High-throughput data

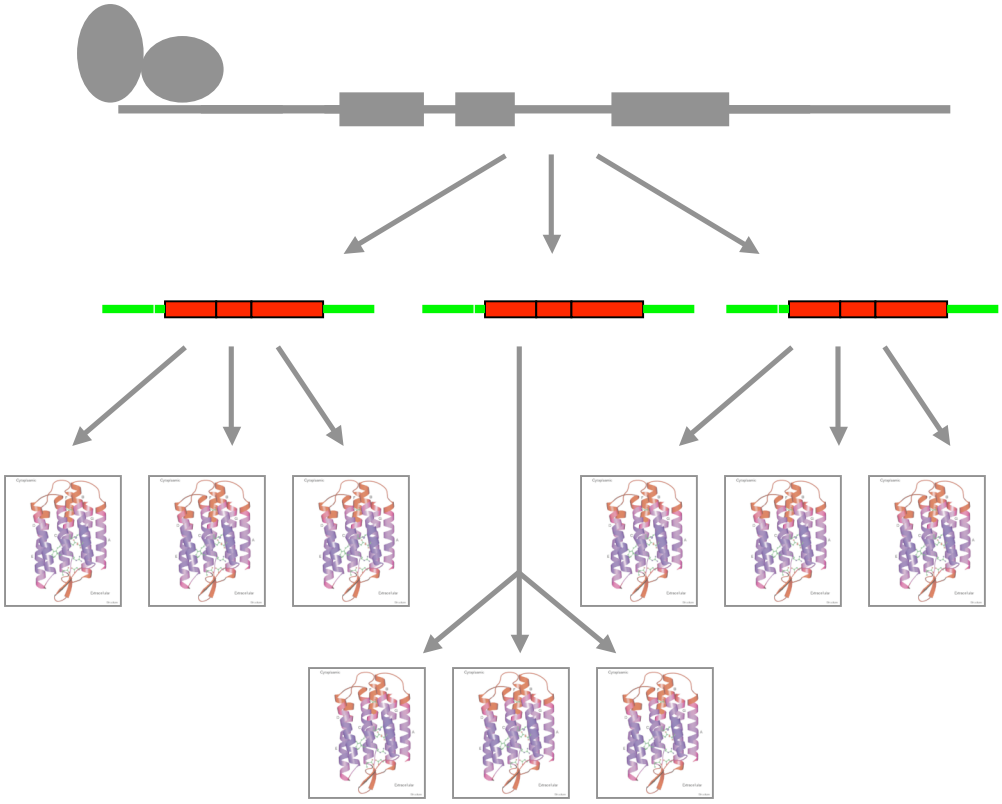
BBSI 2006: Lecture #(χ+4)

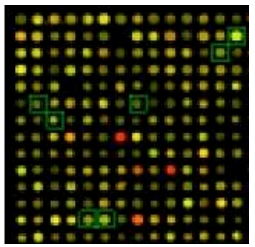
Takis Benos (2006)

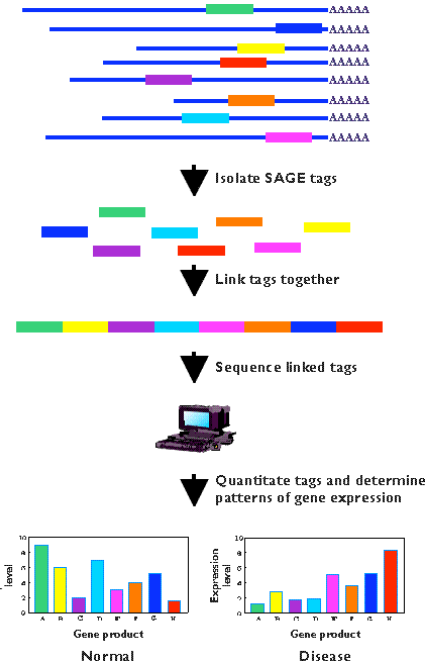
Overview

- Transcriptomics.
  - Microarrays
  - SAGE
- Proteomics.
  - 2D, gels, 2D DIGE
  - Mass-spec

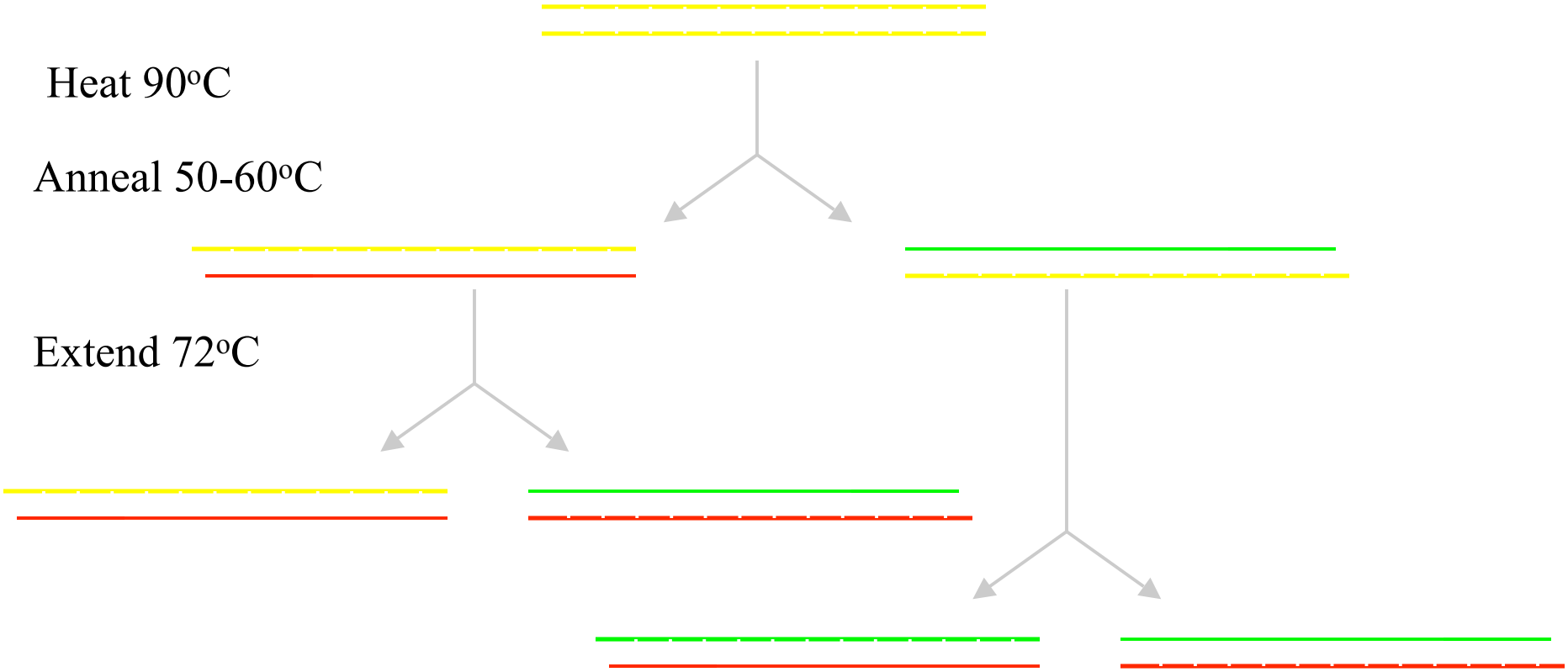
Transcriptomics



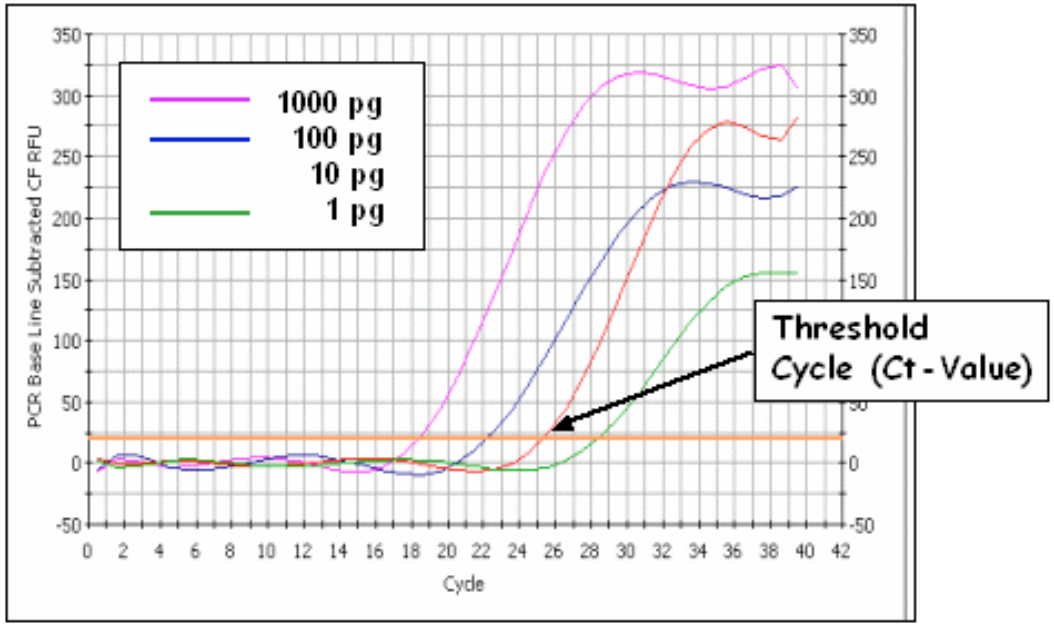




Polymerase Chain Reaction (PCR)



Real-time PCR



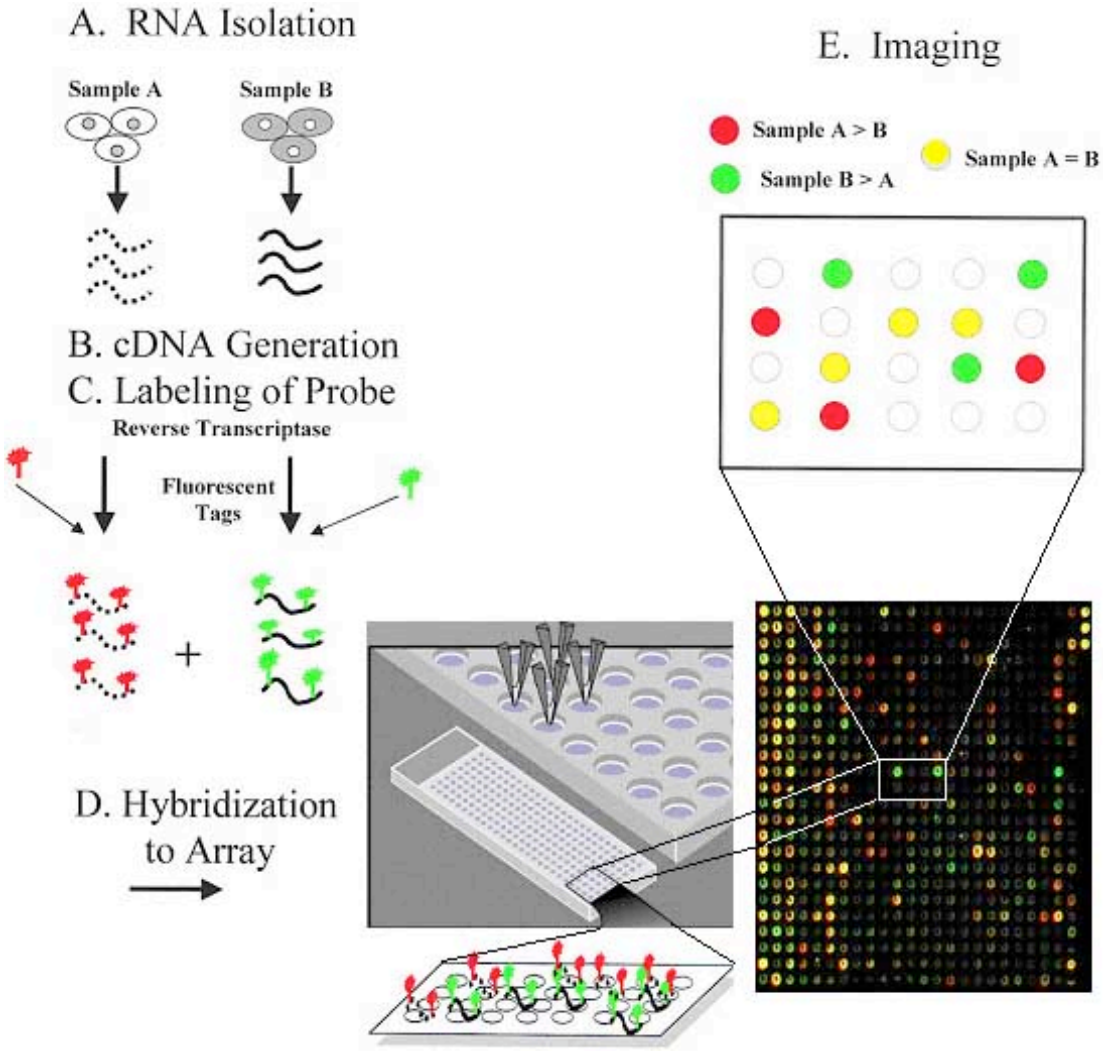
Source: http://wwwuser.gwdg.de/~instphyt/karlovsky/research/

Transcriptomics: questions

Question: which genes or *groups* of genes are differentially expressed between two (or more) cell types/samples?

