Obtain variant constructs one month faster than using manual cloning

Case study: Generation of streamlined nanobody variant library for vaccine development with the BioXp® system

Background

Compared to conventional antibodies, camelids and sharks possess a unique class of antibodies called heavy chain antibodies (HCAB) that are devoid of a conventional light chain. Because of their small size (12–14 kDa), these single-domain antigen-binding fragments — known as VHHs or nanobodies — have received a progressively growing interest in their utility from a biotherapeutic perspective. The Depicker lab[1] initially concentrated their efforts on understanding processes such as integration of transferred DNA into plant cell chromosomes, and the epigenetic regulation of the transferred DNA. In recent years, this expertise has been exploited to uncover novel biotherapeutics focused on nanobodies, to prevent and to treat infectious diseases and improve design efforts for ongoing vaccine development.

Challenge

The Depicker lab set out to address the use of single-domain antibodies/nanobodies as a novel delivery vehicle for more effective vaccine designs. Before they had access to the BioXp system, the lab used manual cloning methods to obtain their variants. Manually building antibody variants can be a relatively slow and labor-intensive process, with a number of steps and a months-long workflow. Additionally, manual methods can induce errors, and are not easily scalable and repeatable, slowing discovery and commercial availability.

Solution

The Depicker lab employed the BioXp system to streamline the construction of variants. The functionality of the system expedites and automates the process, not only saving significant time and resources, but also providing confidence in consistency, and allowing for scalability.

Result and benefits

Using the BioXp system, the Depicker lab successfully built 100% of their nanobody variants more quickly than had previously been possible. Rather than using a manual, multi-step cloning process, spanning several weeks, constructs were built in a single overnight instrument run that directly assembled and cloned the constructs into their custom vector. Adopting the BioXp system allowed them to expedite their research to demonstrate the potential of bivalent VHH-MG fusions as delivery vehicles for vaccine antigens.

Project details

A comparison of steps involved in a manual workflow for the Depicker lab project versus using the BioXp system are shown in figure 1. A manual workflow required two steps, starting with synthesis and cloning of 30 variant fragments (VHH) into vector 1, and synthesis and cloning of the Fc region into vector 2. Next, bacterial transformations were carried out for each construct, followed by plating, picking colonies, growing cultures, plasmid preparations, and sequence verification. The final expression constructs (VHH fragments fused to Fc region resulting in VHH-MG constructs) were assembled in a second round of cloning, in which all of the steps performed in the first round of cloning — including the final sequencing step — had to be repeated before constructs were ready for transformation into *P. pastoris* for protein expression and variant analysis.

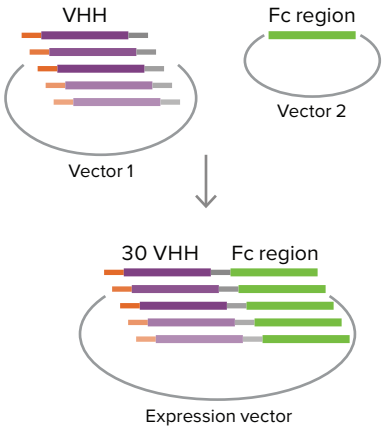
Manual workflow | Total time: 2 months

Steps for VHH region generation
1. Order 30 VHH dsDNA fragments
2. Clone 30 VHH fragments into vector 1
3. PCR amplify 30 VHH regions
4. 30 column purifications
5. Quantify 30 samples
6. Normalize
7. 30 ligations
8. Plasmid prep
9. Sequence

1 MONTH

Steps for final clone construction
1. 30 ligations
2. Transformation
3. Plate
4. Pick colonies
5. Grow culture
6. Plasmid prep
7. Sequence

1 MONTH



Steps for Fc region generation
1. Order Fc dsDNA fragment
2. Clone Fc fragment into vector 2
3. PCR amplify Fc region
4. Column purify
5. Quantify
6. Normalize

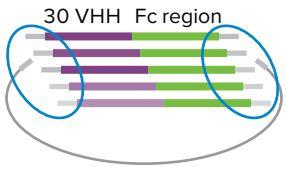
1 MONTH

BioXp® system workflow | Total time: 1 month

Steps for VHH region generation
1. Submit 30 VHH dsDNA fragment sequences
2. Receive BioXp® kit
3. Load and run BioXp system
4. Transform competent cells
5. Pick colonies
6. Grow culture
7. Plasmid prep
8. Sequence

1 MONTH

Complete construct synthesized by the BioXp system



Steps for vector preparation
1. Restriction enzyme digest
2. Gel extraction
3. Quantify
4. Perform Gibson Assembly® control
5. Transform competent cells
6. Calculate colony output for QC
7. Load BioXp custom vector strip

