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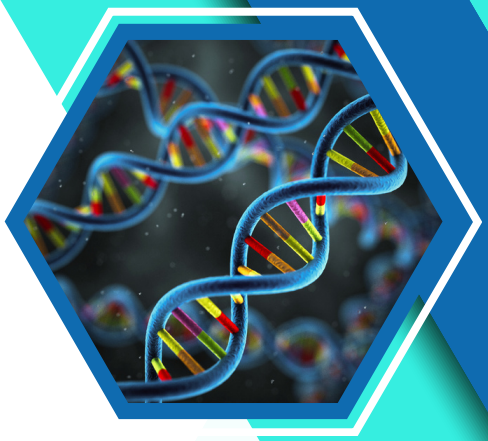
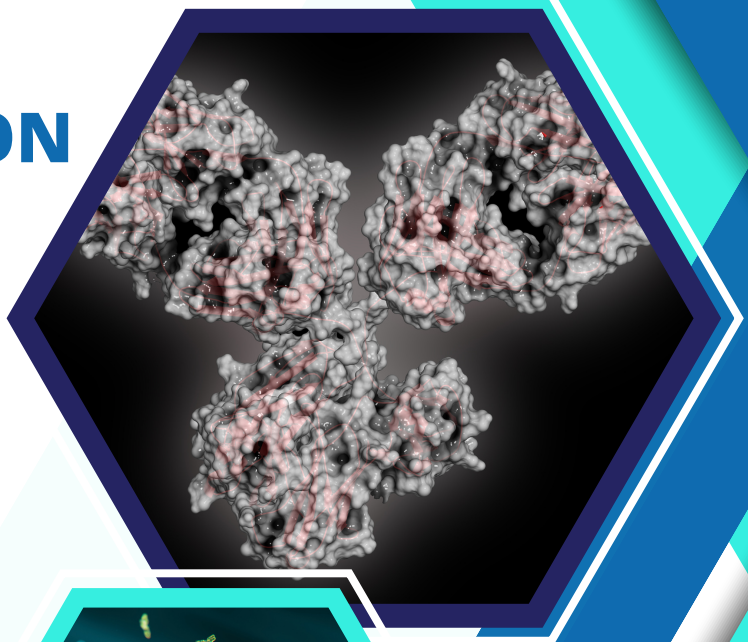
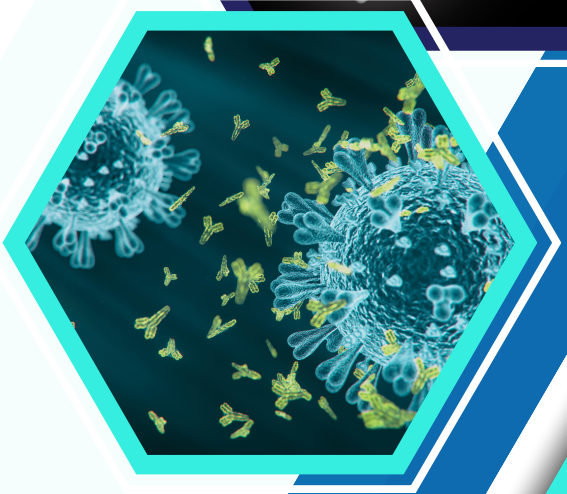


CASE STUDY

ANTIBODY
AFFINITY MATURATION

Effective identification and validation of scFv mutant sequences with enhanced affinity for the target antigen.





