MUNI
MED

Genetics in Dentistry – Practice

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Methods in Molecular Biology I

Biological Materials

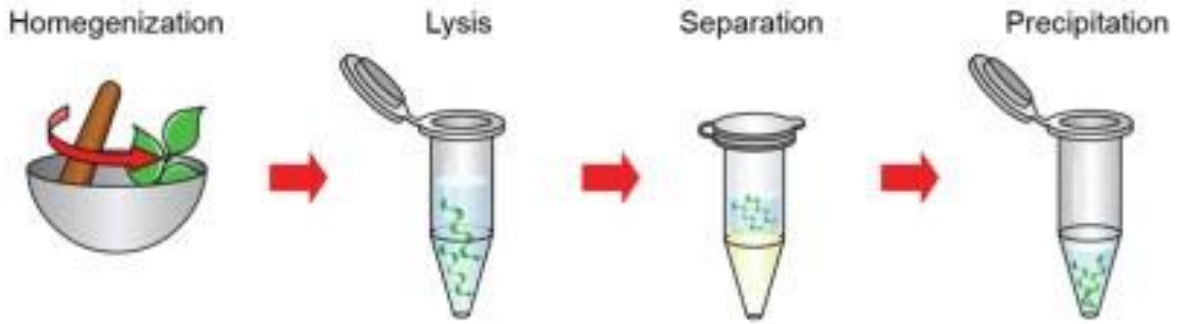
- Biological material is everything that was or is a part or a product of a living organism
  - dried herbal tea mixture
  - apple core
  - oak plank
  - cat droppings/fur
  - tube with SARS-CoV-2 virus
  - bodily fluids – urine, blood, plasma, serum, spit, ejaculate, phlegm
  - tissues, cells

Methods in Molecular Biology

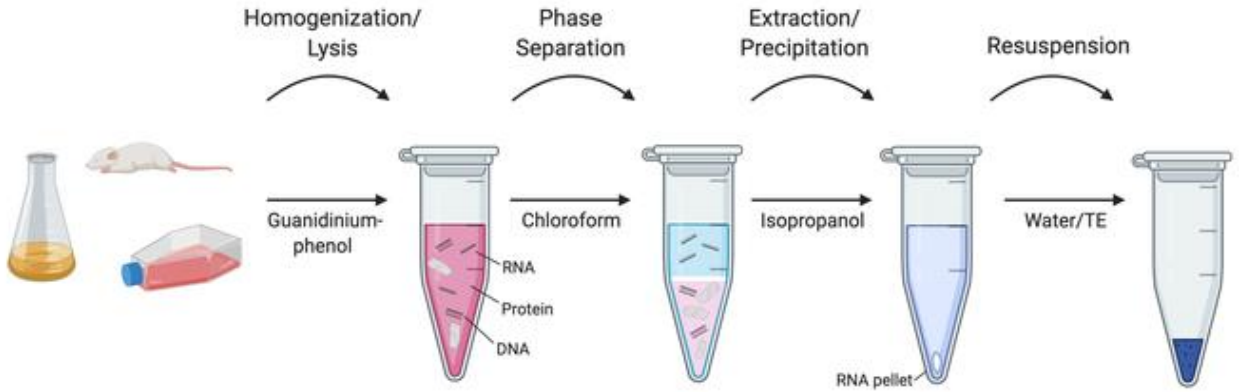
- Determination of nucleic acids
  - PCR, RFLP-PCR – detection by ELPHO
  - Real time PCR
  - Sequencing
- Determination of proteins
  - ELISA
  - Western blot
  - other methods based on antigen-antibody interactions
- Other molecular biology methods

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!

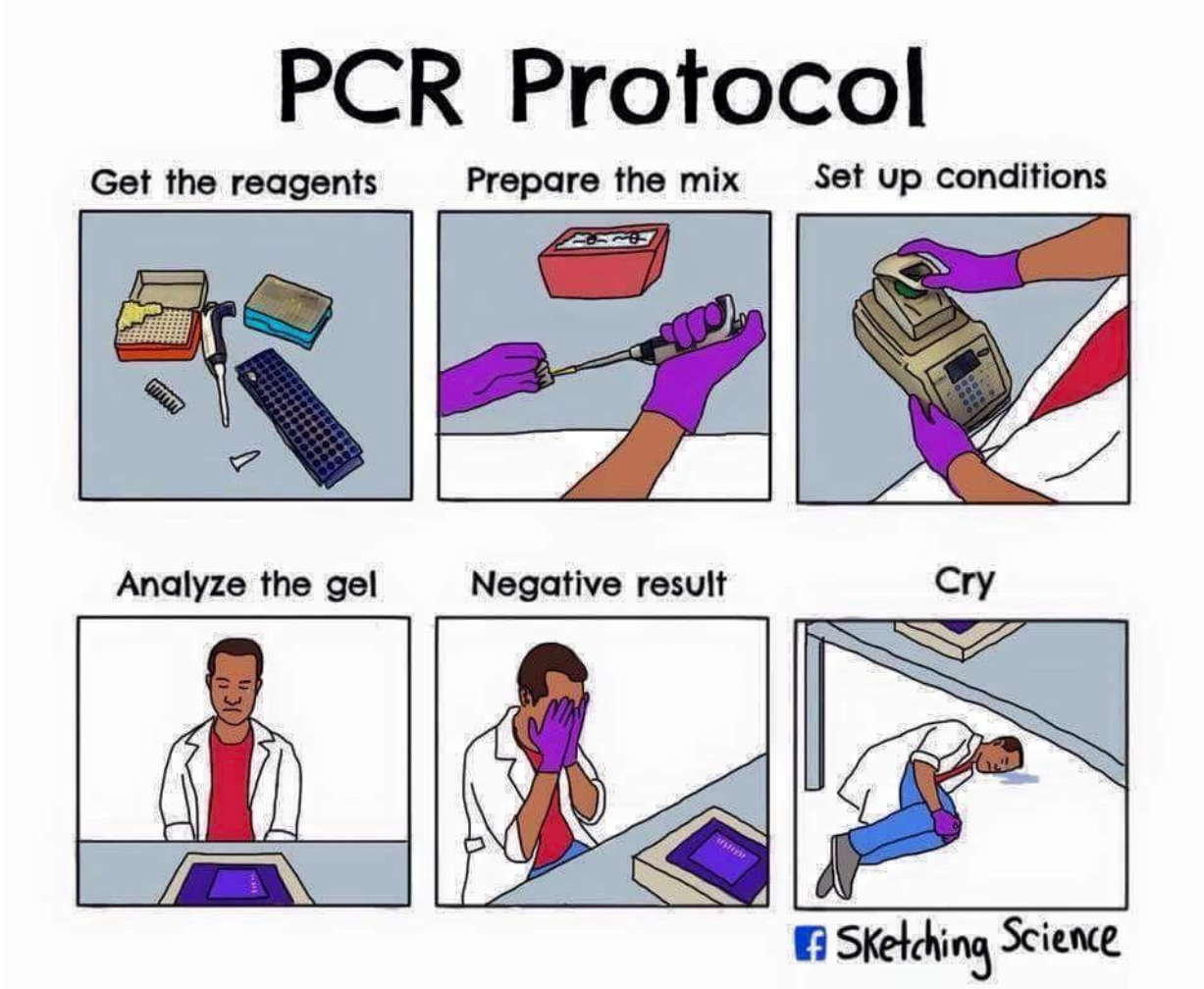


Methods in Molecular Biology II

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)

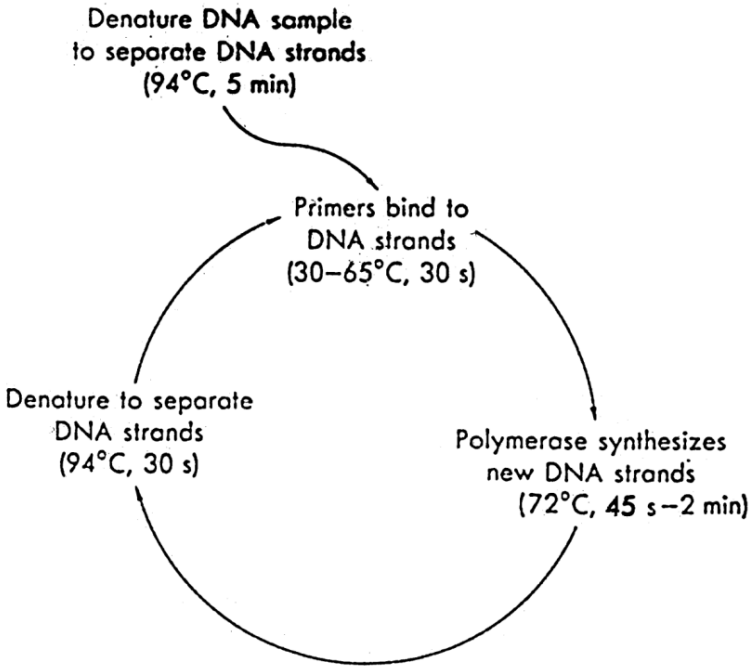
- Thermocycler

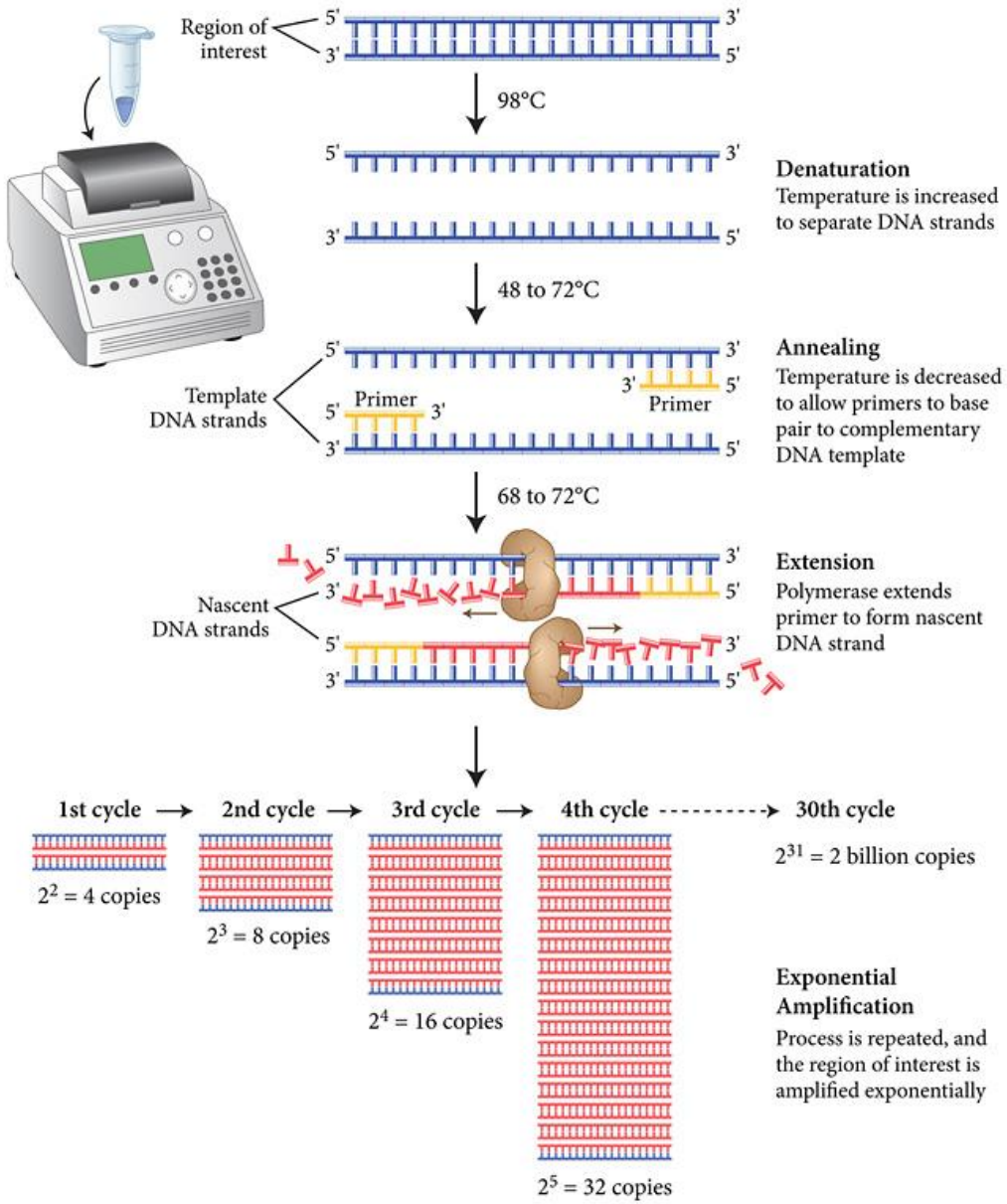


PCR

- **Multiple *in vitro* replication in a tube**
- Chain reaction based on DNA replication

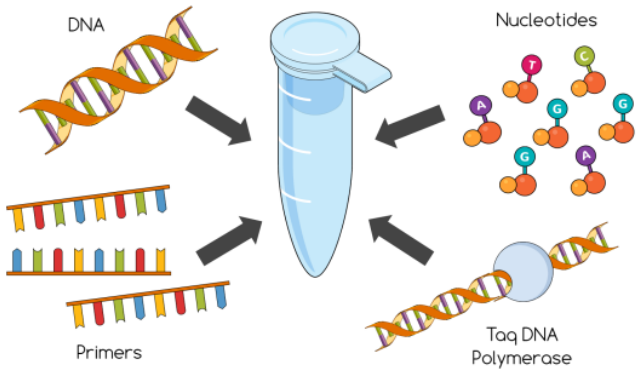
- Repeating cycles:
  - denaturation (separation of dsDNA) – 96 °C
  - annealing – primer binding – 50–65 °C
  - elongation – synthesis of a new DNA strand – 72 °C



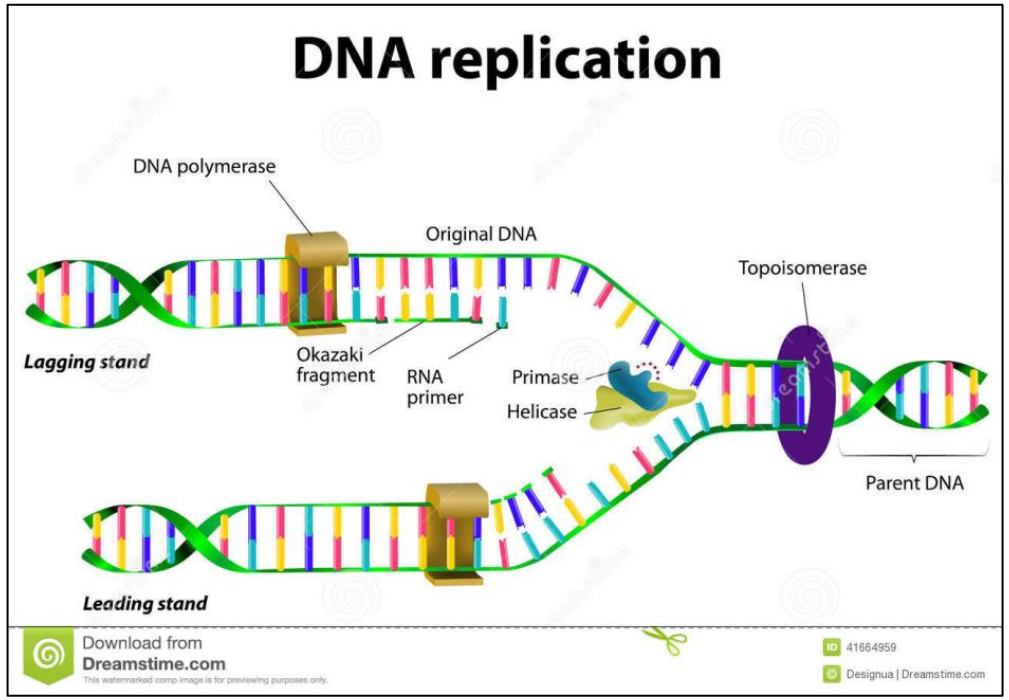


PCR

- **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
  - **Mg^{2+} ions**
    - affect activity and precision of polymerase
  - **template DNA**
  - **dNTP**
  - **buffer** (pH=8)
  - **temperature**



