Case Study - SdAb Development *via* Premade HuSdL-1 Library

Introduction

Single domain antibody (sdAb), is a kind of antibody fragments consisting of a single monomeric variable antibody domain and lacking the light chain and CH domain of the heavy chain in conventional Fab region. In terms of only 12-15 kDa molecular weight, which is much smaller than either full length antibody (150-160 kDa) or other antibody fragments (Fab ~50 kDa, scFv ~25 kDa), sdAb takes great advantages of stability and penetrability, which are essential to the development of several antibody drugs or diagnostic tools.

Creative Biolabs has been a long-term expert in the field of single domain antibody (sdAb) development. Our scientists can employ a series of premade sdAb libraries to screen the target of interest, which is a cost-effective and time-saving manner for specific sdAb development. These libraries were preselected based on the thermostability, which are invaluable resources for isolating human single domain antibody binders for research, diagnostic and therapeutic applications.

Project Objective & Achievement

For this case study, one soluble protein was provided as screening target. Creative Biolabs is entrusted to isolate target-specific sdAb binders from our premade HuSdL-1™ library, a unique human single domain antibody library.

With the provided target, three rounds of biopanning were successfully performed with significant enrichment. 96 clones were randomly picked from the 3rd round enriched pool for validation. 82 positive clones were observed through monoclonal phage ELISA and 13 unique V_HH sequences have been identified *via* subsequent DNA sequencing. All of these 13 sdAb candidates can be expressed properly and presented positive signal against the target in QC soluble ELISA.

Finally, there are 13 unique target-specific sdAbs be discovered in this project.

Introduction of Premade HuSdL-1™ Library

Platform: phage display, M13 pIII fusion
Tag: Myc & VSV
Technique: Enlongated and randomized human HCDR3
Backbone: Camelized human VH3
Capacity: 1.5×10^9

HuSdL-1™ is a synthetic single domain antibody library with the capacity of 1.5×10^9. It was constructed by camelized human VH3 with elongated and sequence-randomized human HCDR3. In brief, the sdAb encoding genes were inserted into pCDisplay-5™ vector, by which sdAbs were fused with phage coat protein III and displayed on the surface of phage virions.

Camelized human sdAbs can be isolated from the HuSdL-1™ library which takes the advantages of the lowest immunogenic potential in humans, especially for long-term and multiple-dose administration because of their human origin. Moreover, this library was preselected based on the thermostability and productability (in *E. coli*) of the displayed antibodies. The antibody repertoire was heat-treated to remove clones that could not withstand heat-induced aggregation. The genetic codons used to encode the antibody binders are also optimized for bacterial preference.

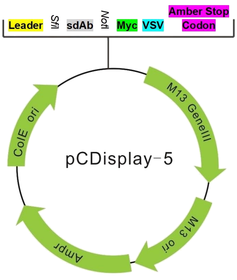

Figure 1. Schematic map of pCDisplay-5™.

Published Works by Using HuSdL-1™ Library

1. Doshi R, Chen B R, Vibat C R T, et al. In vitro nanobody discovery for integral membrane protein targets[J]. Scientific reports, 2014, 4: 6760.
2. Ma L, Gu K, Zhang C, et al. Generation and characterization of a human nanobody against VEGFR-2[J]. Acta Pharmacologica Sinica, 2016, 37(6): 857.

Milestone Overview

Stage 1: Library Screening

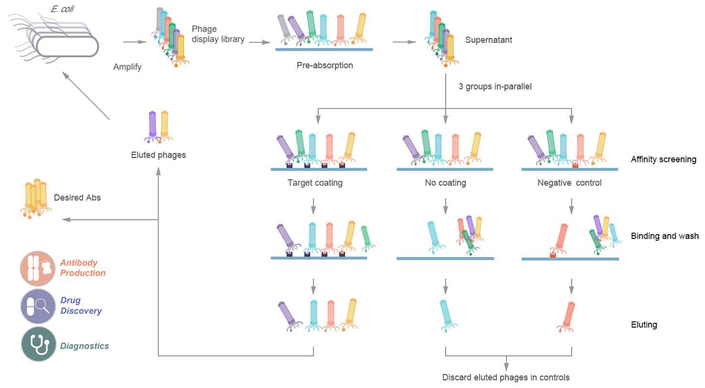

Figure 2. Flow diagram of phage display-based screening.

