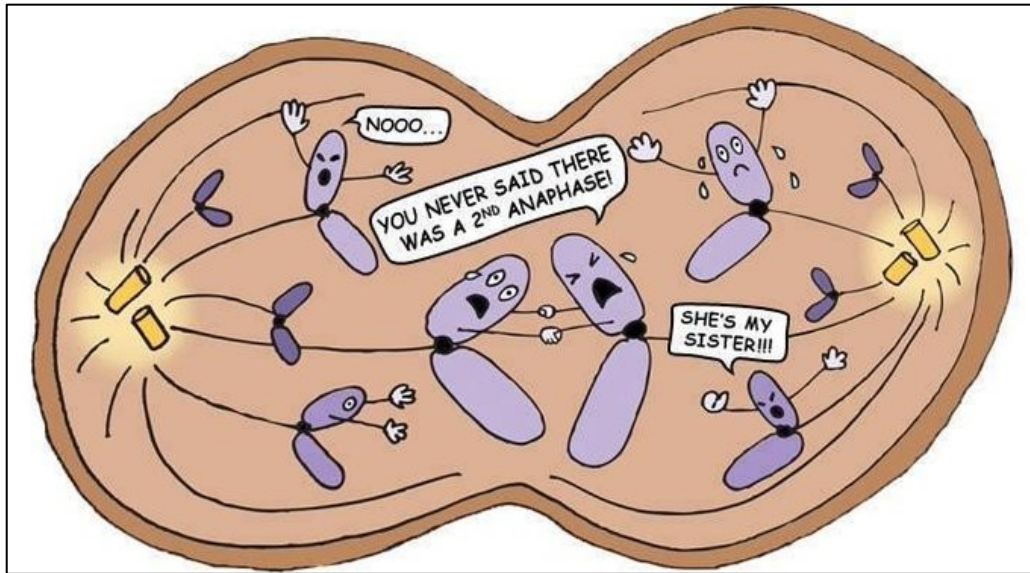
- **Prophase** – 5 stages
 - **Leptotene** – spiralization of chromosomes
 - **Zygotene** – formation of bivalents
 - **Pachytene** – **crossing over** = crossing of non-sister chromatids
 - **Diplotene** – gradual separation of homologous chromosomes
 - **Diakinesis** – dispersion of nuclear membrane, formation of spindle apparatus
- **Metaphase** – formation of equatorial plane
- **Anaphase** – division of $2n$ chromosomes, separation *via* spindle
- **Telophase** – formation of nuclear membrane, breakdown of spindle



Meiosis

Homeotypic division

- **Prophase** – spiralization of chromosomes, formation of the spindle, dispersion of nuclear membrane
- **Metaphase** – formation of equatorial plane, connection to spindle
- **Anaphase** – separation of chromatids from divided chromosomes
- **Telophase** – formation of nuclear membrane, breakdown of spindle, despiralization of chromosomes



Mutation

Mutations are genetic changes on the level of genetic material, which are manifested in the change of the primary structure of the nucleic acid, i.e. change in the sequence of nucleotides. They are related to changes of genotype, but do not have to manifest in phenotype.

- **Significance** of mutations:

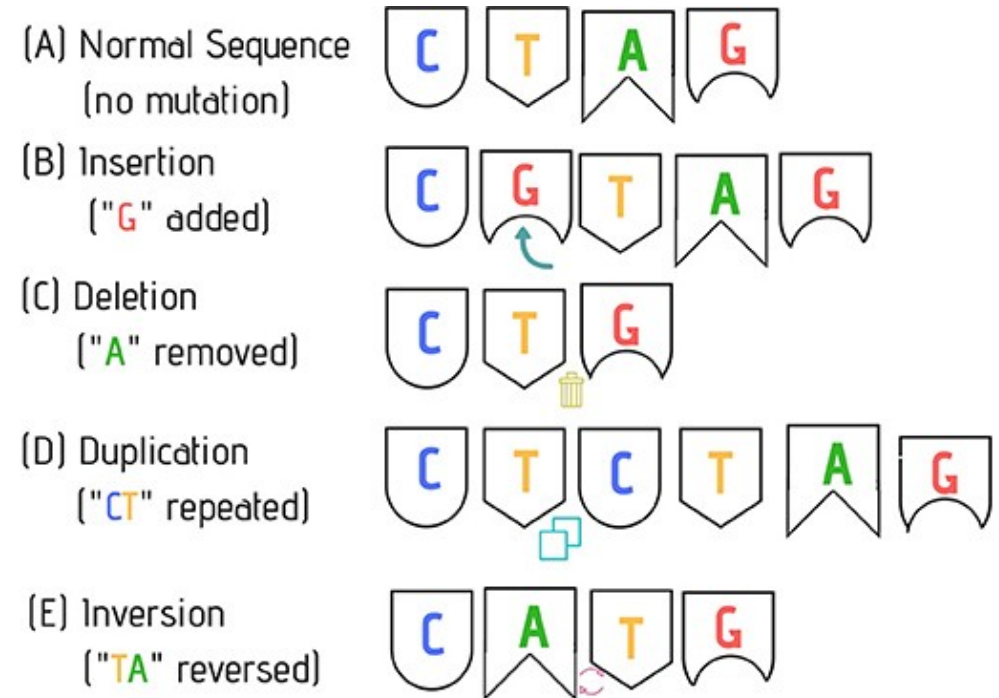
- Positive – source of genetic variability, evolutionary significance
- Negative – cumulation of damaged genes, rise of genetically determined diseases, tumors
- Neutral

- **Classification** of mutations:

- spontaneous (errors in replication) × induced (mutagens)
- gametic × somatic
- dominant × recessive (1:100)
- direct × reverse (mutated genotype reverses to the original genotype)
- vital × lethal
- nuclear × non-nuclear (mt, cp)
- gene × chromosomal (structural CHA) × genomic (numerical CHA, aneuploidy, polyploidy)

Gene Mutations

- Gene (point) mutations
 - base substitution (transition, transversion) – standard allele -> mutant allele -> changed protein
 - deletion/insertion of bases (reading frame shift)
- **Consequences** of point mutations:
 - mutations changing the meaning of a codon (different AA)
 - nonsense mutations (stop codon)
 - silent mutation (different codon, same AA)
- **Sickle cell anemia** – AR inheritance; substitution CTC -> CAC in beta chain (substitution of valine for glutamic acid); resistance to malaria (plasmodium infection) – heterozygous advantage (selective advantage compared to both homozygotes)



Types of genetic variants

The gray cat ran down the hall. **Original**

The gray cat ran down the ball. **Missense**

The gray green cat ran down the hall. **Insertion**

The gray ___ ran down the hall. **Deletion**

The gray cat cat ran down the hall. **Duplication**

The gray. **Nonsense**

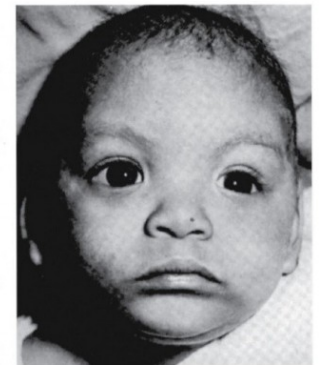
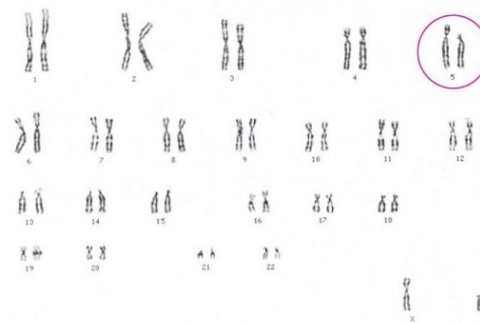
Chromosomal Mutations

Structural chromosomal aberrations are a product of one or more breaks in the DNA.

- **Classification:**

- **balanced** (same amount of genetic material)
 - translocation – e.g. Philadelphia chromosome t(9;22) in CML
 - inversion
 - insertion
- **unbalanced** (portion of genetic material is missing or extra)
 - duplication
 - deletion
 - Cri du chat (deletion on short arm of chr. 5)
 - Prader-Willi syndrome (deletion of paternal chr. 15)
 - Angelman syndrome (deletion of maternal chr. 15)
 - isochromosome
 - ring chromosome

46,XX,del(5)(p14.1)



Genomic Mutations

- **Aneuploidy of gonosomes**

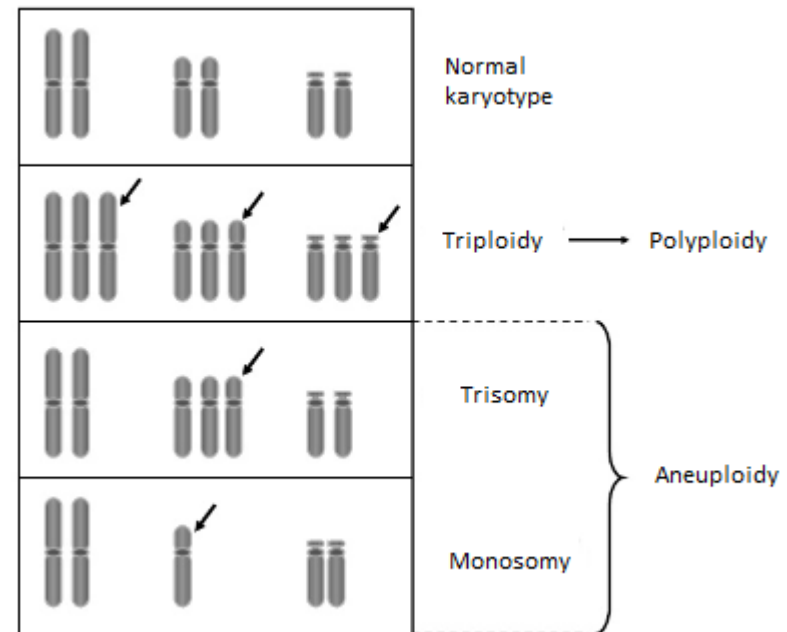
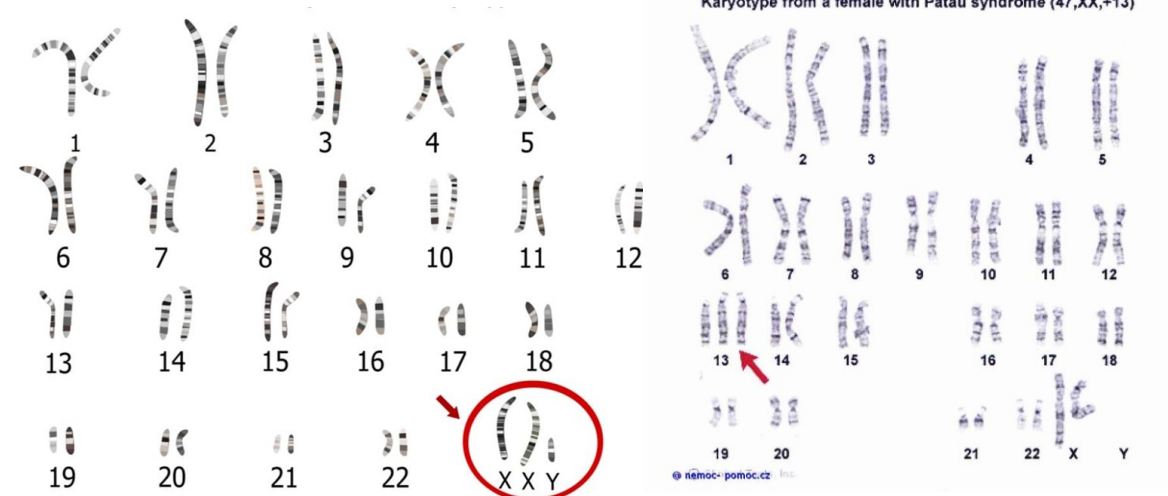
- Turner syndrome 45, X
- Klinefelter syndrome 47, XXY
- XXX syndrome
- XYY syndrome

- **Aneuploidy of autosomes**

- Down syndrome (21)
- Edwards syndrome (18)
- Patau syndrome (13)

