

An effective way to identify conformational epitopes linked to protection

Introduction:

Pepscan has developed a unique and broadly applicable technology, suitable for precision epitope mapping of one single antibody as well as detailed epitope fingerprinting analysis of large sets of polyclonal sera. CLIPS Precision Epitope Mapping provides high resolution results, for linear epitopes, but also for conformational epitopes, which are often involved in neutralizing antibody binding.

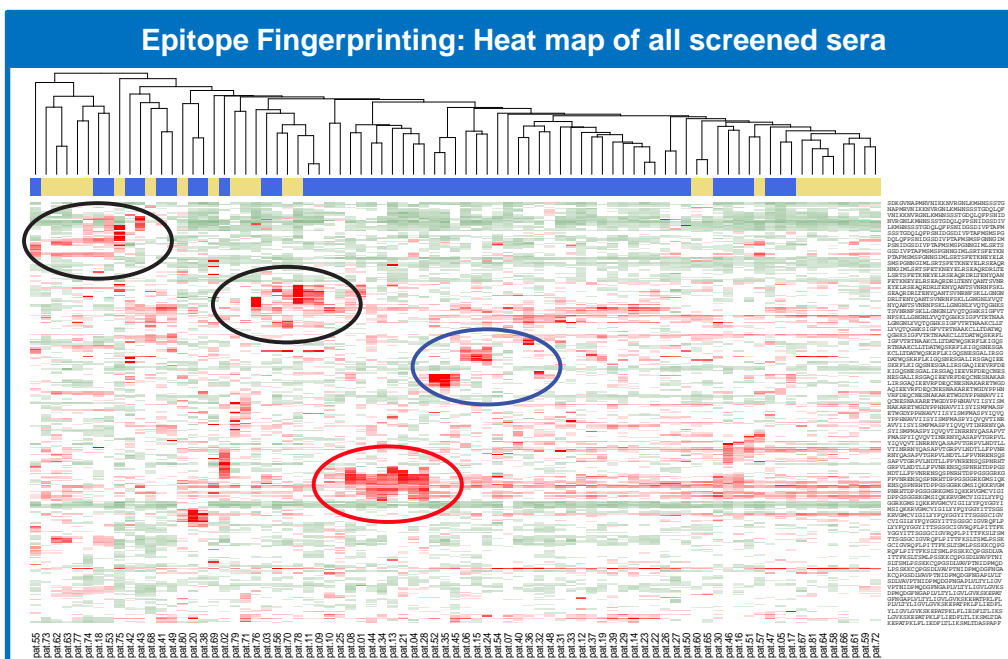
As the CLIPS peptide arrays can be re-used up to 100 times, it is also the most suitable mapping technology for comparative screening of collections of antibodies and sera. For example, profiling the epitope landscapes of large numbers of sera from diseased and healthy origin allows identification of epitopes linked to protection. Therefore, this epitope fingerprinting can be of great value for vaccine development programs.

Method:

A full CLIPS peptide library covering conformational and discontinuous epitopes was designed and synthesized, based on the amino acid sequence of a known protein involved in the disease. Sera from 56 patients and 25 healthy controls were screened on this array and results were compared using a customized bio-informatics approach.

Results:

Comparing sera from patients (blue bars) and healthy donors (yellow bars) identified four defined epitopes regions (see graph)*. Two groups of epitopes could be linked to the disease (red and blue encircled). These two were selected for further vaccine lead development.



The results of screening of 1200 different CLIPS peptides were summarized into the location of the different epitopes along the amino acid sequence of the target protein. The displayed data displays real data of a set of sera, but the shown peptide sequences are scrambled.

The benefits of CLIPS Precision Epitope Mapping

- Works for all types of epitopes:
- Discontinuous, conformational, and linear
- Applicable to all kinds of samples:
- mAbs, antibody-like scaffolds, and polyclonal sera
- Re-usable arrays for multiple screenings
- Comparative mapping of sets of samples
- Unrivalled single-residue resolution
- Solid support for patent claims and FTO assessments

Technical information CLIPS Precision Epitope Mapping

Peptide synthesis	Fmoc chemistry. Maximum peptide length over 40 residues. All amino acids, including D-amino acids and non-natural amino acids.
Capacity	Ten custom high-throughput parallel synthesis robots, each 10.000 peptides per run.
Peptide library format	Proprietary 'Minicard' format with solid phase-bound peptide constructs in 455 microwells. Surface chemistry: proprietary polymeric graft optimized for low non-specific binding and high peptide construct loading.
Combinatorial library complexity	Matrix analysis, e.g. 50 x 50 = 2.500 double loop T3 CLIPS™. All matrix combinations within 40-mers possible. All overlapping single loops, usually 15 - 20-mers. All overlapping peptides of a protein, usually 15 - 20-mers. Full positional scan libraries of all epitopes.
Spatial construct complexity	Single loops on T2 CLIPS. Double loop combinations on T3 or 2 x T2 CLIPS. Sheet-like T2 CLIPS, helix-like T2 CLIPS. All loop structures with 2-6 cysteines and 1 or 2 CLIPS.
Peptide library reusability	At least 20 times, but up to 100 depending on the samples. Library storage and re-use up to years.
Binding detection	Binding of the antibodies to the CLIPS peptides is determined in an ELISA. The resulting color in each well is quantified with a CDD camera.
Binding detection sensitivity	Optimized for epitope mapping, down to $K_d=10^{-3}$
Required material and information	100 µl polyclonal serum or 100 µg antibody Linear sequence of target protein.
Project run-through time	Priority 1.5 months, Standard 3 months.
Reporting	Binding values of all peptides are quantified and stored in the PepLab™ database. A full report is provided including details on binding and specificity for each residue, optimized for registration, regulatory, and/or IP purposes. Full support is offered for IP generation and publishing.



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CLIPS™ Precision Epitope Mapping technology
is covered by one or more of the following
patents: US 7863239 and US 7972993