Modular screening system for protein production in *Aspergillus niger*

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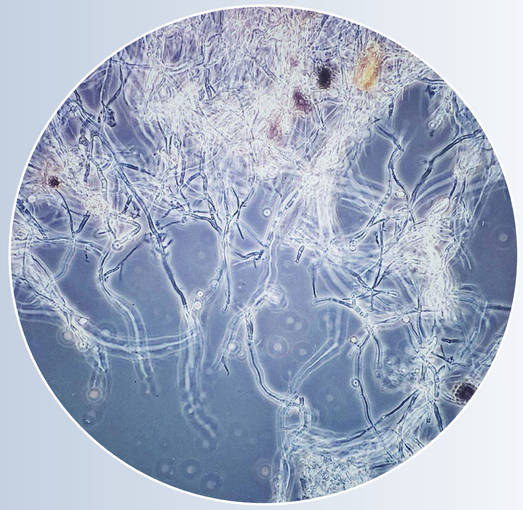

A. NIGER

Filamentous fungi	Robust strains
Organic acids, proteins, enzymes	High yields of products
Food-grade products	Complex proteins
Excellent secretion system	No downstream processing

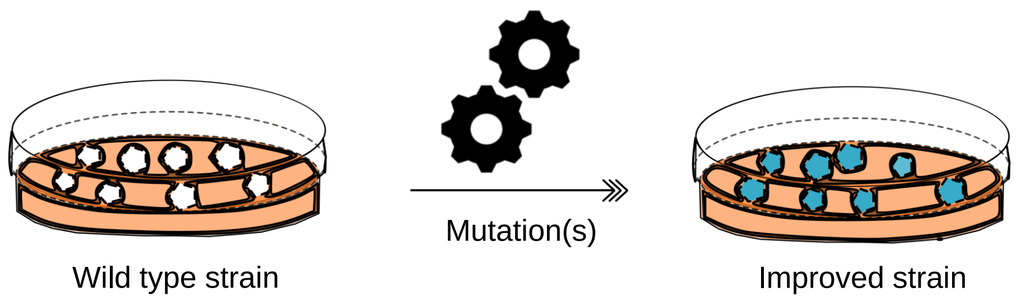

Fig. 1: Engineering of a protein-producing *A. niger* strain useable as universal expression platform.

SCREENING METHOD

Dual-luciferase reporter gene technology

- Microtiterplate scale for high-throughput screening
- Targeted and stable construct integration in *A. niger* genome
- Feasible and quantitative assay
  - Intracellular signal
  - Extracellular signal
- Generation of an *A. niger* secretion mutant library

