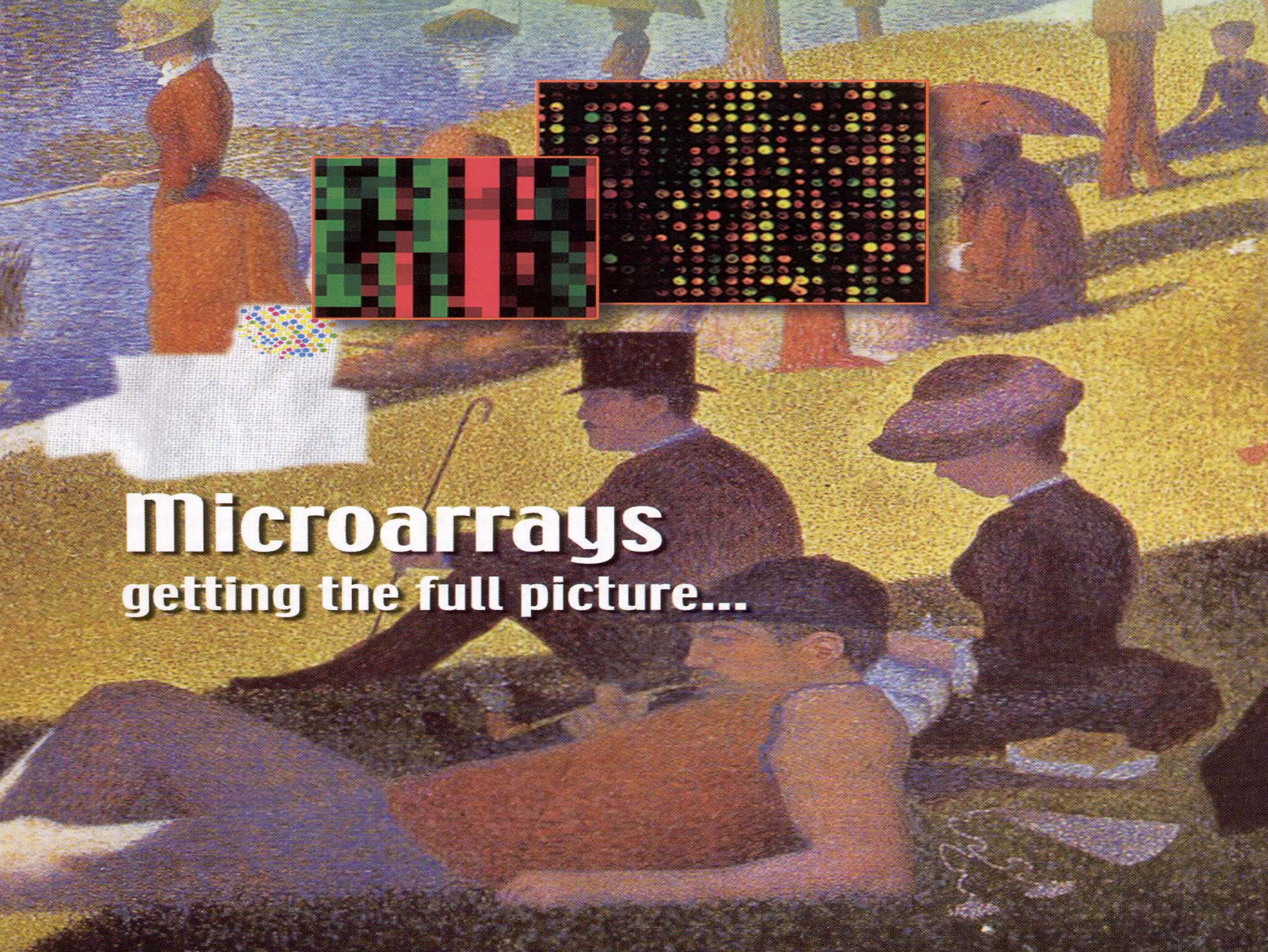
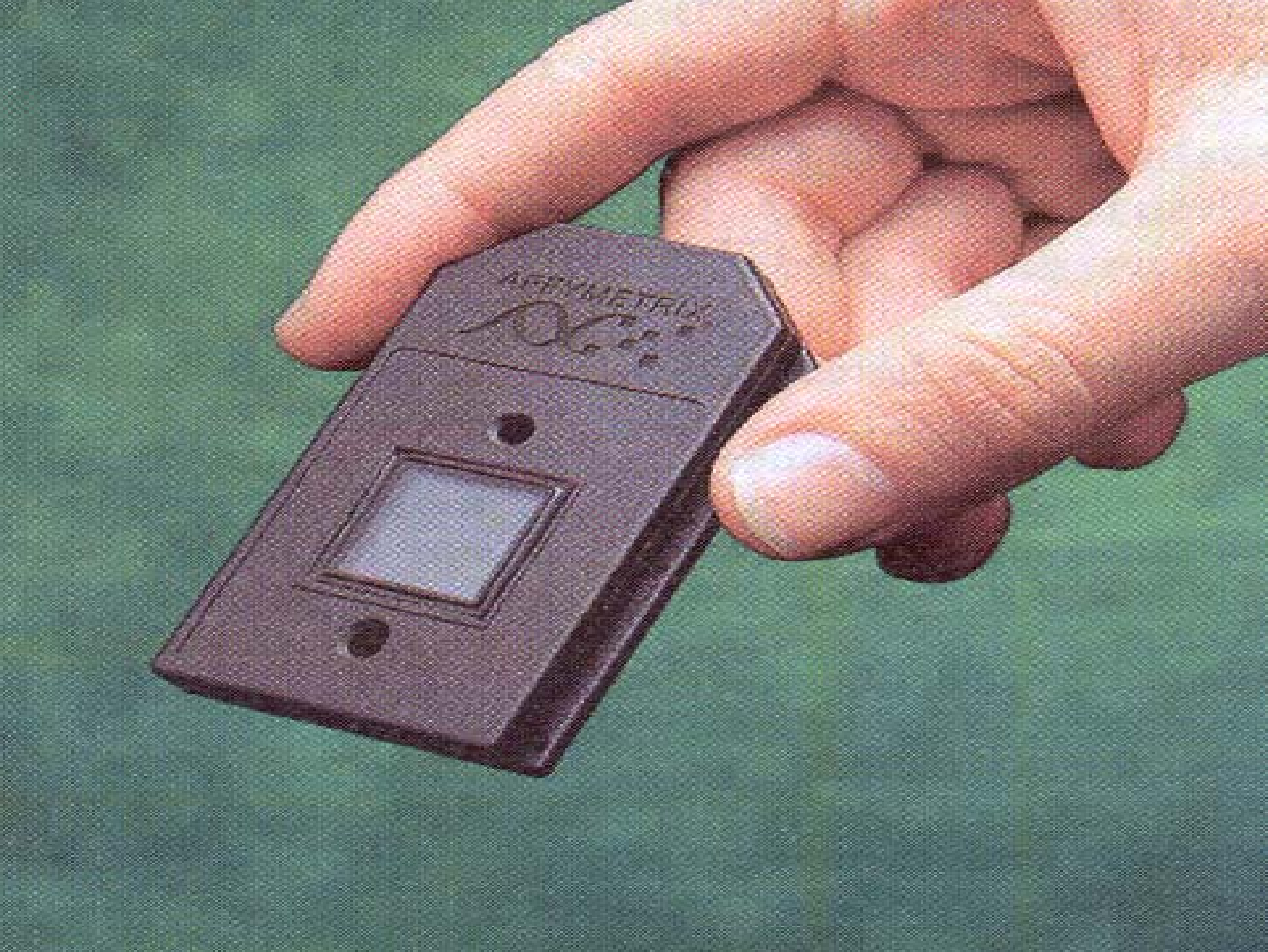
# Introduction to micro-array technology


Microarrays
getting the full picture...


ARTIMETRIX

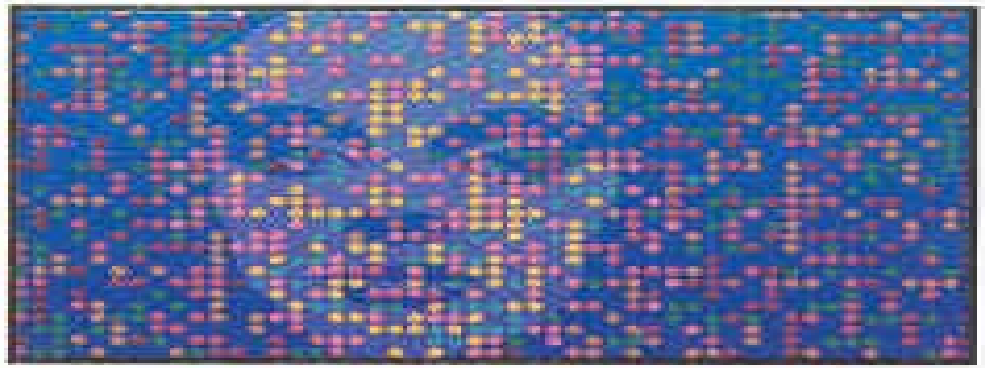
# Definition of micro-array (biochip)

- Device for detecting in one session
- multiple molecules e.g. DNA, RNA, proteins
- using parallel detector probes

