

**Biomanufacturing & Microbiology
Symposium & Workshop**

UCD Conway Institute,
University College Dublin,
Ireland

March 12-14, 2024



**UCD-
BECKMAN
UNIVERSITY**



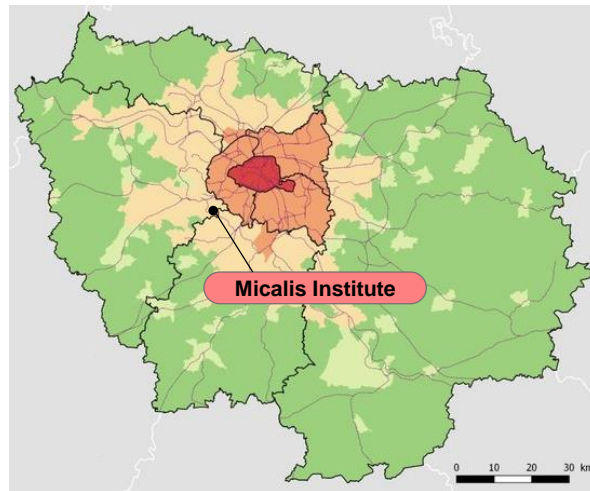
Maximization of production in cell-free systems

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Permanent researcher in Synthetic Biology

Institut Micalis, INRAE - AgroParisTech - Univ. Paris-Saclay, France

Micalis Institute



21 Research Teams

- 340 people
- 130 scientists and engineers
- 140 PhD students and post-docs
- 70 scientific and administrative personnel

Themes

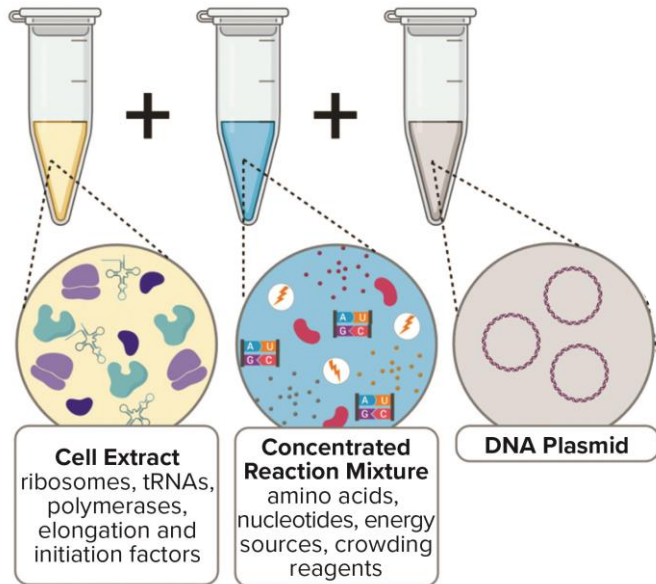
- (1) Emergence, control, and adaptation of pathogenic microorganisms
- (2) Microbial food and intestinal ecosystems
- (3) Synthetic and systems biology

Maximization production in cell-free systems

1. Description of Cell-Free Systems
2. A large-scale exploration of cell-free compositions to **maximize protein production** using active learning
3. A large-scale exploration of cell-free compositions to **maximize mRNA production** using active learning

Cell-free reaction:

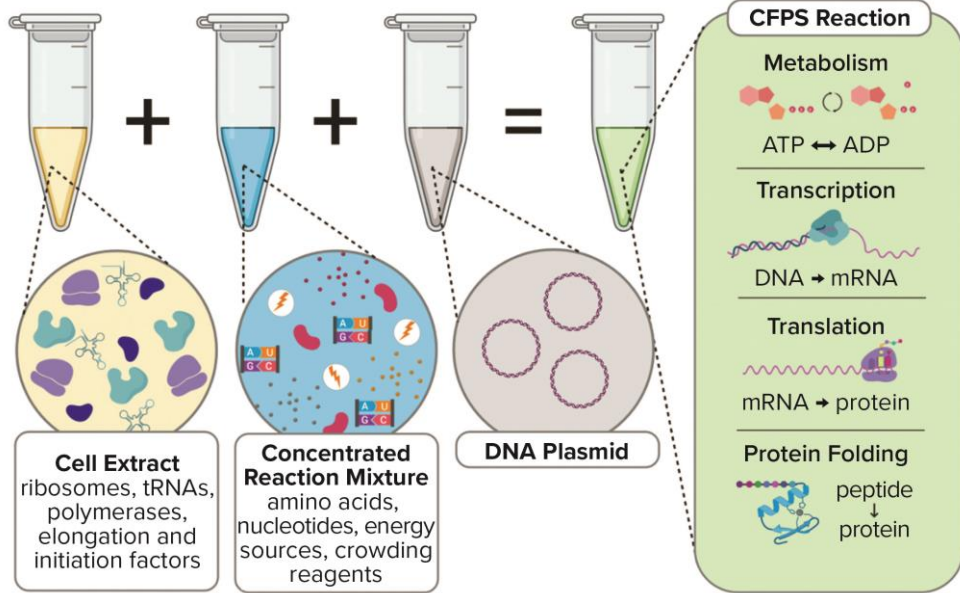
What is the composition of a cell-free reaction?



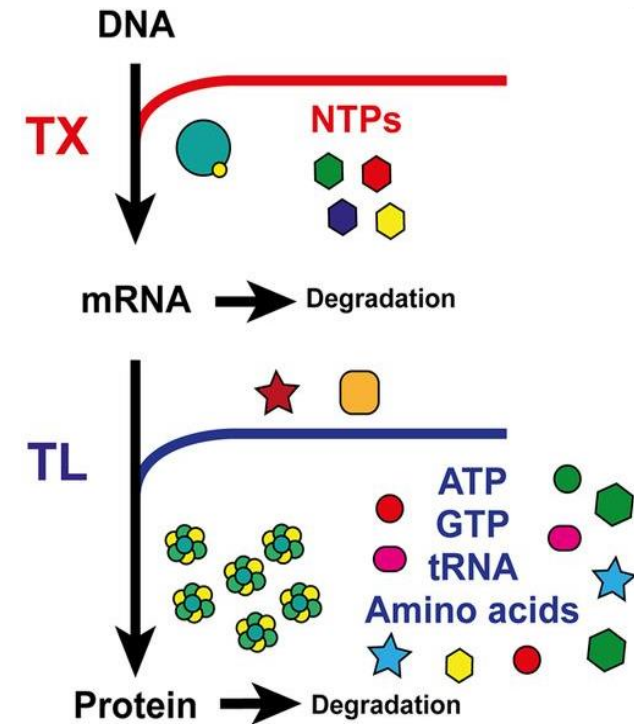
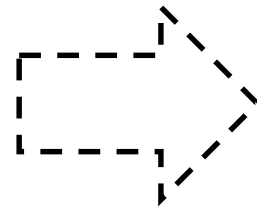
Melinek B, et al. BioProcess International (2020)

Cell-free reaction:

What is the composition of a cell-free reaction?

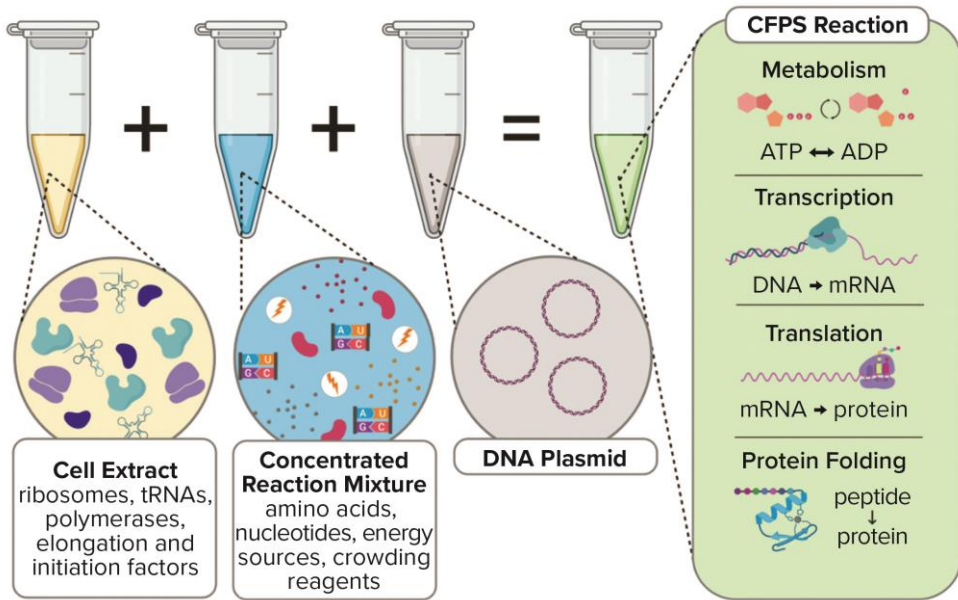


Melinek B, et al. *BioProcess International* (2020)



Seok Hoon Hong et al. *Frontiers in Chemistry* 2014

Cell-free reaction: Cell-extract preparation



Melinek B, et al. *BioProcess International* (2020)