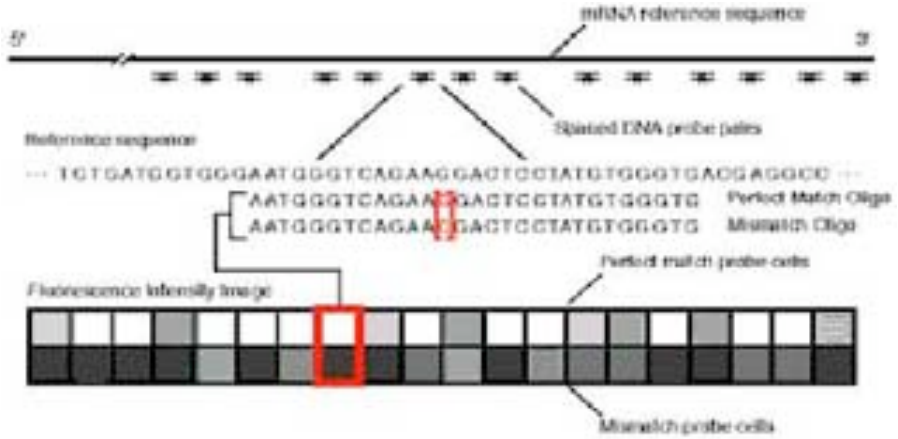
Microarray method: mRNA/cDNA is labeled and hybridizes on an array of genes (cDNAs); the intensity of the signal corresponds to the abundance of the mRNA

cDNA arrays

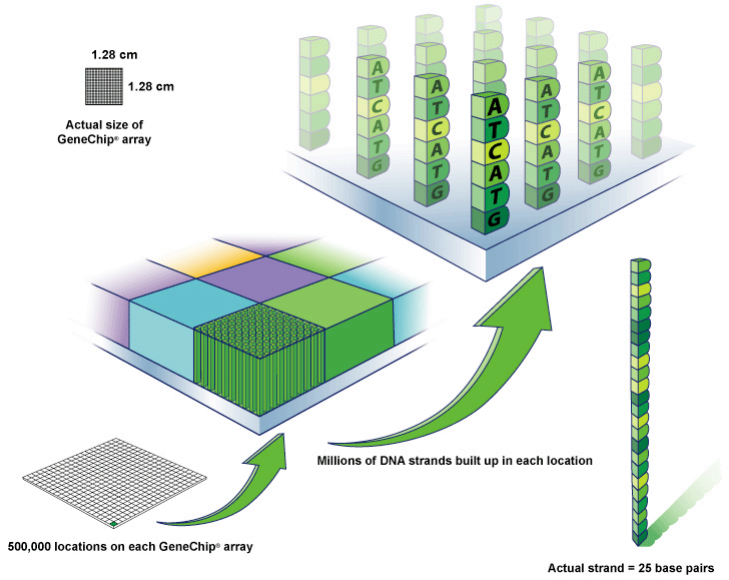


Source: http://www.fao.org/DOCREP/003/X6884E/x6884e00.jpg

Affymetrix microarrays



Affymetrix microarrays (cntd)



Source: http://www.affymetrix.com/

cDNA arrays: points of caution

Variability/noise:

- cross-hybridization variability
- *a priori* knowledge of gene structure
- fluorescence dye variability
- machine printing variability
- exposure variability

Affy chips: general comments

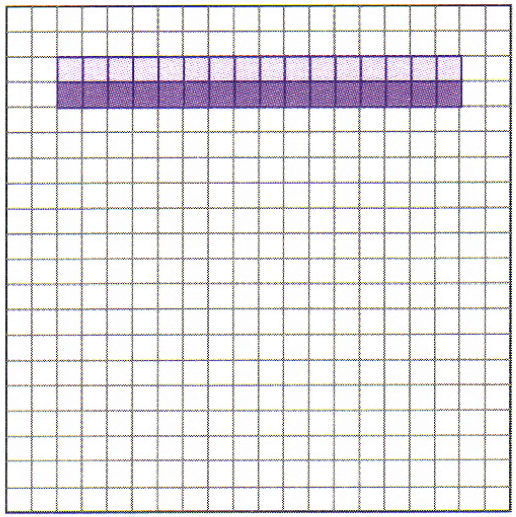
Variability/noise:

- cross-hybridization variability
- *a priori* knowledge of gene structure
- alternative spliced messages?

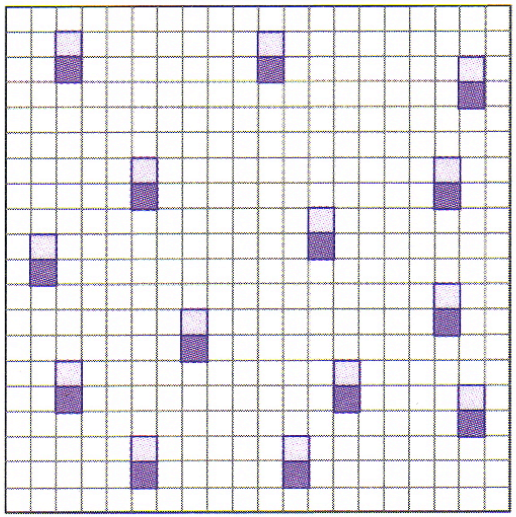
Experimental design

Location/spot variability:

- replicate spots
- distribute them around the array



Grouped Probe Set

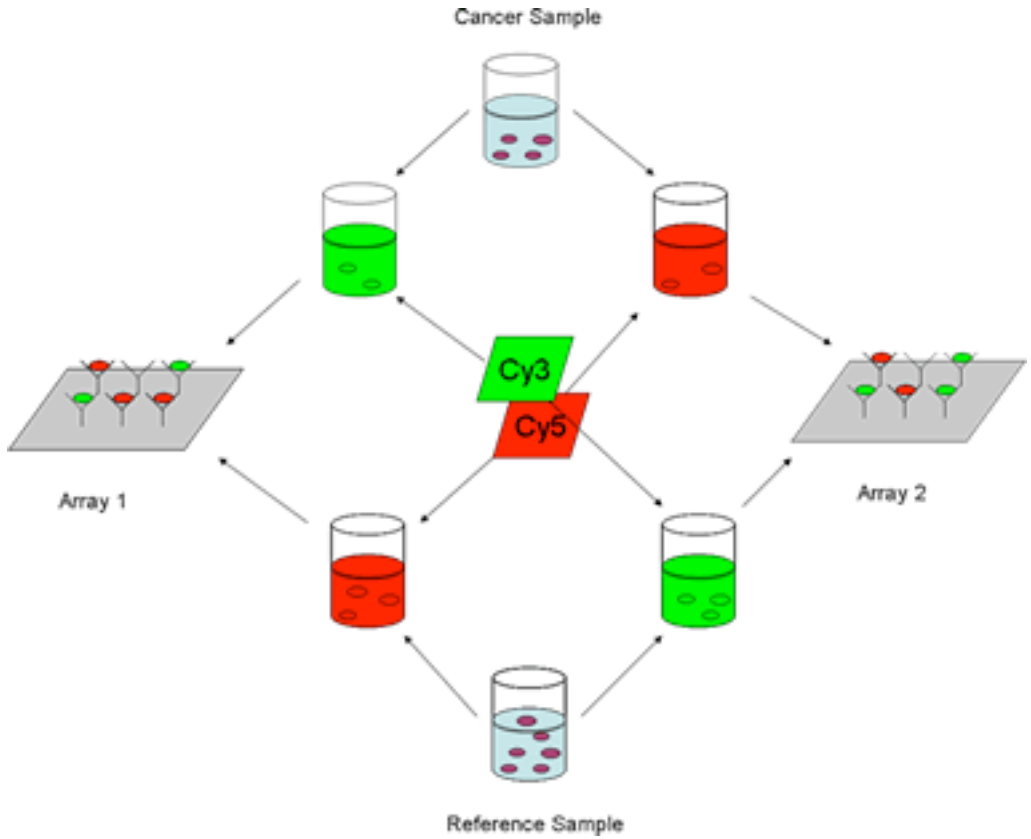


Distributed Probe Set

Experimental design (cntd)

Dye variability:

- dye swap

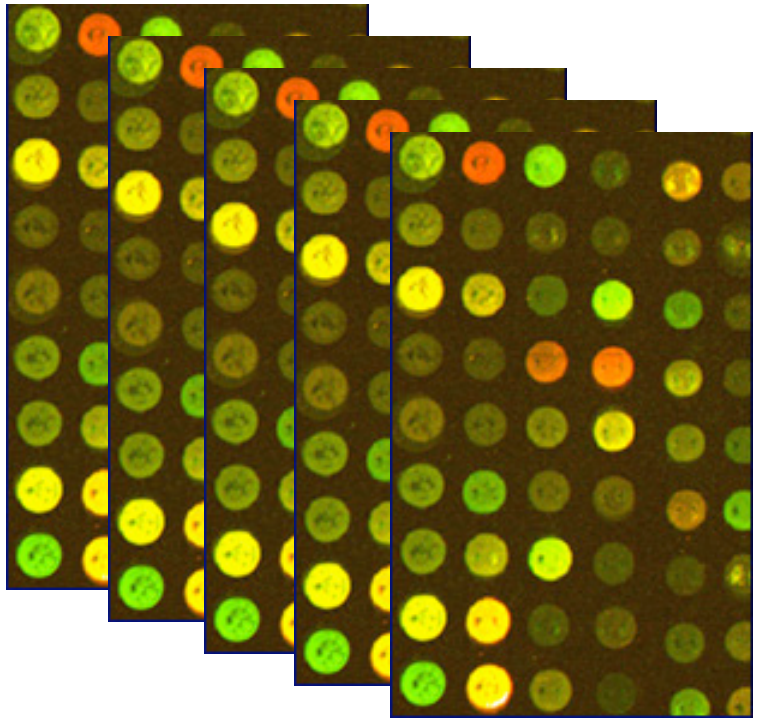


Source:
http://www.stat.purdue.edu/research/coalesce/bioinformatics/Center_for_Bioinformatics/protein_array_analysis.html

Experimental design (cntd)

Array variability:

- replicate whole experiment! (not just technical replicas)



Data pre-processing

Data extraction:

- Identify (and exclude) “damaged” areas
- Spot identification
- Spot quality control
- Quantification

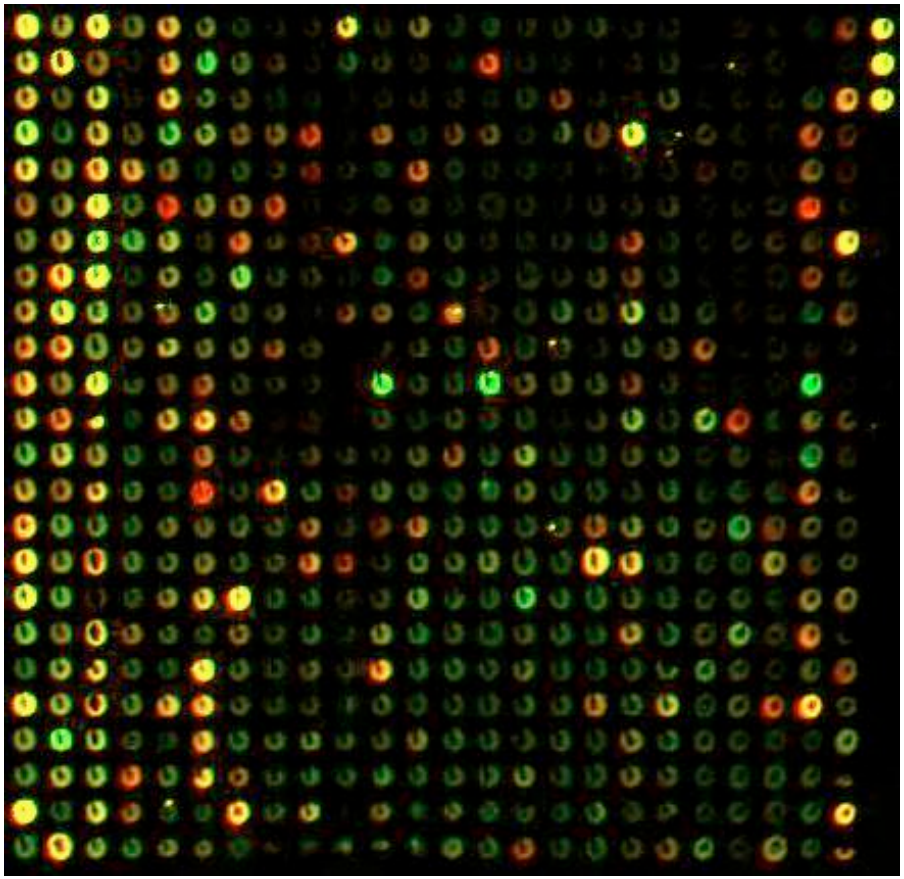
Data transformation:

- Typically, log-values are considered

Data pre-processing (cntd)

Data normalization:

- Within-slide
- Between slides



Data analysis

Supervised learning (classification):

- Main aim: to build robust classifiers
- *k* (known) classes of genes exist
- Examples of expression levels for these genes are available
- Rules are learnt from the examples and applied in new cases (of unknown class)
- Application in *disease classification*, *disease progression*, *response to treatment*, etc

Data analysis (cntd)

Unsupervised learning (clustering):

- Main aim: to identify subsets (clusters) of genes that "behave similarly"
- No labels exist *a priori*
- The number of clusters, *k*, is usually unknown

- *Application in discovery of biological information*

Unsupervised learning

- K-means clustering algorithm :
  1. Start with a guess for the *k* cluster centers
  2. Select *k* centroids at random or at the maximum distance from each other *(Euclidean distance)*
  3. For each point, find the closest cluster centroid
  4. Replace each centroid by the coordinate-wise average of all data points that are closest to it
  5. Repeat steps #3 and #4 until no change in the cluster memberships
  6. Repeat the algorithm for different values of *k*

