

Week 1 – Introduction to protein and cell-free expression

Purpose of the laboratory course

- Introduce engineers to basic concepts in biology, biological engineering, biotechnology, and synthetic biology
- Provide hands on laboratory experience to acquire the necessary skills to conduct biological experiments

Course Content

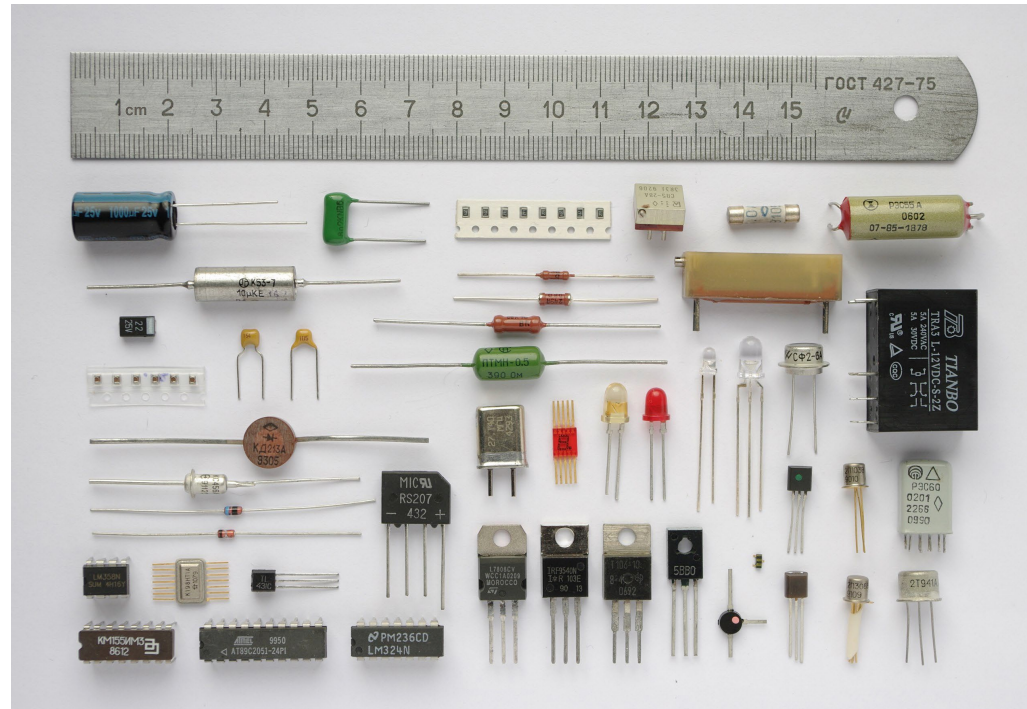
We will cover these main topics:

- Cell culturing techniques
- Molecular Cloning
- PCR
- Protein expression
- Protein purification
- Cell-free Protein expression (cell-free synthetic biology)

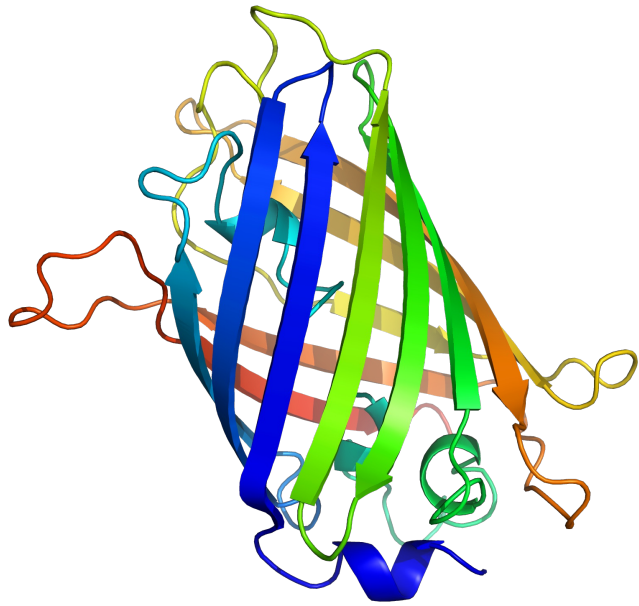
What is protein?

What is protein?

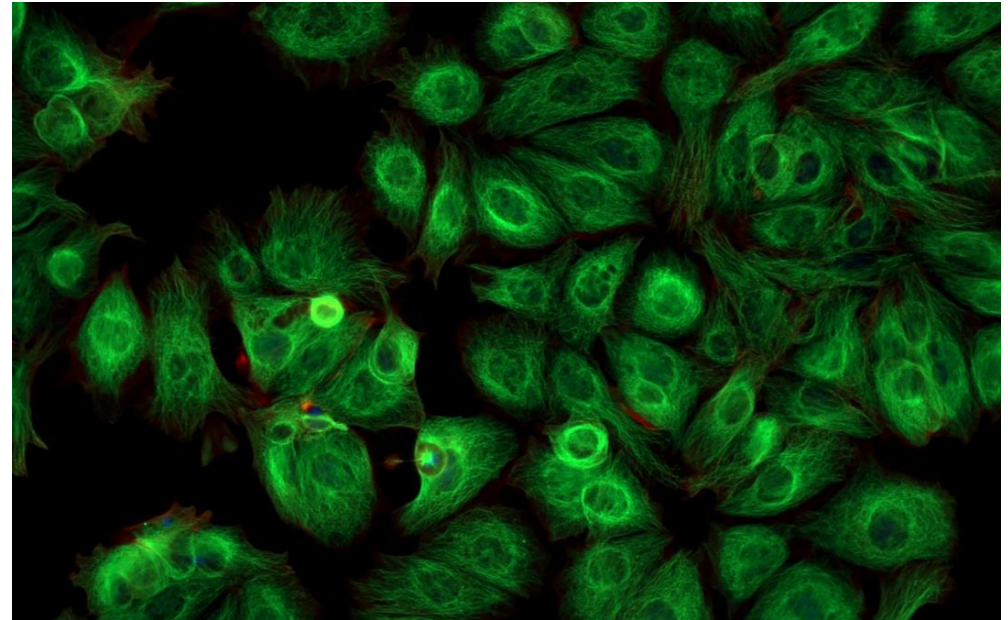
- Polymer made of amino acids.
- Basic functional unit in biological system.

