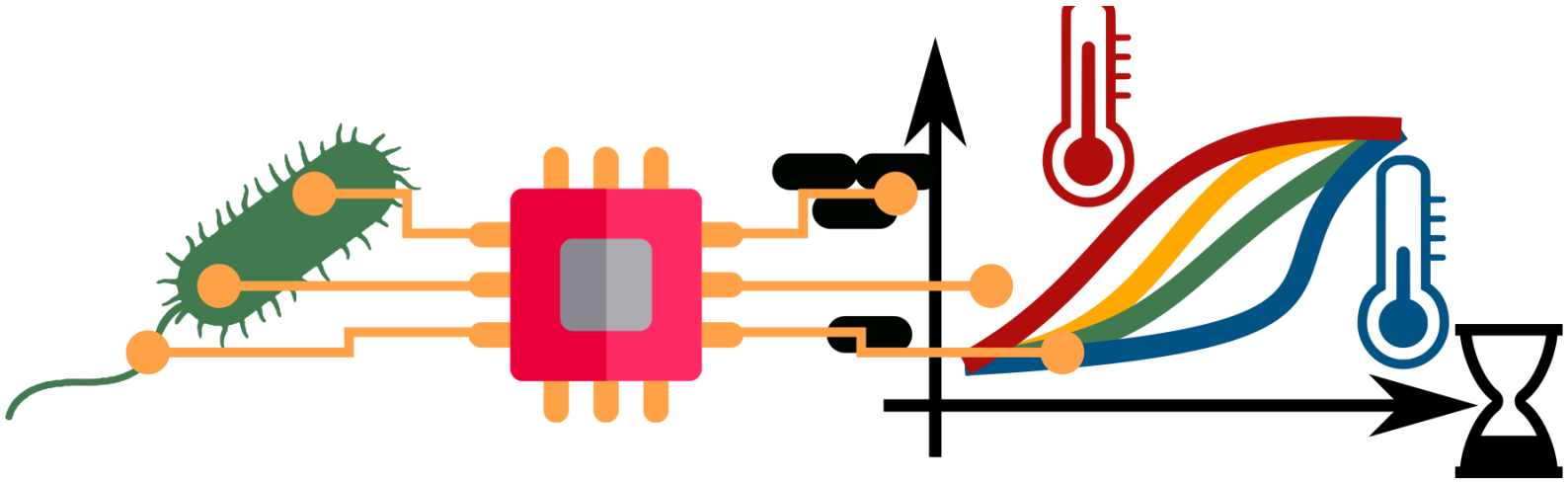
Recombinant Expression Simulation: Theoretical Background

- The BioLabSim.nrw consortium



Outline

1. Theory
    - Microbial Growth
        - » Growth Phenotypes
        - » Mathematical Models
        - » High-Throughput Measurements
        - » Biotechnological Relevance
    - Bacterial Gene Expression
        - » Expression factors
        - » Contribution to expression strength
        - » Expression Strength Prediction
2. Simulation
    - Life presentation of the simulation

Teaching Objectives

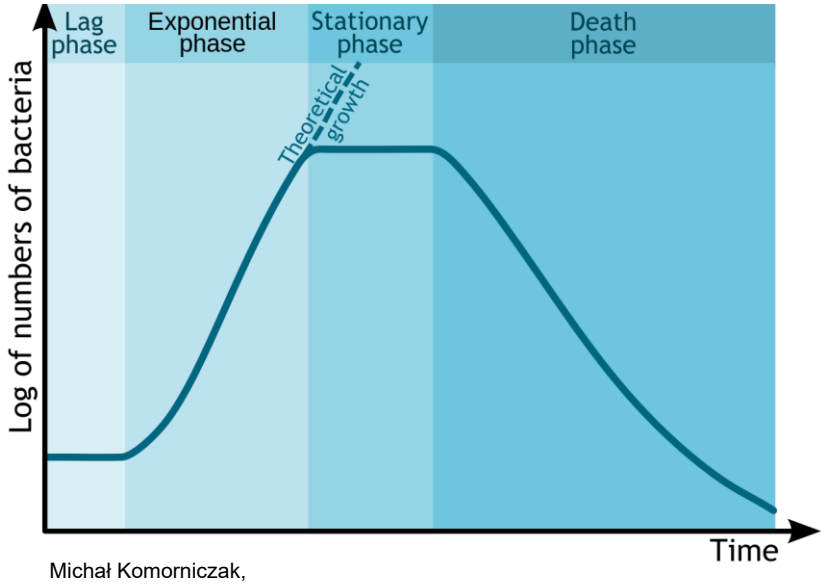
1. Distinguish a suitable growth law for data analysis.
   - Knowledge of growth types
   - Knowledge of equations and their variables

2. Identify factors of gene expression on a DNA sequence.
   - Knowledge of expression factors

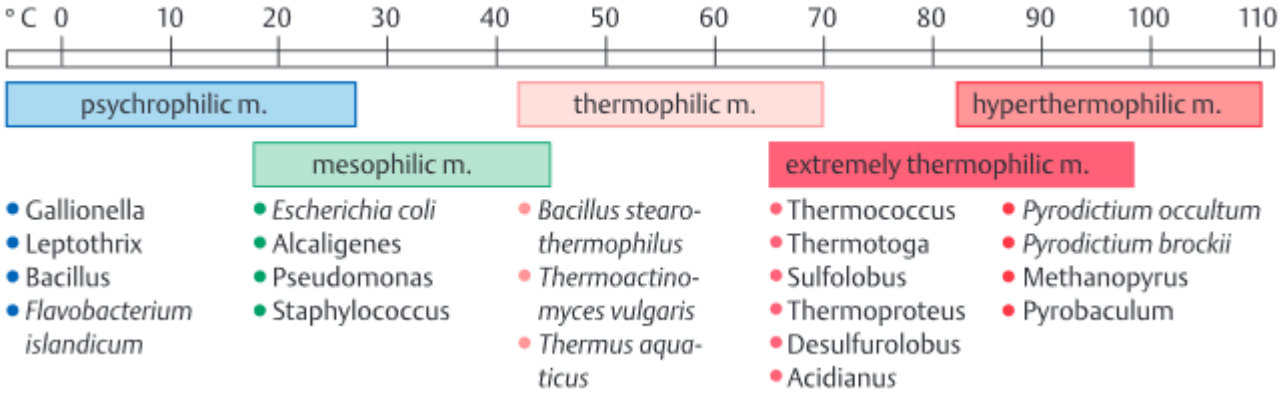
3. Judge whether a phenomenon is better described by a mechanistic or statistic model.
   - Knowledge of modelling principles

