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Synthetic Biology: Implications and Uses*

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Keywords

Synthetic biology
An effort to construct biological systems, which may include entire biosynthetic pathways, synthetic organelles and cellular structures, and whole organisms, that have medical, industrial, and scientific applications. This is achieved via the application of engineering principles, such as hierarchical design, modular reusable parts, the isolation of unrelated functions, and standard interfaces.

Synthetic cell
A cell that is controlled solely by a genome that was assembled from chemically synthesized pieces of DNA.

DNA assembly
The building of larger DNA fragments from smaller DNA fragments.

Circuits
A collection of various modular component parts that responds to an input signal that is then relayed to produce an output signal.

Compartmentalization
The spatial sequestering of substrates, intermediates, products, enzymes, and activities.

Synthetic biology is an effort to construct and engineer biological systems, ranging from individual genetic elements, to biosynthetic pathways, to whole organisms. The results of these engineering efforts can be of great value to human interests such as medicine and industry. In this chapter, advances in DNA assembly technologies are reviewed, and how these advanced DNA assembly technologies, in conjunction with the application of engineering principles such as modular parts, have facilitated the rational engineering of organisms to obtain desired functions or to understand complex cellular behavior, are highlighted. The recent creation of a synthetic cell is also described. Finally, the societal concerns posed by synthetic biology are discussed.

1 Introduction

The field of Synthetic Biology can be considered more as an engineering discipline, and less as an empirical science. Efforts to create artificial life systems, both in biochemical systems [1] and in software environments [2], may also be considered as Synthetic Biology, though these are beyond the scope of this chapter. Synthetic Biology is viewed as the effort to construct and engineer biological systems of value to human interests. Such

efforts can range in scopes far larger than the traditional genetic engineering of genes, to include the engineering of entire biosynthetic pathways complete with the regulation of the genes in that pathway [3, 4], synthetic organelles and cellular structures [5], whole organisms [6–9], and even ecosystems [10–13]. Synthetic Biology has the ambition to apply classical engineering principles such as hierarchical design, modular reusable parts, the isolation of unrelated functions, and standard interfaces. The empirical fields that correlate to Synthetic Biology are Systems Biology, Genetics, and Molecular Biology.

Synthetic Biology is not a new field, but rather extends back into prehistory. For example, it has been determined that the process of engineering maize – a highly optimized domestic agricultural crop plant – from the wild grass teosinte began over 9000 years ago [14]. The method used by the pre-Columbian cultivators of teosinte was simple artificial selection which, as such, is very slow. However, with the discovery of laws of inheritance and natural selection [15–17], and the suggestion that DNA was the chemical medium of inheritance [18], the scene was set to engineer a living system in a far more direct and rapid manner. A prominent example of this is the Dupont *Escherichia coli* strain used for the production of 1,3 propanediol, in which case an entire biosynthetic pathway has been added to *E. coli*, and the metabolism of the bacterium substantially altered to allow for a majority of the carbon feedstock (glycerol) to be converted into the economically valuable chemical 1,3 propanediol [6, 8, 9]. This feat, which was begun prior to the development of most of the Synthetic Biology techniques reviewed in this chapter, took many years and substantial investment to achieve. Yet, with recent advances in the field, such bioengineering projects will become faster to develop, easier to operate, and also much more ambitious.

During recent years, Synthetic Biology has progressed in a manner which is very different from those of other engineering disciplines. This is because, unlike architecture or software engineering, there is already a reservoir of highly sophisticated and complex functional parts to be found in Nature, and consequently most efforts in Synthetic Biology have been focused on harnessing that natural resource base. In general, two basic approaches have been undertaken to achieve this feat. The first approach has been to engineer natural organisms so as to incorporate recombinant pathways and other such desirable attributes. This method has the advantage of not requiring the capability to build – nor require an understanding of – massive biological systems such as genomes and metabolisms. However, it does have the disadvantage of being undefined; that is, whilst certain genes of the organism might be the result of human intervention, most of the genome remains wild-type, and is neither subject to human control nor necessarily operating within the limits of human knowledge.

The alternate approach is to use functional components, originally ''mined'' from Nature, such as promoters, aptamers, protein–protein interaction domains, terminators, or ribosome-binding sites. These functional components can be cataloged and then used to compose larger defined constructions of genes, pathways, and even whole genomes. Because all the components of a synthetic biological system that are constructed in such an approach have precisely defined properties, a high degree of predictive control over the final product is afforded. Indeed,

this more defined approach has become synonymous with advances in Synthetic Biology.

Historically, one of the main limitations in following this defined approach relates to the knowledge of these natural systems that serve as a source of parts. As biology has been characterized, both new components for synthetic biology – and also new tools to utilize those components – have become available. In turn, a new Synthetic Biology capability has driven greater advances in the understanding of biology. This has been most obvious in the development of tools for the synthesis and manipulation of DNA, the first of which were developed via the discovery of restriction endonucleases, DNA ligases, and the creation of recombinant DNA molecules [19–24]. These tools allowed the development of the recombinant DNA cloning and expression techniques that ultimately made possible the exploitation of enzymes with desired activities and properties. Notably, the development of the polymerase chain reaction (PCR) greatly increased the ability to amplify and manipulate DNA [25–27]. The subsequent combination of recombinant DNA cloning techniques with the PCR allowed a much greater exploitation of natural biological components, such as thermostable DNA polymerases, and this in turn made the PCR more robust and practical. Today, the PCR has become an indispensable tool for biology. Thus, knowledge of natural systems has led to an improved technology for exploiting those systems, which has in turn provided an improved knowledge of biological systems in a double feedback loop, thus improving both scientific understanding and technological capabilities. As shown in Fig. 1, as knowledge of the fundamental principles of biology have continued to grow, it has been possible to take a more defined engineering approach.

In the past, advances in synthetic biology have been bounded by the capacity to assemble and modify DNA, as well as knowledge of biological parts and circuits that such DNA might encode. Correspondingly, these two areas are directly addressed in the following sections, with details of synthetic biological pathways, synthetic genomes, synthetic organelles, and even synthetic organisms provided as

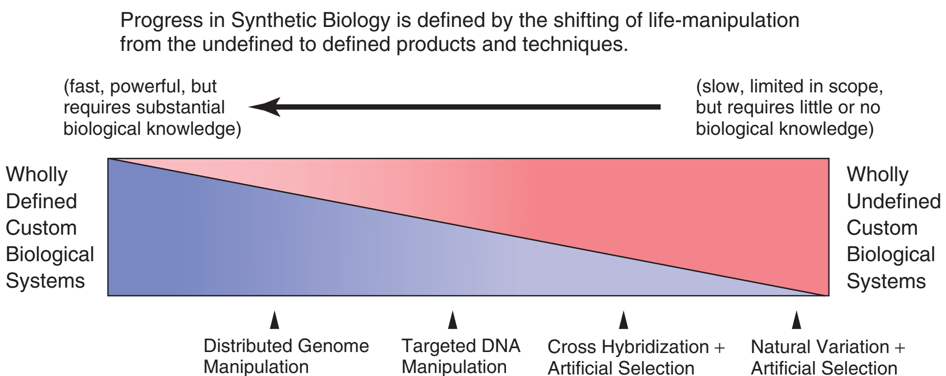


Fig. 1 Increasingly defined biological engineering. A schematic of how biological engineering has emphasized a more defined and rational design as it has advanced, based on a greater knowledge of natural biological systems.

examples of the capabilities that Synthetic Biology has already begun to deliver. These new capabilities create, in turn, new societal challenges, and these are also discussed.

2
DNA Assembly and Modification

As discussed above, one way to view Synthetic Biology is as an engineering discipline aimed at manipulating cellular systems to produce a *de novo*-designed function that does not exist in the natural organism. As with other engineering fields, however, Synthetic Biology is dependent on the tools and techniques available.

Organisms carry out a variety of reactions aimed at their self-sustenance and self-replication, with such reactions being carried out by the proteins and RNAs encoded in the organism's genome. As the sequence of the DNA directs production of the proteins and RNAs of an organism, control of the cellular DNA therefore allows for an ability to direct the functions of a cell. Based on this principle, many of the basic tools utilized in Synthetic Biology are aimed at producing defined sequences of DNA molecules, and easily manipulating the DNA content of an organism. The DNA molecules necessary for Synthetic Biology purposes can vary greatly in length, from individual DNA parts, genes and plasmids (containing tens to thousands of base pairs) to biosynthetic pathways and genetic circuits (thousands to millions of base pairs) to synthesizing whole genomes (viral and bacterial).

