Lecture on

Bioinformatics (part 1)

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Introduction and motivation

There are two different aspects of what is called *Bioinfomatik* in German literature:

- Computational biology: Here one aims to answer questions relevant to **biological research** by using electronic data processing e.g. in order to precess huge sets of biological data.
- Bioinformatics: Here we address questions from **computer science**, mostly of an algorithmic nature, motivated by applications from computational biology.

A **third** aspect of relevance often is disregarded: **Nature as role model** for solving algorithmic problems. Examples are

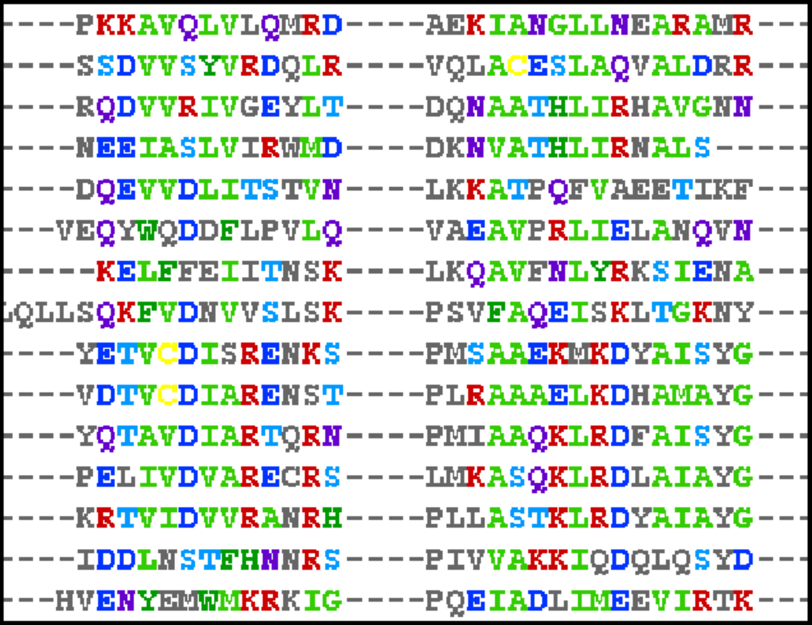
- evolutionary algorithms,
- ant colonies,
- DNA-computing.

In **this lecture** we deal with **bioinformatics** mainly focusing on questions arising from molecular biology. The lecture *Nature Inspired Computing* addresses the before mentioned aspect.

Content

- String Algorithms
  Since **many objects** from molecular biology are modeled as words (DNA-sequences, amino acid sequences,...), efficient string-matching algorithms, i.e. algorithms for finding all occurrences of one string (pattern) within another one (text), are a fundamental component of many software tools in this area. Therefore, we will pay attention to efficient solutions to this problem based on **preprocessing the pattern** and on the data structure *suffix-tree*, which allows for efficient searches by **preprocessing the text**.

- Alignments
  Since **data** processed in computational biology mostly is extracted **in assays** (experimental) it is afflicted with measurement errors. As a consequence, the problem of string-matching has to be adapted i.e. we have to decide whether or not the pattern *approximately* occurs within the text. Accordingly we will address measures of similarity of strings and corresponding algorithms for their comparison. The fundamental idea of these so called *alignment algorithms* is to handle mismatches of the words by inserting gaps in order to align equal parts of the words.



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----SSDVVSYVRDQLR----VQLACESLAQVALDRR---
----RQDVVRIVGEYLT----DQNAATHLIRHAVGNN---
----NEEIASLVIRWMD----DKNVATHLIRNALS-----
----DQEVVDLITSTVN----LKKATPQFVAEETIKF---
---VEQYWQDDFLPVLQ----VAEAVPRLIELANQVN---
-----KELFFEIITNSK----LKQAVFNLYRKSIENA---
LQLLSQKFVDNVVSLSK----PSVFAQEISKLTGKNY---
----YETVCDISRENKS----PMSAAEKMKDYAISYG---
----VDTVCDIARENST----PLRAAAELKDHAMAYG---
----YQTAVDIARTQRN----PMIAAQKLRDFAISYG---
----PELIVDVARECRS----LMKASQKLRDLAIAYG---
----KRTVIDVVRANRH----PLLASTKLRDYAIAYG---
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---HVENYEMWMKRKIG----PQEIADLIMEEVIRTK---
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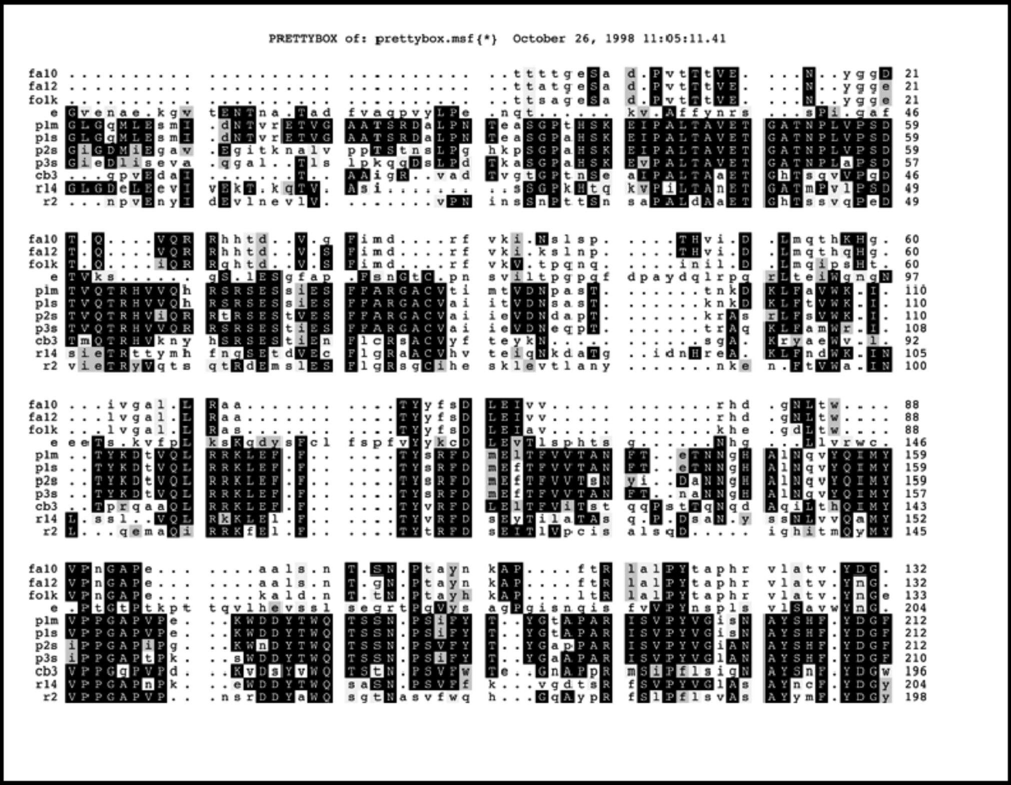

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fa12 .......... .......... .......... ..ttatgeSa d.PvtTtVE. ...N..ygge  21
folk .......... .......... .......... ..ttsageSa d.PvtTtVE. ...N..ygge  21
   e Gvenae.kgv tENTna.Tad fvaqpvyLPe .nqt...... kv.Affynrs ...sPi.gaf  46
 p1m GLGqMLEsmI .dNTvrETVG AATSRDaLPN TeaSGPtHSK EIPALTAVET GATNPLVPSD  59
 p1s GLGqMLEsmI .dNTvrETVG AATSRDaLPN TeaSGPaHSK EIPALTAVET GATNPLVPSD  59
 p2s GiGDMiEgav .Egitknalv ppTStnsLPg hkpSGPaHSK EIPALTAVET GATNPLVPSD  59
 p3s GieDliseva .qgal..Tls lpkqqDsLPd TkaSGPaHSK EvPALTAVET GATNPLaPSD  57
 cb3 ...qpvEdaI .......T.. AAigR..vad TvgtGPtnSe aIPALTAaET GhTsqvVPqD  46
 r14 GLGDeLEevI vEkT.kqTV. Asi....... ..sSGPkHtq kvPiLTAnET GATmPvlPSD  49
  r2 ...npvEnyI dEvlnevlV. .......vPN insSnPttSn saPALdAaET GhTssvqPeD  49