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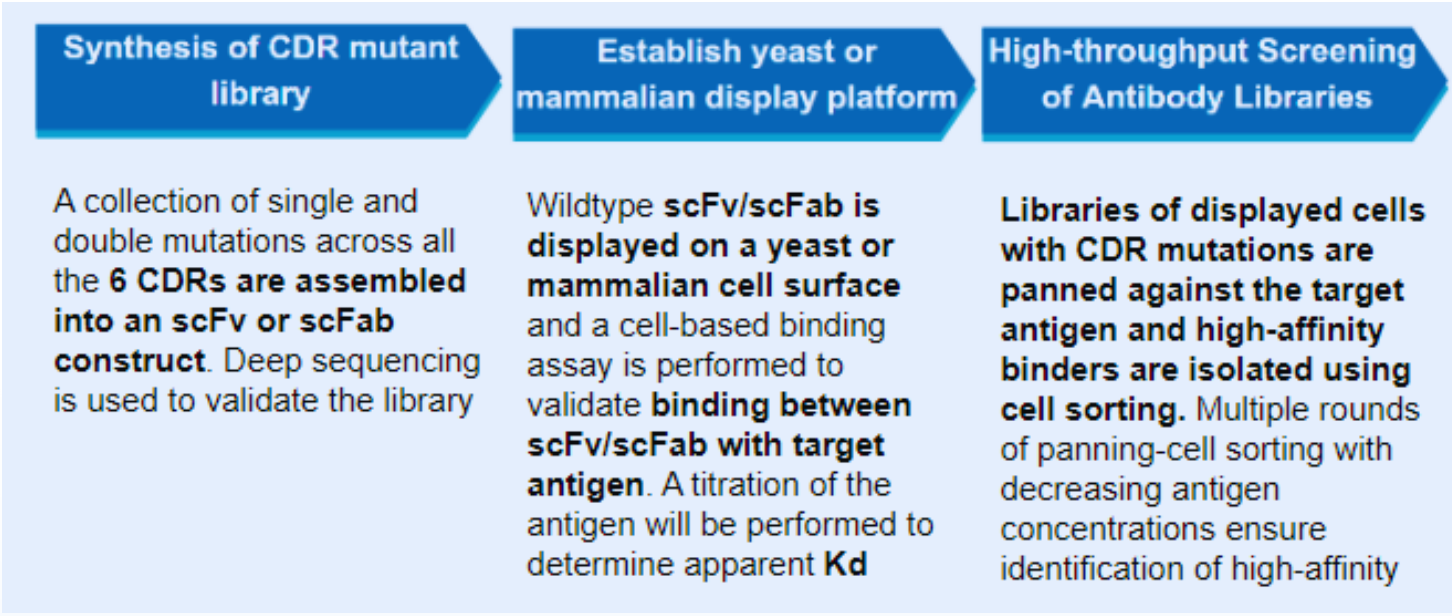
Project summary

A biopharmaceutical company specializing in antibody therapeutics engaged in a collaborative project with Ranomics for the custom affinity maturation of their six CDR sequences. The objective was to synthesize a mutant variant library of these sequences and clone it into Ranomics' scFv yeast display vector. The project aimed to identify mutant CDR sequences with enhanced affinity for the company's target antigen.

Disclaimer: The name of the biopharmaceutical company involved in this case study cannot be disclosed due to a non-disclosure agreement (NDA) with the company.

Ranomics Workflow: CDR engineering & Affinity Maturation



Methodologies & Key Findings:

Design and Construction of scFv Library:

- The scFv sequences were designed with a specific format to facilitate the identification of higher affinity mutants.
- The library included parental CDRs and one mutant CDR, designed for optimal affinity enhancement.
- All single and double mutations across CDR residues on the scFv molecule were synthesized.
- NNK degenerate codons were utilized to encode mutations at each CDR residue.
- The target diversity of the library was set between 1e5 to 1e6.
- The library was successfully cloned into the Ranomics yeast display plasmid.
- Validation of the library was performed using next-generation sequencing (NGS) to confirm that each position was mutated to all 20 amino acids, and any double mutations were identified in a single sequencing amplicon.

Ranomics Yeast Display Vector Design

1. Antigen Titration Experiment:

- Conducted to determine the optimal antigen concentration for downstream screening.
- Results showed a clear dose-dependent increase in binding between the native scFv and the target antigen.
- Established the ideal antigen concentration for subsequent panning experiments.

2. Panning and Cell Sorting:

- Three cycles of panning and cell sorting were performed with varying antigen:scFv molar ratios (1:5, 1:10, and 1:15).
- Flow cytometry analysis demonstrated successful isolation of high-affinity binders.
- Increasing molar ratios led to a higher enrichment of scFv mutant sequences with enhanced binding capabilities.
- The final sorted cell populations exhibited significant improvements in fluorescence signal, indicating successful affinity maturation.

3. Amplicon Deep Sequencing:

- Pre-sorted and sorted cell populations from each cycle of panning were subjected to amplicon deep sequencing.
- Bioinformatics analysis revealed a diverse set of mutant CDR sequences.
- The top-performing mutants were identified based on increased read counts and binding affinity compared to the wildtype scFv.
- The sequences of interest were further analyzed for potential therapeutic applications.