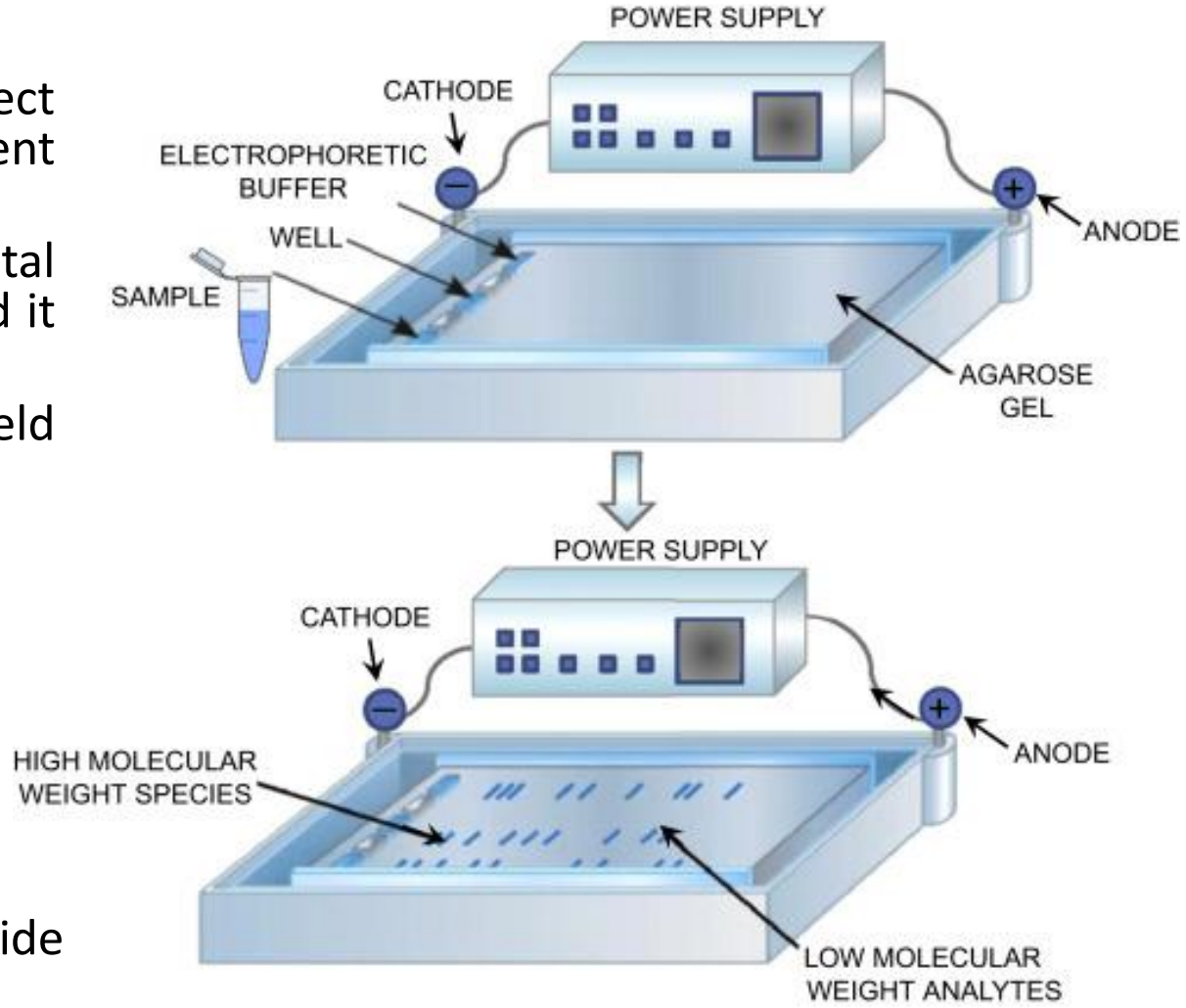
- **DNA replication – *in vivo***
  - **enzymes** – helicase, primase, DNA polymerase, ligase…



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

Gel Electrophoresis

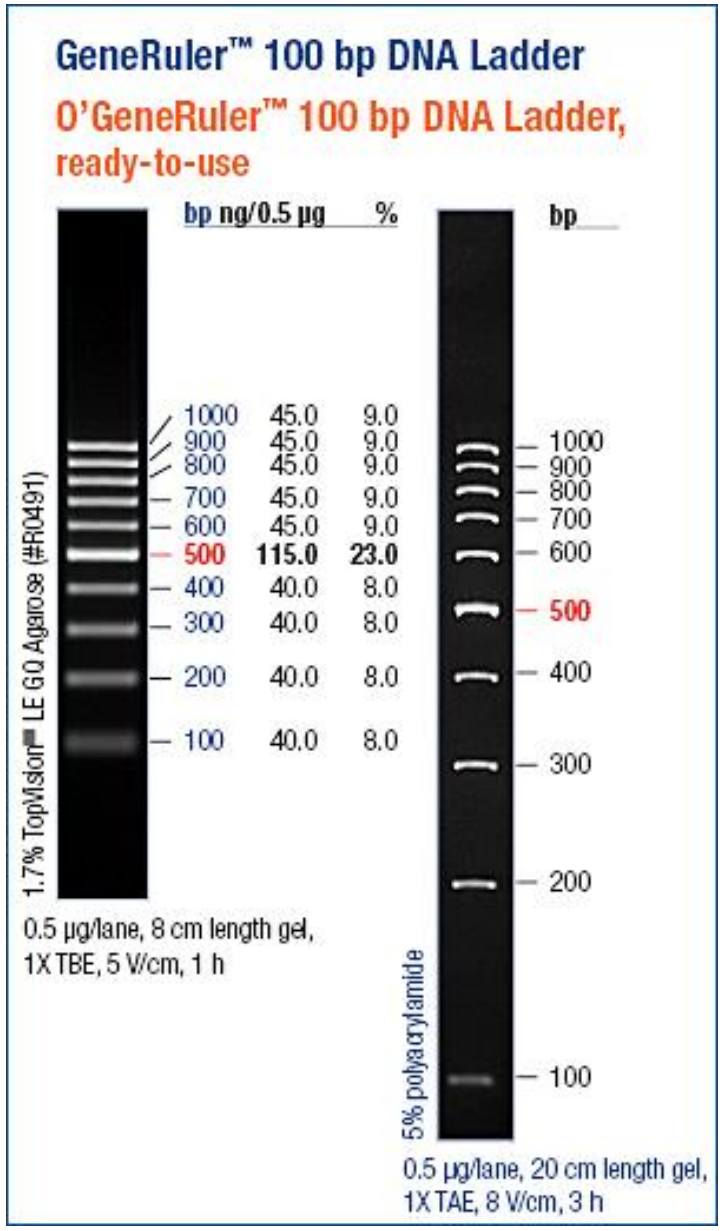
- separation method
- **principle** – movement of charged molecules in direct current field (separation of molecules with different molecular weight)
- speed of movement depends on the size of the total surface charge, size and shape of the molecule and it concentration in solution
- DNA has uniformly 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



https://www.sciencedirect.com/topics/chemistry/agarose-gel-electrophoresis

Gel Electrophoresis

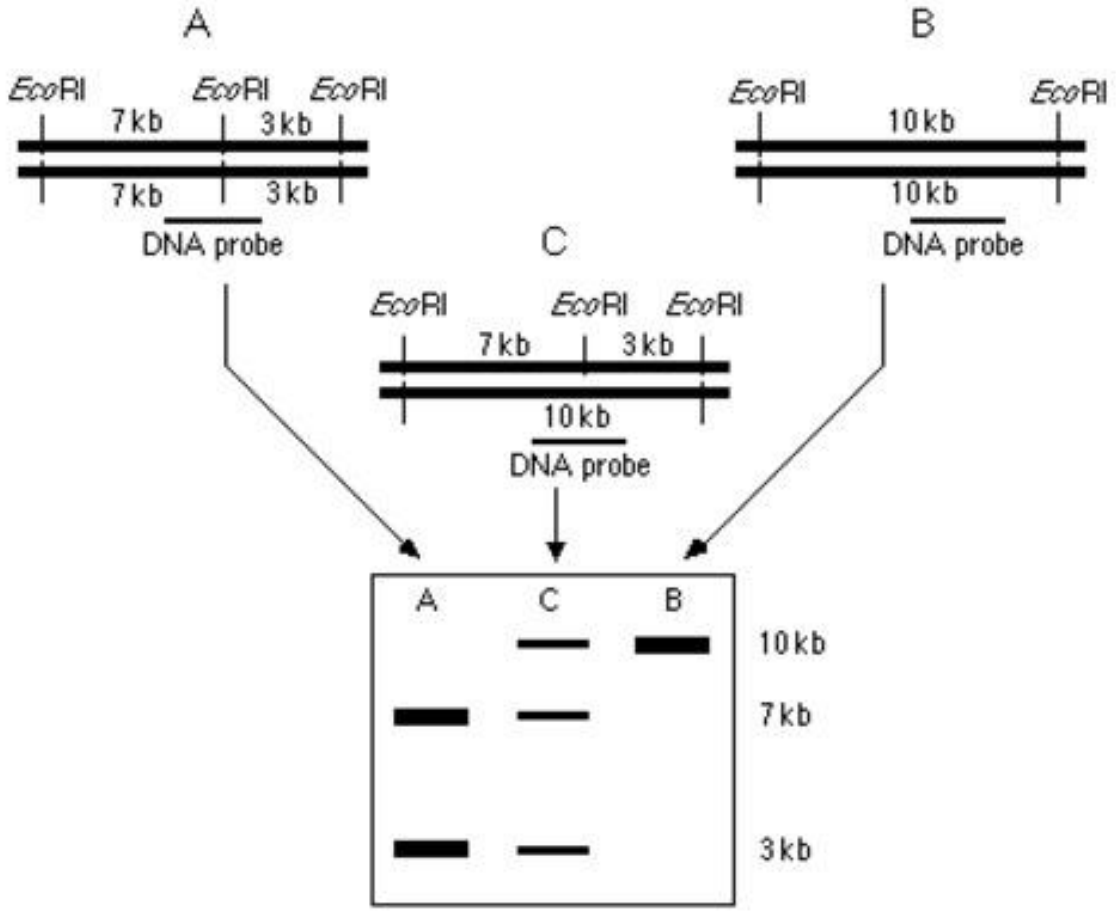
- Size of DNA fragments can be determined with molecular-weight ladders (= restriction fragments of plasmid molecules or genome of bacteriophages, size of which was determined *via* sequencing)



RFLP – Restriction Fragment Length Polymorphism

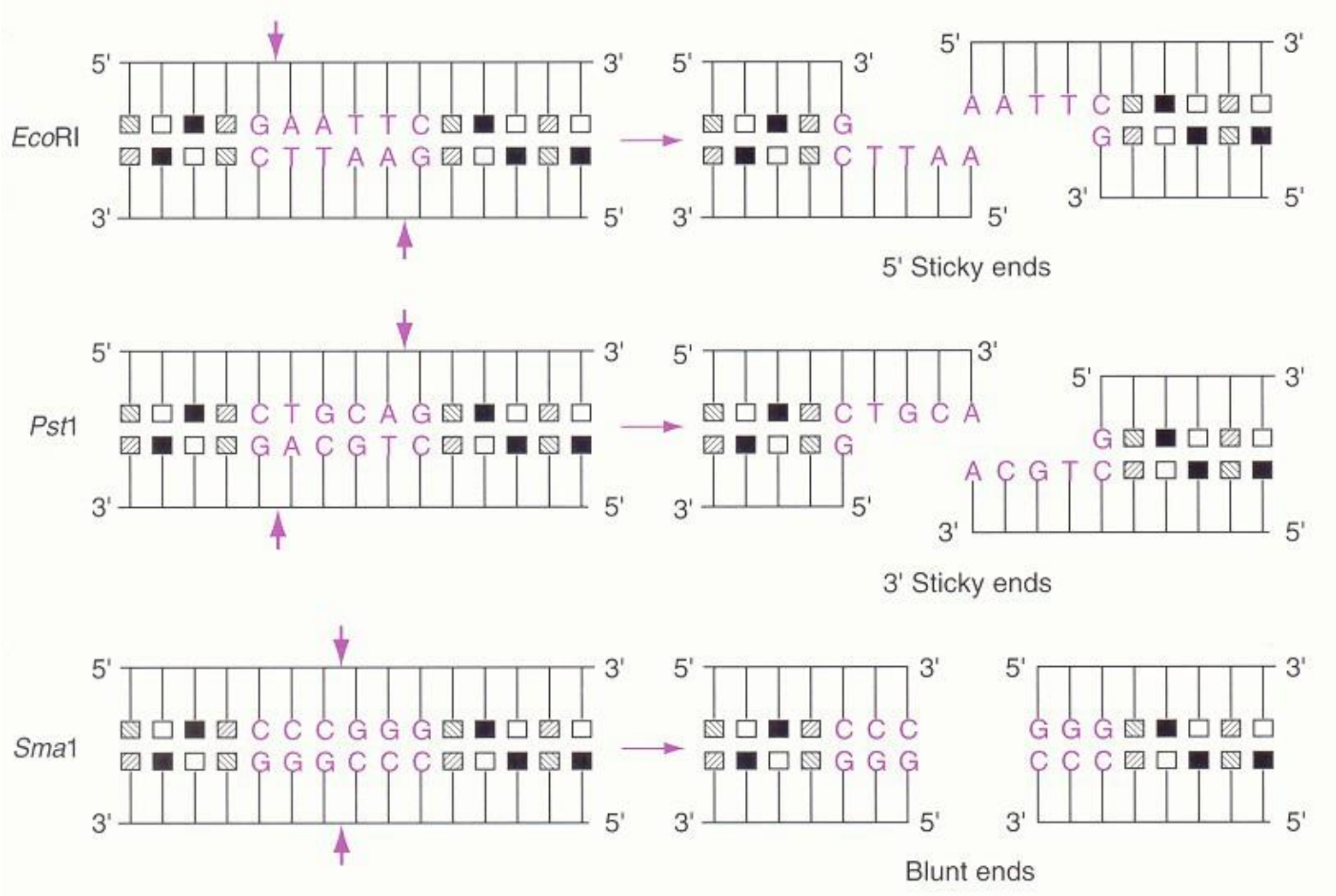
- Enzymatic cleavage of DNA in specific restriction site
- **Restriction endonucleases**
- Production of fragments with different lengths
- Created fragments separated *via* gel electrophoresis

- **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



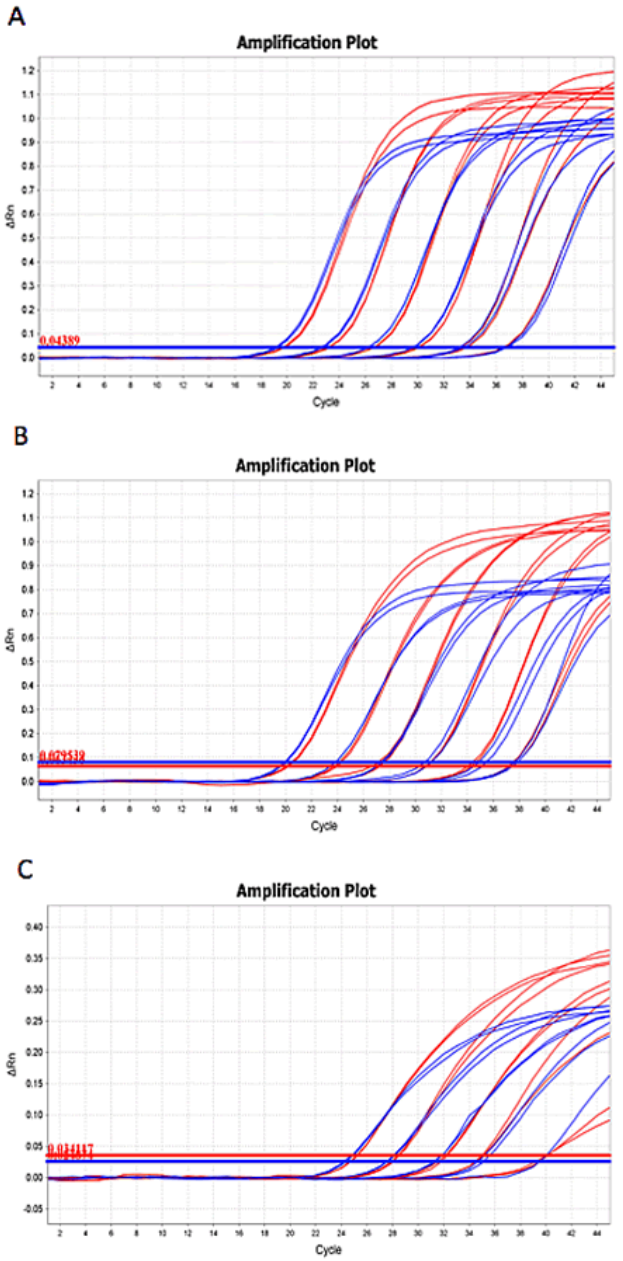
RFLP

- **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



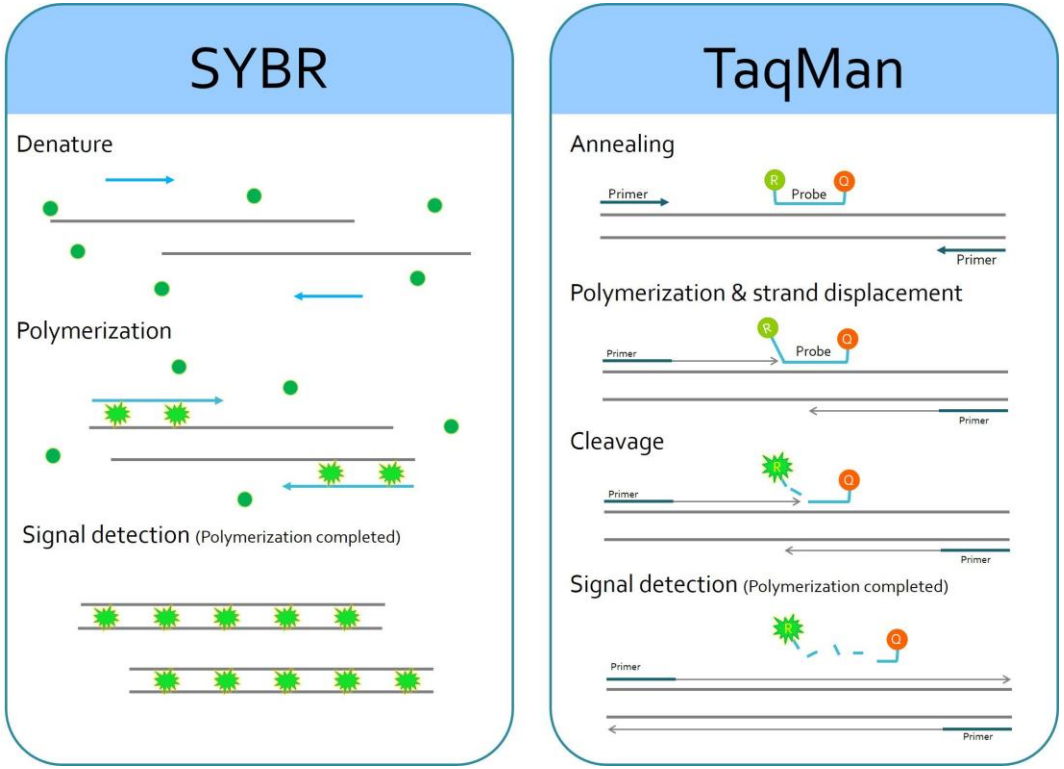
qPCR – Quantitative Real-time PCR

- 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)



