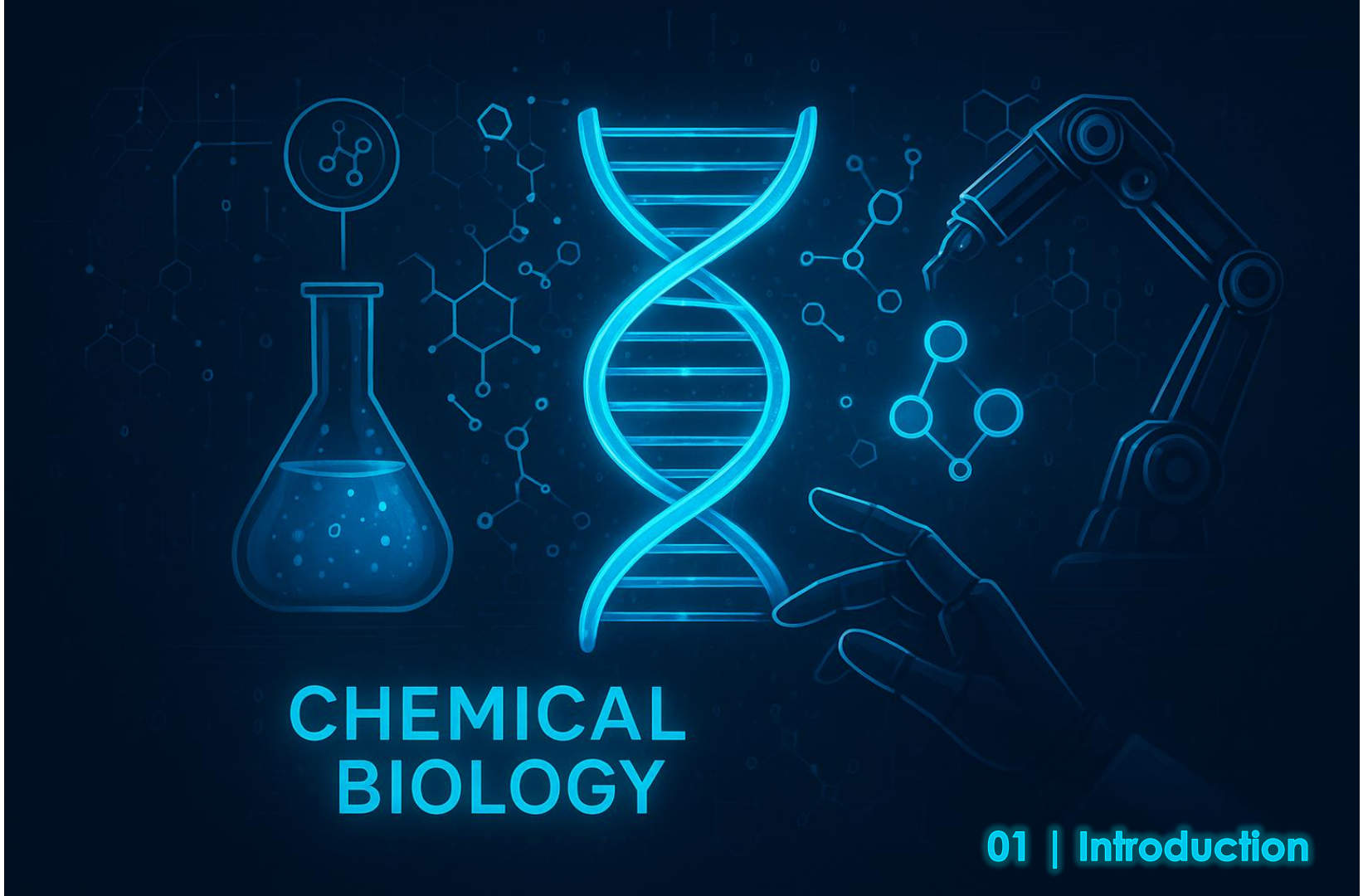

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Brief Intro – LSAM

- About myself…
  - 2005-2011 BSc+MSc | **TU Munich** (D)
  - 2011-2022 PhD+Postdoc+Group Leader | **ETHZ** (D-BSSE)
  - 2019 SNSF Fellow | **Northwestern University** (USA)
  - 2022-2025 Professor (W2) | **University of Regensburg** (D)

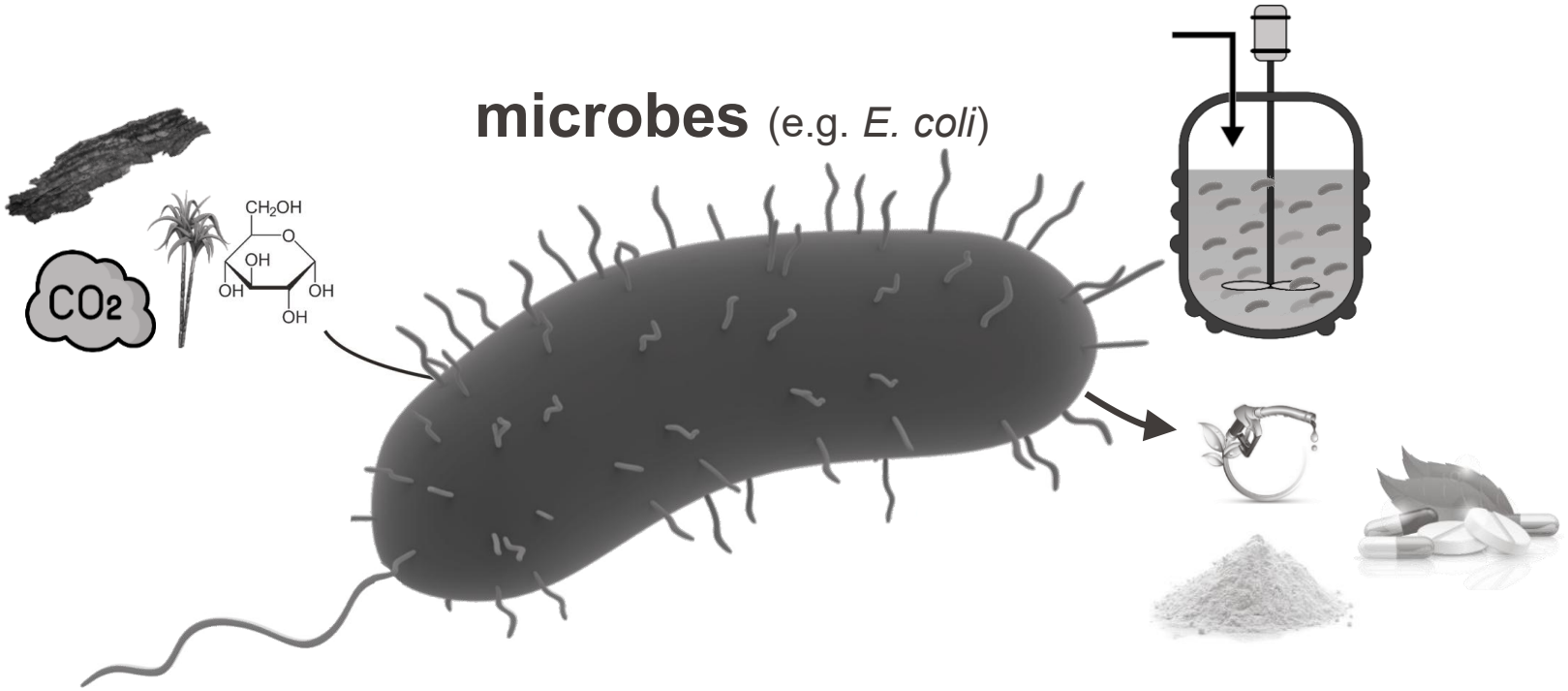
LABORATORY OF SYNTHETIC AND APPLIED MICROBIOLOGY

- **LSAM @EPFL (since June 2025)**
  - "Lonza Chair for Sustainable Biosynthesis"
  - Joint appointment – SB & SV
  - Institutes of Chemical Science and Engineering (ISIC) and Bioengineering (IBI)