fa10 T.Q....VQR Rhhtd..V.q Fimd....rf vki.Nslsp. ....THvi.D .LmqthKHg.  60
fa12 T.Q....VQR Rhhtd..V.S Fimd....rf vki.kslnp. ....THvi.D .LmqthqHg.  60
folk T.Q....iQR Rqhtd..V.S Fimd....rf vkV.tpqnq. ....inil.D .LmqipsHt.  60
   e TVks...... qS.lESgfap .FsnGtC.pn sviltpgpqf dpaydqlrpq rLteiWqnqN  97
 p1m TVQTRHVVQh RSRSESsiES FFARGACVti mtVDNpasT. ......tnkD KLFaVWK.I. 110
 p1s TVQTRHVVQh RSRSESsiES FFARGACVai itVDNsasT. ......knkD KLFtVWK.I. 110
 p2s TVQTRHViQR RtRSEStVES FFARGACVai ieVDNdapT. ......krAs rLFsVWK.I. 110
 p3s TVQTRHVVQR RSRSEStiES FFARGACVai ieVDNeqpT. ......trAq KLFamWr.I. 108
 cb3 TmQTRHVkny hSRSEStiEn FlcRsACVyf teykN..... ......sgA. KryaeWv.l.  92
 r14 sieTRttymh fnqSEtdVEc FlgRaACVhv teiqNkdaTg ..idnHreA. KLFndWK.IN 105
  r2 vieTRyVqts qtRdEmslES FlgRsgCihe sklevtlany .......nke n.FtVWa.IN 100

fa10 ...ivgal.L Raa....... ....TYyfsD LEIvv..... .......rhd .gNLtw....  88
fa12 ...lvgal.L Raa....... ....TYyfsD LEIvv..... .......rhd .gNLtw....  88
folk ...lvgal.L Ras....... ....TYyfsD LEIav..... .......khe .gdLtw....  88
   e eeTs.kvfpL ksKqdysFcl fspfvYykcD LEvTlsphts g......Nhg ...Llvrwc. 146
 p1m ..TYKDtVQL RRKLEF.F.. ....TYsRFD mElTFVVTAN FT..eTNNgH AlNqvYQIMY 159
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 p2s ..TYKDtVQL RRKLEF.F.. ....TYsRFD mEfTFVVTsN yi..DaNNgH AlNqvYQIMY 159
 p3s ..TYKDtVQL RRKLEF.F.. ....TYsRFD mEfTFVVTAN FT..naNNgH AlNqvYQIMY 157
 cb3 ..TprqaaQL RRKLEF.F.. ....TYvRFD LElTFViTst qqPstTqNqd AqiLthQIMY 143
 r14 L.ssl..VQL RkKLEl.F.. ....TYvRFD sEyTilaTAs q.P.DsaN.y ssNLvvQaMY 152
  r2 L...qemaQi RRKfEl.F.. ....TYtRFD sEITlVpcis alsqD..... ighitmQyMY 145