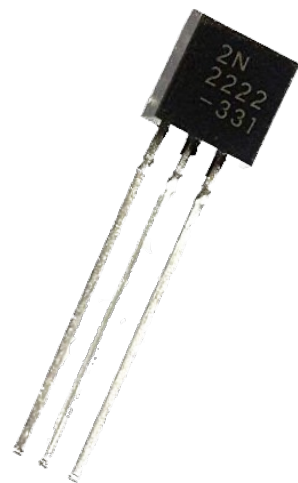


Different proteins

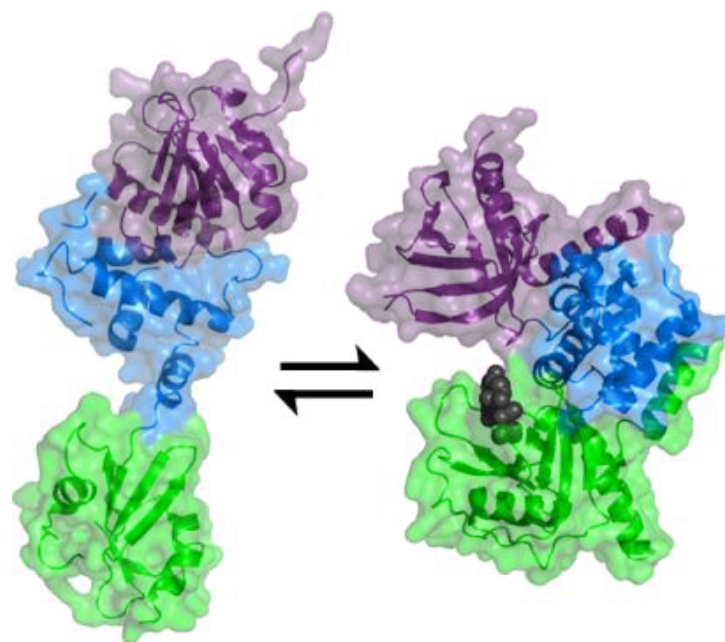


Green fluorescent protein (GFP)

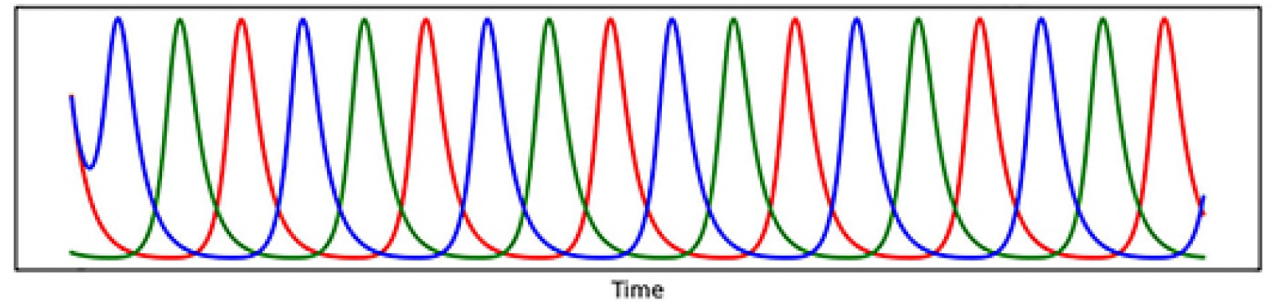
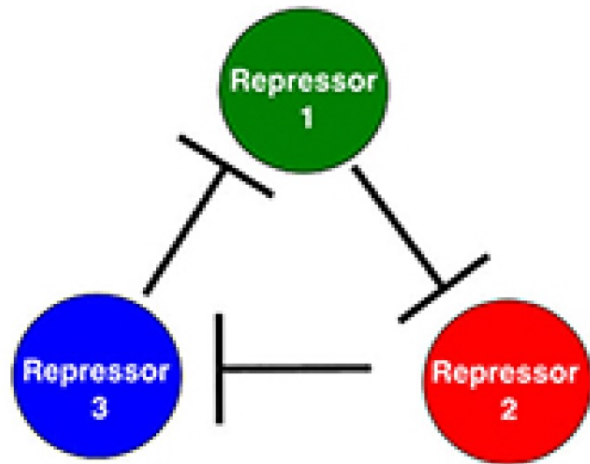
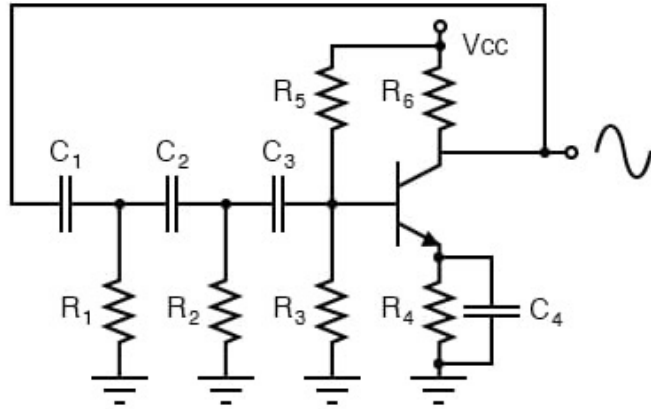


