Front. Chem. Sci. Eng.
DOI 10.1007/s11705-017-1618-2

REVIEW ARTICLE

Genome reprogramming for synthetic biology

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Abstract The ability to go from a digitized DNA sequence to a predictable biological function is central to synthetic biology. Genome engineering tools facilitate rewriting and implementation of engineered DNA sequences. Recent development of new programmable tools to reengineer genomes has spurred myriad advances in synthetic biology. Tools such as clustered regularly interspace short palindromic repeats enable RNA-guided rational redesign of organisms and implementation of synthetic gene systems. New directed evolution methods generate organisms with radically restructured genomes. These restructured organisms have useful new phenotypes for biotechnology, such as bacteriophage resistance and increased genetic stability. Advanced DNA synthesis and assembly methods have also enabled the construction of fully synthetic organisms, such as J. Craig Venter Institute (JCVI)-syn 3.0. Here we summarize the recent advances in programmable genome engineering tools.

Keywords CRISPR, genome engineering, synthetic biology, rational design

1 Introduction

Synthetic biology seeks to develop new organisms through forward genetic engineering. We can develop tools to study complex gene regulatory networks *in silico* [1], however, forward engineering of genetic systems enables us to identify and understand emergent and unexpected phenomena in biology [2,3]. The ability to manipulate DNA is intrinsically linked to our ability to experimentally study and forward engineer regulatory gene networks. Genome engineering tools have allowed us to reprogram life to explore basic science and to engineer novel organisms for biotechnology. The field has progressed from basic molecular cloning to programmable methods for remodeling and constructing new organisms.

Implementations of stable synthetic gene circuits and reengineering of biosynthetic pathways requires reengineering of an organism [4]. Even the scenario of episomal expression of synthetic gene constructs often also requires strains modified from wild-type counterparts. For instance, implementation of the genetic toggle switch requires removal of endogenous lacI repressor via genome editing [5,6]. Library-based investigation of gene network engineering requires efficient genome integration methods [7,8]. A convergence of programmable editing, new directed evolution methods, rational protein engineering, and DNA synthesis have propelled synthetic biology forward. As the field of synthetic biology moves forward, so will the enabling technologies. Genome engineering will require increased specificity to move to therapeutic applications. Evolutionary methods will need to enable large-scale rewriting of organisms to find non-trivial solutions to challenging problems. Proteins that efficiently target DNA recombination will enable large-scale restructuring of organisms. DNA synthesis and assembly methods will enable production of large-synthetic constructs, including whole genomes.

In this review, we highlight newly developed technologies enabling the rational redesign of organisms. Clustered regularly interspace short palindromic repeats (CRISPR) derived technologies have revolutionized our ability to target DNA manipulation *in vivo*. We discuss the state of the art CRISPR based methods for rewriting and implementing synthetic transgenes. We describe methods to conduct large-scale rewriting of genomes, ranging from strategies to target genome reduction, to methods powered by genetic randomization and evolution. Furthermore, we discuss the development of methods to generate *de novo* organisms, such as the recently developed minimal synthetic *Mycoplasma mycoides* genome, JCVI-syn3.0 [9].

Received September 27, 2016; accepted December 2, 2016

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2 RNA-programmable genome engineering

CRISPR and CRISPR-associated (Cas) systems function as a prokaryotic and archaeal immune system [10–13] CRISPR loci express a long non-coding RNA, which is subsequently processed by Cas proteins (e.g., Cas9 of type II CRISPR systems) [14] to form mature targeting CRISPR RNAs (crRNAs). These crRNAs target endonuclease activity of Cas9 (or other Cas proteins) to target DNAs. Watson-Crick base pairing between crRNA and target DNA combined with the presence of a PAM sequence on the target results in Cas9 catalyzed DNA cleavage (Fig. 1 (a)) [15]. Researchers quickly saw the potential of CRISPR systems, in particular, those involving Cas9, as other systems require formation of large multiprotein complexes (e.g., those of type I and III CRISPR systems) [14].