fa10 VPnGAPe... ....aals.n T.SN.Ptayn kAP....ftR lalPYtaphr vlatv.YDG. 132
fa12 VPnGAPe... ....aals.n T.gN.Ptayn kAP....ftR lalPYtaphr vlatv.YnG. 132
folk VPnGAPe... ....kald.n T.tN.Ptayh kAP....ltR lalPYtaphr vlatv.YnGe 133
   e .PtGtPtkpt tqvlhevssl segrtPqVys agPgisnqis fvVPYnspls vlSavwYnG. 204
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 p1s VPPGAPVPe. ..KWDDYTWQ TSSN.PSiFY T..YGtAPAR ISVPYVGisN AYSHF.YDGF 212
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 cb3 VPPGqPVPd. ..KvDsYvWQ TStN.PSVFw Te..GnAPpR mSiPflsiqN AYSnF.YDGw 196
 r14 VPPGAPnPk. ..eWDDYTWQ saSN.PSVFf k...vgdtsR fSVPYVGlAs AYncF.YDGy 204
  r2 VPPGAPVP.. .nsrDDYaWQ sgtNasvfwq h...GqAypR fSlPflsvAs AYymF.YDGy 198
```

- DNA Sequencing

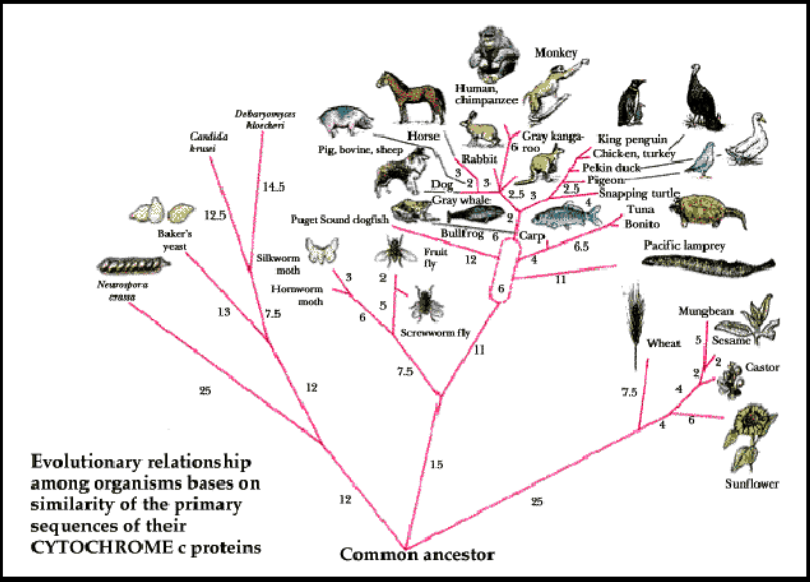
  Sequencing a DNA molecule, i.e. determining its sequence of nucleotides (bases), is a major task of molecular biology. Since lab techniques used for this task are subject to some restrictions, sequencing **cannot be done for the entire molecule in a single step**. Instead one has to divide the molecule into **parts**, which then are sequenced. Unfortunately, the relative order of fragments gets lost during this process and an algorithmic **reconstruction** of the original molecule becomes necessary.

  This lecture will address two different approaches:

  - *Physical mapping*, which has been used for the human genom project, and
  - *shotgun sequencing*, used by **Celera Genomics**.

- Signal Recognition
  By sequencing a DNA molecule we only get knowledge of its sequence of nucleotides (bases) but **nothing is known about the function** (semantics) of this sequence or subsequences. **Signal recognition** thus aims for detecting functional aspects of the molecule like e.g. binding place of proteins or genes within a DNA sequence. In this lecture mostly statistical approaches will be addressed; we will study the relative frequency of substrings of a DNA sequence and so called **Hidden-Markov-Models** which have a wide range of applications in computational molecular biology.

- Phylogenetic Trees (Phylogenies) **(Part 2 of this course)**
  Phylogenetic trees are used for representing **evolutionary interrelationships** among various species or other entities that are believed to have a common ancestor. However, the **structure** of the tree is unknown in advance and **has to be derived** from information about the species available. This can be done based on some notion of distance e.g. of homologous genes of the different species where the tree then is structured such that species with the most similar genotype are placed closest to each other.
  This lecture covers several strategies for constructing a phylogenetic tree using different kinds of information.



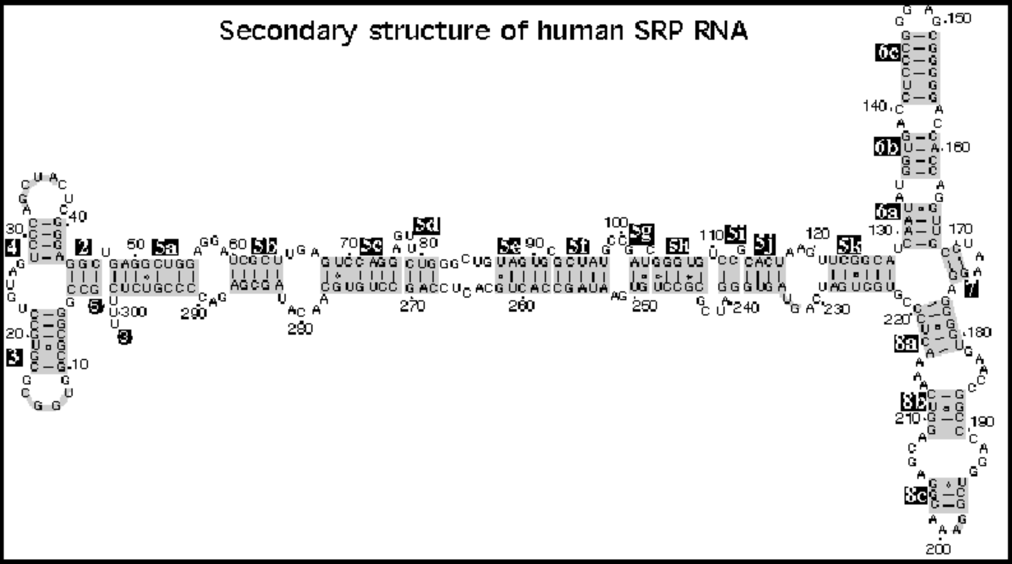