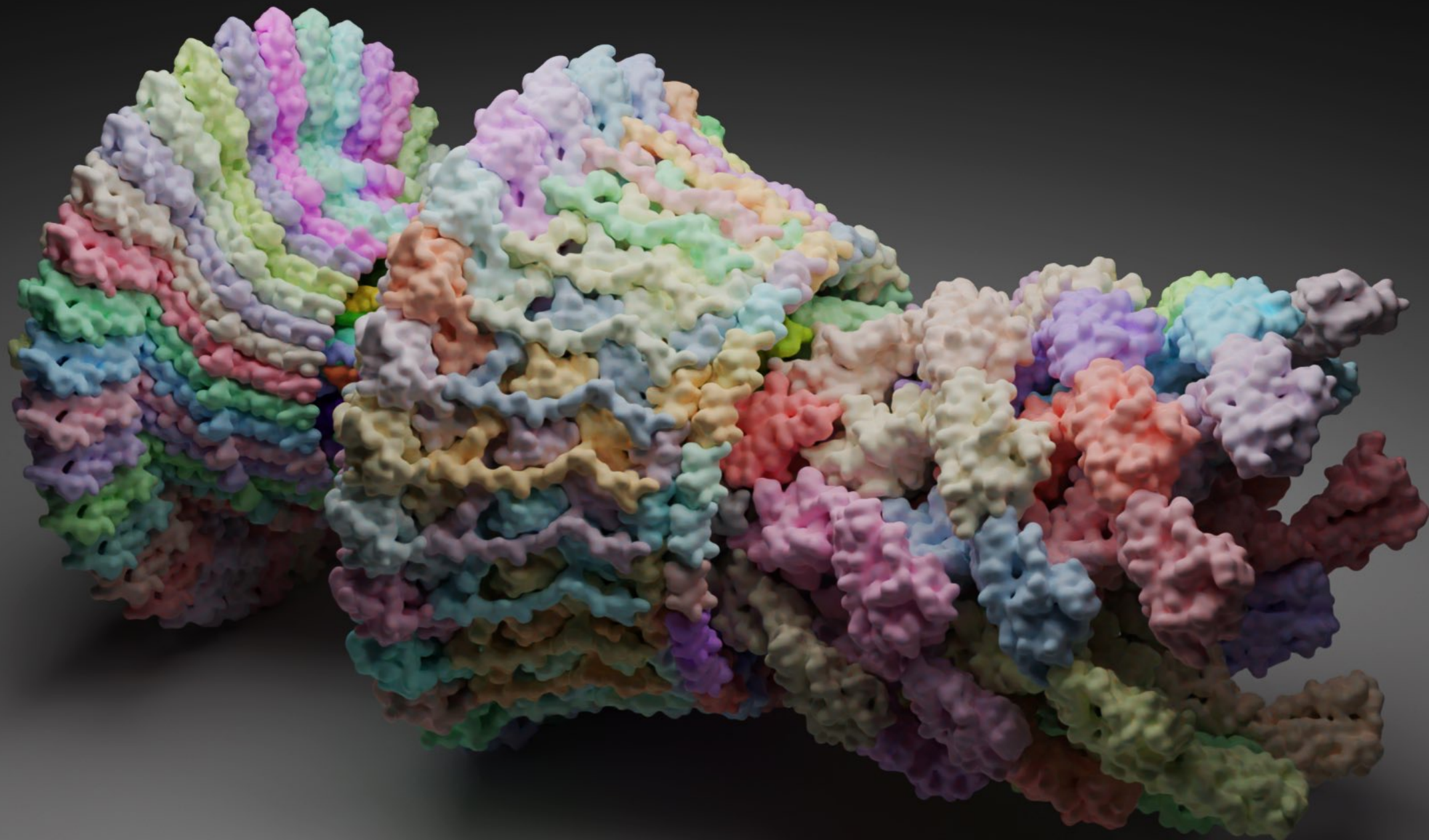


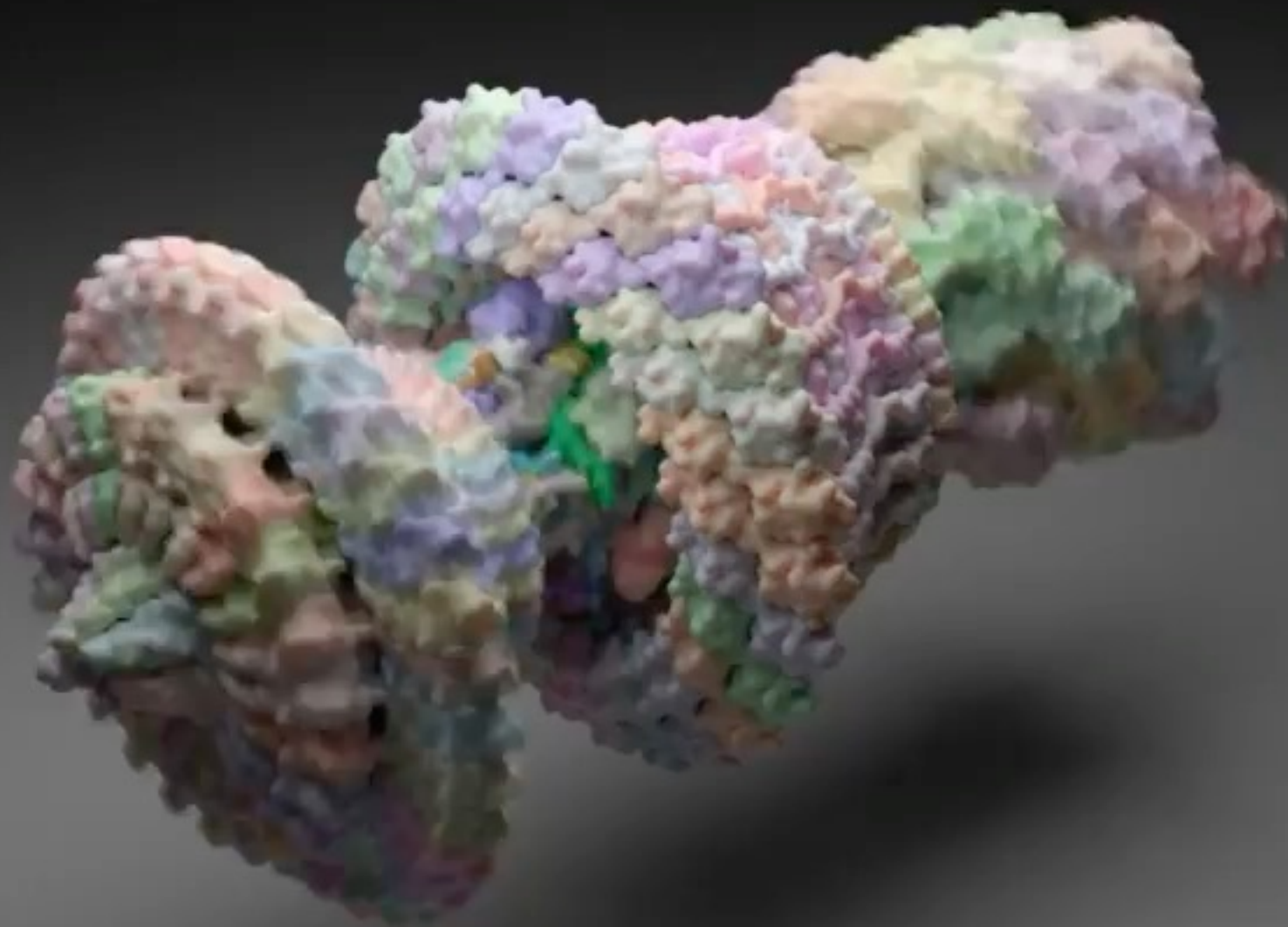
Transistor



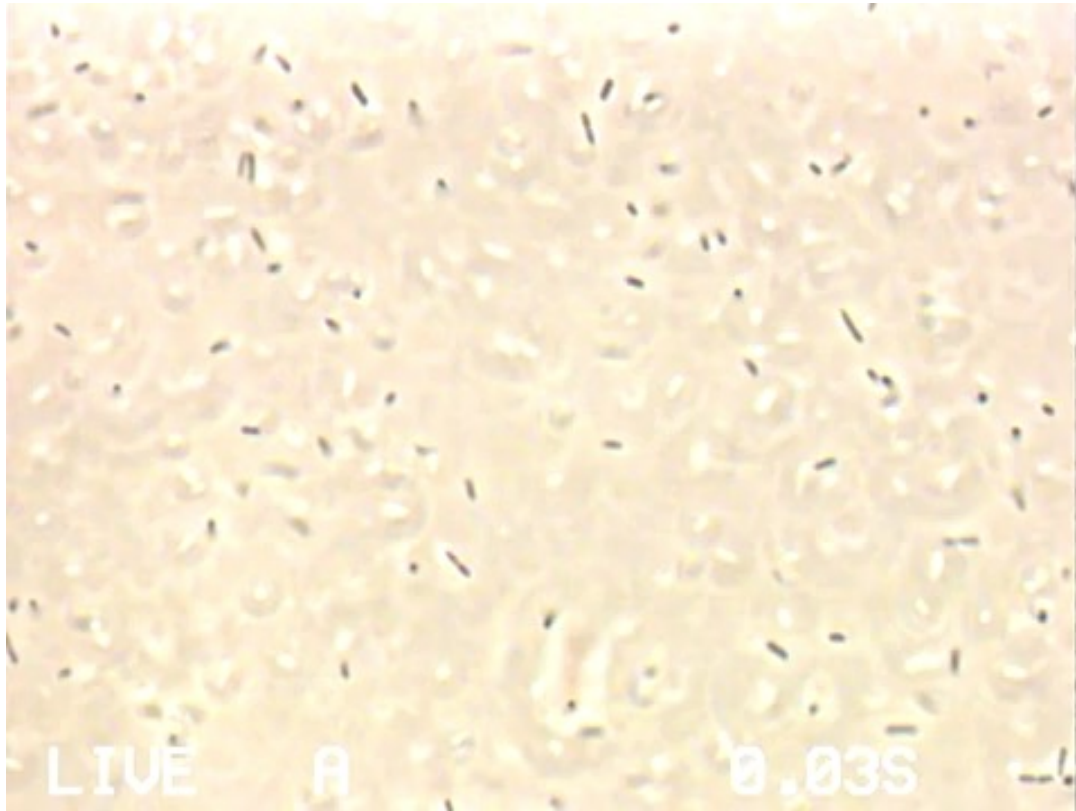
Oscillator