Creative Biolabs can tailor a series of library screening strategies to find the best-fit one of your project. Our scientists are committed to collecting the most reliable data that contribute to understanding the actual situation of each step. For a typical screening process, pre-absorption will be performed before each round of screening to eliminate non-specific binders against the plate surface, corresponding blocking buffer, and negative target (if exists) as much as possible. From the second round, "No Coating" control is also performed in parallel with the "Target Coating" group. If there is any negative target required by the project, an in-parallel test of "Negative" control will be involved as well from the second round.

For this case study, solid-phase screening strategy was performed, which the target was immobilized on the plate surface directly. After three rounds of biopanning, good enrichment was observed for the target and clear difference was found between the "Target Coating" group and "No Coating" control (Figure 3). This indicated some specific binders have been selected for the target.

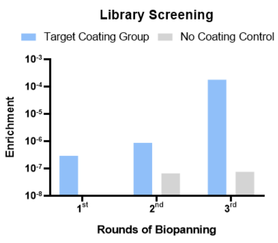

Figure 3. Process monitoring of library screening stage.

(Enrichment is increased round by round and presents significant difference between no coating control.)

Stage 2: Target-Specific Binder Validation & Identification

After the biopanning, 96 clones were randomly picked from the 3rd round output. The monoclonal phage ELISA was then performed against the target. 82 positive clones were observed and then processed for DNA sequencing (Figure 4).

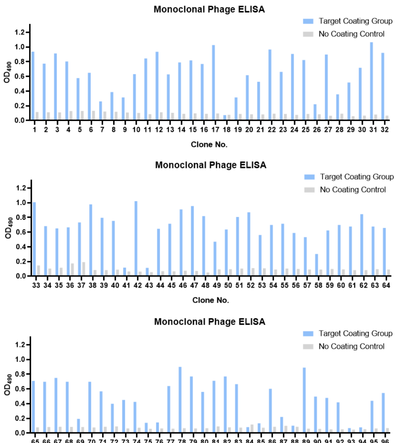

Figure 4. Monoclonal phage ELISA of the 96 randomly picked clones.

Through the sequencing, 77 clones were analyzed successfully. Among of them, 13 unique clones were identified in CDR level. As shown in Figure 5, 2 of the 13 clones presented significant higher abundance. Therefore, these two clones were supposed to play dominate roles to bind the target during screening process.

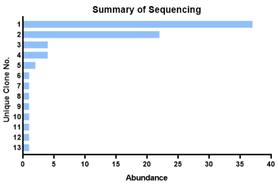

Figure 5. Summary of DNA sequencing results.

(Abundance of each unique clone indicates the number of sequenced clones present the same sequencing information.)

All these 13 unique clones were then prepared as soluble format (phage-free) for the validation of QC soluble ELISA. As shown in Figure 6, all of them were finally confirmed to recognize the target positively.

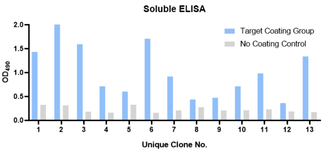

Figure 6. QC soluble ELISA of the 13 unique sdAb candidates.

Conclusion & Key Words

- ✓ **High Purity Target** - Target soluble protein with >95% purity was generated by Creative Biolabs.
- ✓ **Human SdAb Library** - Creative Biolabs' HuSdL-1™ library was screened to isolate high-quality camelized human sdAbs.
- ✓ **High Fidelity Screening** - Solid-phase strategy combined with in-parallel control group, which achieved great enrichment and support the reliability of the screening outcomes.
- ✓ **Two-Step Validation** - 13 target-specific clones were identified and validated through both monoclonal and soluble ELISA, which can avoid potential false positive.
- ✓ **One-Stop Solution** - Extensive experience and integrated procedure enable our scientists to shorten the overall lead time in as little as 30 workdays.

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