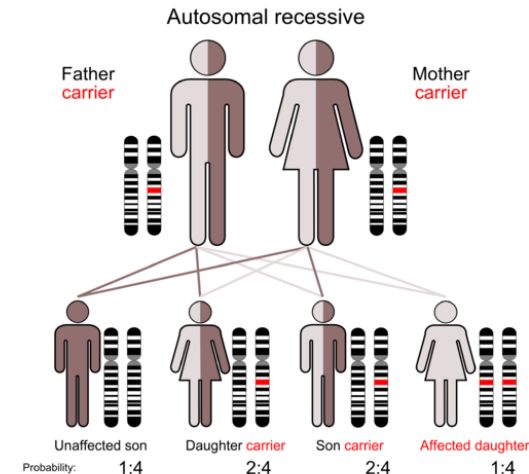
- **Polyploidy**

- rare in vertebrates, more common in plants



DNA Diagnostics

- **Detection of presence of specific nucleic acid sequence**
 - Identification of animal species
 - Paternity
 - Identification of individuals - forensics
 - DNA profile – SNPs
- **Analysis of structure (sequence) of nucleic acid**
- **Determination of genotype**
 - Detection of clinically significant mutations and polymorphisms
 - Hereditary diseases
 - Detection of oncogenes and suppressor genes in tumors
- **Prenatal, preimplantation diagnostics**
- **Quantification of nucleic acid with specific sequence**
 - Evaluation of intensity and changes in gene expression - tumors
- **Quantification of proteins and types of their posttranslational modifications**



Methods in Molecular Biology

Biological Materials

- Biological material is everything that was or is a part or a product of a living organism
 - dried herbal tea mixture
 - apple core
 - oak plank
 - cat droppings/fur
 - tube with SARS-CoV-2 virus
 - bodily fluids – urine, blood, plasma, serum, spit, ejaculate, phlegm
 - tissues, cells



Methods in Molecular Biology

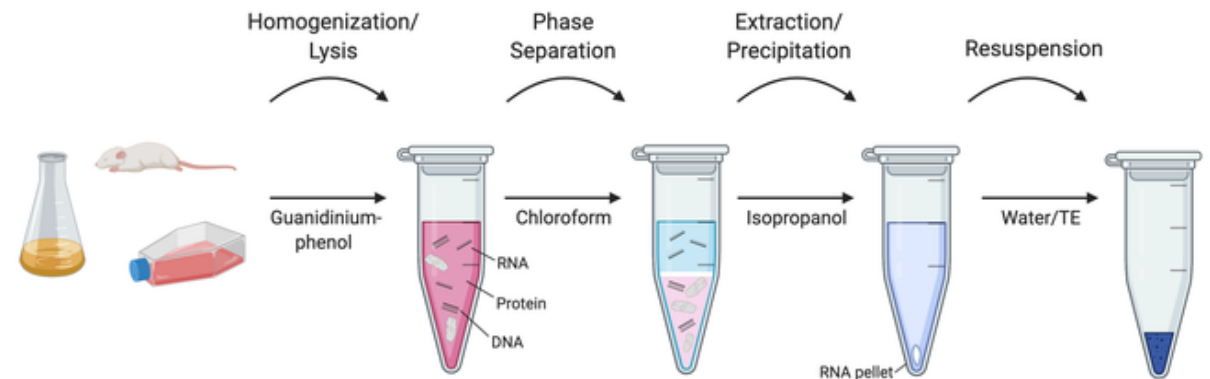
- Determination of nucleic acids
 - PCR, RFLP-PCR – detection by ELPHO
 - Real time PCR
 - Sequencing
- Determination of proteins
 - ELISA
 - Western blot
 - other methods based on antigen-antibody interactions
- Other molecular biology methods

Nucleic Acid Isolation

- In native state from native material – in sufficient quantity and required purity.
- NAs need to be devoid of all substances that would after lysis become a part of the crude substrate and that would impair the specific effects of enzymes used for further analyses.



- Isolation of genomic DNA
- Isolation of RNA – focus on protection against degradation!



PCR – Polymerase Chain Reaction

- **Aim** – acquisition of required specific sequence of genomic DNA without previous cloning
- **Principle** – multiple replications
 - 25 to 35 cycles
 - depends on temperature of reaction mixture
 - amount of replicated DNA grows exponentially (2^n)
- Thermocycler

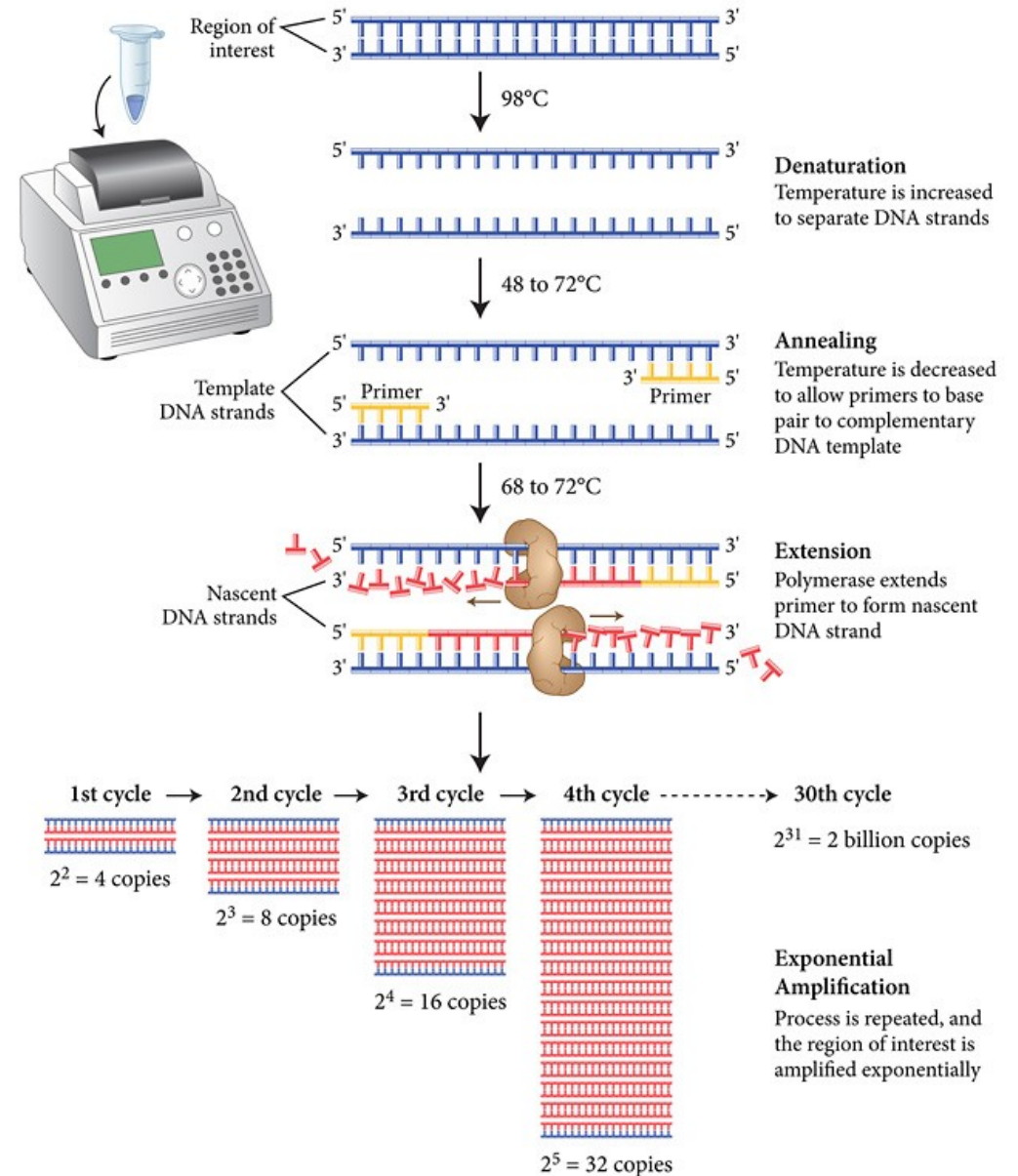
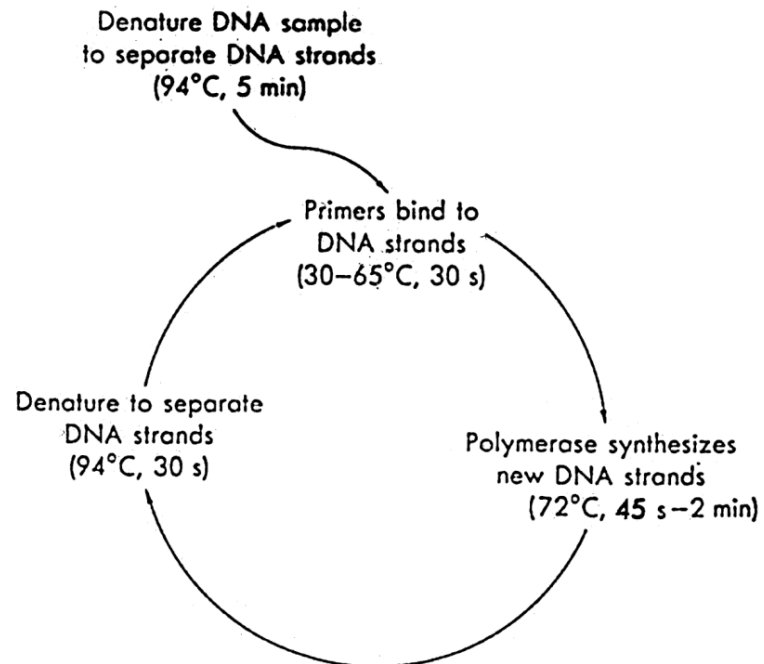


PCR Protocol



PCR

- Multiple *in vitro* replication in a tube
- Chain reaction based on DNA replication
- Repeating cycles:
 - denaturation (separation of dsDNA) – 96 °C
 - annealing – primer binding – 50–65 °C
 - elongation – synthesis of a new DNA strand – 72 °C



PCR

- **DNA replication – *in vitro* (PCR)**

- **DNA polymerase**

- thermostable (resists to temperatures up to 98 °C)
- Taq (*Thermus aquaticus*), Tth (*Thermus thermophilus*)

- **primer**

- short specific segments of DNA
- oligonucleotide 20–25 pb
- limiting the region for DNA amplification

- **Mg²⁺ ions**

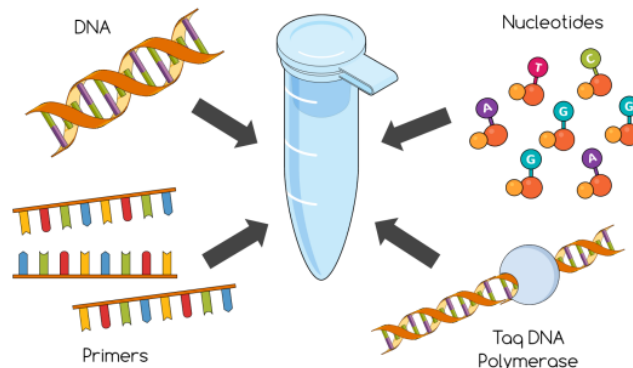
- affect activity and precision of polymerase

