

Factors for consideration in developing research initiatives for DNA microarray development and experimentation.

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The recent development of more robust hardware, chemistry, and software for DNA microarrays has resulted in the promise of a high-throughput technology capable of providing comparative sequence data and insights into gene regulation in a variety of organisms under a variety of stress conditions. Funding agencies developing programs to promote these technologies and their proper application to experimentation must develop relevant program goals and be able to critically evaluate applicants in a variety of areas. This presentation will focus on examining a variety of issues that funding agencies are likely to face in developing program initiatives and evaluating applications. These include the applicants ability to conduct high-throughput microarray experimentation, the technical and experimental limitations of the technology, the types of experimentation to be funded (hypothesis driven vs explorative), funding initiatives currently active or under consideration by other agencies (centralization vs distributing technology), and data access and sharing.

TIGR Array Facility

- **Microarrayers**
 - 2 Intelligent Automations System Robots
 - 2 Molecular Dynamics Robots
- **Scanners**
 - Axon Scanner
 - 2 GSI Lumonics Scanners
- **Software**
 - Image acquisition and analysis
 - Database structure



TIGR Projects

- **Mycobacterium tuberculosis** - Becton Dickinson
- **Porphyromonas gingivalis** - NIDCR, NIH
- **Deinococcus radiodurans**
- **Staphylococcus aureus (drug-resistance)** - NIH and Rockefeller U.
- **Thermatoga maritima** - DOE
- **Methanococcus jannaschii** - DOE
- **Methanobacterium thermoautotrophicum** - DOE
- **Streptococcus pneumoniae** - TIGR, LTI, U. of Alabama
- **Bacillus anthracis** - NIH
- **Potato disease resistance** - NSF Plant Genome Initiative
- **Arabidopsis Chromosome II** - NSF Plant Genome Initiative
- **Human Colon Cancer Metastasis** - NCI, NIH
- **Rodent Models** - NHLBI, NIH (Pending)



Array Project Design

- **Obtain source of genomic information**
 - Genomic sequence data
 - ESTs
- **What is to be arrayed?**
 - PCR amplicons
 - Individual clones
- **Primer design**
- **PCR of amplicons**
- **Analysis of PCR products**
 - Size and specificity
 - Sequence proofing

Array Project Design

- **Optimization of glass surface and diluent**
 - Silanized
 - L-poly-lysine
 - DMSO
 - SSC
- **RNA source/purification and labeling**
 - Experimental design
 - Poly A?
 - Direct cDNA labeling with Cy-3 and Cy-5
 - Indirect labeling using amino-allyl chemistry
- **Hybridization**

Array Project Design

- **Data acquisition**
 - Spot finding
 - Quantitation
- **Linking to database structure**
- **Reproducibility**
- **Validation**
 - Slot blots
 - Northern
 - RNA'ase Protection Assays

Funding Initiatives

- **NIH**
 - **NIAID**
 - ROI's (Strong emphasis on hypothesis driven proposals)
 - Centralization through core distribution
 - **NIDCR**
 - Supplements to current grant holders
 - **NHLBI**
 - **NCI**
- **DOE**
- **NSF**
 - Plant Genome Initiative

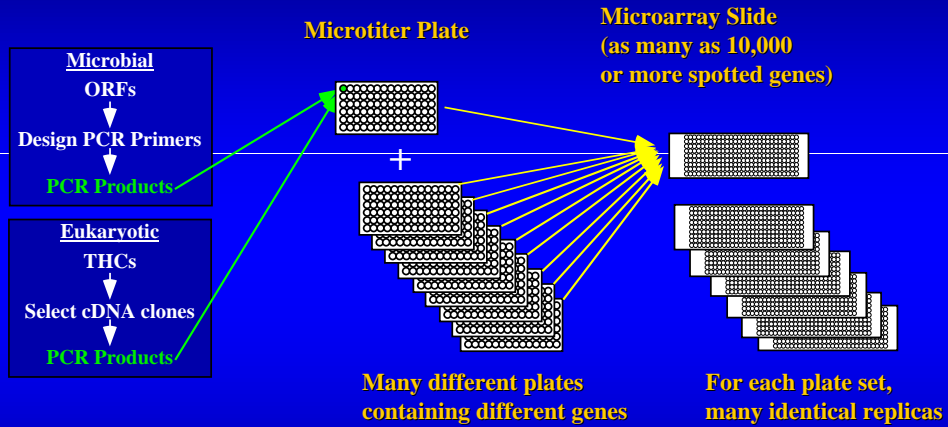
Types of Experimentation

- **Hypothesis driven science**
 - **Propose a specific question**
 - Intellectually superior approach
 - Narrow focus
 - Optimal use of technology??
- **Explorative**
 - **General stress responses**
 - **No proposed hypothesis**
 - **Explore roles of hypothetical ORFs**