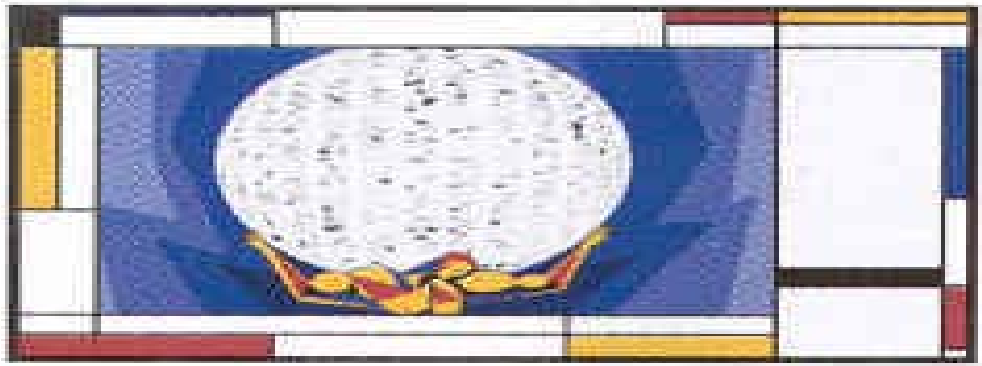
# Components of biochips

- Solid layer
- coating
- activated probe

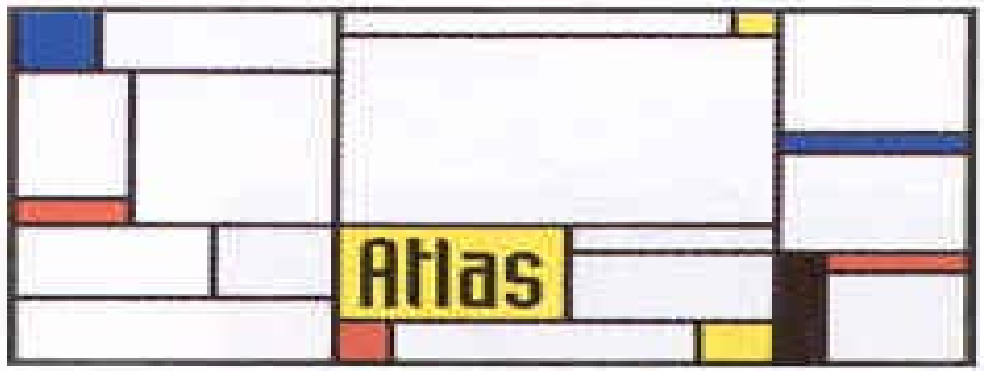
- Glass



- Plastic



- Nylon



| | Glass | | Plastic | Nylon | |
|---|---|---|---|---|---|
| Number of genes | 3,800 | | 8,300 | 1,176 | |
| Targets | Long oligos (80 bp) | | Long oligos (80 bp) | PCR-generated cDNA fragments | |
| Label/detection method | $^{33}P$ | Fluorescence | $^{33}P$ | $^{32}P$ | $^{33}P$ |
| Relative sensitivity | ++++ | ++ | ++++ | ++++ | +++ |
| Relative resolution | +++ | ++++ | +++ | + | ++ |
| Imaging equipment | Phosphorimager or autoradiogram | Fluorescent scanner | Phosphorimager | Phosphorimager or autoradiogram | |
| Reusable | No | | Yes | Yes | |
| Analysis/ease of use | Easy—no membrane deformation | | Easy—no membrane deformation | More difficult | |
| Homologous gene discrimination | ++++ | | ++++ | ++ | |
| Accuracy of spotted material | 100% tested oligos | | 100% tested oligos | 100% sequence-verified | |
| Calibration standards* | Coming soon | | Yes | No | |

# Coating

- Mostly  silanederivatives
- Thin biofilm system

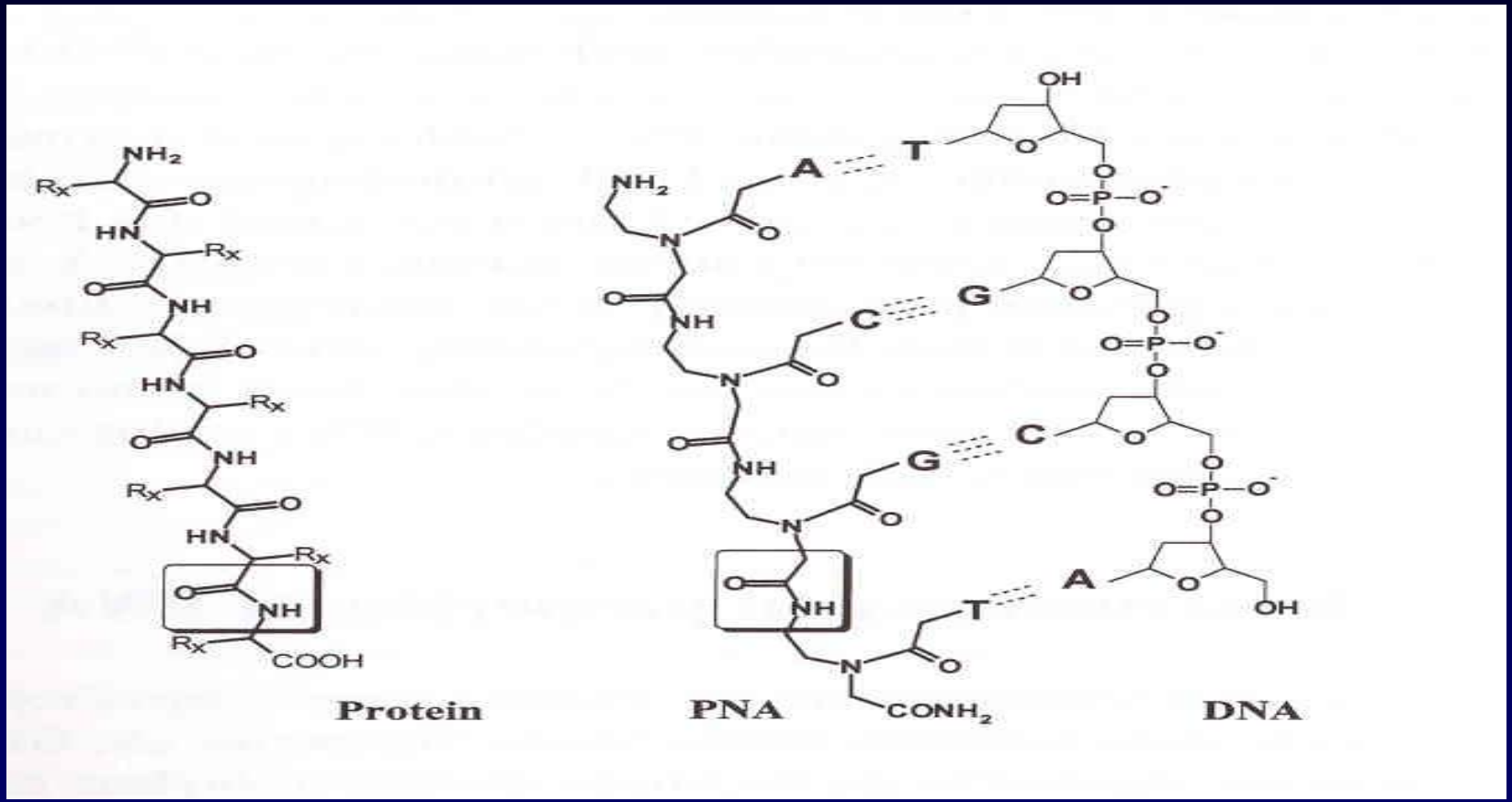
# Probes

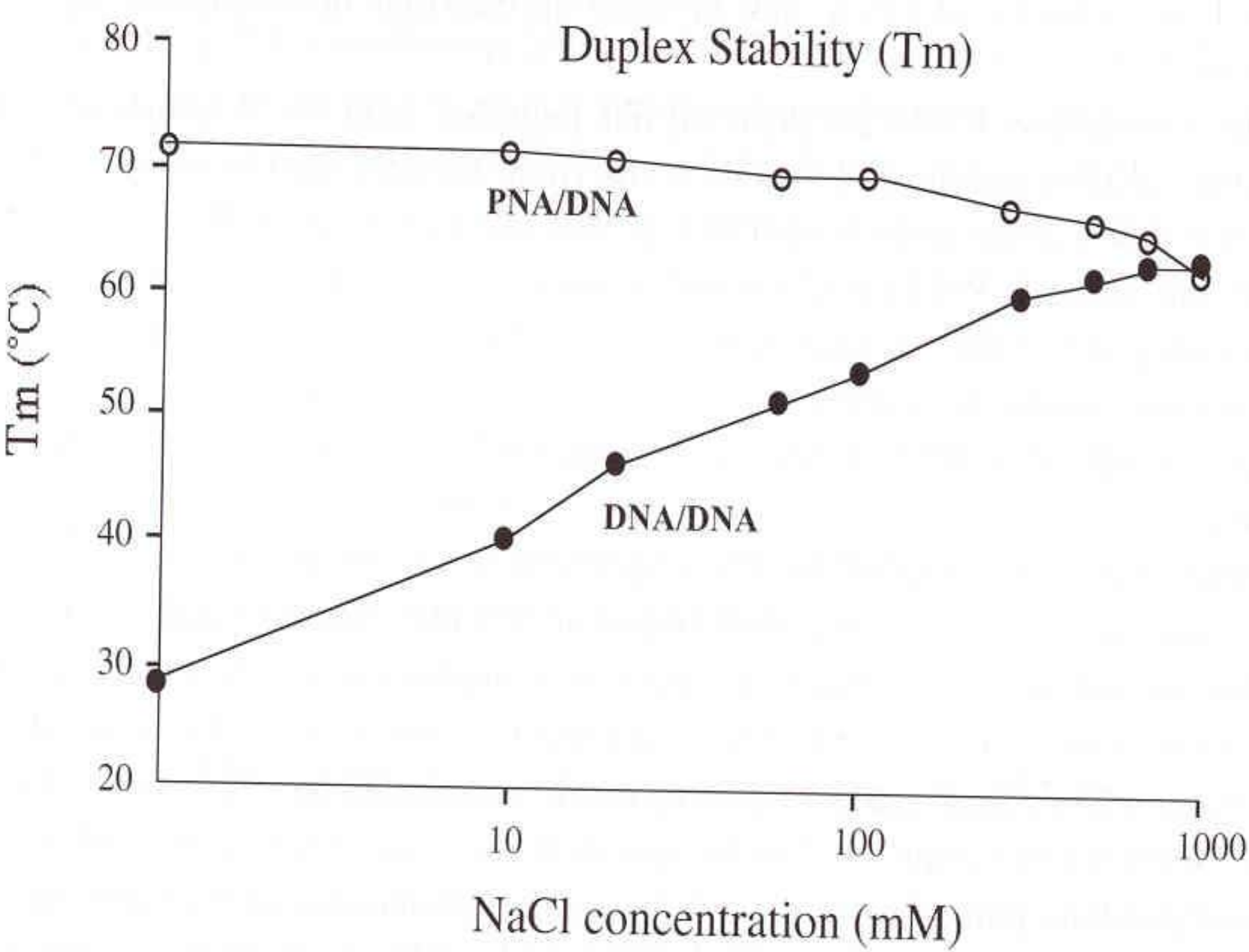
- PCR product
- cDNA or oligonucleotide
- PNA (peptic nucleic acid)
- antibody/antigen

