Advance laboratory techniques

Lecture 2

DNA microarrays

The large-scale genome sequencing effort and the ability to immobilize thousands of DNA fragments on a surface, such as coated glass slide or membrane, have led to the development of DNA microarray technology. An entire microbial genome can be easily represented in a single array, making it feasible to perform genome-wide analysis.

Simply defined, a microarray is a collection of microscopic features (most commonly DNA) which can be probed with target molecules to produce either quantitative (gene expression) or qualitative (diagnostic) data.

There are two major types of DNA microarrays; one is the oligonucleotide-based array and the other is the PCR product-based array. A DNA microarray experiment consists of array fabrication, probe preparation, hybridization and data analysis. DNA microarrays have been developed to perform a large number of different hybridization experiments simultaneously on a single membrane or glass substrate. They are well-suited to comprehensively investigate and quantitatively compare the expression levels of a large number of genes, but they can also be easily used in qualitative studies to detect selected DNA sequences.

With the availability of complete genome sequences of many microorganisms, the DNA microarray technology has become a very powerful tool to explore global gene expression profiles and to measure genome-wide differences in genetic contents.

Major types of DNA microarrays

1- PCR PRODUCT-BASED DNA MICROARRAYS

Steps of PCR product-based DNA microarray.

- **Primer design**

The first step of DNA microarray construction for microbes with known genome sequences is the design of primers to amplify specific regions of interest.

- **PCR amplification**

The goal of the whole genome PCR amplification is to achieve the highest success rate and yield in a high-throughput manner.

- **PCR product purification**

To remove unincorporated nucleotides and primers, it is recommended to purify the PCR product. PCR purification can be done in either 96- or 384-plate format by ethanol precipitation or by commercial purification systems.

- **Spotting**

The purified PCR products are spotted onto membranes or coated glass slides. DNA microarrays on coated glass slides are prepared by printing DNA products with high-speed robots. Aminosilane-coated slides (Corning, Telechem, Amersham Pharmacia Biotech) and poly-L- lysine-coated slides are commonly used. The PCR products are resuspended in an appropriate solution before spotting. The two common solutions are high-salt buffer (3×SSC) and 50% dimethyl sulfoxide (DMSO).

- **Total RNA labeling**

The cDNA probes for array hybridization are synthesized from total RNA by reverse transcriptase. The nucleotides can be labeled with radioisotopes (such as P32 orthophosphate) or fluorescent markers. Either random primers or specific primers are used in the reaction.

- **Hybridization and data acquisition**

Usually, the hybridization solution containing the probe is placed onto the array and covered with a cover slip. The glass slide is then placed in a humidified chamber. The temperature of hybridization and washing conditions depend on the GC content of the organism. After hybridization, the signal intensities of all the spots on a glass slide are captured by scanners (GSI Lumonics, Molecular Dynamics, Genomic Solutions, Axon, and others). For membrane arrays hybridized with P32-labeled probes, a phosphor imaging system (Molecular Dynamics) can be used.

2- OLIGONUCLEOTIDE-BASED DNA MICROARRAYS

Instead of using PCR products, DNA microarrays can be constructed with short oligonucleotides. In the Affymetrix system, Oligonucleotide is synthesized *in situ* on a derivatized glass surface using a combination of photolithography and combinatorial chemistry. The *Escherichia coli* Genome Array system by Affymetrix uses a protocol for the enrichment and labeling of the non-polyadenylated mRNA of prokaryotes. The mRNA is directly labeled so that it represents the natural distri-bution of RNA species within the sample. No reverse transcription or amplification steps are involved. On the other hand, the enrichment procedure could also potentially alter the mRNA population.

