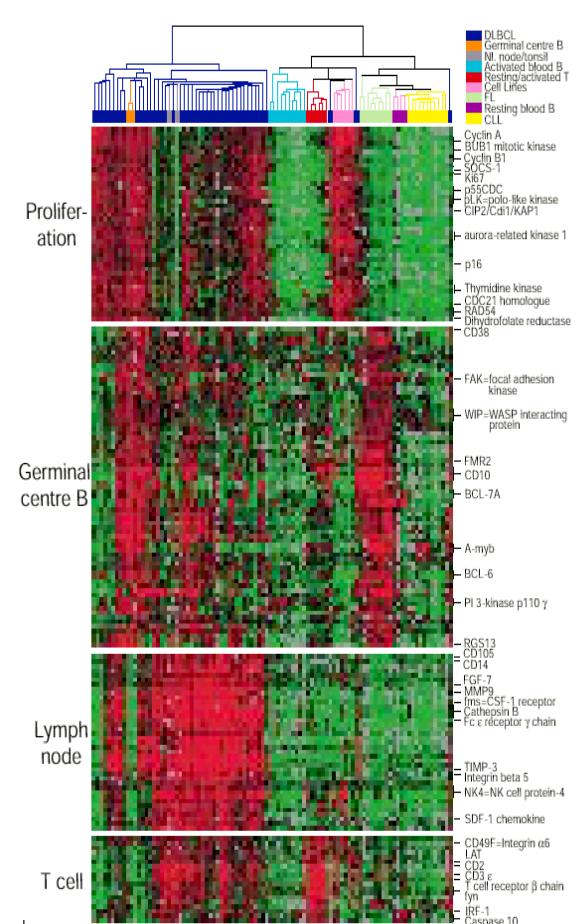
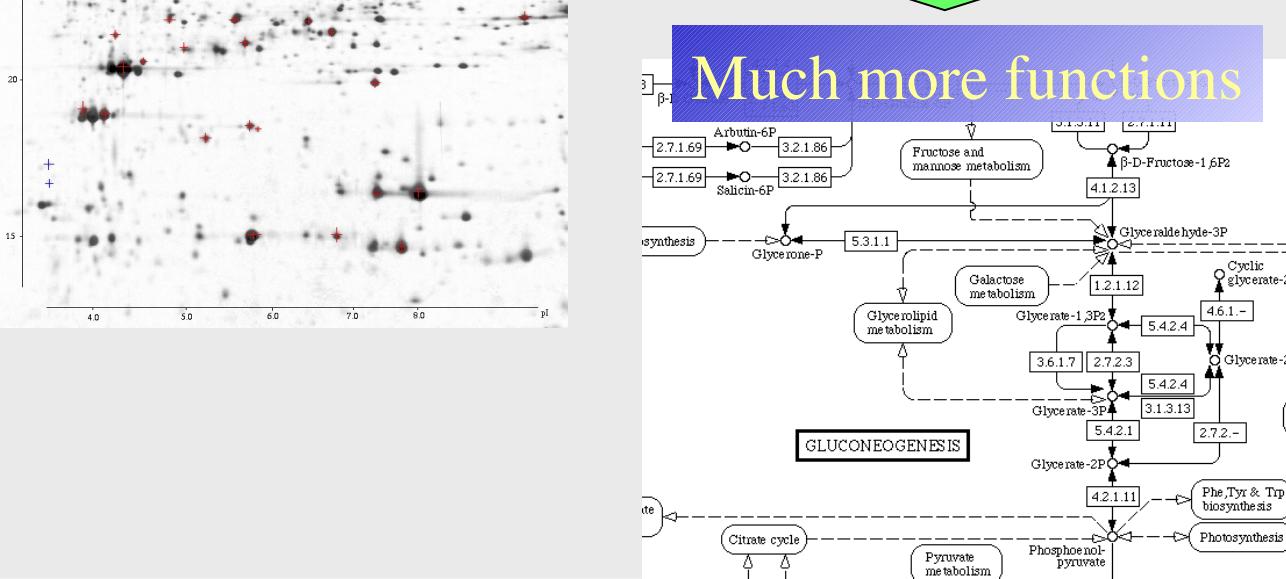
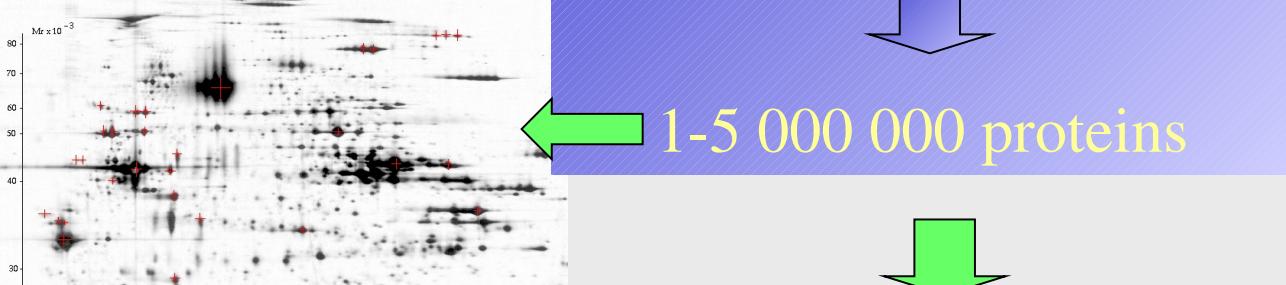
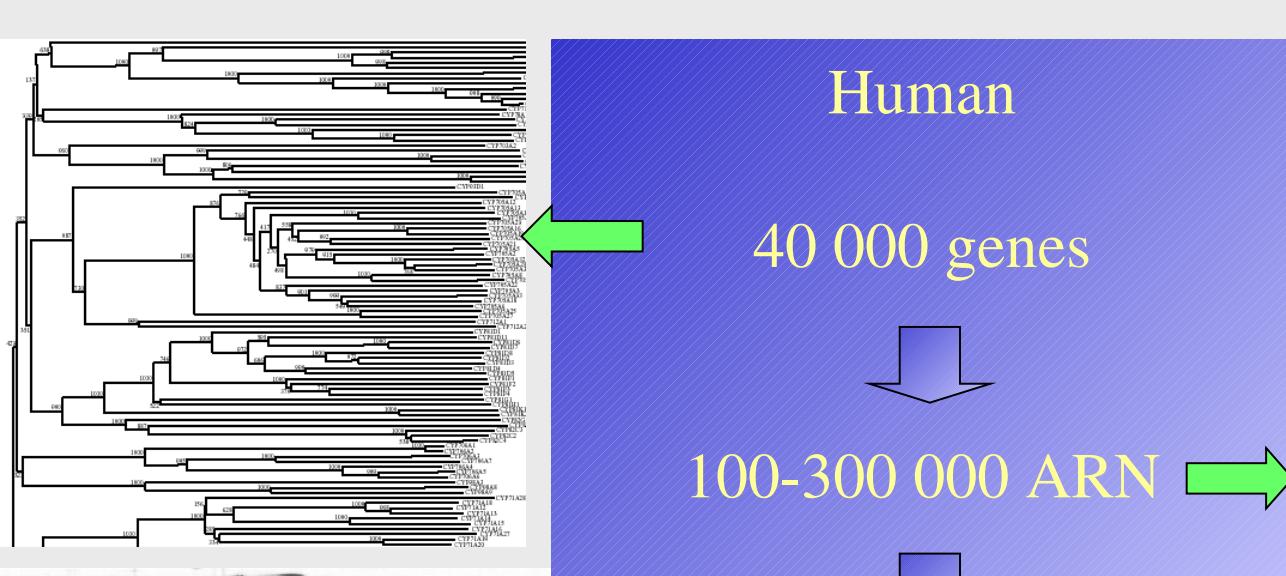


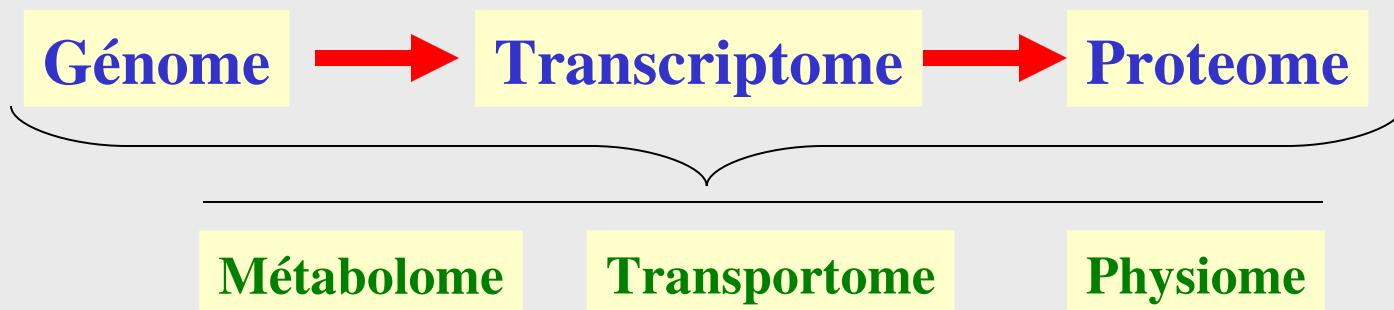
Global approaches for post- economics

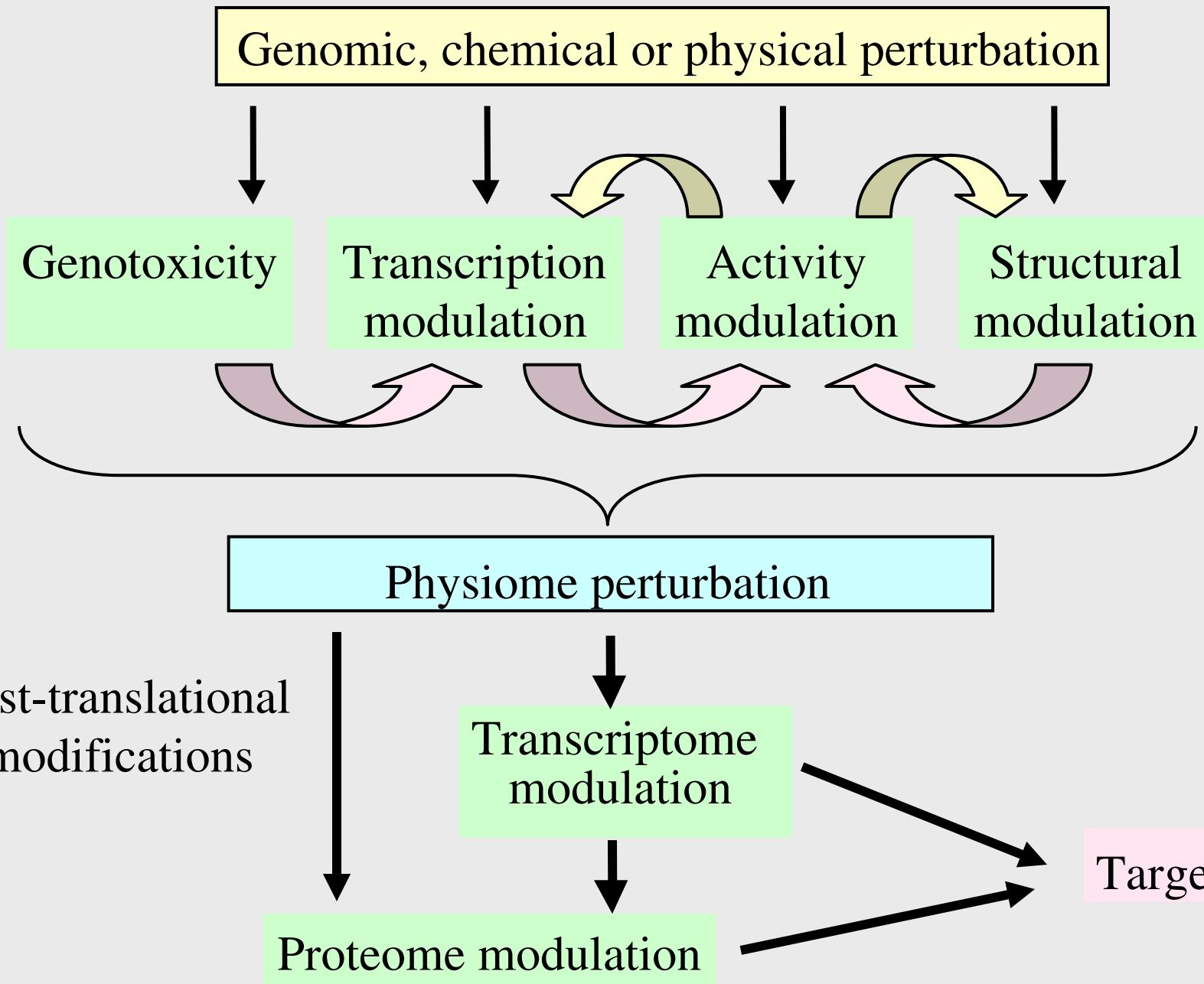


Global approches in post-génomique

new avenues for

- identification of molecular basis of pathologies
- drug discovery
- biotechnologies





Cell culture or organism

No treatment



Treatment

Drugs
Pathogen
Stress / Toxic agents
Transformation

Control cells
or tissue

Treated cells
or tissue

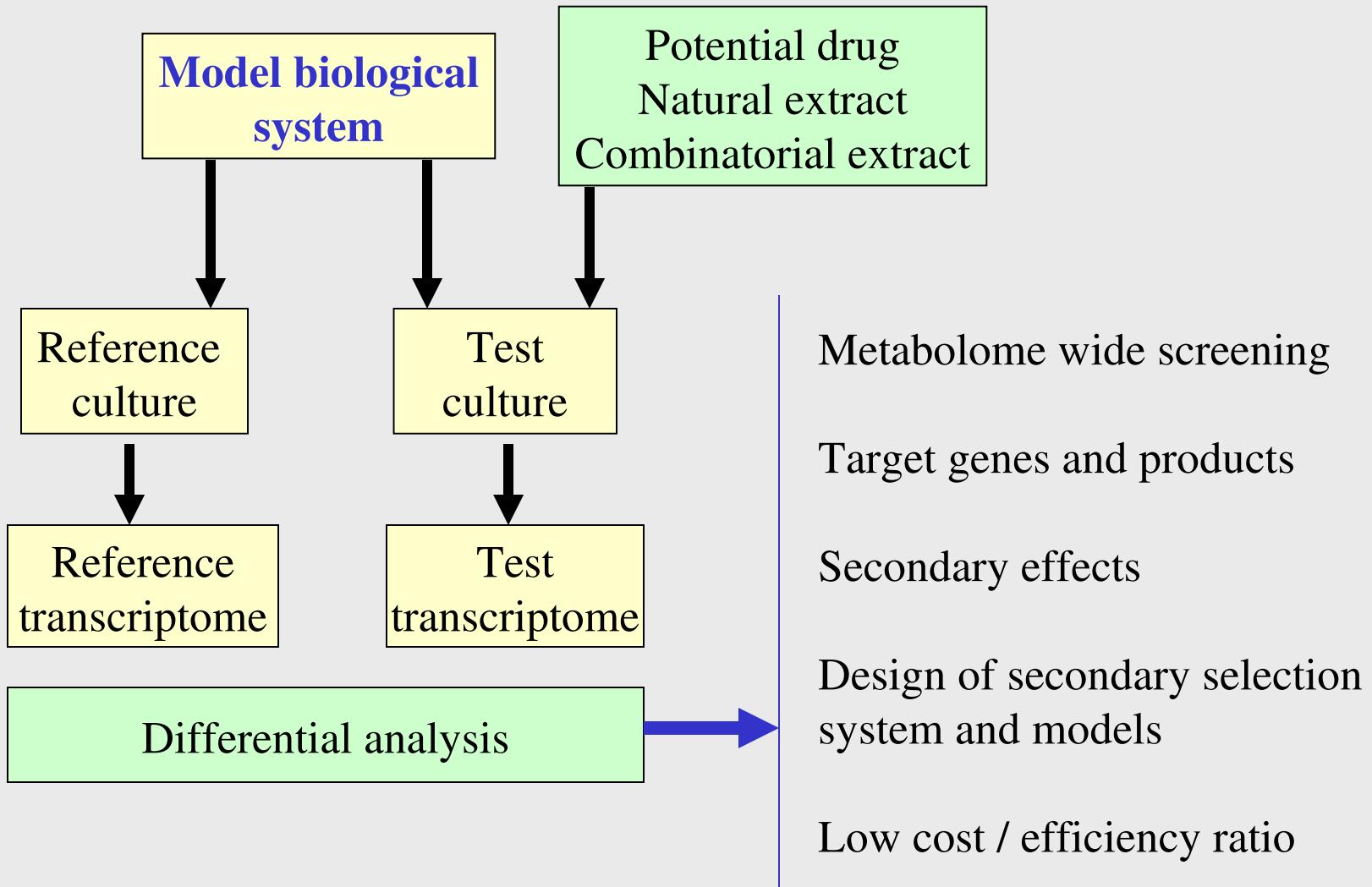


Differential transcriptome or proteome analysis



Molecular target

Differential transcriptome analysis



Nucleic
acids

- Nature (Sequence)
- Quantity (expression)

Proteins

- Sequence
 - Quantity (expression)
 - Processing / modification complexes
 - Folding
 - Catalytic activities
 - Signaling
 - Recognition
- Multiparametric

La transcription



© Sophie Jacopin

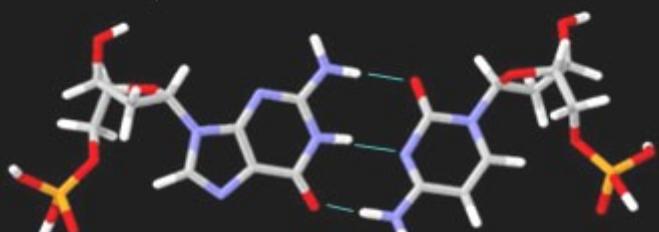
La variation de la transcription est une étape clé de la régulation de l'expression des gènes en réponse à:

- **une adaptation au milieu extérieur (T, P, lumière, absence ou présence d'un nutriment, etc.)**
- **une réponse à un stimulus (nerveux, hormonal, cellulaire)**
- **un devenir cellulaire à respecter (mitose, méiose, différenciation)**

Principe de l'hybridation

Figure B-6: Base Pairing

The chemical structure of each base allows it to match up with another base. The 3D models provide a nice simulation of the shape-dependent base pairing. The actual chemical structures of the bases are shown below, with the bonds drawn in blue.

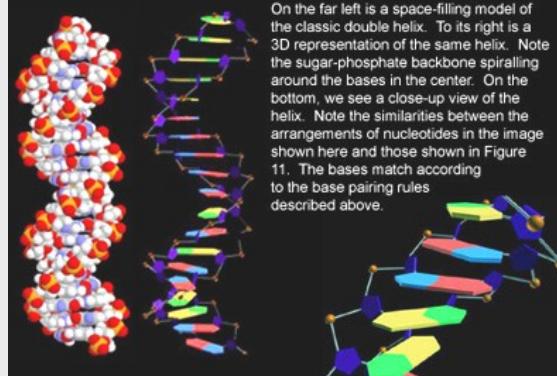


Guanine-Cytosine



Adenine-Thymine

Figure B-7: The Double Helix Revisited



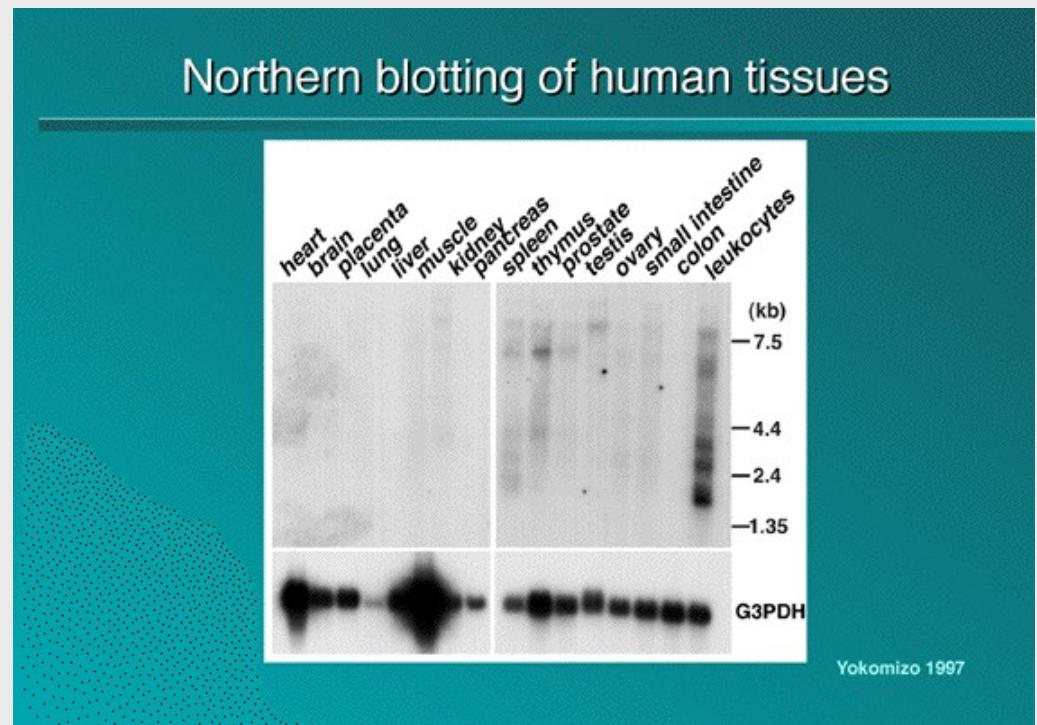
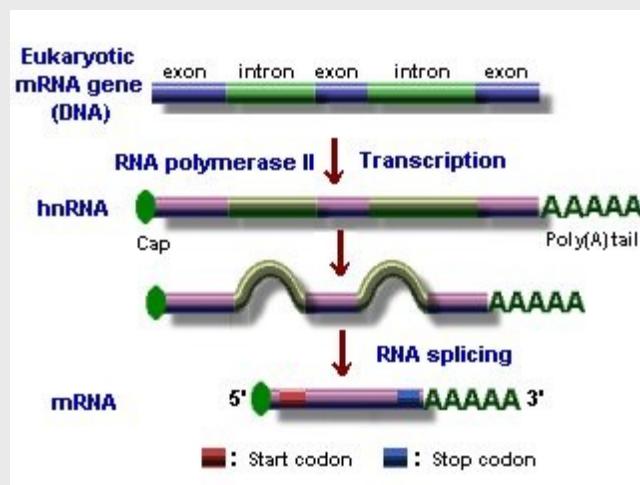
On the far left is a space-filling model of the classic double helix. To its right is a 3D representation of the same helix. Note the sugar-phosphate backbone spiralling around the bases in the center. On the bottom, we see a close-up view of the helix. Note the similarities between the arrangements of nucleotides in the image shown here and those shown in Figure 11. The bases match according to the base pairing rules described above.

Figure B-8: Complementary Strands



DNA strands in a double helix *must* have matching base pairs. Thus, we can figure out the sequence of a single strand's complement simply by picking bases that pair with those in the original sequence.

La technique de Northern Blot



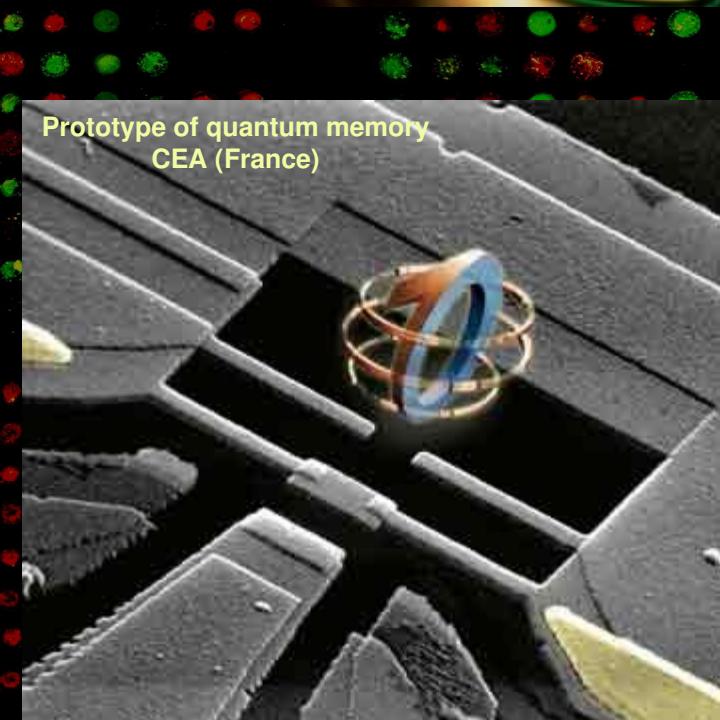
Analyse globale de la transcription

Les techniques classiques de Northern Blot isolent une série de gènes connus, dont on soupconne une régulation. Mais la description des variations transcriptionnelles d'un nombre limité de gènes est-elle suffisante pour analyser la réponse d'un organisme entier?

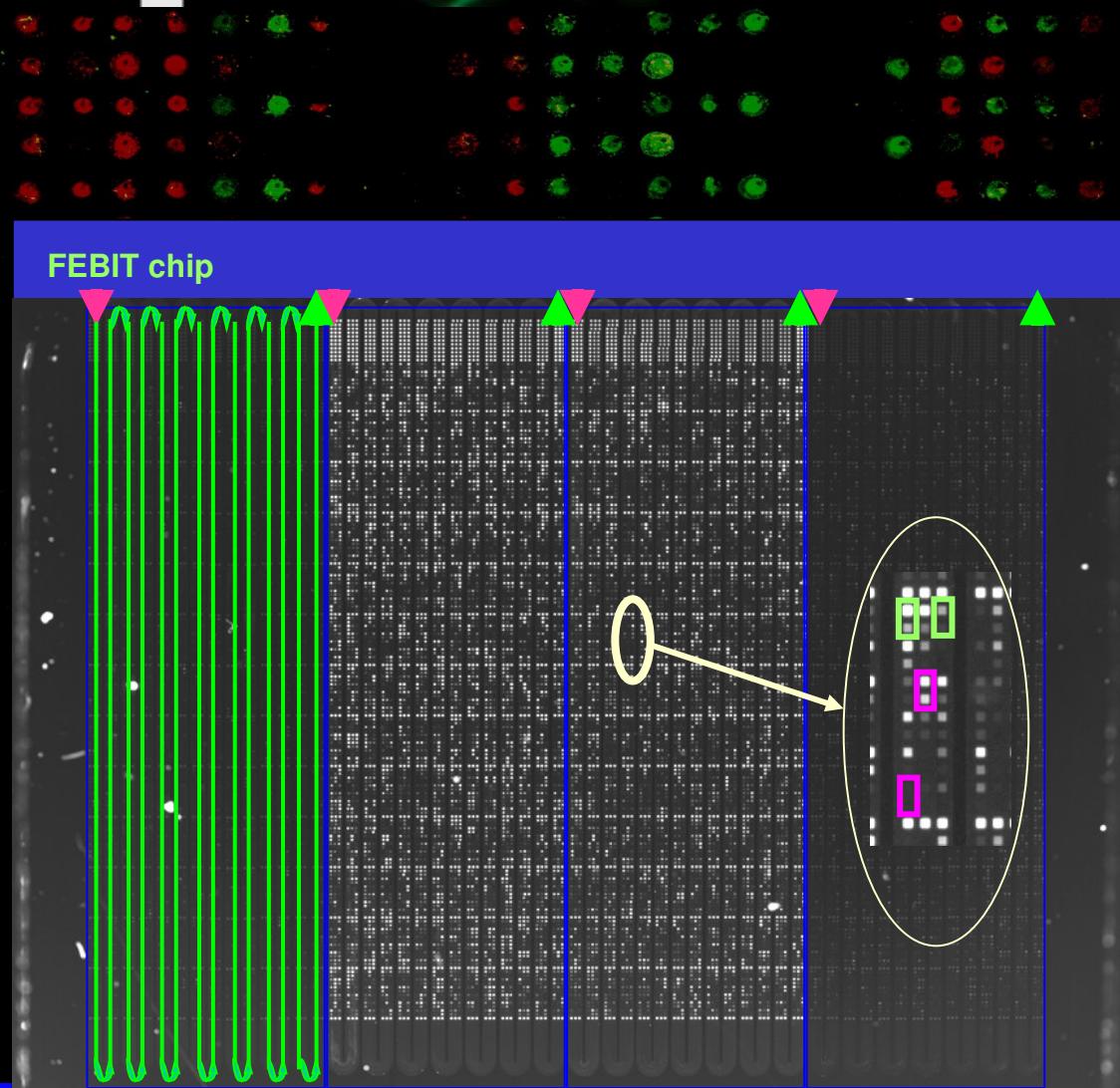
Analyse de la réponse totale des gènes d'un organisme

- **Sans séparer les produits des gènes sur gel.**
- **En miniaturisant l'hybridation pour avoir un volume le plus faible possible (meilleur signal, plus facile à manipuler).**
- **En ayant des conditions d'hybridation identiques pour tous les gènes.**