Physical disruption

Sonication

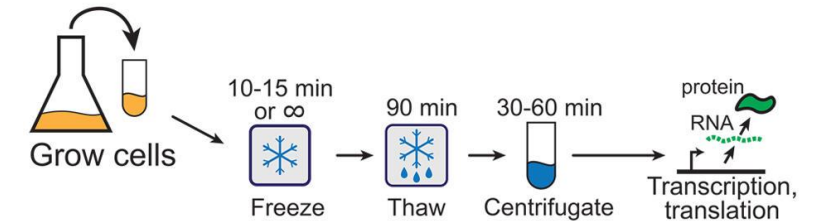


French press

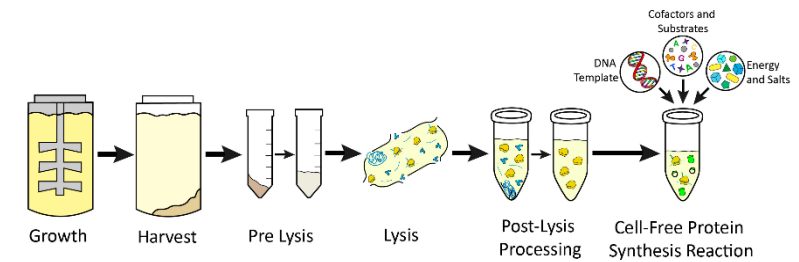


Chemical disruption

Autolysis

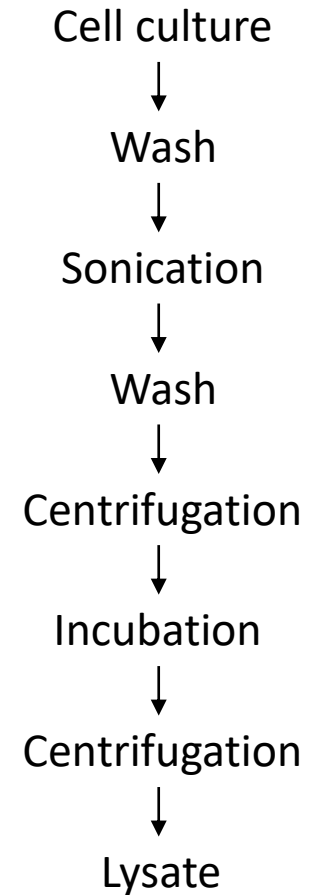
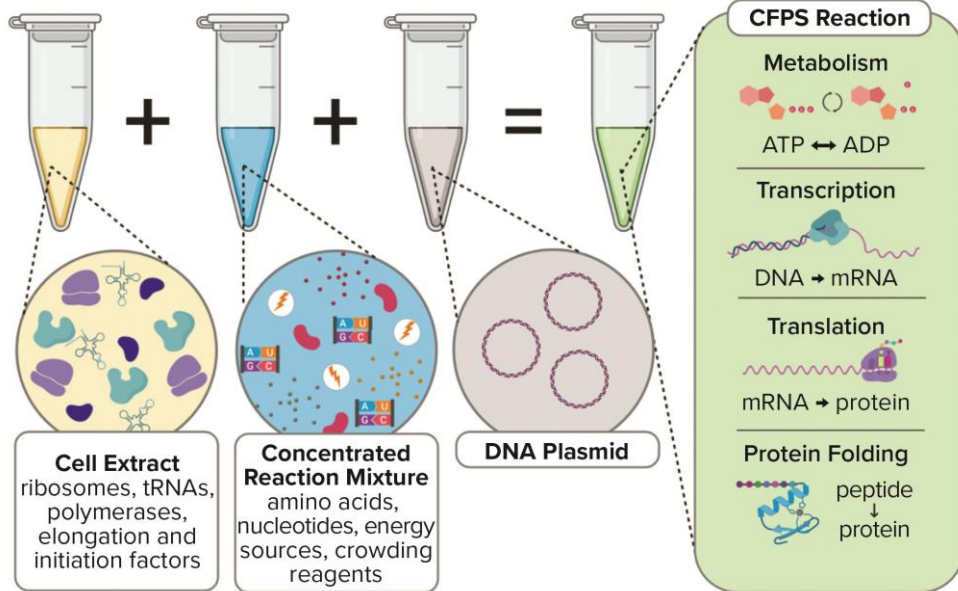


Lysozyme

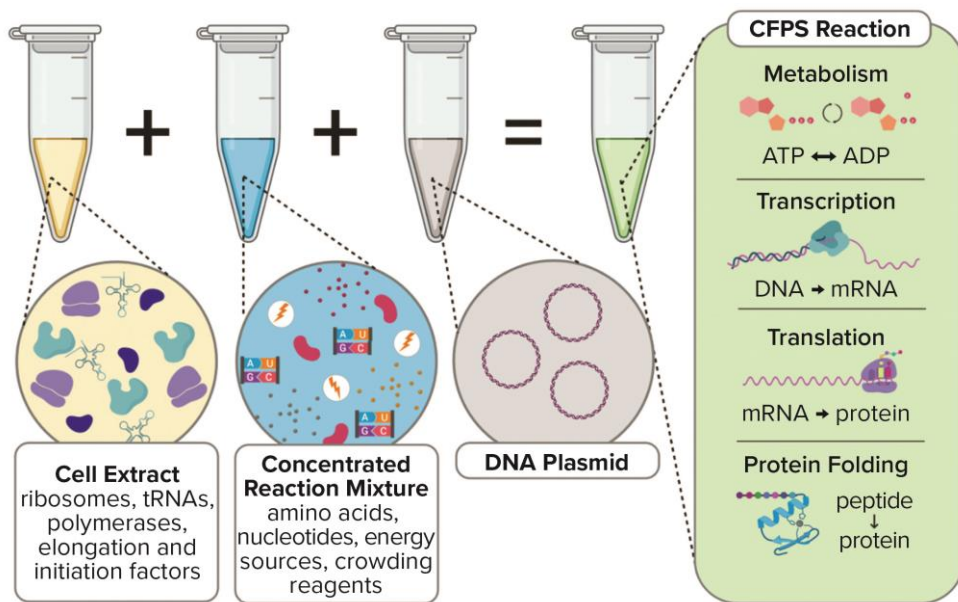


Cell-free reaction:

Protocol for home-made cell-extract preparation



Cell-free reaction: Buffer composition



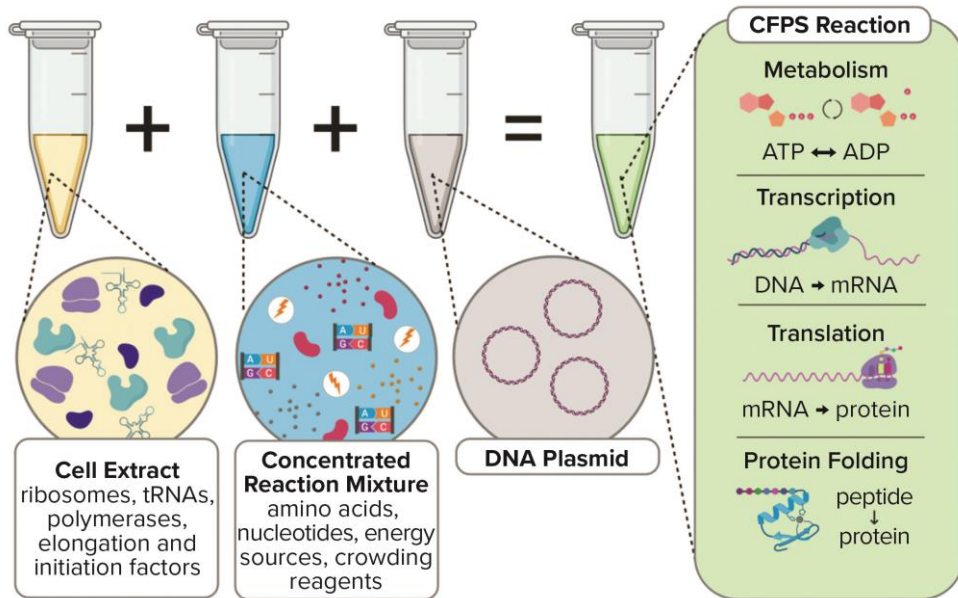
Melinek B, et al. *BioProcess International* (2020)

Amino Acid
tRNA
Mg-glutamate
K-glutamate
Folnic Acid
Spermidine
Coenzyme A
NAD
3-PGA
AMPc
Nucleoside triphosphate (NTP)

Translation
Translation
Ribosome maintenance
Ribosome stability
A precursor for formylmethionine (fMet)
Ribosome activity /RNA stability
Regenerate ATP via glycolysis
Regenerate ATP via glycolysis
Regenerate ATP
regulating glycolysis
Transcription

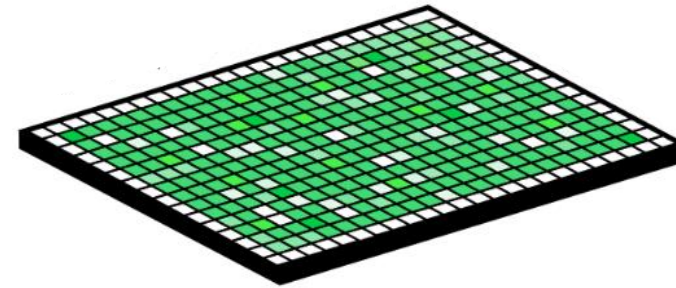
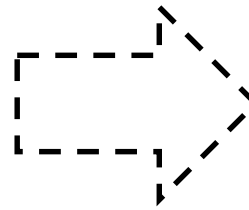
Cell-free reaction:

Measurements in 384-well plate



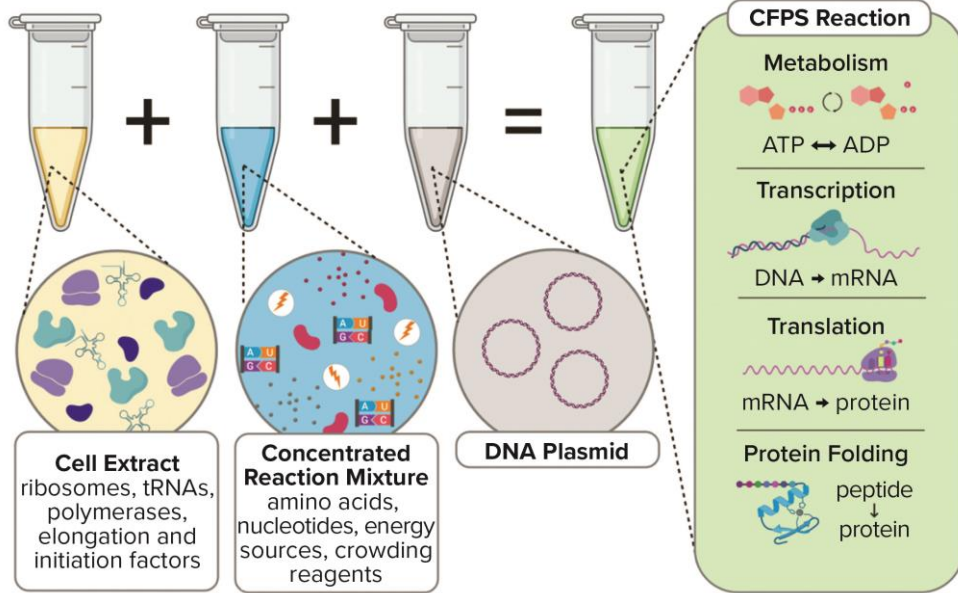
Melinek B, et al. *BioProcess International* (2020)

1 to 20 μ l reactions
(in 384-well)



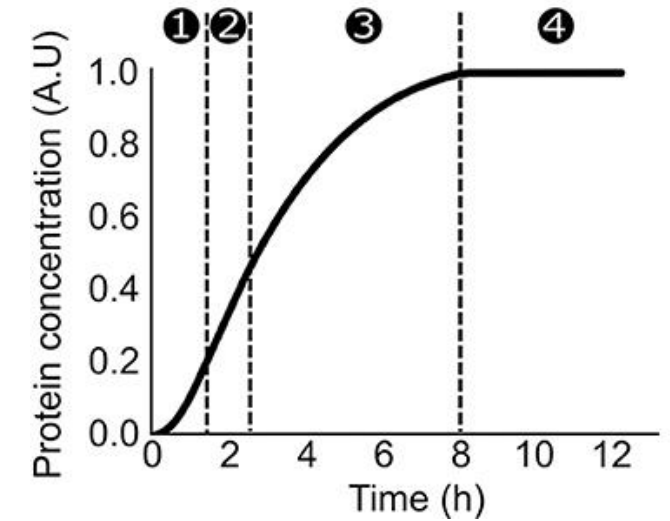
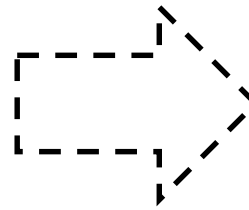
Cell-free reaction:

Measurements in 384-well plate

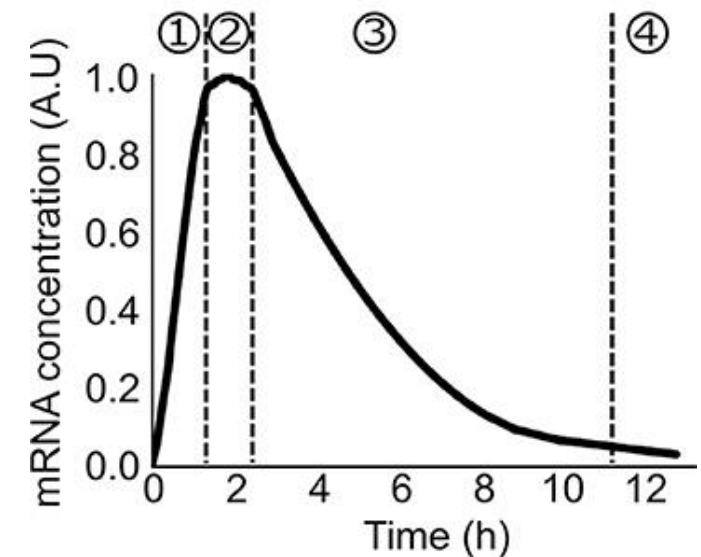


Melinek B, et al. *BioProcess International* (2020)

Translation

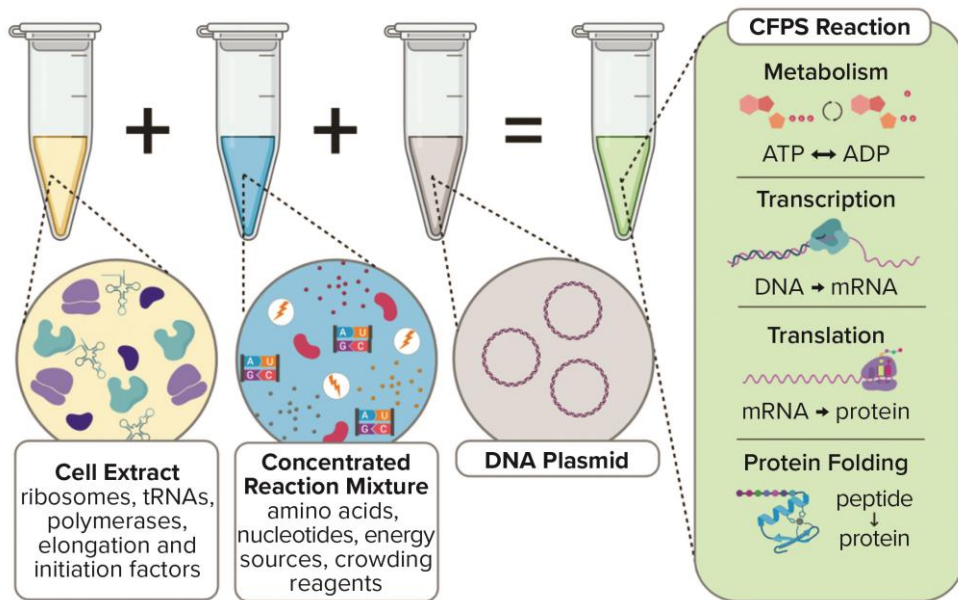


Transcription



Cell-free reaction:

Current existing protocol to produce bacterial cell extract



Melinek B, et al. *BioProcess International* (2020)

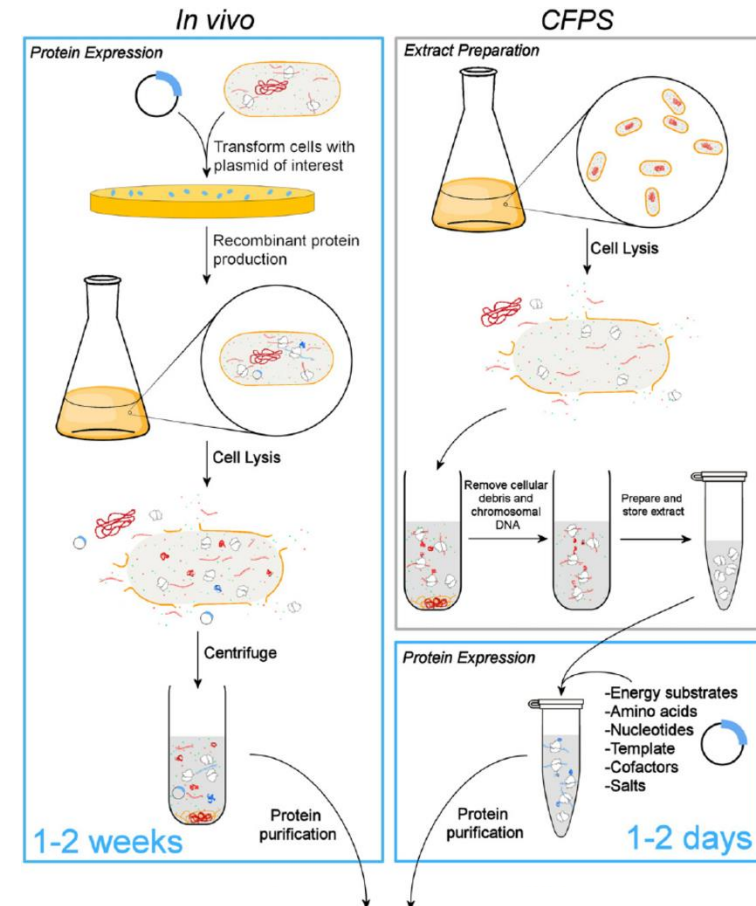
Table 1. Current existing protocols to produce cell-free lysates from bacteria cells

Organism	Bacteria Phylum
<i>E. coli</i>	Proteobacteria
<i>B. subtilis</i>	Firmicutes
<i>L. lactis</i>	Firmicutes
<i>B. megaterium</i>	Firmicutes
<i>C. autoethanogenum</i>	Firmicutes
<i>V. natriegens</i>	Proteobacteria
<i>P. putida</i>	Proteobacteria
<i>E. fergusonii</i>	Proteobacteria
<i>S. enterica</i>	Proteobacteria
<i>K. oxytoca</i>	Proteobacteria
<i>C. glutamicum</i>	Actinobacteria
<i>S. venezuelae</i>	Actinobacteria

Cell-free reaction:

Advantages of producing protein outside of the cell

- Fast reaction to produce protein
- No need for transformation
- No GMO
- No interaction with a living host (No mutation, No burden)
- Decoupling effect (e.g. Metabolic burden vs production burden)
- Low volume of reaction
- Higher level of control: Amount of DNA, Composition of the reaction.
- Work with Linear DNA

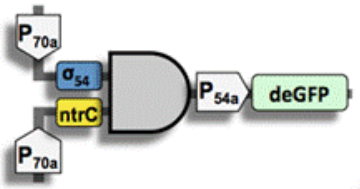


Cell-free reaction:

Example of applications

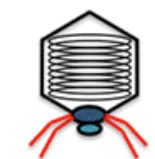
Prototyping

- regulatory parts
- circuits



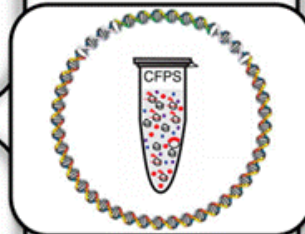
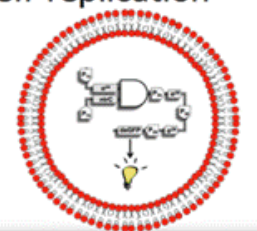
Self-assembly

- phages
- biophysics




Synthetic cell

- sensors
- self-replication



Medicine

- probes
- therapeutics



- Prototyping genetic circuits and pathways
 - Genetic parts
 - Metabolic engineering
- Developing biosensors
 - Optimize cell-free biosensor
 - Biocomputing
 - Materials and cell-free
 - Medical diagnostics
 - Pollution diagnostics
- Bio-machine assembly
 - Phages selection
 - Antibodies

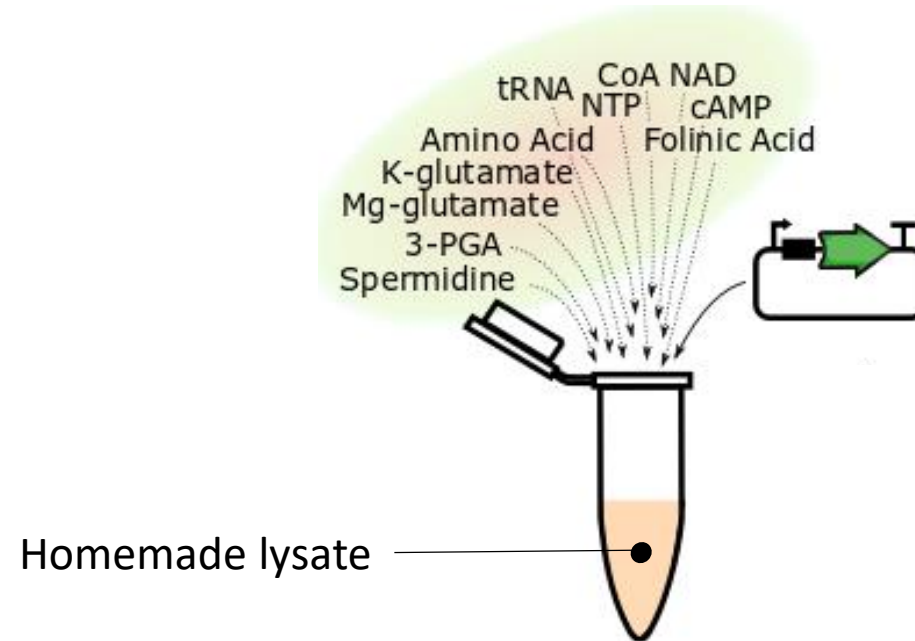
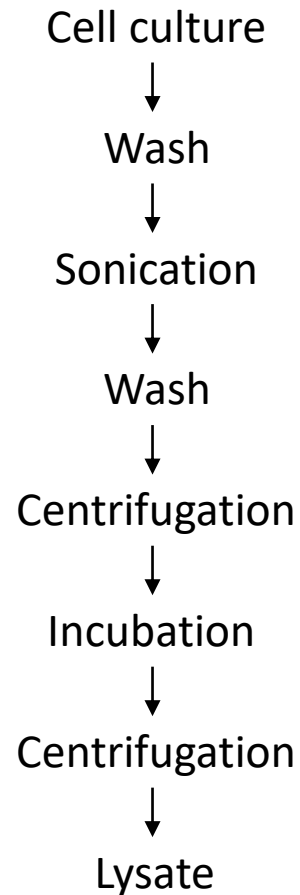


