

Peer **R** Review

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A newsletter for the VUMC research community

microarrays.com

The Vanderbilt Microarray Core Facility is open for business

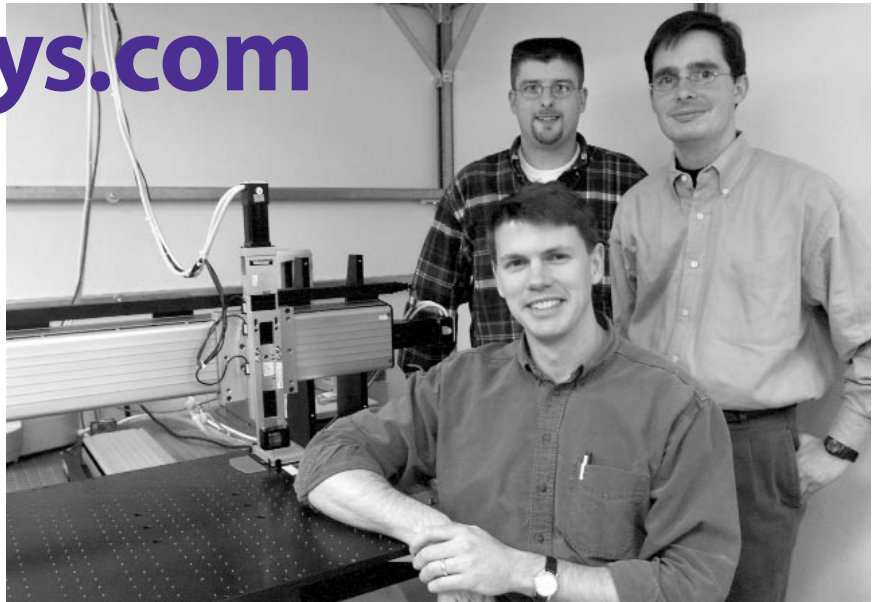
A new core facility is bringing the power of microarray technology to Vanderbilt investigators. DNA microarrays, also known as DNA chips, are orderly arrangements of DNA samples bound to a solid surface—usually a glass chip, and they offer a myriad of experimental possibilities.

Using the arrays involves hybridization of labeled nucleic acids with the DNA on the chip. Hybridization...nucleic acids...if 'Southern' or 'Northern blot' springs to your mind, there is good reason: these blots are the forerunners of the microarray. With DNA microarrays though, investigators can obtain information on thousands of genes simultaneously.

"The whole idea behind the development of this technology is to have some high throughput way to utilize all the genomic information that's coming out," said David Threadgill, PhD, scientific director of the Microarray Core Facility. "Rather than limiting ourselves to looking at how 5 or 10 candidate genes change under experimental conditions, we're going to be able to look at all the genes."

Eventually.

Right now, the Microarray Core Facility offers DNA chips sporting 1152 human or mouse genes, so-called '1K' chips. The core manufactures these gene expression arrays using sequenced verified cDNA clones from Research Genetics. The core plans an expansion to 10K and 30K human and



Dana Johnson

The proud developers of Vanderbilt's 'arrayer'— the robot that produces microarrays. Mark McQuain (seated), a graduate student in Biomedical Engineering, built the arrayer. William Holden (standing right) handles day-to-day operations of the Microarray Core Facility, and Scott Pearsall, PhD, a postdoctoral fellow in Cell Biology, is working on developing standard protocols for array expression analysis.

mouse gene expression arrays, rat gene expression arrays, and human and mouse genome chips containing 400-4000 BACs (bacterial artificial chromosomes).

seeing spots

The goal of most current array-based experiments is to monitor RNA expression levels from a global perspective.

Generally, RNA is isolated from specific tissue samples and is reverse transcribed using oligo-dT primers and fluorescently labeled dNTPs (usually Cy3- or Cy5-dCTP). Labeled cDNAs are hybridized with the chip, which is later washed and imaged with a confocal laser scanner. Because the scanner can excite and detect multiple fluorescent signals, it is possible to hybridize and directly compare at least two different

RNA samples on the same array.

The intensity of a spot's fluorescence determines whether a specific gene is being expressed. When comparing two samples, the ratio of the different fluorescent intensities on the same spot indicates the relative expression level of the gene.

Threadgill plans to use gene expression microarrays to investigate what happens as colon cells become cancerous. His group has been using a genetic approach to study colon cancer susceptibility. They cross specific mouse strains that either do or don't develop colon cancers, producing offspring that have a distribution of response in terms of developing colon cancer.