Evolutionary relationship among organisms bases on similarity of the primary sequences of their CYTOCHROME c proteins

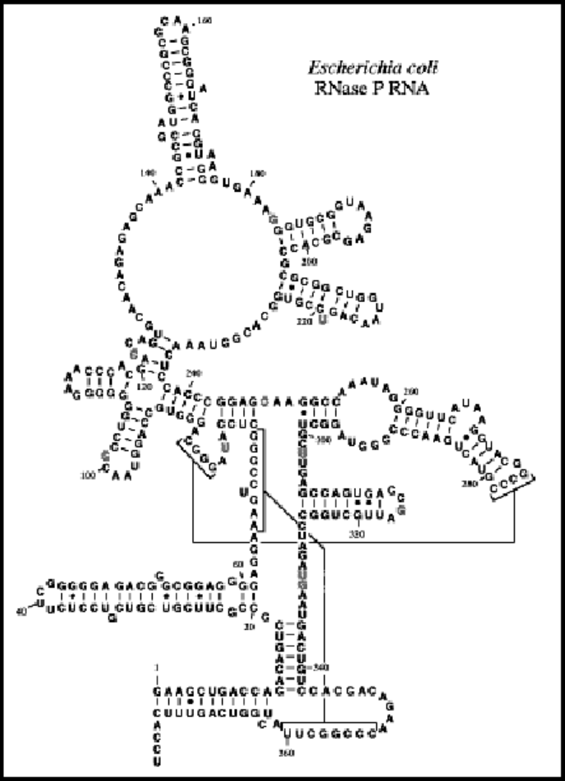
- RNA Secondary Structure Prediction **(Part 2 of this course)**

  From a chemical point of view **RNA molecules** are very similar to DNA molecules with the major difference that RNA is **single stranded**. The same interactions which force the double stranded DNA into its helical structure allow for bonding between different regions of a single RNA molecule. As a consequence, RNA folds into a **3D structure** where different conformations (topologies) are possible. The **function** of numerous kinds of RNA molecules is **determined by its topology**. Therefore we aim to predict the spatial structure of an RNA molecule algorithmically from its known sequence.

  In order to **simplify** the problem one considers the so called *secondary structure* as a first approximation of the molecule's topology by allowing only bonds which let the self-folding remain **planar**. The lecture will present two different approaches of practical importance for predicting the secondary structure, namely

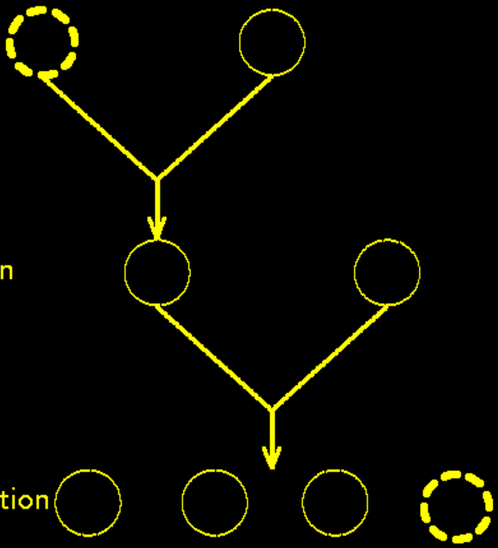
  - the Zuker-algorithm, and
  - stochastic context-free grammars.





Primer on Molecular Genetics

Mendel's Genetic: Gregor Mendel in the 19th century performed the following experiment in order to investigate inheritance:

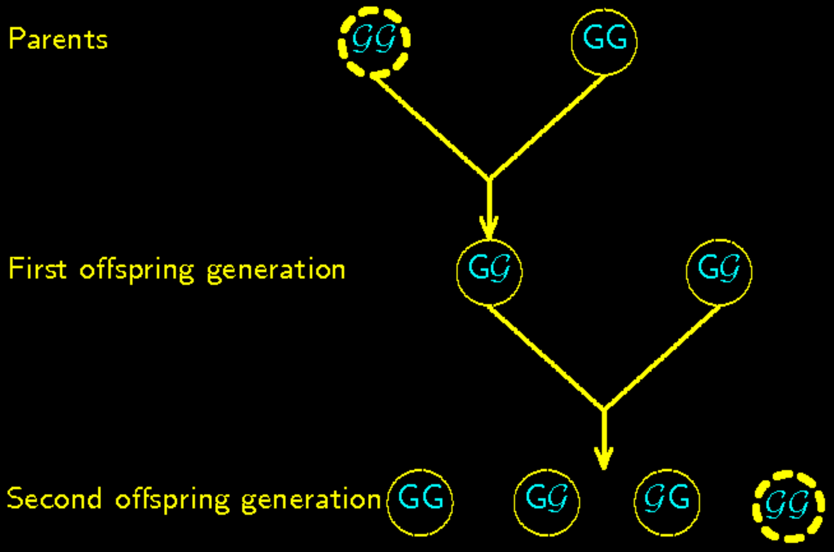


Parents

First offspring generation

Second offspring generation

Explanation: Every offspring (a pea in case of this experiment) inherits one allele (here $\mathcal{G}$ resp. G) **from each parent** for each trait (an allele is one possible version of a gene).



Parents $\mathcal{G}\mathcal{G}$ GG

First offspring generation G$\mathcal{G}$ G$\mathcal{G}$

Second offspring generation GG G$\mathcal{G}$ $\mathcal{G}$G $\mathcal{G}\mathcal{G}$

Details:

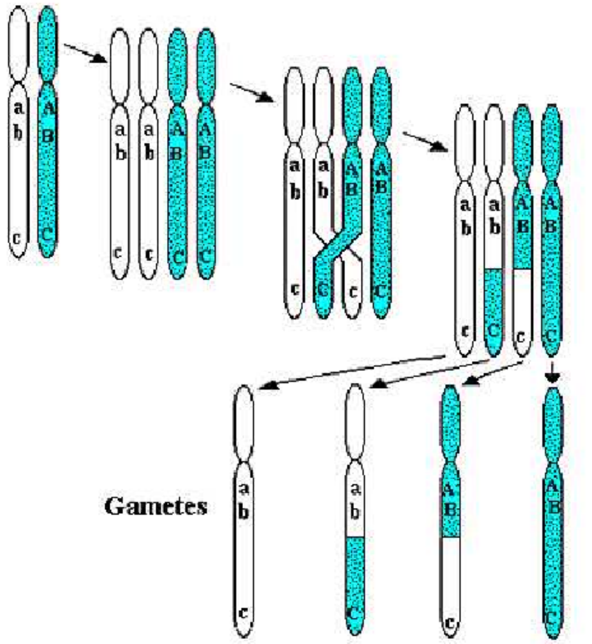
- Genotype (the specific allelic combination for a certain gene or set of genes) and phenotype (literally means “the form that is shown”; it is the outward, physical appearance of a particular trait);
- homozygote (e.g. $\mathcal{G}\mathcal{G}$) and heterozygote (e.g. $\mathcal{G}$G);
- dominant (here G) resp. recessive alleles (here $\mathcal{G}$);
- intermediate phenotype/incomplete dominance (alleles for the colors red and white yield pink);
- co-dominance where neither phenotype is dominant, instead, the individual expresses both phenotypes.

Implications of the experiments: (Mendel’s rules of inheritance)

1. If **two individuals** of one species differing in only one trait (for which both are homozygous) are **mixed** then all individuals of the **first offspring generation** are **equal**.
2. When **mixing** individual of the **first offspring generation**, the **second offspring generation** does not remain uniform but separates according to the **ratio 1:3** (**dominant/recessive**) resp. **1 : 2 : 1** (incomplete dominance).