- **template DNA**

- **dNTP**

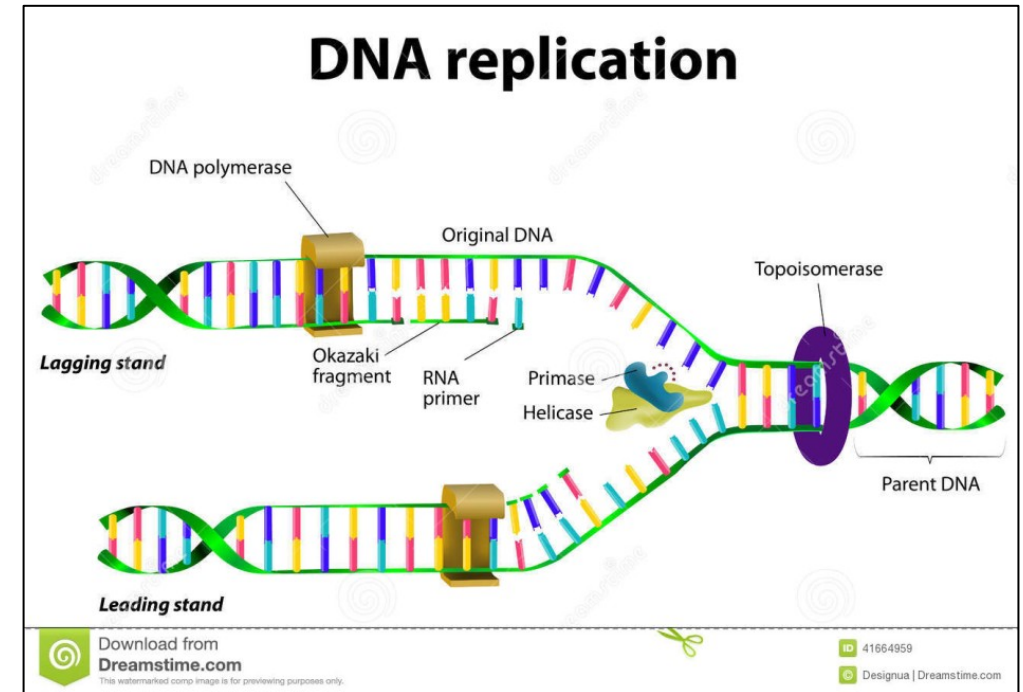
- **buffer (pH=8)**

- **temperature**



- **DNA replication – *in vivo***

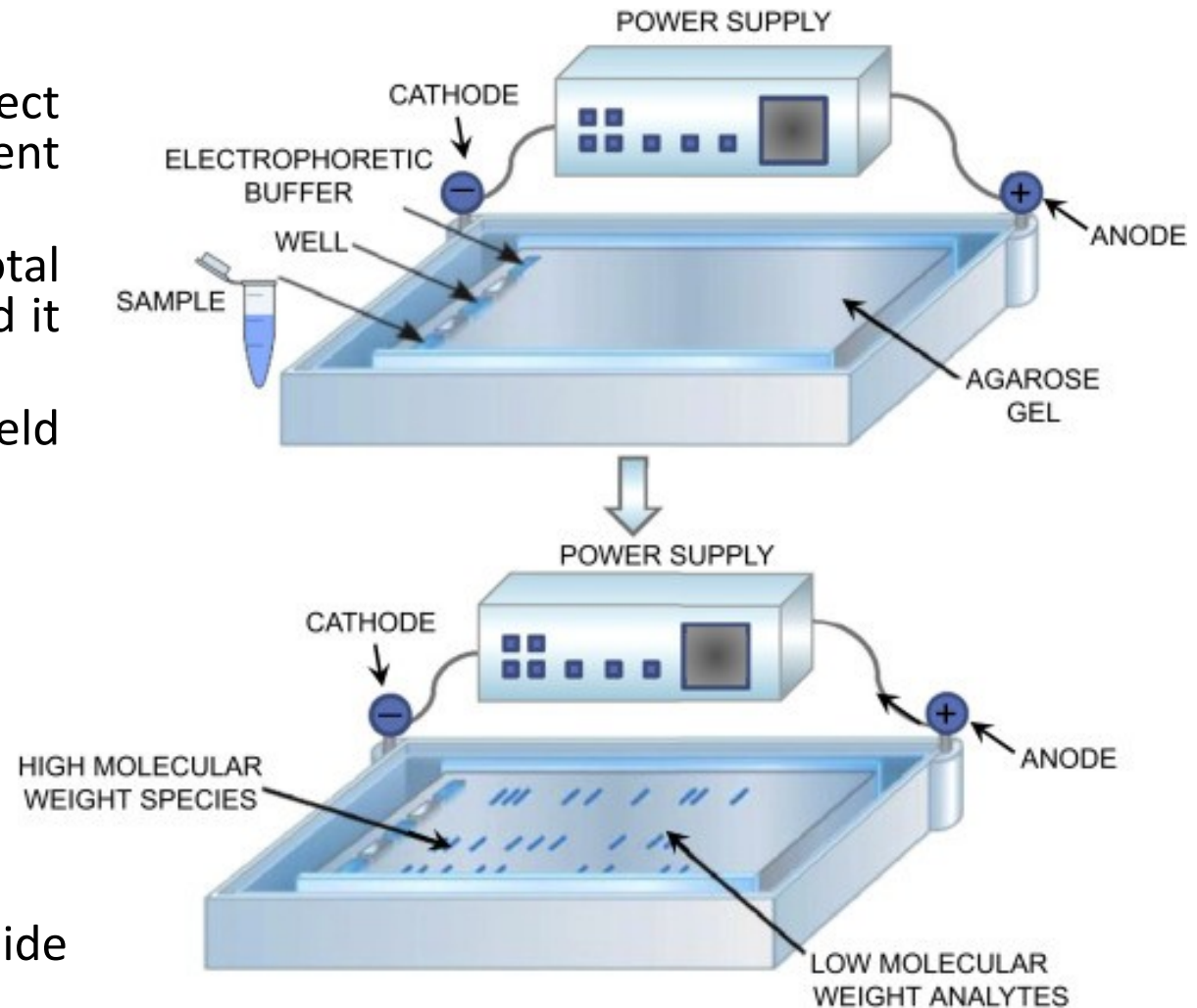
- **enzymes** – helicase, primase, DNA polymerase, ligase...



Video: <https://www.youtube.com/watch?v=matsiHSuoOw> and <https://www.youtube.com/watch?v=oqeV72oYfD0>

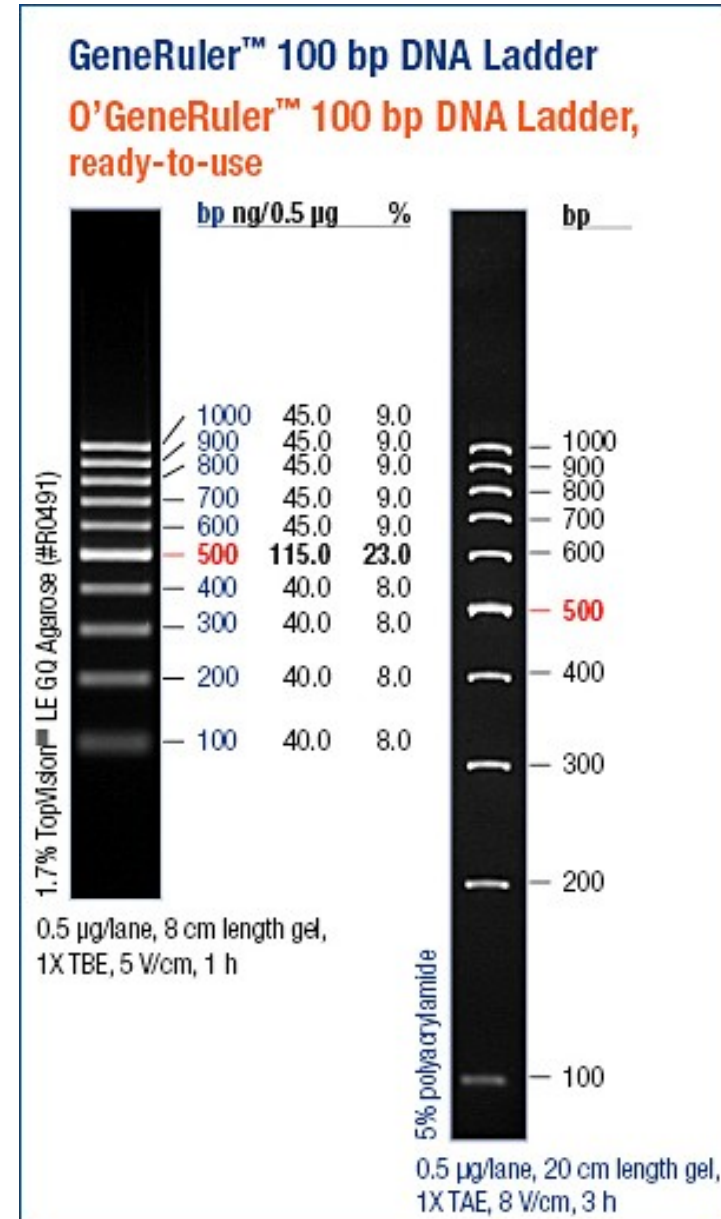
Gel Electrophoresis

- separation method
- **principle** – movement of charged molecules in direct current field (separation of molecules with different molecular weight)
- speed of movement depends on the size of the total surface charge, size and shape of the molecule and its concentration in solution
- DNA has uniformly negative charge → in electric field moves **from cathode to anode**
- parts of **equipment**
 - electrophoretic container
 - separation gel
 - buffer
 - direct current power supply
- **agarose** (produced by seaweed – agar)/polyacrylamide
- EtBr – intercalates between bases, makes DNA visible under UV



Gel Electrophoresis

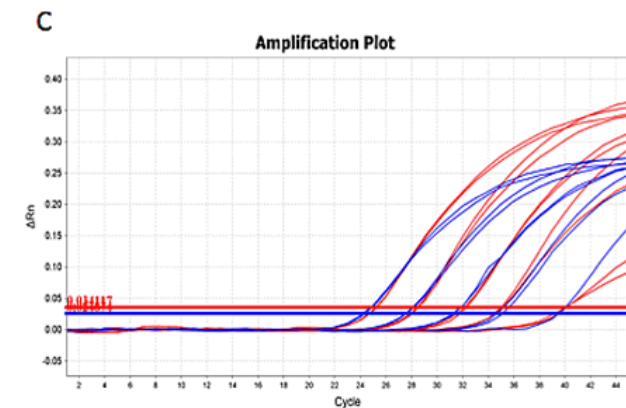
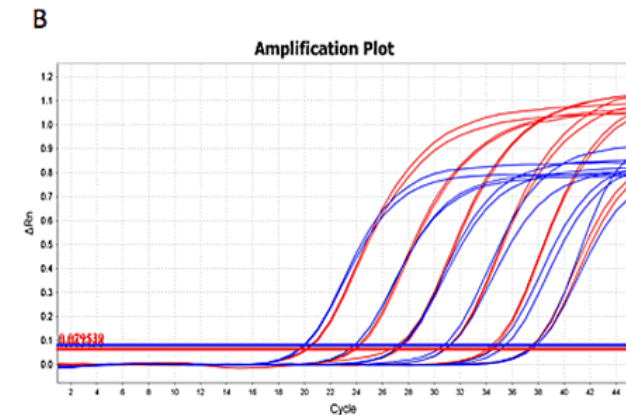
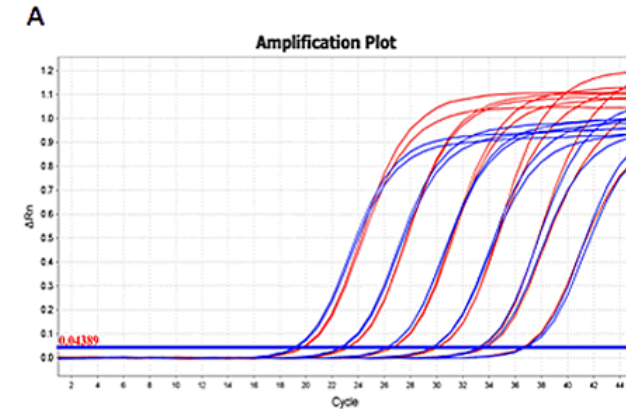
- Size of DNA fragments can be determined with molecular-weight ladders (= restriction fragments of plasmid molecules or genome of bacteriophages, size of which was determined *via* sequencing)



