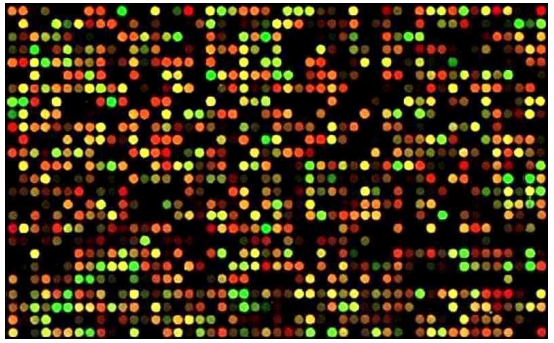
10-810 /02-710

Computational Genomics

Microarrays

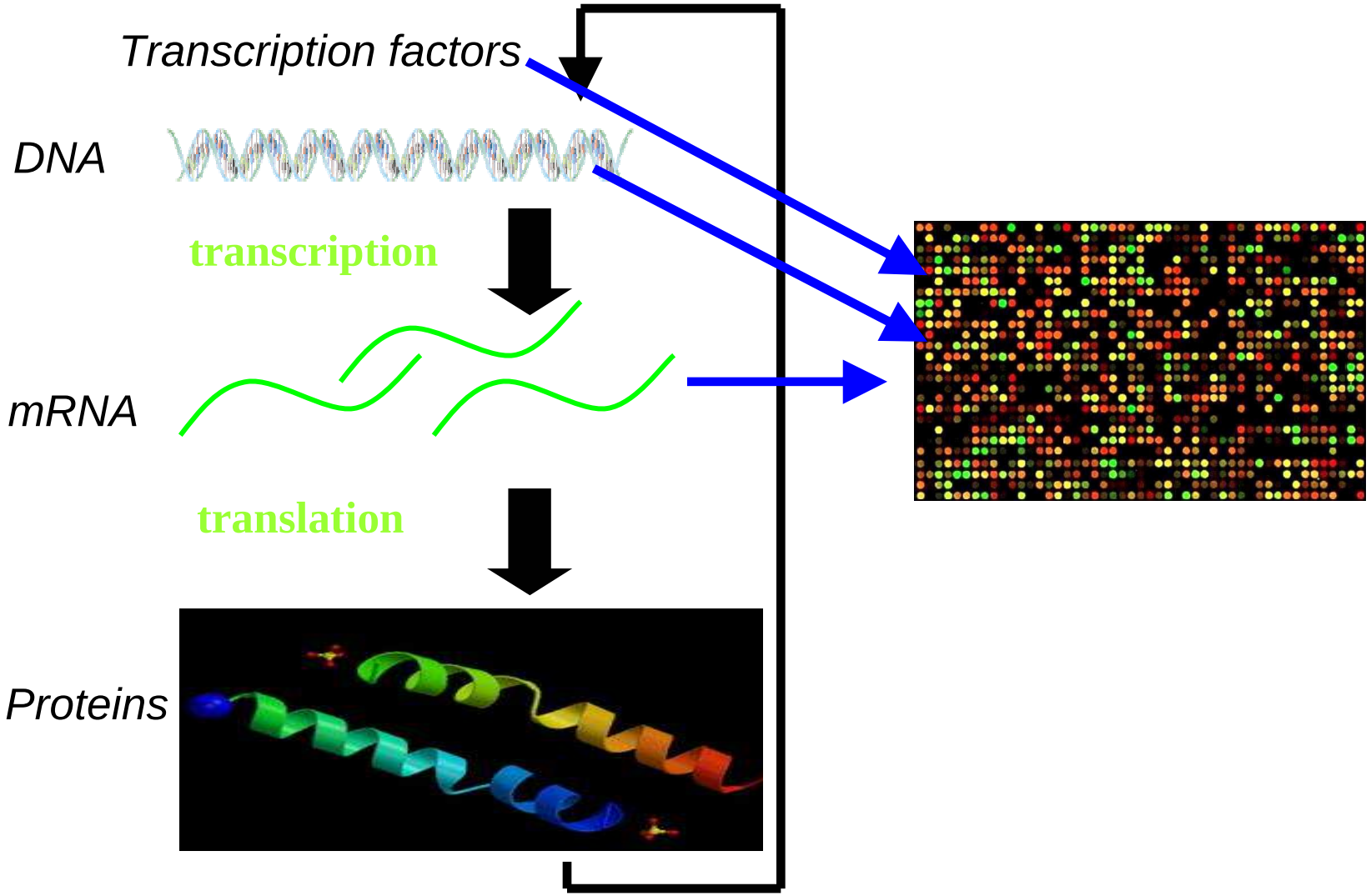


Why sequence is not enough

Identifying genes and control regions is not enough to decipher the inner workings of the cell:

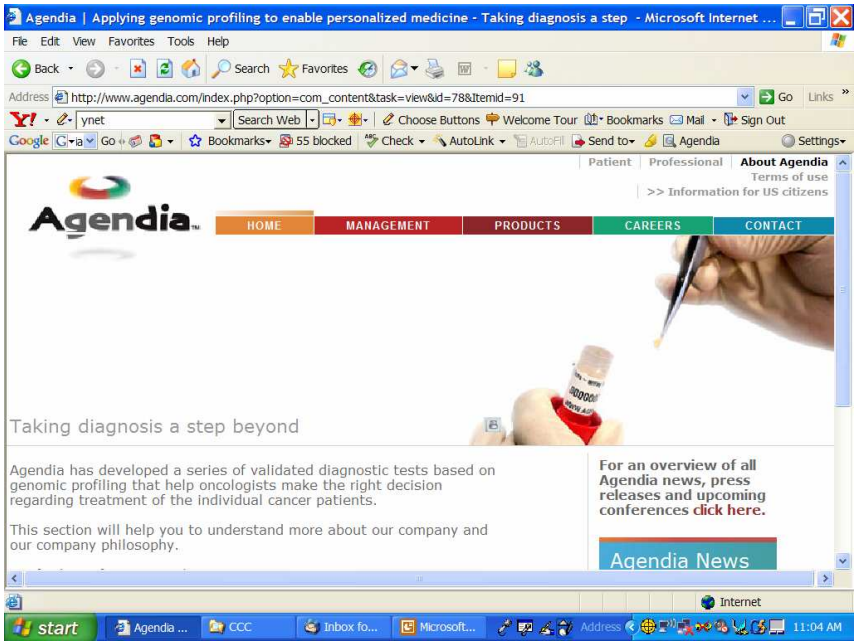
- We need to determine the function of genes.
- We would like to determine which genes are activated in which cells and under which conditions.
- We would like to know the relationships between genes (protein-DNA, protein-protein interactions etc.).
- We would like to model the various dynamic systems in the cell

Microarrays for molecular biology



FDA Approves Gene-Based Breast Cancer Test*

“ MammaPrint is a DNA microarray-based test that measures the activity of 70 genes... The test measures each of these genes in a sample of a woman's breast-cancer tumor and then uses a specific formula to determine whether the patient is deemed low risk or high risk for the spread of the cancer to another site.”



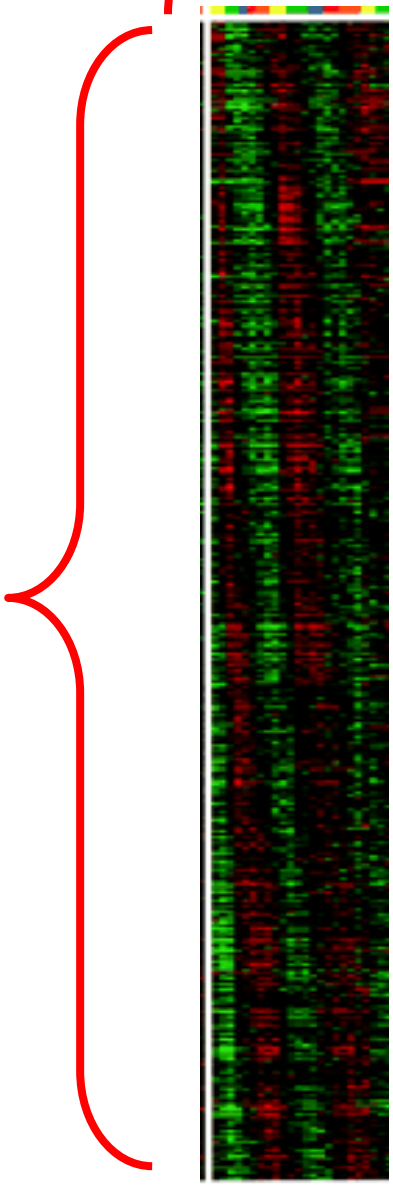
*Washington Post, 2/06/2007

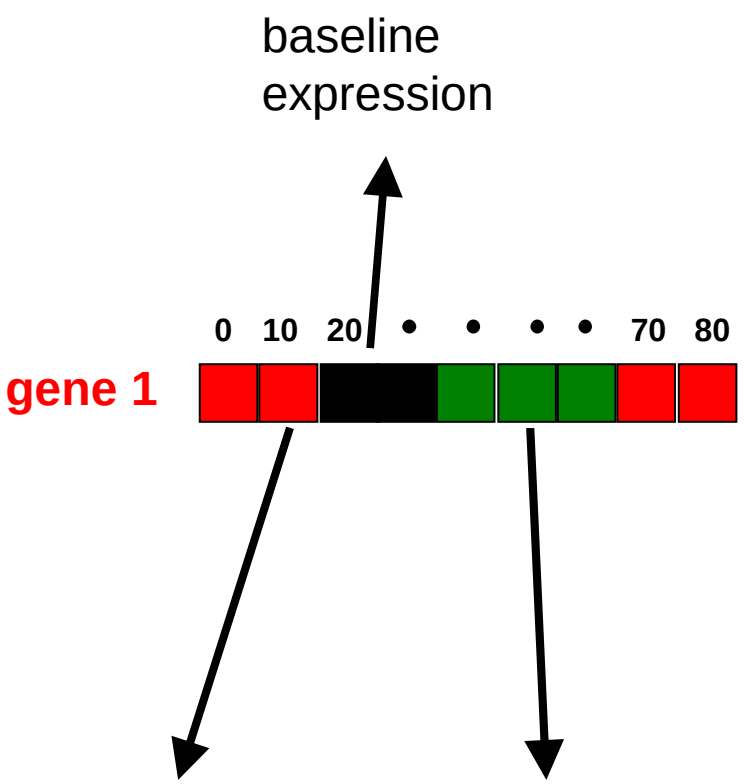
What is gene expression?

