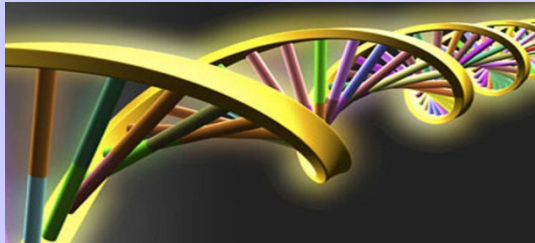




# Introduction to Bioinformatics

Ulf Leser

# Bioinformatics



25.4.1953

Entdeckung der Doppelhelix durch  
Watson/Crick + Wilkins/Franklin



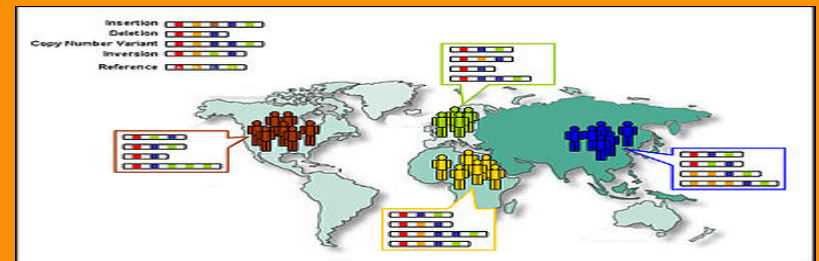
14.4.2003

Humanes Genom zu ~95% sequenziert  
mit ~99% Genauigkeit



2008

Genom of J. Watson finished  
4 Months, 1.5 Million USD



2010

1000 Genomes Project

# Example: Int. Cancer Genome Cons.

- Large-scale, international endeavor
- Planned for 50 different cancer types
- Cancer types are assigned to countries
- Distributed infrastructure
- First federated genome project [HAA+08]

**50 different cancer types, 500 samples per type, always control + cancer**  
**> 50.000 genomes**

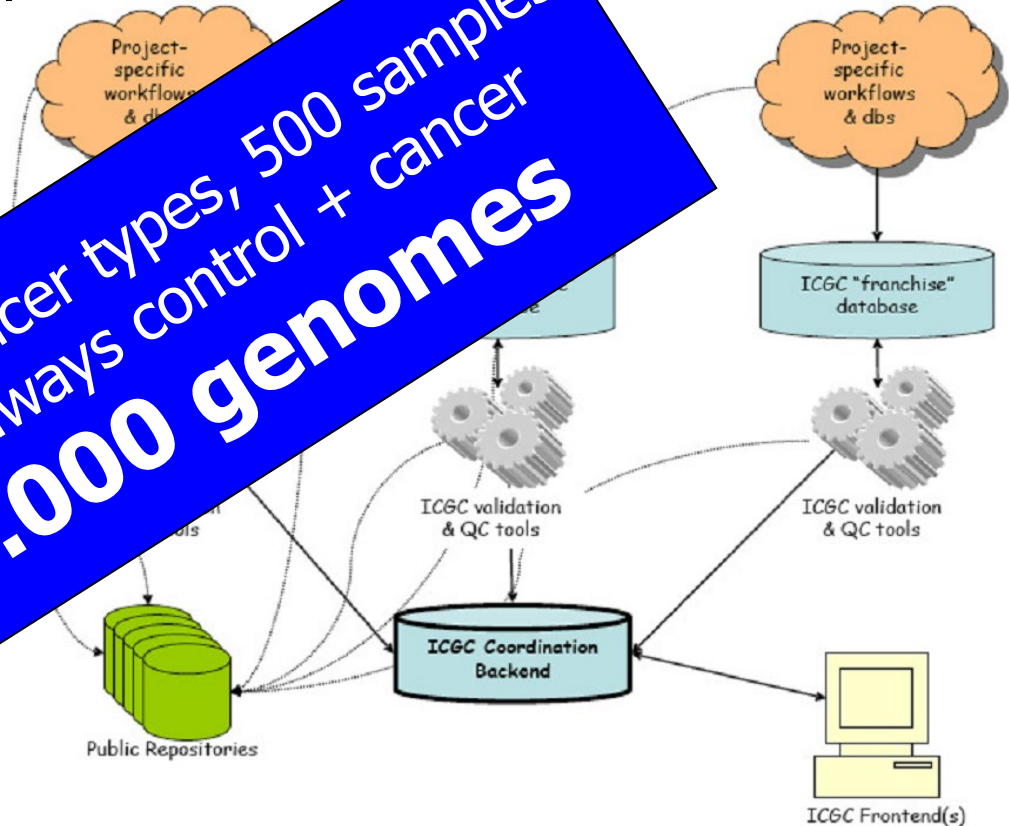


Figure 2: ICGC data coordination as a franchise system

# 2022: Deutschland

watson crick novel prize - Google X Die deutsche Genom-Initiative X

https://www.bundesgesundheitsministerium.de/themen/gesundheitswesen/personalisierte-medizin/genomde.html

Meistbesucht Freqent WBI Lehre Google News Paper Reisen MyStuff hub Berlin Projekte FONDA HU-Zoom Wetter

English Kontakt Gebärdensprache Leichte Sprache

Ministerium Themen Presse Service Suche

## genomDE- Nationale Strategie für Genommedizin

Die Genommedizin hat das Potential, die Prävention, Diagnose und Behandlung von bestimmten Krankheiten entscheidend zu verbessern. Die Strategie genomDE zielt darauf ab, allen Patientinnen und Patienten diese Vorteile langfristig zugänglich zu machen. Auf dem Weg dahin müssen zuerst ethische, regulatorische und sicherheitstechnische Fragen geklärt werden. Nach Schaffung der rechtlichen Grundlage in 2021 steht nun der Aufbau einer entsprechenden Dateninfrastruktur an.

Die grundsätzlichen Ziele und Herausforderungen der nationalen Strategie für Genommedizin genomDE standen zu Beginn - Anfang 2019 - fest. Seit der Sequenzierung des ersten vollständigen menschlichen Genoms im Jahr 2003 hat sich die Forschung rasant weiterentwickelt und gezeigt, dass bestimmte Patientinnen und Patienten erheblich von der Analyse ihrer Genomsequenzen profitieren können. Ärztinnen und Ärzte können dank der Genommedizin Krankheiten inzwischen immer besser diagnostizieren und behandeln sowie individuell angepasste Präventionsmaßnahmen einleiten. Diese personalisierte, auf das individuelle Erbgut eines Menschen angepasste Medizin bietet erhebliche Vorteile, die möglichst bald allen Bürgerinnen und Bürgern zu Verfügung stehen sollten. Allerdings sind Genomdaten auch sensible persönliche Daten, über die auch Aussagen über nahe Angehörige getroffen werden können. Datensicherheit sowie die Aufklärung und Zustimmung der Betroffenen sind daher unerlässlich und somit auch ein prägender Teil der Genom-Initiative.

