Case Study - SdAb Development for Hapten 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 the interested targets. One animal immunized with one antigen is good enough to meet the majority of project requirements, which can offer a cost-effective option for specific sdAb development.

Project Objective & Achievement

For this case study, one hapten (small peptide) (namely Target 1 or T1 for short) was provided. Creative Biolabs is entrusted to immunize one camel with Target 1 and then develop target-specific single domain antibodies. To achieve good immune response, the KLH conjugated format T1 was generated and employed as antigen. And the biotin conjugated format T1 was employed as screening target.

With the generated KLH conjugated T1, one camel was immunized. Promising immune response for Target 1 was observed after 5 injections, which is qualified for library construction. One uniform immune library was then constructed with the capacity of over 10^8, a qualified level for library screening.

With the provided peptide target, three rounds of biopanning were successfully performed with good enrichment. 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 and 24 unique V_HH sequences have been identified and confirmed to specifically recognize Target 1 through DNA sequencing and QC soluble ELISA.

Finally, there are 24 unique T1-specific sdAbs be discovered in this project.

Milestone Overview

Stage 1: Animal Immunization

One native (non-immunized before) camel was employed for this project. The immunization process was designed to last 91 days (5 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 63	4th Injection
Day 0	Primary Injection	Day 70	Bleeding and Titration
Day 21	2nd Injection	Day 84	1st Boost Injection
Day 42	3rd Injection	Day 91	Bleeding and Titration
Day 49	Bleeding and Titration	Day 93	Final Bleed

Table 1. Custom Designed Camel Immunization Schedule.

After the 5th injection, test bleed was collected and 3rd titration was conducted to monitor the immune response. 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 hapten Target 1.

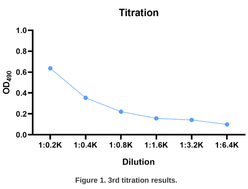

Figure 1. 3rd titration results.

Stage 2: Library Construction

After 5th 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 45 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.

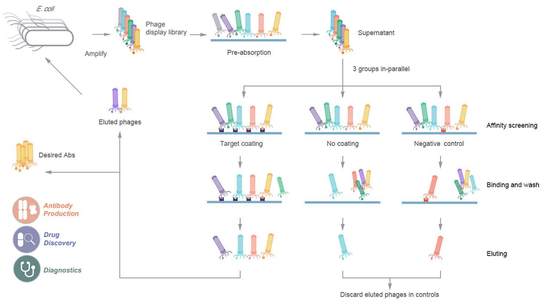

Figure 2. Flow diagram of phage display-based screening.

For this case study, solution-sorting screening strategy was performed, which streptavidin-coated plates were employed during the screening. After three rounds of biopanning, good enrichment was observed for Target 1 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 Target 1.

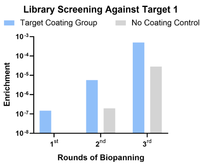

Figure 3. Process monitoring of library screening stage.
(Enrichment is increased round by round and presents significant difference with no coating control.)

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.

For Target 1, 40 positive clones were observed and then processed for DNA sequencing (Figure 4). 24 unique clones were identified (Figure 5). All these 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.

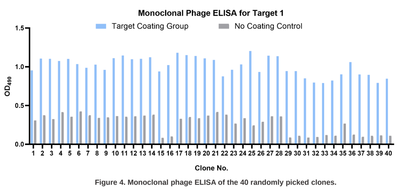

Figure 4. Monoclonal phage ELISA of the 40 randomly picked clones.

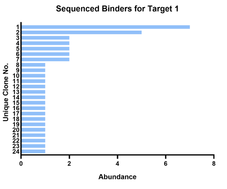

Figure 5. Summary of DNA sequencing results.
(Abundance of each unique clone indicates the number of sequenced clones present the same sequencing information.)

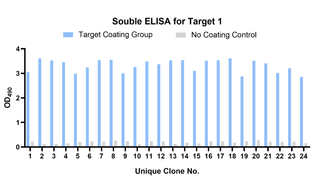

Figure 6. QC soluble ELISA of the unique sdAb candidates.

Conclusion & Key Words

- ✓ **Hapten Target** - Hapten targets can be designed and prepared properly for immunization-based 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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