PCR + ELPHO video

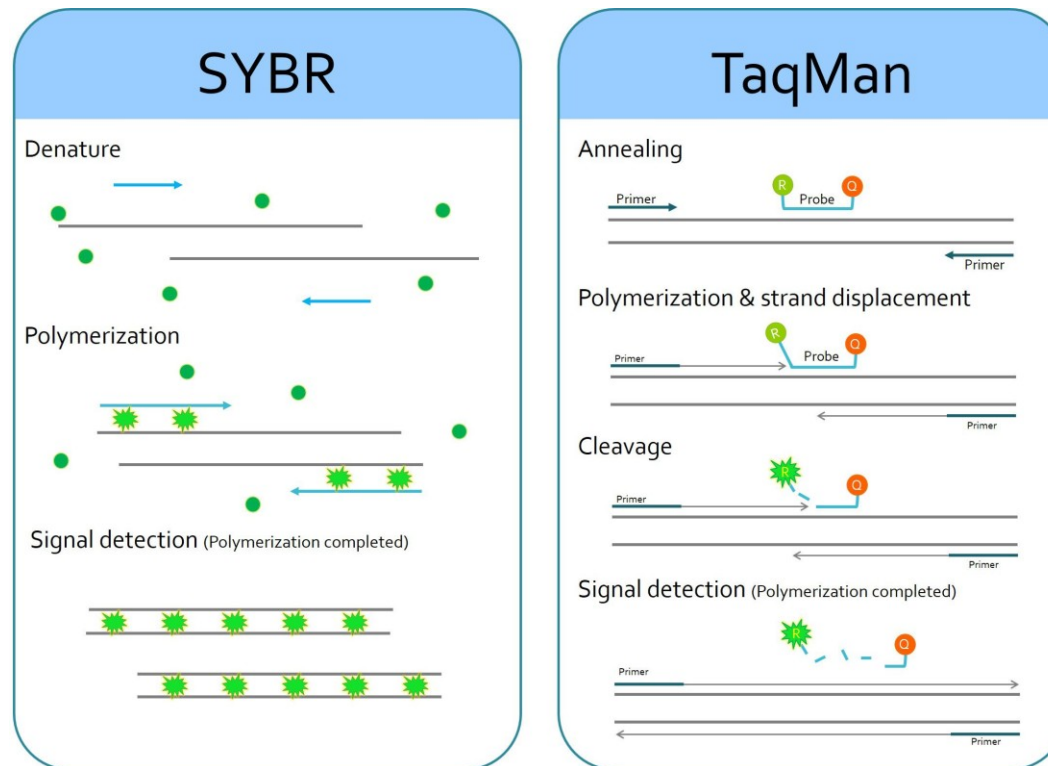
qPCR – Quantitative Real-time PCR

- Polymerase chain reaction monitored in real time
- **Quantification of DNA** – amount of DNA is monitored during each cycle
- Detection of the DNA amount enabled by the presence of **fluorescent substrate**
- Performed in a special cycler, which allows:
 - Cyclical changes of temperature
 - Fluorescence detection
 - Monitoring of PCR progress in real time without the need to detect PCR products *via* electrophoresis
- qPCR is usually performed in 96-well plates, level of fluorescence is monitored in each well
- Highly sensitive and specific method



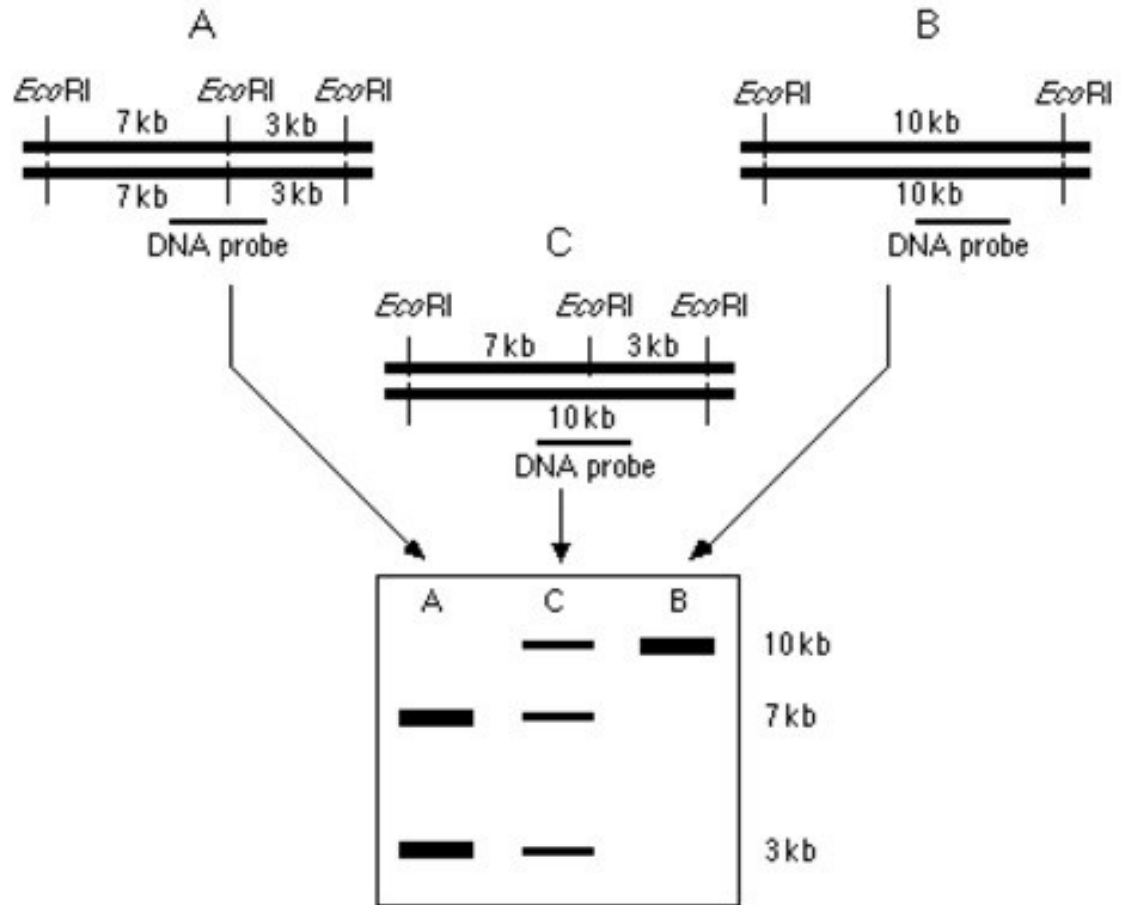
qPCR

- Intensity of fluorescence is **directly proportional to the amount of product created in the reaction**
- Product detection:
 - **intercalating dyes** – SYBR Green – non-specifically binds to dsDNA
 - **sequence specific probe** – short oligonucleotide with a dye and a quencher (TaqMan) – after its breakdown during DNA synthesis – rise of fluorescent signal (uses 5'-3' exonuclease activity of DNA polymerase)



RFLP – Restriction Fragment Length Polymorphism

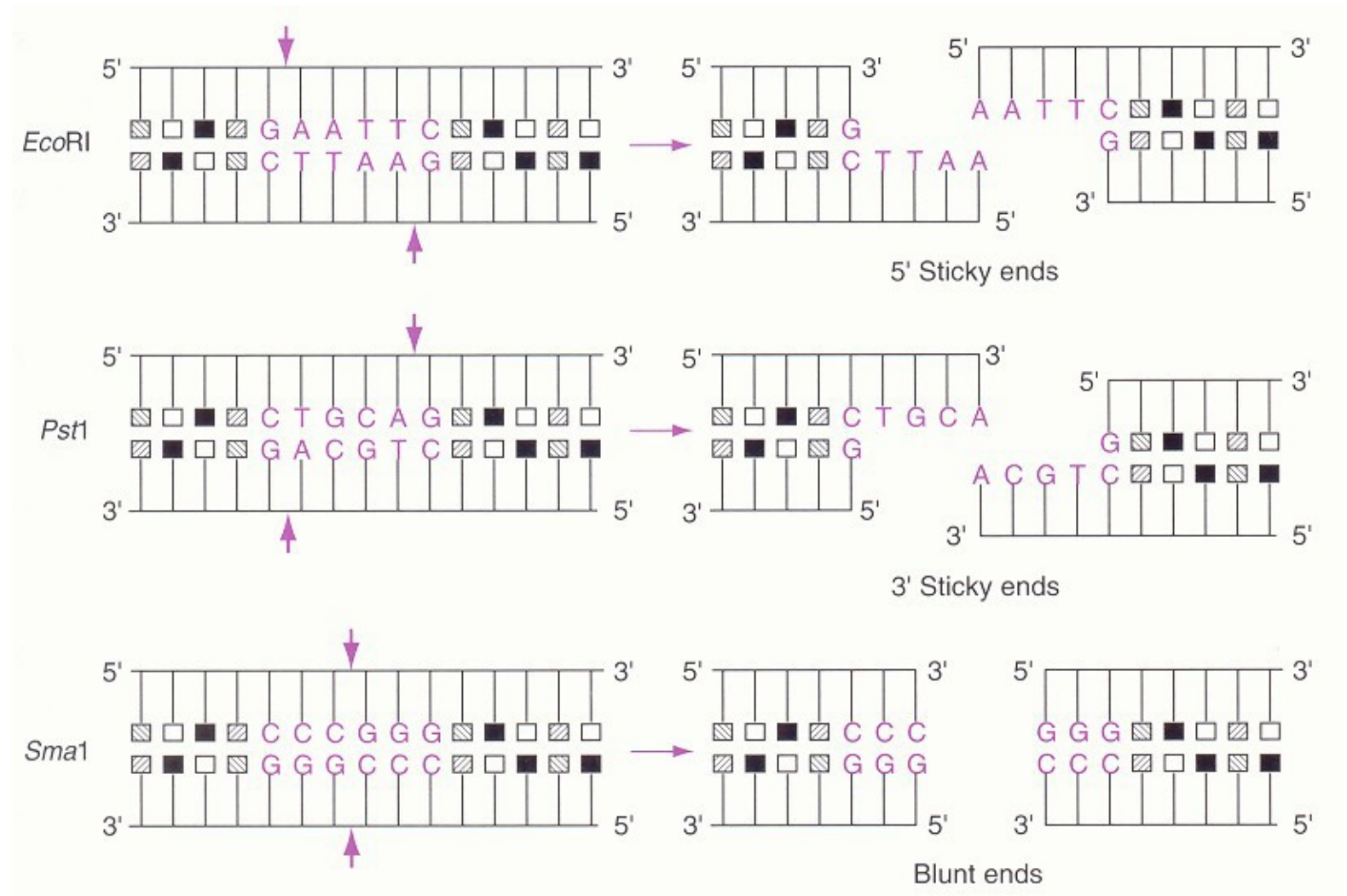
- Enzymatic cleavage of DNA in specific restriction site
- **Restriction endonucleases**
- Production of fragments with different lengths
- Created fragments separated *via* gel electrophoresis
- **Use:**
 - DNA mapping, analysis of DNA modifications, preparation of mutants
 - based on length and number of fragments we can observe differences in studied sequences, so called **polymorphisms** (polymorphisms are created by reconstruction of DNA strand, e.g. insertion, deletion, base substitution)
 - kinship analysis, determination of paternity, identification of persons



RFLP

- **Restriction endonuclease**

- sequence specific endonucleases (originated from bacteria)
- EcoRI (*Escherichia coli*), HindIII (*Haemophilus influenzae*)
- blunt/sticky ends
- function:
 - **recognition of specific dsDNA sequence and subsequent restriction** (hydrolysis of phosphodiester bonds)
- recognition sequence
 - 4–8 bp long
 - character of **palindrome** = same sequence of bases in both directions



DNA Sequencing

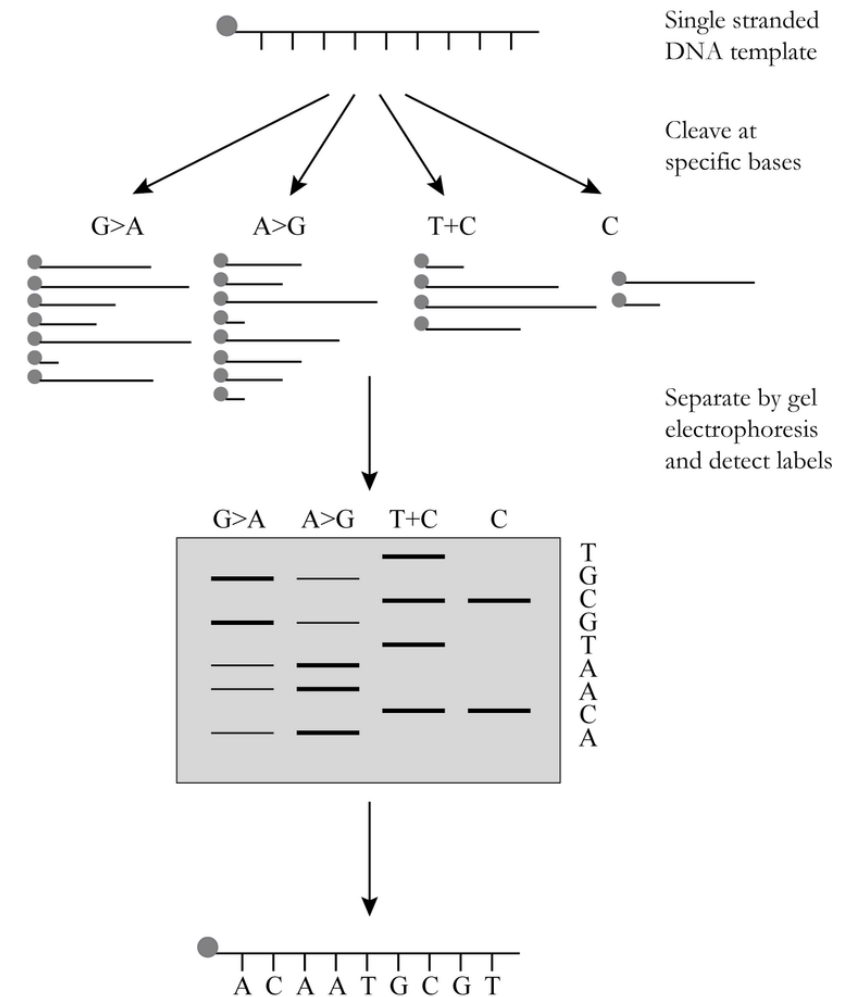
- determination of **primary structure of DNA** (sequence of nucleotides)
 - a) **chemical method** – degradation of nucleic acid chains *via* chemical agents (dimethyl sulfate, NaOH, hydrazine,..)
 - b) **enzymatic method** – specific inhibition of enzymatic synthesis of DNA
 - c) modern large format applications based on e.g. pyrosequencing (**next generation sequencing**)
- **Product** – strands of ssDNA, their relative sizes differing by one base (evaluation using ELPHO)
- **Input material** – fragment of DNA with both defined ends