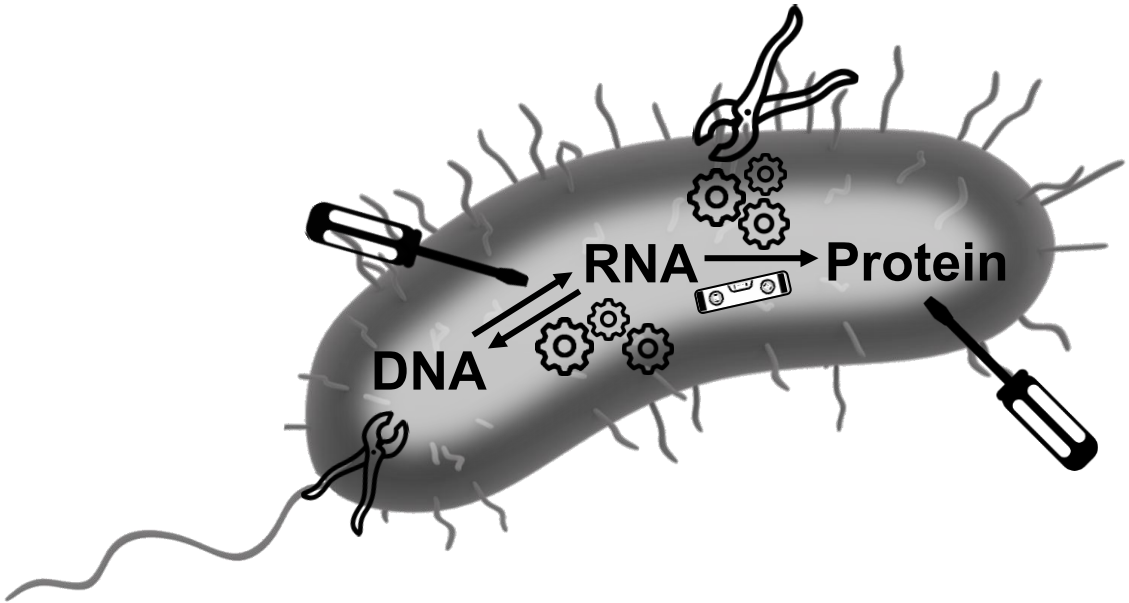
- Teaching
  - Chemical Biology (BSc, CH-313)
  - Specialized Lecture "Synthetic and Applied Microbiology" (MSc/PhD)
  - Internships/theses works

Our Motivation

LABORATORY OF SYNTHETIC AND APPLIED MICROBIOLOGY



Engineering Microbes



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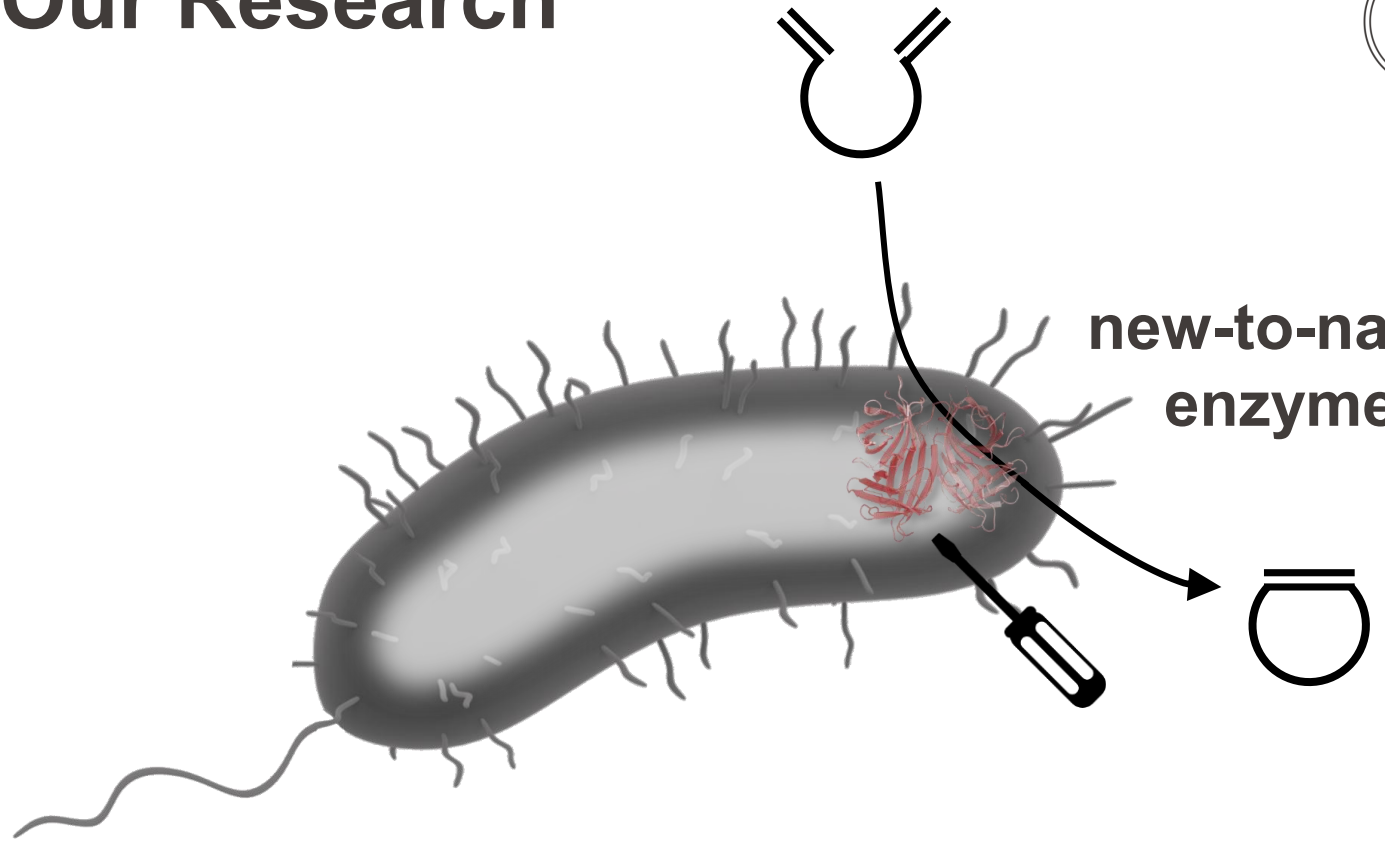
Our Research



L. Huber, T. Kucera, S. Höllerer, K. Borgwardt, S. Panke, M. Jeschek. *Nat. Commun.* 5466 (2025)
T. Vornholt, M. Mutný, G.W. Schmidt, C. Schellhaas, R. Tachibana, S. Panke, T.R. Ward, A. Krause, M. Jeschek. *ACS Central Sci.* 10, 1357-1270 (2024)
T. Vornholt, F. Christoffel, M. Pellizzoni, S. Panke, T.R. Ward, M. Jeschek. *Sci. Adv.* 7, eabe4208 (2021)
M. Jeschek, R. Reuter, T. Heinisch, C. Trindler, J. Klehr, S. Panke, T.R. Ward. *Nature* 537:661-665 (2016)
M. Jeschek, S. Panke, T. R. Ward. *Trends Biotechnol. 36*, 60-72 (2018)

Our Research

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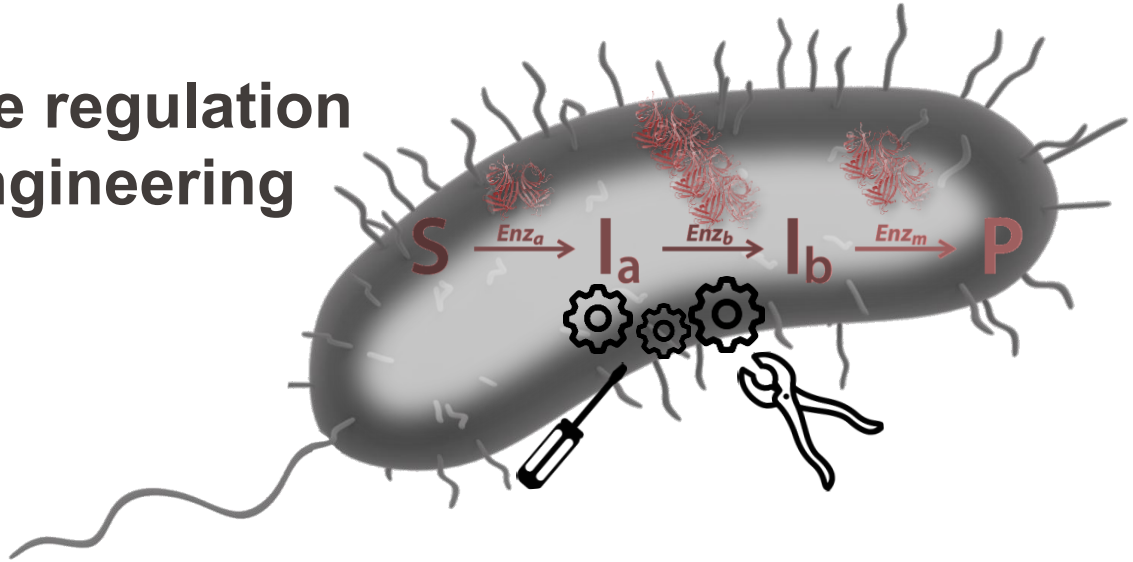
gene regulation engineering