Video: https://youtu.be/YhXj5Yy4ksQ

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

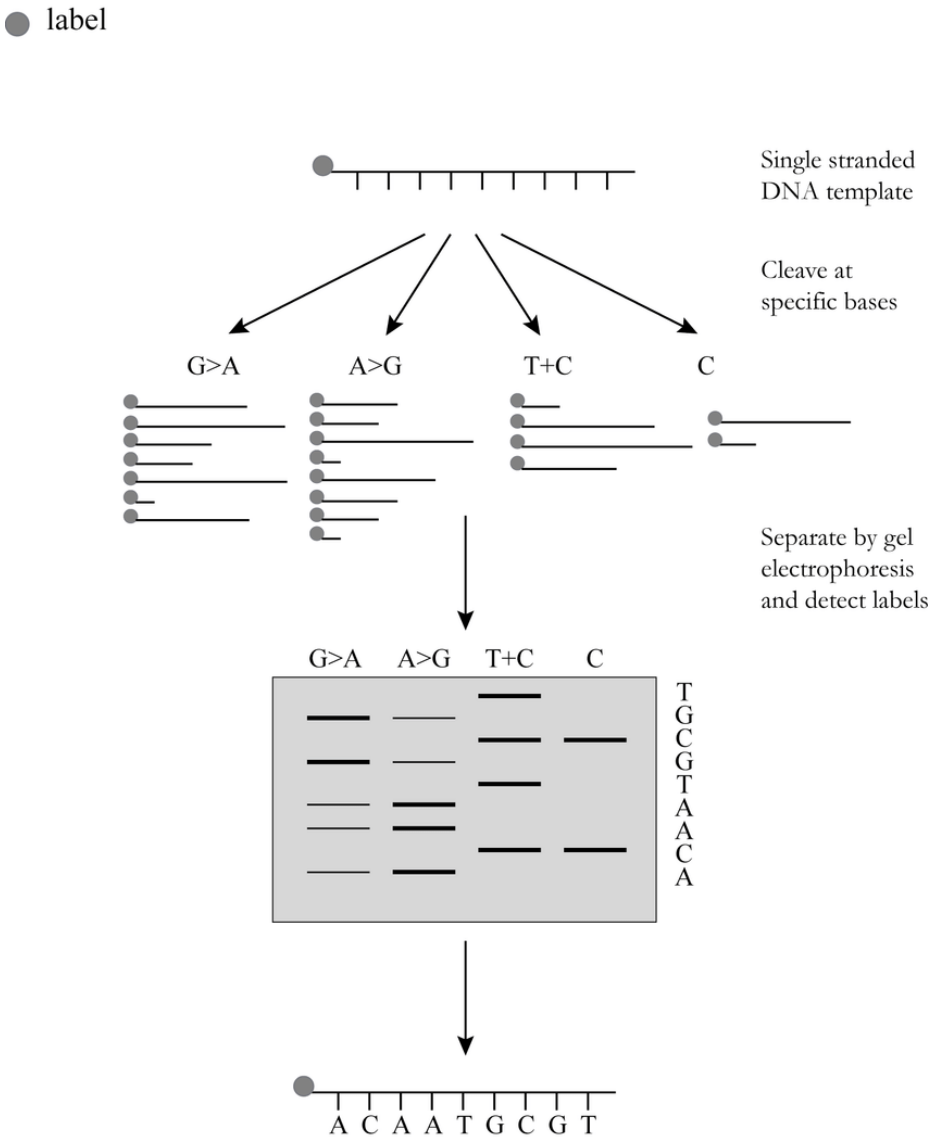
c) modern large format applications based on e.g. pyrosequencing (**next generation sequencing**)

- **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

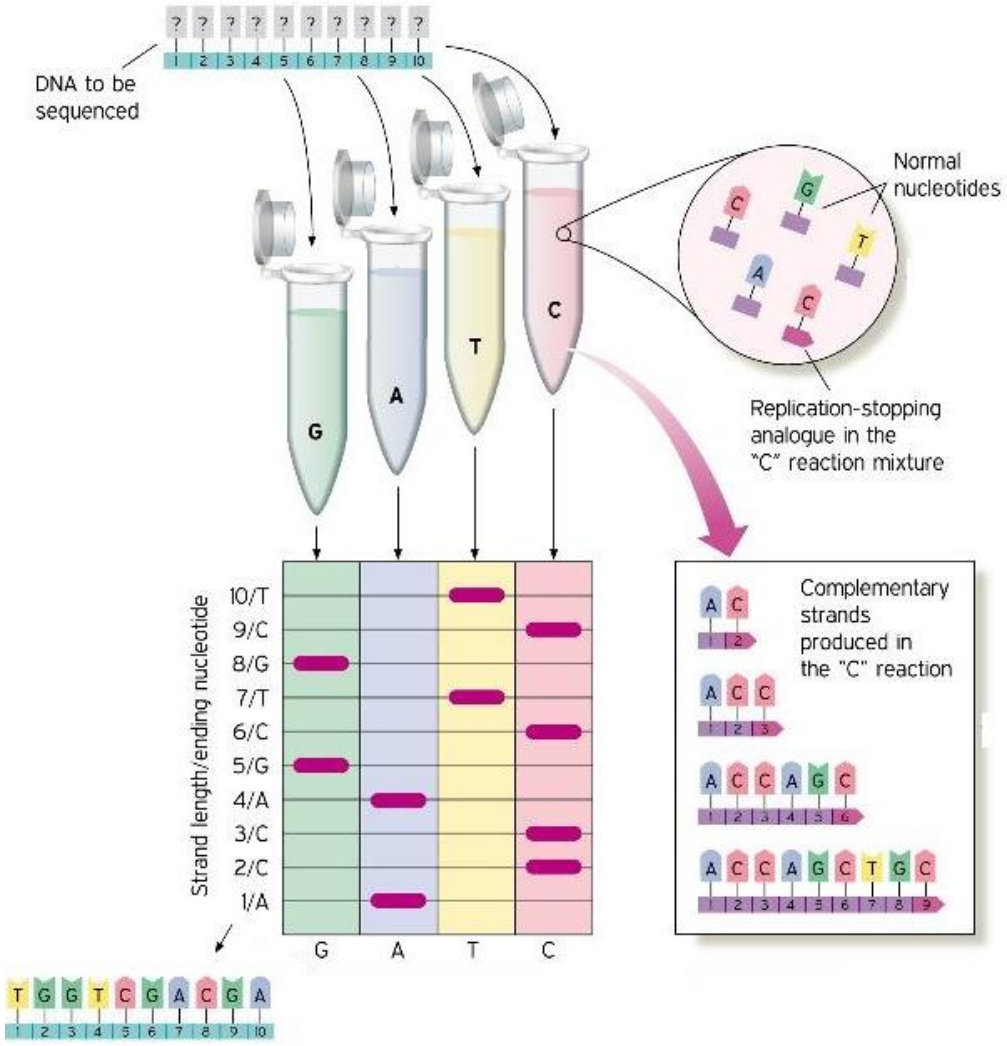
- Sequence is derived from a DNA molecule which chemically degrades to fragments in places with a base of a specific type. These are subsequently separated using ELPHO.
- **Chemical agents** – example:
  - piperidine breaks glycosidic bond of A and G (A + G)
  - hydrazine in the presence of NaCl reacts 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.



Video: https://www.youtube.com/watch?v=_B5Dj8PL4E0

Sanger Sequencing

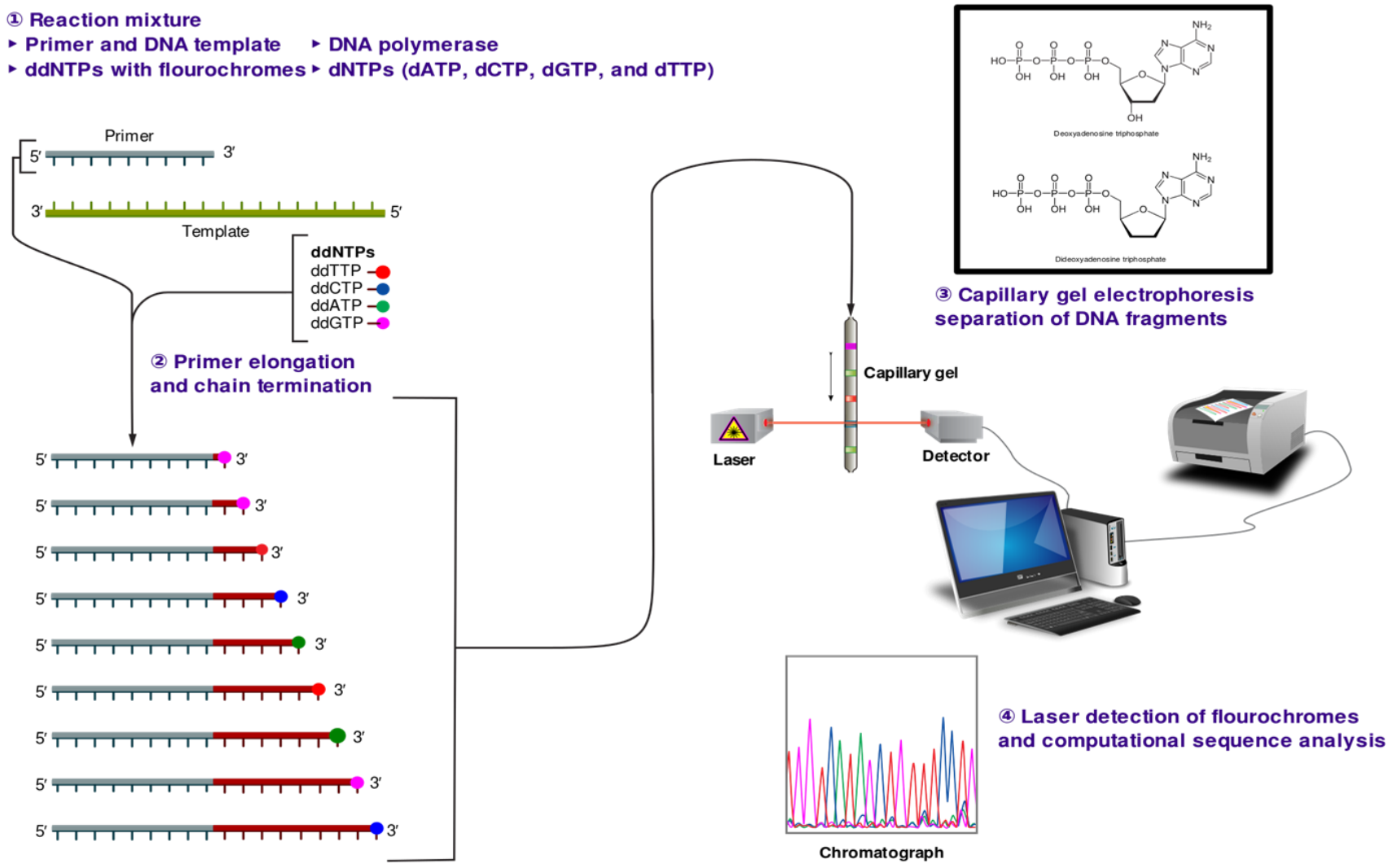
- Enzymatic method
- Based on **principle of replication** – end of DNA synthesis in the moment ddNTP is incorporated instead of dNTP
- **ddNTP** = analogue of dNTP, but lacks 3'-OH group on carbon
- ddNTP – terminator
- **Reaction mixture (4x)**
  - DNA template
  - primer
  - Taq DNA polymerase – synthesis 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



Video pro názornost: https://www.youtube.com/watch?v=wdS3j0TgbjM

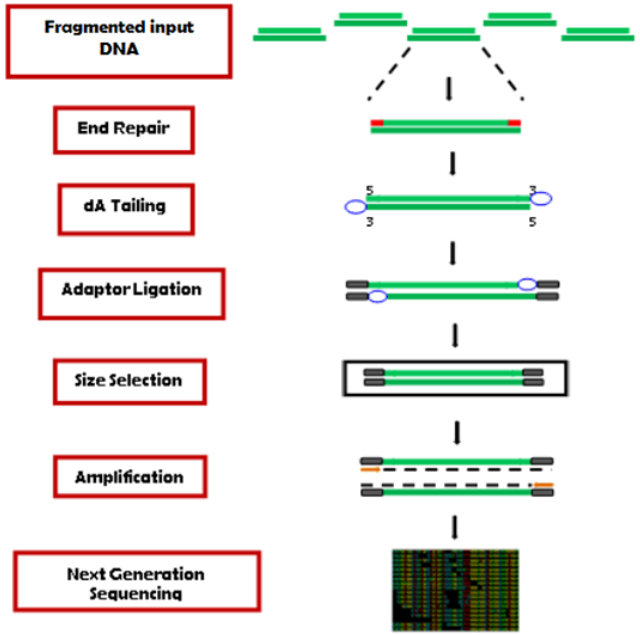
Sanger Sequencing

- Capillary sequencing of DNA with fluorescently labeled ddNTP



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

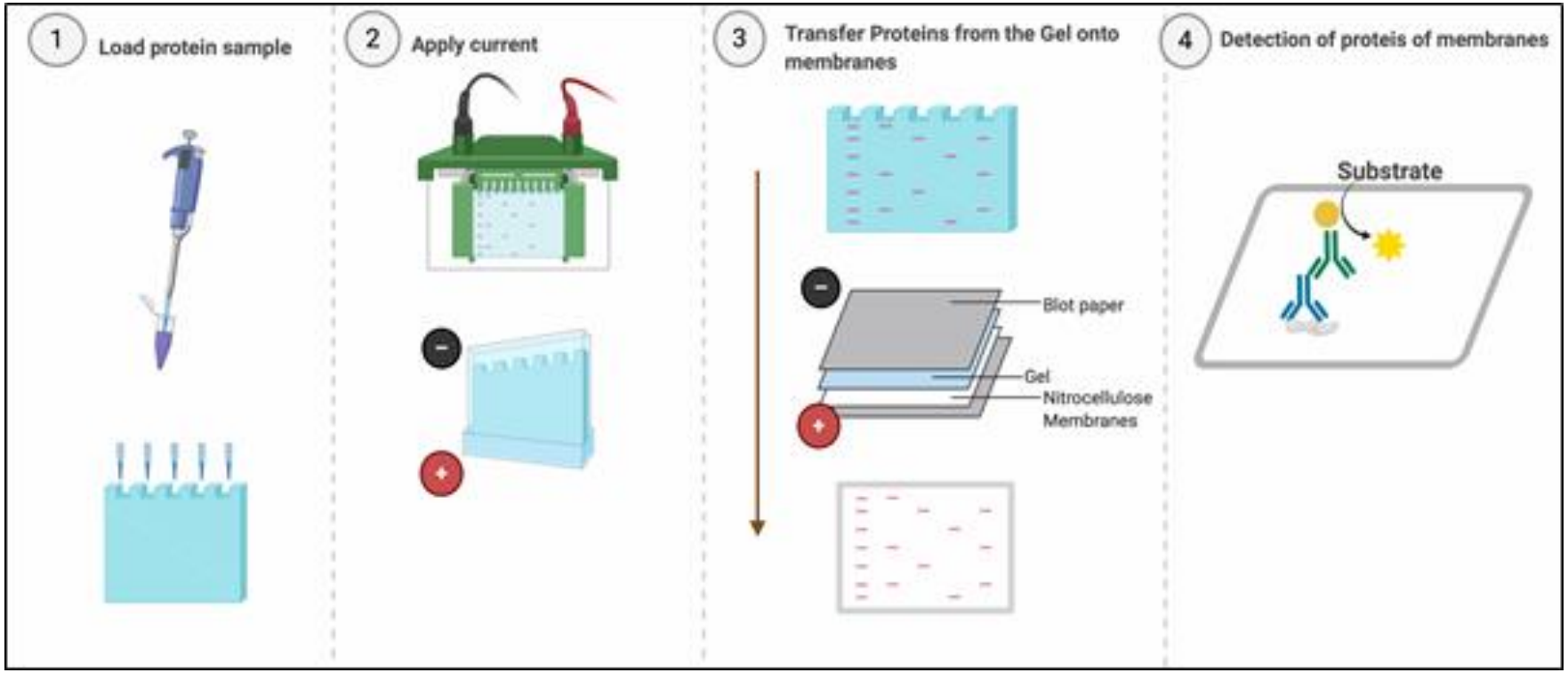


- Use:
  - whole genome sequencing
  - sequencing of chromosomes, plasmids, mt
  - study of genetic variability, mutational analysis
  - transcriptome analysis

Video:
https://www.youtube.com/watch?v=shoje_9IYWc
https://www.youtube.com/watch?v=CZeN-IgjYCo
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



Practical Part

Practical Part

1. PCR
2. ELPHO
3. Evaluation of ELPHO

Practical Part 1

Practical implementation of PCR

volume in microtube: 25 µL each sample

Composition (1 sample):

MASTER MIX

- 2 Primers 10 pmols (1.25 µL)
- $MgCl_2$ 25mM (4 µL)
- dNTP mix (0.5 µL)
- Taq polymerase 1U (1 µL)
- Buffer DYNEX (2.5 µL)
- PCR H_2O (12.5 µL)

- Template DNA 50ng (2µL)

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

35 ×

5. 72°C…..7 minutes
6. 10°C…..10 minutes

Practical implementation of PCR

1) Prepare Master Mix for all samples you have by multiplying prescription by the number of samples (plus 1 extra sample).

2) Divide Master Mix to each microtubes by 23 µL.