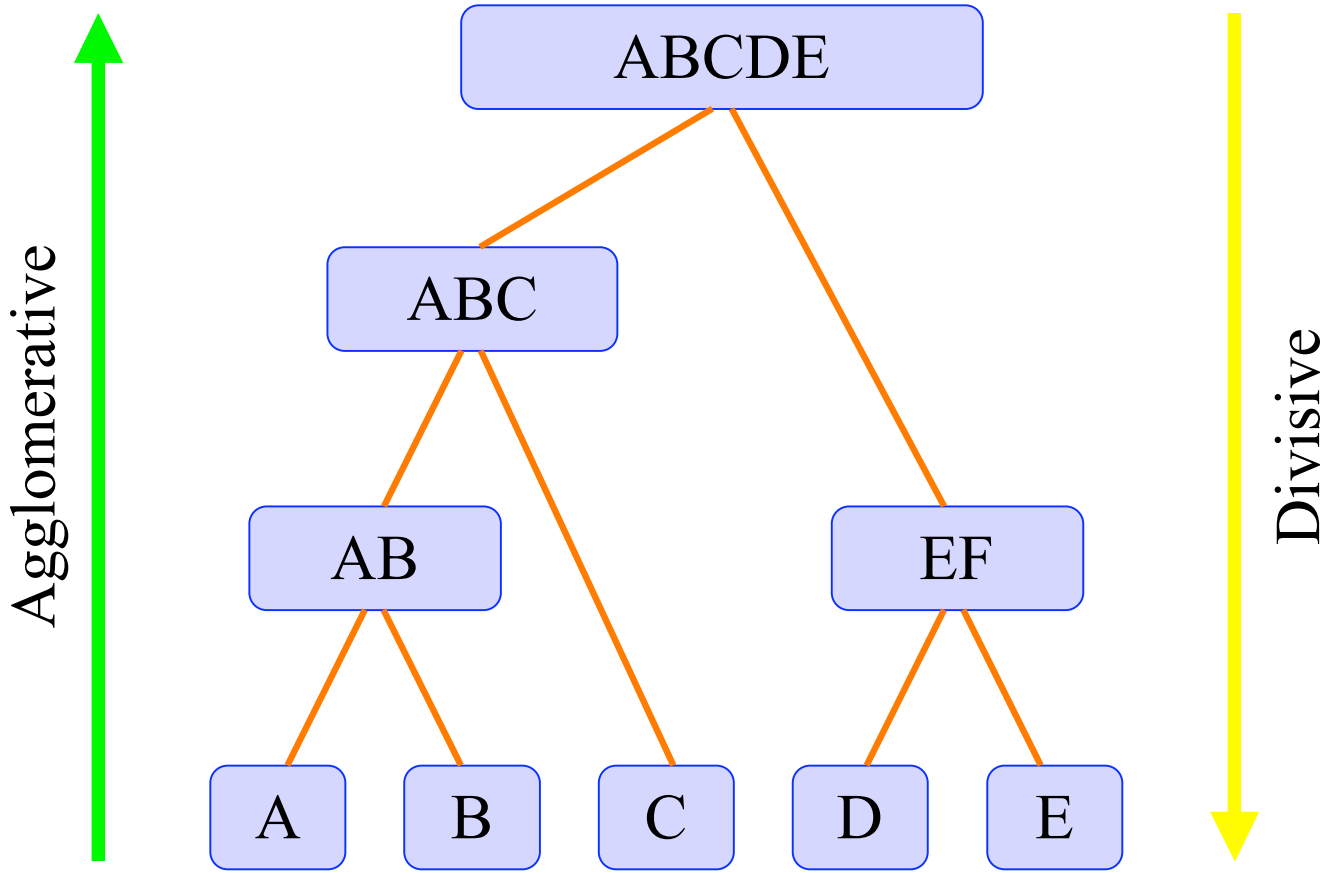
Unsupervised learning (cntd)

- Hierarchical clustering algorithm (divisive):
  1. Calculate all pairwise distances between data points
  2. The two closest points are joined into a cluster
  3. Calculate the centroid of the cluster and calculate the pairwise distances from this point to all other points
  4. Repeat steps #2 and #3 until no points left

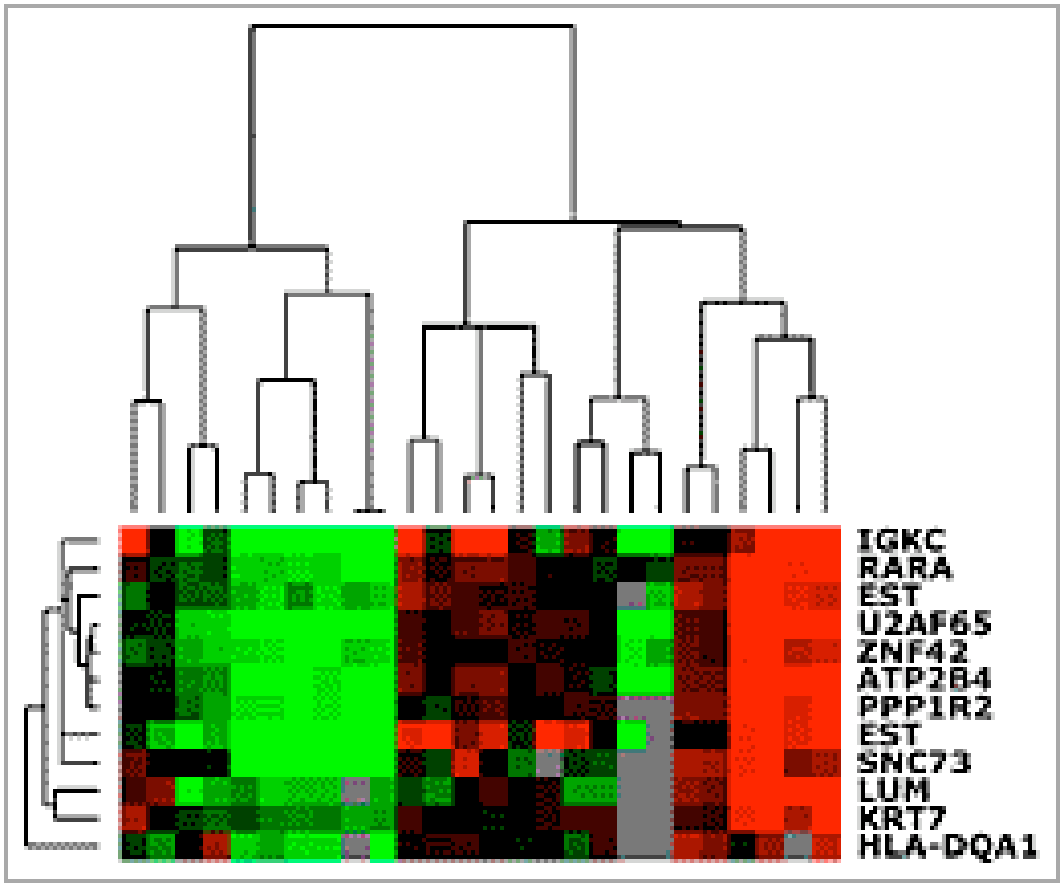
- Hierarchical clustering algorithm (agglomerative):

  The reverse of the divisive algorithm.

Hierarchical clustering



Hierarchical clustering (cntd)

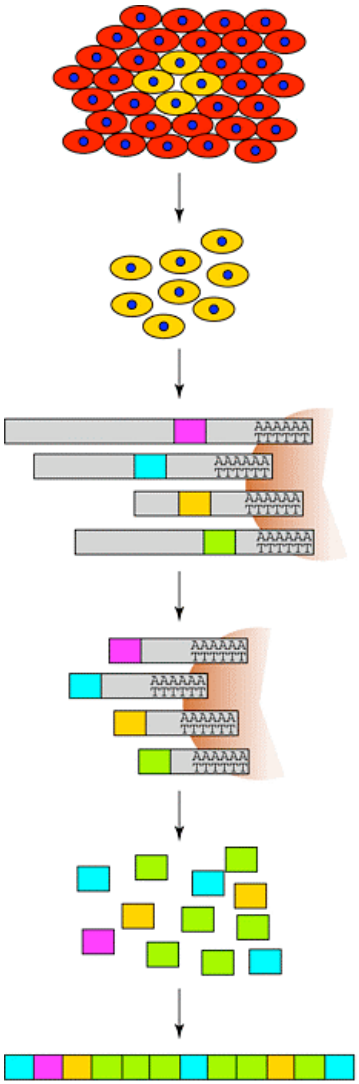


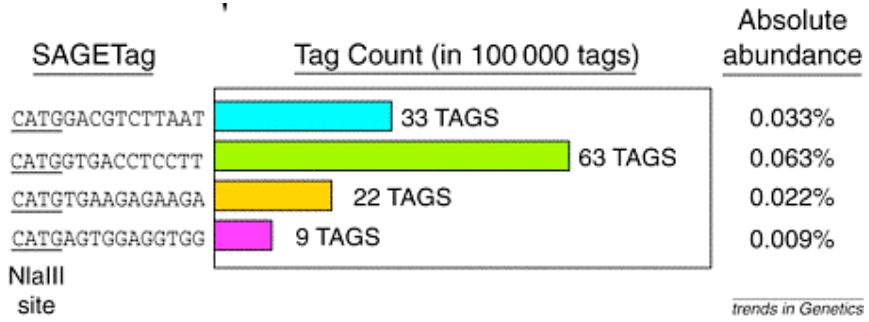
Source: http://www.oncology.cam.ac.uk/images/JB2.gif

SAGE

Serial **A**nalysis of **G**ene **E**xpression

Velculescu *et al.*, *Science* (1995)





Microarrays vs. SAGE

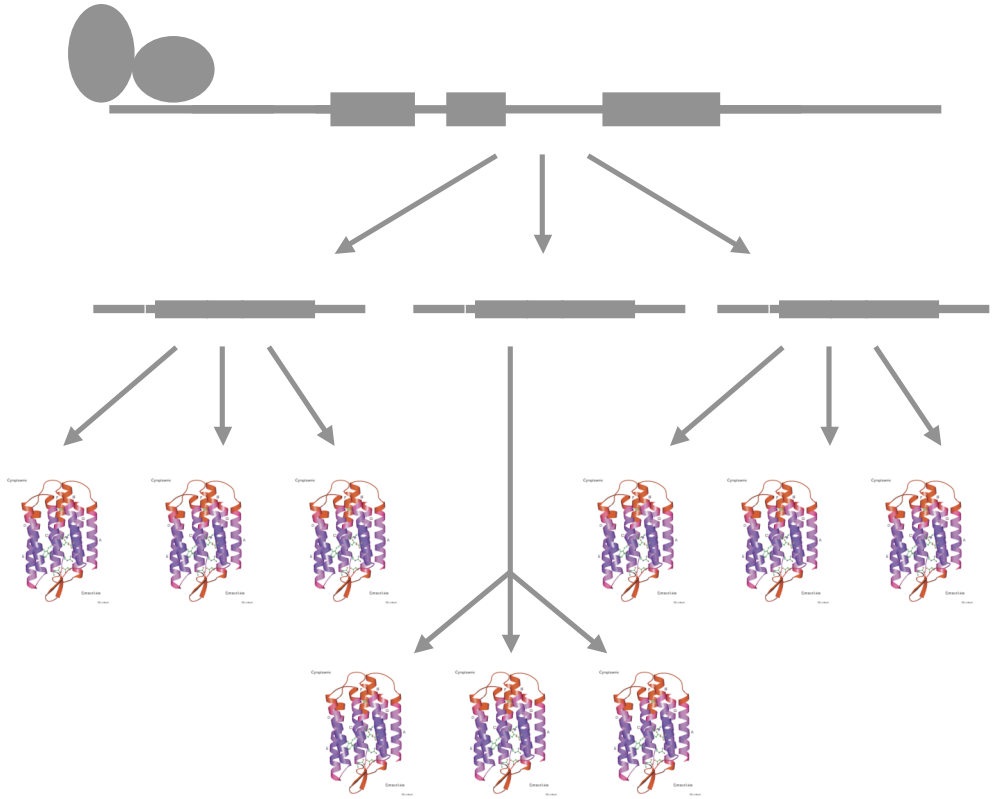
Microarrays:

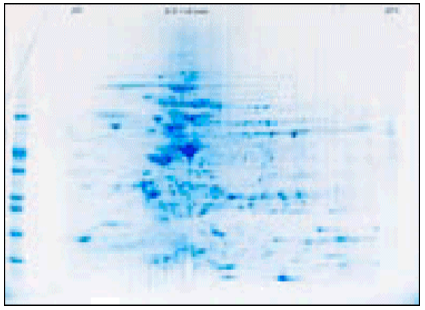
- hybridization variability
- *a priori* knowledge of the genes (exact or non-exact structure)

SAGE:

- time/resource consuming
- sequencing errors decrease efficiency

Proteomics

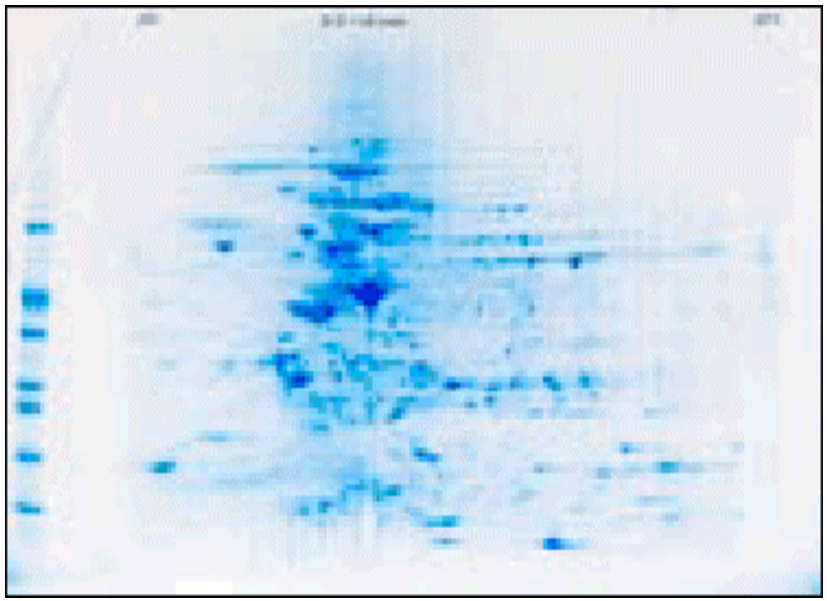


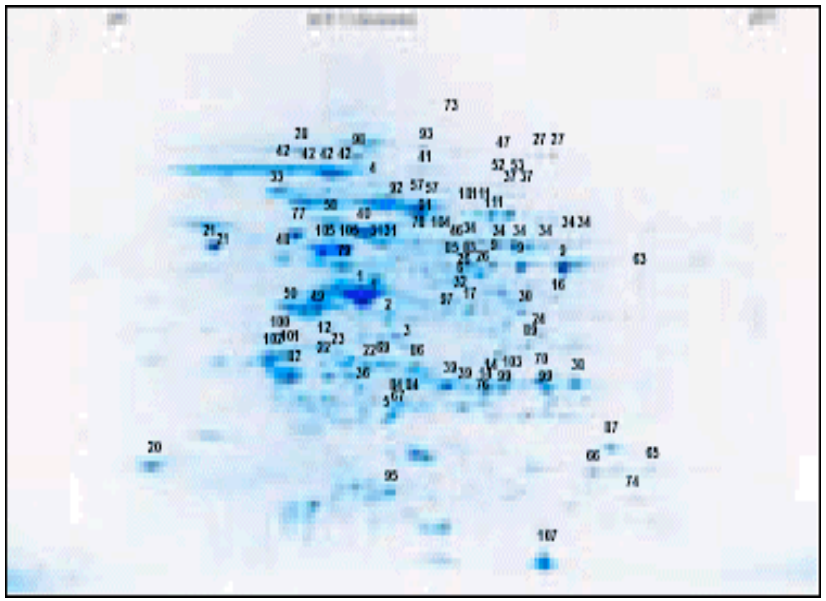


Proteomics technologies

- 2D gels (classical)
- 2D Difference gel electrophoresis (DIGE)
- Mass fingerprinting (e.g., MALDI-TOF)
- Antibody arrays
- Multi ligand arrays

