

iGEM 2009: THE BIOBYTES METHOD

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Foreword

The following work was completed by the 2009 University of Alberta iGEM team. iGEM, or the internationally genetically engineered machines jamboree is a challenge to build biological systems and operate them in living cells. iGEM is a great opportunity for undergraduates to become involved in synthetic biology.

Introduction

Synthetic biology needs more than minor modifications to existing evolutionary plans. We've developed a method of gene assembly allowing complete genome re-design, so termed BioBytes. The speed and automation of the BioBytes method makes possible the maximization of modularity on a grand scale. Imagine a synthetic genome grouping common pathway components and components with similar levels of expression. This degree of organism control would be a milestone marking synthetic biology as a mature field. The BioBytes method of gene assembly allows us to efficiently test, optimize, and correct genome scale design principles. There are currently two alternatives for gene assembly. The first, BioBricks, is modular but slow. The second, the use of unique long sticky ends for each piece, is fast but non-modular.

The BioBytes Method

The BioBytes method begins with BioBytes: segments of DNA ranging from tens to hundreds of base pairs, each encoding a specific cellular instruction. Both ends of a BioByte have 12bp single stranded overhangs. Single stranded

overhangs only anneal if they have complementary sequences. Our method uses only two pairs of end sequences, A/A' and B/B', and BioBytes are assembled with alternating A and B junctions. Only A ends may attach to each other and likewise only B ends may link to each other. To control which end of a growing DNA construct a BioByte is added to, one end of the construct is anchored to a paramagnetic streptavidin coated bead through a DNA anchor (See Figure 1).

Once completed, the construct can be released from the bead by enzyme digestion and, if a terminator BioByte was used, will circularize. As the terminator is added last, only full length constructs will circularize and can be selected for by transformation into *E.coli*. The BioBytes themselves are produced by ligating DNA into a BioBytes universal plasmid, performing PCR on plasmid using our universal primers, and digesting the product with the USER system to produce single stranded ends.

To develop the BioBytes method, we performed optimization experiments on assembly conditions, bead binding capacity, anchor release, BioByte production and overhang construction. We successfully produced a five byte construct

using the BioBytes method on the benchtop, and repeated this construction at a microfluidic scale. Moreover, we constructed and tested a robot for automation, determined operon position using the BioBytes method, and began multiplexing BioByte constructs.

A novel algorithm for predicting essential genes

Next, we explored the BioByte method in its application to the construction of an artificial *E.coli* genome. Using a novel computer modeling approach, we determined which genes would need to be retained for a functional metabolism in different environments, identifying 117 genes never previously considered essential. We designed and tested primers for hundreds of essential genes, the first step of making them BioBytes. These primers and all components needed for the BioBytes method were documented and submitted to the BioBricks Foundation for public use. In total, we submitted 442 parts.

Conclusion

BioBytes has the potential to accelerate synthetic biology toward the grand vision of an artificial cell. Unlike the previously developed BioBrick DNA assembly method, genes produced in the BioByte format can be assembled rapidly in vitro, in any desired order, with great precision and yield. With cycle times approaching 20 minutes for the addition of each new gene, BioByte assembly rates exceed their BioBrick counterparts by 200-fold. This level of improvement immediately opens the door towards the synthesis of simple chromosomes that can be tested and optimized at unprecedented speed. Finally, the BioByte assembly system requires a fraction of the equipment found in a conventional gene lab. This advantage, combined with sheer simplicity, greatly extends its utility. The BioBytes method is already in use by researchers at the National Institute for Nanotechnology.

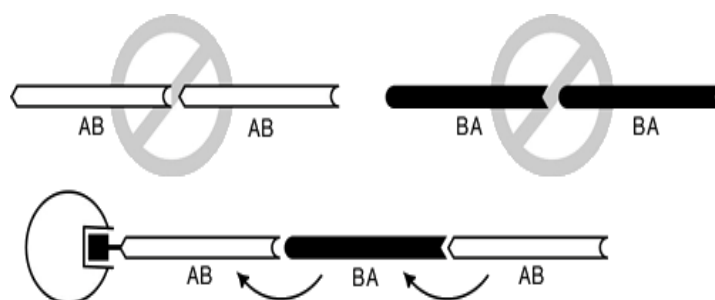


Figure 1. Each end of a BioByte has 12bp single stranded DNA overhangs with alternating ends termed A or B. The BioByte assembly is initiated through a streptavidin anchor, and then alternating AB-BA-AB ligations extend the sequence.