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TIU Phage Display Platform (PanSeq) v1.0

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TIU - Public Protocols

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We use this collection and it's working

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Abstract

The Translational Immunopsychiatry Unit (TIU) at the National Institute of Mental Health (NIMH) is engaged in autoantibody discovery and anti-pathogen antibody profiling in diverse neuropsychiatric populations.

One tool we use is Phage Display Immunoprecipitation Sequencing (PhIP-Seq), a powerful method for profiling antibody specificities, enabling high-throughput identification of epitopes recognized by antibodies in biological samples.

Our PanSeq library incorporates relatively longer, 64 amino-acid peptides, increased tiling density, a modular pool design that encompasses the entire human proteome, and a broad sampling of relevant microbial antigens. Improvements in DNA oligonucleotide synthesis have yielded a library with low intrinsic error rates.

Furthermore, our PhIP-Seq protocol implements technical replicates and a carefully calibrated PCR protocol for sequencing library preparation. We expect that these design and protocol modifications will allow for finer epitope mapping, improved detection of novel and cryptic epitopes (including those arising from alternative splicing or rare genetic variants), and fewer false positives.

In designing PanSeq, we prioritized:

- A 2/3 peptide overlap tile design for reference human proteins that enhances redundancy and facilitates finer epitope mapping;
- Inclusion of all predicted neoepitopes arising from single intron retention or exon exclusion splice events;
- Inclusion of non-canonical autoantigens (e.g., experimentally predicted microproteins, human endogenous retroviral elements, and LINE-1 elements);
- Reuse of existing microbial libraries for backwards compatibility and comparability with prior studies;
- Supplementation of existing microbial libraries with a novel immune epitope database (IEDB) library
- Modularity for subpool screening

In this protocol series, we detail the design, construction, and validation of PanSeq using commercial antibodies. This protocol series will serve as the primary reference for TIU publications that employ PhIP-Seq.

This protocol series is a living document that will be updated and amended as the protocol matures.

We hope these resources will facilitate robust, reproducible antibody profiling for researchers across disciplines.

Materials

See each subprotocol for materials

Troubleshooting

Files

SEARCH

Protocol

NAME

I. PanSeq library design

VERSION 1

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Protocol

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II. PanSeq: Synthesis and construction of the physical mid-copy T7-phage display library

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III: Evaluation of library quality

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IV. PhIP-Seq: immunoprecipitation and phage amplification methods

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V: Data processing

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Protocol references

Larman HB, Zhao Z, Laserson U, Li MZ, Ciccia A, Gakidis MA, Church GM, Kesari S, Leproust EM, Solimini NL, Elledge SJ. Autoantigen discovery with a synthetic human peptidome. Nat Biotechnol. 2011 May 22;29(6):535-41. doi: 10.1038/nbt.1856. PMID: 21602805; PMCID: PMC4169279.

O'Donovan B, Mandel-Brehm C, Vazquez SE, Liu J, Parent AV, Anderson MS, Kassimatis T, Zekeridou A, Hauser SL, Pittock SJ, Chow E, Wilson MR, DeRisi JL. High-resolution epitope mapping of anti-Hu and anti-Yo autoimmunity by programmable phage display. Brain Commun. 2020 Aug 3;2(2):fcaa059. doi: 10.1093/braincomms/fcaa059. PMID: 32954318; PMCID: PMC7425417.

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