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A review of the benefits of using synthetic biology in molecular biology

Mohammed Radhi Mohaisen[*1,2]

[1]*College of Dentistry, University of Anbar, Iraq*

[2]*Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom*

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Abstract

A synthetic biology system can be used for useful purposes by building artificial components. For instance, by genetically encoding a synthetic pathway in DNA, we can harness the power of enzymatic chemistry. Modern molecular biology has been enabled by synthetic biology methods, which enable the learning, testing, designing, building and repeating the cycle. In synthetic biology, cellular systems are genetically engineered from the ground up, creating new biomolecules, networks, and pathways, while rewiring and reprogramming them. A brief review of some of the most common DNA synthetic biology techniques is presented here, with a particular focus on recent advancements that have attempted to reduce the cost of synthesis and enhance DNA sequence accuracy.

***Corresponding Author:** Mohammed Radhi Mohaisen ✉ den.m.radhi@uoanbar.edu.iq

Introduction

An important part of synthetic biology is the genetic engineering of cellular systems in order to construct novel biomolecule components, networks, and pathways so that the biological systems can be rewired and reprogrammed based on the constructs developed through synthetic biology (Khalil and Collins 2010). Synthetic biology is concerned mainly with the use of tools in order to Engineer it more easily and perform biological systems that are much more complex as they are required. Among synthetic biology goals, determining the nucleotides of DNA sequences that would be required for enzyme activity to occur is applicable.

DNA homology and DNA Recombination

A number of previous studies have reviewed the role that DNA homology plays in DNA recombination performed due to site-specific recombinase activity, as well as the fact that DNA recombination itself depends on homology. In recombination, homology between the strands of DNA in relation plays an important role, forming synapsis between two mating DNA strands and making the Holliday junction (HJ) intermediate (Fig. 1). However, it does not play a role in HJ resolution during recombination. This requirement of DNA homology has an effect on how DNA interacts with one another during synapsis, which is how a four-stranded helix is formed during recombination (Kikuchi and Nash 1979; Nash and Pollock 1983). There is, however, evidence of an overlap between lambda phage DNA sites and bacterial DNA sites that contain the right side, sequence mismatching (3-4 bp) of the bacterial DNA and lambda phage DNA sites, which can lead to the production of unfinished HJ intermediates, since it is only possible to produce the Holiday molecule structure through a single pair of DNA strand exchanges (Fig. 1) (Kitts and Nash 1987).

A number of experiments were conducted on DNA substrates that comprised half of both side of lambda phage DNA sites and the heterologous crossover regions. The DNA synapsis or even the initial event of strand exchange could not take place due to sequence mismatching (Nunes-Düby *et al.*, 1989).

There seems to be a relationship between the sequence mismatch location and the output of each DNA recombination incident that is performed by a site-specific recombinase enzyme. It was observed in recombination events performed by lambda recombinase enzyme, also called integrase enzyme (Int), the Holliday junction was accumulated when mismatching nucleotides of the DNA sequence were lying left the core of the crossover region (Fig. 1), however, when mismatches were located on the opposite side, as the first DNA strand exchange is performed, the results were different (Kitts and Nash 1987). The outcome of the recombination events that have DNA sequences with mismatches occurring along the crossover region in lambda Integrase-mediated is the backwards resolve of the HJs to the parental products backwards to the main DNA sequences (de Massy *et al.*, 1989).

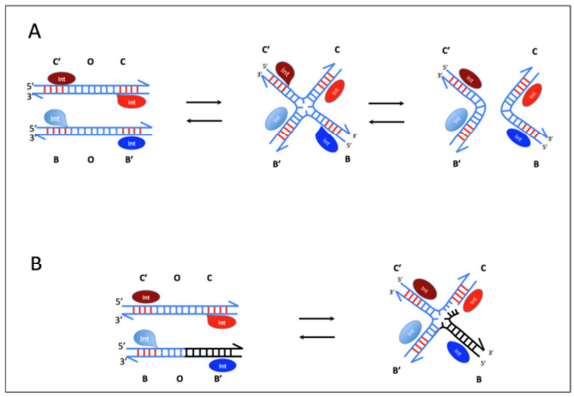

Fig. 1. Homology of DNA sequences and their effect on lambda Bacterial (BOB') and Phage (C'OC) sites recombination outcomes. A. when DNA sites have complete matching sequence of the sharing overlap region (C'OC-BOB'). In this case, the resulted DNA sequences are hybrid sharing part from each parent DNA sequences. B. A parent DAN site with a mismatching shared overlap region (B') (Black sequence) on the bacterial sequence right side can produce uncomplete holiday structure as the exchange appears by one pair of DNA only. Resolving lambda Int-mediated HJ that have mismatches in the crossover region goes snap back to the parent product.

Gibson Assembly

It has been nearly four decades since the ability to join DNA sequences together was achieved. The result of this process is that they are capable of producing DNA segments that aren't found naturally.