For many years, gene cloning and DNA assembly were dominated by the use of restriction endonucleases and DNA ligases [19–24]. While some well-designed restriction enzyme-based methods are still commonly in use [28, 29], these methods are gradually being superseded by the development of very rapid, more robust and less limited DNA assembly techniques. For example, although BioBricks were originally designed to be assembled with a restriction enzyme/ligation method [30], more recently an *in vitro* homologous recombination was adapted to increase the flexibility and speed of BioBrick assembly [31].

The starting materials for DNA sequence construction may include chemically synthesized DNA oligonucleotides (oligos), natural DNA fragments, PCR products, or a combination of all three sources. Defined short single-stranded oligos have been commercially available as a commodity for many years, usually for use as primers in PCR, mutagenesis, and sequencing reactions. Since chemically synthesized DNA oligos are of a user-defined sequence, this allows for a nucleotide level control of gene sequences and even entire genome sequences – a firm requirement when designing new functions in organisms. The idea of synthesizing genes from DNA oligos is not new; previously, oligos have been used to synthesize genes such as the alanine tRNA from yeast [32] and the human leukocyte interferon gene [33]. Likewise, the gene encoding a mammalian hormone, somatostatin, was synthesized and expressed in *E. coli* [34]. It is only recently that the lower costs of oligonucleotide synthesis and DNA sequencing have been combined to allow the development of more cost-effective and rapid methods for assembling groups of oligos into synthetic pieces of DNA or genes [35–39]. Today, several commercial gene synthesis companies exist that are able to produce custom genes/DNA at an accessible cost per base pair, although such costs

can quickly become prohibitive if numerous different DNAs are required. The cost prohibition of large-scale gene synthesis can, in part, be overcome by utilizing natural DNA fragments and PCR products in the DNA assembly for those sections of DNA that do not need to be created synthetically.

Typically, the process of constructing DNA is hierarchical (Figs 2 and 3). Briefly, groups of smaller DNAs (single- or double-stranded) are mixed and assembled into larger DNA pieces. Figure 2 shows double-stranded DNAs being assembled into a larger construct, but the process can begin with single-stranded oligos as the substrates. These larger pieces (subassemblies) are then grouped and assembled. These steps can be repeated until the final full-length DNA construct is obtained, whether it is a gene or genome. The DNA pieces to be assembled must have homologous overlapping ends, the overlaps being important because the DNA assembly techniques utilize homologous recombination. For example, if three pieces of DNA (A, B, and C) are to be assembled into a single DNA molecule, then one end of piece A must have an overlap with piece B, and the other end of B must overlap piece C. This configuration will result in a linear DNA molecule, A–B–C. In order to generate a circle from these pieces, the end of C must overlap piece A. The assembly of DNA into a circle is most often achieved with a DNA piece which contains sequences that enable the final construct to be cloned into a desired host (e.g., *E. coli*, *Saccharomyces cerevisiae* or *Bacillus subtilis*) [37, 38, 40–42]. The DNA homologous recombination reaction can be carried out completely *in vitro* in one step, either with an enzyme mix [37] or by using the PCR [35]. The reaction can also be performed *in vivo* by utilizing the natural homologous recombination activity of an organism, such as *S. cerevisiae* (yeast) and *B. subtilis* [7, 40–44]. Occasionally, a method will include an *in vitro* step to perform a partial reaction (DNA chewback/DNA annealing/DNA extension), and an *in vivo* step to complete the reaction (DNA repair) [38, 45, 46].

The various *in vitro* homologous recombination methods used to assemble double-stranded DNA or single-stranded oligos share the same general mechanism (Fig. 2). The nucleotides are first removed from one strand of the overlapping ends of the adjacent double-stranded DNAs, thus creating single-stranded ends of the DNA (Step 1). This process is analogous to a restriction enzyme digestion creating complementary sticky ends of DNA, except that the single stranded ends are typically 20–60 nucleotides long. Depending on the method used, the nucleotides can be removed by applying an exonuclease activity from either the 5′ or 3′ ends of the DNA [37, 38]. The creation of single-stranded overhangs (Step 1) is not necessary if oligos are used as the starting material for DNA assembly, because the oligos are already single-stranded. The single-stranded DNA overhangs are complementary to each other on adjacent molecules, and thus are able to anneal (Step 2). If the ends of the molecule being constructed are complementary, then the final construct will be circular (this is the normal method used when DNA is being assembled into a cloning or expression vector). The final step to the reaction is repair of the DNA. In the *in vitro* reaction, a DNA polymerase is used to fill the gaps, while a DNA ligase seals the nicks so as to create the larger assembled DNA molecule. The DNA repair activity of *E. coli* can be utilized to complete the reaction after the annealing step

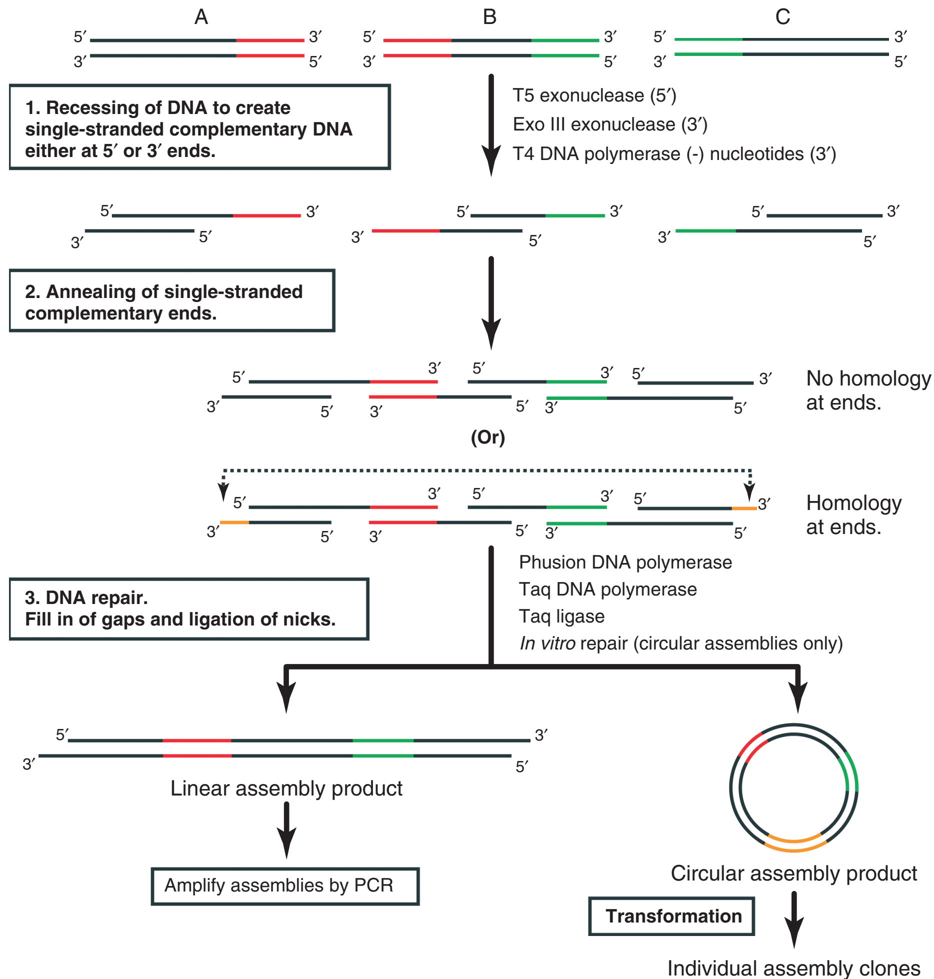
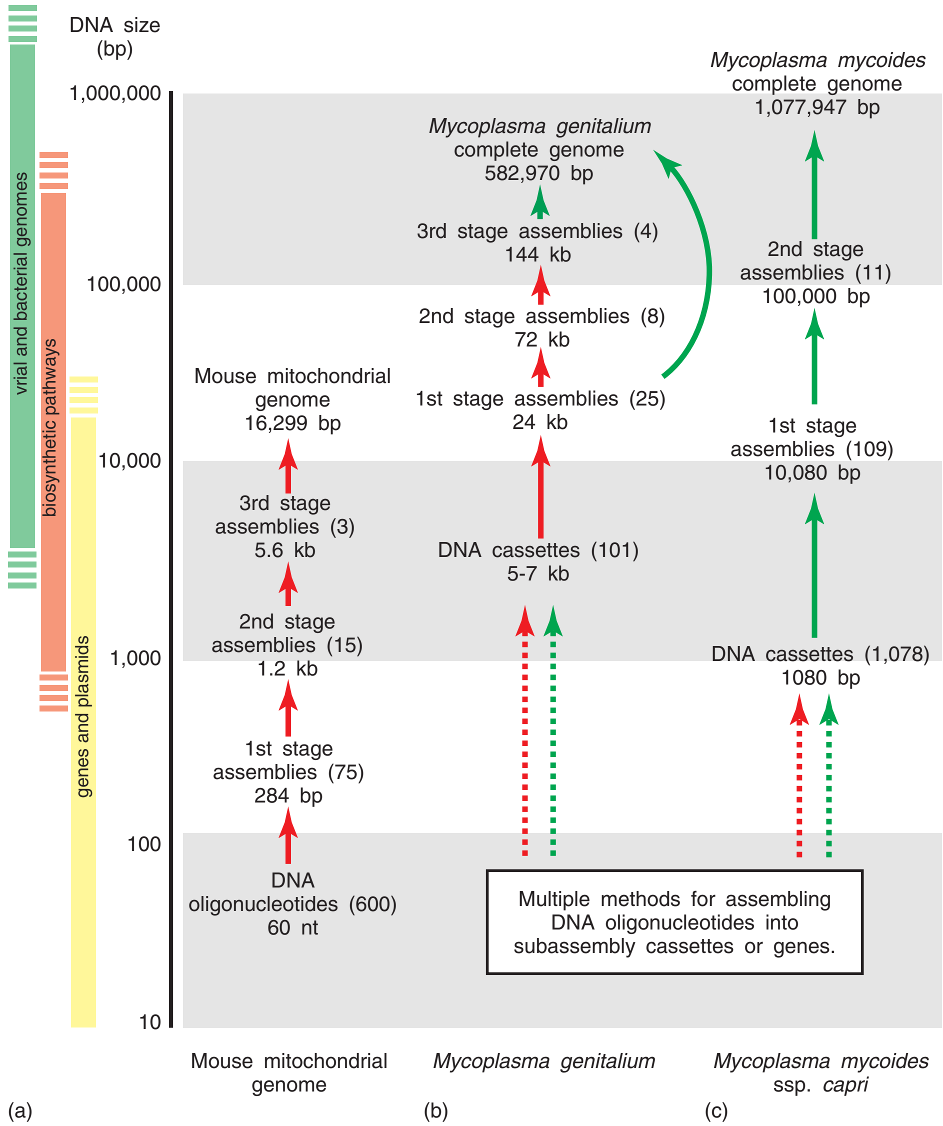


