Establishing Saccharomyces cerevisiae as platform for isoprenoid production

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Directed Evolution towards Increased Isoprenoid Production in *Saccharomyces cerevisiae*

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Introduction

Organic synthesis of isoprenoids often results in low yields due to their complex structure. This makes production in large scale, by organic synthesis economically unfeasible. Microbial production can easily be scaled to meet current demands and it is also an environmental benign production method compared to organic synthesis. Thus it would be attractive to engineer a microorganism to produce high amounts of IPP and other immediate prenyl precursors such as geranyl pyrophosphate, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, thereby establishing it as a microbial platform for heterologous expression of isoprenoid genes (see figure 1).
This project is focused on creating diversity within a lycopene producing *S. cerevisiae* strain by construction of gDNA-, cDNA-, and transposon-libraries. The diversified population of *S. cerevisiae* clones will afterwards be screened using the isoprenoid molecule lycopene as a model compound, hereby enabling the isolation of phenotypes producing higher amounts of isoprenoid. This will elucidate novel genetic targets for increasing isoprenoid production in *S. cerevisiae*.

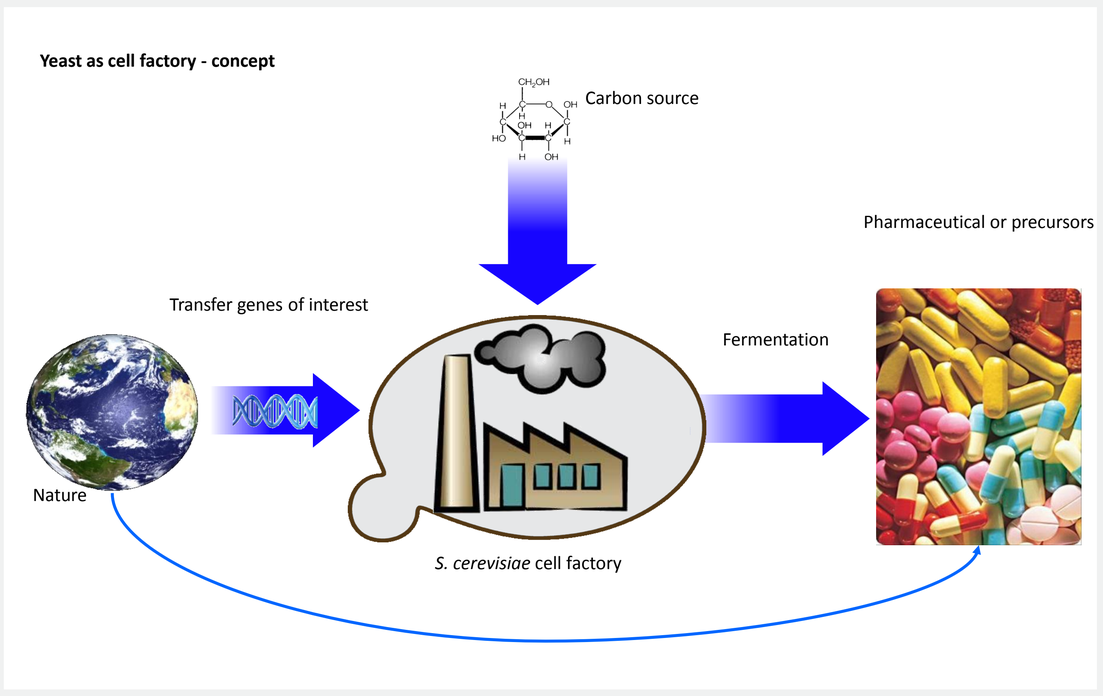

Figure 1: Setup of *S. cerevisiae* as microbial production platform to produce secondary metabolites. By transferring the genes encoding the isoprenoids of interest from its native host to a *S. cerevisiae* strain expressing them heterologous, it becomes possible to construct a microbial production platform for production of isoprenoids, which could be used as pharmaceuticals or precursors instead of having to rely on extraction from native sources (thin curved arrow). By fermentation of the constructed strain it will be possible to convert cheap carbon sources such as glucose into valuable products.