Microbial Growth Phenotypes

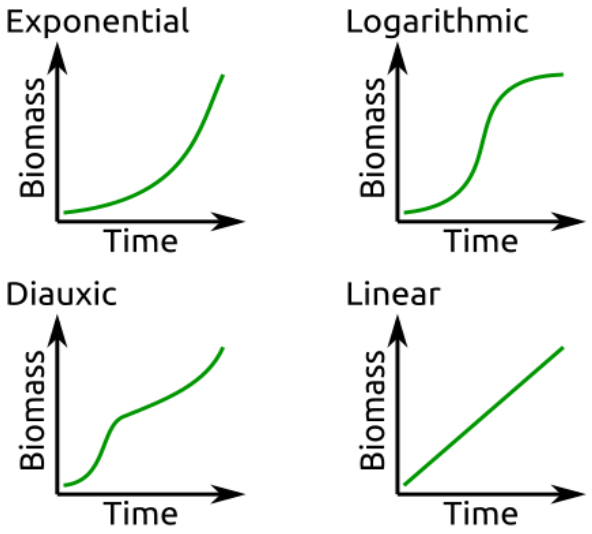
- Environmental conditions determine growth rate:
  - ➢ Temperature, Substrate, pH, etc.
- Growth is separated in different phases
- In non-optimal conditions and environmental changes microorganisms adapt their growth.



Michał Komorniczak, https://en.wikipedia.org/wiki/Bacterial_growth#/media/File:Bacterial_growth_en.svg

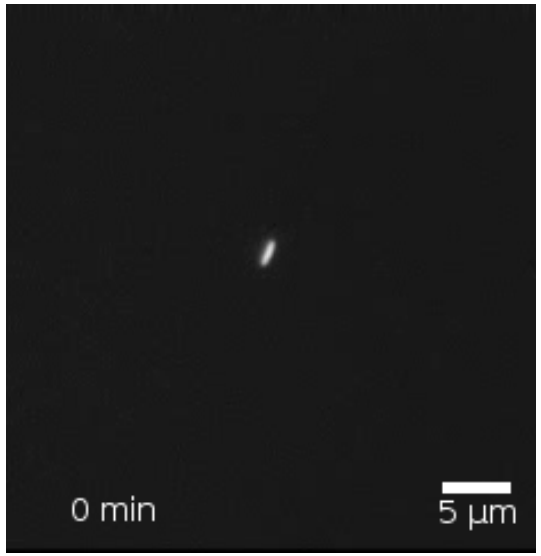


Schmid, Schmidt-Dannert, Hammelehle (2014) Biotechnology, An illustrated primer, ISBN-10: 9783527335152



Growth Models: The Exponential Function

- In optimal conditions microorganisms growth exponentially
- Many processes initially follow exponential dynamics:
  - Covid19 spread, Computing power, etc.
- Growth rate remains constant.



Stewart EJ, Madden R, Paul G, Taddei F (2005) (https://commons.wikimedia.org/wiki/File:E.coli-colony-growth.gif), https://creativecommons.org/licenses/by-sa/4.0/legalcode

Mechanistic Growth Model:

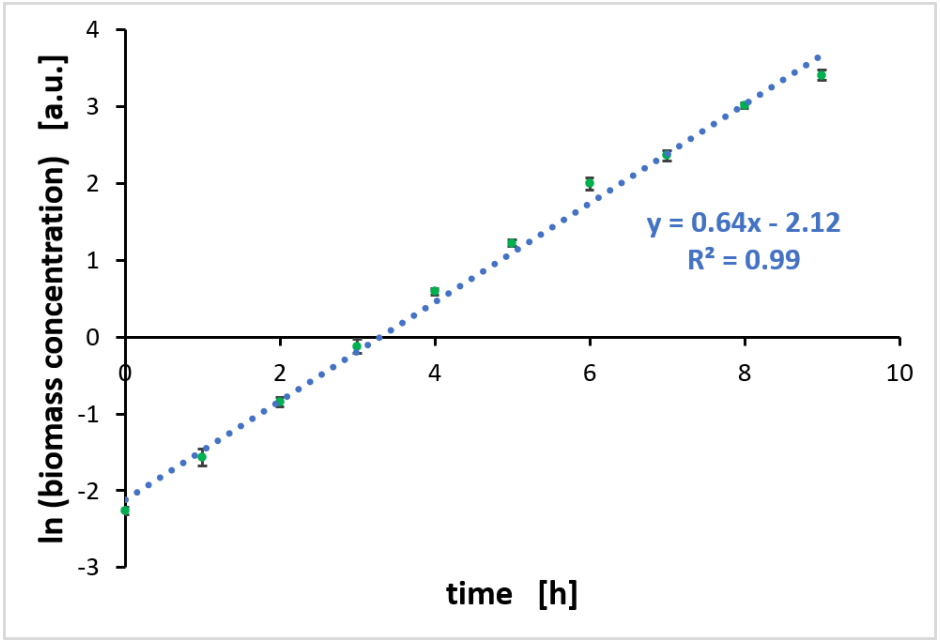
$$X \xrightarrow{\mu} 2X$$

X: Biomass, gDW/L
μ: growth rate, /h
t: time, h

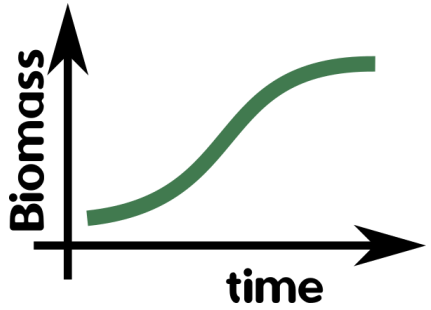
$$\frac{dX}{dt} = \mu X$$

$$X(t) = X_0 e^{\mu t}$$

$$\ln(X(t)) = \ln(X_0) + \mu(t - t_0)$$



Growth Models: The Logistic Function



- Environmental capacity limits cause transition from exponential to logistic growth.
- Capacity limits: substrates, space, waste accumulation, etc.
- Growth rate remains constant