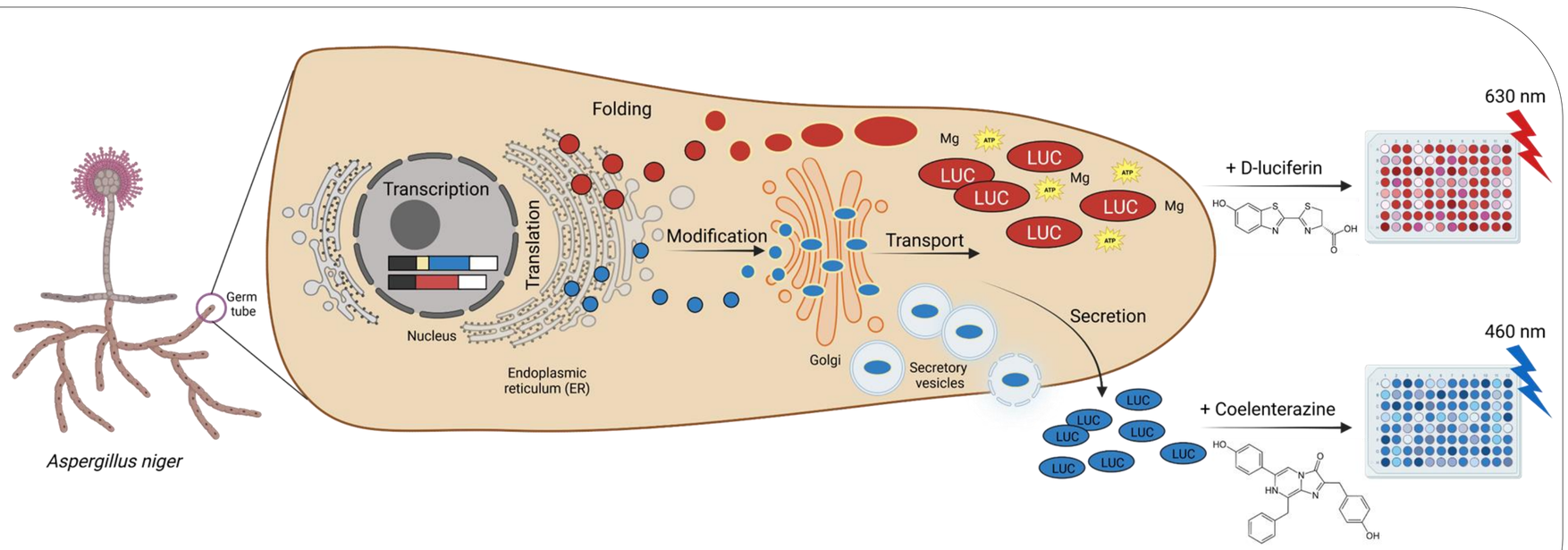

Fig. 2: High-throughput technology to screen improved *A. niger* strains and expression elements using two different luciferases (Luc). Red: Intracellular luciferase; Blue: Extracellular luciferase (created with BioRender.com and modified from Li *et al*. 2020 and Wang *et al*. 2020).

OPTIMIZATION

Strategies for optimizing expression of recombinant proteins in *A. niger*

- Luciferase expression analysis in glucoamylase (*glaA*) locus
- Signal peptide (SP) modfications using directed evolution approaches
- Rational promoter replacement and mutations
- Modifications of the open reading frame (protein fusions, codon optimizations)

SIGNAL PEPTIDE ANALYSIS

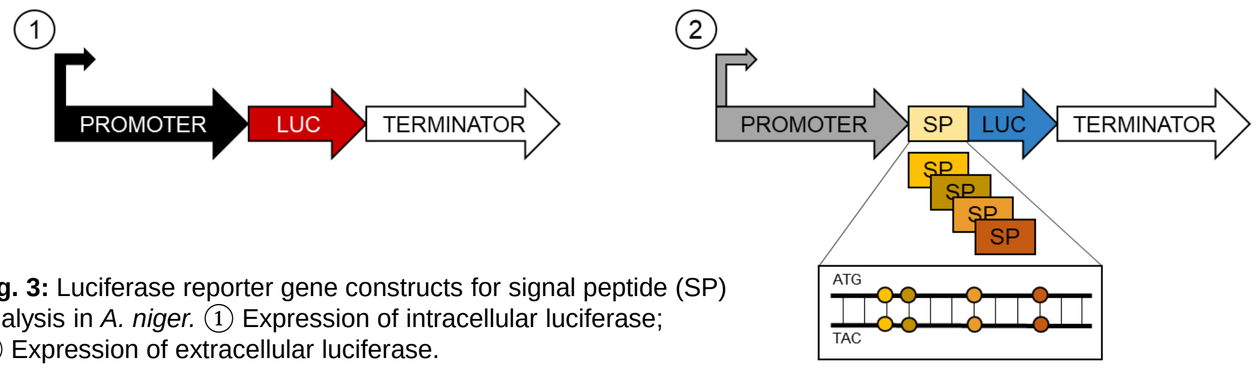

Fig. 3: Luciferase reporter gene constructs for signal peptide (SP) analysis in *A. niger.* ① Expression of intracellular luciferase; ② Expression of extracellular luciferase.