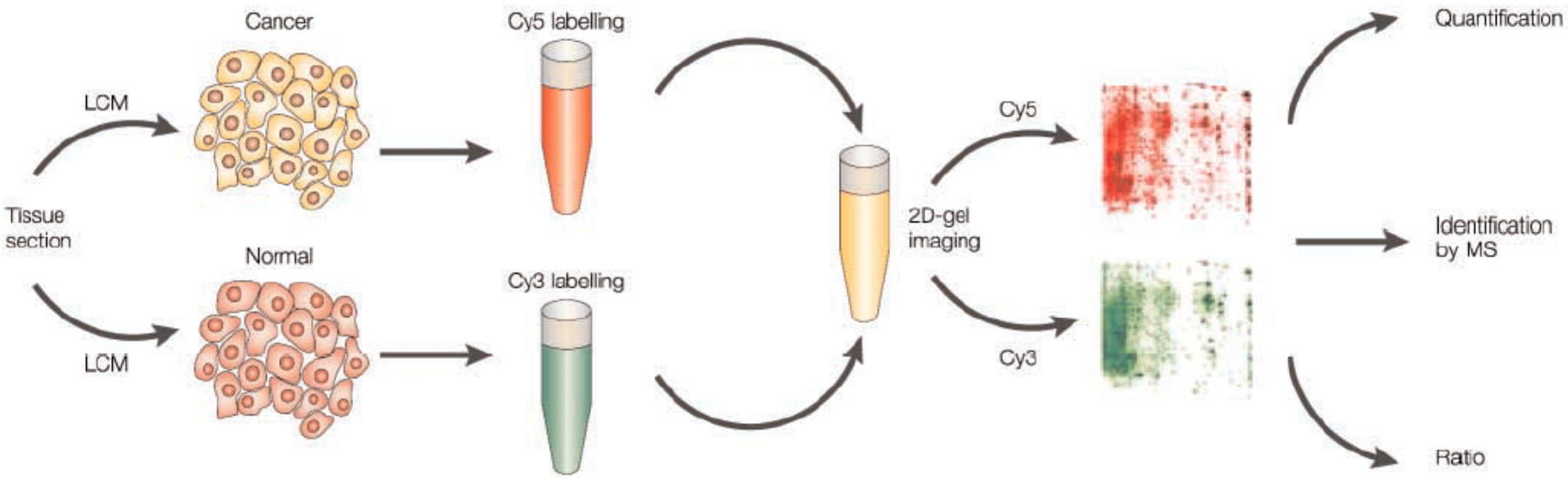
2D gels





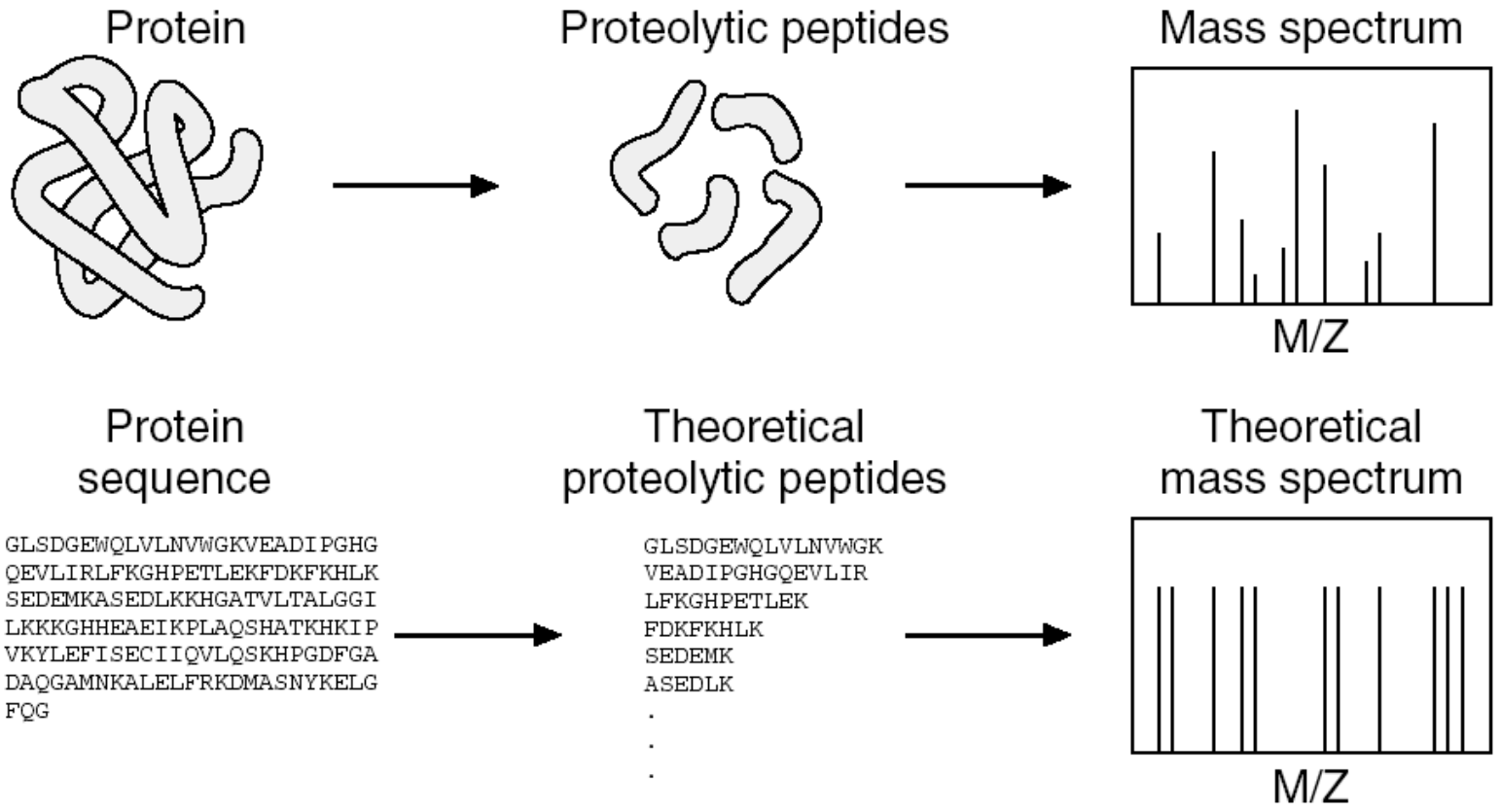
Source: http://www.millipore.com

2D DIGE



Source: Petricoin et al. (2002) Nature Rev Drug Discov. 1:683

Mass-spec fingerprinting

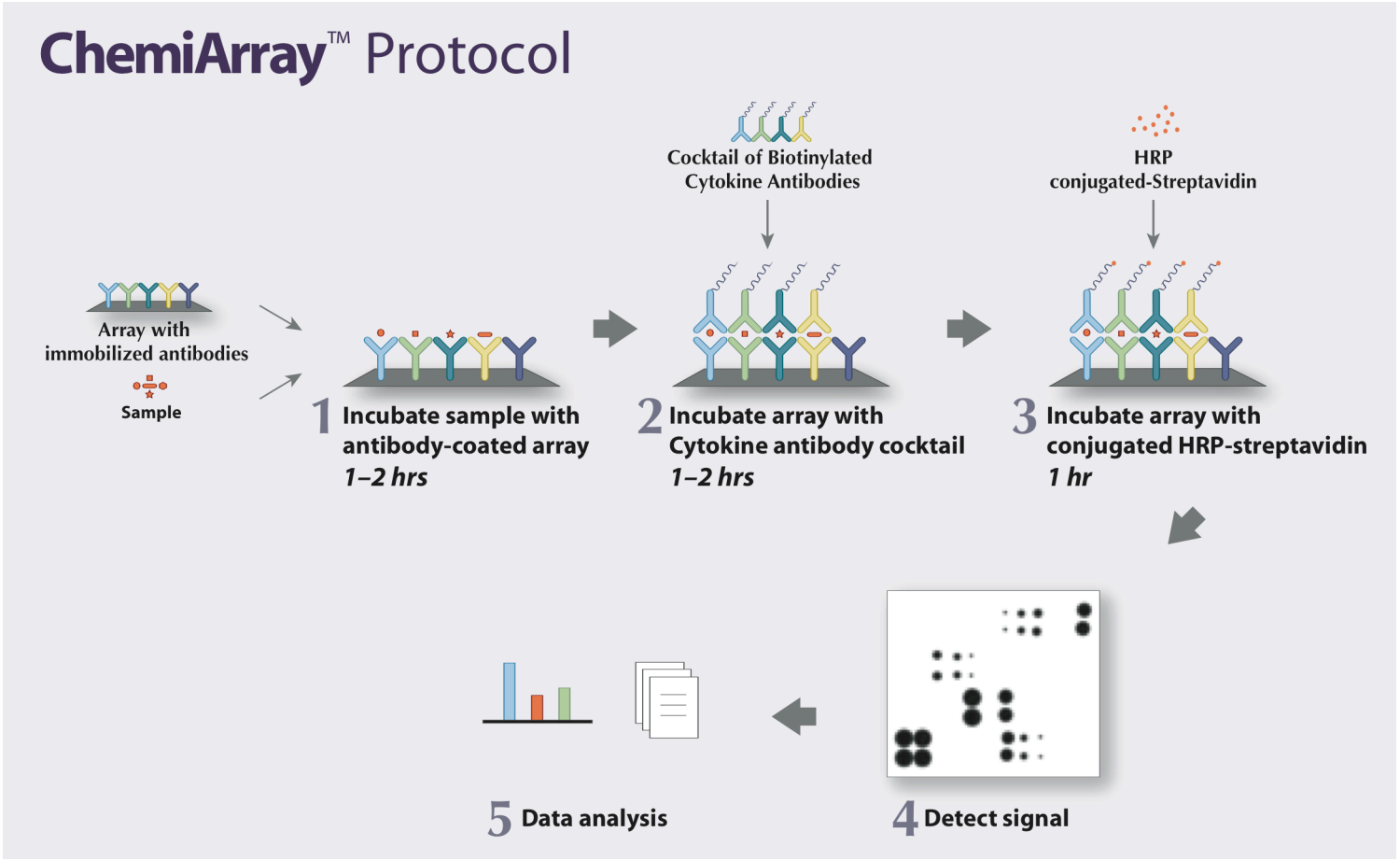


Courtesy: Steve Ringquist PhD, RANGOS Research Center

2D gels & mass-spec

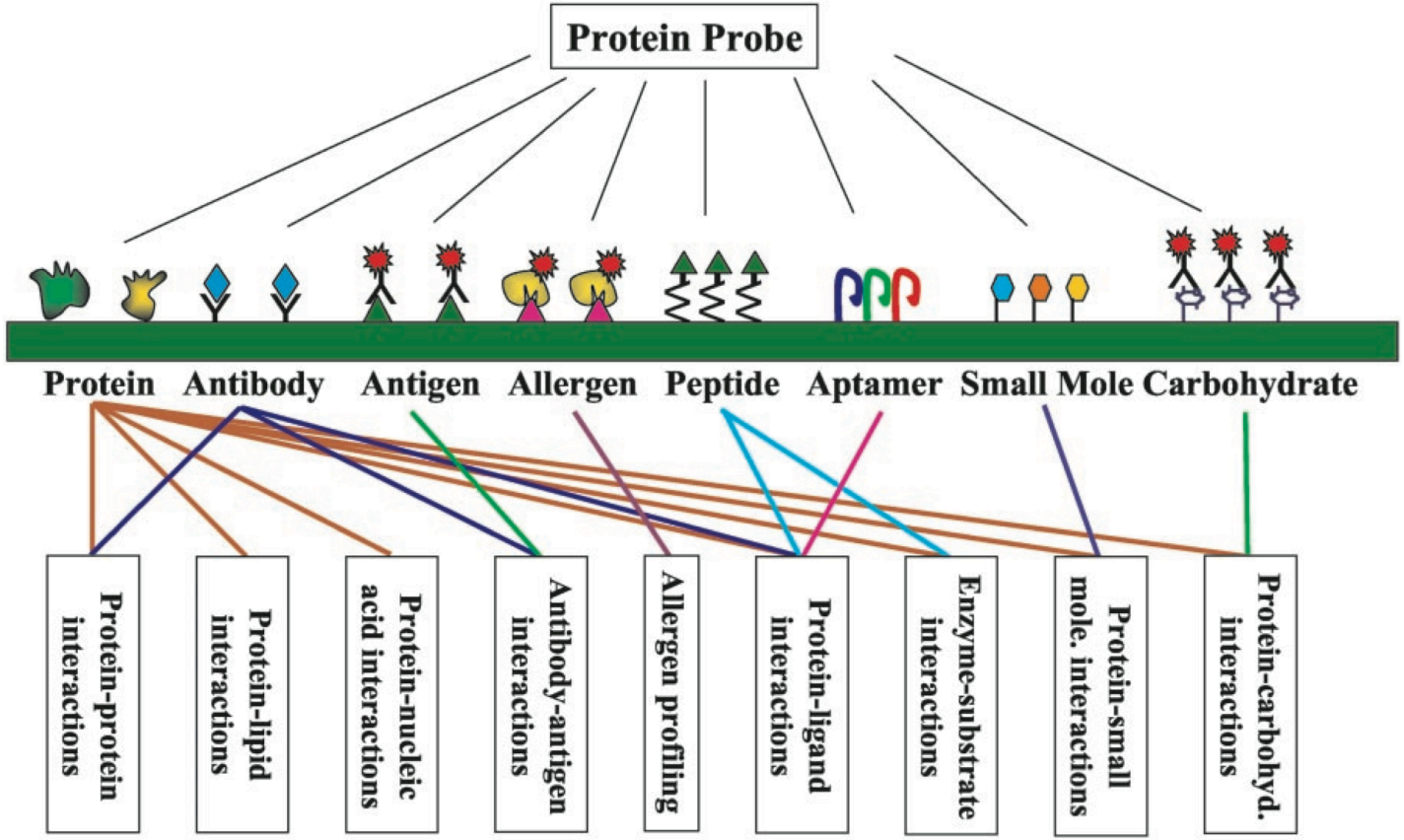
Source: http://www. amershambiosciences.com

Antibody arrays



Source: http://www.chemicon.com

Multi ligand arrays



Source: Zhu (2003) Ann Rev Biochem

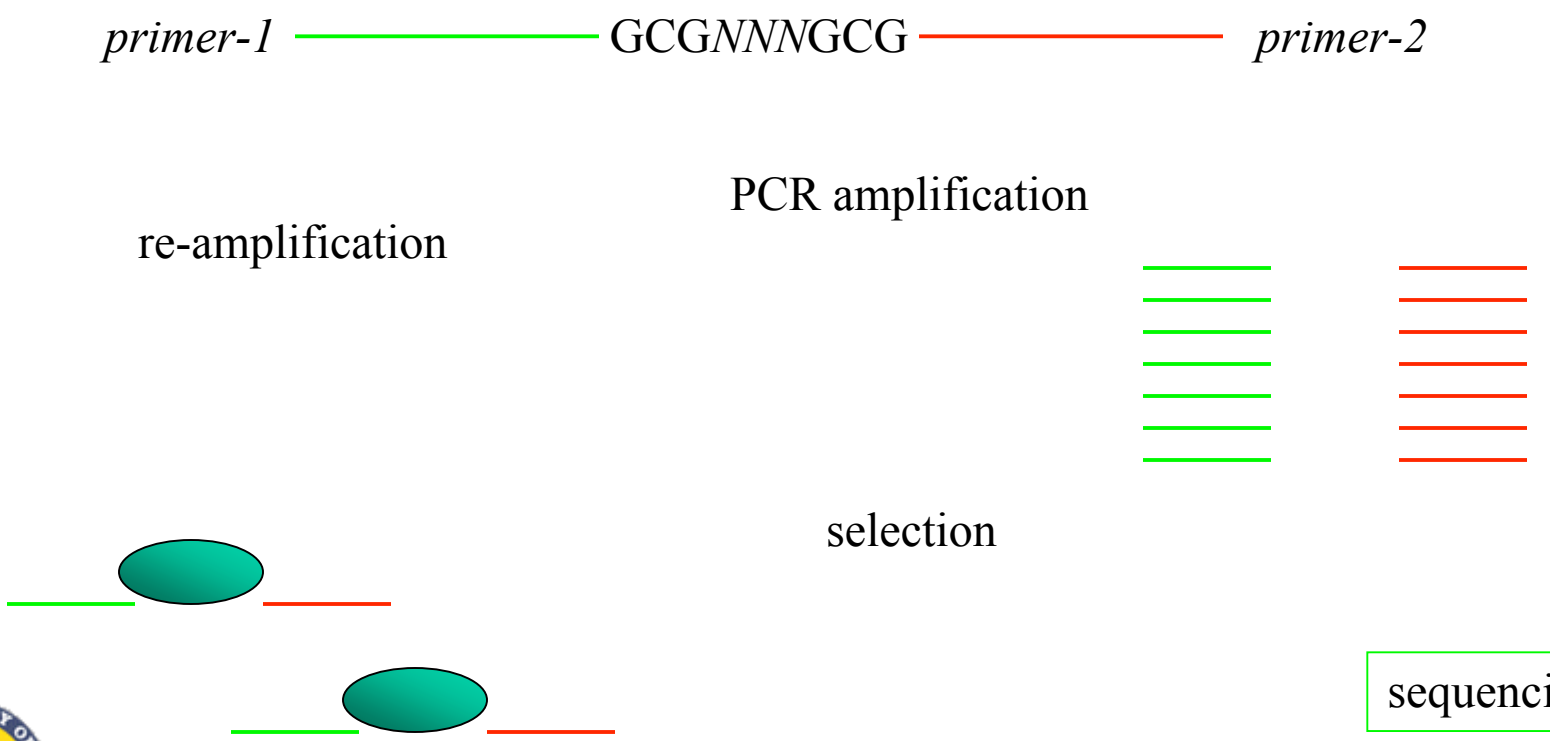
Technologies for protein-DNA interactions

Overview

- *In vitro* target identification.
  - SELEX
  - Phage display
  - Protein-DNA interaction chips
  - Band-shifts, QuMFRA
- *In vivo* target identification.
  - ChIP, ChIP-on-chip
  - STAGE

SELEX

Systematic **E**volution of **L**igands by **EX**ponetial enrichment
Tuerk and Gold, *Science* (1990)



SELEX (cntd)

```
s r s d h l t t h i r
5' g c g  g g g  g c g
5' g c g  g g g  g a g
5' g c g  g g t  g c g
5' g c g  t g g  g c g
5' g a g  g g g  g c g

s r s d E l t R h i r
5' g c g  g g g  g c g
5' g c g  t g g  g c g
```

Phage display

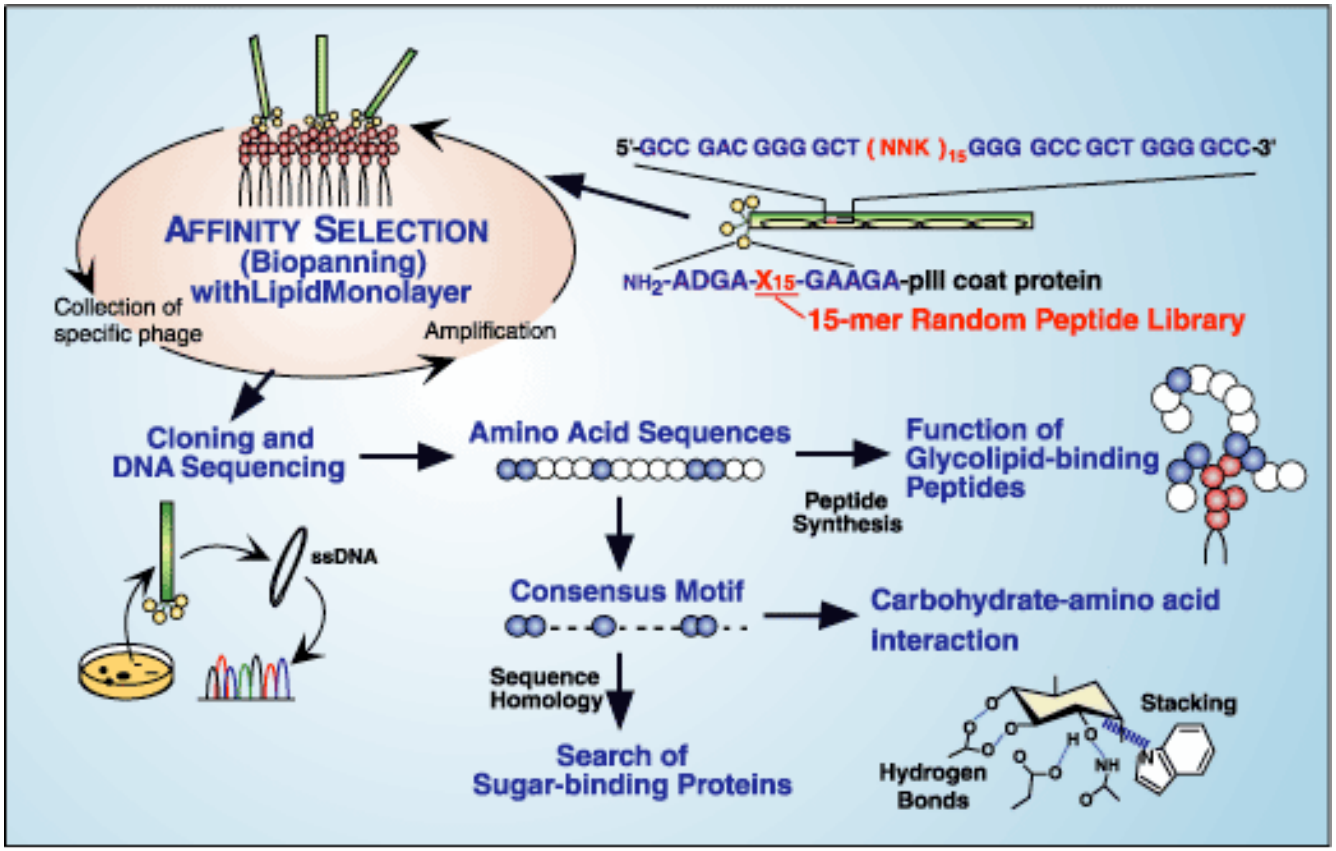


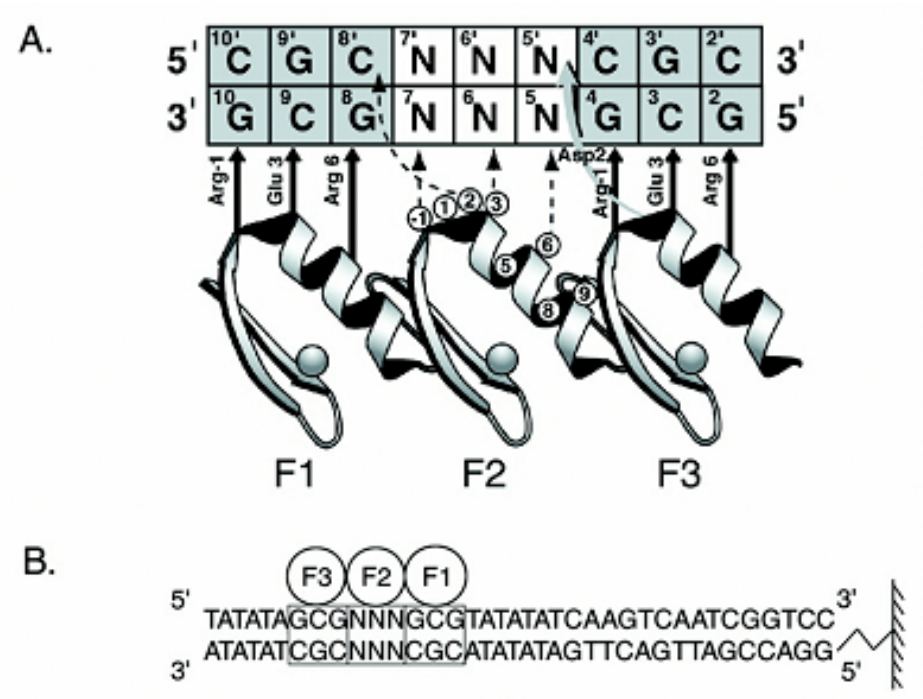
Figure Selection of ganglioside-binding peptides from phage peptide library and the analysis of carbohydrate-peptide interaction

