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 thousands of specific (non-degenerate)  oligodeoxynucleotides. 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® Microfluidic Oligo Synthesis Platform

Three components are at the core of the µParaflo® Technology:
1. Photogenerated reaction chemistry (PG Pharmacy).
2. Digital photolithography directed (programmable) synthesis.
3. Microfluidic devices containing high density 3D reaction chambers of pico-liter volumes.

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

Microfluidic Reactions Device

A novel solution photothermal approach employing light directed parallel synthesis and deprotection with a photogenerated reagent enables high yield parallel synthesis using standard DNE protected phosphoramidites.

Designed Library Diversity with Specific Oligo Sequences

Library Design & Diversity

Diversity is the variability carried by the amino acid sequences of a synthetic antibody library

- Synthetic libraries are diversified by design - in vivo using one of many published algorithms. Naturally occurring antibodies provide guidance on how to limit synthetic diversity.
- Diversity design can be created in one or more of the light and/or heavy chain variable domains (VH and VL) and in varying the length of the hypervariable loops present in the variable domain (length diversity) to allow diverse structural conformations to form in this region and further increase the diversity of the library.
- Often randomized or tailored randomization of oligonucleotides sequences is employed to introduce diversity.
- The ability to precisely define the final diversity of a library facilitates the process of isolating, characterizing, and optimizing an antibody lead.

Recombinant Monoclonal Antibody Library

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

Antih-ErbB2 Antibody A21

ErbB2 (also called HER2/neu and p185) is a 185 KDa trans-membrane epidermal growth factor receptor (EGFR) family protein with intrinsic tyrosine kinase activity. ErbB2 contains several pleiotropic 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.

Bioinformatics Based Library Design

Naturally occurring antibodies provide guidance on how to limit synthetic diversity. An algorithm helps us to determine advantageous codons. Pseudo-codons are introduced such that the mutant library contains every conceivable combination of mutations (determined to be advantageous) at a stochastically even frequency of occurrence while minimizing the introduction of undesired mutations and those infrequently observed in natural antibodies.

Library Construction

Eukaryotic expression vector: mutant antibodies with human IgG Fc region protein

Results

Screening – Library construction and screening over 100,000 clones

Screening – ELISA on library antibodies and polyclonal sera

Eukaryotic expression vector: mutant antibodies with human IgG Fc region protein

It works in vitro!