# Peptide Nucleic Acids

- Synthetic oligonucleotides
- base A,T,G,C coupled with aminoacids
- chemically = oligopeptide
- PNA behaves as DNA oligonucleotide

# COMPOSITION OF PNA

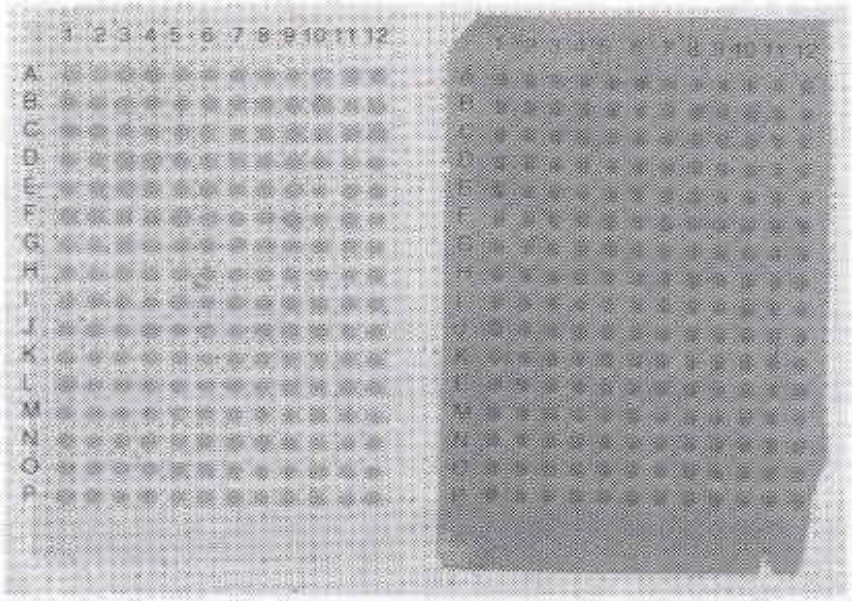



Duplex Stability (Tm)
Tm (°C)
80
70
60
50
40
30
20
PNA/DNA
DNA/DNA
10
100
1000
NaCl concentration (mM)

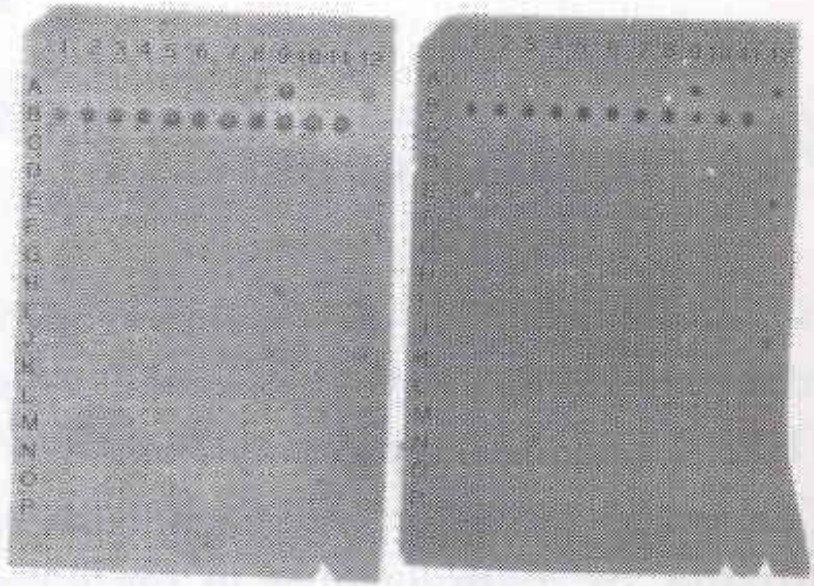
# Advantages PNA

- Very stable substance
- insensitive to nucleases
- specific binding
- very sensitive to mismatch
- DNA binding insensitive to ion concentration

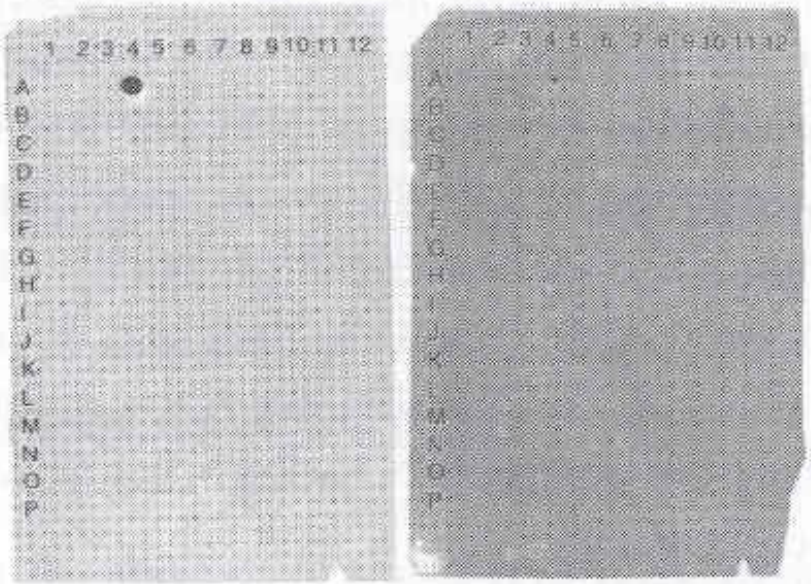
A



B



C

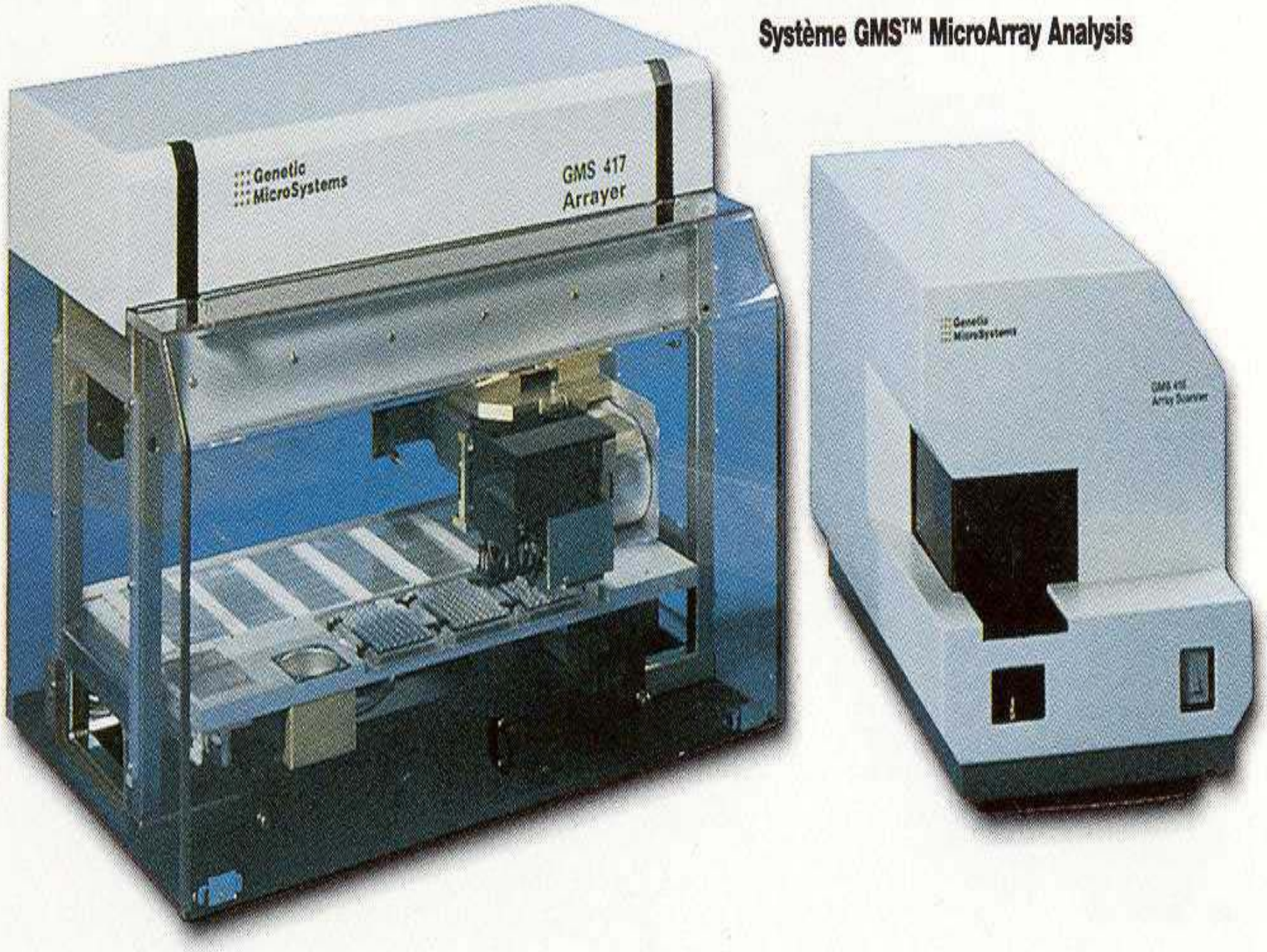


| Coordinate | support bound PNA-Sequence |
|---|---|
| A04 | AcNH- TTTTTTTTTTTTTTTTTTTT |
| A09 | AcNH- AACCGTCTTCTGGTCG |
| A12 | AcNH - GATTCTGGCATGTTCT |
| B01 | AcNH - GATTCTGGTATGTTCTAGCGC |
| B02 | AcNH - GATTCTGGTATGTTCTAGCG |
| B03 | AcNH - GATTCTGGTATGTTCTAGC |
| B04 | AcNH - GATTCTGGTATGTTCTAG |
| B05 | AcNH - GATTCTGGTATGTTCTA |
| B06 | AcNH - GATTCTGGTATGTTCT |
| B07 | AcNH - GATTCTGGTATGTTC |
| B08 | AcNH - GATTCTGGTATGTT |
| B09 | AcNH - GATTCTGGTATGT |
| B10 | AcNH - GATTCTGGTATG |
| E12 | AcNH - GATTGTGGTATGTTCT |
| J12 | AcNH - GAGGCTGGTATGTTCT |