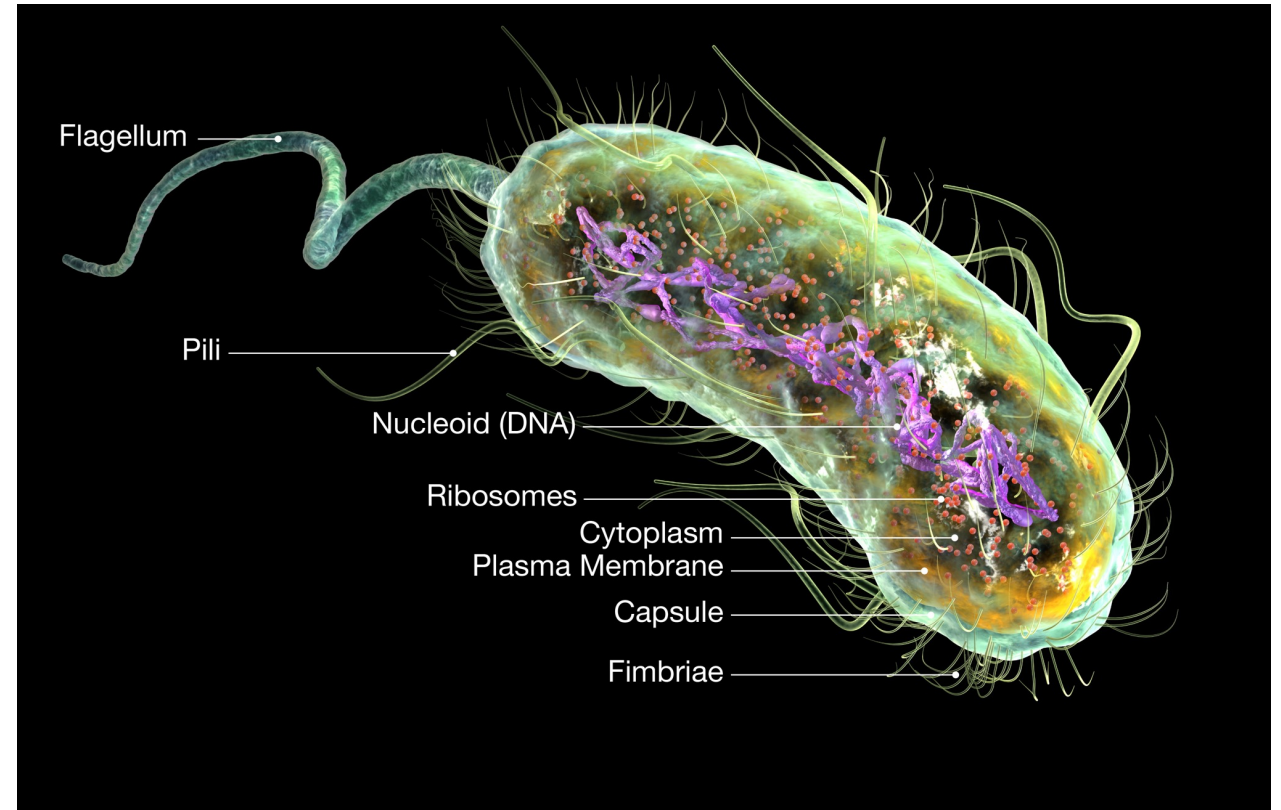




E. Coli motor protein

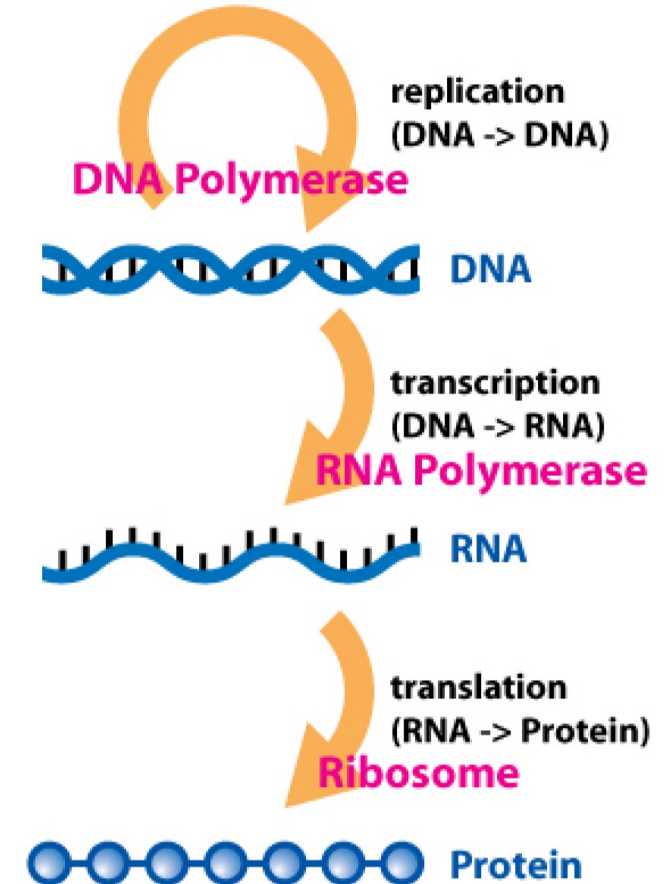
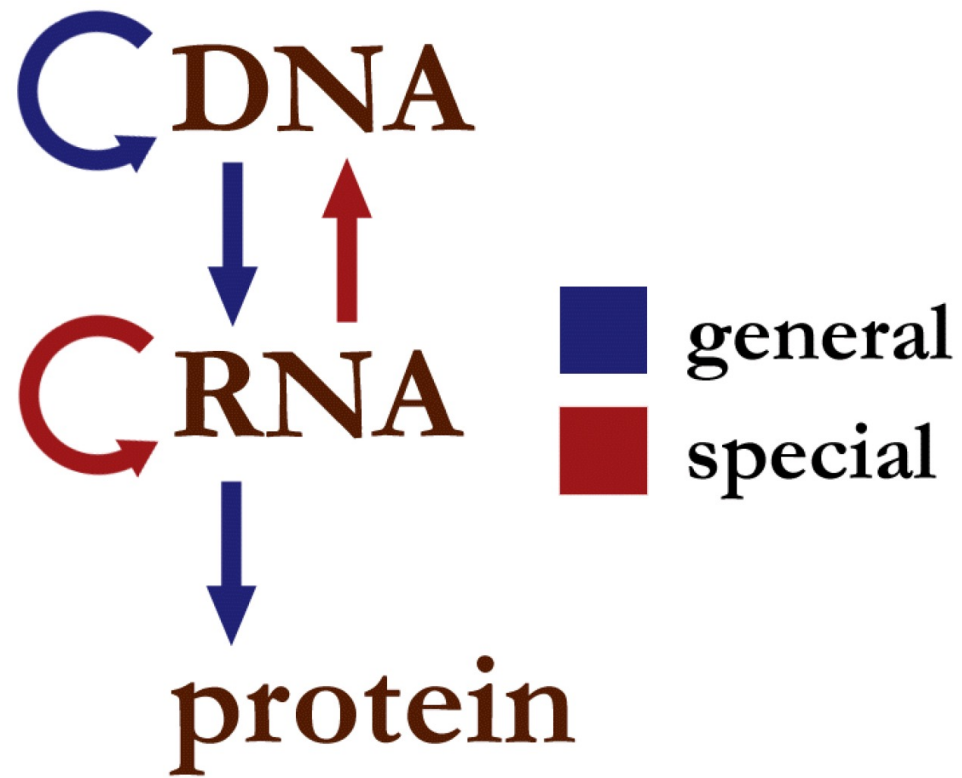


