DNA Microarrays: A Powerful Tool for Pathogen Detection

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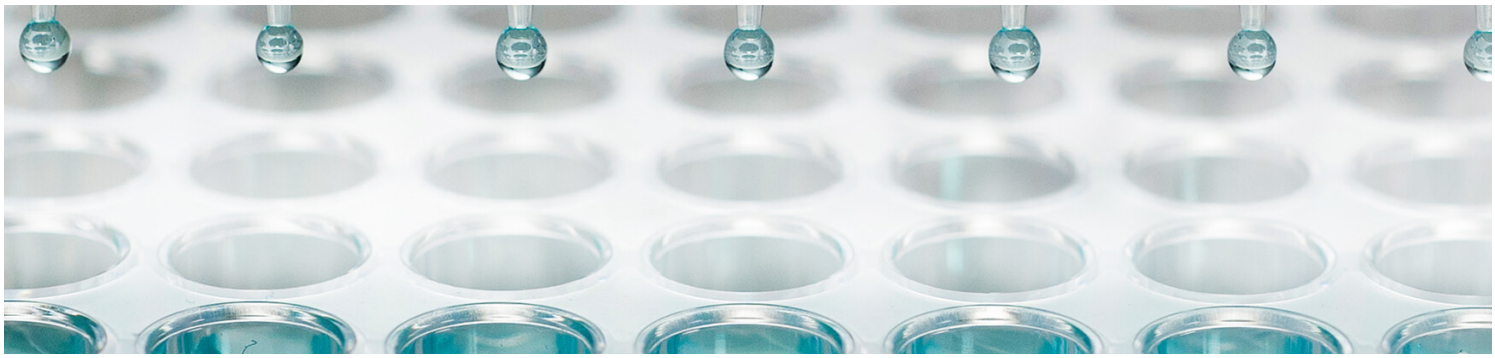
PathogenDx
Setting the standard in DNA testing



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- DNA
- Microarrays

INTRODUCTION

DNA microarray technology is a high-throughput diagnostic tool for the detection and identification of pathogenic organisms. The DNA microarray platforms are extremely flexible, highly sensitive and very specific. Thus, they have been referred to as "labs on a chip" due to the incredible number of unique target DNA sequences that may be probed for simultaneously.
The development of DNA microarrays is divided into two sections:

1. **In-silico Design** – The in-silico microarray work includes the process of primer and probe designing using bioinformatic analysis of an organism's genomic DNA or RNA.

2. **In-vitro Testing** – The wet laboratory work includes slide cleaning and preparation, probe spotting, labeling of the DNA, PCR amplification, and hybridization of the labeled PCR products to the DNA microarray.

DNA microarrays contain a defined pattern of single stranded oligonucleotide sequences (probes) arranged in a grid and bound to a planar surface such as glass microscope slides. These probe sequences, similar to PCR primers and qPCR probes, are complementary to the target sequence being analyzed.
The utilization of DNA microarrays as a diagnostic tool requires the lysis and extraction of pathogenic genomic DNA or RNA, amplification and labeling of the PCR product that is specific for your organisms being tested, and hybridization of the PCR amplified product to the species-specific probes on the microarray surface. Through this brief primer on DNA microarrays we will discuss each of these steps and how they contribute to the design, sensitivity, and specificity achievable using this versatile technology.

DNA MICROARRAY DESIGN AND ASSAY PROCEDURE

DNA microarray are constructed by designing single-stranded (ssDNA) probes that are immobilized on a solid surface using an automated spotting machines. DNA probes are immobilized through chemical modifications of the ssDNA by different chemical groups such as -NH_2. The solid surface in many cases is a glass microscope slide but, the surface of a slide may be treated with different types of chemicals to improve the stability of the covalent bonds between the ssDNA probe and the surface. The chemically modified probes and the chemically coated slide surface ensure the stability of the probe to the surface. Each chemical group in a modified probe needs its specific chemical substrate on the treated slides. For example, the -NH_2 groups in a modified probe binds best with epoxy silane covered slide, creating a powerful bond.

After the microarrays have been designed and validated for probe performance, they are ready to be utilized as a diagnostic tool for the identification of pathogen organisms. A vast array of sample matrices can be processed and utilized for diagnostic purposes on a DNA microarray. No matter what sample matrix your laboratory is concerned with the same general process needs to be performed to extract DNA from the pathogenic organisms. The scientist performing the assay will take the sample of interest and perform a series of cell lysis steps according to the manufacturers recommendations in order to extract DNA from the pathogens. The extracted DNA samples will be used as the starting template for subsequent PCR reactions.