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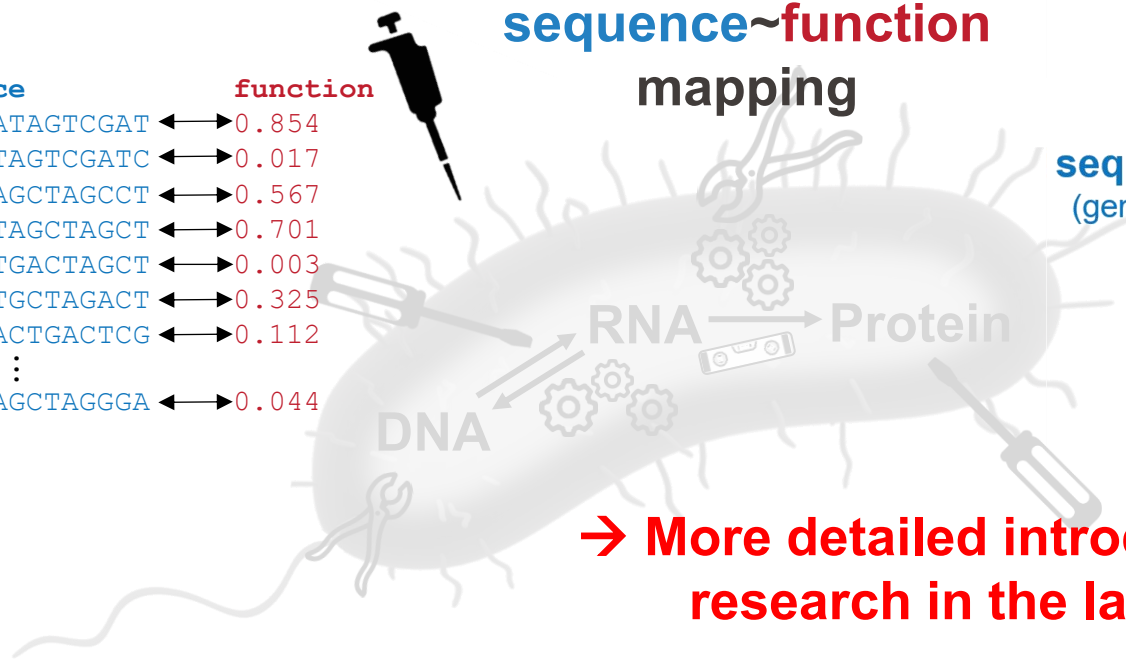
Höllerer & Jeschek. *Nucleic Acids Res.* 51:2377-2396 (2023)
Höllerer, Papaxanthos, Gumpinger, Fischer, Beisel, Borgwardt, Benenson, Jeschek. *Nat. Commun.* 11:3551 (2020)
M. Jeschek, D. Gerngross, S. Panke. *Nat. Commun.* 7:11163 (2016)
M. Jeschek, D. Gerngross, S. Panke. *Curr. Opin. Biotechnol.* 47:142-151 (2017)

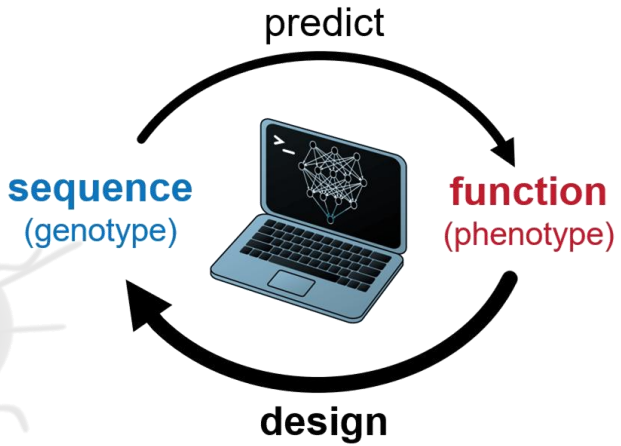
Methodology

LABORATORY OF SYNTHETIC AND APPLIED MICROBIOLOGY

high-throughput sequence~function mapping

#	sequence	function
1	ACTGCGATAGTCGAT	0.854
2	CTAGACTAGTCGATC	0.017
3	CCCTAGAGCTAGCCT	0.567
4	CATGGCTAGCTAGCT	0.701
5	CTAGCATGACTAGCT	0.003
6	CAAGCATGCTAGACT	0.325
7	GGGCTGACTGACTCG	0.112
⋮		
10^8	CTAGCTAGCTAGGGA	0.044





→ More detailed introduction on our research in the last lecture!

L. Huber, T. Kucera, S. Höllerer, K. Borgwardt, S. Panke, M. Jeschek. *Nat. Commun.* 5466 (2025)
T. Vornholt, M. Mutný, G.W. Schmidt, C. Schellhaas, R. Tachibana, S. Panke, T.R. Ward, A. Krause, M. Jeschek. *ACS Central Sci.* 10, 1357-1270 (2024)
Höllerer & Jeschek. *Nucleic Acids Res.* 51:2377-2396 (2023)
T. Vornholt, F. Christoffel, M. Pellizzoni, S. Panke, T.R. Ward, M. Jeschek. *Sci. Adv.* 7, eabe4208 (2021)
Höllerer, Papaxanthos, Gumpinger, Fischer, Beisel, Borgwardt, Benenson, Jeschek. *Nat. Commun.* 11:3551 (2020)

General Course Information

- Moodle: https://go.epfl.ch/CH-313
  - Lecture slides (evening before the lecture)
  - Distributed presentation topics (assignments)
  - Forum (for questions and announcements)

- Examination (written, graded, detailed information will follow)
- Contact:
  - Moodle forum (for questions)
  - markus.jeschek@epfl.ch

- **“Concepts over details!”**
- **Interact! Ask! Discuss! Anytime!**

Group Presentations

- Critical discussion of primary literature
- Illustrative examples for topics from the lecture

- Why?
  - Repetition of core concepts, techniques etc.
  - Presentation skills and critical discussion of research
  - Insight into current research topics

- How?
  - Two students per group
  - Assignments distributed one week before delivery of presentation (via Moodle)
  - **Send slides: markus.jeschek@epfl.ch (Mon evening before presentation)**
  - **15 min presentation (both group members should present!)** + Q&A

Tipps for Group Presentations

- Rough structure
  - Short intro on general topic
  - Main presentation according to assignment
  - Brief outlook incl. points of criticism/open questions/personal opinion as kick-starter for the discussion
- Everybody should participate in the discussion, incl. constructive(!) feedback on presentation style
- Questionnaires with different points, feedback by peers
- Typical assignment:
  - You will receive a certain topic including a related publication
  - Introduce the topic using the publication
  - present the motivation behind the research, methodology, key results (not every graph!)
  - Additional questions will be provided hinting towards central points
  - Be encouraged to look/present beyond the questions and the provided paper

Group Presentations – Schedule

#	Name1	Name2	Presentation on…	Assignment on…
1			**Sep 23, 2025**	Sep 16, 2025
2			**Sep 30, 2025**	Sep 23, 2025
3			**Oct 7, 2025**	Sep 30, 2025
4			**Oct 14, 2025**	Oct 7, 2025
5			**Oct 28, 2025**	Oct 14, 2025
6			**Nov 4, 2025**	Oct 28, 2025
7			**Nov 11, 2025**	Nov 4, 2025
8			**Nov 18, 2025**	Nov 11, 2025
9			**Nov 25, 2025**	Nov 18, 2025
10			**Dec 2, 2025**	Nov 25, 2025
11			**Dec 9, 2025**	Dec 2, 2025

Introduction

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What is “Chemical Biology”?

- ChatGPT says…

“Chemical biology is an **interdisciplinary field** at the interface of **chemistry** and **biology**. It uses the tools, concepts, and techniques of **chemistry to study and manipulate biological systems**.”

