Protein arrays

UHN Microarray Centre

Protein Microarray Platform: Abundance Arrays

The protein microarray platform can be divided into two general strategies, one that is used to predict protein abundance and the other to identify protein function[1]. Abundance-based protein microarrays can be used for basic molecular biology research, disease marker identification, toxicological response profiling and pharmaceutical target screening.

Antibody Arrays

Capture microarrays, such as antibody microarrays, are an example of the abundance-based strategy. These arrays, on which antibodies are spotted, can be used to examine the expression of multiple proteins in complex solutions by binding with specific proteins. In terms of assessing protein-protein interactions, antibody microarray experiments can qualitatively be compared with the yeast two-hybrid assay.

Protein profiling entails measurement of binding specificity, affinity or protein abundance in a sample. The results of high-throughput methods require subsequent validation, and, much like the way quantitative PCR validates gene expression results obtained from DNA microarrays, western blot analysis is used to validate antibody microarray data[1].

Using antibody arrays

Proteins can be detected by direct labelling or sandwich assays. Direct labelling involves adding a fluorescent tag to all analytes. The advantage of this method is that multiple samples can be assayed simultaneously; for example, two proteins could be compared by labelling each with a different dye[1]. The disadvantage of this method is that samples are chemically modified by the addition of the fluorescent marker and this could produce cross-reactive analytes that may lead to false readings and could result in higher background. Sandwich assays involve the use of two antibodies, each recognizing different epitopes of the same protein. The advantage of this method is that specificity is increased and, since it is not necessary to label the sample, the likelihood of cross-reactivity decreases. The disadvantage of this method is that two antibodies are required for detection of each protein and it is more difficult to multiplex.

Antibody microarrays can be used to monitor protein abundance in cancer cells following radiation treatment or used to identify potential biomarkers. Mathur *et al*. used an antibody array to identify DNA repair proteins that were upregulated by ischemic preconditioning in a myocardial infarction model[4].

Antibody Array Production

Some of the challenges in the development of antibody microarrays include the availability of high-affinity/high-specificity antibodies, high-throughput antibody production and purification, inter- and intra-slide variability of protein concentration, and the stability of the arrayed antibodies.

Another consideration is the type of slide used for printing. Olle and colleagues report that epoxy-silane-modified slides are an effective substrate for the application of antibodies[3]. These slides can be used in conjunction with spotting solutions containing glycerol which has been found to normalise spot size, decrease intra-spot variability, reduce drying effects, and increase antibody-spotting density[3]. However, other slide types, like nitrocellulose, allow for use of many buffer types without pH modification or need for tertiary amine-free buffers[3].

Peptide Arrays

To avoid the complexities involved with antibody array production, capture microarrays can also be created by immobilising protein or protein fragments on the slide. The benefits of peptide microarrays include automated synthesis and purification, less expensive than recombinant proteins, can be synthesized with non-natural functionalities (ideal for adding linkers to immobilise peptides in a certain orientation) and more resistant than proteins to harsh conditions which may improve post-printing stability[5].

Andresen and colleagues have shown that peptide arrays, comprised of biologically active small synthetic peptides in a high-density format, are another approach for molecular immune diagnostics[5]. The described manufacture of peptide arrays involves site-specific solution-phase coupling of biotinylated synthetic peptides onto activated glass slides[5]. Antibodies in the sample are then captured by the surface immobilised peptides and detected by fluorescently labelled secondary antibodies[5].

Peptide ligands can be modelled for the binding sites of many proteins. Espejo and colleagues purified and arrayed more than 200 protein fragments and were able to demonstrate distinct and reproducible binding to subsets of protein domains that would allow domain-binding profiles to be made for cellular proteins[6].

References:

1. LaBaer, J. & Ramachandran, N. Protein microarrays as tools for functional proteomics. Current Opinion in Chemical Biology (2005) Vol.9;14-19.
2. Bertone, P. & Snyder, M. Advances in functional protein microarray technology. FEBS Journal (2005) Vol.272;5400-5411.
3. Olle, E.W. *et al*. Comparison of antibody array substrates and the use of glycerol to normalize spot morphology. Experimental and Molecular Pathology (2005) Vol.79;206-209.
4. Mathur, P. *et al*. Antibody-array technique reveals overexpression of important DNA-repair proteins during cardiac ischemic preconditioning. Journal of Molecular and Cellular Cardiology (2005) Vol.38:99-102.
5. Andresen, H. et al. Functional peptide microarrays for specific and sensitive antibody diagnostics. Proteomics (2006) Vol.6:1376-1384.
6. Espejo, A. *et al*. A protein-domain microarray identifies novel protein-protein interactions. Biochem. J. (2002) Vol.367:697-702.