Synthetic Genomics focuses on the construction of rationally designed chromosomes and genomes and offers novel approaches to study biology and to construct synthetic cell factories. Currently, progress in Synthetic Genomics is hindered by the inability to synthesize DNA molecules longer than a few hundred base pairs, while the size of the smallest genome of a self-replicating cell is several hundred thousand base pairs. Methods to assemble small fragments of DNA into large molecules are therefore required. Remarkably powerful at assembling DNA molecules, the unicellular eukaryote *Saccharomyces cerevisiae* has been pivotal in the establishment of Synthetic Genomics. Instrumental in the assembly of entire genomes of various organisms in the past decade, the *S. cerevisiae* genome foundry has a key role to play in future Synthetic Genomics developments.

**Keywords:** synthetic genomics, yeast, *Saccharomyces cerevisiae* (Baker’s yeast), genome foundry, DNA assembly, synthetic cells

**INTRODUCTION**

Synthetic Genomics (SG) is a recent Synthetic Biology discipline that focuses on the construction of rationally designed chromosomes and genomes. SG offers a novel approach to address fundamental biological questions by restructuring, recoding, and minimizing (parts of) genomes (as recently reviewed by (Coradini et al., 2020)). SG is now spurring technological developments in academia and has a strong future potential in industry (Schindler, 2020; Zhang et al., 2020). Humankind’s best microbial friend, the baker’s yeast *Saccharomyces cerevisiae*, has played, and continues to play a key role in SG advances, both by enabling the construction of chromosomes for other hosts, and in the refactoring of its own genome. This mini review explores the reasons for this strategic positioning of *S. cerevisiae* in SG, surveys the main achievements enabled by this yeast and reflects on future developments.

**CURRENT LIMITATIONS OF GENOME ASSEMBLY**

While small-sized viral chromosomes were the first to be chemically synthetized, the breakthrough in the field of SG came with the synthesis and assembly of the 592 kilobase (kb) chromosome of *Mycoplasma genitalium* (Gibson et al., 2008a; Gibson et al., 2008b). The unicellular eukaryote *Saccharomyces cerevisiae* has made a key contribution to this famous milestone. To understand how this microbe, commonly used in food and beverages, contributes to the assembly of synthetic genomes, let us recapitulate how synthetic chromosomes can be constructed (Figure 1).

It starts with the customized synthesis of short DNA molecules called oligonucleotides. Oligonucleotides are mostly synthetized using phosphoramidite chemistry, a 40 year-old method...
(Beaucage and Caruthers, 1981) that, despite decades of
 technological developments, struggles to deliver error-free
 oligonucleotides longer than 200 base pairs (bp). While the
 implementation of microarrays has substantially decreased the
 synthesis cost, it has not increased oligo length, an achievement
 that requires new synthesis methods (Hughes and Ellington,
 2017). Enzymatic alternatives for DNA synthesis are under
 development (Lee H. H. et al., 2019; Lee et al., 2020), but still
 have considerable shortcomings regarding automation and
 scalability that must be overcome before commercial scale can
 be considered (reviewed in (Ostrov et al., 2019; Eisenstein, 2020a;
 Hao et al., 2020; Paul et al., 2021)). Considering that a theoretical

![FIGURE 1](image-url)

**FIGURE 1** | *In vivo* and *in vitro* approaches for DNA assembly in synthetic genomics (A) Simplified overview of chromosome construction using *Saccharomyces cerevisiae* for genome assembly and production (B) Strengths and weaknesses of *in vitro* and *in vivo* assembly methods. (1) Assembly of fragments in *B. subtilis* is performed by integration into the host genome. (2) Between rounds of sequential assembly, transformation into *E. coli* is conventional for selection and amplification of constructs. (3) Requires *in vivo* amplification and selection in a microbial host.
minimal genome would be around 113 kb long (Forster and Church, 2006) and that the first fully synthesized genome of M. genitalium contains 583 kb (Gibson et al., 2008a), thousands of oligos must be stitched together to construct a complete synthetic genome. These DNA oligos can be assembled into longer DNA fragments by using a plethora of in vitro methods (reviewed in (Chao et al., 2014; Casini et al., 2015; Paul et al., 2021)). A method that has gained tremendous popularity since its development is the homology-based Gibson isothermal assembly (Gibson et al., 2009), devised to assemble the M. genitalium genome. As all in vitro methods, Gibson assembly is limited by the number of fragments that can reliably be stitched together in one reaction, usually around a dozen, requiring a stepwise assembly procedure of increasingly large genomic DNA constructs (Gibson et al., 2010b). DNA must be recovered from the reaction, amplified and verified in each round, to allow further processing. Selection and amplification of correctly cloned DNA is routinely performed in Escherichia coli, however, maintenance of large constructs of exogenous DNA, especially from prokaryotic origins, in this bacterium is often limited by expression and toxicity of gene products (Karas et al., 2015). In vitro alternatives for efficient and faithful selection and amplification of correctly assembled DNA are under development, but these are currently limited in length of amplified DNA and scalability (Su'etsugu et al., 2017; van Nies et al., 2018; Libicher et al., 2020; Mukai et al., 2020). While in principle stepwise in vitro assembly can lead to a DNA molecule of any size, and selection and amplification in E. coli worked well for DNA constructs up to 72 kb, E. coli had great difficulties maintaining quarter M. genitalium genomes, causing Gibson and others to turn to baker’s yeast (Gibson et al., 2008a; Gibson et al., 2008b).