"Genetics will reveal the root cause of the difference between these strains," Threadgill said, "but it won't

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necessarily tell us the consequences of those differences. That's where array technology will come in and let us get at the molecular events that lead to recognizable tumors."

By isolating RNA from the colonic epithelium at various time points during its transition from a non-cancerous to a cancerous state, Threadgill's group will be able to follow changes in the gene expression profile that accompany this process.

a new way to genotype

Microarrays can also be used to examine DNA sequence for heterogeneity and genotype.

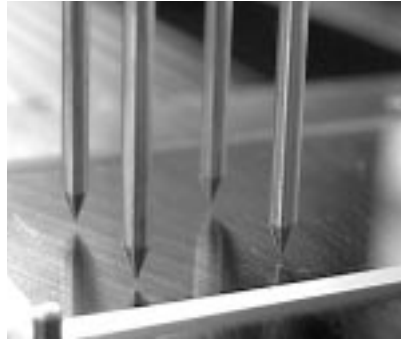
"In tumorigenesis, for example, one of the most frequent changes is the loss or gain of specific regions of DNA," Threadgill said, "but since those regions are pretty small, it is difficult to find them."

A microarray to identify such regions would contain small segments of DNA that span the genome instead of individual genes.

"By hybridizing labeled genomic DNA to this type of array, you could ask: am I losing or gaining copies of specific DNA fragments?," Threadgill said. "This approach queries the genomic DNA itself rather than mRNA."

Special 'genotyping arrays' are still under development at the Microarray Core Facility. They will contain oligonucleotides representing the various sequence possibilities at specific loci. Under the right conditions, labeled genomic DNA will hybridize only to perfect match oligos.

For example, in the case of a single nucleotide polymorphism (SNP), where one base differs at the locus, homozygous genomic DNA would hybridize to either of two different oligonucleotides whereas heterozygous DNA would



Microquill arraying pins printing a cDNA microarray. Photograph courtesy of Eric Duncavage and Mark McQuain.

hybridize to both.

"This is a potential way to genotype DNA at a lot of different loci at the same time," Threadgill said.

made to order

For investigators who are not as interested in a global genetic perspective, the Microarray Core Facility will manufacture both 'boutique' and 'custom' arrays. Both types are custom-made to include only those DNA fragments of interest to the investigator, either provided by the investigator or selected from the core's clone sets.

Boutique arrays will focus on specific biological processes.

"For example, if you are studying vasculogenesis, you might select out a few hundred genes related to vasculogenesis and have a boutique array produced with just those clones," Threadgill said.

data deluge

With thousands of signals of varying color and intensity, analyzing microarray data is no easy task.

The Microarray Core Facility includes a computer lab where several workstations have data analysis packages from both commercial and academic sources. All of the available programs attempt to cull meaningful results from the flood of data.

"The problem is that the technology is still so nascent that no one really knows how best to deal with the data," Threadgill said. "We feel like it's better to have several programs available so that the same data set can be analyzed using a few different approaches."

In addition to providing workstations and data analysis expertise, the core will permanently archive all of the raw scan data.

using the core

The Microarray Core Facility owes its existence to the scientific guidance of Threadgill, Robert Coffey, MD, and biomedical engineer Frederick Haselton, PhD. Coffey gained first-hand experience using microarrays during a sabbatical at Stanford, where Pat Brown and colleagues developed the robotic DNA spotting method for producing arrays. Haselton has been integral to the development of the Vanderbilt core's production machinery.

Support for establishment of the core was provided by the Robert J. and Helen C. Kleberg Foundation, the Vanderbilt-Ingram Cancer Center, and the Vanderbilt Diabetes Research and Training Center. Members of the sponsoring centers receive discounted prices on microarrays.

To find out more about products, services, and pricing, go to the core's website: www.microarrays.com. While you're there, you can register for updates on product availability, share ideas and ask questions using the 'microarray forum,' and visit links to other microarray laboratories, companies, and information.



Bioinformatics overload

With the explosion of genome sequencing efforts, databases are becoming massive and more numerous. They include everything from gene and protein sequences to structure-function information, disease correlations, and population variations. In fact, they include every piece of information being gathered by the biological sciences.

The challenge is to put all of this information to use. That's where bioinformatics comes in.

Bioinformatics is a discipline that uses information technology and mathematical techniques to study patterns and datasets in biology, with the goal of determining characteristics of specific biological systems. The field is also referred to as biocomputing, computational biology, or genomics.

