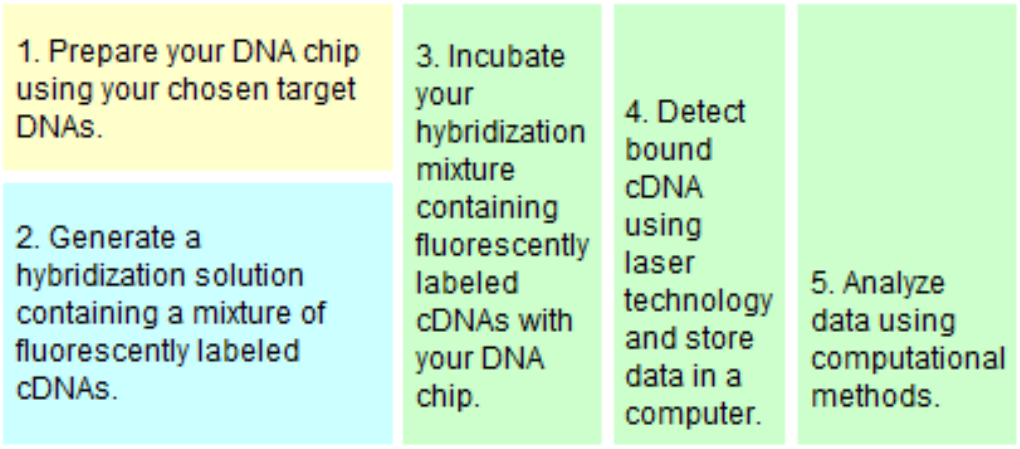
Designing a Microarray Experiment:

The Basic Steps



How does a scientist extract information about a disease condition from a dime-sized glass or silicon chip containing thousands of individual gene sequences? The whole process is based on **hybridization probing**, a technique that uses fluorescently labeled nucleic acid molecules as "**mobile probes**" to identify **complementary molecules**, sequences that are able to base-pair with one another. Each single-stranded DNA fragment is made up of four different nucleotides, adenine (A), thymine (T), guanine (G), and cytosine (C) that are linked end to end. Adenine is the complement of, or will always pair with, thymine, and guanine is the complement of cytosine. Therefore, the complementary sequence to G-T-C-C-T-A will be C-A-G-G-A-T. When two complementary sequences find each other, such as the immobilized target DNA and the mobile probe DNA, cDNA, or mRNA, they will lock together, or **hybridize**.

Now, consider two cells: cell type 1, a healthy cell, and cell type 2, a diseased cell. Both contain an identical set of four genes, A, B, C, and D. Scientists are interested in determining the expression profile of these four genes in the two cell types. To do this, scientists isolate mRNA from each cell type and use this mRNA as templates to generate cDNA with a "**fluorescent tag**" attached. Different tags (red and green) are used so that the samples can be differentiated in subsequent steps. The two labeled samples are then mixed and incubated with a microarray containing the immobilized genes A, B, C, and

D. The labeled molecules bind to the sites on the array corresponding to the genes expressed in each cell.

After this hybridization step is complete, a researcher will place the microarray in a "reader" or "scanner" that consists of some lasers, a special microscope, and a camera. The fluorescent tags are excited by the laser, and the microscope and camera work together to create a digital image of the array. These data are then stored in a computer, and a special program is used either to calculate the red-to-green fluorescence ratio or to subtract out background data for each microarray spot by analyzing the digital image of the array. If calculating ratios, the program then creates a table that contains the ratios of the intensity of red-to-green fluorescence for every spot on the array. For example, using the scenario outlined above, the computer may conclude that both cell types express gene A at the same level, that cell 1 expresses more of gene B, that cell 2 expresses more of gene C, and that neither cell expresses gene D. But remember, this is a simple example used to demonstrate key points in experimental design. Some microarray experiments can contain up to 30,000 target spots. Therefore, the data generated from a single array can mount up quickly.

What Exactly Is a DNA Microarray?

DNA Microarrays are small, solid supports onto which the sequences from thousands of different genes are immobilized, or attached, at fixed locations. The supports themselves are usually glass microscope slides, the size of two side-by-side pinky fingers, but can also be silicon chips or nylon membranes. The DNA is printed, spotted, or actually synthesized directly onto the support.

The American Heritage Dictionary defines "**array**" as "to place in an orderly arrangement". It is important that the gene sequences in a microarray are attached to their support in an orderly or fixed way, because a researcher uses the location of each spot in the array to identify a particular gene sequence. The spots themselves can be DNA, cDNA, or **oligonucleotides**.

An oligonucleotide, or oligo as it is commonly called, is a short fragment of a single-stranded DNA that is typically 5 to 50 nucleotides long.

From: www.ncbi.nlm.nih.gov/About/primer/microarrays.html