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{C}\right)$$

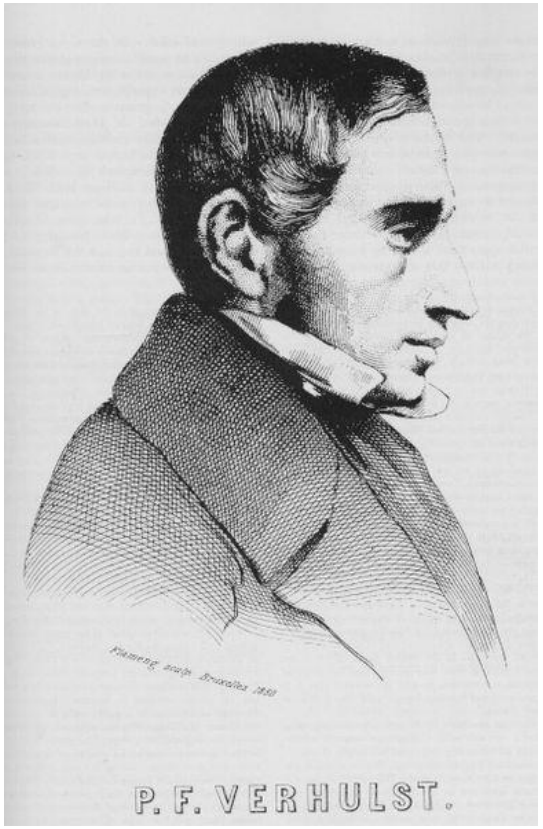
X: Biomass, gDW/L

μ: growth rate, /h

C: capacity limit, gDW/L

t: time, h

$$X(t) = \frac{C}{1 + \left(\frac{C - X_0}{X_0}\right)e^{-\mu t}}$$



source: Pierre Francois Verhulst.jpg, https://commons.wikimedia.org/w/index.php?title=File:Pierre_Francois_Verhulst.jpg&oldid=141824277

- Growth rate is estimated with parameter search to minimize error.

Growth Models: The Monod Equation

- Growth rate is variable and depends on environment, e.g. substrate level, temperature.
- Monod assumed that growth is substrate limited by enzymatic actions.
- Similar to Michaelis-Menten equation.

$$X + S \xrightarrow{\mu} 2X$$

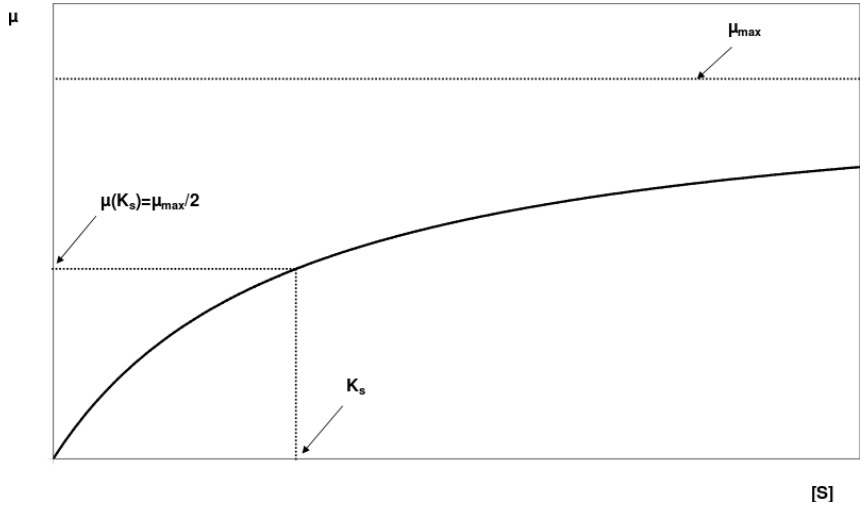
$$\mu = \frac{\mu_{max} S}{K_S + S}$$

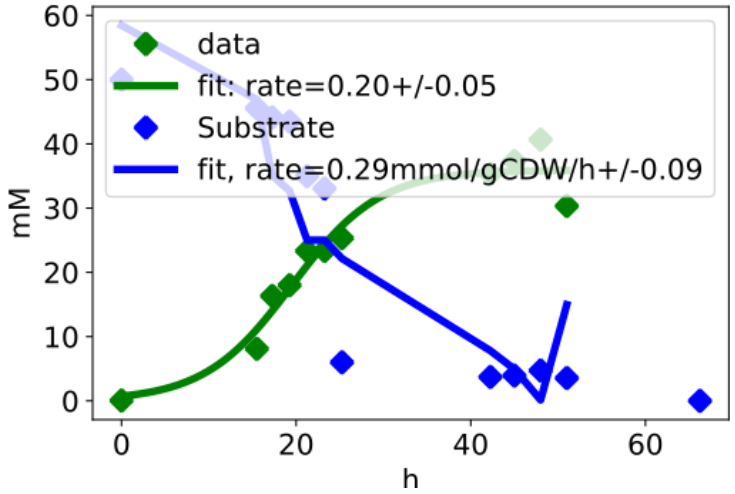
μ: growth rate, /h

μ_{max}: max rate, /h

S: substrate, mM

K_S: substrate affinity, mM



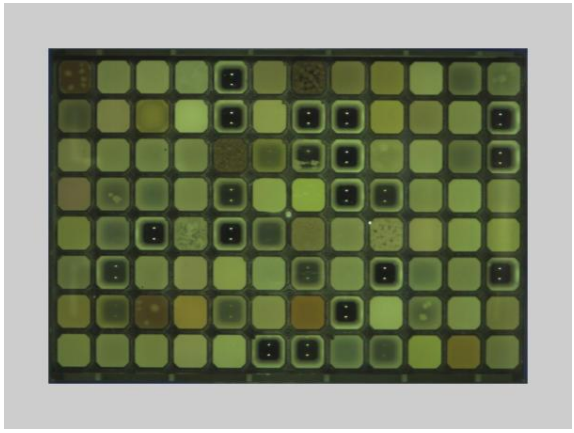


- Substrate measurements required for parameter estimation.

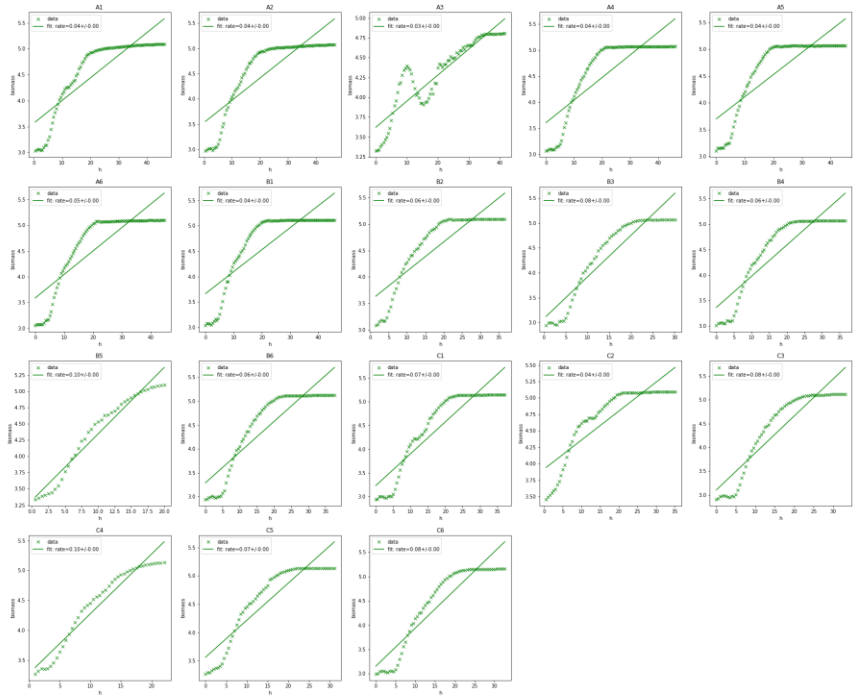
File:Monod 3.svg, https://commons.wikimedia.org/w/index.php?title=File:Monod_3.svg&oldid=427056782 (last visited April 20, 2022).

