The BioXp™ System and Gibson Assembly® cloning accelerate the construction and transfection of a mutational phage display library

Proof-of-concept project demonstrates a methodology for constructing and transfecting a phage display library 5 to 7 days faster than conventional methods

Steve Riedmuller, Senior Director Field Applications, and Kurt R. Klimpel, Field Application Scientist
SGI-DNA, Inc., San Diego, CA

Introduction

Phage display libraries are used extensively to investigate protein-protein interactions and to identify novel therapeutic monoclonal antibodies. Synthetically generated mutational libraries for phage display allow for biopanning: high-throughput screening for antibodies that demonstrate high-affinity binding to target molecules in whole cells. Preparing libraries for phage display biopanning is time-consuming and requires approximately 12 days of preparative time. Steps involved in library preparation include DNA synthesis and mutagenesis, screening for full-length clones, transformation, and plasmid preparation. These steps must be completed before the library is validated and ready for transfecting cells for phage display. Phage display libraries are typically generated one of two ways: (A) at the bench using PCR methods (a labor-intensive process) or (B) by ordering a custom synthetic library from a service provider (which we refer to as the "traditional workflow" in this application note).

Here, we describe novel methods for library construction that can be performed 5 to 7 days faster than conventional methods: (1) an expedited process utilizing the BioXp System and (2) a BioXp/scaled-up Gibson Assembly process (which we refer to as the streamlined BioXp method). The streamlined BioXp method is of particular importance because this workflow allows for same day transfection of constructs, which further accelerates turnaround time. This streamlined process for creating phage display libraries utilizes the BioXp™ System, a personal genomic workstation that builds DNA, and the Gibson Assembly® method, a rapid, highly efficient seamless assembly method. The streamlined BioXp library production workflow not only enables faster library builds, but also requires less sample processing and manual benchwork, thereby dramatically accelerating the design-build-test cycle.

Using the BioXp System to rapidly generate library constructs

In this section, we describe accelerated methods of generating constructs for library construction using the BioXp System. Typically, generating a phage display library begins with DNA synthesis of the desired heavy and light chain sequences that will encode single-chain variable fragments (scFv, Figure 1A). As indicated, there are many ways to obtain these heavy and light chain constructs, including creating constructs from PCR, using a synthesis service, or as we describe in this application note, by building the constructs on the BioXp System (see Figure 1B).

Using PCR to build a library is labor intensive and the accuracy of constructs is dependent on both the polymerase used and cycling conditions. For medium- and high-throughput labs, synthesizing constructs is often preferred. Synthesized constructs must still be processed and screened, as depicted in the "traditional workflow" in Figure 1B. Following synthesis of the DNA sequences, restriction enzyme sites present in the synthetic DNA must be removed. The DNA is then column-purified and quantified before it is subcloned into an appropriate vector. However, building constructs on the BioXp System streamlines this process as BioXp Tiles (linear dsDNA fragments) do not require restriction digests or PCR modifications and are immediately ready for Gibson Assembly cloning with an appropriate vector.

In addition to accelerating the library construction workflow, building constructs with the BioXp System also reduces the amount of required hands-on time. Placing a BioXp order, receiving custom reagents, and running the instrument are steps that require no more than 30 minutes of hands-on time, providing a more efficient and faster library production workflow.

