Appendix A: Microarray Methodology Overview

DNA microarrays allow an investigator to measure simultaneously the relative activity of every gene in a genome. A microarray consists of a glass slide with DNA from every gene in the genome spotted in precise locations in a grid of spots, one for each gene. The single-stranded DNA printed on the microarray is the target bound by fluorescently labeled cDNA probe from two different sources. One probe sample is obtained from the control condition (e.g. yeast cells grown with unlimited glucose, or healthy human tissue biopsy) where mRNA is isolated, converted to cDNA, and tagged with a fluorescent dye (e.g. green dye such as Cy3). The other probe sample is taken from the experimental condition (e.g. yeast with reduced glucose, or human cancer biopsy) where the mRNA is converted to a different color cDNA (e.g. red dye such as Cy5). These two populations of cDNA probes are mixed and allowed to hybridize with the spotted DNA on the slide. After washing, the amount of bound cDNA is detected and quantified (e.g. 3000 units of green and 500 units of red for a particular gene). The intensity of the two colors is converted into a ratio that indicates the relative steady state transcript levels of the gene in the experimental condition compared to the control condition (e.g. 6-fold repressed in the experimental condition; 500 ÷ 3000). Microarrays can also be used to in comparative genome hybridization (CGH) experiments to measure chromosomal abnormalities due to amplification or deletion of chromosome segments. For CGH, genomic DNA is isolated from wild-type parental and derived aneuploid cells. Each DNA sample is labeled a different color and used to probe the microarray. In this case, color ratios indicate gene amplification or deletion.