Precise Control of Diversity for Synthetic Antibody Library Design & Construction

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Synthetic antibody libraries have proven to be effective tools for drug discovery and development through the generation of functional, high-affinity antibodies against a wide variety of antigens. They are an expanding alternative to standard hybridoma technology especially for application to particularly difficult therapeutic challenges that cannot be addressed with antibodies from the natural repertoire. The performance of a synthetic antibody library depends in large part on the diversity of the library which must be designed based on thorough understanding of the antibody structure and function. Focused diversity can provide an efficient path to antibody candidates designed for exceptional performance in specialized applications if precise control over design and construction is exercised. The use of degenerate oligos and other standard methods of diversity introduction lack this precise control and can introduce unwanted or useless codons into the library, thus limiting its performance. Fully designed library diversity is enabled through parallel in-situ (on-chip) synthesis of hundreds of specific (non-degenerate) oligonucleotide sequences. We demonstrate the bioinformatics-based design and high-throughput synthesis of a mutant phage display library to improve affinity of anti-erbB2 single chain monoclonal antibody A21.

µParaflo® Microfluidics Oligo Synthesis Platform

Three components are at the core of the µParaflo® Technology:
1. Photogenerated reactor chemistry (PGR-chemistry).
2. Digital phototransformation directed (programmable) synthesis.
3. Microfluidic devices containing high density 3D reaction chambers of picoliter volumes.

Digital Light Projection drives light directed chemical reactions at specific sites in an array format and eliminates the need for expensive, inconvenient microfabricated photomasks. A computer generates the digital mask and a Digital Light Projector (DLP) projects the light beam very accurately into the microreaction chambers where a photogenerated reagent is produced.

Microfluidic Reaction Devices

Other Methods Have Limitations Too

- TBEA - Tricistine Mutagenesis - The codon-based tricistine synthesis for building oligonucleotides provides specific codon for each amino acid, but it is expensive to be of routine use and suffers from biased or low efficiency coupling issues.
- Split-Mix-Split Method - Offers better control of amino acid distribution and composition of randomized sequences, however, there is significant complexity and potential for human error as synthesis resists are mixed multiple times.
- A2 or Custom Histories of building blocks can combat codon biases but also are limited by the potential for imprecise mixing/handling.

Synthetic Antibody Library Construction Process

Application Ex - Directed Evolution for Increased Affinity

Bioinformatics-based design and high-throughput microfluidic synthesis of mutant phage display library to improve affinity of single chain antibody A21

Anti-erbB2 Antibody A21

008 (also called HB2 and pSU8) is a 185 KDa trans-membrane epidermal growth factor receptor (EGFR) Family protein with intrinsic tyrosine kinase activity. ERBB2 contains several plausible tyrosine phosphorylation sites and is capable of associating with other EGFR proteins to enhance a cascade of kinase activities in signal transduction pathways known to be active in cell proliferation and/or cell apoptosis. ERBB2 is shown to have up-regulated expression in several malignant human tumors such as breast and ovarian cancer compared to normal cells, making the protein an intensely pursued target as cancer therapeutics and as a biomarker for tumor cell detection.

Antibody A21 is a predominant growth inhibitor of cells over expressing the ERBB2 gene with significant in vivo work done toward producing higher affinity mutants. However, antibody affinity maturation in vitro often fails to produce antibody drugs of the targeted potency making further in vitro affinity maturation by computational design and directed evolution necessary.

Library Construction

Oligonucleotide Synthesis
Library construction
Helper phage rescue
Express and purify

Results

It works in vivo!

Eukaryotic expression vector:
ontogenous antibodies with human IgG Fc fusion protein

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