DNA chip technology

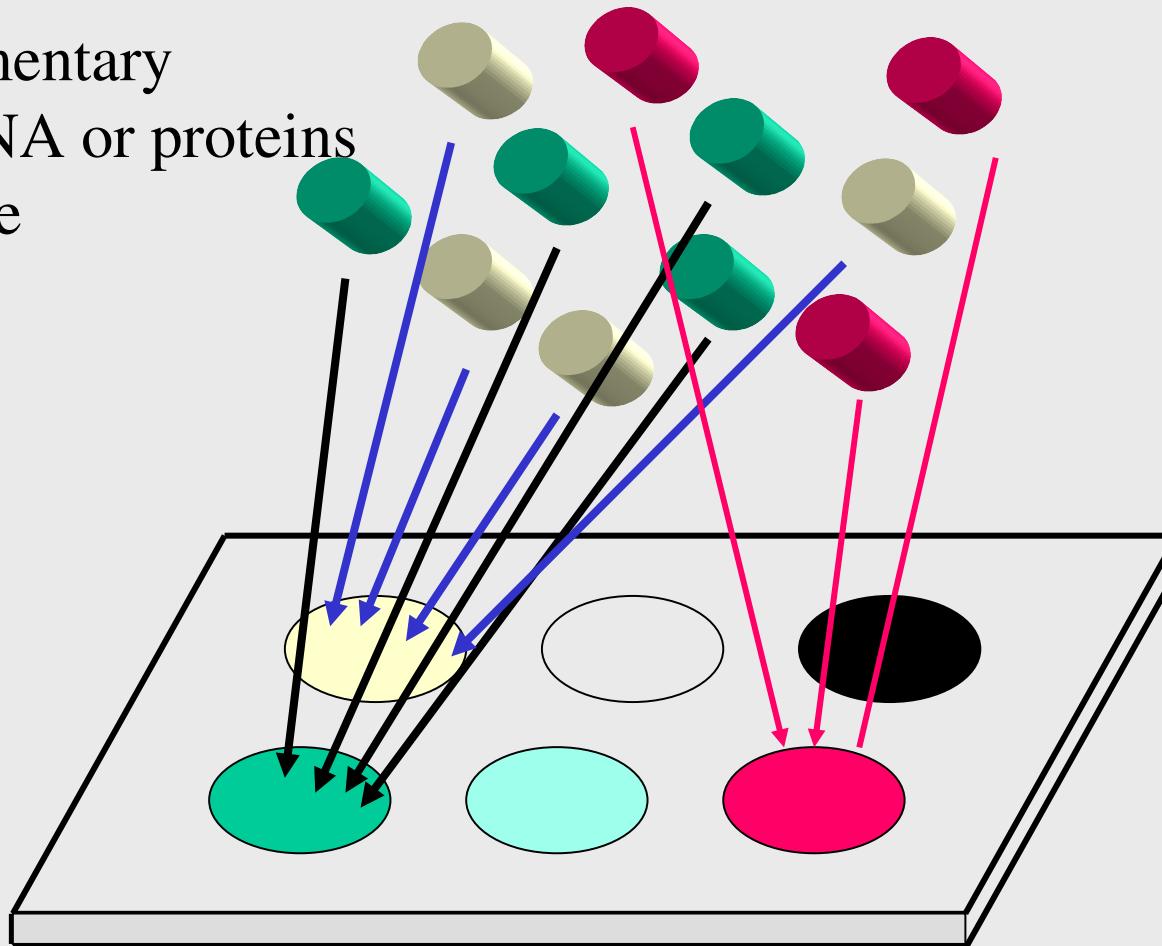


Prototype of quantum memory
CEA (France)

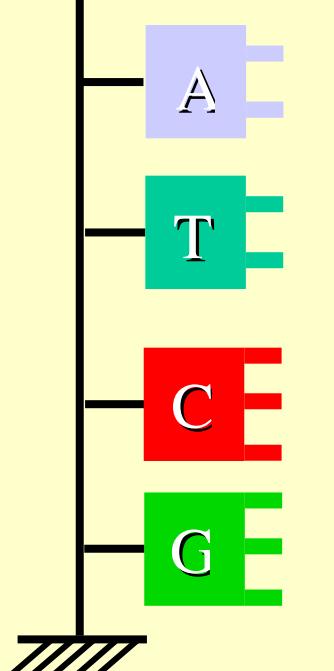
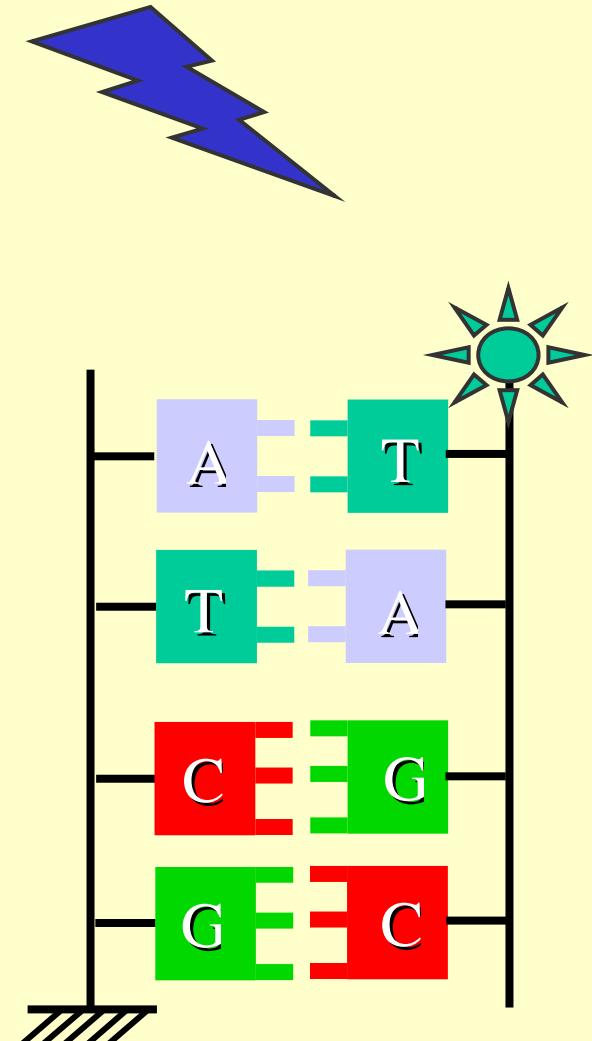


How do DNA and protein chips work ?

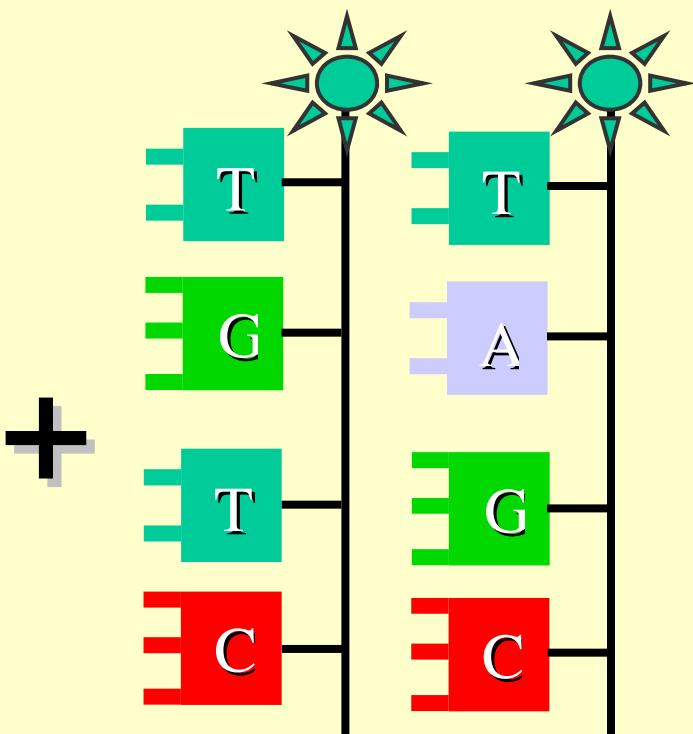
Complementary
DNA, RNA or proteins
to analyze



**DNA from different genes or corresponding
proteins attached on a support**



Sonde fixée
sur la lame de
verre



Fragments d'ARN cible marqués

Formation d'un duplex

Common features to DNA chips

Basic principle

Base pairing recognition between surface supported DNA and DNA/RNA in solution

Density

High density from several hundred to millions of hybridization spots per device

Detection

Nucleic acid labeling
radioactive, direct fluorescence,
energy transfer, chemiluminescence

Chip embedded detection
mass detection, refraction index, electric field, conduction

Usages

Transcriptome analysis
Sequence mapping, pseudo-sequencing
Detection, cloning, interaction analysis

Technologies

spotting of oligonucleotides or PCR products

Commercial & custom chips

Best adapted to genome subset

Inexpensive to customize

- medium density
- medium cost / spot
- medium global cost
- short & medium & long probes

in situ synthesis

Commercial solution

Adapted to full genomes

Plug and play

- high to very high density
- low cost / spot
- high global cost
- short oligos only

self-probing and nano-chips

Still mainly experimental

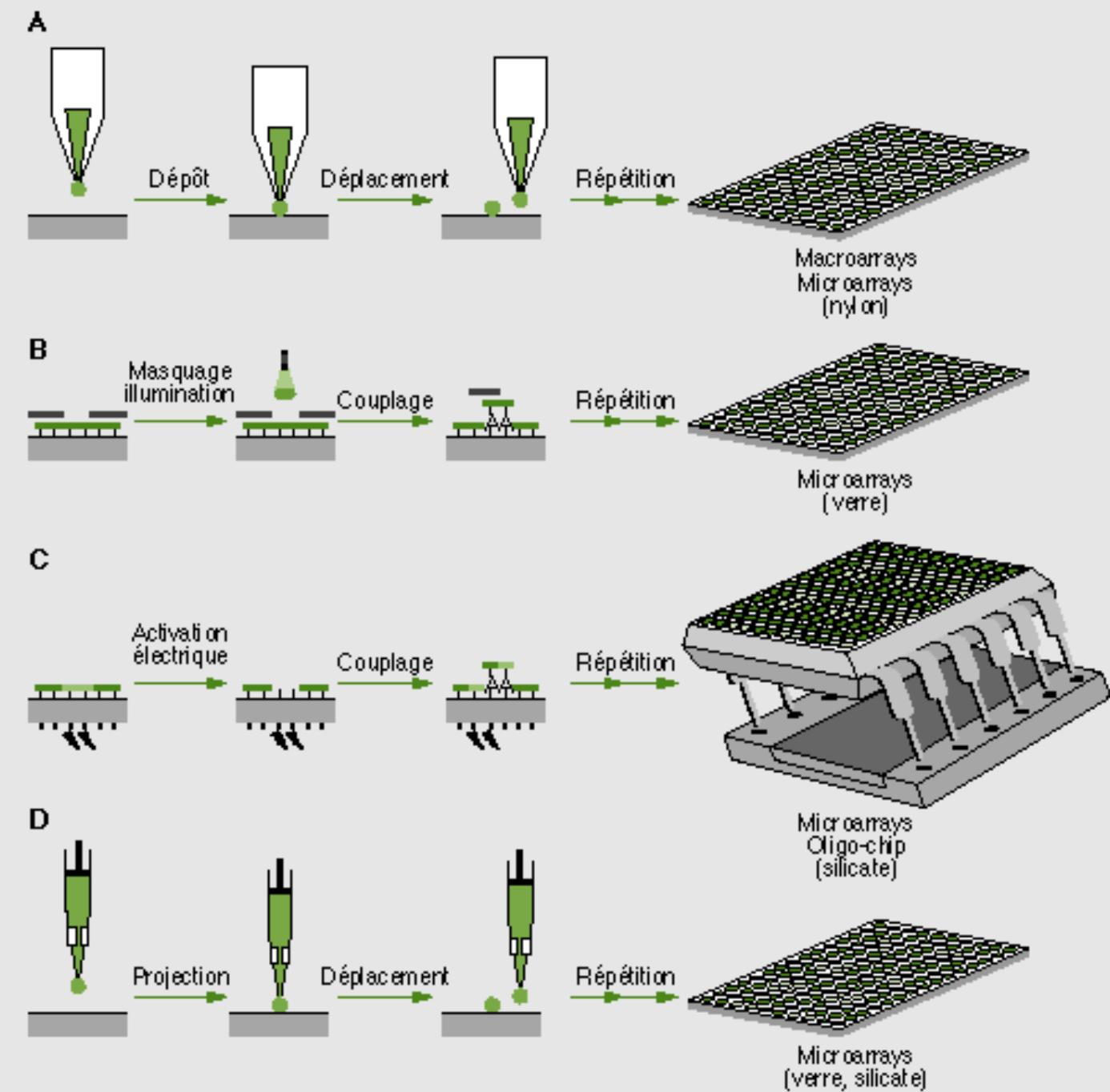
Today low but target high density

Dedicated chips

- unique features
- oligo or non-conventional probes

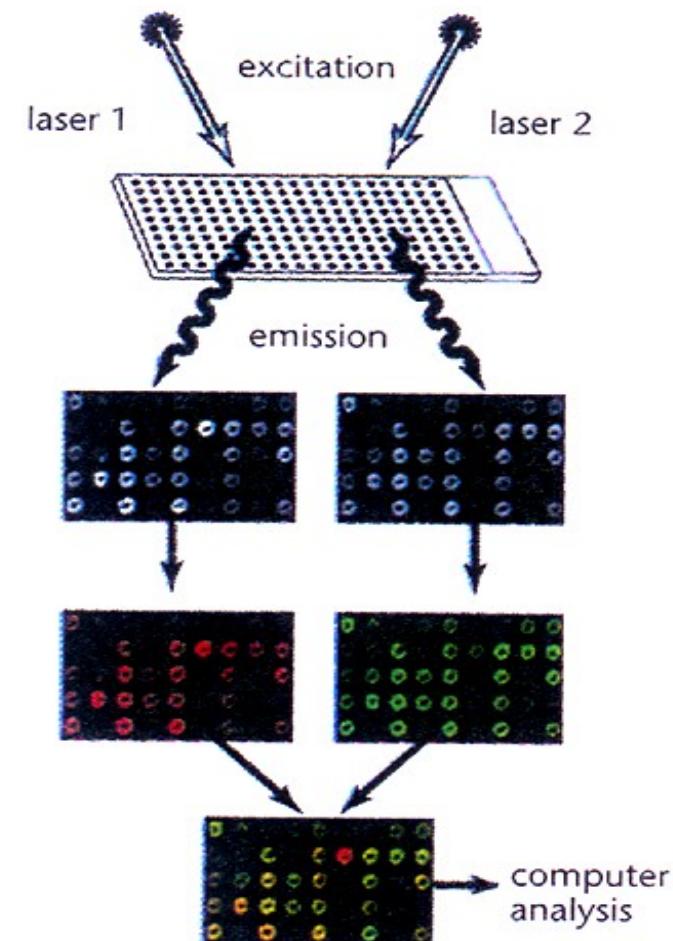
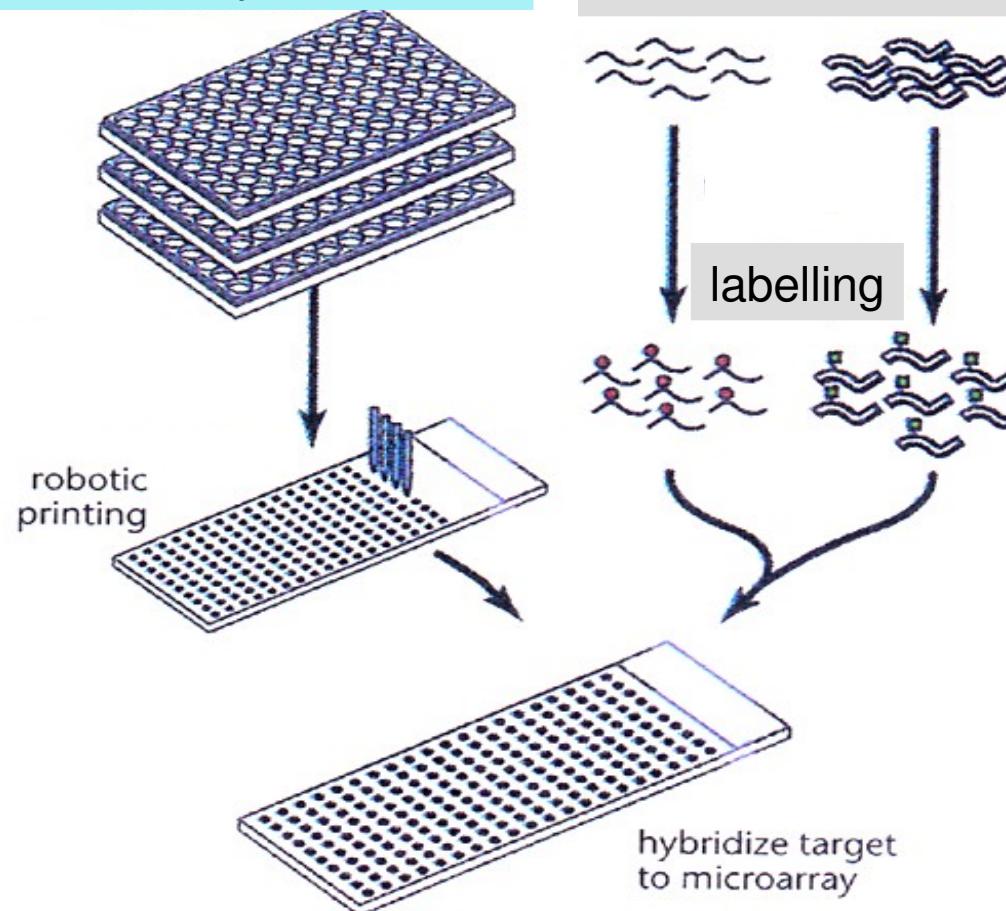
Fabrication des puces

- A base d'oligonucléotides: (type Affymetrix)
 - ✓ Adressage mécanique
 - ✓ Adressage photochimique
 - ✓ Adressage électrochimique
- A base d'ADN provenant de PCR (Pat Brown)

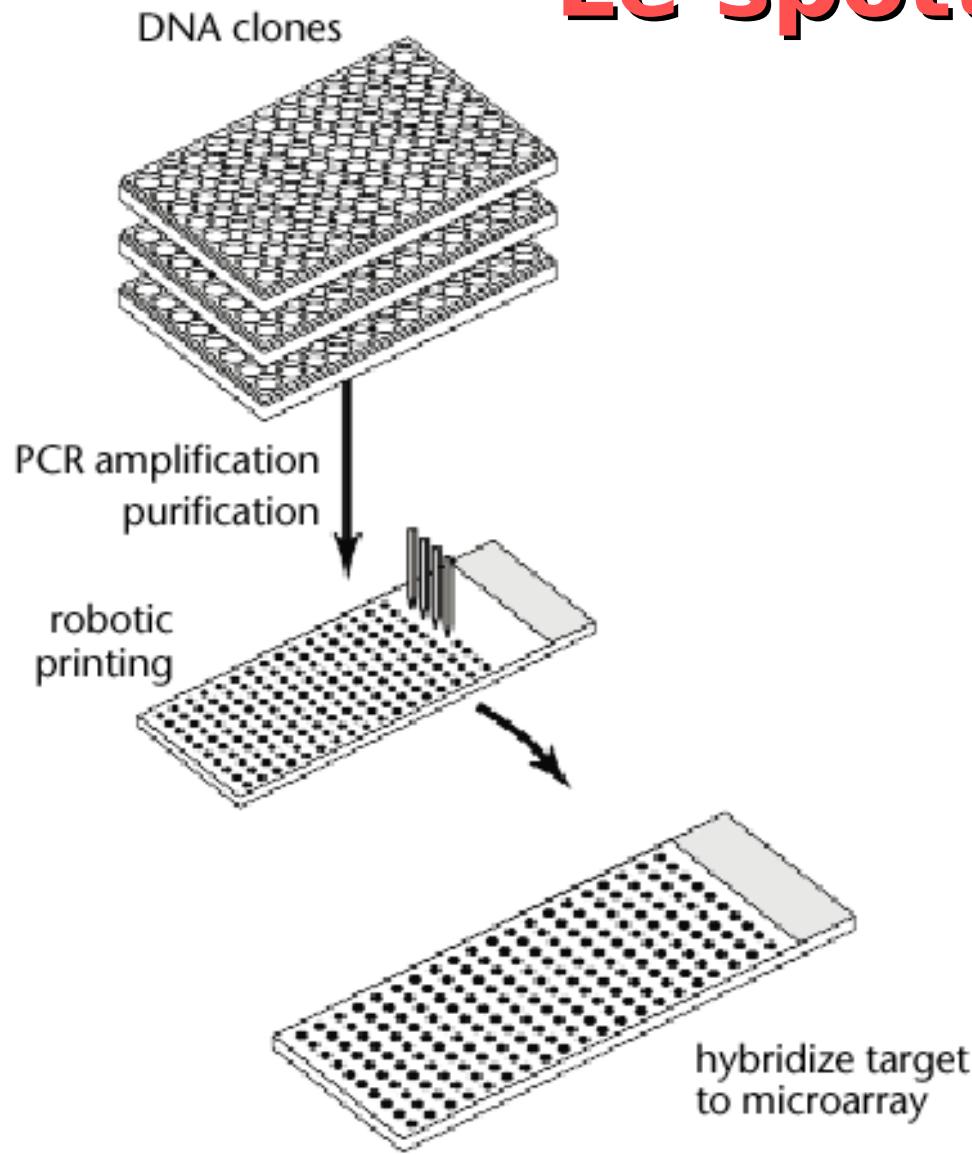


ARN
(condition A) ARN
(condition B)

Probes : oligonucleotides
or PCR products

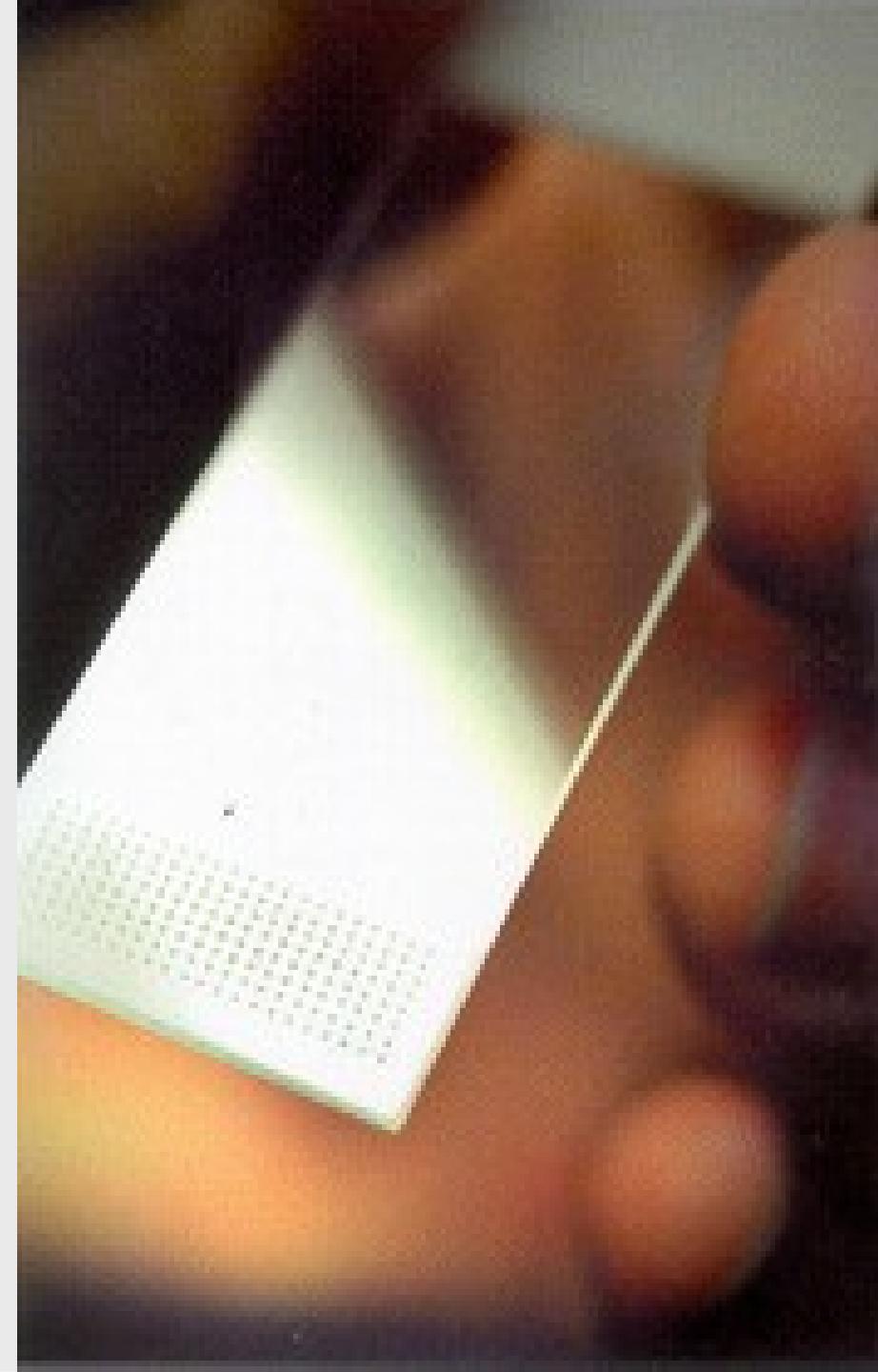


Le spotting



On dépose de l'ADN sur des lames de verre:

- oligonucléotides synthétisés sur la lame (Affymetrix et variations)
- PCRs déposés sur la lame par un spotter (Pat Brown et dérivés)

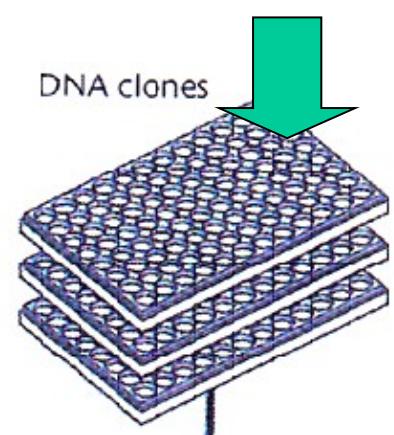
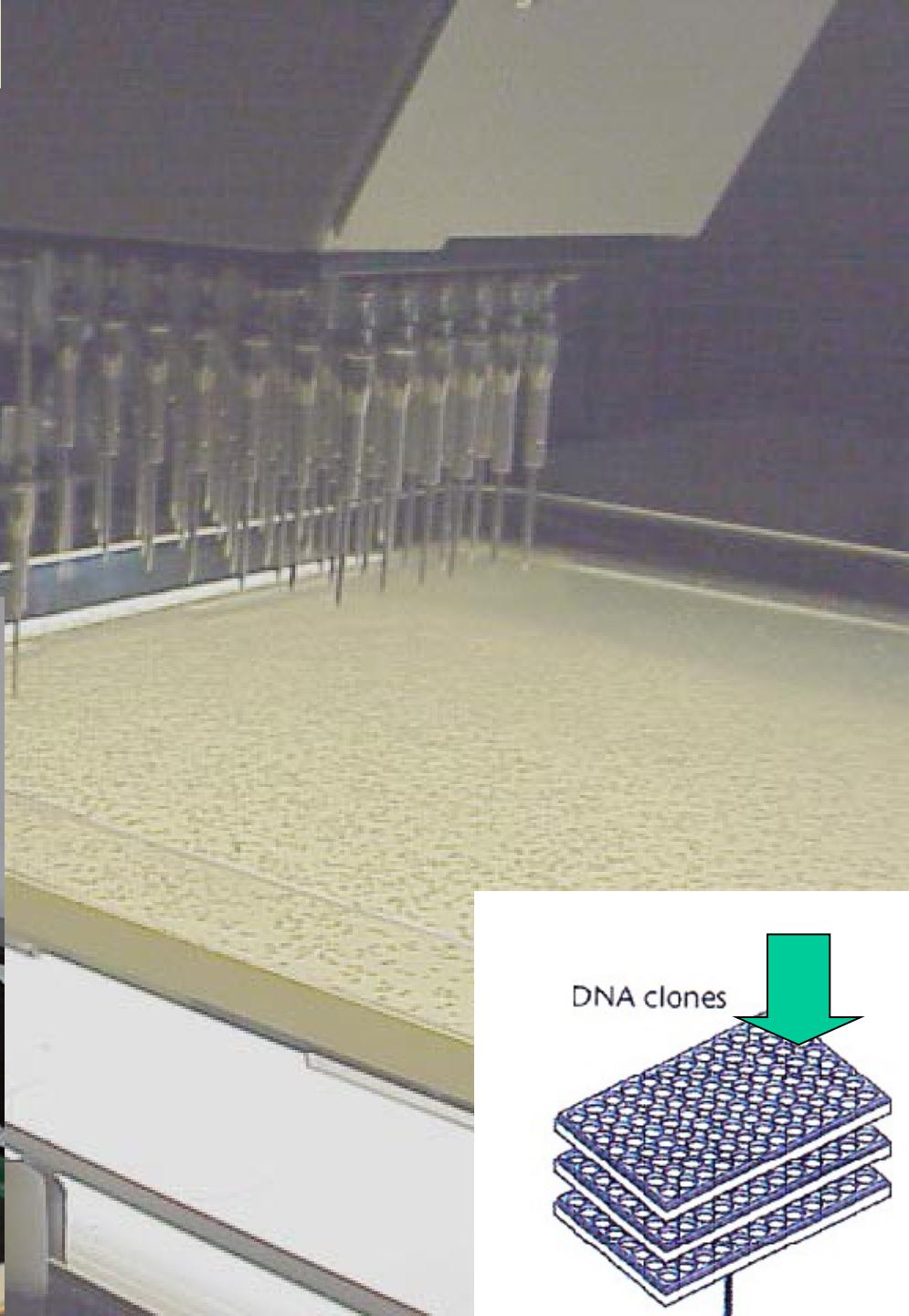
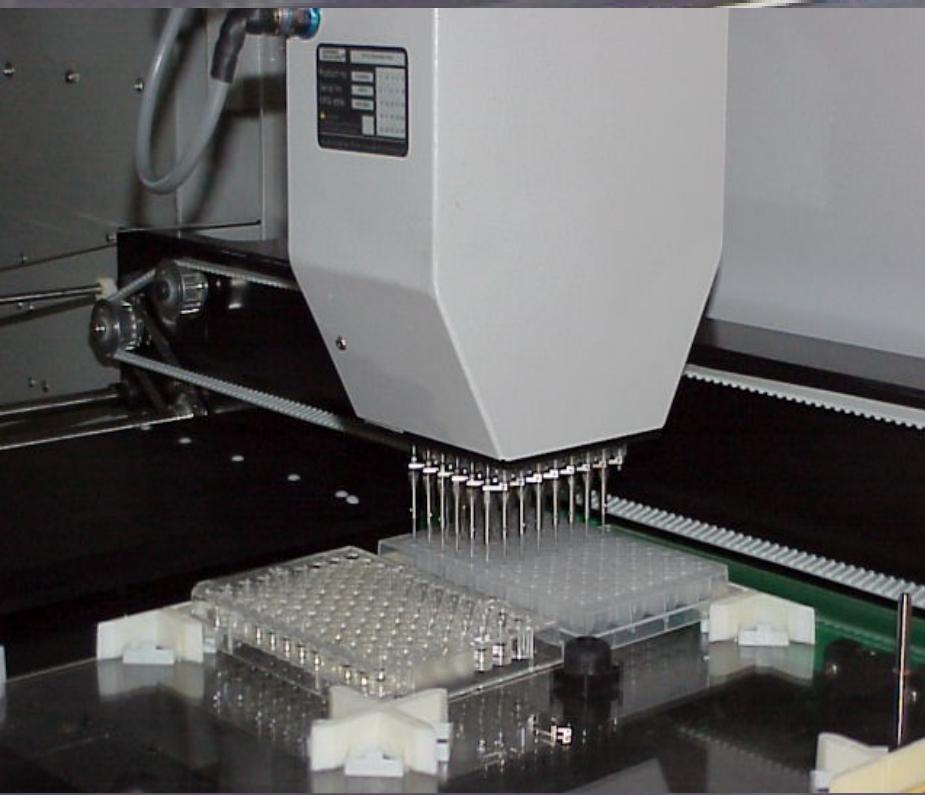