3. **Mixing** individuals of **one species** which homozygoty differ according to **several traits**, rules 1 and 2 hold for each single trait. Besides combinations of traits existing in the parent generation, the **second offspring** generation poccesses **new combinations**.

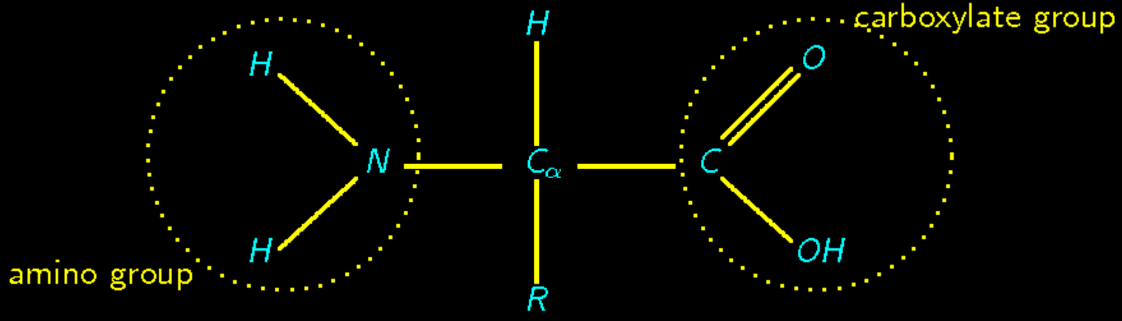
Remark: The **set of all genes** of an organism ist called *genom*, which may be distributed across several *chromosomes*. The **third rule** holds only for traits of genes located on **different chromosomes** resp. of genes residing on a single chromosome but with enough distance such that so-called *crossing-over-mutations* are sufficiently probable.



Crossing-over and recombination during meiosis

Proteins:

Proteins are a kind of **molecule** of high importance to creatures. They work as **enzymes** to catalyze biochemical reactions, are important in **cell signaling** and serve as **building material**. Each protein is made of *amino acids* arranged in a linear chain and joined together by peptide bonds. The chemical structure of a single amino acid is pictured below:



Here *R* is the so called **side chain**, in which the various amino acids differ.

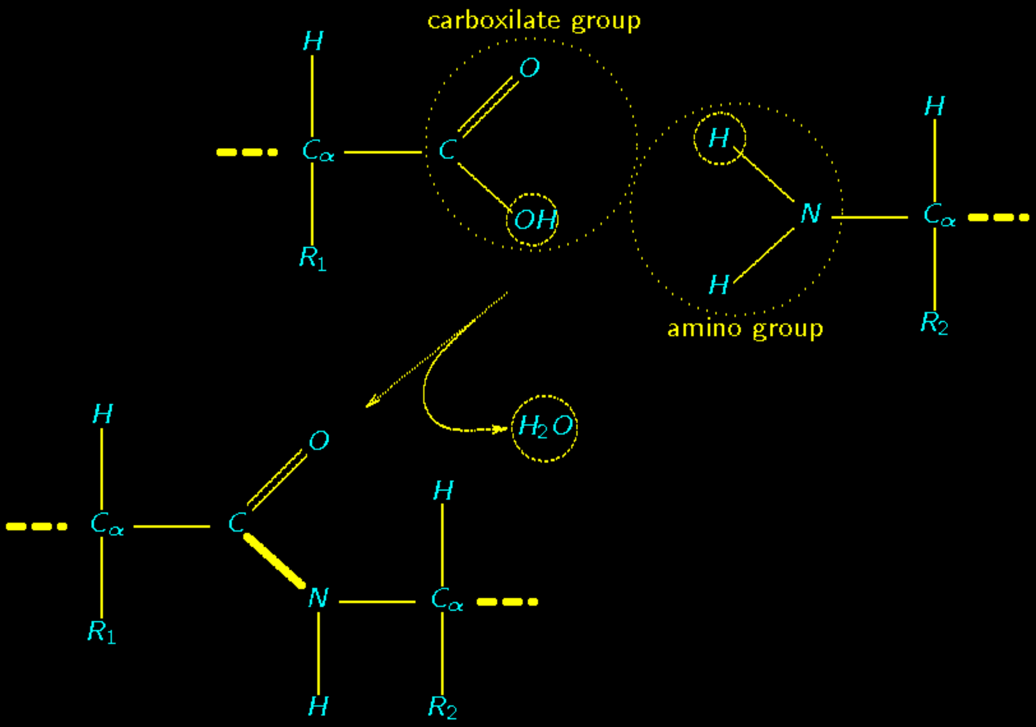
There are **20** different amino acids which occur as **building blocks of proteins**; the table to the right lists their names and abbreviations:

amino acid	abbreviation	code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

As already mentioned amino acids may combine by a chemical reaction between the amino group of one and the carboxylate group of the other acid ($\rightsquigarrow$ peptide bond).

$\Rightarrow$ Several amino acids may build a long chain (polypeptide) whose backbone is build by the peptide bonds between the different amino and carboxylate groups and the carbon atom C_α.

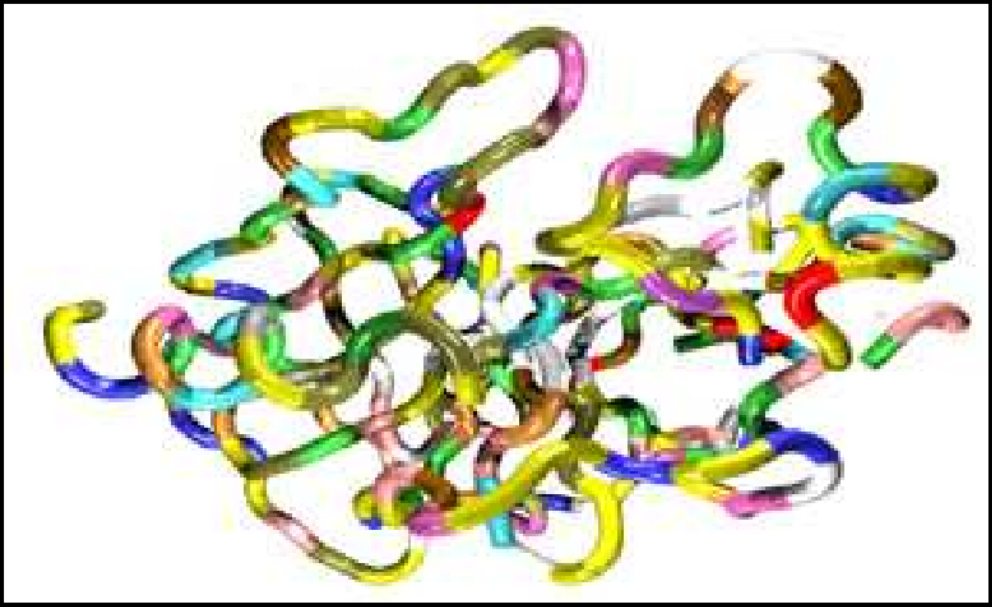
Our image of a protein: **polypeptide-chain**.

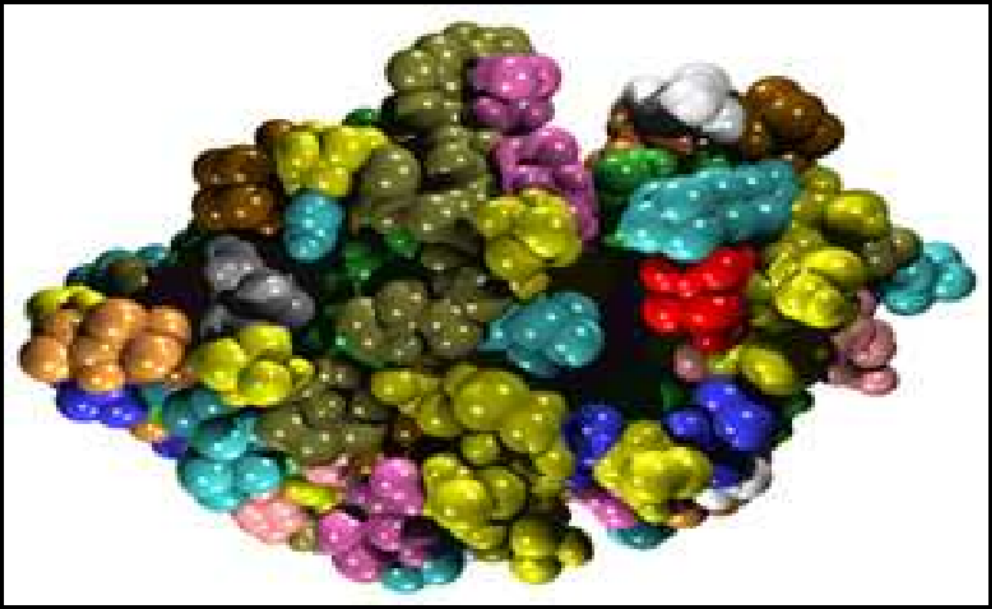


For **many applications** it will be **sufficient**, to represent this chain as a **word** over the alphabet

$$\{A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y\}.$$

However: (see next slides).

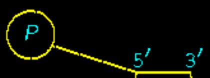


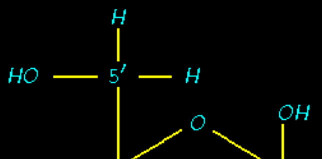


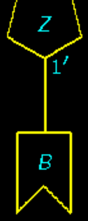
Nucleic acids:

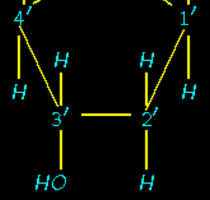
For all species nucleic acids are used to **store** the **genome** and thus they provide the structural design (building plan) of the proteins. Furthermore, they are used to **transfer genetic information** between generations. Additionally, they play an important rôle for the **biosynthesis of proteins**.

Nucleic acids are build of so called **nucleotides** which are composed of a sugar Z, a phosphate group P and a base B.



