Last advances in pathway engineering

Metabolic engineering and synthetic biology

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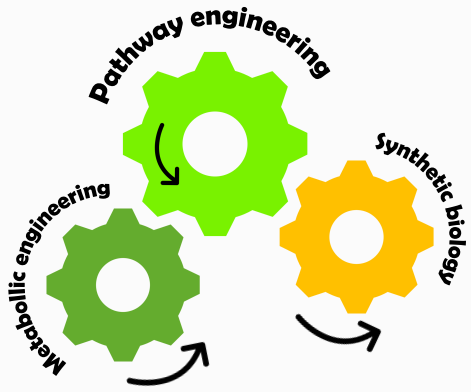
What is Pathway engineering?

The use of biological knowledge to design or re-design microorganisms' biochemical pathways to obtain biochemical or biofuels in a more sustainable way.



Metabollic engineering

Improvement of cellular processes by the modification of specific reactions and functions within the cell, or the introduction on new ones with recombinant DNA technology.

Synthetic biology

Design and engineering of biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems.

DBTL cycle

BIOINFORMATICS

Design: GOALS & LIMITATIONS

Build: MIX & MATCH

Genome	Cre-lox inducible system
Transcription	Promoter strength Toggle switch Sensor-Actuator system
Translation	Synthetic RBS Riboswitches
Protein enhancement	Directed evolution *de novo* design
Spatial organisation	Substrate channeling Synthetic scaffold Compartimentation

Test: APPLY

Learn: ADJUST & OPTIMIZE

Rational design of a synthetic Entner–Doudoroff pathway for improved and controllable NADPH regeneration

Chiam Yu Ng, Iman Farasat, Costas D. Maranas & Howard M. Salis. (2015)

Organism: *Escherichia coli*

Goal: NADPH cofactor regenaration rate improvement, which is a rate limiting compound in many anabolic reactions

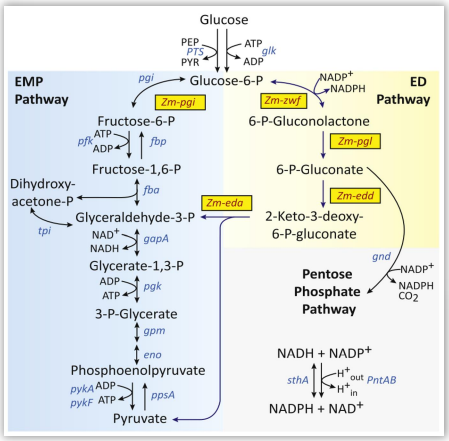


Figure 1

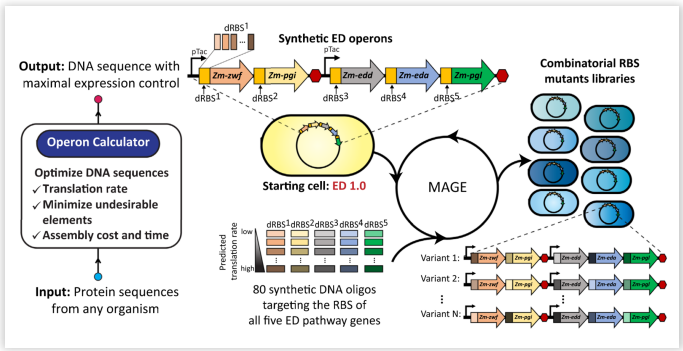


Figure 2

D Heterologous enzyme screening for efficient pathway → Entner–Doudoroff pathway *Zymomonas mobilis* heterologous genes (Zm-pgi, Zm-zwf)

→ Pathway with lower ATP production → more exergonic

→ Operon Calculator creates a drop-in module

→ RBS library calculator

B MAGE (multiplex automated genome Engineering) targets many locations on the chromosome for modification in a single cell or across a population of cells

T Strain screening

L Coupled to Carotenoid biosynthesis (97% increase)

Small expression changes of high turnover enzymes might lead to big changes

Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories

Yongjin J. Zhou, Nicolaas A. Buijs, Zhiwei Zhu, Jiufu Qin, Verena Siewers & Jens Nielsen. (2016)

Organism: *Saccharomyces cerevisiae*

Goal: Free fatty acid production (for alkane and fatty alcohol production)

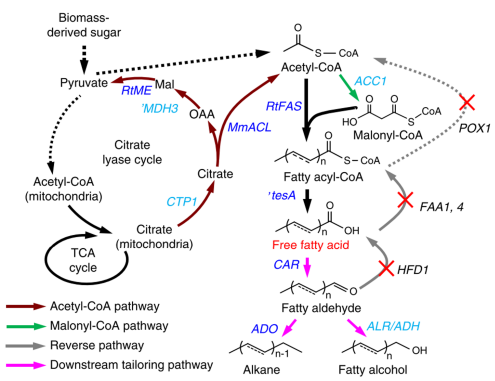


Figure 3

D From basic metabolism to specific product

→ Fatty acid activation and degradation disabled

→ Gene upregulation (ALR/ADH, ACC1)

→ Heterologous enzyme screening for efficient pathway (CAR, ADO)

B Integration in the genome

Pathway monitored and modified to avoid intermediate metabolite accumulation

L Failed enzyme fusion (CAR-ADH susbtrate channeling) due to low enzyme activity

T Gene deletion screening

Native promoters used

References

Ng, C. Y., Farasat, I., Maranas, C. D., & Salis, H. M. (2015). Rational design of a synthetic Entner–Doudoroff pathway for improved and controllable NADPH regeneration. Metabolic Engineering, 29, 86–96. https://doi.org/10.1016/j.ymben.2015.03.001

Zhou, Y. J., Buijs, N. A., Zhu, Z., Qin, J., Siewers, V., & Nielsen, J. (2016). Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories. Nature Communications, 7(May), 11709. https://doi.org/10.1038/ncomms11709

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