Microarrays

A ***microarray*** is a pattern of ssDNA probes which are immobilized on a surface (called a chip or a slide). The probe sequences are designed and placed on an array in a regular pattern of spots. The chip or slide is usually made of glass or nylon and is manufactured using technologies developed for silicon computer chips. Each microarray chip is arranged as a checkerboard of 10^5 or 10^6 spots or ***features***, each spot containing millions of copies of a unique DNA probe (often 25 nt long).

Like Southern & northern blots, microarrays use ***hybridization*** to detect a specific DNA or RNA in a sample. But whereas a Southern blot uses a *single* probe to search a complex DNA mixture, a DNA microarray uses *a **million** different* probes, fixed on a solid surface, to probe such a mixture. The exact sequence of the probes at each feature/location on the chip is known. Wherever some of the sample DNA hybridizes to the probe in a particular spot, the hybridization can be detected because the target DNA is labeled (and unbound target is washed away). Therefore one can determine which of the million different probe sequences are present in the target.

{NOTE: In a Southern, the target DNA is immobilized on a membrane; in a microarray, the probes are fixed to the slide or chip. In a Southern, the probe is labeled; in a microarray, the DNA being studied is labeled.}

Additionally, the amount of signal directly ***depends on the quantity of labeled target DNA***. Thus microarrays can give a **quantitative** description of how much of a particular sequence is present in the target DNA. This is particularly useful for studying gene expression, one common application of microarray technology.

Obviously, microarrays must be read mechanically, using a laser and detector. Good software for interpreting the raw data is crucial (as one can imagine a long list of sources of error in reading the individual spots, including nonspecific hybridization and background fluorescence).

To study gene expression, mRNA is isolated from the cells of interest and converted into labeled cDNA. This cDNA is then washed over a microarray carrying features representing all the genes that could possibly be expressed in those cells. If hybridization occurs to a certain feature, it means the gene is expressed. Signal intensity at that feature/spot indicates how *strongly* the gene is expressed (as it is a sign of how much mRNA was present in the original sample). One can therefore study gene expression in an entire cell (not just for one or two genes) under various conditions, over time, or in normal vs. diseased cells.

Microarrays are **sensitive enough to detect single base differences**, mutations, or SNPs (single nucleotide polymorphisms). This makes them useful for a wide range of applications, for example: identifying strains of viruses; identifying contamination of food products with cells from other plants or animals; detecting a panel of mutations in a patient's cancer cells that may influence the disease's response to treatment.

Protein microarrays are also being developed to allow massive screening for interactions between proteins on the microarray, and other proteins, substrates, or ligands.

From Affymetrix, makers of the GeneChip brand DNA microarrays: "Monitoring gene expression lies at the heart of a wide variety of medical and biological research projects, including classifying diseases, understanding basic biological processes, and identifying new drug targets. Until recently, comparing expression levels across different tissues or cells was limited to tracking one or a few genes at a time. Using microarrays, it is possible to simultaneously monitor the activities of thousands of genes (see Figure 1).

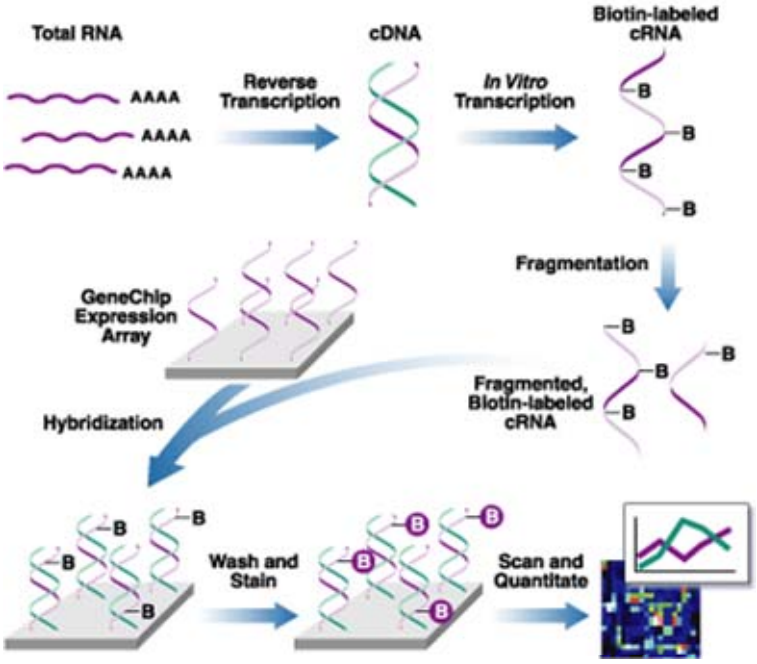
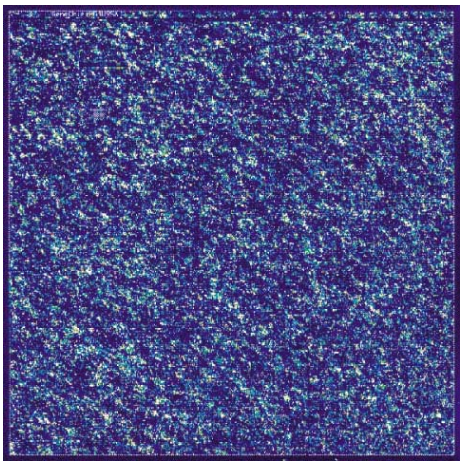