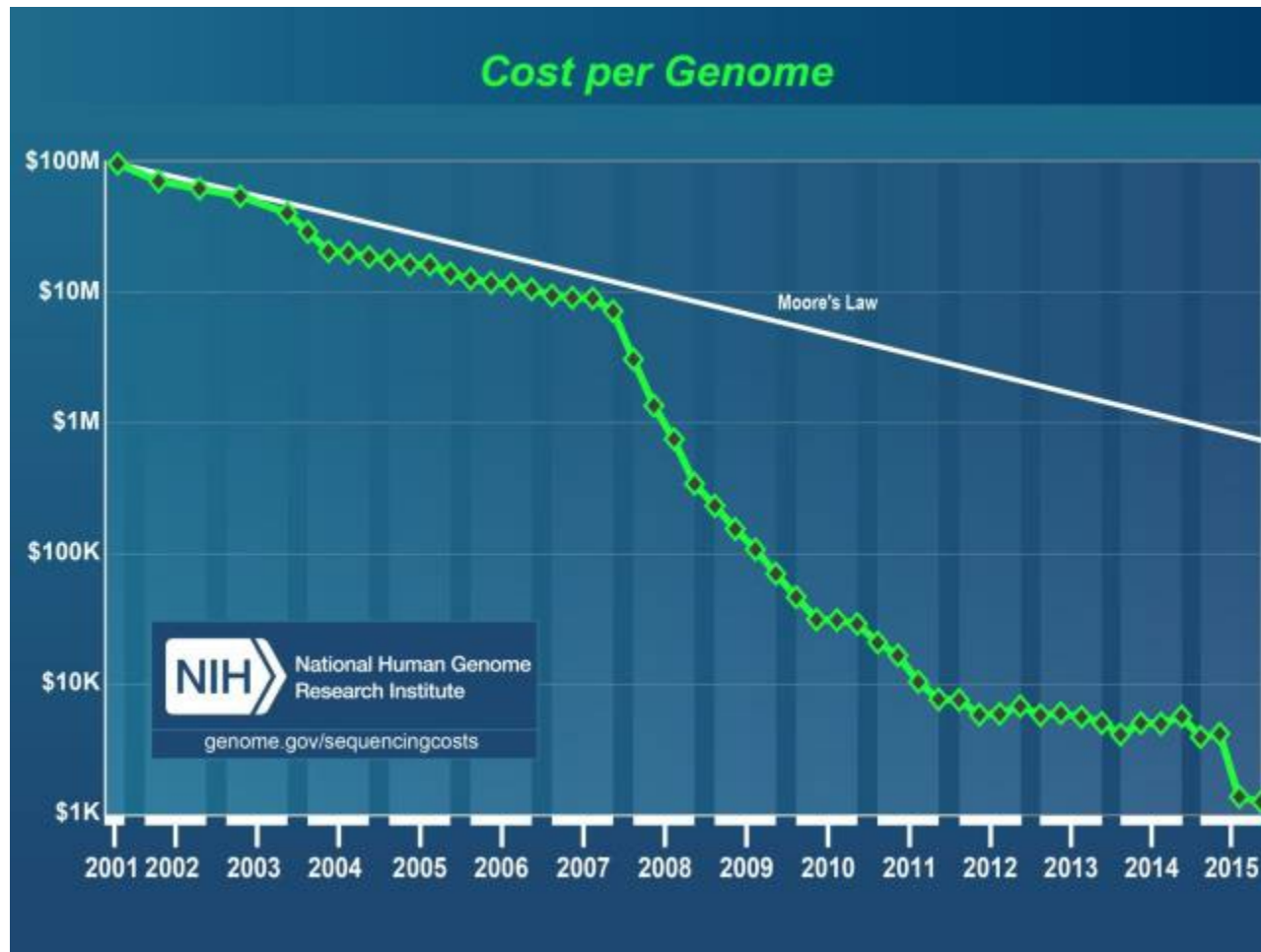
### Strategie genomDE im Überblick

The timeline shows the following milestones:

- Februar 2019: Start Nationale Strategie genomDE
- Januar 2020: Beitritt 1+ Million Genomes
- Juli 2020: Start EU-Strukturprojekt
- November 2020: Erste Vorstellung genomDE
- Juli 2021: Inkrafttreten § 64e SGB V Modellvorhaben
- Oktober 2021: Start Initiative genomDE
- Januar 2023: Start Modellvorhaben nach § 64e SGB V

Im Rahmen der Strategieentwicklung hat das Bundesministerium für Gesundheit (BMG) zunächst eine erste

# Possible Through Cost Reduction



What  
does  
this  
mean  
?

<http://www.genome.gov>

# Things you can do with it

- 2002
  - 2 companies
  - 32 Tests
  - Price: 100–1400€

Indikation*	Anbieter**	Untersuchungsgegenstand	Preis (inkl. MwSt.)
Alkoholverträglichkeit	2	keine Angaben (k. A.)	207,79 €
Alzheimer	2	k. A.	134,06 €
Alzheimer	1	E4-Allel des Apolipoprotein-E-Gens auf Chromosom 10	650,00 €
Angelman-Syndrom <sup>21</sup>	1	Deletion auf dem Chromosom 15	850,00 €
Anti-Aging-Risikoprofil	2	k. A.	653,61 €
Arteriosklerose/Herzinfarkt/Schlaganfall	2	k. A.	512,81 €
Asz	1	31 Mutationen einschließlich einer 5T-Variante auf dem CFTR-Gen auf dem Chromosom 7	850,00 €
Bluthochdruck	2	k. A.	127,40 € 439,24 €
Cholester Typ II	2	k. A.	127,40 € 194,39 €
Dickdarmkrebs <sup>31</sup>	1	MLH1- und MSH2-Mutationen	1600,00 €
Entgiftungsfähigkeit	2	k. A.	811,10 €
Faktor V Leiden-Mutation	1	Gerinnungsfaktor-V auf dem langen Arm von Chromosom 1	400,00 €
Familiäre Hypercholesterinämie	1	Mutationen im Low-Density-Lipoprotein-Rezeptor-Gen und im Exon 26 Apolipoprotein-B-Gen	850,00 €
Familiäre Hyperlipoproteinämie Typ III	1	E2-Allel des Apolipoprotein-E-Gens auf Chromosom 19	500,00 €
Familiärer Brustkrebs <sup>30</sup>	1	BCRA1- und BCRA2-Mutationen	1400,00 €
Fettgen/Adipositas	2	k. A.	241,35 € 576,44 €
Fettstoffwechsel/Cholesterin	2	k. A.	395,48 €
Fragiles X-Syndrom <sup>41</sup>	1	FMR1-(fragile X mental retardation-)Gen des X-Chromosoms (Region Xq27.3)	950,00 €
Hämochromatose	2	k. A.	207,84 €
Hämochromatose	1	Austausch der DNS-Basen Guanin zu Adenin an der Position 845 und von Cytosin zu Guanin an der Position 187 des HFE-Gens auf dem Chromosom 6	500,00 €
Hyperhomocysteinämie	1	k. A.	550,00 €
Mukoviszidose (Cystische Fibrose)	1	Mutation eines Gens auf Chromosom 7	850,00 €
Muskeldystrophie	1	Deletionen (Verlust von DNA-Teilsequenzen) im Dystrophin-Gen auf dem X-Chromosom	850,00 €
Osteoporose	2	k. A.	103,89 € 191,01 €
Osteoporose	1	Mutation (Basenaustausch von Guanin zu Thymin) im Intron 1 des Kollagen Typ I Alpha 1-Gens	650,00 €
Ovarialkarzinom <sup>30</sup>	1	BCRA1- und BCRA2-Mutationen	850,00 €
Persönliches Ernährungsprofil	2	k. A.	841,32 €
Prader-Willi-Syndrom	1	Deletion oder Translokation auf dem langen Arm des Chromosoms 15 (15q11)	850,00 €
Prothrombinogen	1	Austausch der DNS-Basen Guanin zu Adenin an der Position 20210 des Prothrombingens auf dem Chromosom 11	550,00 €
Risiko Alkohol- und Drogenabhängigkeit	2	k. A.	274,86 €
Thrombose	2	k. A.	134,06 € 281,52 €

# Situation Today

---

- Precision oncology: Determine therapies depending on **molecular characterization** of an individual patient
- Tumors are driven by genomic variants (“mutations”)
- **Different variants** – different prognosis & treatment
- **Targeted therapies**: Drugs whose applicability depend on the presence / absence of certain variants
  - 1/3 of all new anti-cancer drugs in 2019 (FDA)
  - **90% of all current late-stage anti-cancer drugs** in developments

