

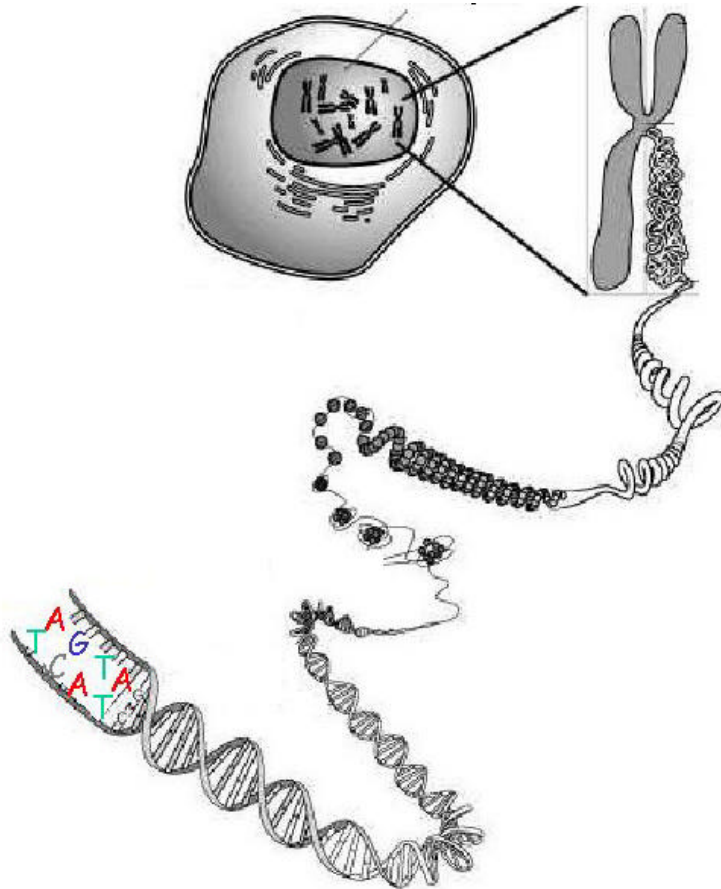
From GeneChips to regulatory networks

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The Genomics Platform

- Core facility providing various technologies for quantification of RNA and DNA
- Proposes complete solution, from advice in experimental design to data analysis

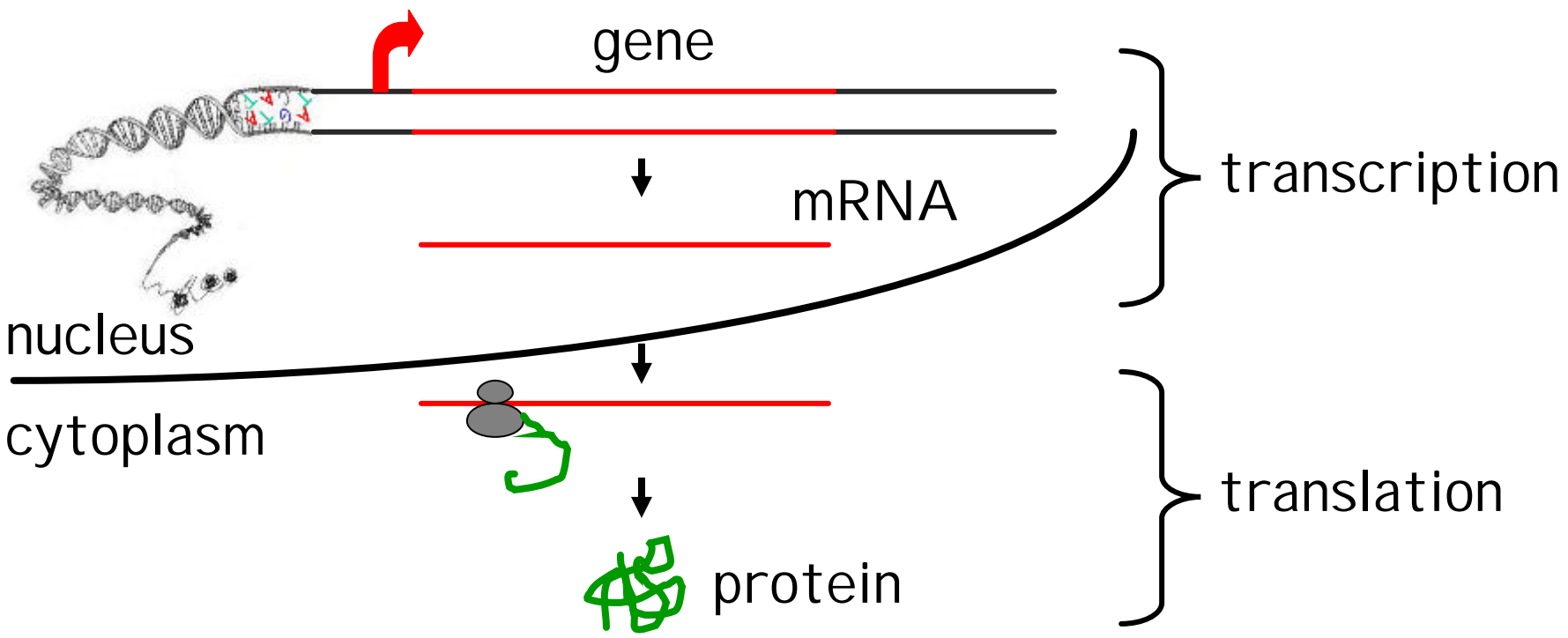
The human cell



- 23 pairs of chromosomes
- About 3×10^9 bases
- About 25'000 genes

- The chromosomes carry the genes, which contain information necessary for the cell to function in the organism

Flow of information



gene → protein → function

The transcriptome

a few numbers and facts:

- 25'000 protein coding genes
- about 15'000 are expressed in a given cell type (tissue)
 - some expressed at high level (globin)
 - some expressed at low level (telomerase)
- 300'000 mRNA molecules/cell
- 2 categories of genes:
 - house keeping
 - tissue-specific

The transcriptome is a direct reflection of the identity and the metabolic status of a cell at a given moment

The transcriptomic

- Measure of the level of expression of the genes in various situations
- Identify modification in expression
- Correlate these changes with the physiological status of the situations

The methods

- Before:
 - Northern blotting
- Curently
 - Microarrays
- Same principle: HYBRIDIZATION

Hybridization



complementarity

T C A A G T G A T A T A G
 +
 A G T T C A C T A T A T C



T C A A G T G A T A T A G
 A G T T C A C T A T A T C

non-complementarity

T C A A G T G A T A T A G
 +
 A G **A** T **G** A C **G** A **C** A T C

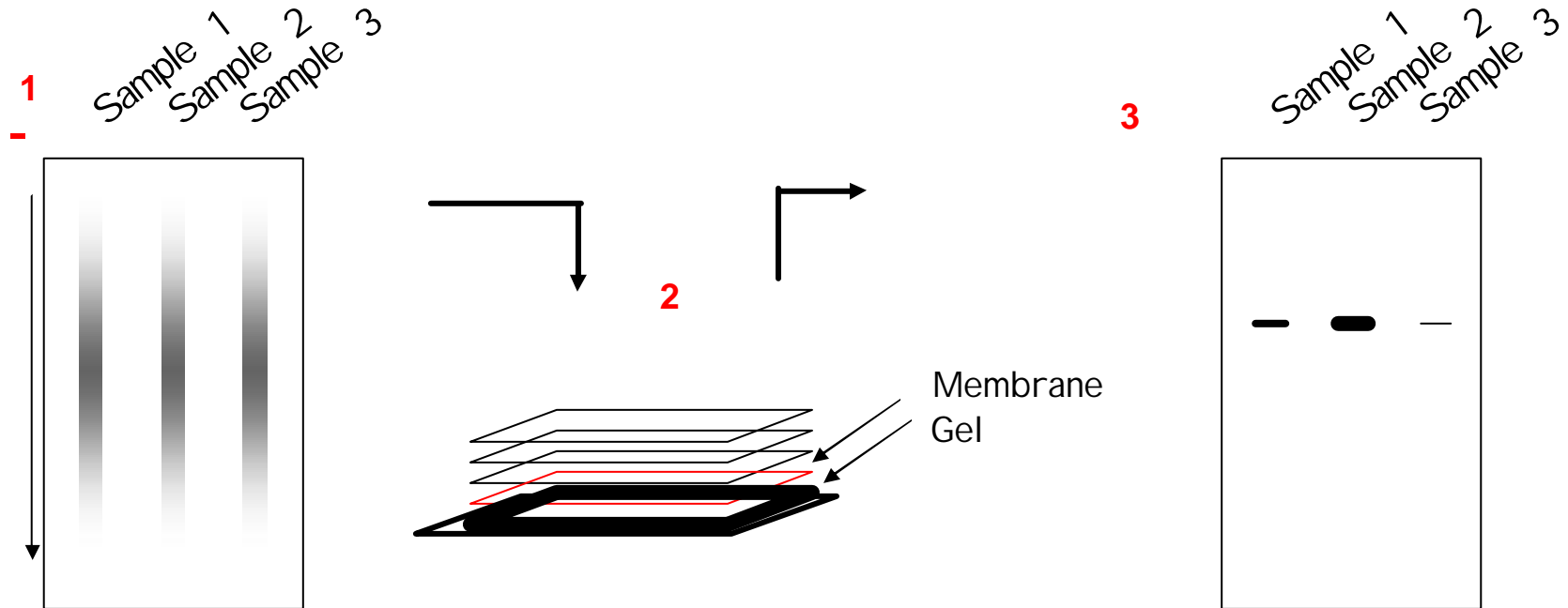


T C A A G T G A T A T A G
 A G **A** T **G** A C **G** A **C** A T C

Influenced by:

- Degree of complementarity
- Temperature
- Salts concentration
- Concentration of the two complementary strands

Northern blotting (ancestor of arrays)

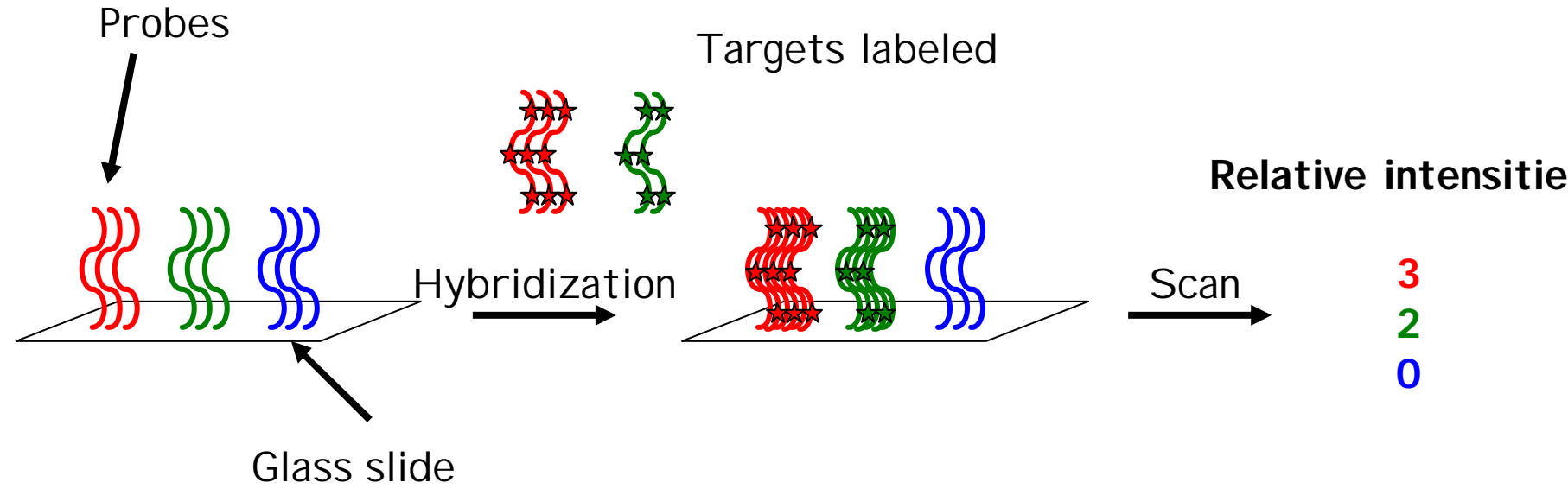


+

1. separate RNA by size
2. transfert RNA onto membrane
3. hybridize labeled probe

Measures only ONE gene

Microarrays



Highly parallel measure of the expression level of all the genes in a single experiment

Global vision

A profiling experiment

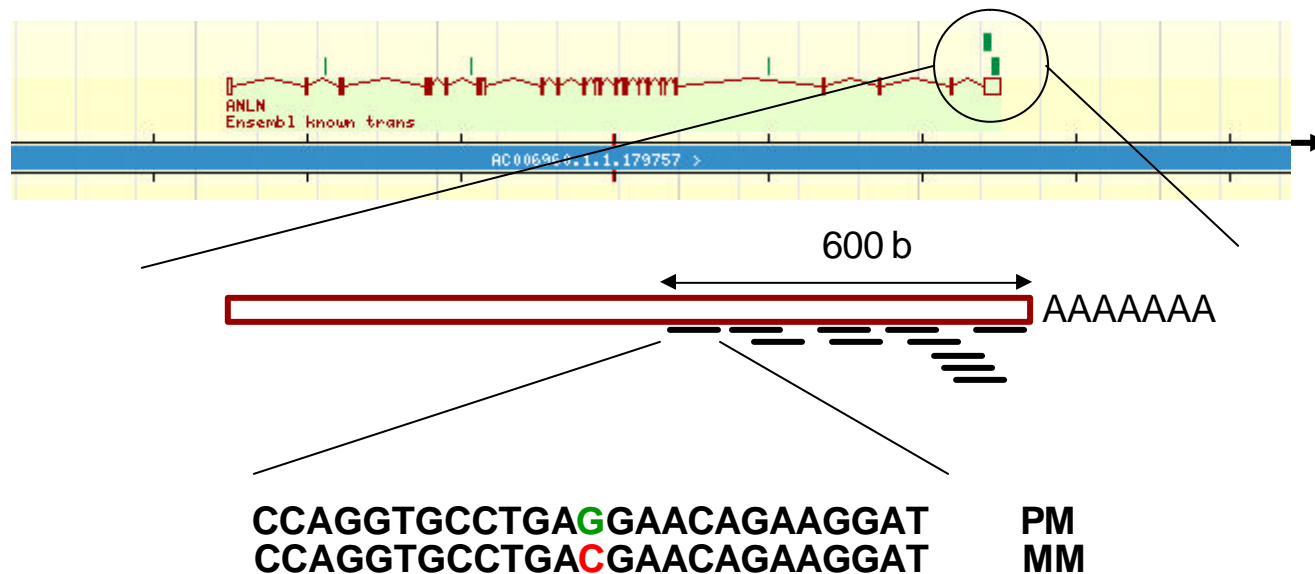
- The question (biological-medical)!
- Choice of the microarray
- Experimental design
- RNA extraction and labeling
- Hybridization and image acquisition
- Data analysis and interpretation

A profiling experiment

- The question (biological-medical)!
- **Choice of the microarray**
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- **Hybridization and image acquisition**
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GeneChips® (Affymetrix microarrays)

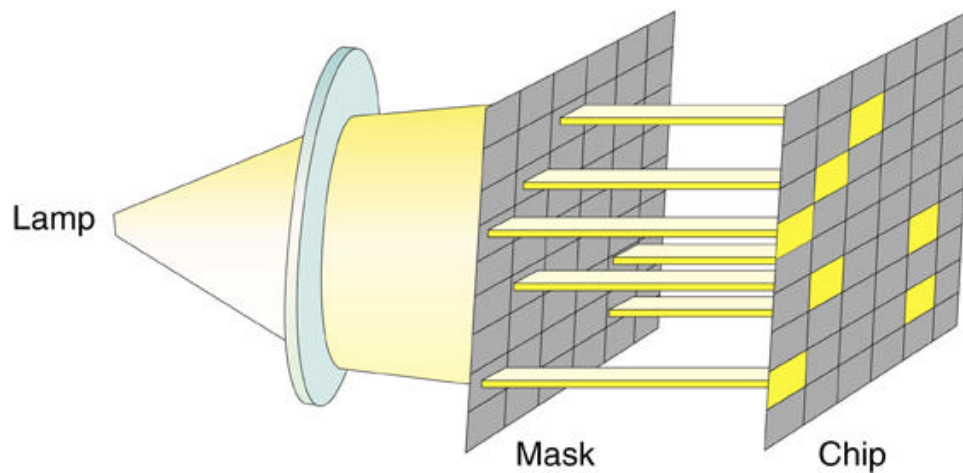
- Selection of probes (specificity and melting temp)
Bioinformatics and experimental



- **11 pairs of oligos/transcript measured**
- **45'000 transcripts, so more than 1.5×10^6 oligos**