Cong and colleagues along with Mali and coworkers co-published initial reports demonstrating the application of engineered CRISPR systems in human cells [16,17]. They demonstrated that CRISPR RNAs can be engineered to target Cas9 nuclease activity to endogenous target sites. These reports spurred the development of CRISPR based technologies.

Initial reports suggested Cas9 activity was highly specific to target sites, requiring nearly 20-out-of-20 nucleotides matching between crRNA and target DNA, however a subsequent report showed CRISPR can readily induce off-target mutations [18]. To circumvent, this numerous strategies have been developed to increase Cas9 specificity. Ran and colleagues developed a paired-nickase system for targeting non-homologous end joining (NHEJ), homology directed repair (HR) and non-HR mediated integration [19]. Individual single-stranded DNA breaks (nicks) to the chromosome are repaired without mutagenesis. However, paired CRISPR-targeted nicks in close proximity and in a 5' overhang orientation result in efficient mutagenesis (Figs. 1(b) and 1(c)). Alternatively, Tsai et al. and Guilinger et al. concurrently utilized protein engineering to increase specificity. They showed fusion of catalytically inactive Cas9 fused to a FokI endonuclease domain dramatically increase DNA cleavage specificity [20,21]. This system enables cooperative genome targeting, wherein double-stranded DNA cleavage requires dimerization of FokI domains. This increases the specifi-