High-Throughput Measurements

- Organisms with high growth rates need to be selected based on mutations and medium.
- 96-well plate measurement of growth by Enzyscreen GrowthProfiler

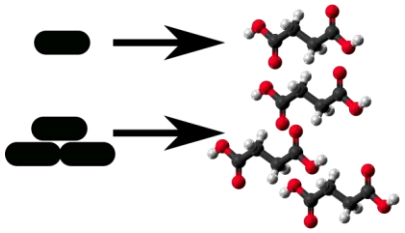


- Data export as csv-files
- Automated analysis in Jupyter Notebooks

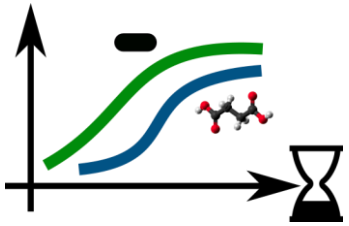


Source: https://www.enzyscreen.com/growth_profiler.htm

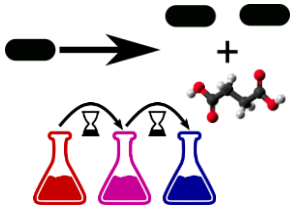
Biotechnological Relevance



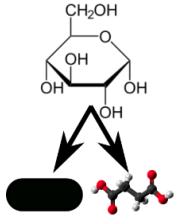
High cell densities lead to high productivity.



Target products produced in different growth phases.



Growth-coupled production enables optimization with ALE.



Efficiency tradeoff by substrate use in biomass and product.

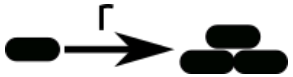
Optimal growth requires energy for heating/cooling.

Biomass waste disposal strategy for GMOs.

Growth Curve Data Analysis Example

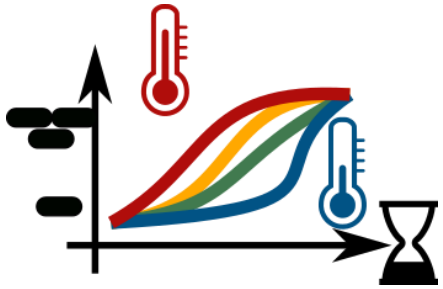
Objective

Optimal Temperature



Growth Rate & Max Biomass

Procedure



Analysis

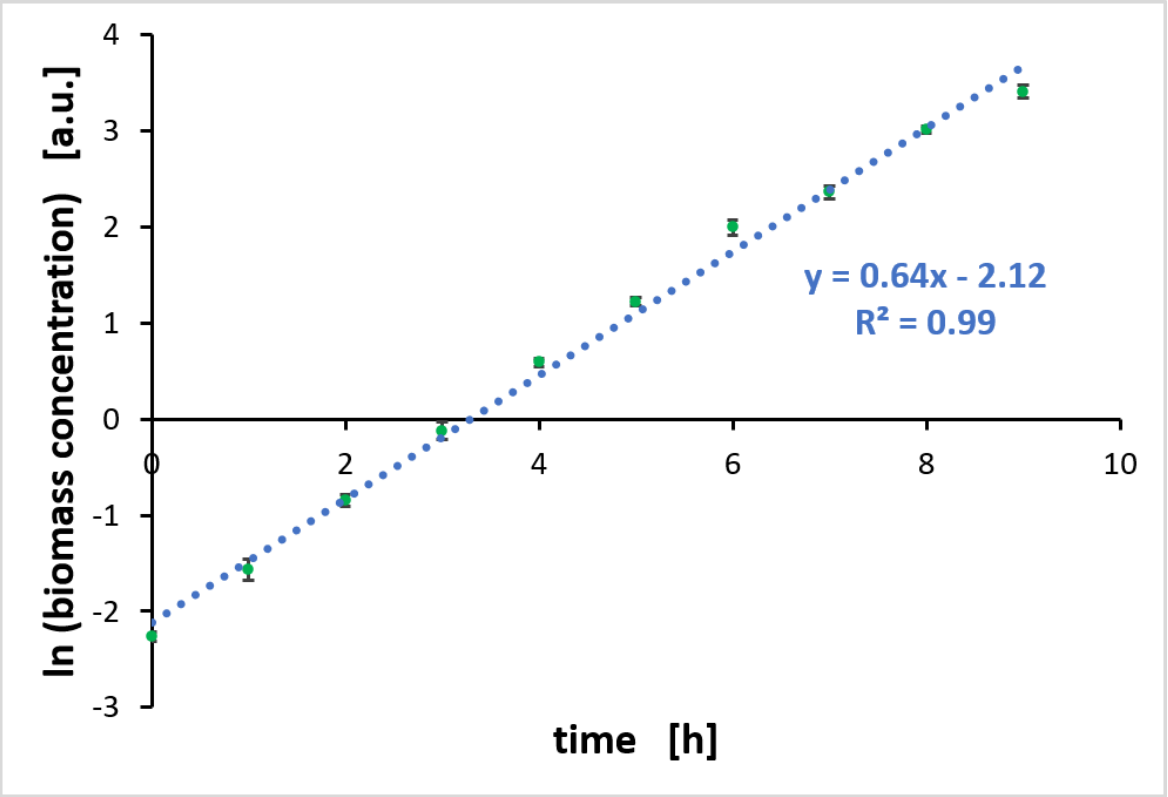
- Scrambled code-lines provided
- User rearranges line order for functional code

- Standard for quick laboratory data analysis.
- Users implement Excel linear regression.

Excel-basee Growth Rate Calculation

1. Determine mean value and standard deviation (only necessary for biological replicates)
2. Plot the Logarithm of biomass concentrations against time
3. Perform a linear regression with the linear range

→ The slope of the regression line is equal to the growth rate



Example cultivation from BioLabSim at 35 °C.
Shown is the mean value and the standard deviation of three biological replicates.

