Overview of Microarray Types

DNA Microarrays

DNA microarrays (also called Gene Chips) are devices not much larger than postage stamps. They are based printed on a glass substrate containing as many as 400,000 tiny cells each containing a microscopic spot of DNA. Each microscopic spot holds a short, synthetic, single-stranded DNA sequence from a different human gene. This makes it possible to carry out a very large number of genetic tests on a sample at one time. The devices are used in pharmaceutical laboratories to investigate what genes are involved in various normal and disease processes.

DNA microarrays are created using high-speed robotics. The substrate is usually glass but sometimes nylon is used. Though the terms have been used interchangeably by the scientific community, the convention is that the DNA of known identity, on the surface of a glass chip are called “probes” and the cDNA or cRNA sample tagged with fluorescent dyes are called “targets”. The targets are hybridized to the “probes” and are used to detect the abundance of mRNA. This abundance of mRNA is directly proportional to the signal intensity of each spot on the array. The base-pairing of A-T and G-C for DNA; A-U and G-C for RNA is the underlining principle of the DNA microarray.

There are two types of DNA microarrays.

**Expression Microarrays** which analyze the expression levels of many genes simultaneously.

**Diagnostic Microarrays** have the capability of examining many different genes from a single small tissue sample to determine which are normal and which may contain mutations.

The Environmental Microarray simulation is an Expression Microarray.
Protein Microarrays can be based on any ligand-binding assay that relies on the formation of product with an immobilized capture molecule and a target molecule present in the solution. These assays can be miniturized and placed on a protein microarray chip.

Protein microarrays are now becoming very popular due to their possible future applications in the study of nucleic acid-protein, protein-protein, ligand-receptor, drug-protein target, and enzyme-substrate interactions.

Types of Capture Molecules for Protein Microarray Chips. To analyze protein interactions with other molecules, protein microarrays can have various types of molecules immobilized on the slide surface to act as capture molecules in the protein microarray assay.

Figure 1 illustrates the most common protein array, the antibody microarray. Antibodies are spotted onto a glass slide. Two cell lysates with proteins labeled with Cy3 and Cy5 (for example, cancer vs normal) are added as a solution onto the protein array. Signals are detected from the two different signals. Data is computed allowing an analysis of how much dye-labeled protein the antibodies on each spot are binding. This information allows one to determine whether the protein expression is up-regulated in cancer, down-regulated or unchanged.

Figure 2. a) Demonstrates protein arrays which are based on microarray analysis of antigen-antibody interactions. Antigens are spotted onto glass slides. Antibodies which are tagged bind to antigens and emit fluorescent signal (shown as the yellow star) which can then be detected from the spot on the array.

b) Shows a protein microarray composed of spots which act as sandwich immunoassays. Antibodies are spotted onto the chips and cell lysate or a solution is applied to the slide. Antibodies are incubated with the solution, with later washing to remove non-specific binding. The sandwich complexes formed emit a detectable signal from the spot.

c) A protein-protein interaction protein microarray where proteins are spotted onto a protein chip, and labeled proteins are added to the chip and incubated to determine where they may bind and interact. A detectable signal is emitted from binding spots.

d) Nucleic acid-protein interaction array, (here a DNA-protein interaction chip). Nucleic acids such as DNA are spotted onto a glass slide. This could be transcriptional binding sites for example. Proteins such as fluorescently labeled DNA-binding transcription factors in solution are added to the chip and incubated on the array. A detectable signal is emitted from the DNA spots where the proteins bind.

e) If you have a set of ligands and you want to find the receptors the bind to, you can use a receptor-ligand protein chip as displayed. Receptor proteins are spotted on glass slides. Fluorescently labeled ligands are added to the chip. Binding of ligand to receptors causes the emission of a detectable signal which pinpoints the interacting spots (i.e. which protein did the ligand bind to).

f) To test for enzyme-substrates specificity one can employ an enzyme-substrate protein microarray. This chip is composed of discrete nano-wells in which enzymes are bound. Substrate is added into the nano-wells and signal emission is detected from the nano-wells in order to assay for enzyme function.
Microarray versus ELISA

ELISA is short for "Enzyme Linked Immunosorbent Assay". Let’s dissect that name to see what it means and how it relates to microarrays.

We will do this from back to front.

An ASSAY is a procedure in molecular biology for testing and/or measuring the activity of a drug or biochemical in an organism or organic sample.

Immunosorbent is the binding of antigen or antibody to a solid support such as the plastic surface at the bottom of a well of a 96 well plate or to the glass surface on a glass slide. Enzyme Linked refers to an enzyme attached to an antibody that then attaches to the antigen being assayed for. This enzyme is used to react with a colorless indicator added to the well to create a colored solution which can then be observed quantitatively.

The procedure for an ELISA test in a 96 well plate calls for 1) the attachment of an antibody to the plastic surface of the well. 2) An antigen is then added that may or may not attach to the antibody. 3) A second antibody is added that has an enzyme attached to it. If the antigen was captured by the first antibody attached to the plastic, 4) the second antibody will attach to the antigen. 5) The enzyme will then cause the colorless indicator solution to become colored.
Sandwich ELISA Microarray

The sandwich ELISA microarray is a powerful screening tool in biomarker discovery and validation because of its ability to simultaneously probe for multiple proteins in a miniaturized assay.

