Engineering transcription in *S. cerevisiae* through random assembly of an UAS library

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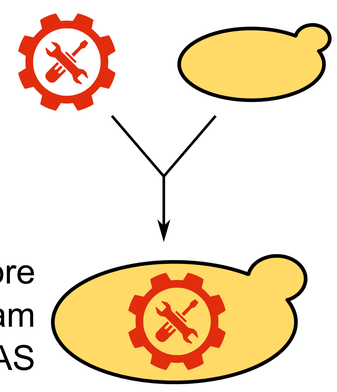
Introduction

Need for tools to fine tune pathways in yeast

Developing 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.

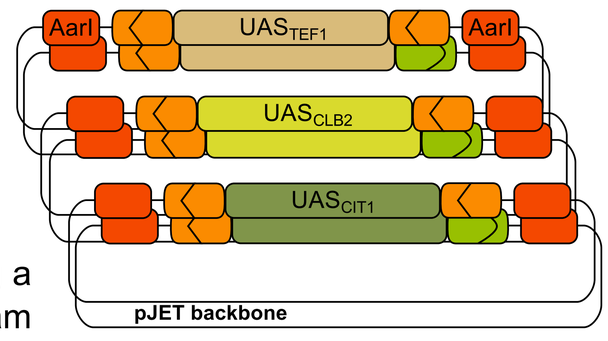


Varying gene transcription levels

The yeast promoter is typically divided in an upstream part, carrying the transcription factor binding sites, and a downstream core promoter needed for binding RNA polymerase II. Gene transcription can be modified by varying the numbers and types of upstream activating sequences (UASs) [1]. Here, we present a one pot reaction, based on Golden Gate (GG) [2], for generating a random UAS library upstream of the *TEF1* core promoter. The library is made of three earlier defined parts [1]: UAS_{TEF1}, UAS_{CLB2} and UAS_{CIT1}.

Methods

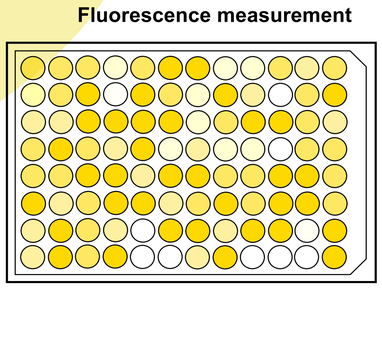
GG carrier vectors containing the UAS parts and type IIs AarI restriction enzyme sites.

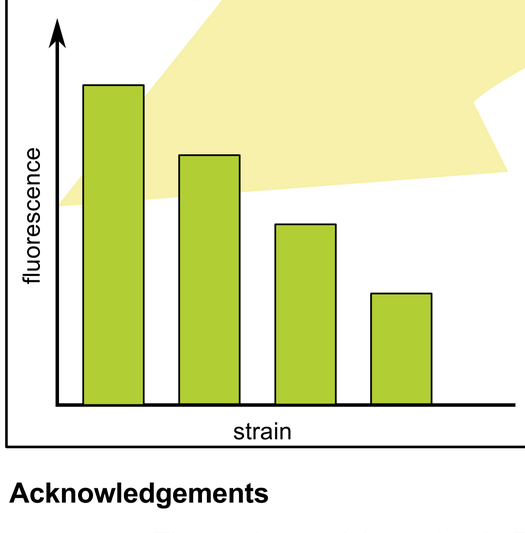


GG destination vector containing a SacBR counterselection upstream of the *TEF1* core promoter.

In a one pot GG reaction, AarI delivers the CEN/ARS backbone and UAS parts, T4 DNA ligase ligates the sticky ends to the final expression vectors.