Gene cloning

PCR a

Gene isolation and amplification



Slide (DNA chips) printing

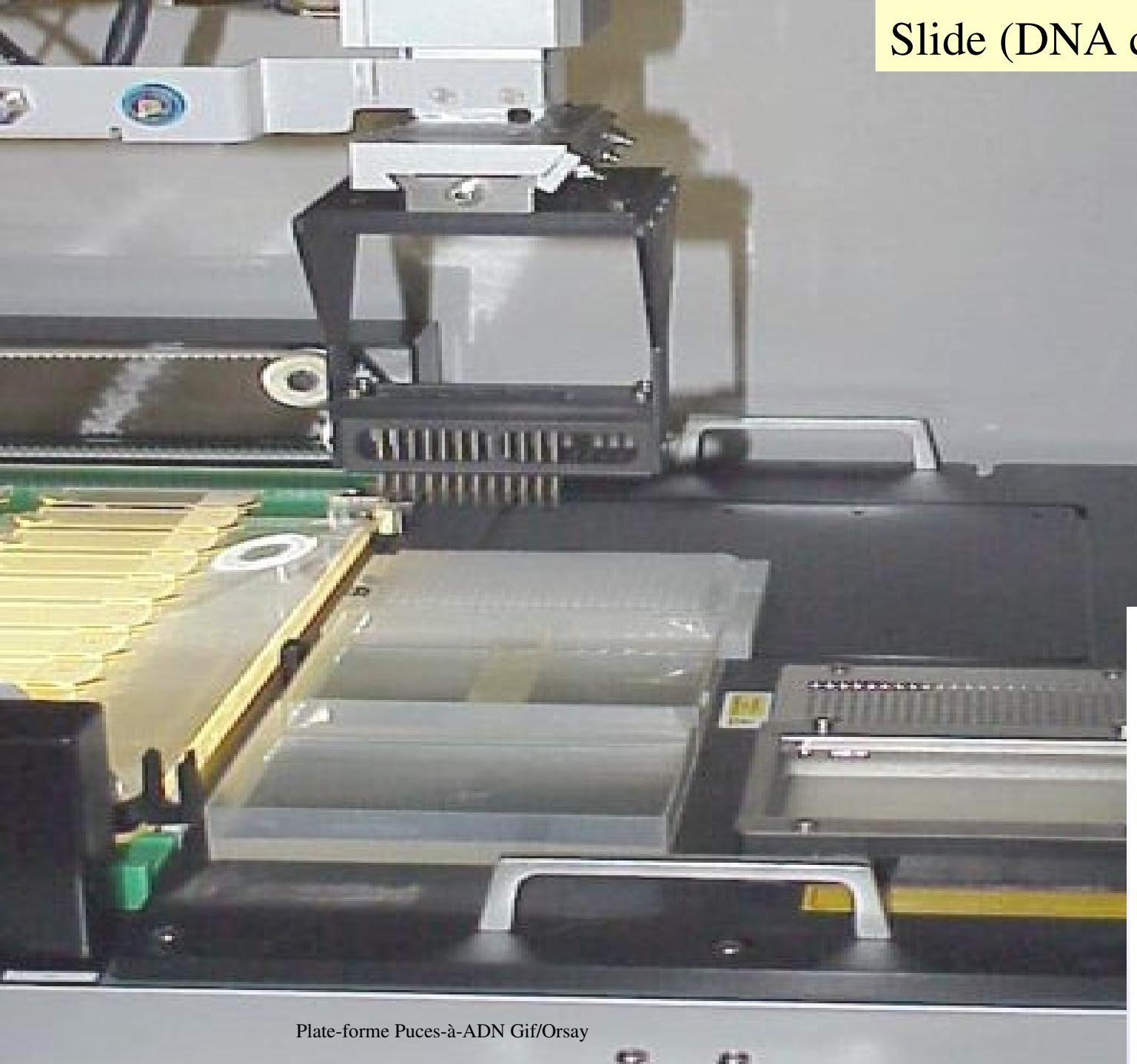
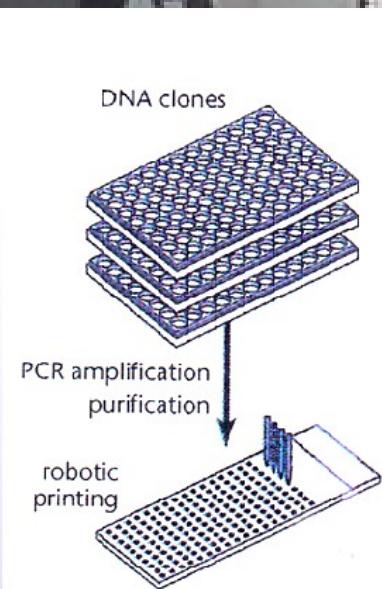


Plate-forme Puces-à-ADN Gif/Orsay



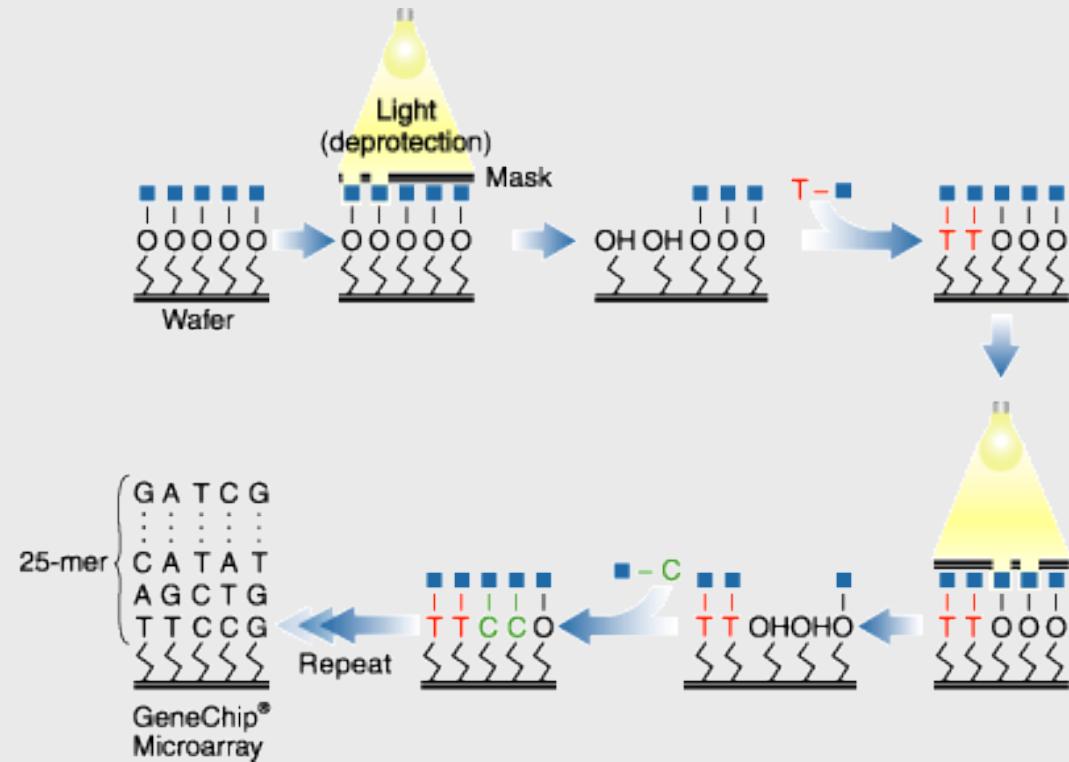
- *In situ* synthesis of short oligoprobes
- High density
- Industry standard
- High cost

Direct photodeprotection based chemistry

Although each position in the sequence of an oligonucleotide can be occupied by 1 of 4 nucleotides, resulting in an apparent need for 25×4 different masks per wafer, the synthesis process can be designed to significantly reduce this requirement.

Uses photolithography and solid-phase chemistry to produce arrays containing hundreds of thousands of oligonucleotide probes packed at extremely high densities.

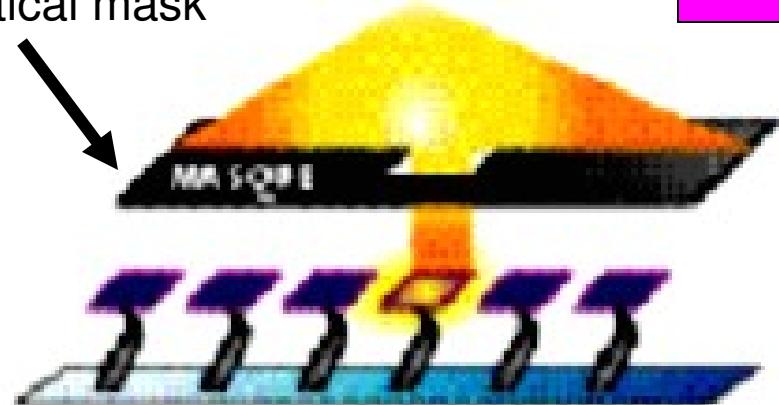
The probes are designed to maximize sensitivity, specificity, and reproducibility, allowing consistent discrimination between specific and background signals, and between closely related target sequences.



Photodeprotection

Light

Optical mask



Coupling



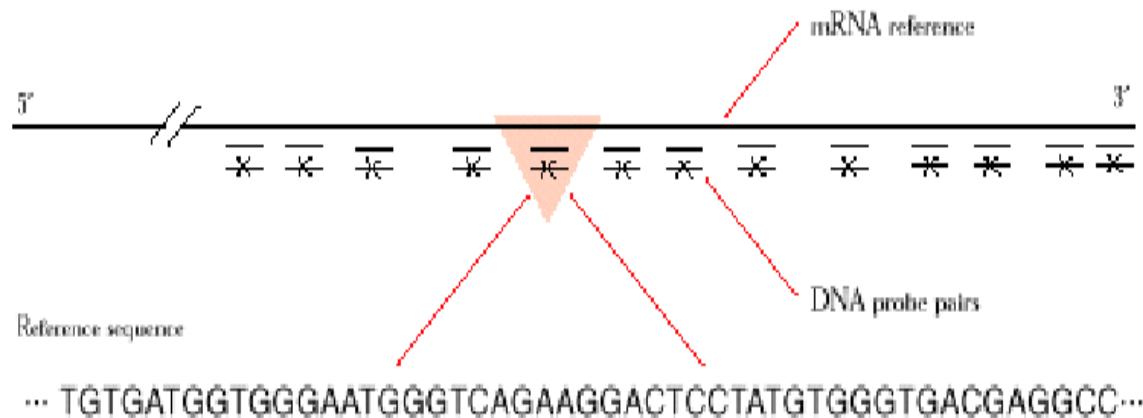
Photodeprotection

Coupling



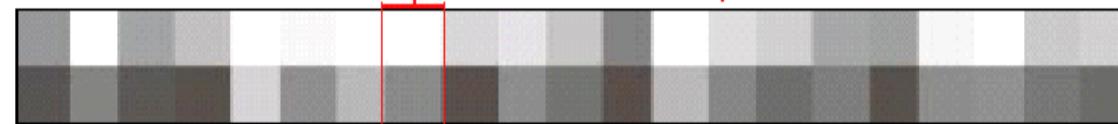
AFFYMETRIX TECHNOLOGY

Reference sequence



Probe sequences

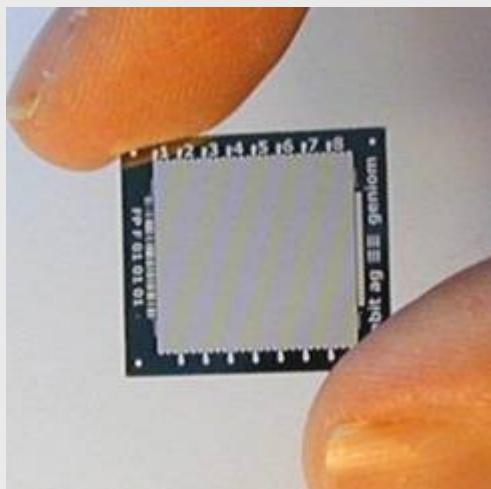
Fluorescence Intensity Image



Perfect match probes
Mismatched probes

Fluorescence intensity

Mismatch probe cells

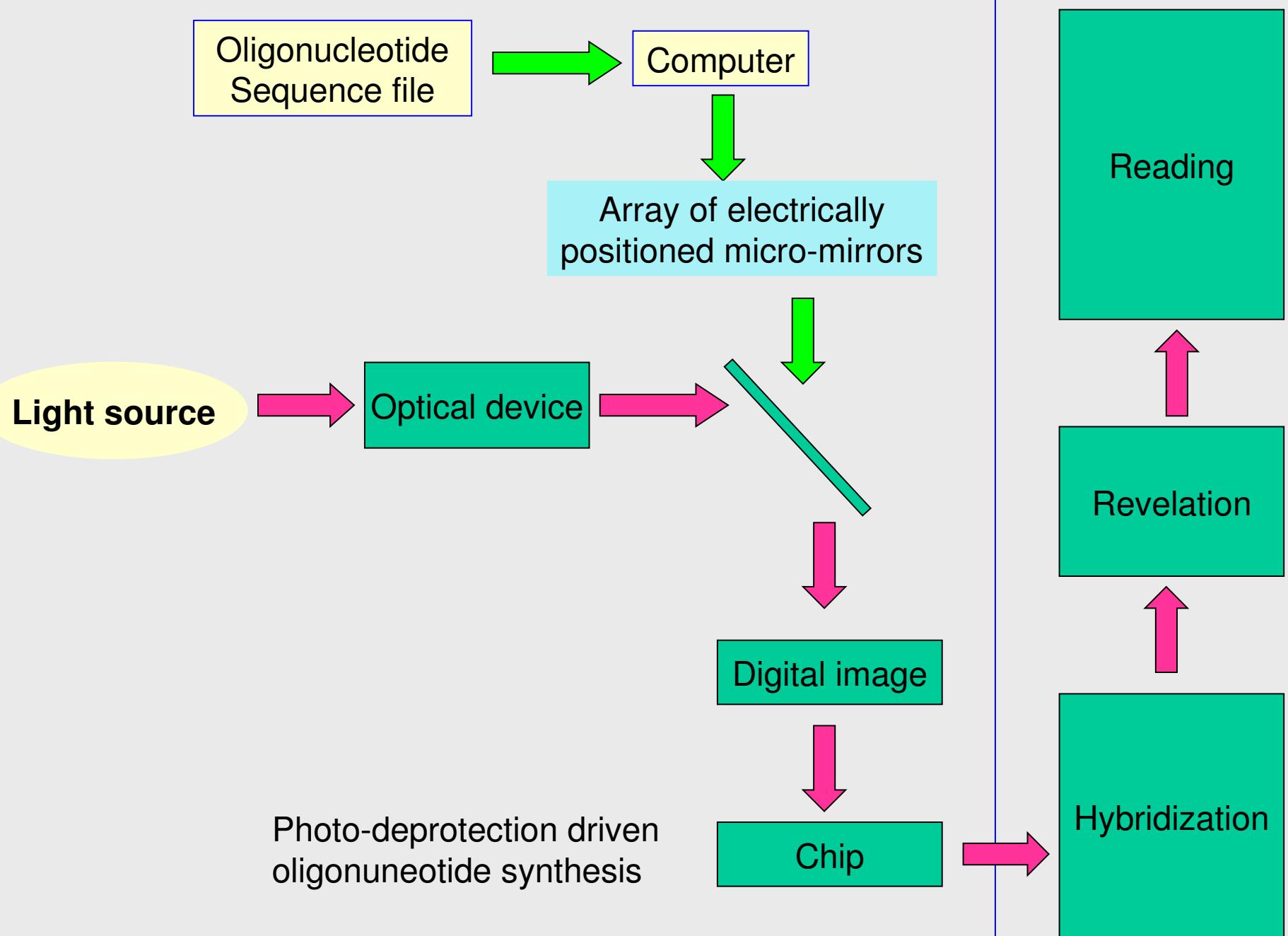


The DNA processor TMCentral element of the geniom® technology is the DNA processor TM, a unique 3-D microchannel structure. It is subdivided into eight segments with individual fluid control resulting in eight independent microarrays.

Microarrays are build up by *in situ* oligonucleotide synthesis inside the DNA processor TM. A digital projector and proprietary phosphoramidite chemistry allow maskless light activated synthesis. This results in spots of defined oligonucleotides with a size of 34 µm x 34 µm.

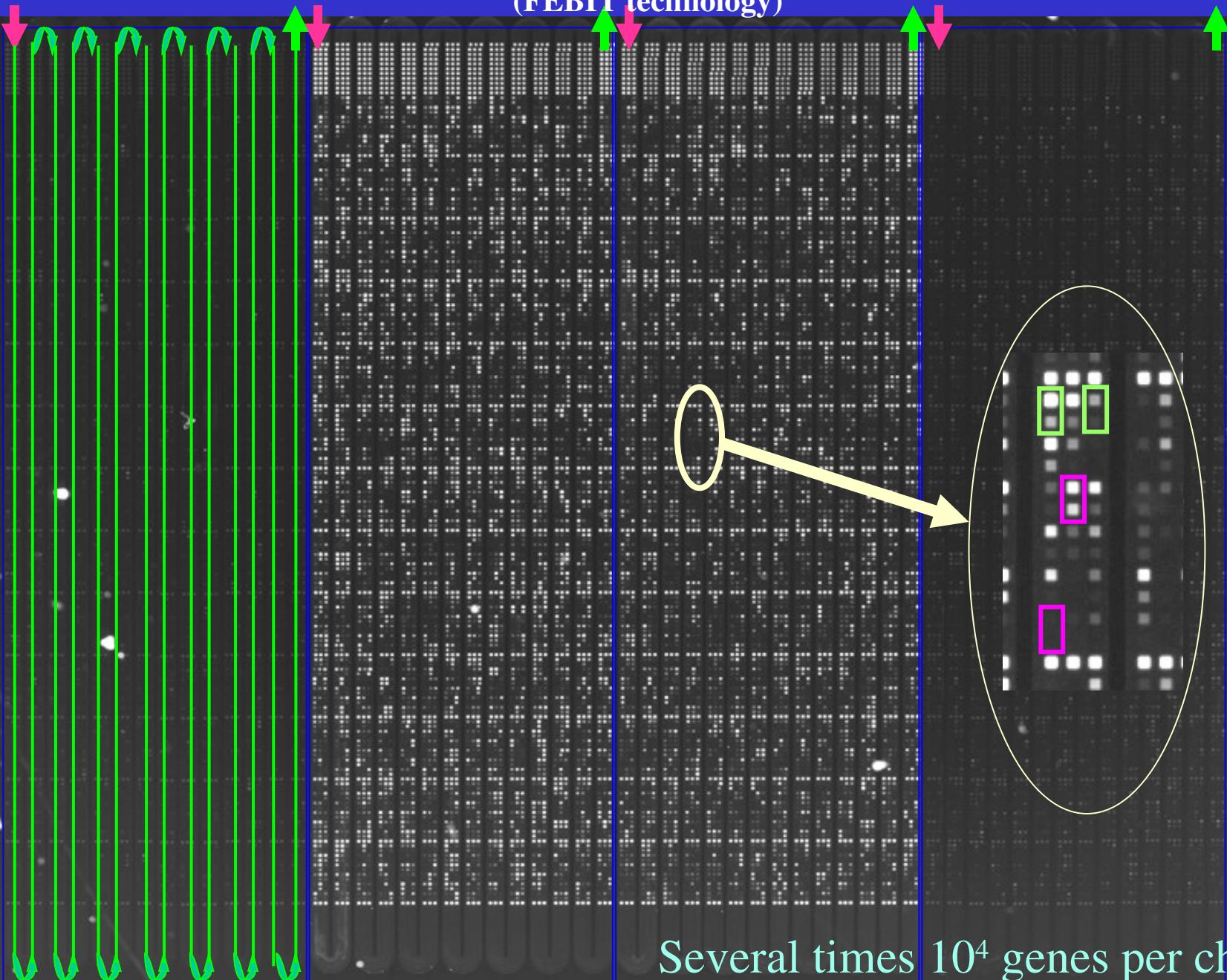
The transparent reactive surface is about 1 cm² in total and allows parallel synthesis of up to eight arrays with a minimum of 6,000 oligonucleotide probes each.

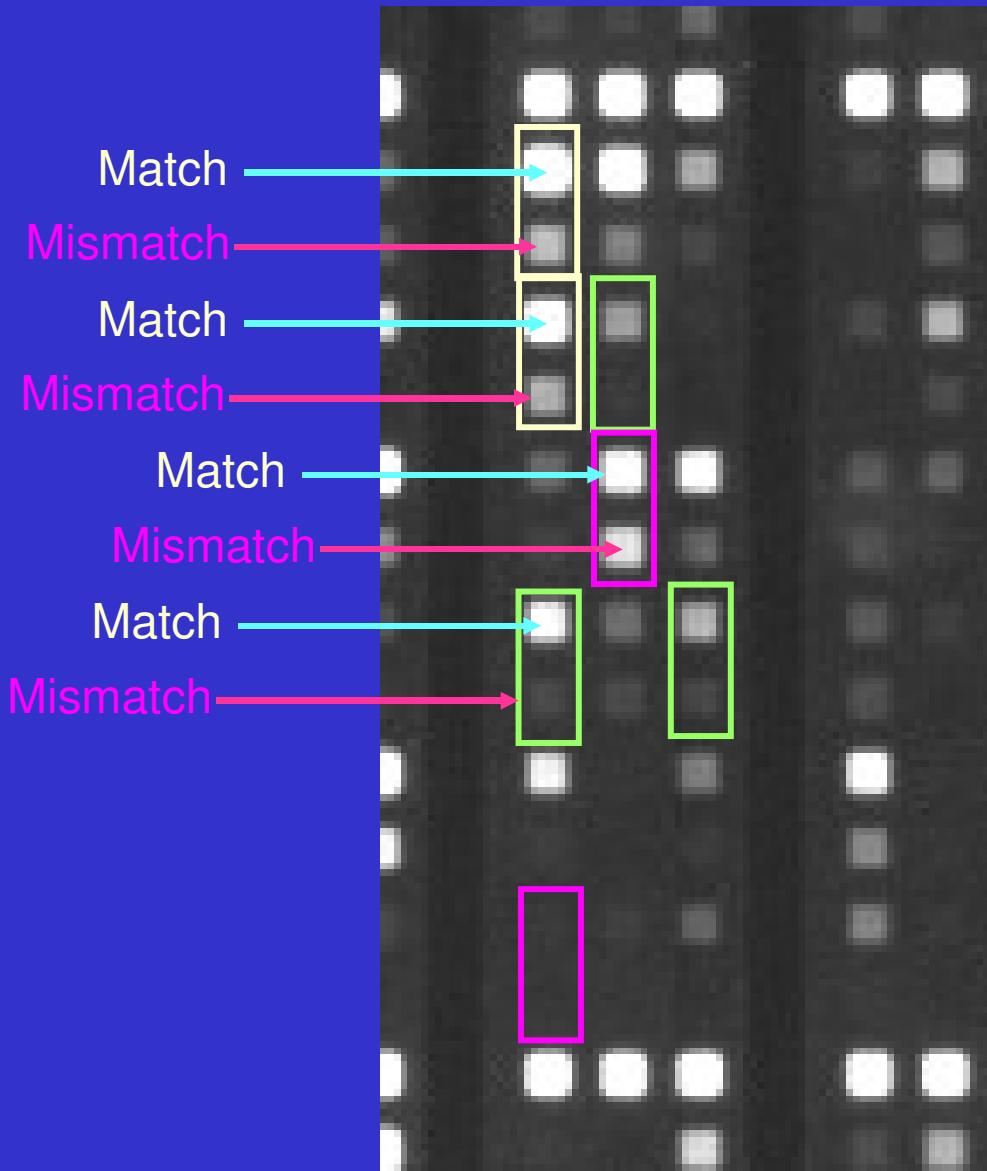
The disposable DNA processorTM is held in a cartridge for easy handling and auto alignment in the instrument's fluid system and optical path. A memory module in the cartridge assures correct identification and history tracking.



Optically addressed *in situ* synthesis of oligonucleotides

(FEBIT technology)





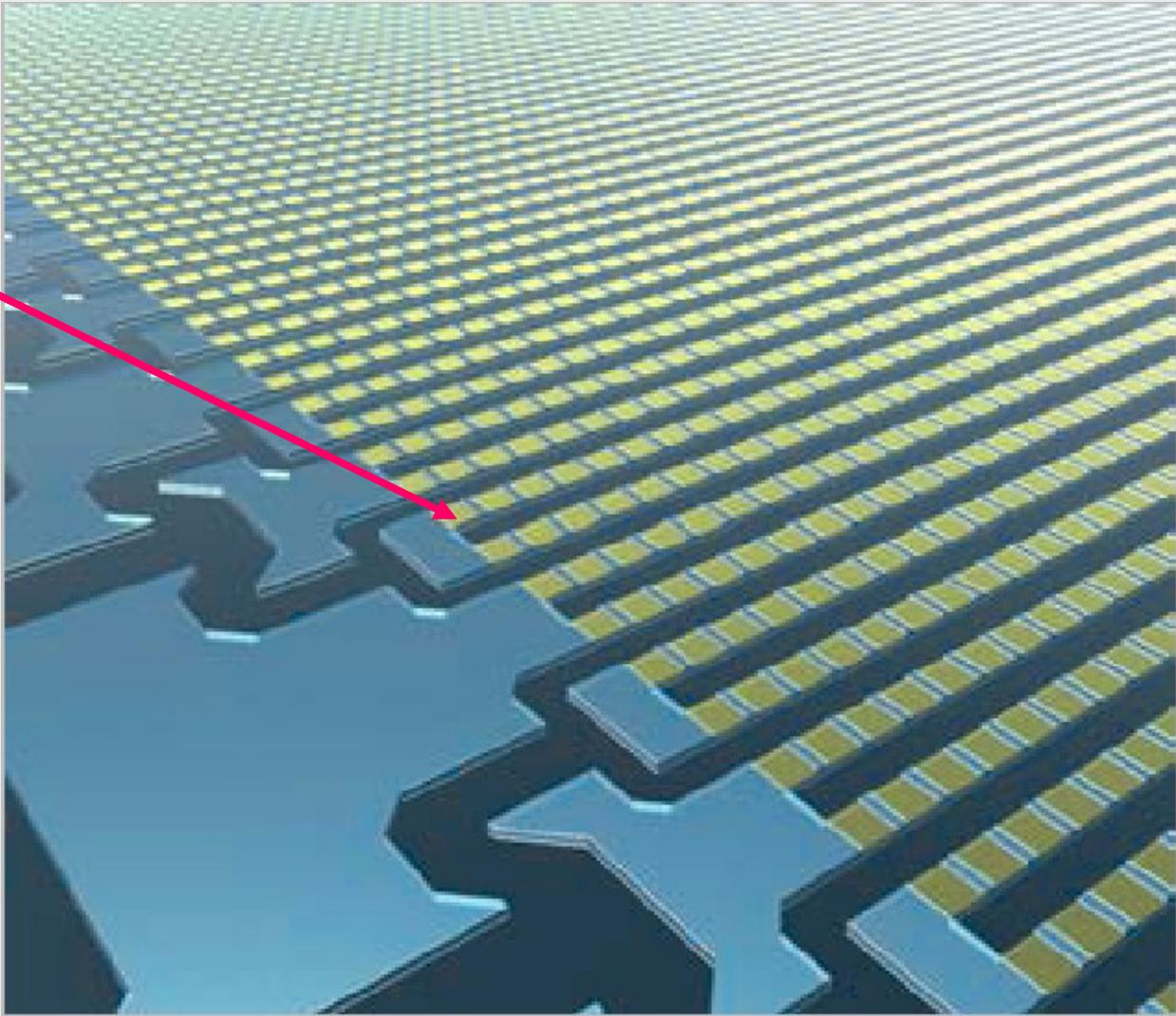


XeoChip™ (Houston , USA)
is proprietary platform technology for
manufacturing nano-chamber micro-array
biochips .

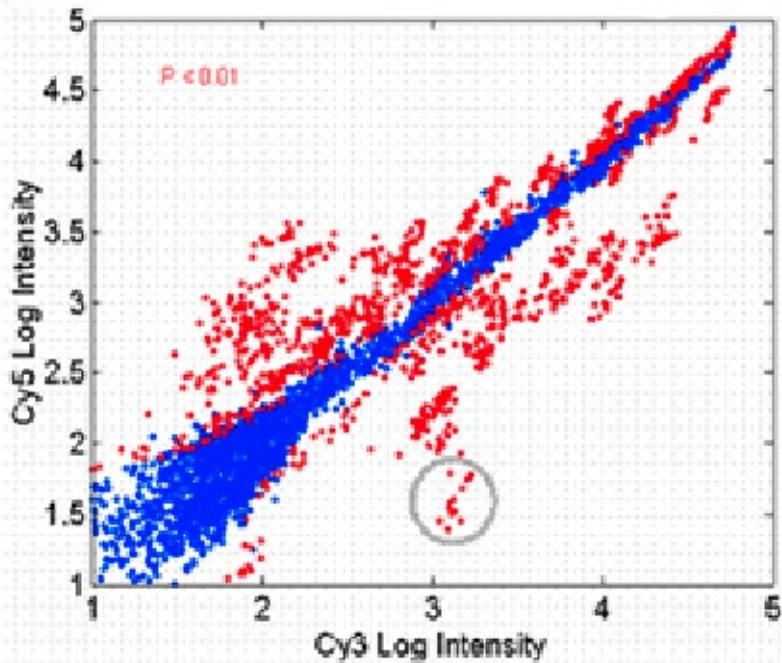
Optically addressed (micro-mirrors) are used to direct oligonucleotide synthesis using photo-generated acid for deprotection in preformed micro-slots.

