ApplicationForum

Successful Diatom Transcription Factor Synthesis and Downstream Cloning Using the BioXp™ 3200 System

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Overview

A limiting factor in large-scale gene family studies is the time and labor involved in generating gene constructs for downstream analysis. Often, laboratories embarking on these studies are not set up as high-throughput gene synthesis facilities, making the generation of constructs for gene analysis studies linear and methodical (i.e., cloning a small subset of genes of interest at once before progressing to the next batch for study), rather than high-throughput (allowing for the acquisition of a large number of constructs simultaneously). As part of a pilot program for beta testing of the BioXp™ 3200 System, a personal bench top work station that provides rapid, high-quality, linear DNA fragments from custom designed oligonucleotide pools, we employed a strategy that could be universally applied to the synthesis and expression analysis of any group of genes. We identified 49 diatom transcription factor genes, from the *Phaeodactylum tricornutum* genome (1), to target for synthesis and subsequent analysis. Here, we describe the BioXp™ 3200 System workflow, our synthesis strategy for downstream application and transcription factor analysis, as well as results and observations of our participation in the pilot program.

The BioXp™ 3200 System Workflow

Preparing for a BioXp™ 3200 System synthesis project is straightforward, consisting of submitting an order online, entering sequence information, receiving custom-built reagents, loading and running the instrument, and collecting the synthesized DNA fragments. The BioXp™ 3200 System workflow is outlined in Figure 1. Currently, DNA fragments synthesized on the BioXp™ 3200 System are recommended to be 40% to 60% GC rich and 400–1800 bp in length. Additionally, sequences must pass SGI-DNA sequence complexity analysis.

Strategy for Downstream Cloning of BioXp™ Constructs in Gateway® Vectors

BioXp™ constructs contain universal ends to enable modification-free, streamlined assembly into the SGI-DNA pUCGA 1.0 vector (2). Additionally, by specifying adapter sequences flanking sequences of interest prior to BioXp™ synthesis, constructs can be universally modified to enable cloning into any vector. Here, we demonstrate a strategy for cloning BioXp™ constructs into the Gateway® vectors to allow for immediate downstream expression studies.

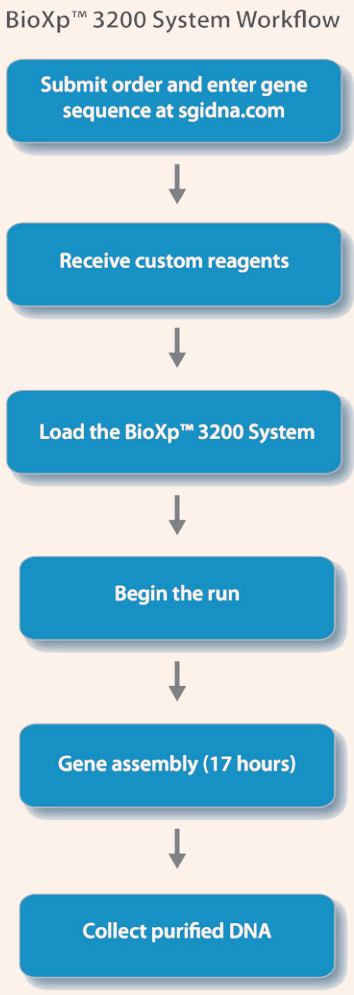

Figure 1. Required steps for synthesizing DNA using the BioXp™ 3200 System.

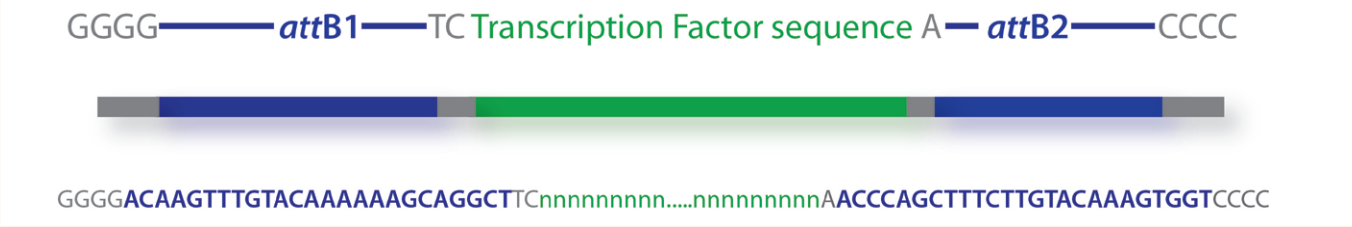

Figure 2. *P. tricornutum* diatom transcription factor construct design. This design strategy is for construct synthesis with the BioXp™ System and downstream Gateway® cloning. To facilitate Gateway® cloning, each construct was designed to contain a string of four guanines, followed by the *att*B1 sequence, a thymine and cytosine insertion, the transcription factor sequence (with stop codon removed), a single adenine, the *att*B2 sequence, and a string of 4 cytosines.

