Module 2: Expression Engineering

20.109

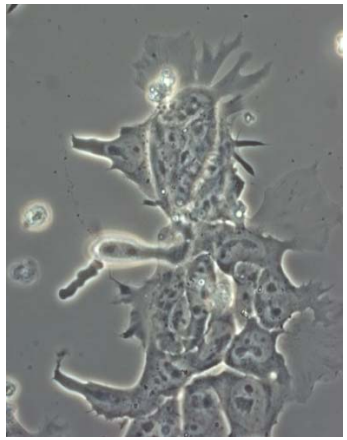
Lecture 5

October 25th, 2007

Expression Engineering Experiment

Day 1

Day 2



Day 3

Image of glowing luciferase in a microcentrifuge tube, removed due to copyright restrictions.

Day 4

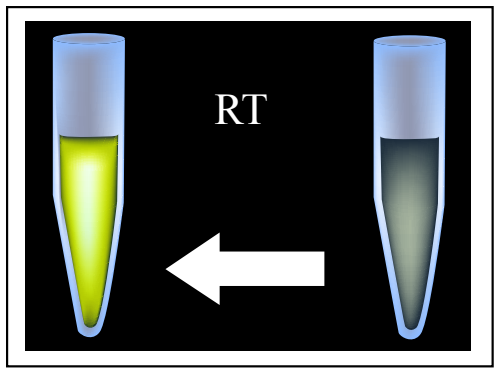
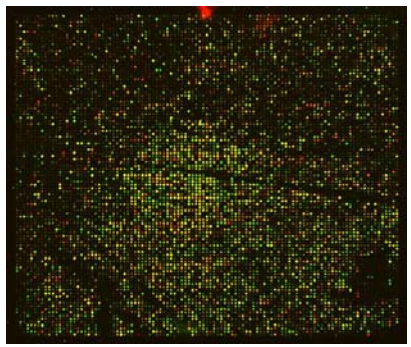


Figure by MIT OpenCourseWare.

Day 5



Day 6

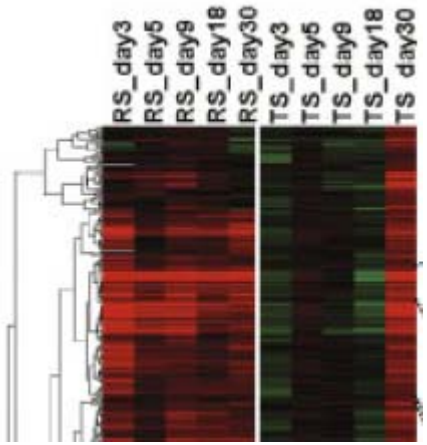
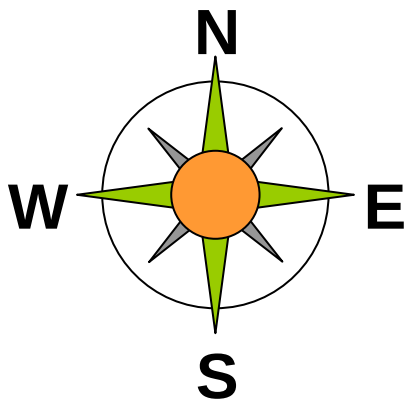


Image credits: Day 1 - Courtesy of Arkitek Studios. Used with permission. Day 2 - Courtesy of The Exploratorium. © The Exploratorium, http://www.exploratorium.edu.
Day 4 – Figure by MIT OpenCourseWare. Day 5 – Dr. Natalie Kuldell. Day 6 – Courtesy of NIH.

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



DNA/RNA/Protein Compass

Figure 2 Western Blot

Sample	Marker	M13k07 (positive control)	M13 Candidate 1 (colony #3)	M13 Candidate 2 (colony #4)
Volume loaded (ul)	5	25	25	25

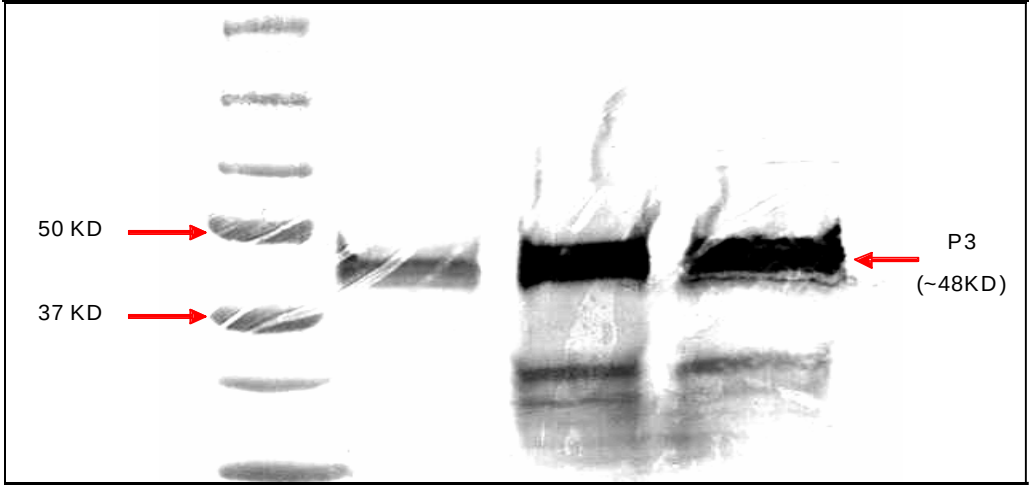
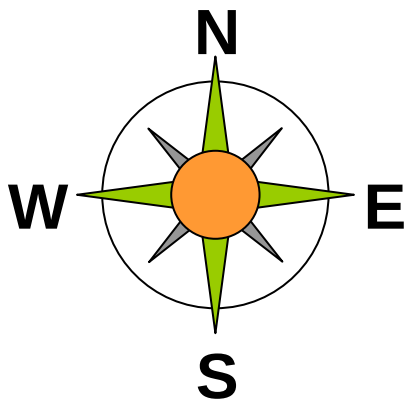


Figure 2 Protein samples from bacteria infected with modified and unmodified M13 virus ran in a polyacrylamide gel. Antibodies with alkaline phosphatase were used to identify virally encoded protein p3. We see strong bands at the expected length of p3 for our control and experimental samples which suggests our modified viruses successfully induced p3 production in their hosts. ABs used: Primary: mouse anti-P3, Secondary: Goat anit-mouse with alkaline phosphatase.