<https://youtu.be/EWj2TGsTQEI>

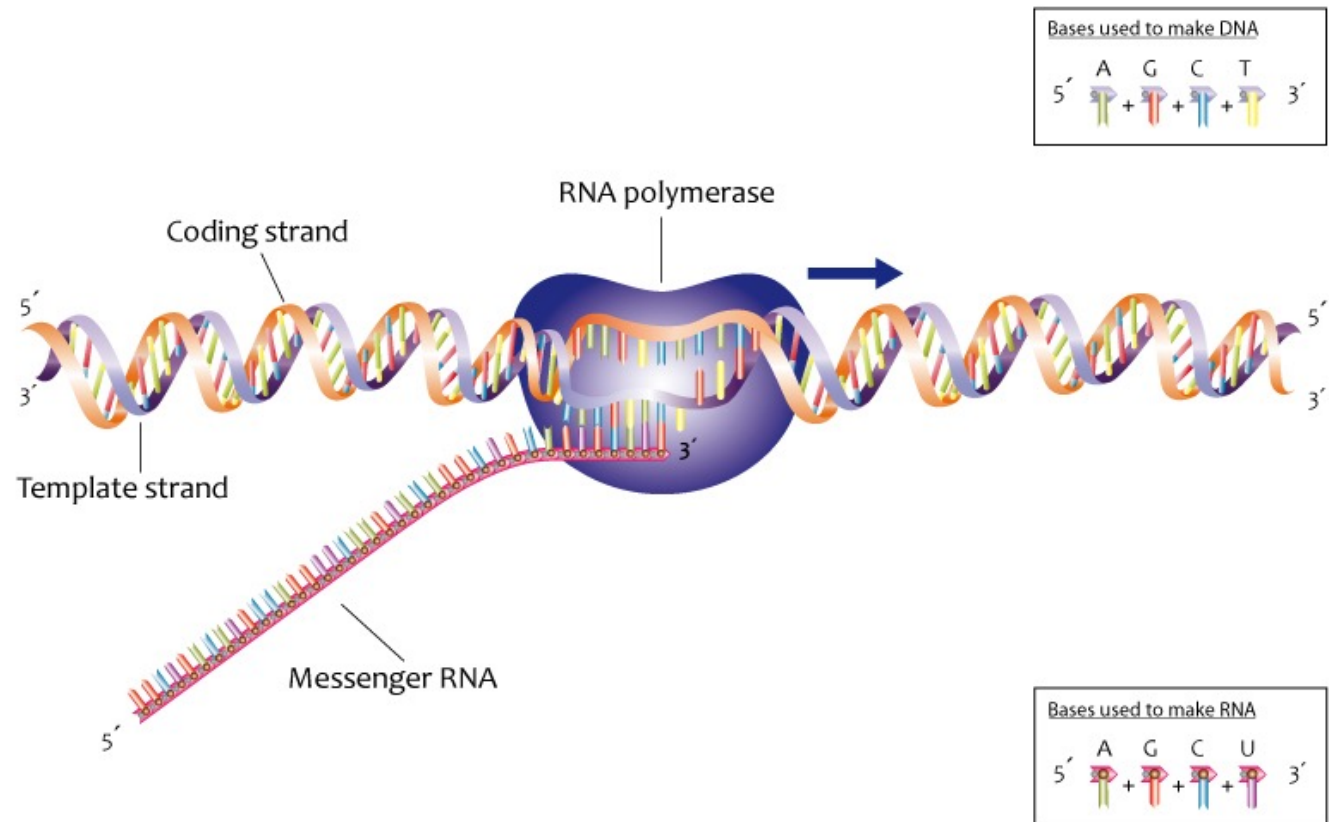


How proteins are made?

Central Dogma



Transcription



What are the components required for trascnription translation?

- DNA, RNA
- tRNA
- Amino acids
- ATP
- Ribosome
- RNA polymerase

How to use bacteria to produce protein

- Transform DNA into bacteria
- Plasmid transformation

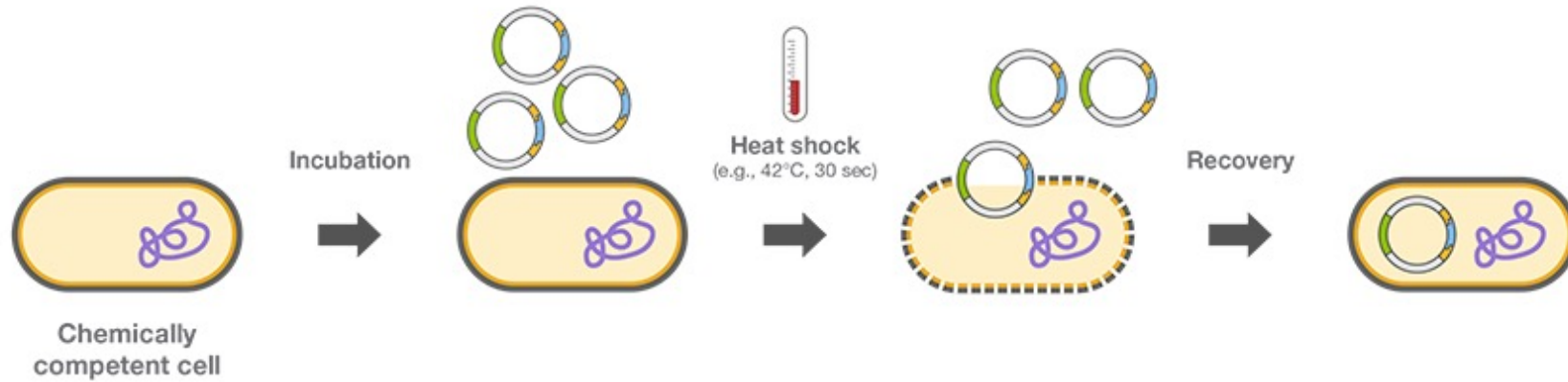
Plasmid

- Small circular DNA molecule
- Separated from chromosomal DNA
- More stable than linear DNA