Help wading through the various bioinformatics tools is just a phone call (2-0855) or email (bioinformatics@vanderbilt.edu) away. On the other end of the line will be Charles Alexander, a bioinformatics consultant who arrived last year as part of the Program in Human Genetics computing/bioinformatics core. The division of Genetic Medicine and the Office of Research also participated in creating the position and recruiting Alexander.

"My job is to make bioinformatics software tools available, to show researchers how to use them, and to consult on the best strategies to answer different kinds of questions—to maximize usage of the tools and

databases," Alexander said.

Alexander has set up a one-stop shopping bioinformatics website that is loaded with information about and links to various biocomputing resources. Get rid of all your individual bookmarks to Blast, FASTA, Entrez, NCBI, GenScan, ExPaSy, EBI/EMBLNet....and bookmark just one site instead: www.vanderbilt.edu/bioinformatics/.

The major focus of current support at Vanderbilt is the Genetic Computer Group's (GCG) Wisconsin package. The package, referred to as GCG or the "Wisconsin" package, has over 100 programs that perform DNA/RNA and protein analyses.

The package can be accessed by a user-friendly web interface (SeqWeb) and by more versatile and comprehensive interfaces: SeqLab (X- Windows interface) and GCG shell commands. To set up a **free** account to access GCG, go to the bioinformatics website.

Through the core, investigators can also take advantage of reduced prices (below the standard academic discount price) for specific bioinformatics software, such as Vector NTI and OMIGA 2.0.

In addition to installing and maintaining the various bioinformatics packages and consulting one-on-one, Alexander offers regular workshops on how to use the packages.

Alexander recognizes that his efforts are a first step in biocomputing at Vanderbilt.

"We're trying to give researchers a



Anne Rayner

Bioinformatics consultant Charles Alexander

basic repertoire of tools and software that they can use—that's our first goal," Alexander said. "Next we hope that they will come to us with specific kinds of questions that these tools don't help them solve, and we will write custom programs and packages to solve those questions."

The Program in Human Genetics computing/bioinformatics core offers additional services for genetic studies, including access to genetic pedigree analysis programs, high-end computing resources, and research database development and support.

Alexander received a degree in Biotechnology at the Rochester Institute of Technology. A self-professed "danger in the wet lab," he switched to bioinformatics and has essentially taught himself the computing side of the field. He is eager to see bioinformatics blossom at Vanderbilt and hopes that a training program will develop here.



Office of Research takes shape

Deep inside the maze of Medical Center North, a metamorphosis has occurred. What used to be the Office of Biomedical Sciences is now a new entity: the Office of Research.

This is more than just a name change. It is a complete reorganization of research-related support entities. Alastair J. J. Wood, MD, and Mark A. Magnuson, MD, newly named assistant vice chancellors for Research, are overseeing various components of the office.

Efforts are underway to establish structures that provide greater support for the research enterprise.

“We are working to identify Vanderbilt’s needs for an outstanding clinical research program and to take the steps to make it happen,” Wood said at the first Research Enterprise meeting in January.

Magnuson echoed these sentiments. “Our efforts are focused on improving the institutional infrastructure necessary for performing top-tier science,” he said.

Wood, clinical research initiatives:

- **Training/Career Development in Clinical Trials Research**
- **General Clinical Research Center**
Director, David Robertson, MD
- **Institutional Review Board / Regulatory Issues**
Medical Director, Gordon Bernard, MD
- **Clinical Trials Center**
Director, Italo Biaggioni, MD
- **Research Contracts Office**
Under development

- **Core Facility Development for Clinical Research**

Examples: Biostatistics; Patient capture of DNA; Gene therapy; Clinical research informatics

Magnuson, strategic planning and core facility infrastructure:

- **Research Resources (Cores)**

Examples: Transgenic/ES cell; Confocal; Electron microscopy; DNA sequencing; Mouse pathology; Animal physiology
Director to be determined

- **Division of Animal Care**

Director, Joan Richerson, DVM

- **Institutional Animal Care and Use Committee**

Chair, Jeff Holt, MD
Program Coordinator, Anna Marie Alderson

- **Program Development**

Director, Barbara Meyrick, PhD

- **Metrics Development, Systems Support and Web Site Management**

Rick Stotler

- **Grants Management**

Director, Tom Barnes
Associate Director, Elaine Barnes

For more information:

www.mc.vanderbilt.edu/vumc/biosci/

Peer Review

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Editor and writer: Leigh B. MacMillan, PhD
Editorial board: Lee E. Limbird, PhD
Mark A. Magnuson, MD
Medical Art Group
Design and layout: Wayne Wood
Director of publications for VUMC:

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