# This Lecture

---

- Formal stuff
- A very short introduction in Molecular Biology
- What is Bioinformatics?
  - And an example
- Topics of this course



# This course

---

- Bachelor computer science, Wahlpflichtbereich
- 5 SP, lecture / exercises are 2+2
- We assume 4<sup>th</sup> semester knowledge in [computer science](#)
  - Programming, algorithms, complexity
- We do not assume knowledge in [biology](#)
- Introductory – many topics, often not much depth
  - Visit “Algorithmische Bioinformatik” afterwards ...
- Ask questions! [leser \(a\) informatik.hu ... berlin...](#)

# Exercises

---

- Taught by Ulf Leser
- There will be 4-5 assignments
- We build teams
- System
  - First week: 2-3 presentations of results of previous assignment and discussion of new assignment
  - Next week: Questions
  - ...
- Group needs to **pass all assignments** for exam admission

# Exams

---

- Written examination
- Dates to be announced
  - July, October

# Literature

---

- For algorithms and data structures
  - Gusfield (1997). „Algorithms on Strings, Trees, and Sequences“, Cambridge University Press
  - Böckenhauer, Bongartz (2003). „Algorithmische Grundlagen der Bioinformatik“, Teubner
- For other topics and overviews
  - Lesk (2005/2019). „Introduction to Bioinformatics“, Oxford Press
  - Cristianini, Hahn (2007). "Introduction to Computational Genomics - A Case Study Approach", Cambridge University Press
  - Merkl, Waack (2009). "[Bioinformatik Interaktiv](#)", Wiley-VCH Verlag.
  - Dandekar, Kunz (2017) „Bioinformatik: Ein einführendes Lehrbuch“, Springer
- For motivation and relaxation
  - Gibson, Muse (2001). "A Primer of Genome Science", Sinauer Associates.
  - Krane, Raymer (2003). "Fundamental Concepts of Bioinformatics", Benjamin Cummings.

# Web Side

The screenshot shows a web browser window displaying the website 'Vorlesung Grundlagen der Bioinformatik'. The browser's address bar shows the URL: [https://www.informatik.hu-berlin.de/de/forschung/gebiete/wbi/teaching/archive/ss16/vl\\_bioinfo/](https://www.informatik.hu-berlin.de/de/forschung/gebiete/wbi/teaching/archive/ss16/vl_bioinfo/). The website header includes the Humboldt-Universität zu Berlin logo and the text 'HUMBOLDT-UNIVERSITÄT ZU BERLIN'. The main content area is titled 'Vorlesung Grundlagen der Bioinformatik' and is authored by 'Prof. Dr. Ulf Leser'. The page describes the course content, which covers fundamental questions in bioinformatics, including sequencing, comparison, and search in DNA sequences, as well as gene expression experiments, proteomics, and protein-protein interaction networks. It also mentions that the course is accompanied by exercises and that participants must write a paper. The page lists prerequisites, stating that basic knowledge in algorithms and Java is required. It also includes information about exams and credits, mentioning a written exam on September 29, 2016, and that the course is credit-bearing for various degrees. A list of literature for the course is provided, including books by Gusfield, Lesk, and Xiong. Finally, the page lists the topics of the lectures, starting with 'Introduction'.

Mathematisch-Naturwissenschaftliche Fakultät  
Institut für Informatik  
**Wissensmanagement in der Bioinformatik**

People  
Lehre  
Studien- und Diplomarbeiten  
Archiv  
SoSe 18  
WS 17/18  
SoSe 17  
WS 16/17  
SS 16  
Vorlesung Informationsintegration  
Übung Informationsintegration  
**Vorlesung Grundlagen der Bioinformatik**  
Übung Grundlagen der Bioinformatik  
Proseminar Wissenschaftliches Arbeiten  
Übung Algorithmen und Datenstrukturen  
Forschungsseminar  
WS 15/16  
SS15  
WS 14/15  
SS14  
WS 13/14  
SS13  
WS 12/13  
SS12  
WS 11/12  
SS 11  
WS 10/11  
SS 10  
WS 09/10

HUMBOLDT-UNIVERSITÄT ZU BERLIN

Humboldt-Universität zu Berlin | Mathematisch-Naturwissenschaftliche Fakultät | Institut für Informatik | Wissensmanagement in der Bioinformatik | Lehre | Archiv | SS 16 | Vorlesung Grundlagen der Bioinformatik

**Vorlesung Grundlagen der Bioinformatik**  
**Prof. Dr. Ulf Leser**

Die Vorlesung behandelt grundlegende Fragestellungen der Bioinformatik. Sie vermittelt zunächst die notwendige Grundkenntnisse in der Molekularbiologie und behandelt dann ausgewählte Themen der Bioinformatik, wie Sequenzierung von Genomen, Vergleich und Suche in DNA Sequenzen, Messung und Interpretation von Genexpressionsexperimenten, Proteomics, Analyse von Protein-Protein-Interaktionsnetzen etc. Sie ist grundlegend konzipiert und führt in die Themen nur ein.

Die Vorlesung wird durch eine [Übung](#) begleitet, in die sich Teilnehmer über [Goya](#) einschreiben müssen. Die Übung vertieft ausgewählte Methoden der Vorlesung durch deren praktische Umsetzung. Bestehen der Übung ist Voraussetzung zur Prüfungsanmeldung.

**Voraussetzungen**  
Voraussetzung für den Besuch sind grundlegende Kenntnisse in Algorithmen und gute Kenntnisse in Java.

**Prüfungen und Anrechenbarkeit**  
Die Prüfungen erfolgt schriftlich per **Klausur am 29.7.2016**, Raum 3.001, von 11.30 - 13.30 (Einlass ab 11.00 Uhr). Voraussetzung für die Prüfungsanmeldung ist das Bestehen der Übung.  
Das Modul ist anrechenbar für

- Monobachelor Informatik, Wahlpflichtbereich, 5 SP
- Kombibachelor Informatik Erstfach, Wahlpflichtbereich, 5 SP
- Bachelor Biophysik, Pflichtvorlesung im Modul Bioinformatik (5 SP)

**Literatur zur Vorlesung**

- Gusfield, D. (1997). "Algorithms on Strings, Trees and Sequences", Cambridge University Press.
- Lesk, A. M. (2008). "Introduction to Bioinformatics", Oxford University Press.
- Xiong, J. (2006). "Essential Bioinformatics", Cambridge University Press.

**Themen der Vorlesung**  
Diese Liste wird ständig aktualisiert. Folien zu den Vorlesungen und notwendige Daten werden hier veröffentlicht.

- Introduction

# My Questions

---

- Bachelor Informatik?
  - Kombibachelor?
  - Other?
- 
- Semester?
  - Prüfung?
  - Spezielle Erwartungen?