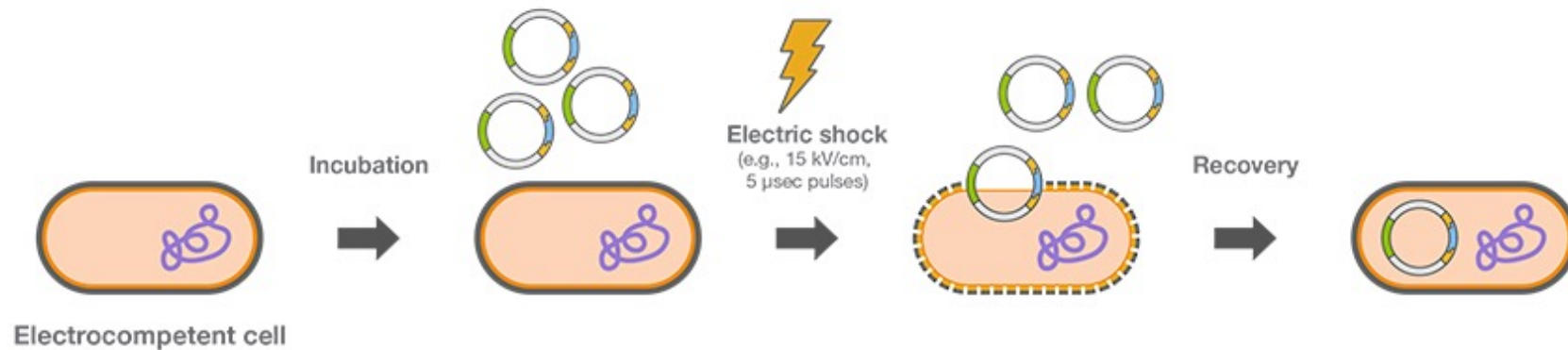


Transformation

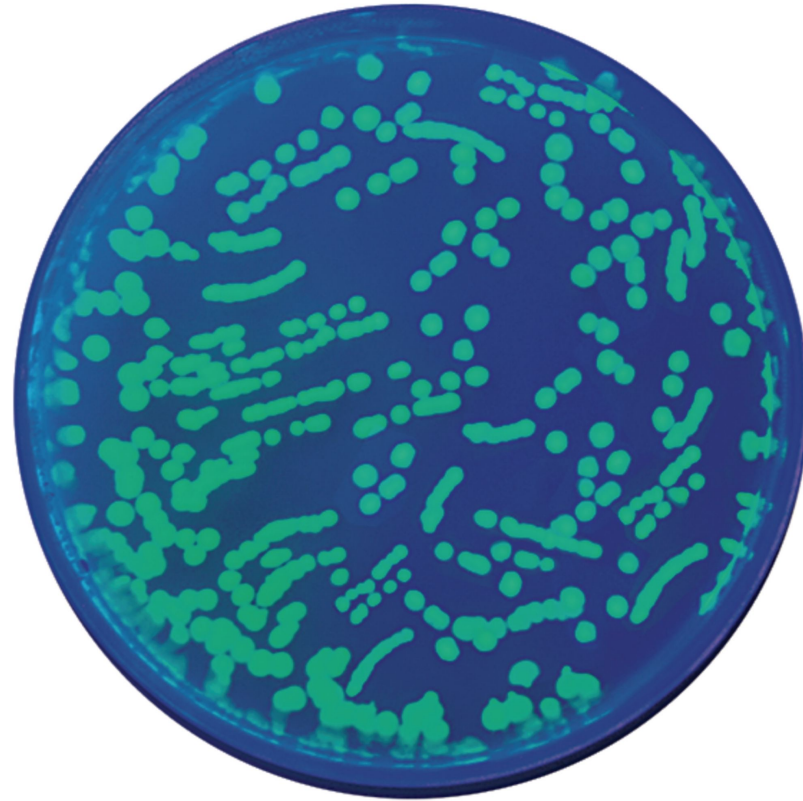
Chemical transformation



Electroporation

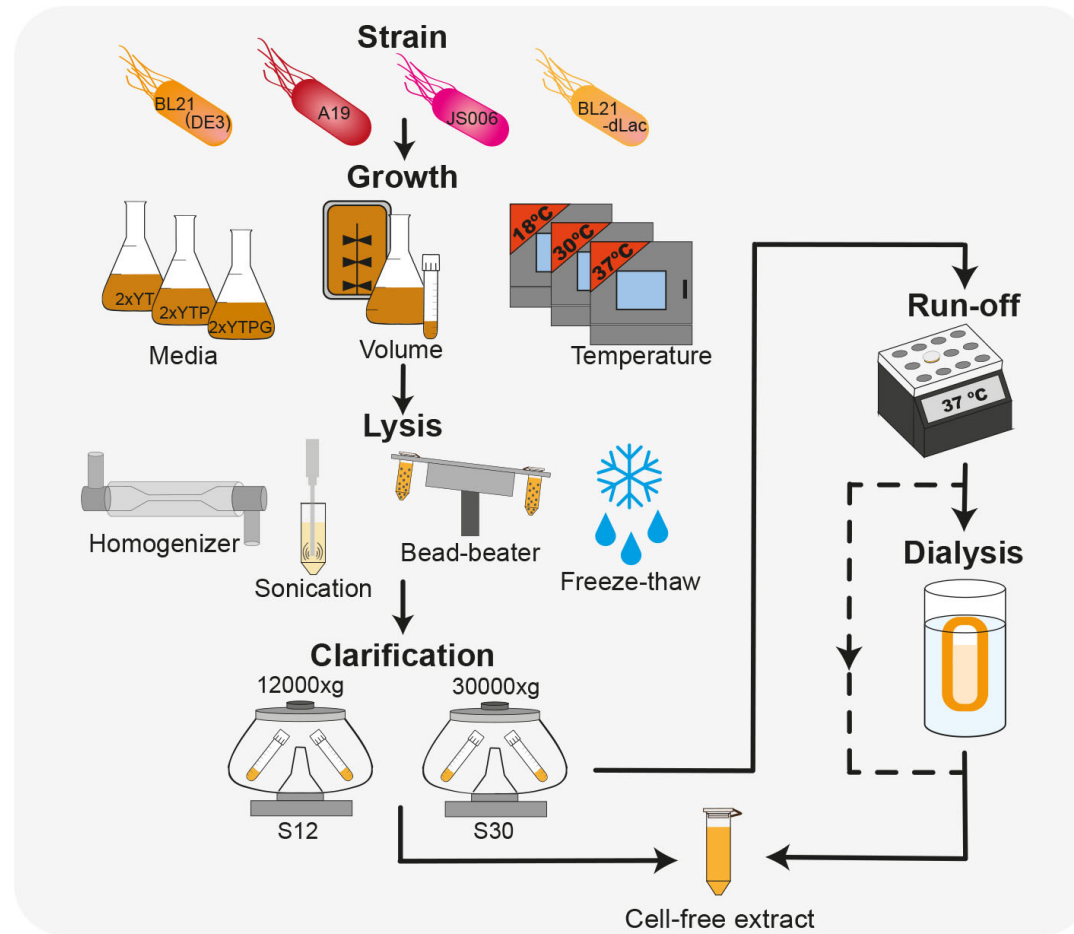


E coli with GFP DNA



Can we produce protein without cell?