Fig. 2 Schematic depicting *in vitro* homologous recombination DNA assembly. First, nucleotides are removed from either the 5′ or the 3′ ends of the DNA pieces (5′ removal depicted). This step can be performed by several enzymes. The newly exposed single-stranded homologous ends (red, green, or yellow regions) on the adjacent pieces are complementary, and can anneal. Providing homology at the ends of the DNA pieces will result in the assembly of a circular DNA molecule. Following the annealing step, the DNA is repaired by filling in the gaps with a DNA polymerase and sealing any nicks with DNA ligase. A linear assembly product can be amplified by using the PCR and used in further assembly reactions. The circular assembly products are transformed into the appropriate host in order to isolated individual clones with the final assembled molecule. The assemblies can also be repaired *in vivo* after transformation by the native activities of *E. coli*.


DNA size (bp)
1,000,000
100,000
10,000
1,000
100
10
vrial and bacterial genomes
biosynthetic pathways
genes and plasmids
Mouse mitochondrial genome 16,299 bp
3rd stage assemblies (3) 5.6 kb
2nd stage assemblies (15) 1.2 kb
1st stage assemblies (75) 284 bp
DNA oligonucleotides (600) 60 nt
Mycoplasma genitalium complete genome 582,970 bp
3rd stage assemblies (4) 144 kb
2nd stage assemblies (8) 72 kb
1st stage assemblies (25) 24 kb
DNA cassettes (101) 5-7 kb
Mycoplasma mycoides complete genome 1,077,947 bp
2nd stage assemblies (11) 100,000 bp
1st stage assemblies (109) 10,080 bp
DNA cassettes (1,078) 1080 bp
Multiple methods for assembling DNA oligonucleotides into subassembly cassettes or genes.
Mouse mitochondrial genome
Mycoplasma genitalium
Mycoplasma mycoides ssp. *capri*
(a)
(b)
(c)

[38] and after other enzymatic steps that remove errors [36]. The final assembly can be amplified by using the PCR and, if necessary, used in another round of assembly to generate even larger DNA molecules. If the final assembly is circular. it can be transformed into the appropriate host in order to generate individual assembly clones.

The construction of DNA from oligos can also be performed *in vivo* using the recombination activity of an organism. The yeast *S. cerevisiae* has robust homologous recombination activity, as well as an ability to take up multiple pieces of double- or single stranded DNA [47–51]. Previously, Gibson and colleagues have shown that yeast can assemble at least 38 overlapping single-stranded 60-mer oligos directly into a plasmid, thus forming a 1170 bp DNA insert. Alternatively, fewer – but longer – oligonucleotides (up to 200 nt in length) that overlap by as little as 20 bp can also be used to assemble 1100 bp assemblies directly into the desired plasmid [43]. The ability of yeast to support at least 2 Mb of cloned DNA also makes yeast a good host for assembling large constructs.

At this point, a discussion of gene synthesis and DNA assembly methods, within the context of the synthesis of three different genomes, will be used to highlight and demonstrate a variety of DNA assembly methods that have been used to synthesize DNA sequences, starting from single-stranded oligos, and the hierarchical assembly of the DNA subfragments into complete genomes. This is not meant to be a comprehensive list of all available methods and techniques; rather, the intention is to demonstrate the flexibility of recently used methods in DNA assembly. Whilst the discussion will be within the framework of whole genome synthesis, these techniques can also be used to synthesize and/or assemble any DNA of interest, from a few base pairs in length to over a million.

Gibson and colleagues have synthesized three genomes using both *in vitro* and *in vivo* assembly techniques [7, 40, 41]. Figure 3 shows a schematic flow of the hierarchical synthesis of the mouse mitochondrial genome (16 299 bp) (this has also been assembled, using a different method, by Itaya *et al.*; see below),

Fig. 3 Synthesis of mitochondrial and bacterial genomes. The hierarchical assembly of three genomes is depicted with the sizes of the intermediate subassemblies and final products on a logarithmic scale. The red arrows represent *in vitro* assembly, and the green arrows *in vivo* assembly in yeast. Values in parentheses indicate the number of pieces at that stage in the assembly. The colored bars on the left represent the several different DNA molecule classes that can be produced, and their relative sizes. (a) The mouse mitochondrial genome was synthesized starting from 60 nucleotide-long oligonucleotides in four stages. All of the assembly steps were performed *in vitro*; (b) The *Mycoplasma genitalium* genome was assembled from 5 to 7 kb cassettes purchased from a custom DNA synthesis company. Both, *in vitro* and *in vivo* DNA assembly techniques were utilized in the genome construction. The final stage of the assembly was performed *in vivo*, using yeast. The ability of yeast to take up multiple DNA pieces can eliminate several rounds of construction, as demonstrated by the 25-piece *in vivo* assembly of the genome; (c) The *Mycoplasma mycoides* ssp. *capri* genome was assembled from over one thousand 1080 bp purchased DNA cassettes. The three assembly steps for this genome were all performed *in vivo*, with yeast as the host. The dotted red and green lines represent the availability of several *in vitro* and *in vivo* DNA synthesis methods that can be applied to produce the DNA cassettes for use in hierarchal DNA assemblies.

the *Mycoplasma genitalium* genome (582 970 bp), and the *Mycoplasma mycoides* ssp. *capri* genome (1 077 947 bp). The basis of these DNA assembly methods is the DNA homologous recombination of overlapping DNA fragments.

The mouse mitochondrial genome was synthesized starting from 600 overlapping 60 nt-long single-stranded DNAs (60-mers), using an *in vitro* one-step assembly method (as discussed above) and PCR [52]. The first stage of genome construction produced 284 bp subassemblies by assembling groups of eight oligos directly into pUC19 (Fig. 3a). Assembling the oligos directly into a cloning vector allowed the individual assemblies to be cloned, isolated, and sequence-verified before continuing with the construction process. Following sequence verification, the correct 284 bp first stage assemblies were amplified by PCR in order to generate more material. Following amplification, the first-stage assemblies were pooled into overlapping groups of five, and again assembled *in vitro* to produce the 1.2 kb second stage assembly intermediates. The second stage assemblies were not propagated in a host organism, but rather were PCR-amplified immediately following the *in vitro* assembly reaction. These 15 PCR products were pooled into groups of five and then joined to form the 5.6 kb third stage assembly intermediates. The assembly products were amplified by PCR (as before), and these three PCR products were then assembled to form the complete synthetic mouse mitochondrial genome. The final assembly reaction with the three PCR products also contained a bacterial artificial chromosome (BAC), so that the finished mitochondrial genome could be cloned into *E. coli*. This case demonstrates not only the ability to perform large-scale DNA construction almost entirely *in vitro*, but also that the process is amenable to automation.

