DNA Assembly and Cloning in an Overnight Run with the BioXp™ 3200 System

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Abstract

The BioXp™ 3200 System is an automated, personal genomic workstation that builds and clones DNA fragments in a process that is virtually hands-free. In an overnight run, the instrument generates cloned DNA from custom-designed oligonucleotide pools and reagents engineered from sequence information. Here we discuss highlights and advantages of the BioXp System and the two modules currently available– the assembly module and the assembly and cloning module.

Introduction

As the scale of genetic analysis trends away from single gene studies toward gene family, genomic, or metagenomic studies, the demand for large-scale DNA synthesis services and technologies has grown. Moreover, with newer cloning and sequencing technologies, the pace of molecular biology research is accelerating. SGI-DNA, Inc. has developed the BioXp System (Fig. 1) as an in-house laboratory solution to the increased interest and demand for rapid DNA synthesis. With an assembly module, the BioXp System generates high-quality, linear DNA fragments from custom designed oligonucleotide pools and reagents across a meaningful section of the complexity continuum in an overnight run. Now, with the introduction of the assembly and cloning module, the BioXp System has the additional capability to deliver cloned DNA from custom DNA sequence information in an overnight run (Fig. 2).

Figure 1. The BioXp 3200 System, a Genomic Workstation.

Since its launch in early 2015, commercial and research laboratories have been reaping the advantages of having access to the BioXp System, whether in an individual laboratory or in a core facility. On-site access to the automated BioXp System liberates researchers from the time-consuming, tedious steps needed to obtain DNA fragments to instead focus on new discoveries and DNA analytics.

The BioXp System Assembly and Cloning Module

With the assembly module, the BioXp System builds linear, blunt-end, double-strand DNA fragments. Now, with the introduction of the assembly and cloning module, the instrument has the added capability to build and clone DNA fragments of interest into the SGI-DNA pUCGA 1.0 vector. The pUCGA 1.0 clones generated from the BioXp System are immediately ready for transformation and further downstream analysis.

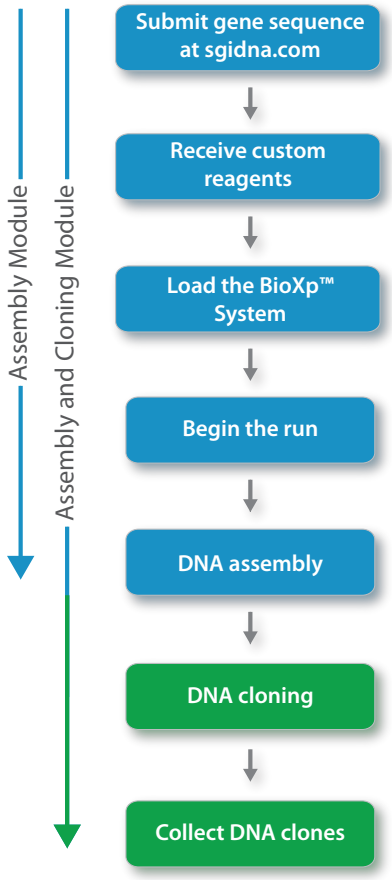

Figure 2. Module workflows of the BioXp 3200 System. The assembly module delivers linear DNA fragments. The assembly and cloning module delivers DNA cloned into a plasmid vector. Both modules build DNA in an overnight run.

BioXp System pUCGA 1.0 Clones

DNA clones obtained from the BioXp System consist of a DNA fragment of interest (400 to 1800 bp, 40% to 60% GC content) cloned into the 2.7 kb pUCGA 1.0 vector. The pUCGA 1.0 vector map and additional information are available at sgidna.com. Utilizing Gibson Assembly® technology, homologous overlap regions are automatically designed into the termini of the DNA fragments and are present in the pUCGA 1.0 vector to facilitate cloning. These homologous regions, referred to as GA ends, are 30 bases that have minimal sequence homology to naturally occurring genes. Highly accurate and robust automated cloning on the BioXp System is performed using the Gibson Assembly method (see Figure 3A). Clones obtained from the BioXp System are ready for transformation and subsequent downstream analysis.

