Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Budding yeast as a factory to engineer partial and complete microbial genomes

Sanjay Vashee¹,a, Yonathan Arfi²,a and Carole Lartigue²,a

Abstract
Yeast cells have long been used as hosts to propagate exogenous DNA. Recent progress in genome editing opens new avenues in synthetic biology. These developments allow the efficient engineering of microbial genomes in Saccharomyces cerevisiae that can then be rescued to yield modified bacteria/viruses. Recent examples show that the ability to quickly synthesize, assemble, and/or modify viral and bacterial genomes may be a critical factor to respond to emerging pathogens. However, this process has some limitations. DNA molecules much larger than two megabase pairs are complex to clone, bacterial genomes have proven to be difficult to rescue, and the dual-use potential of these technologies must be carefully considered. Regardless, the use of yeast as a factory has enormous appeal for biological applications.

Addresses
¹ J. Craig Venter Institute, Rockville, MD, USA
² Univ. Bordeaux, INRAE, Biologie du Fruit et Pathologie, UMR 1332, F-33140, Villenave d’Ornon, France

Corresponding author: Lartigue, Carole (carole.lartigue-prat@inrae.fr)
a These authors contributed equally to this work.

In this review, we will discuss the ever-expanding use of yeast as an efficient propagating and editing factory for the genomes of various microbial species. This process involves the cloning or assembly of a full or partial genome into yeast, its engineering, and its rescue into a suitable recipient cell to rescue the designed function or live cells (Figure 1). This approach can be a novel method to (1) study intractable organisms, (2) genetically edit intractable organisms or (3) build new living systems for basic and applied biology. Each component of the yeast factory cycle is detailed in the following. Potential barriers as well as the risks/benefits of such an approach are discussed.

In-yeast cloning of whole, native, and synthetic microbial genomes
Yeast has long been used as a host to clone DNA molecules, either as yeast artificial chromosomes (YACs) or yeast centromeric plasmids (YCps), from a wide range of donor organisms. Many of the early examples involved cloning genomic DNA fragments from a range of eukaryotic [5,6] and prokaryotic species [7] as well as viruses [8,9] for genome analysis, including physical maps of complex genomes and gene function studies. However, several issues of chimeras and instability of some cloned heterogeneous DNA in yeast reduced its use, while vectors in bacterial systems such as cosmids and bacterial artificial chromosomes (BACs) gained favor for genome analysis and development of reverse genetics tools.

Over the past decade, yeast has re-emerged as an attractive genome engineering host, bolstered by a groundbreaking experiment to assemble and boot-up the first ‘synthetic cell’ [1] and, subsequently, by the cloning of several partial and full bacterial or eukaryotic genomes as well as assembly of viral genomes (Table 1).

Multiple approaches can now be used to clone large DNA fragments in yeast, including complete megabase-sized genomes. Depending on the characteristics of the donor organism or downstream applications, some approaches enable the cloning of native genomes, whereas others permit the simultaneous cloning, editing or assembly of entire genomes from polymerase chain reaction (PCR)–amplified, fully synthetic, or transformation-associated-recombination (TAR)-cloned...
Figure 1

Overview of the in yeast cloning and editing process and associated techniques. (a) Specific genetic elements derived from yeast must be added to the target genomes to ensure proper replication and segregation. (b) Multiple strategies can be used to introduce the yeast element in the genome, and to introduce the genome in the yeast cells. (c) The target genome can be fully synthesized chemically or biochemically and assembled in yeast cells. (d) A wide array of tools are available in yeast to perform genome editing. (e) Biological entities can be reconstituted from the edited microbial genomes using diverse strategies depending on their viral or bacterial nature.
fragments. All of these methods require the presence of certain yeast genetic elements, including an autonomously replicating sequence (ARS), a centromere, and a selection marker to replicate and maintain the cloned DNA. An ARS is not necessarily required for genomes with low G + C% (<40%) as the AT-rich consensus motif may naturally occur in their sequence (Figure 1a). These elements can be added before cloning, as a plasmid integrated in a bacterial genome. Then, the newly marked genome is isolated and transferred intact into yeast spheroplasts by the conventional yeast transformation procedure [10,11] or by fusing the bacterial