APPLICATIONS OF DNA MICROARRAYS

- **Microbial detection and identification**

microarrays have emerged as potential tools for bacterial detection and identification given their high parallelism in screening for the presence of a wide diversity of genes. The most commonly used gene targets have been the 16S bacterial and 28S fungal and intergenic transcribed spacers (ITSs) in rRNA genes, and microarray technology has been incorporated to compensate for the time-consuming sequencing identification procedure (Tang et al., 1998). An oligonucleotide microarray targeting the 16S rRNA gene was developed for the detection of a panel of 40 predominant human intestinal bacterial pathogens in human fecal samples or blood cultures. A similar procedure was developed and used for the rapid diagnosis of bloodstream infections caused by common bacterial pathogens in the pediatric and general populations.

- **Comparative genomics and microbial typing**

Genomic hybridization of a whole genome array detects the presence or absence of similar DNA regions in other microorganisms, allowing genome-wide comparison of their genetic contents. It is an effective way to conduct a comparative genomic study in the absence of complete genome sequences. DNA microarrays can facilitate a better understanding of the genetic differences between closely related organisms, providing useful information for the identification of virulence factors, exploration of molecular phylogeny, improvement of diagnostics and development of vaccines.

Numerous studies that use DNA microarrays for microorganism typing by taking advantage of its simultaneous detection of a variety of genomes have been reported. The accurate identification and prompt typing of pathogens causing diarrheal diseases are critical for directing clinical intervention, including appropriate antibiotic administration, and facilitating epidemiological investigations.

- **Determination of virulence factors**

Many genes associated with virulence are regulated by specific conditions. One way to determine the candidate virulence factors is to investigate the genome-wide gene expression profiles under relevant conditions, such as physiological changes during interaction with the host.

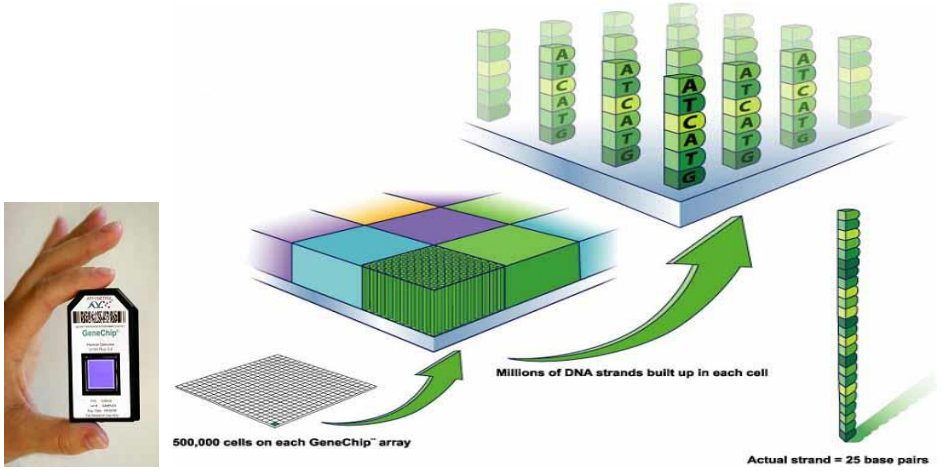
- **Gene expression profiles of drugs, resistance, inhibitors and toxic compounds**

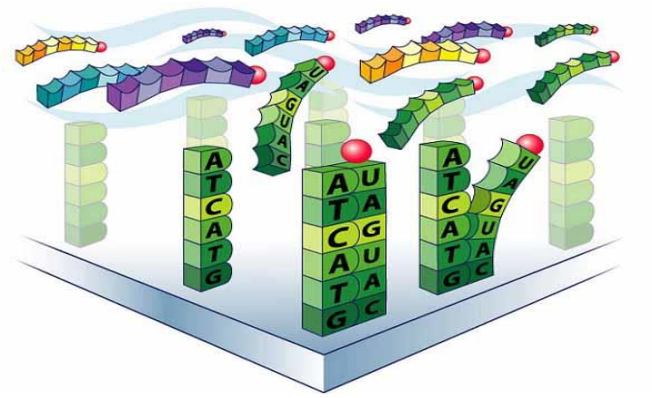
Inhibition of a particular cellular process may result in a regulatory feedback mechanism, leading to changes in gene expression patterns. Exploring the gene expression profiles with DNA microarrays may reveal information on the mode of action for drugs, resistance, inhibitors or toxic compounds.

