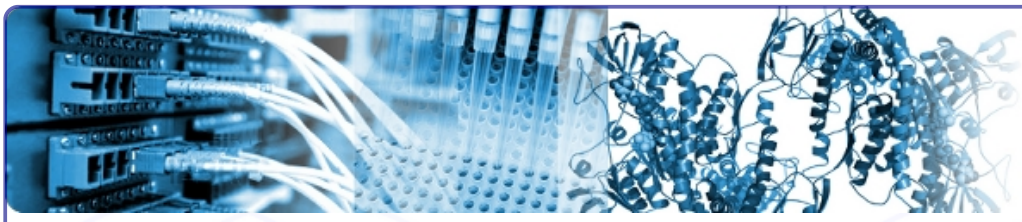

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CODONEWS
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PepID

The smart way of building rational peptide libraries

PepID: application in diagnostics and research

Virally transmitted diseases are a major health hazard, especially once they go epidemic. Whether you look at influenza, noroviruses, West Nile (WNV) - all of them have a significant health and economical impact.

Thus, research tools that will result in diagnostic procedures and potentially also therapies, are in great demand. **Peptide libraries** have been successfully used in biomedical research for decades, mostly in the field of immunology. **Epitope mapping** serves to identify (continuous or discontinuous) protein-derived epitopes that mediate or initiate biochemical or phyiological processes, e.g. **binding of high-affinity antibodies** or stimulation/attenuation of an immune response. **Peptide epitopes** can also be **biological signatures** for certain pathogens. Identification of such epitopes is the basis for diagnostic and, ideally, therapeutic applications, e.g. protective **epitope-based vaccines**.

A recent publication in PLOS ONE is an application example of ATG's biopeptide technology in research on WNV variants.

CHECK OUT OUR DESIGN TOOL AND START DEVELOPING YOUR OWN PEPTIDE LIBRARY!

Finding suitable epitopes becomes harder for closely related pathogens that vary only slightly in their protein sequences. Epitopes undergoing rapid mutation (a prerequisite for immune evasion) and thus leading to variant new strains also make development of vaccines with a broad effeciency spectrum difficult.

In this case, a method for

- a) **discriminating between viruses/strains**, and
- b) **detecting conserved**, yet still immunogenic or diagnostically relevant epitopes that cover different strains,

would benefit research efforts.

Generally, one can either use huge numbers of peptides with random sequences which require little or no background information on the biology of the virus or pathogen and hope to get a lucky punch. Alternatively, one can use a more **rational approach** that also incorporates knowledge about the system one is studying. **PepID** is such a rational-design system for generating and expressing a multitude of potentially biologically relevant peptides.

Rational Design of Peptide Libraries

While random approaches jumble strings of DNA whether they are biologically relevant or not, PepID follows a different path. As microorganismal and viral genomes have become available in public databases, a **rational design approach** is used to partition any protein or protein-coding DNA from a given organisms or virus into fragments of freely definable but uniform length (see fig. 1).

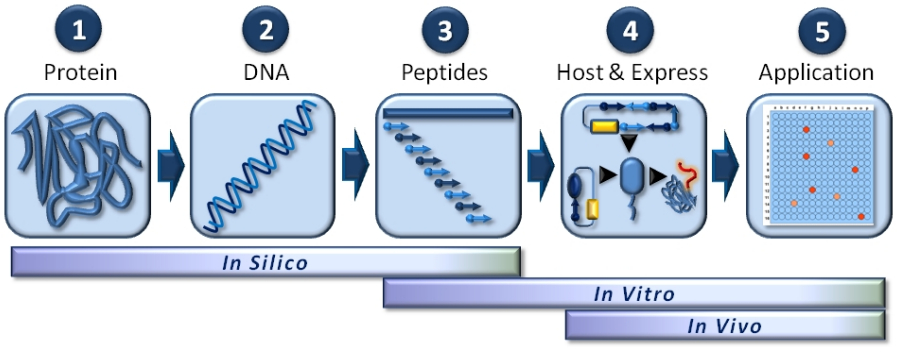

Figure 1: PepID Workflow. (1+2) Electronic backtranslation of protein into DNA, (3) Electronic dissection of proteins CDS into multi-peptide-coding concatemers / library construction, (4) hosting of plasmid-encoded library in host organism, individualization of peptides into tagged single-peptide construct, expression, (5) application, e.g. ELISA, peptide-array, etc.

Potentially diagnostically or therapeutically interesting proteins from pathogens (bacteria, viruses, etc.) are either electronically backtranslated into DNA or their protein-coding DNA sequence extracted from sequence databases and directly used for constructing the peptide-CDS library (fig. 1, step 1 and 2).

Target protein sequences are entered into the PepID submission form and are then electronically backtranslated into DNA sequences. These are then further dissected into peptides of defined length and overlap (fig. 1, step 3).

PepID lets you **adjust the length and overlap of the peptides** you wish to scan thus allowing you to **change the coverage of the protein** as required. In addition, if you have extensive knowledge of your protein, you can simply leave out certain biologically irrelevant amino acid stretches and just focus on the important structures and motifs (hot spots or (hyper)variable regions) which you can zoom in on and cover in more detail by e.g. increasing the overlap and thus the scanning resolution.