Claimed advantage is the used of classical chemistry (more efficient) for oligonucleotide synthesis. Oligonucleotides are synthesized on a in a matter of few hours with a stepwise yield higher than 98.5%.

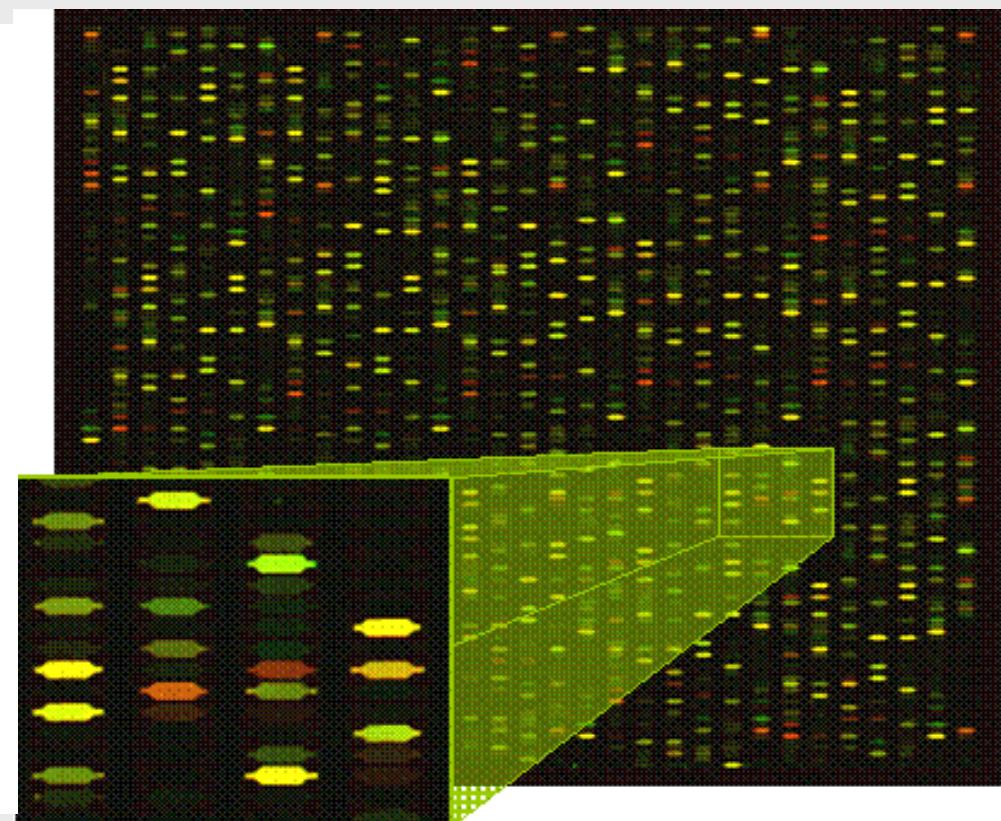
Up to 150 base probe can be synthetized



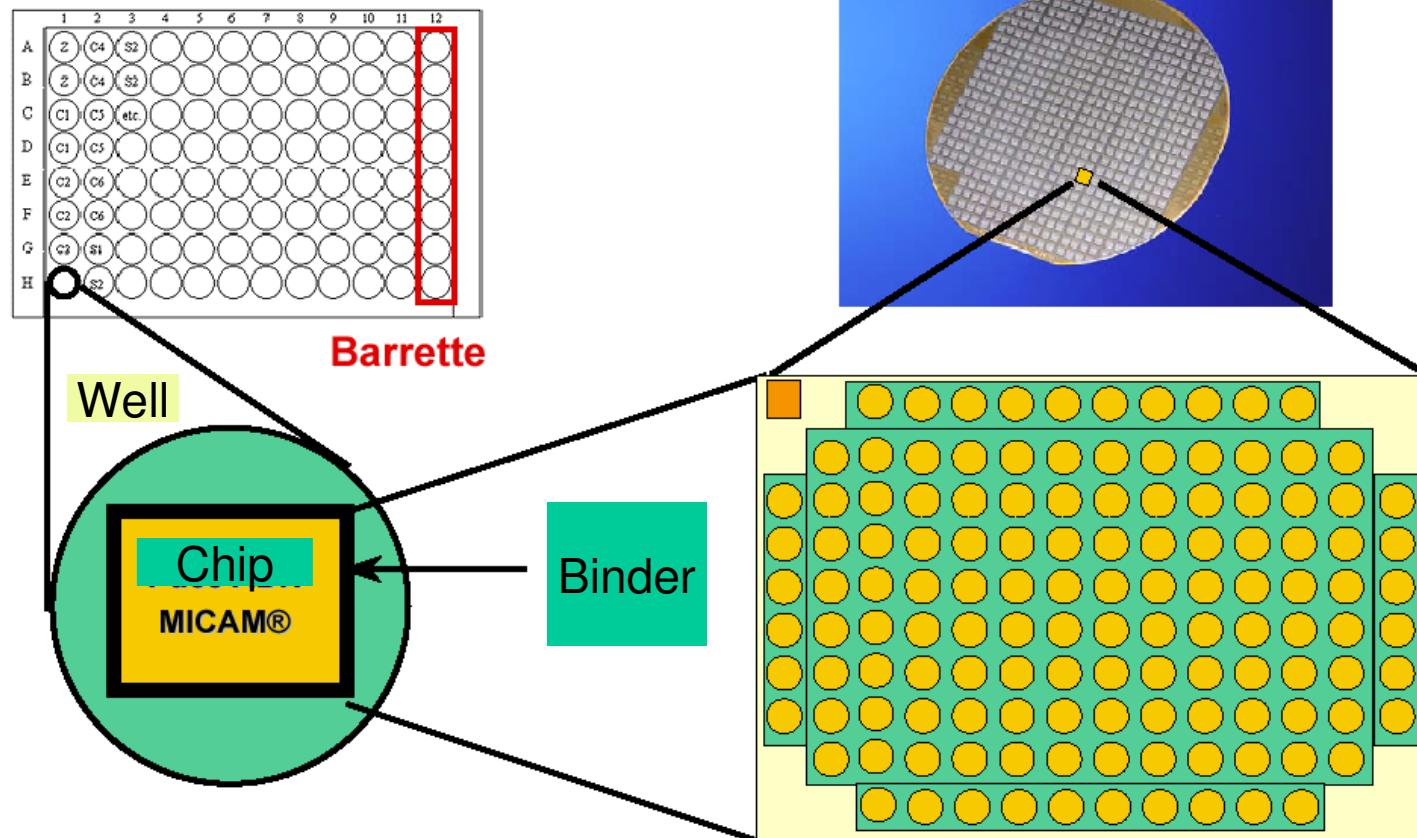
Representative log plot of the differential expression of gene in skeletal muscle RNA (Cy3) vs brain RNA (Cy5)

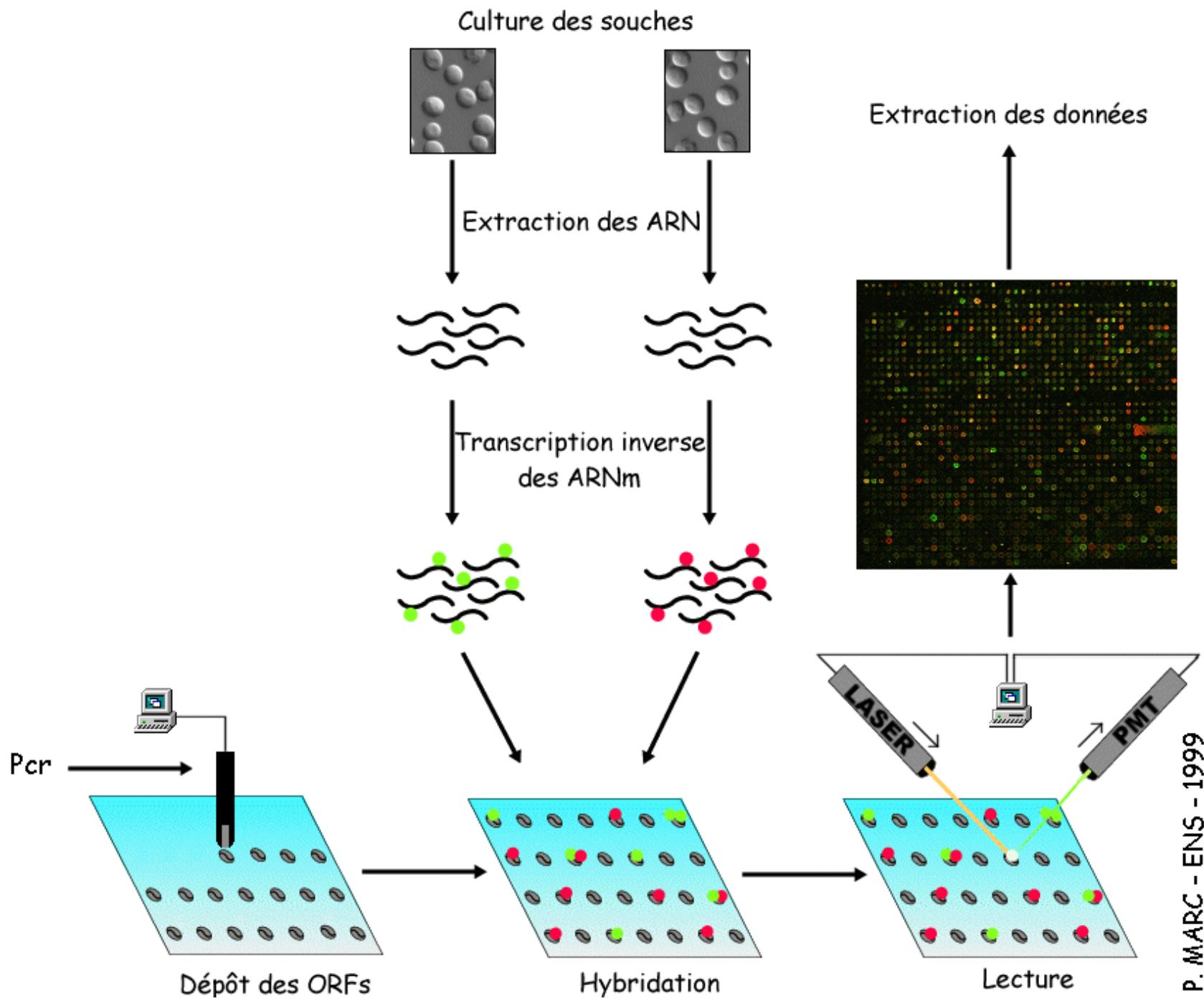


Example of a XeoChip™ used in differential gene expression of brain (green color) versus skeletal muscle (red color) samples. This XeoChip™ contains 253 cancer-related genes in 15 replicates throughout the chip.

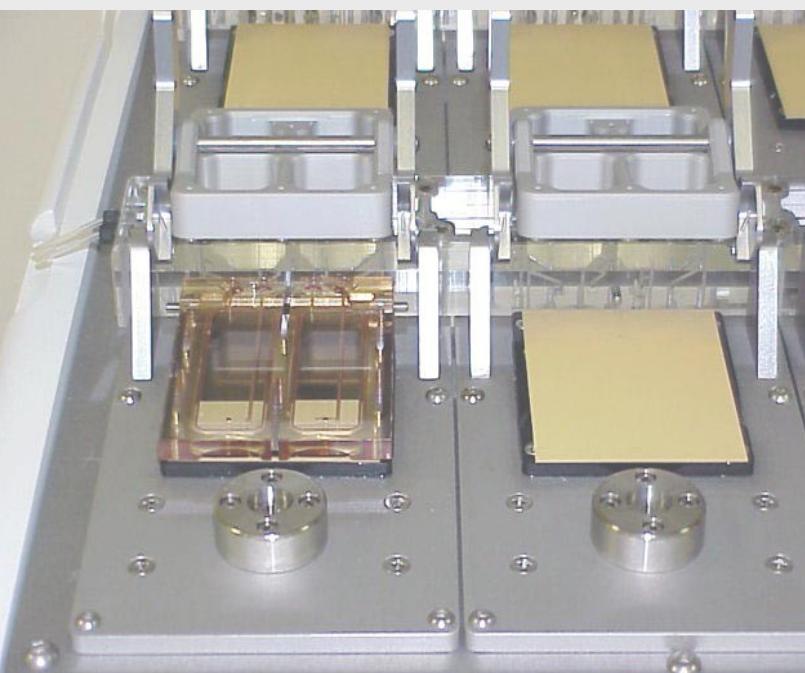
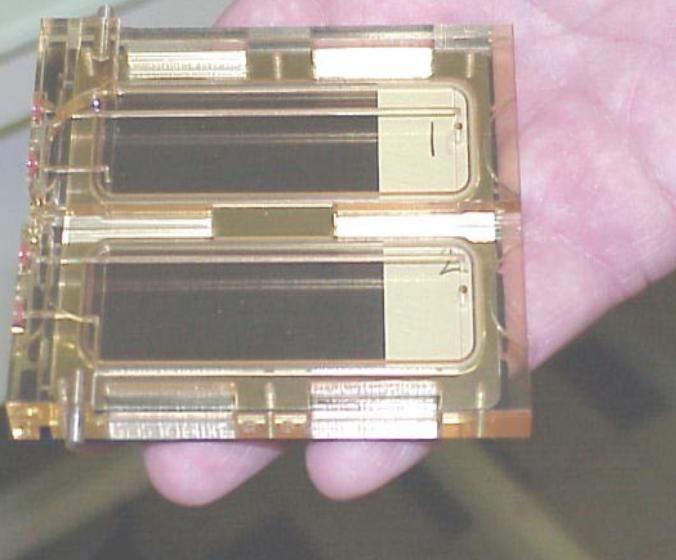


Adaptation to high throughput screening





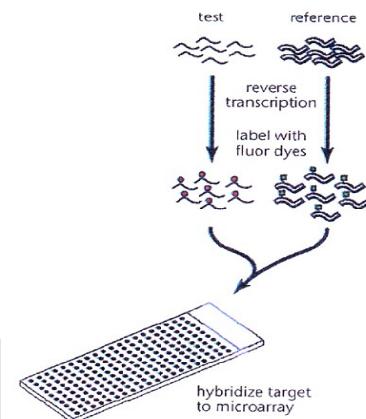
DNA chip ready to hybridize



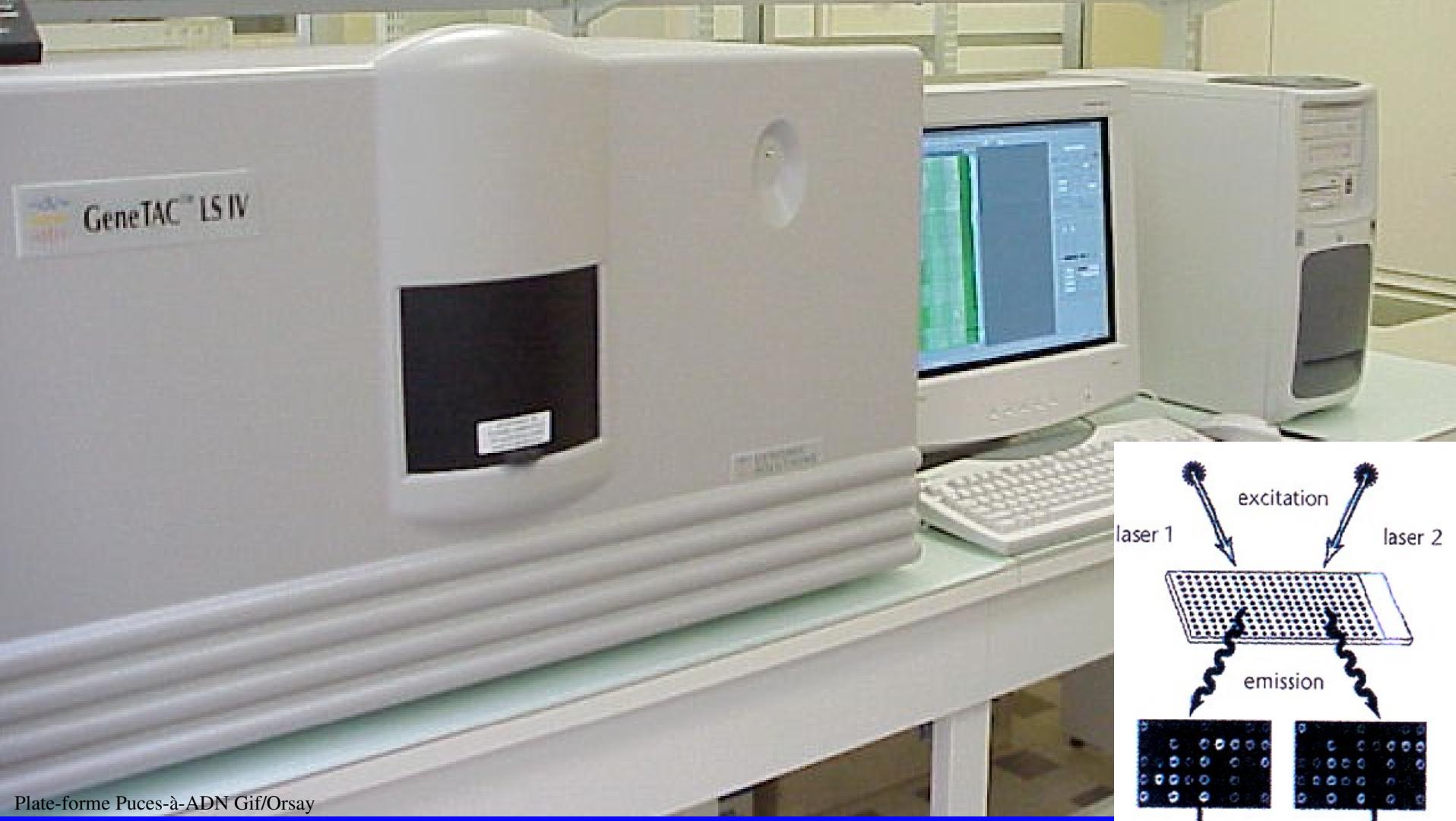
The automated hybridization step



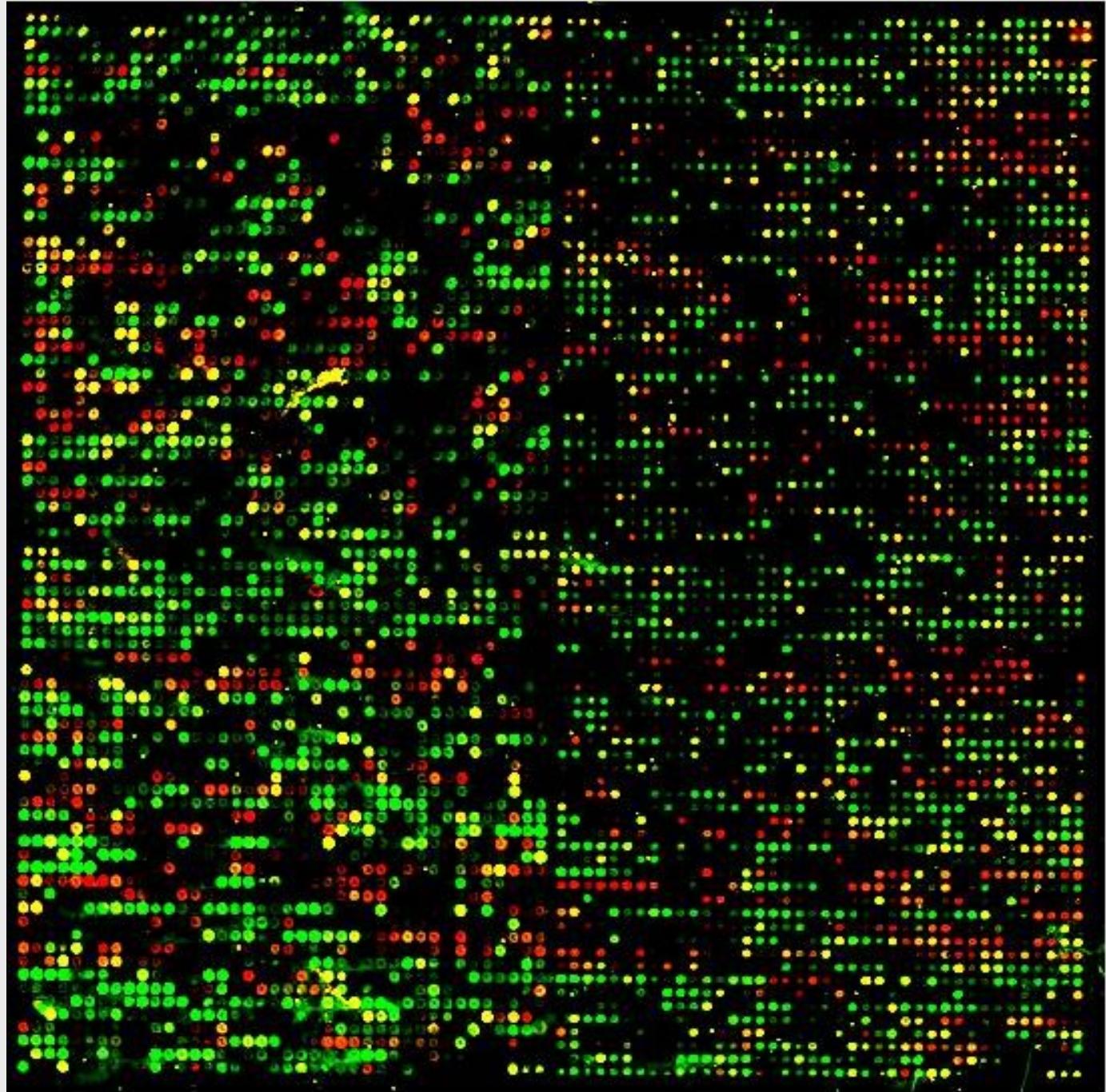
CDNA hybridization



The four LASER DNA chip reading machine



Red = reference
Green = modified
transcriptome



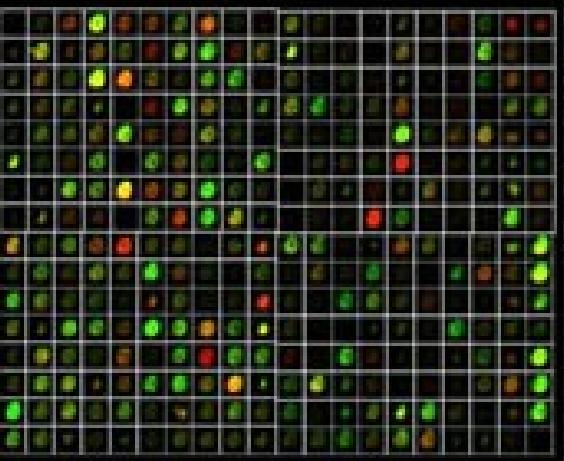
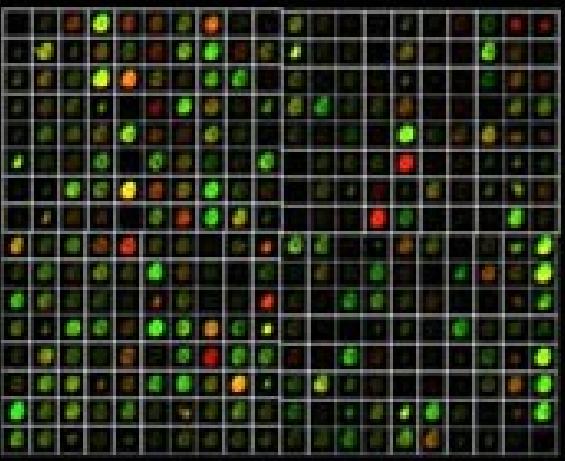
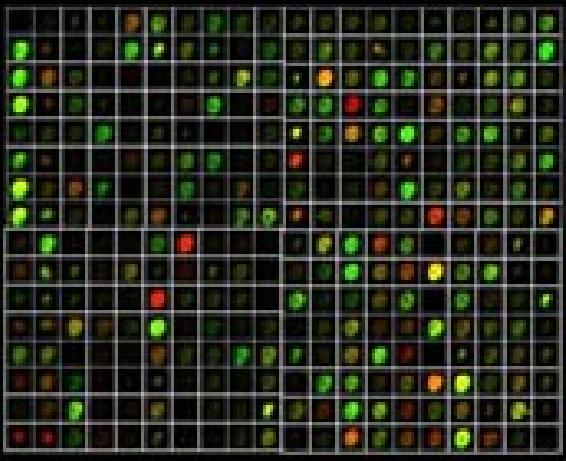
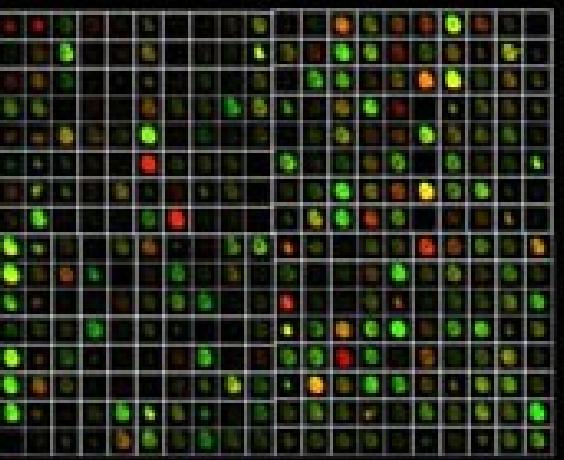
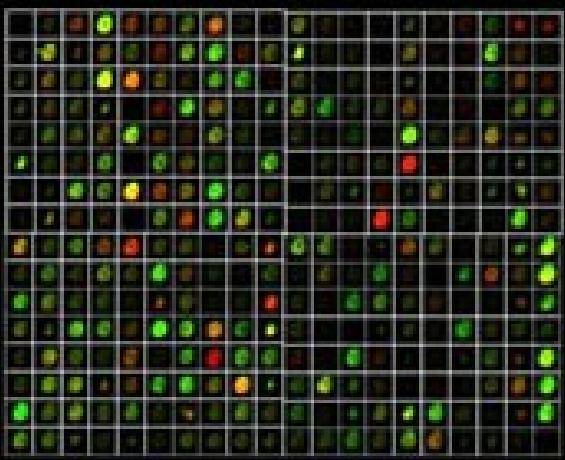
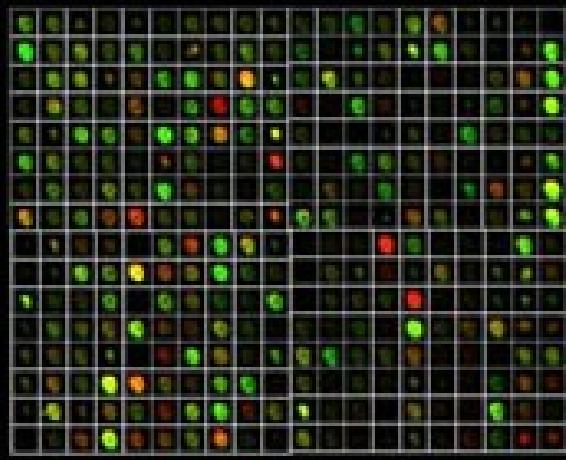
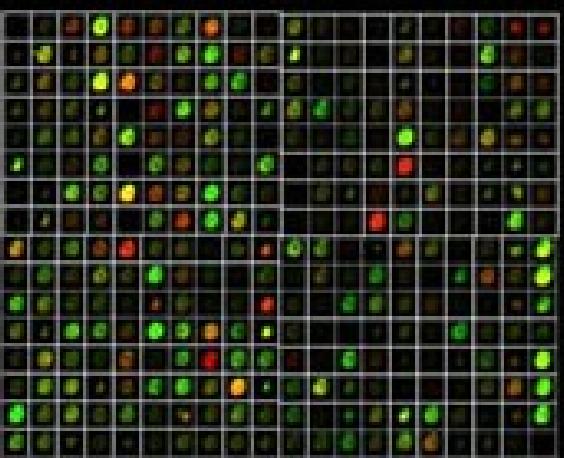
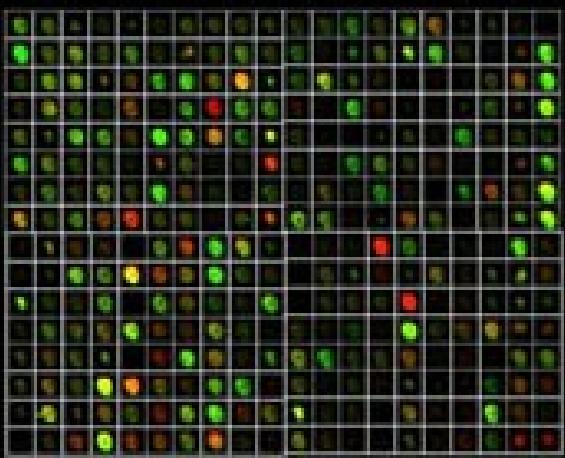
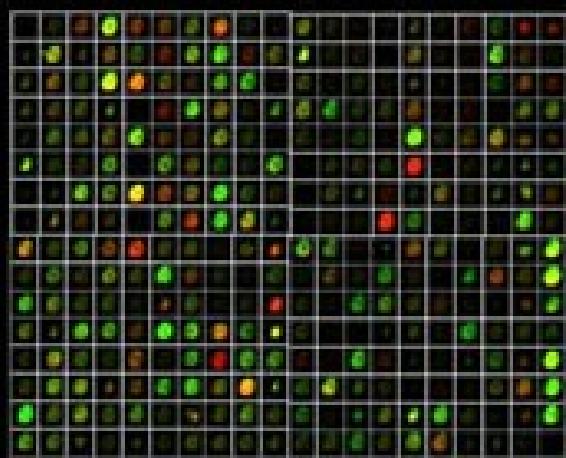




Image | Histogram | Lab Book | Results | Scatter Plot | Report |

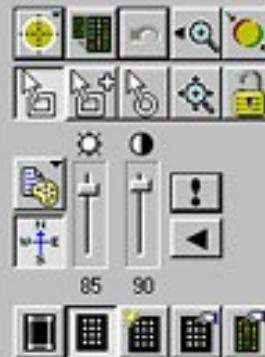
Image

- Preview (635/532)
- Preview (635/488)
- Preview (635/594)

- Wavelength 635
- Wavelength 532
- Wavelength 488
- Wavelength 594

- Ratio (635/532)
- Ratio (635/488)
- Ratio (635/594)

Tools



Feature Viewer

X,Y (μm): Block: 5
5680.33820 Feature: 5.6
Name:

Ratio (635/532)



Rp: 0.684
Rm: 0.598
mR: 0.588
fR: 0.583

Ratio (635/488)



Rp: 1
Rm: 1
mR: 1
fR: 1

Ratio (635/594)



Rp: 0.684
Rm: 0.598
mR: 0.588
fR: 0.583

Wavelength 635



P: 1167
F: 1309
B: 87

Wavelength 532



P: 1705
F: 2101
B: 57

Wavelength 488



P: 1167
F: 1309
B: 87

Wavelength 594



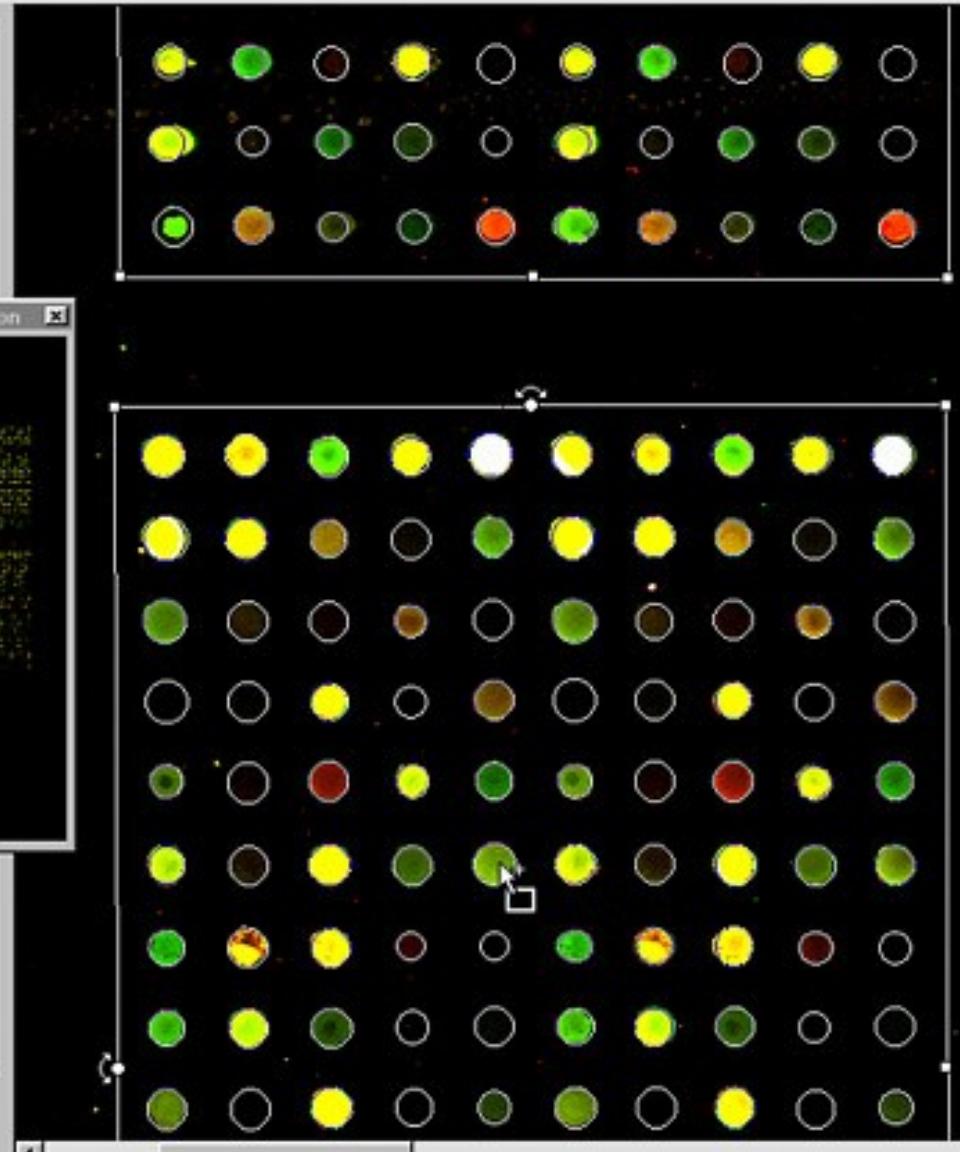
P: 1705
F: 2101
B: 57

FP=540, BP=2720, CPI=3590

Navigation



8 blocks selected.