Expression = level of gene (protein) in this experiment

genes

Experiments (over time)





Higher expression compared to baseline

Lower expression compared to baseline

Spellman *et al Mol. Biol. Cell* 1998

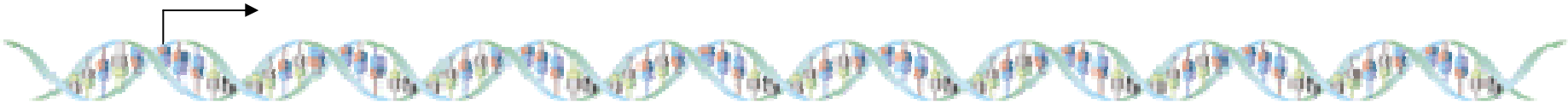
Genes and Gene Expression

Technology

Display of Expression Information

What is a gene?

Promoter Protein coding sequence Terminator

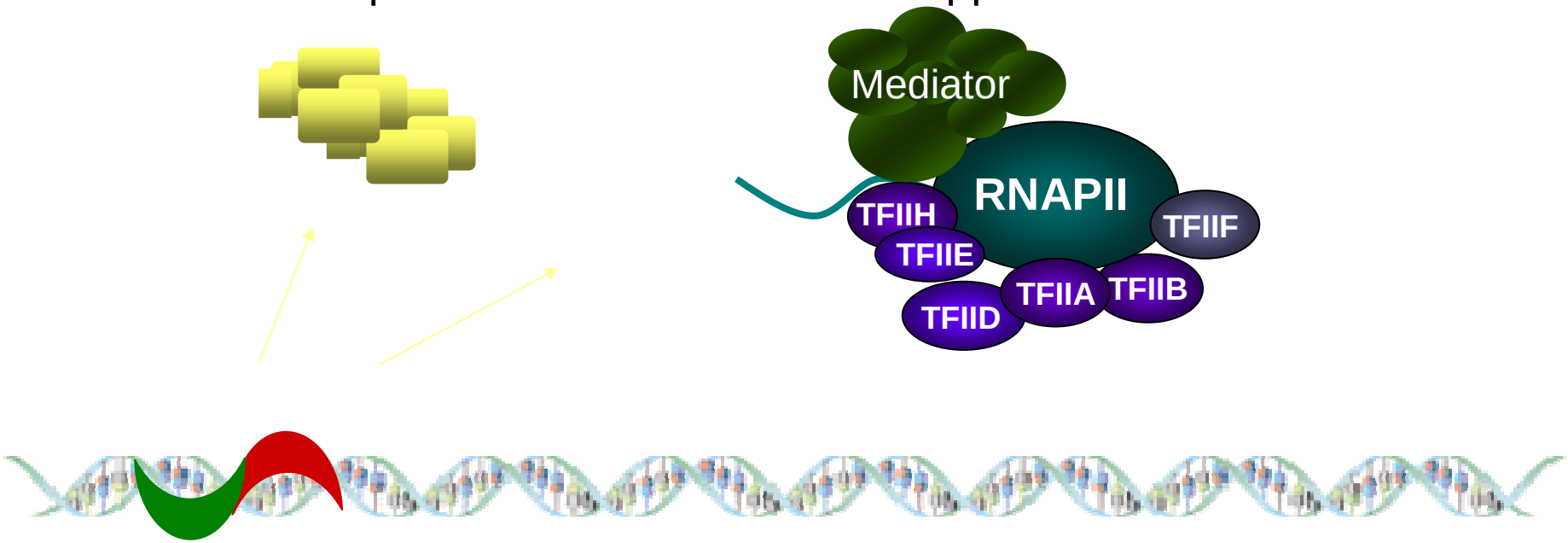


Genomic DNA

How are Genes Regulated?
DNA-binding Activators Are Key To Specific Gene Expression

Chromatin modification complexes

Transcription initiation apparatus



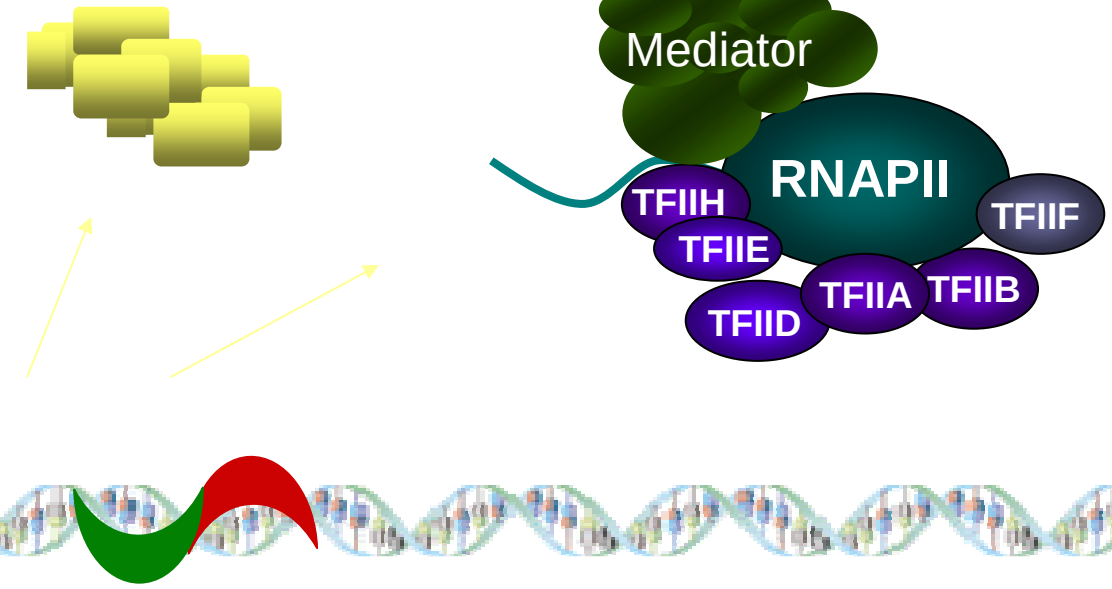
Activators

Gene

How are Genes Regulated?
DNA-binding activators are key, but there are additional factors