3) Add one sample (2 µL) to each microtube.

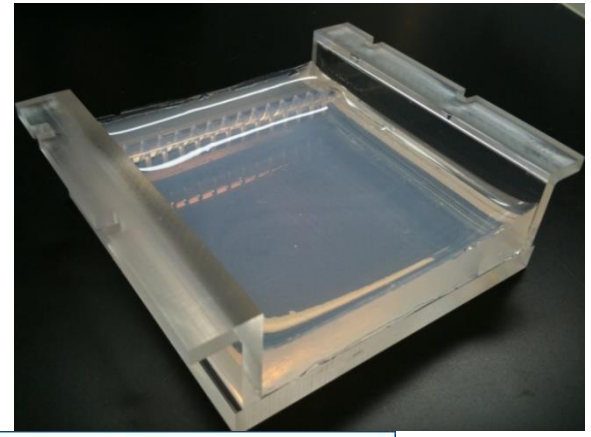
How to Use a Micropipette

https://www.youtube.com/watch?v=aSeod1Y5MRc

Practical Part 2

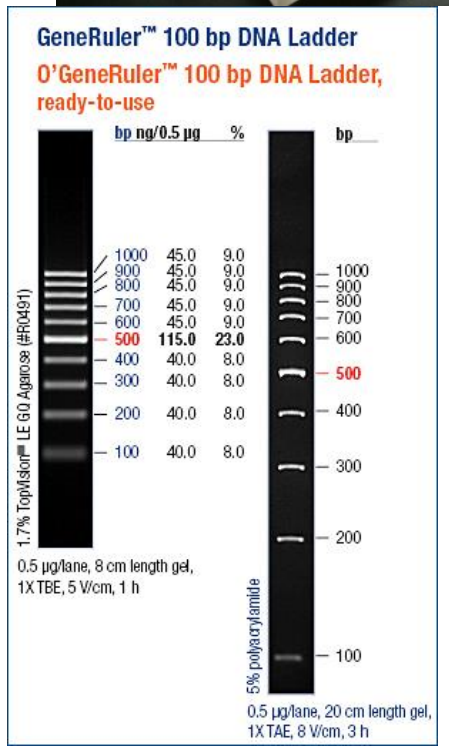
Practical implementation of ELPHO

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



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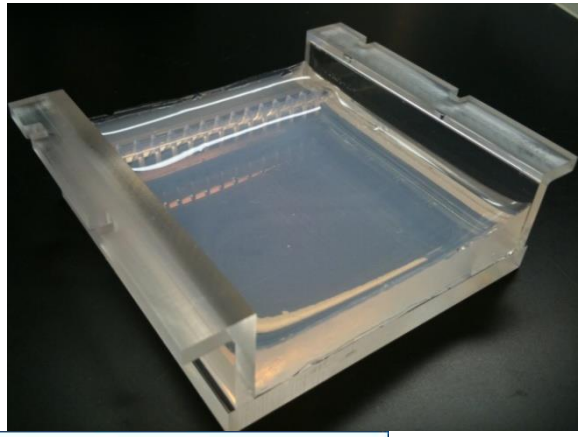

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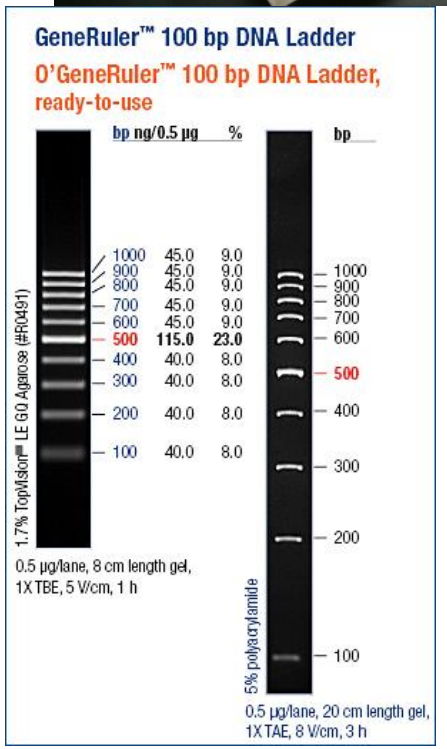
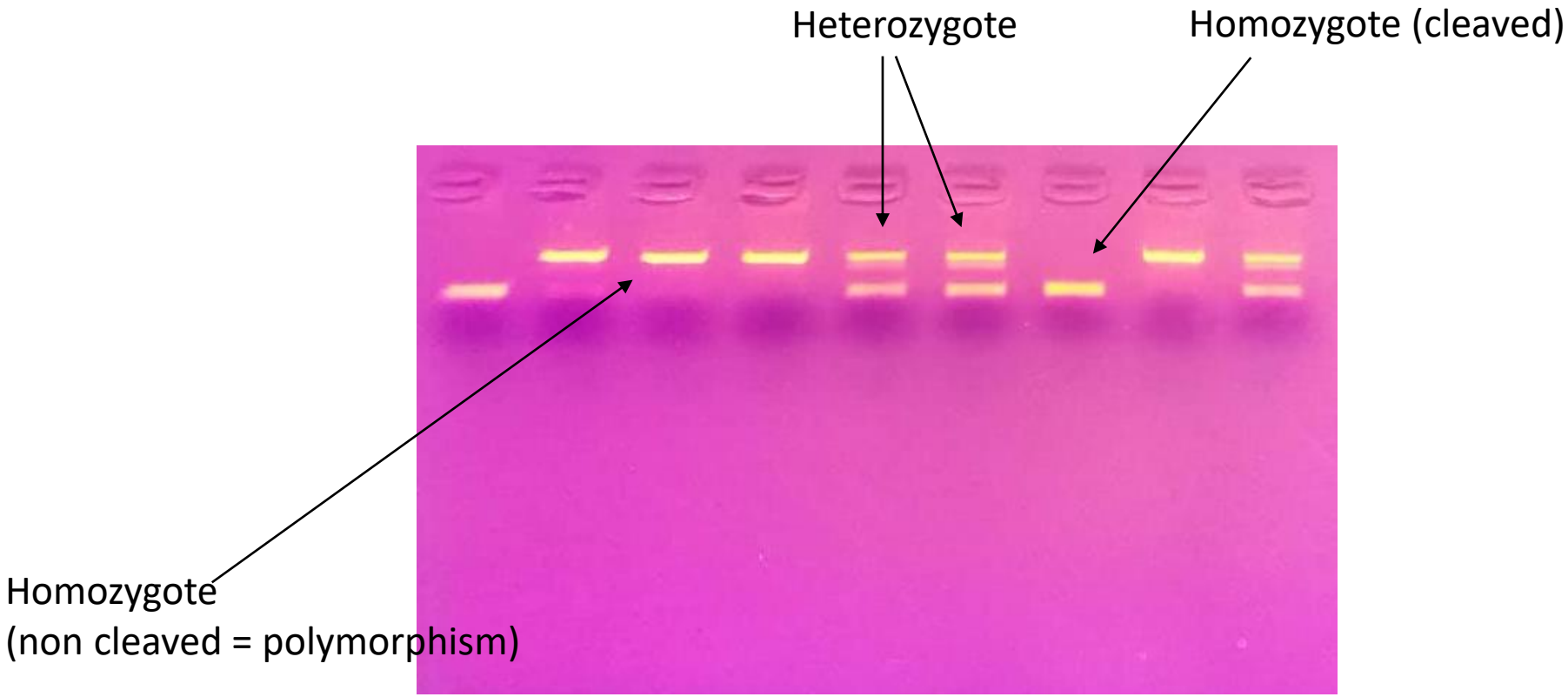


Practical Part 3

Practical implementation of ELPHO

2. Electrophoresis of restrictive fragments after TaqI cleavage



General Genetics

Johann Gregor Mendel

* 20. 7. 1822, Heizendorf
† 6. 1. 1884, Brno

- 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

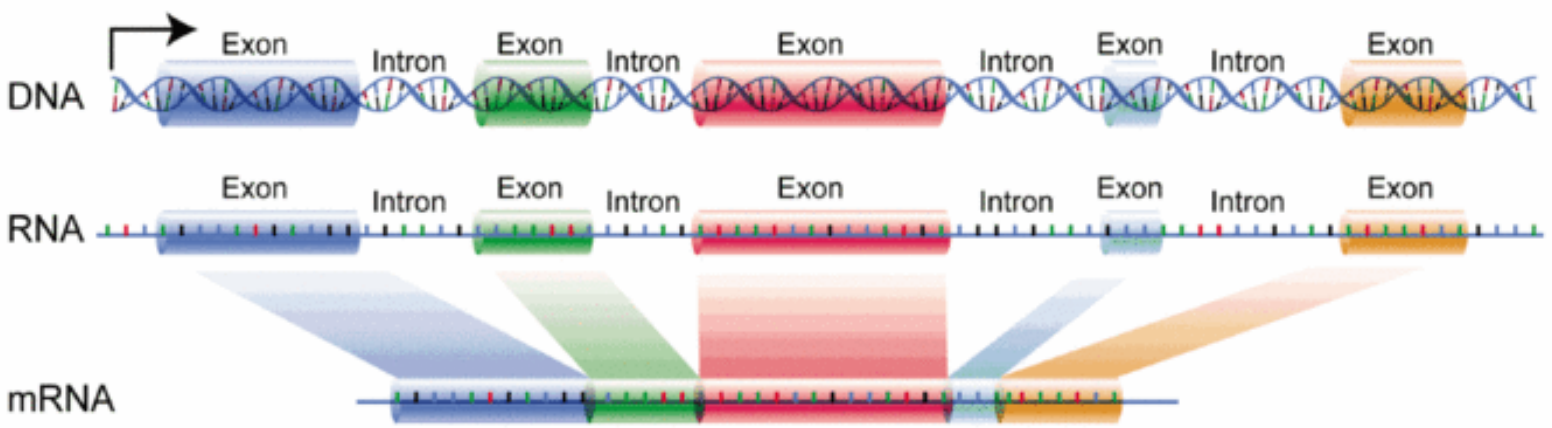
	Flower Colour	Plant Height	Seed Color	Seed Shape	Pod Colour	Pod Shape	Flower Position
Dominant Trait	Purple	Tall	Yellow	Round	Green	Inflated (full)	Axial
Recessive Trait	White	Short	Green	Wrinkled	Yellow	Constricted (flat)	Terminal

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

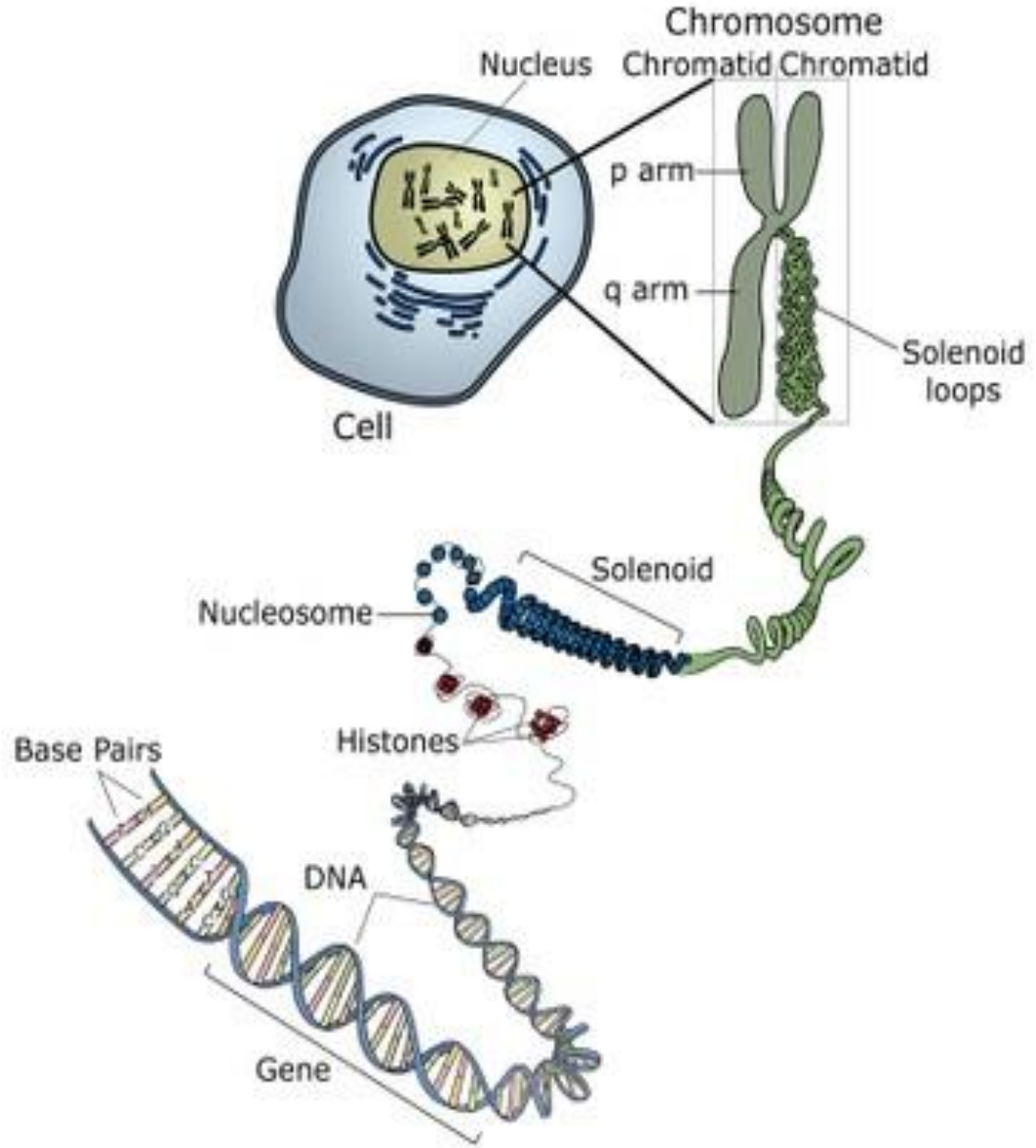
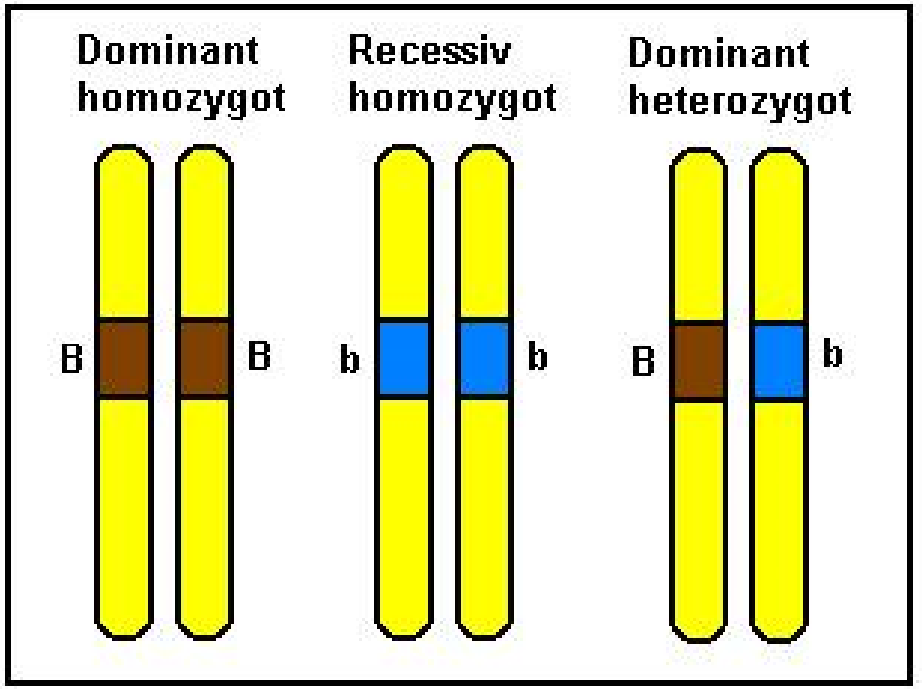


Image adapted from: National Human Genome Research Institute.

