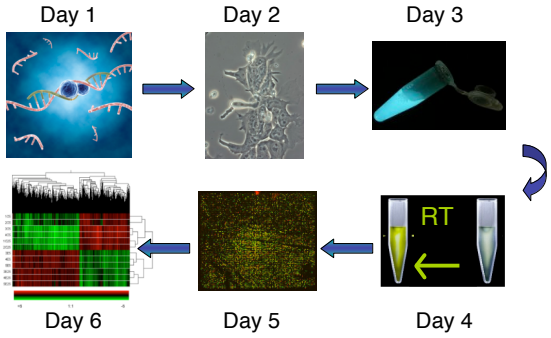
Module 2: Expression Engineering

20.109
Lecture 5
October 25th, 2007

Expression Engineering Experiment



Expression Engineering Experiment

Lecture 1 • intro to cell culture • intro to gene exp'n/RNAi	Lecture 2 • transfection • luciferase
Lecture 3 • off-target/nonspecific RNAi	Lecture 4 • Writing lecture (Neal Lerner)
Lecture 5 • measuring gene express'n	Lecture 6 • microarray analysis (Rebecca Fry)
Lecture 7 • high throughput technologies or RNAi applications (no lab)	Lecture 8 • review of your data

DNA/RNA/Protein Compass

Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

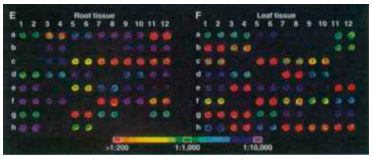
Mark Schena,* Dari Shalon,*† Ronald W. Davis,
Patrick O. Brown‡

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

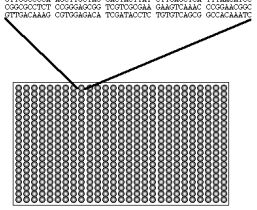
the array

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

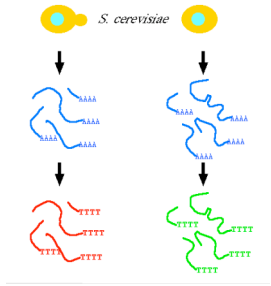
Microarray

sample preparation

two samples to compare

isolate RNA

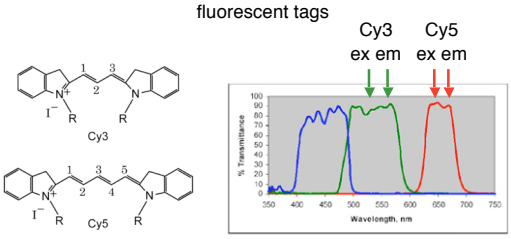
reverse transcribe and label



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

Microarray

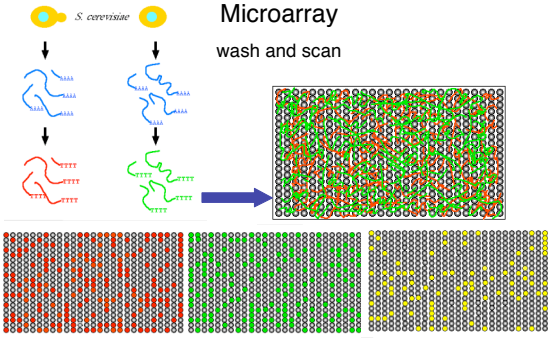
fluorescent tags



www.qubitsystems.com

Microarray

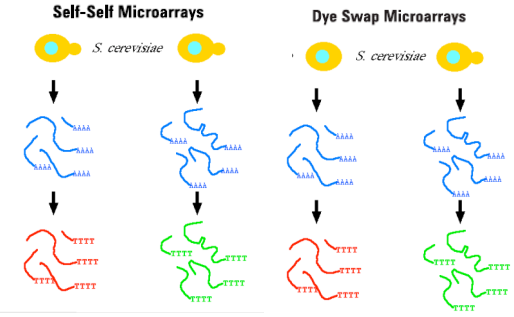
wash and scan



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

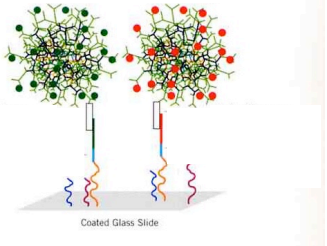
Microarray

controls



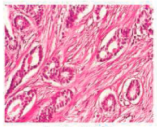
Microarray

indirect labeling method

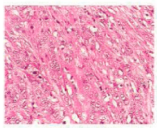


http://www.genisphere.com/about_3dna.html

Diagnostic Tool: is it cancer?



Low-grade



High-grade

1 in 3 women will develop a cancer in their lifetime, 1 in 8 breast cancer

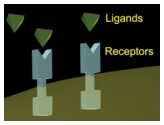
Of all breast cancers diagnosed in the U.S., only 5 to 10% are related to genetics and family history of breast cancer.

Available treatments
surgery, chemo, radiation, hormone

Science **2007** 315:924

Cancergeek.com

Treatment Evaluation Tool: will tumor be hormone-responsive?



~75% of all breast cancers are ER+ (estrogen receptor positive), with remaining 25% negative or an unknown status

~ About 65% of all ER+ are also PR+ (progesterone receptor positive)

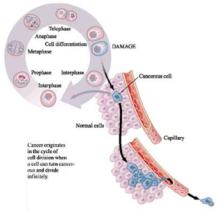
~ 10% of breast cancers are ER+ and PR-

~ 5% of breast cancers are ER- and PR+

Cancergeek.com

Treatment Evaluation Tool: how likely is it to spread?

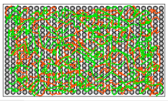
Most commonly spreads to lymph tissue

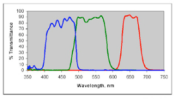
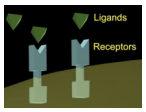
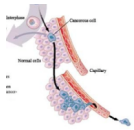


"Chemotherapy and/or hormonal therapy reduce the risk of distant metastases by approximately one-third; however, 70-80% of patients receiving this treatment would have survived without it."
Agendia

Summary

1. Mechanics of microarrays





2. Microarrays for molecular medicine