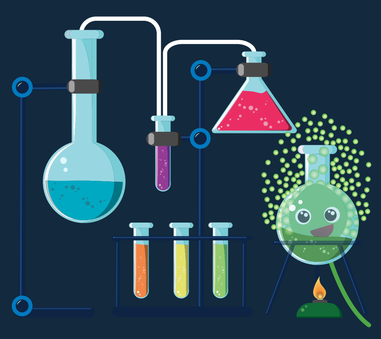

Library of Engineered Aspergillus Promoters
LEAP

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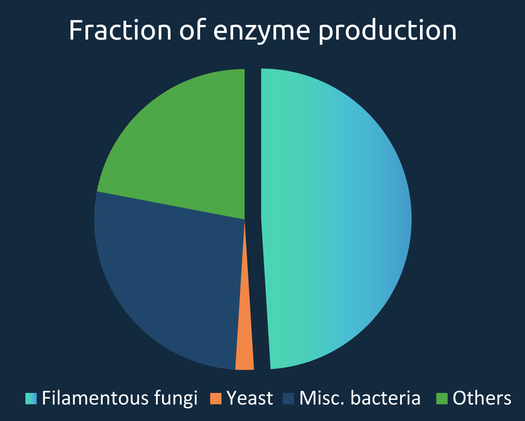
Introduction

Filamentous fungi are tremendously useful production organisms [1][2], yet very few basic parts, e.g. promoters, are publically available in academia, iGEM or industry… Until now!
We have designed a software tool that can generate promoters for any given organism - or multiple organisms, and tested it in *Aspergillus niger* as a proof-of-concept.



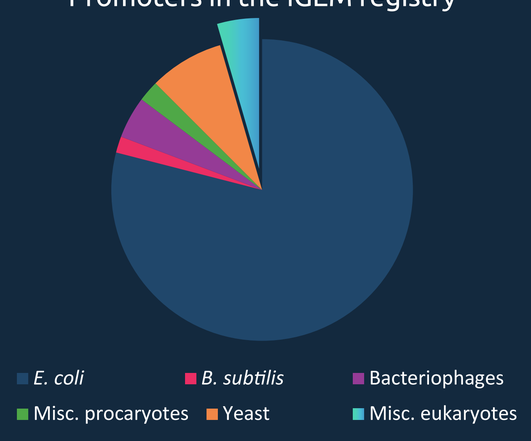


Fig 1: The pie charts illustrate the scarcity of fungal promoters in the Registry compared to the predominance of enzymes produced by filamentous fungi.

Methods

The synthetic promoters were inserted on a test platform (BBa_K3046009) and evaluated in *A. niger*. From this, the fluorescence of mCherry was measured to quantify the expression of the promoter.

Model & Software

Expression data analysis was conducted which led to the selection of a number of genes of interest, shown in Figure 6. To produce synthetic promoters with the attributes illustrated in Figure 7, our software, `proHMMoter`, constructed a sequence model of these genes' promoters across *Aspergillus* spp., and used this model to generate the promoter sequences. Figure 8 displays the process the program follows.

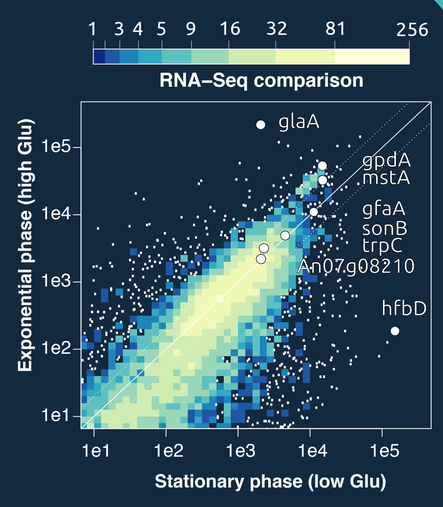


Fig 6: This heatmap visualizes the RNA-seq data used[3], showing read counts at stationary and exponential phase and the final selection of genes.

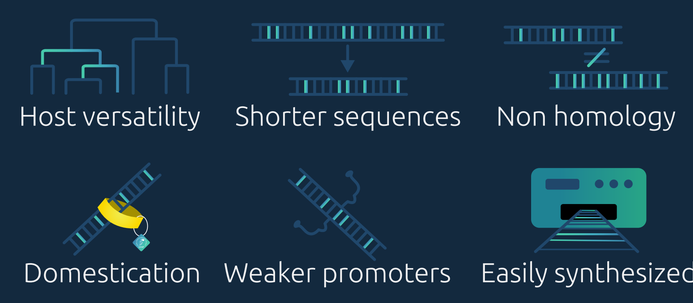


Fig 7: This figure illustrates some of the benefits of our approach to synthetic promoter generation, and synthetic promoters in general, as enabled by our software.