Basic Concepts

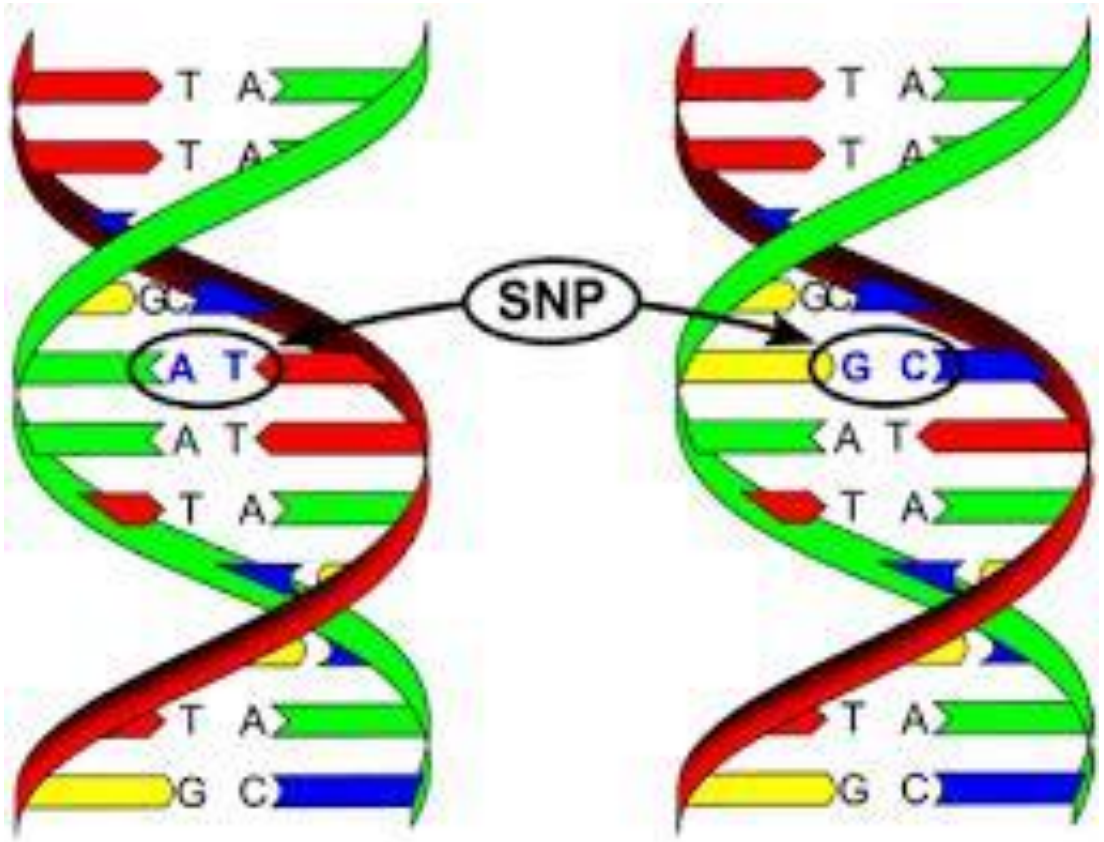
- **Genomics**
  - Field of genetics trying to determine the whole genetic information of an organism and to interpret it in terms of life processes
- **Heterozygote**
  - Two different variants (alleles) of a particular gene or its part
- **Homozygote**
  - Two same variants (alleles) of a particular 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 **≥ 1 %**

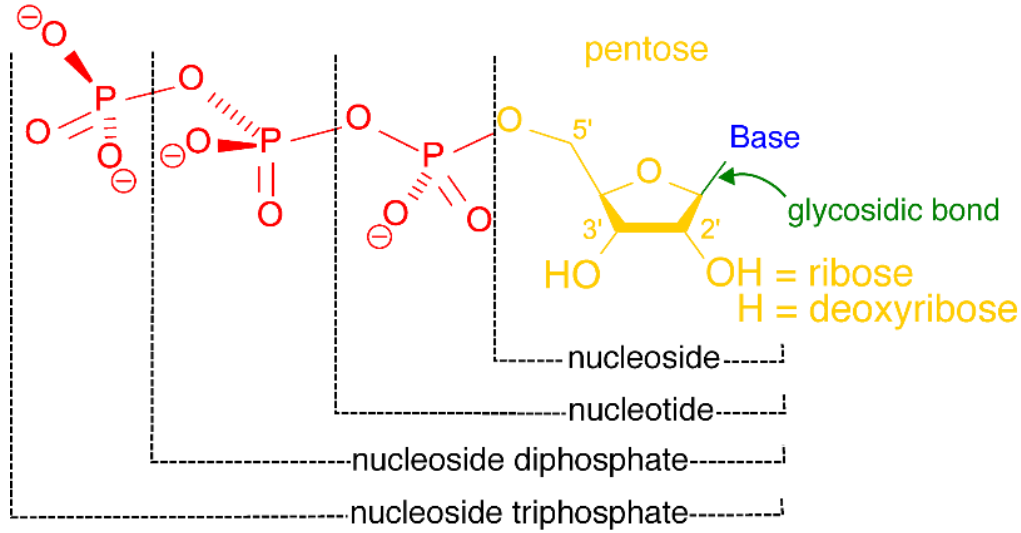
- **Mutation**
  - Processes, during which changes in genotype occur due to different environmental factors
  - Less common allele has population frequency **< 1 %**

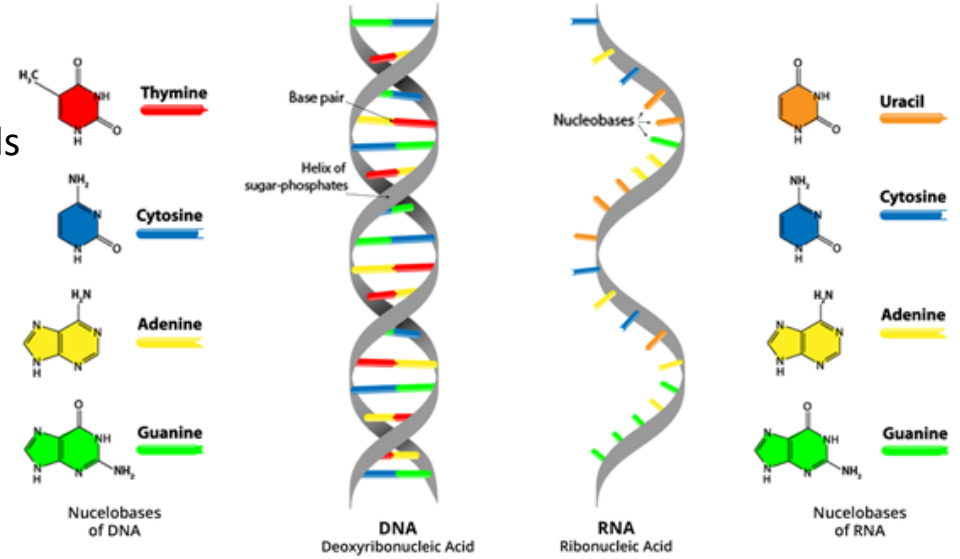


DNA vs. RNA

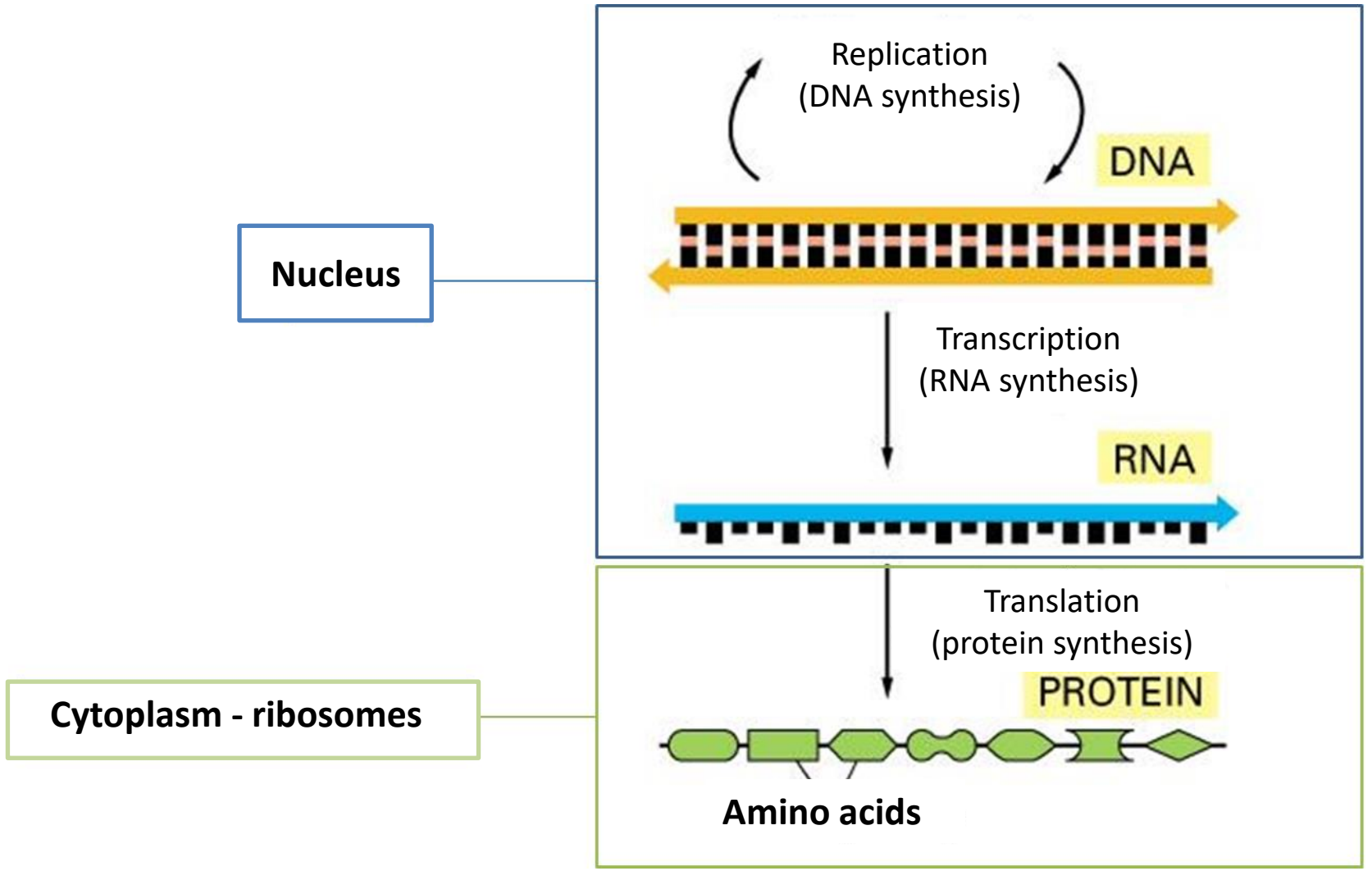
- DNA molecule = **deoxyribonucleic acid**
  - Double helix – 2 strands in opposing directions
  - Polynucleotide chain
    - Nitrogenous bases (T, A, C, G) connected with hydrogen bonds
    - Phosphate group
    - Sugar – deoxyribose

- 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

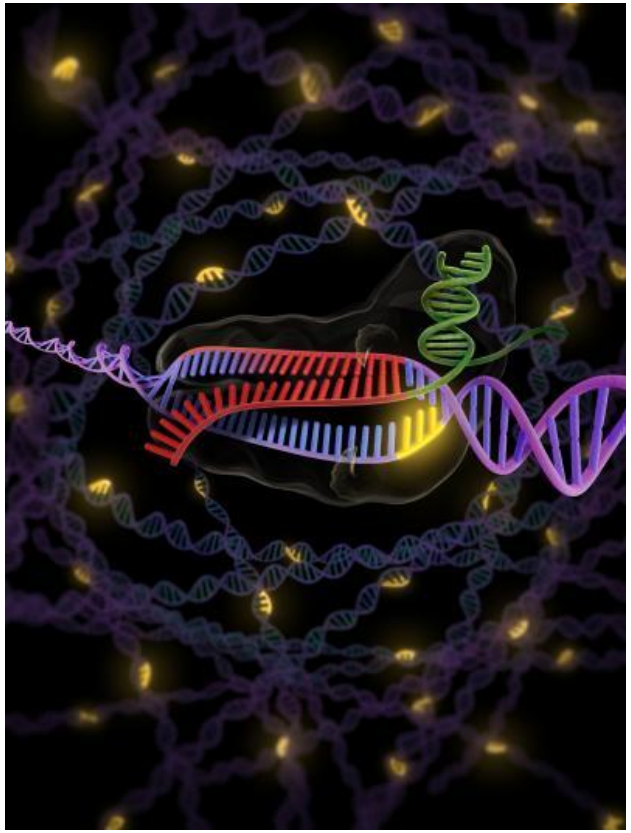


DNA Replication

= **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

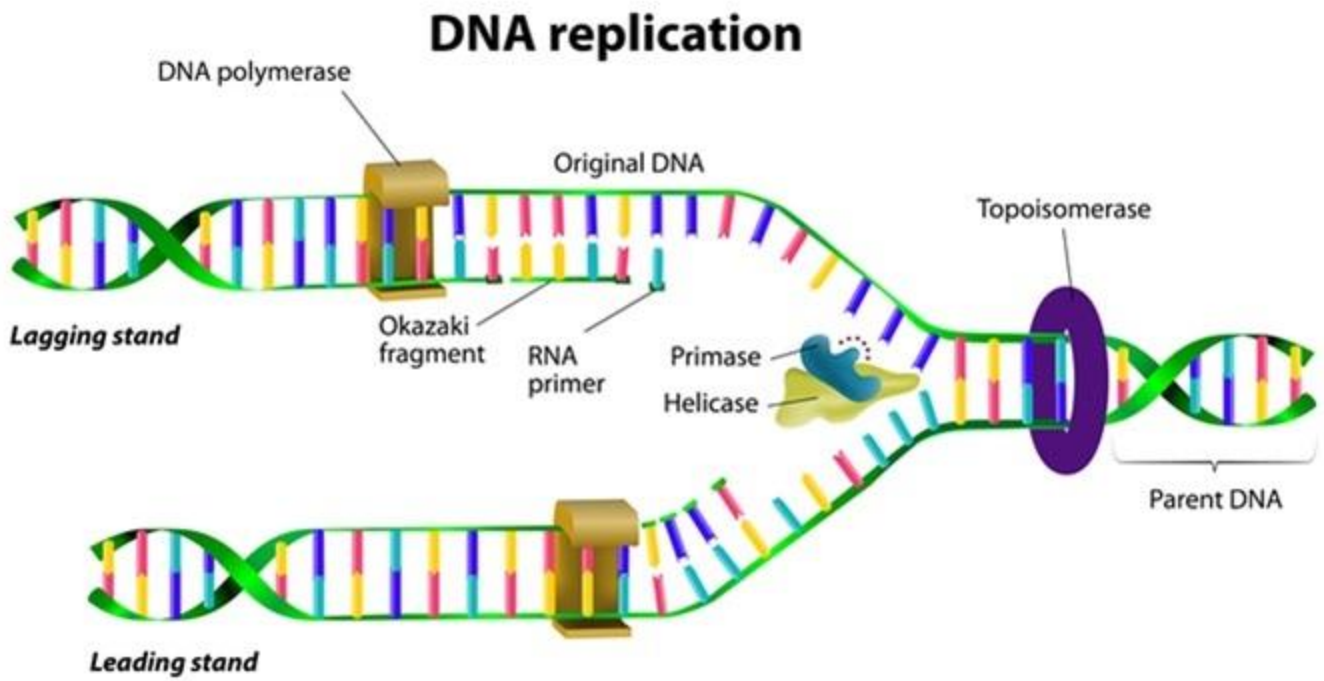


DNA Replication

- 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

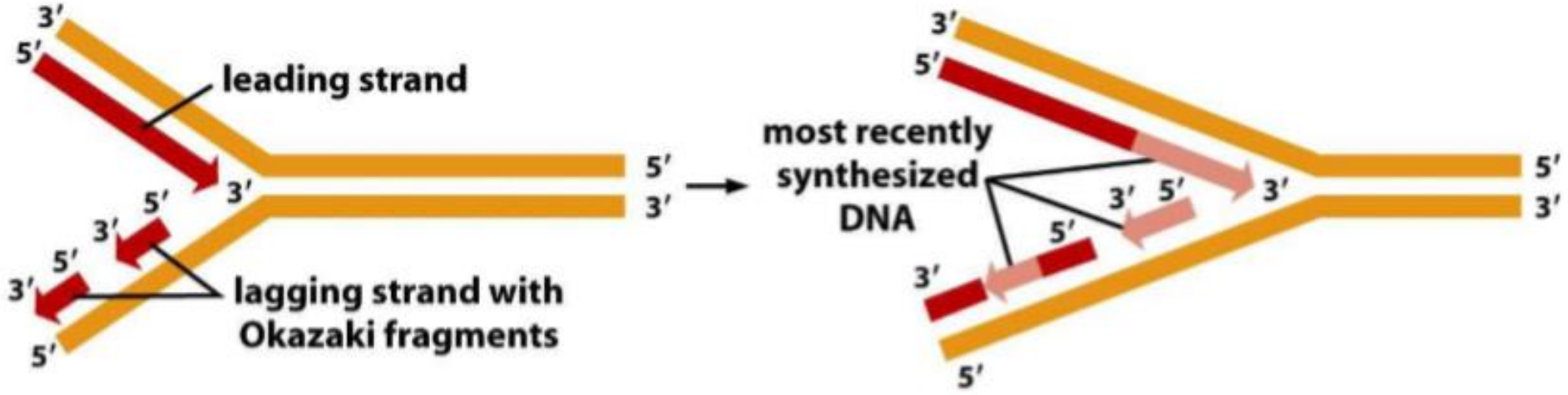
- Replication is started in specific locations - **replication origins**

- **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

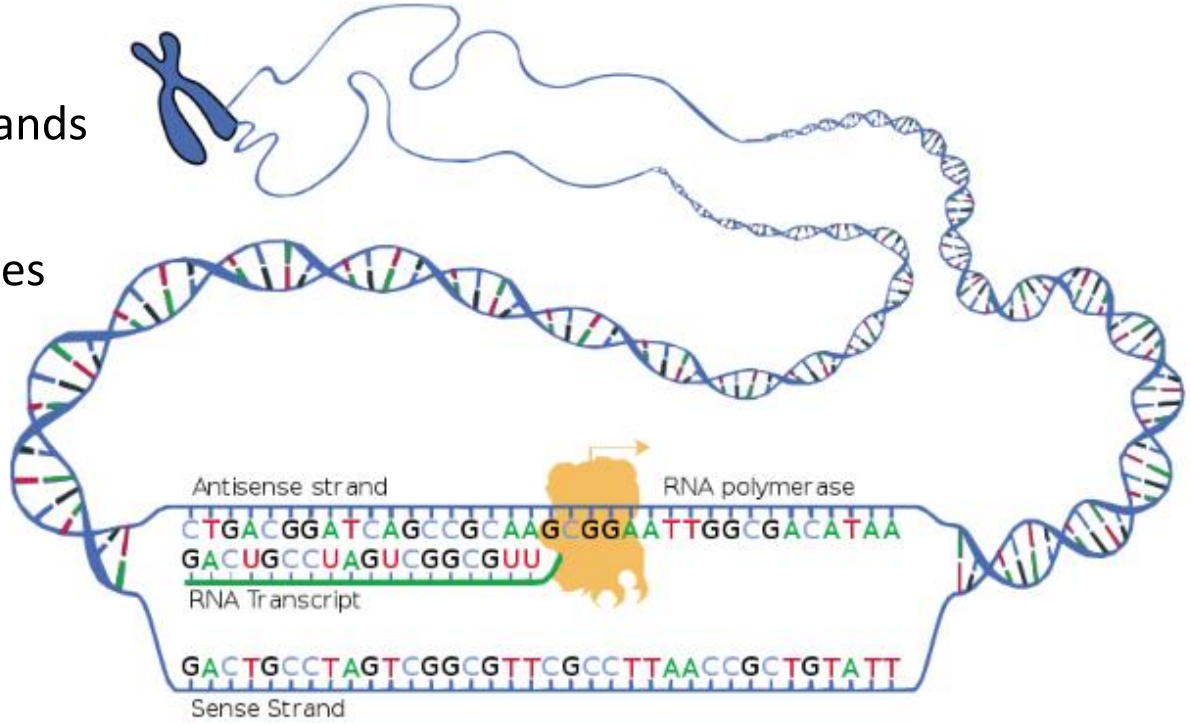
- Template strands are **antiparallel** – one strand is lagging
  - Leading strand – one RNA primer at the beginning, replication without interruption
  - Lagging strand – in direction 5'- 3' are discontinuously produced short **Okazaki fragments** (every from a new RNA primer), later connected by **DNA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