Maxam-Gilbert Sequencing

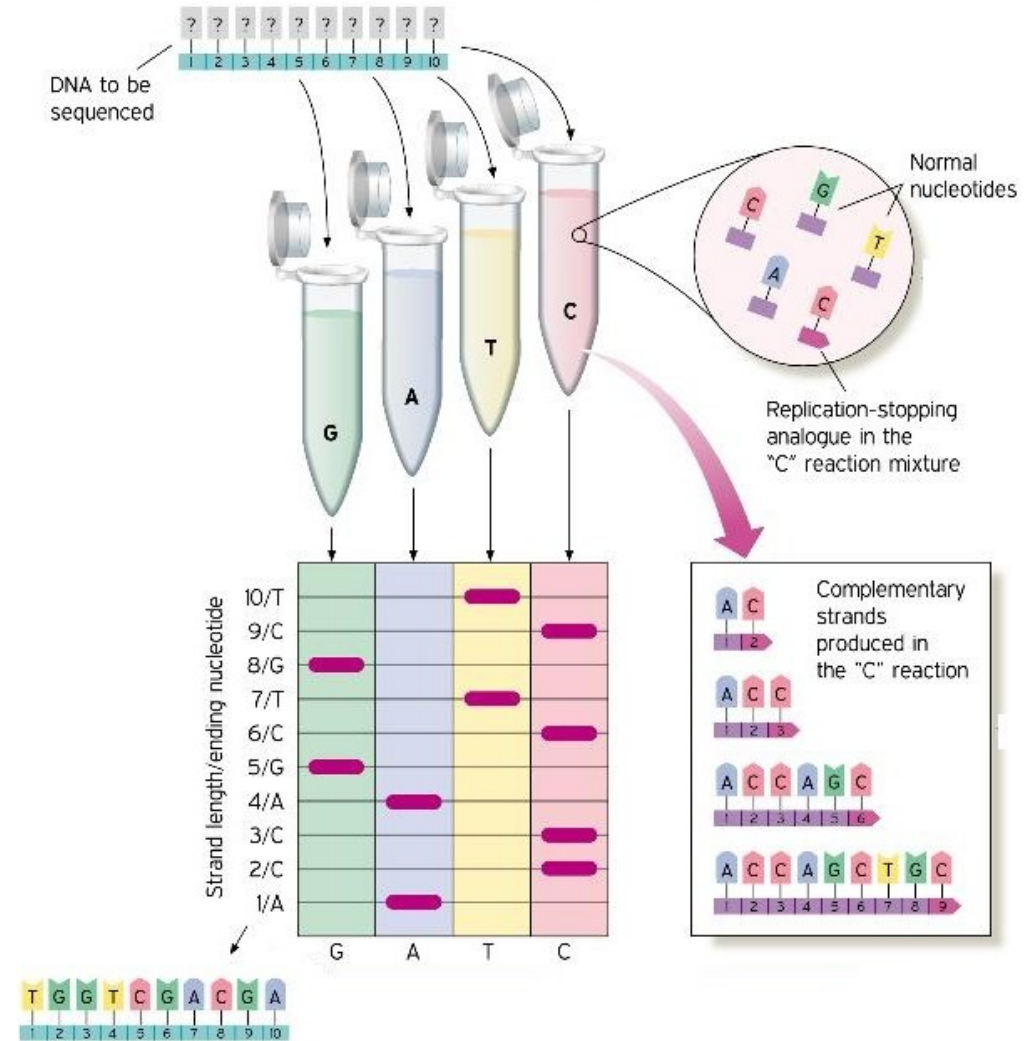
- Sequence is derived from a DNA molecule which chemically degrades to fragments in places with a base of a specific type. These are subsequently separated using ELPHO.
- **Chemical agents** – example:
 - piperidine breaks glycosidic bond of A and G (A + G)
 - hydrazine in the presence of NaCl reacts only with C
 - NaOH at 90 °C strongly cleaves A and weakly cleaves C (A > C)
- Requires radioactive labelling on one end of ssDNA.
- Reaction is done in 4 tubes – in each only some types of bases are cleaved.
- Mixture of differently long fragments ending in a place of a specific base is created → evaluation using ELPHO, determination of a sequence of a given section.

● label



Sanger Sequencing

- Enzymatic method
- Based on **principle of replication** – end of DNA synthesis in the moment ddNTP is incorporated instead of dNTP
- **ddNTP** = analogue of dNTP, but lacks 3'-OH group on carbon
- ddNTP – terminator
- **Reaction mixture (4x)**
 - DNA template
 - primer
 - Taq DNA polymerase – synthesis of DNA from 5' to 3' end
 - buffer
 - dNTP – abundant (to get fragments of all possible lengths)
 - ddNTP – low concentration
- **Evaluation** – electrophoresis
- Modification → fluorescently labeled ddNTP (4 different color labels) – reaction performed in one tube

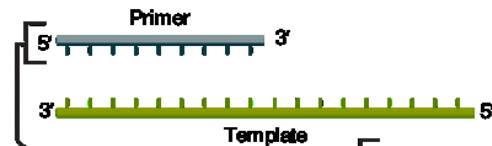


Sanger Sequencing

- Capillary sequencing of DNA with fluorescently labeled ddNTP

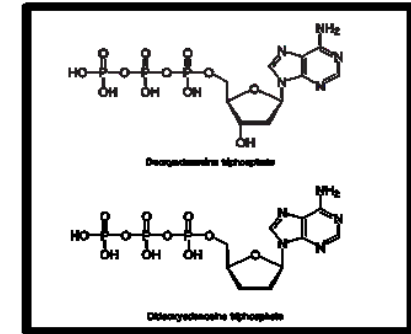
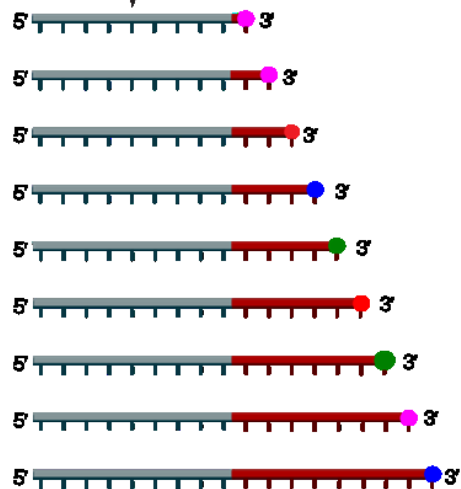
① Reaction mixture

- ▶ Primer and DNA template
- ▶ DNA polymerase
- ▶ ddNTPs with fluorescent labels
- ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)

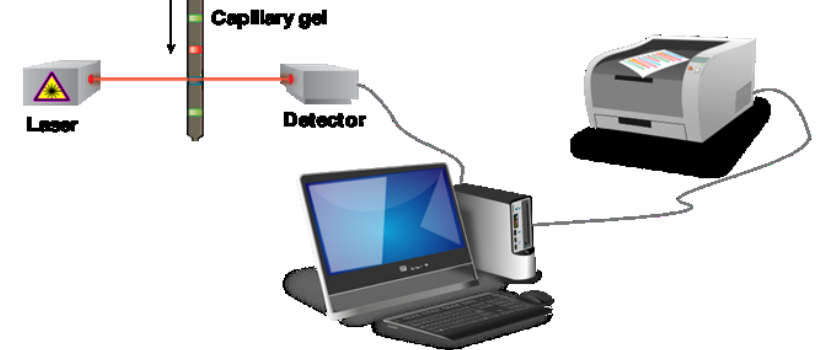


- ddNTPs
- ddTTP (red)
- ddCTP (blue)
- ddATP (green)
- ddGTP (magenta)

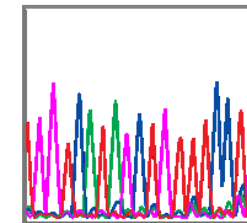
② Primer elongation and chain termination