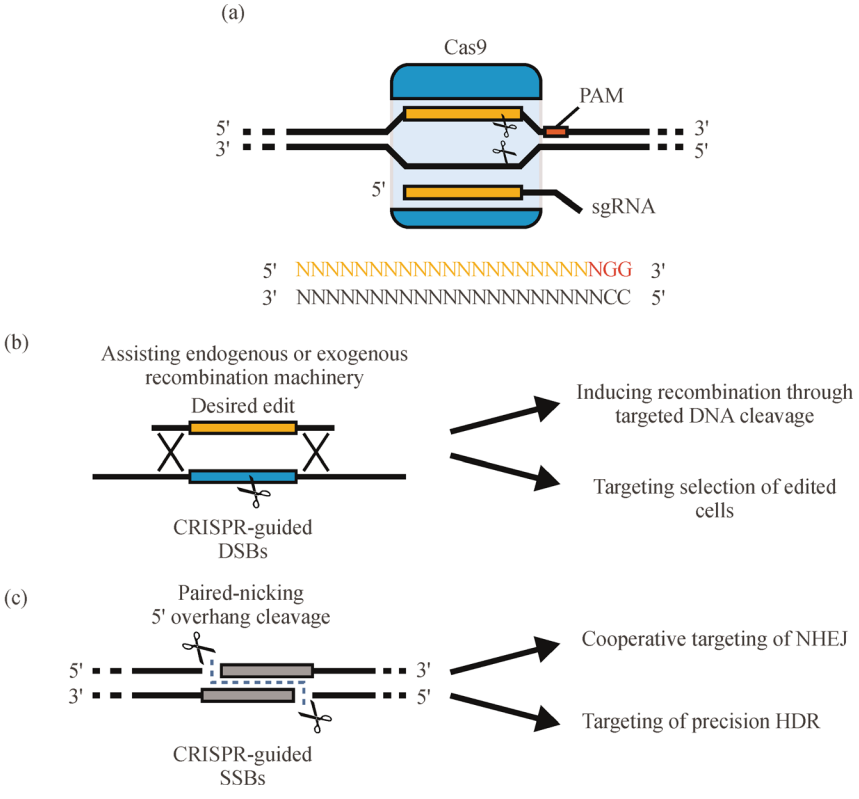

Fig. 1 Programmable editing of genomes. (a) A schematic of CRISPR-directed targeting with wildtype *S. pyogenes* Cas9. Cas9 (blue) is targeted to a DNA sequence based on the presence of a protospacer adjacent motif (PAM, red) nucleotides matching those in a short guide RNA (sgRNA). The target DNA sequence is written beneath. The strand matching the 20 nucleotide guide of the sgRNA is orange and the complementary strand is black; (b) CRISPR-guided double stranded DNA breaks (DSBs) involving a recombination template have various modalities. DSBs either induce host homology directed repair (HDR) or DSBs kill cells that have not acquired the desired edit, wherein the full CRISPR target site is not present in the desired edit. HDR-mediated editing can be either a function of one or both of these modalities; (c) novel mutant versions of Cas9 that mediate single-stranded DNA cleavage have been developed to target recombination in a broad range of organisms

city of genome editing over 140 fold over wild-type *Streptococcus pyogenes* Cas9. This approach is simultaneously versatile and highly specific. Fu and colleagues found an interesting alternative to increase accuracy of CRISPR editing methods: decreasing the length of short guide RNAs (sgRNAs) from 20 to 18 nucleotides increases targeting specificity [22]. Furthermore truncating sgRNAs to 14 nucleotides enables targeted DNA binding of Cas9 while avoiding DNA cleavage [23]. This enables the development of multi-targeted Cas9 editing and regulatory fusions. These methods will likely be greatly useful with therapeutic application allowing simultaneous function of Cas9 in genetic circuitry and gene knockout [23,24]. Slaymaker and coworkers developed an enhanced specificity Cas9 (eSpCas9) through rational protein reengineering [25]. Mutation of various positively charged amino acids in the non-target DNA binding groove of *S. pyogenes* Cas9 confers higher specificity of Cas9 mediated cleavage. Similarly, Kleinstiver and colleagues described the engineering of a high fidelity version of Cas9 (spCas9-HF) through rational protein reengineering [26]. Likewise, Kleinstiver and coworkers demonstrated in a separate piece the directed evolution of *S. pyogenes* and *Staphylococcus aureus* Cas9 to generate novel variants with altered PAM requirements [27,28]. This may be advantageous if alternative (non-NGG) PAMs are desired. Likewise, increasing the size of the requisite PAM region may be useful in the creation of therapeutically relevant highly specific Cas9s, This may be more likely to make it into therapeutic and disease modeling use.

Synthetic biology seeks to forward engineer novel cellular behaviors and phenotypes. This can be accomplished through both rewiring of endogenous gene networks or through integration of synthetic DNAs. He and coworkers demonstrate potential utility for CRISPR targeted genome integration in human cell lines. They show that double stranded DNA break (DSB) induced NHEJ can target chromosomal integration of fluorescent markers [29]. The system described by He and colleagues is capable of integrating 4.6 kb of DNA with relative high efficiency (20%). This is accomplished by simultaneous cleavage of a genomic target along with cleavage of a transfected donor plasmid. NHEJ results in incorporation of the synthetic reporter at the location of sgRNA targeted cleavage [29]. This technique, along with other editing modalities will be useful in the implementation of genetic circuitry or differentiation state reporters.