| Organism                      | Size (Mbp) | % G + C | Genetic code     | Cloning strategy                        | Rescue strategy                  | References  |
|-------------------------------|------------|---------|------------------|-----------------------------------------|----------------------------------|-------------|
| **Mollicutes**                 |            |         |                  |                                         |                                  |             |
| Mycoplasma genitalium         | 0.58       | 32      | Nonstandard      | Synthesis and assembly                  | N/A                               | [66]        |
| Mycoplasma mycoides subsp. capri | 1.1       | 24      | Nonstandard      | Cloning                                | Transplantation                   | [10]        |
| Mycoplasma pneumoniae         | 0.81       | 41      | Nonstandard      | Cloning                                | N/A                               | [10,17]     |
| JCVI Syn 1.0                  | 1.1        | 24      | Nonstandard      | Synthesis and assembly                  | Transplantation                   | [1]         |
| Acholeplasma laidlawii        | 1.5        | 32      | Universal        | Cloning                                | N/A                               | [51]        |
| JCVI Syn 3.0                  | 0.53       | 24      | Nonstandard      | Synthesis and assembly                  | Transplantation                   | [2]         |
| Mycoplasma mycoides subsp. mycoides | 1.2     | 24      | Nonstandard      | Cloning                                | Transplantation                   | [45]        |
| Mycoplasma capricolum subsp. capricolum | 1.1   | 25      | Nonstandard      | Cloning                                | Transplantation                   | [45]        |
| Mycoplasma leachii            | 1          | 24      | Nonstandard      | Cloning                                | Transplantation                   | [45]        |
| Mycoplasma putrefaciens       | 0.8        | 27      | Nonstandard      | Cloning                                | Transplantation                   | [45]        |
| Spiroplasma citri             | 1.8        | 26      | Nonstandard      | Cloning                                | N/A                               | [45]        |
| Mycoplasma hominis            | 0.66       | 27      | Nonstandard      | Cloning                                | N/A                               | [67]        |
| Mesoplasma florum             | 0.79       | 27      | Nonstandard      | Cloning                                | Transplantation                   | [46]        |
| Mycoplasma capricolum subsp. capripneumoniae | 1     | 24      | Nonstandard      | Cloning                                | Transplantation                  | Pers. com. 2020 |
| Mycoplasma feriruminatoris    | 1.2        | 24      | Nonstandard      | Cloning                                | Transplantation                   | Pers. com. 2019 |
| **Proteobacteria**            |            |         |                  |                                         |                                  |             |
| Haemophilus influenzae        | 1.8        | 38      | Universal        | Cloning                                | N/A                               | [12]        |
| Escherichia coli (reduced genome) | 1.03     | 51      | Universal        | Synthesis and assembly                  | N/A                               | [48]        |
| Escherichia coli (recoded genome) | 3.98   | N/A     | Universal        | Synthesis and assembly                  | Partial replacement               | [68]        |
| Salmonella typhimurium (recoded genome) | 4.47 | N/A     | Universal        | Synthesis and assembly                  | Partial replacement               | [47]        |
| Escherichia coli (recoded genome) | 3.98   | N/A     | Universal        | Synthesis and assembly                  | Partial replacement               | [3]         |
| Caulobacteur ethensis 2.0 (reduced/ recoded genome) | 0.78 | 57      | Universal        | Synthesis and assembly                  | N/A                               | [4]         |
| **Cyanobacteria**             |            |         |                  |                                         |                                  |             |
| Prochlorococcus marinus       | 1.6        | 31      | Universal        | Cloning                                | N/A                               | [69]        |
| Synechococcus elongatus (fragments) | 2.7     | 55      | Universal        | Cloning                                | N/A                               | [70]        |
| **Diatoms**                   |            |         |                  |                                         |                                  |             |
| Phaeodactylum tricornutum Chromosome 25 | 0.5 | 48      | Universal        | Cloning                                | N/A                               | [71]        |
| Phaeodactylum tricornutum Chromosome 26 | 0.44 | 48      | Universal        | Cloning                                | N/A                               | [71]        |
| **Viruses**                   |            |         |                  |                                         |                                  |             |
| Dengue virus type 2           | 0.011      | 46      | Universal        | Cloning                                | RNA transfection                  | [72]        |
| MERS-CoV                      | 0.029      | 41      | Universal        | Cloning                                | DNA transfection                  | [73]        |
| AcMNPV                        | 0.14       | 45      | Universal        | Synthesis and assembly                  | DNA transfection                  | [74]        |
| HCMV                         | 0.23       | 49      | Universal        | TAR cloning and assembly                | DNA transfection                  | [35]        |
| Herpes simplex virus type 1   | 0.15       | 68      | Universal        | TAR cloning and assembly                | DNA transfection                  | [36]        |
| Horsepox virus                | 0.21       | 33      | Universal        | Synthesis and assembly                  | DNA transfection                  | [42]        |
| SARS-CoV-2                    | 0.03       | 38      | Universal        | Synthesis and assembly                  | RNA transfection                  | [39]        |
| MHV                           | 0.032      | 42      | Universal        | Synthesis and assembly                  | RNA transfection                  | [39]        |
| MERS-CoV                      | 0.03       | 41      | Universal        | Synthesis and assembly                  | RNA transfection                  | [39]        |
| HCoV-229E                     | 0.027      | 38      | Universal        | Synthesis and assembly                  | N/A                               | [39]        |
| ZIKA virus                    | 0.011      | 51      | Universal        | Synthesis and assembly                  | N/A                               | [39]        |
| Human RSV-B                   | 0.015      | 34      | Universal        | Synthesis and assembly                  | N/A                               | [39]        |