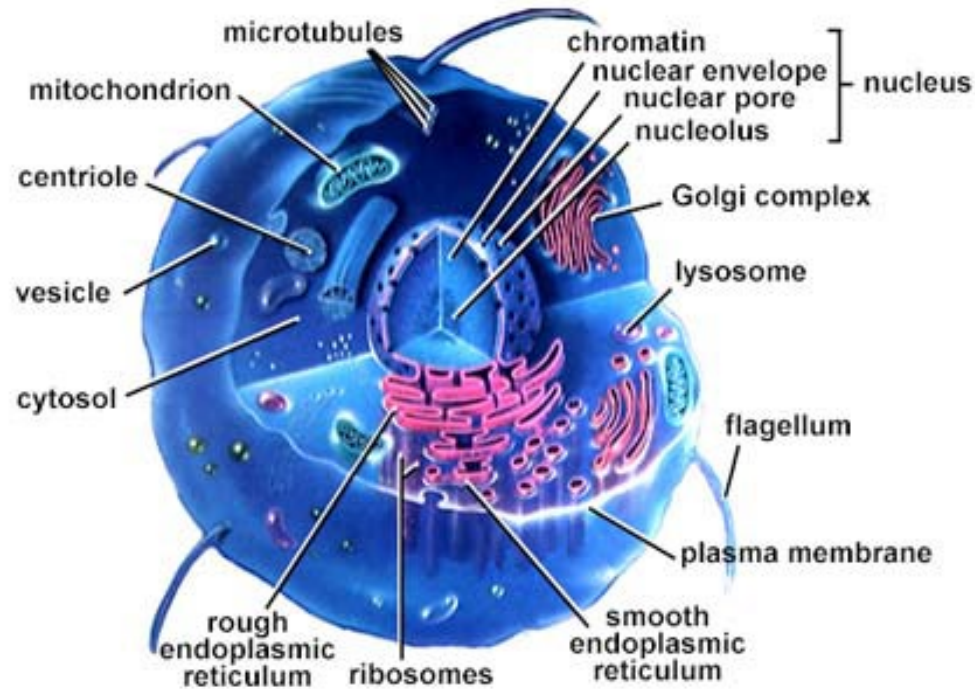
# This Lecture

---

- Formal stuff on the course
- A very short introduction in Molecular Biology
- What is Bioinformatics?
- Topics of this course

# Cells and Bodies

---

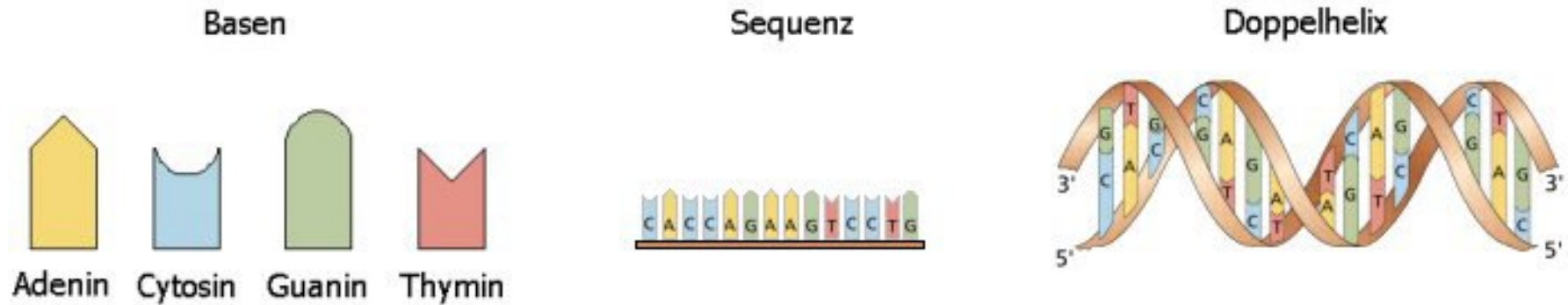


- App. 75 trillion cells in a human body
- App. 250 different **types**: nerve, muscle, skin, blood, ...



# DesoxyriboNucleicAcid

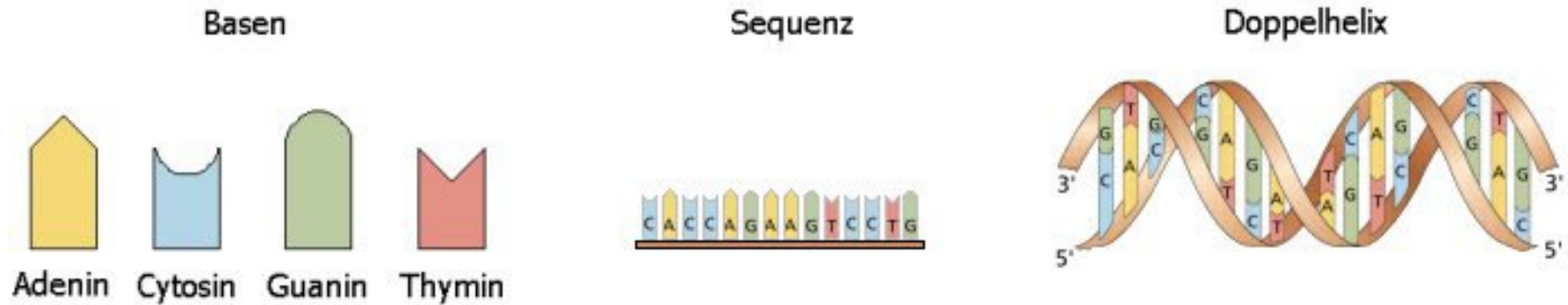
---



- DNA: Desoxyribonukleinsäure
  - Four different molecules
  - The DNA of all chromosomes in a cell forms its genome
  - All cells in a (human) body carry the same genome
  - All living beings are based on DNA for proliferation
  - There are always always **always exceptions**

# DesoxyriboNucleicAcid

---

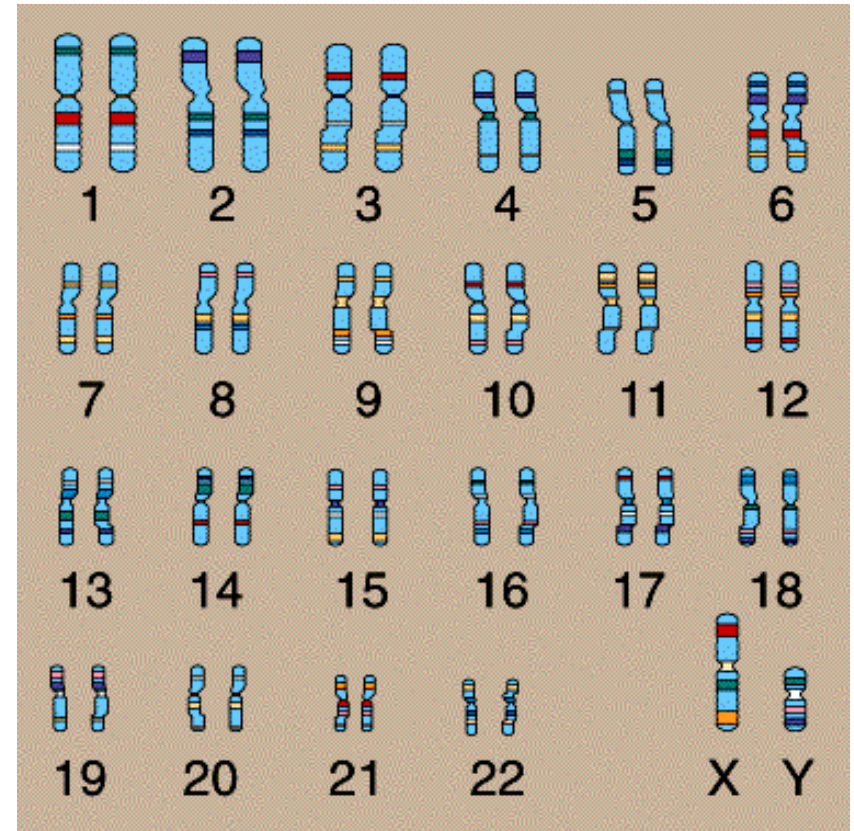


- DNA: Desoxyribonukleinsäure
  - Four different molecules (one replaced in RNA)
  - The DNA of all chromosomes in a cell together with the mitochondria-DNA forms its genome
  - Almost all cells in a (human) body carry almost the same genome
  - All living beings are based on DNA or RNA for proliferation

# The Human Genome

---

- 23 chromosomes
  - 22 in pairs
- ~3.000.000.000 letters
- ~50% are repetitions of 4 identical subsequences
  - ~~~100.000 genes~~
  - ~~~56.000 genes~~
  - ~~~30.000 genes~~
  - ~~~24.000 genes~~
- ~22.000 genes