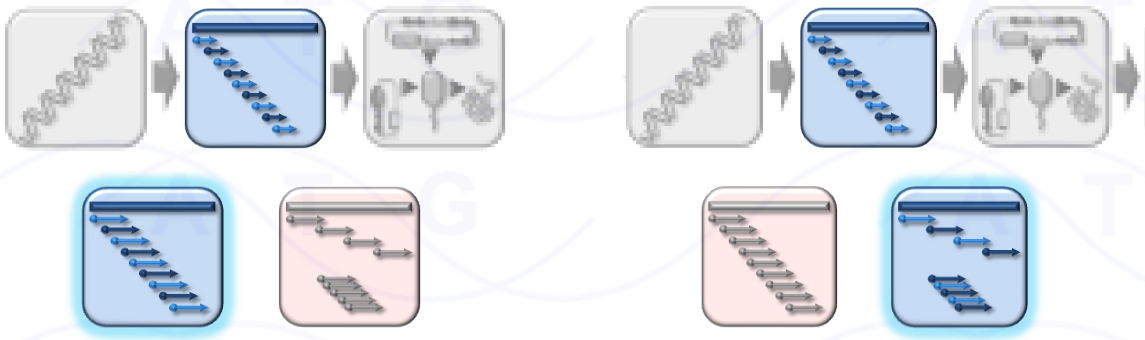

Figure 2: Modifying **peptide length** (left panel) and **overlap** (right panel) when designing peptide libraries with the PepID system.

PepID biopeptide libraries are **codon-optimized to avoid repeats** and to adhere to **codon usage** in the host organism (usually **E.coli**). In addition, the individual peptides are arranged for a **compact design** without excess linkers etc.
This **stringent biologically-oriented design** sets it apart from chemically synthesized random libraries with peptide sequences that are permutated.

In addition, PepID separates hosting and expression into 2 subsystems. It is a ***bio-peptide* system** because the peptides are produced in a biological system, usually a bacterial host organism (but it may

be any other microorganism as well), from a plasmid DNA construct.

Individual peptides are released from their maintenance (host) plasmids by a simple restriction and then cloned into the specifically designed expression vector (fig. 1, step 4). This expression vector can carry any custom-tailored functional elements relevant to your research agenda. The peptides will be expressed as protein-peptide fusions as peptides usually act as antigens in a protein or functional context (MHC, adjuvants, etc).
This is a bulk process and individual clones need to be sequence-verified. The expression constructs are then hosted in the individual bacterial clones and can be regenerated and retrieved from them (fig. 1, step 4).

These fusion proteins can then be used in various formats (in vitro, phage display, etc.; fig. 1, step 5) to probe the peptides with antibodies, immune cells, etc. and assay for any relevant output. Highly reactive/stimulatory peptide epitopes can thus be identified and matched against bioinformatically predicted epitopes. In addition, combinations of functionally diverse epitopes can also be tested.

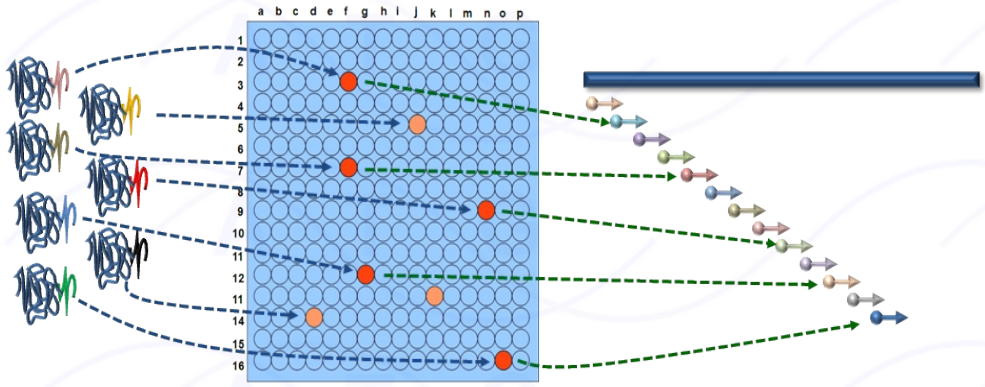

Figure 3: Application of PepID-derived peptides. If fused to a suitable tag and bound to an appropriate surface, the peptides can be used to e.g. screen antibody affinities in an ELISA set-up or similar technologies. Peptides to which antibodies bind strongly, can then be identified and their position in the protein determined.

The technology may also be used to probe differential reactions of patient sera (e.g. infected vs. naive) against certain peptides. This can help identify peptides that can then be used as markers of specific infection, e.g. in a diagnostic tests.

PepID can potentially be used with other technologies to:

- introduce multiple peptide-fusions (color-tagging) into mammalian cells (via MultiLabel plasmids) and monitor distribution, trafficking, interactions, etc.
- work as a modified yeast-2-hybrid assay with protein domains (e.g. as in Boxem et al, 2008)
- perform target identification *in vitro*

Advantages of using PepID:

- more efficient for longer peptides (30 aa and up)
- can potentially reflect small structural epitopes and protein domains
- easily replenished from maintenance / source vectors
- biological system, better ecological fingerprint
- initial rapid screening as prerequisite for chemically synthesized variants, e.g. mimotopes

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