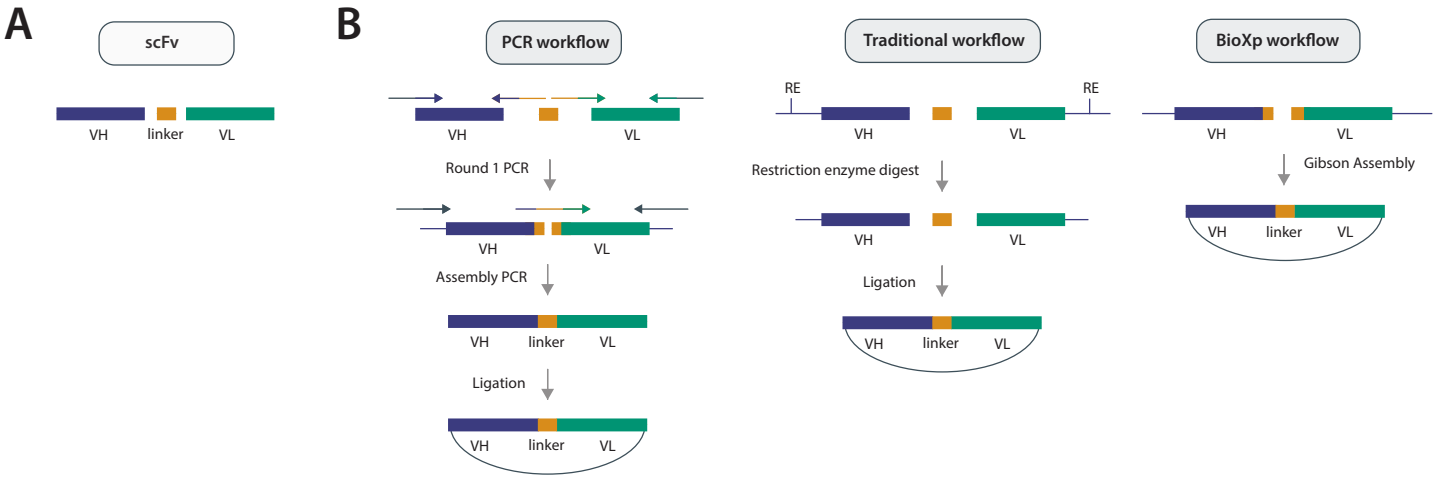

Figure 1. The BioXp System can be used to create library constructs in a single, overnight run. (A) The scFv construct for phage library display is composed of sequence encoding a variable heavy (VH) and variable light (VL) chain with intervening linker sequence. **(B)** The scFv-encoding sequence must be assembled with vector for phage display. Three ways to build scFv constructs are outlined. In the PCR workflow, two rounds of PCR are necessary. This methodology may require 7 primers and optimization to prevent preferential amplification of one domain over another. In the traditional workflow, DNA may be built by PCR or built synthetically. In either case, constructs often require downstream processing (restriction enzyme digestion and subcloning). **(C)** In the BioXp workflow, constructs are built in an overnight run on the instrument. After obtaining BioXp DNA fragments, constructs are immediately ready for Gibson Assembly, which takes less than 80 minutes.

Accelerated workflows with the BioXp System

Steps involved in preparing constructs for library display are shown in Figure 2. The first two steps of the workflows outlined in Figure 2 are necessary to build the scFv construct. In the traditional workflow, additional purification and subcloning steps may be necessary. The traditional workflow requires a second round of column purification before the construct is electroporated into competent cells. After an overnight culture, a plasmid prep is performed. Then, following plasmid purification, appropriate phage display library cells are transfected. Using the traditional workflow, the entire process of library preparation from sequence data to transfection takes approximately 12 days.

To expedite and streamline phage display library production, DNA fragments were built on the benchtop BioXp System. Building fragments on the BioXp System allows users to build DNA fragments at the bench instead of relying on a gene synthesis provider. DNA fragments or libraries built on the instrument do not require any downstream modifications and are immediately ready for cloning. To assemble the fragments with a vector, BioXp library fragments were assembled using Gibson Assembly cloning. This workflow (BioXp construct assembly followed by Gibson Assembly cloning) built constructs 5 days faster than the traditional workflow.

When coupled with a scaled-up Gibson Assembly reaction, the BioXp workflow can be accelerated further, reducing the overall workflow duration by an additional 2 days. The BioXp System routinely produces high quantities of DNA. Furthermore, the Gibson Assembly method is a high cloning efficiency reaction. High-efficiency Gibson Assembly cloning of sufficient quantities of DNA allowed for the direct transfection of the synthetic plasmids into CHO cells. In other words, BioXp-generated fragments assembled with library vector using the Gibson Assembly method produced enough full-length product to bypass plasmid preparation methods typically used in library preparation methods (see Figure 2, BioXp workflow, same day transfection). This streamlined library construction protocol reduces the amount of time necessary for library construction by two additional days and allowed for the direct transfection of the assembled variants, following a column purification step, into CHO cells. To obtain sufficient quantities of fully assembled DNA for transfection, Gibson Assembly reactions were scaled up. Typically, for Gibson Assembly cloning with a 7-kb vector, approximately 25 ng of vector is used in the assembly reaction. For this library scale-up reaction, 700–900 ng of vector with 250 ng of the Hc-encoding and 750 ng of the Lc-encoding fragments were used in the assembly reactions. This scaled-up reaction produced sufficient quantities of scFv-encoding constructs to allow for direct transfection into CHO cells following column purification, which translated into a substantial reduction of hands-on time required for the library construction. After DNA fragments are built by the instrument, only two additional hands-on steps (Gibson Assembly and column purification, each totaling 10 minutes of hands-on time) were required prior to CHO cell transfection.