Courtesy of Augusto Tentori. Used with permission.

from Augusto Tentori

DNA/RNA/Protein Compass

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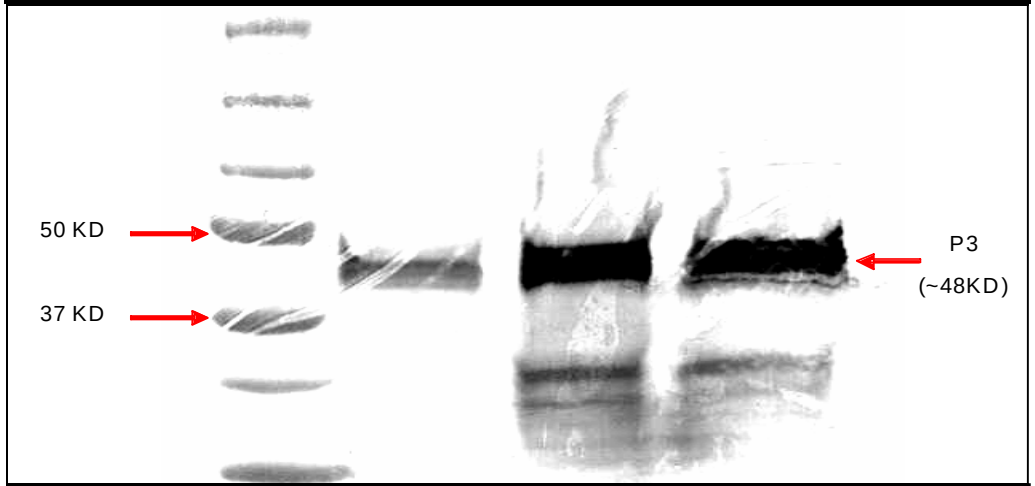

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Courtesy of Augusto Tentori. Used with permission.

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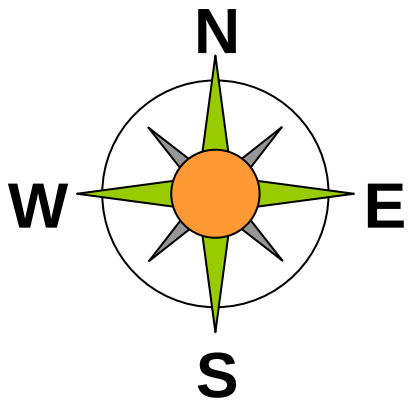

Blot: DNA

Probe: DNA (P^{32})

Measures: _____

DNA/RNA/Protein Compass

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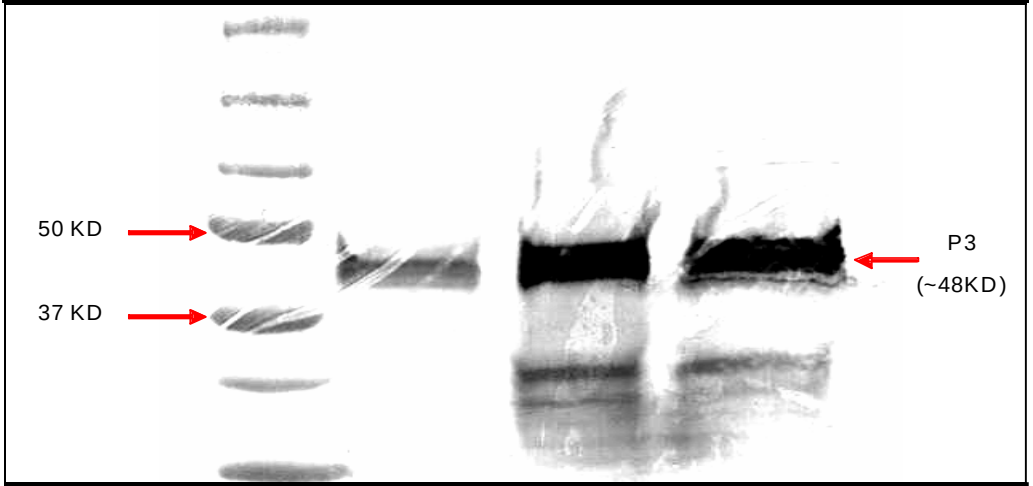

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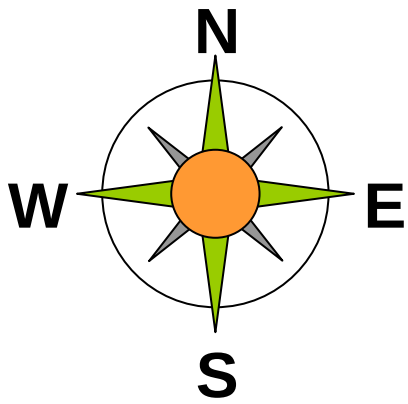
Courtesy of Augusto Tentori. Used with permission.

from Augusto Tentori

Blot: RNA

Probe: DNA (P^{32})

Measures: _____



Blot: DNA

Probe: DNA (P^{32})

Measures: _____

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-->

Image of microarray removed due to copyright restrictions.

