Protein Engineering

20.109 Module 2 Day 7
Tuesday Oct 28th, 2008

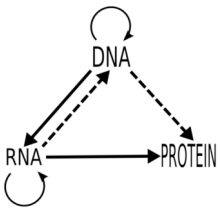
Little things mean a lot

Genetic variation

Development

Touchstone for understanding gene expression:

"central dogma"



Measures of central dogma
DNA/RNA/Protein

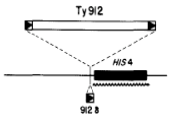
Touchstone for understanding gene expression:

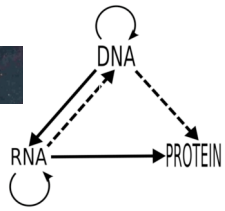
"central dogma"

NY420

SC-his

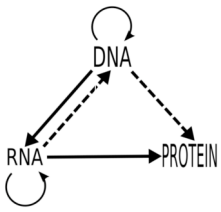
SPT3+TAP





Touchstone for understanding gene expression:

"central dogma"



https://www.23andme.com/you/gen101/01/

Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

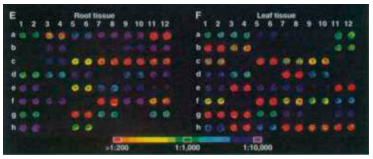
Mark Schena,* Dari Shalon,*† Ronald W. Davis, Patrick O. Brown‡

A high-capacity system was developed to monitor the expression of many genes in parallel. Microarrays prepared by high-speed robotic printing of complementary DNAs on glass were used for quantitative expression measurements of the corresponding genes. Because of the small format and high density of the arrays, hybridization volumes of 2 microliters could be used that enabled detection of rare transcripts in probe mixtures derived from 2 micrograms of total cellular messenger RNA. Differential expression measurements of 45 Arabidopsis genes were made by means of simultaneous, two-color fluorescence hybridization.

each ~1kb long

fluorescein (root)
lissamine (leaf)

2 scans +pseudocolor-->



Science **1995** 270:467

Microarray

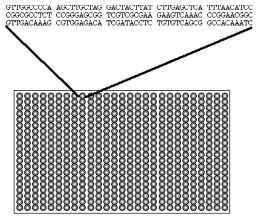
the array

Microarray printing

http://www.youtube.com/watch?v=S8Cwy71nMNU

Spot diameter: 10-150 um

Content: ~10^9 molecules/um2



http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

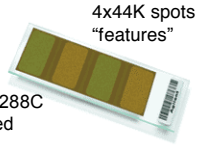
Microarray : the arrays we'll use

Catalog Oligo Microarrays

Agilent's non-contact industrial inkjet printing process uniformly deposits oligo monomers onto specially-prepared glass slides. Both the catalog and custom microarrays are manufactured using Agilent's non-contact in situ synthesis process of printing 60-mer length oligonucleotide probes, base-by-base, from digital sequence files. This is achieved with an inkjet process which delivers extremely small, accurate volumes (picoliters) of the chemicals to be spotted. Standard phosphoramidite chemistry used in the reactions allows for very high coupling efficiencies to be maintained at each step in the synthesis of the full-length oligonucleotide. Precise quantities are reproducibly deposited "on the fly." This engineering feat is achieved without stopping to make contact with the slide surface and without introducing surface-contact feature anomalies, resulting in consistent spot uniformity and traceability.

Agilent's *in situ* Oligonucleotide Microarray

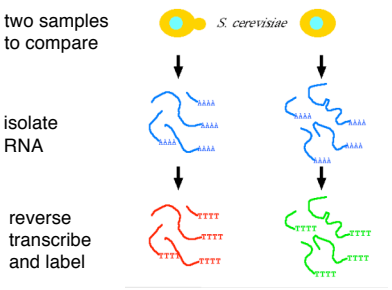
4x44K spots "features"



6,256+ *S. cerevisiae* (S288C strain) ORFs represented

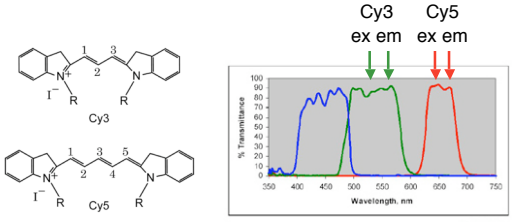
Each 60-mer in length

Microarray: sample preparation



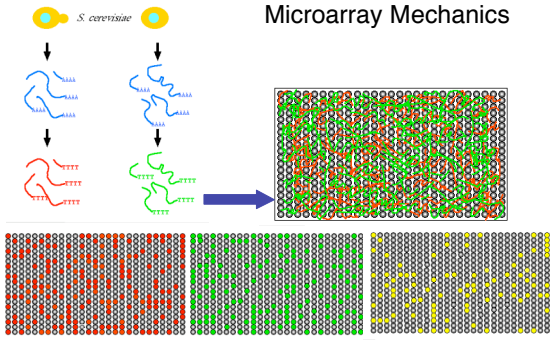
http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

Microarray: fluorescent labels



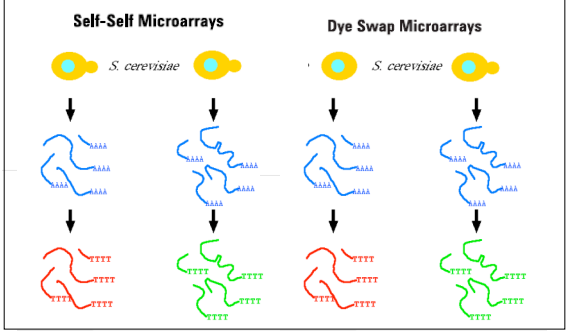
www.qubitsystems.com

Microarray Mechanics

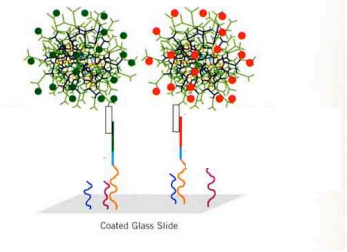


http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

Microarray: controls



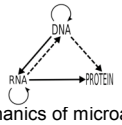
Microarray: indirect labeling method



http://www.genisphere.com/about_3dna.html

Summary

Control of gene expression



Mechanics of microarrays