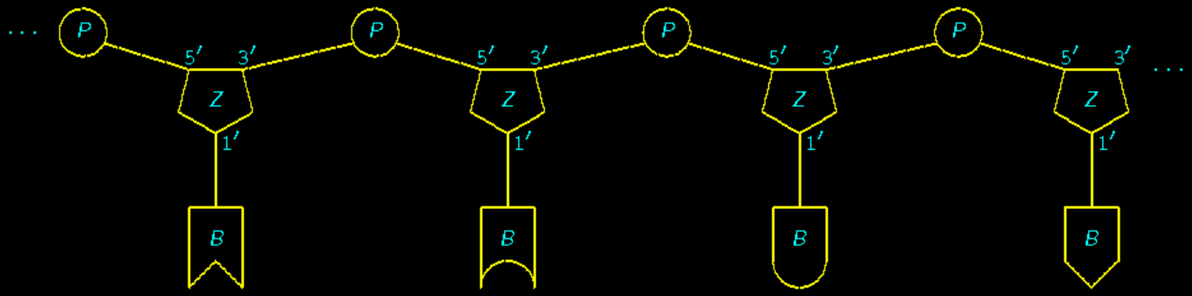






Like the amino acids in proteins, **nucleotides** in nucleic acids build long **chains**. This is done by a reaction between the phosphate group of one and the 3′-end of the sugar of the other nucleotide.

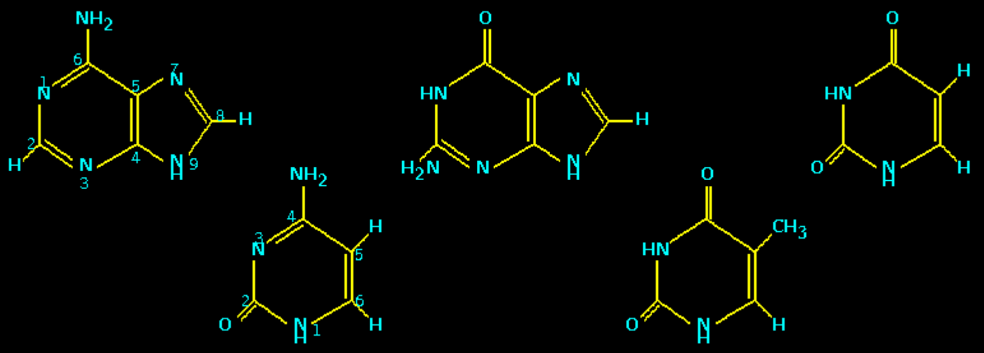
If this chain is used for encoding, it has to be read starting with the free 5′-end towards the free 3′-end.



Primary structure: Word over the alphabet Σ.

We distinguish **two types** of nucleic acids:

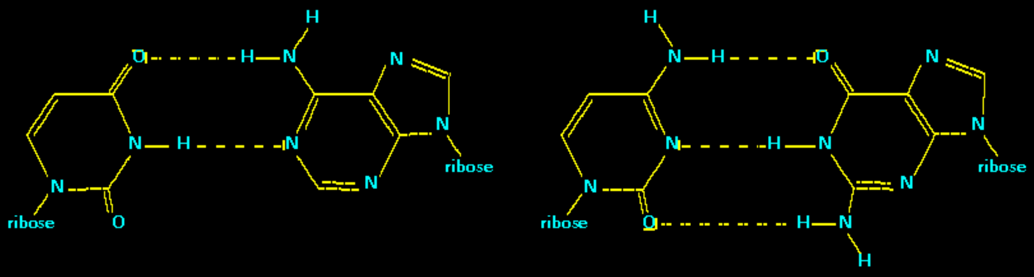
- Deoxyribonucleic acid (DNA for short), where the sugar is a **deoxyribose** and adenine, cytosine, guanine and thymine occur as bases ($\Sigma = \{A, C, G, T\}$);
- Ribonucleic acid (RNA for short), where the sugar is a **ribose** und uracil replaces thymine ($\Sigma = \{A, C, G, U\}$).



Base pairing:

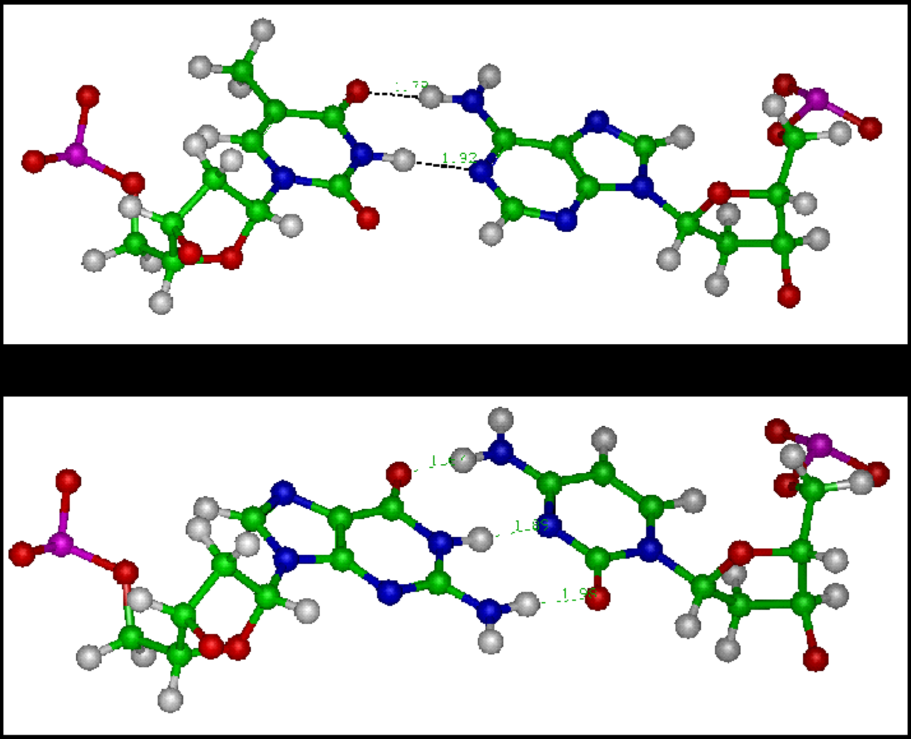
C and *G* as well as *A* mit *T* (resp. *U* in case of RNA) may form a hydrogen bond with each other.

Example from the RNA world: (*U* and *A* on the left, *C* and *G* on the right)



For simplifying the presentation, hydrogen atoms at the free places of the rings were omitted.

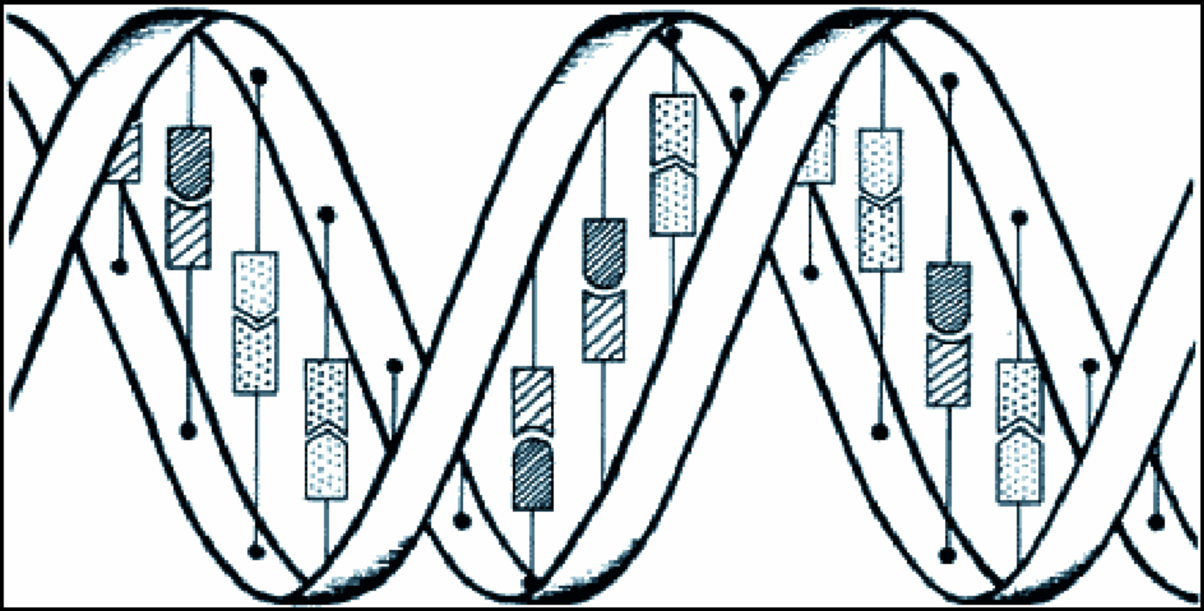
DNA world (*T* and *A* on top, *G* and *C* bottom):



DNA:

- We always observe **two complementary strands** (chains of nucleotides).
- If position i (direction $5' - 3'$) of one strand consists of base $x \in \{A, C, G, T\}$, then the other strand has the base complementary to x at position i (direction $3' - 5'$); a double stranded molecule results from the hydrogen bonds between the complementary bases.
- This *ladder-like* structure is twisted by effects which will not be discussed here; a double helical structure results.

Illustration:

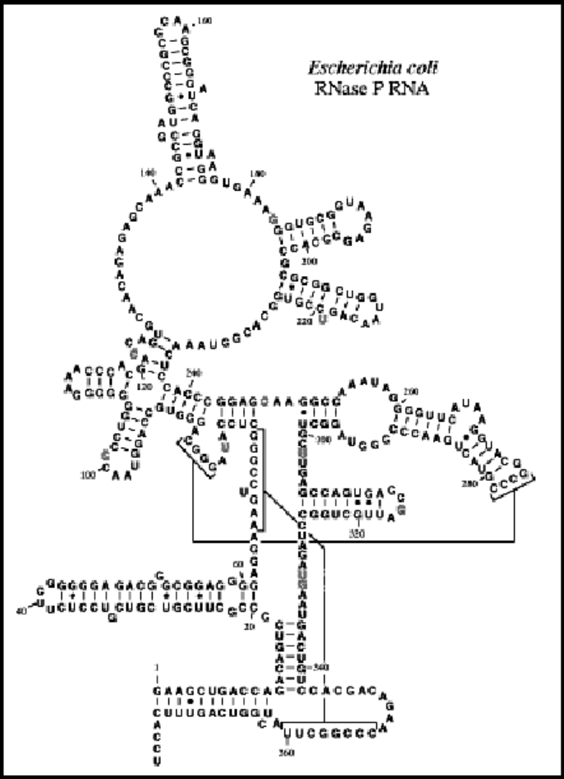


RNA:

- RNA molecules are **single stranded**, however
- **some kinds** of RNA are folded with themselves by means of hydrogen bonds between **complementary** nucleotides of the **same strand**.
- The subsequences involved need **not** be the **exact complement** of each other; by means of **bulges** and **loops** parts which find no counterpart remain unpaired.

$\Rightarrow$ molecule gets a fixed spatial structure and may act e.g. as enzyme.

Illustration:

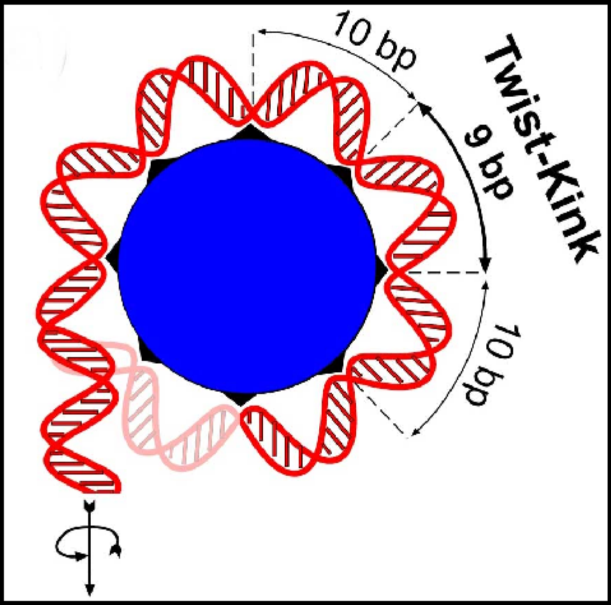


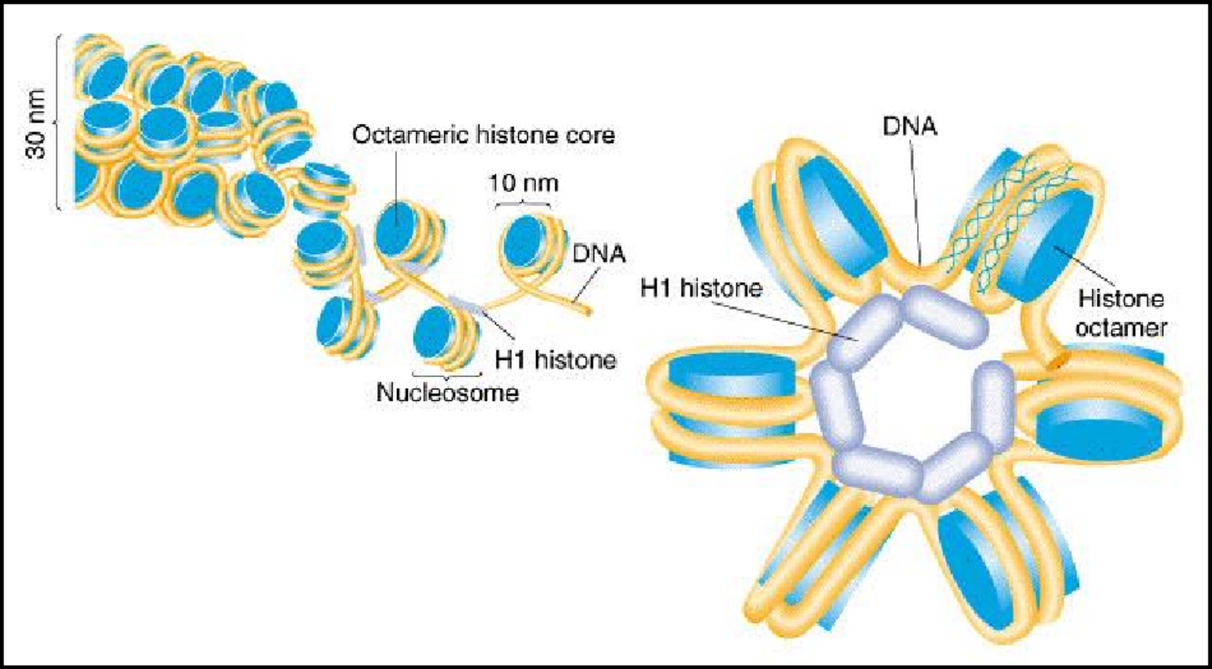
Genetic information and biosynthesis:

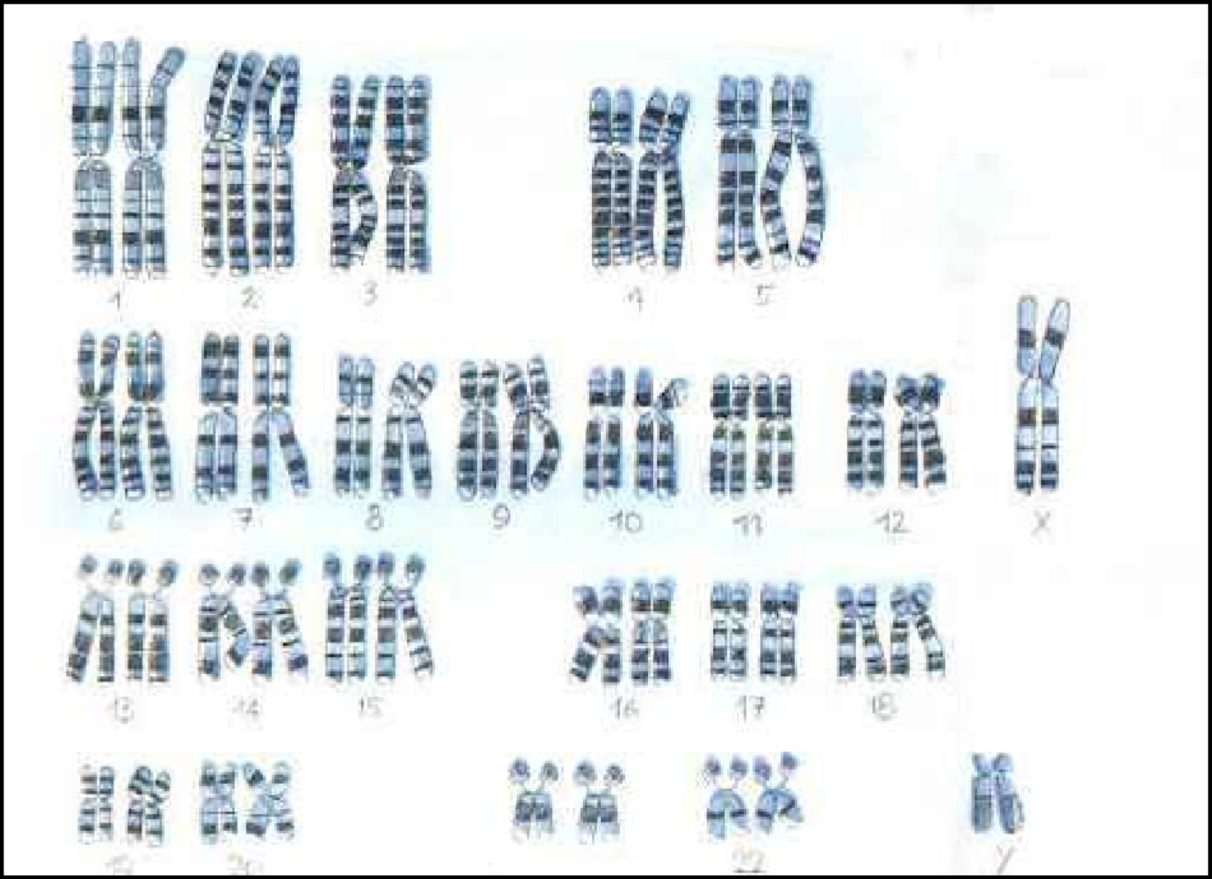
- **Chemical design** of a protein (1 gen = 1 protein) is stored in the DNA which (for eukaryotes such as animals and plants) resides in the **cell nucleus**.
- DNA is subdivided into chromosomes (DNA double helixes wrapped around histones (chromatin proteins)) each of which carries quit a number of genes[1].
- Chromosomes cannot leave the nucleus but the ribosomes where biosynthesis takes place are located outside the nucleus.

