Case Study - SdAb Development for 3 Targets *via* 1 Camelid Immunization

Creative Biolabs
Single Domain Antibody

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 1 immunized host animal to generate high-specific sdAbs for multiple antigens. This is a cost-effective and time-saving option for specific sdAb development, especially when you need to investigate different targets with low homology.

Project Objective & Achievement

For this case study, THREE different targets were provided as antigens and screening targets. Creative Biolabs is entrusted to immunize only ONE camelid host animal with these targets and then develop antigen-specific single domain antibodies, respectively.

With the provided antigens (namely Target 1, Target 2, and Target 3 or T1, T2, T3 for short), one camelid was immunized with mixed antigens. Promising immune response for each antigen was observed after 4 injections, which is qualified for library construction. One uniform immune library was then constructed with the capacity of over 10^8. Three rounds of biopanning were successfully performed against each of the three targets respectively with significant good enrichment. 40 clones were randomly picked from the 3rd round enriched pool of each target for validation.

For Target 1, all the 40 clones were observed as positive through monoclonal phage ELISA and 7 unique V_HH sequences have been identified and confirmed to recognize the target specifically. For Target 2, all the 40 clones were observed as positive through monoclonal phage ELISA and 5 unique V_HH sequences have been identified and confirmed to recognize the target specifically. For Target 3, 22 of the 40 clones were observed as positive through monoclonal phage ELISA and 19 unique V_HH sequences have been identified and confirmed to recognize the target specifically.

Finally, there are 7 unique T1-specific sdAbs, 5 unique T2-specific sdAbs, and 19 unique T3-specific sdAbs be discovered in this project.

Milestone Overview

Stage 1: Animal Immunization

One native (non-immunized before) camelid animal was employed for this project. The immunization process was planned to last 70 days (4 injections with 3-week interval) and performed *via* multiple sites subcutaneous immunization strategy, which contributes to triggering immune response for all the three targets.

Date	Steps	Date	Steps
Day 0	Pre-bleed	Day 49	Bleeding and Titration
Day 0	Primary Injection	Day 63	4th Injection
Day 21	2nd Injection	Day 70	Bleeding and Titration
Day 42	3rd Injection	Day 72	Final Bleed

Table 1. Typical Camelid Immunization Schedule.

After the fourth injection, test bleed was collected and 2nd titration was conducted to monitor the immune response. The three targets were coated separately and tested in-parallel with pre-immune sera (negative control) and antisera. As shown in Figure 1, good immune response was observed for all the three targets: the titer of T1 and T3 was over 1:128,000, and T2 reached 1:32,000.

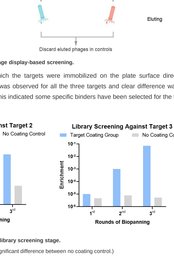

Figure 1. 2nd titration results.

Stage 2: Library Construction

After 4th 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 (Figure 2). It was then desalted and subjected to electrotransformation with *E. coli* TG1 as the host strain to form the original bacteria library. Based on the QC colony PCR and DNA sequencing analysis, a qualified immune library with capacity of over 10^8 has been generated successfully.

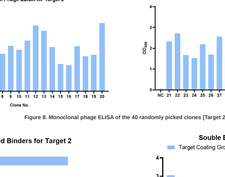

Figure 2. QC colony PCR of random clones from the end library

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.

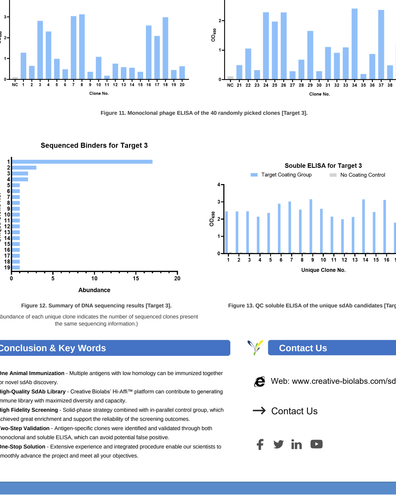

Figure 3. Flow diagram of phage display-based screening.

For this case study, solid-phase screening strategy was performed, which the targets were immobilized on the plate surface directly and screened separately. After three rounds of biopanning, good enrichment was observed for all the three targets and clear difference was found between the "Target Coating" group and "No Coating" control (Figure 4). This indicated some specific binders have been selected for the targets.

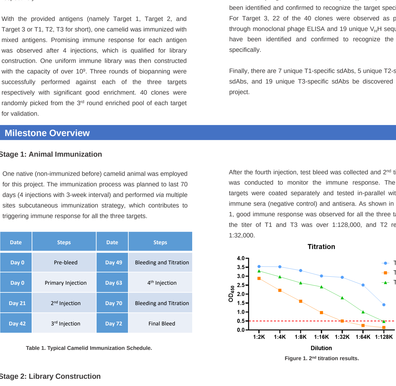

Figure 4. Process monitoring of library screening stage.

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

Stage 4: Binder Validation

After the biopanning, 40 clones were randomly picked from the 3rd round output of each 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). 7 unique clones were identified in CDR level (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, all 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].

For Target 2, 40 positive clones were observed and then processed for DNA sequencing (Figure 8). 5 unique clones were identified in CDR level (Figure 9). All these unique clones were then prepared as soluble format (phage-free) for the validation of QC soluble ELISA. As shown in Figure 10, all of them were finally confirmed to recognize the target positively.

Figure 8. Monoclonal phage ELISA of the 40 randomly picked clones [Target 2].

Figure 9. Summary of DNA sequencing results [Target 2].

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

Figure 10. QC soluble ELISA of the unique sdAb candidates [Target 2].

For Target 3, 22 positive clones were observed and then processed for DNA sequencing (Figure 11). 19 unique clones were identified in CDR level (Figure 12). All these unique clones were then prepared as soluble format (phage-free) for the validation of QC soluble ELISA. As shown in Figure 13, all of them were finally confirmed to recognize the target positively.

Figure 11. Monoclonal phage ELISA of the 40 randomly picked clones [Target 3].

Figure 12. Summary of DNA sequencing results [Target 3].

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

Figure 13. QC soluble ELISA of the unique sdAb candidates [Target 3].

Conclusion & Key Words

- **One Animal Immunization** - Multiple antigens with low homology can be immunized together for 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** - Solid-phase strategy combined with in-parallel control group, 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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