a Sorting is done by year of publication.
b Cloning refers to any method described in the text.
c The assembly was performed in mammalian cells.
cell to yeast [12] (Figure 1b). The advantage of this approach is the selection of vector insertion sites that do not interfere with bacterial viability, which is convenient for genomes that are meant to be transplanted into a recipient cell to produce live cells. Another approach, TAR-cloning, exploits yeast’s ability to efficiently recombine DNA fragments if they contain ends (~60 bp) that are homologous to a target sequence. In this case, the genome is isolated, linearized in vitro by a restriction enzyme or using the CRISPR-Cas9 system, and cotransformed into yeast together with a linear yeast vector containing homology sequences [13–16] (Figure 1b). A variation of this approach is CReasPy-Cloning which enables the simultaneous cloning and engineering of megabase-sized genomes in yeast [17] (Figure 1b). The TAR-cloning approach can be extended so that the yeast transformation is carried out with multiple overlapping fragments, either PCR-amplified, synthetic, or previously TAR-cloned (Figure 1c), allowing for genome-wide engineering of microbial genomes.

Using these methods, many bacterial and viral genomes, both native and synthetic, have been cloned or assembled in yeast. Key examples are shown in Table 1. For future target genomes, certain considerations can be factored into the choice of the cloning method. These include whether the organism is cultivable, is transformable, and/or has genetic tools. If the organism has all of these characteristics, then any of the outlined approaches can be used. For other organisms lacking one or more characteristics or for large-scale editing, the in vitro or assembly methods are more appropriate.

In-yeast genome engineering

Over the last decade, the cost of DNA synthesis has drastically reduced, almost reaching the 0.01$/base bar. Such low costs have enabled the engineering of organisms with fully synthetic DNA, with recent examples of recoded or reorganized genomes [3,4]. As a result, genome editing can now be performed by the assembly of synthetic fragments in yeast. This approach remains nonetheless costly at the megabase scale and may be excessive for small, localized editing tasks. Therefore, depending on the need, it may be more appropriate to use one of the many genome engineering tools already available in yeast to modify the native cloned genome. Particular examples are TREC [18], CRISPR-Cas9 [19–22], and Cre-Lox [23] (Figure 1d). The first system was developed for the scar-less edition of mycoplasma genomes cloned in yeast [24,25] and was later improved in TREC-IN [26,27]. Cas9, the well-known and broadly used RNA-guided endonuclease, has been adapted to a wide array of organisms, including yeast [28]. Due to the very high efficiency of this system, it opened the door for marker-less genome edition, with the ability to delete, add, or replace genomic loci in the kbp range. Given its efficiency, it has become the engineering method of choice for precisely altering genomes cloned in yeast [29,30]. Finally, Cre-Lox has also been extensively used for targeted editing, but interestingly, it is the basis of SCRaMbLE, a system enabling massive chromosome rearrangements to produce strains with large genotypic diversity [31,32]. Regarding microbial genomes cloned in yeast, the Cre-Lox system was notably used during the construction of the ‘minimal cell’ [2,33].

**Rescue of genomes cloned in yeast: transplantation, transfection or in vitro approaches**

Once a microbial genome has been modified in yeast, it can be ‘rescued’ using various approaches. For this review, ‘rescue’ is defined as the process by which the cloned genome isolated from yeast is converted into the biological entity it encodes.

Because viruses are generally simpler systems, they are relatively easy to rescue (Figure 1e, right panel). In many cases, viruses can be reconstituted by transfecting their modified genomes or fusion into host cells [34–38]. For RNA viruses, the modified genomes can be transcribed in vitro using purified RNA polymerase and the resulting RNAs transfected into host cells [39]. Significantly, the Noireaux laboratory has shown the capacity to package bacterial viruses in vitro using the TXTL system [40]. In other cases, it is necessary to use helper genes or viruses to boot-up the recombinant genomes [41–43].

For modified bacterial genomes, the rescue is more difficult, due in part to larger genome size, more complicated pathways, and cellular structure. One possibility to rescue a whole genome is to isolate intact edited microbial chromosomes from yeast and transfer them into recipient cells (Figure 1e, left panel) [10,44–46]. This process is known as genome transplantation (GT) and yields live cells driven by the donor recombinant genomes. It is for now limited to a small set of mycoplasma species.

For non-mycoplasma bacterial species, it is convenient to use yeast to clone and/or engineer large subgenomic fragments and then integrate them into native target bacterial genomes for desired applications. For example, Fredens et al. have used assembly of synthetic E. coli 100-kb fragments in yeast as an intermediate to generate an E., coli strain that uses only 61 codons for protein synthesis [3], instead of the native 64 codons. A similar approach was used by Lau et al. to recode large segments of the *Salmonella typhimurium* genome, using iterative genomic integration of 10- to 25-kb chunks assembled in yeast [47].
Bottleneck and future developments
Although potentially extremely powerful, the in-yeast cloning and editing of microbial genomes comes with a few drawbacks and bottlenecks.

