Biology | Molecular Biology

DNA Microarray

General Aim

Gene expression profiling.

Method

Microarray hybridization: whole human genome (4*44) oligo microarray and dual color labelling kits.

Learning Objectives (ILOs)

- Perform the steps for RNA amplification.
- Practice the laboratory protocols to synthesize cDNA, cRNA and fragment cRNA prior to hybridization.
- Practice the technique for dual labelling of samples for microarray hybridization.
- Apply the skills and precautions required for microarray slide handling and sample application.
- Identify the benefits of positive displacement pipette.
- Practice the process of microarray slide wash prior to detection.

Theoretical Background/Context

The identification of differential gene expression between two samples can be easily and rapidly done by DNA microarrays. This requires high quality RNA samples, proper labeling and hybridization.

A microarray is a technique used to detect the expression of thousands of genes, at the same time, on microarray slides. These small slides are originally printed with thousands of spots, each with a known DNA sequence. These sequences are probes that help detect gene expression upon hybridization with samples.

During sample preparation, two fluorescent dyes are added, cy3 (green fluorescence) to the 'to be studied group' and cy5 (red fluorescence) to the 'reference group'. Thus; samples that contain DNA sequences complementary to array slide probes hybridize and fluoresce when scanned.

Good quality arrays should produce high signal at relatively low PMT values. Massive or widely spread differential gene expression is probably non significant. Thus; a picture of the array should look more yellow, rather than green or red. Good quality signals should also be in a dynamic range, where the signal histograms fully overlap.

Quality control of RNA samples applied to microarrays is important and can be detected using bioanalyzer 2100. It is worth mentioning that good quality total RNA samples should produce two major peaks corresponding to the 2 major ribosomal RNA species. Severely degraded, low quality RNA will show a broad peak or a series of peaks at low retention times, while the 2 ribosomal RNA peaks will be of very low intensity or not identifiable at all. An RNA Integrity Number (RIN) higher than 9 is preferably used.

Other application of DNA microarray:

1. Transcriptomes and proteomes.
2. Gene chips to diagnose several pathogenic and genetic diseases in man.
3. Species specific probes
4. Single nucleotide polymorphism (SNP) analysis.

Principle of Work

In this protocol, Two RNA samples were prepared: cancerous & healthy (reference).

A- Amplification and labelling of extracted RNA:

1)Synthesizing cDNA:

- Using T7 RNA polymerase by reverse transcription.

2)Synthesizing cRNA:

- cDNA is used in an in vitro transcription reaction to generate cRNA. This reaction is performed in the presence of labeled ribonucleotides, producing microgram quantities of labeled RNA for array hybridization.

B- Microarray hybridization:

1)The generation of fluorescently labeled RNA using Agilent's Quick Amp labeling kit.

- A hybridization sample is prepared and added to the cRNA prior to array hybridization. During this stage, cRNA is fragmented and labelled. cyanine 3 (cy3-fluoresces green) is added to the 'to-be studied' cells and cyanine 5 (cy5-fluoresces red) is added to the reference cells.

2)Sample upload into the array slide with positive displacement pipette. The slide is then put in the hybridization station.

C- Microarray slide wash prior to scan.

- The scanner has a laser which causes the hybrid bonds to fluoresce.