After initial reports of CRISPR editing in human cells, numerous reports came out employing engineered CRISPR systems in other organisms. Jiang and colleagues demonstrated CRISPR-Cas9 systems can direct recombination between the genome of *Streptococcus pneumoniae* and exogenous editing templates. This enabled selection marker free editing of multiple genomic targets. Likewise, they showed CRISPR-Cas9 can assist the lambda Red recombineering system for *Escherichia coli* by selecting for desired edits of the genome [30]. More recently it was demonstrated that CRISPR-Cas9 systems could augment potential sizes of genome integrations. Building off work that demonstrated use of SceI meganuclease can work cooperatively with the lambda Red recombination machinery to integrate large, 7 kb, synthetic constructs [31]. Bassalo and coworkers demonstrated cooperative use of lambda Red and CRISPR target DNA cleavage. They show CRISPR increases DNA editing efficiency up to 95%, wherein 50 out of 50 clones contain the correct integration. Furthermore they demonstrate integration of a 10 kb isobutanol biosynthetic pathway, using CRISPR combined with lambda Red [32]. Rapid implementation of full biosynthetic pathways, such as that for isobutanol described in Bassalo et al., was not possible through basic lambda Red mediated recombination. However, incorporation of CRISPR targeted DNA cleavage with lambda Red can target integration with ease and efficiency. The ability for single step integration of large-synthetic constructs is necessary for synthetic biologist to create complex new cellular functions. In *E. coli*, multiple works have demonstrated CRISPR working cooperatively with the lambda Red homologous recombination machinery, however, we took a different approach and developed a system to target endogenous *E. coli* homologous recombination. We demonstrated that nicking Cas9 mutant ($Cas9^{D10A}$) can be easily guided to genomic loci and, when dual-targeted, can efficiently direct large-scale recombination across the bacterial genome [33].

CRISPR-guided genome engineering has become an indispensible tool for non-model bacteria. For instance, Wang et al. and Li et al. demonstrate CRISPR as a tractable genome-engineering tool for *Clostridium beijerinckii* [34,35]. *C. beijerinckii* is an industrially useful organism for the production of acetone, butanol, and ethanol that previously lacked easily programmable methods for genome engineering [36]. Wang et al. show targeted gene deletion in *C. beijerinckii* using plasmid delivered 1 kb homology sequences. Because homolgous recombination rates are low in *C. beijerinckii* , the use of Cas9 enables high efficiency selection of edited clones [35]. Similarly, Li and colleagues show Cas9 nickase can target gene deletion via HR. They generate deletions ranging from 20 to 1149 bp in multiple clostridium species [34]. Mougiakos and colleagues provide an extensive review focusing on CRISPR's development from a bacterial immune system to a prokaryotic genome engineering technology [37]. Likewise, Choi and Lee provide a comprehensive description of the published methods using CRISPR systems for bacterial genome engineering [38]. Collectively CRISPR has functioned so reliably as a genome engineering platform in bacteria, it will expedite combinatoric reverse genetics studies and forward engineering of new organisms for synthetic biology [39].

CRISPR-guided genome engineering's influence extends beyond bacteria into fungi also. *Saccharomyces*

cerevisiae (yeast) is a model fungus with tremendous biotechnological potential. Likewise, the eukaryotic cell physiology of yeast has allowed it to serve as a "proxy-organism" for the development of genome engineering tools [40]. It was not long after initial development of CRISPR systems that Dicarlo and coworkers demonstrated CRISPR-targeted double stranded DNA breaks can be used for simultaneous induction and selection of genome edits via homologous recombination [41]. Bao et al. demonstrated up to 3 simultaneous edits at a time in *S. cerevisiae* [42]. Subsequent works have demonstrated multi-pathway assembly employing CRISPR systems with up to 6 exogenous DNA sequences combined simultaneously or conversely removal of large genetic fragements [43]. Work from Jakociunas and colleagues demonstrated the power of CRISPR genome engineering strategies in yeast, enabling the assembly of 15 parts simultaneously [44]. They demonstrate assembly of a multipart carotenoid pathway generating *S. cerevisiae* capable of producing red pigment. The authors also demonstrate engineering tyrosine production through simultaneous pathway assembly and deletion of competing metabolic processes [44]. Tsarmpopoulos and coworkers demonstrated in an interesting study that exogenous bacterial genomes can be edited inside *S. cerevisiae* [45]. Similarly Kannan et al. demonstrated CRISPR-guided editing in yeast combined with genome transplantation can be employed to study 16S rRNA structures in *M. mycoides* [46]. The methods described offer an efficient way to reengineer the minimal genomes and organisms recalcitrant to manipulation for basic science and forward genetic engineering. CRISPR systems have become an indispensible molecular instrument for the combinatoric rewriting and construction of new genetic systems.