Transcription

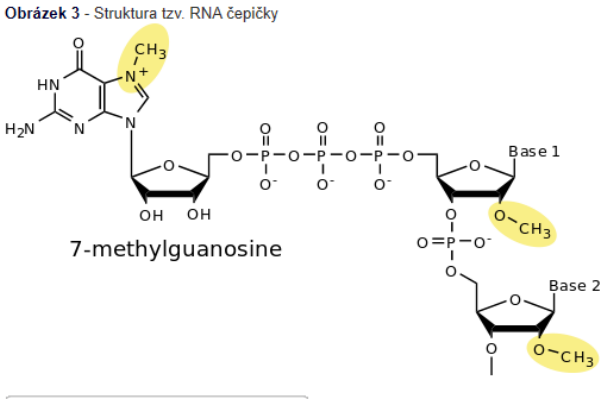
- **transcription** of the information in a form of DNA sequence to RNA sequence
- cell nucleus
- **template** - DNA strand
- transcripts are from the template released as single strands
- **DNA-dependent RNA polymerase**
  - 3 types (similar structure, transcribing different types of genes)
    - RNA 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

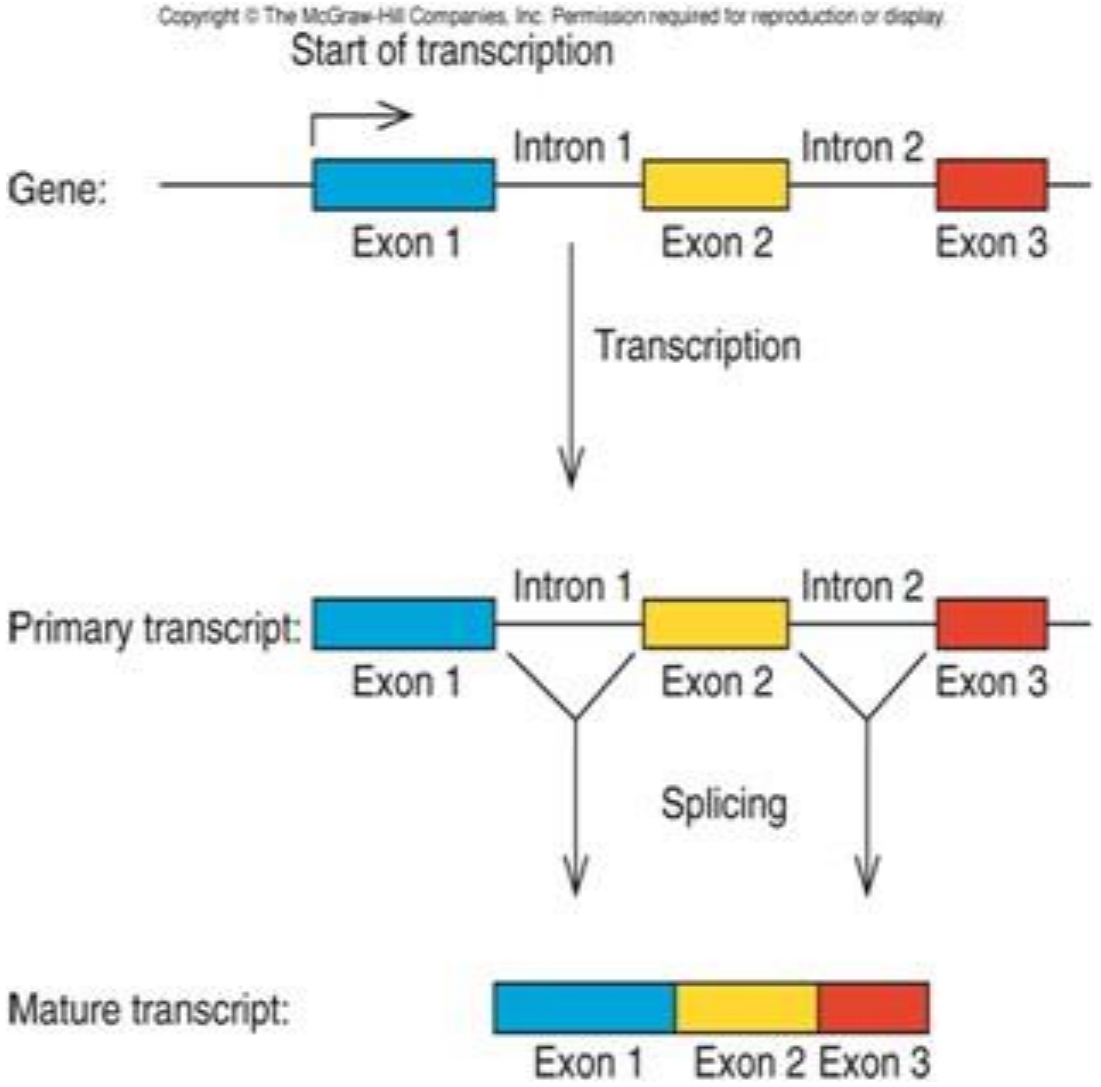


Post-transcriptional Modification

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



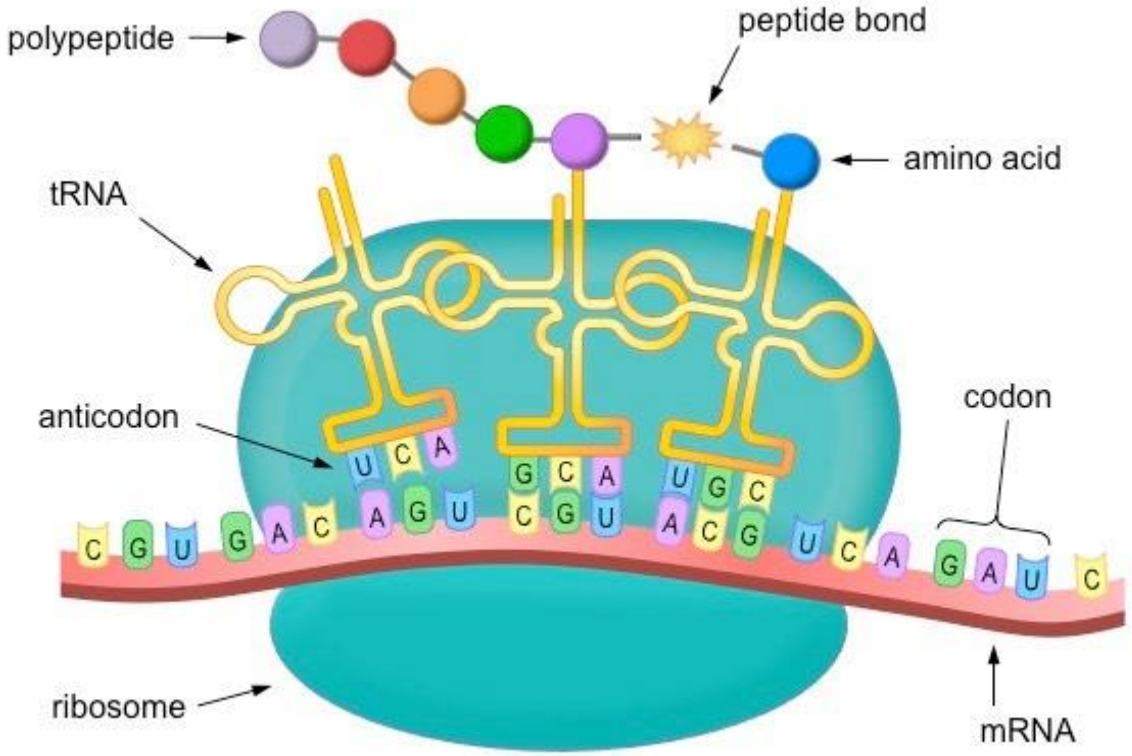
  - connection of **polyadenylic chain** to 3'end
- **RNA splicing** – cutting out of introns to form mature mRNA



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**

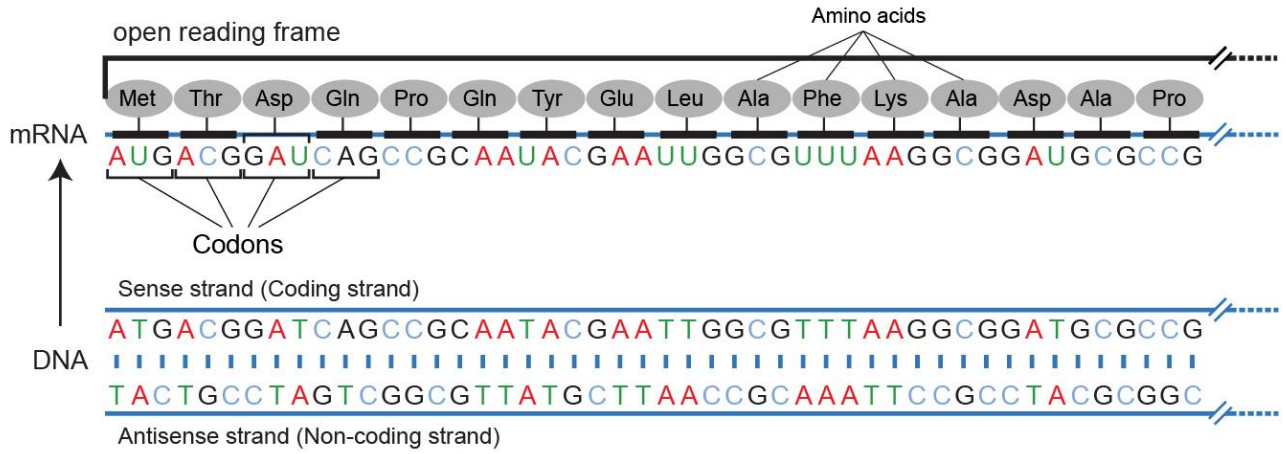
RNA codon table

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

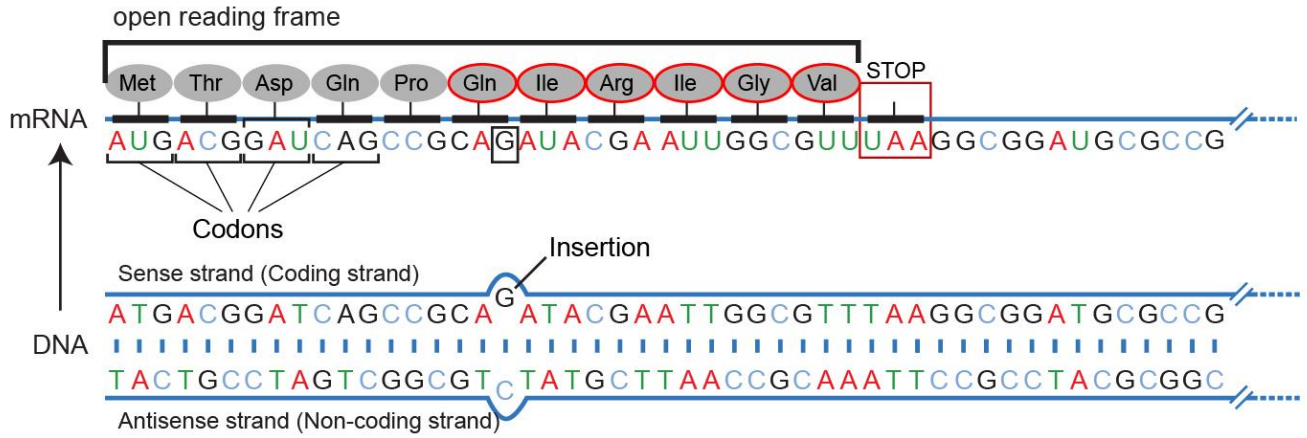
Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp:Aspartic acid
Cys:Cysteine
Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine
Leu: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
Thr: Threonine
Trp: Tryptophane
Tyr: Tyrosisne
Val: Valine

Genetic Code – Reading Frame Shift

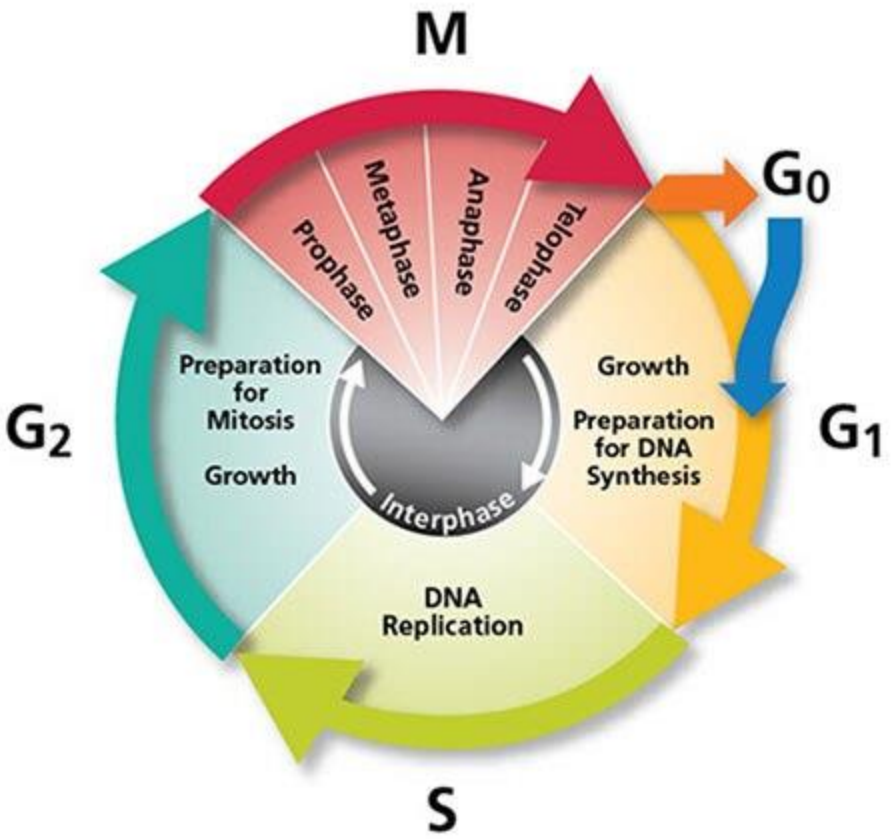
Normal



Frameshift mutation - single nucleotide insertion



Cell Cycle



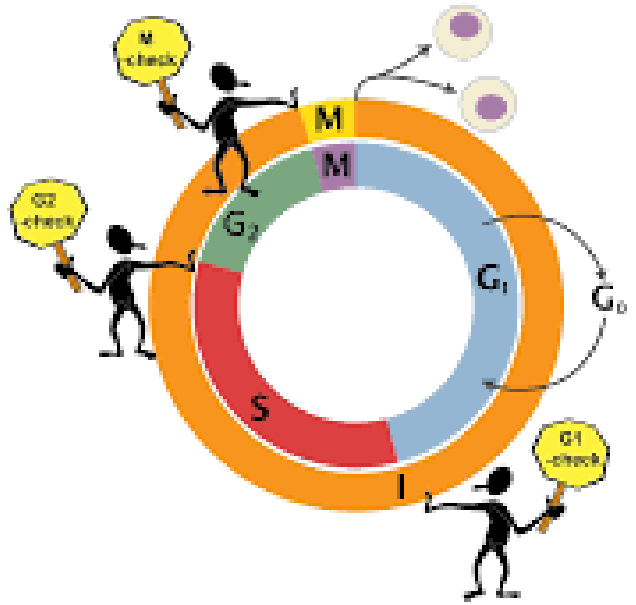
= organized sequence of processes during which the cell doubles its content and subsequently divides into two daughter cells (both of them carry the same chromosomes)

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

Cell Cycle

- **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



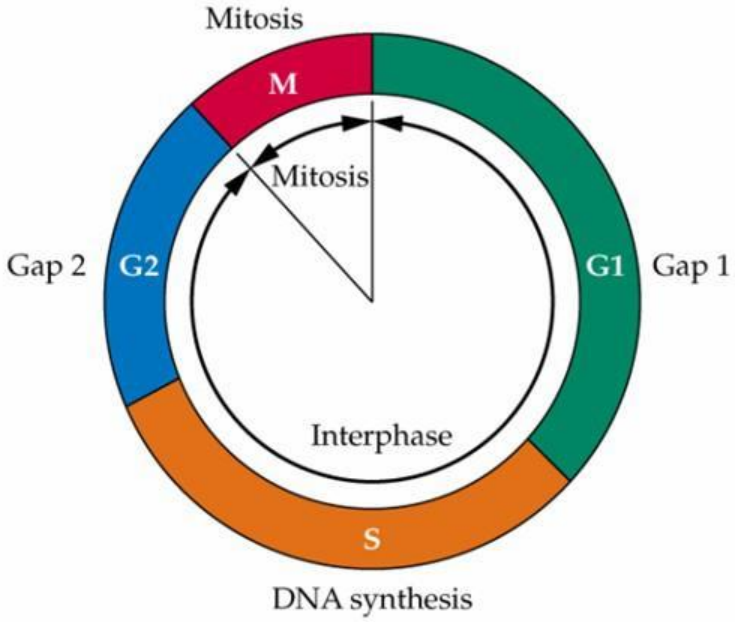
Cell Cycle - Interphase

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)