Python-based Growth Rate Calculation

1. Rearrange code lines

```
# Rearrange the correct code sequence for plotting the growth curves with the logarithm of biomass.
Time, Biomass = my_data[:,0], my_data[:,1:]
DataFile = 'Strain_characterization_1.csv'
LnBiomass = np.log(Biomass)
plt.scatter(Time, X, label=Exp) for Exp,X in enumerate(LnBiomass.T)]
plt.legend(bbox_to_anchor=(1.05, 1), loc='upper left')
my_data = np.genfromtxt(DataFile, delimiter=',', skip_header=1)
```

2. Extract the highest growth slope for ln(biomass)
3. Extract the range of linear slope for ln(biomass)
4. Rearrange code lines for linear regression and max biomass extraction

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    - **Bacterial Gene Expression**
        - » Expression factors
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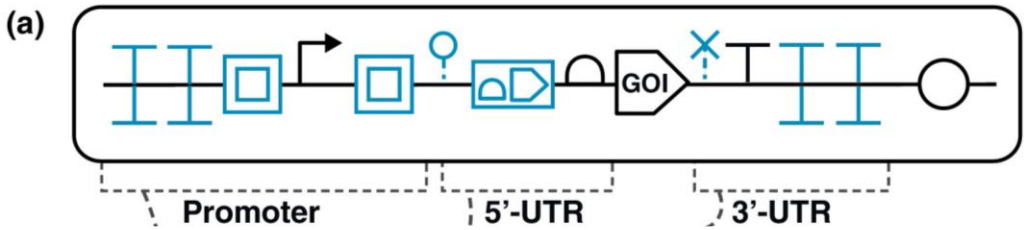
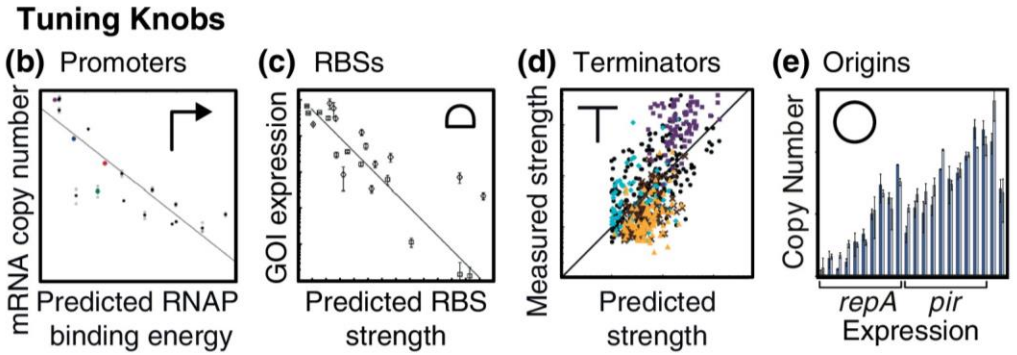
Bacterial Gene Expression



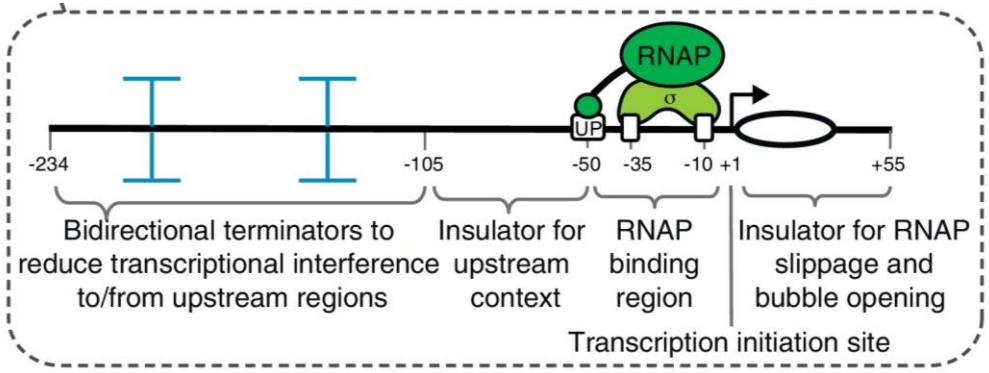
- Three levels of gene expression regulation:
  - Transcription initiation by the promoter
  - Translation initiation by the 5' UTR
  - mRNA stability by the 3' UTR

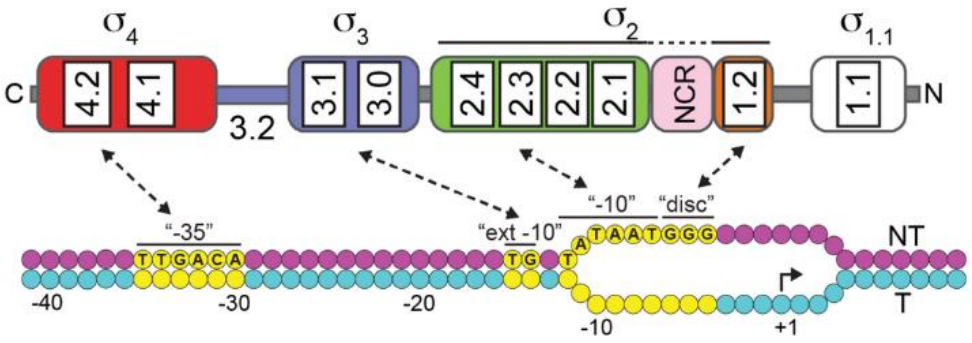


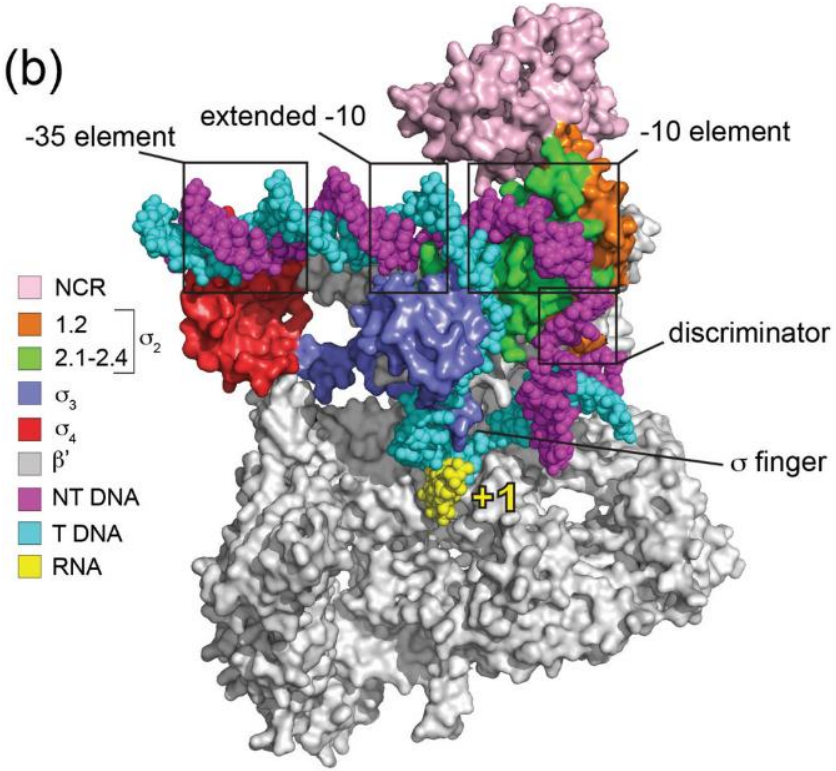
Nielsen, A., Voigt, C. (2013).
Advances in genetic circuit design: Novel biochemistries, deep part mining, and precision gene expression.
Current Opinion in Chemical Biology, *17*(6), 878–892. doi: 10.1016/j.cbpa.2013.10.003

