**Expression Profiling with Microarrays**

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| Chapter title and time code | Transcript |
| 0:00: Overview | DNA microarrays are widely used tools to simultaneously measure the expression of many different genes. They consist of thousands of probes—each representing a different gene—immobilized on “chips” or slides, and rely on complementary hybridization to evaluate gene expression in different biological conditions.  This video will cover the basic principles of microarray technology, a protocol for gene expression profiling using microarrays, and some current applications. |
| 0:42: Principles of Microarray Technology | Let’s begin by discussing the principles of gene expression profiling and microarray technology.  One of the earliest methods developed to assess gene expression in biological samples is Northern blotting, which involves “probing” for specific RNA molecules immobilized on nitrocellulose membranes. “Free-floating” probes recognize complementary RNA sequences in the sample, and are typically labeled with radioactive or fluorescent molecules so that they can be visualized.  Advances in microfabrication, genome sequencing, and other technologies have led to the development of the microarray biochip. Like Northern blots, microarrays are based on the principle of complementary binding between probe and sample nucleic acid sequences. Unlike Northerns, however, in microarrays it is the oligonucleotide probes that are immobilized on a glass slide or chip. The “free-floating” samples are generated from RNA isolated from cells or organisms of interest, which is reverse transcribed to complementary or “c”-DNA. This can either be directly labeled with fluorescent molecules, or their amounts may be further amplified by *in* *vitro* transcription into cRNA. The sample is then hybridized to the chip. Because probes on microarrays designed for different applications may either be “sense,” which means their sequences are in the same direction as an organism’s expressed RNA, or “antisense,” researchers must ensure that the strand directionality of the sample is complementary to that of the probes.  The “raw” fluorescence intensity data for each gene-specific dot on the chip can then be quantified and processed. The data can be subjected to further statistical tests, like the Student’s t test, to determine if the fluorescence signals—and thus expression levels—for a gene of interest are significantly different between two cell types or experimental conditions.  Researchers can also use this data to “cluster” or group genes based on similar patterns of expression. For example, when comparing expression patterns between two cell populations, certain genes may be found to demonstrate expression changes by roughly equivalent amounts in the same direction, and would thus be grouped together. Researchers can depict these relationships in a type of tree diagram or “dendrogram” where heights and arrangements of “branches” indicate how similar—or dissimilar—gene expression patterns are. This type of analysis can provide insight into gene networks, as “clustered” genes may participate in the same biological pathways. |
| 3:32: General Protocol for a Microarray Experiment | Now that we’ve discussed the principles of microarray methodology, let’s take a look at a typical microarray experiment.  To ensure the quality of the isolated RNA, workspaces and equipment should be treated with chemicals that inactivate RNases—enzymes that would otherwise destroy RNA. The RNA is then isolated from samples of interest and purified, and its concentration and integrity are determined through spectrophotometry or microcapillary electrophoresis.  This sample RNA is converted to cDNA, and then cRNA. Next, the sample is labeled with fluorescent molecules and fragmented, and its quality and quantity can again be checked, at which time the extent of fluorescent labeling can also be assessed.  The labeled cRNA is then mixed with “hybridization solution” before loading onto a microarray. To facilitate successful hybridization, a “mixer” is placed onto the chip to form “hybridization chambers.” The hybridization mix is then slowly added onto the array. Care must be taken to avoid air-bubbles, as these can interfere with the binding of the sample to specific regions on the microarray, and result in a false negative signal. Once the sample is added, chips are incubated at the appropriate temperature for up to 24 hours.  After hybridization, the mixer is removed from the chip, unbound sample is washed off, and the array is thoroughly dried by centrifugation in a specialized, slide-holding centrifuge. The dried chip is inserted into a microarray scanner, and the machine is adjusted so that the brightest signals observed on a chip are not over-saturated. The microarray is then scanned, and an image of the entire chip produced.  Once the chip has been scanned, the image file is loaded into data-extraction software and assessed for any signal irregularities. Data extracted from the microarray image is subjected to a number of statistical manipulations, including log2 transformation, which allows researchers to numerically depict data in terms of fold increases or decreases in gene expression; as well as normalization, which accounts for signal differences between microarray chips. This processed data can then be further analyzed. |
| 6:02: Applications | Now that we’ve demonstrated how expression profiling with microarrays is performed, let’s look at how microarrays can be used in specific experiments.  Researchers often employ microarrays to evaluate how gene expression changes throughout a biological process, such as cellular differentiation.Here, scientists assessed the levels of microRNAs, which are 22-nucleotide small RNAs involved in fine-tuning gene expression, in three human cell types representing different stages of retina development. By comparing microRNA expression between these cells, researchers were able to identify genes potentially involved in retinal tissue differentiation and development.  Microarrays can also be used to assess expression differences between different cells or tissue types. In this experiment, a rodent model of post-traumatic stress disorder, or PTSD, was established by exposing rats to electric shocks. Neurons were collected from different brain regions and RNA was isolated. Microarrays were then used to identify differential expression of mitochondria-associated genes in these neurons, providing insight into the complex molecular mechanisms behind PTSD.  Finally, researchers are also applying microarrays to cancer studies, in the hope that new disease biomarkers can be identified. As a result of infections by viruses throughout our evolution, human genomes contain viral genetic sequences referred to as “endogenous retroviruses” or ERVs, some of which are still actively expressed. Here, the expression of ERVs in cancerous and normal prostate tissues were compared using microarrays. This method allowed researchers to pinpoint several ERVs that were upregulated in prostate cancer, making them potential biomarkers that can be used to diagnose disease. |
| 8:07: Summary | You’ve just watched JoVE’s video on gene expression profiling using microarrays. This video covered the basic principles of microarray technology, a protocol for expression profiling, and applications of this technique. As always, thanks for watching! |