PROMOTER ANALYSIS

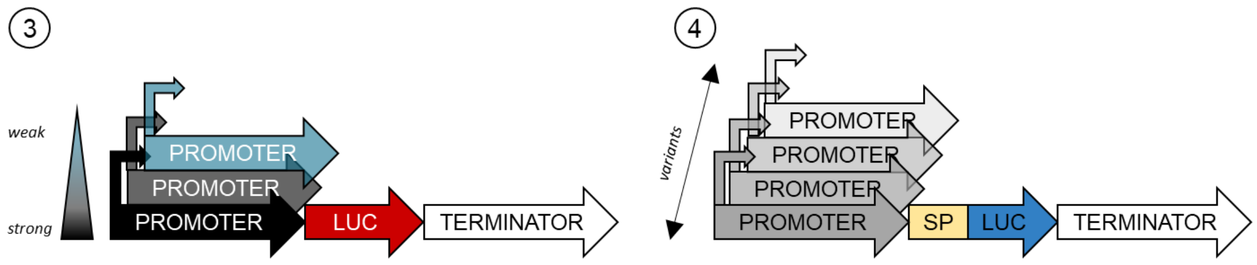

Fig. 4: Luciferase reporter gene constructs for promoter analysis in *A. niger.* ③ Expression of intracellular luciferase with different promoter strength; ④ Expression of extracellular luciferase with different *glaA* promoter variants.

PROTEIN PRODUCTION

Constructing a smart multipurpose microbial cell factory:

Producing *novel* recombinant proteins successfully often is challenging, resource- and time-consuming.
Therefore, *A. niger* mutant libraries are needed to understand the "adjusting screws" to produce high yields of recombinant proteins.
After generating an *A. niger* secretion mutant library as case study, the system will be transferred and tested to further proteins of interest.
The technology can be integrated into bio-regenerative life support systems for the autonomous production of e.g., food, food proteins, food enzymes and other complex biocatalysts on earth as well as in deep-space.

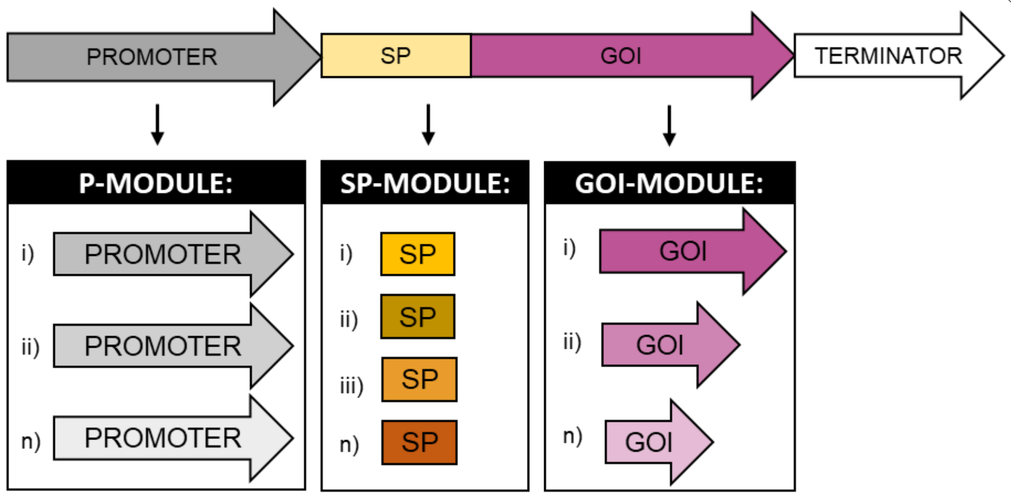

Fig. 5: Modular gene expression cassettes for recombinant expression of proteins of interest in *A. niger.* GOI = gene of interest.

Li, C., Zhou, J., Du, G., Chen, J., Takahashi, S., & Liu, S. (2020). Developing Aspergillus niger as a cell factory for food enzyme production. *Biotechnology Advances*, *44*, 107630.
Wang, Q., Zhong, C., & Xiao, H. (2020). Genetic engineering of filamentous fungi for efficient protein expression and secretion. *Frontiers in Bioengineering and Biotechnology*, *8*, 293.

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