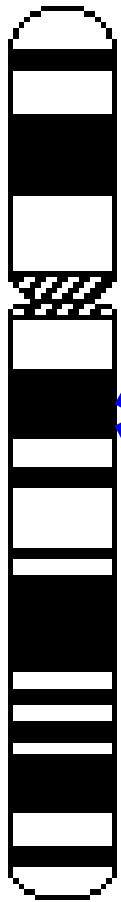
# (Protein-Coding) Genes

Chromosome

RNA

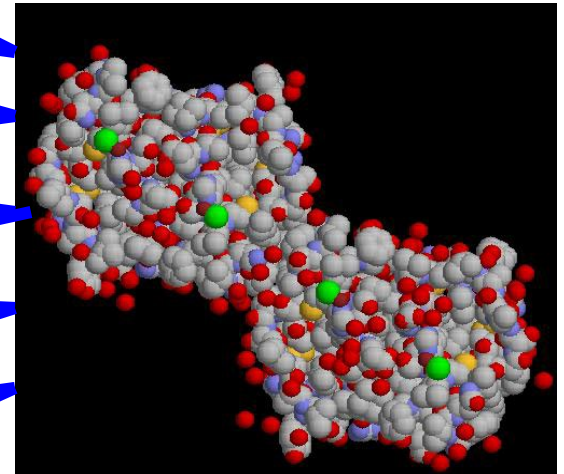
mRNA

Proteine

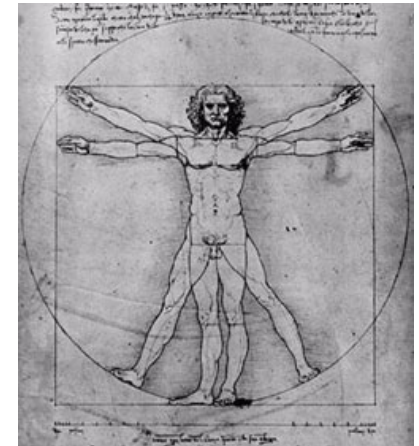
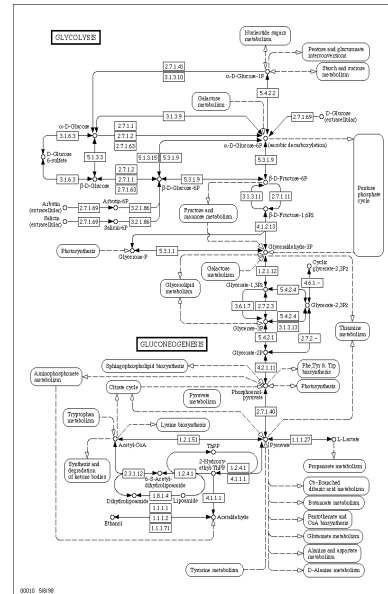
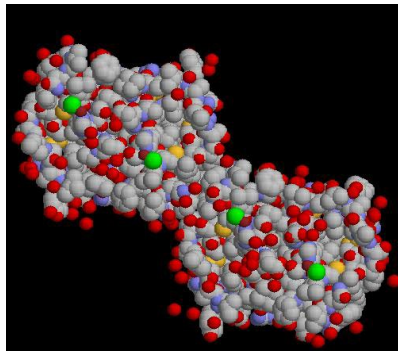
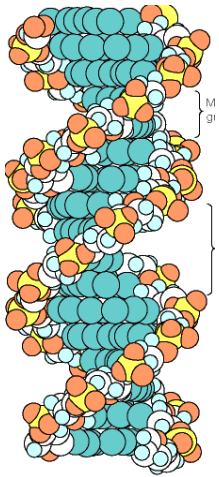
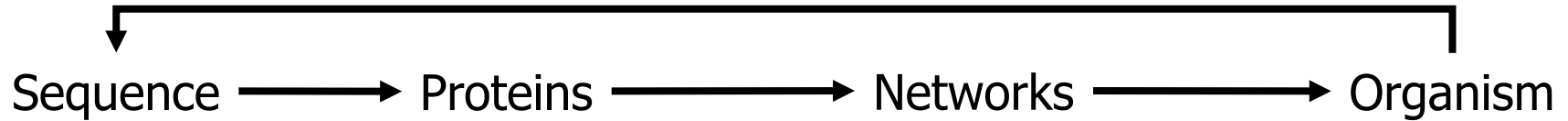


A  
C  
G  
U  
U  
G  
A  
U  
G  
A  
C  
C  
A  
G  
A  
G  
C  
U  
U  
G  
U

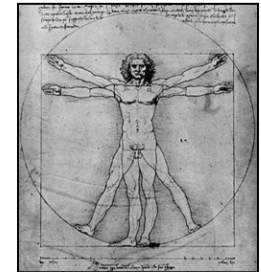
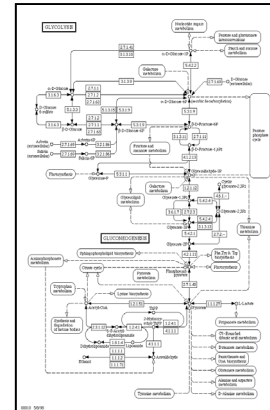
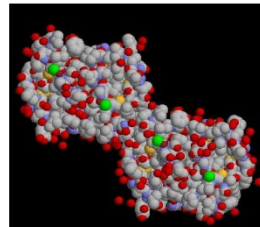
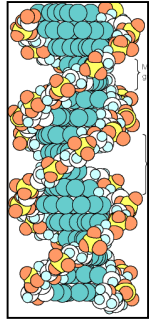
A  
C  
G  
U  
U  
G  
A  
C  
A  
G  
A  
G  
C  
U  
U  
G  
U



# Proliferation



# This Lecture



## Genomics

Sequencing

Gene prediction

Evolutionary  
relationships

Motifs - TFBS

Transcriptomics

RNA folding

...

## Proteomics

Structure prediction

... comparison

Motives, active sites

Docking

Protein-Protein

Interaction

Proteomics

...

## Systems Biology

Pathway analysis

Gene regulation

Signaling

Metabolism

Quantitative models

Integrative analysis

...

## Medicine

Phenotype –  
genotype

Mutations and risk

Population genetics

Adverse effects

...

# This Lecture

---

- Formal stuff on the course
- A very short introduction in Molecular Biology
- What is Bioinformatics?
  - And an example
- Topics of this course

# Bioinformatics / Computational Biology

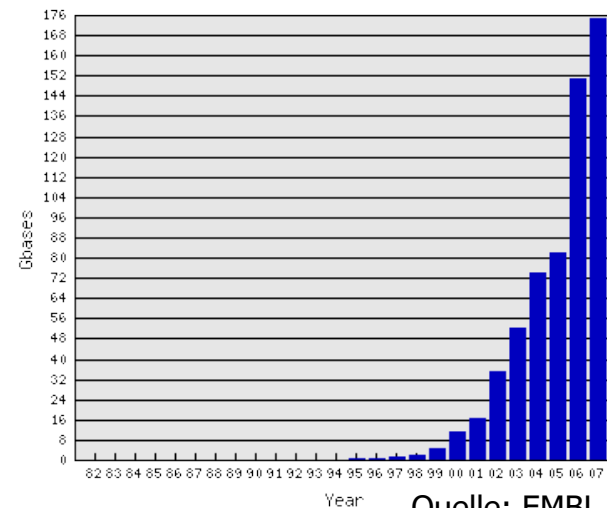
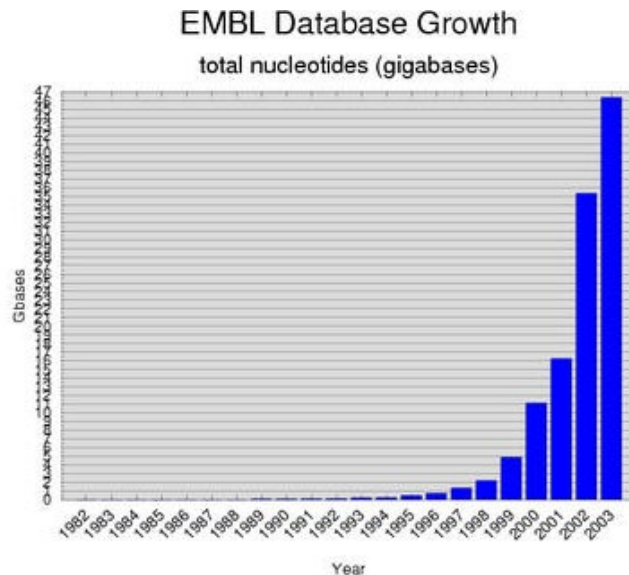
---

