Engineering eukaryotic transcription in yeast: towards the minimal core promoter

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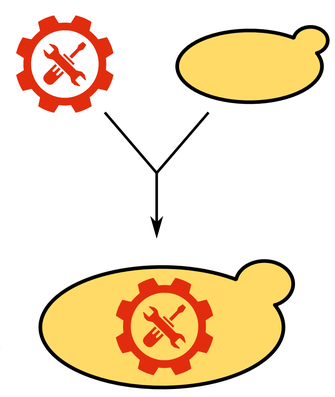
Introduction

Need for tools to fine tune pathways in yeast

Developping robust industrial production strains remains challenging. The transfer of heterologous pathways in microorganisms often leads to metabolic burden and changes in metabolic fluxes. To circumvent these issues, the native metabolism and the introduced heterologous pathway have to be properly balanced. Especially in eukaryotic hosts, good techniques to fine tune (heterologous) pathways remain a bottleneck.

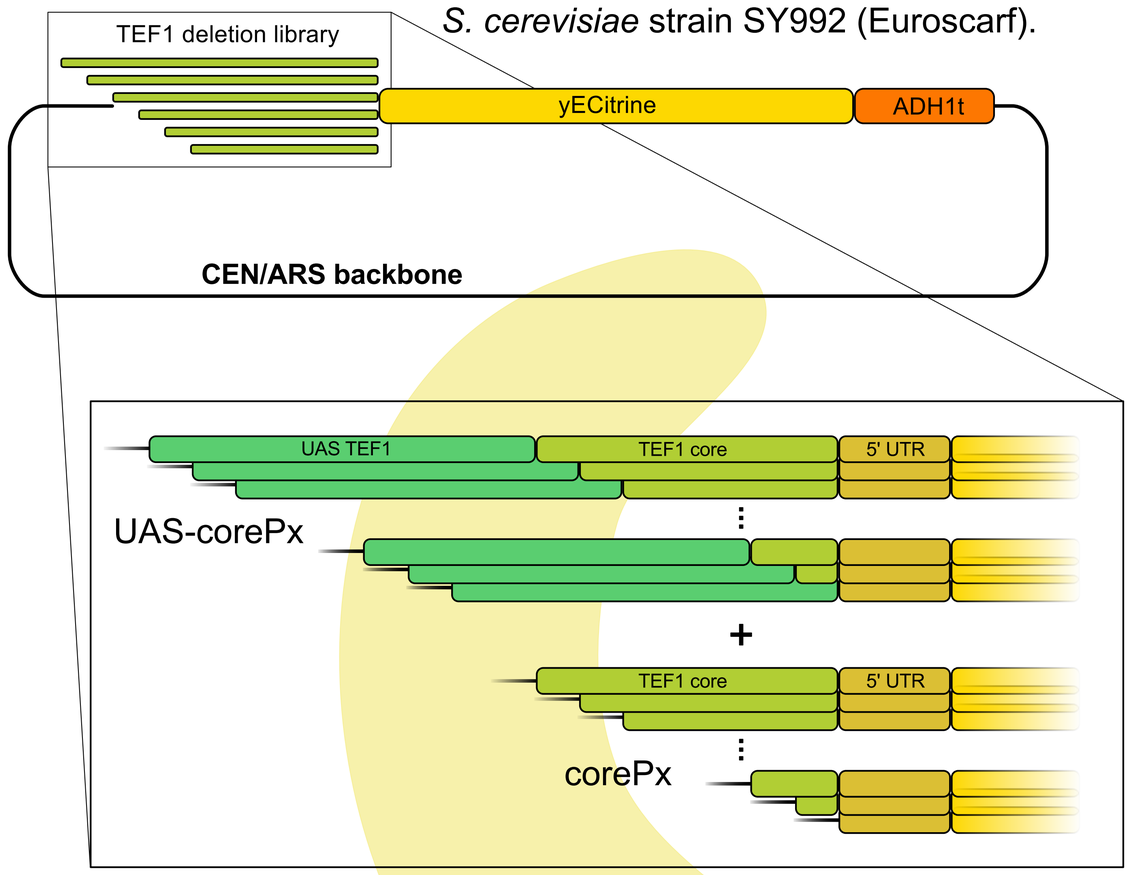


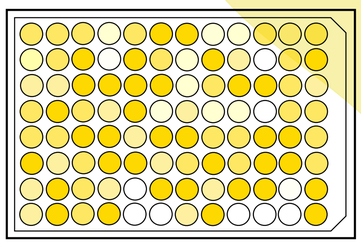
Varying gene transcription levels

Pathway finetuning is often performed by the creation of promoter libraries. However, borders and structures of eukaryotic promoters are mostly not well defined making the modification and search for 'easy to handle', standardizable promoters difficult. Therefore, we are working on a 'minimal length' promoter library to facilitate eukaryotic synthetic biology. The strong constitutive *TEF1* promoter of *Saccharomyces cerevisiae* was used as start point to determine the minimal core promoter length which gave sufficient expression.