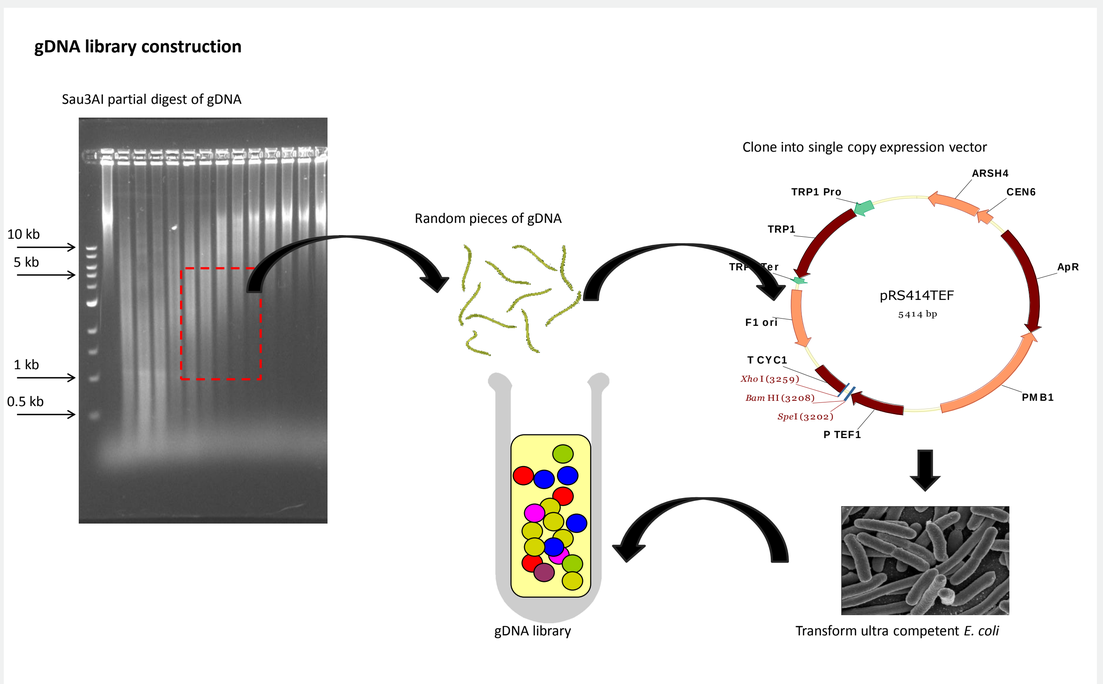

Figure 2: Method for construction of gDNA library. In order to construct gDNA libraries it is essential to obtain randomly digested gDNA. This can be done by treating high concentration gDNA with a 4 bp recognition endonuclease for a defined period of time. To find the correct amount of enzyme needed to obtain a partial digest a dilution series is setup and the reactions with the desired size range is subsequently pooled wherefrom the size selected gDNA pieces are purified. The random pieces are then ligated into an expression cassette (*TEF1p*-gDNA-*CYC1t*) in a single copy vector (pRS414TEF from Mumberg et al. 1994) followed by transformation of ultra competent *Escherichia coli* cells. The final gDNA library is purified from the *E. coli* transformants. This library can be used for transformation of *Saccharomyces cerevisiae*. This method is based on Jauert et al. 2005 and Liu 2002.

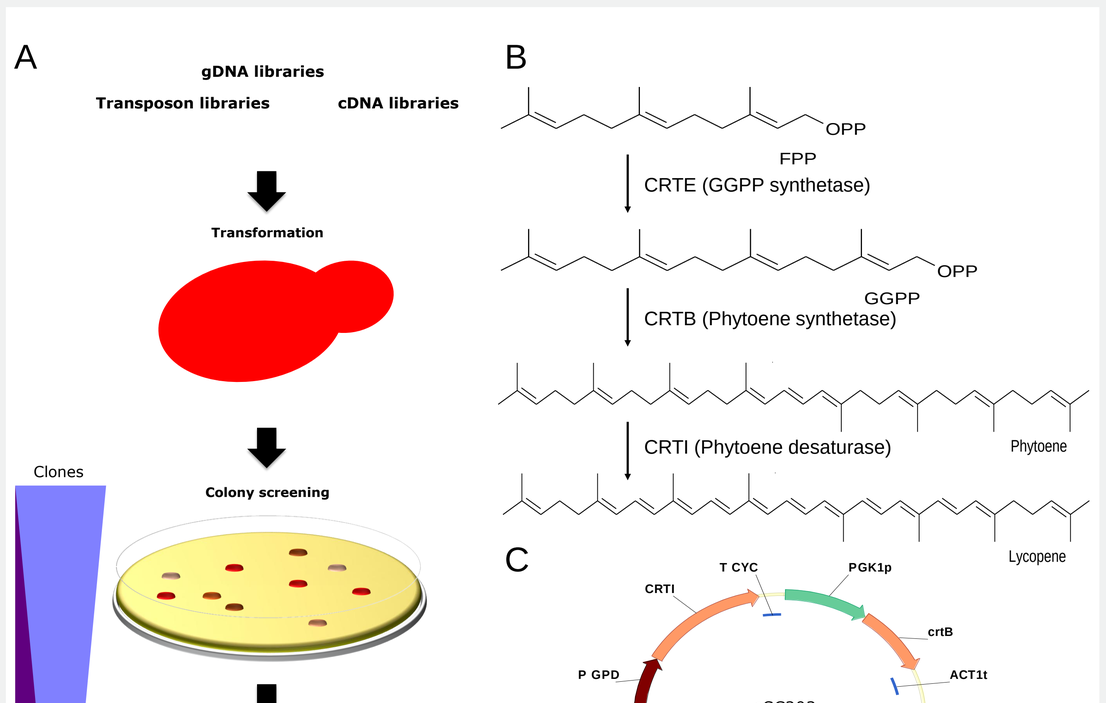

Figure 3: A. Library screening based on lycopene colony coloration. The colonies exhibiting phenotypes with more intense red coloration compared to the wild type are producing more lycopene than the wild type, hence the underlying genotype could result in a higher flux towards and through the mevalonate pathway hereby increasing the precursor availability or in another way be beneficial for isoprenoid production. The transformants which shows intensified coloration can be further analysed resulting in identification of the best candidates from which the underlying genotype can be elucidated. **B.** Biosynthetic pathway for converting farnesyl pyrophosphate into lycopene. **C.** pSC203 plasmid for chromosomal integration of the *CrtE*-, *CrtB*-, and *CrtI*-gene from *Erwinia herbicola* under the control of the *TEF1*-, *PGK1*-, and *GDP*-promoter respectively. **D.** UV-Vis absorption spectrum for lycopene. **E.** *S. cerevisiae* transformed with the pSC203 plasmid.

Future perspectives

When different genetic perturbations that increase the production of isoprenoids have been identified, the underlying metabolic mechanisms will be sought identified through further analysis. We foresee that some perturbations will be straight forward to identify e.g. overexpression of genes which are directly linked to isoprenoid precursor biosynthesis. The mechanisms which are harder to unravel will be the ones encoded by ORFs with unassigned functions or regulatory mechanisms. Finally systematic combinations of the identified perturbations will be engineered aiming at the construction of a *S. cerevisiae* platform for high titer production of isoprenoids.

References

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