Chromatin modification complexes

Transcription initiation apparatus

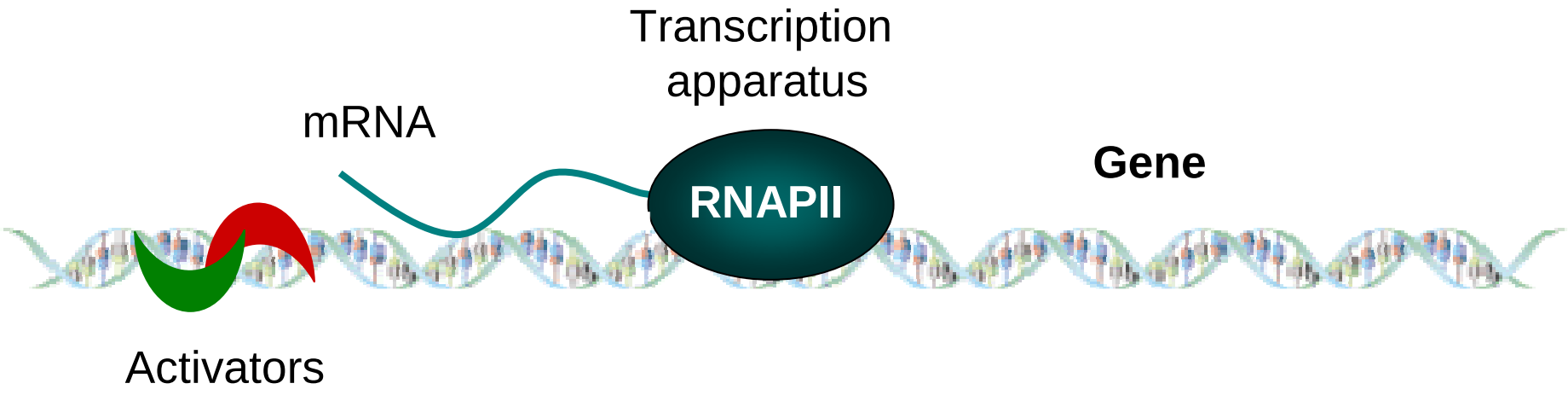


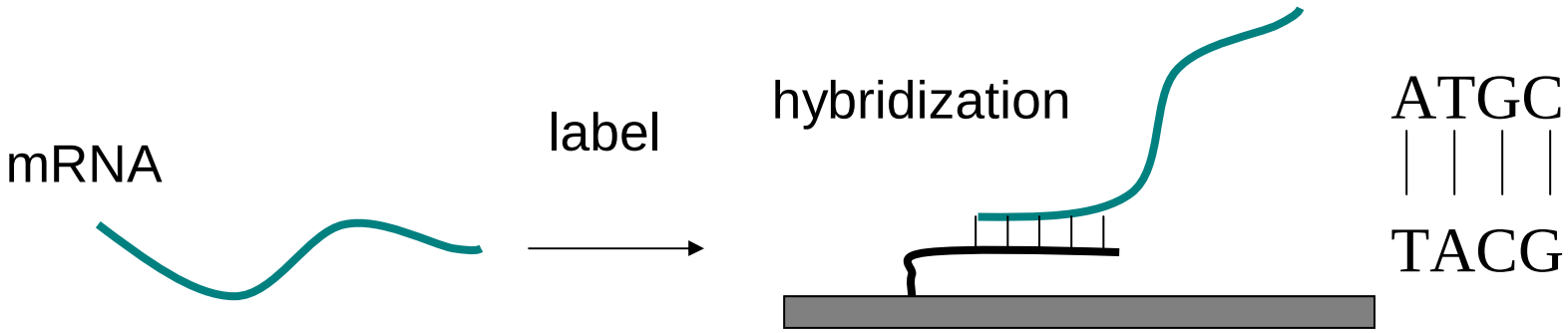
activators
repressors
coactivators
corepressors
transcription apparatus
chromatin factors
RNA processing
RNA transport
RNA degradation

Activators

Gene

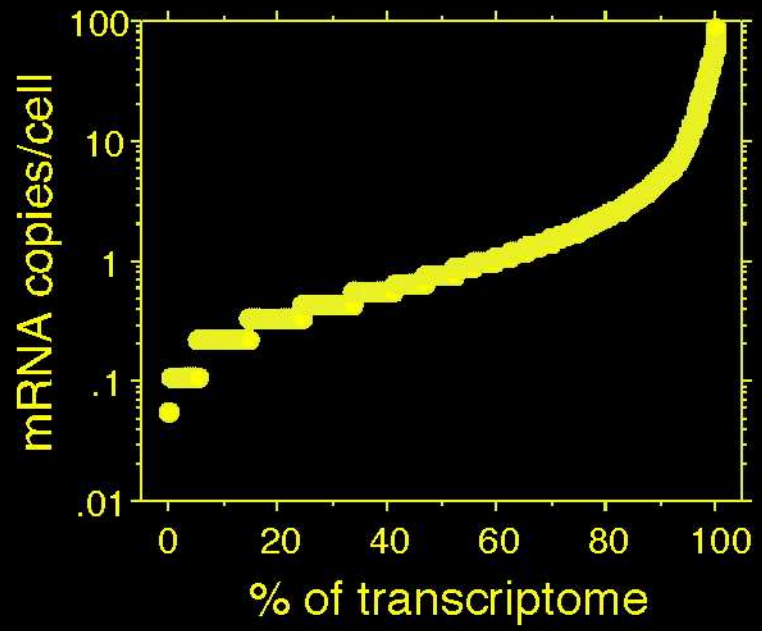
Genome-wide Gene Expression (mRNA) can be Measured with DNA Microarrays





Yeast Transcriptome (Glucose)

5460 mRNA species
average level: 2.8 copies/cell
median level: 0.8 copies/cell



80% of the transcriptome is
expressed at 0.1 - 2 mRNA copies/cell

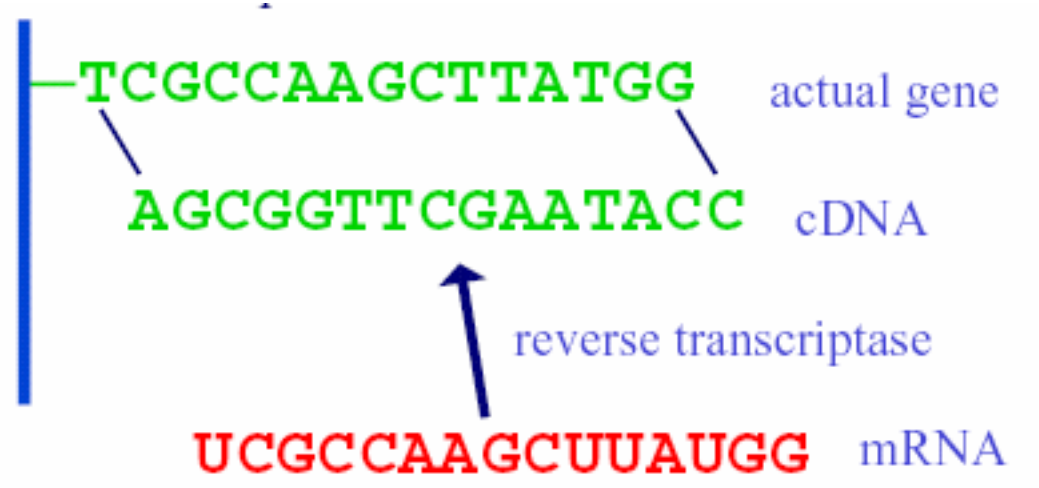
Genes and Gene Expression

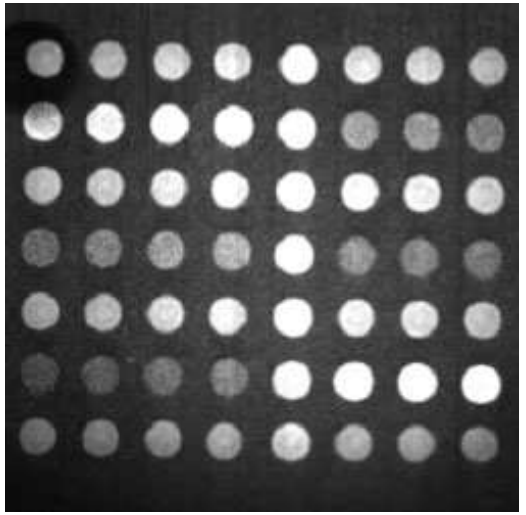
Technology

Display of Expression Information

Microarray Hybridization

- Watson-Crick base pairing of complementary DNA sequences.





- Microarrays have thousands of spots, each representing a piece of one gene, immobilized on a glass slide.
- The intensity (or intensity ratio) of each spot indicates the amount of labeled cDNA hybridized, thus, representing the starting mRNA transcript abundance.

Two major technologies

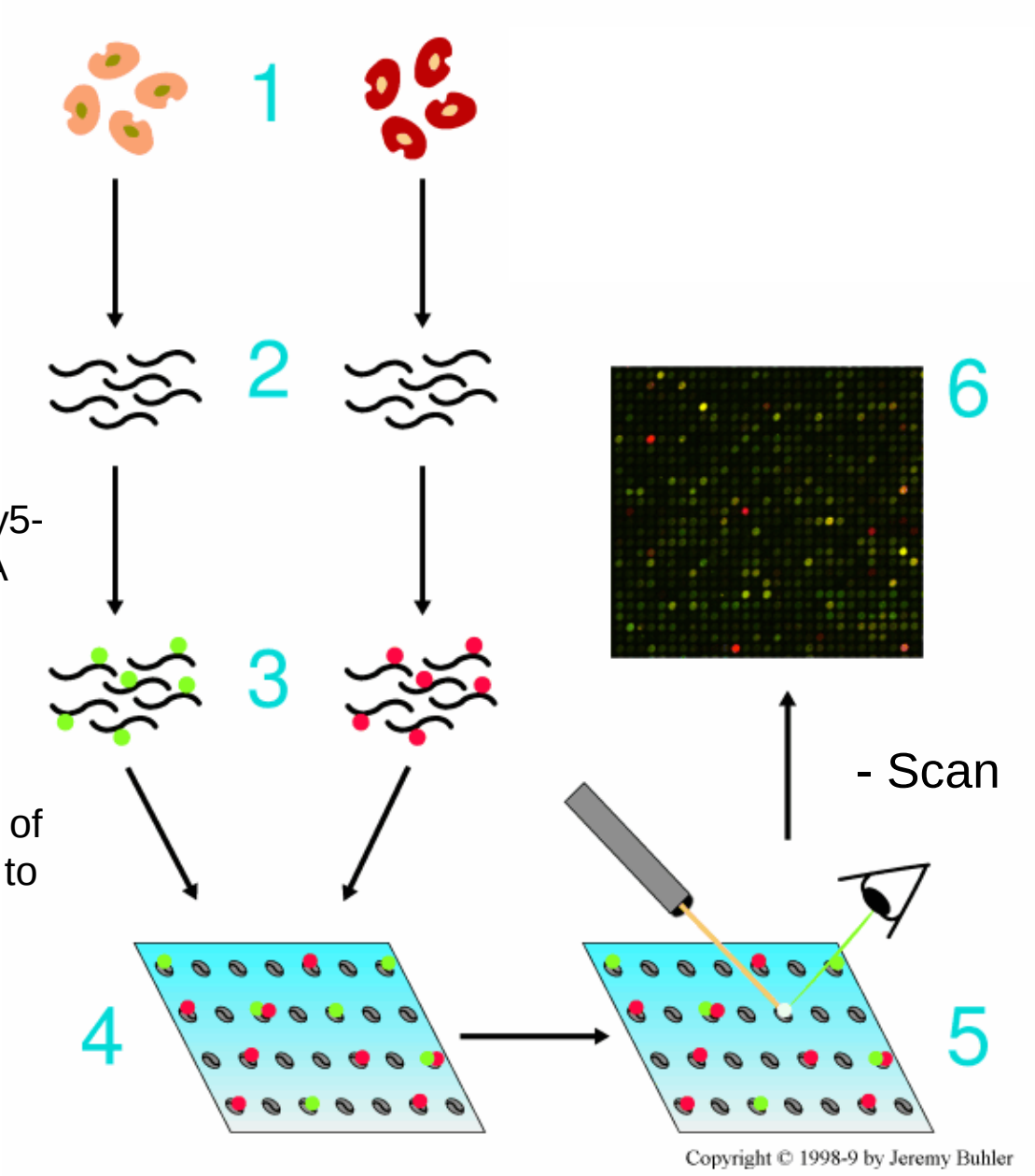
- cDNA arrays
  - probes are placed on the slides
  - allows comparison of different cell types