Data Access and Sharing

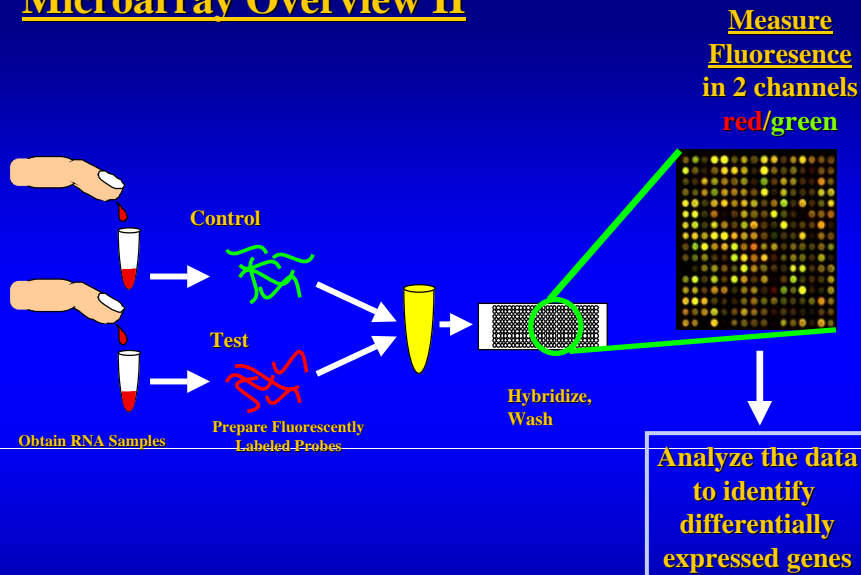
- **Data release policies - role of funding agency**
- **Should array data be treated like genomic data?**
 - **Public release of all array images?**
 - Web pages
 - NCBI, others
 - **To what end?**
 - Others can extract “untapped” information
 - Quality control

Array Fabrication



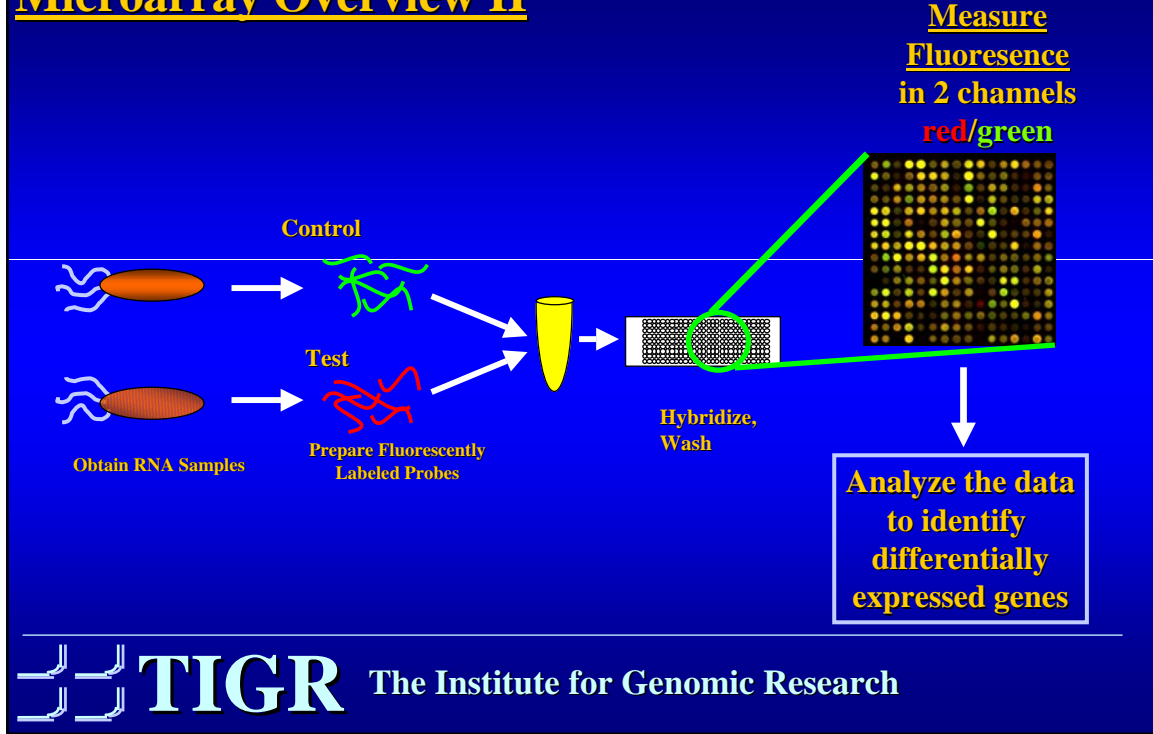
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Microarray Overview II



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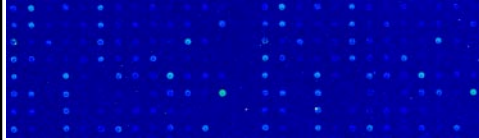
Microarray Overview II



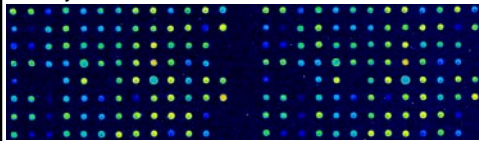
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Cy3: RNA



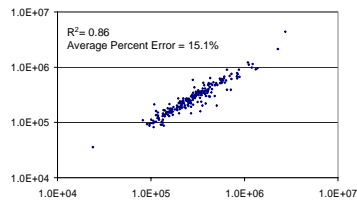
Cy5: Genomic DNA



0 Fluorescence Intensity 65535

Using Ratios Decreases Error

Cy3 Intensity Integral for Each Duplicate



Cy3/Cy5 Ratio for Each Duplicate