- Important aspects
  - understanding biology…
  - changing biological properties…
  - diagnostics and therapeutics…
  - …through chemical tools

- Timely and impactful field! (e.g. see Nobel Prizes in Chemistry)

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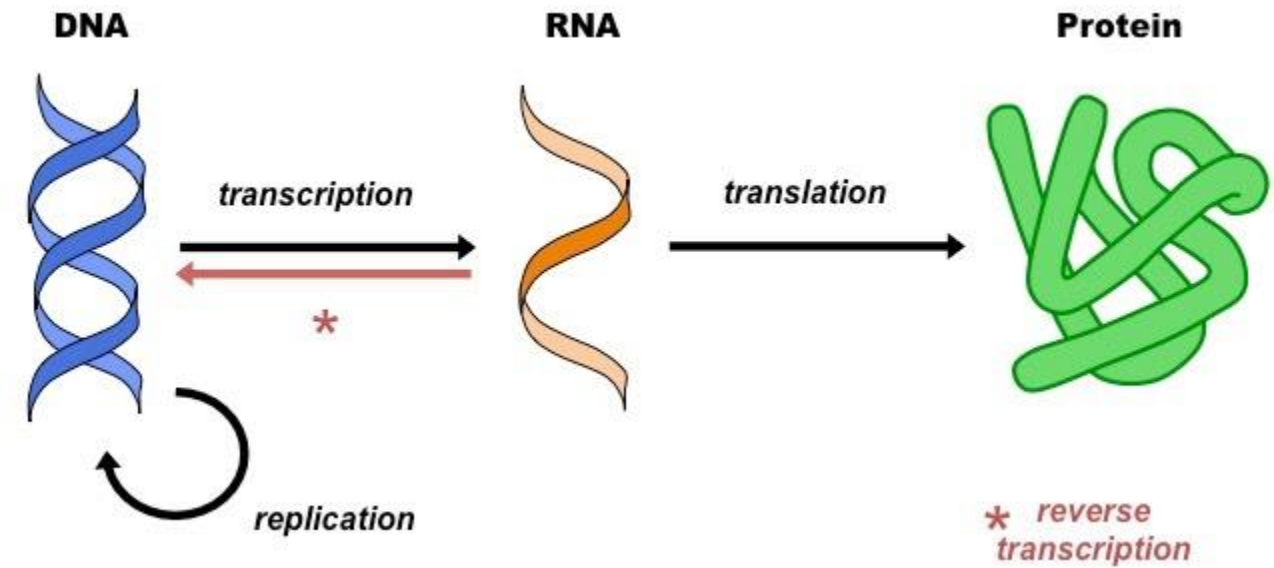
The Central Dogma

- …of Molecular Biology



Q: What are the “biological machines” responsible for the different processes?

Q: What is the genetic code?

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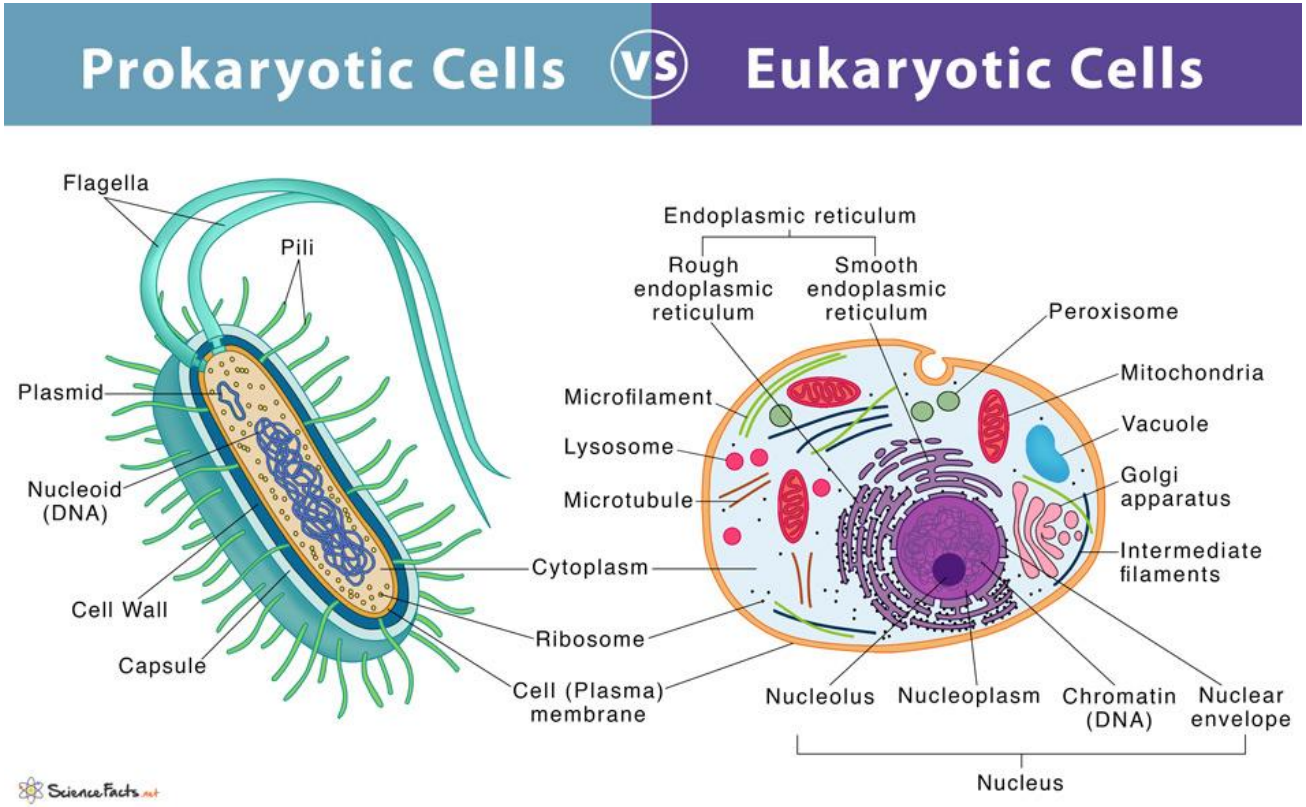
The Cell



Q: What are key components of cells? How do they differ between pro- and eukaryotes?

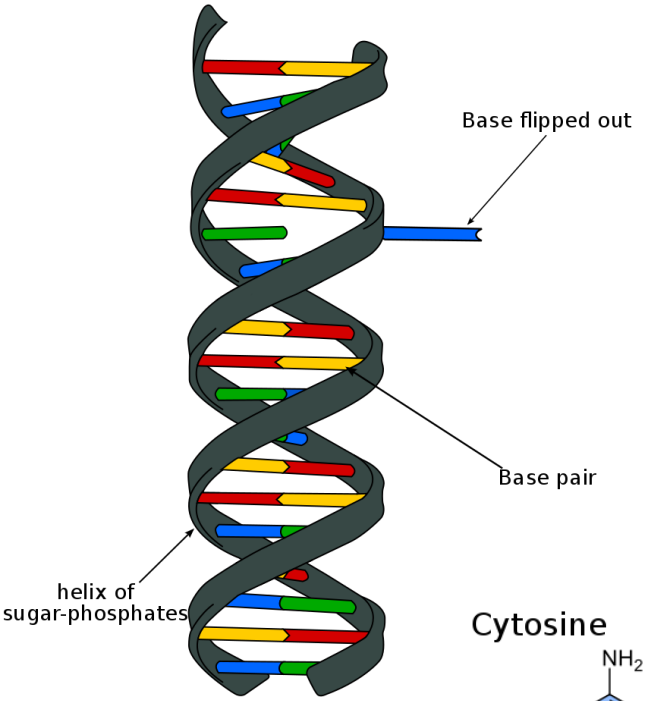
Course Topics – Overview

- **Week 1 | Introduction + DNA**
- Week 2 | DNA
- Week 3 | DNA
- Week 4 | RNA
- Week 5 | Protein/Enzymes
- Week 6 | Enzymes
- Week 7 | Enzymes
- Week 8 | Membranes
- Week 9 | Metabolism
- Week 10 | Metabolism
- Week 11 | Engineering
- Week 12 | Engineering
- Week 13 | Engineering
- Week 14 | LSAM Intro + Exam Preparation

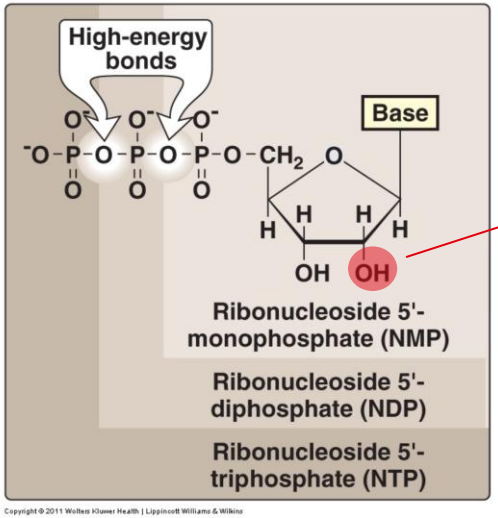
[tentative schedule]

DNA

Deoxyribonucleic acid (DNA)