- Computer Science methods for
  - Solving biologically relevant problems
  - Analyzing and managing experimental data sets
- **Empirical**: Data from high throughput experiments
- Focused on algorithms and statistics
- Problems are typically complex, data full of errors – importance of **heuristics and approximate methods**
- Strongly **reductionist** – Strings, graphs, sequences
- **Interdisciplinary**: Biology, Computer Science, Physics, Mathematics, Genetics, ...



# History

- First protein sequences: 1951
- Sanger sequencing: 1972
- **Exponential growth** of available data since end of 70<sup>th</sup>
  - Bioinformatics is largely **data-driven** – new methods yield new data requiring new algorithms



Quelle: EMBL, Genome  
Monitoring Tables

# History 2

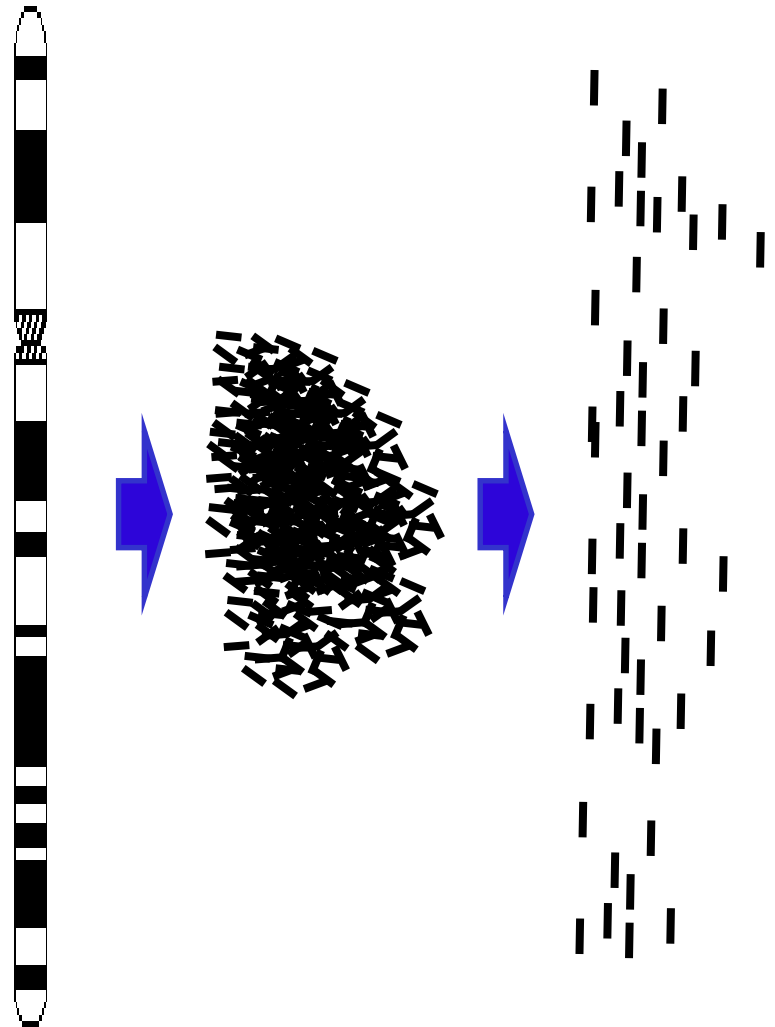
---

- First papers on sequence alignment
  - Needleman-Wunsch 1970, Gibbs 1970, Smith-Waterman 1981, Altschul et al. 1990
- Large impact of the **Human Genome Projekt** (~1990)
- Only 14 mentions of „Bioinformatics“ before 1995
- „Journal of Computational Biology“ since 1994
- First **professorships** in Germany: end of 90's
- First university programs: ~2000
- First German book: 2001
- Commercial hype: 1999 – 2004
- Indispensable for medical research: 2010 –
- **Regular sequencing** for some cases (cancer): 2016 -

# A Concrete Example: Sequencing a Genome

---

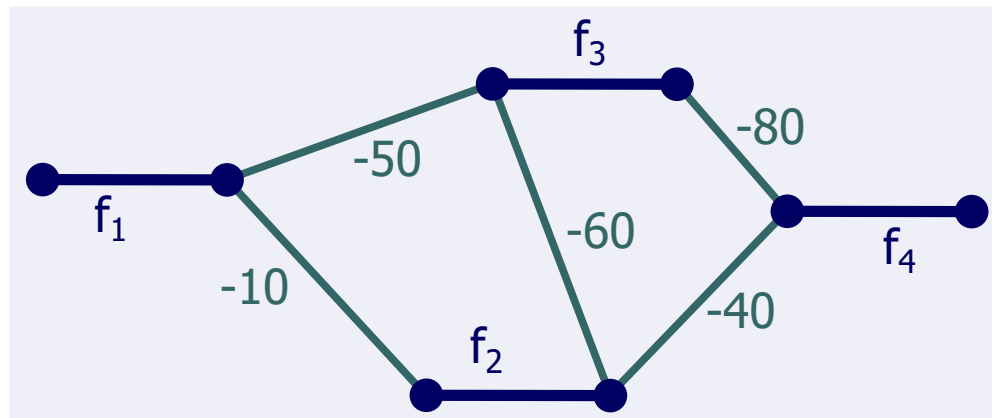
- Chromosomes (still) cannot be sequenced entirely
  - Instead: Only **small fragments** can be sequenced
- But: Chromosomes cannot be cut at position X, Y, ...
  - Instead: Chromosomes only can be cut at **certain subsequences**
- But: We don't know where in a chromosome those subsequences are
  - **Sequence assembly** problem



# Problem

---

- Given a large set of (sub)sequences from randomly chosen positions from a given chromosome of unknown sequence
- Assembly: Determine **sequence of the original** chromosome
  - Everything may overlap with everything to varying degrees
  - Let's forget about orientation and sequencing errors



# Abstract Formulation

---

- SUPERSTRING

- Given a set  $S$  of strings
- Find string  $t$  such that
  - (a)  $\forall s \in S: s \in t$  (all  $s$  are substrings of  $t$ )
  - (b)  $\forall t'$  for which (a) holds:  $|t| \leq |t'|$  ( $t$  is minimal)