Science **1995** 270:467

Microarray

the array

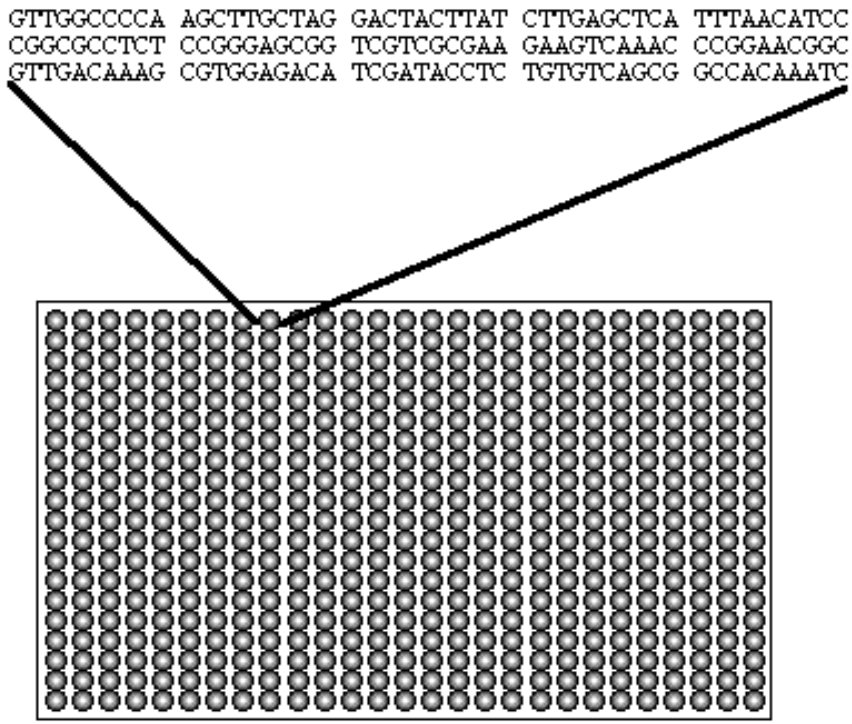
Microarray printing

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

Courtesy of André Silva. Used with permission.

Spot diameter: 10-150 um

Content: ~10^9 molecules/um2



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

Courtesy of A. Malcolm Campbell. Used with permission.

Microarray

the arrays we'll be using

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

Traditional "In-lab"
Pin Spotted Microarray

Courtesy of Agilent Technologies, Inc.
Used with permission.

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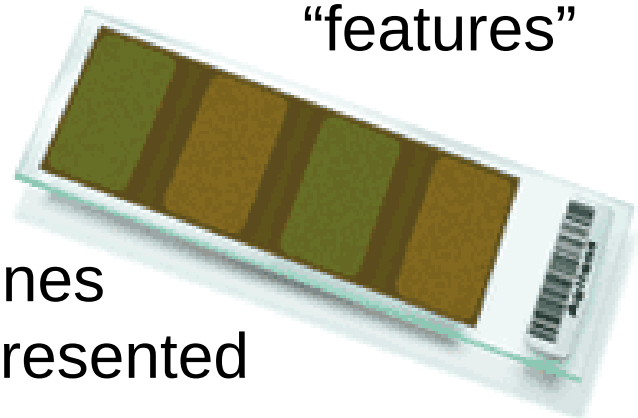
Agilent's *in situ*
Oligonucleotide Microarray

4x44K spots
"features"



41,000+ mouse genes
and transcripts represented

Each 60-mer in length

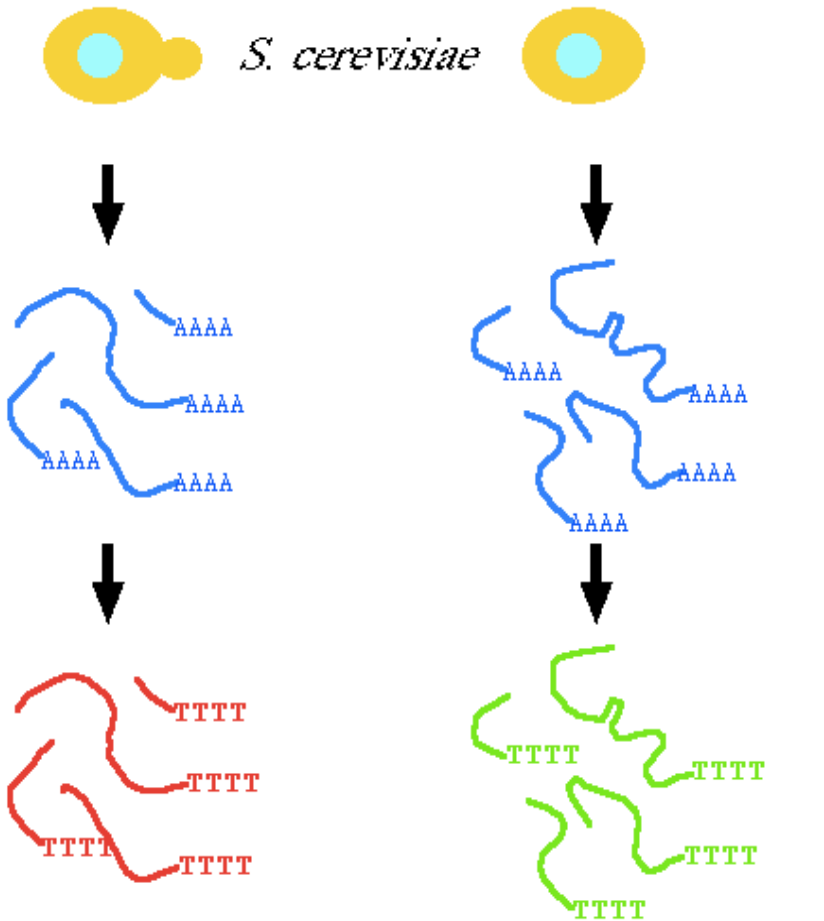
Microarray

sample preparation

two samples
to compare

isolate
RNA

reverse
transcribe
and label



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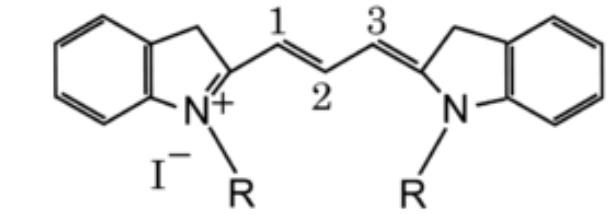
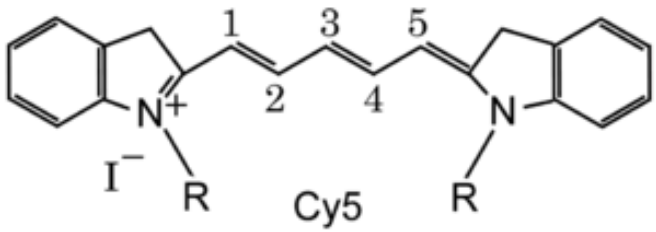
Microarray