Maximize protein production for bio-production

Olivier Borkowski*, Mathilde Koch*, Agnès Zettor, Amir Pandi, Angelo Cardoso Batista, Paul Soudier, Jean-Loup Faulon

Protocol for homemade lysate

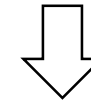
Protocol for home-made cell lysate



Cell-free composition: A vast combinatorial space to explore

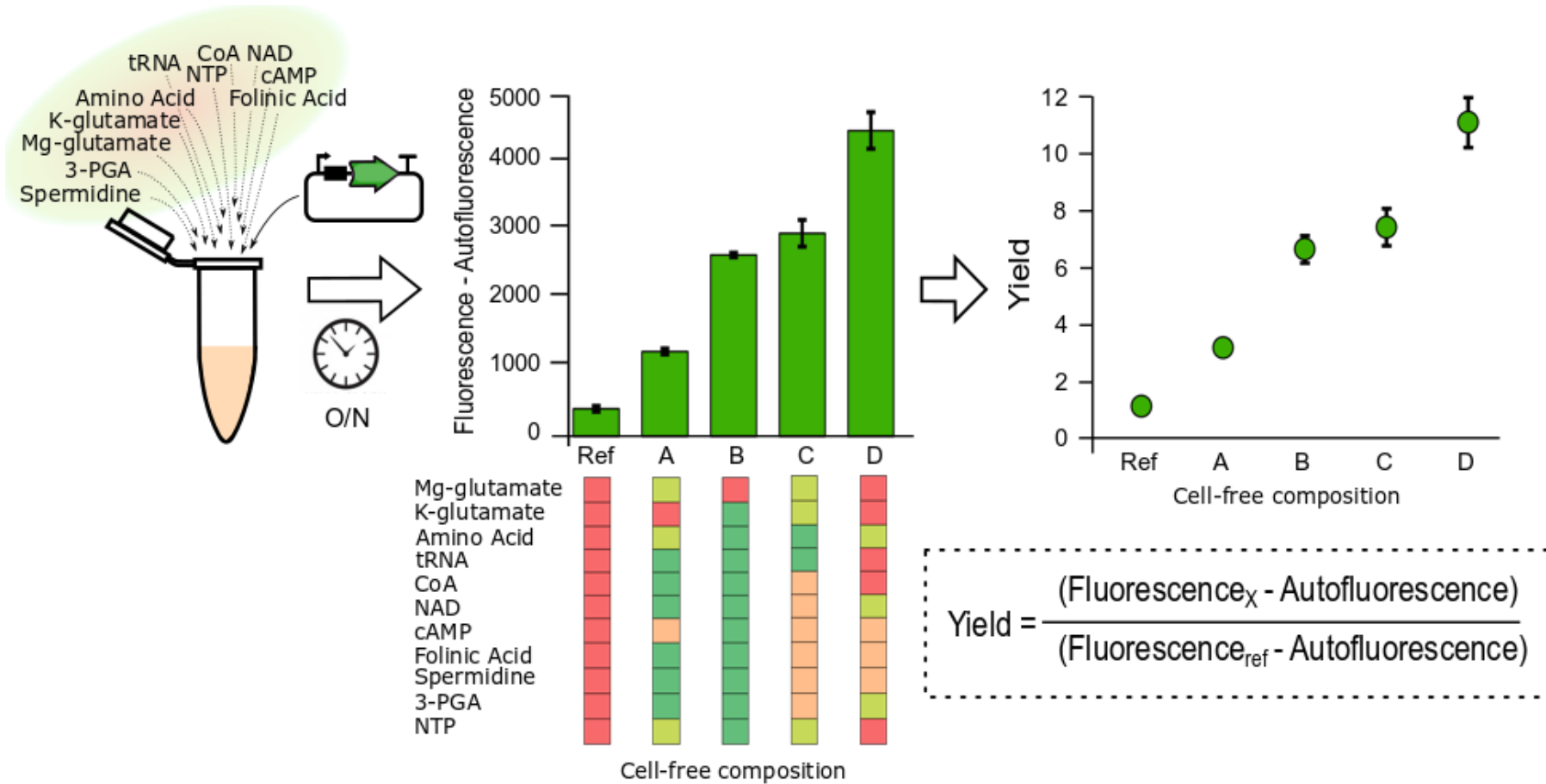
Component	Concentration			
Mg-glutamate (mM)	0.4	1.2	2	4
K-glutamate (mM)	8	24	40	80
Amino Acid (mM)	0.15	0.45	0.75	1.5
tRNA (mg/ml)	0.02	0.06	0.1	0.2
CoA (mM)	0.026	0.078	0.13	0.26
NAD (mM)	0.033	0.099	0.165	0.33
cAMP (mM)	0.075	0.225	0.375	0.75
Folinic Acid (mM)	0.0068	0.0204	0.034	0.068
Spermidine (mM)	0.1	0.3	0.5	1
3-PGA (mM)	3	9	15	30
NTP (mM)	0.15	0.45	0.75	1.5

Combinatorial space = $4^{11} = 4\,194\,304$ compositions

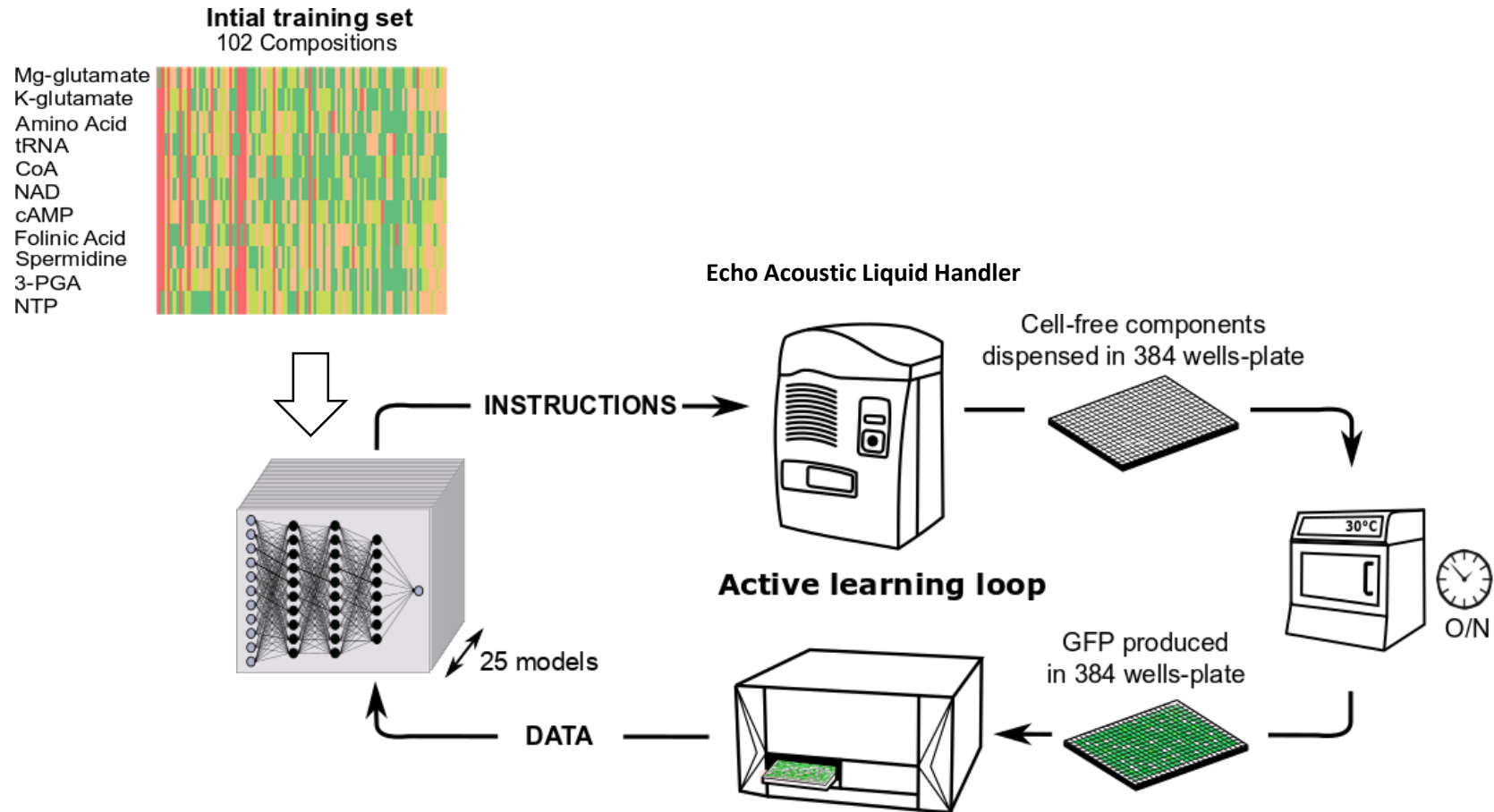


- Can we improve protein production for a specific lysate without increasing the price of cell-free reaction?
- Can we provide efficient predictions of protein production?
- Can we highlight the critical parameters involved in protein production?