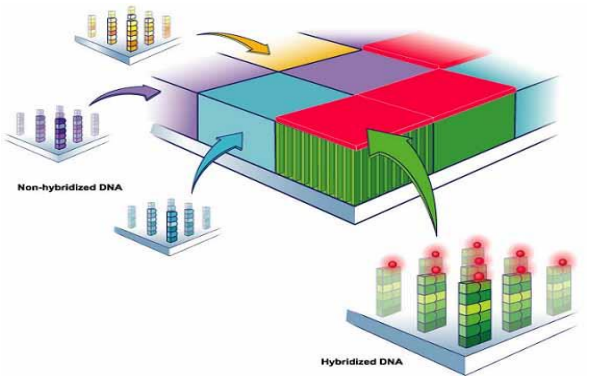
Another successful application of DNA microarray techniques in medical microbiology is the determination of antimicrobial resistance by simultaneously detecting a panel of drug resistance-related mutations in microbial genomes.

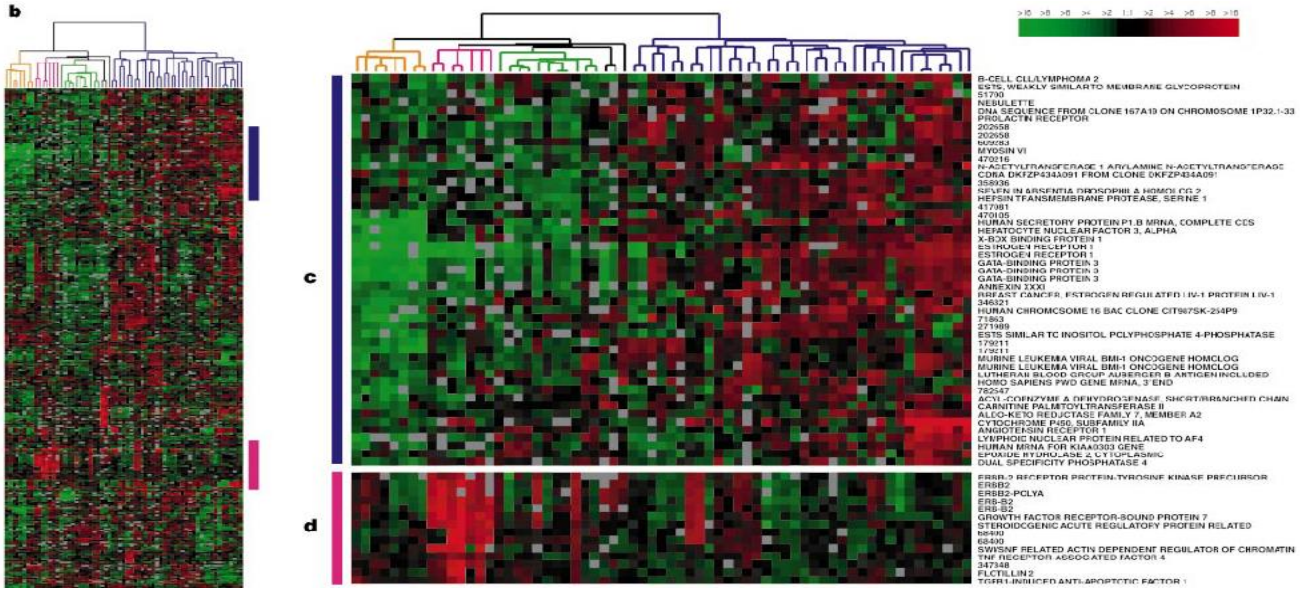
- **Analyses of microbial evolution and epidemiology**

DNA microarrays can be used to explore the variability in genetic content and in gene expression profiles within a natural population of the same or related species and between the ancestor and the descendents. As a result, it provides very rich information on the molecular basis of microbial diversity, evolution and epidemiology.