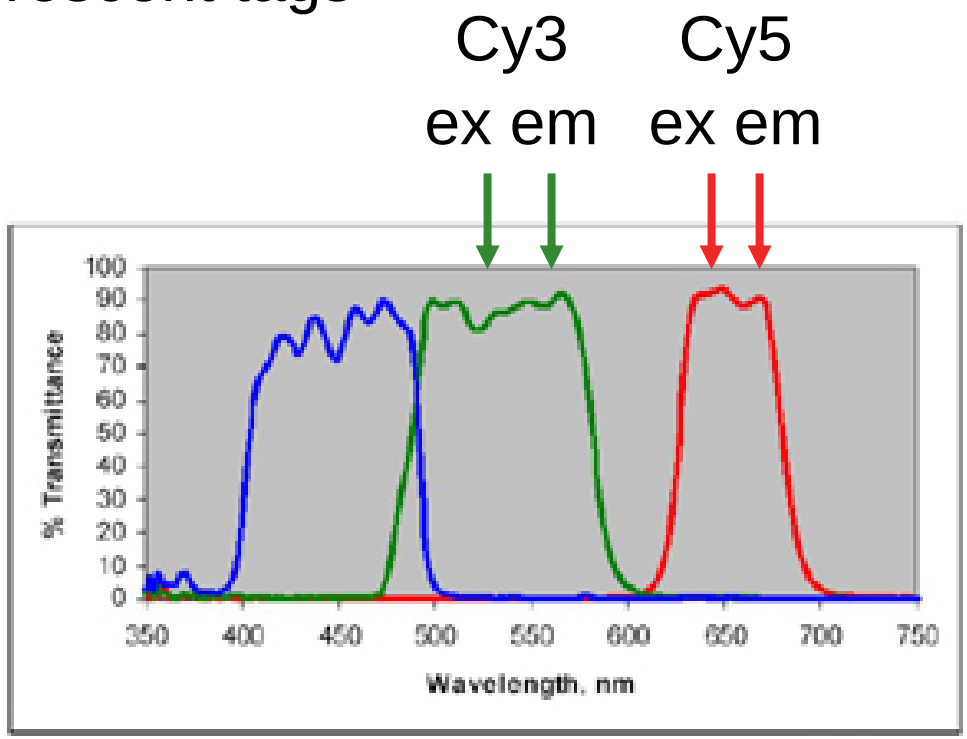
fluorescent tags



Cy3



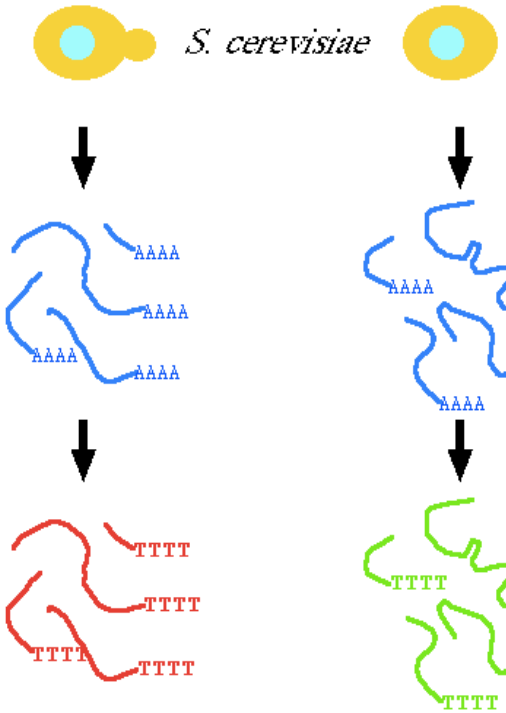
Cy5



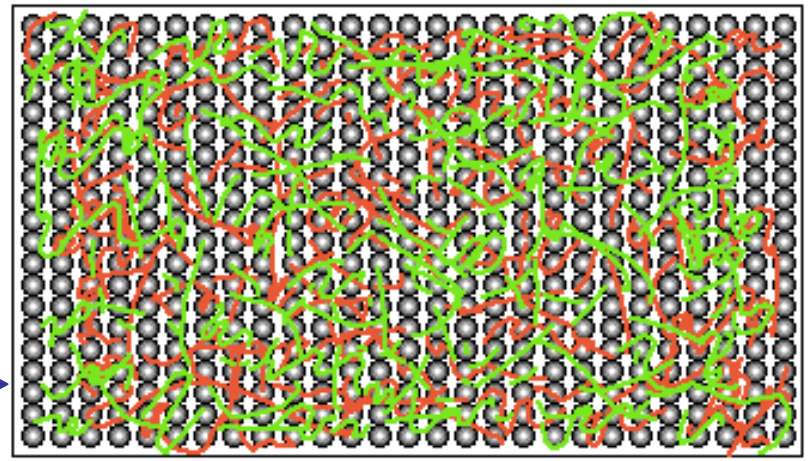
Courtesy of Qubit Systems. Used with permission.