In 2008, the first synthetic bacterial genome was synthesized at the J. Craig Venter Institute [40]. The process to assemble the synthetic 582 970 bp *Mycoplasma genitalium* genome was hierarchical – similar to the mouse mitochondrial genome assembly (although the *M. genitalium* genome is about 35-fold larger). The synthetic *M. genitalium* genome was assembled from 101 synthetic DNA cassettes each of about 5–7 kb in length (Fig. 3b). In this case, the cassettes were synthesized from oligos by several different gene synthesis companies, and verified by sequencing. The cassettes overlapped their adjacent neighbors by an average of about 80 bp. In order to allow the formation of the circular genome, cassette 1 overlapped cassette 101.

The main challenge in the synthesis of the *M. genitalium* genome was the assembly and cloning of synthetic DNA molecules larger than those previously known. In the first stage, sets of four adjacent cassettes were assembled by *in vitro* recombination into a BAC vector to form circularized recombinant plasmids with about 24 kb inserts that were then released by restriction enzyme-mediated digestion in preparation for the next assembly stage. The 25 first-stage assemblies were taken three at a time to form the 72 kb second-stage assemblies, again by *in vitro* recombination. In the third stage, the 72 kb second-stage assemblies were taken two at a time to produce four third-stage assemblies, each of approximately one-quarter-genome (144 kb) in size. The first three stages of assembly were performed by *in vitro* recombination and cloned into *E. coli* in order to generate more DNA for the subsequent rounds of assembly. The final stage of

the *M. genitalium* genome assembly was carried out *in vivo* by utilizing the homologous recombination activity of *S. cerevisiae*. The last stage of the genome assembly consisted of six overlapping pieces of DNA to generate the complete *M. genitalium* genome (one yeast vector, two fragments of quarter 3, and quarters 1, 2, and 4). The final step was performed *in vivo* because limitations of the cloning host and *in vitro* assembly reaction became apparent. It is possible that larger assemblies (280–580 kb) are not stable in *E. coli*, but it is also possible that the circularization of large DNA molecules may be inefficient during the *in vitro* recombination reaction, and/or that handling large DNA molecules in solution leads to breakage of the DNA before transformation.

Subsequently, the powerful ability of yeast to take up and assemble multiple large fragments of DNA was demonstrated by taking the 25 first-stage assemblies and assembling them in one step by using yeast [41]. This proved to be significant because it allows for fewer assembly steps, and thus greatly reduces the time required to construct large DNAs.

By leveraging the DNA uptake and recombination capability of yeast, a three-stage hierarchical strategy was designed to assemble the 1 077 947 bp *M. mycoides* ssp. *capri* genome. In this case, the assembly steps were performed entirely *in vivo* by transformation and homologous recombination in yeast, following the initial DNA cassette constructions (Figs 3c and 4) [7]. This differs from the strategy used to construct the mouse mitochondrial and *M. genitalium* genomes, which used in part an *in vitro* homologous recombination reaction. The cassettes designed to assemble into the complete genome were generally each of 1080 bp, with 80 bp overlaps to adjacent cassettes. As with *M. genitalium*, the 1078 cassettes (each 1080 bp long) were all produced commercially by the assembly of chemically synthesized oligos. To assist in the assembly process, DNA cassettes and assembly intermediates were designed to recombine in the presence of vector elements to allow for growth and selection in yeast. During the first stage of assembly, groups of 10 of the 1080 bp DNA cassettes and a vector were recombined in yeast to produce circular subassembly plasmids; these were then transferred to *E. coli* in order to easily generate the quantities of subassembly DNA required for the second-stage assembly step.

For the second-stage assemblies, 10 of the 10 kb assemblies were pooled and their respective cloning vectors transformed into yeast to produce 100 kb assembly intermediates. Circular plasmid DNA was extracted from yeast in order to proceed to the final assembly stage. In the final stage, 11 of the second-stage assemblies (100 kb each) were pooled, and the yeast transformation procedure was repeated a final time to produce the circular *M. mycoides* ssp. *capri* genome.

Recently, various alternatives to using yeast to clone and assemble large DNAs *in vivo* have been described. For example, Itaya and colleagues also constructed the complete recombinant mouse mitochondrion (16.3 kb) and rice chloroplast (134.5 kb) genomes from their small contiguous DNA pieces. In these cases, the starting DNAs for the assembly were derived via a PCR and assembled in *B. subtilis* [53]. The latter bacterium has a very large capacity to uptake and assemble DNA, as demonstrated by cloning of the 3.5 Mb genome of the photosynthetic bacterium *Synechocystis* into the *B. subtilis* genome [42, 54]. This was accomplished

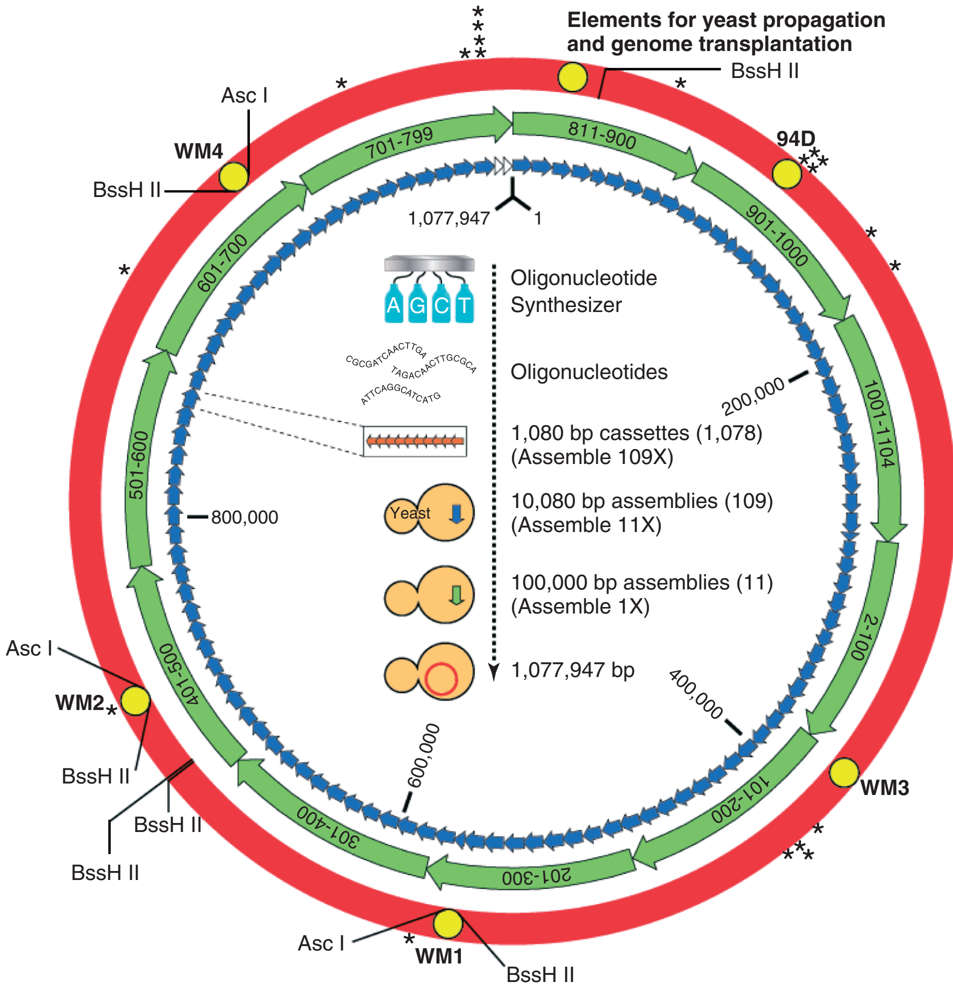


Fig. 4 The assembly of a synthetic *M. mycoides* ssp. *capri* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 approximately 10 kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 approximately 100 kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle). With the exception of two constructs that were enzymatically pieced together *in vitro* (white arrows), assemblies were carried out by *in vivo* homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include four watermarked regions (WM1 to WM4), a 4 kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* ssp. *capri* sequence. The designed sequence is 1 077 947 bp.

by progressively assembling and editing contiguous DNA regions that cover the entire *Synechocystis* genome. It is important to note that the *Synechocystis* genome was not a circular free molecule (as are the other genomes), but rather was incorporated as two pieces into the *B. subtilis* genome.