# Linker and spacer

- Amino-group
- Acrylic-group
- Tailing

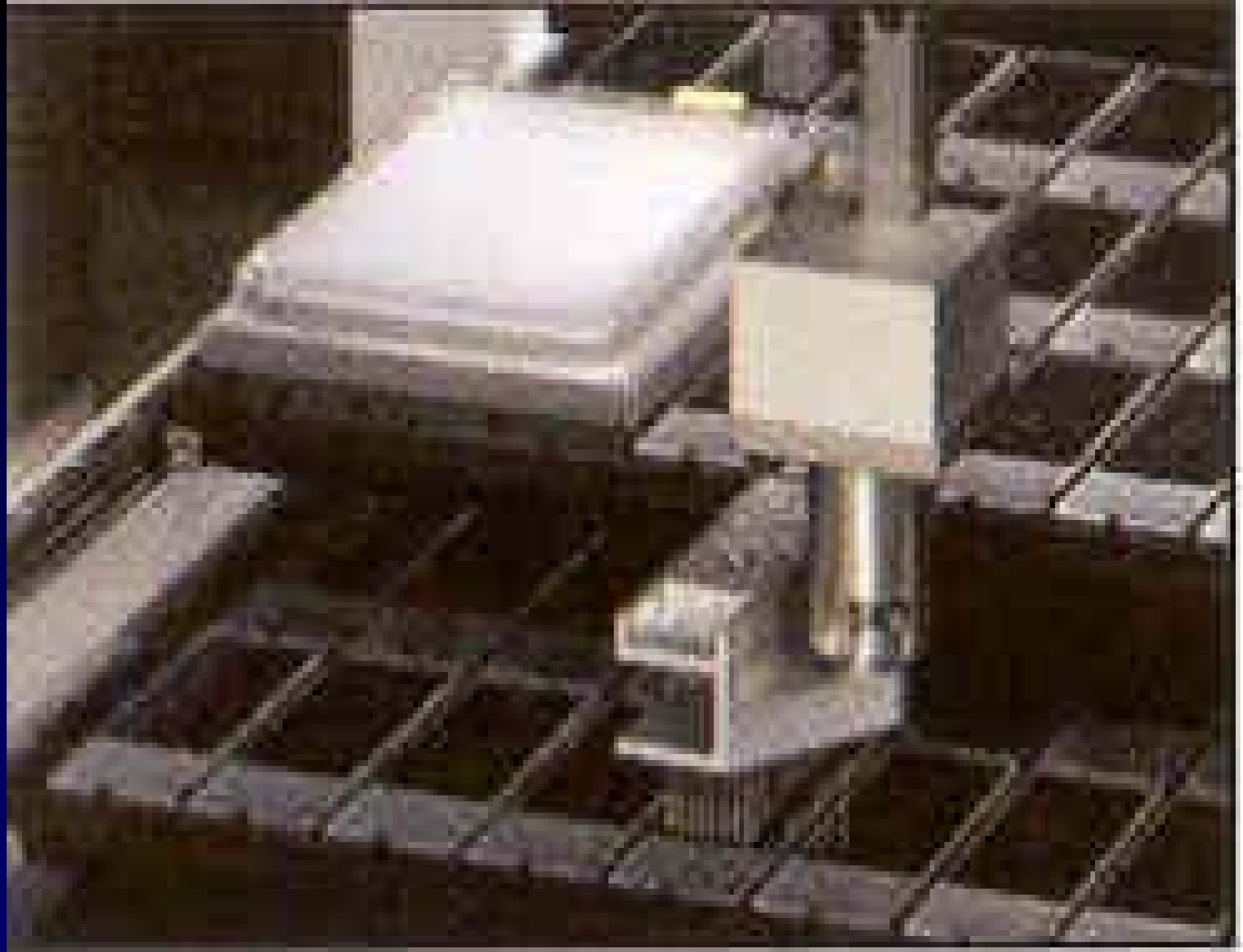
# Système GMS™ MicroArray Analysis

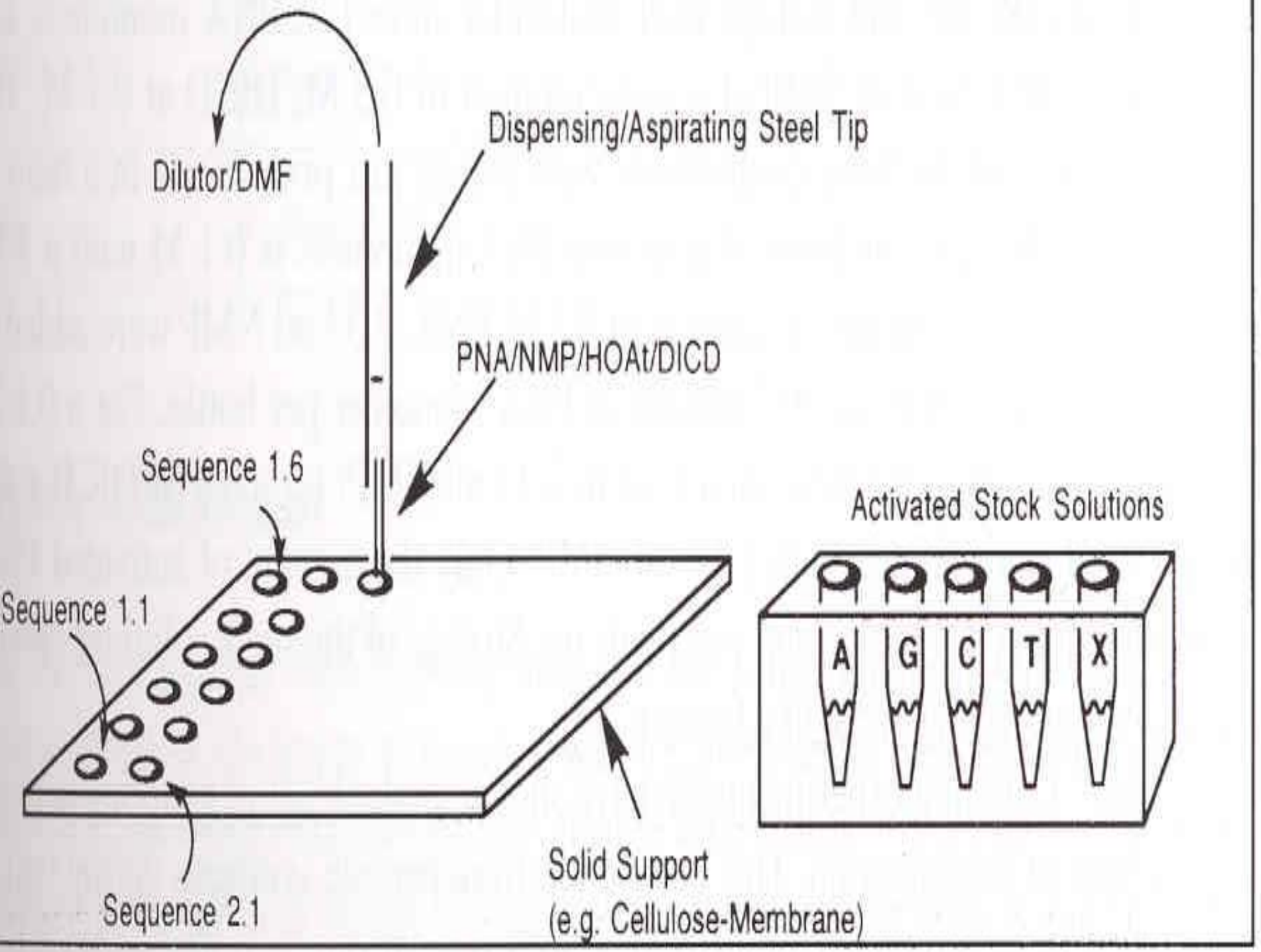


# Application of probes

- Printing with micro-arrayer
- Inktjet printing
- in situ synthesis=photolithography


MicroGrid
Compact
MicroGrid
Compact
BioRobotics




Dispensing/Aspirating Steel Tip
Dilutor/DMF
PNA/NMP/HOAt/DICD
Sequence 1.6
Activated Stock Solutions
Sequence 1.1
A
G
C
T
X
Solid Support
(e.g. Cellulose-Membrane)
Sequence 2.1

