

PENDAHULUAN MICROARRAY

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Apa itu microarray?

*MicccroArray = DNA chip =
Biochip*

microarray merupakan salah satu teknologi terkini dalam dunia biologi molekuler yang membantu para pekerja ilmiah menentukan struktur dan fungsi berbagai macam gen yang ada pada tubuh makhluk hidup khususnya manusia.

Ed Shouther (1975) : Shouter Blot dan DNA hibridisasi
(Bains and Smith, 1988; Drmanac et al., 1989; Khrapko et al., 1989) : Membran Nitroselulosa dan glass plate
Schena (1995) : Memperkenalkan teknik microarray yang diaplikasikan ke genome *Saccharomyces cerevisiae*

Tipe Microarray

1. DNA microarray
2. MMChips: microRNA
3. Protein microarrays
4. Peptide microarrays (analisis dari interaksi protein-protein)
5. Tissue microarray
6. Cellular microarray (transfection microarrays)
7. Chemical compound microarrays
8. Antibody microarrays
9. Carbohydrate arrays (glycoarrays)
10. Phenotype microarrays
11. Reverse Phase Protein Microarrays (microarray of lysates atau serum)
12. Interferometric reflectance imaging sensor (IRIS)

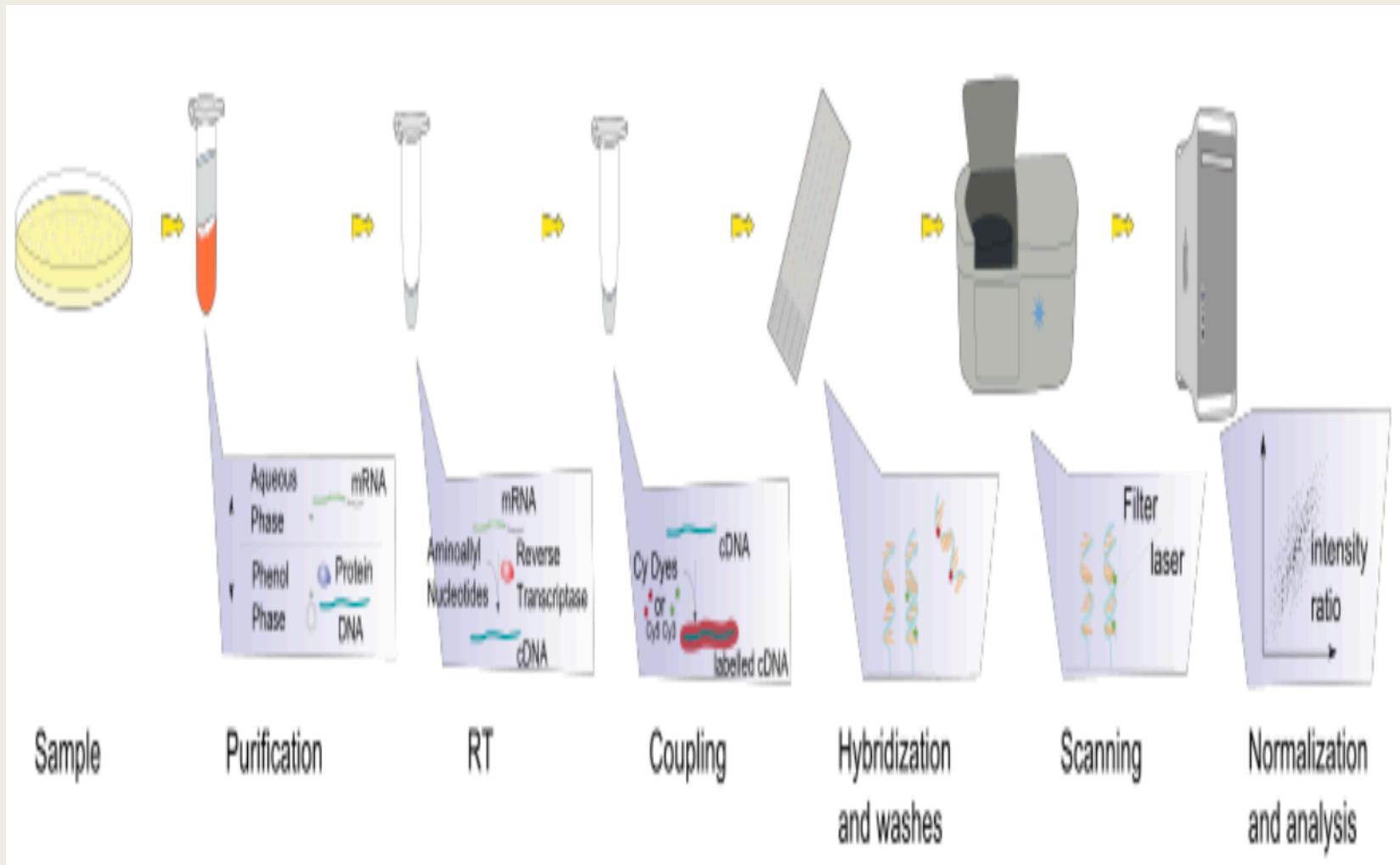
Tujuan Microarray

- Mampu menganalisis ekspresi gen
- Membantu dalam identifikasi gen baru, mengetahui tentang fungsi dan tingkat ekspresi gen pada kondisi yang berbeda.
- Membantu dalam monitoring, mendiagnosa, dan memprediksi penyakit.
- Deteksi SNP dan Mutasi