Several general features have been identified that might be desirable in a DNA synthesis method. Although, ideally, the method should have a low cost per base pair of DNA synthesized, the present cost of DNA synthesis is dominated by the price of the starting oligos. Oligonucleotides obtained from DNA microarrays can greatly reduce the cost of gene synthesis (estimated to be as much as an order of magnitude), because thousands of oligos can be synthesized on a single chip on a small scale. Gene synthesis from DNA microarrays can be hampered by the small quantity and complex mixture of the oligos obtained [55–57]. Regardless of the source of oligos, the cost of DNA synthesis can be greatly impacted by the amount of sequencing required to find a correct clone, and this leads to the importance of accuracy in gene synthesis. Errors in gene synthesis are unavoidable because the process of creating the starting DNA oligos is not perfect, and some fraction of the oligos will inevitably contain errors. Whilst the starting oligos can be purified by using different methods in order to minimize the number of error-containing oligos, such processes are expensive and time- consuming, and also are not compatible with high-throughput gene synthesis. The sequencing of multiple clones is sometimes sufficient to identify the correct synthesized DNA, depending on the efficiency of the assembly method used and the size of the DNA construct. Several error-correction methods have been developed, however, in attempts to reduce the amount of sequencing required [36, 58, 59].

The goals of Synthetic Biology often require DNA to be manipulated from the nucleotide to the genome level. Although the methods available to generate synthetic DNAs from genes to genomes have been discussed, in many cases a genome may need to be modified by inserting, deleting, or replacing a gene or sequence. These modifications may be necessary in only a few places, either individually or simultaneously in several noncontiguous places. Consequently, several DNA manipulation techniques have been developed to allow these types of change.

Recombineering uses the activity of lambda phage enzymes to catalyze highly efficient homologous recombination *in vivo* [60]. The lambda Red system allows an easy and rapid modification of the genome of a compatible host. However, because only short homologous overlaps are required for recombination with the lambda Red system, it is possible to use PCR to easily generate the modifying DNA by incorporating the homologous overlaps into the primer design. Recombineering can be used to insert, delete or replace a gene or sequence. Moreover, if multiple modifications are needed in the same organism the process can be repeated sequentially, although the time required to make more than a few changes can become significant. This system is available for *E. coli* [61, 62], *Pseudomonas* [63], and *Salmonella* [64], while a similar system based on a different phage has been developed for *Mycobacterium tuberculosis* and *M. smegmatis* [65]. Based on the studies with *M. tuberculosis*, it is not unreasonable to speculate that a similar recombineering system can be implemented in many more organisms by exploiting their native phages.

By using the same lambda recombination proteins, Wang and colleagues developed the technique of multiplex automated genome engineering (MAGE) for the large-scale programming and evolution of *E. coli* cells [66]. MAGE employs a mixture of single-stranded oligos to simultaneously target many locations on the chromosome for modification either in a single cell, or across a population of cells. Selection markers are not necessary because the process is efficient, iterative, and cumulative. The highest efficiencies of MAGE are observed when small changes are being made to the genome (a few base pairs), but the efficiency is much reduced when larger changes such as insertions (>20 bp) or deletions (>1000 bp) are attempted. The MAGE process is able rapidly to produce combinatorial genomic diversity. Indeed, the power of MAGE has been demonstrated by tuning the translation of 20 endogenous genes and optimizing the production of lycopene in *E. coli*. Warner *et al.* have combined a molecular barcode technology with recombineering to develop trackable multiplex recombineering (TRMR) [67], such that thousands of specific genetic modifications can be produced simultaneously, by recombineering. In this case, each modification is associated with a molecular barcode, and the barcode sequences and microarrays can then be used to quantify the allele frequency in the population; this, in turn, allows mapping of the genetic modifications that affect a trait of interest. This technique may be useful when engineering a trait for which there is limited genetic knowledge.

A method for the rapid engineering of multiple genetic changes in yeast was developed by Suzuki and colleagues [68]. This method, referred to as "*Green Monster*," employs an inducible green fluorescent protein (GFP) reporter gene to create individual deletions in separate yeast strains. The deletions in the individual strains are then combined by repeated rounds of mating, meiosis, and flow cytometry-based enrichment. In each sexual cycle, progeny bearing an ever-increasing number of altered loci are enriched on the basis of gene dosage. If it could be adapted to extrachromosomal DNAs, the Green Monster might represent a valuable technique for altering large DNAs or bacterial genomes which are cloned in *S. cerevisiae*. Although the method has been demonstrated using *S. cerevisiae*, it should be possible to extend the technology to bacteria with a mating equivalent such as bacterial conjugation (e.g., *E. coli*).

In some cases, when the expression from a high gene dosage is desired, the use of plasmids may not be a feasible option. In this case, copies of the gene of interest could be inserted into the genome by using some of the above-described methods, but this may be both labor- and time-intensive if a high gene dosage is required. In an attempt to overcome this problem, Tyo and colleagues used a plasmid-free, high-gene copy expression system termed chemically inducible chromosomal evolution (CIChE) to evolve an *E. coli* chromosome with about 40 copies of a recombinant pathway [69]. This was achieved by creating a cassette that contained the genes of interest, along with a gene encoding antibiotic resistance for chloramphenicol. When the strain was then grown in increasing concentrations of chloramphenicol, the selective pressure of the increasing antibiotic concentration resulted in duplications of the cassette and, therefore, also of the antibiotic resistance gene by *recA*-dependent homologous recombination. When the desired cassette copy number was reached, *recA* could

be deleted to prevent any homologous recombination that could alter the cassette copy number.

As shown above, many good methods have been devised for constructing and assembling either synthetic or natural DNA. However, several factors must be considered and balanced when deciding on a DNA assembly strategy; these should include the cost of the method, the time required, the individual's experience with the different host organisms, and whether there is a need for vast combinatorial numbers. Fortunately, the different assembly methods are diverse and robust, so that a large range of requirements can be met. In general, it appears that yeast may be the preferred host when constructing large DNAs, although several of the methods available to generate genes or smaller (ca. 1 kb) subassemblies for the construction of larger DNAs are equally suitable, depending on the user's preferences.

3
Modular Parts and Circuits

One core component of Synthetic Biology is to apply engineering-based approaches of modularization, rationalization, and modeling to control cellular behavior, in order to obtain desired functions or to understand complex biological systems [70, 71]. As a result, an increasing number of synthetic biologists have begun to apply electrical circuit analogies to biological pathways as a means of designing and generating synthetic genetic devices which can then be placed into cells to control their behavior [72]. The first examples of genetic circuits – the toggle switch and the repressilator – were demonstrated about a decade ago [73, 74], since when there has been an ever-expanding number of reports of various types of biological circuit, including additional genetic switches [75, 76], other oscillators [77–80] and memory networks [81, 82], as well as other electronic-inspired genetic devices [70] such as pulse generators [83], logic gates [75, 84], filters [85], and communications modules [86, 87]. Today, these devices have begun to be used for practical applications in biosensing, therapeutics, and in the generation of important industrial products. As the scope of this chapter is very broad, it is only possible here to discuss these synthetic genetic devices and their uses with limited representative examples. A number of excellent recent reviews will provide more detailed information on these synthetic gene networks [70, 71, 88–90].

The basic design of circuits is the assembly of various modular component parts that respond to an input signal that is then relayed to produce an output signal. For biological circuits, the component parts have their origins in the vast amount of basic research in all aspects of the biological functions of organisms. The combined effort of many research groups has led to great understanding of the various pathways that organisms use to respond to environmental signals, such as light or quorum sensing. Accordingly, many of the components of these pathways – such as signaling proteins and transcription factors, as well as the promoters they control – have been identified from a wide variety of organisms [71, 89, 91]. In order to create some biological devices, synthetic biologists have taken components from one organism and placed them in a different model organism. For example, Danino *et al.* synthesized synchronized genetic oscillators by placing elements of the quorum sensing machineries of *Vibrio fischeri* and *Bacillus thurigensis* into the model organism, *E. coli* [77]. Given

the difference in GC (guanine–cytosine) content, codon usage, transcription and translation processes between the various organisms, it was not initially clear whether the devices created from the different organism-derived component parts would function as intended. However, to overcome this difficulty several methods have been adapted.

First, web applications such as GeneDesign [92] have been developed that enable synthetic biologists to design the component parts according to the specifications of the host organism (see Table 1). In addition, as discussed above, the recent advances in gene synthesis, as well as the decrease in the cost of oligos and the proliferation of gene synthesis companies, have allowed synthetic biologists simply to purchase the component parts.