# Probes

- Length
- %GC

# Hybridisation mixture

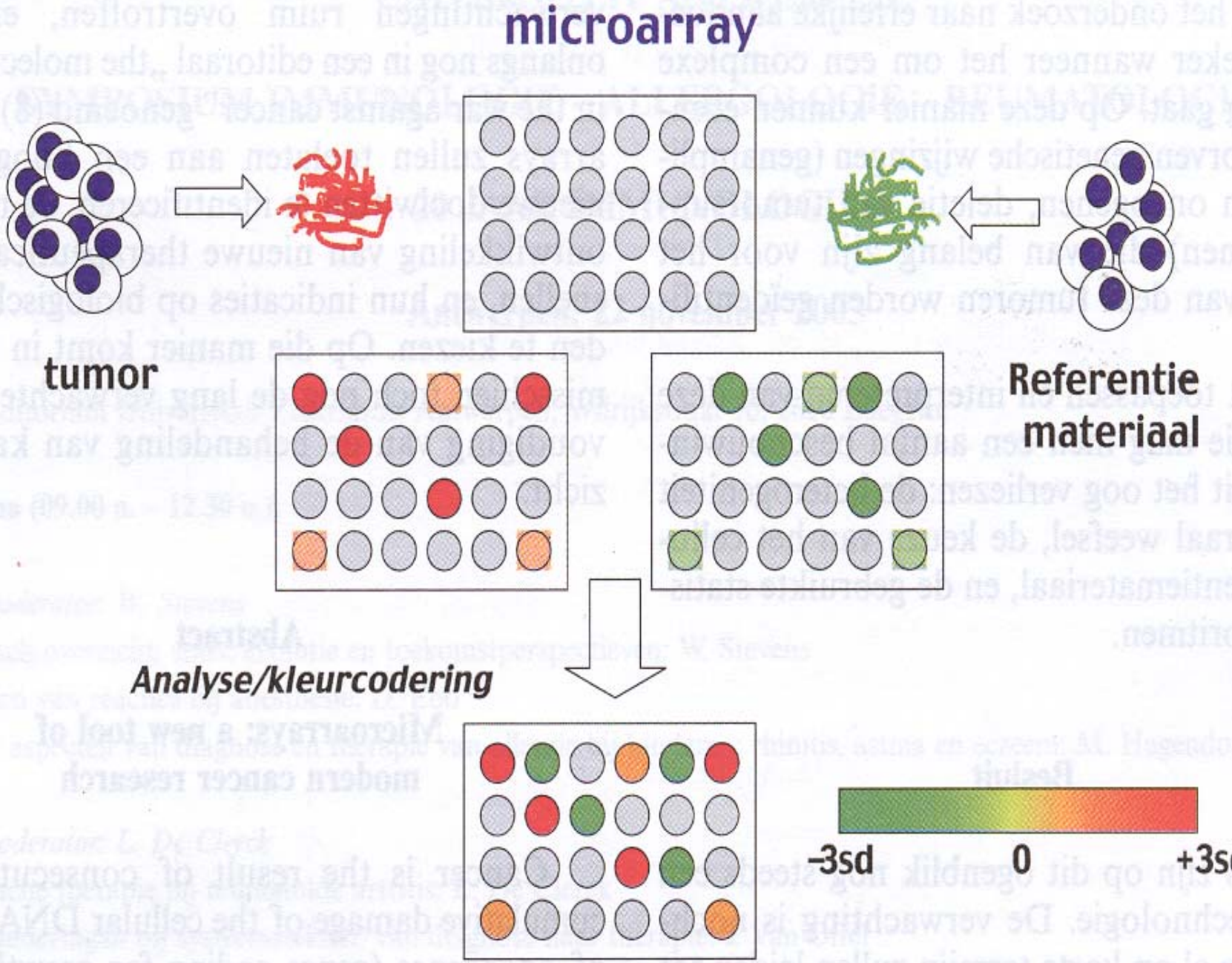
- SSC (sodium citrate)
- SDS (sodium dodecylsulphate)
- Carrier DNA

# Hybridisation

- Tagged  nucleic acid or protein
- classical tags: Cy3 and Cy5

# MICRO-ARRAY: RNA

- Solid support
- oligonucleotides
- up to 65.000 genes
- fluorescent labels
  - Cy3
  - Cy5


microarray
tumor
Referentie materiaal
Analyse/kleurcodering
-3sd
0
+3sd

Control RNA sample (Normal tissue/cell)

**Preparation of labeled cDNA probes by Reverse transcriptase**
(dT, random or specific primers and 33P, CY3, or CY5)

**Test RNA sample (Diseased or treated tissue/cell)**

Control cDNA Probes

**Hybridization with array and revelation**
(Phosphorimager, microscope, plus annexed software)

**Test cDNA Probes**

**Analysis**
(Internal and external standards)

**Expression profile**

| | control | test |
|---|---|---|
| Gene A | 1 | 1 |
| Gene B | 1 | 5 |
| ... | | |
| ... | | |
| Gene Z | 1 | 3 |

# Practical approach

- Extraction of RNA to be investigated
- Labelling of this RNA with Cy3
- Reference RNA extraction
- Labelling of this RNA with Cy5

# Practical approach

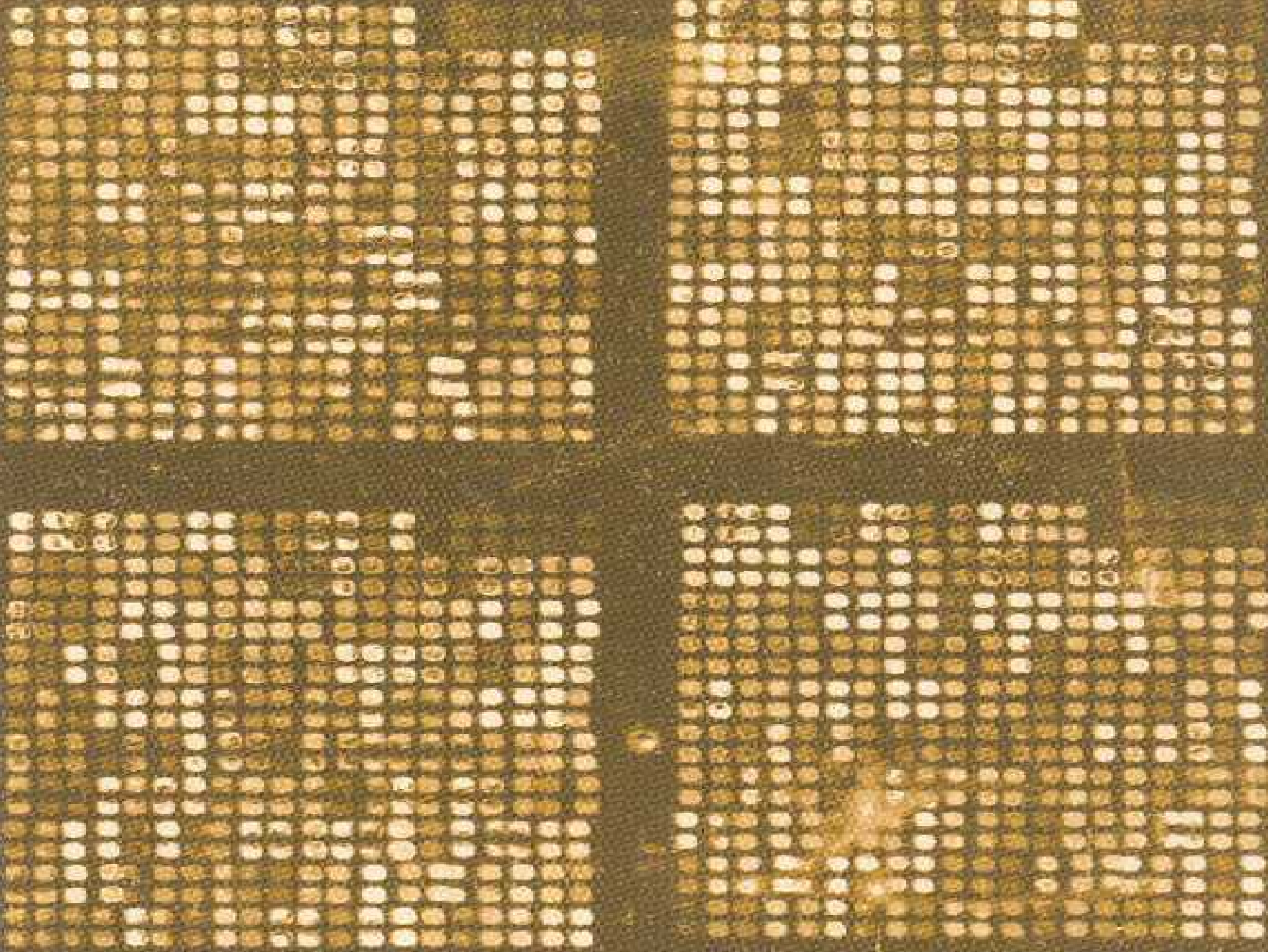
- Co-hybridization of the labelled RNA on the micro-array