SACCHAROMYCES CEREVISIAE AS A GENOME FOUNDRY

S. cerevisiae seems a logical host for SG as it naturally maintains a 12 Mb genome consisting of 16 chromosomes ranging from 230 to 1,500 kb in its haploid version, lives as polyploid in natural environments, and is extremely robust to changes in genome content and architecture (Shao et al., 2018). The extreme robustness of S. cerevisiae to supernumerary, chimeric chromosomes, a key feature for SG, was already demonstrated in the late ’80s (Burke et al., 1987; Larianov et al., 1996). A second key feature of S. cerevisiae is its preference for homologous recombination (HR) to repair double-strand DNA breaks (Kunes et al., 1985), a rare trait among eukaryotes. S. cerevisiae ability to efficiently and with high fidelity stitch together linear DNA molecules that present homologous regions as short as 40 bp (Noskov et al., 2001) at their ends, was rapidly valorized for genetic manipulations and assembly of heterologous DNA. Recently renamed in vivo assembly, this cloning technique (Figure 1) contributes to the remarkable genetic tractability and popularity of S. cerevisiae as model and industrial microbe (Larianov et al., 1994; Gibson et al., 2009). The combination of S. cerevisiae’s HR efficiency and fidelity, chromosome maintenance and propagation enabled the construction of the full Mycoplasma genome. Reflecting that “in the future, it may be advantageous to make greater use of yeast recombination to assemble chromosomes”, this study propelled S. cerevisiae as powerful ‘genome foundry’ (Gibson et al., 2008a). In the challenge to synthesize genomes, Ostrov and others rightfully identified assembly of these long DNA constructs as “the most critical hurdle” (Ostrov et al., 2019).

To date, S. cerevisiae has been key to assembling entire or partial genomes in most synthetic genome projects (Table 1). For instance, the entire 785 kb refactored Caulobacter crescentus (renamed C. ethensis) genome was assembled in vivo from 16 fragments (Venetz et al., 2019), while the recoded E. coli genome was split over 10 fragments of 91–136 kb which were individually assembled in yeast, and then sequentially integrated in the E. coli chromosome to replace native segments (Fredens et al., 2019) (Table 1). In vivo assembly also proved to be powerful in assembling and modifying genomes of organisms that are poorly amenable to genome editing; the rapid and faithful HR-based assembly of S. cerevisiae recently enabled the reconstruction of a synthetic SARS-CoV-2 genome in a single week (Thao et al., 2020), and has been shown to be a promising host for in vivo assembly and modification of other viral genomes (Vashee et al., 2020) as well as the genomes of various pathogens (Benders et al., 2010) and even a 101 kb human gene, which was transplanted into mouse embryonic cells (Mitchell et al., 2021) (Table 1). Moreover, S. cerevisiae was selected for the construction of the first synthetic eukaryotic genome. The international Sc2.0 consortium, spearheaded by Jef Boeke, undertook less than 10 years ago the daunting task of synthesizing recoded versions of the 16 yeast chromosomes. Via stepwise, systematic replacement of 30–40 kb (using ca. 12 DNA fragments of 2–4 kb) of the native yeast sequence, the consortium is close to the completion of the largest synthetic genome to date (Pretorius and Boeke, 2018; Eisenstein, 2020b), with the ambition to reshape and minimize the S. cerevisiae genome (Dai et al., 2020).