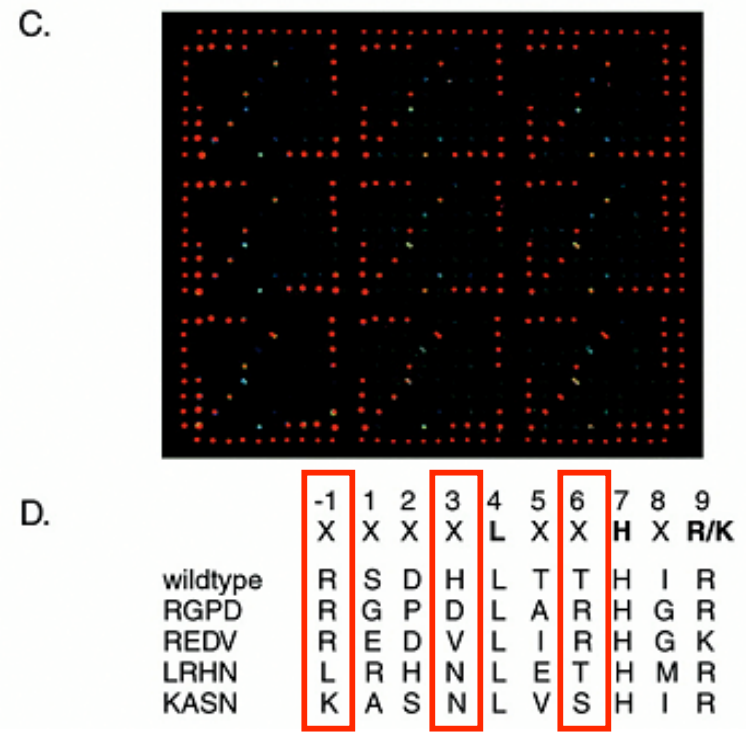
Source: http://www.glycoforum.gr.jp/science/word/glycotechnology/GT-C08E.html

Phage display (cntd)

SELEX	*Phage display*
s r s d h l t t h i r	5' g c g g a a g c g
5' g c g g g g g c g	s Q G G N l V R h L r
5' g c g g g g g a g	s N G G N l G R h M k
5' g c g g g t g c g	s A R S N l L R h T r
5' g c g t g g g c g	s L Q S N l V R h Q r
5' g a g g g g g c g	s I A S N l L R h Q r
s r s d E l t R h i r	5' g c g c a g g c g
5' g c g g g g g c g	s R G D H l K D h I k
5' g c g t g g g c g	s R S D H l T T h I r

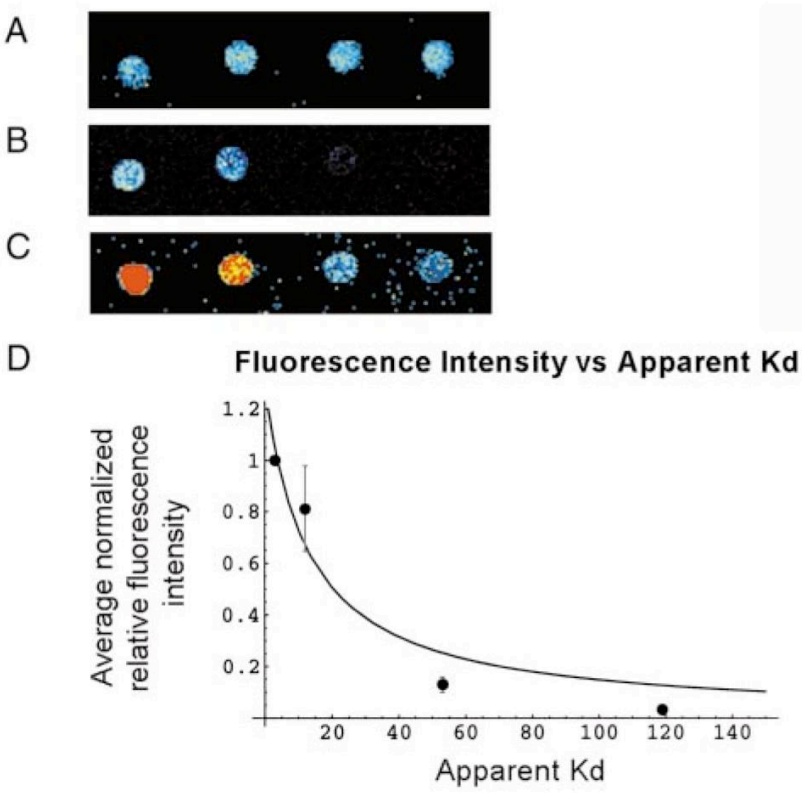
Protein-DNA chips



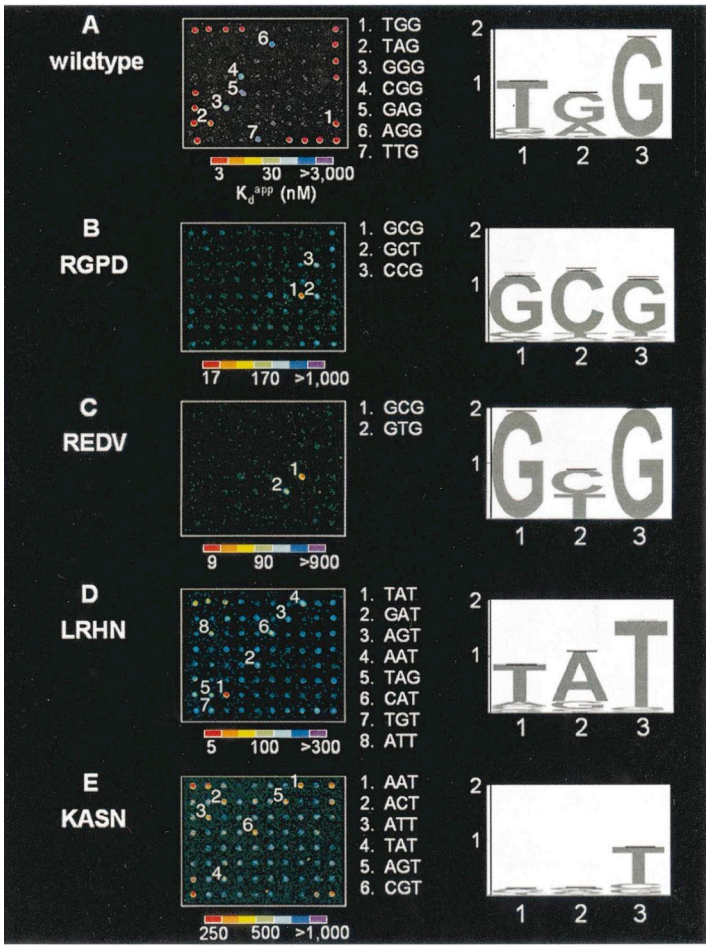


Source: Bulyk *et al.*, *Nucl Ascids Res (2002)*

Protein-DNA chips (cntd)