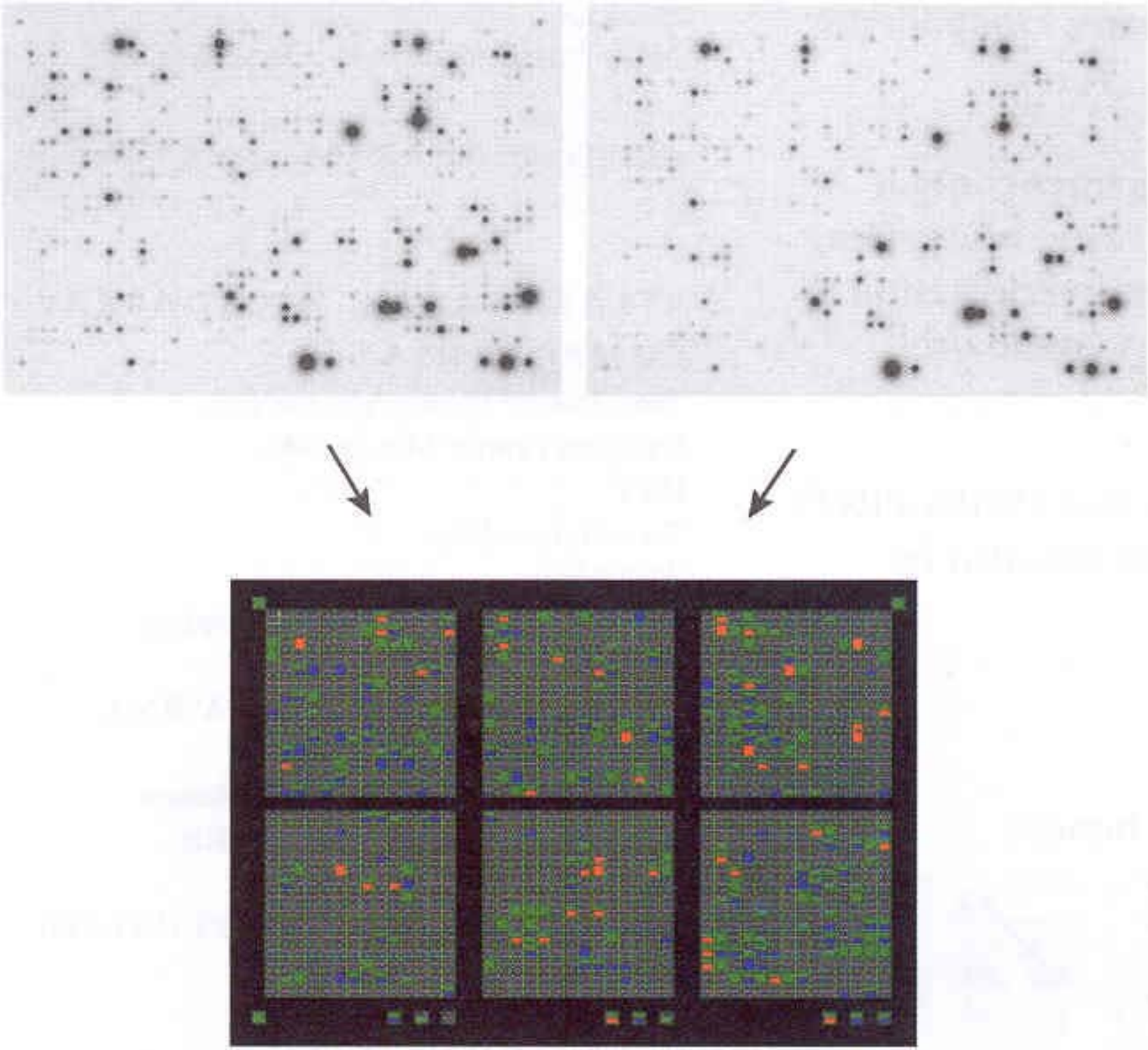
# Practical approach

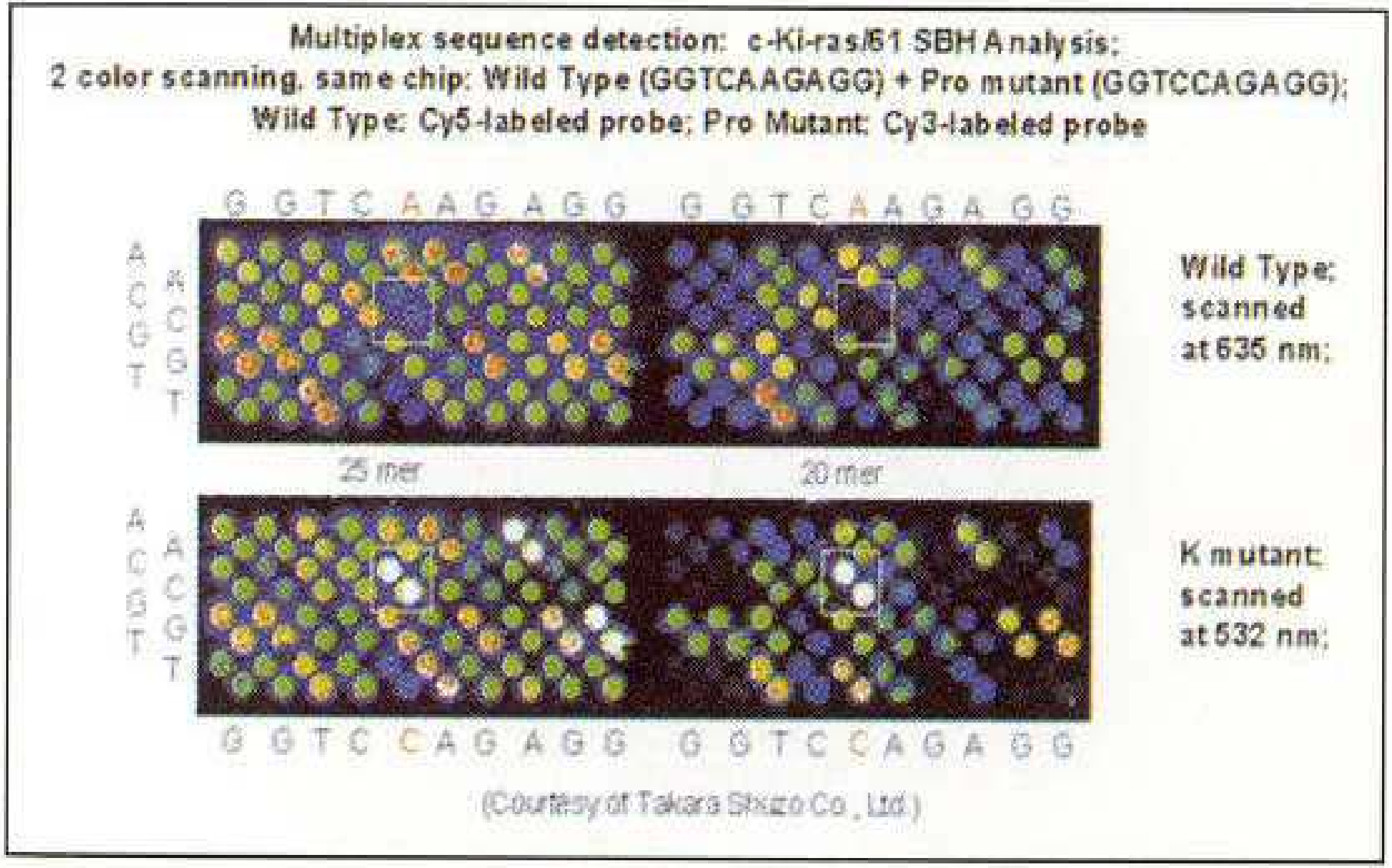
- Co-hybridization of the labelled RNA on the micro-array
- Post -Hybridization washings

# Scanning

- Laser device
- Confocal laser scanner (micro-array reader)
- Interpretation of huge mass of data by computer