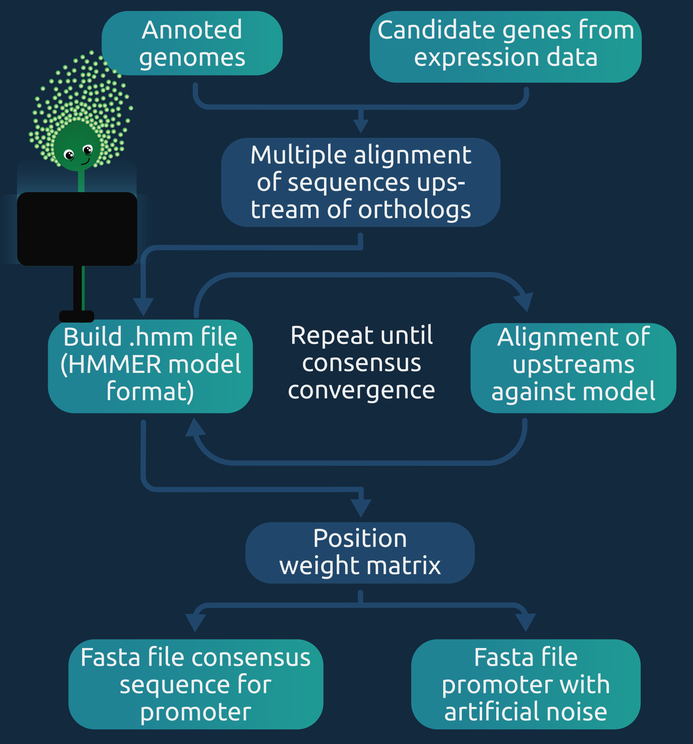


Fig 8: The program identifies orthologs of protein-coding genes. It then performs multiple sequence alignment of their upstream sequences as a starting point for expectation maximization, alternating between building a HMM and aligning the upstream sequences to it, to build a HMM while trimming any nonconserved ends. Finally, it extracts a position-weight matrix and uses that information to generate both sampled and consensus domesticated synthetic promoters.

Results

We designed and validated promoters that function in different growth stages, as seen in Figure 2. Figure 3 demonstrates that our promoters can work consistently across scales. Furthermore, due to the diversity of the generated promoters, we were able to create an entire ladder of promoters with different strengths, which is shown in Figure 4. The promoter strengths were measured by the expression of mCherry, which can be seen in Figure 5.

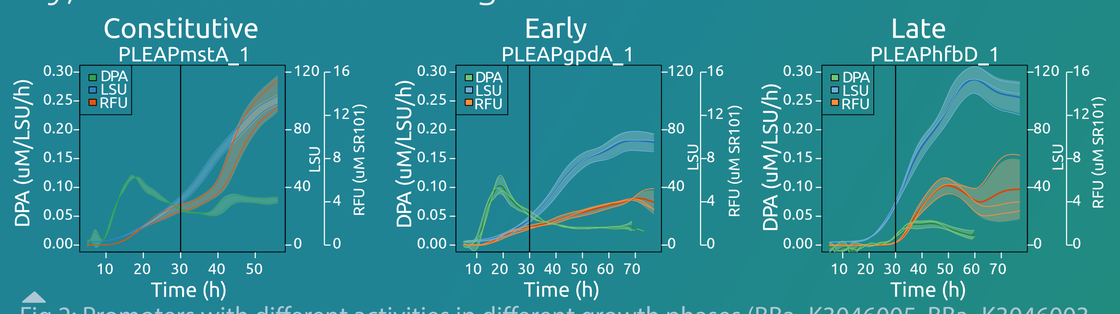


Fig 2: Promoters with different activities in different growth phases (BBa_K3046005, BBa_K3046003, BBa_K3046008)

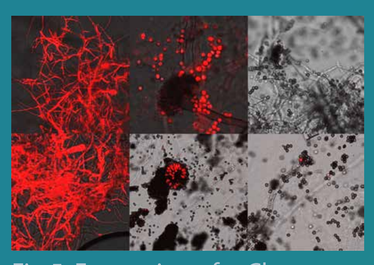


Fig 5: Expression of mCherry as seen in a confocal microscope.

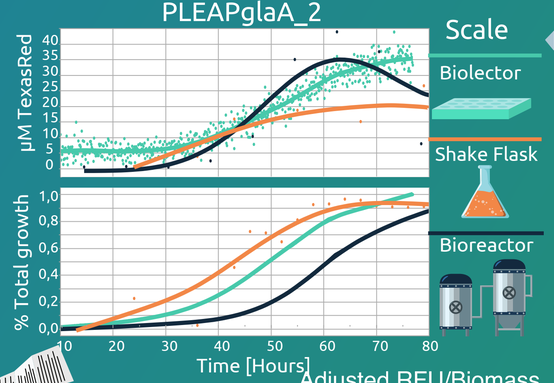
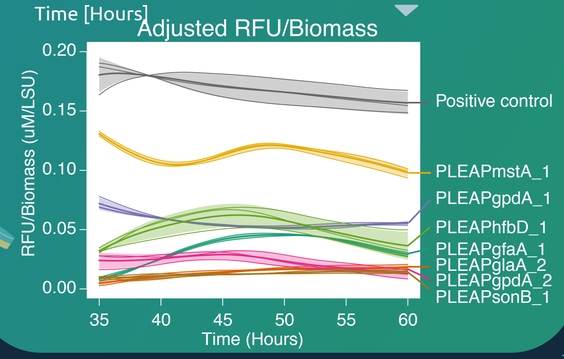