Second, Knight, Rettberg, Endy and others have founded the Registry of Standard Parts (Table 1) to provide a framework for synthetic biologists to devise biological circuits, pathways, and other genetically encoded systems. The idea here is to emulate the engineering principles involved in the construction of such things as electronic devices [93]. The Registry of Standard Parts provides a catalog of a wide variety of biological parts, such as transcription promoters and terminators, ribosome binding sites and regulatory proteins, as well as a variety of chassis in which the parts can function. This facilitates the ability of the synthetic biologists to tailor their biological devices by allowing them to choose from a variety of parts whilst, at the same time, providing a wealth of information on how these parts can function. The Registry also allows for interfacing with other web applications such as modeling tools. As an example, users can input information from the Registry into SynBioSS designer to generate kinetic models for the selected biological constructs, and provide a picture of how these constructs would influence the behavior of the whole [94].

With these advances in place, synthetic biologists are today beginning to use DNA assembly techniques to piece together modular parts into controlled biological pathways as well as biological circuits in a wide spectrum of applications, such as biosensing, the production of therapeutics and biofuels, as well as understanding complex cellular behavior and even ecosystems. For example, synthetic biosensors can be used to detect various environmental signals and then to prompt cells to enter a programmed behavior [70]. In one study, Kobayashi *et al.* generated a genetic device that could detect DNA damage and, through a designed activation of the SOS pathway, program *E. coli* cells to enter a biofilm state [95]. The ability was also demonstrated of designed bacteria to produce invasin from *Yersinia pseudotuberculosis*, upon the detection of an hypoxic environment of tumor cells, which in turn allowed the bacteria to invade the tumor cells [96]. In a particularly innovative experiment, Looger *et al.* computationally redesigned protein–ligand specificities to construct receptors that could bind trinitrotoluene or L-lactate. These receptors were then incorporated into synthetic bacterial signal transduction pathways to regulate gene expression in response to extracellular trinitrotoluene or L-lactate [97]. The results of these studies confirm the promise that genetic biosensors of this type may be useful for detecting any desired environment or signal, and to allow the host organism to respond in a targeted manner.

Biological circuits have also shown their possible value in the antibacterial therapeutic field. For example, bacteriophages

Tab. 1 Resources for synthetic biology.

Resource	***URL***	***Comments***
Synthetic Biology Resources	http://www.istl.org/10-spring/internet1.html	Provides links to various resources on the internet, including synthetic biology associations, centers of research, ethics, training and educational resources, and journals
Synthetic Biology.net	http://www.syntheticbiology.net/index.aspx	Portal for professionals in synthetic biology providing information on news, events, products, suppliers, etc., regarding synthetic biology
Synthetic Biology Project	http://www.synbioproject.org/	Established as an initiative of the Foresight and Governance Program of Woodrow Wilson International Center for Scholars to foster informed public and policy discourse concerning the advancement of Synthetic Biology
BioBricks Foundation	http://bbf.openwetware.org/	Encourages the development and responsible use of technologies based on BioBrick™ standard DNA parts to allow synthetic biologists to program living organisms in the same way a computer scientist can program a computer (see below)
Registry of Standard Biological Parts	http://partsregistry.org/Main_Page	A collection of genetic parts that can be mixed and matched to build synthetic biology devices and systems
SynBERC Synthetic Biology Engineering Research Center	http://www.synberc.org/	Mission is to develop technologies to build biological components and assemble them into integrated systems to perform designed tasks, train engineers for biology, and educate the public on Synthetic Biology
BIOFAB	http://www.biofab.org/	Biological design–build facility that aims to produce useful collections of standard biological parts available to academic and commercial users
JBEI Registry	https://public.jbeir.org/	Also aims to provide standard DNA parts for Synthetic Biology
GeneDesign	http://www.genedesign.org/	Set of web applications that provides public access to a nucleotide manipulation pipeline for Synthetic Biology
SynBioSS Designer	http://synbioss.sourceforge.net/	Software suite for the generation, storage, retrieval, and quantitative simulation of synthetic biological networks

were engineered to suppress the SOS pathway of bacteria and enhance the killing of antibiotic-resistant bacteria, ‘‘persister cells,’’ and biofilm cells [98]. Another area where the ability to easily design and place modular parts into a designed biological pathway is proving useful is in the control of metabolic flux for the production of industrially important materials or chemicals. Recently, the Stephanopoulos group has reported an ability to increase the titer of taxadiene (an intermediate of the potent anticancer drug, Taxol) in an engineered *E. coli* strain [99]. This effect was accomplished by first partitioning the taxadiene metabolic pathway into two modules – a native upstream methylerythritol-phosphate (MEP) pathway forming isopentenyl pyrophosphate, and a yew tree-based heterologous downstream terpenoid-forming pathway – and then varying the module's expression simultaneously to obtain an improved balanced pathway.

Genetic devices are also proving their worth in helping to understand the underlying basic principles of coordinated complex cell behavior. As examples, two relatively recent studies have highlighted efforts to emulate pattern formation that is important for development in higher eukaryotes [85, 100]. The latter study used an *in vitro* approach with DNA-coated paramagnetic beads fixed by magnets in an artificial chamber to form artificial transcription–translation networks that generate simple patterns [100]. In the former study, Basu *et al*. engineered two genetically distinct populations of bacteria (acyl-homoserine lactone senders and receivers) and manually overlaid them in different configurations to produce different patterns [85]. More recently, instead of using two distinct bacterial populations, Tabor *et al*. genetically engineered an isogenic community of *E. coli* cells to sense light, to communicate to identify light-dark edges, and produce an image [101]. Similar biological circuits to those discussed above have also been utilized to construct synthetic ecosystems or biofilms to model and better understand microbial communities [10–13]. While relatively simple genetic devices have been used thus far, it is expected that a thorough characterization of their performance, together with improved predictive mathematical tools, will allow for the design and construction of more elaborate circuits to program cells and cellular communities for functions that mimic those of natural systems.

4
Spatial Regulation

Many of the parts and circuits detailed above involve the regulation of genes or gene-products. For most of the time, when gene regulation is referred to, it is assumed to be a temporal regulation – that is, the increase or decrease of expression of a gene that was, at a previous *time*, in the other state. It is, however, also possible to regulate genes and gene products in space. The ability to compartmentalize or localize enzymatic activities has long been recognized as a powerful means of optimizing catalysis. In Nature, this can take the form of macromolecular complexes that actively channel substrates from one enzymatic active site of a biosynthetic pathway to the next [102–105], organelles and compartments that can physically separate certain enzymes and substrates from the rest of the cellular processes [106, 107], or it may simply be the result of reduced diffusion due to co-localization [108, 109]. Just as compartmentalization and co-localization can take many forms, so too they offer many advantages.

These include the mitigation of toxicity of intermediates of a biosynthetic pathway, the protection of intermediates from diffusion or degradation, the elimination of unproductive side reactions as a consequence of the other biological activities of the host organism, or improving activity by driving kinetics (for an excellent review of both natural and engineered systems of spatial control over cellular processes, see Ref. [108]). In the past, organic chemists have been inspired by these natural systems and have created a wide variety of biomimetic catalysts that incorporate such features as cyclodextrin covalent linkers that allow many enzyme molecules to be tethered to one another, leading to improved kinetics [110]. However, whilst inspired by biology, and often using components derived from living organisms, such systems are not wholly biological and rarely operate in the biological context from which their component enzymes are derived. Consequently, such cell-free systems, which lack the self-replication capability of living cells, often suffer from problems of enzyme stability and purification. An excellent review of biochemical constructs that incorporate compartmentalization and co-localization based around such diverse methods as covalent and noncovalent linkers, of micelles made from both synthetic polymers and lipids, and of vesicles and viral particles used as nanoreactors, has been prepared by Vriezema *et al.* [111].

Here, examples of engineered, cellular compartmentalization strategies will be discussed, with emphasis placed on the design principles that they embody and use.

