

DNA array techniques in gene-expression profiling

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1 DNA arrays

DNA arrays are used for collecting gene-expression data. With DNA array you can measure the amount of gene products, mRNA molecules in cell. So the genes expression level is the measured amount of mRNA in the cell.

DNA array is orderly arrangement of tens to hundreds of thousands of DNA molecules that are called probes. These probes are unique complementary sequences for the measured mRNA or cDNA (cDNA = complementary DNA sequence made from mRNA through reversal transcription). Probes length varies from 25 nucleotides to the length of their complementary genes ORFs (open reading frame). In the DNA array experiment gene sequences under interest hybridize to their complementary target probes. Probes are located to unambiguous addresses and each probe uniquely matches to one gene, so after the experiment each genes expression levels can be measured.

1.1 In situ synthesized arrays

In in situ (in situ = in the natural or original position) methods the probes are synthesized directly to the array, nucleotide by nucleotide. The probes are oligonucleotides which are about 25 bs (basepairs) long. The size is limited because it's technically challenging and expensive to manufacture longer probes. Careful planning is needed cause of the short probe size; otherwise there is danger that the probes will hybridize to several genes.

The most common in situ array is utilizing photolithographically synthesized probes. The photolithographic method is commercialized by Affymetrix (See picture 1).

There are also other in situ methods that won't require photolithography. One method is standard piezoelectric (ink-jet) printing process that uses standard phosphoramidite chemistry for probe synthesization. The ink-jet printing technology is capable of depositing very small volumes of DNA solution very rapidly and very accurately so there's no need for masks.

Another maskless technology (MAS) is using tiny mirrors arranged on the surface of the computer chip for reflecting light to the specific addresses on the array.

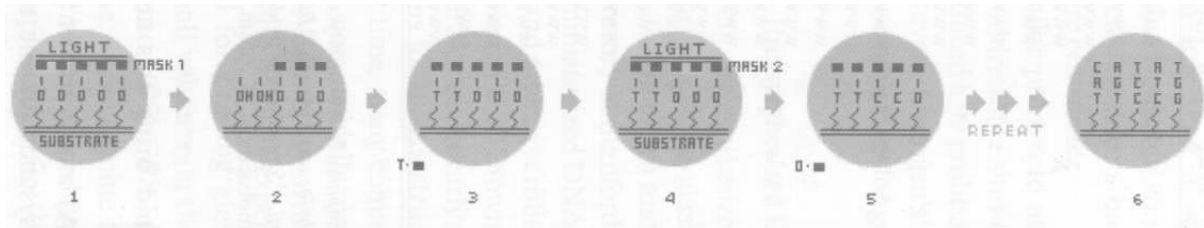


Figure 1: [Bal02] The Affymetrix method for the manufacture in situ synthesized DNA array: 1. A photo-protected glass substrate is selectively illuminated by light passing through a photolithographic mask. 2. Deprotected arrays are activated. 3. The surface is flooded with a nucleoside solution and chemical coupling occurs at photo-activated positions. 4. A new photolithographic mask pattern is applied. 5. The coupling step is repeated. 6. This process is repeated until desired set of probes is obtained.

Electrical addressing systems based on semiconductor technology is also utilized in preparing probes on in situ arrays. Every addressable site on the chip is attached to an electrical conduit. Each DNA probe is synthesized one base at time by flooding nucleosides and activating each electrode where a new base is to be added. Nucleosides combine with each other through electrochemical reaction.

1.2 pre-synthesized DNA arrays

These arrays differ from the synthesized oligonucleotide arrays by the probe arraying mechanism. In pre-synthesized arrays you select and amplify the probes first and then you spot them to the array which is coated with poly-lysine or -amine. Also in pre-synthesized arrays you need two kinds of samples, the control and the case. In Affymetrix arrays the exact amount of probes is known, but in pre-synthesized arrays the amount of probes per spot is unclear. So the control sample reflects the amount in which the case sample is proportioned.

1.3 Filter-based DNA arrays

Besides glass arrays nylon filter-based arrays are also popular format in DNA arraying technology. Cheap and common methods make this format easy to use for molecular biologists. Fluorescent labels are also used in nylon filter-based arrays, but typically radioactive labeling offers greater sensitivity in these kinds of arrays. Nylon filter-based arrays are not amenable to miniaturization cause of their porous nature. Nevertheless, they can be utilized in gene expression profiling studies restricted to organisms with small genome sizes, or in studies which concentrate certain functional subset of an more complex organism's genes.