While S. cerevisiae is not the only microbial host available for the construction of (neo)chromosomes (Figure 1), several key features make it superior to its bacterial alternatives Bacillus subtilis and E. coli as genome foundry: 1) S. cerevisiae has the natural ability to carry large amounts of DNA and therefore to host multiple exogenous bacterial genomes (Benders et al., 2010); 2) E. coli frequently struggles with toxicity caused by the expression of exogenous bacterial sequences (Sorek et al., 2007; Gibson et al., 2008b; Karas et al., 2015), while S. cerevisiae is very robust to the presence of heterologous DNA from prokaryotic or eukaryotic origin (Tagwerker et al., 2012); 3) S. cerevisiae can, in a single transformation, assemble many DNA oligonucleotides into (partial) genomes. B. subtilis can also maintain large exogenous DNA constructs, but requires a stepwise method for DNA assembly, in which each DNA part is integrated sequentially into B. subtilis genome (Itaya et al., 2018). This approach is intrinsically more labor-intensive and time-consuming than S. cerevisiae single transformation assembly.

Surprised by S. cerevisiae genetic tractability, Gibson and others wondered "how many pieces can be assembled in yeast
| Donor DNA | Number of transformed fragments | Approximate size of transformed fragments | Approximate size of final construct | Aim of yeast assembly | References |
|-----------|-------------------------------|----------------------------------------|-----------------------------------|-----------------------|------------|
| Viruses   |                               |                                        |                                   |                       |            |
| Herpes simplex type 1 | 11                           | 14 kb                                  | 152 kb                            | Assembly and modification of viral genome, transfection and reconstitution in mammalian cells | Oldfield et al. (2017) |
| Autographa californica nucleopolyhedrovirus | 4                           | 45 kb                                  | 145 kb                            | Assembly and modification of viral genome, transfection and reconstitution in insect cells | Shang et al. (2017) |
| Cytomegalovirus isolate Toledo | 3                           | 116 kb                                 | 230 kb                            | Assembly and modification of viral genome, transfection and reconstitution in mammalian cells | Vashee et al. (2017) |
| Prokaryotes |                               |                                        |                                   |                       |            |
| Mycoplasma genitalium | 6                           | Up to 144 kb                           | 592 kb                            | Assembly of synthetic M. genitalium genome which could not be stably maintained in E. coli | Gibson et al. (2008a) |
| Mycoplasma genitalium | 25                          | 17–35 kb                              | 592 kb                            | Assembly of synthetic M. genitalium genome from short fragments, exploring assembly capacity in yeast | Gibson et al. (2008b) |
| Mycoplasma mycoides | 11                          | 100 kb                                 | 1 Mb                              | Assembly of synthetic M. mycoides genome, transplantation to a recipient cell to create the first bacterial cell controlled by a synthesized genome | Gibson et al. (2010a) |
| Mycoplasma pneumonia | 2                           | 10–816 kb                             | 826 kb                            | Insertion of yeast regulatory elements in the full M. pneumonia genome to allow for cloning and engineering of the genome | Benders et al. (2010), Ruiz et al. (2019) |
| Mycoplasma hominis | 2                           | 5–665 kb                              | 670 kb                            | Insertion of yeast regulatory elements in the full M. hominis genome to allow for cloning and engineering of the genome | Rideau et al. (2017) |
| Acholeplasma laidlawii | 33                         | 121–897 kb                            | 1.38 Mb                           | Exploring potential toxicity when assembling bacterial genomes in yeast | Karas et al. (2012) |
| Escherichia coli | 3                           | 185–660 kb                            | 1.03 Mb                           | Assembly of a minimal E. coli genome by Cas9-induced recombination of partial genomes | Zhou et al. (2016) |
| Escherichia coli | 7–14                        | 6–13 kb                               | 100 kb                            | Assembly of recoded E. coli partial genomes, used to replace the E. coli genome by a recoded synthetic genome | Fredens et al. (2019) |
| Caulobacter crescentus | 16                        | 38–65 kb                               | 785 kb                            | Assembly of a minimized and synthetic C. crescentus genome, recoded to be compatible with chemical DNA synthesis and transplanted in a recipient cell | Venetz et al. (2019) |
| Prechlorococcus marinus | 2                        | 580–675 kb                             | 1.66 Mb                           | Exploring assembly capacity and DNA stability of exogenous genomes in yeast | Tagwerker et al. (2012) |
| Synechococcus elongatus | 4                        | 100–200 kb                             | 454 kb                            | Exploring the ability to clone genomes with high G/C-content in yeast | Noskov et al. (2012) |
| Algae      |                               |                                        |                                   |                       |            |
| Phaeodactylum tricomutum | 5                        | 106–128 kb                             | 497 kb                            | Assembly of DNA with a moderate G + C content as a case study for assembly and modification of eukaryotic chromosomes in yeast | Karas et al. (2013) |
| Chlamydomonas reinhardtii chloroplast genome | 6                        | 34–129 kb                             | 230 kb                            | Assembly of a partial C. reinhardtii chloroplast genome to create genetic diversity at multiple loci at once | O’Neill et al. (2012) |
| Yeasts     |                               |                                        |                                   |                       |            |
| Yeast chromosome XII | 33<sup>a</sup>                    | 26–39 kb                             | 976 kb                            | Assembly of a megabase synthetic yeast chromosome harboring the highly repetitive ribosomal DNA locus | Zhang et al. (2017) |
| Single-chromosome yeast | 15<sup>b</sup>                | 230–1,500 kb                          | 11 Mb                             | Assembly of all sixteen S. cerevisiae chromosomes into a single chromosome | Shao et al. (2018) |
| Yeast neochromosome | 44                       | 2.5 kb                                  | 100 kb                            | Assembly of a circular supernumerary S. cerevisiae neochromosome that can act as a platform for modular genome engineering | Postma et al. (2021) |
| Yeast neochromosome for pathway engineering | 43                       | 2.5–6 kb                              | 100 kb                            | Assembly of circular and linear supernumerary S. cerevisiae | Postma et al. (2022) |