Sigma Factors and Sequence Boxes

- The RNA-polymerase function is extending a nucleotide sequence based on homology.
- A σ-factor guides the RNA-polymerase to the promoter of a gene.
- Different σ-factors have different recognition sequences and expression efficiencies.
- A useful biotechnical used σ–factor in *E. coli* and *P. putida* is σ70.
- Important σ70 recognition sites are the boxes:
  - -35: TTGACA
  - -10: TATAAT

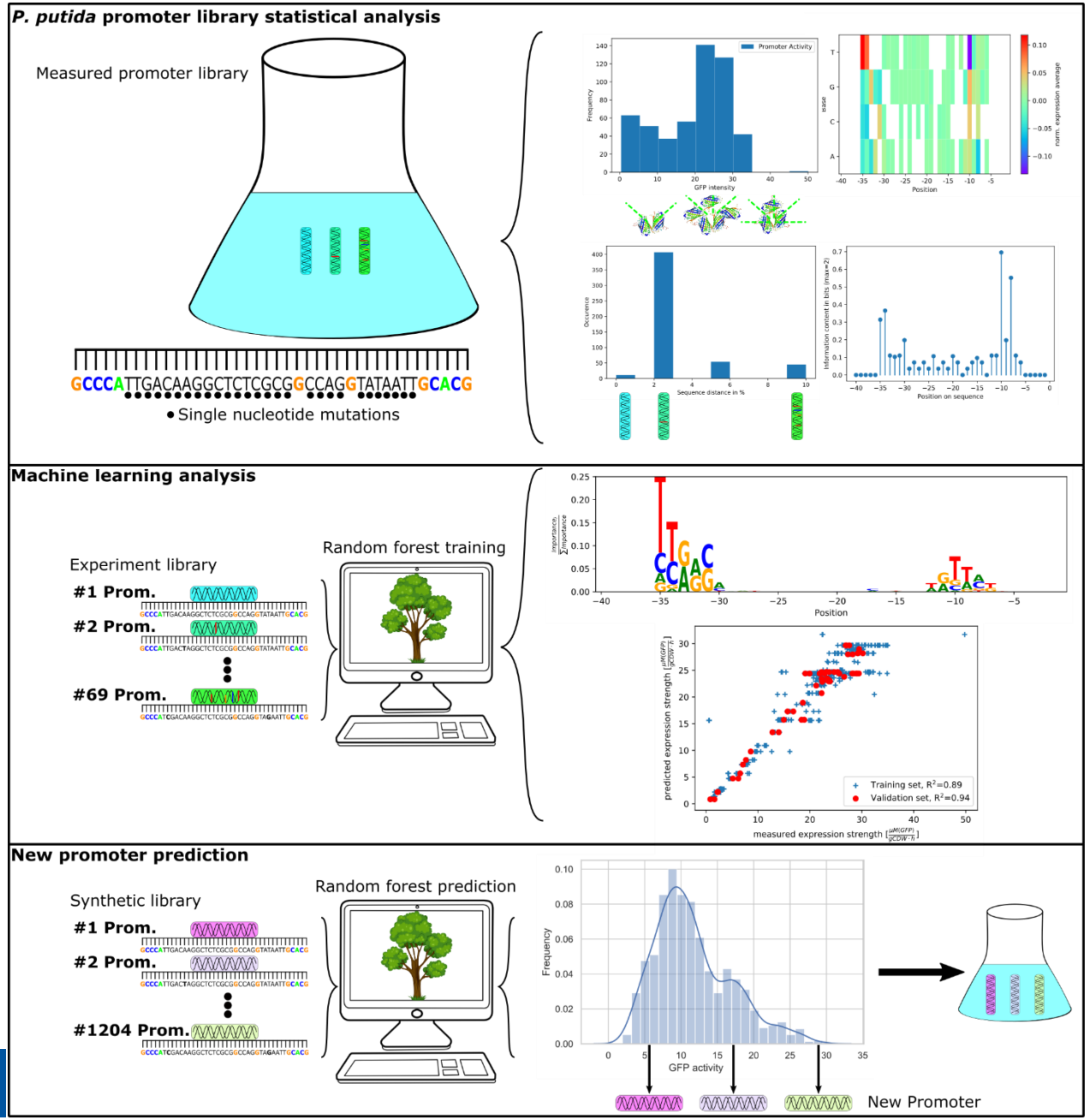






M.S. Paget (2015). Bacterial Sigma Factors and Anti-Sigma Factors: Structure, Function and Distribution *Biomolecules*, 5(3), 1245–65. doi: 10.3390/biom5031245