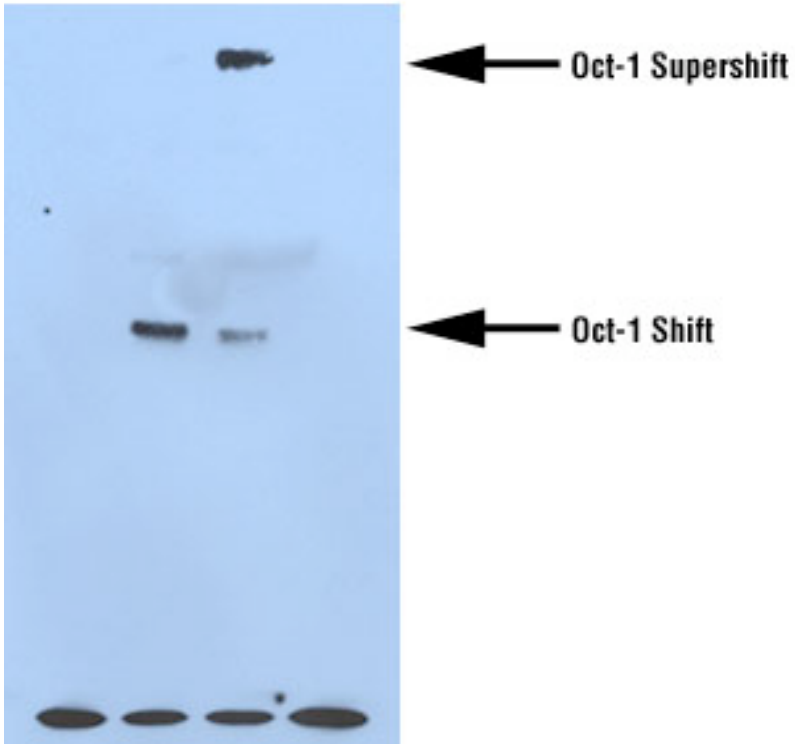


Source: Bulyk *et al.*, *Proc Natl Acad Sci USA (2001)*

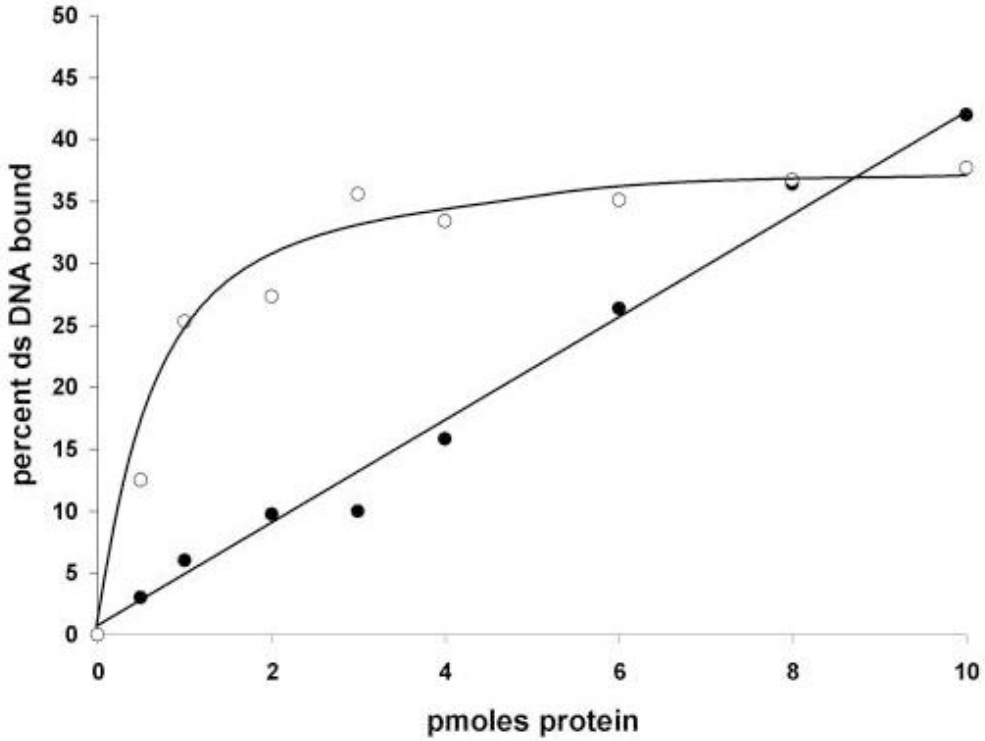


Source: Bulyk *et al.*, *Nucl Ascids Res (2002)*

Quantitative data: band-shifts



Source: http://www.piercenet.com/media/super_oct1.jpg

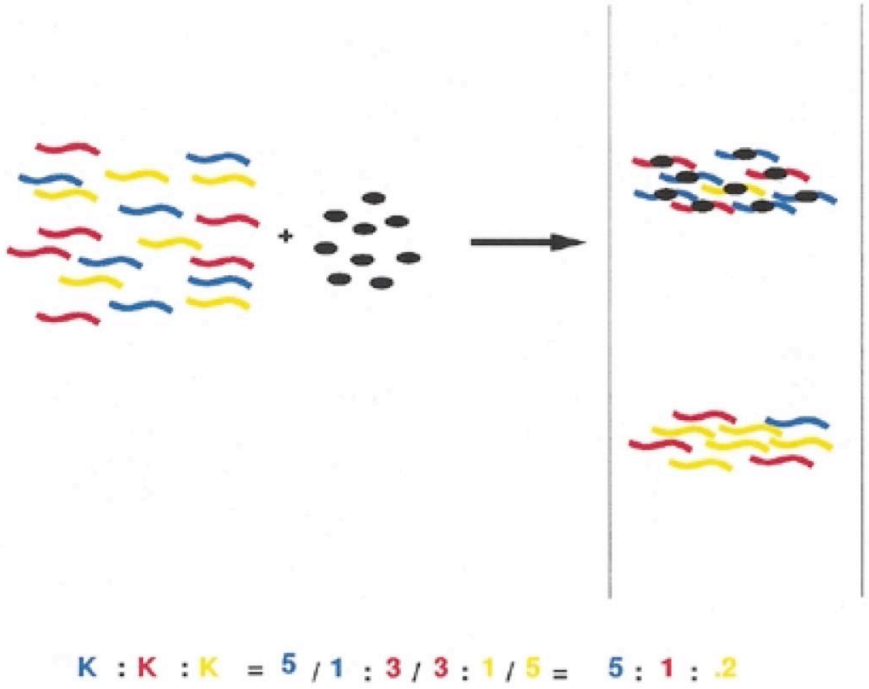


Source: http://www.biomedcentral.com/content/figures/1471-2091-3-13-3.jpg

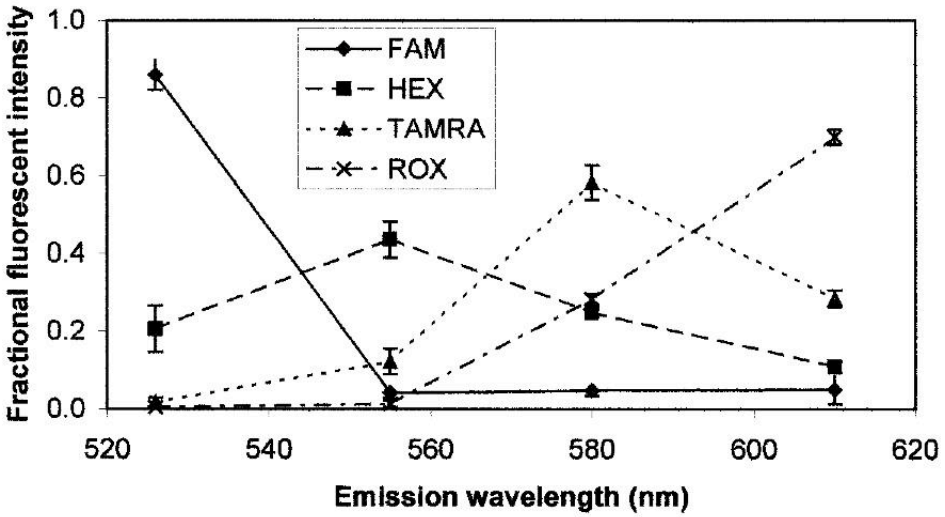
Quantitative data: QuMFRA

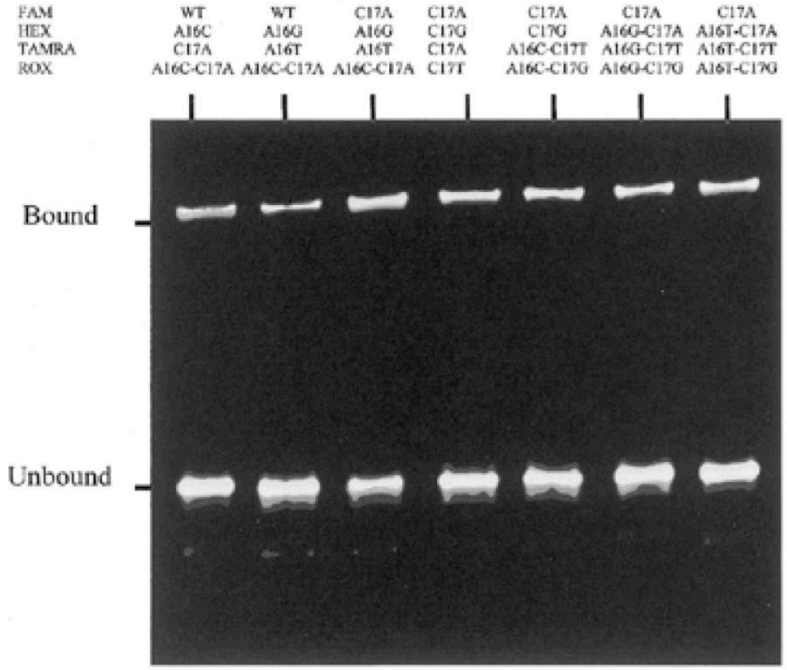
Quantitative **M**ultiple **F**luorescence **R**elative **A**ffinity

Man and Stormo, *Nucl Acids Res* (2001)



Quantitative data: QuMFRA (cntd)





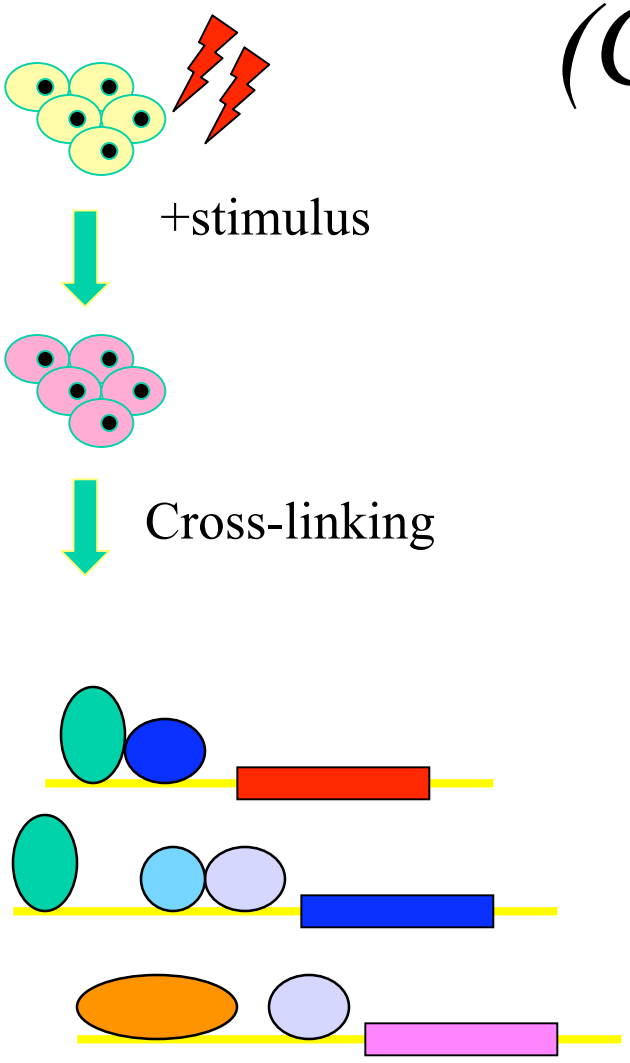
$$\frac{K_A(D_1)}{K_A(D_2)} = \frac{[P \cdot D_1]/[P] \cdot [D_1]}{[P \cdot D_2]/[P] \cdot [D_2]} = \frac{[P \cdot D_1]/[P \cdot D_2]}{[D_1]/[D_2]}$$

Source: Man and Stormo, *Nucl Acids Res* (2001)

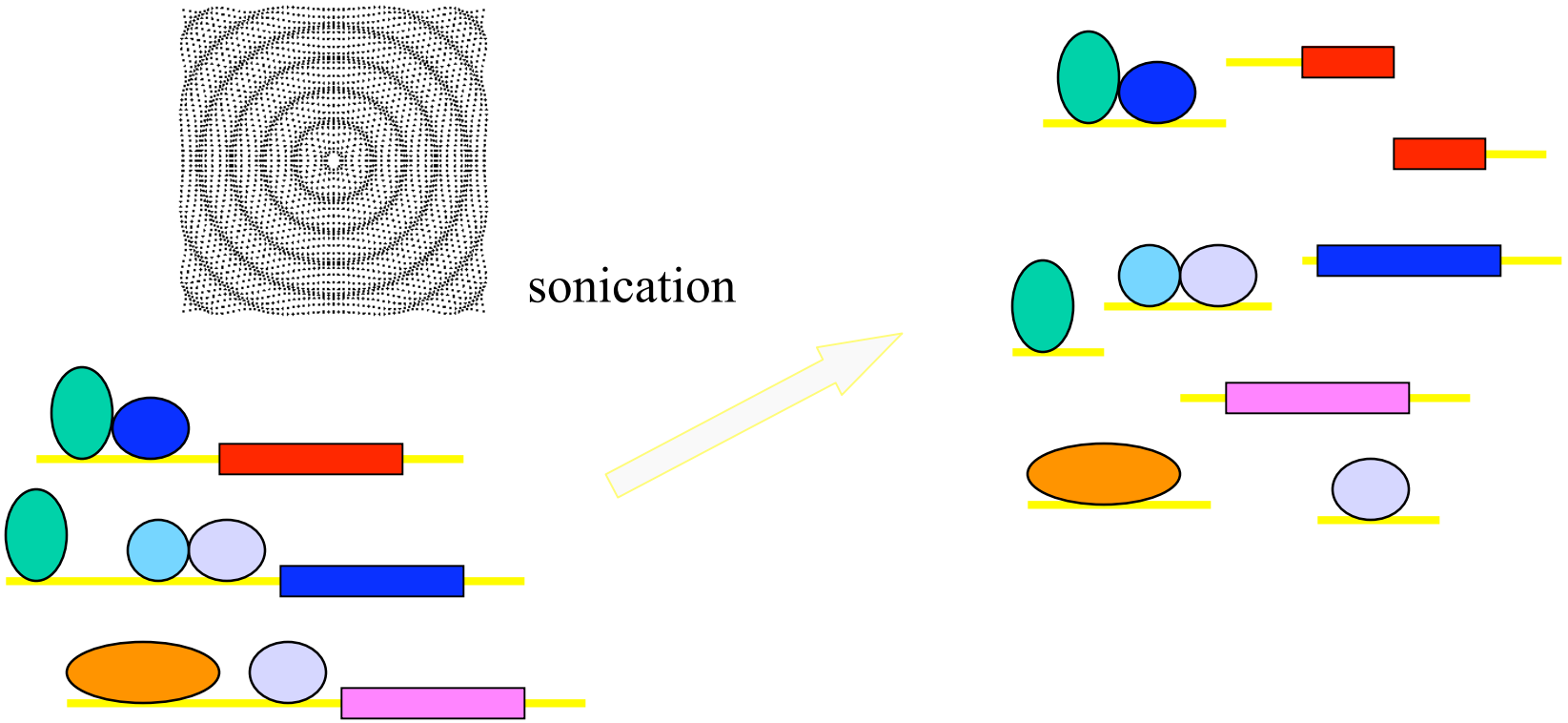
Overview

- *In vitro* target identification.
  - SELEX
  - Phage display
  - Protein-DNA interaction chips
  - Band-shifts, QuMFRA
- *In vivo* target identification.
  - ChIP, ChIP-on-chip
  - STAGE

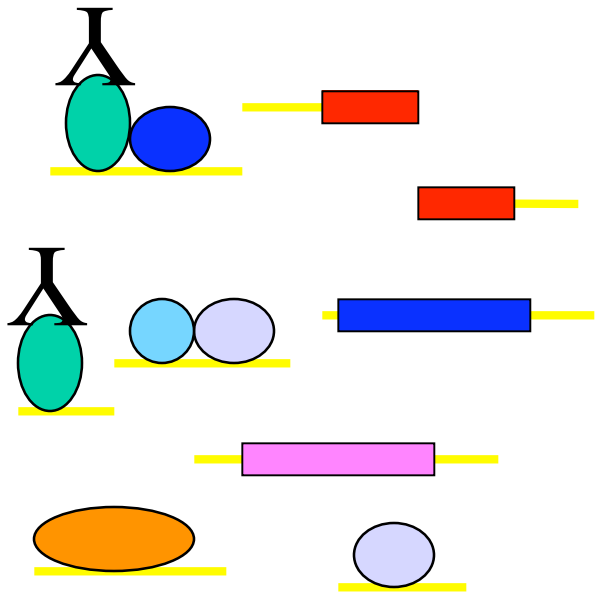
Chromatin immunoprecipitation (ChIP)



Chromatin immunoprecipitation (ChIP)



Chromatin immunoprecipitation (ChIP)



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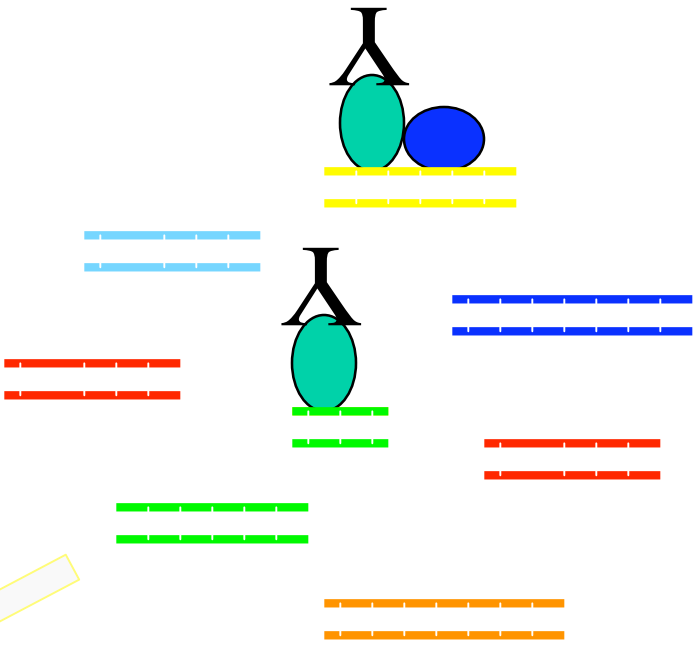
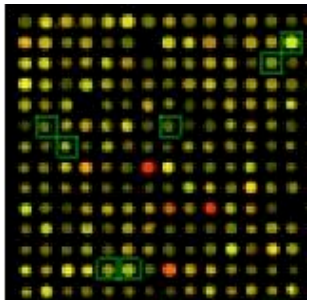
Chromatin immunoprecipitation (ChIP)