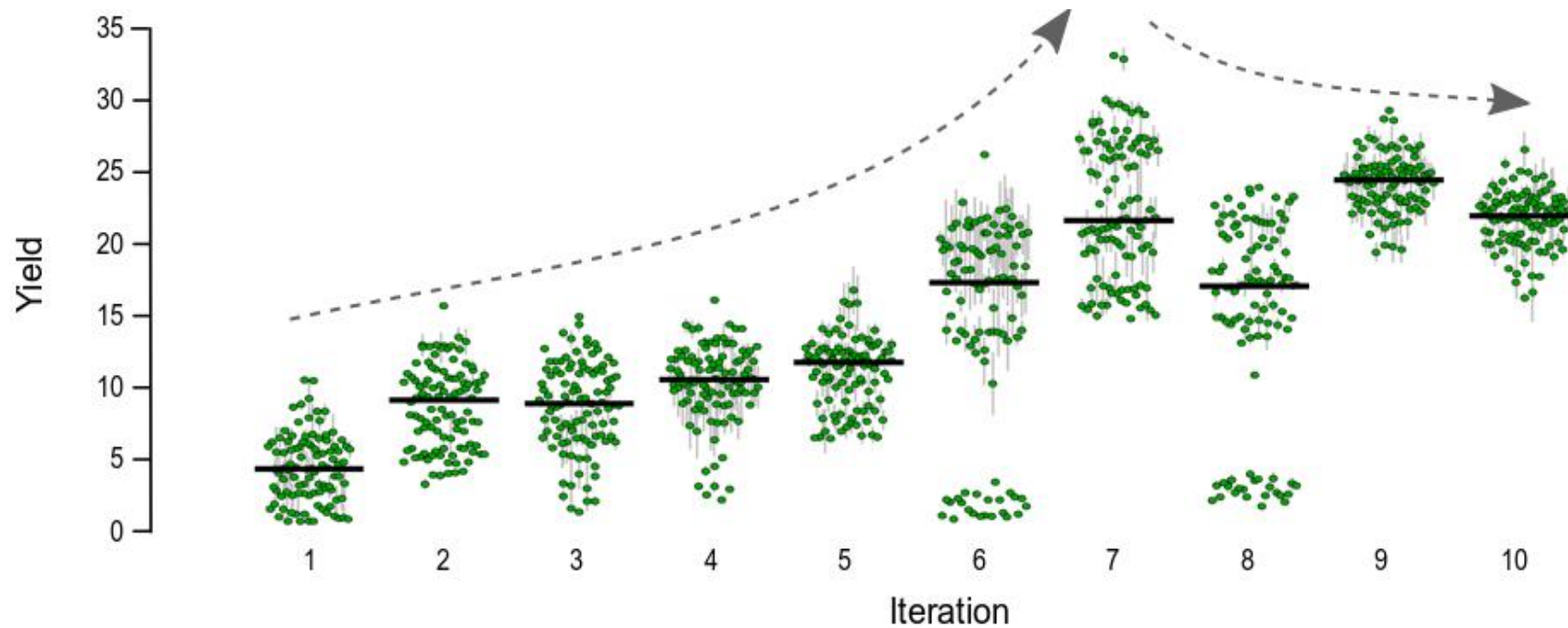
A relative measure of efficiency: Yield



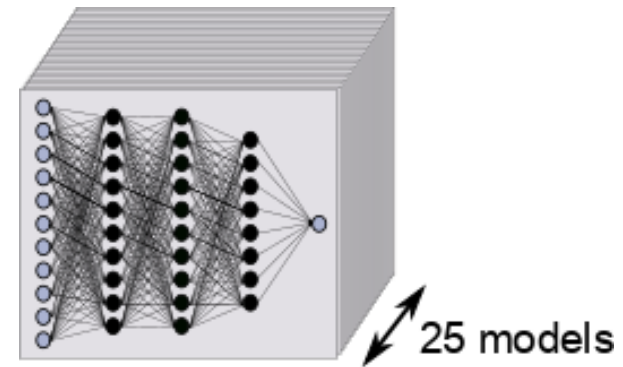
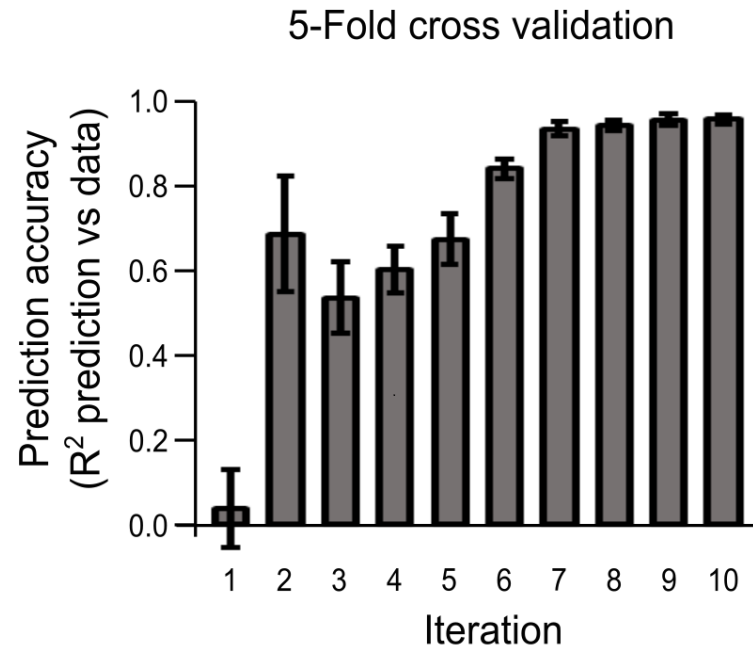
Exploration using active learning



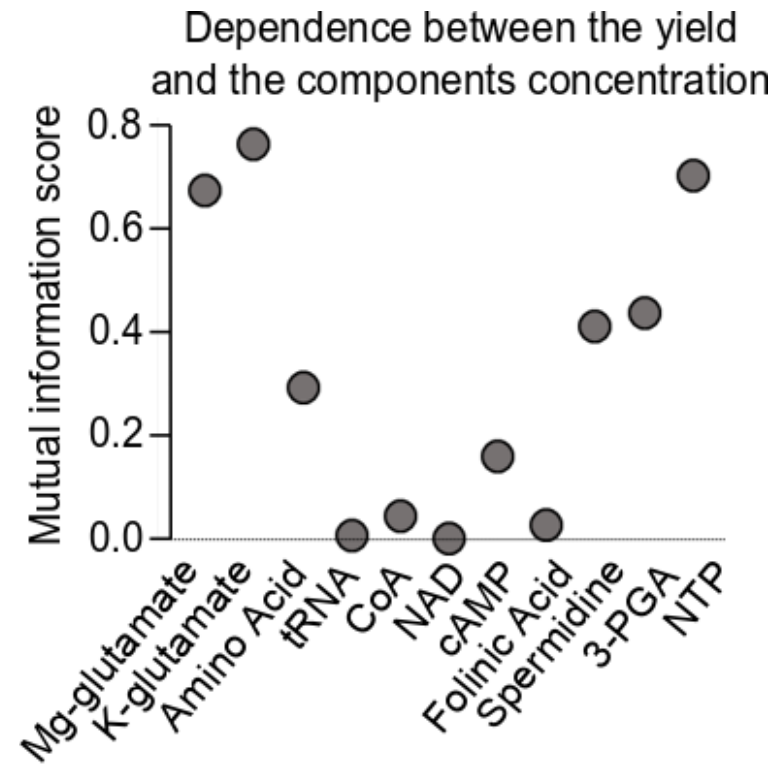
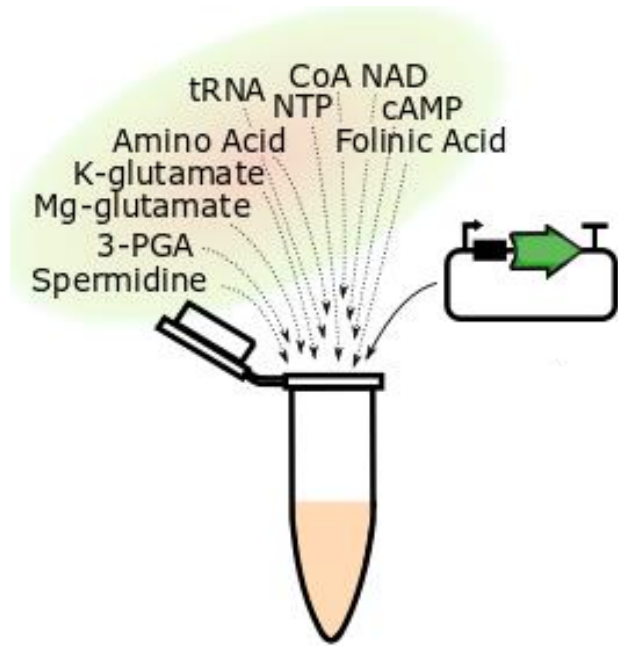
A 34 time Yield improvement using an home-made lysate



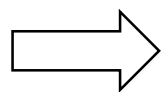
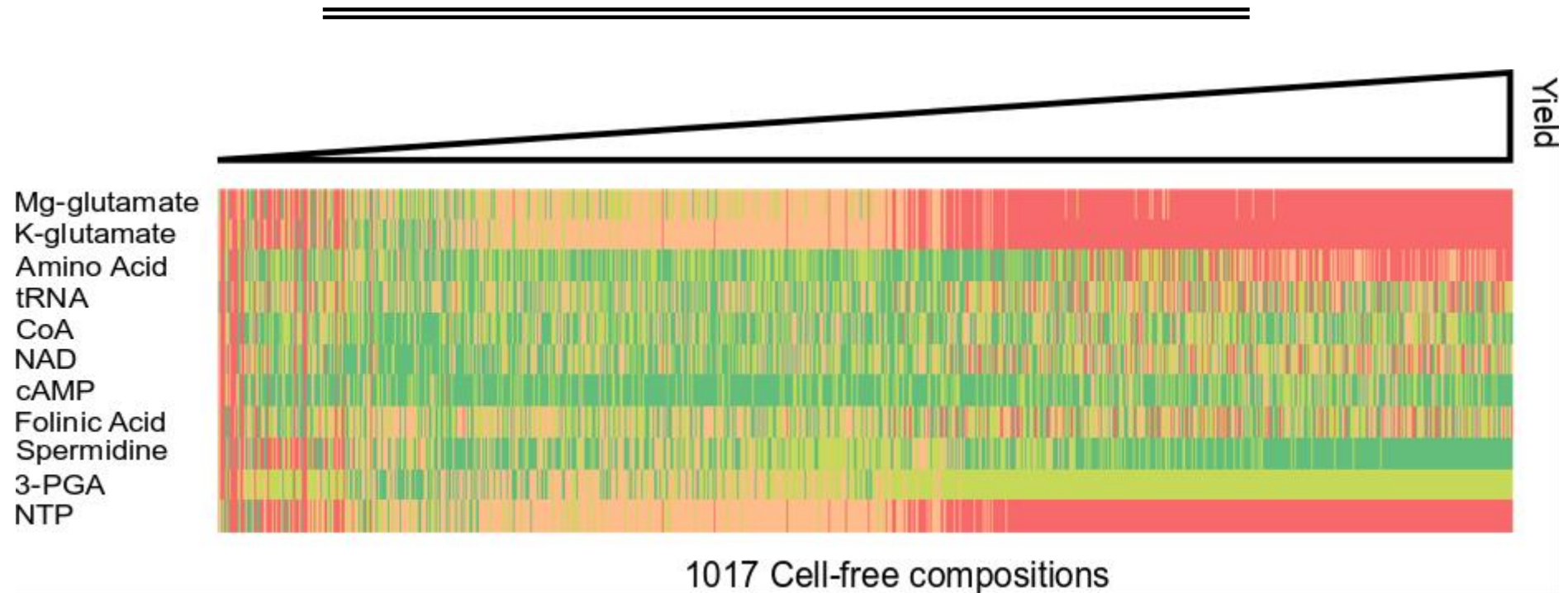
Prediction accuracy of our models



Impact of each component on cell-free efficiency



Impact of each component on cell-free efficiency



Components that improve yield at high concentration:

Mg-glutamate, K-glutamate, Amino Acids and NTPs

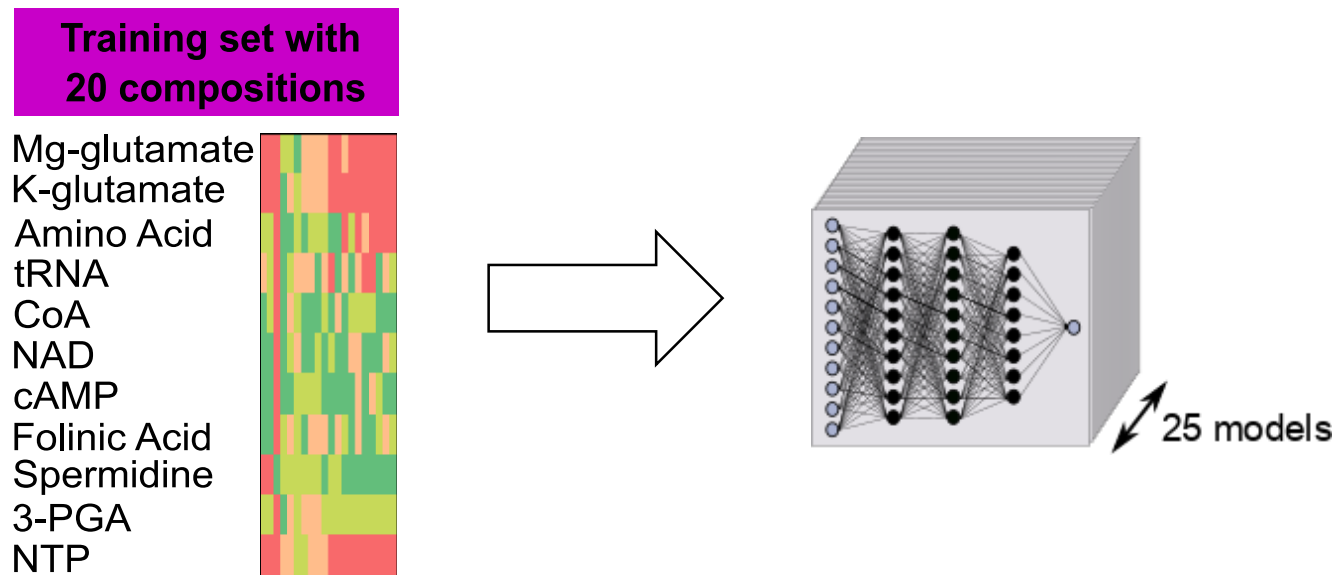
Components that improve yield at low concentration:

Spermidine, 3-PGA

A one-step method to maximize Yield with home-made lysates

Lysate quality is user-dependent, strain-dependent, etc...

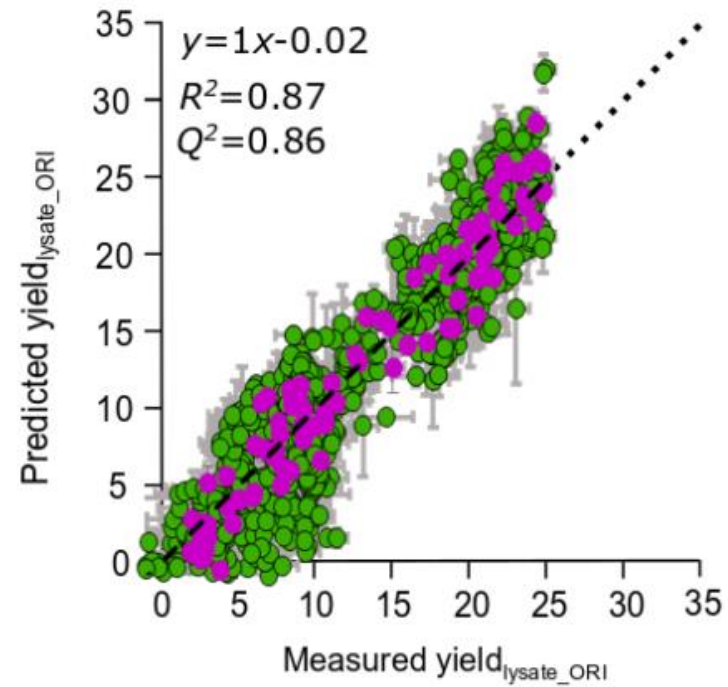
We provide a quick method to maximize protein production with any lysate



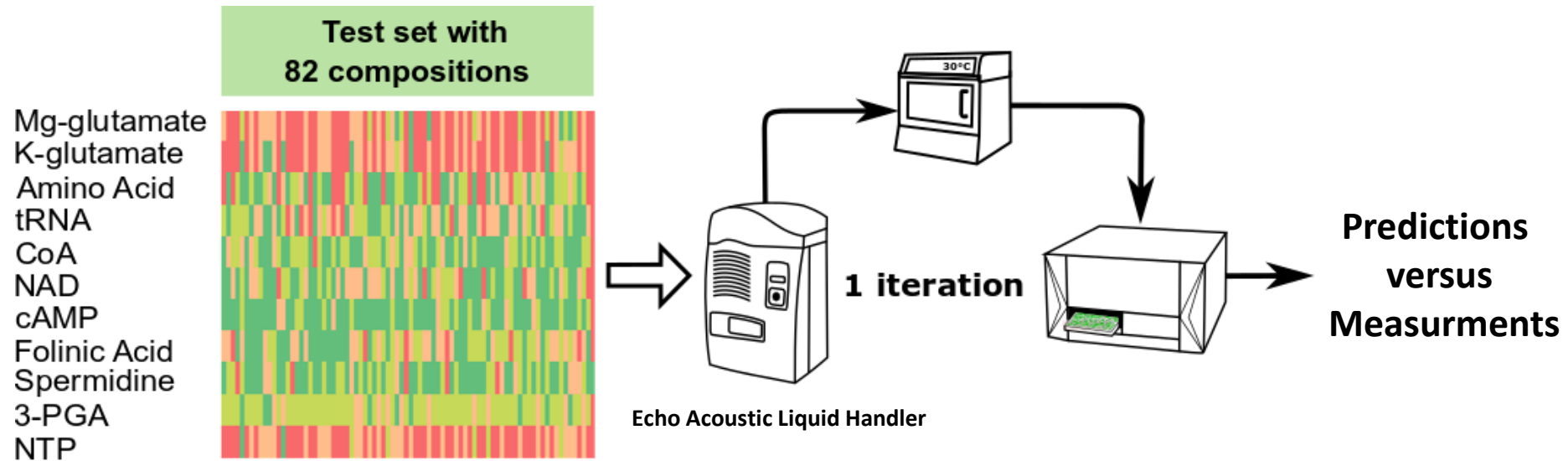
Training a machine learning algorithm with the most informative compositions

<https://github.com/brsynth>

102 cell-free compositions
to train (20) and test (82) our models efficiency

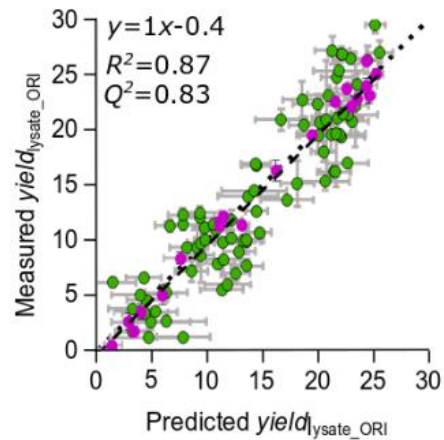


Prediction of the Yields in cell-free using home-made lysates

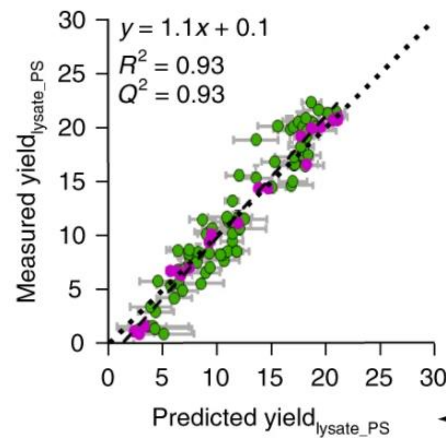


Predictions of cell-free efficiency (lysates from other experimentalists)

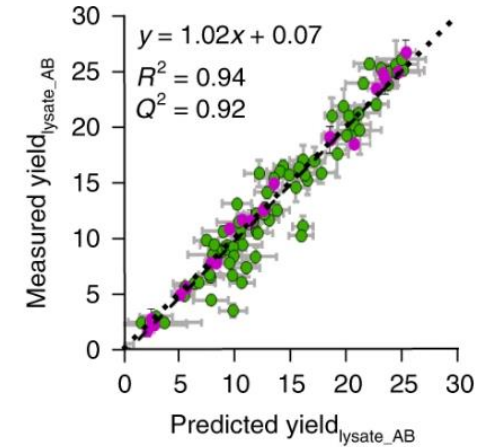
Lysate BL21 Ori



Lysate BL21 PS

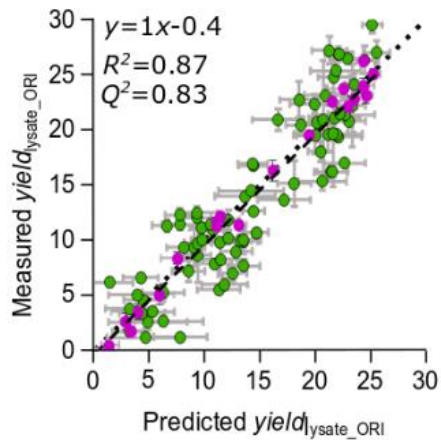


Lysate BL21 AB

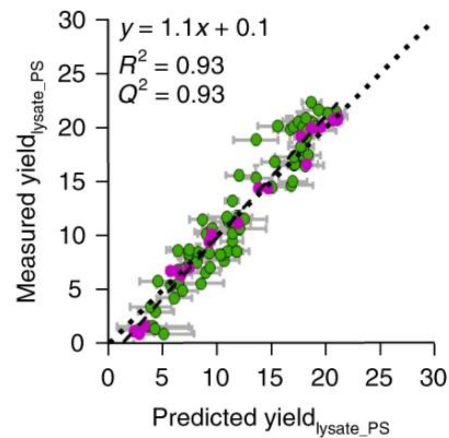


Predictions of cell-free efficiency (lysates from other experimentalists)

