Phage Display

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Phage display is a technique used for the study of protein or peptide interaction with different ligands or other proteins. In phage display a peptide or protein library is fused to a phage coat protein and exposed on the surface of phage particles. Displayed proteins can be screened for binding to target while the gene encoding this protein fusion is contained in the phage genome, guaranteeing the link between genotype and phenotype allowing that interacting proteins in the library can be easily identified by DNA-sequencing.

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Protein fusion is restricted to the surface-exposed coat proteins. Display of peptides or proteins is most often realized by fusion with minor coat protein gIIIp or major coat protein gVIIIp. Different display vector systems are in use: Phage vectors with a unique copy of the coat protein genes in which insertion of a protein sequence or library results in an obligate fusion with all copies of the coat protein. These systems are referred as 3 or 8 systems, depending of which of the coat proteins was used to make the fusion. A second type of phage vectors contains two copies of a coat protein gene in which insertion of a protein sequence or library in one of the copies of the gene results in both wild type coat protein and coat protein bearing the fused proteins. This systems are referred as 33 or 88 systems. Finally, a third kind of system involves two different elements, A phagemid, which is plasmid that contains a packaging signal and a copy of the coat protein fused with a protein sequence or a library, and helper phage, which is normal M13 phage with a defective packaging signal. When a host cell containing phagemid is infected by a helper phage, phage particles containing a mixture of wild type and fused coat proteins are produced. This systems are referred as 3+3 or 8+8 systems.

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Library construction is a key step in phage display. Multiple approaches can be considered in ordered to generate a library of protein variants, but in general, methods can be divided in three main categories. The first category comprise the methods that intend to randomly change the wild type sequence, These methods include the use of physical and chemical mutagens, mutator strains, which are *E.coli* strains with deficient DNA repair pathways and introduces mutations to cloned plasmids, and error-prone PCR. The second category comprises the methods that intend to randomize only a specific region of the protein, these methods are called directed methods and involve the use of synthetic randomized oligonucleotides to generate variants via PCR or direct cloning.

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Finally, in the third category of methods, the main goal is not to generate sequence diversity, but to combine, to take portions of existing sequences and mix them in novel combinations, these are the recombination techniques.

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Once a library of a desired protein is displayed on phage particles, they are subjected to multiple rounds of selection in which the variants with stronger binding to the desired target are selected. This process is called bio-panning. In bio-panning, a biotinylated ligand or target is immobilized on a streptavidin coated matrix, like a microtiter plate. Afterwards, the phage particles displaying the library are added to the plate. Phages that bind to the target will remain attached while non-binders will be removed by subsequent washing steps. Bound phages are eluted and amplified by allowing them to infect new *E.coli* cells. New phages containing selected variants are submitted to subsequent rounds of bio-panning. Enrichment of specific phage typically is in the order of 20 to 1000 fold per cycle. This explains the need for several panning rounds to achieve useful enrichment of binding phages from large libraries. After multiple rounds, strong binder variants can be identified by DNA sequencing.

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Phage display is a powerful technique that allow the parallel screening of thousands of peptides or protein variants for enhanced or new binding capabilities not only at the level of protein ligand interactions but also in the generation of protein-protein, protein-DNA interactions. This robustness and its relatively easy implementation have made phage display a useful technique for basic and applied research, especially in the field of clinical research, in the generation of recombinant antibodies and in the identification of clinically relevant peptides.