(Continued on following page)
in a single step” (Gibson et al., 2008a). Pioneering a SG approach for metabolic engineering based on modular, specialized synthetic chromosomes, Postma et al. probed this limit recently in our lab by constructing 100 kb artificial linear and circular neochromosomes from 44 DNA parts in a single transformation (Postma et al., 2021; Postma et al., 2022). The remarkable efficiency of in vivo assembly (36% of assemblies faithful to design) revealed that its limit has clearly not been reached yet, and that future systematic studies are required to evaluate the true potential of S. cerevisiae as a genome foundry. The supernumerary chromosomes were shown to stably maintain complete heterologous pathways as well as the yeast’s central carbon metabolism, underlining the potential of yeast synthetic genomics in the development of optimized cell-foundries. Once assembled, synthetic chromosomes could be easily edited in S. cerevisiae thanks to its efficient HR and rich molecular toolbox.

**CHALLENGES IN GENOME ASSEMBLY USING YEAST**

While S. cerevisiae is natively proficient for SG, several aspects of in vivo assembly in yeast are still far from optimal. Firstly, compared to bacterial alternatives, S. cerevisiae cells grow slowly with a maximum specific growth rate around 0.4–0.5 h⁻¹ and are hard to disrupt due to their sturdy cell wall. Considering that large DNA constructs above a few hundred kilobases are sensitive to shear stress, chromosome extraction and purification from S. cerevisiae is possible, but remains tenuous and inefficient, leading to low DNA yields and potentially damaged chromosomes (Blount et al., 2016). Secondly, the strength of S. cerevisiae can become its weakness, as the HR machinery can be overzealous and recombine any (short) DNA sequence with homology within or between the (neo)chromosomes, which may lead to misassemblies. Lastly, non-homologous end joining and microhomology-mediated end joining, DNA repair mechanisms that assemble pieces of DNA with no or minimal homology, are present in S. cerevisiae with low activity (Ranjha et al., 2018; Lee K. et al., 2019), and can also cause misassemblies. Similar to how E. coli was engineered to become a lab tool for DNA amplification, these shortcomings could be alleviated by engineering S. cerevisiae into a more powerful genome foundry.

Are there future alternatives to S. cerevisiae? Naturally, B. subtilis and E. coli could also be engineered. However, considering the minute fraction of the vast microbial biodiversity that has been tested for genetic accessibility and DNA assembly, it is likely that microbes yet to be discovered are even better genome foundries. Environments causing extreme DNA damage (high radiation, toxic chemicals, etc.) might be a source of HR-proficient organisms (e.g. (Albarracín et al., 2012; Sato et al., 2020)) better suited for SG.