③ Capillary gel electrophoresis separation of DNA fragments

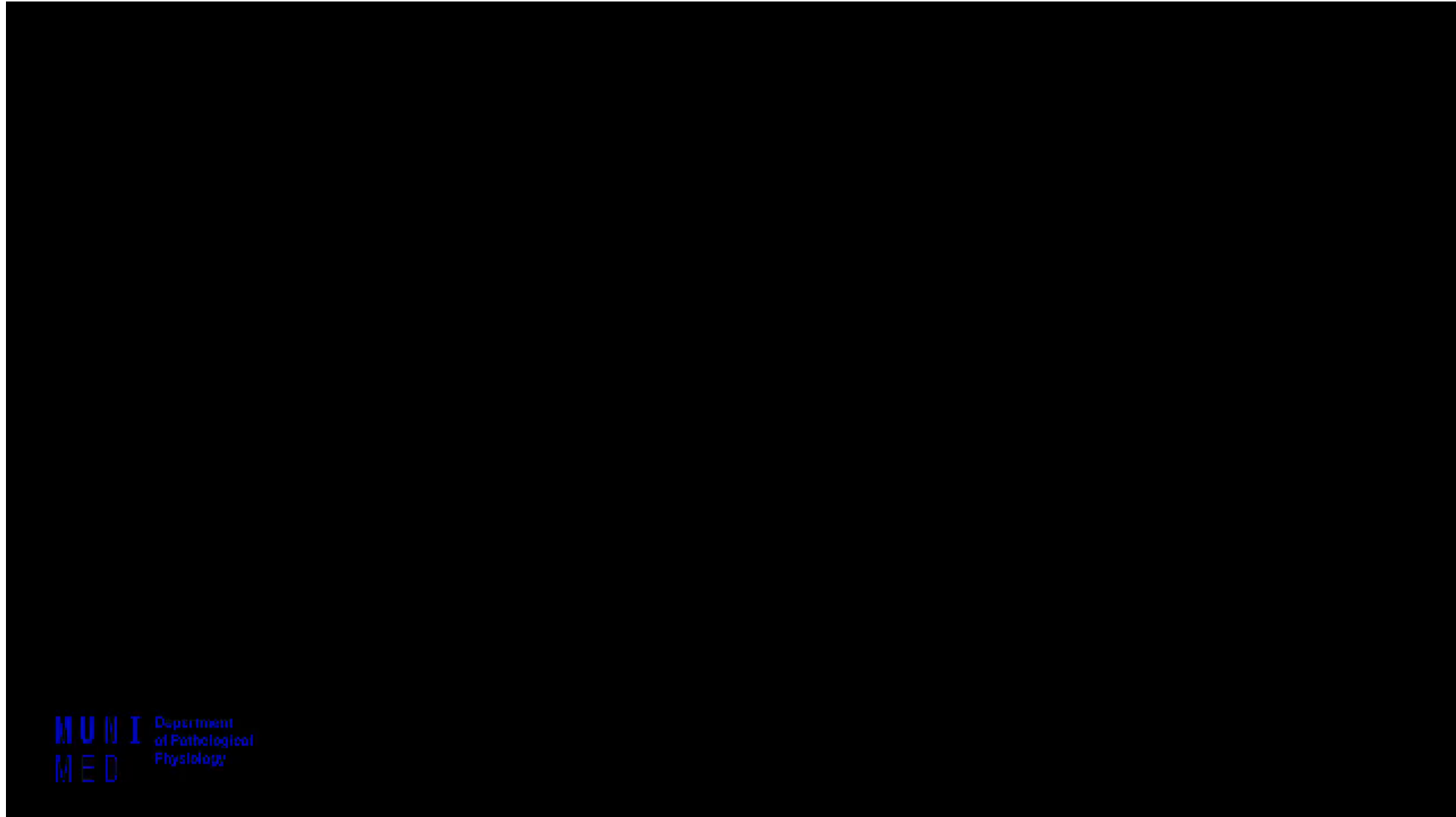


④ Laser detection of fluorescent labels and computational sequence analysis



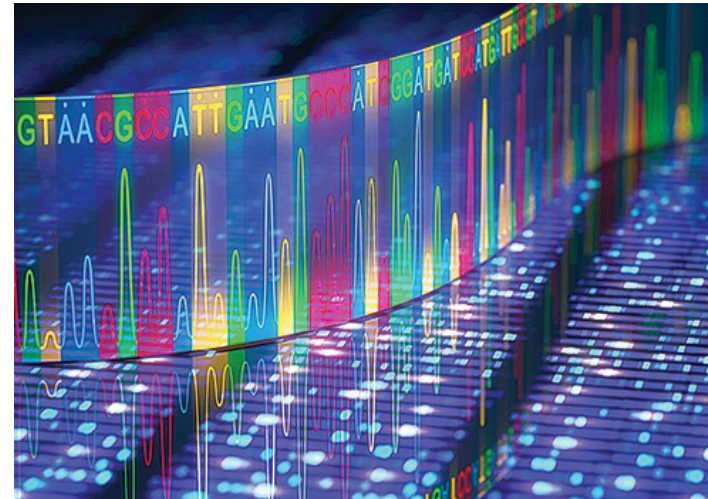
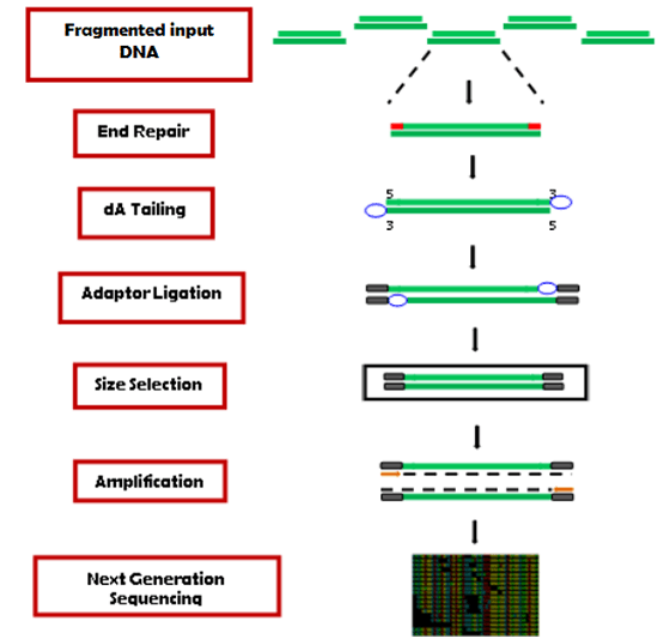
Chromatograph

DNA Sanger Sequencing - video



NGS – Next Generation Sequencing

- Sequencing of thousands to millions of sequences at the same time
- Template DNA are fragmented sections few hundred bases long
- Ends of fragments are enzymatically blunted and connected to oligonucleotides of specific sequence (= adapters)
- Single fragments are separately amplified *via* PCR and in the next step sequenced in parallel
- Use:
 - whole genome sequencing
 - sequencing of chromosomes, plasmids, mt
 - study of genetic variability, mutational analysis
 - transcriptome analysis



Video:

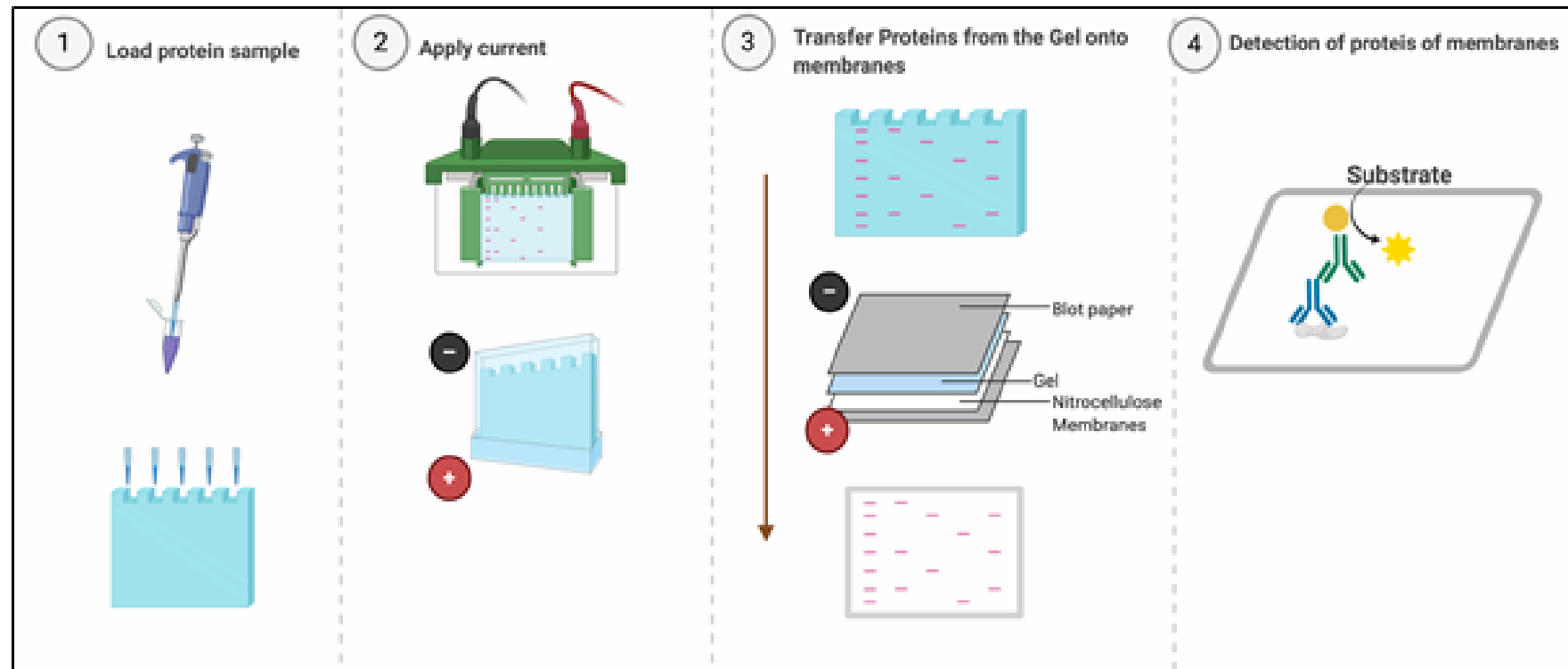
https://www.youtube.com/watch?v=shoje_9IYWc

<https://www.youtube.com/watch?v=CZeN-lgjYCo>

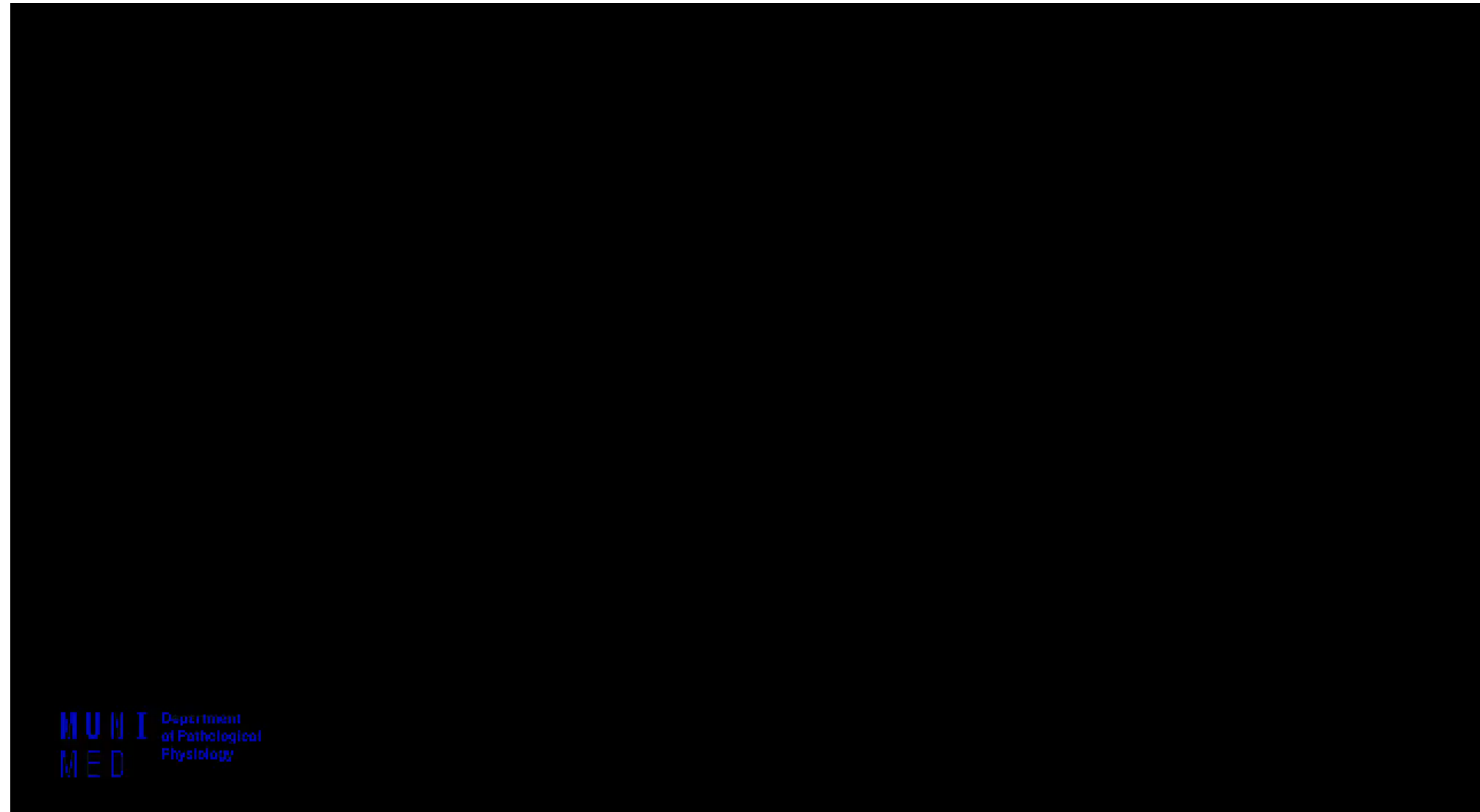
<https://www.youtube.com/watch?v=fCd6B5HRaZ8>

Western Blot

- Qualitative or semi-quantitative detection of proteins
- Principle – detection of protein in gel in electric field utilizing antigen-antibody bonding
- 3 phases
 1. Electrophoretic separation of proteins (polyacrylamide gel)
 2. Transfer of separated proteins
 3. Detection of proteins