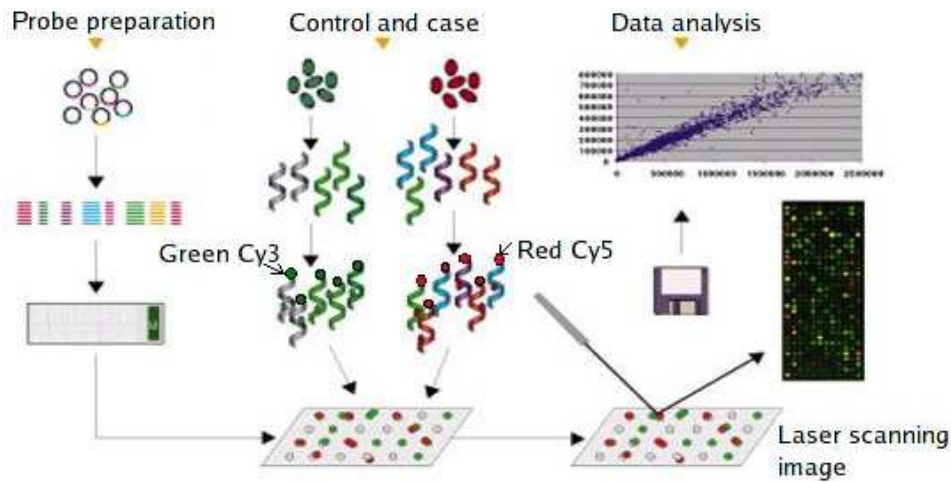


Figure 2: [Dob] Pre-synthesized DNA array.

2 DNA array readout methods

After the DNA array experiment is done, the data must be extracted and analyzed. For each gene the expression level is measured. There are several methods to extract and analyze the data cause there are several kinds of array surfaces, hybridization methods and labeling techniques.

2.1 Reading data from a fluorescent signal

One widely used sample labeling method is fluorescent labeling. Most commonly used labeling dyes are cyanine dyes, Cy3 and Cy5 for the control and the case samples (see picture 2). Fluorescent dyes are excited with light source and the emitted light is collected with some kind of detector. One commonly used method for reading fluorescent signals is Confocal Scanning Laser Microscopy. The method is described more precisely in the following picture 3.

2.2 Reading data from a radioactive signal

Radioactive labeling is older but more sensitive method than fluorescent labeling technique. Radioactive labeling is not suitable for high-density DNA arrays cause the radioactive signals coming from neighboring spots can contaminate each other. Method also includes health risks.

Samples are labeled with beta-emitting radioisotopes. Radioactive emission is extracted from DNA arrays with phosphorimage scanning instruments which are utilizing phenomenon called photostimulated luminescence (PSL).

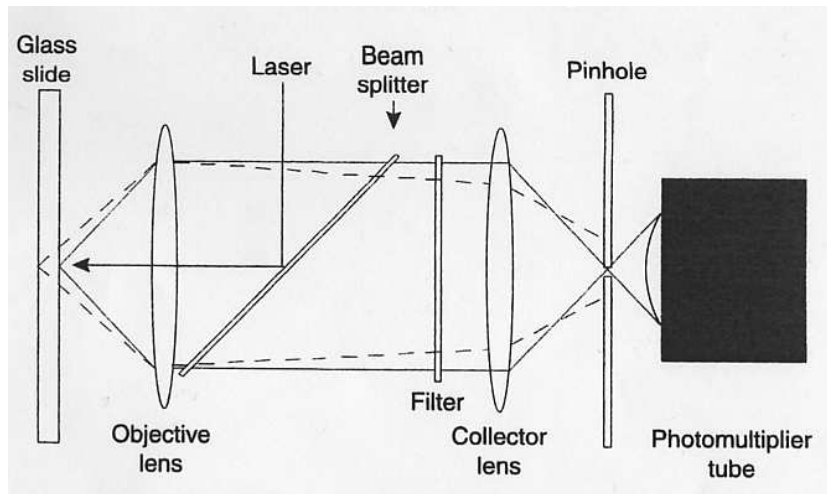


Figure 3: [Bal02] Laser beam is reflected by the beam splitter and focused to the spot on the surface of the glass slide by an objective lens. Fluorescent emissions are collected by the objective and the objective directs rays (also reflected laser rays) towards the collector lens. The laser rays are reflected away by the beam splitter and remaining rays excluded by the filter. The fluorescent rays are collected by the collector lens and directed through the pinhole to the detector (Photomultiplier tube). The pinhole permits only the light coming from a exact point on the surface of the glass.

3 Limitations

Microarray technologies are improving all the time and with these methods one can efficiently collect gene expression data. Still there are several possibilities to go fail in the process. Difficulties and mistakes can occur in any phase from designing the experiments to the data analysis. It's often necessary to repeat the experiments cause of these uncertainties. Also researchers might have to use less accurate but cheaper methods cause of the financial reasons.

References

- [Bal02] Baldi, P. Hatfield, G. W., *DNA microarrays and Gene Expression*. Cambridge, 2002.
- [Dob] Dobbins, D. J. J., *Preparation of DNA Microarray Biochips for the Classroom*. Bellarmine University. URL <http://www.kbrin.louisville.edu/education-tools/fellows/dobbins.html>.