Based on previous experience, we expect that the cloning of genomes in yeast to be more readily achievable than the subsequent rescue of the genomes. In addition, viral genomes have also proven much easier to clone and rescue than their bacterial counterpart.

In-yeast cloning
For bacterial genomes, the nature of the cloned DNA, as well as its genetic content should be taken into consideration. First, size might matter. To date, the Haemophilus influenza and the Spiroplasma citri chromosomes are the largest DNA molecules cloned in yeast (1.8 Mb) [12,45]. However, it is still not yet clear whether much larger genomes such as B. subtilis (4.2 Mb) can be transformed intact in yeast. Approaches that allow the construction of a genome inside the yeast cell [48] or based on bacterial/yeast fusion could alleviate this problem [12]. Moreover, results from the SC2.0 consortium and others suggest that replicating up to a 12-Mbp chromosome may not be an issue other than, potentially, the cumulative size of the yeast and the cloned genome [49,50]. With this in mind, using a yeast cell with a minimized genome could be key for increasing the amount of ‘cargo’ DNA that it could carry. The G + C% of the cloned genome also appears to be a relevant problem. While the cloning of the A + T rich mycoplasma genomes (0.58–1.8 Mb; G + C%<40%) is routine, bacterial genomes with much higher G + C% require adding an ARS to the target genomes for maintenance in yeast [4,48]. Another issue is ectopic expression of the cloned genome that may be toxic to yeast [51]. This can be solved by empirical identification of the culprit toxic gene(s), or through the engineering of new host cells that are genetically isolated from their cargo (e.g., using orthogonal promoters and ribosome binding site (RBS), or having a nonstandard genetic code). Finally, the presence of repeat sequences in target genomes may present issues in yeast, especially if they are in the overlapping homologous sequences during TAR assembly. However, in our experience, if the repeat sequences are buried within the fragments or genomes to be assembled, they have not caused problems [10,11,35,36].

In-yeast engineering
Current methods are effective to perform a few modifications at a time. TAR assembly alleviates this issue to some extent, but it is somewhat limited by the number of fragments that can be used as well as the efficiency of homologous recombination. A potential improvement may be the use of yeast mutants impaired in competing repair pathways, such as non-homologous end-joining, as engineering hosts for microbial genomes. Another possibility is the use of other yeasts as hosts, such as Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris and Kluyveromyces marxianus. In addition, development of improved technology should increase the speed and widen the scale of microbial genome engineering in yeast [31,52–54].

Rescue of viral genomes
For the most part, rescue of viral genomes is not a major concern. However, there are still a few viruses, such as African swine fever virus (ASFV), whose genomes are not infectious or for which there are no known helper genes or helper viruses to reconstitute live virus from recombinant genomes [55]. In addition, novel dangerous viruses may emerge as humans encroach into new environments for which reverse genetics would need to be developed. Thus, for these types for viruses, generalized methods to boot them up would need to be developed.

Rescue of bacterial genomes
Currently, the most broadly applicable strategy is to transfer sections of the engineered genome back to the original cell and proceed in an incremental manner to completely replace the original genome [3,47]. Alternatively, GT can be attempted to transfer in one step the entirety of the engineered genome. However, this strategy has only been achieved for a small cluster of Mollicutes and appears to have multiple hurdles that limit its broad application. First, the recipient cell should be closely related to the donor genome, to process and replicate the donor’s genetic information [10,45]. Therefore, to apply GT to other species, one needs to develop a specific set of recipient cells and transplantation methods. The recipient cell may also be engineered to remove a number of natural systems that might limit the efficiency of GT. For instance, secreted or membrane-bound nucleases [56], internal defense mechanisms against foreign DNA, such as restriction-modification systems [57–59] or CRISPR-Cas9 [60,61], may degrade unprotected donor genomes before or after entry in the recipient cell. A recipient cell with a strong recombination activity may be problematic for GT. It could result in increased frequency of illegitimate exchanges between the donor and recipient genomes, leading to the transfer of the selection marker to the recipient’s genome or the emergence of chimeric chromosomes and thus, to hybrid cells rather than the desired outcome. Using ghost cells devoid of the resident DNA as recipient cells or using DNA-damaging agents to make the resident genome nonfunctional for recombination may overcome this issue. Another important concern is that DNA uptake may be limited by transformation efficiency and cell surface structure.

To bypass these obstacles, improvement of methods to make spheroplasts/protoplasts in target organisms may
be used to remove cell walls to increase DNA uptake. In addition, other DNA transfer methods, such as conjugation, can be used to transfer a genome from the donor species to the recipient.

Conclusions and perspectives: benefits and risks of such technologies

The combination of genome transplantation/transfection and genome engineering in yeast is an exciting approach to manipulate synthetic and native genomes. This approach could be of importance for genetically intractable yet medically and industrially important organisms, such as *Chlamydia*, *M. leprae*, and *Clostridia* and ASFV for which it would provide convenient tools to better understand their biology. However, there are still many unanswered questions regarding the process of GT and at a lower degree back transfection. More investigation in understanding the process would facilitate its expansion to other organisms.