Non-hybridized DNA
Hybridized DNA


b
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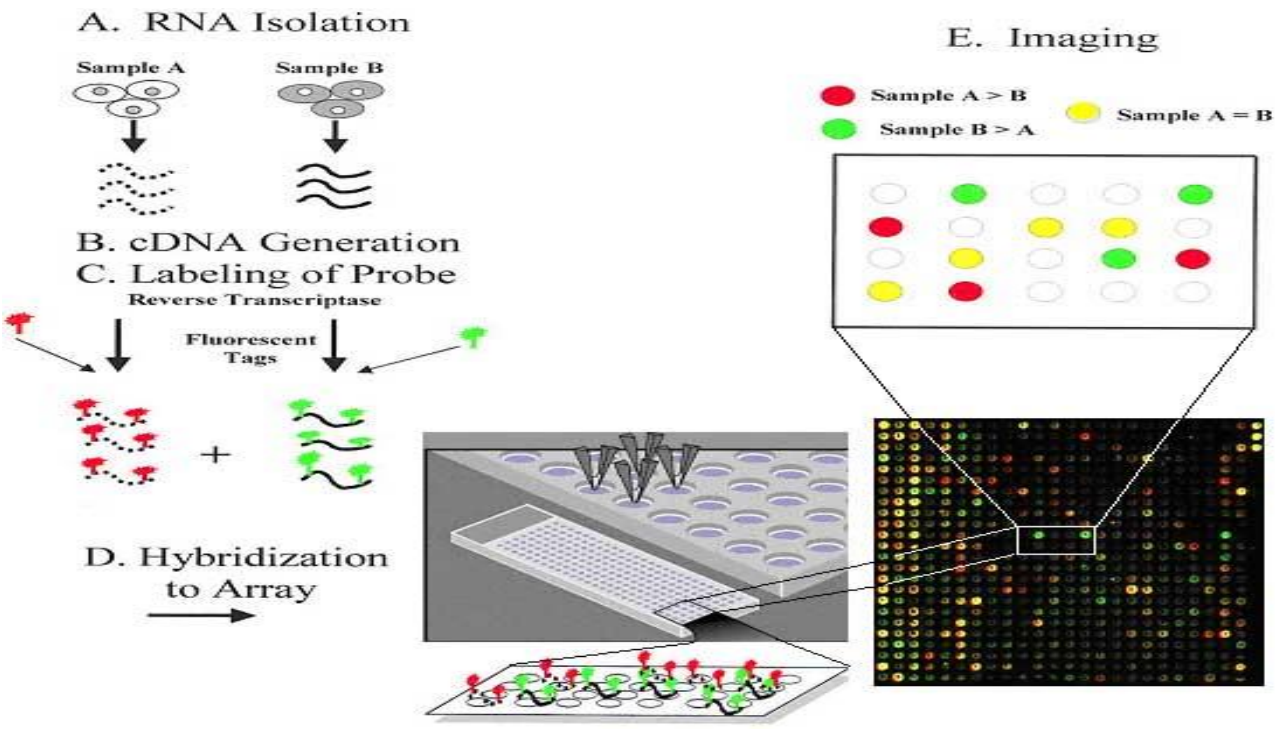
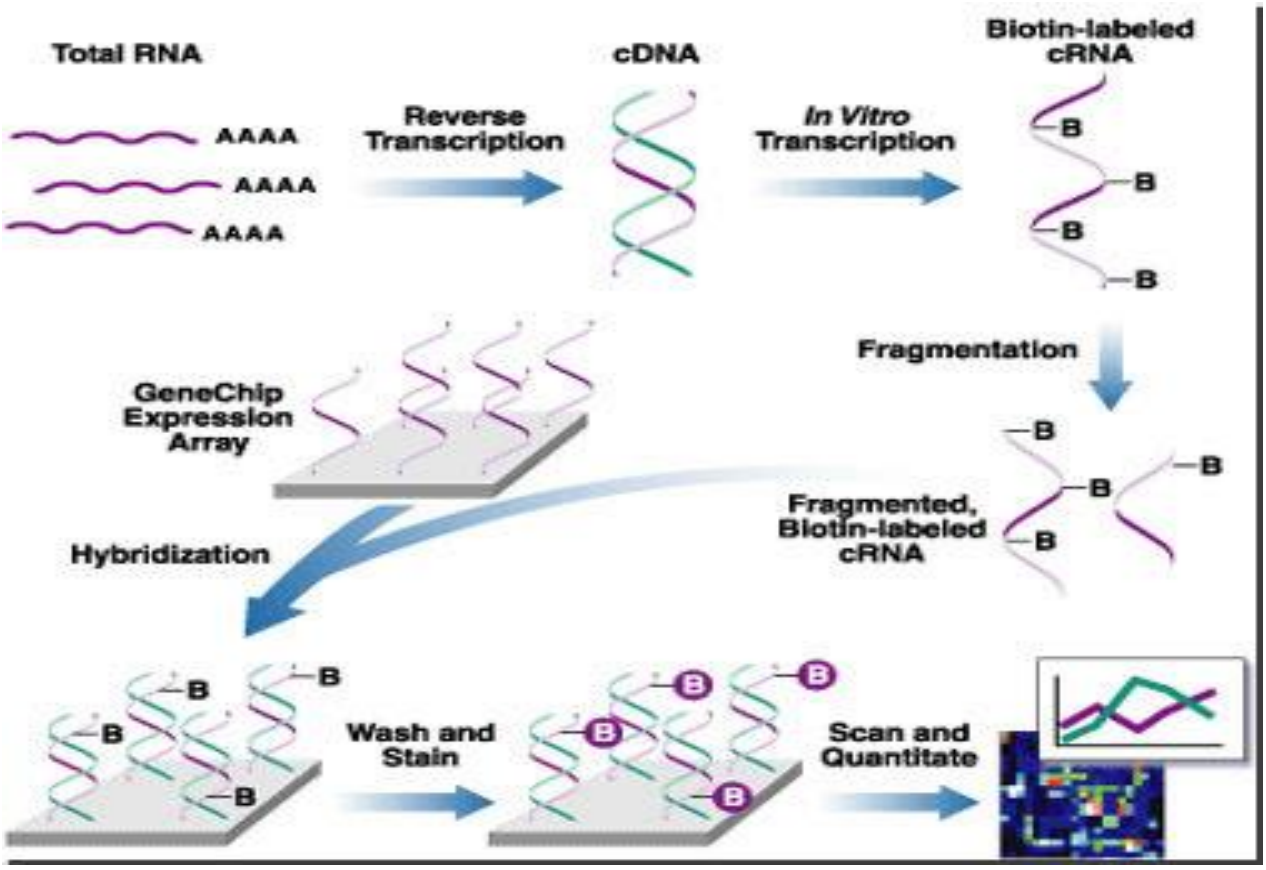

A. RNA Isolation
Sample A
Sample B
B. cDNA Generation
C. Labeling of Probe
Reverse Transcriptase
Fluorescent Tags
D. Hybridization to Array
E. Imaging
Sample A > B
Sample B > A
Sample A = B


Total RNA
AAAA
AAAA
AAAA
Reverse Transcription
cDNA
In Vitro Transcription
Biotin-labeled cRNA
B
B
B
B
Fragmentation
GeneChip Expression Array
Fragmented, Biotin-labeled cRNA
Hybridization
Wash and Stain
Scan and Quantitate