- Oligonucleotide arrays
  - partial sequences are printed on the array
  - measure values in one tissue type

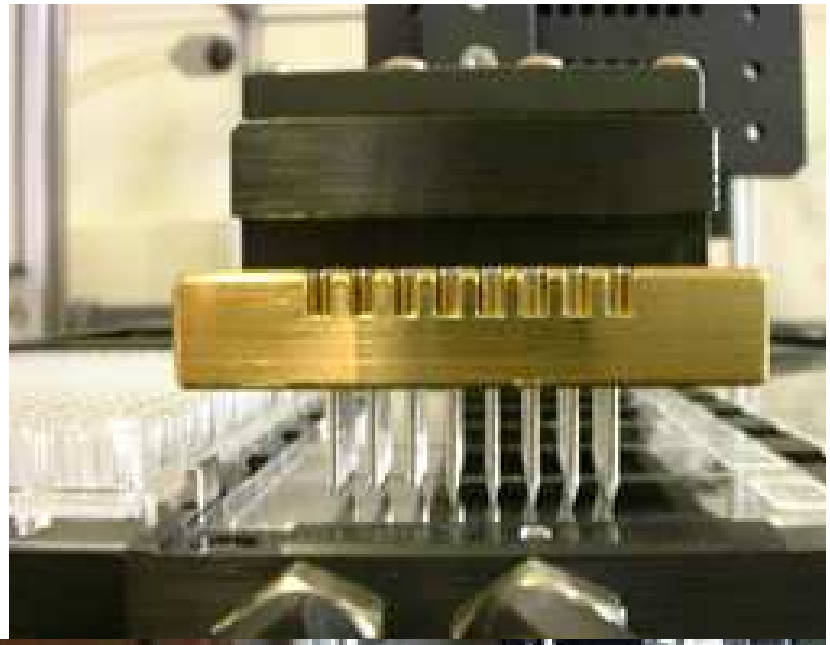
Hybridization and Scanning— cDNA arrays

- Prepare Cy3, Cy5-labeled ss cDNA

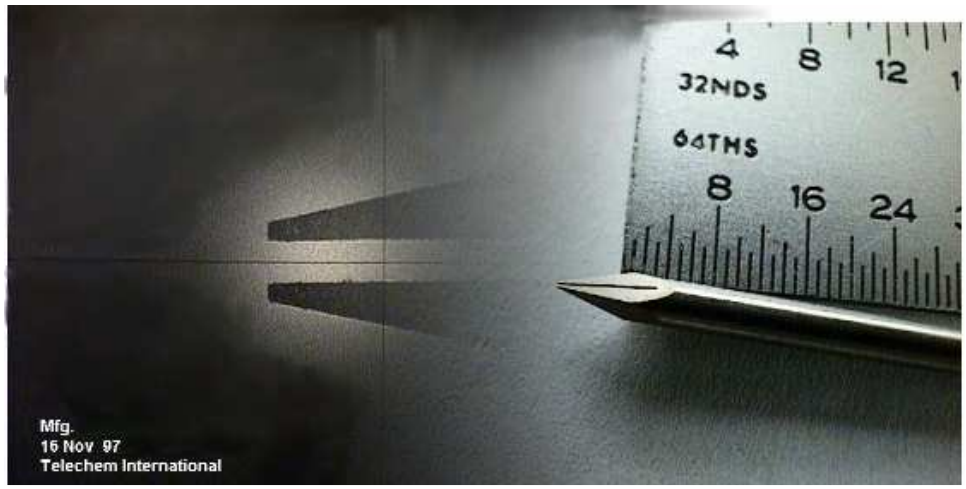
- Hybridize 600 ng of labeled ss cDNA to glass slide array



Cartesian PixSys 5500 with quill printing technology



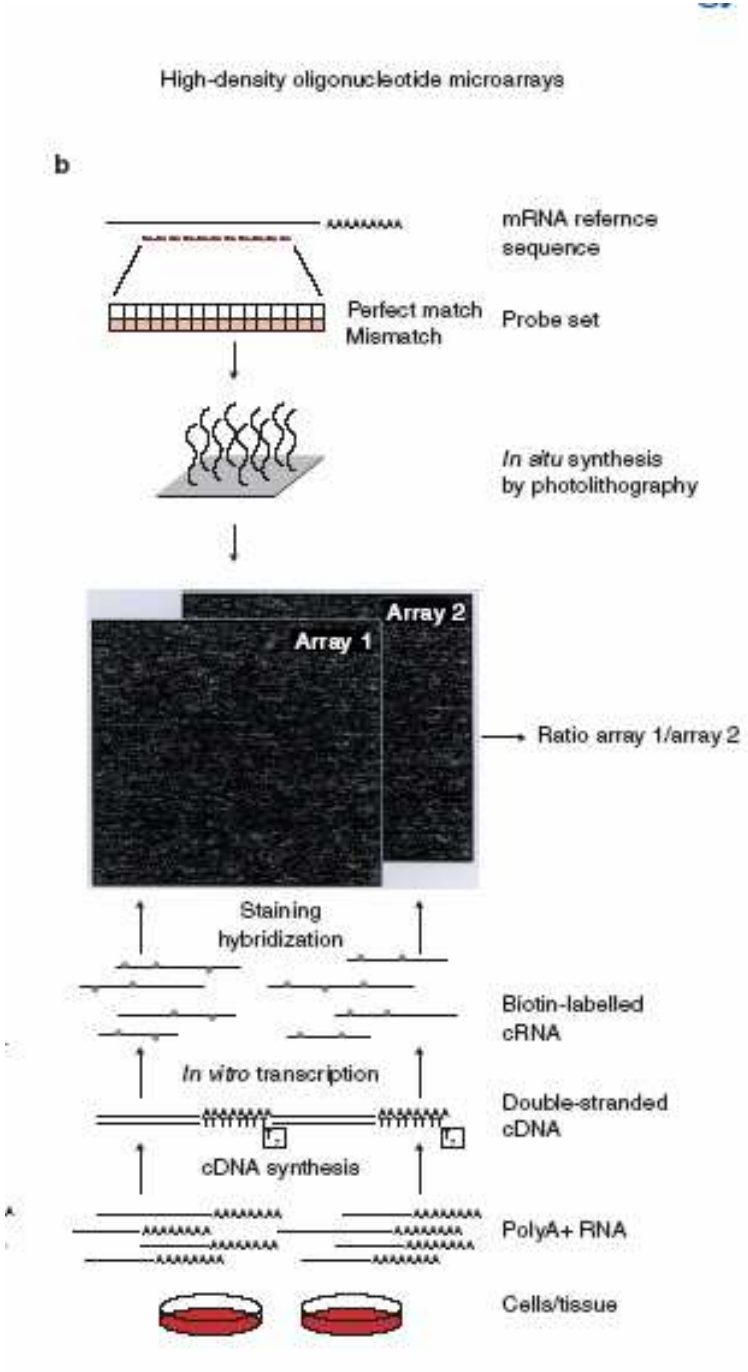
- Complete subsequences are printed on the array
- 10,000 spots/slide
- Spots are 100-200 µm in diameter
- Hybridization volumes: 20-100ul



Array Scanning

Laser based - fluorescent emission

Hybridization and Scanning—oligo arrays



cDNA vs. Oligo: Pros and Cons

cDNA

- Does not require sequence
- Cheap
- Direct comparisons
- Inaccurate
- Cannot measure individual samples

Oligo

- Can be designed to minimize cross hybridization
- Allows for internal control
- Both lead to better accuracy
- expensive
- limited to certain species

Errors

Microarrays introduce many errors which should be taken into account when working with measured expression values:

- Scanning errors
- Spotting errors
- Cross hybridization
- Errors related to day / reading device / experimentalist
- Background differences between slides

Error types

Microarrays introduce many types of errors which should be taken into account when working with measured expression values:

- Scanning errors additive + multiplicative
- Spotting errors multiplicative
- Cross hybridization multiplicative
- Errors related to day / reading device / experimentalist

additive + multiplicative

- Background differences between slides additive

Handling the Different Errors

- Scanning errors
- Spotting errors
- Cross hybridization
- Errors related to day / reading device / experimentalist
- Background differences between slides

Analysis of image data (we assume it was performed)

Handling the Different Errors

- Scanning errors
- Spotting errors
- Cross hybridization
- Errors related to day / reading device / experimentalist
- Background differences between slides

Use ratio instead of individual values:

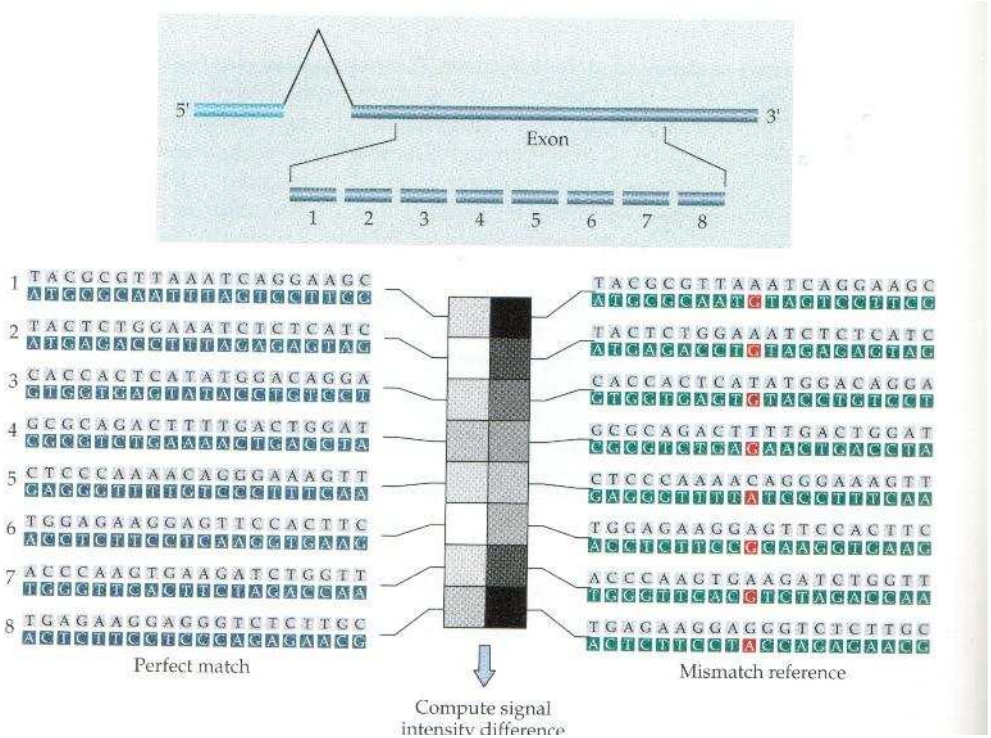
$Y_i = R_i / G_i$

Handling the Different Errors

- Scanning errors
- Spotting errors
- Cross hybridization
- Errors related to day / reading device / experimentalist
- Background differences between slides

For Oligo arrays, use the match / mismatch spots

Match / Mismatch



• Presence and absent calls can be made using the Match / Mismatch information.

• However, it has been reported that in some cases the mismatch was higher than the match.

Handling the Different Errors

- Scanning errors
- Spotting errors
- Cross hybridization
- Errors related to day / reading device / experimentalist
- Background differences between slides

Normalization (later)

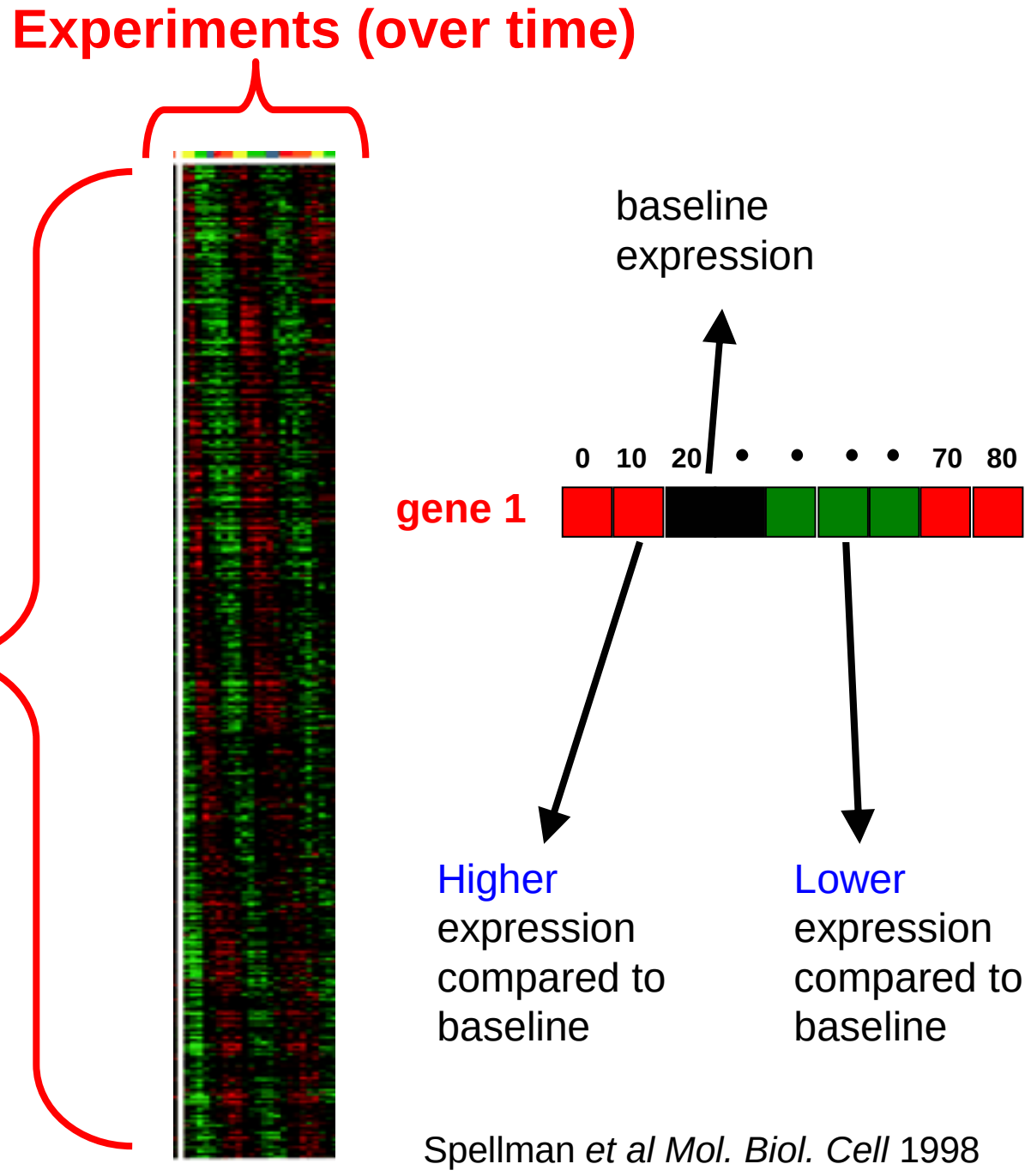
Binding arrays

- Instead of printing the genes on the microarray, we can print the intergenic region (an area upstream of the gene).
- We tag a protein of interest (a transcription factor) and fuse all proteins to DNA.
- Next, we hybridize the extracted portions of DNA onto the array, resulting in areas that are bound by the TF being spotted on the microarray.