The PCR reactions are used in two ways:

1. to amplify the specific genes of interest so that they are detectable and
2. to label the DNA with fluorescent proteins that are used for detection

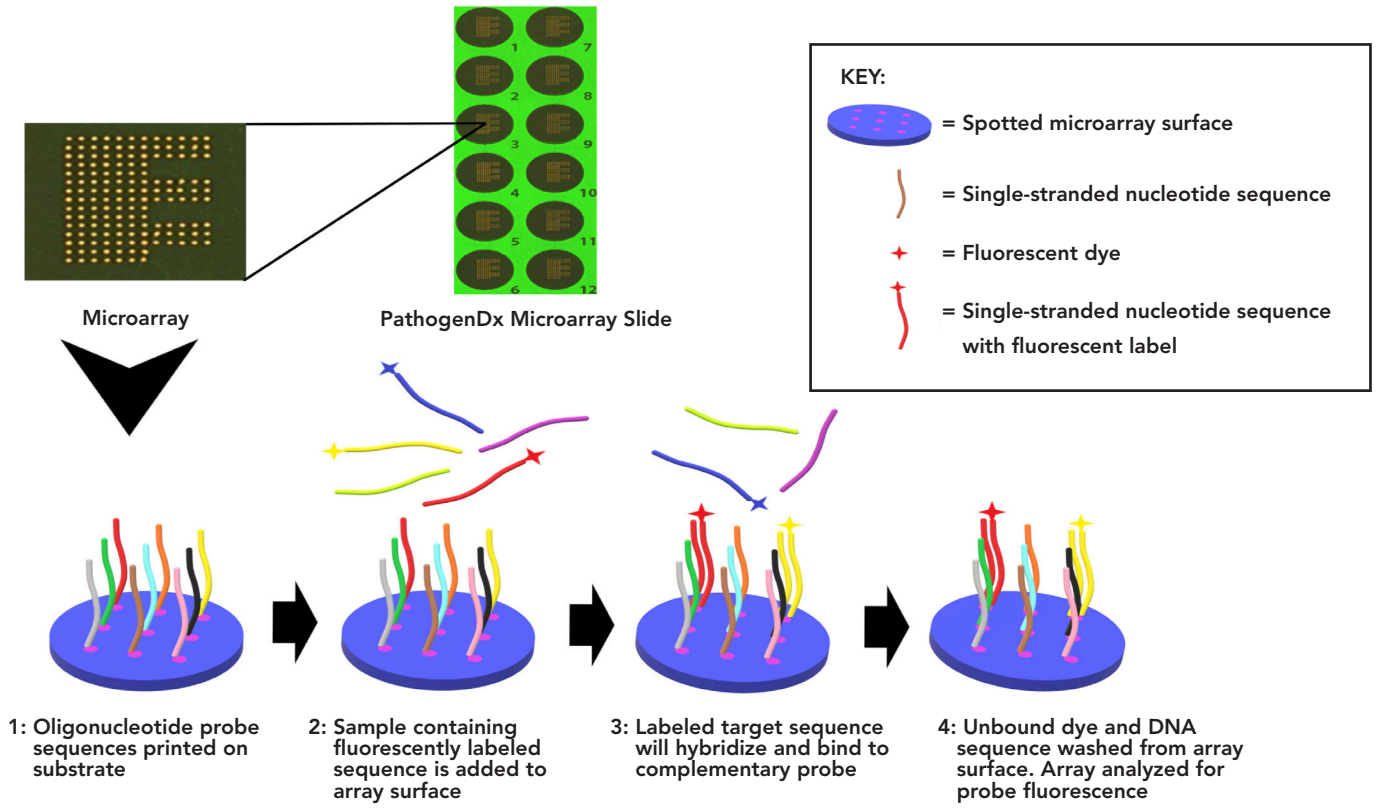
Once the ssDNA is amplified and labeled, they are ready for hybridization to the DNA microarray. For sample analysis, the mixture containing any number of fluorescently labeled target single strand DNA sequences from a prepared sample may be placed on the microarray. Under high stringency conditions, the fluorescently labeled target sequences then bind simultaneously to the target's complementary probe strands on the microarray in a process known as hybridization. Unbound dye and sequence are then washed off of the array. The presence or absence of fluorescent signal on a probe position is determined during microarray analysis. This probe position fluorescence corresponds to the presence or absence of the target sequence, and thus organism or species in the analyzed sample.

BENEFITS OF DNA MICROARRAYS

DNA microarray offers several unique advantages when applied to the field of microbial detection and identification. This includes:

1. **Multiplexed Array Layout** – the dynamic nature of DNA microarrays allows for a highly flexible platform for the inclusion of additional organisms, ultimately broadening the diagnostic sensitivity and specificity.
2. **Probe Design** – probe design and study of highly conserved genomic sequence allows a great degree of control in organism detection. Family, genus, species, and genotype level detections and identifications are all possible with stringent levels of specificity. This makes it possible to simultaneously identify possible pathogenic species of organisms, and whether the tested sample contains a known pathogenic genotype.
3. **Sensitivity and Specificity** - DNA based technology relies on the positive identification of unique DNA sequence, as opposed to qualitative observation of the organism or its metabolism as in culture-based methods. This also avoids the false positives encountered in immunological based assays due to possible cross-reactivity among proteins and antibodies. The use of PCR also allows for a high degree of pathogen detection sensitivity: 1 CFU/g for the PathogenDx Detect^{X} assay. DNA microarray is also more rapid in processing time as compared to other methods in microbiology.
4. **Low Cost** – in comparison to traditional plate-based diagnostics as well a most qPCR platforms the overall cost from sample collection to identification is vastly lower.

This has many applications in the laboratory, not limited to transcription factor and gene expression research, genotyping, and microbial detection/identification.



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