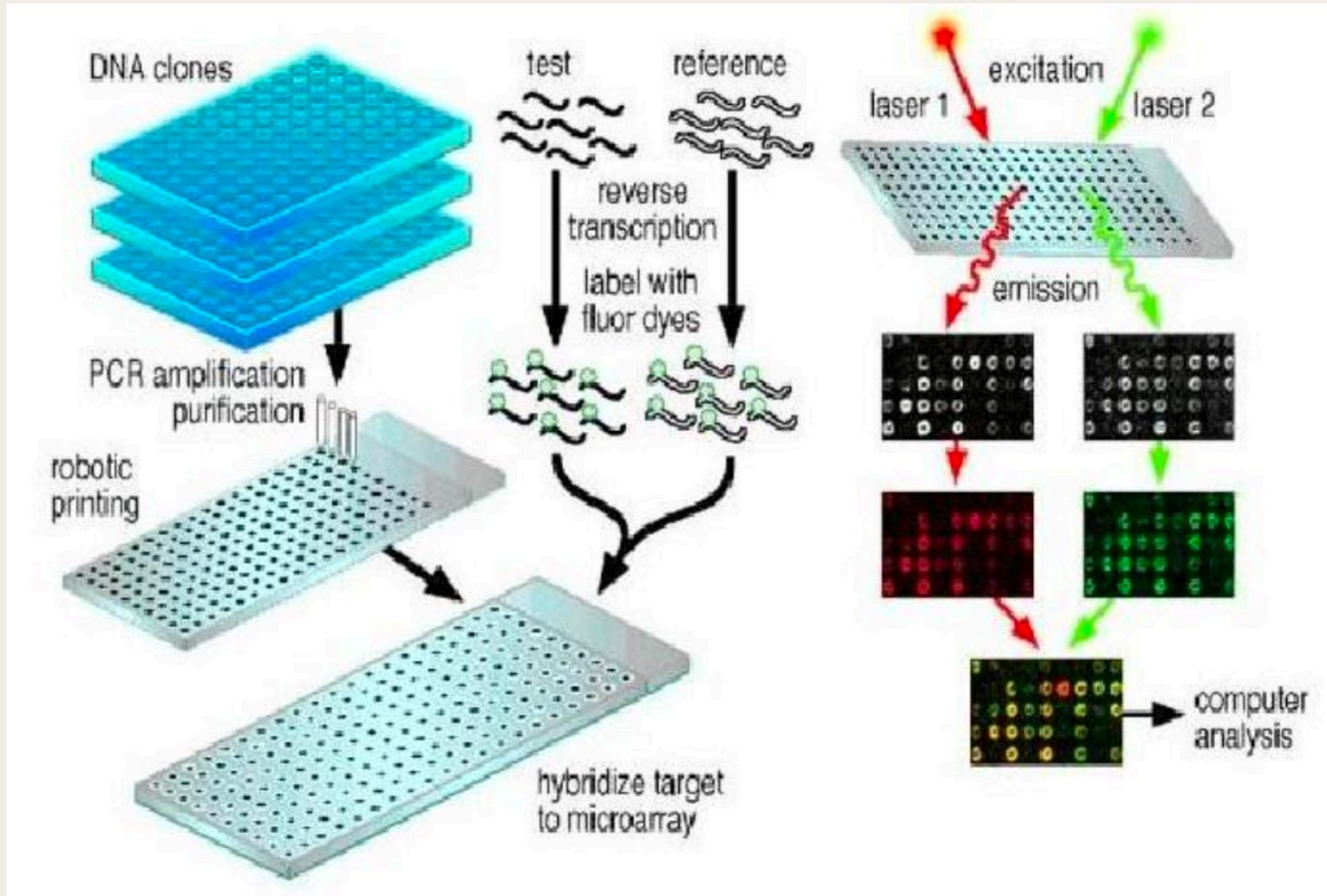
Prinsip Dasar

1. Mengumpulkan sel atau jaringan yang akan dianalisis (kultur sel)
2. Isolasi mRNA dengan menggunakan primer poly-T atau primer yang spesifik
3. Membuat cDNA dengan bantuan enzim reverse transkriptase dan diberi label fluoresens (Cy3 dan Cy5)
4. Hibridisasi cDNA dengan proba pada chip
5. Pembacaan dengan menggunakan scanner untuk membaca reaksi fluoresensi
6. Hasil scanner diamati danolah secara statistika