GeneChips® fabrication

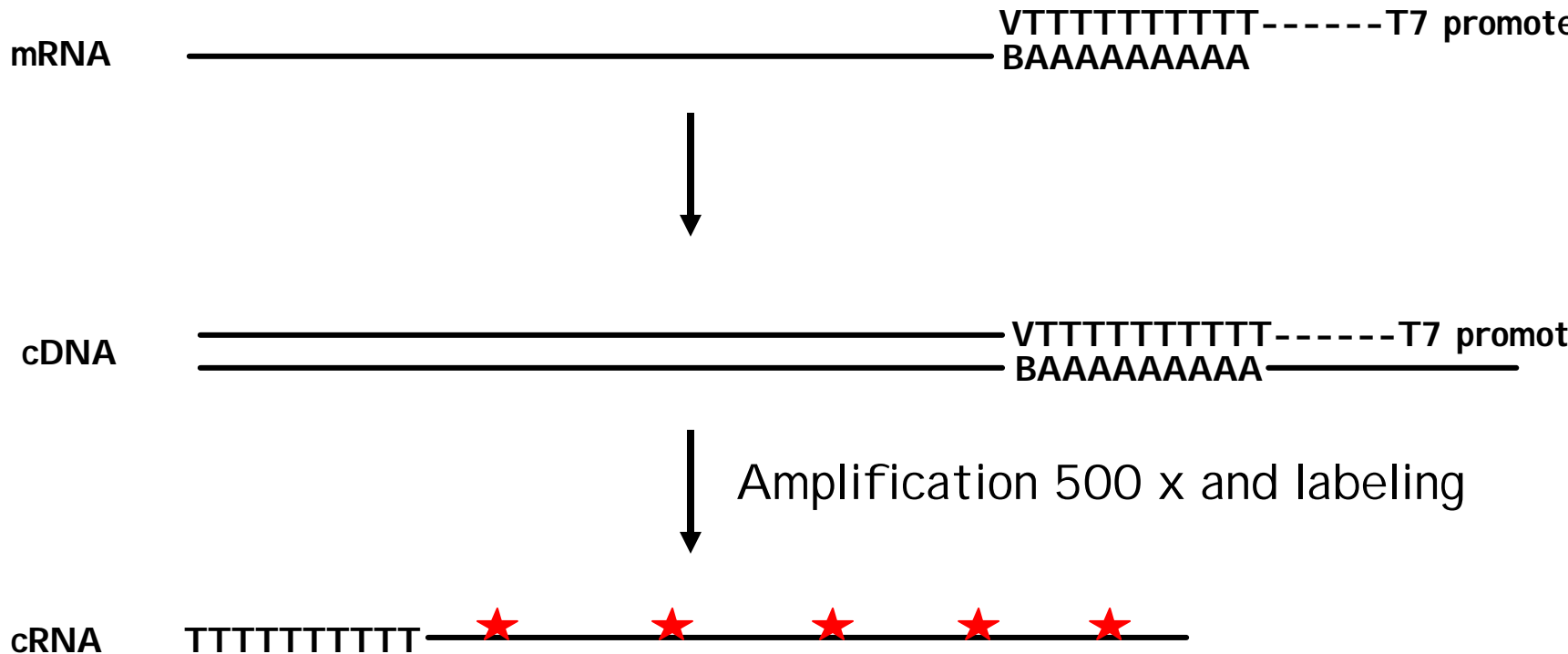
Photolithographic Synthesis



Film

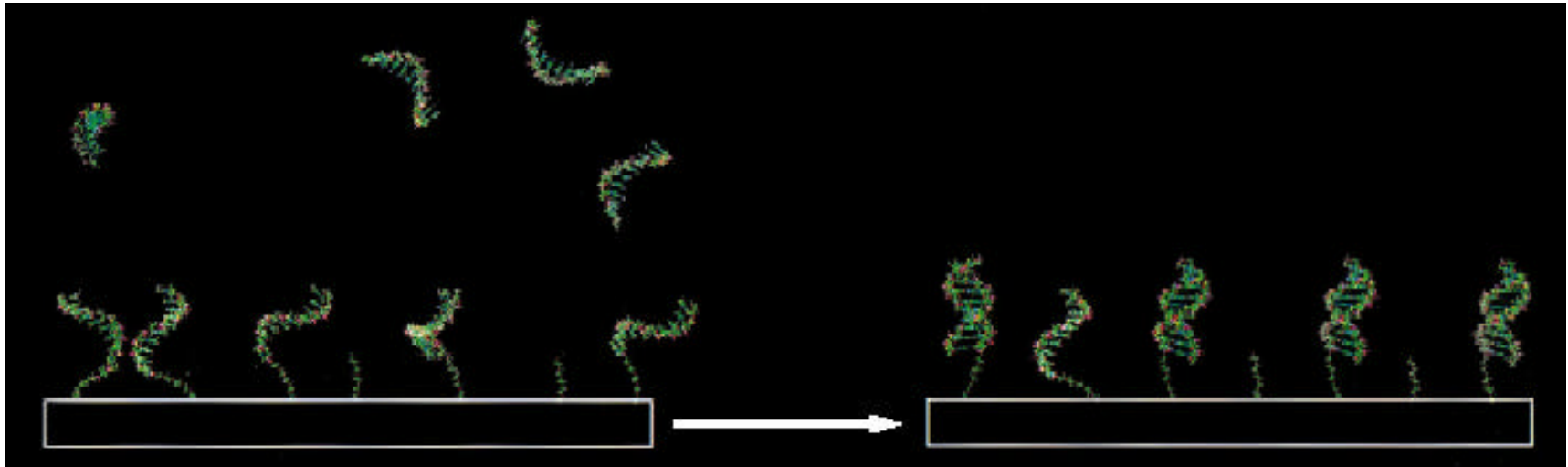


Target preparation

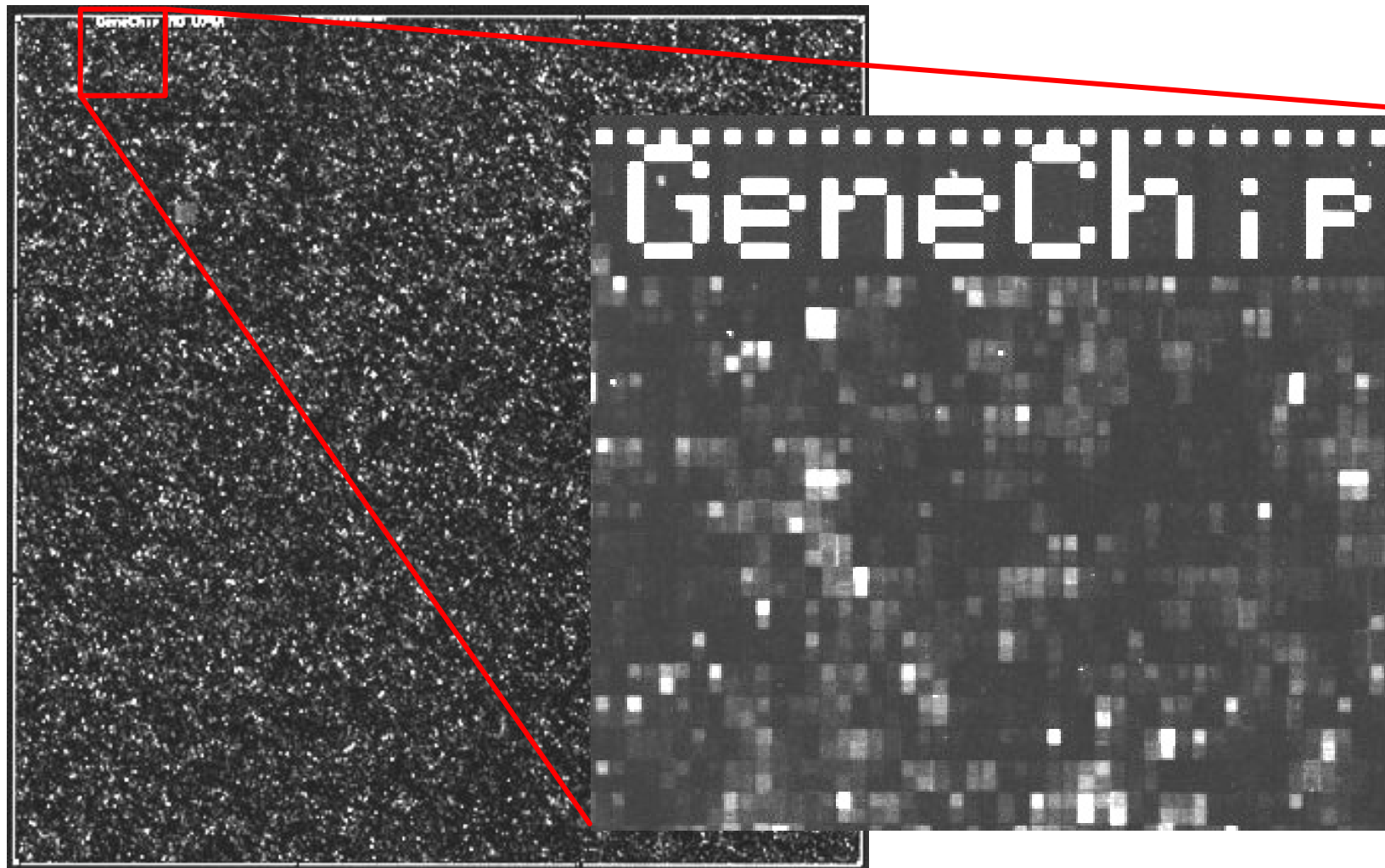


Hybridization

- In conditions that favor signal/noise (temperature, target concentration, time)