Western Blot - video

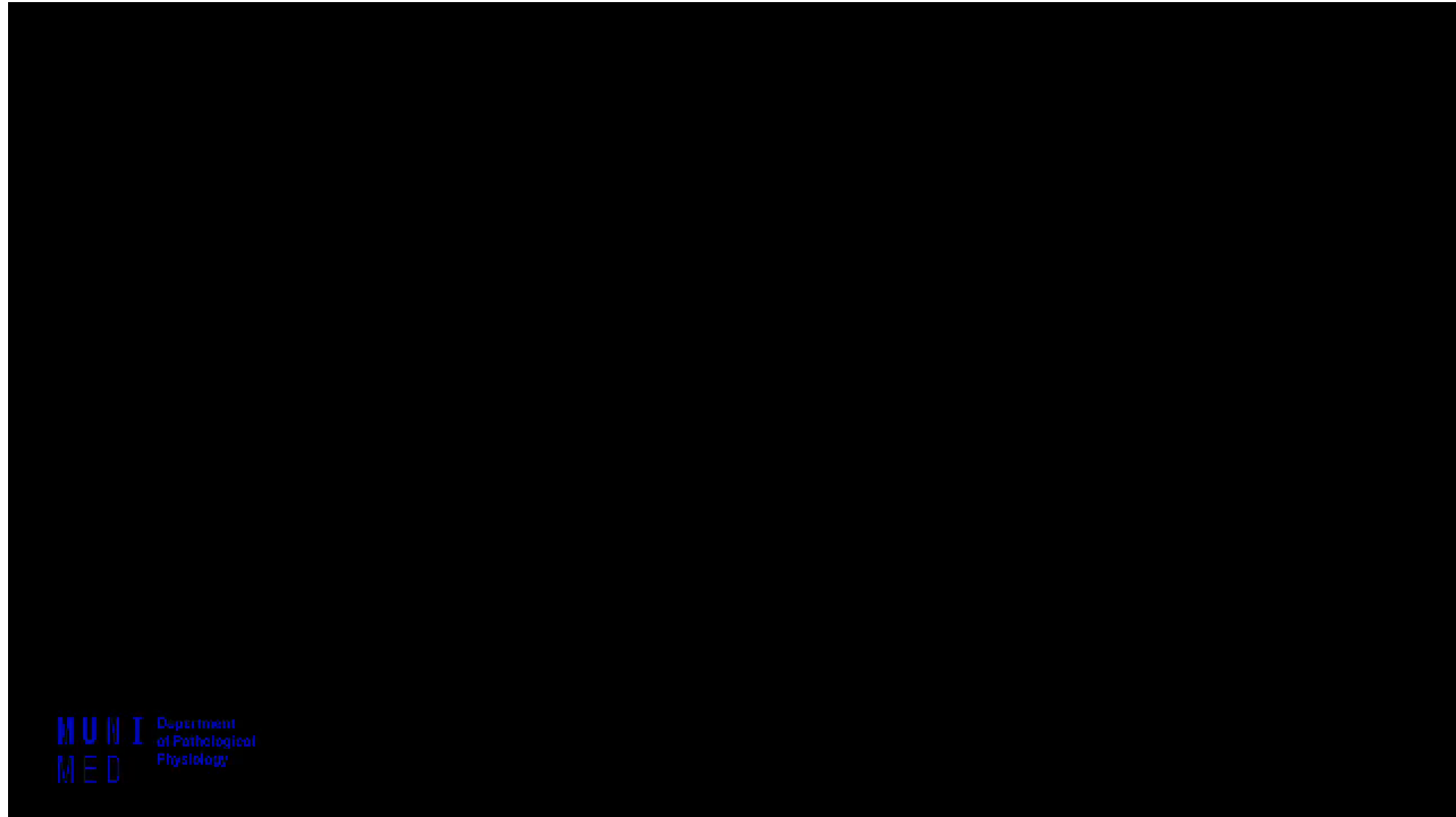


Cell Culture Laboratory

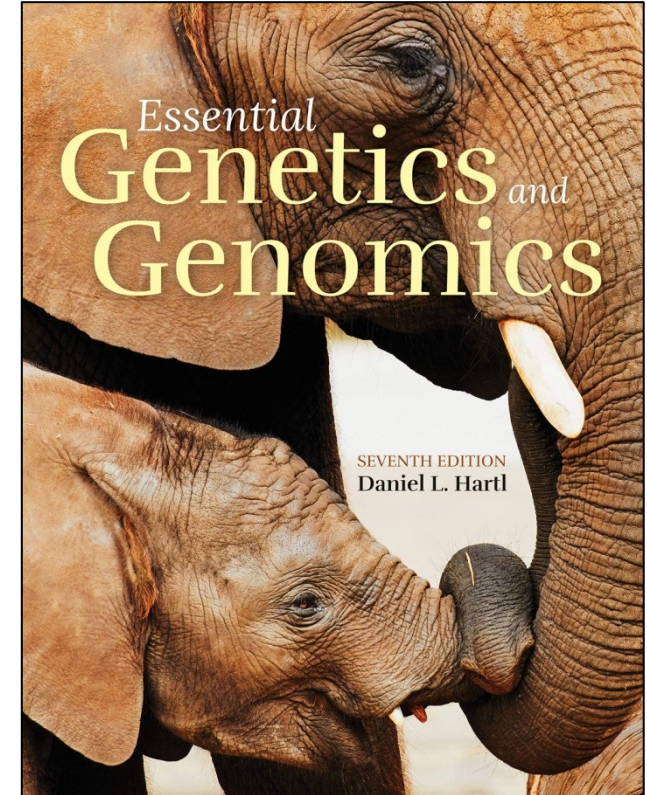
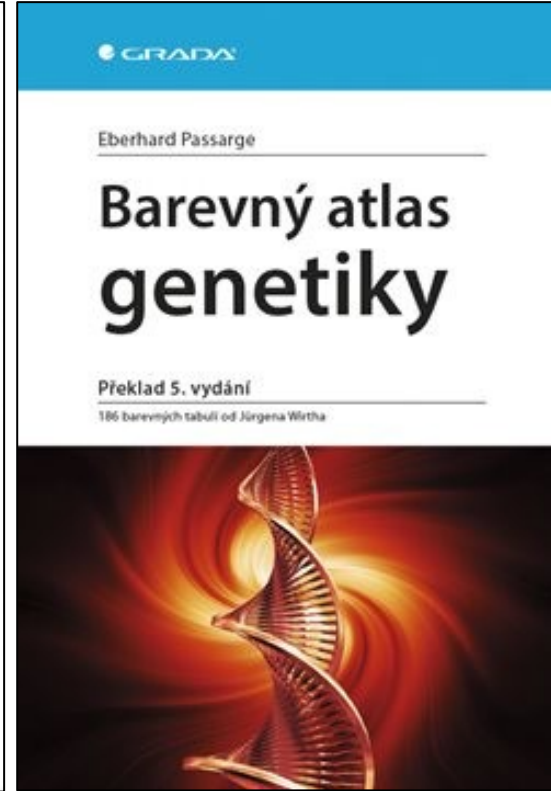
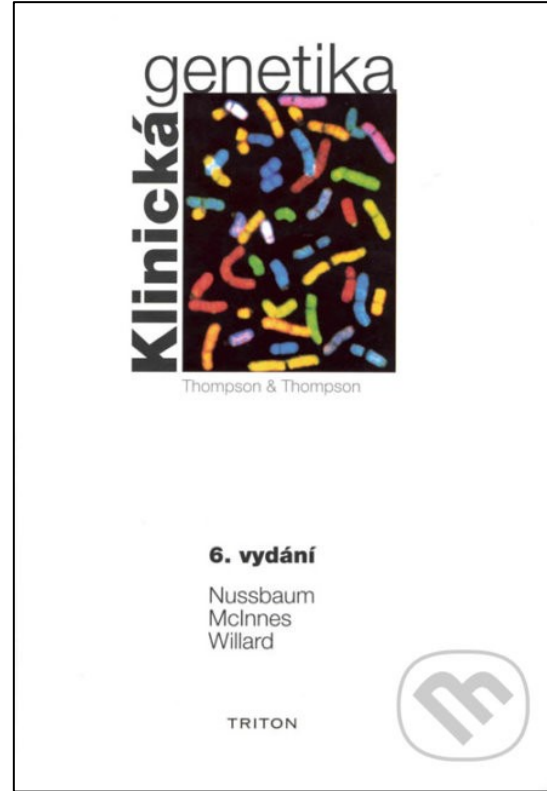
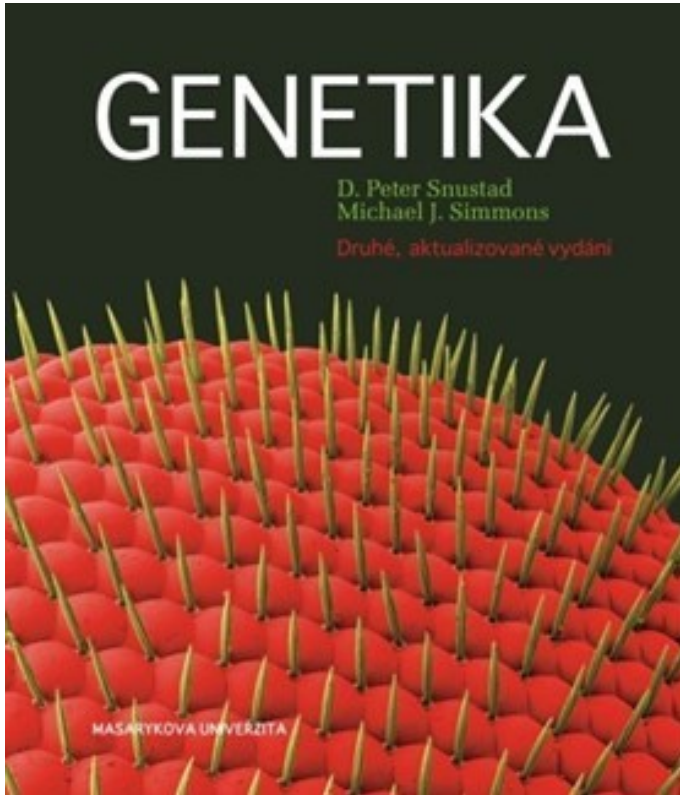
In vitro experiments are used in biology, medicine and other related fields. They are based on cultivation of cell/tissue cultures outside of the organism from which they come.



Cell Culture Laboratory - video



Recommended Literature for Self-study



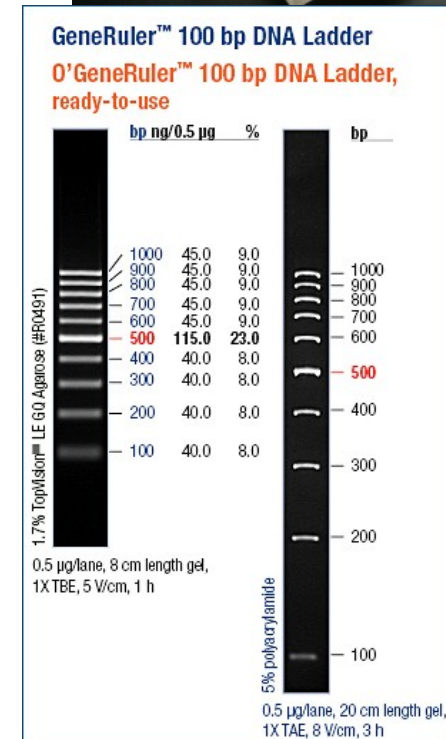
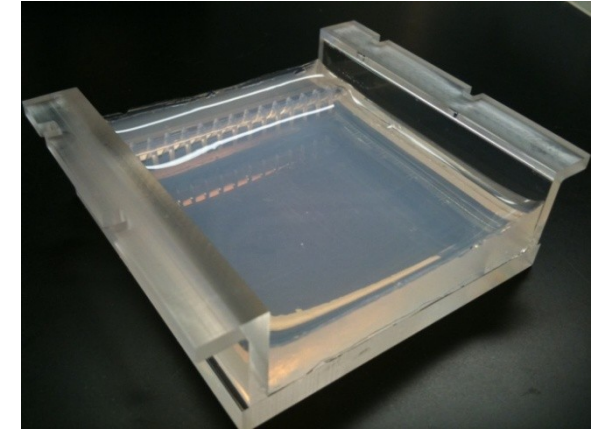
Practical Part

Practical Part

1. Gel preparation
2. PCR
3. ELPHO

Practical implementation of ELPHO

1. Prepare casting tray – combs and tape
2. Prepare gel (2%): weigh agarose and add it to Erlenmeyer flask, add TBE buffer (200 ml)
3. Weigh and boil in microwave.
4. After cooling to cca 40 °C add EtBr (1 µl/10 ml)
5. Cool down the gel (cca 30 min)
6. Remove combs and put into ELPHO container with TBE buffer (electrolyte)
7. Add size standard ladder (DNA + dye)
8. Prepare drops of loading dye on paraffin paper and mix with DNA samples.
9. Load samples into wells (max 15 µl)
10. Connect to power supply
11. Track the progress of DNA through the gel (40 min)
12. Visualisation under UV and evaluation



Practical implementation of PCR

volume in microtube: 25 μ L

Composition (1 sample):

- Template DNA 50ng (2 μ L)

- MASTER MIX
- 2 Primers 10 pmols (1.25 μ L)
 - MgCl₂ 25mM (4 μ L)
 - dNTP mix (0.5 μ L)
 - Taq polymerase 1U (1 μ L)
 - Buffer DYNEX (2.5 μ L)
 - PCR H₂O (12.5 μ L)