⇒ We need a means of transportation for genetic information.

[1] In most organisms the different (homologous) chromosomes occur in pairs where one is inherited from the mother and one from the father.


10 bp
Twist-Kink
9 bp
10 bp


30 nm
Octameric histone core
10 nm
DNA
H1 histone
Nucleosome
DNA
H1 histone
Histone octamer

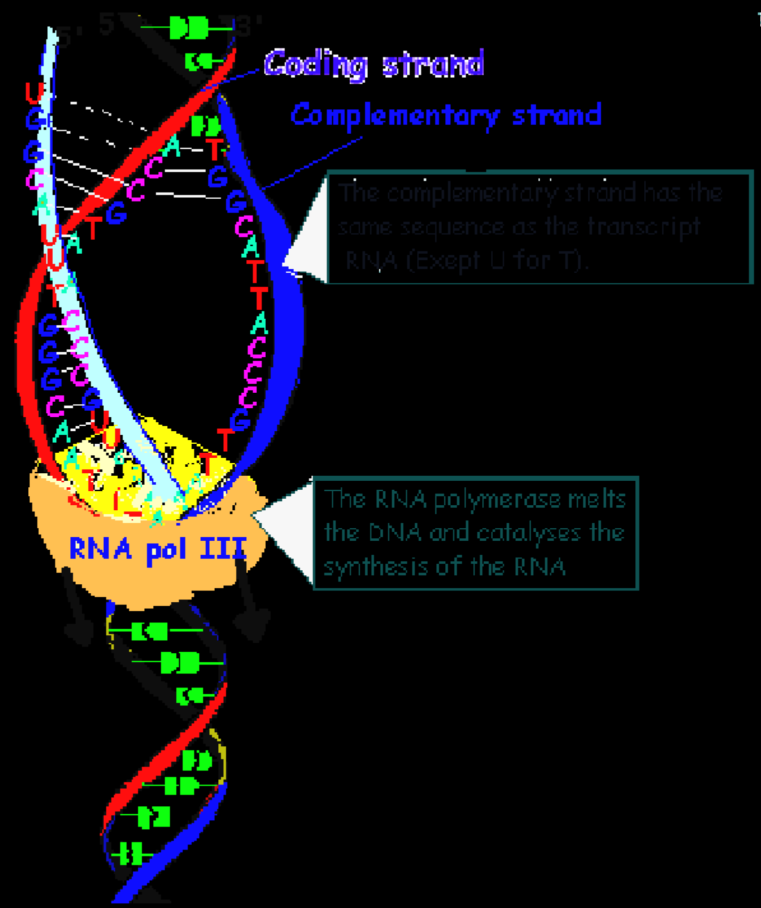


Transcription:

(Task: build a copy of the genetic information)

- RNA polymerase (an enzyme) binds to a promoter in DNA and forces it to unwind and to produce a small open complex;
- synthesis begins on only the template strand and a (complementary) copy of the gene is produced;
- **non-coding regions** within the resulting RNA are **removed** (splicing);

⇒ Messenger RNA (**mRNA** for short), which leaves the nucleus and carries the information needed to the ribosomes.

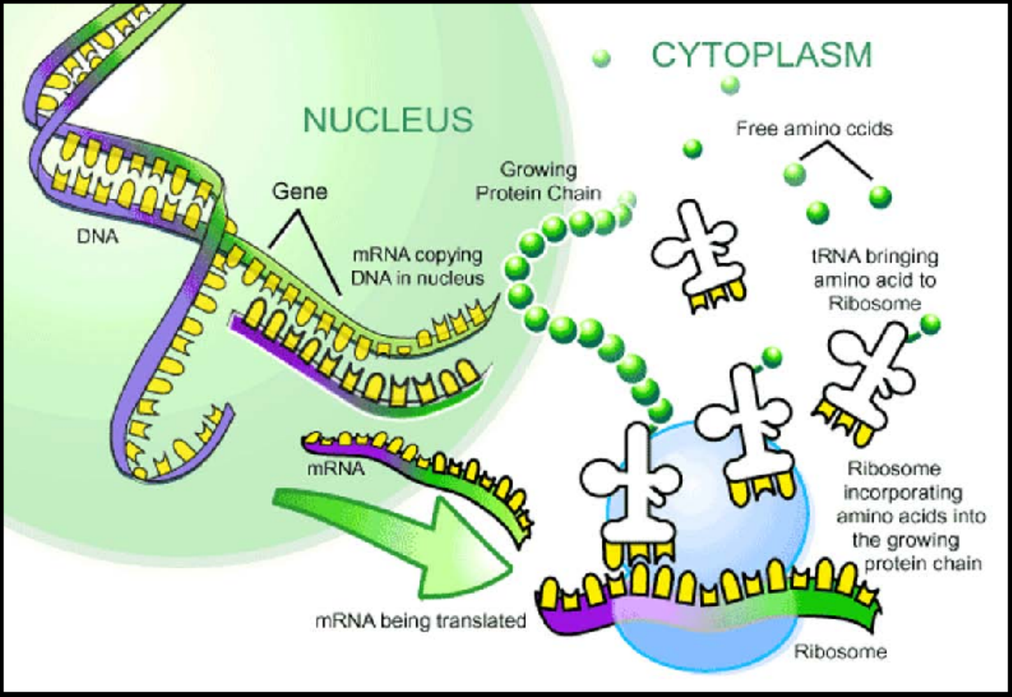

5'
3'
Coding strand
Complementary strand
The complementary strand has the same sequence as the transcript RNA (Exept U for T).
The RNA polymerase melts the DNA and catalyses the synthesis of the RNA
RNA pol III

Translation:

(Task: *translate* mRNA into sequence of amino acids)

- In each case, **three** successive **bases** (called a codon) are coding a single **amino acid**;
- the **transfer RNA** (tRNA for short) – another type of RNA – possesses a base triple complementary to the codon called anticodon;
- tRNA is **folded onto itself** and may **bind an amino acid** according to its anticodon;
- within the **ribosomes** the mRNA is processed **codon after codon**, while a **tRNA-molecule** always **furnishes** the next **amino acid** needed;
- this process **ends**, once a codon corresponding to the **STOP-signal** occurs.

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



Lab techniques

Basic methods:

- **Heating**: Divides a DNA-molecule in its two strands (denaturation as the reverse process to hybridation);
- Cutting the **sugar phosphate backbone**: **sonic waves** or **vibrations** are breaking the backbone at random positions, **restriction enzymes** are cutting predefined positions (every enzyme recognizes a specific sequence of nucleotides near which it cuts the DNA in blunt or sticky way).

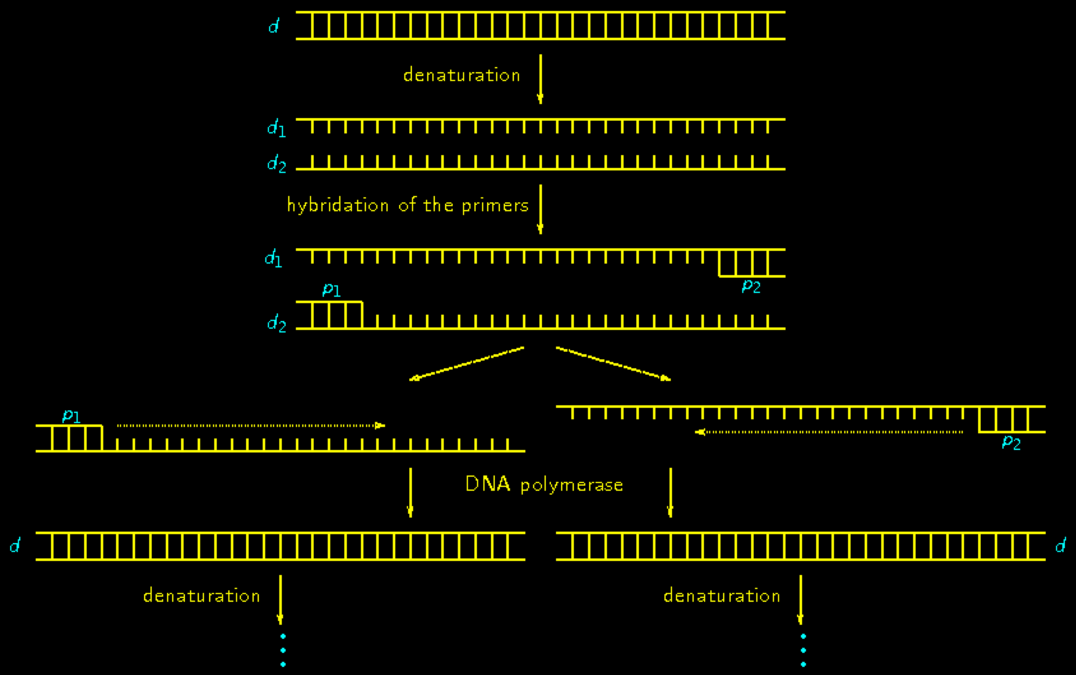
Amplifying (copying) DNA:

(polymerase chain reaction)
Step 1: Fill into a test-tube:

- The **DNA** d to be copied,
- the **primer** p_1 and p_2, equal resp. complementary to the beginning resp. ending of d's template strand,
- **all nucleotides** in sufficient magnitude, and
- **DNA polymerase**.

Step 2:

- **Denaturate** the DNA by heating.
- Let the DNA cool down. Along the way the primers **hybridate** at one end of each single strand.
- The **DNA polymerase** makes sure that both single strands are completed to two identical double-stranded copies of d.



Gel electrophoresis:

(Task: Sort DNA-molecules according to their length)

Background: **DNA**-molecules are **negatively charged** (due to their sugar-phosphate backbone) and therefore run towards the anode (positive pole) of an electric field.

- Place DNA samples into holes at one end of a *gel*;
- add an electrical current by which the DNA starts moving;
- short strands move through the holes in the gel more quickly than long strands (due to a smaller friction). Over time, the shorter strands therefore will move farther away from the starting point than the longer strands.
- by adding molecules of known sizes to a distinguished hole of the gel not only sorting is possible but we are able to determine the molecules sizes as well.

Dideoxy sequencing (Sanger method):

(Determine the primary structure of d)
Step 1: Generate many **copies** of the DNA single strand d.
Step 2: Distribute these copies among **four test-tubes** A, C, G and T.
Step 3: Fill into test-tube $X \in \{A, C, G, T\}$ **all** nucleotides **beside** the one with base X.
Step 4: Fill into test-tube $X \in \{A, C, G, T\}$ in an appropriate **ratio**

- **nucleotides**, with base X, and
- **nucleotides**, with a **chemically modified** version of base X, at which the DNA polymerase **cannot continue** with completing the strand.

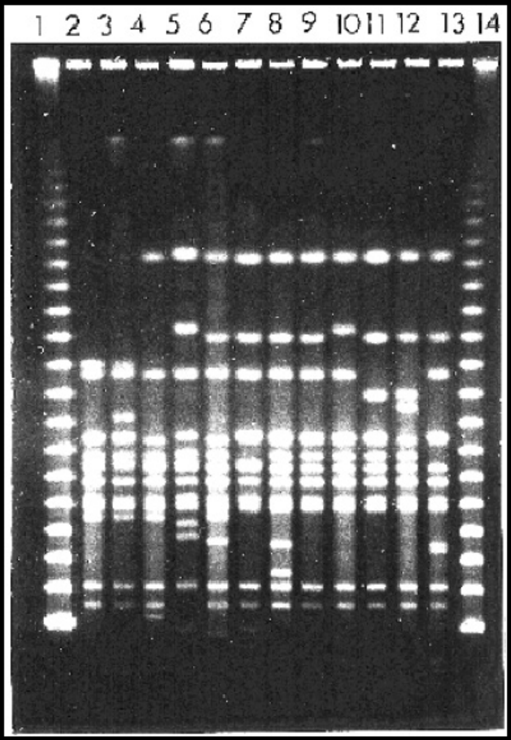
Step 5: Fill into all the test-tubes the primer for d and DNA polymerase.

Since test-tube $X \in \{A, C, G, T\}$ contains the modified nucleotides with base X the tube will eventually contain all the incomplete double strands ending with X with high probability.

Step 6:

- Place the content of the four test-tubes into four different holes of a gel and run a gel electrophoresis.
- Since the molecules with **different completion** run on **different speed** through the gel, we can determine for each test-tube X, at which position hybridation has been canceled by means of the modified nucleotide with base X.
- If a molecule of test-tube X_1 has moved furthest, d starts with the complement of X_1; if a molecule of tube X_2 went second furthest the second nucleotide of d is of type $\overline{X_2}$ ...

⇒ We know wich bases show up within d at all the different positions.



DNA microarrays:

(Is t a subsequence of the unknown sequence s?)
Idea: Perform a hybridation experiment for testing whether $\bar{t}$ binds to s or not.

- For performing **numerous** such **experiments at the same time**, one uses so called DNA micro arrays, to which one can tie the complements $\bar{t}_1, \dots, \bar{t}_n$ at distinguished positions.
- Afterwards, marked (fluorescent) **copies of** s are added; finally those not bonded to any of the t_i are washed away.
- The marking makes it possible to identify the subsequences showing up in s.

Attention: Experiments might be faulty (false positives and false negatives occur)!