Scan: raw image



Instruments

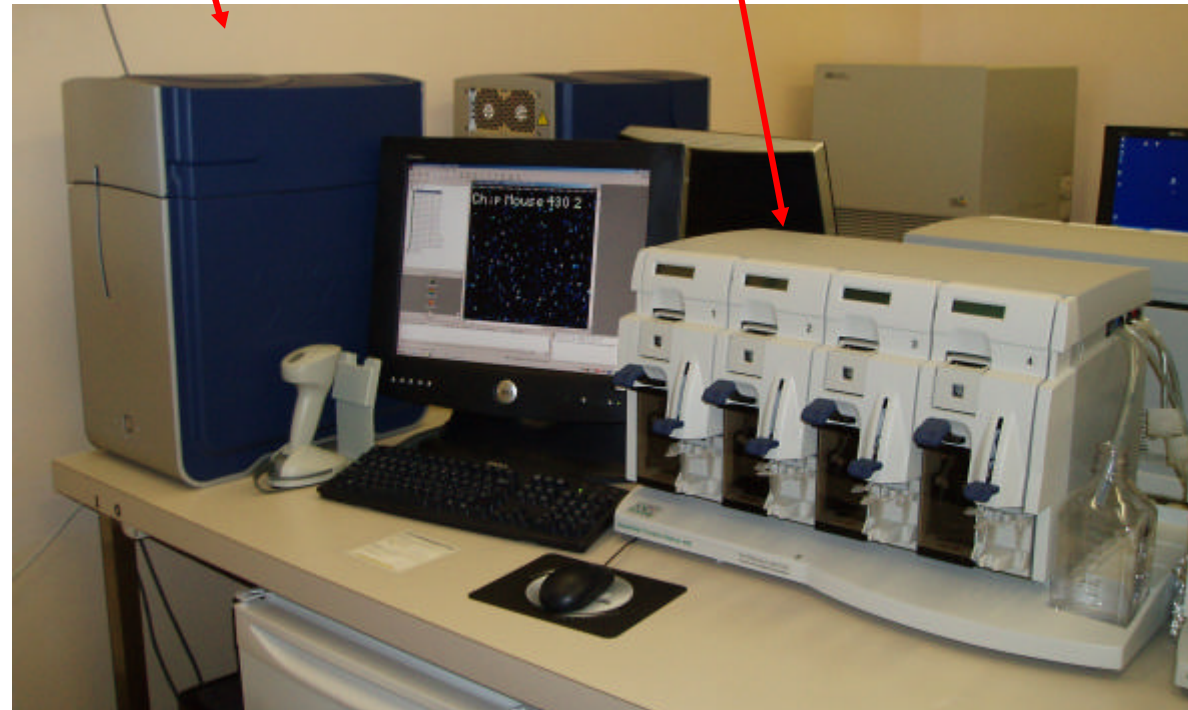
- Affymetrix Genechip System

Hybridization oven



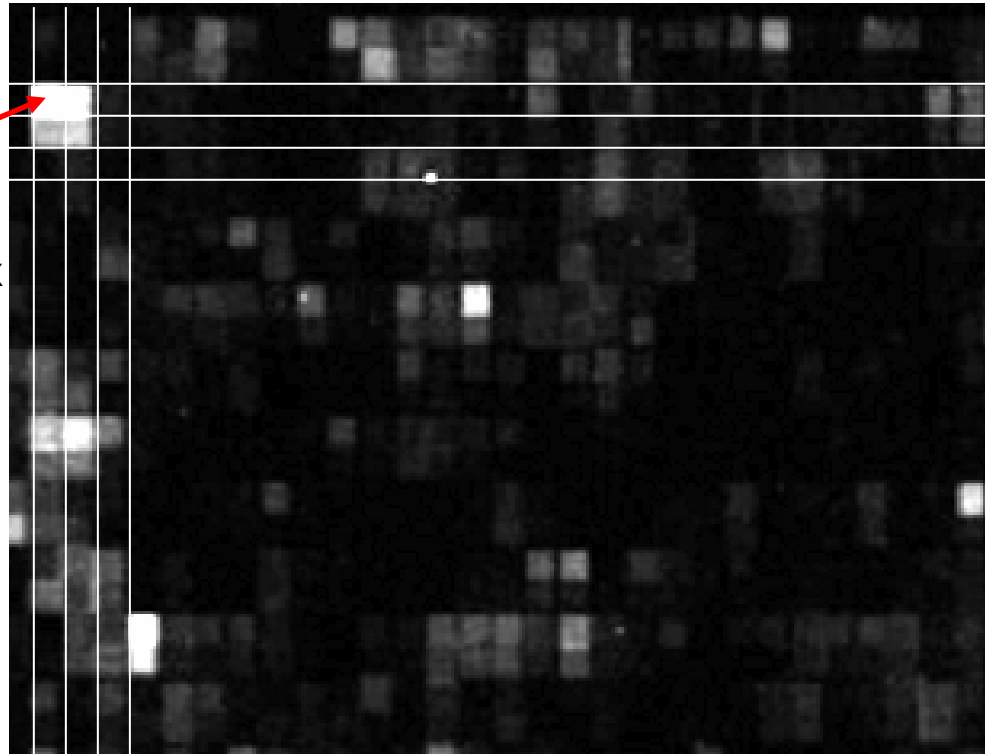
Scanner

Staining/washing station



Grid alignment for probe ID

Oligo Xi for gene x



Data normalization

Objective: minimize the effects of technical noise or technical variability, in order to increase our ability to detect meaningful biological variability

Sources of technical variability:

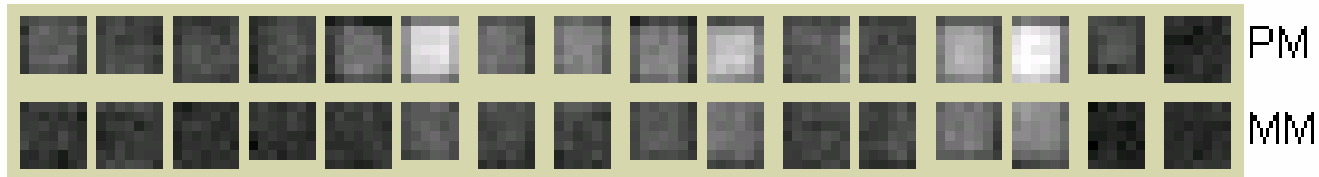
- Amount of starting material
- Enzymatic efficiencies
- Differences between cells types or tissues

In most microarrays experiments, the vast majority of transcripts will not display differential expression.. nothing changes!

Therefore, **global** normalization can be applied.

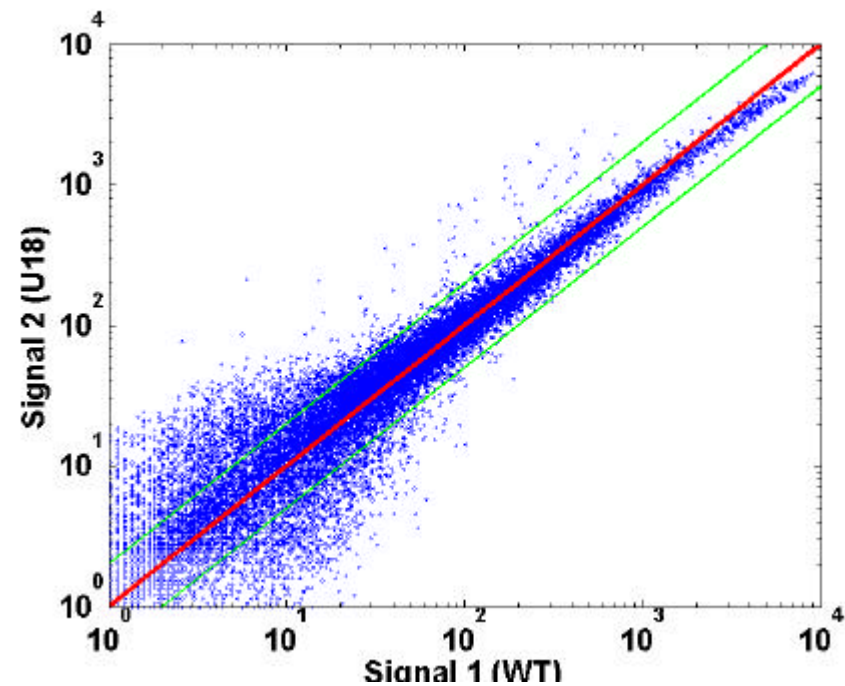
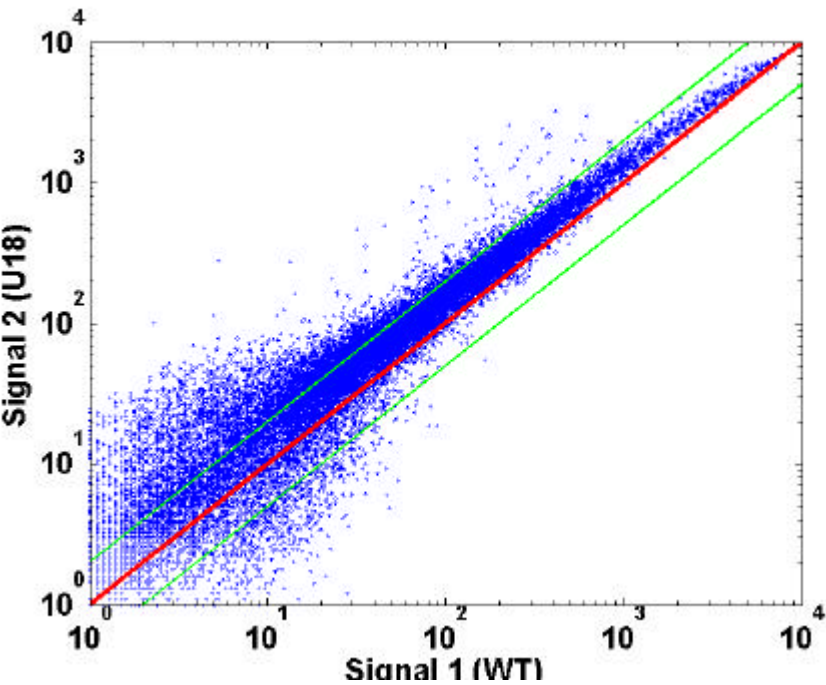
Signal