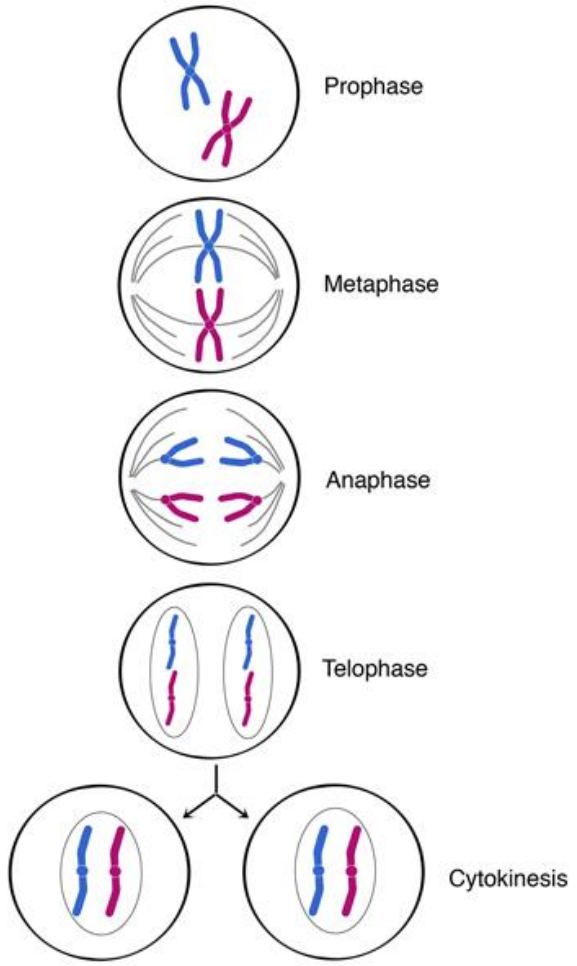
Cell Cycle - Mitosis

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

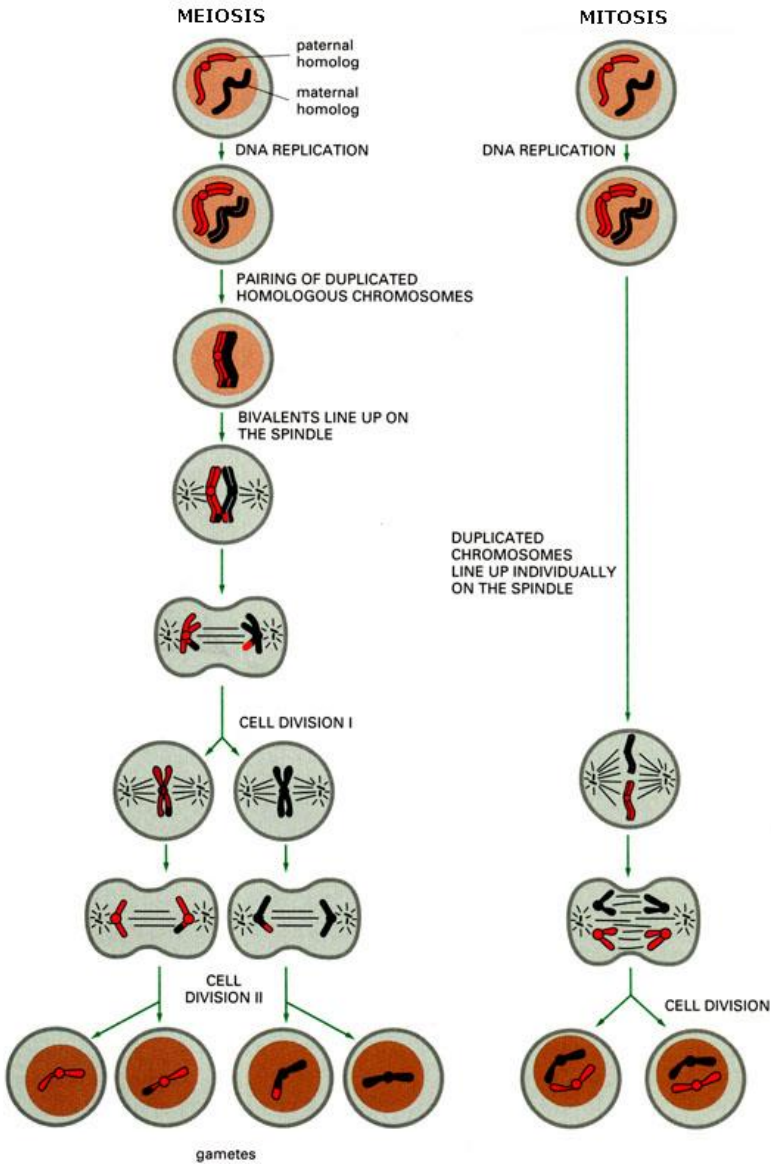
Mitosis

- 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 kinechotore 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.

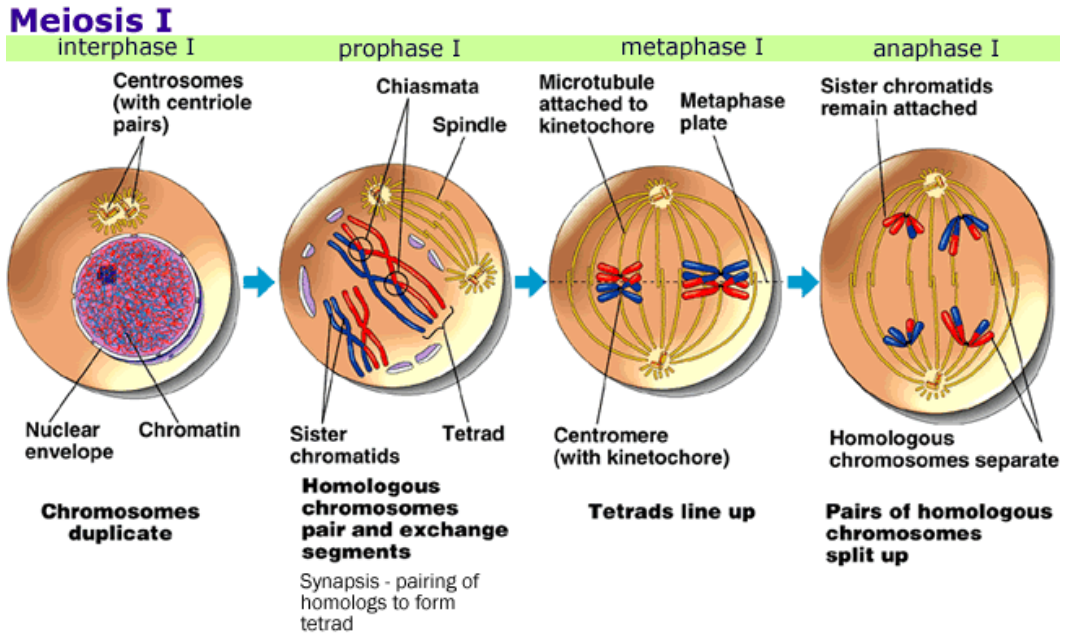
second meiotic division – separation of daughter chromatids -> 2 daughter cells with haploid number of chromosomes, formation of germ cells (sperm, egg), further swapping of genetic material by crossing-over

Meiosis

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

Heterotypic division

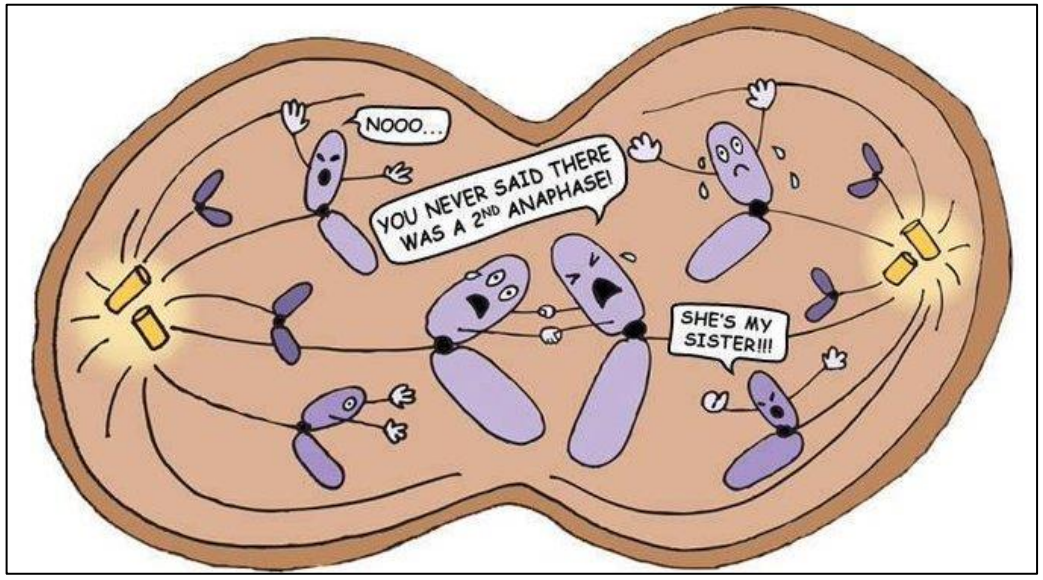
- **Prophase** – 5 stages
  - **Leptotene** – spiralization
  - of chromosomes
  - **Zygotene** – formation of bivalents
  - **Pachytene** – **crossing over** = crossing of non-sister chromatids
  - **Diplotene** – gradual separation of homologous chromosomes
  - **Diakinesis** – dispersion of nuclear 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

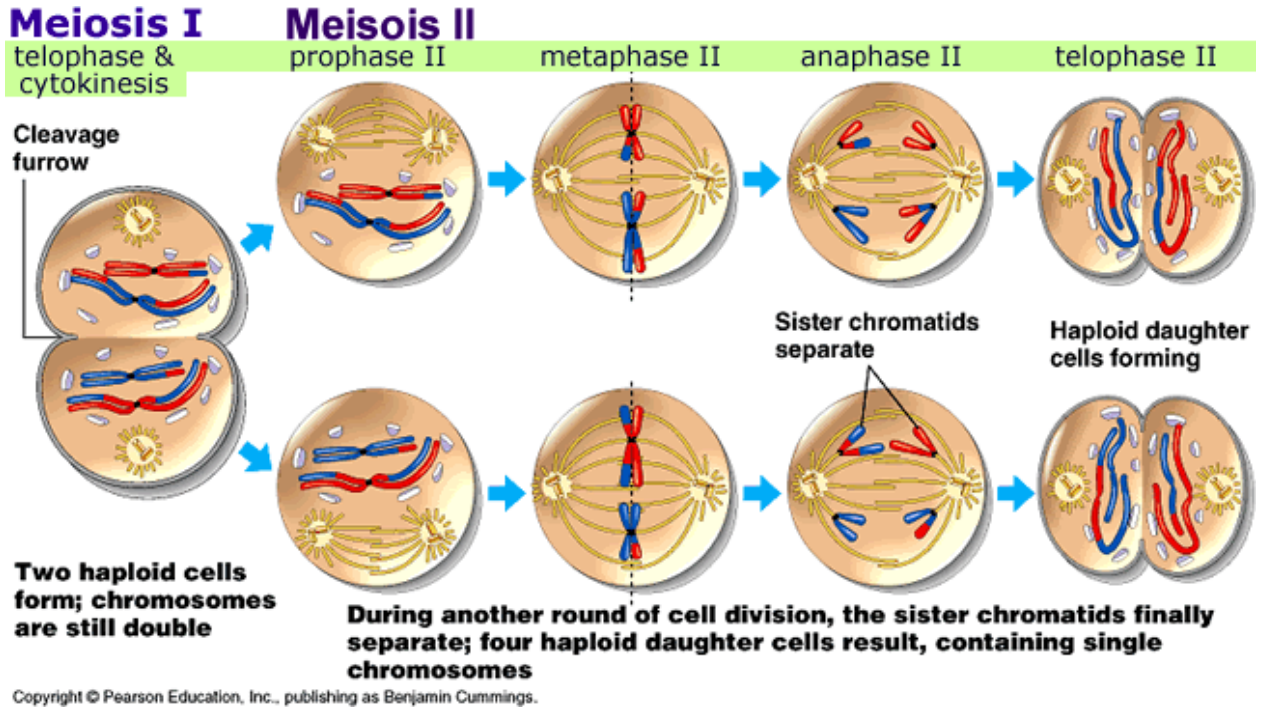


Meiosis

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.

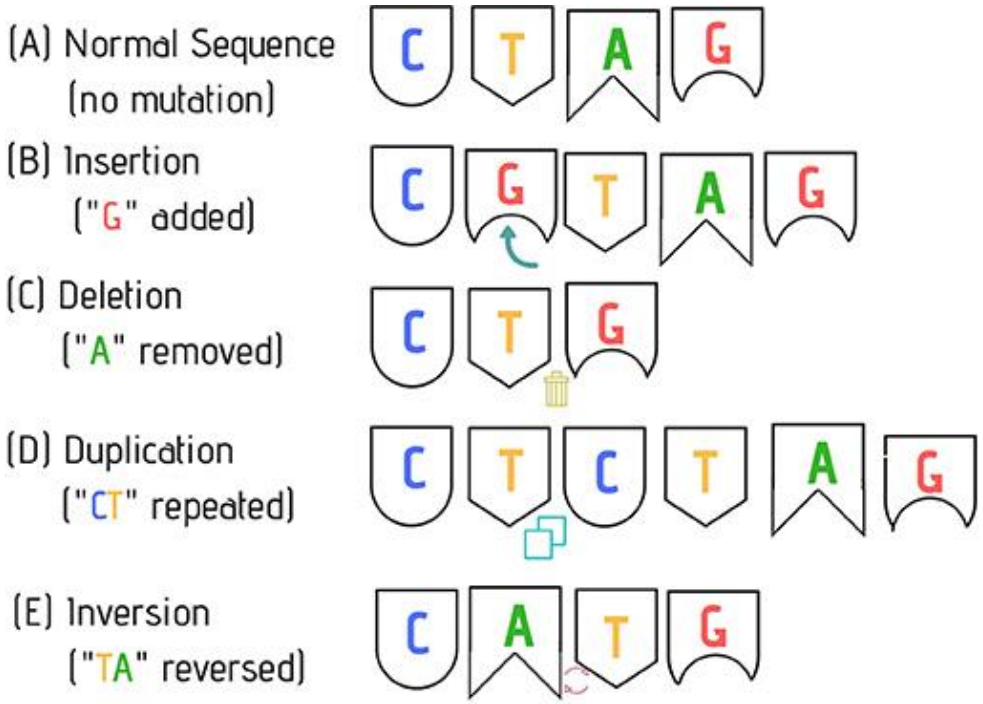
- **Significance** of mutations:
  - Positive – source of genetic variability, evolutionary significance
  - Negative – cumulation of damaged genes, rise of genetically determined diseases, tumors
  - Neutral
- **Classification** of mutations:
  - spontaneous (errors in replication) × induced (mutagens)
  - 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)

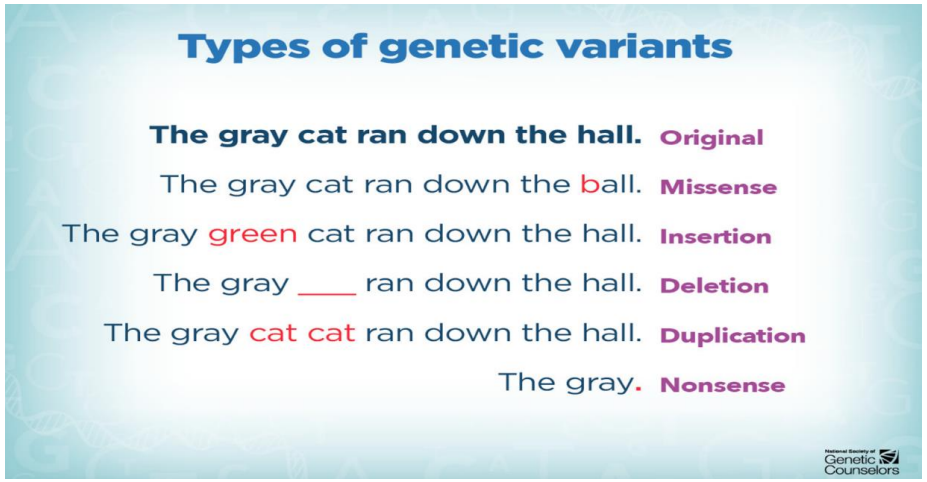
Gene Mutations

- 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)



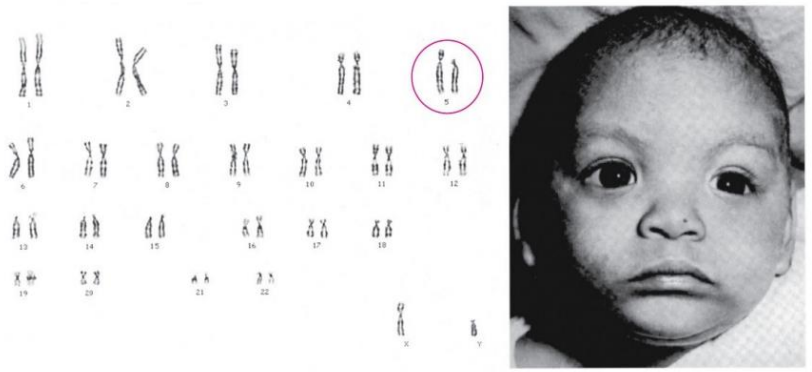


Chromosomal Mutations

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)