4. Assay Validation:

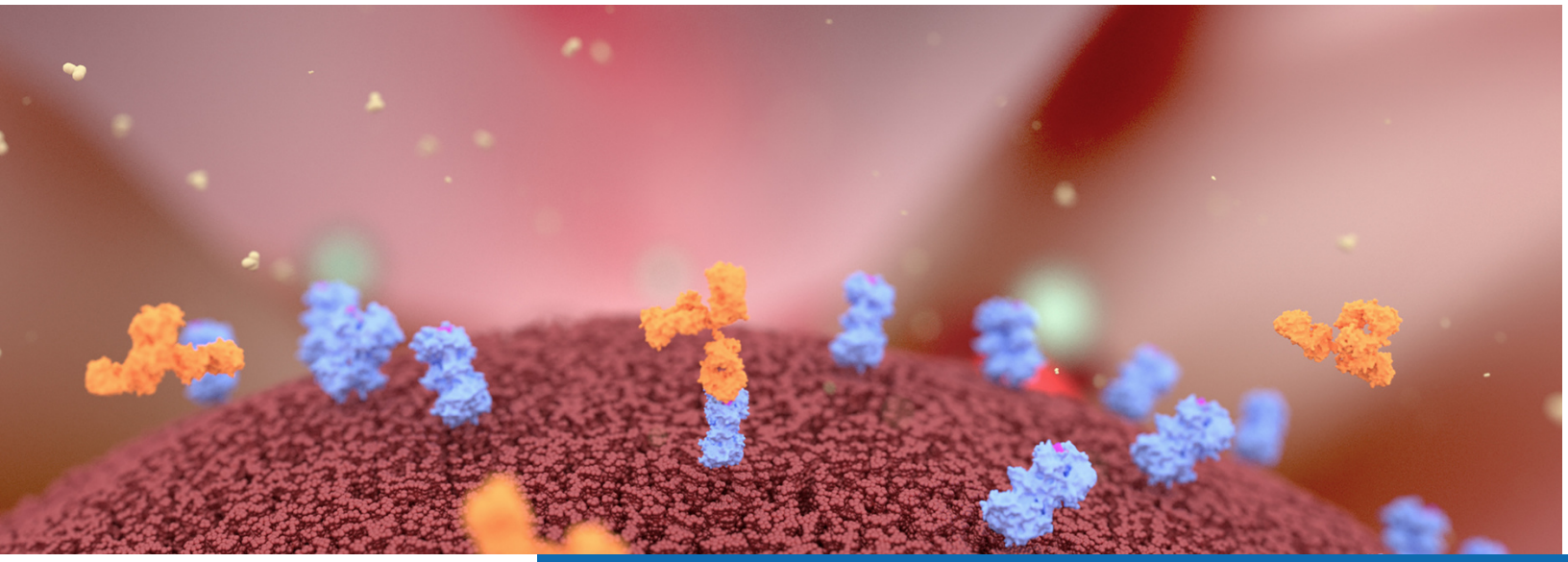
- Transformation of control sequences (wildtype vs. non-binder) confirmed the specificity of the binding assay.
- Flow cytometry analysis demonstrated a clear distinction between wildtype and non-binder sequences, validating the assay.
- Binding assays with a titration of target antigen confirmed the enhanced binding of mutant yeast display cells compared to controls.

5. NGS and Data Analysis:

- NGS library building from sorted cells generated extensive data.
- Illumina sequencing with NovaSeq PE 2x250bp lane produced 325 to 400 million reads per sample.
- Bioinformatics analysis provided detailed insights into read count determination for each mutation in sorted cell populations.
- The top 10 performing scFv sequences were identified for further investigation.

Summary

The collaborative efforts between the biopharmaceutical company and Ranomics resulted in a successful custom affinity maturation project. The results showcase the effective identification and validation of scFv mutant sequences with enhanced affinity for the target antigen. These results lay the foundation for potential therapeutic candidates, demonstrating the feasibility of Ranomics' affinity maturation approach in antibody discovery and development.



Ranomics: Excelling in Antibody Engineering Services

As a leader in the field of genetic engineering, Ranomics stands out for its unparalleled expertise in antibody engineering services. Leveraging cutting-edge technologies, Ranomics provides comprehensive solutions for epitope mapping, mutagenesis studies, and high-throughput screening. With a commitment to innovation and precision, Ranomics empowers researchers and biopharmaceutical companies to accelerate their antibody development programs, ultimately advancing the frontier of therapeutic discovery. Ranomics' proficiency in integrating diverse techniques ensures that clients receive tailored and insightful results, making them a preferred partner in the pursuit of novel and effective antibody therapeutics.