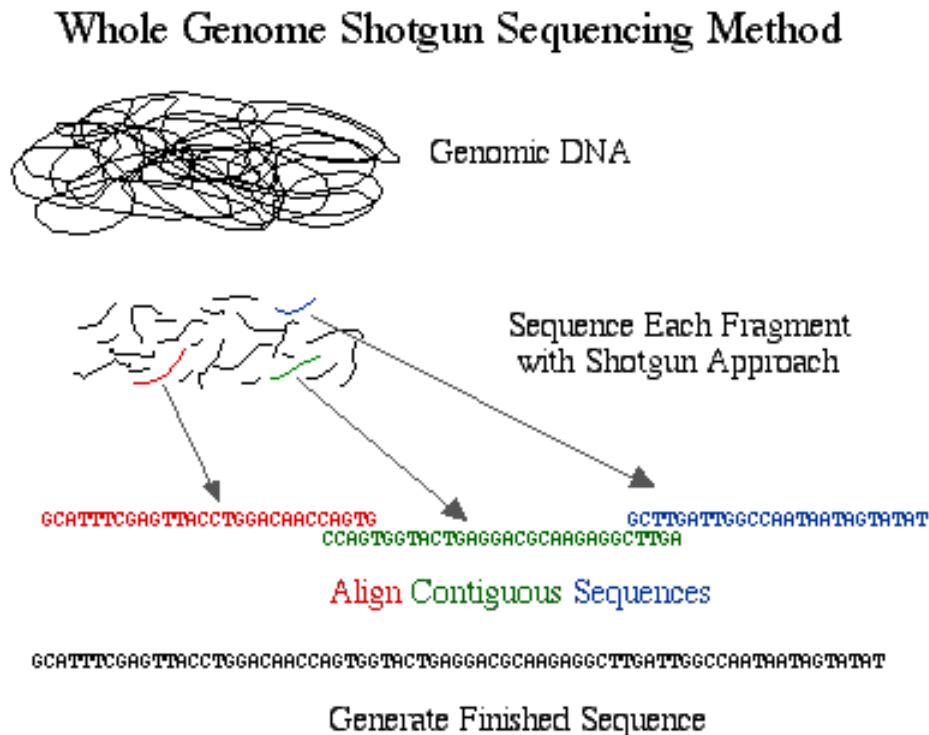
- Problem is NP-complete

- Very likely, there is no algorithm that solves the problem in less than  $k_1 * k_2 2^n$  operations, where  $k_1, k_2$  are constants and  $n = |S|$

- Bioinformatics: Find clever heuristics

- Solve the problem “good enough”
- Finish in reasonable time

# Dimension



- Whole genome shotgun
  - Fragment an entire chromosome in pieces of 1KB-100KB
- Sequence start and end of all fragments
  - Homo sap.: 28 million reads
  - Drosophila: 3.2 million reads
- Eukaryotes are very difficult to assemble because of repeats
  - A random sequence is easy

# Why is this “reductionist”?

---

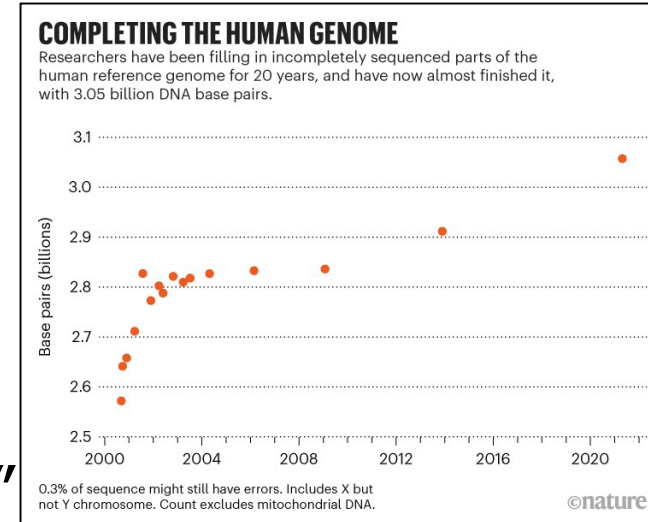
- Real alphabet is larger: N, AorT, ...
- Errors need not be errors: Humans have two alleles
- Errors are not equally distributed but accumulate at begin / end of read
- Paired-end sequencing
- Substantial parts of a genome cannot be sequenced – wholes in the genome
- Why sequence everything when only genes count?
  - Whole genome, whole exome, RNASeq, ...

# Sequencing Today

A month ago!



- 2003: 1st **human reference** sequence
  - Continuously refined since then
- Two human genomes are >99% identical
  - And ~98% identical to a mouse genome
- Today: Sequencing by “**read mapping**”
  - Create reads as before
    - 2<sup>nd</sup> generation: Many more yet shorter reads (~100bp)
    - 3<sup>rd</sup> generation: Much larger reads (15K bp); still highly error prone
  - “Mapping”: **Find position of reads** in reference genome
    - String matching with few errors
  - Much faster and cheaper
  - Cannot detect larger (“structural”) variations



Nature, 6/2022



# This Lecture

---

- Formal stuff on the course
- A very short introduction in Molecular Biology
- What is Bioinformatics?
  - And an example
- Topics of this course

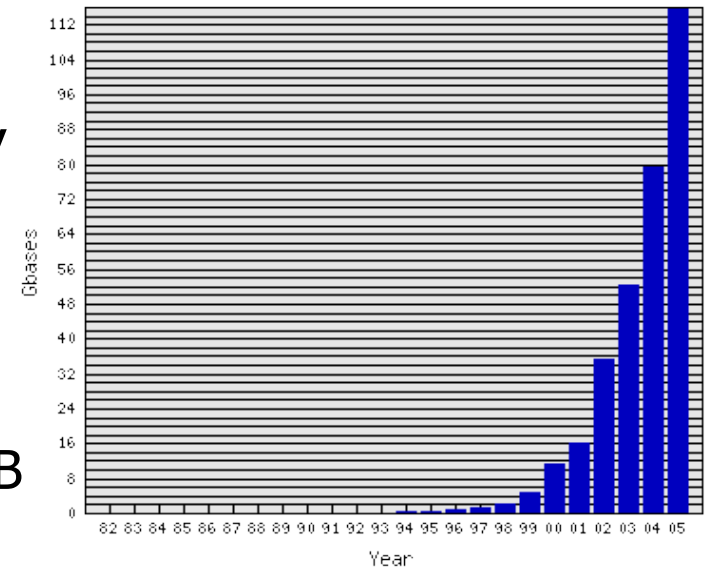
# Searching Sequences (Strings)

---

- A chromosome is a string
- Substrings may represent **biologically important areas**
  - Genes on a chromosome
  - Transcription factor binding sites
  - Similar gene in a different species
  - ...
- Exact or **approximate string search**

# Searching a Database of Strings

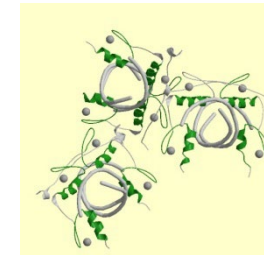
- Comparing two sequences is costly
- Given  $s$ , assume we want to find the **most similar  $s'$  in a database** of all known sequences
  - Naïve: Compare  $s$  with all strings in DB
  - Will take years and years
- **BLAST**: Basic local alignment search tool
  - Ranks all strings in DB according to similarity to  $s$
  - Similarity: High if  $s, s'$  contain substrings that are highly similar
  - Heuristic: Might **miss certain similar sequences**
  - Extremely popular: You can “blast a sequence”



# Multiple Sequence Alignment

- Given a set  $S$  of sequences: Find an arrangement of all strings in  $S$  in columns such that there are (a) few columns and (b) **columns are maximally homogeneous**
  - Additional spaces allowed

YVCR...	LCN...	FAP	KTR	GNL	TKH	MKS	K..	AH
YRC	PR.	ENC	D...	RTY	TTK	FN	LKSH	ILT..
FR	CGY.	K	CGG...	RLY	TTA	HHL	KVHER	A...
YR	CE...	K	CG...	KMY	KTER	CL	KVHNL	V...
F	SCS...	Q	CD...	ESF	VQ	RSE	LEL	HRQL...
F	P	CE...	Q	CD...	EKF	KTE	KQ	LERHVKT...
F	Q	CN...	Q	CG...	ASF	TQ	KNLL	RHIKL...
F	K	CH...	L	CV...	RCF	G	QQT	NLDRHLKK...
F	R	CK...	R	CR...	TRF	RQ	SEL	KKHMKT...
F	E	CN...	V	CG...	S	A	FRL	QLYLSEHQKT...
M	S	CKV...	C	D...	R	V	FY	RDLNLRSHLQK...
F	S	CQ...	H	CH...	R	A	F	ADRSNLR
F	R	CG...	Y	CG...	R	A	F	TVKDYL
H	V	CWV...	P	CGH...	R	A	F	S
L	T	CAH...	C	D...	W	S	F	D



