Serimmune

A Bacterial Display Library-based Method to Rapidly Discover Disease-associated Epitopes for Immunoassay Development

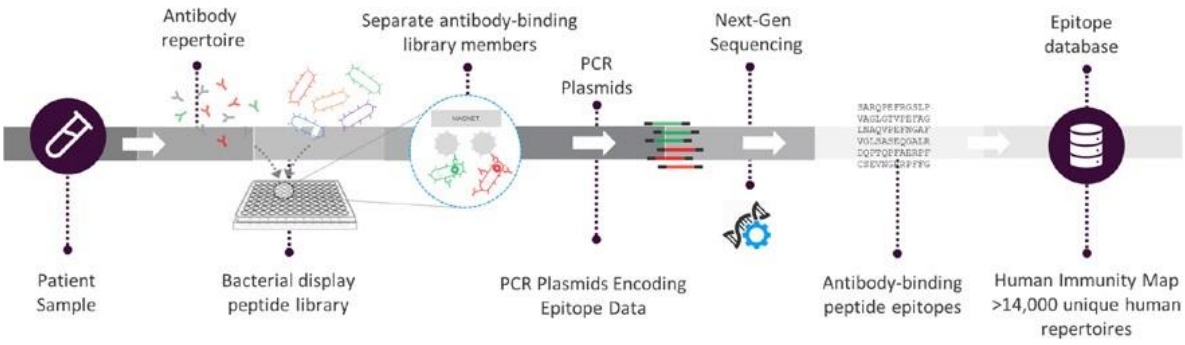
The detection of disease-specific antibodies remains a cornerstone of disease diagnosis and staging. Still, many tests in clinical use suffer from low sensitivity or specificity [1,2] and for some diseases serological tests have yet to be developed. Protein arrays have been used successfully for high-throughput antigen screening [3-5], however, for large proteomes, protein production is time-consuming and costly. Additional limitations include relatively low throughput, poor protein expression or folding, and the need to generate a custom array for each organism.

Serimmune has developed a high throughput serum epitope repertoire analysis platform, SERA, with demonstrated usefulness in the discovery of conserved disease-associated antigens and epitopes[6,7]. SERA utilizes a bacterial display random peptide library with a diversity 10^{10}, over 10,000-fold greater than that of the largest planar arrays[4].

The random nature of the library enables discovery of antibody-based biomarkers for all types of diseases without prior knowledge of the antigen or proteome sequence. Additionally, the database allows for in silico specificity testing against thousands of other repertoires to remove epitopes that may exhibit cross-reactivity with similar proteins organisms. We have discovered and validated epitope panels for a viral, parasitic, bacterial and fungal diseases as well as a number of antibody biomarkers associated with autoimmunity and cancer. Here we present a workflow for the rapid discovery and selection of candidate antigens and peptides that can be used in near-patient immunoassay development.

- Rapid discovery of candidate antigens and epitopes – The whole process including sample screening can be completed in a month
- Identification of both mapping and non-mapping motifs – Unlike peptide arrays, SERA can identify highly disease- specific library-derived peptides
- In silico cross-reactivity and specificity analysis – identifies and removes epitopes that may be shared between similar organisms or look-a-like diseases.

The Serum Epitope Repertoire Analysis (SERA) Workflow



Serum is incubated with bacterial display 12mer peptide library and antibody-bound bacteria are separated from unbound bacteria with protein A/G magnetic beads. The resulting bacterial pool, representing the peptide epitopes of individuals' antibody repertoire, is propagated, plasmids encoding the peptides are purified, and the peptide encoding regions are amplified by PCR. DNA is sequenced, translated back to amino acid sequences and uploaded to the epitope repertoire database.

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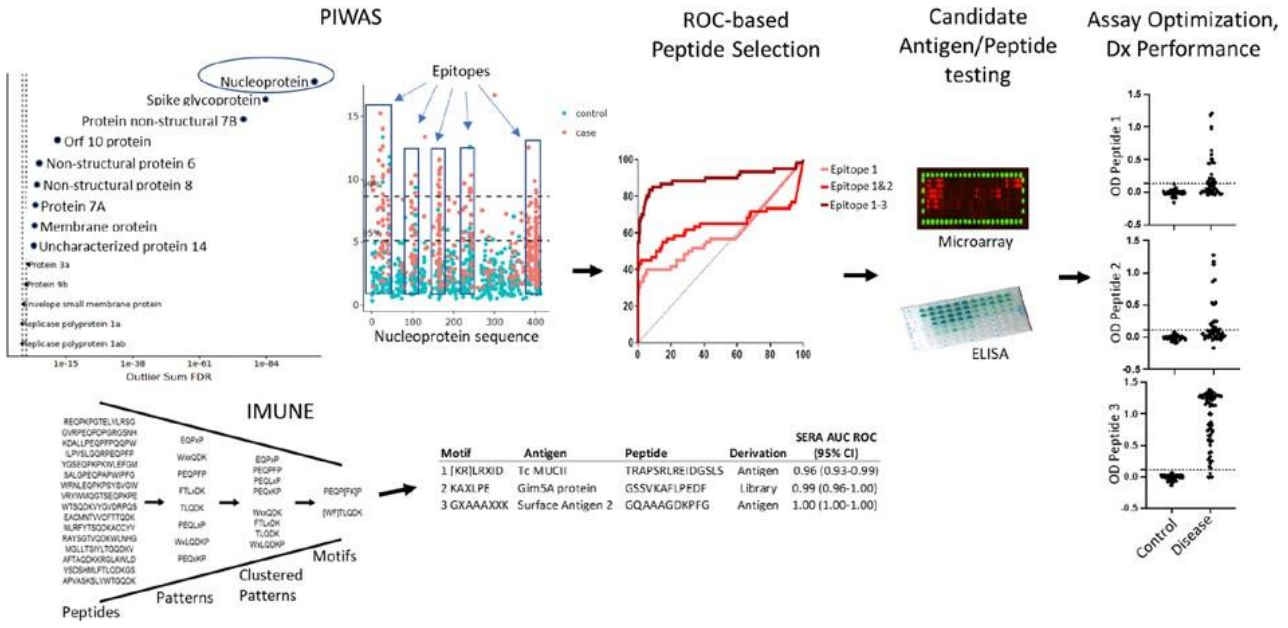
Proprietary Informatics Leverage a Large Epitope Repertoire Database to Identify Immunodominant Disease Antigens and Epitopes

Epitope repertoires for a given disease are compared with non-diseased repertoires using two complementary informatics algorithms. IMUNE identifies peptides patterns that are enriched in disease repertoires and absent from controls. These patterns reflect epitopes that map to linear regions of the proteome of interest, as well as mimotopes that serve as highly disease-specific biomarkers. PIWAS tiles 5 and 6mers derived from the 12mer peptides across any proteome of interest and identifies candidate linear epitopes based on regions where kmers are enriched relative to a large group of controls[8,9]. PIWAS also generates a ranked list of antigens based on the number of distinct epitopes on a protein as well as the effect size or sensitivity of the signal across the protein. The entire discovery process can be completed in less than a month.

Informatics-based Discovery and Testing of Antigens and Peptides for Immunoassay Development



Candidate antigens and epitopes are selected based on their combined sensitivity within the disease cohort and specificity as compared to thousands of database controls (including other diseases with known or predicted cross-reactivity). This in silico specificity assessment provides a means to filter out cross-reactive antigens or epitopes prior to further testing6. Peptides selected for synthesis may be derived from the antigen sequence or from the library and can be ported to any standard immunoassay platform for testing including microarray, ELISA or lateral flow.

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