Probe set: group of oligos used to interrogate a given transcript

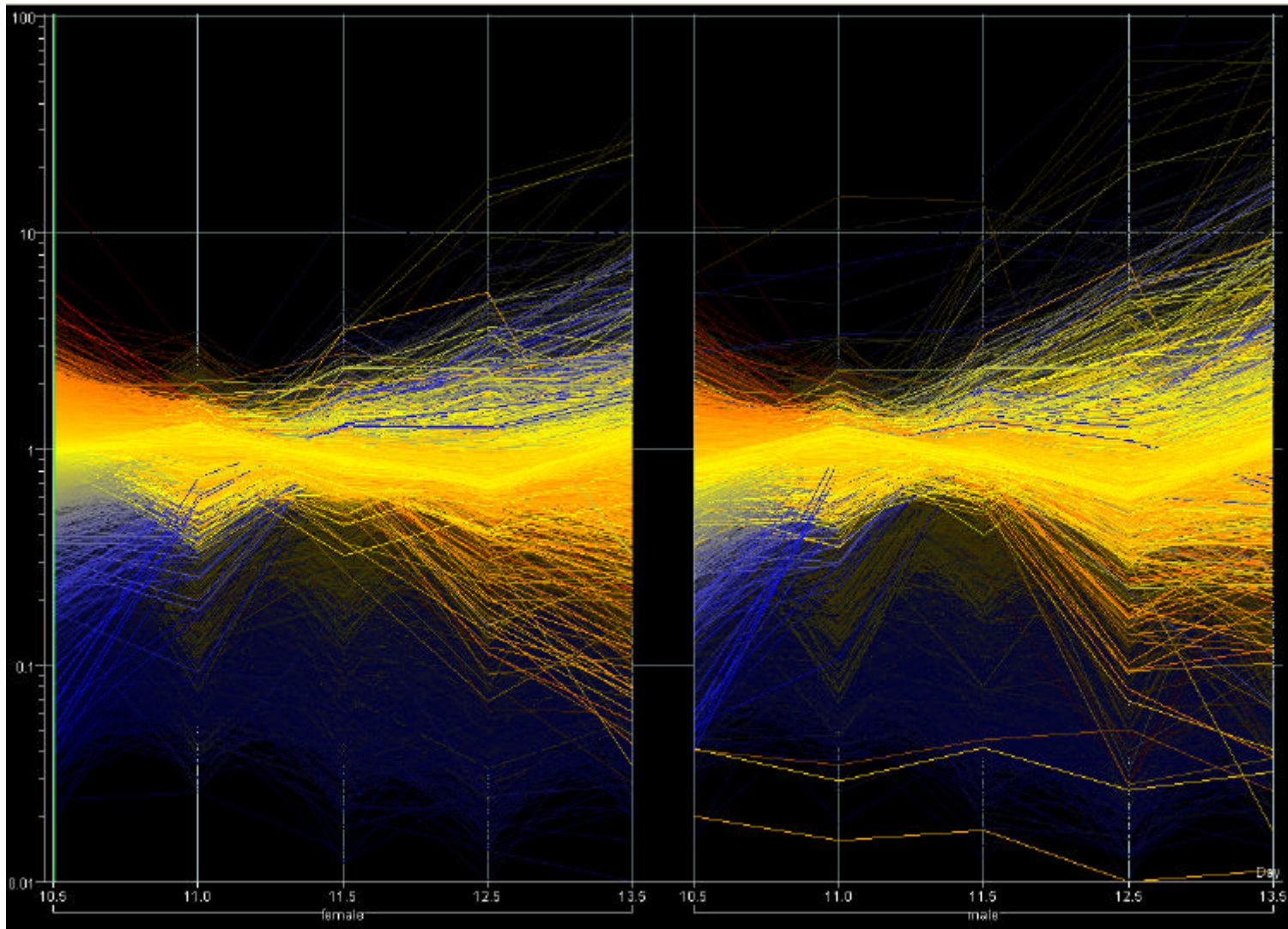


The difference PM-MM is calculated for each pairs of PM and MM
 The signal is calculated using a robust average method of
 all PM-MM differences using a Tukey biweight test