505 Antibodies on one slide

Fig. 6: Detection of multiple cytokines utilizing a sandwich assay and fluorescence detection. Monoclonal antibodies to cytokines were printed in triplicate in Whatman Arraying Buffer with a non-contact printer (triplicates from upper left to bottom right: Donkey anti-goat positive control, GM-CSF, IL-10, IL-1β, IL-12p70, IL-2, MCP-1, IL-4, RANTES, IL-6, Donkey anti-goat positive control).
The Citrus, Virus and Water Microarray simulations are Sandwich ELISA Microarrays.

Our first-generation microarray, the Ab Microarray 380 (Cat. No. 631785), consists of 378 distinct, well-characterized monoclonal antibodies. On this array, each antibody is printed in duplicate—the side-by-side spots provide an added measure of confidence to your results (Figure 2). The Ab Microarray 380 detects a wide variety of intracellular proteins—both cytosolic and membrane-bound—representing a broad range of functional classes (Figure 3). The targets include proteins involved in signal transduction, cell-cycle regulation, gene transcription, apoptosis, and oncogenesis. Though the antibodies were raised against human proteins, many of the monoclonals recognize homologous proteins in mice and rats.

The procedure for using a sandwich ELISA microarray is similar to the ELISA test except that you can screen large numbers of subjects at once. 1) The attachment of an antibody to the glass surface on each of the spot of the microarray. 2) An antigen is then added that may or may not attach to the antibody. 3) A second antibody is added that has a fluorescent marker attached. This fluorescent marker does nothing in the dark or in normal light. 4) The microarray is then illuminated with ultraviolet (UV) light. Each spot on the microarray that has the antibody with the fluorescent marker will

Antibody Microarray

An antibody microarray is a specific form of protein microarrays, a collection of capture antibodies are spotted and fixed on a solid surface, such as glass, plastic and silicon chip for the purpose of detecting antigens. Antibody microarray is often used for detecting protein expressions from cell lysates in general research and special biomarkers from serum or urine for diagnostic applications.

Background
The theoretical background for protein microarray-based ligand binding assays was initially developed by Ekins et al. in the late 1980s.[3] According to the model, antibody microarrays not only would permit simultaneous screening of an analyte panel, but would also be more sensitive and rapid than conventional screening methods. Interest in screening large protein sets only arose as a result of the achievements in genomics by DNA microarrays and the Human Genome Project.

The first array approaches attempted to miniaturize biochemical and immunobiological assays usually performed in 96-well microtiter plates. 96-well antibody arrays were first created with 144 elements each for "standard enzyme-linked immunosorbent assays" (ELISA). Similar arrays were used to measure prostate-specific antigen (PSA) and cytokines.

Filter membranes were also initially used because of their superior protein binding capacity. They were mostly probed with antibodies using ELISA techniques. A low density array of 48 purified proteins involved in transcription was developed for the investigation of specific interactions of proteins with radiolabeled DNA, RNA, ligands, and other small chemicals. A membrane-based high density array was developed for the purpose of screening a human fetal brain cDNA expression library consisting of 37830 clones. Purified proteins were spotted onto PVDF membranes at a density of 300 samples/cm2. Other filter based arrays were constructed but the limitations were the low resolution and considerable background making it difficult to use them in applications with limiting sample quantities such as protein expression profiling of tumor biopsies.

Protein arrays are comprised of a library of proteins or antibodies immobilized in a 2D addressable grid on a chip. Protein microarray biochips extract and retain targets from liquid media are distinct from microfluidic biochips, which separate and process proteins in a transport medium in situ using microfluidic devices. A typical array may contain 103–104 spatially distinct elements within a total area of 1 cm².
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Figure 2. Format and layout of Ab Microarrays. The layout shown is that for the Ab Microarray 380. Yellow spots correspond to Cy3/Cy5-labeled RSA spots, which serve as orientation markers.


505 Antibodies on one slide

Fig. 6: Detection of multiple cytokines utilizing a sandwich assay and fluorescence detection. Monoclonal antibodies to cytokines were printed in triplicate in Whatman Arraying Buffer with a non-contact printer (triplicates from upper left to bottom right: Donkey anti-goat positive control, GM-CSF, IL-10, IL-1β, IL-12p70, IL-2, MCP-1, IL-4, RANTES, IL-6, Donkey anti-goat positive control).


Fig. 3: Protein microarrays can be used for many different applications. (A) Antibody arrays can be used in sandwich ELISA-like assays or (B) to capture specific antigens which are directly labelled with a hapten. (C) Purified or recombinant proteins can be arrayed to study protein-protein interaction or (D) to probe serum samples for antibodies (e.g. to study allergy or autoimmune diseases). (E) Reverse-phase arrays are used to profile dozens or hundreds of arrayed samples (e.g. cell lysates) for the presence of a small number of antigens.

Virus Microarray = Protein (Antigen) Microarray

Environmental Microarray = DNA Microarray

Citrus Microarray = Antibody Microarray
Water Microarray = Antibody Microarray