3 Engineering through evolution

Evolution is the fundamental force that has driven the development of all life. Engineering through evolution has tremendous potential to enable researchers to identify non-intuitive and non-trivial solutions to biological problems [48]. For instance, biosynthetic pathways and genetic circuitry may require evolutionary optimization to reach a desired function [49]. Wang and colleagues demonstrated a method that can employ MAGE to rewrite the *E. coli* genome [47]. Using lambda Red recombineering machinery, this system integrated recoding oligonucleotides into the *E. coli* genome [50,51]. The MAGE system facilitates efficient diversification and rewriting of the genome. This generates populations of cells with diversified phenotypes, which can be leveraged to identify and select organisms with desired traits (Fig. 2(b)). For instance, in the initial implementations of MAGE the authors developed an *E. coli* strain capable of better producing lycopene, a commercially useful pigment. Following the first development of MAGE, the same group reported removal of all UAG stop codons in *E. coli* via combining MAGE and bacterial conjugation in a method called Conjugative Assembly Genome Engineering (CAGE) [52]. Removal of all endogenous UAG stop codons renders *E. coli* resistant to various bacteriophages and frees the codon for researchers to study *in vivo* incorporation of new amino acids. Generating novel codon variants will be useful for engineering proteins with synthetic amino acids. Recoded organisms are resistant to bacteriophages and are genetically orthogonal to their natural counterparts, making sharing traits by horizontal gene transfer highly unlikely [53]. This suggests great biotechnological and *ex vitro* potential for recoded organisms.

More recently, Farzadfard and Lu developed a novel genome-rewriting platform for *E. coli* called synthetic cellular recorders integrating biological events (SCRIBE) [54]. The SCRIBE system utilizes a reverse transcriptase along with a retron template RNA cassette to generate single stranded DNAs and the lambda Beta gene to facilitate DNA incorporation in lagging strand synthesis. This enables targeted and chemically controllable bacterial genome rewriting. The system was originally applied to generate *in vivo* analog memory in bacterial populations. The population of bacterial cells function as "recorders" where genome rewriting is linearly proportional to time of retron induction. It is foreseeable that this system becomes useful in the detection of specific compounds or pathogens and perhaps most interestingly as a tool for inducible genome editing and evolution of organisms. More recently, Perli and colleagues demonstrated human cell genetic recording [55]. Self-targeting sgRNAs (stgRNAs) form indel mutations in response to environmental stimuli. This enables detection and quantification of inflammation response to lipopolysccharide. This suggests potential application of mammalian SCRIBE as a biological recorder and for the investigation of DNA sequence evolution. Fundamentally, SCRIBE systems demonstrate how genome engineering technologies can be utilized in synthetic biology as "recorders" and analog memory units in gene circuitry.

Evolution of organisms also employs large-scale genetic rearrangements and genome minimization if advantageous. Work by Richard Lenski and colleagues on *E. coli* has demonstrated reductive genome evolution over years in laboratory culture [56–58]. Tools to target this have been developed to enable *de novo* generation of bacteria and other microorganisms with large-scale changes from their progenitors. Genome reduction strategies may be advantageous to synthetic biology by removing non-essential genetic and metabolic burden to cells (Fig. 2(a)). Lambda Red recombineering tools can be employed to target genome reduction. Posfai and colleagues have demonstrated that 15% of the *E. coli* genome can be removed [59]. This leads to emergent phenotypes such as increased transformation efficiency and increased genome stability