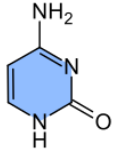
DNA
Deoxyribonucleic acid



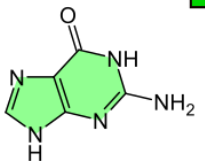
-OH → ribose (RNA)
-H → deoxyribose (DNA)

nucleotides
= nucleoside
+phosphate(s)

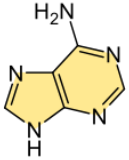
Cytosine **C**



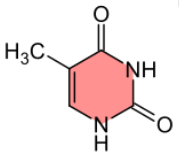
Guanine **G**



Adenine **A**



Thymine **T**



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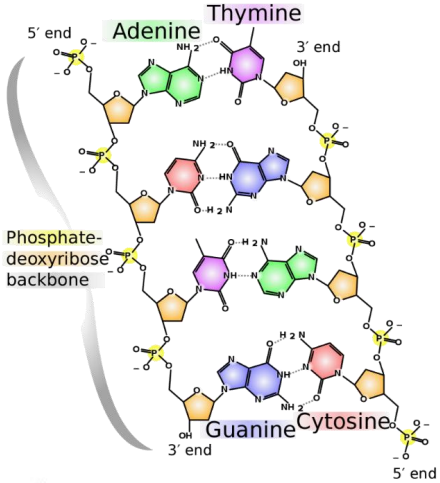
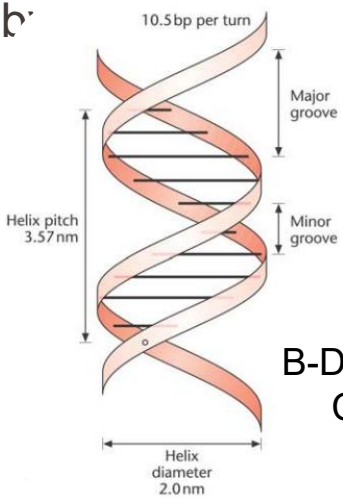
Key Properties of DNA

- Long polymer
  - backbone: alternating phosphate and deoxyribose groups
  - negatively charged
  - double-helix structure

- Extremely stable (high T, solvents, hydrolysis etc.)
- Highly dense information storage (e.g. 215 petab
- Codes for structure and function
  - non-coding RNAs
  - mRNAs → proteins

- **Directionality! (3‘-end, 5‘-end)**
- **Complementarity!**





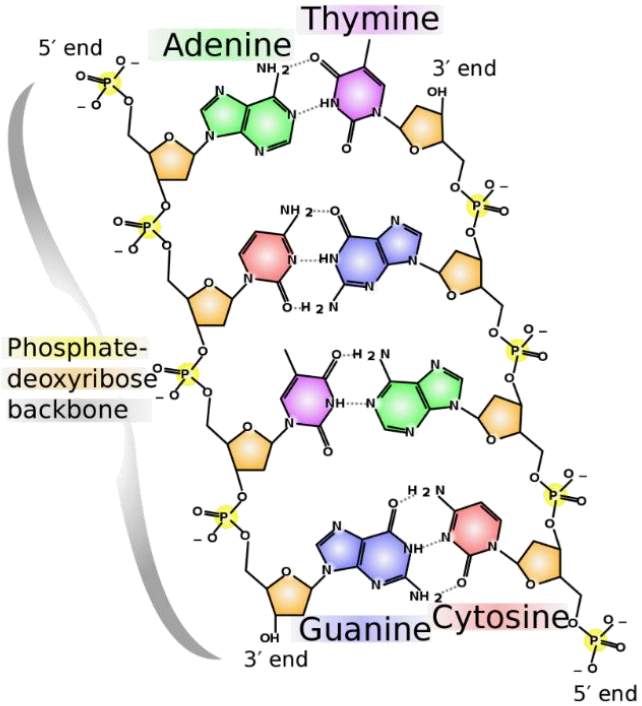
B-DNA (Watson & Crick, 1953)

Complementarity of DNA

- Through non-covalent interaction, “base pairs“ (bp)
  - A – T (two hydrogen bonds, ~4 kcal/mol),
  - C – G (three hydrogen bonds, ~6 kcal/mol)

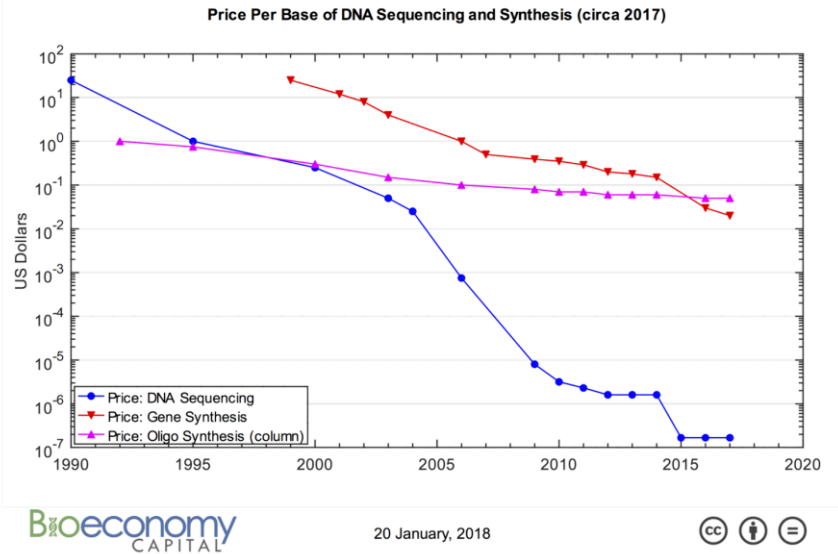
- Essential biological property
  - DNA replication
  - transcription, Translation (genetic code)
  - gene regulation
  - etc.

- Basis for numerous biotechnological techniques and applications
  - PCR
  - gene synthesis, DNA assembly
  - sequencing
  - nanotechnology (e.g. DNA origami)
  - etc.



How to “write and read” DNA?

- DNA synthesis and sequencing
  - essential every-day tools across the entire life sciences
  - chemical tools play key roles in both
  - **knowing how it works is critical for correct use and applications!**
- DNA sequencing
  - “classical” (Sanger method)
  - “next-generation” techniques
- DNA synthesis
  - chemical synthesis of oligonucleotides
  - assembly of larger DNA molecules



Q: Why would you want to synthesize and sequence DNA? Name examples!

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De Novo Synthesis of DNA

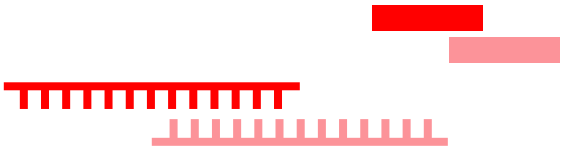
- Oligonucleotides
  - first step of DNA synthesis
  - single stranded!
  - 50 – 100 nt (rarely up to 300 nt)

DNA oligonucleotide (“oligo”)

5′-ACGTACGTTTACTAG-3′

Oligo duplex

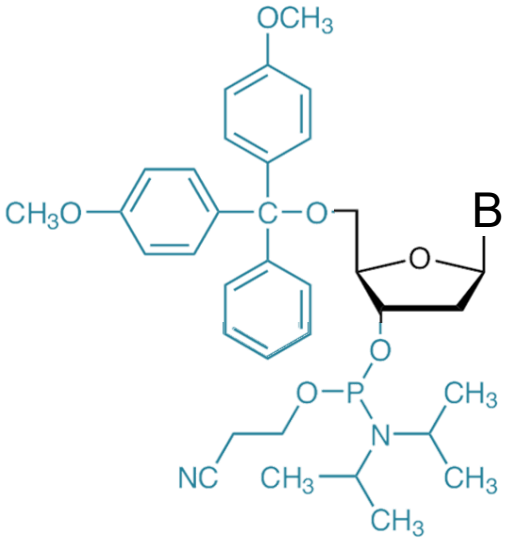
5′-ACGTACGTTTACTAG-3′
||||||||
3′-AAATGATCTTACTAG-**5′**