Lysate



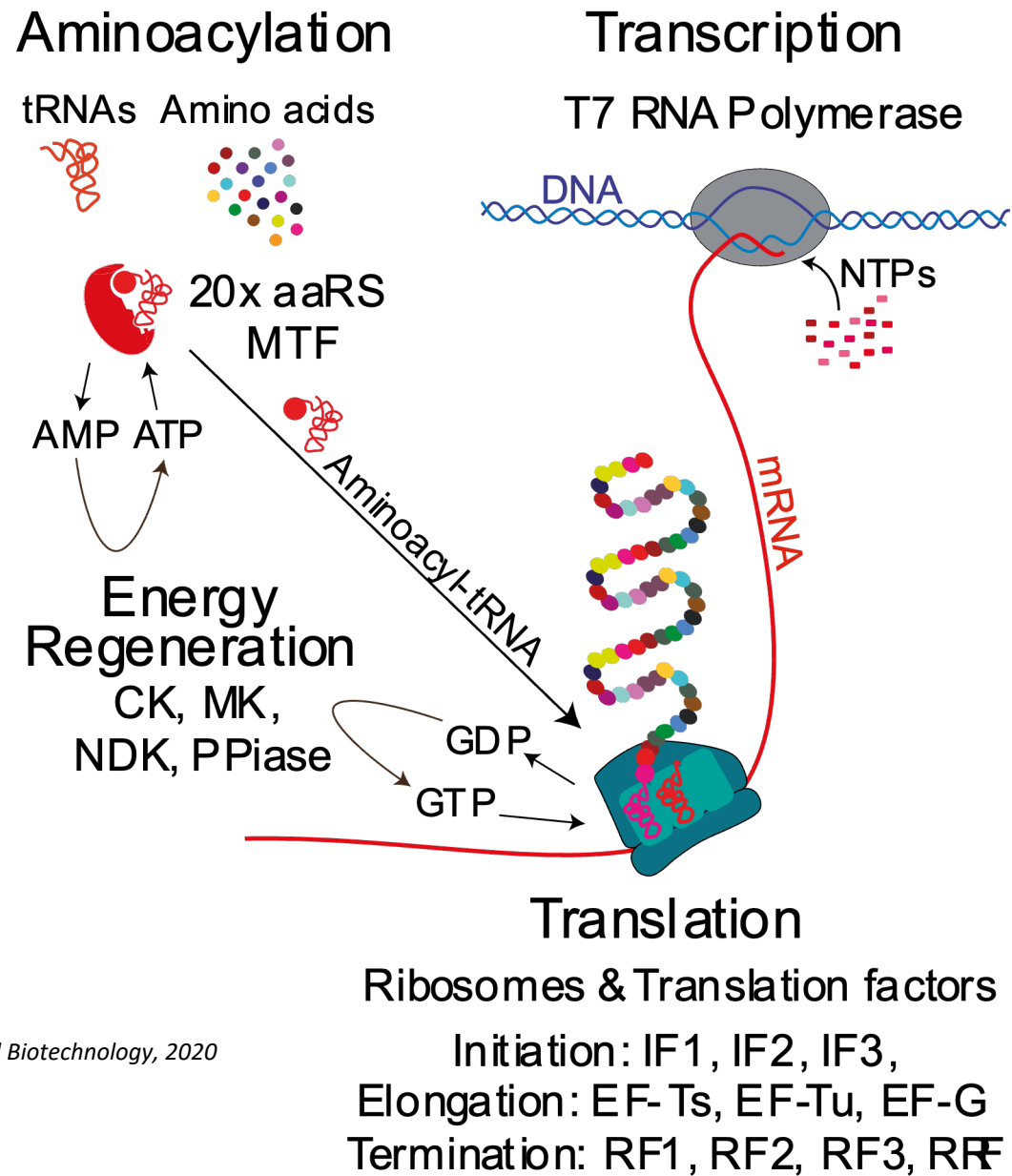
adapted from: Laohakunakorn et al., Frontiers in Bioengineering and Biotechnology, 2020

Lysate system

- Simple cell-free system
- No need to maintain a living system
- Black box with undefined components

Can we minimize the system?