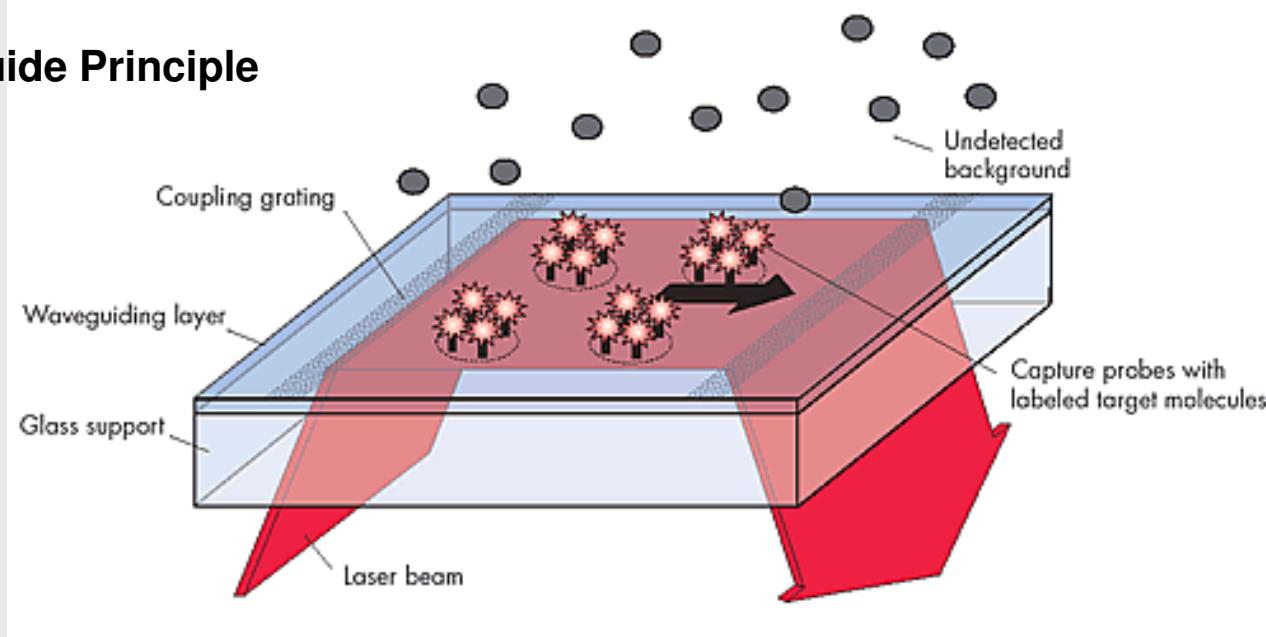


SensiChip Array Detection System

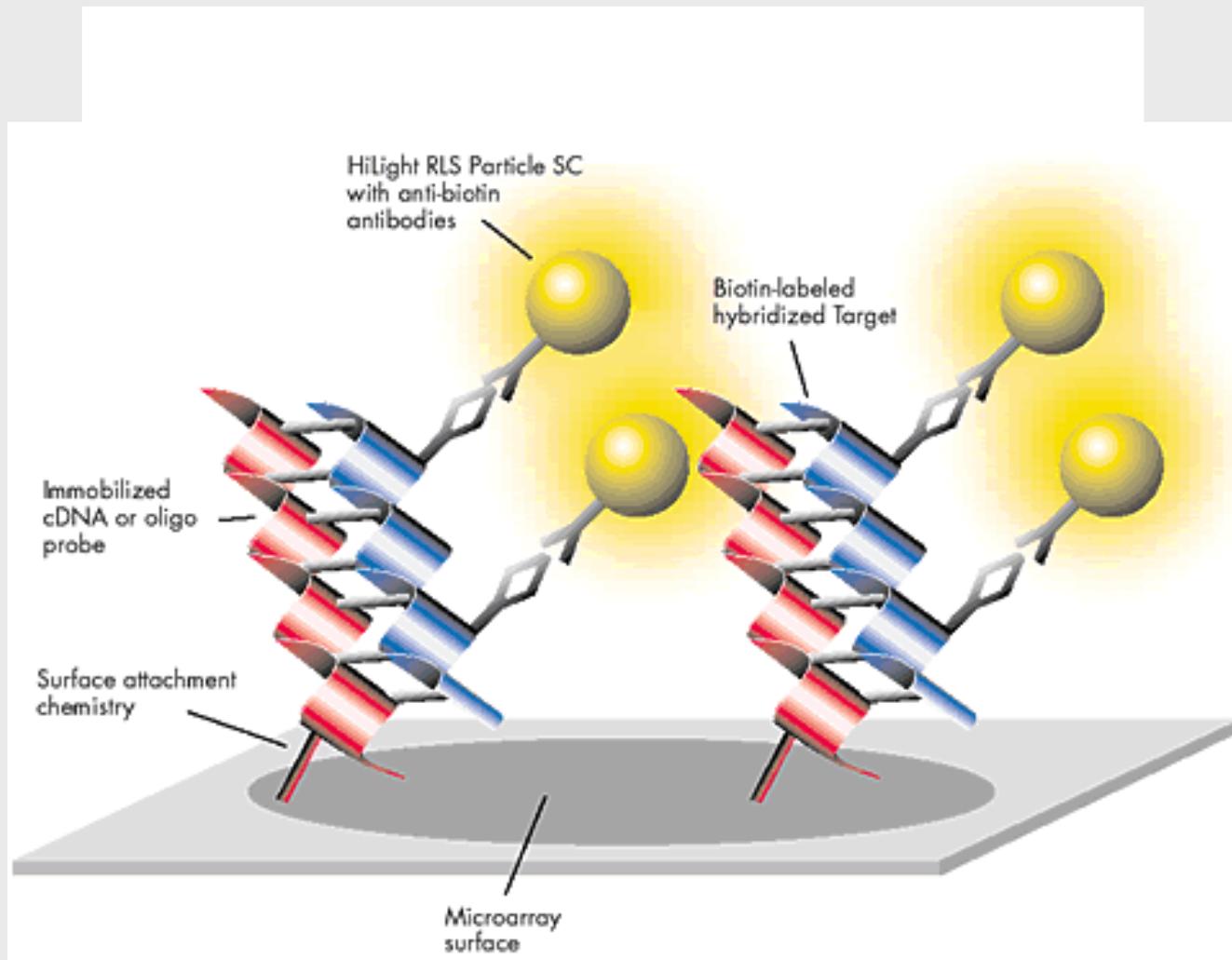
Principle

PWG technology provides highly sensitive detection with low background and high signal-to-noise ratios. A laser beam is directed into the thin PWG layer by a coupling grating. The light propagates within this PWG layer and generates a strong electromagnetic field. This evanescent field decays exponentially in relation to the distance to the PWG layer, limiting its penetration depth to approximately 300 nm. Detection of fluorescent molecules is restricted to the sensing surface with the capture probes and their bound target molecules with no background noise beyond the penetration depth of the field.

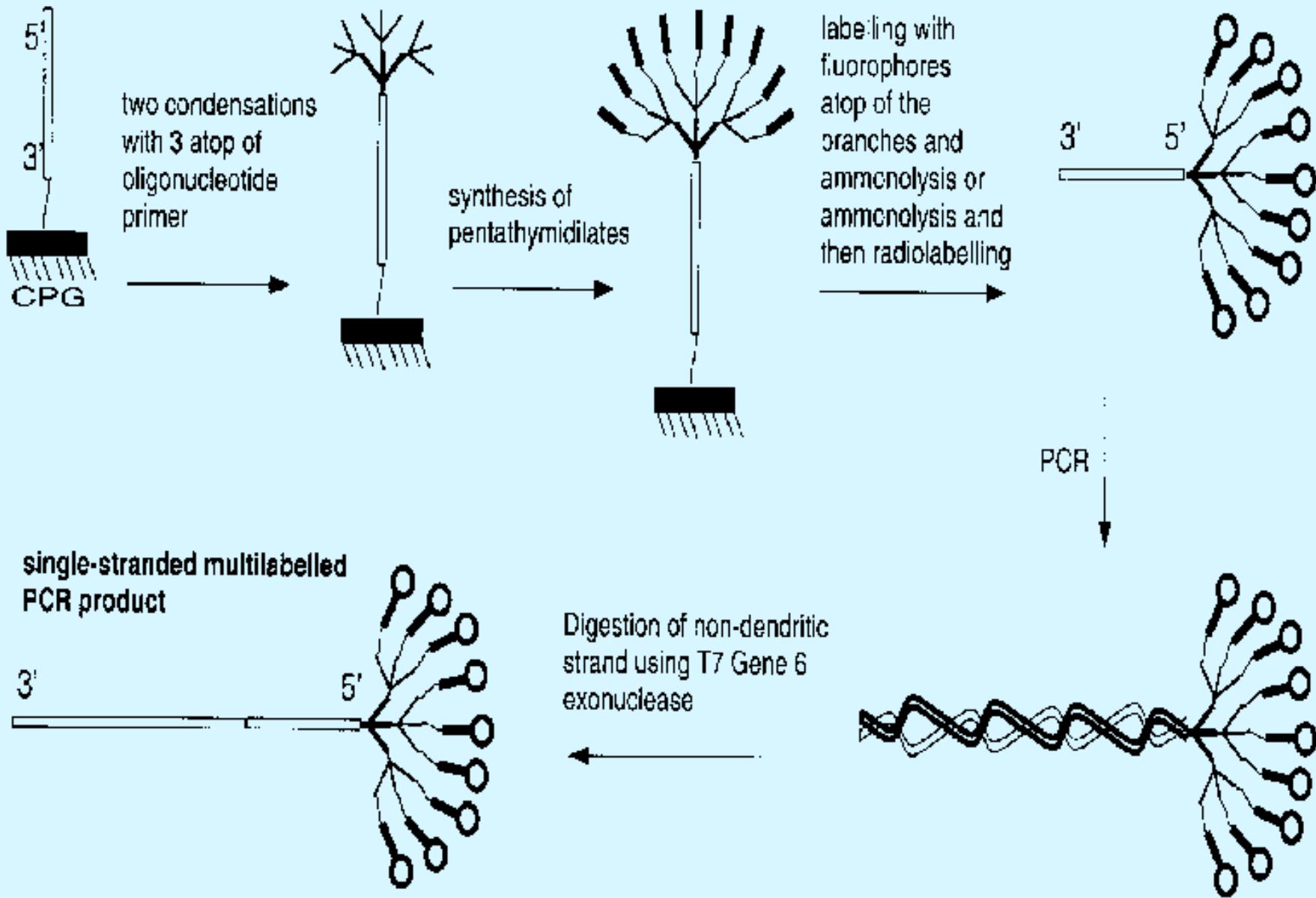
Planar Waveguide Principle



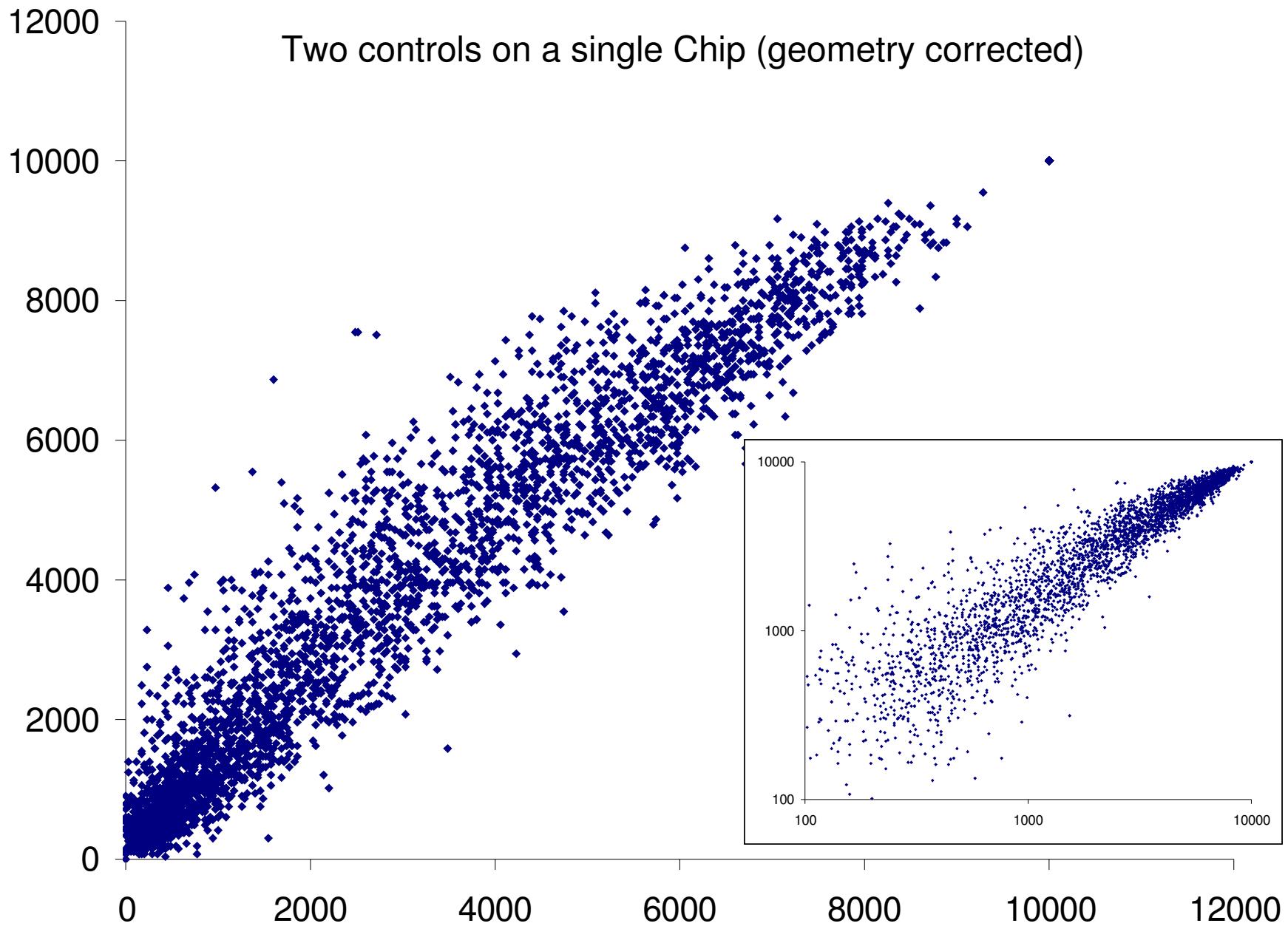
HiLight ® Detection System

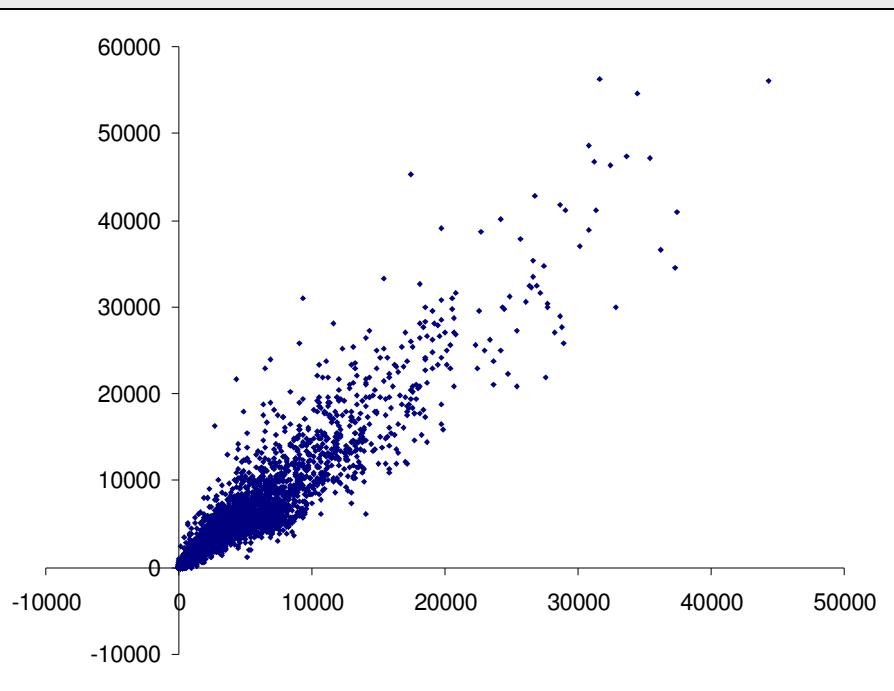


Dendrimer based amplification

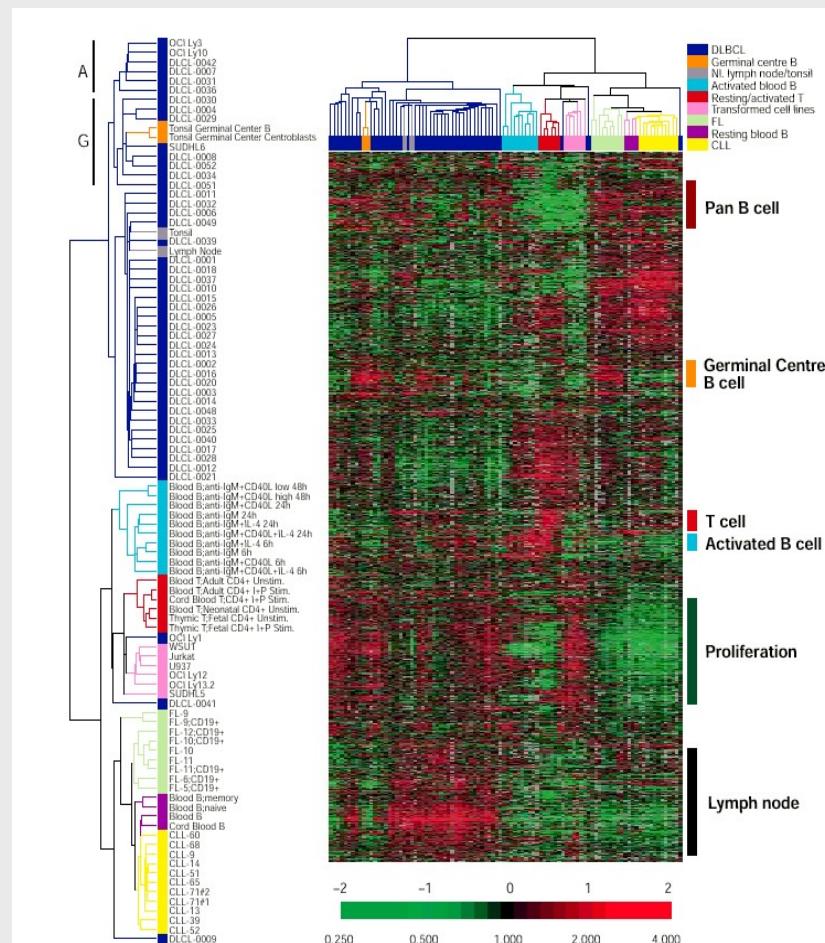
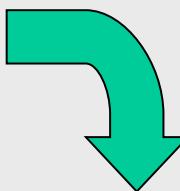


Two controls on a single Chip (geometry corrected)





The bioinformatic step



List

Transfer

- Refresh
- diff mismatch raw
- Rep. Merge

Input

- Set >> A
- Set >> AF
- Set >> AD

Transfers

- AD >> A
- T >> A
- M >> A
- A >> AF
- A >> M
- AF >> A

Data

Chanel	Gene	YGL145W
A	Chip	a2616_t1_m2
Index	Value	254.776993
Search gene		

Normalize (A>AF)

- YDR213W
- YBR301W
- YJR104C

Preprocess

- Go Use SQRT Use offset
- Half mode
- Activate renorm
- Post normalize
- Log and non-parametric
- Magic_Log
- Magic_Sort
- Local dispersion standardized
- Sigma
- Calculate AR
- AD as ratio
- Set Filter
- GO
- Rebuild (SVD >> AR)

SVD

Active vectors

- SVD_0 100.00000
- SVD_1 24.36492
- SVD_2 16.57439
- SVD_3 11.48136
- SVD_4 7.14528
- SVD_5 5.79461
- SVD_6 4.72456
- SVD_7 3.69999

Convert

- Operate A % AF>AD
- Substrat
- Execute
- Half mode
- Transform A>T
- Th value
- Gene name convert
- Reset
- Load
- View
- More

Control

- Gene name convert
- Reset
- Load
- View
- More

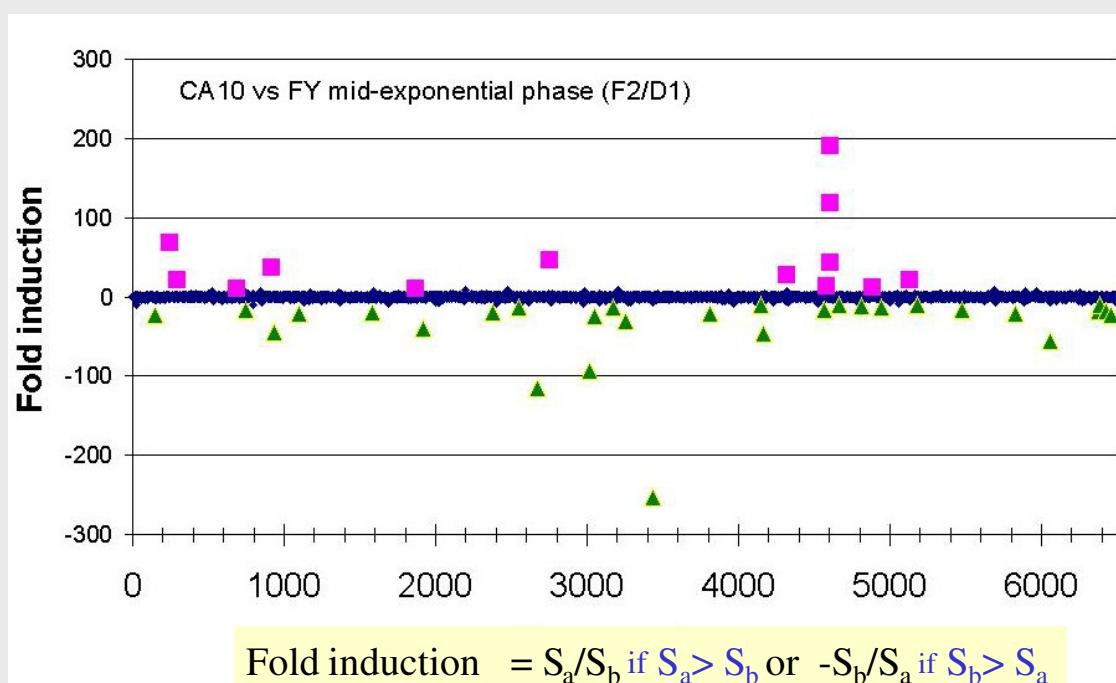
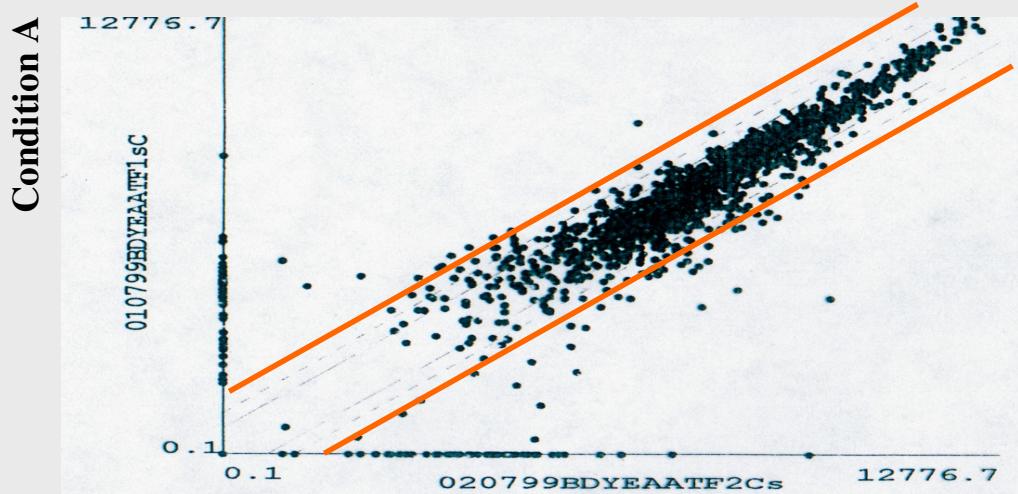
FEAT Generate mask