Moreover, the ability to quickly synthesize or modify viral or bacterial genomes might be a critical factor to respond to emerging pathogens [62—64]. Indeed, while acquiring genomic information is now a matter of days due to (meta)genome sequencing, creating new microbial strains is much longer. These new strains can be used as vaccines, or to decipher the virulence of pathogens. The design of such strains is often not the limiting step but rather, the actual manufacturing of the modified biological entity. However, progress made in DNA synthesis and now extremely short turnaround times of commercial suppliers, suggest that this bottleneck may soon disappear. Recently, it was shown that only 30 days were necessary to go from a publicly released sequence of SARS-CoV-2 to a functional, rescued recombinant virus, using yeast to assemble synthetic DNA fragments [39]. This example highlights the potential of in-yeast methods and indicates that it can be highly beneficial to the global population.

Nevertheless, as discussed elsewhere, advances in synthetic genomics methods, including methods described herein, raise several dual-use concerns [36,64,65]. A number of measures can be adopted to ensure biological control: some are inherent to the organism (engineered auxotrophy; use a non-standard genetic code), while other devices can be added (genetically encoded kill-switches, incorporation of unnatural amino acids into essential proteins ...).

In conclusion, while it is clear that budding yeast is a powerful engineering factory, there is still room for improvement to fulfill its use for synthetic biology applications.

Funding

This work was supported in part by the National Institutes of Health [Grant numbers 1R01AI137365, R03AI146632], the IDRC [Grant number 109212] and the French National Funding Research Agency [No ANR-18-CE44-0003-02].

Conflict of interest statement

Nothing declared.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Gibson DG, Glass JI, Lartigue C, Noskov VN, ChuangRY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, et al.: Creation of a bacterial cell controlled by a chemically synthesized genome. Science 2010, 329:52–56.

2. Hutchison CA, Chuang RY, Noskov VN, Assad-Garcia N,Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma LL, et al.: Design and synthesis of a minimal bacterial genome. Science 2016, 351:6253–6253.

3. Fredens J, Wang K, da Torre D, Funke LFH, Robertson WE, Christova Y, Chia T, Schmied WH, Dunkelmann DL, Berisé V, et al.: Total synthesis of *Escherichia coli* with a recoded genome. Nature 2019, 569:514–518.

Fredens et al. have used assembly of synthetic E. coli 100 kb fragments in yeast as an intermediate to generate an E. coli strain that used only 61 codons for protein synthesis, instead of the native 64 codons. In total, more than 18 000 codons were rapidly re-coded.

4. Venetz JE, Del Medico L, Wölfe A, Schächle P, Bucher Y,Appert D, Tschann F, Flores-Tinoco CE, van Kooten M,Guennoun R, et al.: Chemical synthesis rewriting of a bacterial genome to achieve design flexibility and biological functionality. Proc Natl Acad Sci USA 2019, 116:8070–8079.

The authors report the chemical synthesis and testing of an essential genome of Caulobacter crescentus. This is a computer-designed reduced and recoded genome.

5. Schlessinger D: Yeast artificial chromosomes: tools for mapping and analysis of complex genomes. Trends Genet 1999, 6:248–258.

6. Larionov V, Kouprina N, Graves J, Chen XN, Korenberg JR,Resnick MA: Specific cloning of human DNA as yeast artificial chromosomes by transformation-associated recombination. Proc Natl Acad Sci USA 1996, 93:491–496.

7. Kuspa A, Vollrath D, Cheng Y, Kaiser D: Physical mapping of the Myxococcus xanthus genome by random cloning in yeast artificial chromosomes. Proc Natl Acad Sci USA 1989, 86:8917–8921.

8. Kelner G, Spencer F, Tugendreich S, Connelly C, Hieter P: Efficient manipulation of the human adenovirus genome as an infectious yeast artificial chromosome clone. Proc Natl Acad Sci USA 1994, 91:6186–6190.

9. Garcia-Ramirez J, Ruchti F, Huang H, Simmen K, Angulo A,Ghazal P: Dominance of virus over host factors in cross-species activation of human cytomegalovirus early gene expression. J Virol 2001, 75:26–35.

10. Lartigue C, Vashee S, Algire MA, Chuang-R-Y, Benders GA, Ma L, Noskov VN, Denisova EA, Gibson DG, Assad-Garcia N,et al.: Creating bacterial strains from genomes that have been cloned and engineered in yeast. Science 2009, 325:1693–1696.

11. Benders GA, Noskov VN, Denisova EA, Lartigue C, Gibson DG,Assad-Garcia N, Chuang R-Y, Carrera W, Moodie M, Algire MA,et al.: Cloning whole bacterial genomes in yeast. Nucleic Acids Res 2010, 38:2558–2569.
Yeast as a factory to engineer microbial genomes Vashee et al. 7

12. Karas BJ, Jablonsievic J, Sun L, Ma L, Goldgof GM, Ramon A, Manary MJ, Winzeler EA, Venter JC, Philip D, et al.: Direct transfer of whole genomes from bacteria to yeast. Nat Methods 2013, 10:410–412.