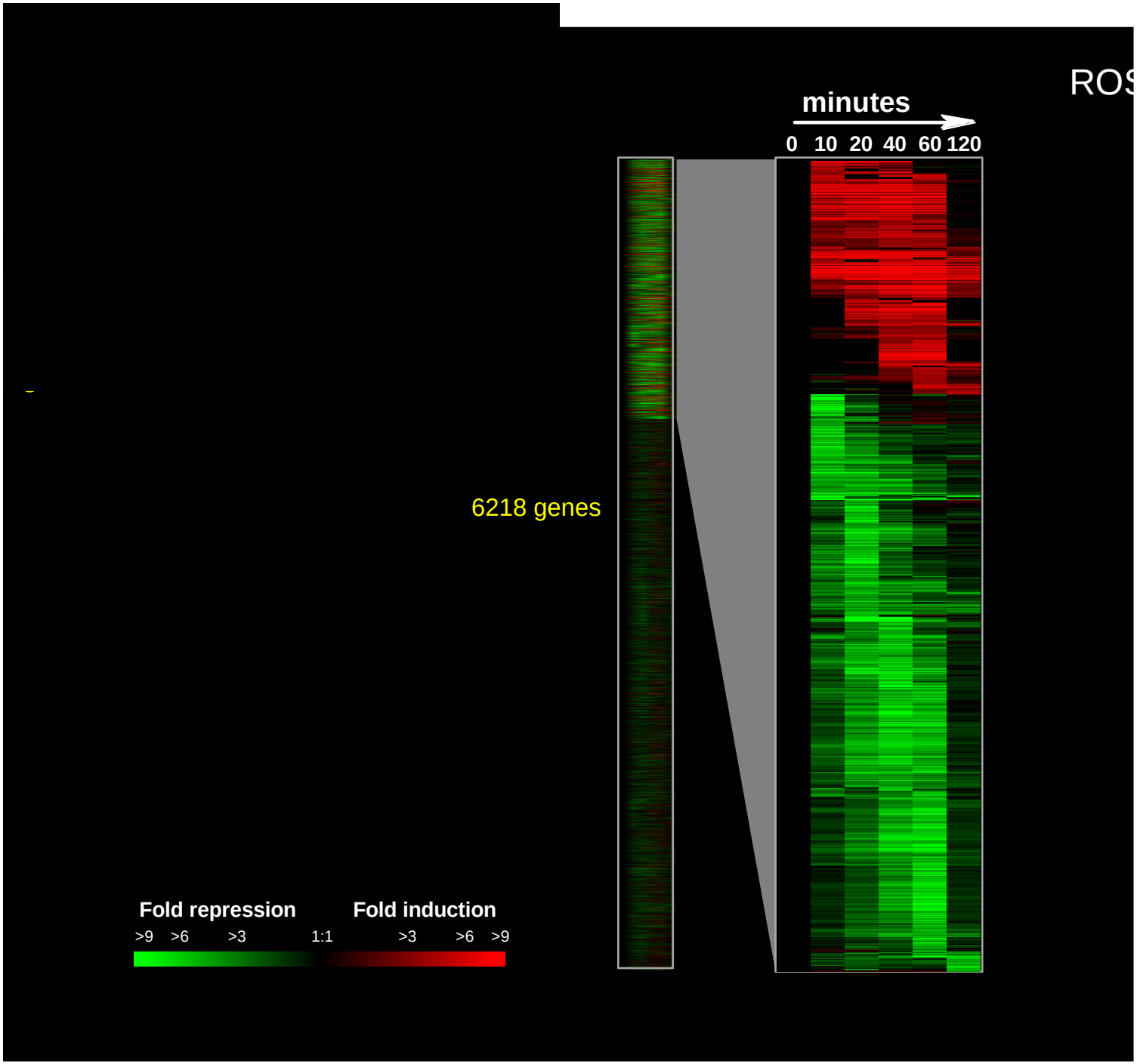
Genes and Gene Expression Technology

Display of Expression Information

Yeast cell cycle expression program

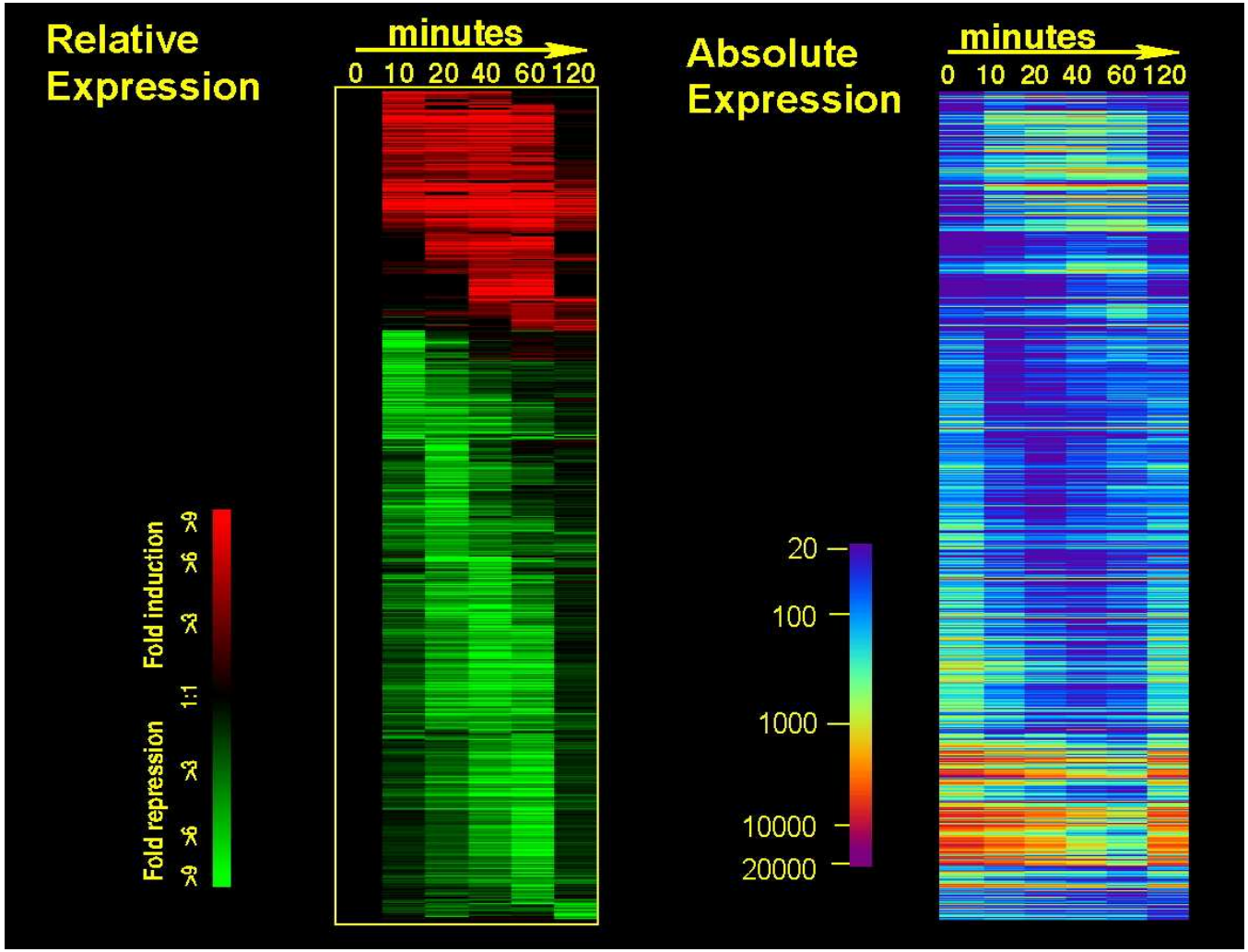


Spellman *et al Mol. Biol. Cell* 1998

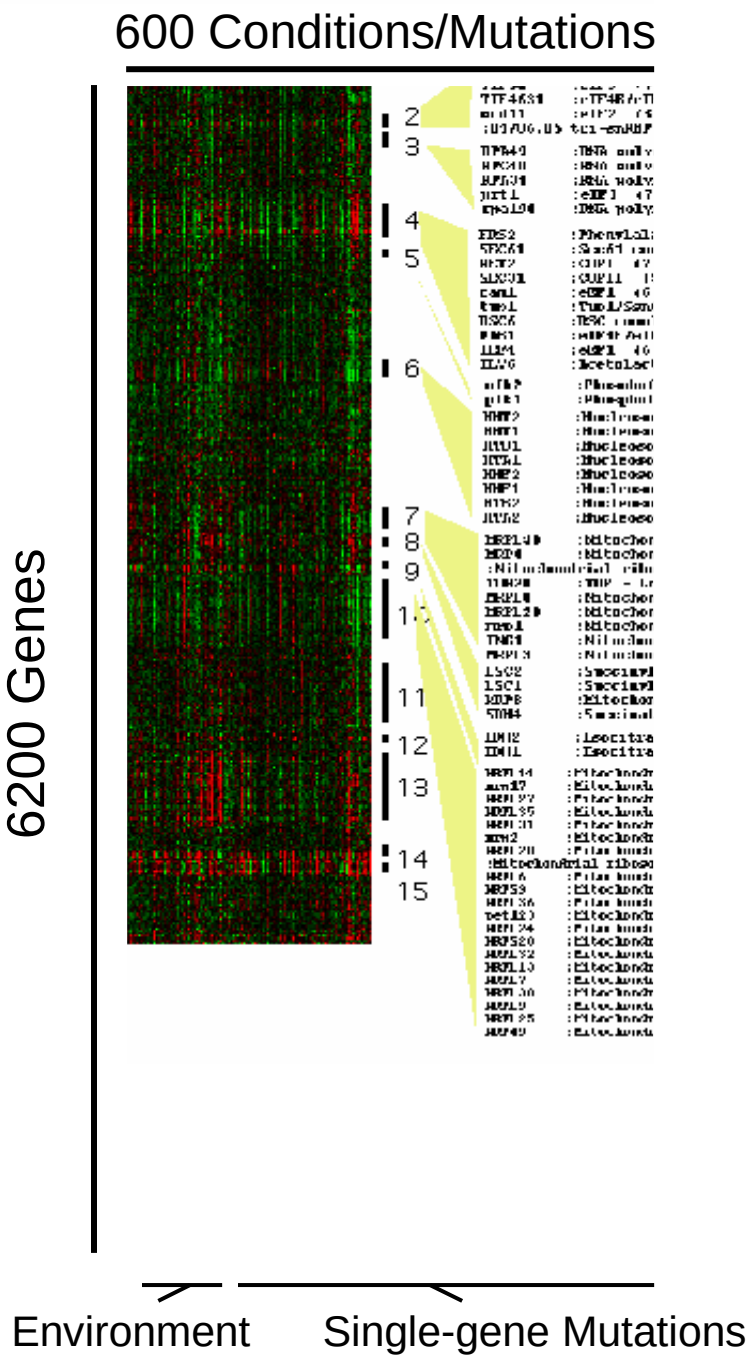

ROS
minutes
0 10 20 40 60 120
6218 genes
Fold repression
Fold induction
>9 >6 >3 1:1 >3 >6 >9