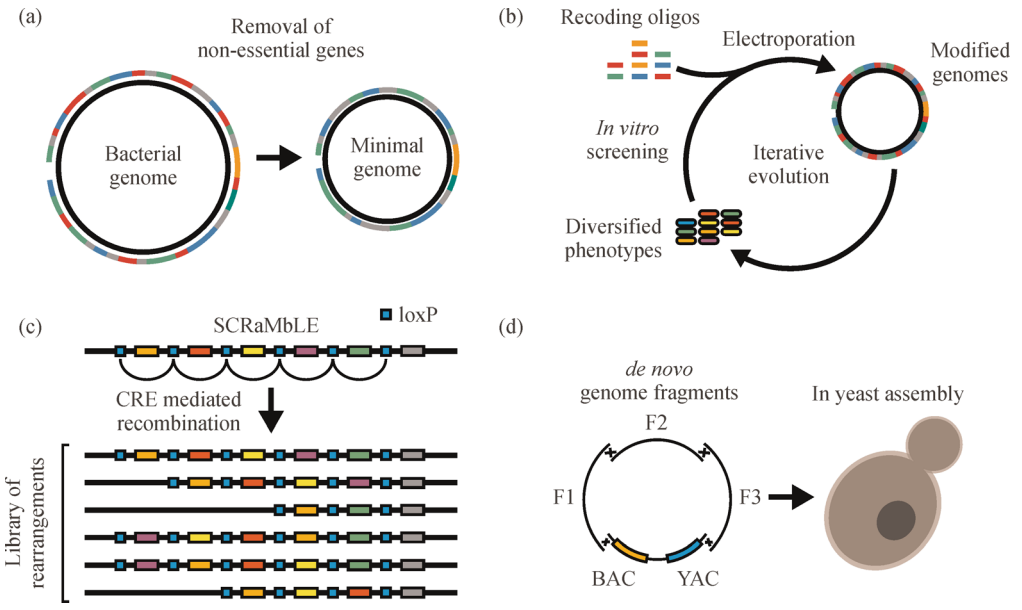

Fig. 2 Large-scale reengineering of organisms. (a) Genome reduction methods, such as methods employing CRE recombinase, lambda Red recombineering, and CRISPR-nickases, have enabled large-scale reductions to the *E. coli* genome. Genome reduction methods look to investigate the emergent phenotypes by removal of large numbers of non-essential genes. These methods may identify novel organisms and phenotypes for synthetic biology; (b) multiplex automated genome engineering (MAGE) offers itself as a powerful tool for coupling DNA synthesis, targeted editing, and evolution. MAGE functions as an iterative process. Recoding oligo nucleotides are electroporated into *E. coli*, which are then screened for a desired phenotype. This process is repeated to maximize output from a biosynthetic pathway or to systematically replace DNA sequences (adapted from [47]); (c) synthetic chromosome rearrangement and modification by loxP-mediated evolution (SCRaMbLE) is a promising tool for investigating evolution and combinatorial genetics. loxP sites (blue squares) are placed around genes (various color rectangles), induction of CRE recombinases leads to recombination between loxP sites resulting in deletions, inversions, duplications, and translocations. Resulting clones from this method can be screened for desired phenotypes; (d) forward genome construction methods such as yeast assembly enabled construction of large-subgenomic fragments. The efficiency of yeast homologous recombination enables connection of multiple fragments. Homolgoue fragments are connected via yeast HDR to a bacterial artificial chromosome (BAC, orange) and yeast artificial chromosome (YAC, blue) sequence. These circular fragments can measure up to 1 megabase and be propagated in *S. cerevisiae*. These assemblies can be transferred to recipient organisms via various methods

[60]. This, like the minimal mycoplasma genome, will likely become a valuable tool for the generation of organisms with stable genetic content (i.e., lacking transposable elements, and perhaps reduced mutation rates) devoted to production of various bio-compounds. Alternatively, our group has demonstrated that CRISPR-guided nicking can target endogenous homolgous recombination. This enabled removal of 133 kb, 3%, of the *E. coli* genome via a single plasmid transformation [33]. Collectively these methods provide research and development tools for the creation of novel organisms. Concurrently, these tools are useful for the investigation of systems level reengineering of organisms [61].