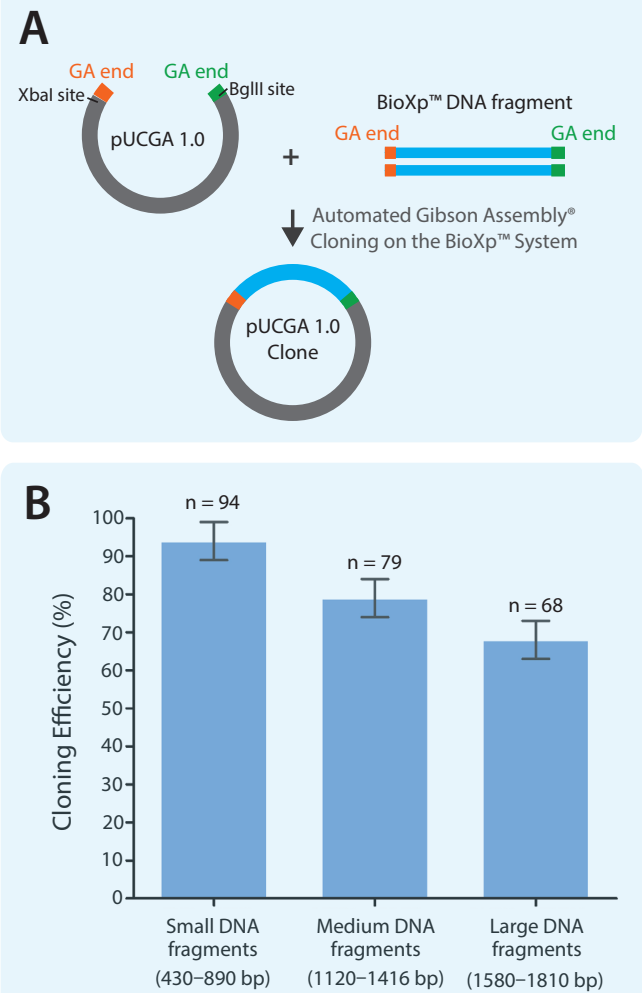

Figure 3. The BioXp 3200 System utilizes high efficiency Gibson Assembly cloning to deliver DNA in the pUCGA 1.0 vector. (A) Overview of Gibson Assembly cloning on the BioXp 3200 System with a BioXp fragment and the pUCGA 1.0 vector. **(B)** High cloning efficiency from the BioXp 3200 System. As expected, highest cloning efficiencies are from smaller DNA fragments. The total cloning efficiency (CE) for full length inserts is calculated using the formula:

CE (%)= (# white colonies / total) x (# full insert colonies / total) x 100.

The sample number (n) is shown above each respective bar; error bars represent ±s.d.

Transforming pUCGA 1.0 Clones

Once clones have been collected from the BioXp System deck after an assembly and cloning run, transformation is the next downstream step required for error-free clone identification and further analysis. The pUCGA 1.0 vector contains *lacZ* (the gene encoding the N-terminal fragment of β-galactosidase), which is disrupted by insertion of the BioXp DNA fragment, allowing for blue-white screening of recombinant clones. To assess clone quality, we performed transformation and calculated the cloning efficiency of pUCGA 1.0 clones. Briefly, an assembly-and-cloning run was repeated thirteen times on five different BioXp instruments. Twenty clones were randomly collected from the instruments after each run, transformed into TransforMax™ EPI300™ electrocompetent *E. coli*, and plated onto LB plates containing 100 µg/mL carbenicillin with 40 µg/mL X-Gal, and 0.1 mM isopropyl β-D-1-thiogalactopyranoside. After an overnight incubation, colonies were counted, picked, grown overnight, and plasmid DNA was prepared.

High Cloning Efficiency of pUCGA 1.0 Clones

The cloning efficiency of constructs generated with the BioXp System assembly-and-cloning module is shown in Figure 3B. The overall cloning efficiency of full-length pUCGA 1.0 clones was 83%. Additionally, we grouped clones according to fragment size for further cloning-efficiency analysis. As expected, the highest cloning efficiency (>90%) is observed for the smallest DNA fragments (<900 bp).

Full-length inserts were identified from double enzyme digestion with XbaI and BglII (see Figure 3A), which leaves partial or full GA end sequence intact at the termini of the BioXp fragment. For excision of only the fragment of interest from pUCGA 1.0, the BioXp fragment may be pre-engineered with restriction enzyme sites internal to the GA ends. Alternatively, PCR amplification with a high-fidelity DNA Polymerase may also be used to isolate the fragment of interest or to subclone the fragment in an alternate vector (e.g., an expression vector).

Conclusion

The BioXp System brings a new, rapid, automated method of DNA synthesis and cloning directly to the laboratory benchtop. The instrument can currently assemble and clone 24 DNA fragments of interest simultaneously in an overnight run. The pUCGA 1.0 DNA clones obtained from the BioXp System exhibit high cloning efficiencies, greater than 90% for DNA fragments less than 900 bp in length, and 83% overall. Laboratories using the BioXp System have the capability for virtually hands-free assembly and cloning of genes into vectors in an overnight run.

Additional information and resources are available at **sgidna.com/pages/bioxp-3200-system-1**.

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