Visualization:

Relative vs. absolute expression

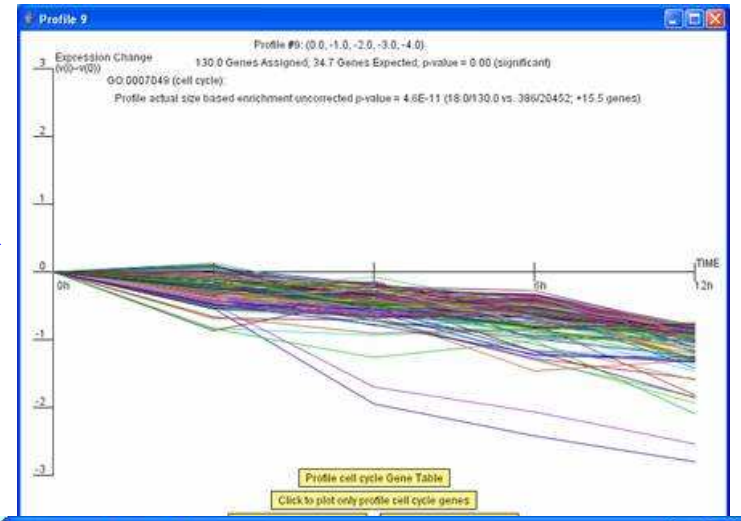


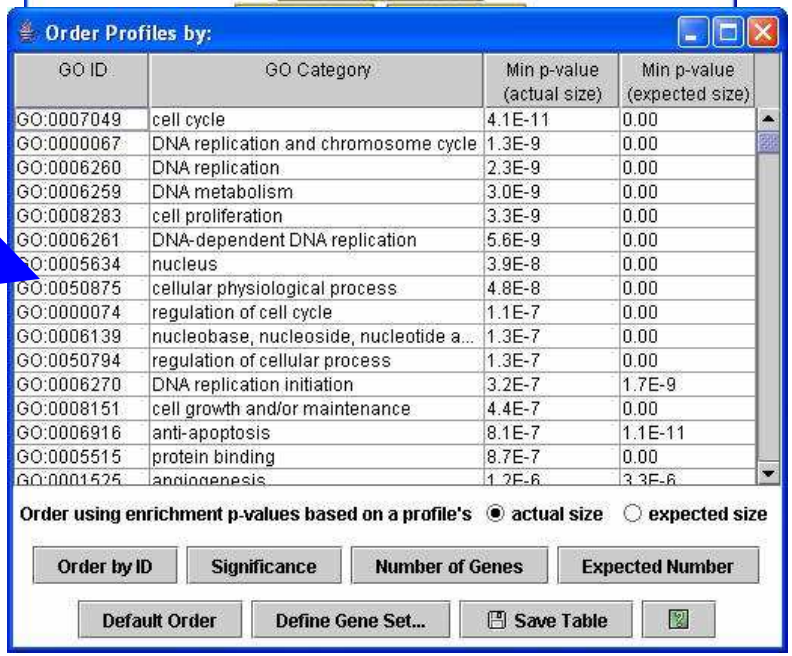
Exercising the Genome

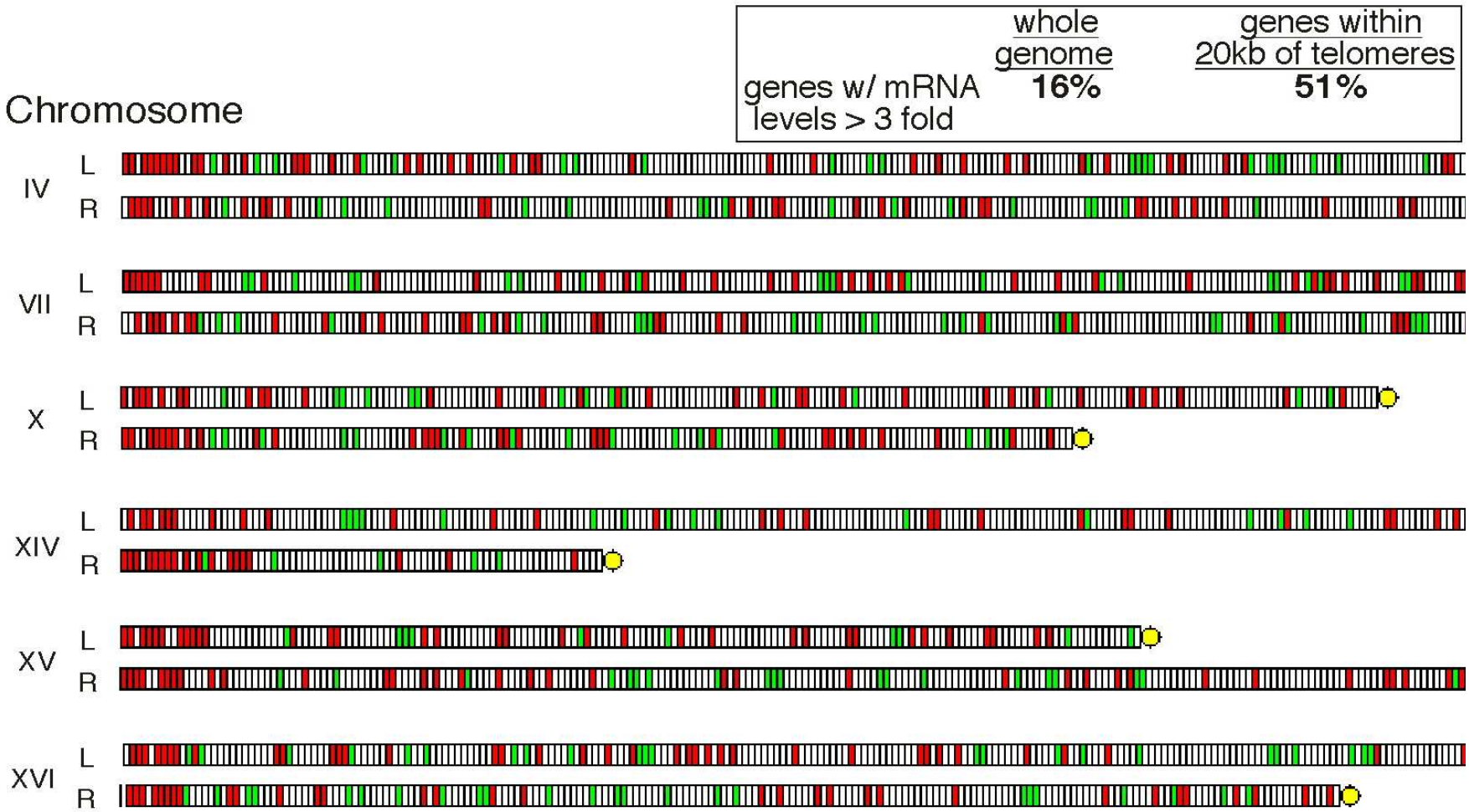


Using annotation databases

- Statistical tests to identify the overlap with various functional categories



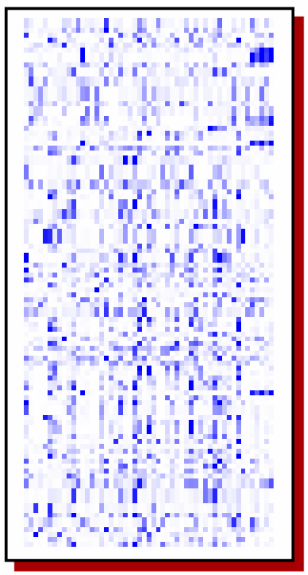


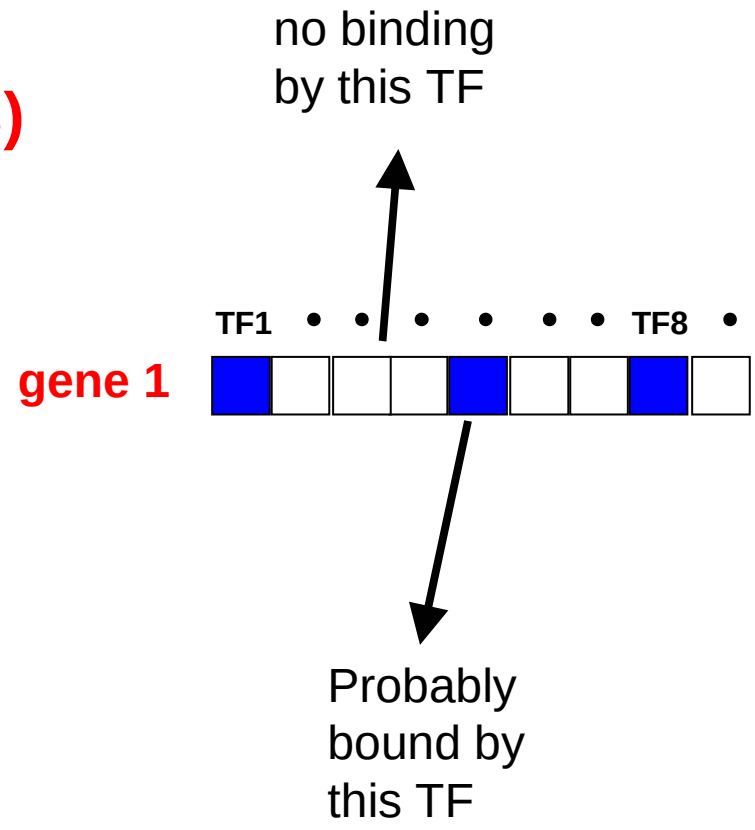

genes w/ mRNA levels > 3 fold
whole genome
16%
genes within 20kb of telomeres
51%
Chromosome
IV
L
R
VII
L
R
X
L
R
XIV
L
R
XV
L
R
XVI
L
R