In a more distant future, in vitro alternatives might replace the need for live DNA foundries altogether, thereby accelerating and simplifying genome construction. However, this will require major technological advances in in vitro DNA assembly and amplification. Already substantial efforts have led to the development of methods for DNA amplification, such as rolling circle amplification by the phage φ29 DNA polymerase (Dean, 2001; Lau et al., 2017), recently implemented for the amplification of a 116 kb multipartite genome (Libicher et al., 2020) and the in vitro amplification of synthetic genomes using the E. coli replisome, which already demonstrated to be capable of amplification of 1 Mb synthetic genomes (Mukai et al., 2020). Targets for improvement of these methods are the maximal length of amplified DNA fragments, the yield of amplification, the need for restriction of the amplified, concatenated molecules or the formation of non-specifically amplified products. The development of an in vitro approach that can parallel S. cerevisiae in vivo assembly capability seems even more challenging. While an interesting avenue might be to transplant S. cerevisiae HR DNA repair in vitro, it presents a daunting task considering that all players and their respective role have not been fully elucidated yet (Kwon et al., 2017; Ranjha et al., 2018). Still, considering that highly complex systems such as the transcription and translation machineries have been successfully

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**TABLE 1** | (Continued) Overview of the contribution of S. cerevisiae in synthetic genomics by the assembly of large (>100 kb) DNA constructs.

| Donor DNA | Number of transformed fragments | Approximate size of transformed fragments | Approximate size of final construct | Aim of yeast assembly | References |
|-----------|-------------------------------|----------------------------------------|-----------------------------------|----------------------|------------|
| Other     | Human HPRT1 gene              | 13                                    | 3–83 kb                           | 125 kb               | Mitchell et al. (2021) |
|           | Artificial data storage       | 5                                     | 40 kb                             | 254 kb               | Chen et al. (2021)    |

*In case of a sequential assembly, the fragment number and size of the last assembly is used.

*Short backbones containing regulatory elements such as CEN/ARS, and markers not included.

*Initial assembly of the entire genome failed due to gene toxicity.

*Assembly was performed by stepwise integration in multiple rounds.
implemented in vitro and are commercially available (Shimizu et al., 2001), cell-free \textit{S. cerevisiae} HR might become a reality in the coming years.

**OUTLOOK**

Since the first genome synthesis in 2008, relatively few genomes have been synthetized. Low-cost, customizable construction of designer genomes, currently accessible for small viral, organelar or bacterial constructs, is still out of reach for large (eukaryotic) genomes. There are still numerous technical, financial, and computational hurdles that must be overcome on the road to microbial designer genomes, tailored to applications in bio-based industry. Here we reviewed why the yeast \textit{S. cerevisiae} is a key organism in the field of SG, however, the spectrum of available hosts is expected to increase as research in SG advances. For example, a recent study shows improving the HR capacity of the industrially relevant yeast \textit{Yarrowia lipolytica} could greatly expand the potential applications of SG in bio-based processes (Guo et al., 2020).

In the near future, SG is anticipated to contribute to various fields, such as a platform technology for industrial biotechnological processes (Schindler, 2020; Postma et al., 2022), as a new means for data storage (Chen et al., 2021) and for the development of new cell therapies and other medical applications, which is the ambition of the Genome Project-Write (Boeke et al., 2016). In parallel, worldwide bottom-up approaches endeavor to construct synthetic cells from scratch, such as the European consortia BaSyC (http://www.basyc.nl), MaxSynBio (https://www.maxsynbio.mpg.de) and the Synthetic cell initiative (http://www.syntheticcell.eu) and the US-based Build-a-cell initiative (http://buildacell.io) (reviewed in Mutschler et al., 2019). Looking further ahead, SG may even assist in understanding and engineering entire ecosystems by assembly of a metagenomes in a single cell (Belda et al., 2021). SG, albeit still in its infancy and mostly limited to academic research, has bright days ahead, and \textit{S. cerevisiae} is foreseen to remain a valuable, if not indispensable, SG tool for the coming decade.

**AUTHOR CONTRIBUTIONS**

All authors contributed to the work presented in this article. CK, EP, and PD-L conceived and drafted the manuscript, CK, EP, and CC performed literature research and wrote sections of the manuscript, PD-L edited and revised the manuscript. All authors read and agreed to the final manuscript.

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