13. Kouprina N, Larionov V: Exploiting the yeast Saccharomyces cerevisiae for the study of the organization and evolution of complex genomes. FEMS Microbiol Rev 2003, 27:629–649.

14. Lee NCO, Larionov V, Kouprina N: Highly efficient CRISPR/Cas9-mediated TAR cloning of genes and chromosomal loci from complex genomes in yeast. Nucleic Acids Res 2015, 43:55.

15. Kouprina N, Larionov V: Transformation-associated recombination (TAR) cloning for genomics studies and synthetic biology. Chromosoma 2016, 125:621–632.

16. Kouprina N, Noskov VN, Larionov V: Selective isolation of large segments from individual microbial genomes and environmental DNA samples using transformation-associated recombination cloning in yeast. Nat Protoc 2020, 15:734–749.

The authors describe an extension of the transformation-associated recombination (TAR) cloning protocol, enabling selective isolation of any DNA segments from microbial genomes or from environmental DNA samples. The TAR method is a reference method for cloning genomes of various nature and set the stage for using yeast as a factory to engineer genomes.

17. Ruiz E, Talenton V, Dubrana M-P, Guesdon G, Lluch-Senar M, Salin F, Sirand-Pugnet P, Arfi Y, Lartigue C: CReasPy-cloning: a method for simultaneous cloning and engineering of megabase-sized genomes in yeast using the CRISPR-Cas9 system. ACS Synth Biol 2019, 8:2547–2557.

18. Noskov VN, Segall-Shapiro TH, Chuang RY: Tandem repeat coupled with endonuclease cleavage (TREC): a seamless modification tool for genome engineering in yeast. Nucleic Acids Res 2010, 38:2570–2576.

19. Hsu PD, Lander ES, Zhang F: Development and applications of CRISPR-Cas9 for genome engineering. Cell 2014, 157:1262–1278.

20. Rath D, Amlinger L, Rath A, Lundgren M: The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie 2015, 117:119–128.

21. De La Fuente-Núñez C, Lu TK: CRISPR-Cas9 technology: applications in genome engineering, development of sequence-specific antimicrobials, and future prospects. Integrative Biology 2017, 9:109–122 (United Kingdom).

22. Brooks AK, Gaj T: Innovations in CRISPR technology. Elsevier Ltd, 2018.

23. Missiriis PI, Smailus DE, Holt RA: A high-throughput screen identifying sequence and promiscuity characteristics of the loxP spacer region in Cre-mediated recombination. BMC Genom 2006, 7:73.

24. Lartigue C, Lebaudy A, Blanchard A, Yacoubi BE, Rose S, Grosjean H, Douthwaite S: The flavoprotein Mcap0476 (RimF0) catalyzes mU1939 modification in Mycoplasma capricolum. Nucleic Acids Res 2014, 42.

25. Schiek E, Lartigue C, Frey J, Vozza N, Hegermann J, Miller RA, Valguarnera E, Muriuki C, Meens J, Nene V, et al.: Galactofuranose in M. yacopila myoides is important for membrane integrity and cell wall adhesion but does not contribute to serum resistance. Mol Microbiol 2016, 99:55–70.

26. Chandran S, Noskov VN, Segall-Shapiro TH, Ma L, Whiteis C, Lartigue C, Jores J, Vashee S, Chuang R-Y: TREC-IN: gene knock-in genetic tool for genomes cloned in yeast. BMC Genom 2014, 15:1180.

27. Jores J, Ma L, Sasiakambwe P, Schiek E, Liljander A, Chandran S, Staff Ch, Cippa V, Arli Y, Assad-Garcia N, et al.: Removal of a subset of non-essential genes fully attenuates a highly virulent mycoplasma strain. Front Microbiol 2019, 10.

28. DiCarlo JE, Norville JE, Mall P, Rios X, Aach J, Church GM: Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic Acids Res 2013, 41:4336–4343.

29. Kannan K, Tsvetanova B, Chuang R-Y, Noskov VN, Assad-Garcia N, Ma L, Hutchison III CA, Smith HO, Glass JJ, Merryman C, et al.: One step engineering of the small-subunit ribosomal RNA using CRISPR/Cas9. Sci Rep 2016, 6:30714.

30. Tsamopoulos I, Gourgues G, Blanchard A, Vashee S, Jores J, Lartigue C, Sirand-Pugnet P: In-yeast engineering of a bacterial genome using CRISPR/Cas9. ACS Synth Biol 2016, 5:104–109.

31. Jones S: SCRaMbLE does the yeast genome shuffle. Nat Biotechnol 2018, 36:503.

32. Blount BA, Gowers GOF, Ho JCH, Ledesma-Amaro R, ** Joviciev D, McKiernan RM, Xie ZX, Li BZ, Yuan YJ, Ellis T: Rapid host strain improvement by in vivo rearrangement of a synthetic yeast chromosome. Nat Commun 2018, 9:1–10.