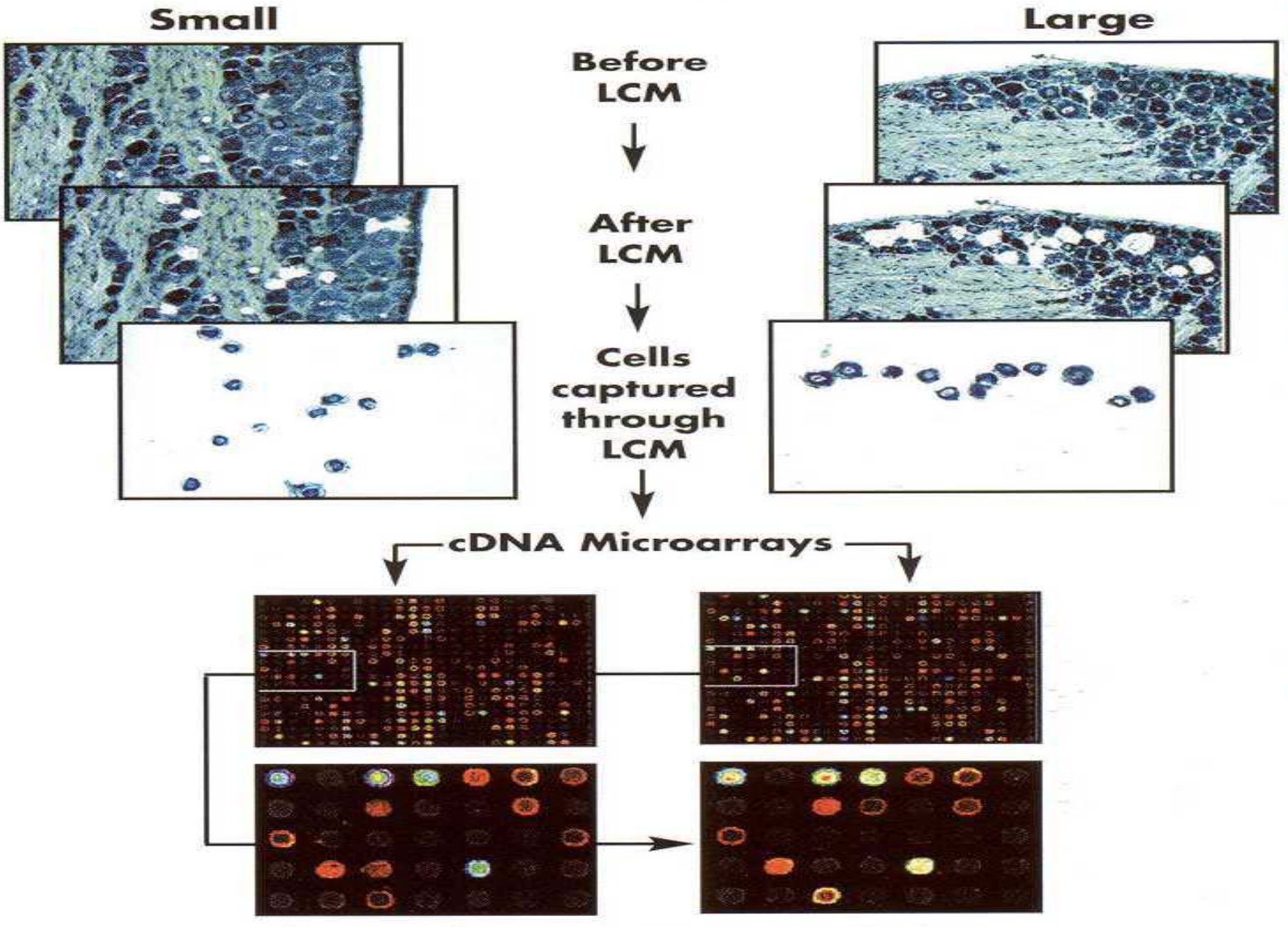




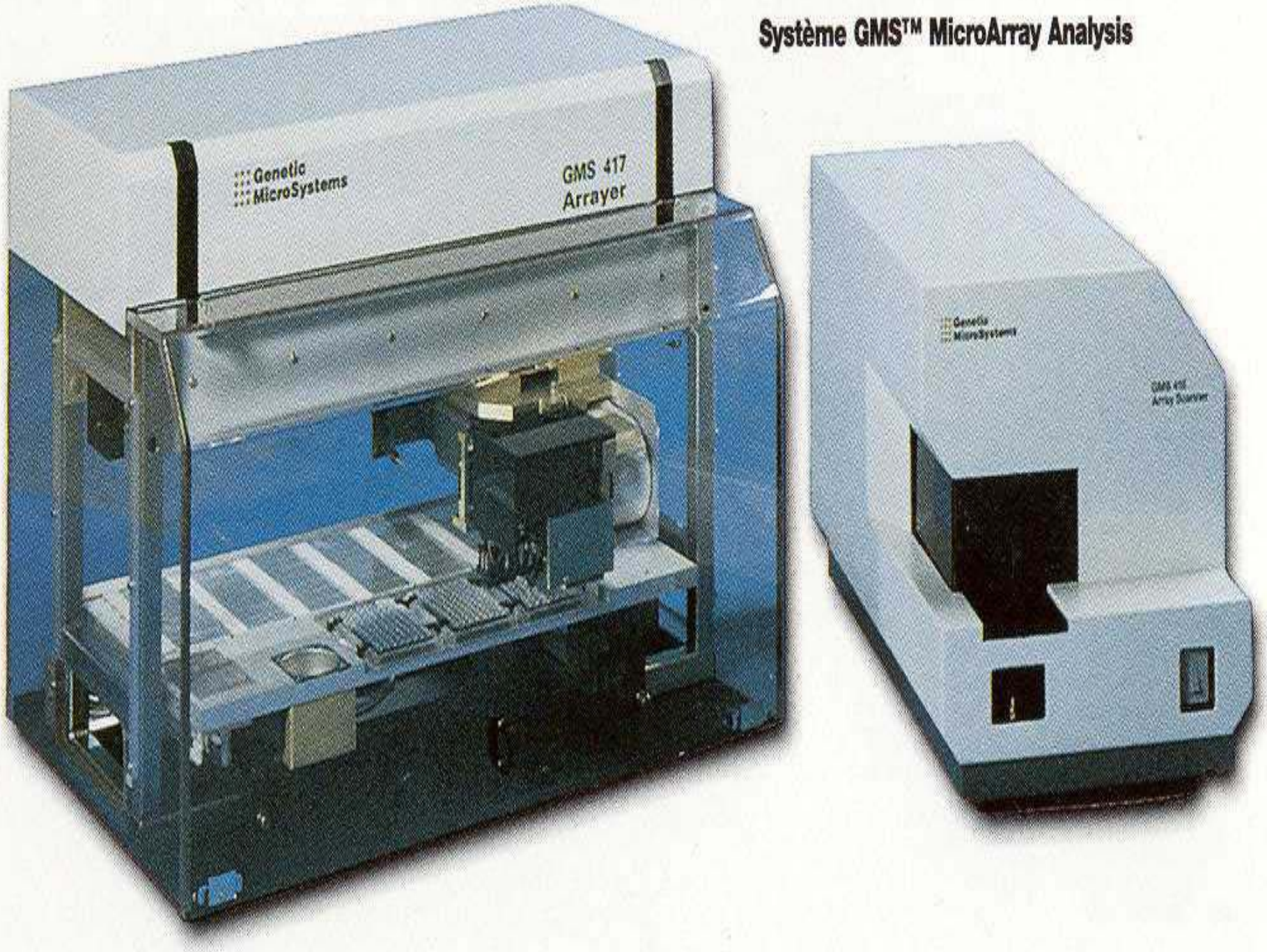



# Multiplex Sequence Detection

# Dorsal Root Ganglion Neurons



# Système GMS™ MicroArray Analysis

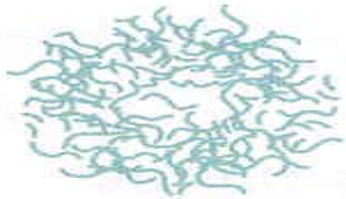


## Plastic or Nylon Macroarrays

## Glass Microarrays

Atlas™ Pure Total RNA Labeling Kit (#K1038-1)

Atlas SMART™ Probe Amplification Kit (#K1034-1)



Atlas™ Glass Fluorescent Labeling Kit (#K1037-1)

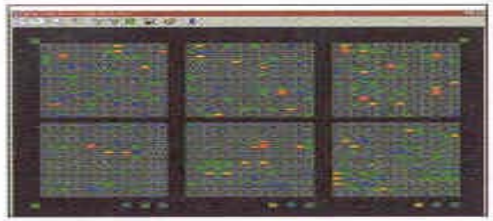
**Generate probes**

Atlas™ Plastic or Nylon Arrays

Atlas™ Glass Microarrays

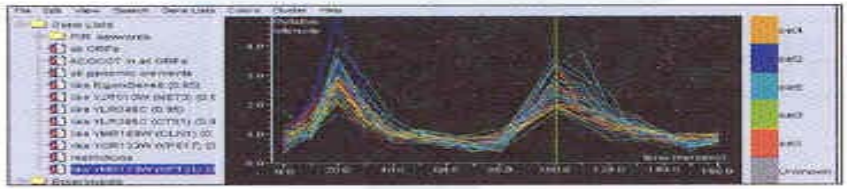
**Profile gene expression**

AtlasImage™ (#V1211-1)