There is a process known as recombinant DNA technology that involves cutting DNA sequences and rejoining them together. In the 1970s, restriction enzymes and DNA ligases were discovered, which led to the development of recombinant technology (Gellert 1967; Smith and Wilcox 1970). The scientists' group of Daniel Gibson, in 2009, created a new *in vitro* method of DNA recombination unlike the conventional methods that involve cutting DNA sequences and ligating, but instead, they used a single isothermal reaction to perform ligating DNA sequences (Fig. 2) (Gibson *et al*., 2009).

A few enzymes, with minor manipulations, are required to mediate the Gibson system's DNA combination reaction. The Gibson reaction is a reaction that requires the activity of three enzymes: A DNA polymerase, an exonuclease and a DNA ligase. There is the possibility of combining up to 15 fragments of DNA at the same time. It is imperative that two DNA fragments intersect at their adjacent ends by a minimum of 20-40 bp, so they can be combined. The activities described above could be achieved with large bond DNA fragments (over 500 kilobytes). All reagents and enzymes that are required for the experiment are readily available on the market.

For cloning, a master mix (Gibson *et al*., 2009), is incubated at 50 C for a few minutes with DNA fragments that share complementarity regions at each end (the time varies depending on how the desired outcome is achieved and how many enzymes and reagents are required), which makes it easier than ever to create biological systems based on the small constituent parts used in the process.

Specifically, exonucleases, such as 5' T5, are enzymes that are responsible for cleaving DNA fragments on each 5' end of polynucleotide chains. The outcome of the exonuclease activity is the formation of an end region with a single-stranded DNA fragment (Fig. 2), Consequently, these DNA fragments are annealed together for the next process involved in the cloning procedure. Nucleotides are added to the gaps by DNA polymerase. Ligase enzyme, in the final step, allows the DNA sequences in the DNA products to be joined together resolving the DNA segments' nicks (Fig. 2) (Gibson *et al*., 2009).

Gibson assembly approaches are known by two types: an assay with one step or with two steps. The one-step assay is assembling multiple DNA fragments in one step; it is also called the isothermal process. An hour of incubation at 50 C is conducted after DNA fragments and enzyme master mix are mixed together. On the other hand, two-step Gibson methods are employed for the formation of DNA outcomes consisting of 15 or more DNA fragments. Rather than using a master mix, the relevant enzymes need to be added separately. Exonuclease as the first enzyme removes the 5' termination and starts the annealing process. Then adding the polymerase and ligase. DNA assembly is performed at different temperatures depending on the step (Gibson 2011).

Bio-Bricks

A BioBrick is a DNA sequence that functions similarly to a plastic or wooden building block. BioBricks are utilized to build biochemical circuits of greater size, strength, and complexity. A biological system can be artificially constructed from these building blocks because they have a defined structure and function. Their parts have many examples including promoters, ribosomal binding sites, coding sequences and terminators. It is possible to combine these functional parts into new combinations to create *devices* comprised of complementary BioBricks.

Now biological systems can be created by connecting devices together. The reliability of synthetic higher biological systems can be enhanced because each component and device of the system can be tested independently (Shetty *et al*., 2008). As of right now, every BioBrick component is a physical DNA sequence in a circular plasmid available from the Registry of Biological Parts (Sleight *et al*., 2010).

The assembly of these biological systems can be performed using several BioBricks assembly standards. As reported by Tom Knight in 2003, the first BioBrick assembly standard had been published

(Shetty *et al.*, 2008); when it was reported as "standard sequence assembly of BioBricks".

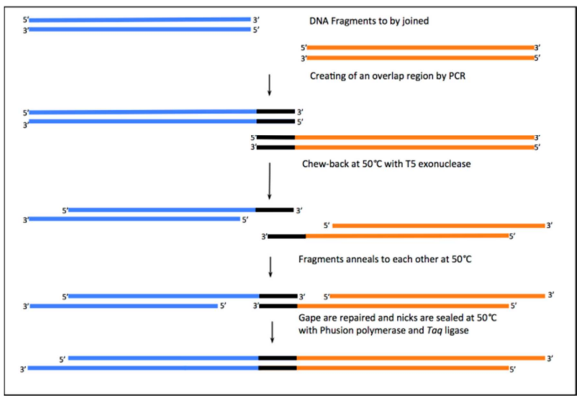

Fig. 2. An overview of Gibson assembly. In the black region, both DNA fragments have a short region of homology, so they share an area of complementarity. Both fragments are annealed together as the exonuclease cut DNA sequences on 5' ends. DNA ligase then heals the remaining nicks on the sequences after DNA polymerase closes all gaps (Gibson *et al.,* 2009).

Nevertheless, There have been many other assembly techniques reported since 2003 when the first assembly approach was discovered (Røkke *et al.*, 2014). Standard assembly involves digestion and ligation of restriction endonucleases via restriction endonuclease enzymes in order to join two BioBricks (Canton *et al.*, 2008). In the BioBrick assembly standard, a major innovation is the ability to combine two BioBrick parts together and still define it as a BioBrick component that can gather any other BioBrick part. The result is a unit that is compatible to gather any other BioBrick component.