1 drop of mineral oil

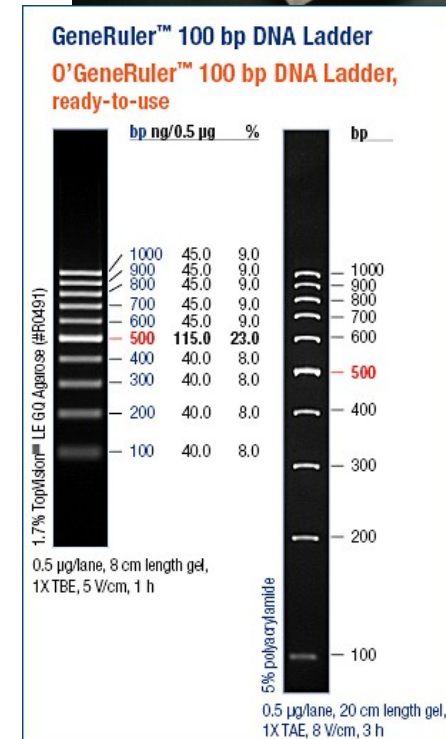
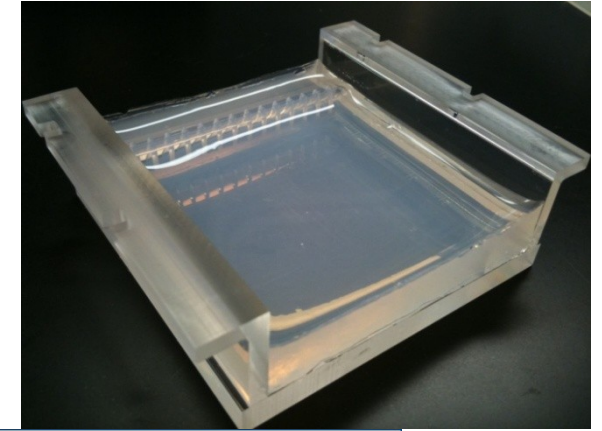


1. 95°C.....5 minutes
2. 95°C.....1 minute
3. 60°C.....1 minute
4. 72°C.....1 minute
5. 72°C.....7 minutes
6. 10°C.....10 minutes

35 x

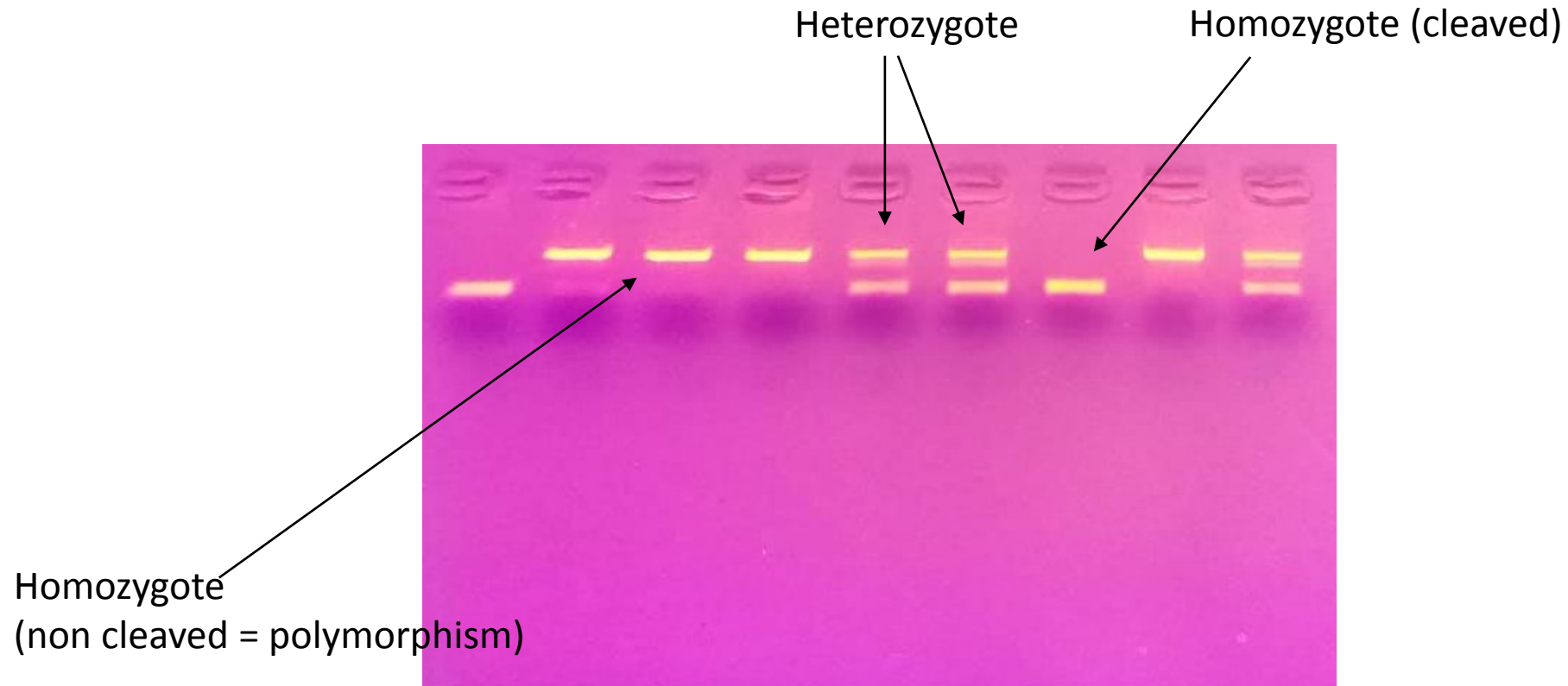
Practical implementation of ELPHO

1. Prepare casting tray – combs and tape
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4. After cooling to cca 40 °C add EtBr (1 μ l/10 ml)
5. Cool down the gel (cca 30 min)
- 6. Remove combs and put into ELPHO container with TBE buffer (electrolyte)**
- 7. Add size standard ladder (DNA + dye)**
- 8. Prepare drops of loading dye on paraffin paper and mix with DNA samples.**
- 9. Load samples into wells (max 15 μ l)**
- 10. Connect to power supply**
- 11. Track the progress of DNA through the gel (40 min)**
- 12. Visualisation under UV and evaluation**

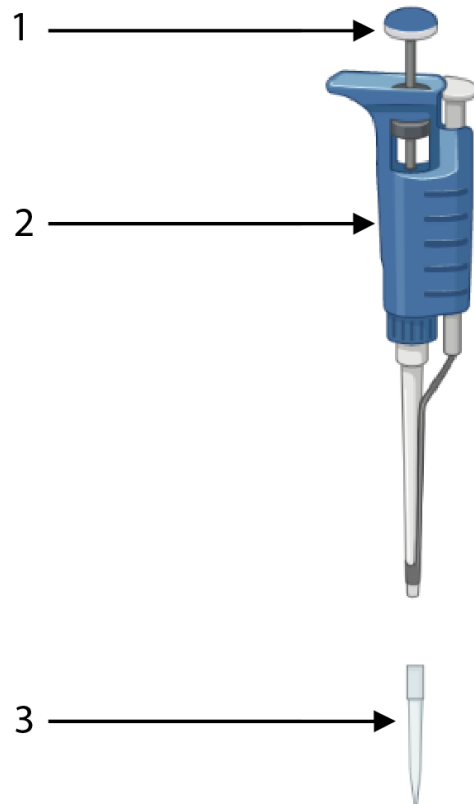


Practical implementation of ELPHO

2. Electrophoresis of restrictive fragments after TaqI cleavage



How to Use a Micropipette



Micropipette

1 – two-position plunger

2 – handle with a finger rest

3 – single-use tip

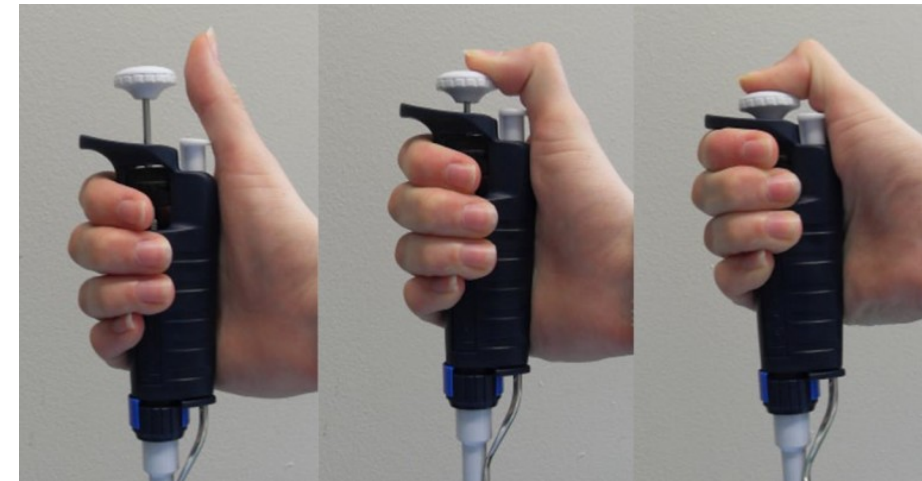
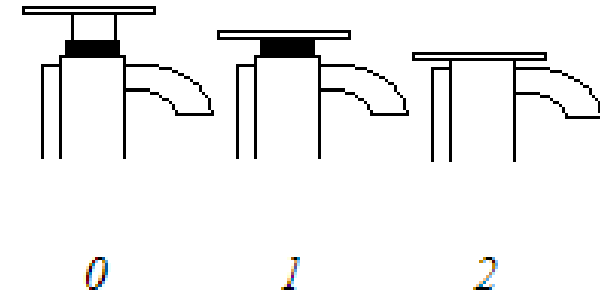
- Always hold the pipette **vertically** (tip pointing down).
- Hold the pipette in a palm hanging from an index finger and operated by a thumb.
- **Choose optimal volume range!!! Never set the pipette outside of the range in either direction!!!**
- Before pipetting, use a corresponding **tip** (according to the volume range of the pipette).
- Always use a new **sterile tip**.
- While pipetting the same solution multiple times, use the same tip during the whole time.
- Tip ejector button on the side of the handle is for ejecting a used tip.

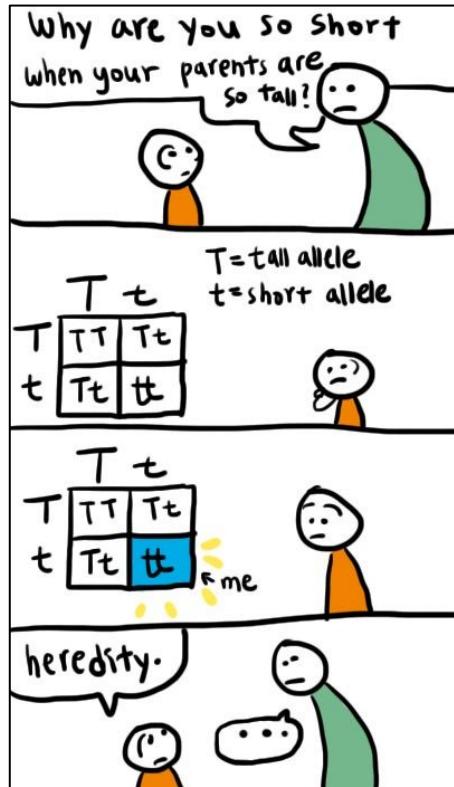
How to Use a Micropipette

Procedure:

- Set desired volume on the pipette. Horizontal line on display is a decimal point.
- Put a tip on a pipette (seal thoroughly) – **not by hand!**
- Pick up the pipette so that the finger rest of the handle is on the index finger and thumb can operate the two-position plunger.
- **Aspirating:** Press the plunger into the position „1“ (pipette tip is in the air), immerse the tip into the liquid, **slowly** release the plunger.
- **Dispensing:** Immerse the pipette into the solution into which you want to dispense the aspirated liquid. Dispense by pressing the plunger into the position „1“, finalize by pressing into the position „2“ and take the tip out of the solution (still in position „2“).
- Release the plunger, throw away the tip.

Plunger positions





Thank you for you attention

FUCK THE CELL CYCLE.



JUST KIDDING THE TEST IS NEXT WEEK...

meme-generator.net