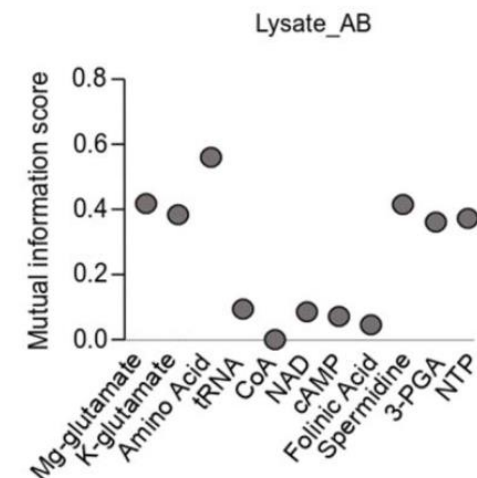
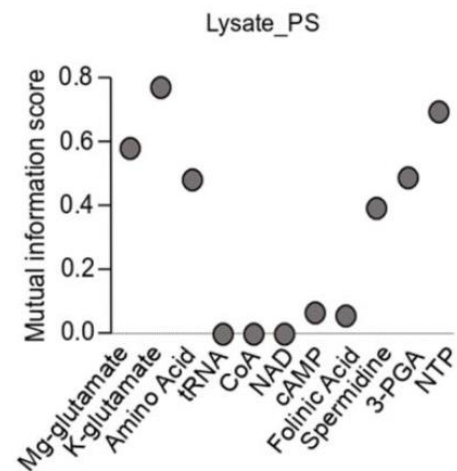
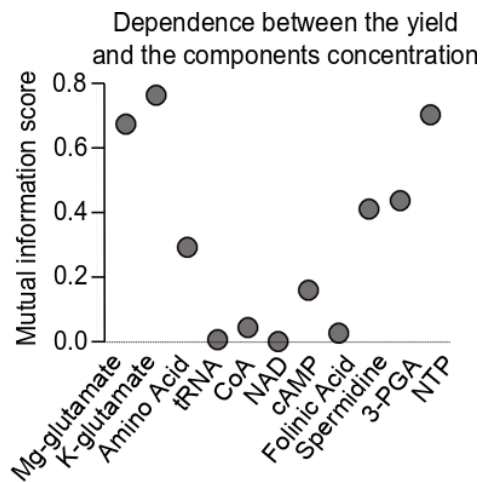
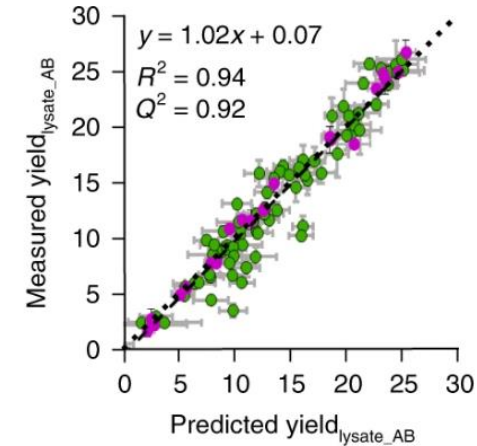
Lysate BL21 Ori



Lysate BL21 PS

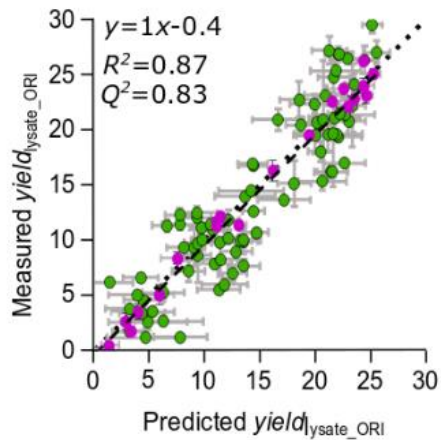


Lysate BL21 AB

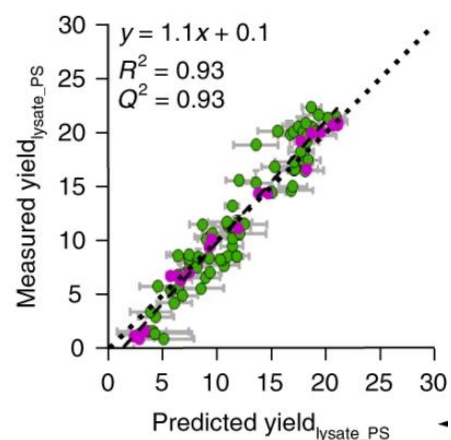


Predictions of cell-free efficiency (lysates with antibiotics and a different strain)

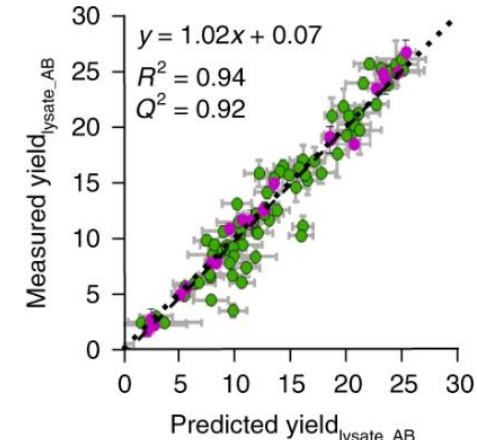
Lysate BL21 Ori



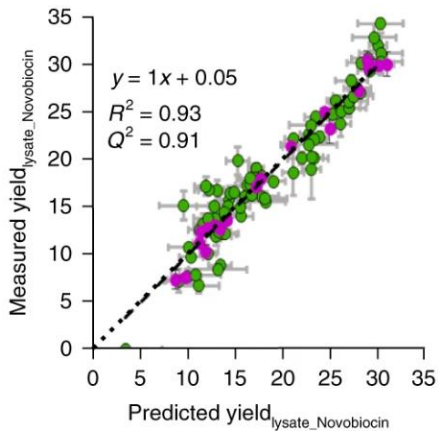
Lysate BL21 PS



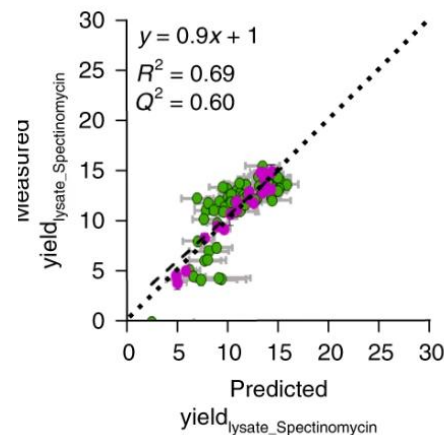
Lysate BL21 AB



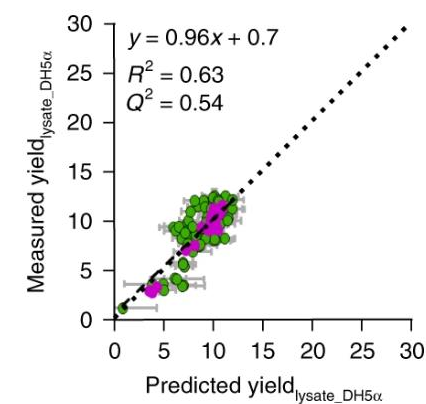
Novobiocin (impact on TX)



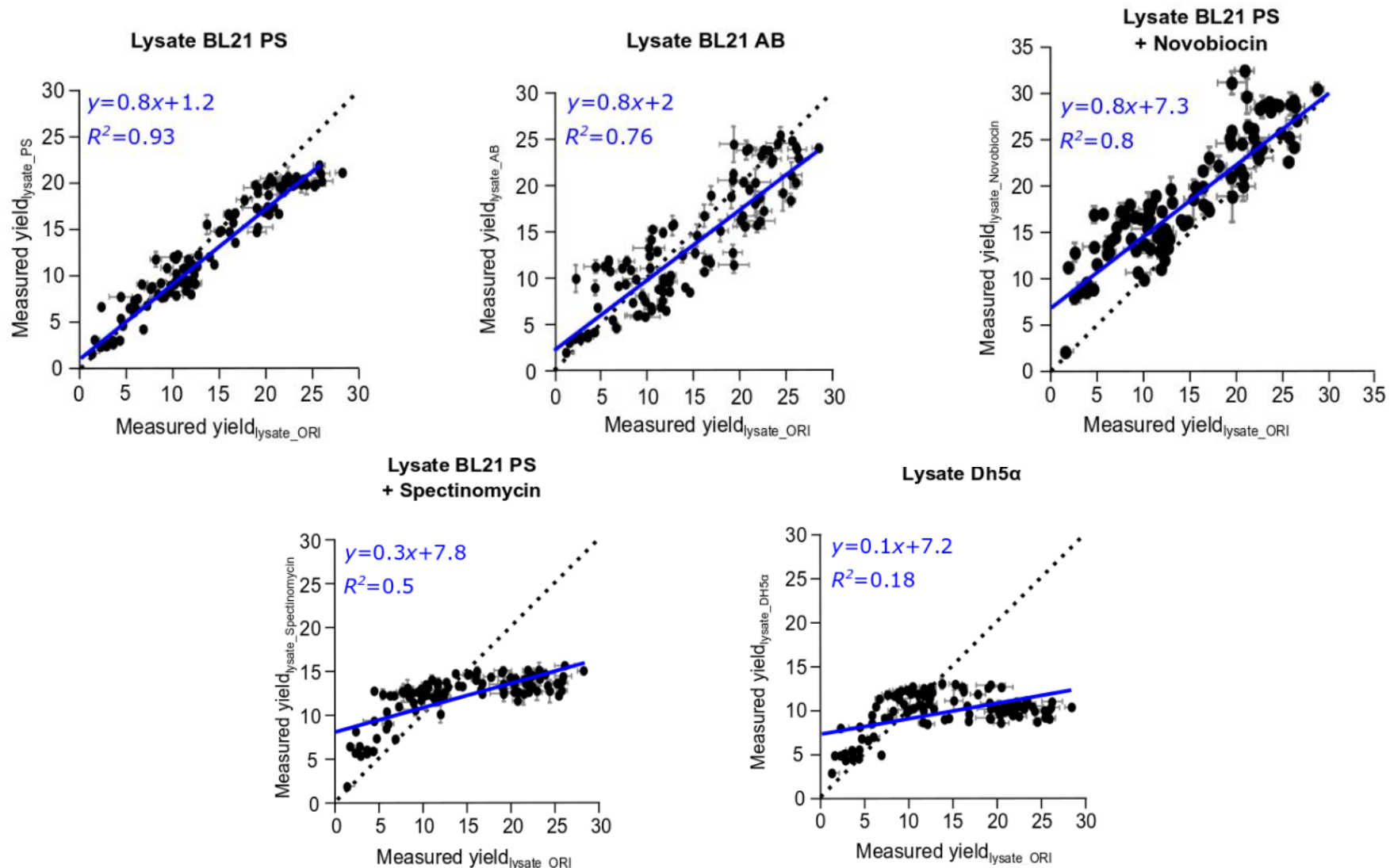
Spectinomycin (impact on TL)



DH5a



Lysates efficiencies vs original lysate efficiencies



Conclusion

Cell-free composition:

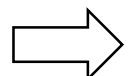
- We improved production of an home-made cell-free reactions without increasing the price
- We provided efficient predictions of protein production
- We highlighted the critical parameters involved in protein production

Lysate-independent maximization of protein production:

- A one step method to maximize protein production for home-made cell-free

Transcription vs translation:

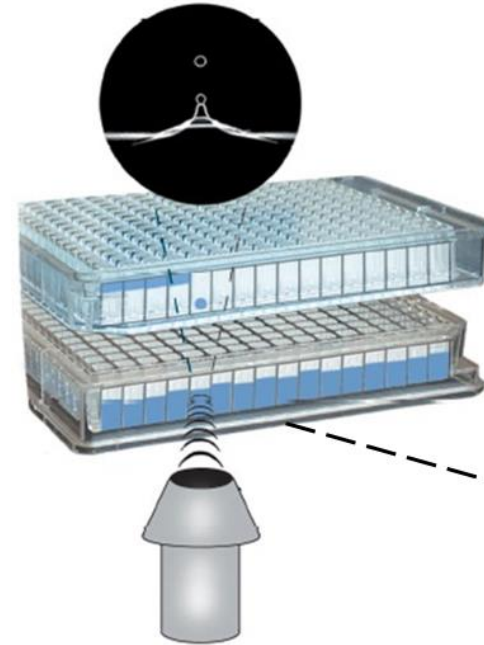
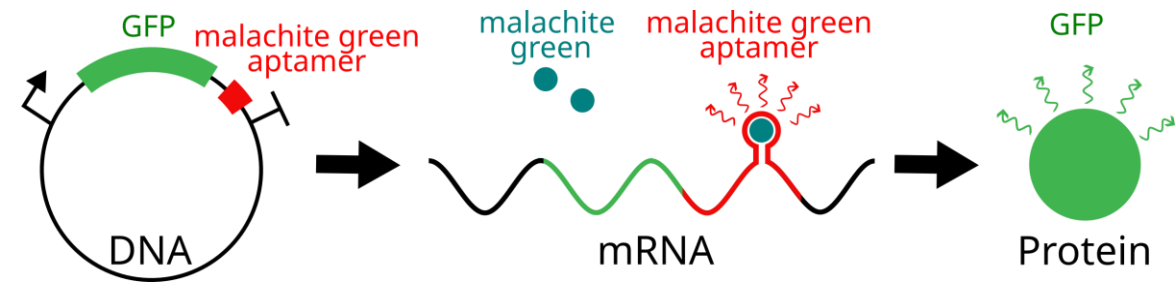
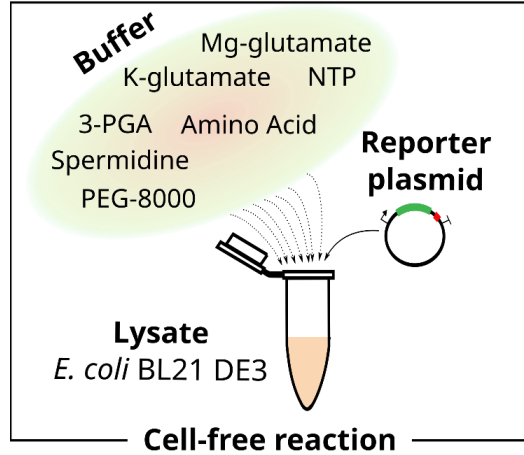
- Our data suggests that we mainly improved translation via composition optimization

 Our method can be extended to other bacterial-based cell-free (*B. subtilis*, *V. natrigens*...)

Maximize mRNA production for genome expression

Léa Wagner, An Hoang, Mahnaz Sabeti Azad, Olivier Delumeau, Jean-Loup Faulon, Matthieu Jules, **Olivier Borkowski***

Measurements of mRNA level in cell-free



4000 pipetting per plate
30 min to fill a plate
Volume of the order of nL

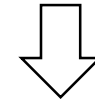


Combinatorial space to explore

Historical composition
from literature

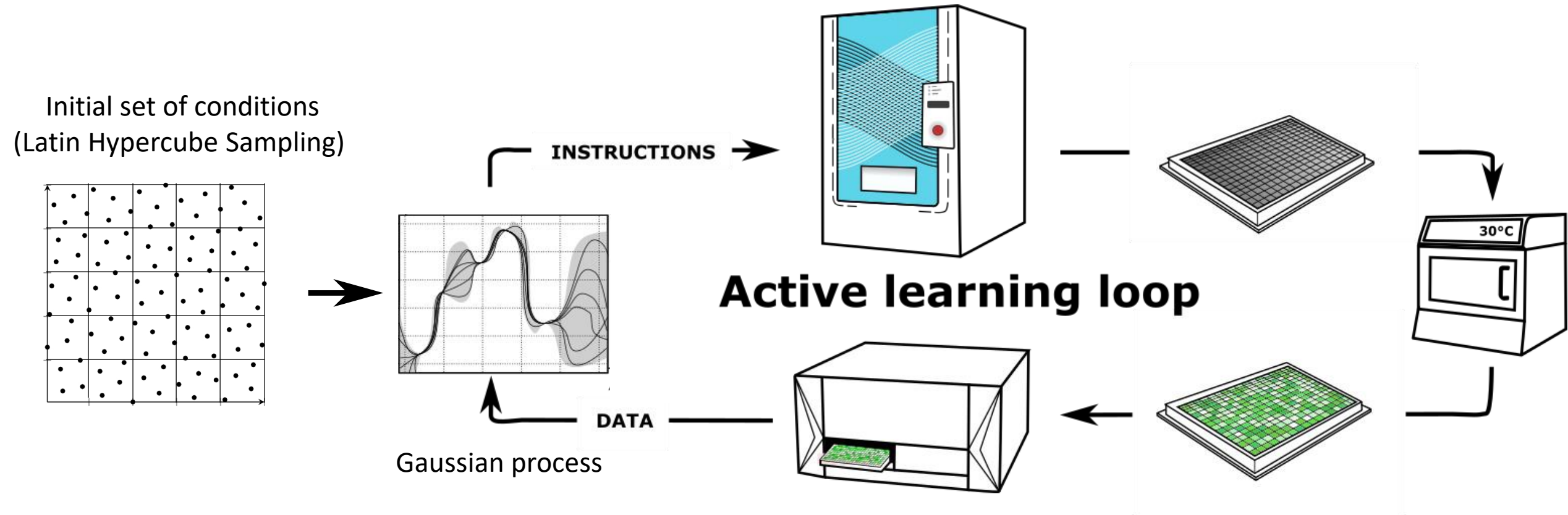
Component	Concentrations						
Mg- glutamate (mM)	0	1.5	3	4.5	6	9	
K- glutamate (mM)	0	40	80	120	160	240	
Amino acid (mM)	0	0.375	0.75	1.125	1.5	2.25	
Spermidine (mM)	0	0.25	0.5	0.75	1	1.5	
3- PGA (mM)	0	7.5	15	22.5	30	45	
NTP (mM)	0	0.375	0.75	1.125	1.5	2.25	
PEG- 8000 (%)	0	0.5	1	1.5	2	3	
ADN (nM)	1	2.8	4.6	6.4	8.2	10	

Combinatorial space = $4^{11} = 4\,194\,304$ compositions



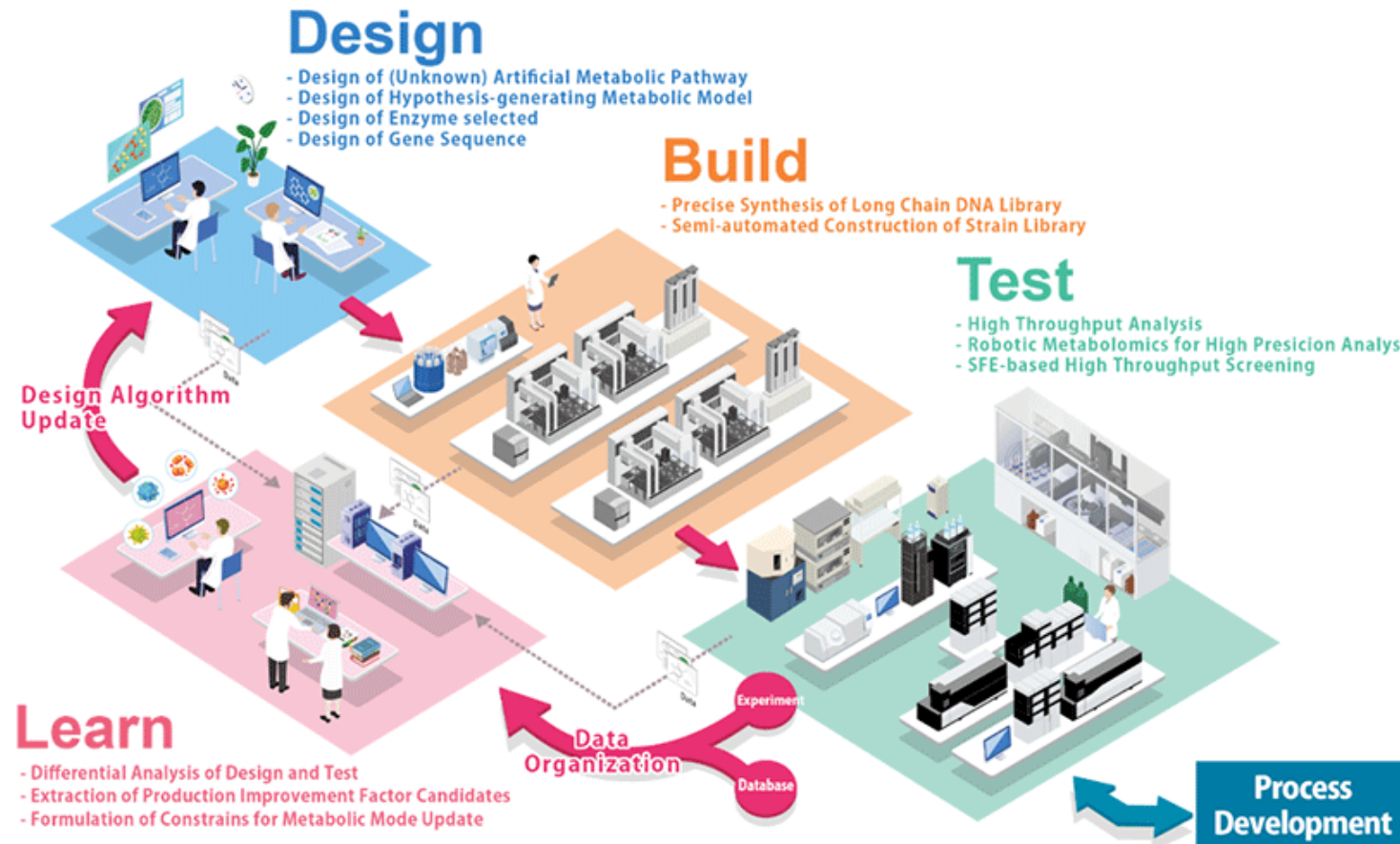
- Can we improve mRNA production as we did with the protein production?
- Does mRNA production correlated with protein production?
- Can we highlight the critical parameters involved in mRNA production?
- Can we obtained sufficient improvement for RNAseq analysis?

Exploration using active learning



Cell-free Biofoundry at INRAE jouy-en-josas

Part of the **Bioconvergence Paris Biofoundry** :
INRAE/Paris-Saclay Université + Sorbonne Université + Institut Curie + Genopole Evry



Domaine de Recherche et d'Innovation Majeur



Acknowledgements



Matthieu Jules

Léa Wagner

Olivier Delumeau



Bio-RetroSynth

Jean-Loup Faulon

Mathilde Koch

Amir Pandi

An Hoang



Institut Pasteur

Fabrice Agou

Agnès Zettor

TOM ELLIS LAB

Imperial College
London

Tom Ellis

Guy-Bart Stan Lab

Brooke Rothschild-Mancinelli

Francesca Ceroni

Michela Murgiano

Carlos Bricio