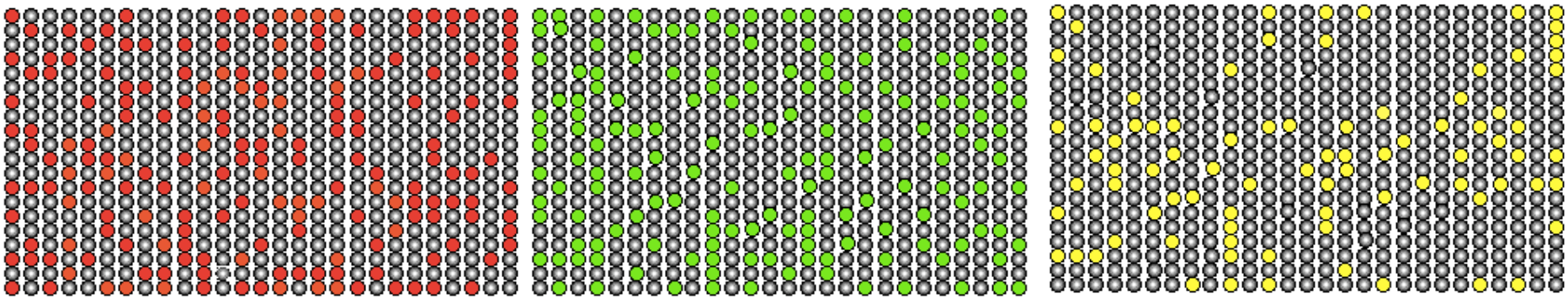
www.qubitsystems.com

Microarray



wash and scan





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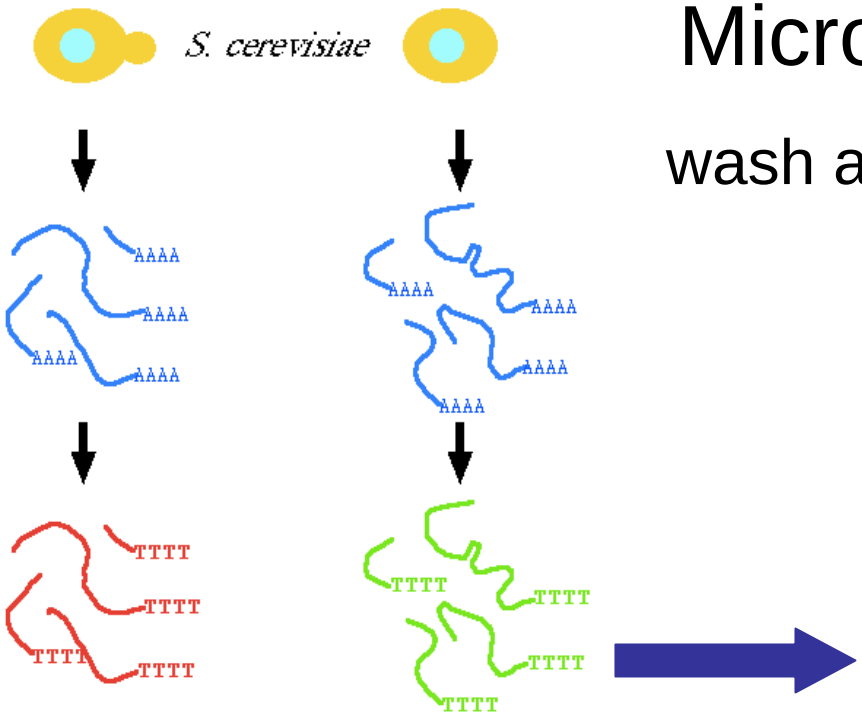

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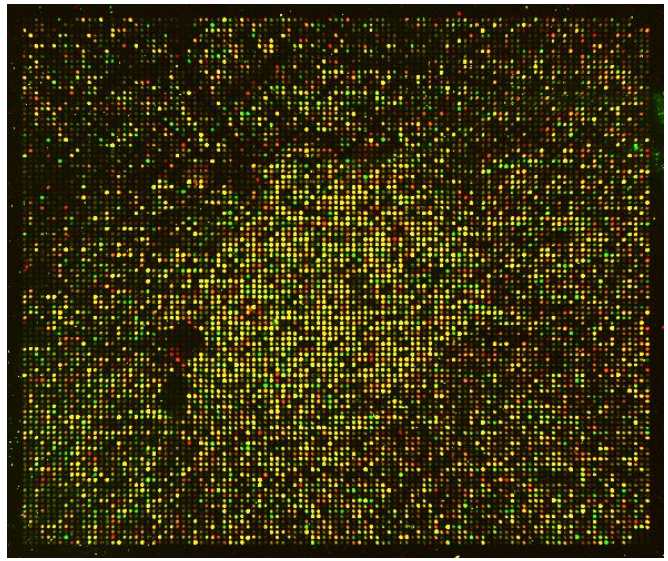