Figure 1. Standard eukaryotic gene expression assay. The basic concept behind the use of GeneChip microarrays for gene expression is simple: labeled cDNA or cRNA targets derived from the mRNA of an experimental sample are hybridized to nucleic acid probes attached to the solid support. By monitoring the amount of label associated with each DNA location, it is possible to infer the abundance of each mRNA species represented. Although hybridization has been used for decades to detect and quantify nucleic acids, the combination of the miniaturization of the technology and the large and growing amounts of sequence information, have enormously expanded the scale at which gene expression can be studied.

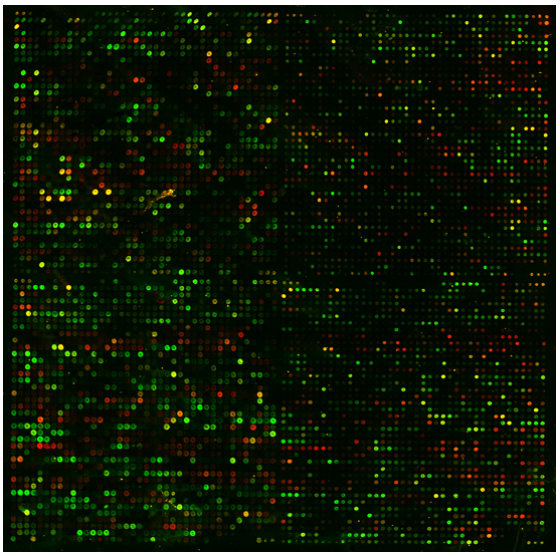
Global views of gene expression are often essential for obtaining comprehensive pictures of cell function. For example, it is estimated that between 0.2 to 10% of the 10,000 to 20,000 mRNA species in a typical mammalian cell are differentially expressed between cancer and normal tissues. Understanding the critical relative changes among all the genes in this set would be impossible without the use of whole-genome analysis. Whole-genome analyses also benefit studies where the end goal is to focus on small numbers of genes, by providing an efficient tool to sort through the activities of thousands of genes, and to recognize the key players. In addition, monitoring multiple genes in parallel allows the identification of robust classifiers, called "signatures", of disease. Often, these signatures are impossible to obtain from tracking changes in the expression of individual genes, which can be subtle or variable. Global analyses frequently provide insights into multiple facets of a project. A study designed to identify new disease classes, for example, may also reveal clues about the basic biology of disorders, and may suggest novel drug targets."

http://www.bio.davidson.edu/courses/genomics/chip/chip.html



LEFT: Affymetrix GeneChip raw data

RIGHT: Actual data for a yeast gene expression microarray



IMPORTANT TO UNDERSTAND:

The yeast gene expression microarray above (with yellow, green & red spots) is an example of a comparison of gene expression between two conditions (in this case, yeast grown in the presence and absence of oxygen). This microarray would tell you about changes in gene expression during fermentation vs. oxidative respiration.

- Isolate mRNA from yeast grown aerobically; make cDNA and label RED
- Isolate mRNA from yeast grown anaerobically; make cDNA and label GREEN
- Wash BOTH cDNAs onto appropriate yeast microarray
- Analyze data

***Red spot** = this gene was expressed **ONLY** under aerobic conditions
***Green spot** = this gene was expressed **ONLY** under anaerobic conditions
***Yellow spot** = this gene was expressed under **BOTH** conditions
***Black spot** = **no** gene expression under either condition

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If you are interested in how Affymetrix makes their GeneChips (proprietary name for Affymetrix product) using **photolithography**, you'll find an easy to read students' description at:

http://www.affymetrix.com/corporate/outreach/lesson_plan/downloads/student_manual_activities/activity3/activity3_manufacturing_background.pdf

Note that there is a competing method for microarray synthesis, pioneered by Stanford University. You can probably find information at their Stanford Microarray Database.