The authors used SCRaMbLE to rapidly generate new, improved host strains with genetic backgrounds favorable for desired applications, including violatein and penisillin biosynthesis and for xylose utilization.

33. Noskov VN, Ma L, Chen S, Chuang R-Y: Recombine-mediated cassette exchange (RMCE) system for functional genomics studies in Mycoplasma mycoides. Biol Proced Online 2015, 17:6.

34. Ando H, Lemire S, Pires DP, Lu TK: Engineering modular viral scaffolds for targeted bacterial population editing. Cell Systems 2015, 1:187–196.

35. Vashee S, Stockwell TB, Alperovich N, Denisova EA, Gibson DG, Cady KC, Miller K, Kannan K, Malouli D, Crawford LB, et al.: Cloning-assembly, and modification of the primary human cytomegalovirus isolate Toledo by yeast-based transformation-associated recombination. mSphere 2017, 2.

36. Oldfield LM, Grzesik P, Voorhies AA, Alperovich N, MacMath D, Najera CD, Chandra DS, Prasad S, Noskov VN, Montague MG, et al.: Genome-wide engineering of an infectious clone of herpes simplex virus type 1 using synthetic genomics assembly methods. Proc Natl Acad Sci USA 2017, 114:E8885–E8891.

37. Kilcher S, Studer P, Muesener C, Klumpf J, Loessner MJ, Adhya S: Cross-genus reboothting of custom-made, synthetic bacteriophage genomes in L-form bacteria. Proc Natl Acad Sci USA 2018, 115:567–572.

38. Brown DM, Chan YA, Desai PJ, Grzesik P, Oldfield LM, Vashee S, Way JC, Silver PA, Glass JI: Efficient size-independent chromosomal delivery from yeast to cultured cell lines. Nucleic Acids Res 2017, 45:50.

39. Thi Nhu Thao T, Labrousseau F, Ebert N, Vkovski P, Stalder H, Portmann J, Kelly J, Steiner S, Holwerda M, Kratzel A, et al.: Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. Nature 2020, 582:561–565.

The authors demonstrate the possibility to reconstruct, within one week of receipt of synthetic DNA, in the laboratory, emerging viruses (such as the SARS-CoV2). This method is of major importance as it accelerates our capacity to understand emerging viruses and find potential cures.

40. Rustad M, Eastlund A, Jardine P, Noireaux V: Cell-free TXTL synthesis of infectious bacteriophage T4 in a single test tube reaction. Synthetic Biol 2018, 3.

In this work, the authors present the complete synthesis and reconstruction of the phage T4, one of the largest enterobacteriophages that infect E. coli, from its 169-kbp genome in one-pot TXTL reactions. This work provides an in vitro approach to engineering complex bacteriophages.
bacteria: changing one species to another. Science 2007, 317: 632–638.

45. Labroussefa S, Lebady A, Baby V, Gourgues G, Matteau D, Vashee S, Sirand-Pugnet P, Rodrigue S, Larrique C: Impact of donor-recipient phylogenetic distance on bacterial genome transplantation. Nucleic Acids Res 2016, 44.

46. Baby V, Labroussefa S, Brodeur J, Matteau D, Gourgues G, Larrique C, Rodrigue S: Cloning and transplantation of the mesoplasma florum genome. ACS Synth Biol 2018, 7:209–217.

47. Lau YH, Stirling F, Kuo J, Karrenbelt MAP, Chan YA, Baby V, Labroussaa F, Brodeur J, Matteau D, Gourgues G, et al.: Large-scale recoding of a bacterial genome by iterative recombining of synthetic DNA. Nucleic Acids Res 2017, 45: 6971–6980.

48. Zhou J, Wu R, Xue X, Qin Z: CasHRA (Cas9-facilitated homologous recombination assembly) method of constructing megabase-sized DNA. Nucleic Acids Res 2016, 44. e124–e124.

49. Shao Y, Lu N, Wu Z, Cai C, Wang S, Zhong L, Zhou F, Xiao S, Liu L, Zeng X, et al.: Creating a functional single-chromosome yeast. Nature 2018, 560: 331–335.

50. Luo J, Sun X, Cormack BP, Boeke JD: Karyotype engineering by chromosome fusion leads to reproductive isolation in yeast. Nature 2018, 560:392–396.

51. Karas BJ, Tagwerker C, Yonemoto IT, Hutchinson CA, Smith HO: Cloning the Acholeplasma laidlawii PG-8A genome in Saccharomyces cerevisiae as a yeast centromeric plasmid. ACS Synth Biol 2012, 1:22–28.

52. Jakociunas T, Bonde I, Herrgard M, Harrison SJ, Kristensen M, Pedersen LE, Jensen MK, Keasing JD: Multiplex metabolic pathway engineering using CRISPR/Cas9 in Saccharomyces cerevisiae. Metab Eng 2015, 28:213–222.