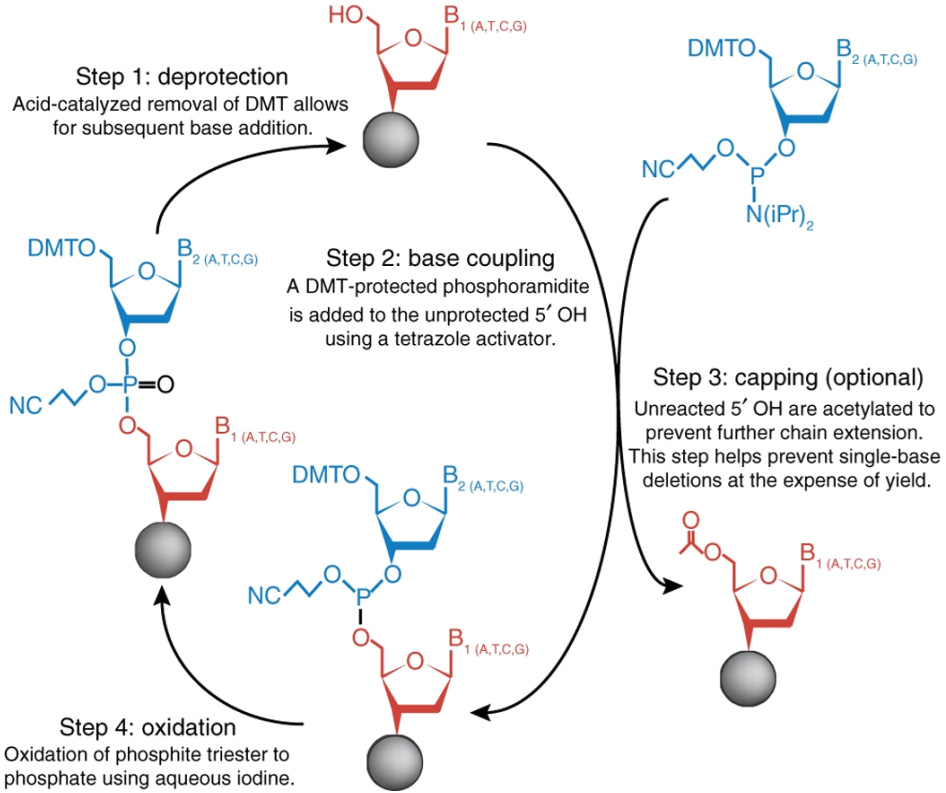
- Chemical synthesis
  - Sequential coupling of single nucleotides
  - On solid support
  - 3‘ → 5‘ direction
  - Chemical protecting groups to avoid multiple couplings

Chemical Oligo Synthesis

dimethoxytrityl (DMT)



phosphoramidite



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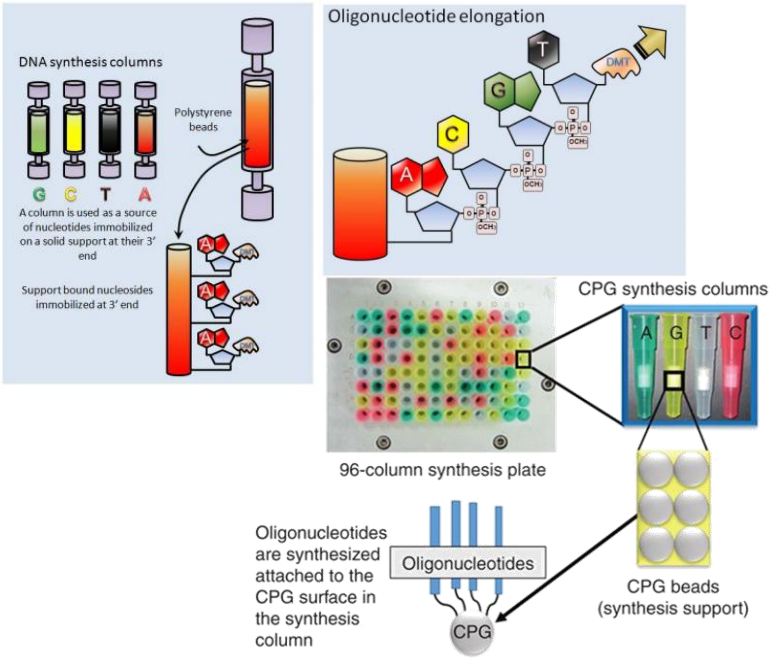
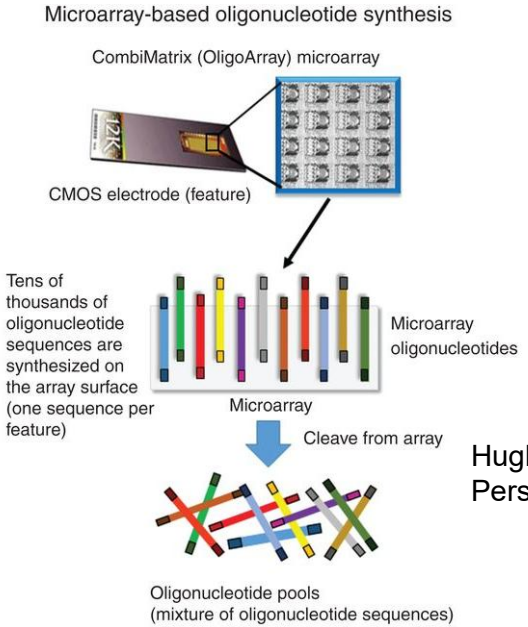
Oligo Synthesis – Low/High Throughput



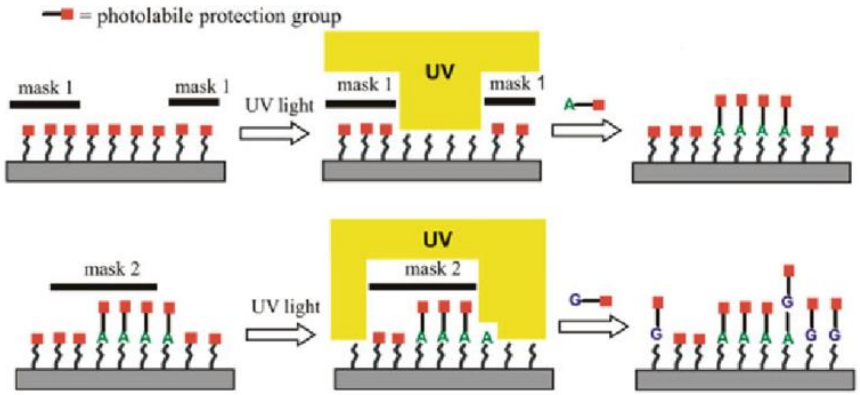


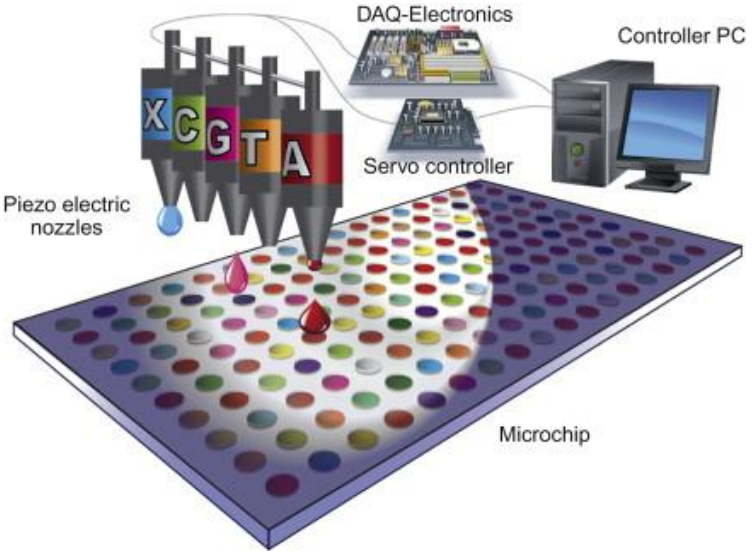
Hughes & Ellington, CSH Perspectives, 2017

- “classical” , column-based
  - approx. 1-20 US-$ per oligo
  - one sequence per column

- high-throughput , microarray-based
  - < 0.05 US-$ per oligo
  - sequence pools/“libraries“ (max. 250,000 per chip)
  - lower quantities
  - e.g. photolithography or ink-jet technology

Microarrays - Photolithography & Ink-Jet Technology



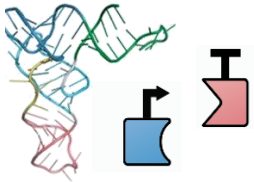


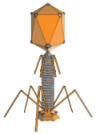
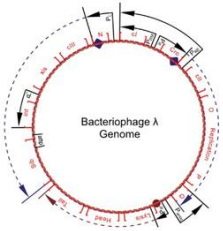
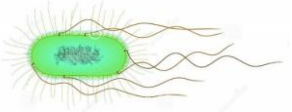
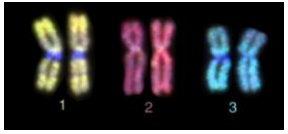
Synthesis Errors

- Deletions, depurination (A/G) → shortened product, cleavage
- Error rates: approx. 0.5-2% per nucleotide position
- Higher error rates for microarray synthesis
- Purification possible (electrophoresis, chromatography; expensive!)