PURE

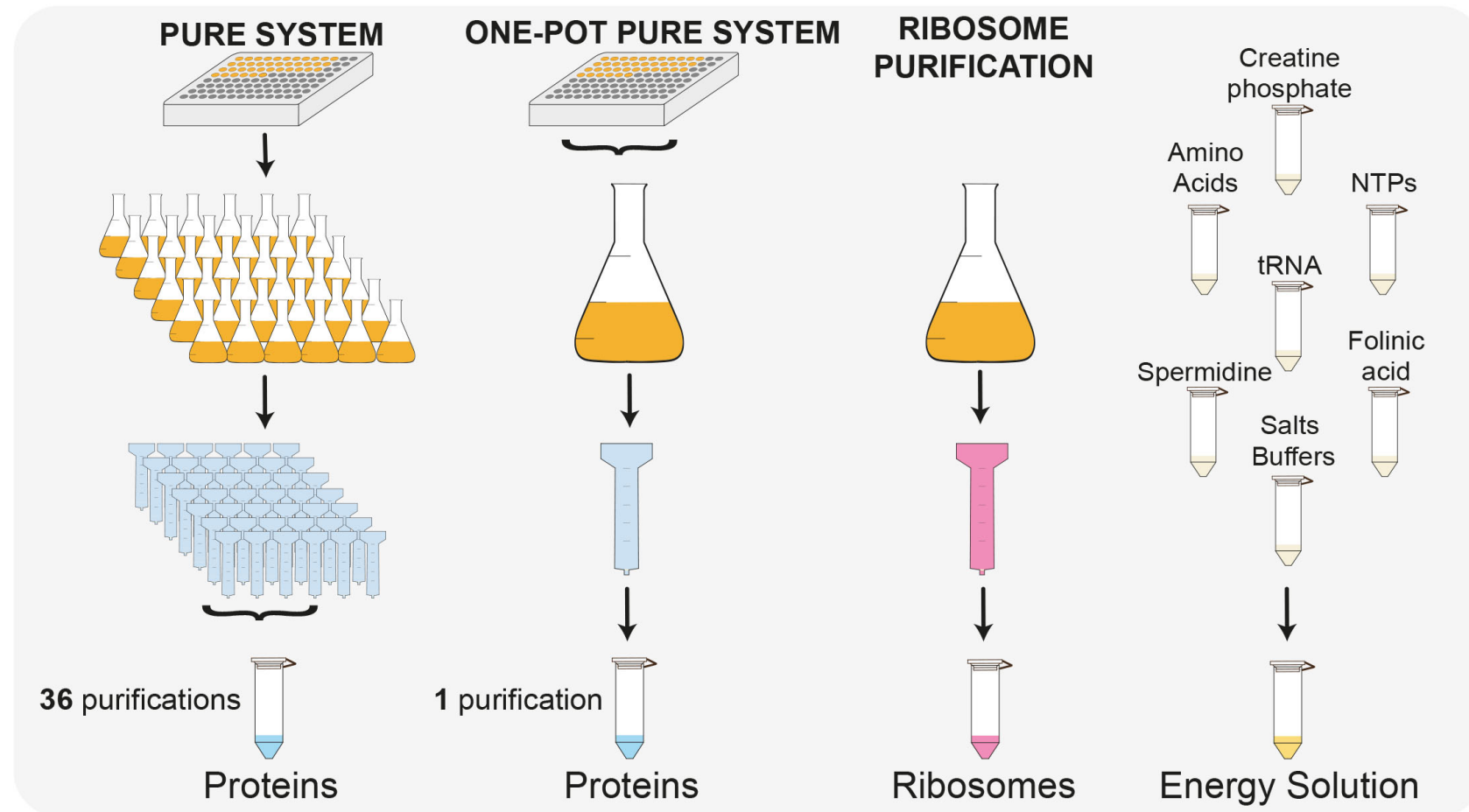


adapted from: Laohakunakorn et al., Frontiers in Bioengineering and Biotechnology, 2020

What are the components required in the trascnription translation?

- DNA
- NTPs
- tRNA
- Amino acids
- Ribosome
- RNA polymerase, aaRS.... In total **36** proteins

PURE



adapted from: Laohakunakorn et al., Frontiers in Bioengineering and Biotechnology, 2020

Lysate vs PURE

Lysate

- Has been around forever
- High yield
- Cheap and easy to make
- Many different systems available
- Undefined and complex

→ Black box

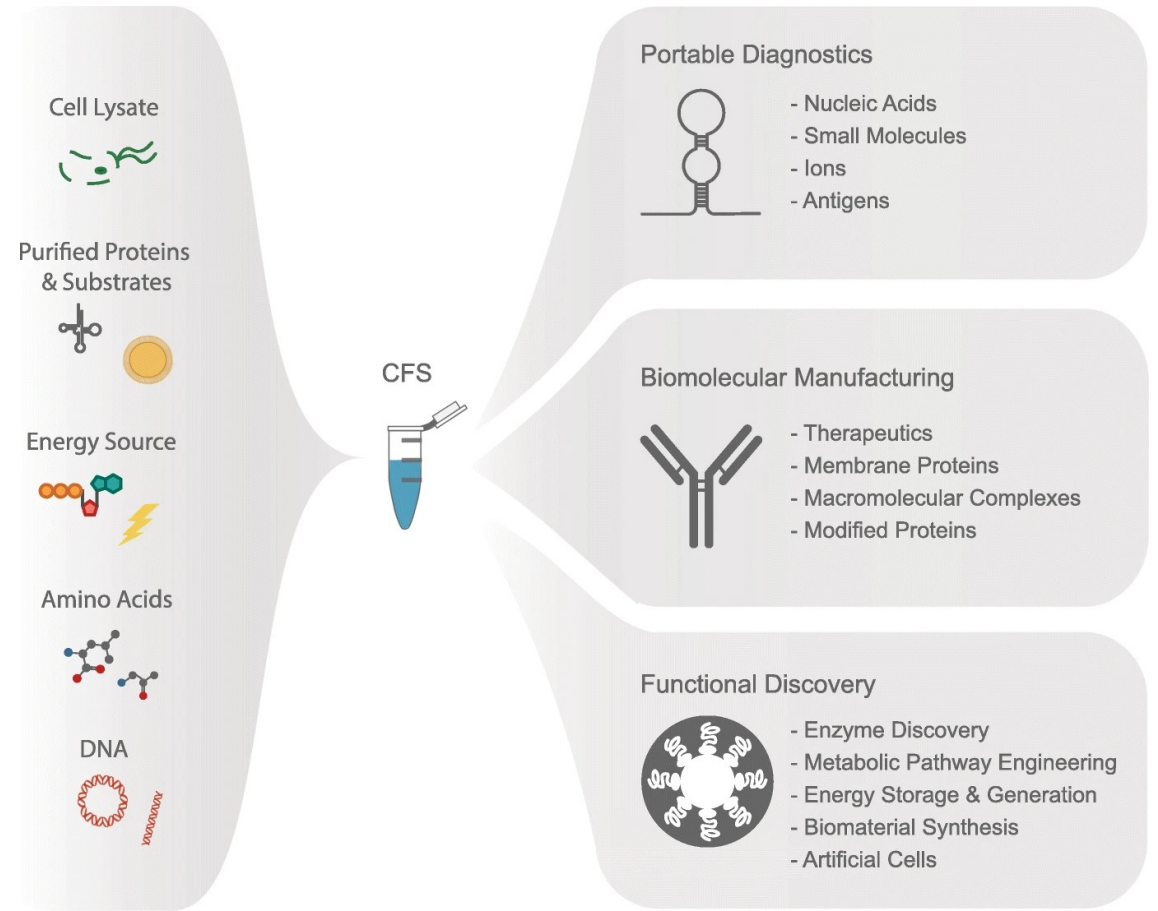
PURE

- Since 2001
- Less active
- Expensive and laborious to make
- Only E. coli based
- Well-defined and comparably well understood

→ Less of a black box

Applications

- Basic science: understanding biological systems better
- Rapid prototyping
- Diagnostics
- Therapeutics
- Metabolic engineering
- Building a synthetic cell



Goal of the course

- Make a cell-free system that can produce protein

Week 1	19.09.23	Introduction to PURE
Week 2	26.09.23	Microbiology
Week 3	3.10.23	Genetics
Week 4	10.10.23	Genetics
Week 5	17.10.23	Onepot PURE protein production
Week 6	24.10.23	Onepot PURE protein purification
Week 7	31.10.23	Onepot PURE protein adjustment
Week 8	7.11.23	Ribosome production
Week 9	14.11.23	Ribosome purification
Week 10	21.11.23	Ribosome adjustment
Week 11	28.11.23	SDS PAGE
Week 12	5.12.23	Onepot PURE reaction
Week 13	12.12.23	Backup
Week 14	19.12.23	Final Exam

Grading method	
Attendance	25%
Log Book	30%
Experiment Results	20%
Final exam	25%