Creative Biolabs®
Single Domain Antibody

Case Study - SdAb Development for Low Immunogenic Target

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 have extensive experience in immunizing camelid animals with the target of interest to generate novel sdAbs. In terms of our advanced Hi-Affi™ phage display platform, we can use the immunized host animal to generate high-specific sdAbs for low immunogenic targets. This is a cost-effective and time-saving option for specific sdAb development, especially when you need to investigate the targets with low immunogenicity.

Project Objective & Achievement

For this case study, one low immunogenic protein (namely Target 1 or T1 for short) was provided as antigen and screening target, another protein with over 90% homology with Target 1 was also provided as negative control (namely Negative Target). Creative Biolabs is entrusted to immunize one llama with Target 1 and then develop T1-specific single domain antibodies which do not cross-react with Negative Target.

With the provided antigen, one llama was immunized. Although the immune response for the Target 1 was pretty low after 6 injections, a qualified uniform immune library was still generated by our seasoned scientists, which the overall capacity reached 10^8, a qualified level for library screening.

After three rounds of biopanning, good enrichment can be observed for Target 1 with distinguished difference between Negative Target, which indicated the library screening was performed successfully. 40 clones were then randomly picked from the 3rd round enriched pool for validation. All the 40 clones were observed as positive through monoclonal phage ELISA, which clear difference can be found between Target 1 and Negative Target. Thereafter, 23 unique V_HH sequences have been identified and confirmed to specifically recognize Target 1 through DNA sequencing and QC soluble ELISA.

Finally, there are 23 unique T1-specific sdAbs be discovered in this project.

Milestone Overview

Stage 1: Animal Immunization

One native (non-immunized before) llama was employed for this project. The immunization process was designed to last 105 days (6 injections with 3-week interval) and performed *via* multiple sites subcutaneous immunization strategy with increased antigen dosage, which contributes to triggering immune response for Target 1.

Date	Steps	Date	Steps
Day 0	Pre-bleed	Day 70	Bleeding and Titration
Day 0	Primary Injection	Day 84	1st Boost Injection
Day 21	2nd Injection	Day 91	Bleeding and Titration
Day 42	3rd Injection	Day 105	2nd Boost Injection
Day 49	Bleeding and Titration	Day 108	Final Bleed
Day 63	4th Injection		

Table 1. Custom Designed Llama Immunization Schedule.

During immunization, three titrations were performed separately. Target 1 was coated and tested in-parallel with pre-immune sera (negative control) and antisera. As shown in Figure 1, the 3rd titration still indicated relevantly low immune response, which was an expected outcome for the low immunogenic Target 1.

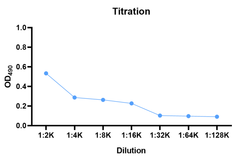

Figure 1. 3rd titration results.

Stage 2: Library Construction

After 6th injection, the antisera were collected and subjected to PBMC isolation, RNA extraction, and cDNA preparation, freshly on the same day. The V_HH genes were then PCR amplified by using our species-specific primers. The phagemid library was constructed with high-quality phagemid vectors and optimized ligation strategies to achieve 100% correct insertion rate. It was then desalted and subjected to electrotransformation with *E. coli* TG1 as the host strain to form the original bacteria library. 20 random clones were selected for QC colony PCR to identify the insertion of sdAb repertoire. Then 40 clones from the library were randomly picked and subjected to DNA sequencing and aligned, the results (omitted here) showed that no common sequences could be found among them. Based on the QC colony PCR and DNA sequencing analysis, a qualified immune library with the capacity of over 10^8 has been generated successfully even the titer is pretty low.

Stage 3: Library 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.

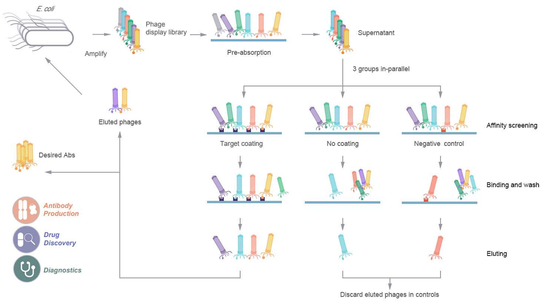

Figure 2. Flow diagram of phage display-based screening.

For this case study, solution-sorting screening strategy was performed, which Dynabeads MyOne Streptavidin T1 was coated with the target. Before the biopanning process, the biotinylation efficiency was determined by ELISA (Figure 3).

After three rounds of biopanning, good enrichment was observed for the target and clear difference was found between the "Target Coating" group, "Negative Coating" control, and "No Coating" control (Figure 4). This indicated some specific binders have been selected for Target 1 but not Negative Target.

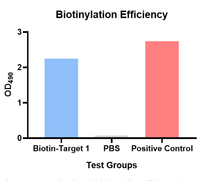

Figure 3. Determination of biotinylation efficiency by ELISA.

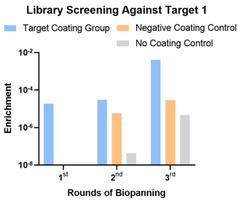

Figure 4. Process monitoring of library screening stage.

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

Stage 4: Binder Validation

After the biopanning, 40 clones were randomly picked from the 3rd round output of the target group. The monoclonal phage ELISA was then performed against the target, respectively.

For Target 1, 40 positive clones were observed and then processed for DNA sequencing (Figure 5). 25 unique clones were identified (Figure 6). All these unique clones were then prepared as soluble format (phage-free) for the validation of QC soluble ELISA. As shown in Figure 7, 23 of them were finally confirmed to recognize the target positively.

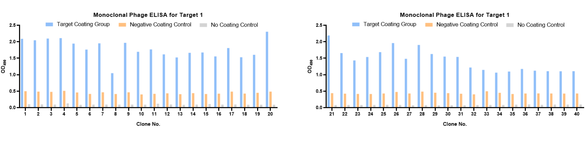

Figure 5. Monoclonal phage ELISA of the 40 randomly picked clones [Target 1].

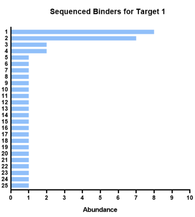

Figure 6. Summary of DNA sequencing results [Target 1].

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

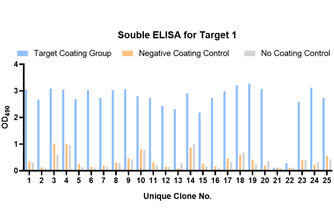

Figure 7. QC soluble ELISA of the unique sdAb candidates [Target 1].

Conclusion & Key Words

- **Low Immunogenic Target** - Antigens with low immunogenicity can be immunized for phage display library generation and novel sdAb discovery.
- **High-Quality SdAb Library** - Creative Biolabs' Hi-Affi™ platform can contribute to generating immune library with maximized diversity and capacity.
- **High Fidelity Screening** - Solution-sorting strategy combined with in-parallel control groups, which achieved great enrichment and support the reliability of the screening outcomes.
- **Two-Step Validation** - Antigen-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 smoothly advance the project and meet all your objectives.

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