53. Dicarlo JE, Conley AJ, Penttila M, Jantti J, Wang HH, Church GM: Yeast oligo-mediated genome engineering (YOGE). ACS Synth Biol 2013, 2:741–749.

54. Mosbach Y, Poggi L, Viterbo D, Charpentier M, Richard GF: TALEN-induced double-strand break repair of CTG trinucleotide repeats. Cell Rep 2018, 22:2146–2159.

55. Revilla Y, Pérez-Núñez D, Richt JA: African swine fever virus biology and vaccine approaches. In Advances in virus Research. Academic Press Inc.; 2018:41–74.

56. Sharma S, Tivendale KA, Markham PF, Browning GF: Disruption of the membrane nuclease gene (MBOVPG45_0215) of Mycoplasma bovis greatly reduces cellular nuclease activity. J Bacteriol 2015, 197:1549–1558.

57. Tock MR, Dryden DTF: The biology of restriction and anti-restriction. Curr Opin Microbiol 2005, 8:466–472.

58. Vasu K, Nagaraja V: Diverse functions of restriction-modification systems in addition to cellular defense. Microbiol Mol Biol Rev 2013, 77:53–72.

59. Roberts RJ, Vincze T, Posfai J, Macelis D: REBASE-a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res 2015, 43.

60. Horvath P, Barrangou R: CRISPR/Cas, the immune system of bacteria and archaea. Science 2010, 327:167–170.

61. Makarova KS, Wolf YI, Akhmashi OS, Costa F, Shah SA, Saunders SJ, Barrangou R, Brouns SJ, Charpentier E, Haft DH, et al.: An updated evolutionary classification of CRISPR–Cas systems. Nat Rev Microbiol 2015, 13:722–736.

62. Dormitzer PR, Suphaphiphat P, Gibson DG, Wentworth DE, Stockwell TB, Aligre MA, Alperovich N, Barro M, Brown DM, Craig S, et al.: Synthetic generation of influenza virus for rapid response to pandemics. Sci Transl Med 2013, 5.

63. Dormitzer PR: Rapid production of synthetic influenza vaccines. Curr Top Microbiol Immunol 2015, 386:237–273.

64. Wimmer E, Mueller S, Tumpey TM, Taubenberger JK: Synthetic viruses: a new opportunity to understand and prevent viral disease. Nat Biotechnol 2009, 27:1163–1172.

65. Wimmer E: The test-tube synthesis of a chemical called poliovirus the simple synthesis of a virus has far-reaching societal implications. EMBO Rep 2006, 7.

66. Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveni J, Stockwell TB, Brownlee A, Thomas DW, Aligre MA, et al.: Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. Science 2008, 319:1215–1220.

67. Rideau F, Le Roy C, Descamps E, Renaudin H, Larrique C, Bébéar C: Cloning, stability, and modification of mycoplasma hominis genome in yeast. ACS Synth Biol 2017, 6:891–901.

68. Ostrov N, Landon M, Guell M, Kuznetsov G, Teramoto J, Cervantes N, Zhou M, Singh K, Napolitano MG, Moosburner M, et al.: Design, synthesis, and testing toward a 57-codon genome. Science 2016, 352:819–822.

69. Tagwerker C, Dupont CL, Karas BJ, Ma L, Chuang RY, Benders GA, Ramon A, Novotny M, Montague MG, Venevally P, et al.: Sequence analysis of a complete 1.66 Mb Prochlorococcus marinus MED4 genome cloned in yeast. Nucleic Acids Res 2012, 40:10375–10383.

70. Noskov VN, Young L, Chuang R-Y, Gibson DG, Lin Y-C, Stam J, Yonemoto IT, Suzuki Y, Andrews-Pfannkoch C, Glass JI, et al.: Assembly of large, high G+C bacterial DNA fragments in yeast. ACS Synth Biol 2012, 1:267–273.

71. Karas BJ, Molparia B, Jablanovic J, Hermann WJ, Lin Y-C, Dupont CL, Tagwerker C, Yonemoto IT, Noskov VN, Chuang R-Y, et al.: Assembly of eukaryotic algal chromosomes in yeast. J Biol Eng 2013, 7.

72. Polo S, Ketner G, Levis R, Falgout B: Infectious RNA transcripts from full-length dengue virus type 2 cDNA clones made in yeast. J Virol 1997, 71:5365–5374.

73. Nikiforuk AM, Leung A, Cook BWM, Court DA, Kobasa D, Theriault SS: Rapid one-step construction of a Middle East Respiratory Syndrome (MERS-CoV) infectious clone system by homologous recombination. J Virol Methods 2016, 236: 178–183.

74. Shang Y, Wang M, Xiao G, Wang X, Hou D, Pan K, Liu S, Li J, Wang J, Art BM, et al.: Construction and rescue of a functional synthetic baculovirus. ACS Synth Biol 2017, 6:1393–1402.