Strategy, continued

To facilitate downstream cloning using Gateway® vectors, each transcription factor sequence (ranging in size from 334–1807 bp) was flanked with *att*B1 and *att*B2 sequences as shown in Figure 2. Additionally, the 5' end of the sequence contained a string of four guanines and the 3' end of the constructed sequence contained a string of four cytosines. To maintain the correct reading frame in the vector, the transcription factor sequence was flanked with an insertion of thymine and cytosine at the 5' end and a single adenine at the 3' end. This design strategy was used universally for all 49 constructs.

Results

Following synthesis, BioXp™ constructs were analyzed by gel electrophoresis. A gel image from the electrophoresis of 8 of the 49 fragments is shown in Figure 3. Each lane shows a distinct band of the expected size. Although secondary bands are evident, in all instances the expected fragment was recovered after introducing the constructs separately into a vector via the Gateway® BP reaction, transforming *E. coli* cells with the plasmids, picking only one or two colonies, and sequencing purified plasmid. Of these 8 transcription factor constructs, we obtained perfect sequences for 6 constructs (75%). For the two colonies exhibiting single nucleotide insertion or deletions, we only analyzed a single colony from each plate of transformants. Analysis of additional colonies may yield perfect sequences.

For the entire set of 49 transcription factors, perfect sequences were obtained for 29 (59%) constructs. Of the remaining 20 constructs, 10% did not meet BioXp™ System specifications. For 50% of the remaining 20 constructs, we analyzed only 1 or 2 colonies, suggesting that recovering additional clones with perfect sequences after further screening is likely. Of note, when we examined the data after categorizing samples, we observed that of the 19 constructs ranging in size from 958–1267 bp, 16 (84%) yielded perfect sequences.

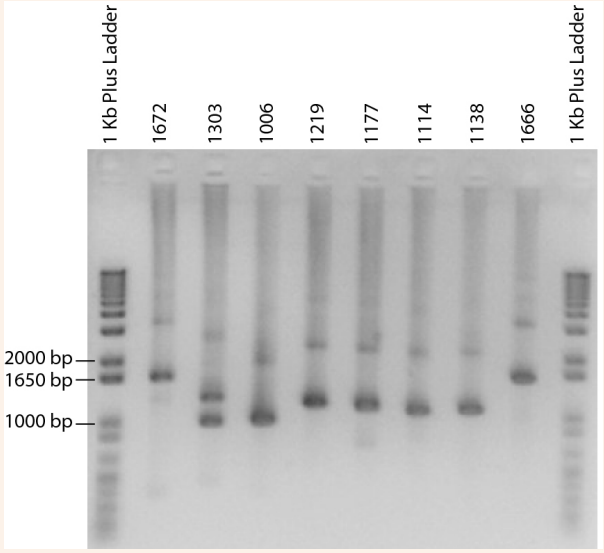

Figure 3. Gel electrophoresis of 2 µL of 8 representative BioXp™ constructs on a 1 % agarose gel for 40 minutes at 100 V. Sample lanes are designated with the expected fragment size.

Conclusions

Access to a BioXp™ 3200 System in a core facility enabled our laboratory to synthesize diatom transcription factor constructs in two days, rather than months that manual methodologies would have dictated. Assembly of our Gateway®-compatible constructs with the BioXp™ instrument was straightforward and successful, enabling us to embark on expression studies of 31 diatom transcription factors from 29 perfect clones, plus two additional clones containing single nucleotide substitutions that did not alter the amino acid sequence.

Summary
• 49 *Phaeodactylum tricornutum* diatom transcription factor genes were synthesized by the automated BioXp™ System
• The capacity of the BioXp™ System is 31 samples per run; therefore, 2 batches (31 samples in one batch and 18 samples in the second batch) were run
• Combined, > 60 kb of DNA sequence was synthesized (Maximal capacity of the instrument for two runs is 112 kb)
• Transcription factor genes ranged in size from 334–1807 bp
• Highest sequence fidelity (84%) was obtained for the construct subgroup ranging in size from 958–1267 bp
• Overall, perfect sequences were obtained for 59% of constructs after screening only a single colony for the majority (65%) of constructs

References

1. Bowler et al. 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456: 239–244.

2 For information about the pUCGA 1.0 vector, email techservices@sgidna.com.

To learn more about the BioXp™ 3200 System, please visit sgidna.com/bxp3200.

This research is funded in part by the Gordon and Betty Moore Foundation through Grant GBMF3828 to Scripps, A.E. Allen, Synthetic Genomics Inc., the United States Department of Energy Genomics Science Program grant DE-SC0008593 for biodesign to JCVI, A.E. Allen, and the United States Department of Energy cooperative agreement DE-EE0006109 to JCVI, Y. Suzuki.,

BioTechniques 59:46-47 (July 2015) doi 10.2144/000114311