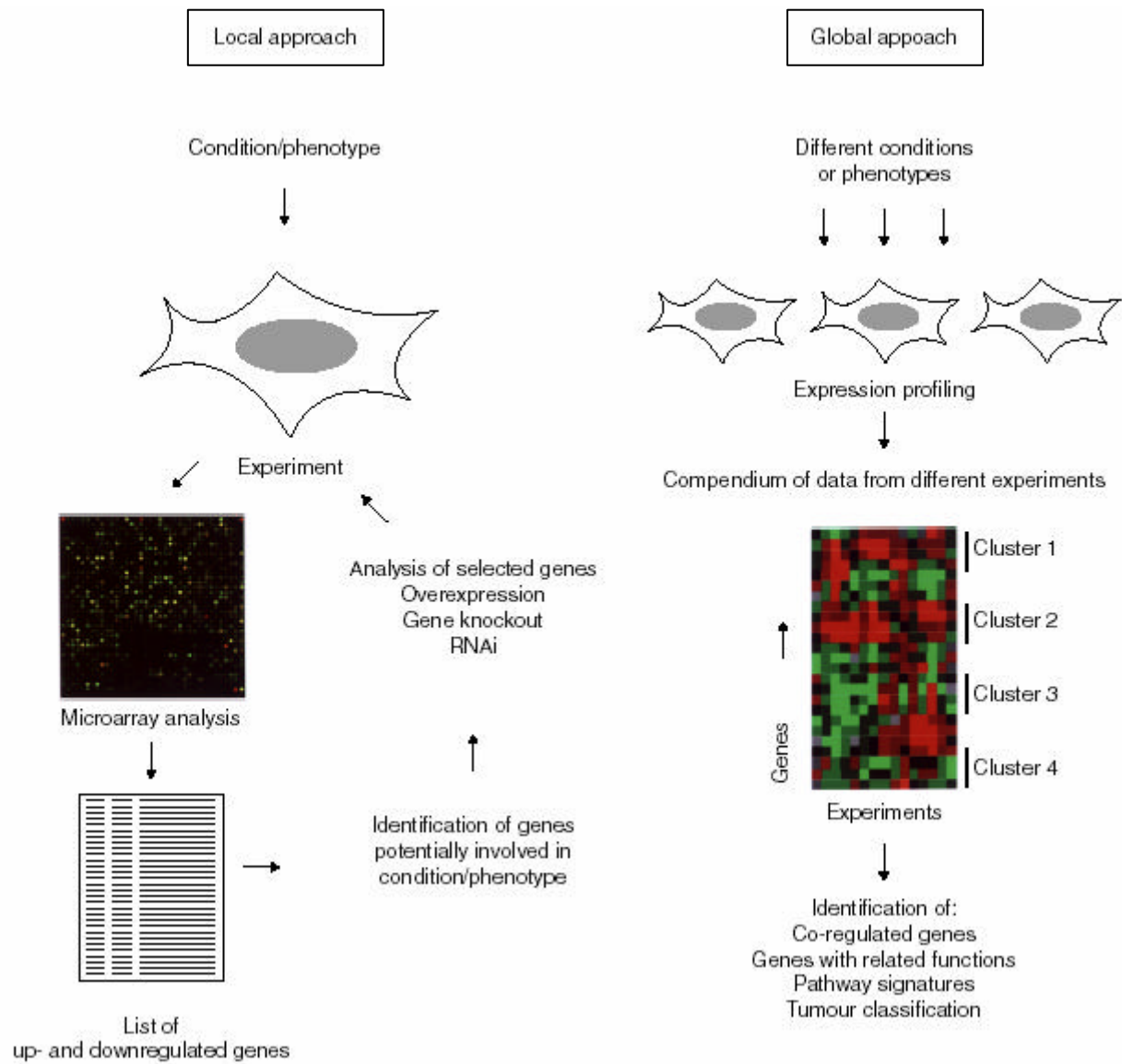
Normalization-scaling



Expression profile

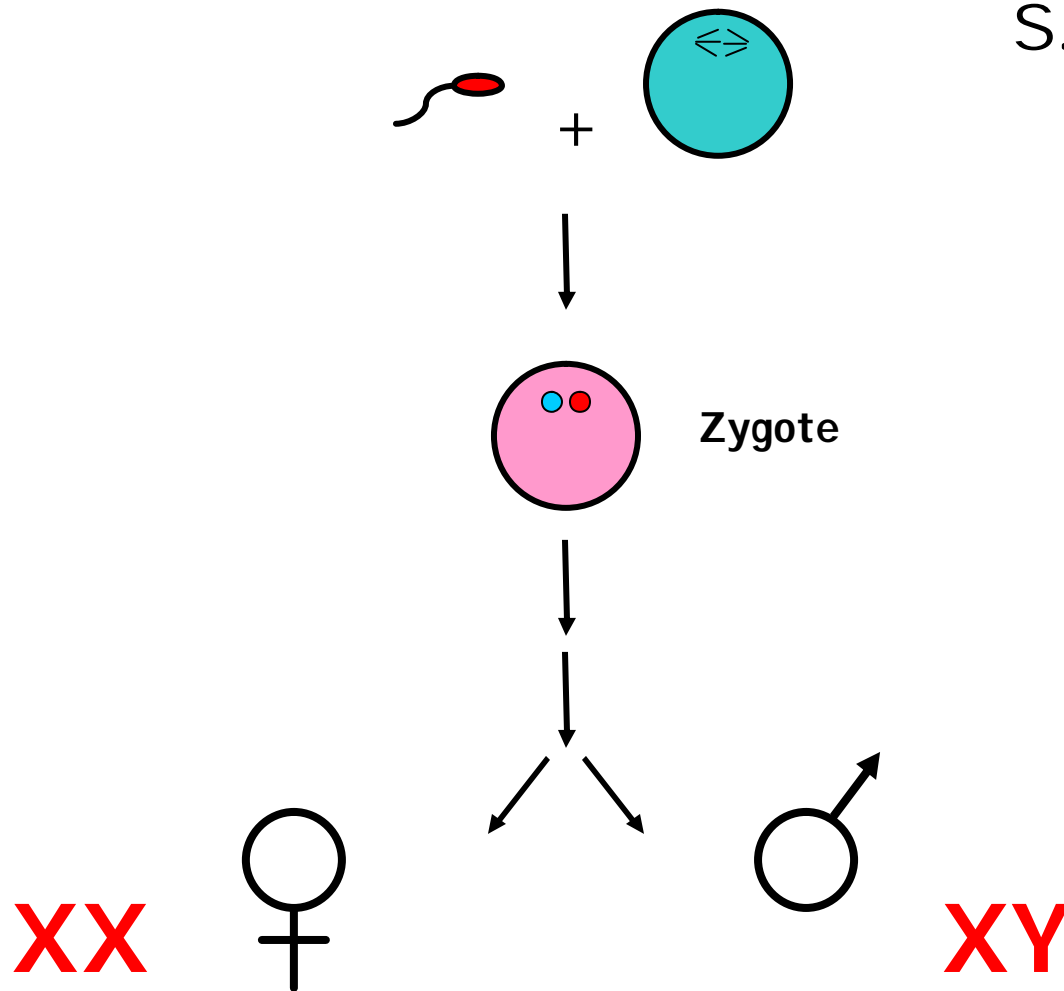


Different purposes for microarrays experiments:



Example 1: sex determination

S. Nef, Uni GVA

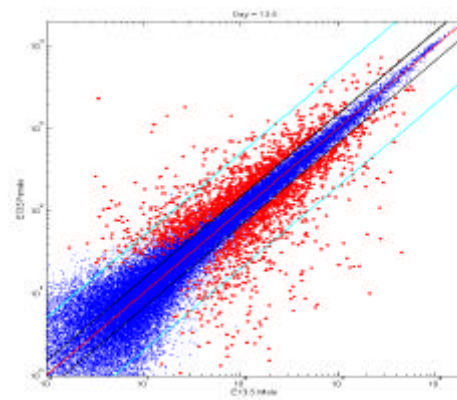
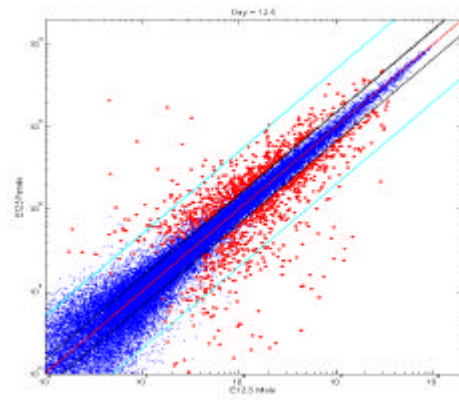
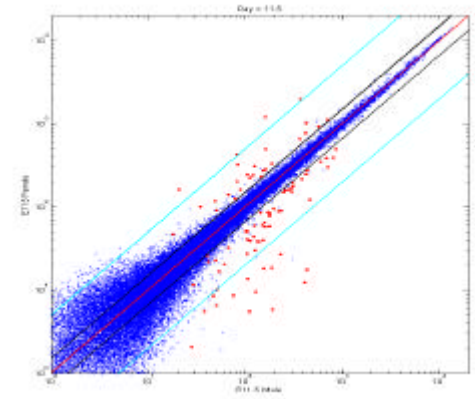
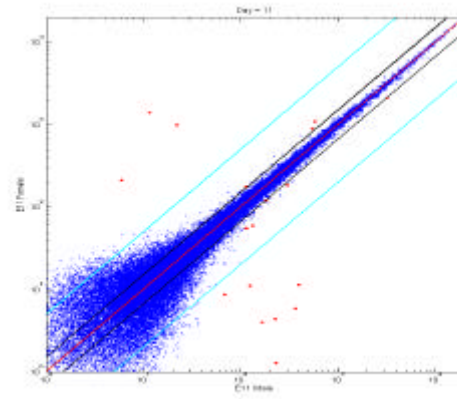
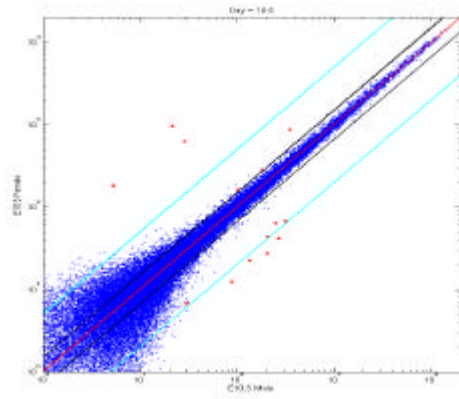