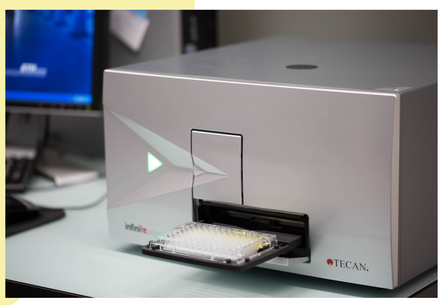
Methods

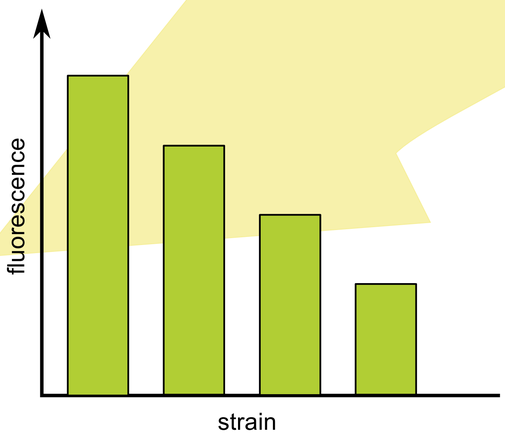
Promoter library cloned in a YCp expression plasmid with CPEC [1] and transformed in *S. cerevisiae* strain SY992 (Euroscarf).





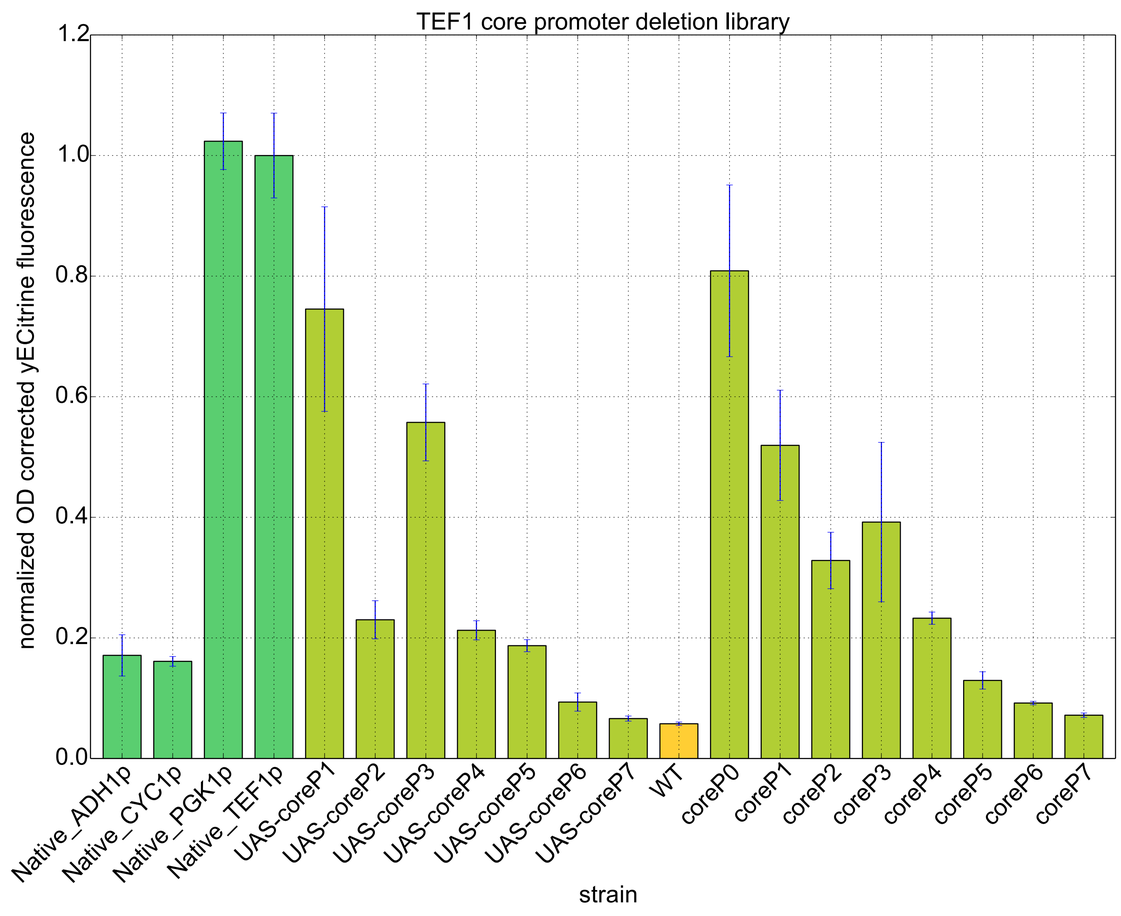
OD at 600 nm (OD_{600}) and yECitrine fluorescence (ex. 502 nm, em. 532 nm) are measured in 96 well plates with an MTP reader.





Afterwards, fluorescence is corrected by OD_{600} for every strain. This value can be used as a measure for promoter strength.

Results



OD corrected yECitrine fluorescence for the constructed *TEF1* core promoter library. The data was normalized to the *S. cerevisiae* native *TEF1* promoter. Also the weak *ADH1* and *CYC1* promoter, together with the strong *PGK1* promoter were used as references. The strain with an empty YCp plasmid is defined as wild type (WT).

Conclusions

A decline in promoter strength is visible when reducing the length of the *TEF1* core promoter.

No major difference is visible in the presence or absence of the *TEF1* UAS. Optional, UASs from other promoters can be placed in front of the *TEF1* core promoter library to create a larger expression range.

The coreP5 has more or less the same strength as the *ADH1* and *CYC1* promoter and is the shortest promoter giving a fluorescence significantly higher than the 'WT'. By randomizing this core promoter sequence, a minimal length promoter library with a range in expression levels will be made.

In the future, this promoter library can be used to vary and balance expression levels in heterologous pathway building in *S. cerevisiae*.

Acknowledgements

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References

[1] Quan, J., & Tian, J. (2009). Circular polymerase extension cloning of complex gene libraries and pathways. PloS One, 4(7), e6441