Q: How high is the yield of full-length product for a 100 nt oligo at 1.5% per-position error?

Typical Size of Genetic “Parts”



Genetic «Part»	Approximate size range
promotors, terminators, tRNAs	50 – 200 bp ***de novo* synthesis**
genes, gene fragments 	0.2 – 3.0 kbp
operons, genetic circuits 	5 – 20 kbp
gene clusters, phage genomes, BACs  	20 – 500 kbp
yeast chromosomes, small bacterial genomes 	500 – 2000 kbp
common bacterial genomes (*E. coli* !) 	2.0 – 5.0 Mbp
human chromosomes 	50 – 250 Mbp

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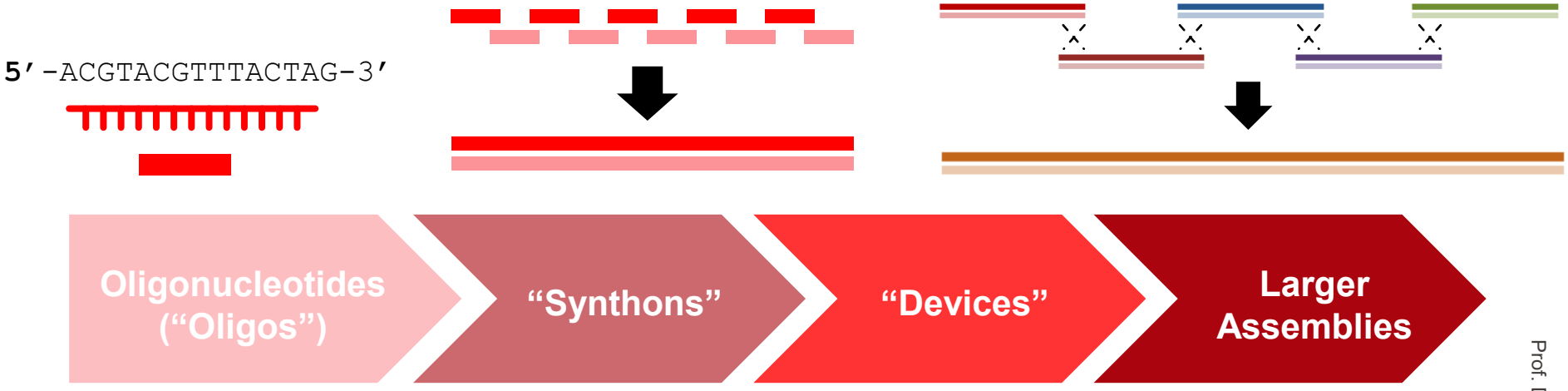
DNA Synthesis – Overview



chemical synthesis (*de novo*)

PCR-based

enzymatic DNA assembly → *in vitro* assembly → *in vivo* assembly

50 – 100 nt

500 – 5000 bp

10 – 200 kb

up to 1 Mbp
(few Mbps with tricks)

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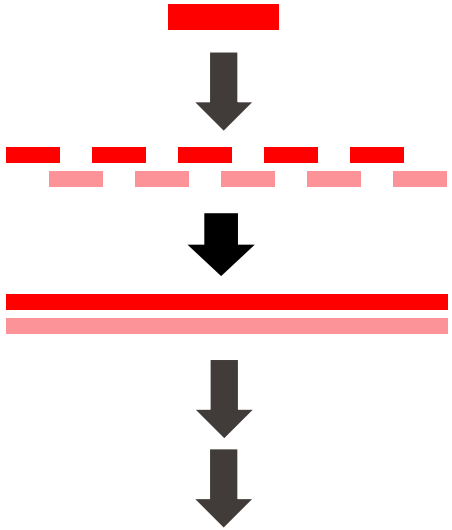
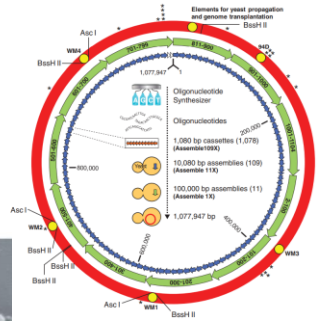
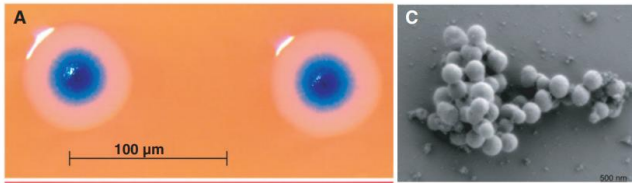
DNA Assembly

- Synthetic procedures to obtain pieces of DNA > 200 bp
- Step-wise joining of smaller pieces
- Verification by sequencing!
  - errors from *de novo* synthesis
  - assembly errors
- Modular process (construction of DNA libraries possible)
- General trend towards high throughput
  - parallelization, miniaturisation
  - total length, number of pieces ↑
  - price per assembly ↓







Pushing the limits…

Generating a synthetic genome by whole genome assembly: ϕX174 bacteriophage from synthetic oligonucleotides

Hamilton O. Smith, Clyde A. Hutchison III†, Cynthia Pfannkoch, and J. Craig Venter‡

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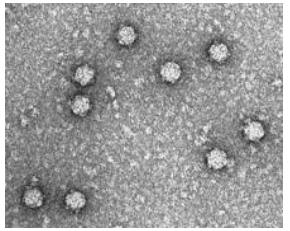

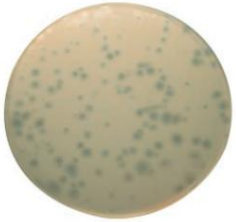

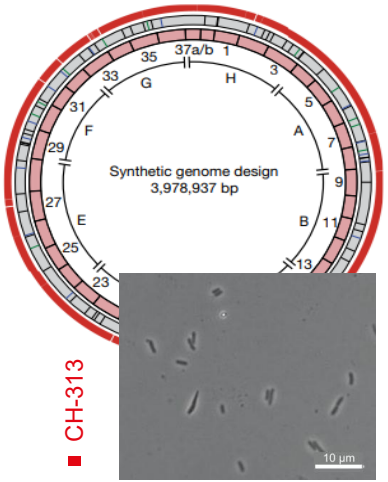

nature

Total synthesis of *Escherichia coli* with a recoded genome

Julius Fredens[1,4], Kaihang Wang[1,2,4], Daniel de la Torre[1,4], Louise F. H. Funke[1,4], Wesley E. Robertson[1,4], Yonka Christova[1], Tiongsun Chia[1], Wolfgang H. Schmied[1], Daniel L. Dunkelmann[1], Václav Beránek[1], Chayasith Uttamapinant[1,3], Andres Gonzalez Llamazares[1], Thomas S. Elliott[1] & Jason W. Chin[1]*

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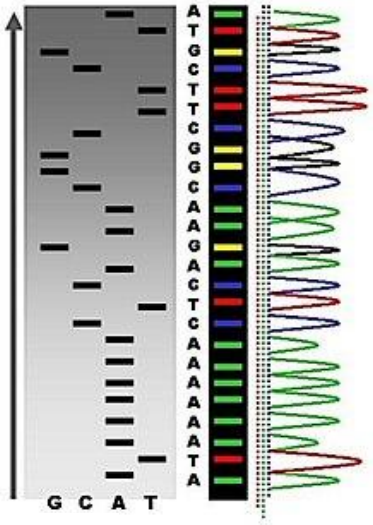
DNA Sequencing – Overview

- Analytical process to determine the sequence of nucleotides (nucleobases) in a DNA molecule

- Key technology in molecular biology/biotech (“era of genomics“)
  - early methods: 1970s
  - wider availability: 1980s/1990s
  - “next-generation“ sequencing: late 1990s/early 2000s, ongoing!

- Selected applications
  - molecular biology
  - evolutionary biology
  - epidemiology, virology
  - quality control (DNA synthesis, cloning etc.)
  - diagnostics, forensics
  - etc. etc.