Genome wide binding

experiments (transcription factors)

genes





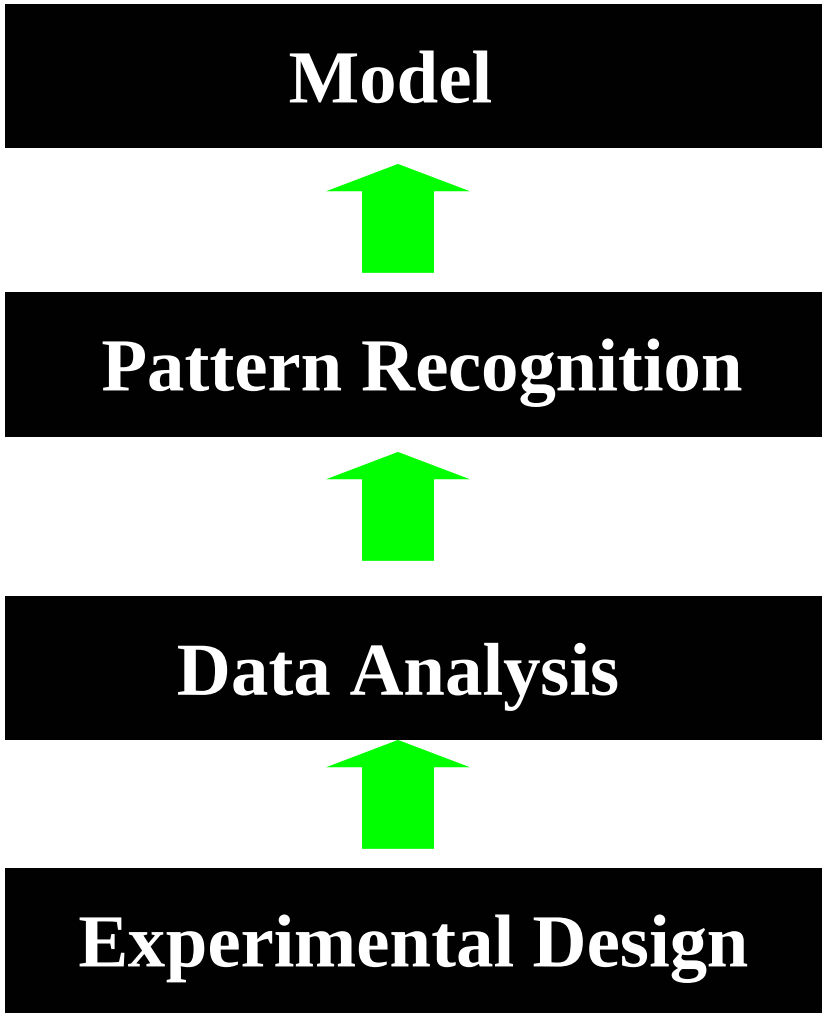
Lee *et al Science* 2002

What you should know

- The basic idea behind microarray profiling
- The two different microarray technologies
- Pros and cons for each
- Noise factors in microarray experiments (more next time)

Gene expression analysis

Gene Expression Analysis



Computational	Biological
information fusion	regulatory networks
clustering, classification	functional assignment, response programs
normalization, miss. value estimation	diff. expressed genes
array design, number of repeats	experiment selection

Experiment design

A number of computational issues should be addressed:

- Selecting short subsequences for oligo arrays to minimize cross hybridizations
- Determining the number of replicates for each sample
- Sampling rates for time series experiments

Data analysis

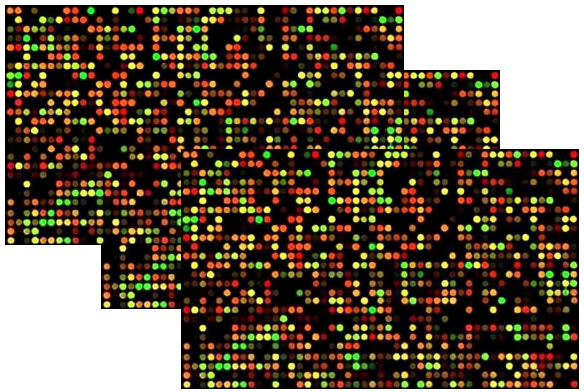
- Normalization
- Combining results from replicates
- Identifying differentially expressed genes
- Dealing with missing values
- Static vs. time series

Data analysis

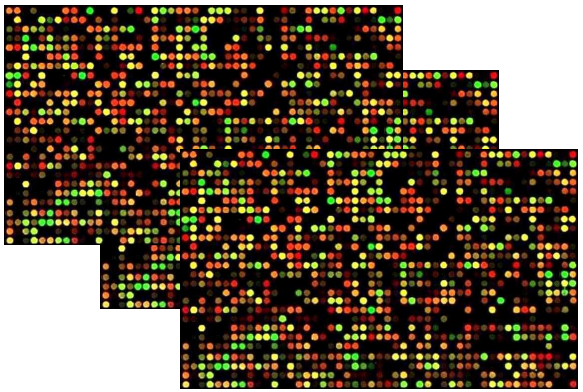
- Normalization
- Combining results from replicates
- Identifying differentially expressed genes
- Dealing with missing values
- Static vs. time series

Typical experiment: replicates

healthy



cancer



Technical replicates: same sample using multiple arrays

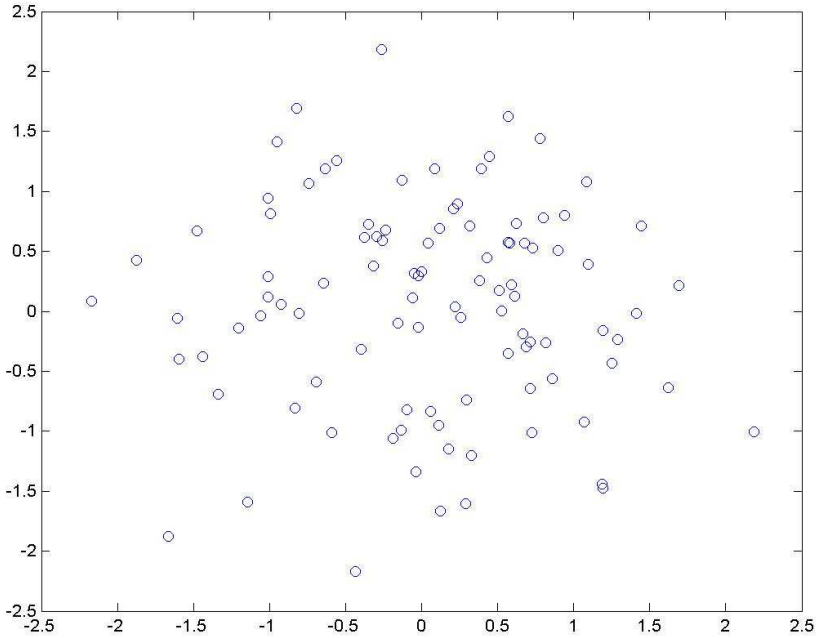
Dye swap: reverse the color code between arrays

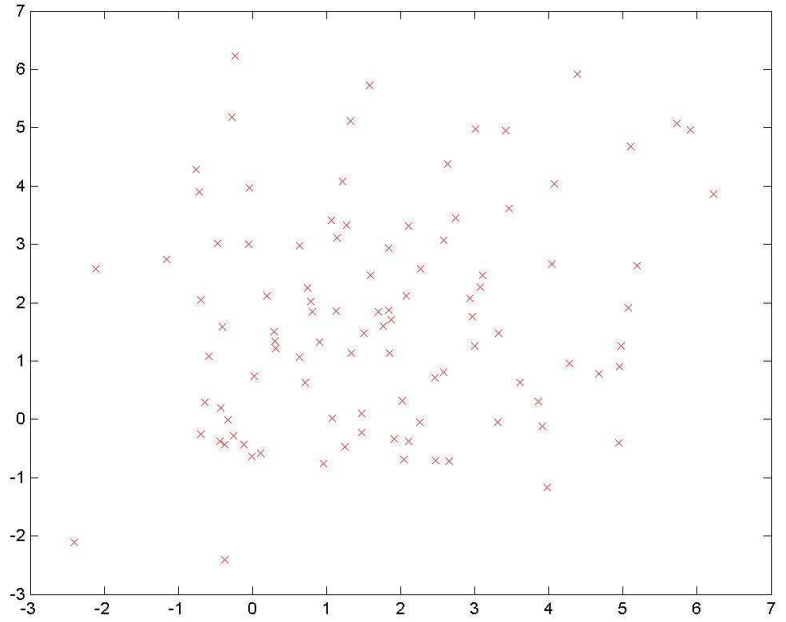
Clinical replicates: samples from different individuals

Many experiments have all three kinds of replicates

Normalizing across arrays

- Consider the following two sets of values:





Lets put them together …