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DNA Microarray: Applications and Limitations

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Description

A DNA microarray (also known as a DNA chip or a biochip) is a solid-surface collection of tiny DNA patches. DNA microarrays are used by scientists to concurrently evaluate the expression levels of a multiple genes or genotype multiple regions of a genome. Each DNA patch comprises probes, which are picomoles (10-12 moles) of a specific DNA sequence (or reporters or oligos). A fragment of a gene or other DNA element can be utilised to hybridise a cDNA or cRNA (also known as anti-sense RNA) sample (called target) under high-stringency conditions.

Applications of Microarrays

Gene expression analysis

The most common use of DNA microarrays is to determine gene expression levels. RNA is collected from the cells of interest and either labelled directly, converted to labelled cDNA, or converted to a T7 RNA promoter tailed cDNA, which is then turned to cRNA by the Eberwine amplification process. Incorporation of fluorescently labelled nucleotides during synthesis, incorporation of biotin labelled nucleotide that is then stained with fluorescently labelled streptavidin, incorporation of a modified reactive nucleotide to which a fluorescent tag is added later, and a variety of signal amplification methods have all been developed for labelling of cDNA or cRNA. The integration of fluorescently labelled nucleotides in the reaction and the usage of fluorescently labelled nucleotides in the reaction are the two most commonly utilized approaches.

Transcription factor binding analysis

Microarrays have also been used in conjunction with chromatin immune precipitation to identify transcription factor binding locations. In a nutshell, Transcription Factors (TFs) are cross linked to DNA using formaldehyde, resulting in DNA fragmentation. The TFs of interest (together with the DNA to which they were bound) are affinity purified using either a TF antibody or a peptide that can be tagged with an affinity chromatography-friendly peptide. The DNA is extracted from the TF, amplified, tagged, and hybridized to the array after purification. This method is known as "ChIP-chip," which stands for Chromatin Immuno-Precipitation on a "chip" or microarray.

Genotyping

Microarrays have long been used to genotype Single-Nucleotide Polymorphisms (SNPs). Allele discrimination by hybridization, as used by Affymetrix, allele specific extension and ligation to a "bar-code" oligo which is hybridised to a universal array, or approaches in which the arrayed DNA is extended across the SNP in a single nucleotide extension reaction are the most commonly used approaches to detect SNPs. Due to non-specific hybridization in complex genomes, allelic discrimination via hybridization suffers from background. Affymetrix developed a PCR-based technique to minimise genomic complexity in order to reduce this background.

Limitations of DNA Microarrays

Microarrays are essential devices that measure the relative concentrations of several distinct DNA or RNA sequences at the same time. While they have shown to be quite effective in a wide range of applications, they do have some drawbacks. For starters, arrays can be used to calculate relative concentrations in an indirect manner. For example, the signal recorded at a particular spot on a microarray is usually considered to be proportional to the concentration of a single species in solution that can hybridise to that spot. The signal intensity at a given point on the array, however, is not directly proportional to the concentration of the species hybridizing to the array due to the kinetics of hybridization. The array will become saturated at high concentrations, while equilibrium prefers low values.