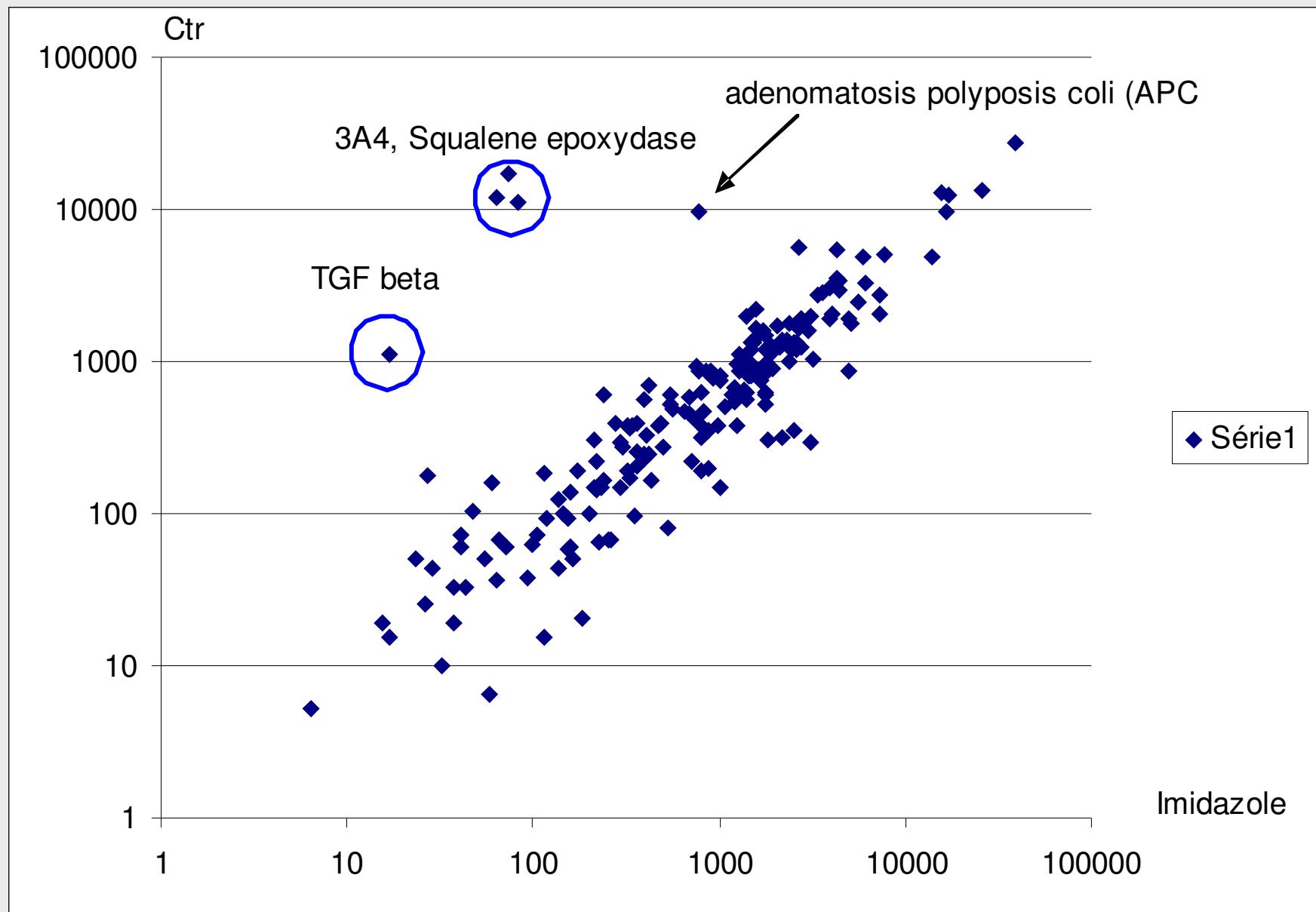
- moy > 0.2 n
- Cor > 0.7
- AF SVD > AD
- No negative
- Avoid neg
- Rk moy<4
- Rk Cor<4
- SVD use SQR
- SVD use mode 2
- SVD use variance

Buttons

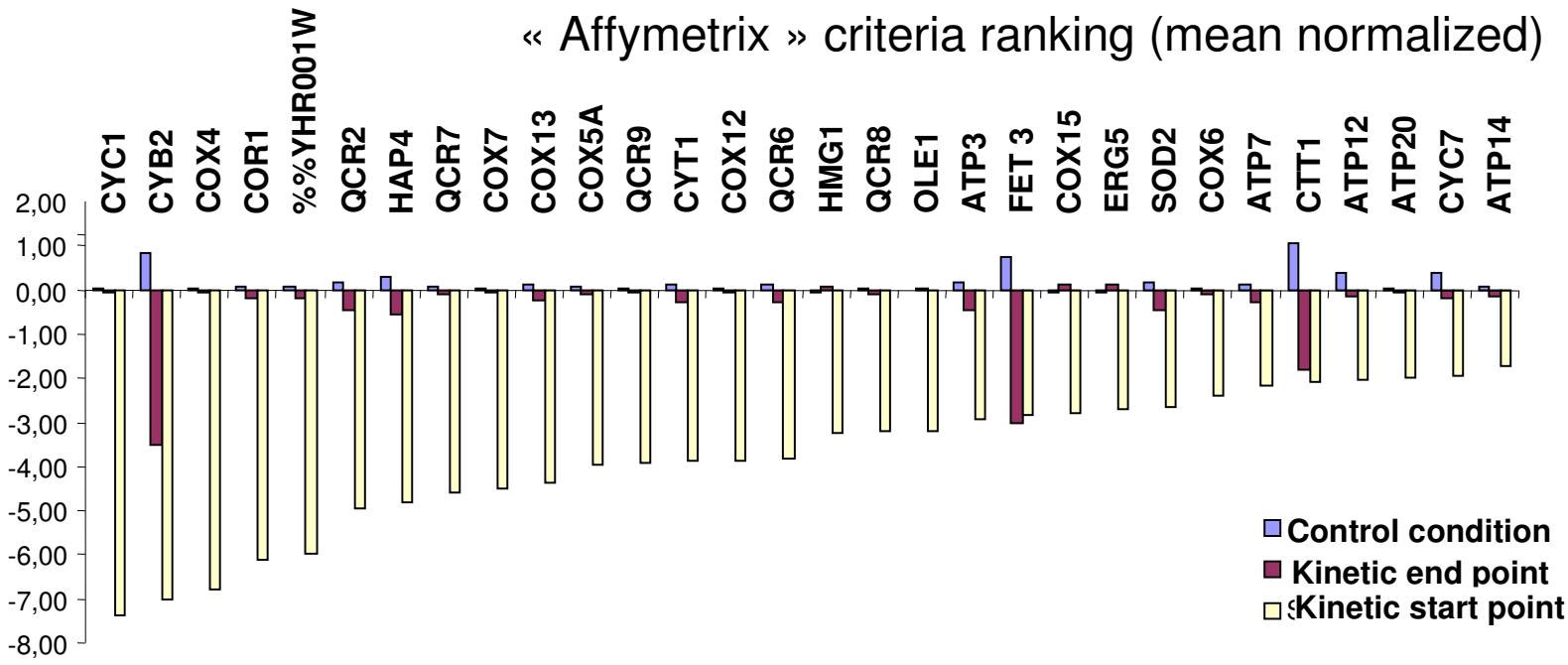
- Add Ref.
- Save
- Weight
- Norm to 1
- Del Ref.
- Load
- Select Chip
- SDV Norm
- Intern. Norm
- Extern. Norm
- Use Half mode
- List
- List A
- List AD
- List AF
- List T
- List M
- List SVD

Affymetrix DNA chip analysis of yeast transcriptome (whole genome)

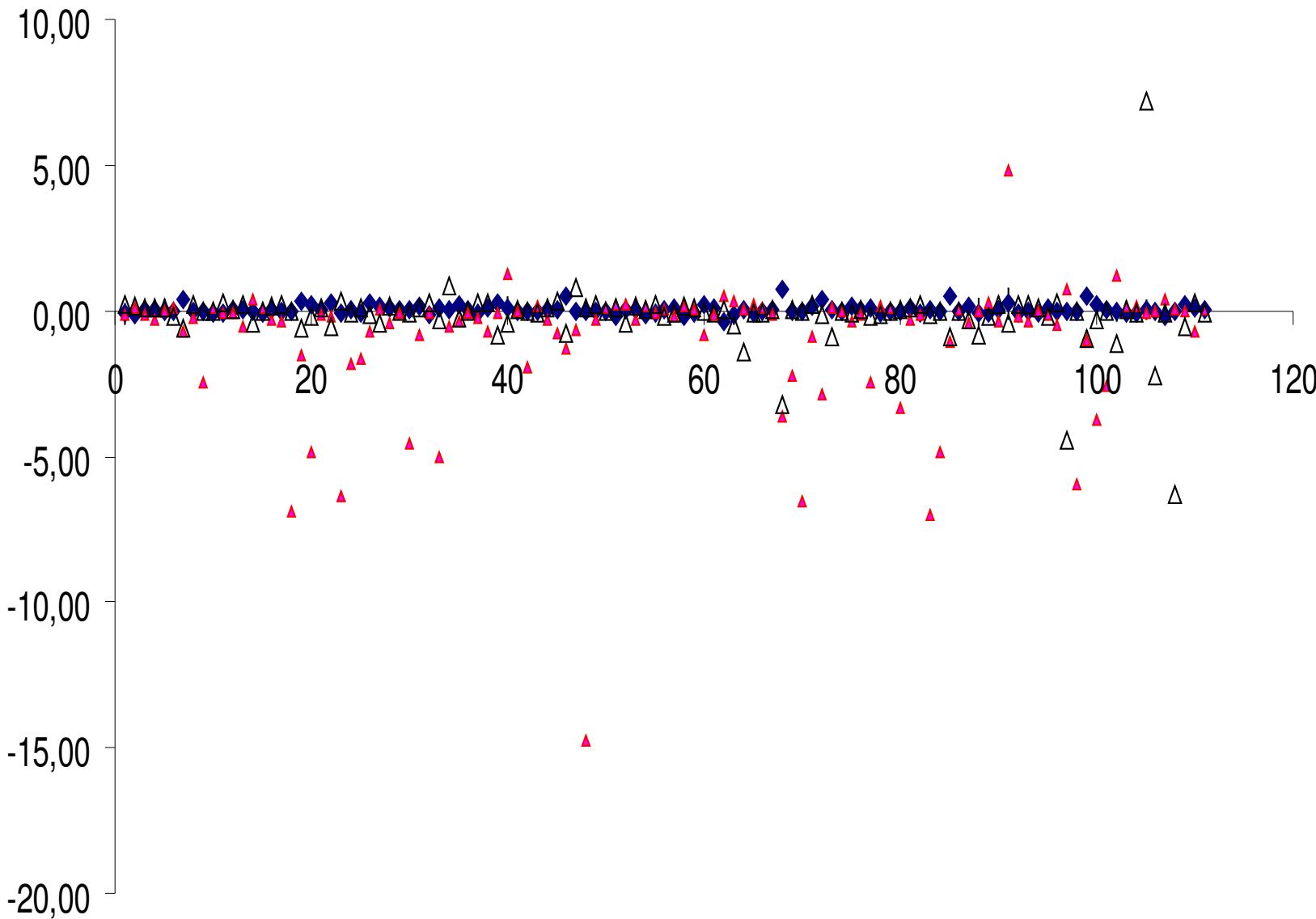




« Affymetrix » criteria ranking (mean normalized)

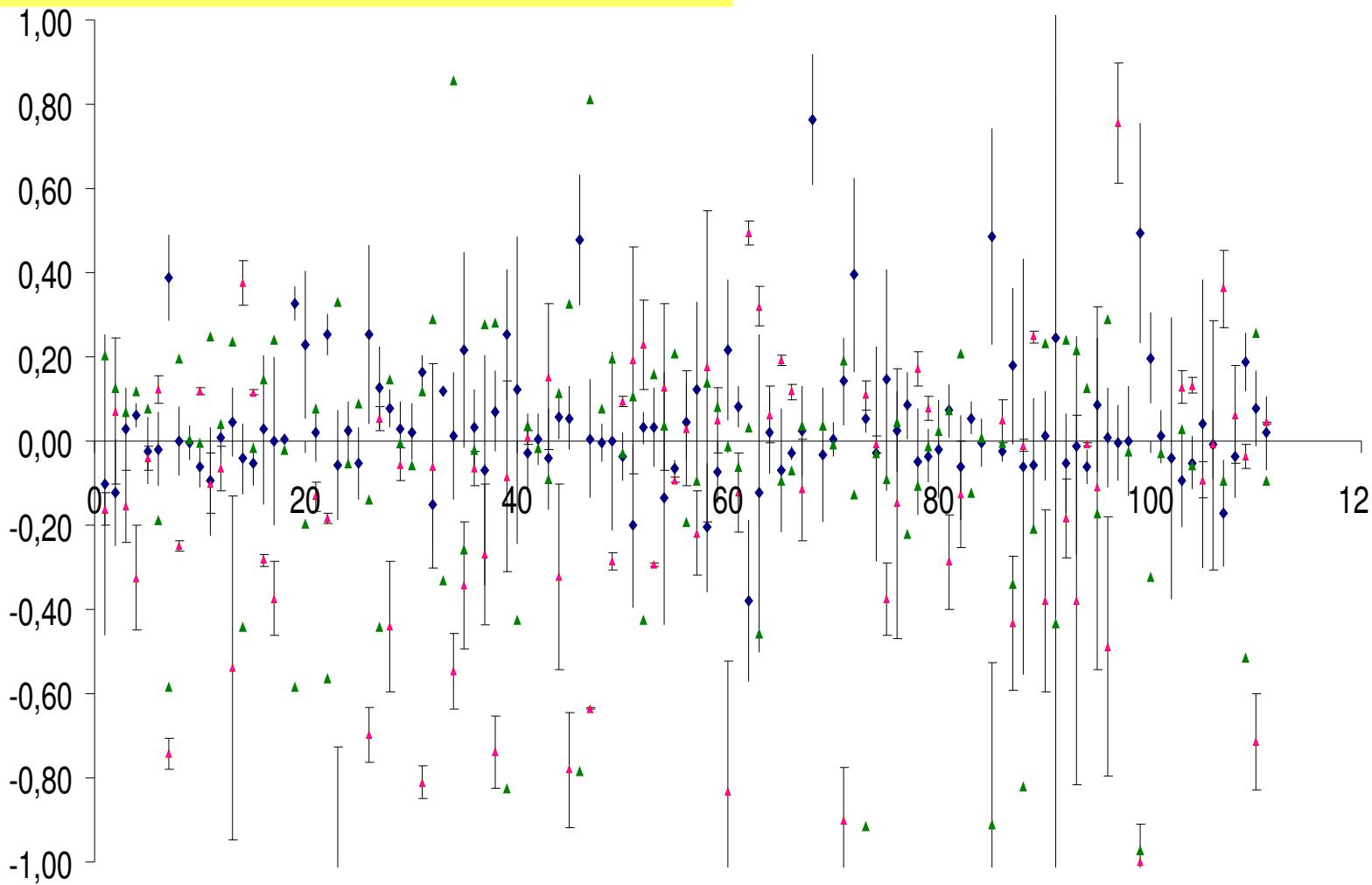


Control Time 1 Time 15

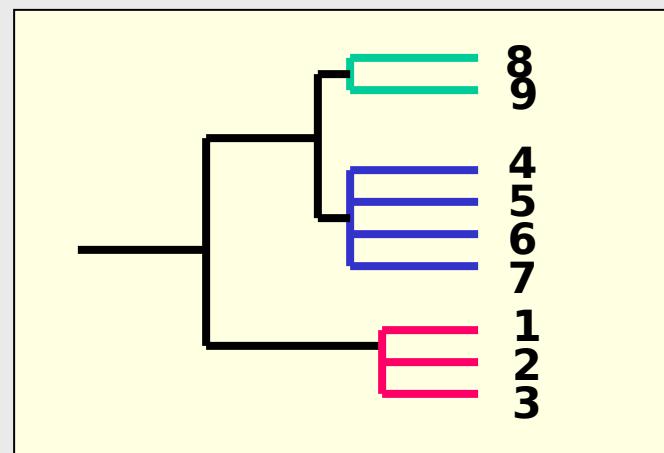
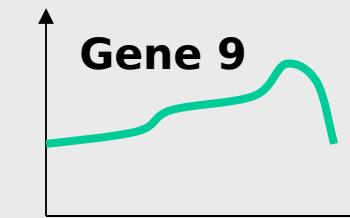
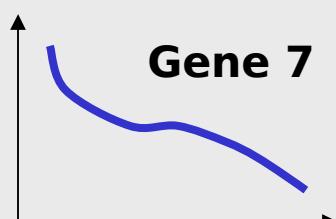
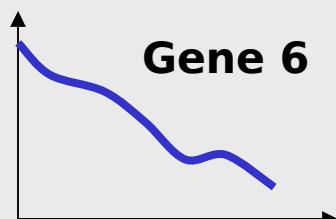
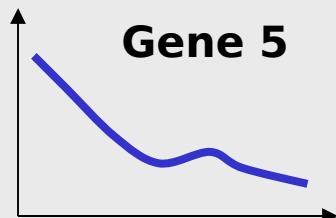
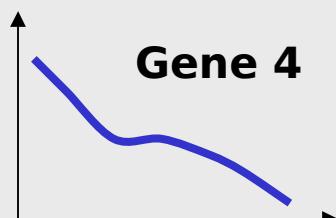
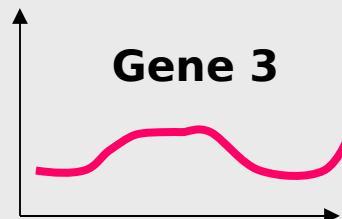
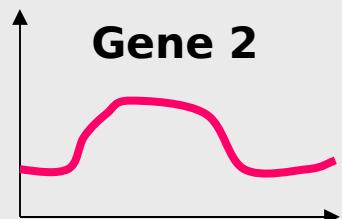
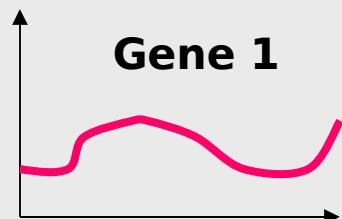


█ Control █ Time 1 █ Time 15
 $N = (A + L_{std}) / (B + L_{std}) - 1 \quad (A \geq B)$
 $N = 1 - (B + L_{std}) / (B + L_{std}) \quad (B > A)$

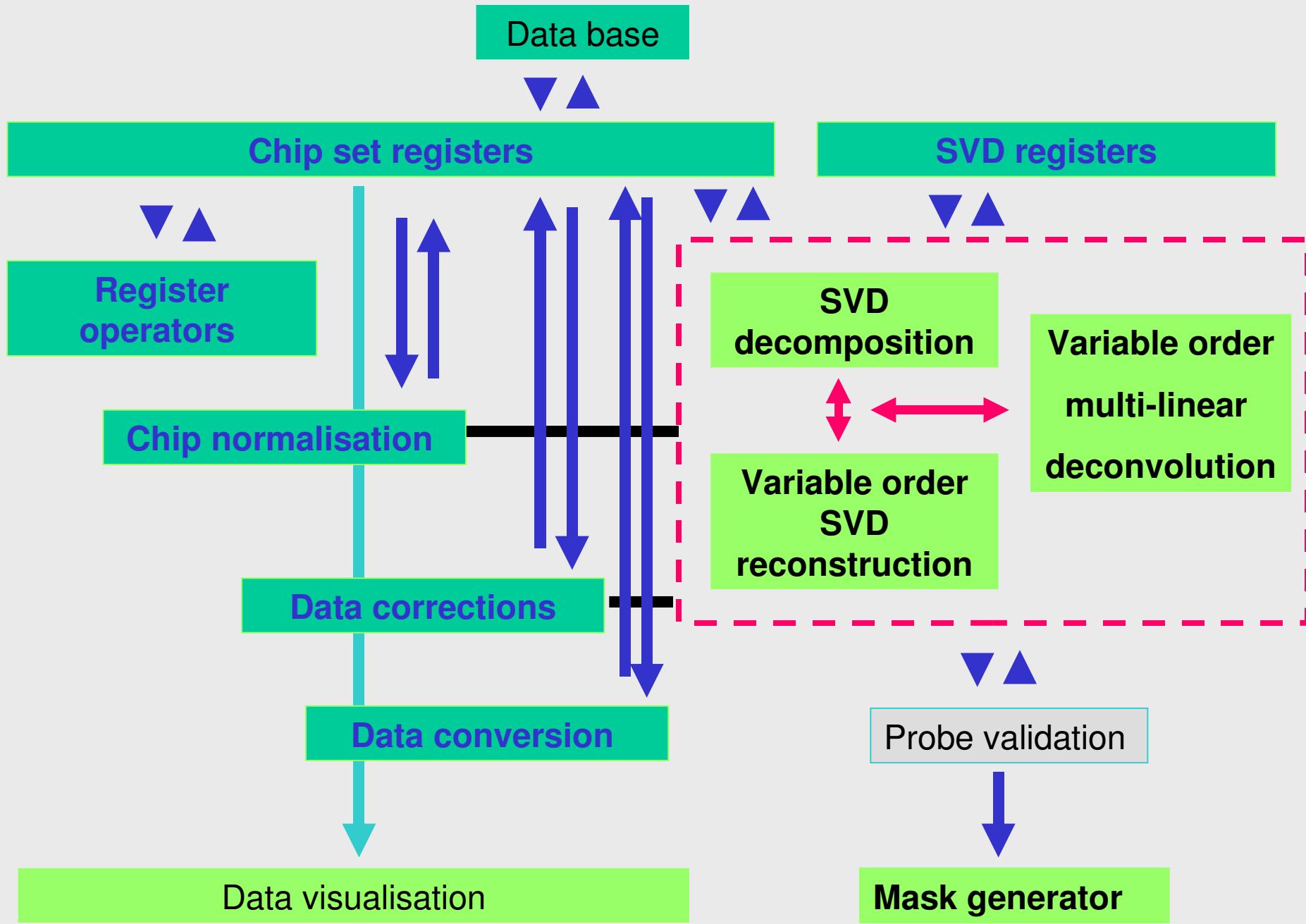
Normalized induction factors (AFM like)

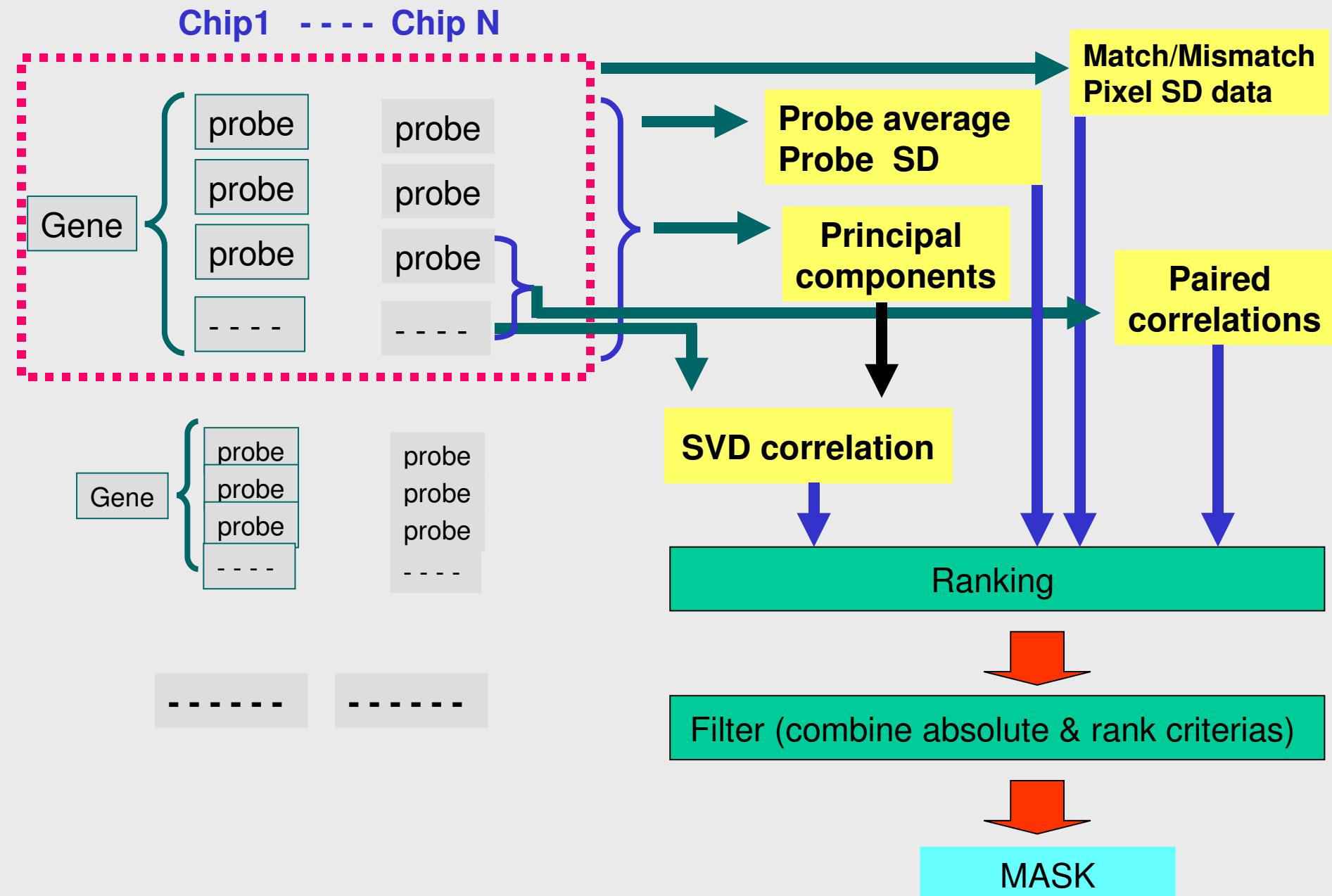


Clustering : regrouper les gènes selon leur pattern d'expression

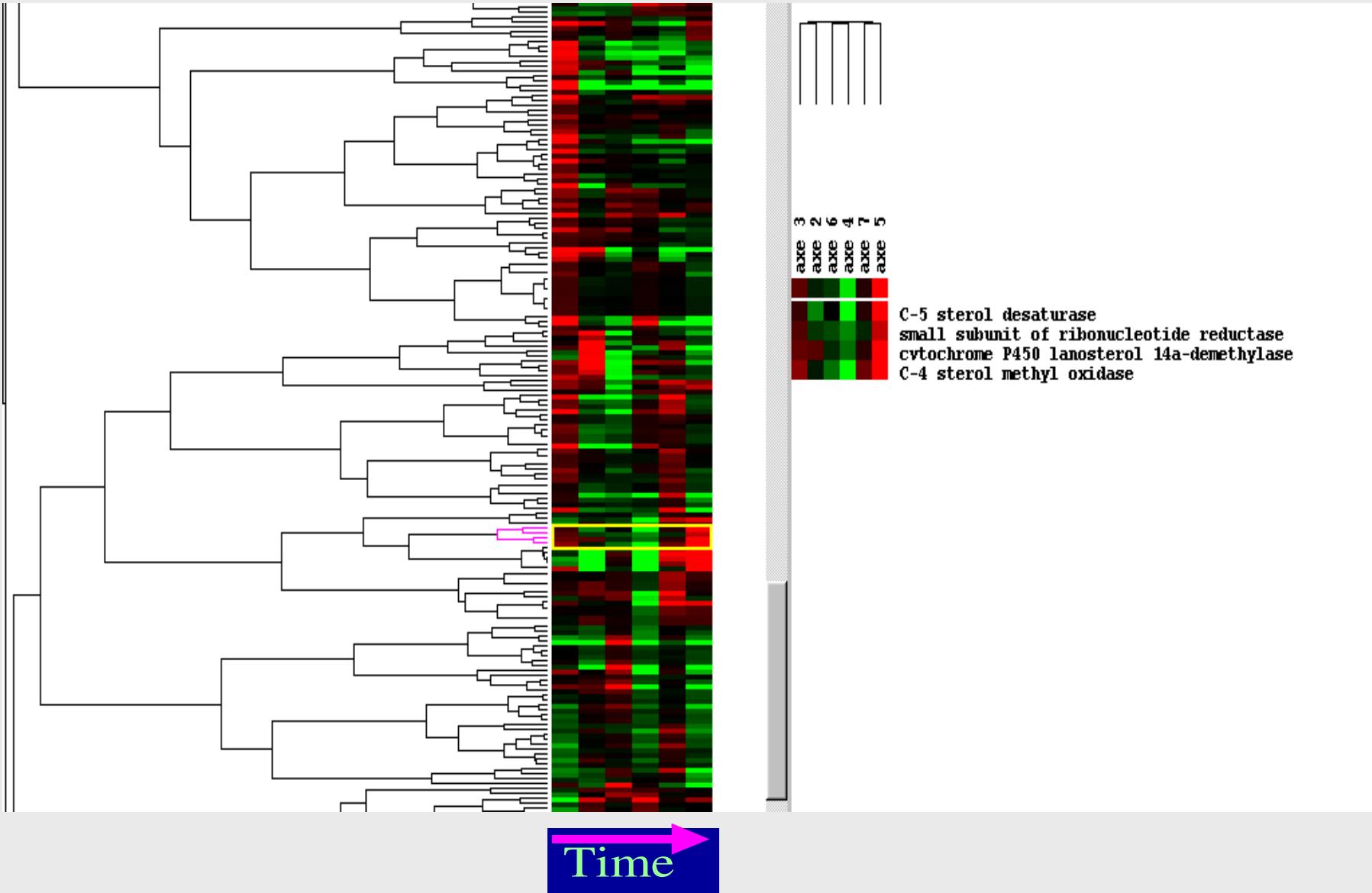


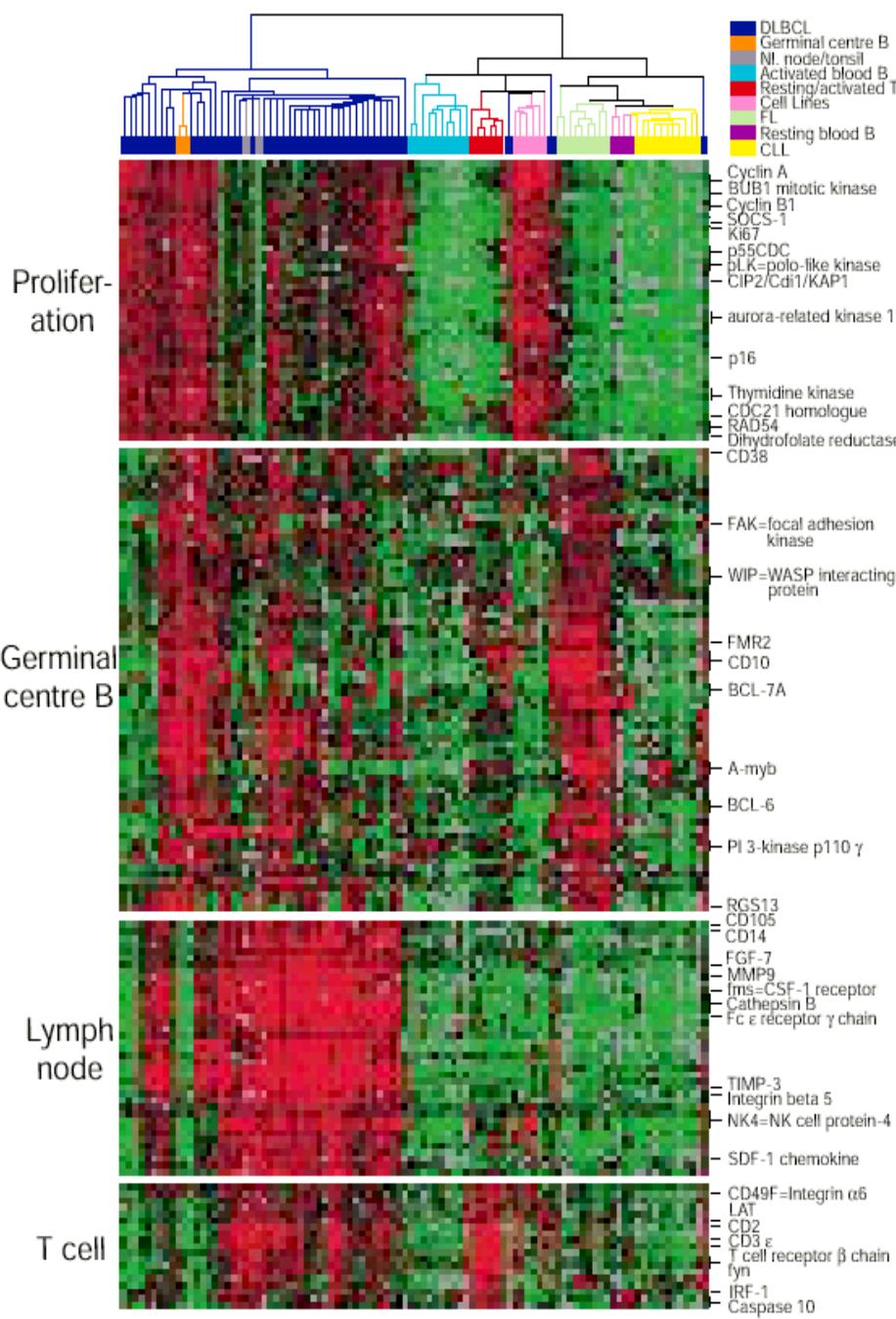
Algorithms for data analysis, correction & normalization