Strategy

In early development, no visible difference

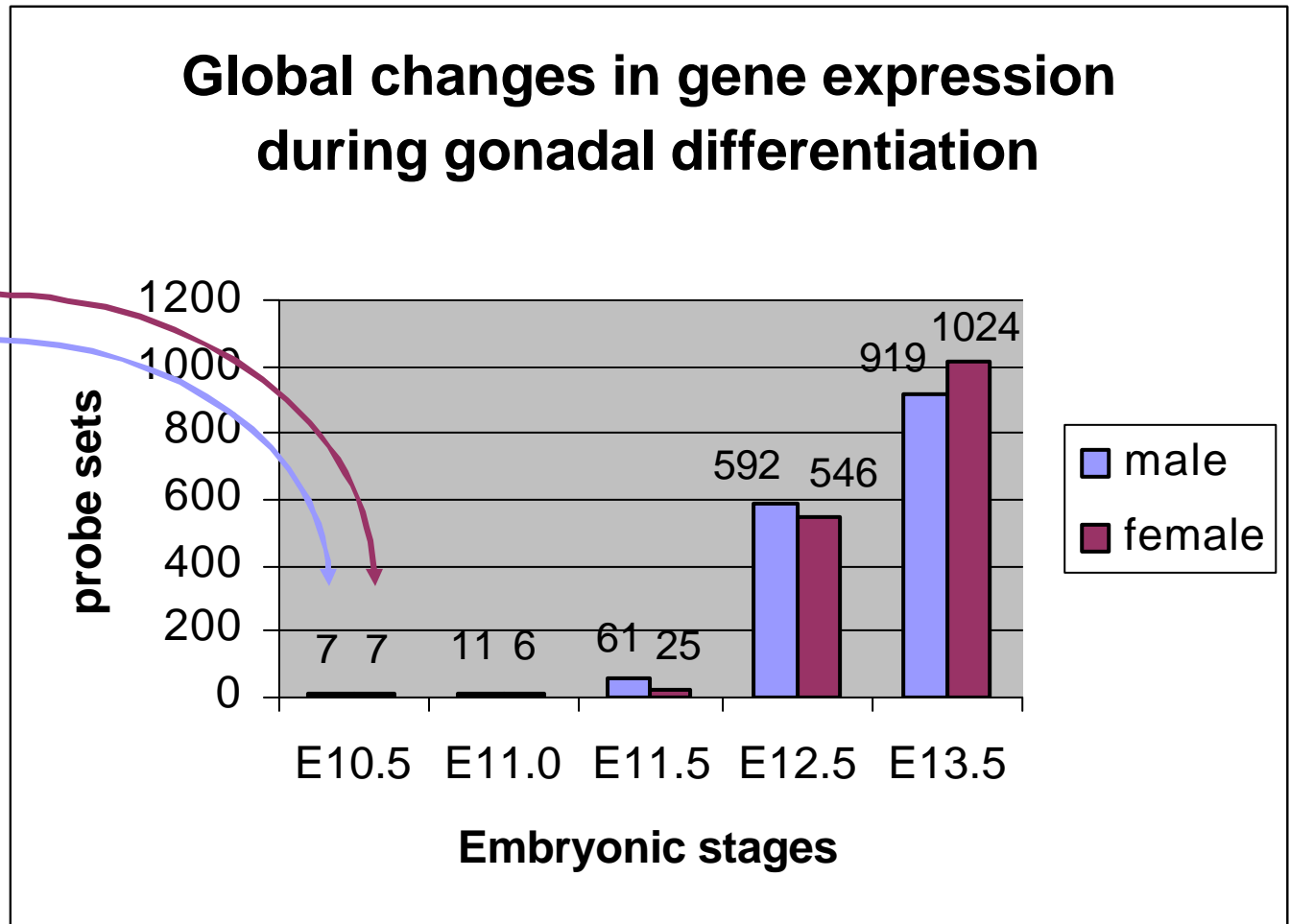
- I solate RNA from male and female gonads before and after sexual differentiation
- I dentify genes showing dimorphic expression

