

Antibody Engineering to Increase Affinity — Two Novel Approaches: Domain Engineering and Comprehensive CDR Mutagenesis

- *Domain Engineering* offers a fast, efficient service to obtain modified antibody clones.
- *Comprehensive CDR Mutagenesis* allows you to test every possible change to antibody CDRs and subsequently every combination of the best changes in two quick, comprehensive experiments.

Introduction

Blue Heron Biotechnology offers two novel gene synthesis custom services to improve antibody affinity and/or specificity — ***Domain Engineering and Comprehensive Mutagenesis***. Domain Engineering simplifies the process of making a series of directed changes to an antibody gene. Comprehensive Mutagenesis can be thought of as a highly efficient synthetic version of affinity maturation.

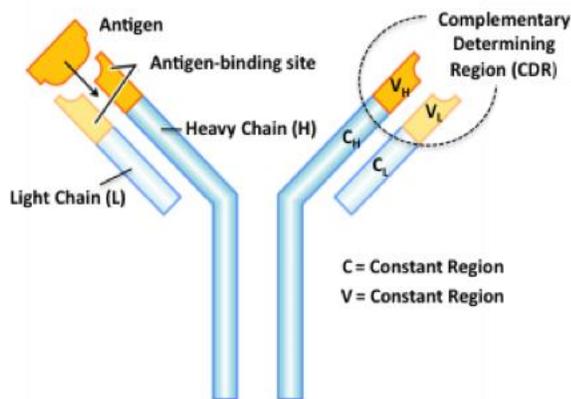


Fig. 1 Antibody Structure

Background

Antibodies form the basis for many diagnostics and therapeutics due their ability to achieve high-affinity binding to the antigen of choice. This binding is critical to expanding detection limits, extending dissociation half-times, decreasing therapeutic dosages, and increasing their overall efficacy. Antibody humanization can result in lower affinity and/or specificity. Lower affinity can increase the cost of product development and manufacturing since, in the case of a therapeutic, a higher dose may be required to compensate for the lower affinity.

Domain Engineering

Make simple or complex variable region changes needed to improve downstream antibody affinity.

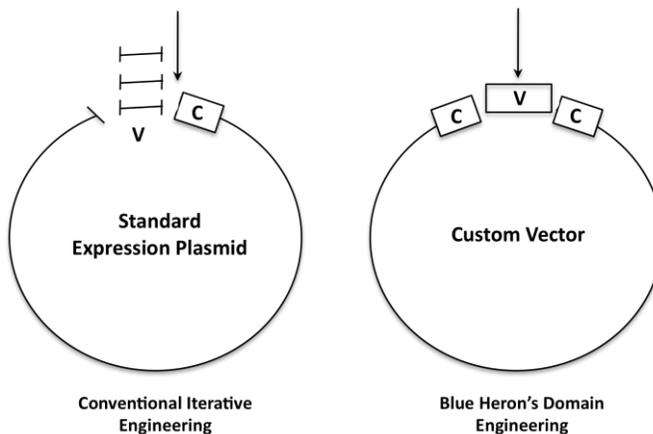


Fig. 2 Domain Engineering

Process

- You submit the full CDR plus Variable region DNA sequence and the expression vector.
- We create a Custom Vector containing the Constant regions specific for your project.
- You simply order versions of the variable region in this Custom Vector.
- We build the variants, insert the variable region Cassettes into the Custom Vector.
- Deliverable: Full-length, sequence-verified, individual antibody clones in your expression vector (Fig. 2).

Advantages

- Flexibility to make single or multiple amino acid variants within the variable region Cassette.
- Delivery reliability
- Faster than using traditional mutagenesis methods

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Comprehensive CDR Mutagenesis

Test any modification to the CDR regions in a single experiment.

Process

- You specify the base sequence of the antibody gene, the amino acid positions that are to be modified (typically the CDRs), and a list of substitutions at each position.
- We deliver a library of individual, sequence-verified clones or a pooled library representing the specified changes.

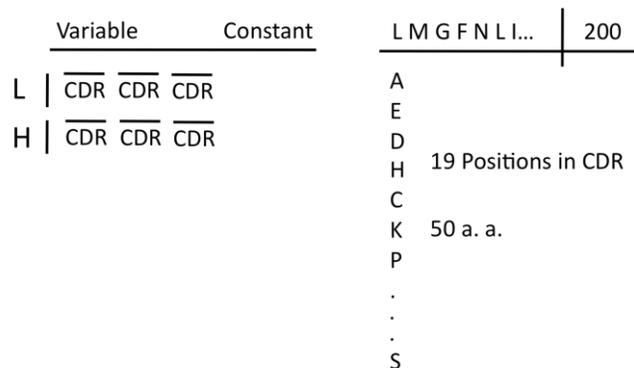


Fig. 3 Comprehensive CDR Mutagenesis

Example Library: Test 19 amino acid

substitutions at 50 CDR positions in a heavy and light chain pair. Blue Heron will deliver 950 sequence verified individual clones in your expression vector of choice.

- Delivered individually or in a 96-well plate in the positions you specify, including positive and negative controls.
- Once you have tested every possible change in the antibody CDRs, Blue Heron can combine the best variants into a multi-site library of your design.
- Blue Heron will then generate and deliver a multi-site variant library with all possible combinations of the second tier variants. Example Second-Tier Library: a single amino acid change made at ten different positions will generate 1,024 clones each with a unique combination of the specified variants ($2^{10} = 1,024$).

Additional Benefits

- Either approach also can be applied to developing enzyme variants with optimal catalytic functionality.
- Throughout the development process, Blue Heron works closely with you to provide you with the clones you need to achieve your research objective.

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