4.1 Co-Localization

Dueber *et al.* were able to use the synthetic biology principles of modular parts and co-localization to create a heterologous synthetic protein complex in *E. coli* of three enzymes: acetoacetyl-CoA thiolase (A to B); hydroxy-methylglutaryl-CoA synthase (HMGS); and hydroxymethylglutaryl-CoA reductase (HMGR) [3]. These three genes, derived from yeast, compose a pathway that produces mevalonate from acetyl-CoA. Mevalonate is a precursor for the production of chemicals in the industrially and medically valuable large isoprenoid family [112]. However, due to very different levels of activity, these enzymes – even if expressed at optimal levels – result in a build-up of the toxic intermediate hydroxymethylglutaryl-CoA (HMG-CoA). To overcome this, Dueber *et al.* organized the three-enzyme pathway into a synthetic complex of enzymes on a ‘‘scaffold’’ by using the well-characterized signal processing protein–protein interaction domains of the metazoan cells [mouse SRC Homology 3 (SH3) and Post Synaptic Density protein, *Drosophila* Disc Large Tumor Suppressor, and Zonula Occludens-1 protein (PDZ) domains and the rat GTPase protein Binding Domain (GBD domain] with their corresponding ligands. Each ligand is a small tag-like sequence that can be added to either end of a protein, and which binds specifically to its corresponding domain. The SH3, PDZ and GBD domains were then expressed as a fusion protein that would recruit the three ligand-tagged enzymes of the mevalonate pathway into a single complex or ‘‘scaffold.’’ The main aspects of the scaffold optimization and design involved the order of the binding domains (and, consequently, of the pathway enzymes with their ligands), and whether the ligand sequence was fused to the N or C terminus of each enzyme. Another aspect to be optimized was the number of each enzyme that was recruited to the

scaffold. Typically, a scaffold might be designed to have three PDZ domains, but only one SH3 and one GBD domain; this caused three copies of one of the pathway enzymes to be recruited to the complex, but only one each of the other enzymes. When these optimizations were made, the result was a dramatic 77-fold increase in the yield of mevalonate due to a reduction in the metabolic load on the cell and toxicity associated with HMG-CoA build up. The general nature of the method was also proved by its application to the pathway for glucaric acid synthesis [3].

The principle of synthetic complexes and co-localization can be used to improve not only synthesis pathways but also degradative pathways. For example, the fungal cellulase Cel6A has been docked onto a bacterial mini-cellulosome to achieve a greater cellulose degradation and, as with the mevalonate biosynthetic pathway discussed above, issues of geometry and organization of the final synthetic scaffold strongly affected the efficacy of the result [113, 114]. As the technology of scaffold design improves, the development may begin of synthetic pathways that resemble tryptophan synthesis [102], polyketide synthesis [104] or carbamoyl phosphate synthetase [105], where the enzymes are not merely co-located but actually channel the substrate down a tunnel.

4.2 Compartmentalization

Instead of recruiting enzymes to a scaffold and achieving a degree of control over the diffusion of substrates from enzyme to enzyme of a pathway, it might be advantageous to create a separate cellular compartment to physically encapsulate specific enzymes and substrates. An impressive example of this has been seen in the investigations of Parsons *et al.* [5], who characterized the genes of a natural bacterial organelle, which they called a bacterial microcompartment (BMC). BMCs are polyhedral protein shells that are associated with specific biosynthetic pathways. The most well-studied BMC is the carboxysome, which is associated with the fixation of carbon in Cyanobacteria [115]. Parsons *et al.* focused their attention on the *pdu* operon which contains a number of genes, some of which are implicated in the construction of a BMC, while others are associated with *Salmonella enterica* serovar. *typhimurium* LT2's pathway for the conversion of 1,2-propanediol into propionaldehyde, 1-propanol, and propionyl-CoA. These enzymes, and the reactions they catalyze, are localized within the BMC that the *pdu* operon encodes [106]. Based initially on the sequence similarity to carboxysome proteins, and later on the results of experiments where certain proteins were subtracted, Parsons and coworkers identified a set of five genes (*PduA*, *PduB*, *PduJ*, *PduK*, and *PduN*) which produced six proteins (*PduB* produces two versions of the protein) that are necessary and sufficient to produce empty BMC compartments in *E. coli*, similar to those produced by the native *pdu* operon in *S. enterica* serovar. *typhimurium* LT2. Although, not necessary, PduU was shown to help regulate the size of the BMC to the approximately 100 nm dimensions it has natively. If the full set of required genes for BMC construction was not present, then large intracellular structures such as sheets, filaments, or hexagonal lattices were observed. To prevent these structures from forming, the wild-type order and orientation of the remaining *Pdu* genes needed to be maintained. Lastly, by using the N-terminal region of a gene in the *Pdu*

operon, *PduV*, it was possible to create a tag that localized GFP into the BMC.

Although Parsons *et al.* failed to demonstrate any enzymatic activity inside the resulting BMC, this was achieved inside a compartment that was similar in some ways, but prepared from a cowpea chlorotic mottle virus capsid, albeit with the very simple single enzyme system of horseradish peroxidase [116]. It was also shown possible to recruit compartments that are already present in a cell, such as the periplasmic space [117].

In keeping with the modular approach central to most Synthetic Biology methods, Parsons *et al.* and Deuber *et al.* devised systems by which tags could be fused to enzymes, allowing for their localization and thus the spatial regulation.

5 The Synthetic Cell

The organisms that have been engineered thus far for industrial or other purposes have had the advantage of being easily manipulated genetically, because of high transformation efficiencies and good recombination activities. Unfortunately, many industrially relevant organisms do not possess these characteristics and therefore, cannot easily be engineered; for these and other intractable organisms, novel engineering methods are necessary. One approach, as adopted by the research team at the J. Craig Venter Institute, has been to build a minimal cell that contains only essential genes, the functions of which have been characterized, in an attempt to understand the basic principles of life. Such a cell may also provide a base into which various pathways can be placed to synthesize industrially important products, but in a more energy efficient manner than is possible with the presently engineered organisms.

During their efforts to build a minimal cell, the J. Craig Venter Institute group recently reported the creation of a bacterial cell that could be controlled by a chemically synthesized genome [7]. The research group described in detail the design, synthesis, and assembly of the 1.08 mega-base pair *M. mycoides* JCVI-syn1.0 genome (see Fig. 4), starting from digitized genome sequence information, and its transplantation into a recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. To distinguish the synthetic genome from the natural genome, the researchers placed ‘‘watermark’’ sequences and included other designed gene deletions and polymorphisms in the synthetic genome (see Fig. 4). Even though the cytoplasm of the recipient cell is not synthetic, the research team referred to the cells produced after the transplantation process as ‘‘synthetic cells,’’ because they are controlled solely by a genome that was assembled from chemically synthesized pieces of DNA. These synthetic cells have expected phenotypic properties, although the JCVI-syn 1.0 transplants grew slightly faster than a control strain.

These studies, using several *Mycoplasma* species, were the culmination of extensive efforts over a number of years that led to the development of several novel technologies (as summarized in Fig. 5). First, as discussed above, the team developed a strategy of assembling viral-sized pieces to produce large DNA molecules that allowed them to assemble bacterial genomes in *S. cerevisiae* [40, 41, 43]. Second, the team established additional methods to clone whole bacterial genomes as centromeric plasmids in yeast [118]. Third, they developed methods to transplant the genome of one bacterial species, *M. mycoides* ssp.

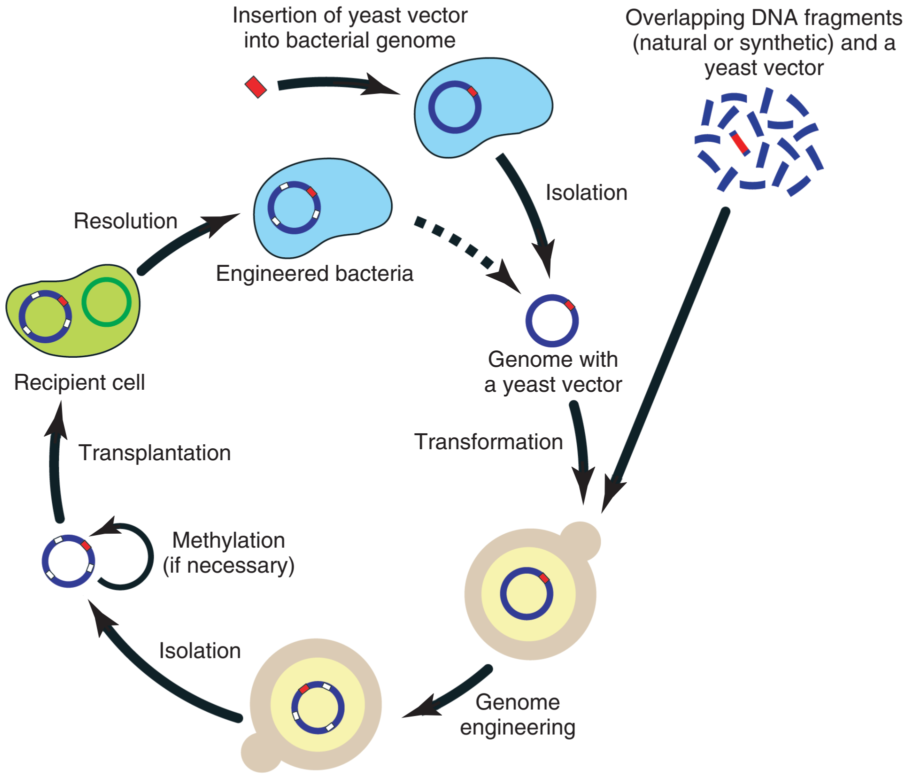


Fig. 5 Moving a bacterial genome into yeast, engineering the genome, and its re-installation into a bacterium, by genome transplantation. A yeast vector is inserted into a bacterial genome by transformation, and the genome is then cloned into yeast. Alternatively, a bacterial genome is cloned by transforming overlapping DNA fragments (natural or synthetic) together with a yeast vector into yeast and allowing the host's homologous recombination system to assemble an intact genome. After cloning, the repertoire of yeast genetic methods is used to create insertions, deletions, rearrangements or any combination of modifications in the bacterial genome. This engineered genome is then isolated and transplanted into a recipient cell to generate an engineered bacterium. Prior to transplantation, it may be necessary to methylate the donor DNA in order to protect it from the recipient cell's restriction system(s). This cycle can be repeated starting from the newly engineered genome (dashed arrow).