The standard assembly of BioBrick enables biomedical components from a variety of sources to be assembled in a similar way. It is possible to optimize and automate this assembly, in contrast to traditional *ad hoc* molecular approaches (Shetty *et al.*, 2008).

There are two short functional sequences that flank the BioBrick components called prefixes and suffixes. Prefixes and suffixes contain restriction endonuclease digestion sites, so they are able of connecting two BioBrick components. There have been a number of different assembly standards developed over time.

As a result, there are a wide variety of custom prefixes and suffixes that are offered for every assembly. In addition, the BioBricks rates for each fusion protein are different, leading to the recent development of more recent assemblies (Røkke *et al.*, 2014).

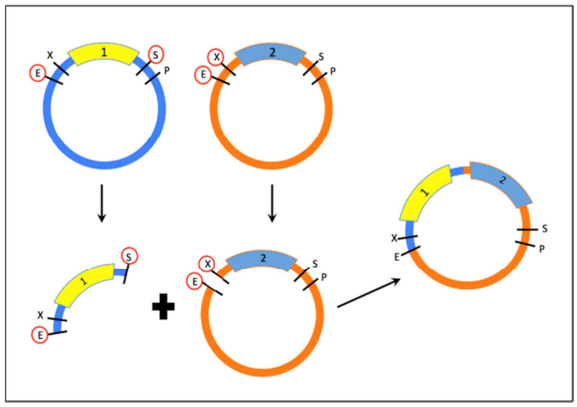

Fig. 3. An illustration showing how standard assembly of BioBrick is used to assemble two BioBricks. The first part (1) is digested by the restriction enzymes *Spe*I and *Eco*RI, and the restriction enzymes utilized to cut the second part (2) are *Xba*I and *Eco*RI. As a result of ligation, two DNA molecules that have been digested become a new BioBrick.

BioBrick Standard Assembly

In 2007, Tom Knight proposed The standard assembly of BioBrick, or Assembly Standard 10 (Røkke *et al.*, 2014). BioBricks are comprised of DNA sequences that are carried on circular plasmids. Introducing the Prefix, Suffix, and Specific restriction enzyme sites is the first step towards BioBrick standard assembly (Voigt 2011). There is one standardized suffix in this assembly standard, while two different types of prefixes are acceptable to be used. Based on a BioBrick is a sequence coding protein or not, it will depend on which type of prefix is used. Both sequences of both prefixes contain sites that could be cut by restriction *Eco*RI and *Not* I. As well as these two sites, the prefix, with a non-protein-coding sequence, also has a site that *Xba*I can recognize. The protein-coding prefix that contains *Xba*I site is fused with, ATG starting, sequence that coding protein (Røkke *et al.*, 2014). The suffix sequence has restriction endonuclease sites that could be used by *Pst*I, *Not*I and *Spe*I restriction endonucleases (Radeck *et al.*, 2013).

In a common BioBrick assembly approach, two BioBrick components are commonly combined by the restriction endonucleases *Spe*I and *EcoR*I by following the BioBrick Standard Assembly with the use of the restricted enzymes *EcoR*I and *Spe*I. While *EcoR*I and *Xba*I are used to digest the second BioBrick (Fig. 3) (Sleight *et al.*, 2010). At this point, both of the two BioBricks have sticky ends that could be fused together. These two ends were previously performed by *Eco*RI. A ligated DNA molecule that fuses the two restriction sites formed by *Spe*I and *Xba*I will be a fusion of the two sticky ends constructed by the two endonucleases enzymes (Fig.3) (Liu *et al.*, 2014). The resulting DNA product become unrecognizable by *Xba* I or *Spe* I. As a result of the formation of this new sequence, known as a scar, the second BioBrick will be composed of either TACTAGAG or TACTAG depending on its protein-coding or non-coding characteristics (Røkke *et al.*, 2014).

This common assembly is an example of the standard approaches that BioBricks uses for assembly; other assembly methods include Silver (Biofusion) standard, Freiburg standard (Røkke *et al.*, 2014) and BglBricks standard assembly (Lee *et al.*, 2011), The difference is mainly in their prefixes and suffixes, as well as the restriction sites within them.

Conclusion

The study of biology is primarily concerned with the functions of living organisms. In synthetic biology, new biological substances are designed and developed, such as genes, proteins, organs, or even entire organisms. Synthetic biology, therefore, is the process of designing a new living system built from known chemicals as an alternative to existing living organisms, but easier to understand. There is an immense amount of precision required for synthetic biology, like the precision required for the evolution of mathematical equations that follow a logical course as they grow out of the previous equations. There are many technologies that are needed for synthetic biology, including those that are existing, evolving, and new techniques. PCRs, polynucleotides, and recombinant DNA technologies are some examples of these methods. Creating living organisms from non-living chemicals is the ultimate in synthetic biology so we can answer questions we cannot answer with existing life systems.

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