The Random Forest – Applications

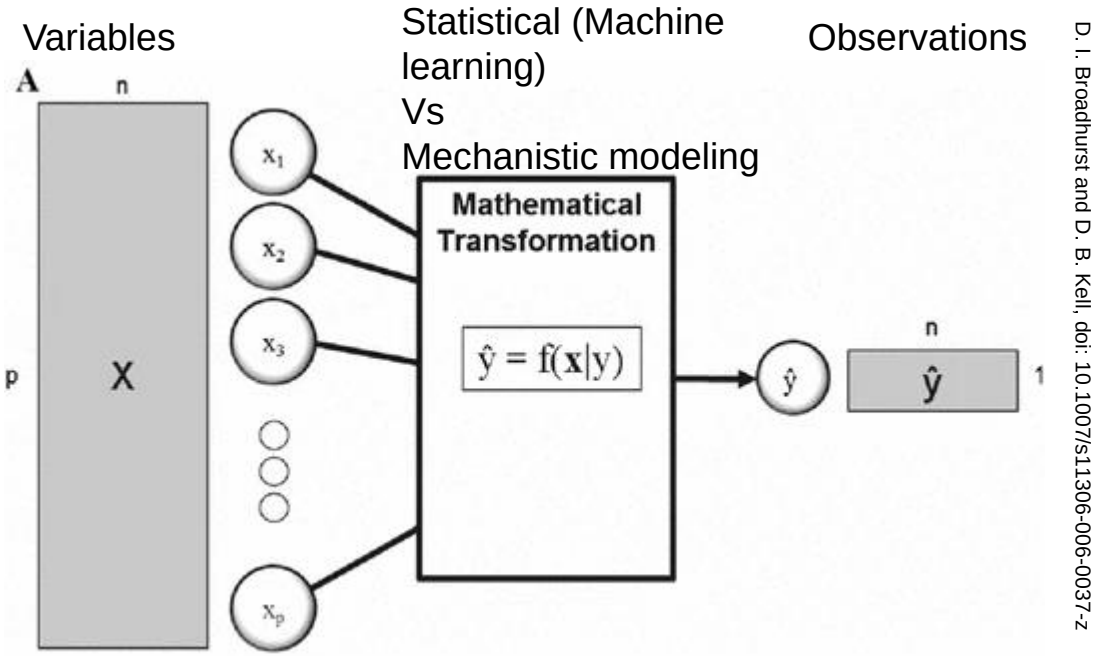


Promoter Library Analysis

1. Promoter library with mutations in promoter sequence and GFP activity.
2. RF-Learning with sequence as binary input, and additional variables, e.g., GC-content.
3. Output is GFP activity.
4. Prediction quality control with R^2 and RMSE
5. Variable importance: nucleotide contribution to the prediction.
6. Identification of novel strong promoters.

U. Liebal et al. (2021) Insight to Gene Expression From Promoter Libraries With the Machine Learning Workflow Exp2Ipynb. *Front. Bioinform.*, doi: 10.3389/fbinf.2021.747428

Principles of Regression



D. I. Broadhurst and D. B. Kell, doi: 10.1007/s11306-006-0037-z

Regression:

- **Prediction** of continuous observations with variables.

Mathematical transformation:

- **Statistical modeling** with generic formula (machine learning, AI).
- **Mechanistic modeling** with biological interactions and laws.

Examples

Statistical Model

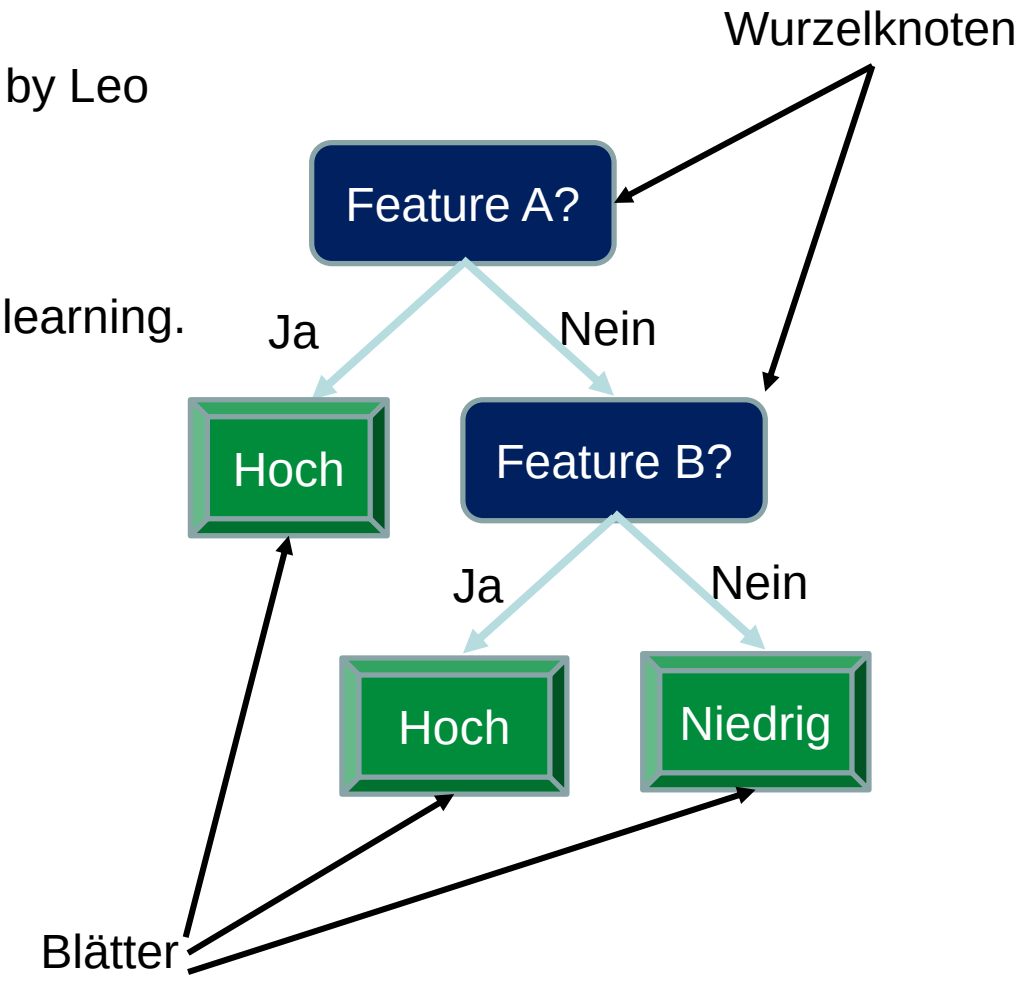
- X: Sequence Library with mutations in promoter region
- Y: Expression rate, measured with GFP
- Regression model: Random Forest

Mechanistic Modeling

- X: time of culture growth
- Y: Biomass
- Regression model: Logistic Function

Promoter Activity Prediction with Random Forest

- Automated method for classification and regression.
- Most famous as ‘CART: Classification And Regression Tree’ by Leo Breiman, 1984.
- Visually ordered overview to formal rules.
- Binary rules at roots lead to leave-outcomes.
- A decision tree can be generated automatically via machine learning.