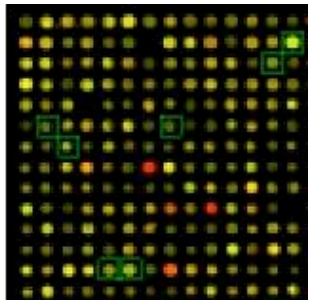


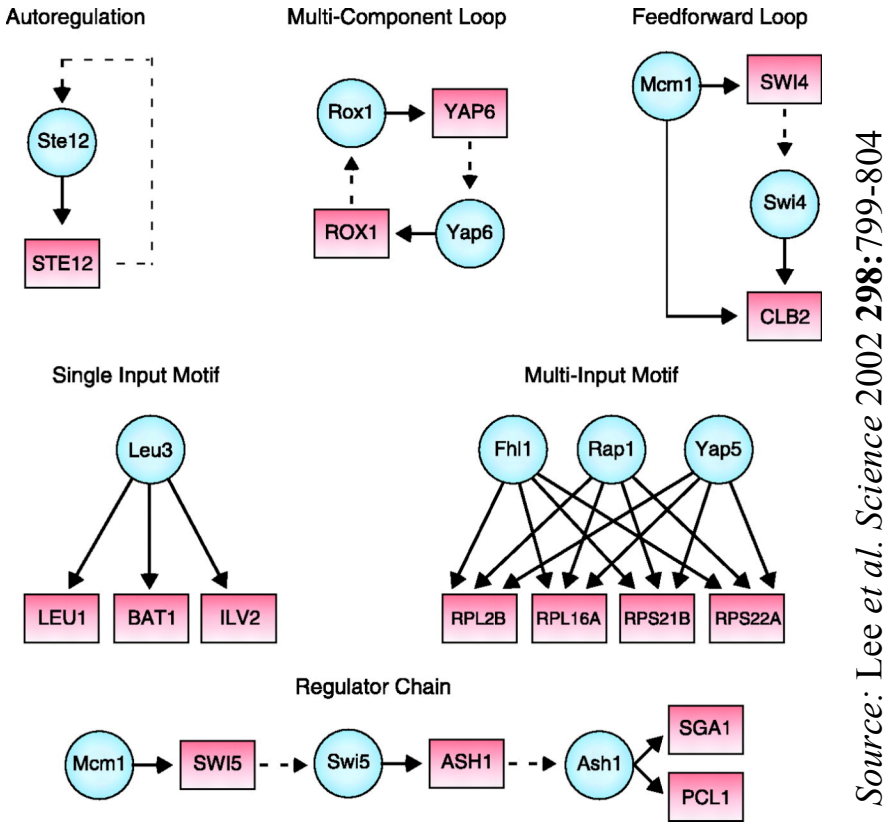
LM-PCR



Proteinase K

ChIP-on-chip





Source: Lee *et al. Science* 2002 **298:**799-804

ChIP-on-chip: yeast study

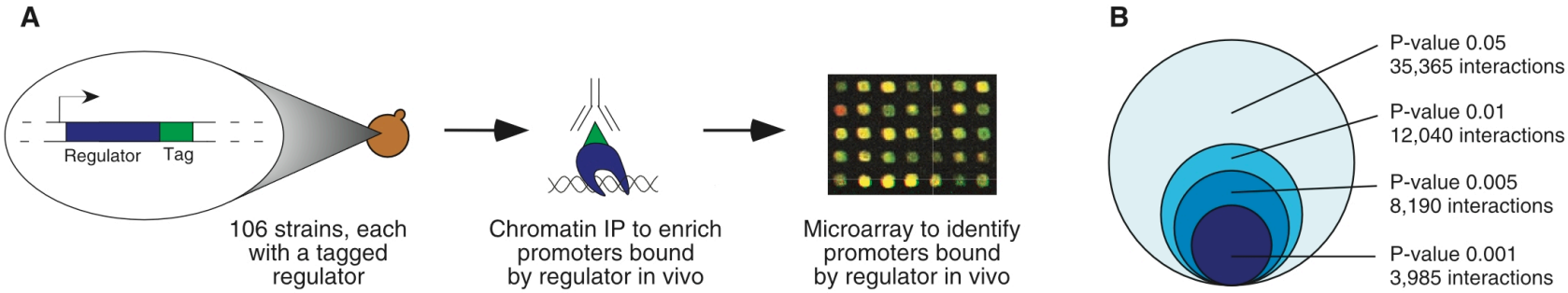
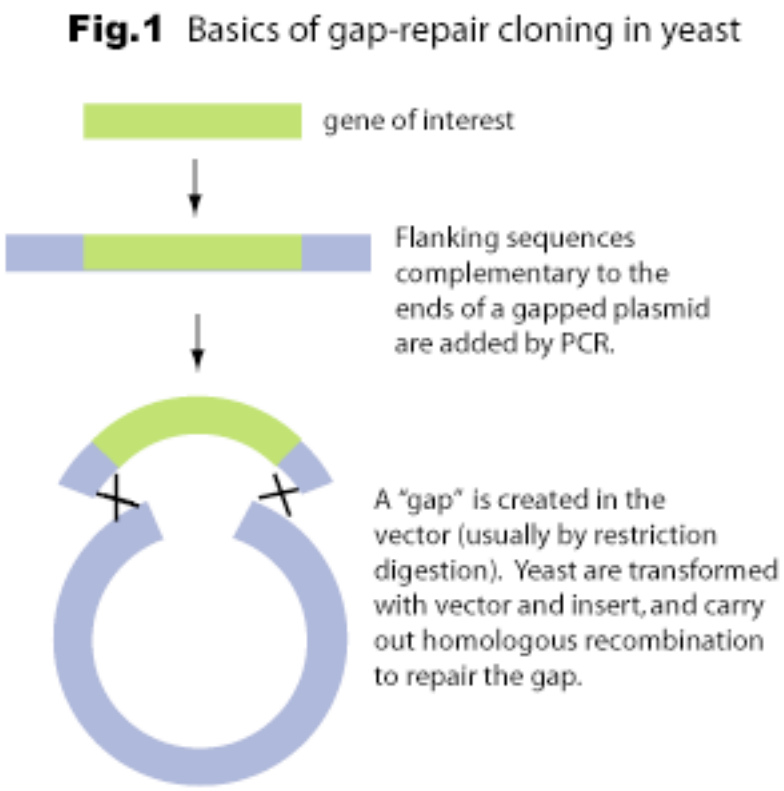


Fig. 1. Systematic genome-wide location analysis for yeast transcription regulators. (**A**) Methodology. Yeast transcriptional regulators were tagged by introducing the coding sequence for a c-*myc* epitope tag into the normal genomic locus for each regulator. Of the yeast strains constructed in this fashion, 106 contained a single epitope-tagged regulator whose expression could be detected in rich growth conditions. Chromatin immunoprecipitation (ChIP) was performed on each of these 106 strains. Promoter regions enriched through the ChIP procedure were identified by hybridization to microarrays containing a genome-wide set of yeast promoter regions. (**B**) Effect of *P* value threshold. The sum of all regulator-promoter region interactions is displayed as a function of varying *P* value thresholds applied to the entire location data set for the 106 regulators. More stringent *P* values reduce the number of interactions reported but decrease the likelihood of false-positive results.

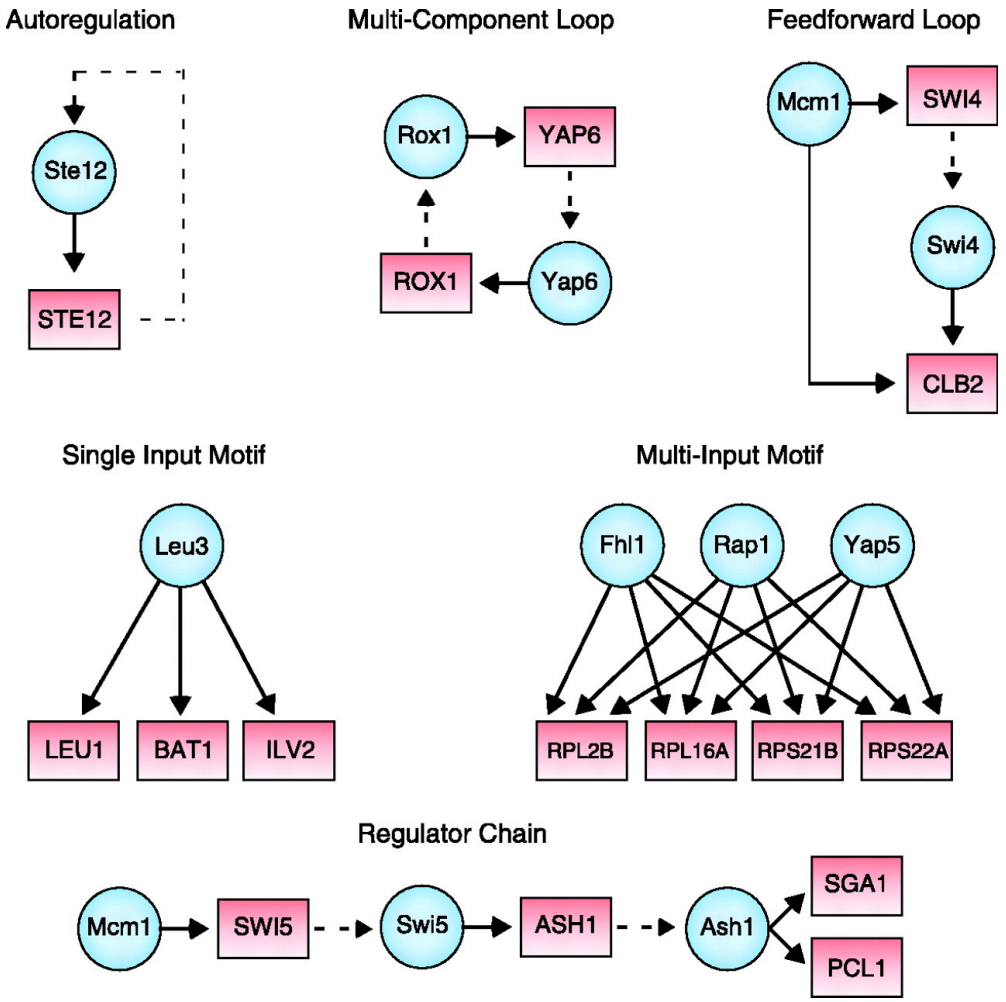
Source: Lee *et al. Science* 2002 **298:**799-804

Homologous recombination



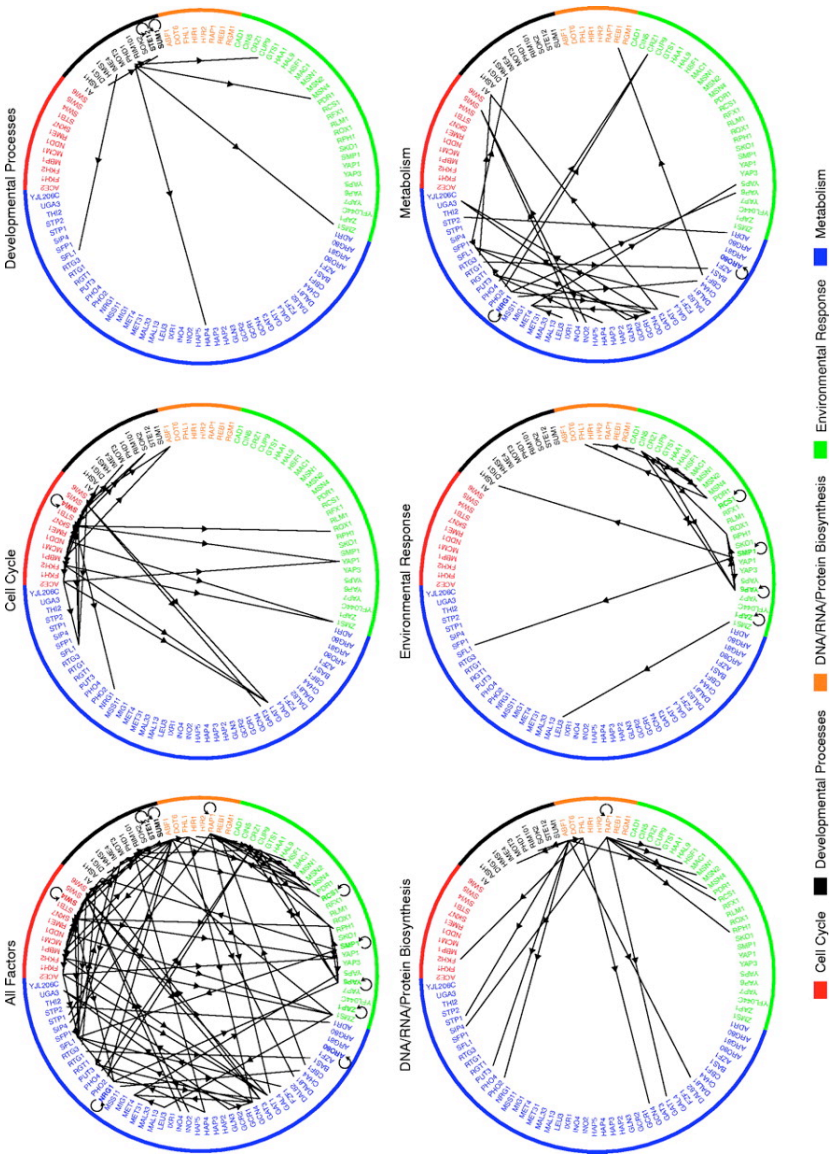
Source: http://www.biology.duke.edu/model-system/ymsg/cloning.html

ChIP-on-chip : yeast study (cntd)



Source: Lee *et al. Science* 2002 **298:**799-804

ChIP-on-chip : yeast study (cntd)



Source: Lee *et al. Science* 2002 **298:**799-804

ChIP-on-chip: spot normalization

Single Array Error Model.

- Intensity spot ratio, X (single array model):

$$X = \frac{a_2 - a_1}{(\sigma_1 + \sigma_2 + f(a_1 + a_2))/2}$$

- X is Normally distributed.
- f, σ_1 and σ_2 are chosen so that Var(X) = 1.

Significance of change of magnitude X.

$$P(X = x) = 2 \cdot (1 - erf(|x|))$$

$$\text{erf}(z) \equiv \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2}\, dt.$$

ChIP-on-chip: multiple measurements

Uncertainty od each normalization.

$$\sigma(\log\text{–}\,ratio) = \frac{\log_{10}(a_2 / a_1)}{X}$$

Average measurement ratio.

$$\bar{x} = (\sum_{i=1}^{3} w_i \cdot x_i) / (\sum_{i=1}^{3} w_i)$$

Weights (w_i).

- *Method:* minimum variance weighted average

Acknowledgements

- *Special Thanks to…*
  - Massimo Trucco MD & Steve Ringquist PhD, Children's Hospital and RANGOS Diabetes Research Center, University of Pittsburgh *(for proteomics pictures)*
  - Eleanor Feingold PhD, Human Genetics, GSPH, University of Pittsburgh *(for slides)*

Enjoy the rest of the BBSI experience!