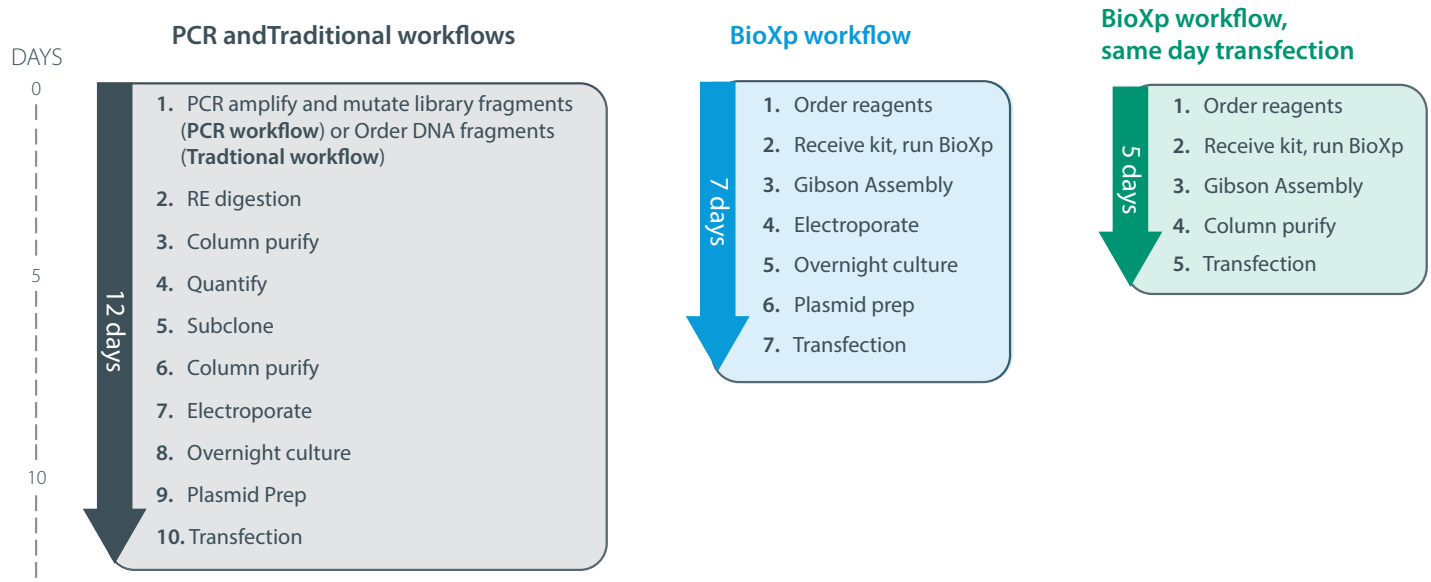

Figure 2. The BioXp System reduces turnaround time. Streamlined processes for building monoclonal antibody libraries using the BioXp System result in substantial time savings over conventional library production methods.

The BioXp System generates high-quality phage libraries

To evaluate the quality of the phage display libraries, transformation, colony PCR, and sequencing of assembled constructs built on the BioXp instrument and assembled using Gibson Assembly cloning were performed. Sequencing demonstrated the presence of full-length constructs with no mutations in the conserved regions 70% to 82% of the time. After transfection into CHO cells, another round of sequencing was performed. Following the second round of sequencing, 80% to 100% of the colonies analyzed were positive for the expected sequence. These results demonstrate that using the BioXp System and Gibson Assembly cloning, followed by plasmid preparation to build and assemble a mutational phage display library produced a high-quality, accurate library.