Second generation sequencer: 454 GS20

Prof. Dr. Markus Jeschek, LSAM

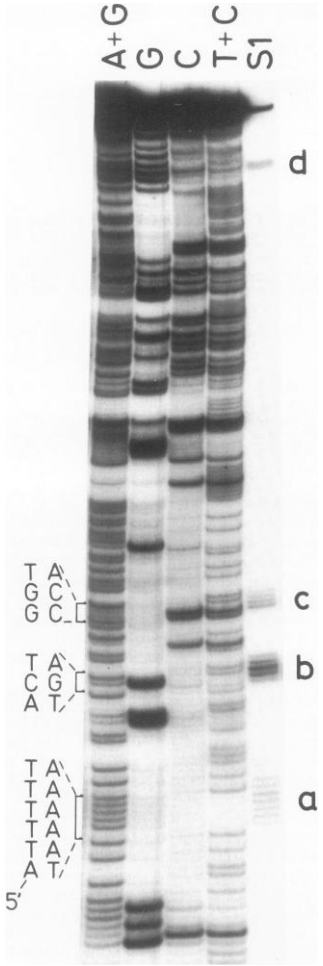
Maxam-Gilbert Sequencing

5′ ^{32}PGCTACGTA 3′

- Allan Maxam & Walter Gilbert (1976–1977)
- Chemical modification of nucleobases → selective cleavage
  - (1) labelling of target DNA on 5‘- or 3‘-end (radioactive, dyes etc.)
  - (2) splitting into four samples and base-specific modification
    - A+G: depurination with formic acid
    - G: methylation with dimethyl sulfate
    - C+T: hydrolysis with hydrazine
    - C: hydrolysis with hydrazine in presence of NaCl
  - (3) cleavage at the modified base with hot piperidine
  - (4) fragments are separated/resolved in acrylamide gel electrophoresis
- Base modifications (step 2) occur stochastically

  → lower efficiency at greater lengths! (max. 200-300 bp)
- No longer in use!

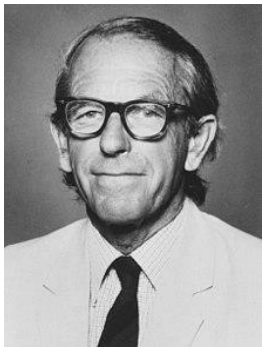
Maxam-Gilbert Sequencing

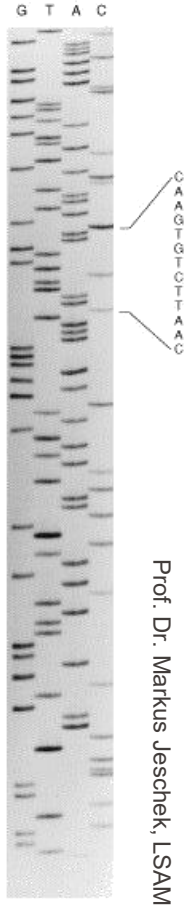


Prof. Dr. Markus Jeschek, LSAM

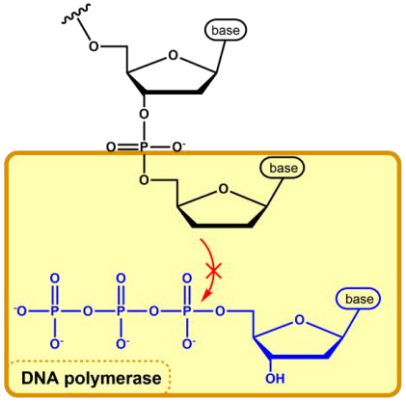
The Gold Standard – Sanger Sequencing

- Chain-termination/dideoxynucleotide method
- Simpler, faster, less hazardous, automatable
- History:
  - developed in 1977 by Frederick Sanger
  - first commercialized in 1986
  - still in use today!
  - standard low-throughput sequencing in “every lab“





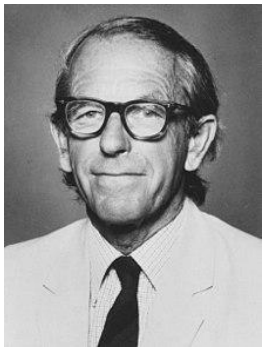
- PCR-based method
- Premature termination of DNA polymerase reaction
- So-called “stop nucleotides”

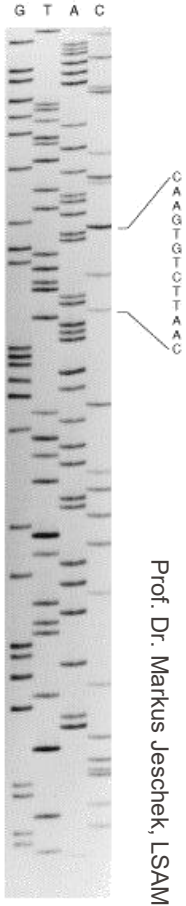


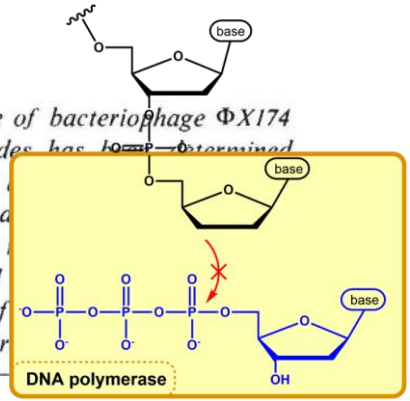
Q: Why does the polymerization stop?

The Gold Standard – Sanger Sequencing

- Classical procedure:
  - (1) splitting of DNA into four samples
  - (2) addition of one di-deoxynucleotide triphosphates (ddNTP) to each sample (together with mix of all four dNTPs)
  - (3) addition of primer and DNA polymerase → elongation and stochastical (!) incorporation of ddNTP → termination
  - (4) separation of fragments in acrylamide gel electrophoresis (visualization through radioactively labelled primer or nucleotides)







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articles

Nucleotide sequence of bacteriophage ΦX174 DNA

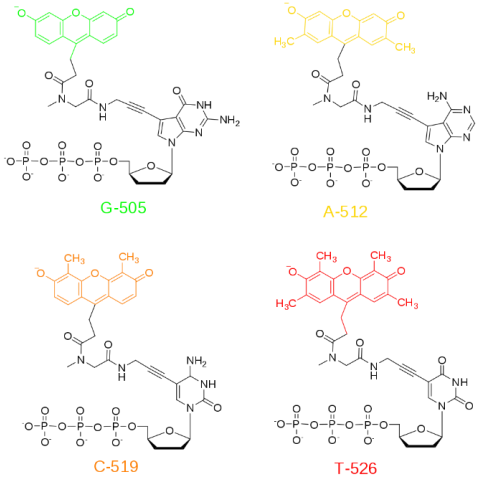
F. Sanger, G. M. Air*, B. G. Barrell, N. L. Brown†, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III‡, P. M. Slocombe§ & M. Smith'

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus sequence identifies many of the fea production of the proteins of the organism, including initiation and proteins and RNAs. Two pairs of same region of DNA using differ

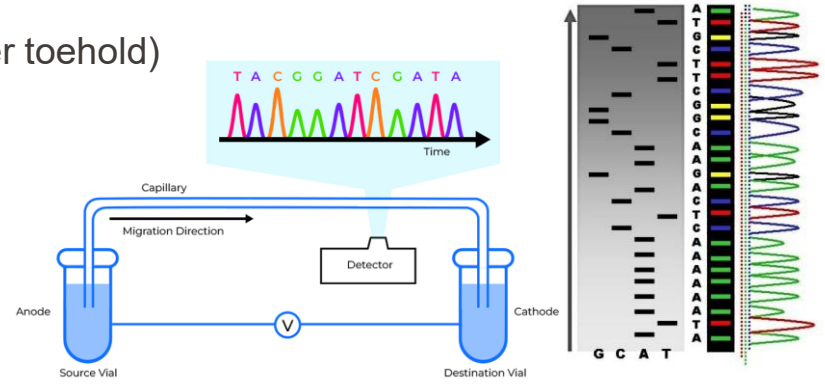
Sanger Sequencing – Pushing the Price

- Fluorescently labelled ddNTPs (“dye-terminator sequencing“)
  - → no radioactive labels/autoradiography
  - → one instead of four reactions
- Capillary electrophoresis
- Automation, miniaturization etc.
- **600 – 1200 bp, < 4 CHF per sample, <24 h result delivery**
- high accuracy (> 99.9% per positions)
- Limitations:
  - weak signal in the beginning (~50 bp from primer toehold)
  - length limit
  - repetitive sequences



Q: What could be reasons for the length limitation?

How can one sequence fragments larger than ~ 1kbp?



The Human Genome Project

- 1990-2003
- consortium involving USA (coordinating), UK, Japan, France, Germany, China
- **3 billion US-$ and 20 universities** (NIH funded)
- Based on Sanger sequencing and primer walking
- “End point” in 2003: ~85% of the genome

(i.e. ~2.7 of 3.2 Mbp of the haploid genome)

- In parallel: privately funded project, Craig Venter (shotgun sequencing!)
- Jan 2022: final gapless assembly
- **Today: <1000 US-$ per human genome (NGS)**