Construction of a decision tree

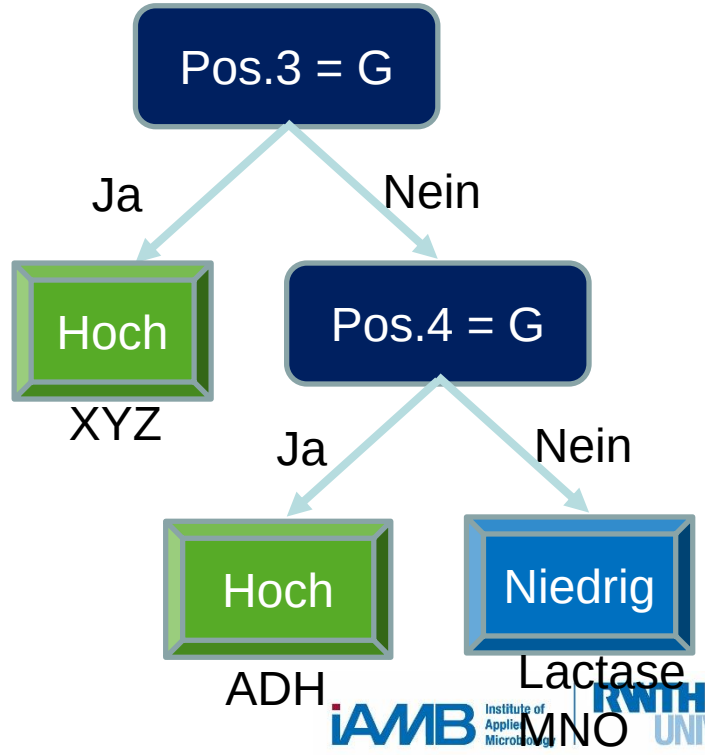
- Step 1: determine starting root

Effective separation at position 3.
If: Pos3 = ‘G’ then expression *High*.
Else: different level of expression → no clear decisions

- Step 2: data separation

If: Pos.4 = ’G’ then expression *High.*
Else: expression *Low*.

Name	Position 1	2	3	4	Expr. Level
Lactase	A	G	T	A	Niedrig
ADH	A	C	T	G	Hoch
XYZ	A	T	G	C	Hoch
MNO	A	G	T	A	Niedrig



The expression of all sequences can be determined following the roots to the leafes.

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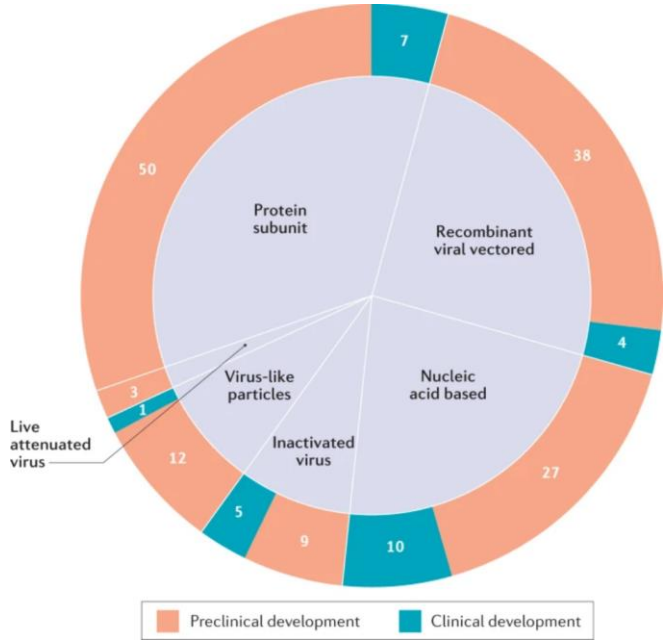
Simulation of Recombinant Expression

Develop an expression system for recombinant production of COVID19 protein subunits for vaccination.

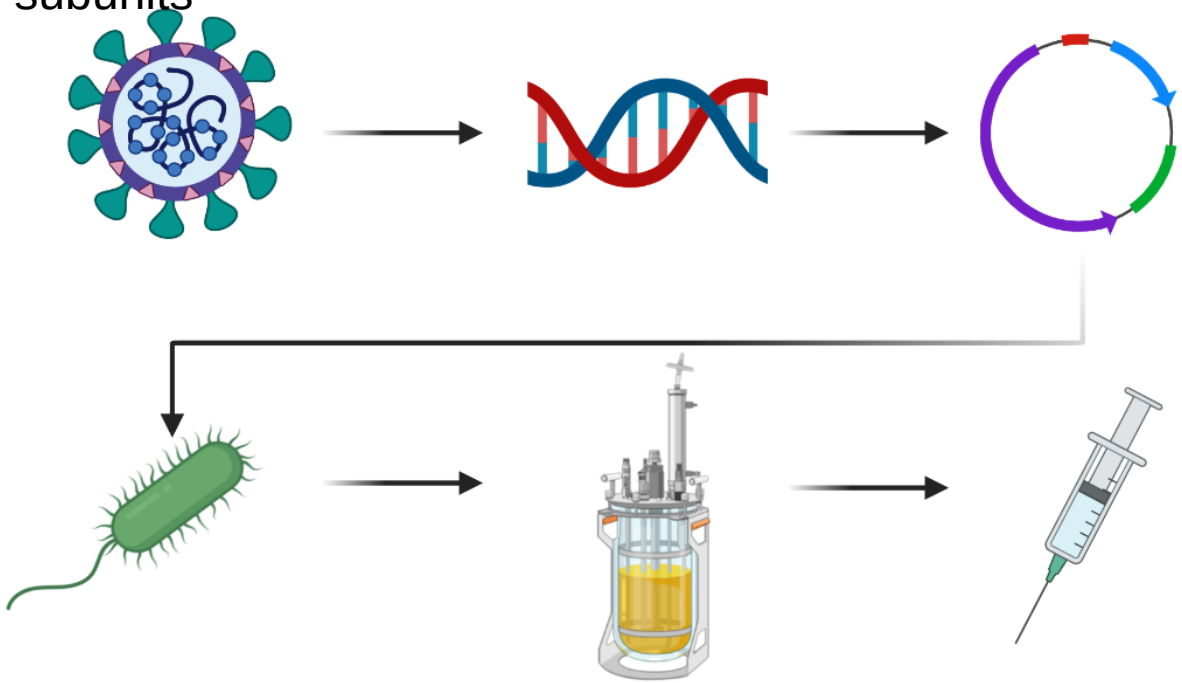


Source: Jeyanathan et al., 2020, https://doi.org/10.1038/s41577-020-00434-6

The majority of vaccine (candidates) is based on recombinant expression of protein subunits.

Your tasks:

1. Characterize two strains for their growth properties
2. Generate promoter sequences
3. Clone promoter sequences into strain
4. Measure expression rate
5. Conduct batch fermentation to harvest protein subunits



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Check the BioLabSim.nrw members at:

https://git.rwth-aachen.de/ulf.liebal/biolabsim/-/blob/91dd40b2f40e6a24263af96c2d5b9817733f4a25/AUTHORS.md