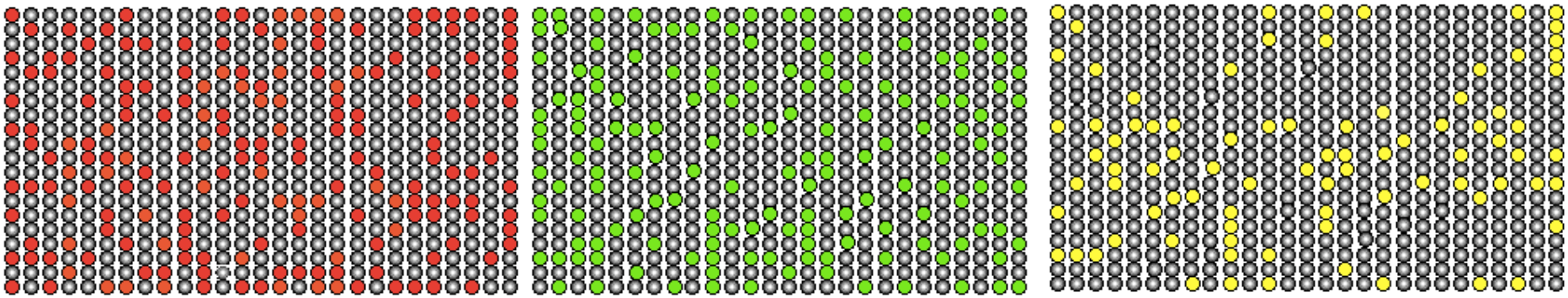

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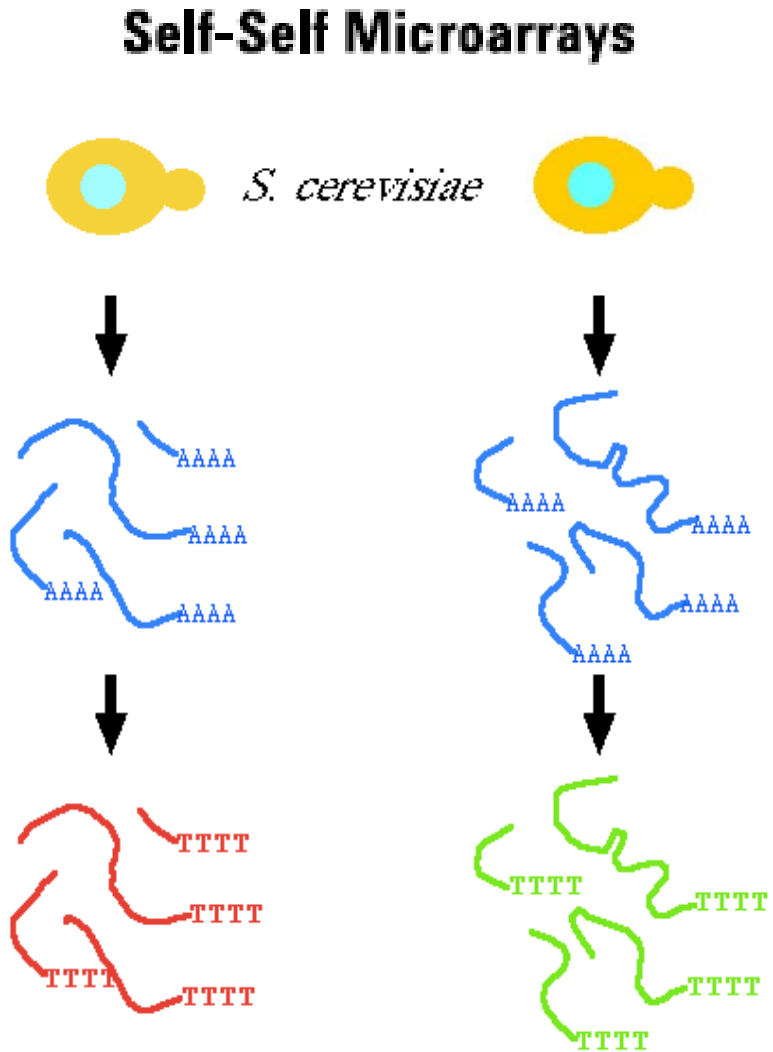
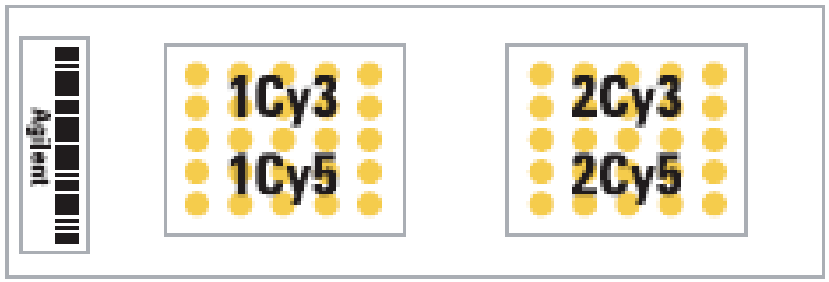
Microarray

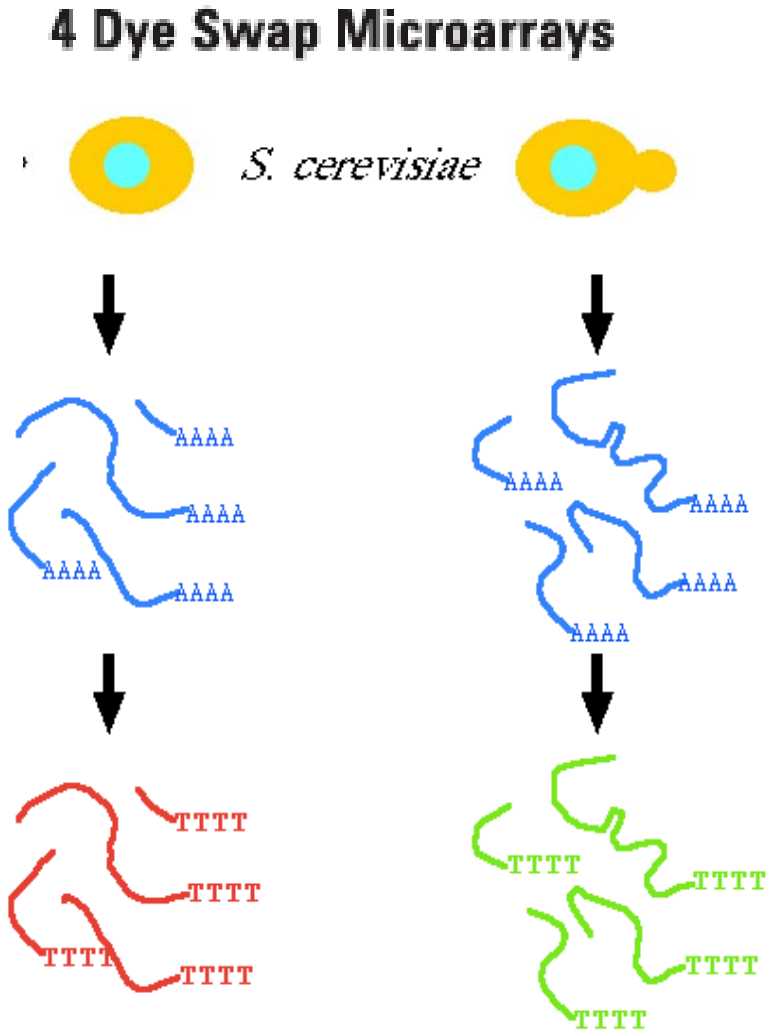
controls



Microarray
controls



Courtesy of Agilent Technologies, Inc. Used with permission.



Courtesy of A. Malcolm Campbell. Used with permission.