capri, into a different recipient bacterial cell species, *M. capricolum* ssp. *capricolum*, to obtain cells of the donor *M. mycoides* ssp. *capri* [119]. These studies were extended when the group was able to transplant the genome of *M. mycoides* ssp. *capri*, which this time was isolated from yeast as a centromeric plasmid, into recipient *M. capricolum* ssp. *capricolum* cells and produced viable *M. mycoides* ssp. *capri* cells [120]. The team also genetically altered the *M. mycoides* ssp. *capri* genome in yeast by using the host's powerful genetic tools and newly developed tools [121] to produce a new strain of *M. mycoides* ssp. *capri* that would not have been possible with the tools currently available for these bacterial species.

Taken together, the Venter Institute research team has developed a series of technologies that enabled them to clone whole bacterial genomes, whether from natural sources or synthetic pieces, to manipulate them, and to transplant them to produce viable bacterial cells (see Fig. 5). It will be interesting to see in the future whether this technology can be utilized for other intractable organisms.

6
Societal Challenges Posed by Synthetic Biology

Along with the potential of significant benefit, all new technologies raise societal concerns. With respect to biotechnology in general, these can be described as concerns about bioterrorism, laboratory safety, harm to the environment, the distribution of benefits, and ethical and religious concerns [122–125].

Synthetic Biology itself – both at the level of research and in the application of such research to the development of new products – also raises a variety of societal concerns, some of which are identical to those raised by all biotechnology, though some may be unique. These new or unique concerns may be especially important for the governance of the new technology. To its credit, the community of research workers that identify as synthetic biologists recognized at a very early stage that these societal concerns were both real and legitimate. A good number of the synthetic biologists have worked with a variety of policymaking and social science research communities, to ensure that the studies being carried out would be performed in a safe and ethical manner. Given much interaction between and among these various communities, concerns that are unique – or that have been recognized as of great importance, even if not unique – have been well analyzed, and while the policy problems have in no way been solved the challenges have been very well articulated.

The first sets of societal concerns that were dealt with in detail by these communities were those of biosecurity and biosafety. There is a constellation of ethical issues (discussed below) that are of no less importance than security and safety. However, it was clear very early on that if Synthetic Biology were to result in hazards that could not be mitigated for the research teams or for society as a whole, then there would need to be a moratorium on such studies. Consequently, these societal concerns regarding security and safety were analyzed first by a variety of policy researchers. Hence, the safety and security analyses remain, for now, somewhat more advanced than the ethics analyses.

In particular as much of the recent Synthetic Biology studies have been conducted in a post ''9–11'' environment, concerns relating to biosecurity were at the forefront of virtually all policy analyses. Specifically, the ability to synthesize genomes means that, at least in some cases, access to pathogens can no longer be physically limited as long as the sequences are publicly available. For now, the concerns are about increasing the ease with which viruses, such as 1918 influenza, smallpox and Ebola, can be obtained [125]. Additionally, Synthetic Biology may eventually provide a relatively straightforward way to construct pathogens with increased virulence, by allowing those with nefarious intent to add a variety of pathogenesis factors directly to a viral genome or bacterial chromosome.

In order to deal with these potential malicious applications, both the United States Government [126] and a consortium of

companies providing synthetic DNA [127] have released guidelines for the screening both of synthetic DNA orders, and of the customers placing these orders. Questions as to whether these guidelines should be legally binding, the full range of what the companies should be screening for, and whether orders for smaller pieces of DNA (oligonucleotides) should also be screened are all currently under discussion.

Current biosafety or laboratory safety concerns are mostly focused on the speed and scale that Synthetic Biology brings to research, and some concerns about workers in the field who have not been trained as microbiologists. At institutes with a formal approach to dealing with biosafety – such as universities, research foundations and scientific companies – institutional biosafety committees and other institutional groups will likely be taking up questions in Synthetic Biology research in the same way that they do all other biological/biotechnological research. In an earlier report [125], policy researchers at the JCVI (Michele Garfinkel and Robert Friedman), MIT (Drew Endy), and the Center for Strategic and International Studies (Gerald Epstein) have described several options for such bodies for educating themselves, and their institutional research teams, about what steps must be taken to ensure that the research is safe. A more generic set of concerns about the research teams, and how to mitigate any possible biosafety dangers, was discussed even earlier in a report from the National Academy of Sciences [128]. Yet another set of people interested in synthetic biology has also raised concern, namely the "do it yourself" (DIY) community [129]. Whilst for the moment, little is being done by the DIY community that is clearly "synthetic biology," there has been much discussion among the group eventually to employ those technologies. In anticipation of this possibility, the Presidential Commission for the Study of Bioethical Issues, which is in the process of completing a report on and recommendations for synthetic biology, has addressed the issues of DIY specifically [130].

Although the safety and security concerns may not be "solved," it appears at least that the great majority of issues have been laid out, and at least for the moment there seem to be no risks that would lead to the conclusion that the research should be banned, or even severely restricted. (This also appears to be the conclusion of the Presidential Bioethical Commission, although its current recommendations are only in draft form.) Thus, the policy- and social science research communities have turned at least some of their attention to other, broader societal challenges brought about by the potential use of Synthetic Biology technologies.

Concerns about harm to the environment from accidental or planned releases of engineered microbes date to discussions at the Asilomar meetings during the mid-1970s. The two critical concerns are that an engineered microbe will grow out of control if released accidentally or as part of a planned release, and that DNA from an engineered organism may be transferred to a related organism. These concerns have been dealt with over time via guidance and regulation dealing with the containment of genetically modified organisms and rules for testing these organisms for release into the environment. Several US Government agencies are currently reviewing several sets of regulations and guidance to understand whether they are sufficient to deal with the use of many new microbes in open environments. For example, the *NIH Guidelines* for working with recombinant DNA are currently being reviewed

to assure that guidance which was written to deal specifically with recombinant DNA applies equally to synthetic DNA [131].

In addition to the potential harm to people or to the environment (as discussed above), there are societal challenges beyond the physical. The distribution of benefits and risks is a very long-standing concern, and one which surfaces for virtually every new technology. Ownership as defined by intellectual property rights, the concentration of knowledge, and resources in a small number of firms or institutes – and how and whether these resources should be shared – are issues that have become particularly acute around research groups (both academic and commercial) who wish to develop products. Interestingly, the Synthetic Biology community, in addition to civil society organizations, has placed this issue at the forefront of many of its own discussions [132]. These discussions could well lead to a better understanding of distribution concerns and possible solutions generally.

Finally, hubris – sometimes called *"playing God"* – might be the major nonphysical concern in Synthetic Biology, even if it is not fully unique in this case. In brief, concerns about hubris are focused on a key issue: Are there actions that human beings simply should not take? In the case of constructing a synthetic cell, these questions arise for many communities, from religious traditions to policymakers. Is constructing a synthetic cell creating life? If it is, is it hubris? If it is not creating life, then what would define creating life? In either case, is this hubris? And how might conducting such experiments as constructing a synthetic cell change how human beings think of themselves, both individually and with respect to other organisms and the environment in general? There are long and thoughtful writings – both fiction and nonfiction, and both recent and deep in history – about hubristic pursuits that will not be reviewed here. However, it should be noted that these questions are being studied in detail with respect to Synthetic Biology by philosophers, ethicists and theologians [133].

7 Concluding Remarks

Today, the capability is available to create any arbitrary DNA sequence, and to express that sequence in a wide variety of living systems. By using modular parts with standardized structures, this DNA assembly capability has allowed the engineering of pathways, organelles, organisms, tissues, and even ecosystems. Unlike previous genetic engineering methods and selective breeding methods, these engineering projects have the potential to be cheap, fast, and easy. As these capabilities are further refined and extended, an era can be anticipated in which biological engineering will impact every industry and activity of humankind.

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