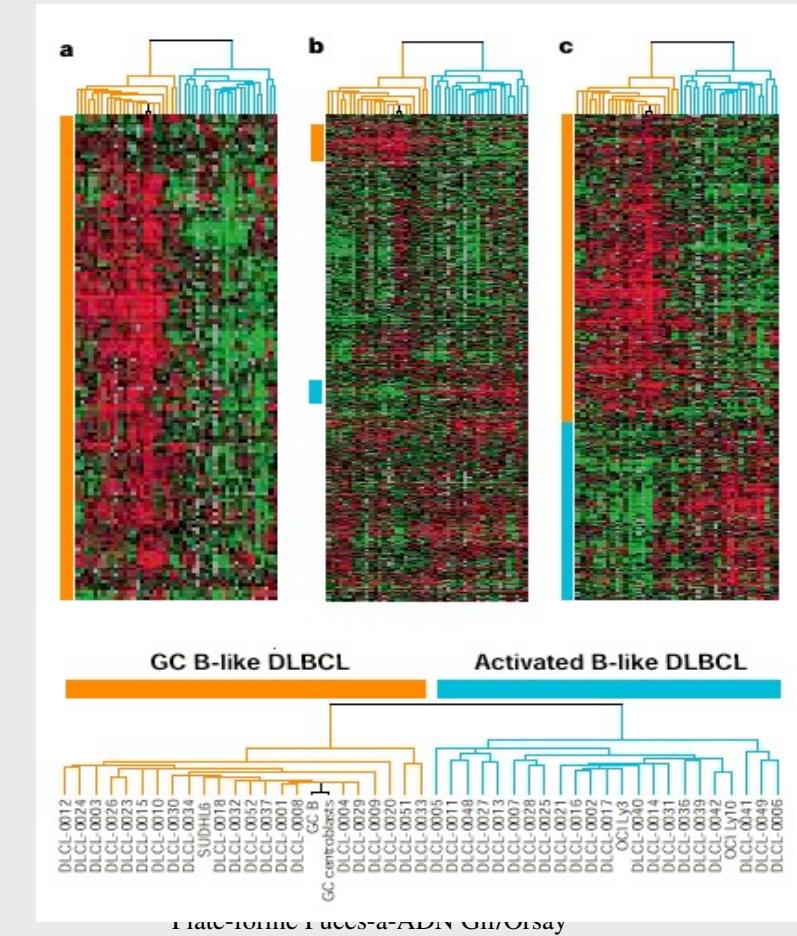


Window in the clustering of differential transcriptome in yeast

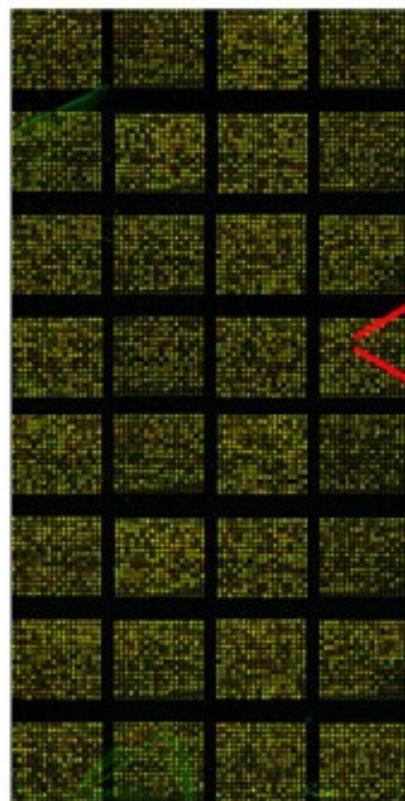




Example of identification of cancer subtypes using DNA chip analysis

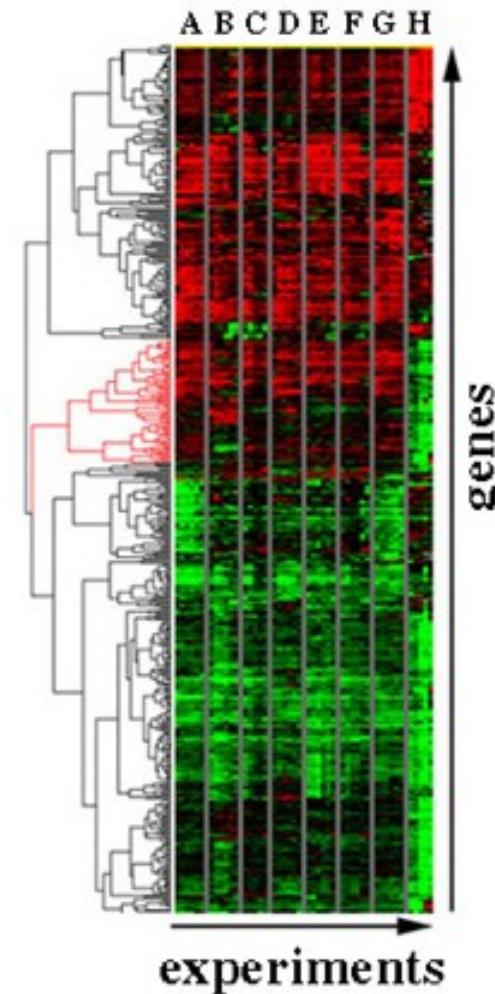


Anopheles gambiae
cDNA microarray



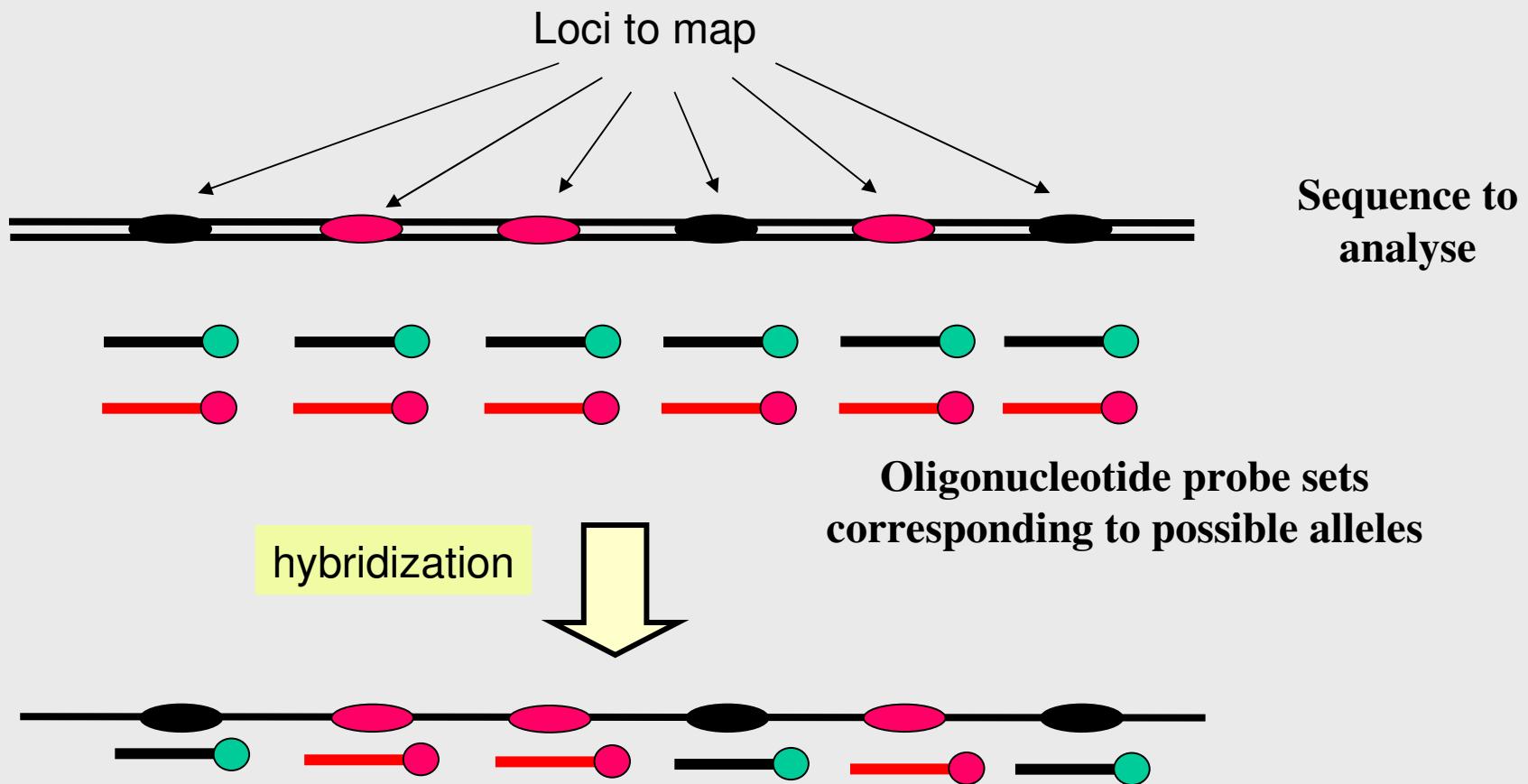
8500 spots

Clustering analysis
of expression data

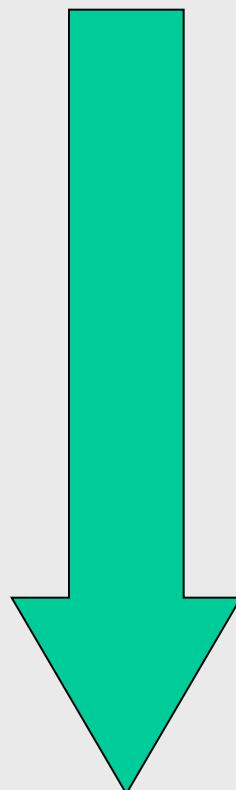
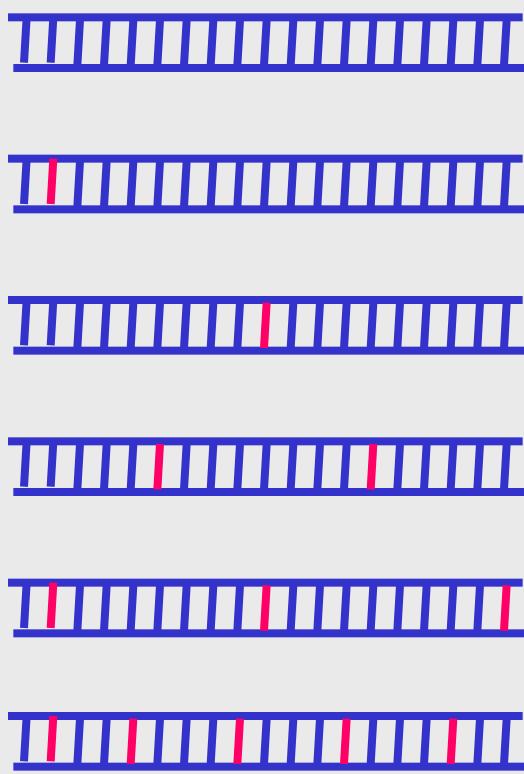


chip for sequencing

DNA chip based sequence mapping

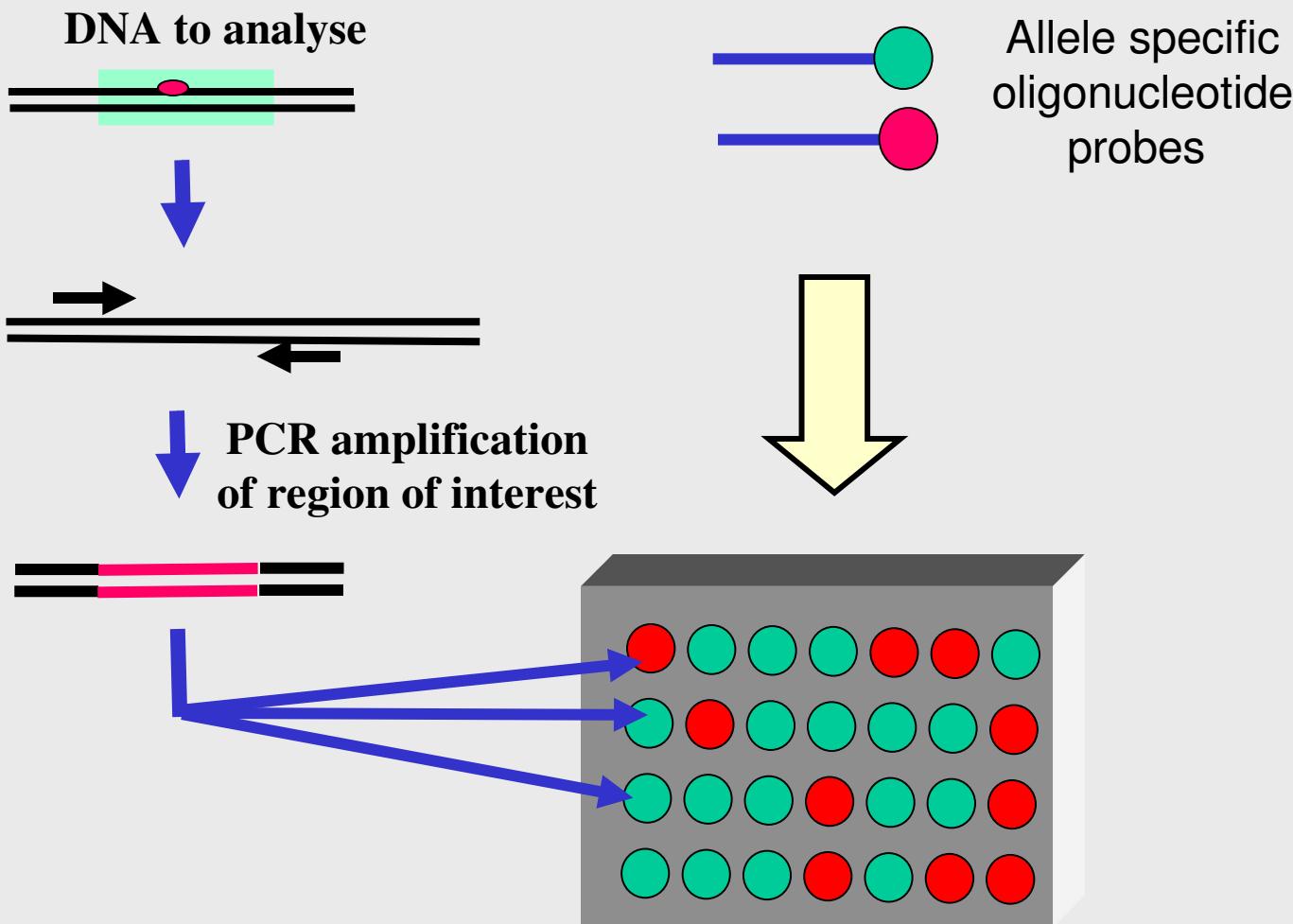


Use of chips for sequence analysis

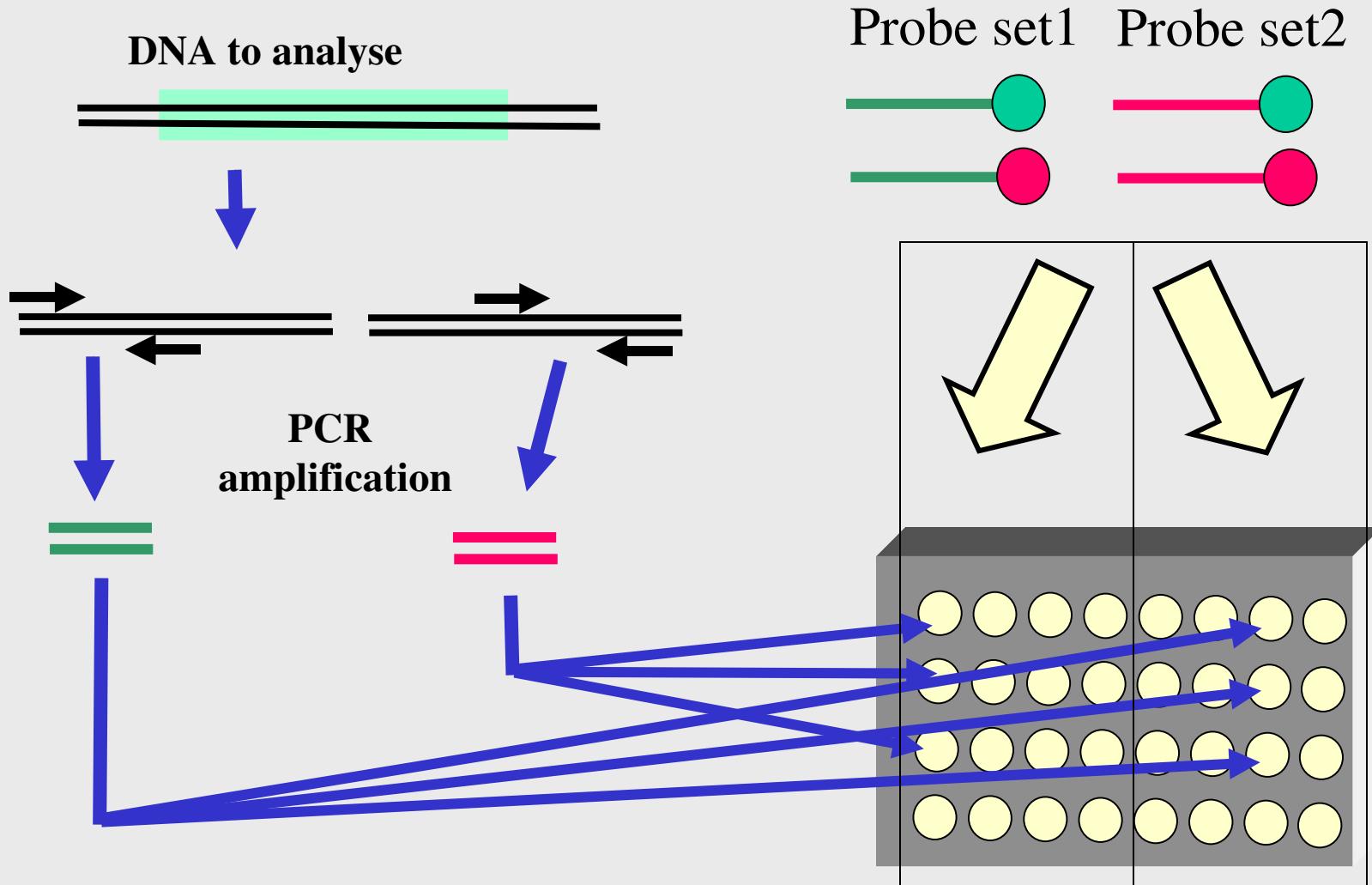


Decreasing stability
of duplex DNA

DNA chip based sequence mapping technology

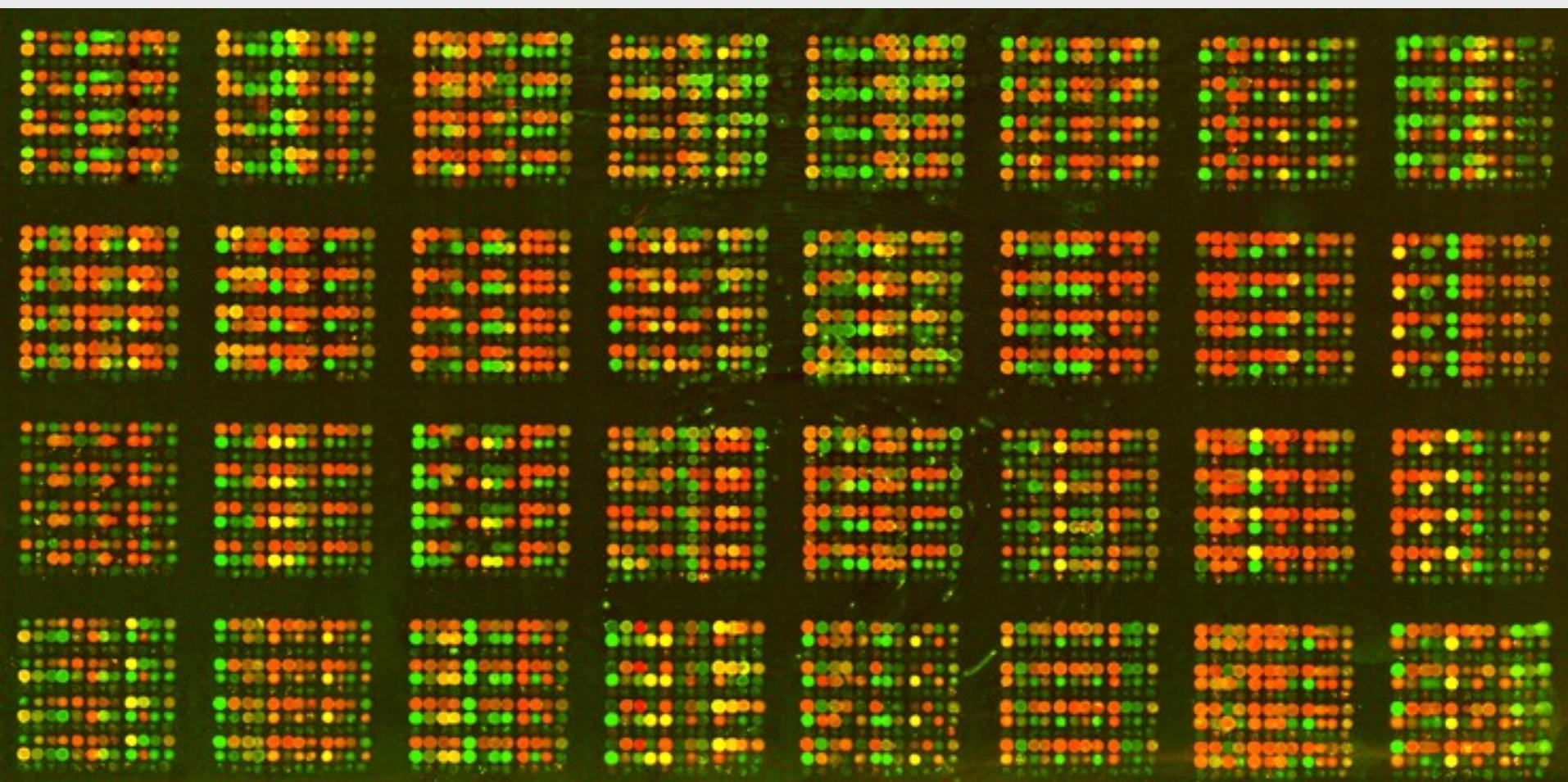


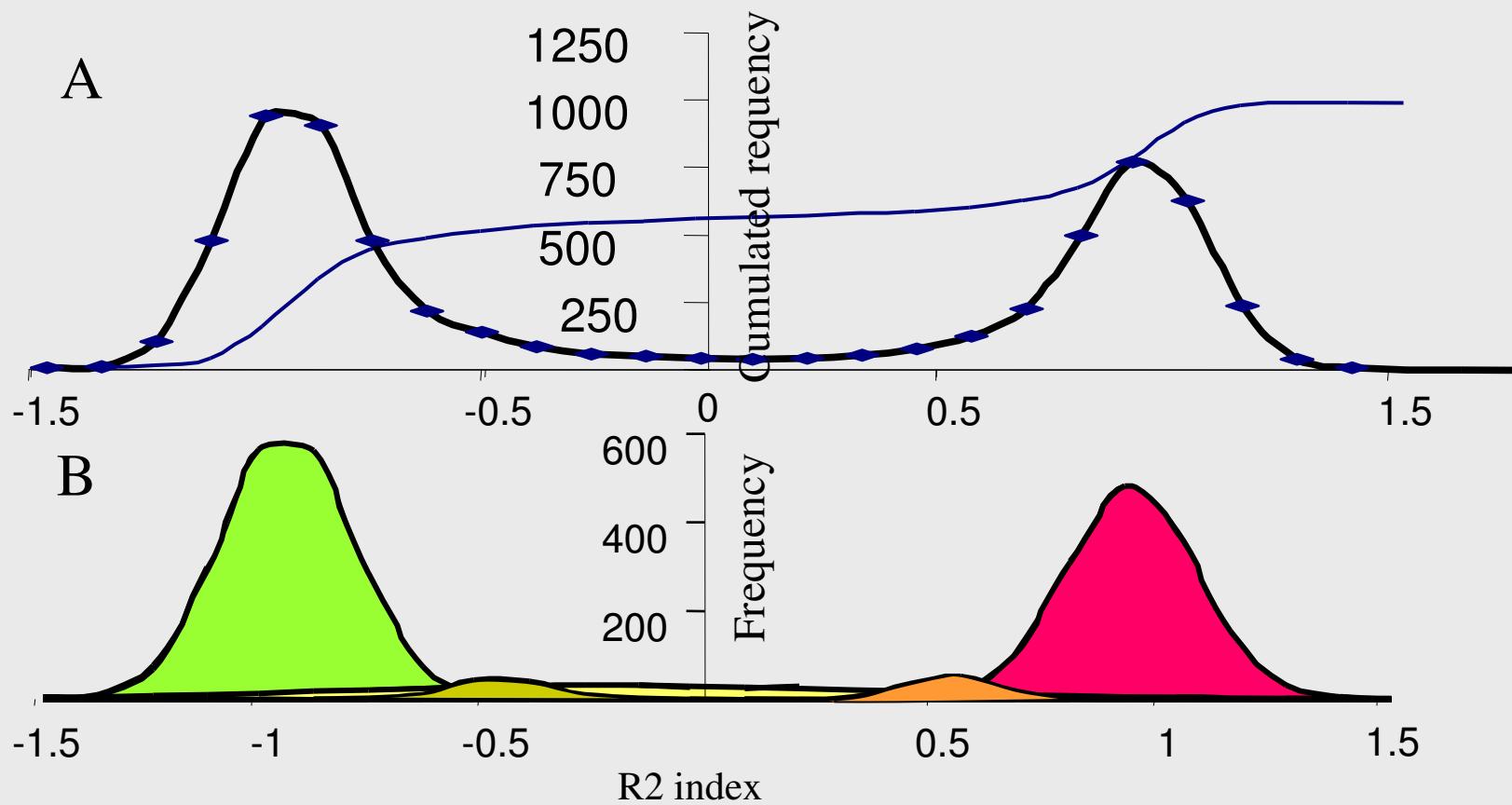
Multiplex sequence mapping



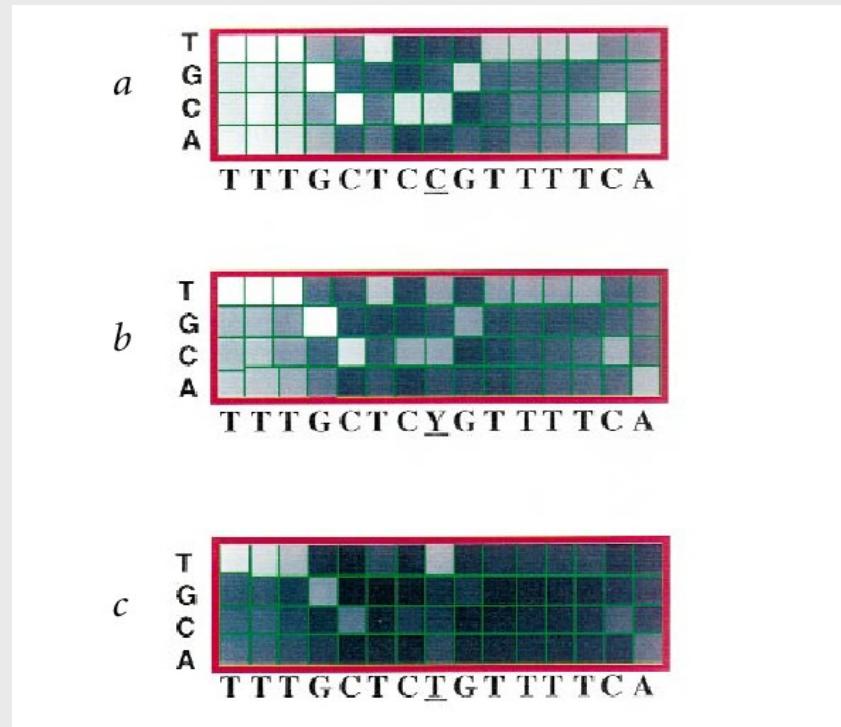
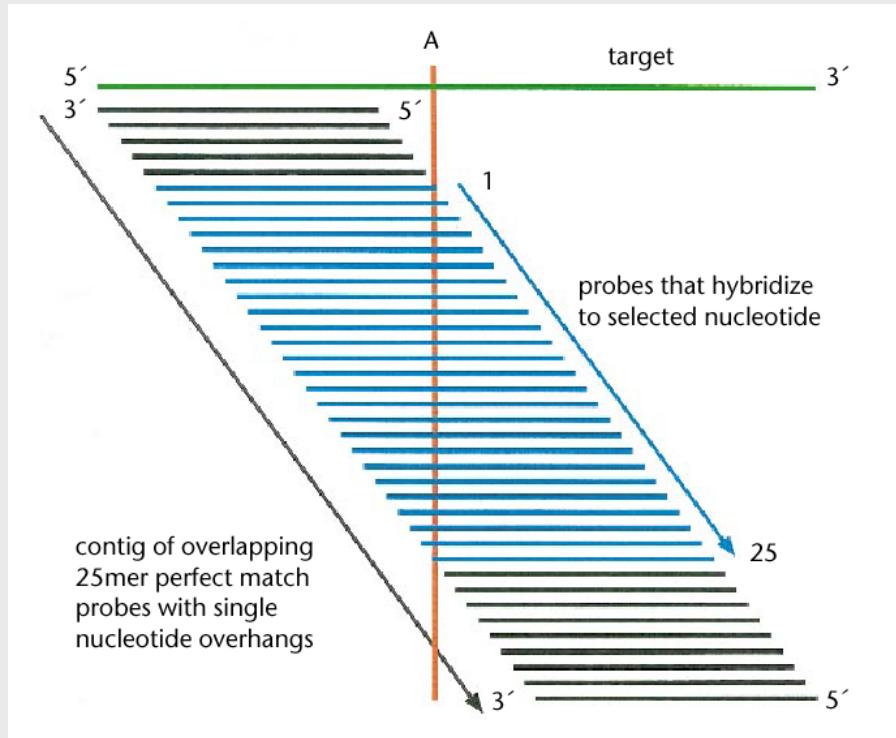
Sequencing with chips

Sequence mapping (MOSECA) of three combinatorial libraries





Inverted geometry using high density chips



Genotyping

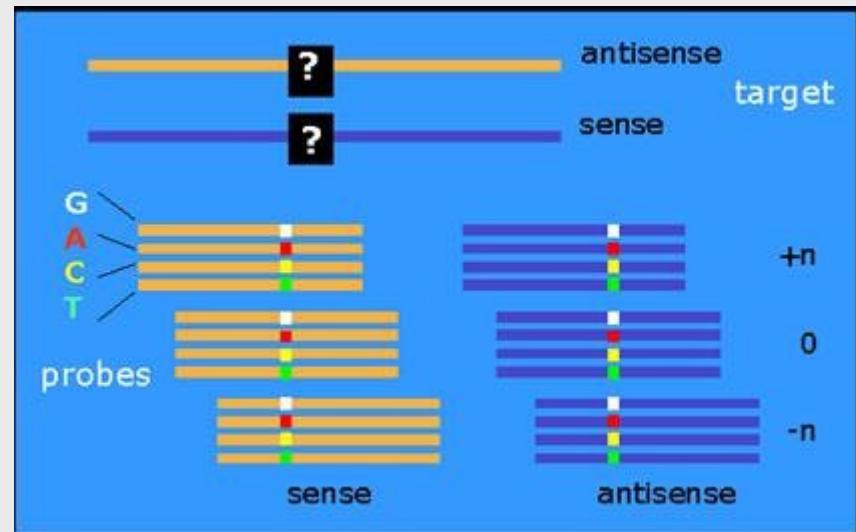
The geniom® technology has been used to identify relevant single nucleotide polymorphism (SNP) patterns in immune regulatory genes. An array has been developed to analyze 94 SNPs in parallel.