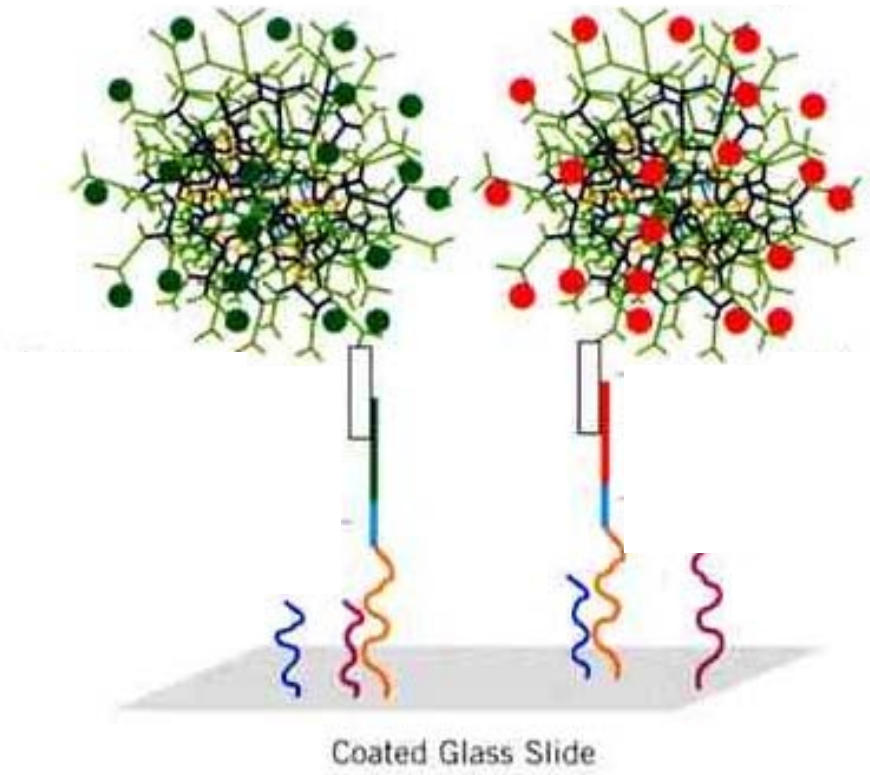
Microarray

controls

Courtesy of Agilent Technologies, Inc. Used with permission.

Microarray

indirect labeling method



Courtesy of Genisphere Inc. Used with permission.

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

MIAME

Minimal Information About a Microarray Exp't

Provide:

1. Raw data for each hybridization
2. Final processed data for the set of hybridizations
3. Experimental factors and their values (e.g., compound and dose in a dose response experiment)
4. Experimental sample relationships (e.g., which raw data file relates to which sample, which hybridisations are technical, which are biological replicates)
5. Array annotation (e.g., commercial array catalog number)
6. Data processing protocols (e.g., normalization method used)

Diagnostic Tool: is it cancer?

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.

Figure removed due to copyright restrictions.
See Couzin, Jennifer. “Amid Debate, Gene-Based Cancer Test Approved.” Science 315 (2007): 924.

Available treatments
surgery, chemo, radiation, hormone

Science **2007** 315:924

Cancergeek.com

Treatment Evaluation Tool: will tumor be hormone-responsive?

Image of ligand and receptor removed due to copyright restrictions.

~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)

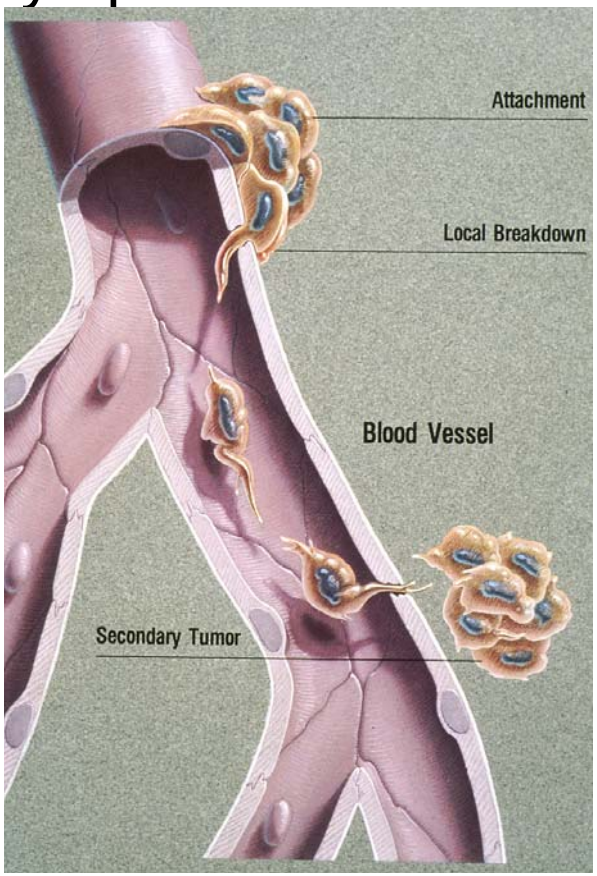
~ 10% of breast cancers are ER+ and PR-

~ 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

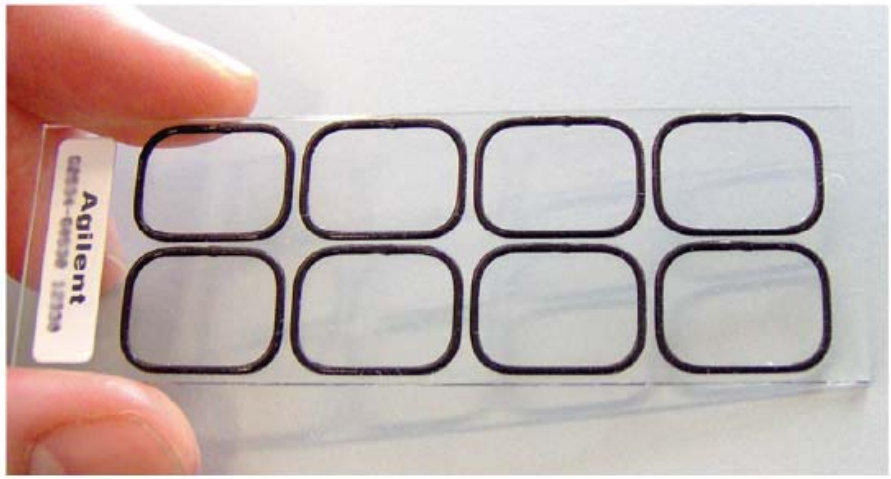


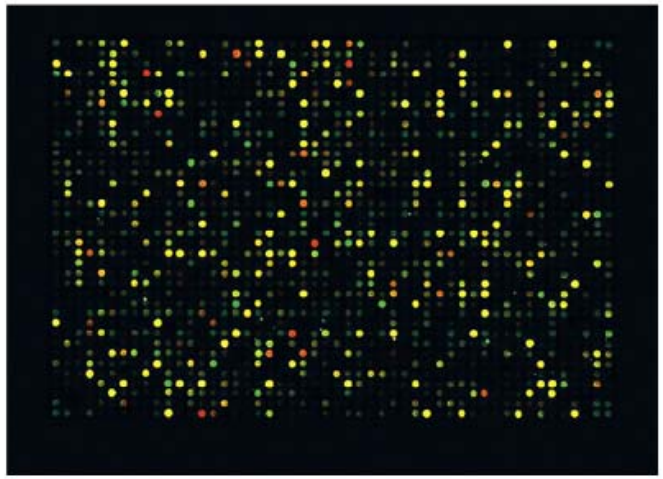
Courtesy of National Cancer Institutes.
Illustration by Jane Hurd.