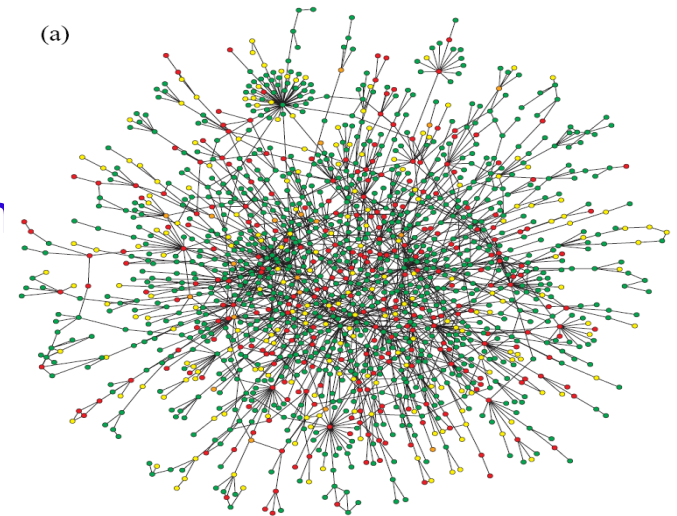
Source: Pfam, Zinc finger domain

- Goal: Find **commonality** between a set of functionally related sequences
  - Proteins are composed of different functional domains
  - Which domain performs a certain function?

# Protein-Protein-Interactions

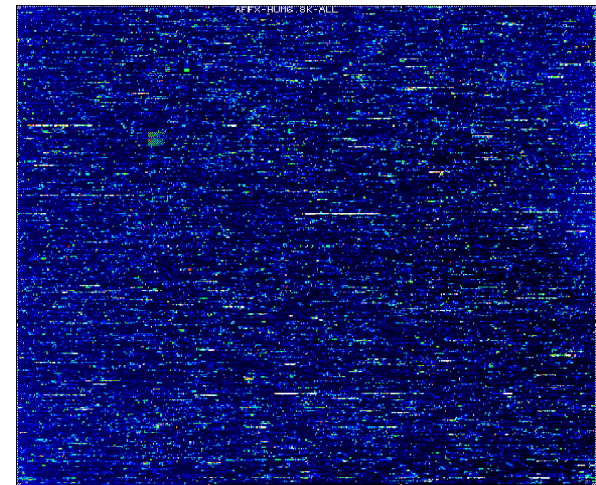
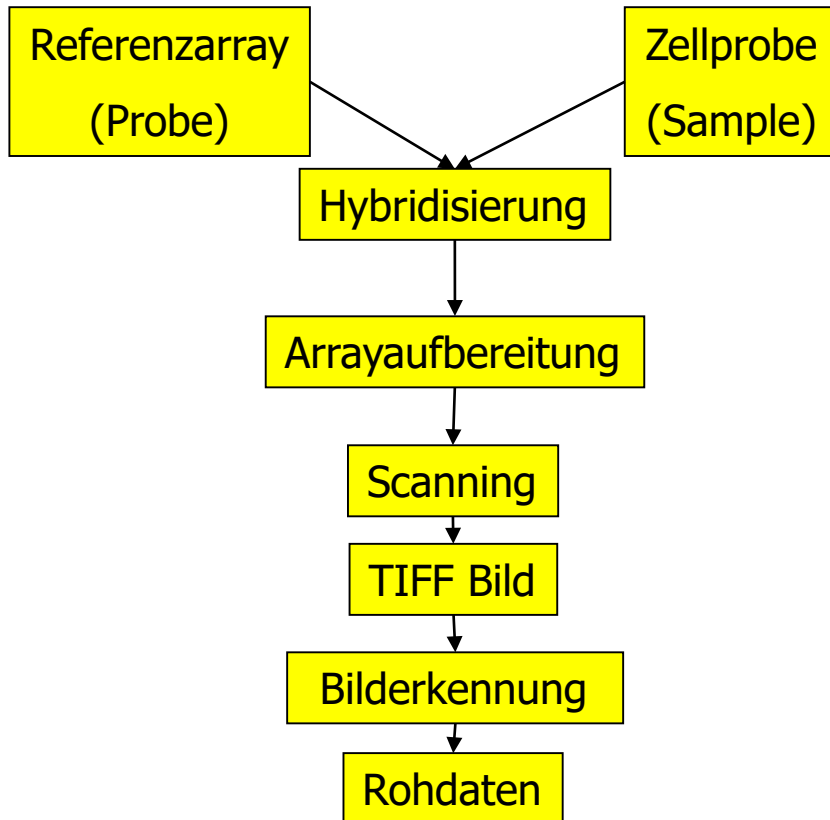
---

- Proteins do not work in isolation but **interact with each other**
  - Metabolism, complex formation, signal transduction, transport, ...
- PPI networks
  - Neighbors tend to have **similar function**
  - Interactions tend to be evolutionary conserved
  - **Dense subgraphs** (cliques) tend to perform distinct functions
  - Are not random at all



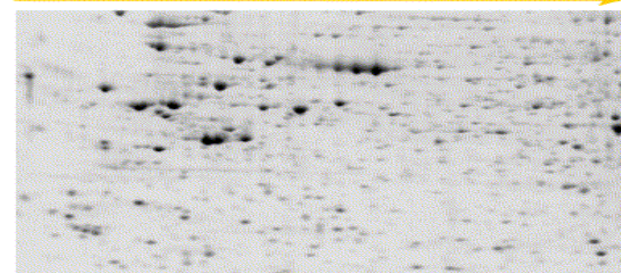
# Transcriptomics

---

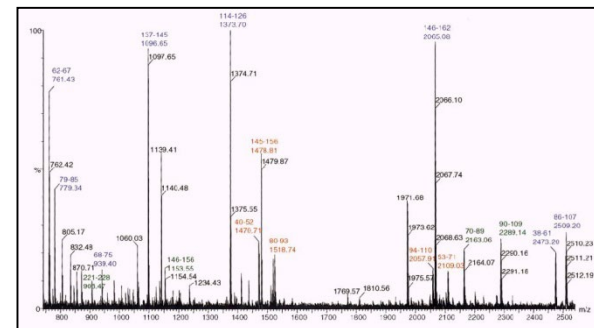


# Proteomics

- The real workhorses in a cell **are proteins**
    - Differential splicing, post-translational modifications, degradation rates, various levels of regulation, ...
  - But: Much more difficult to study (compared to mRNA)
  - Separation of proteins
    - 2D page, GC / LC
  - Identification of proteins
    - **Mass-spectrometry**
- 
- Drug Discovery Today
- 



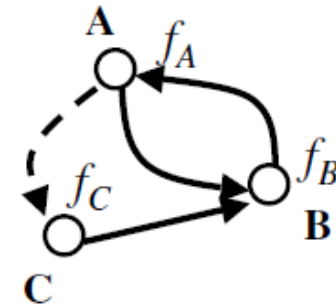
Drug Discovery Today



# Network Reconstruction

---

- Molecules perform functions by means of interactions
- **Regulation**: Networks of genes regulating each other
- Reconstruction: Which gene regulates **which other genes** in **which ways**?
- One approach: Boolean networks



$$f_A(B) = B$$

$$f_B(A, C) = A \text{ and } C$$

$$f_C(A) = \text{not } A$$

Boolean Network