4 Recombinase based engineering

Recombinases have functioned as an indispensible tool for efficient and precise genetic manipulation in a broad range of organisms. Bacterial suicide vectors often employ bacteriophage-derived recombinases to facilitate efficient site-specific integration [62]. These systems enable genome integration of large-synthetic constructs and can be easily designed into experimental workflows for restructuring organisms. Santos and colleagues demonstrated recombinase assisted genome engineering can generate *E. coli* capable of alginate metabolism and ethanol production with higher titers than typical plasmid based expression experimental regimes [63,64]. Enyeart et al. demonstrated that targetron technology and Cre-lox recombinase systems can be used synergistically to restructure bacterial genomes [65]. This system enables large-scale deletion (up to 120 kb), targeted inversion (1.2 Mb), and translocation of targeted loci. Using this system they demonstrated a programmable and efficient way to remodel the genomes of *E. coli*, *S. aureus*, *Bacillus subtilis*, and *Shewanella oneidensis* [65]. Krishnakumar and colleagues at the Craig Venter Institute developed a technology for large-scale bacterial genome restructuring. This system utilizes Cre-lox sites located on a donor vector and the genome. This allows targeted replacement of large genomic fragments with synthetic fragments [66].

Recombinases methods extend beyond application in bacteria into mammalian cell lines and full organisms. Recombinases based methods can work cooperatively with evolutionary genome engineering methods [67]. They have enabled large-scale genome restructuring in *S. cerevisiae*. Dymond and colleagues developed synthetic chromosome rearrangement and modification by loxP-mediated evolution (SCRaMbLE) [67]. The authors systematically placed loxP sites in the 3' UTRs of genes on of the right arm of synthetic yeast chromsome IX (synIXR) and the left arm of semi-synthetic chromsome VI (semi-synVIL). They demonstrated induction of SCRaMbLE generates highly diverse genotypes with numerous genomic deletions, duplications, and transpositions (Fig. 2(c)). The SCRaMbLE method offers itself as a tool for studying higher order combinatorial genetics and for large-scale reengineering of eukaryotic genomes. Cre recombinase is an auspicious protein, and because of comprehensive biochemical understanding from years of research, it is also being investigated as a tool for gene therapy. For example, Karpinksi and colleagues recently created Brec1 recombinase [68]. Brec1 was created by directed evolution of Cre recombinase to target Human immunodeficiency virus (HIV) LTRs. It is shown that expression of Brec1 in patient-derived HIV + cells leads to proviral excision and curing of the virus.

5 Fully synthetic organisms

Perhaps one of the most promising aspects of genome engineering coupled with synthetic biology is the *de novo* design and construction of new organisms. The methods to accomplish this, have in large part been undertaken by Synthetic Genomics, who have developed a series of novel methods for identifying the minimal set of genes needed for a genome and *in vitro* and *in vivo* assembly of large synthetic DNA molecules [69]. To facilitate large-scale *in vitro* DNA assembly, Daniel Gibson et al. developed Gibson DNA Assembly to assemble DNA molecules nearly half a megabase [69]. To accomplish this, T5 exonuclease removes nucleotides from substrate DNA molecules. This reveals single-stranded DNA homologies and allows hybridization between separate DNA molecules. Meanwhile, Phusion DNA polymerase adds nucleotides counter to the exonuclease and Taq ligase catalyzes formation of phosphodiester linkages thus connecting DNA molecules.

To create the synthetic genome, Lartigue and colleagues developed a method to transfer whole genomes between bacteria via digestion of cells in agarose plugs and polyethylene glycol (PEG) mediated transformation [70]. More recently, Lartigue and coworkers described and updated method for genome transplantation wherein whole bacterial chromosomes are transplanted to yeast. *S. cerevisiae* is an extremely effective host for homologous recombination experiments and propagation of large DNA molecules [71]. In this study they demonstrated the utility of yeast HR by removing a type III restriction enzyme gene that renders M. mycoides cells resistant to introduction of exogenous DNA molecules. In addition, Karas et al. recently described a protocol for direct cell-to-cell transfer of genomes [72]. This method utilizes PEG mediated cellular fusion, thus reducing the likelihood of chromosome damage caused during the DNA purification process.