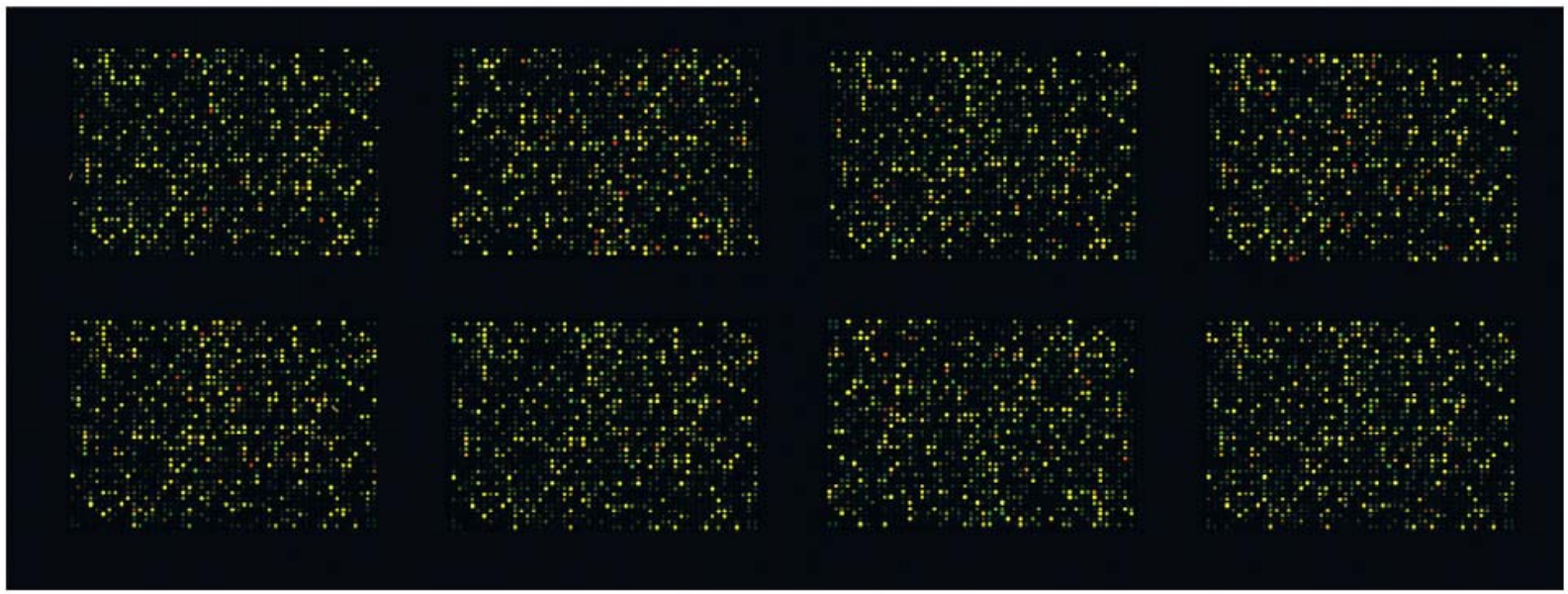
"Chemotherapy and/or hormonal therapy reduce the risk of distant metastases by approximately one-third; however, 70-80% of patients receiving this treatment would have survived without it."

Agendia

FDA approved “MammaPrint” assay





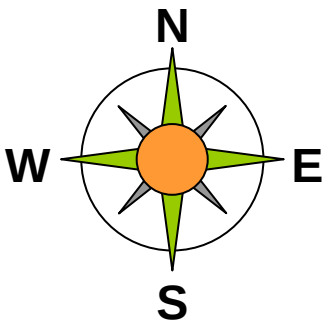


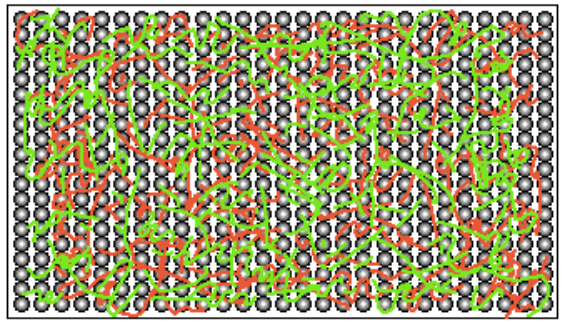
Courtesy of Agendia. Used with permission.

Summary

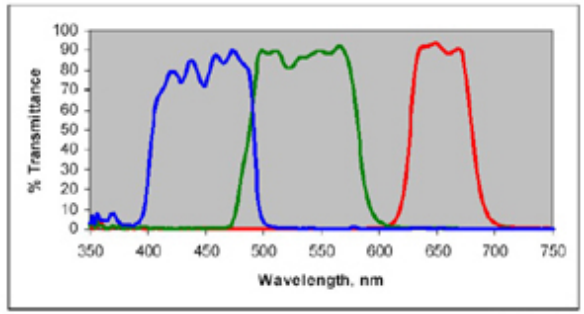
Images of James Watson and Francis Crick
images removed due to copyright restrictions.

1. Mechanics of microarrays





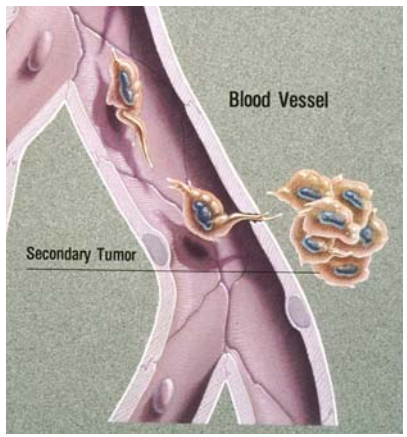
Courtesy of A. Malcolm Campbell. Used with permission.



Courtesy of Qubit Systems. Used with permission.

2. Microarrays for molecular medicine

Image of ligand and receptor
removed due to copyright restrictions.



Courtesy of National Cancer Institute.
Illustration by Jane Hurd.

the end