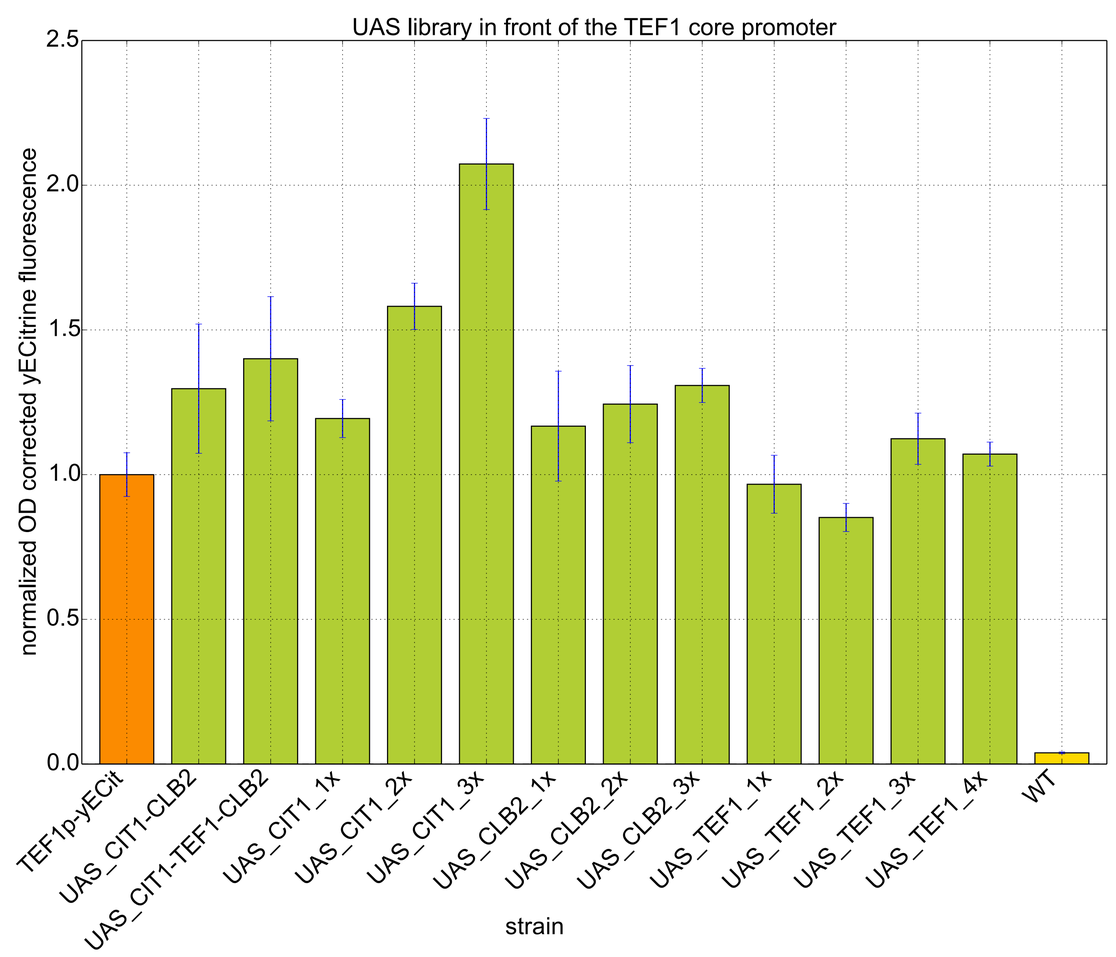
OD at 600 nm (OD_{600}) and yECitrine fluorescence (ex. 502 nm, em. 532 nm) are measured in 96 well plates with an MTP reader.





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

Results



Preliminary results of OD corrected yECitrine fluorescence for a first constructed UAS library in front of the *TEF1* core promoter. The data was normalized to the *S. cerevisiae* native *TEF1* promoter (TEF1p-yECit). The strain with an empty YCp plasmid is defined as wild type (WT). Error bars are standard deviations of the mean (4 biological replicates).

Conclusions

UAS_{CIT1} and UAS_{CLB2} upstream of the *TEF1* core promoter seem to increase expression compared to the WT *TEF1* promoter.

One or more UAS_{TEF1} upstream of the *TEF1* core promoter does not significantly increase expression. This observation is confirmed in the strains with the combination UAS_{CIT1}-UAS_{CLB2}, where an extra UAS_{TEF1} does not make a significant difference.

Adding an extra UAS_{CIT1} significantly increases yECitrine expression. Three UAS_{CIT1} even led to a doubling of promoter strength. UAS_{CIT1} seems to be the strongest building block to enhance expression.

Until now, we were not able to assemble more than four UASs with this method. Also, combining different UASs seems more difficult than fusing identical UASs. Possibly, varying concentrations of carrier and desination vectors can overcome this obstacle.

Acknowledgements

AGENTSCHAP INNOVEREN & ONDERNEMEN

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References

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[2] Engler, C., Kandzia, R., & Marillonnet, S. (2008). PLoS ONE, 3(11), e3647.