Probes

Probe design was supported by the geniom® software generating arrays of 12 probes for both sense and the antisense strand interrogating each SNP position.

Sample preparation

Starting from genomic DNA SNPs were amplified by PCR in a multiplex reaction. PCR products were pooled and labeled by incorporation of e.g. biotinylated dNTPs.

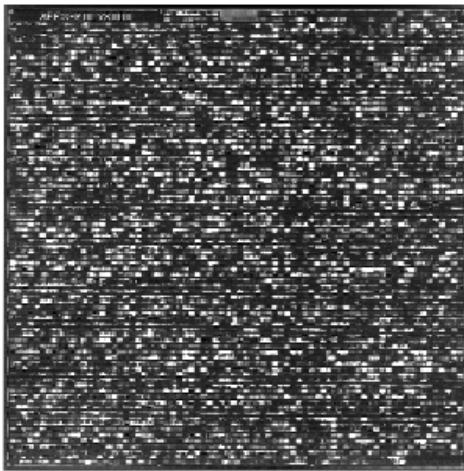


Example of a SNP typing result

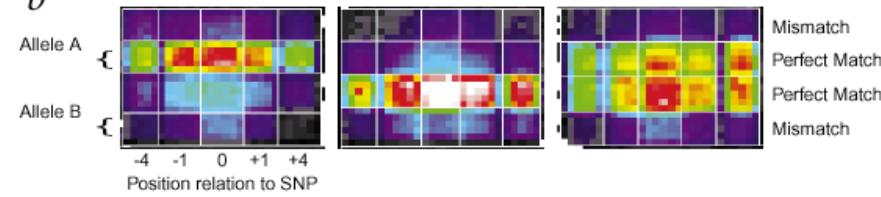


Génotypage avec des puces à ADN

a

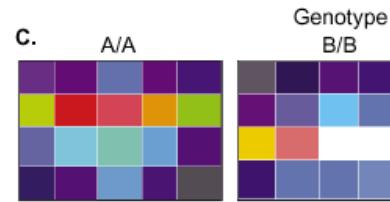


b



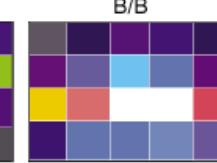
c.

A/A

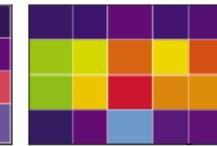


Genotype

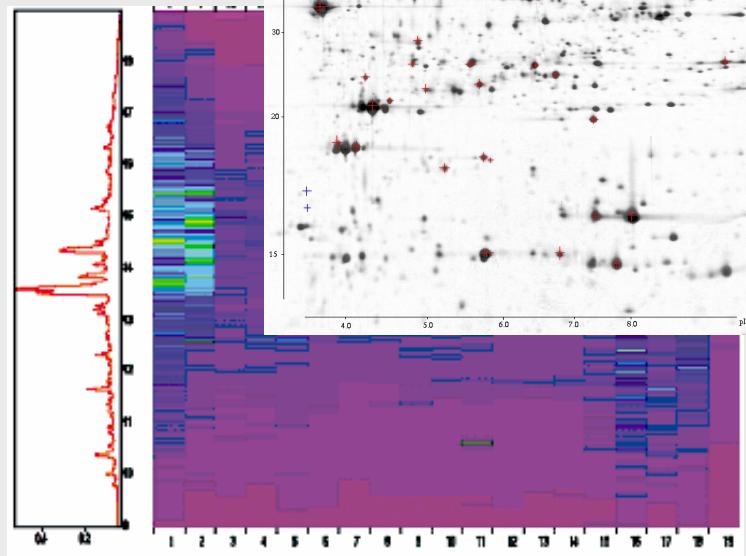
B/B



A/B



Proteome



Multi dimensionnal
resolution

Isolated
proteins

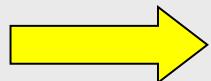
Quantification

Peptides

Mass- spectroscopy



Genomic informations
(sequence)



Identification

The protein chip technology

Still an emerging
technology ...

Human
40 000 genes



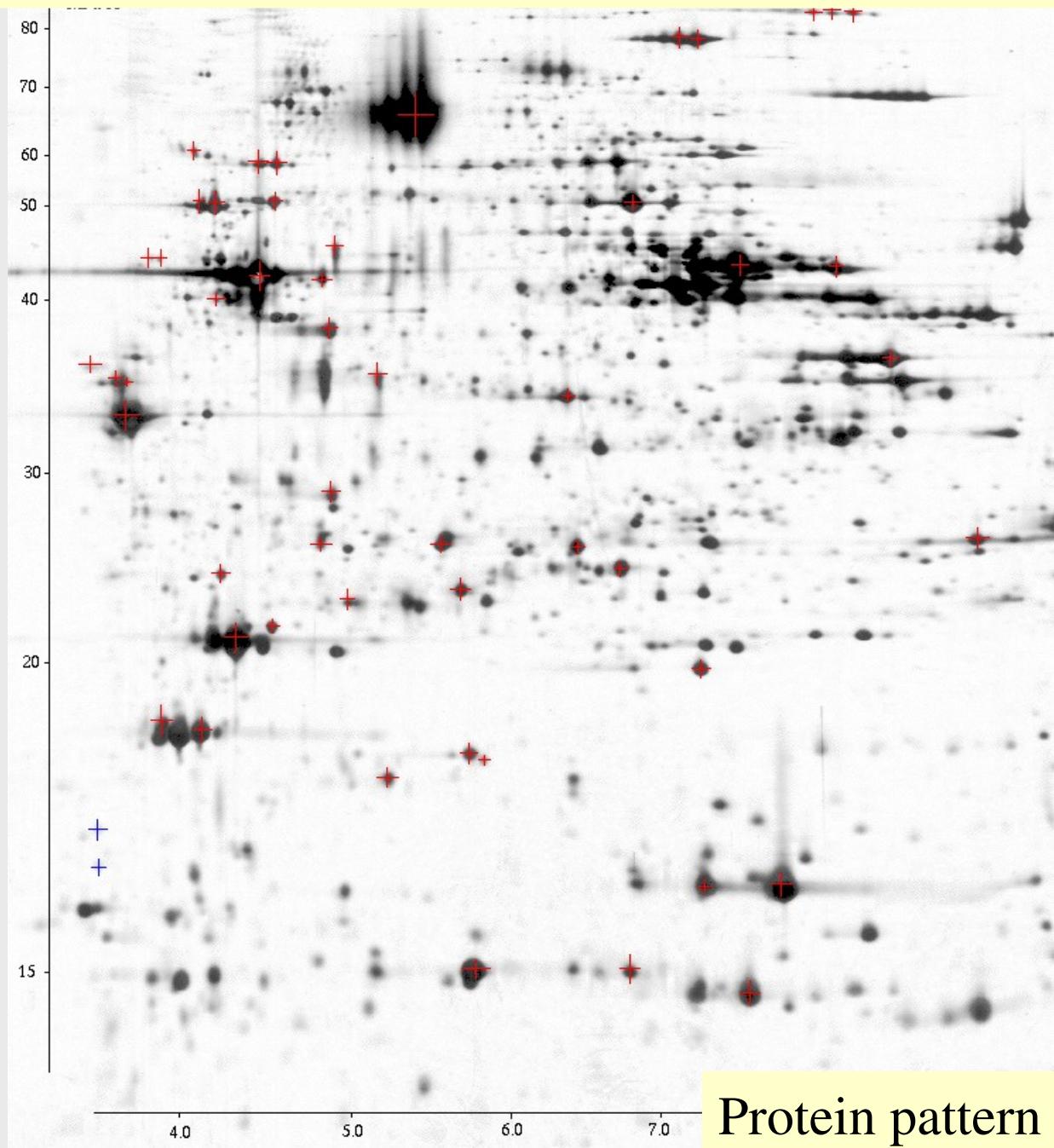
100-300 000 ARN



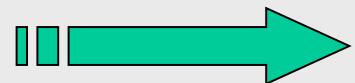
1-5 000 000 proteins



Much more functions



High throughput approaches of protein functions



Catalytic functions



Recognition functions

- Nature & characteristics of activities
- Modified properties
- Search for inhibitor or activator
- Search for partners



Classical approaches involving large robotic facilities



Miniaturisation

Lab-on-Chip

Protein chips



***ab initio* (sequence based) and structural approaches**

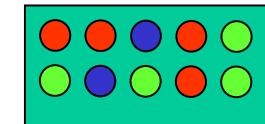
Protein chips

Several hundred of technologies, more than 40 companies in 2002

Bring together on the same device



Spatial resolution
(large set of objects)



Function analysis
-Interaction
-catalysis



Spatial resolution



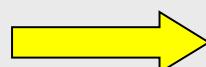
1D- or 2D-
resolution of a
mixture

Micro-chromatographic
Micro-electrophoresis
Micro-fluidic
Affinity (antibodies, molecular imprinting)



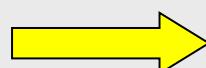
Spotting on a 2D-matrix
Electrical, optical or magnetic addressing
Binding to self-identifying micro-spheres

Function analysis



« Events » detection system

fluorescence
SPR, MS
mechanical
field effect

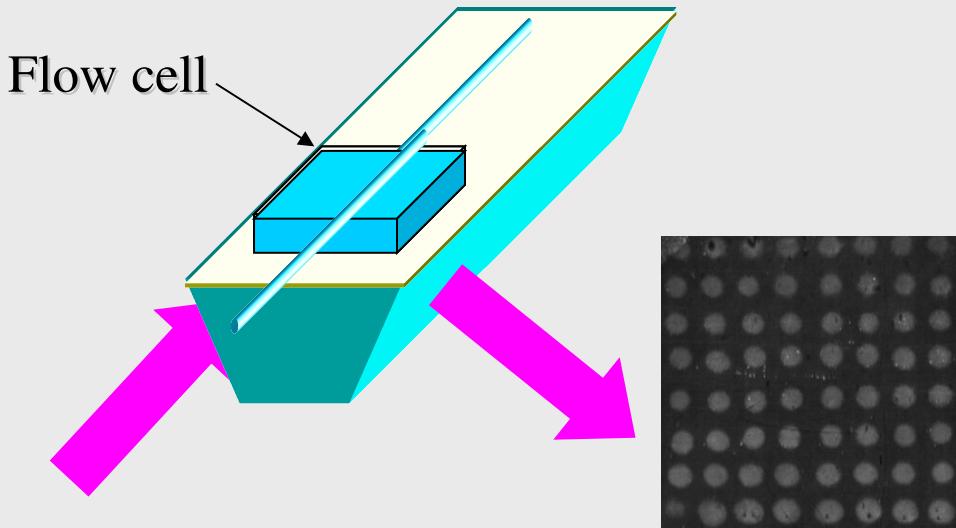


Access to the time dimension (kinetic)



Need for real time systems

Surface Plasmon Resonance imagery

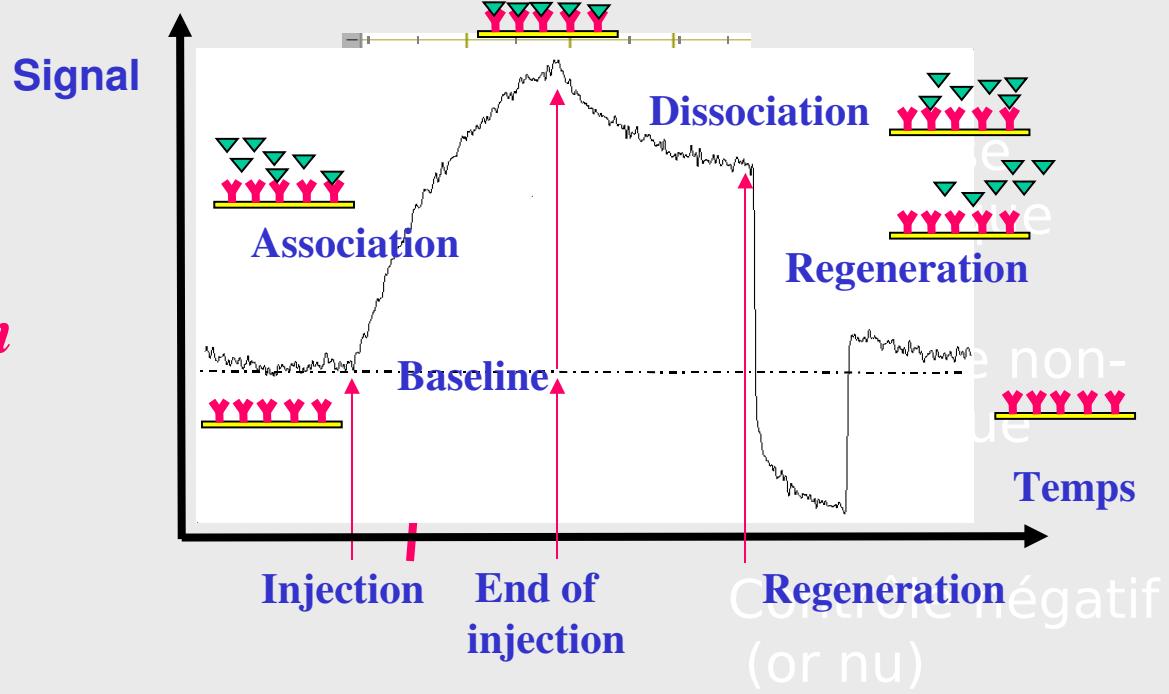


*and
kinetic discrimination*

The first world wide available technology that bring together:

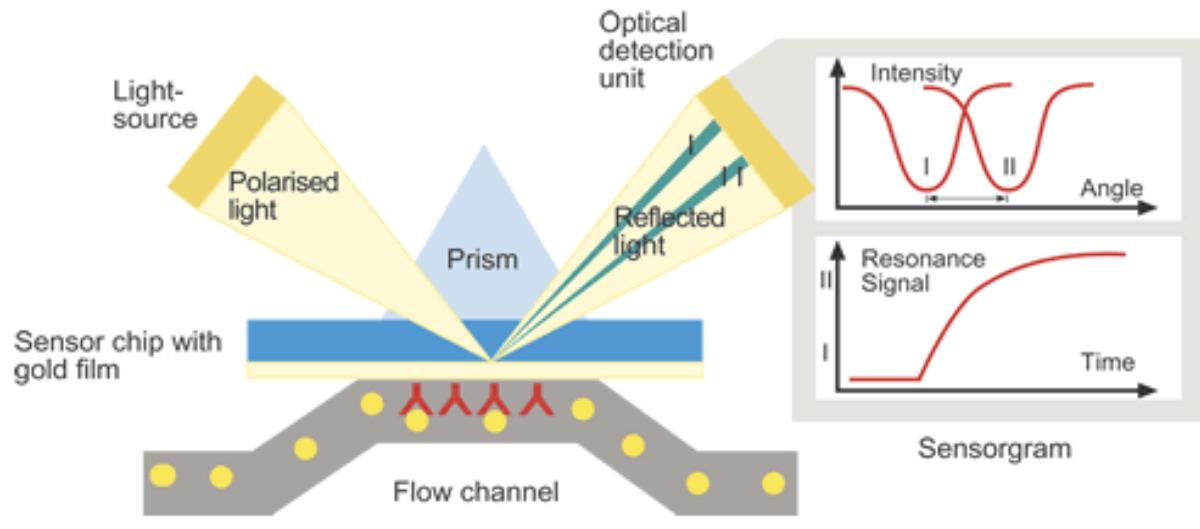
Spatial discrimination

Saturation

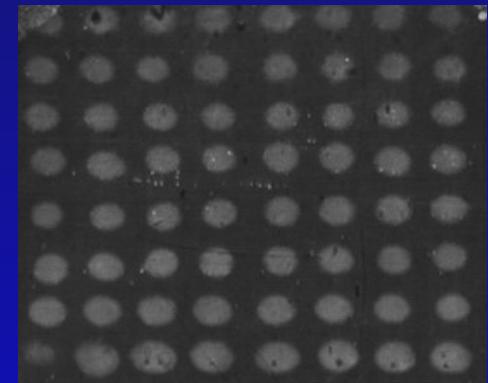


for full and massively parallel functional characterization of proteins

A proprietary unique Hi-Tech technology

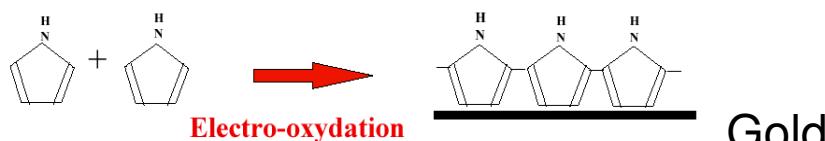


A powerful parallel protein chip approach

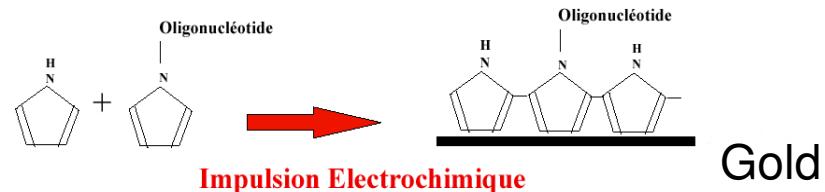


GENOPTIC and CNRS proprietary surface chemistry

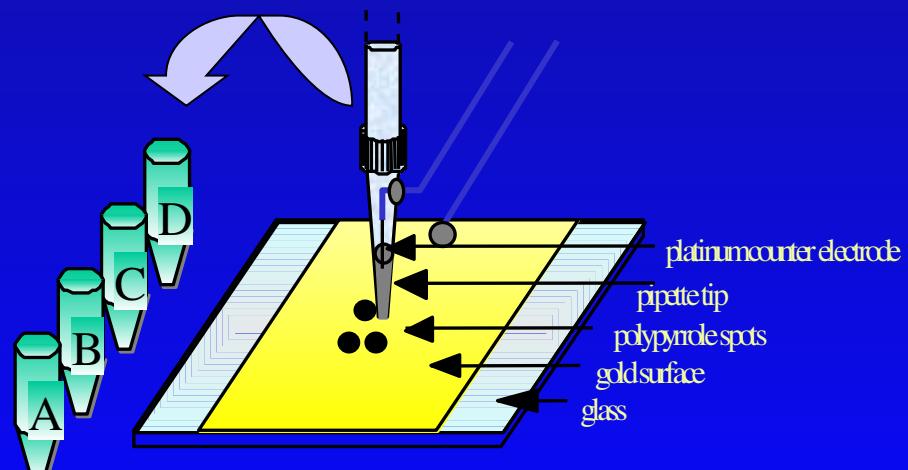
Oxydation Pyrrole \rightarrow Polypyrrole



Co-polymérisation Pyrrole / Pyrrole ODN



Sophisticated robotics



CNRS combinatorial library technologies

HTS screening for accelerated drug discovery

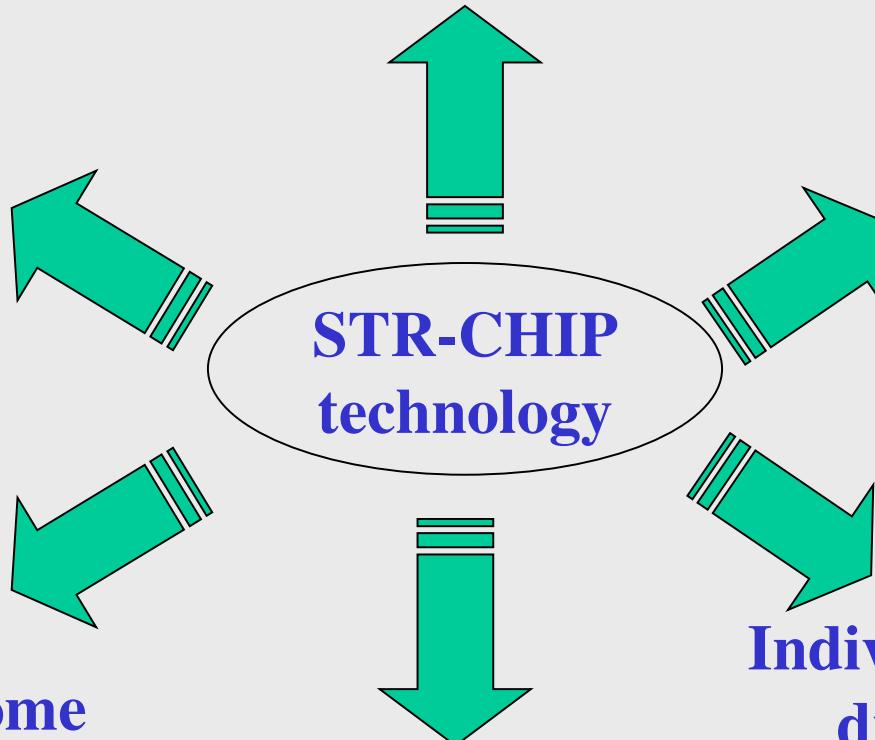
Advanced
diagnostic tool

On chip proteome
analysis

Environmental
monitoring
and hazard
detection

Individually adjusted
drug-treatment

Drug security





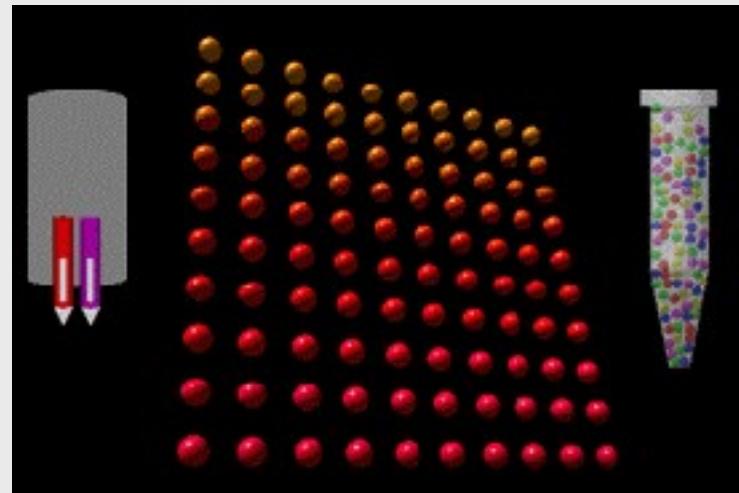
« Homogeneous phase » technology

Substitute single chip by a large number of micro-beads

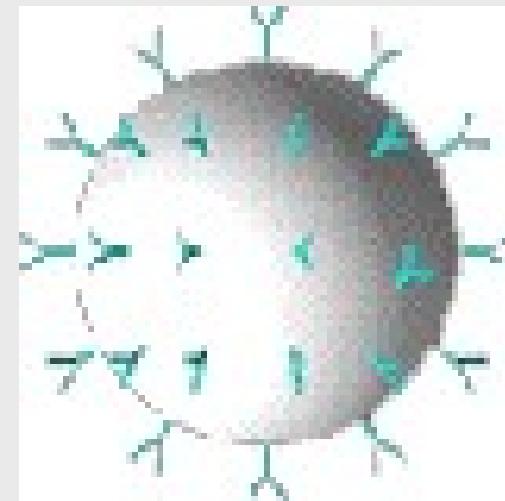
Self-identifying micro-beads

Low density but low cost and easy to implement

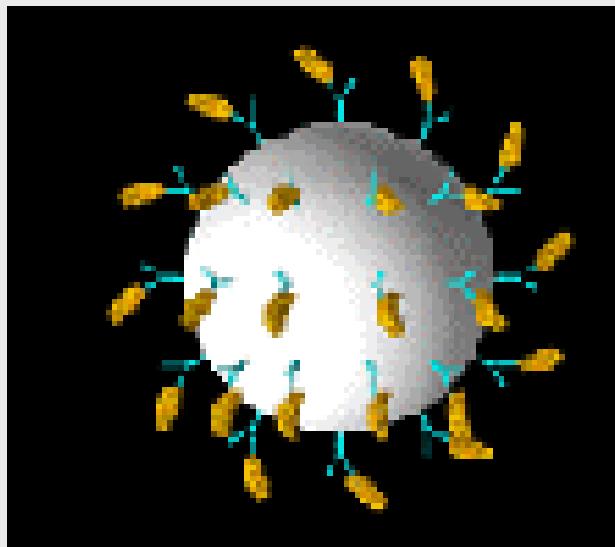
Color coded micro-spheres collection



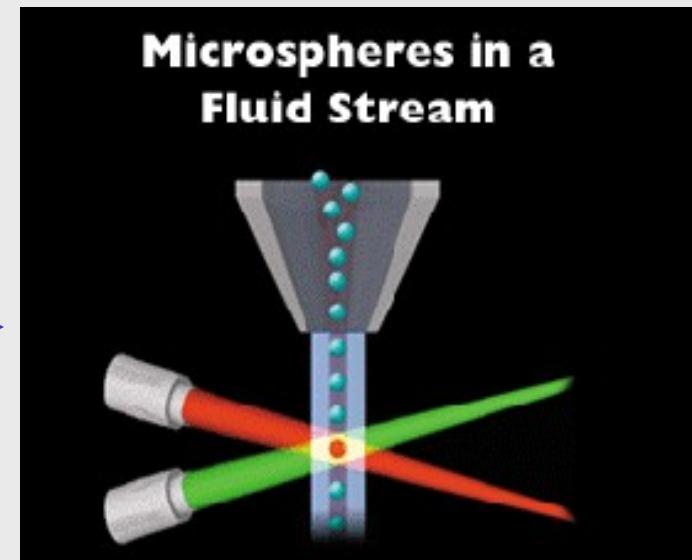
Single microsphere type coupled to probe



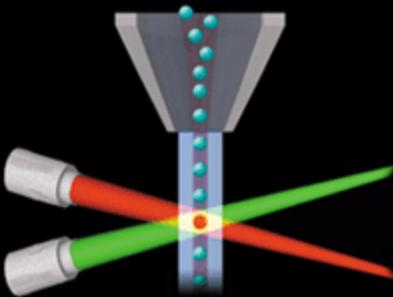
Hybridization (DNA) or
recognition (antibodies)



Microbeads
sorter and reader

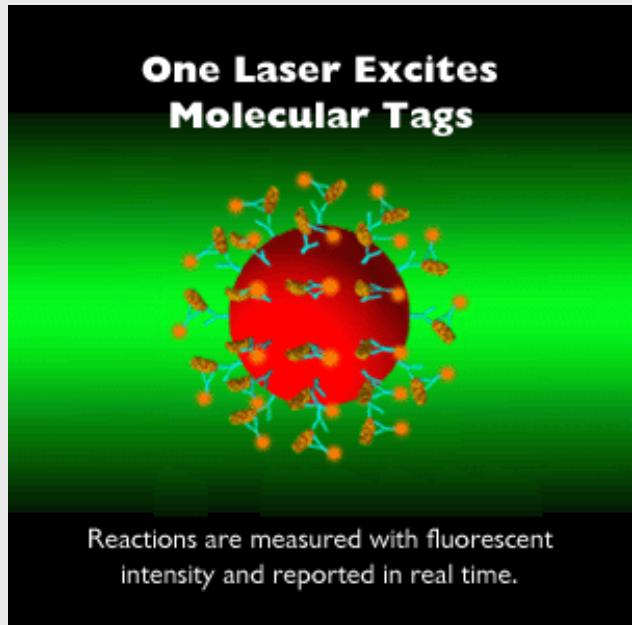


Microspheres in a Fluid Stream



Precision fluidics align the microspheres in single file, and pass them through the lasers one at a time.

Reading hybridization



Reading gene name