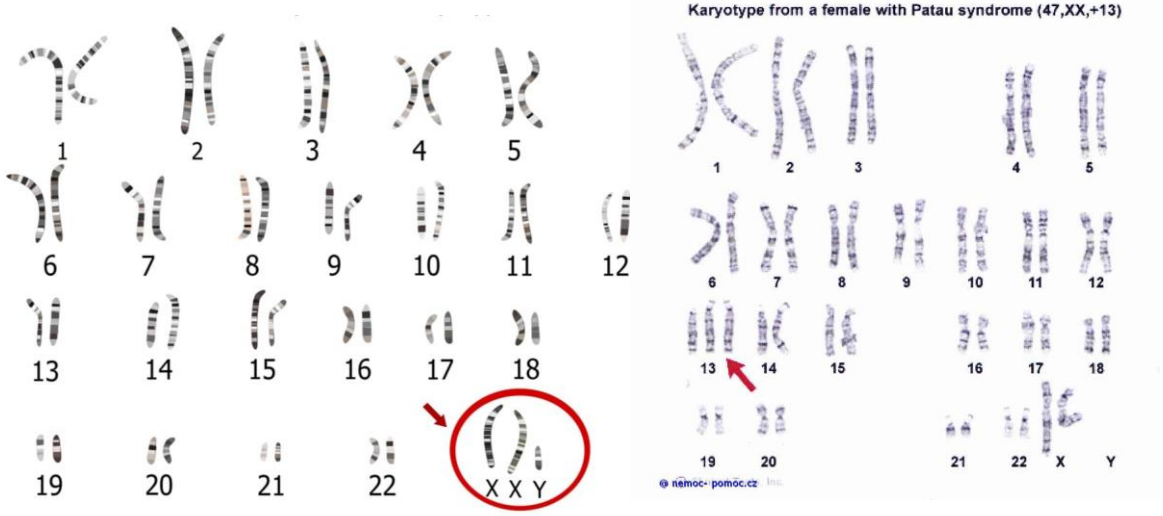


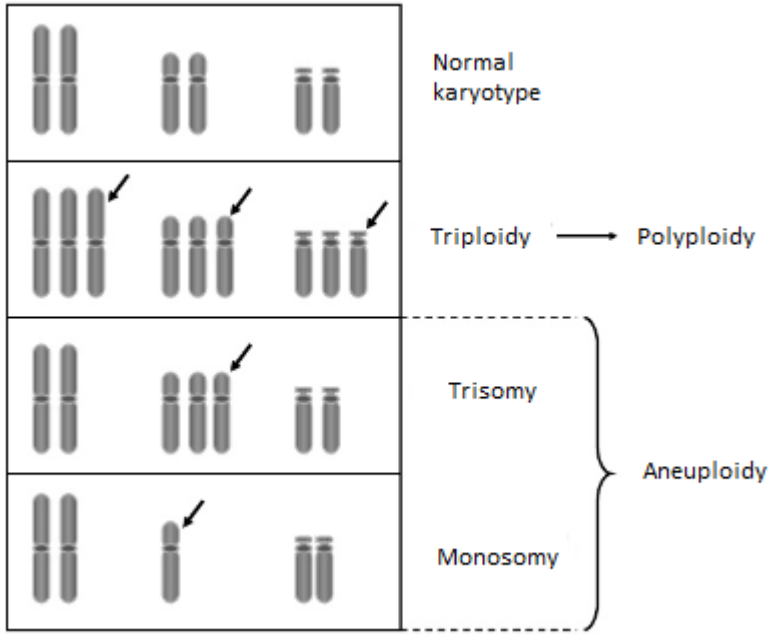
Genomic Mutations

- **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)

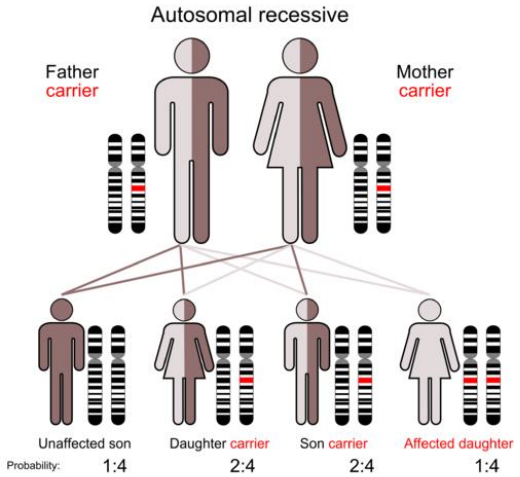
- **Polyploidy**
  - rare in vertebrates, more common in plants



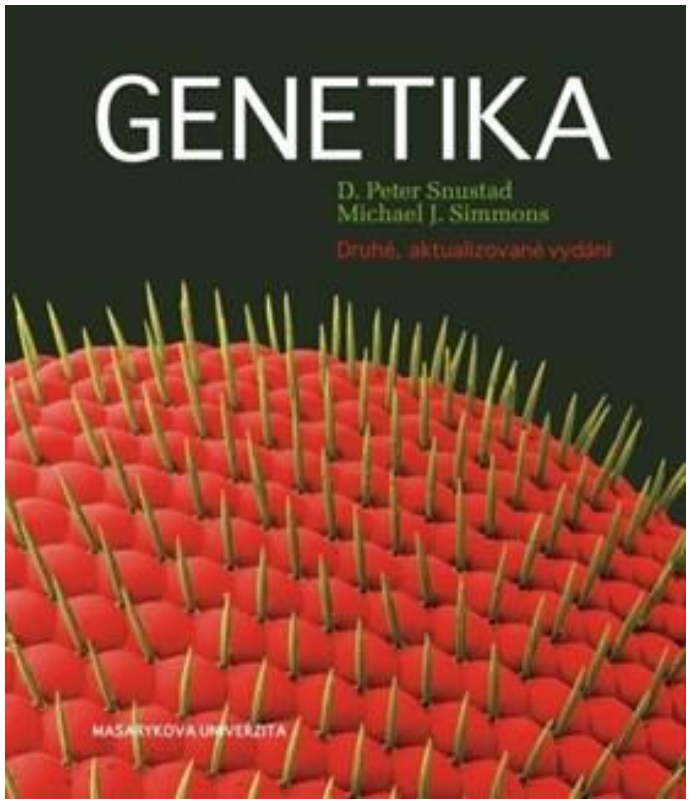


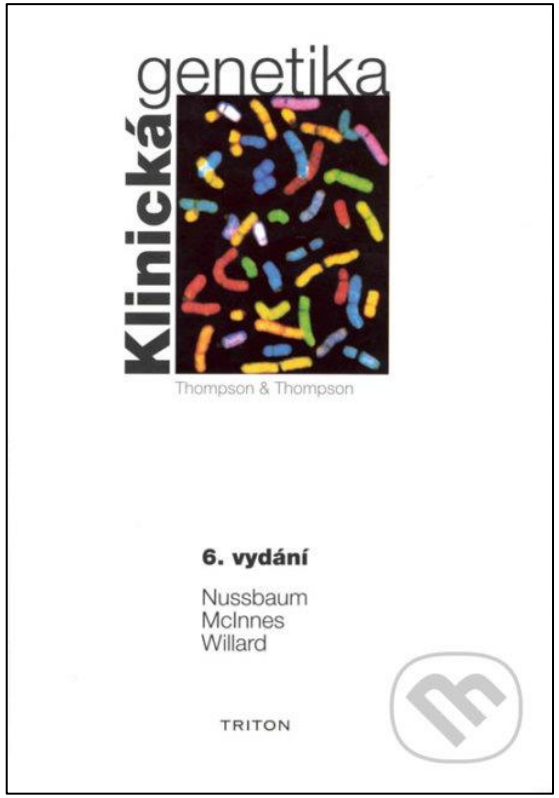
DNA Diagnostics

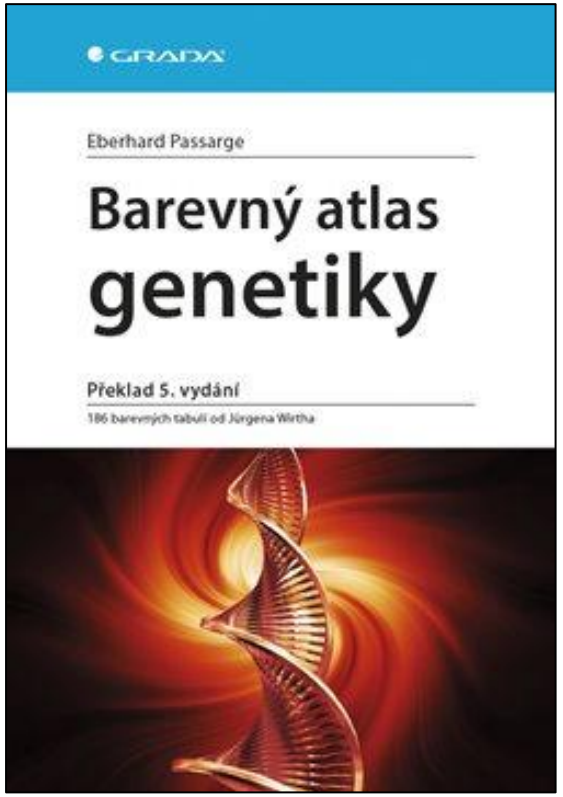
- **Detection of presence of specific nucleic acid sequence**
  - Identification of animal species
  - Paternity
  - Identification of individuals - forensics
  - DNA profile – SNPs
- **Analysis of structure (sequence) of nucleic acid**
- **Determination of genotype**
  - Detection of clinically significant 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**

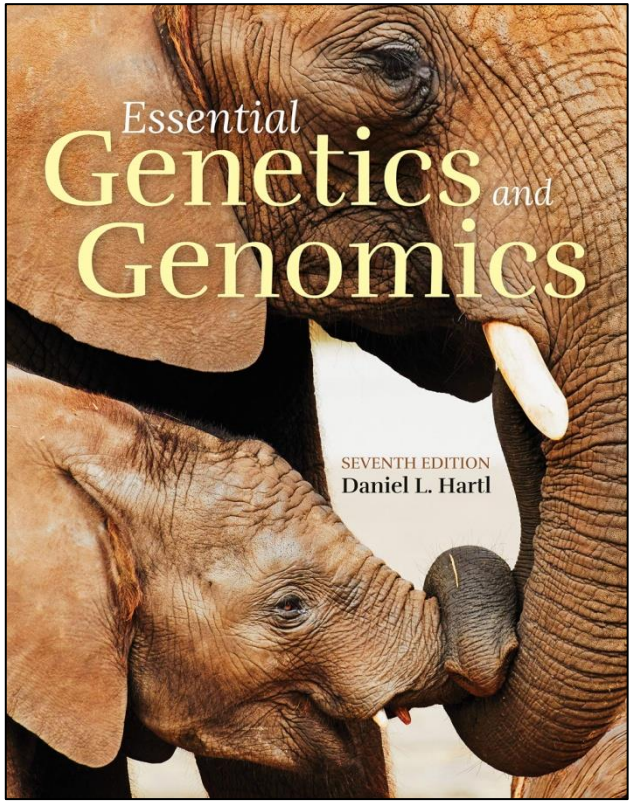


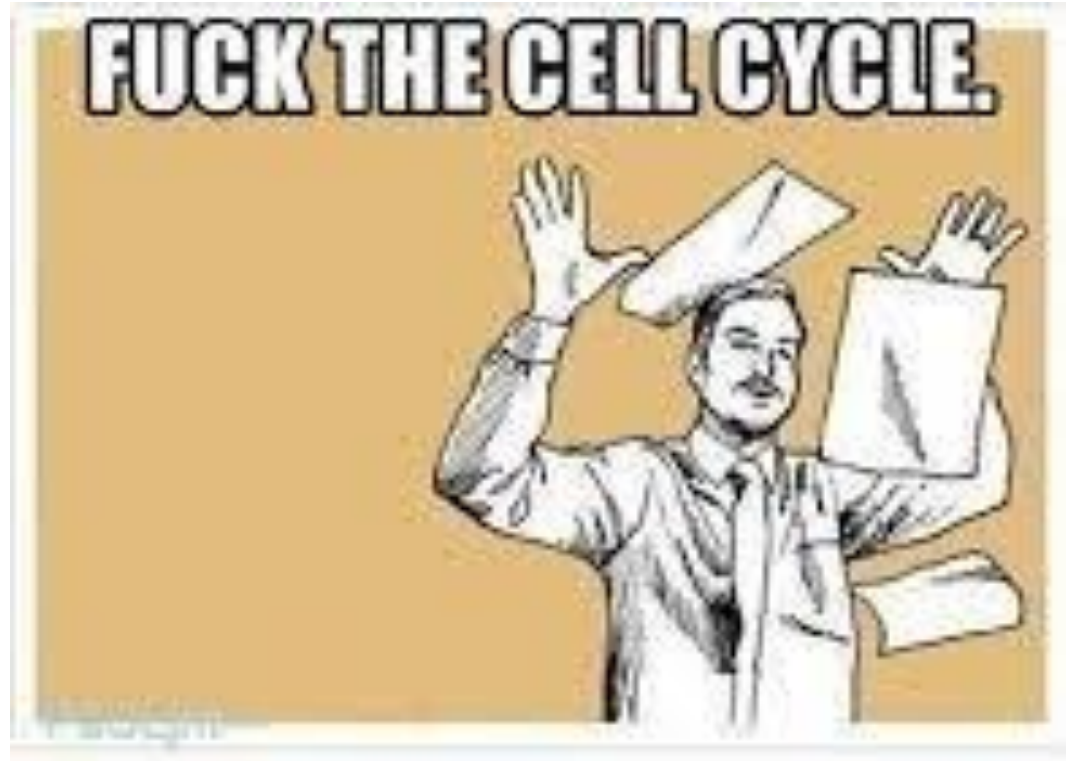
Recommended Literature for Self-study



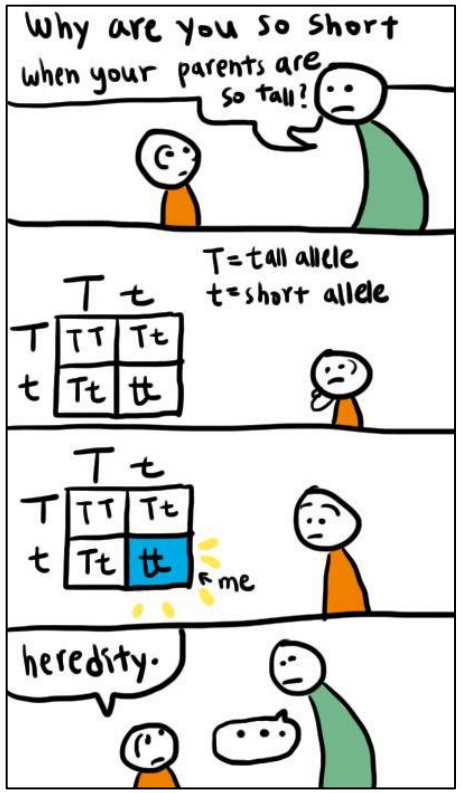


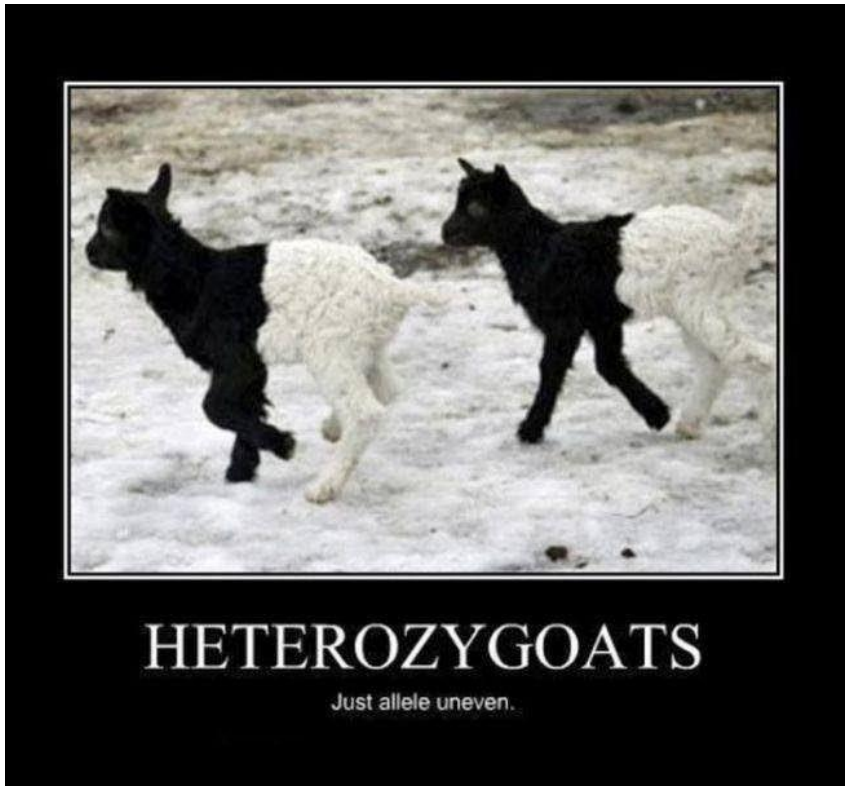





FUCK THE CELL CYCLE.


JUST KIDDING THE TEST IS NEXT WEEK...
memegenerator.net





Thank you for you attention