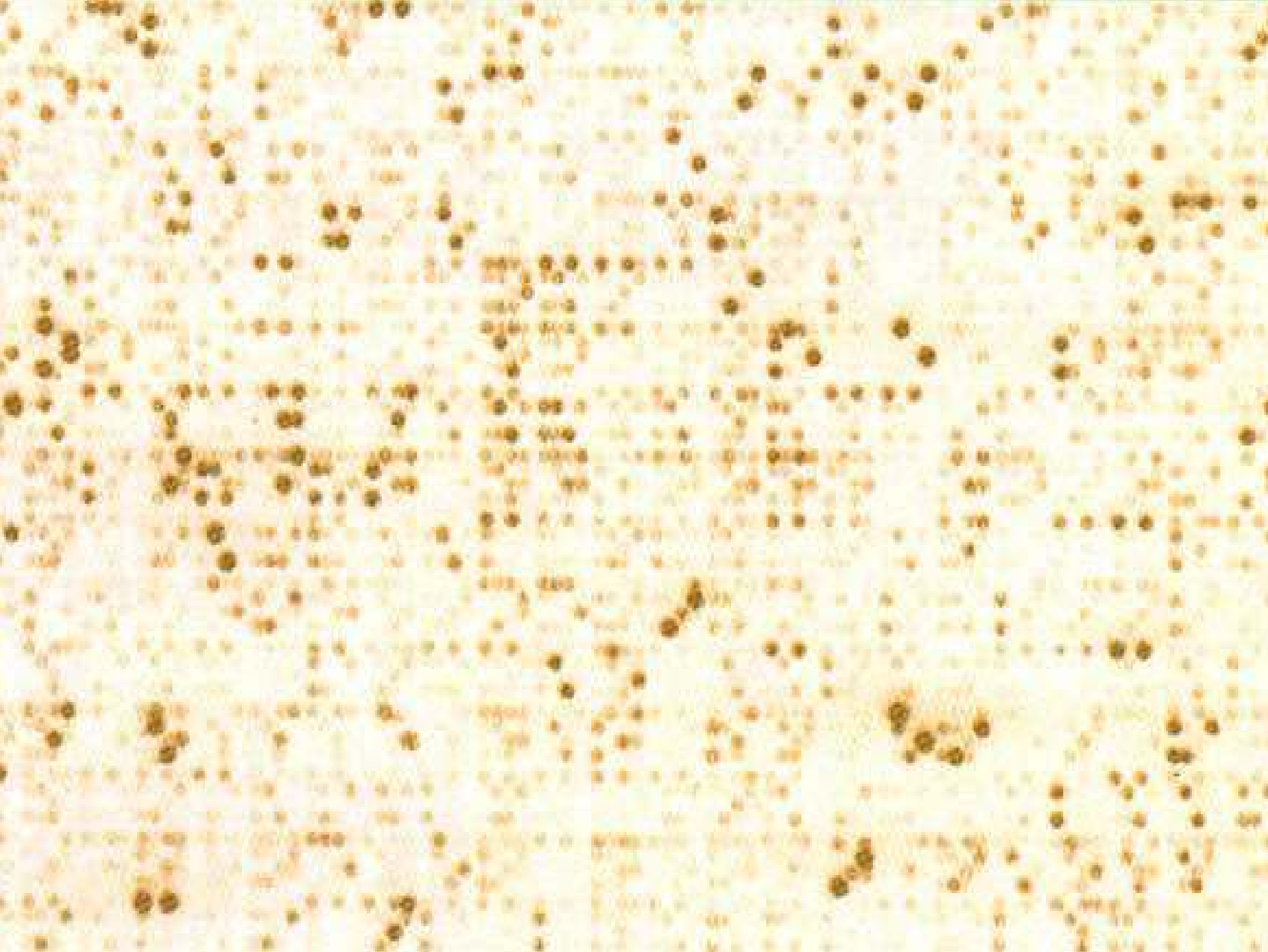


Fluorescent scanning software

**Quantify raw array data**



**Analyze data to observe trends**



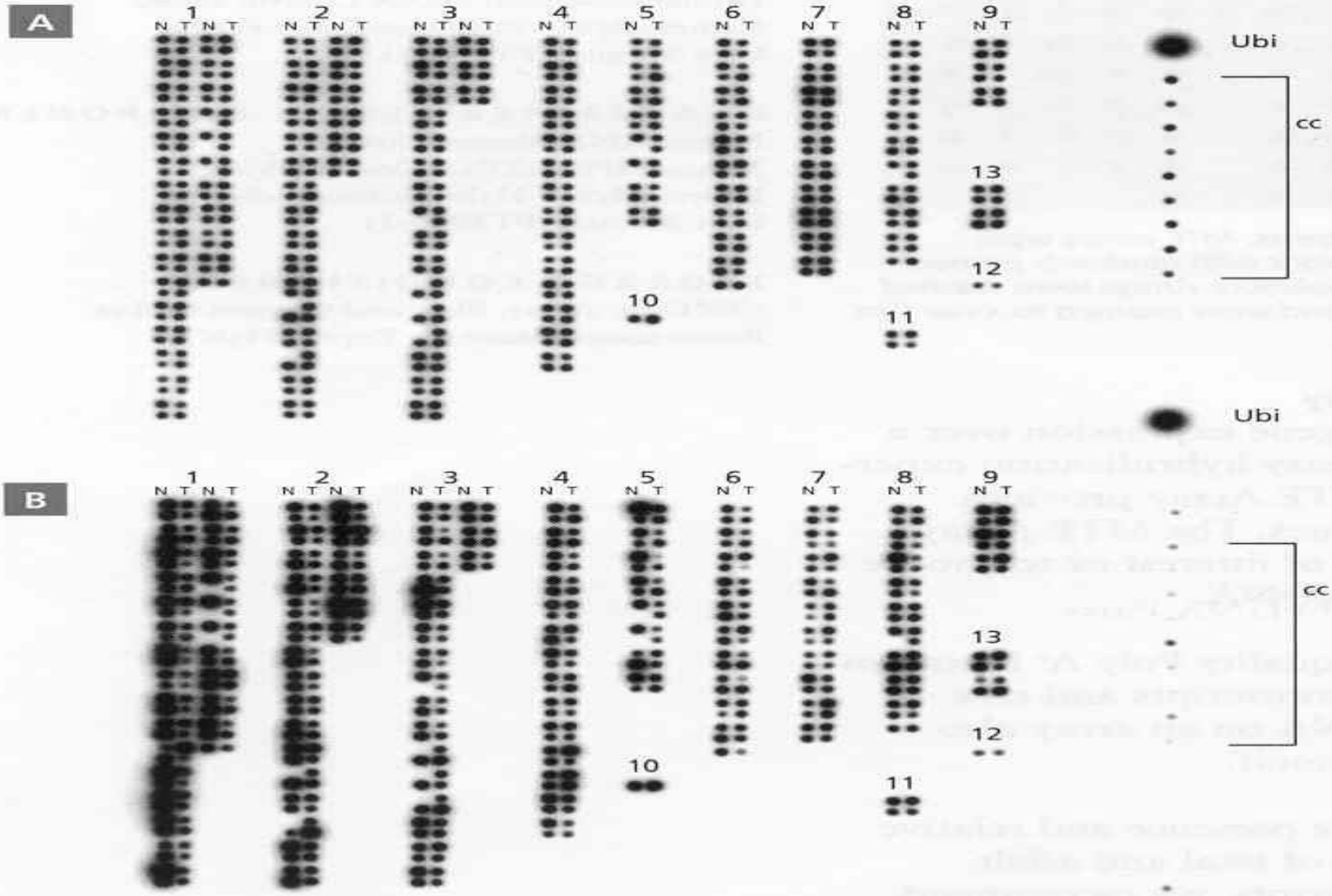
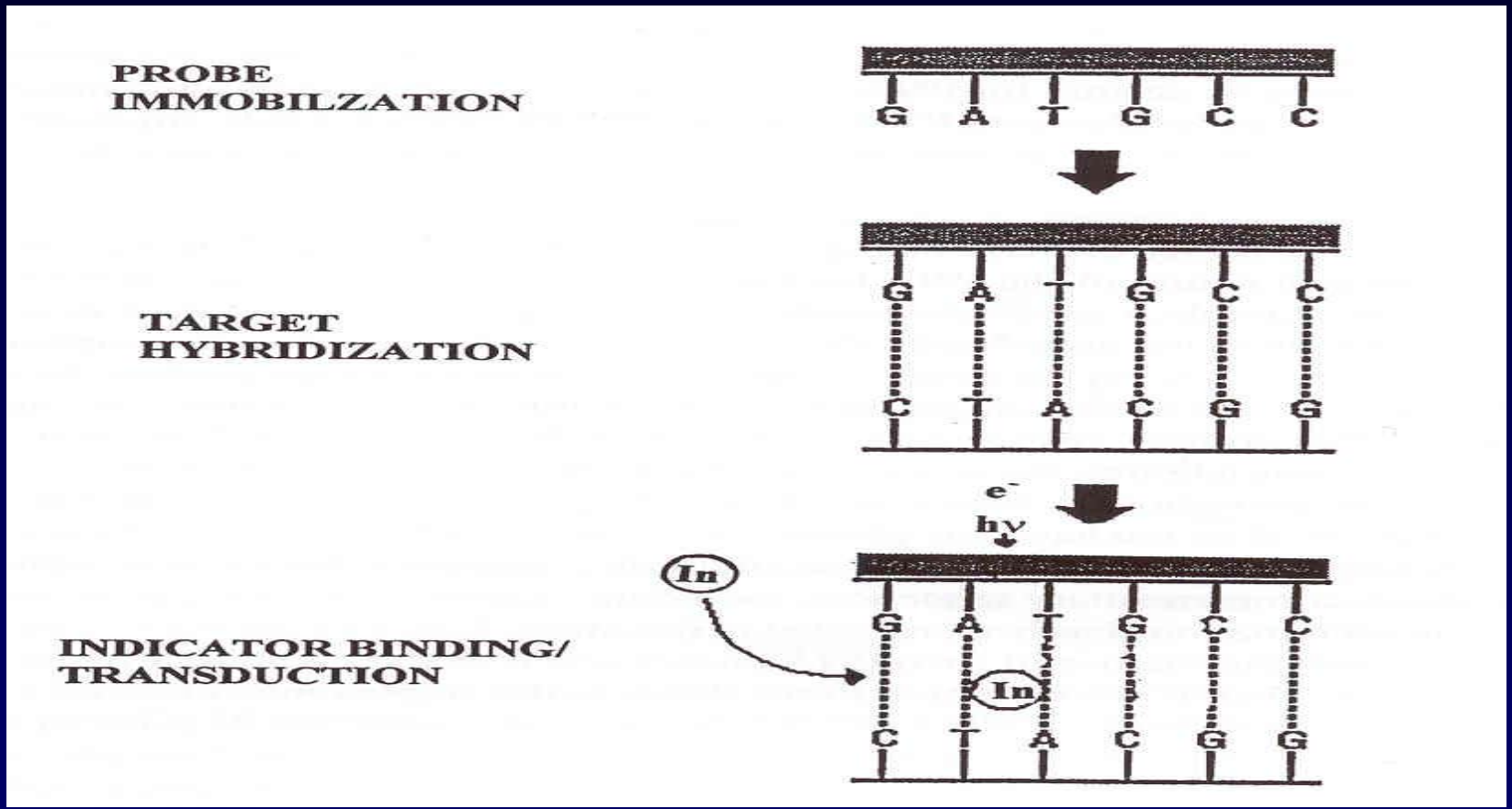



***Figure 1. The Cancer Profiling Array demonstrates tissue-specific expression of gelsolin.*** *The Cancer Profiling Array was hybridized separately with a radiolabeled probe for the housekeeping gene ubiquitin (**Panel A**) and a radiolabeled probe for gelsolin (**Panel B**). Hybridization signals were detected by phosphorimaging. Numbers indicate tissue types in columns. 1: breast. 2: uterus. 3: colon. 4: stomach.5: ovary. 6: lung. 7: kidney. 8: rectum. 9: thyroid gland. 10: cervix. 11: small intestine. 12: pancreas. 13: prostate. N = normal. T = tumor. Ubi = ubiquitin cDNA. cc = cancer cell line cDNAs.*

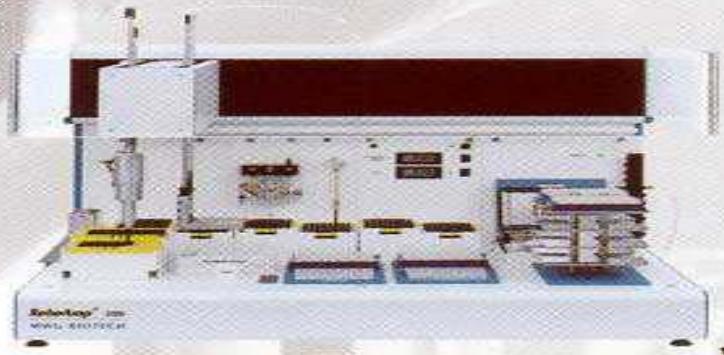
# BIO-SENSORS


PROBE
IMMOBILZATION
G A T G C C
TARGET
HYBRIDIZATION
G A T G C C
C T A C G G
e⁻
hν
In
INDICATOR BINDING/
TRANSDUCTION
In

# Intelligent Microarray Lab Networking

## Universal Automated Platform

for liquid handling, automated PCR and quality control



Primus Multiblock system

Easy and fast spotting of perfect microarrays due to the unique ring and pin system

Salt free HPSF oligos

Direct spotting of amino linked oligonucleotides

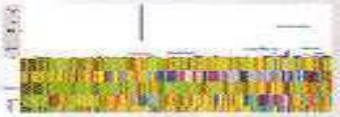
Clone tracking through direct communication with Affymetrix Arrayer

Data acquisition with robust and highly sensitive Affymetrix scanner

Flying objective lens technology

Scanner control

**Integrated Analysis System**



Advanced clustering analysis software

Automated batch mode analysis


OH NO!
NOT MORE
DATA