Next, monoclonal antibody (mAb) production in CHO cells following transfection was evaluated. Three unique mAbs generated using the BioXp workflow and the streamlined BioXp workflow with same-day transfection of synthetic plasmids were analyzed and compared. For the 7-day BioXp workflow, concentrations ranged from 0.45 to 0.79 mg/mL, demonstrating that the BioXp System and Gibson Assembly cloning are effective for building library constructs that can be used to create high-titer mAb libraries (Figure 3). As expected, the amount of antibody detected from the 5-day streamlined method was lower (0.14 to 0.37 mg/mL) than the amount detected from the 7-day protocol. However, the amount of antibody detected from the streamlined samples was sufficient for downstream applications including antibody purification. In all cases, the amount of measurable antibody exceeded the targeted threshold concentration of 0.1 mg/mL.

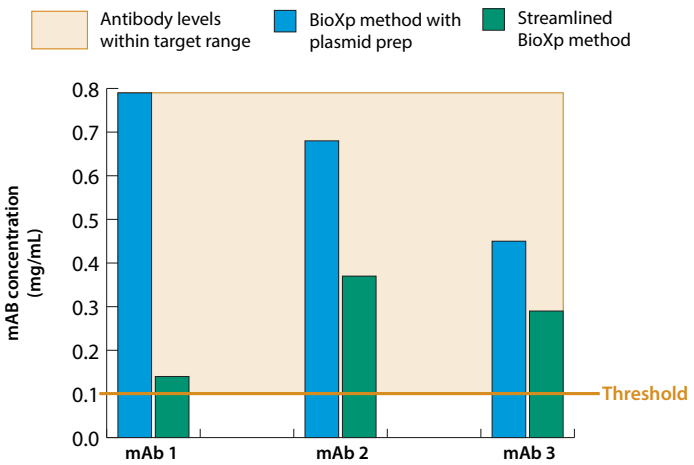

Figure 3. The BioXp System can be used to rapidly generate sufficient quantities of mAbs. DNA fragments generated by the BioXp System and assembled with Gibson Assembly can be used to rapidly generate mAbs. The mAb concentration of three antibodies generated from the BioXp System with plasmid prep (7-day method) is shown in blue; the mAb concentration from the 5-day streamlined method (direct transfection of Gibson-assembled constructs without plasmid preparation) is shown in green.

Discussion

Preparing phage libraries with the BioXp System and Gibson Assembly reduced the total time necessary for mAb library production by 5 to 7 days and required only minimal hands-on time. Traditional phage library construction methodologies require 8 lab bench steps (restriction enzyme digestion, column purification, quantification, subcloning, another round of column purification, electroporation into competent cells, and plasmid preparation following overnight culturing), but the streamlined BioXp workflow only requires 2 steps, totaling 10 minutes of hands-on time (Gibson Assembly cloning and column purification).

Reducing the amount of time required to build libraries is a true benefit of library production with the BioXp System. By using the streamlined BioXp method for library construction, experimental capacity can be expanded. The experimental capacity of any type of drug discovery program, including identifying novel therapeutic monoclonal antibodies, is dependent on a number of factors, including the effort and time required to process samples. With the streamlined method described in this application note, the total amount of benchwork was reduced from 10 hours using traditional methods to 30 minutes. The streamlined method eliminated all the steps of restriction enzyme digestion, quantification, subcloning, electroporation, plasmid preparation, as well as the sub-steps of waiting for cultures to grow. When the time and effort savings using the streamlined BioXp method are reallocated directly into drug discovery experiments and analysis, this reduced benchwork translates into a substantial increase in resources available for the drug discovery testing pipeline. The BioXp System offers the added advantage of automated cloning, which some users may prefer for library construction. This proof-of-concept project utilized linear DNA fragments from the instrument, which were then used for manual cloning in order to maximize the amount of DNA used in the cloning reaction. Downstream plasmid isolation steps may be bypassed in the BioXp workflow when all of the DNA built on the instrument is used in the DNA assembly reaction.

Taken together, the proof-of-concept results presented in this application note demonstrate that streamlined mAb library production from the BioXp System can produce high-quality libraries in adequate quantities for downstream analysis and purification with a substantial savings of time and resources for library production.

SGI-DNA, Inc.
9535 Waples Street, Suite 100
San Diego, CA 92121 USA
North America: (855) 474-4362
Outside North America: (858) 228-4115