To go from synthetic DNA sequences to a full genome, Gibson and coworkers utilized a hierarchical DNA assembly scheme with a mixture of *in vitro* and in yeast DNA construction (Fig. 2(d)). The 1-megabase synthetic *M. Mycoides* genome (JCVI-syn1.0) was transplanted into recipient *Mycoplasma capricolum* cells. This represented the first assembly scheme going entirely from synthetic DNA sequence to full genome of an organism [73]. More recently, Hutchinson and coworkers revealed a new minimal genome, JCVI-syn3.0. Here they reduced the size of the 1 megabase *M. mycoides* genome to 531 kilobases [9]. This substantial reduction of genome size was accomplished by genome redesign informed by TN5 transposon mutagenesis studies of JCVI-syn1.0. Collectively the tools for genome construction developed by the J. Craig Venter Institute provide a framework for synthetic biologist to go from digitized DNA sequence to full genomes.

6 Conclusions and future perspectives

As circuits progress and become more complex, editing and genome redesign schemes will require more and more power. Systematic rational and combinatoric design coupled with evolutionary based engineering methods will enable production of microbes with larger synthetic gene networks [48,75,76]. Technologies that allow us to go from digitized DNA sequence to biological implementation are central to synthetic biology. CRISPR systems with high specificity will become the most tractable for implementing biological devices in human cell systems [77]. CRISPR based genome engineering methods make rapid construction of biosynthetic pathways possible (See Table 1). Genome engineering tools like MAGE enable optimization of biosynthetic pathways and will become more prevalent as automated workflows become more commonplace. Coupling of MAGE with CAGE has made removal of all UAG stop codons from the *E. coli* genome possible (See Table 1). Freeing of various codons is a step towards engineering organisms with orthogonal genetic code from their outside counter parts. This is an important hurdle synthetic biology faces in eventual application outside the laboratory. Large-scale genome reduction and construction of minimal genomes will enable creation of

Table 1 Example applications of genome engineering to obtain certain products

Method	Host	Description	Product	Ref.
MAGE	*Escherichia coli*	Multiplex automated genome engineering: an automated recombineering workflow for directed evolution	Lycopene	[47]
CAGE	*Escherichia coli*	Conjugative assembly genome engineering for hierarchical assembly of genomic mutations	UAG codon replacement [a)]	[52]
RAGE	*Escherichia coli*	Recombinase assisted genome engineering for integration of heterologous pathways into the *E. coli* genome	Ethanol from brown macroalgae	[63]
CRISPR/lambda Red	*Escherichia coli*	Integration of large synthetic constructs into the *E. coli* genome	Isobutanol	[32]
CasEMBLR	*Saccharomyces cerevisiae*	Multiplex assembly of biosynthetic pathways on the yeast genome	Carotenoids and tyrosine	[44]
mCRISTAR	*Saccharomyces cerevisiae*	Combined CRISPR and TAR cloning for construction and refactoring of pathways for application in heterologous organisms	Tetarimycin A	[74]

a) Product will be useful for engineering of synthetic proteins

designer bacterial strains with reduced metabolic burden and increased genetic stability [60].

Acknowledgements Work by the Xiao Wang laboratory has been supported by National Institutes of Health Grant GM106081.

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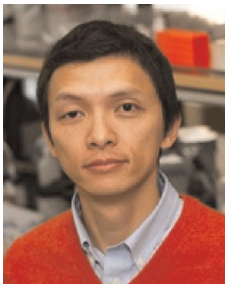

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