Scatterplots



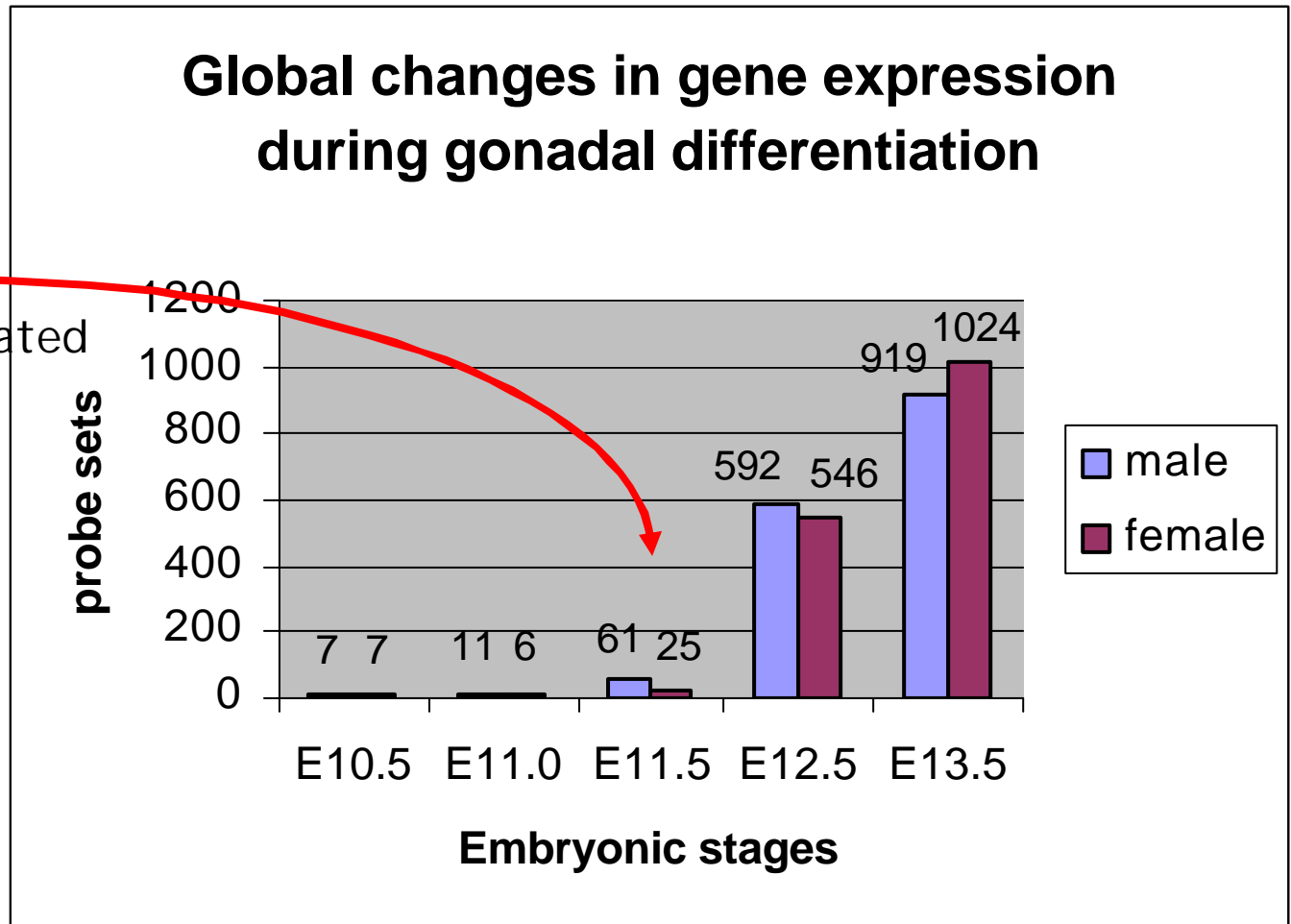
Global changes in gene expression

- X-specific genes
- Y-specific genes

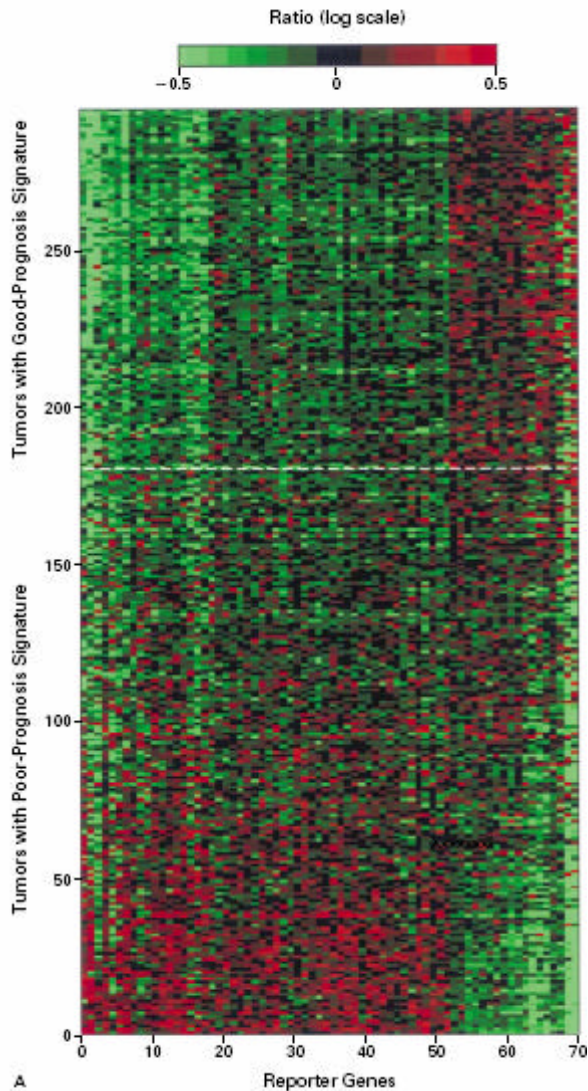


Global changes in gene expression

Male and female
specific genetic
programs are initiated
at E11.5



Example 2: breast cancer

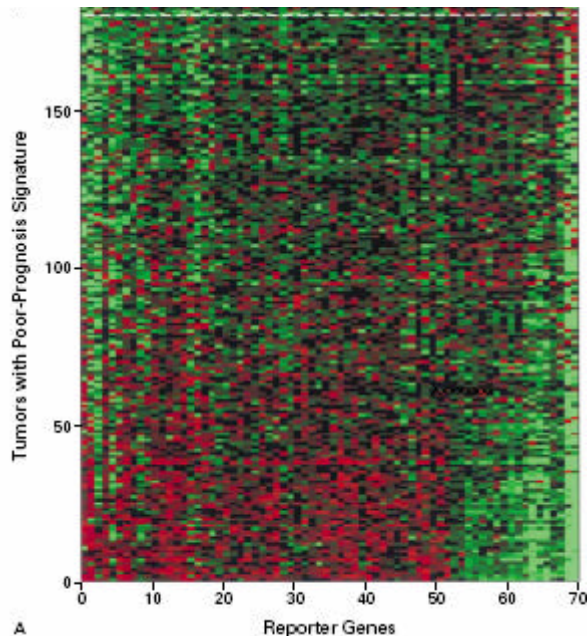


Clustering:
Lines: patients
Columns: genes

Expression profile different depending
on prognostic

From lists of genes to networks

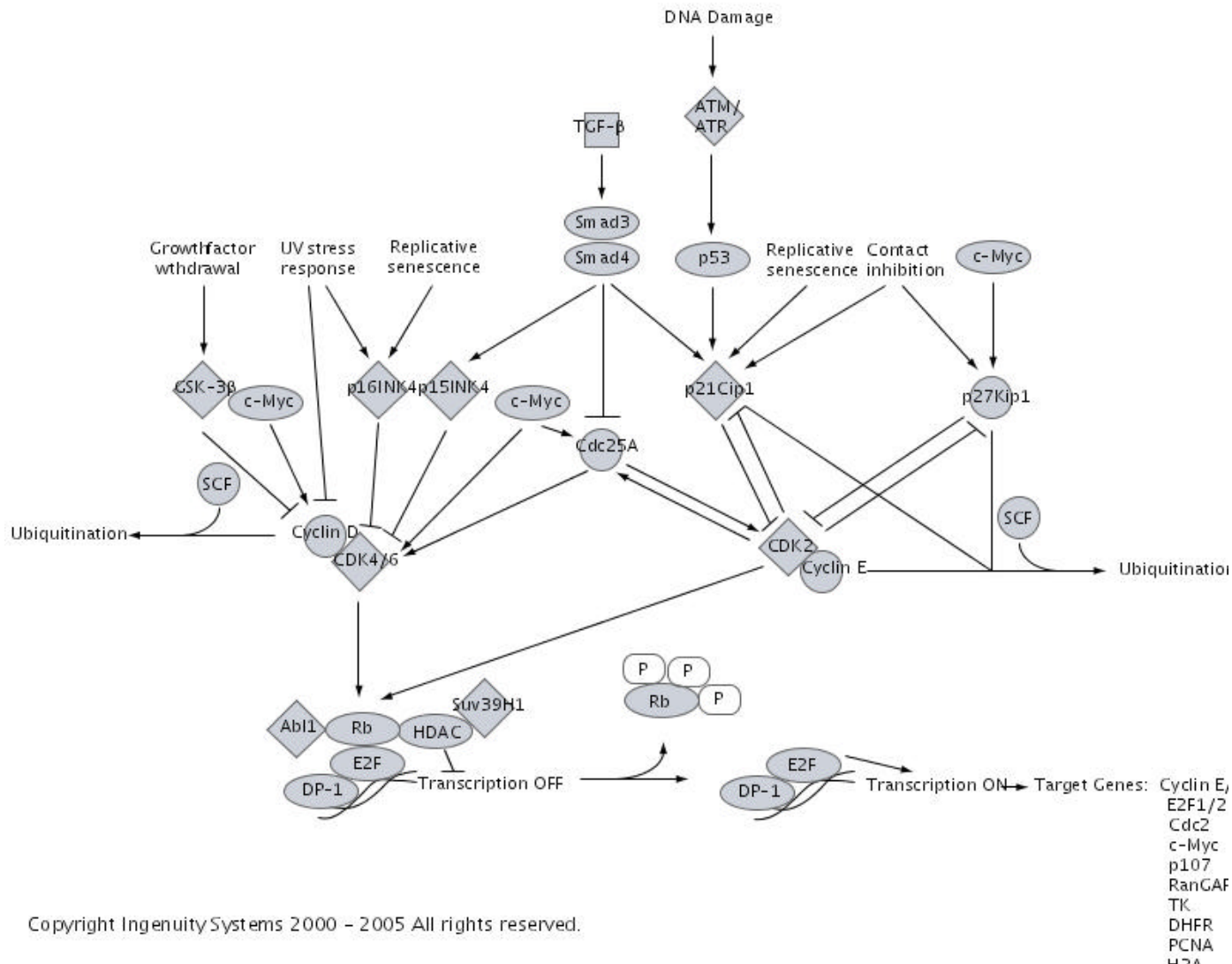
- A) Deduced from similar expression patterns: co-expression may reveal functional relationship



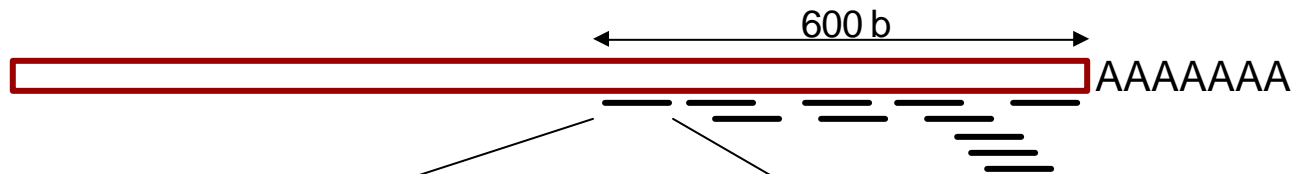
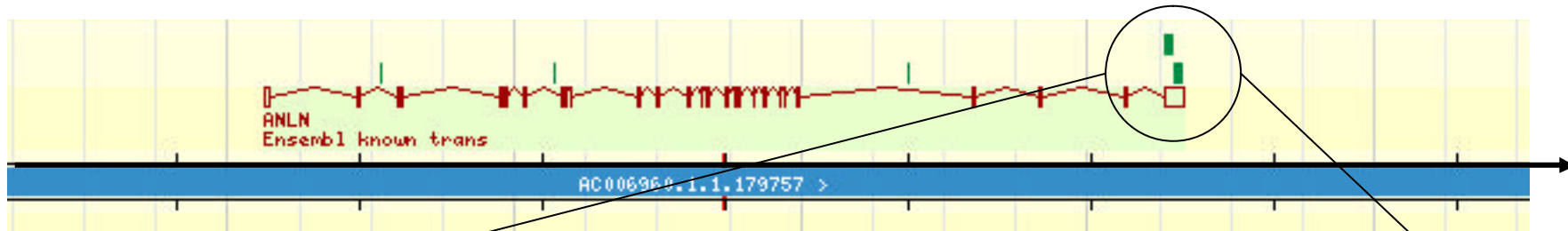
Functional pathways

- B) Use databases of known relationships between proteins-genes to search for genes participating in a similar pathway

Part of the cell cycle pathway

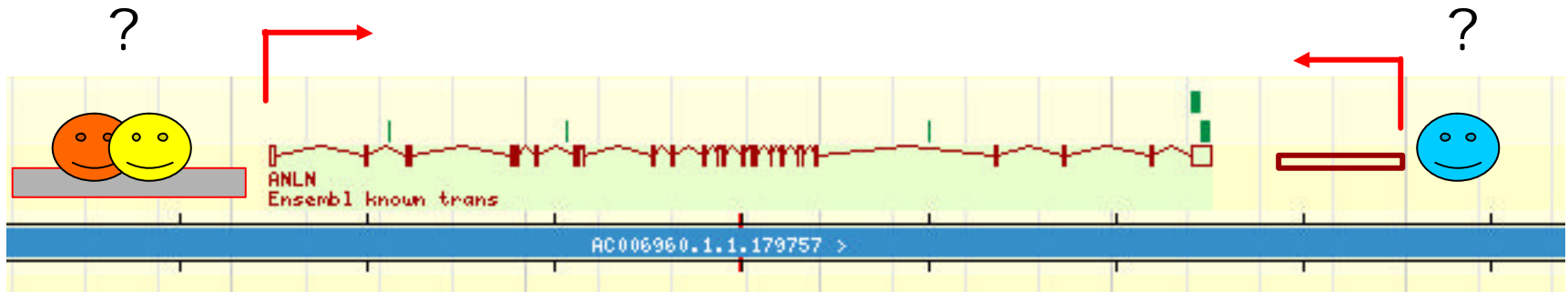


Expression



CCAGGTGCCTGAGGAACAGAAGGAT

Beside known genes ??



Tiling arrays



Oligos walking along the whole chromosome (except repetitive sequences)

Applications: unbiased transcriptome mapping, alternative splicing, ChIP on chip

Currently:

- Human, mouse, drosophila, yeast, arabidopsis whole genome
- Human promoters
- Human exons

GeneChips to networks

“A dictionary contains all the words. So all that Shakespeare had to do was to choose the right words and put them in the right order”

Monty Python

The Genomics Platform

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Mylène DOCQUI ER

Olivier SCHAAD

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<http://genomics.frontiers-in-genetics.org>