Fig 3: Consistably scalable promoters (K3046001)

Fig 4: Promoter ladder (BBa_K3046001-BBa_K3046008)



Integrated Human Practices

From the very beginning of the project, we consulted with experts from academia and industry. The consultations led to a variety of insights, see Figure 9.

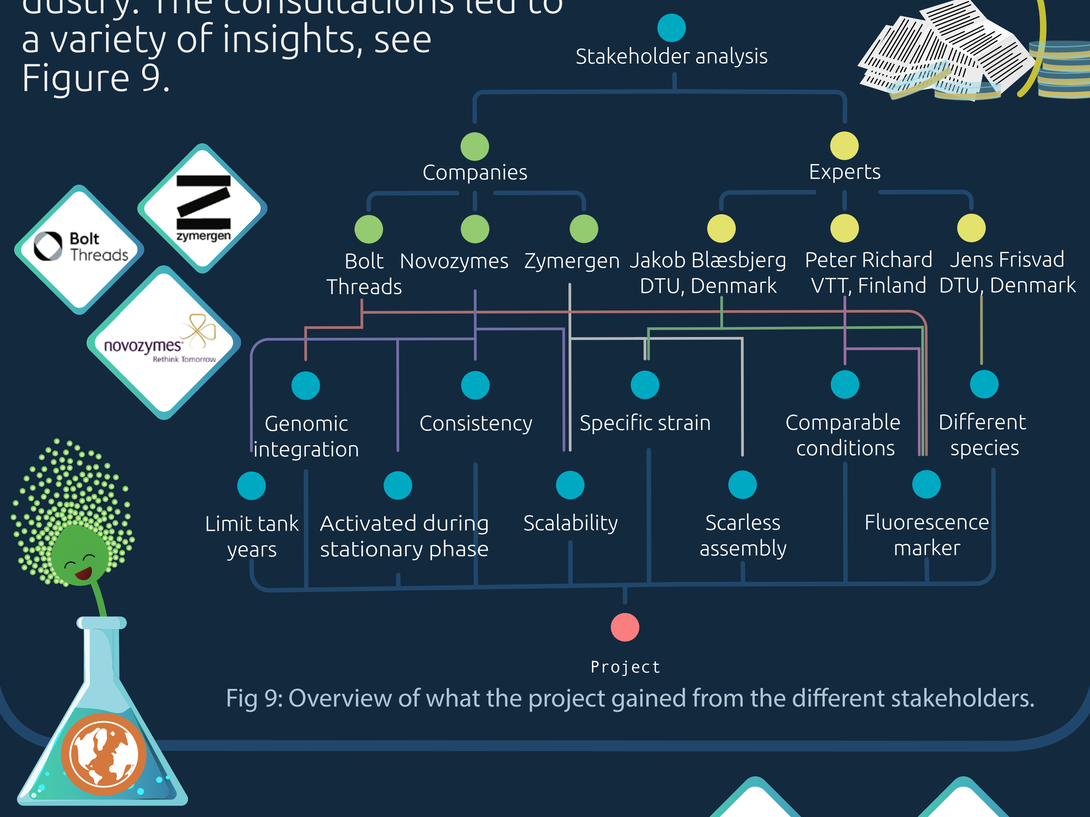


Fig 9: Overview of what the project gained from the different stakeholders.

Achievements

- Developed software to construct promoters based on homology, for any given organism - validated by the Brown-Stanford-Princeton team.
- Expanded the synthetic biology toolbox for filamentous fungi - by making more promoters and protocols available.
- Designed a platform (BBa_K3046009) for promoter testing in filamentous fungi.
- Proved that our synthetic promoters work in vivo in *A. niger*, creating a promoter ladder that everyone can use.
- Tested the consistency of several promoters (BBa_K3046001-BBa_K3046008) of varying strengths and dynamics at multiple scales.

Sources
(1) Novozymes, "The Novozymes Report 2017," Novozymes A/S, 2016.
(2) S. Siddiqui, Protein Production: Quality Control and Secretion Stress Response. Elsevier B.V., 2016.
(3) P. Schape et al., "Updating genome annotation for the microbial cell factory Aspergillus niger using gene co-expression networks.," Nucleic Acids Res., vol. 47, no. 2, pp. 559–569, Jan. 2019.