1 MONTH

Figure 1. Comparison of the manual workflow required to generate VHH Variants to the BioXp system protocol

The BioXp system built families of sequences (variants) with a design approach that utilized sequence homologies to gain efficiencies in synthesis, saving resources and associated costs. After submitting variant sequences to the Telesis Bio ordering portal, customized reagents were prepared and shipped to the end user. These customized reagents had been designed exclusively for automated Gibson Assembly® cloning, and include 40 bp homologous overlaps between the insert variants and the vector, required for seamless assembly of the final construct. A two-step error correction and a purification step in the BioXp system workflow minimizes introduction of sequence errors. Thus, by building constructs on the BioXp system, variants were generated faster, more efficiently, and — importantly — with improved fidelity.

The customer's yeast expression vector pKaiGGr (pre-prepared and designed with the necessary Gibson Assembly® overlaps) was loaded onto the instrument deck, along with the required custom reagents. The complete construct was then assembled and cloned into the vector in a single overnight run on the BioXp system. Following the instrument run, variants were transformed into *E. coli* and four colonies per construct were analyzed by colony PCR or restriction digestion, followed by sequencing to identify clones with the right insert. After the final sequencing step, constructs were ready for transformation into *P. pastoris* for protein expression and variant analysis.

Case study experimental details

Number of variants	30
Gene length	1,160 bp
Shared homology	80%

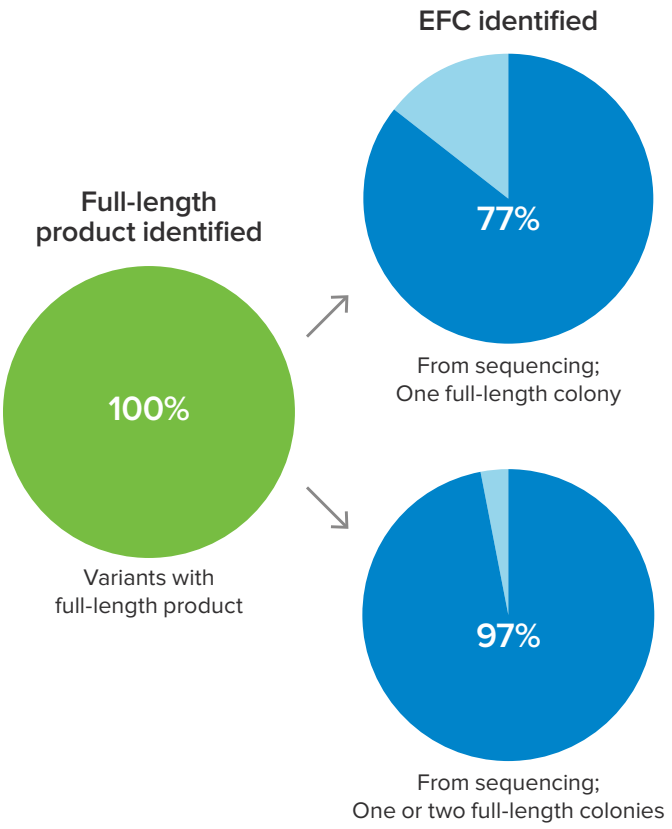


Figure 2. Successful error-free clone (EFC) identification. Full-length product was identified for all 30 variants (100%, green pie chart). EFCs were successfully identified after sequencing DNA. 23 out of 30 VHH-MG constructs (77%) resulted in an EFC by sequencing only one colony, while for six other VHH-MG constructs, two colonies had to be analyzed to obtain the right clone.

All 30 VHH-MG expression constructs were successfully built and cloned into the custom vector and produced full-length product. Of these, 97% were identified to have an error-free clone by sequencing one or two colonies (figure 2). This automated workflow involved far fewer steps, saving a considerable amount of manual benchwork, and thereby expanding the laboratory capabilities and potential discoveries of the research team. Using the BioXp system to build the variant library resulted in substantial savings of time and resources, and enabled a more efficient and productive workflow.

To learn how the BioXp™ system can advance your research, visit telesisbio.com/bioxp or contact help@telesisbio.com.

1. Shruti Bakshi, Raquel Sanz Garcia, Hans Van der Weken, Ashuwini Tharad, Shubham Pandey, Paloma Juarez, Vikram Virdi, Bert Devriendt, Eric Cox, Ann Depicker. 2020. Evaluating single-domain antibodies as carriers for targeted vaccine delivery to the small intestinal epithelium. *Journal of Controlled Release*. 321, 416–429.

Telesis Bio
9535 Waples Street, Suite 100
San Diego, CA 92121 USA
hello@telesisbio.com | 858.228.4115 | telesisbio.com

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