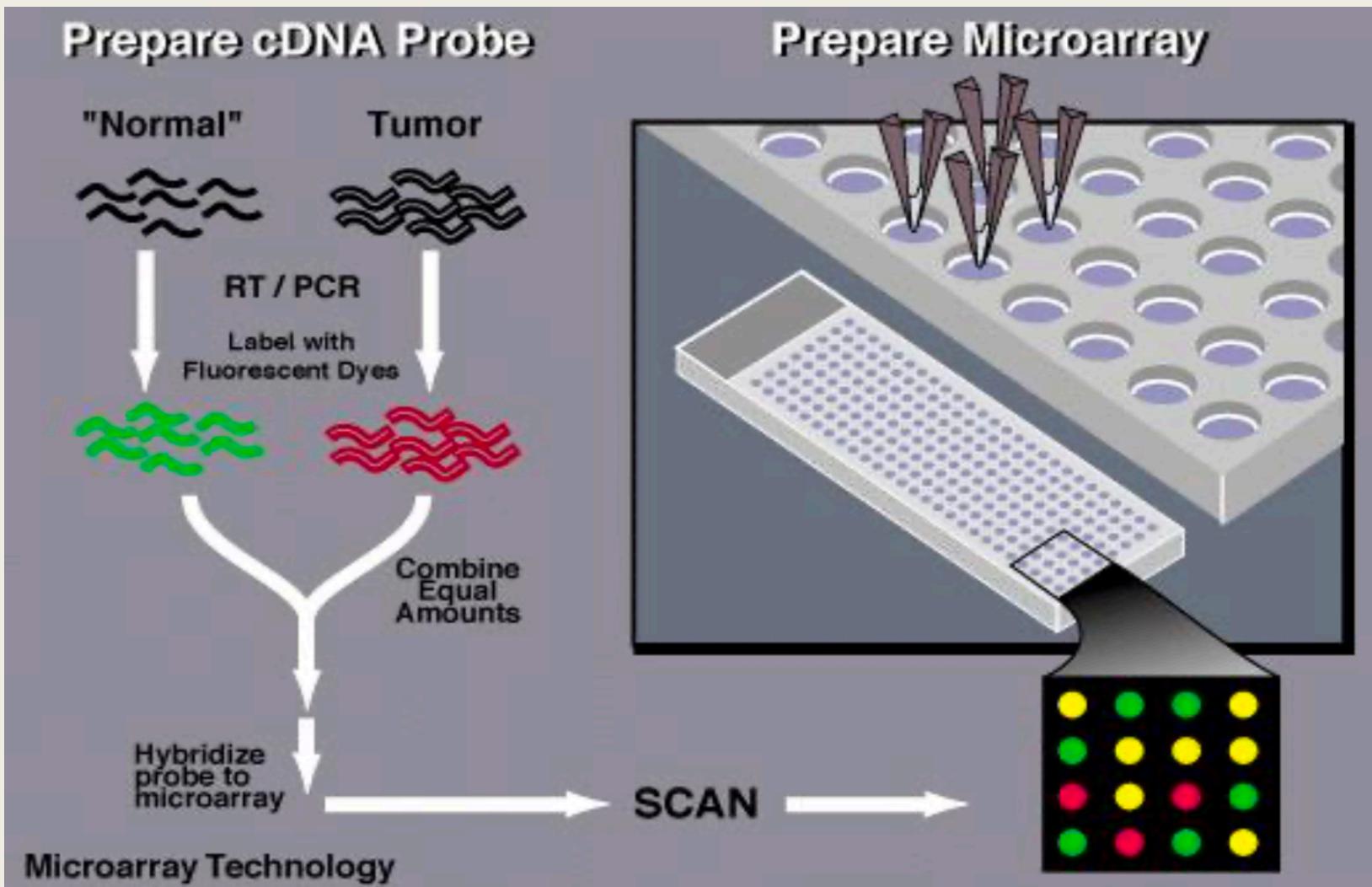
Alur Kerja Microarray



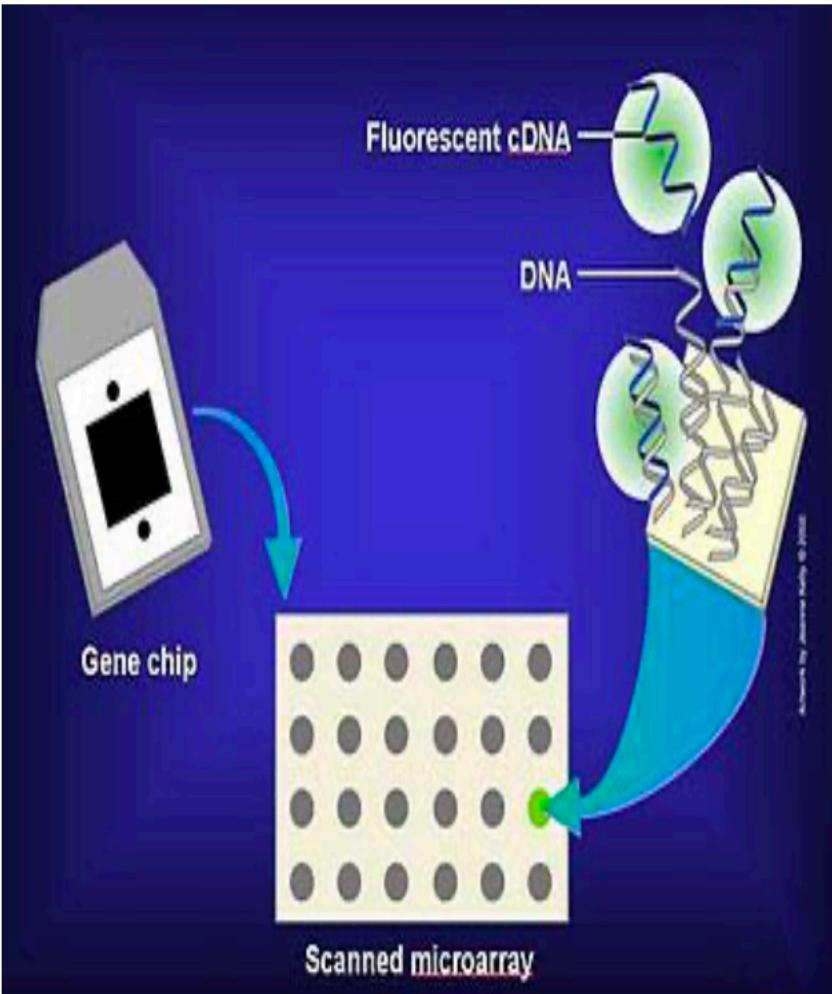
DNA Microarray



DNA Microarray



DNA Microarray



Chip microarray terbuat dari bahan nitrocelulose yang dapat berbentuk silicon chips ataupun glass slide, setiap chips memiliki well yang berisi probe complementer dengan gen yang akan dideteksi

Memiliki kemampuan mendeteksi mRNA
microRNA
Methylation
SNP

DNA Microarray



Gen chip mengandung complementary cDNA yang dapat berupa sekueun komplementer dari gen target atau berupa oligonukeotida

Tipe Microarray

Probe Manufacture

Spotted Arrays

- DNA probes are synthesized and then “spotted” onto the microarray
- Customized for each experiment “in-lab”
- Low cost

Oligonucleotide microarrays

- DNA oligonucleotides are synthesised directly on the microarray
- Commercial arrays (Affymetrix, Nimblegen and CombiMatrix)
- expensive

Detection

Two-color detection

- Spotted Array: Compare two samples by labeling with two different fluorophores and analyzing on the same array
 - Cy5 (red) and Cy3 (green)

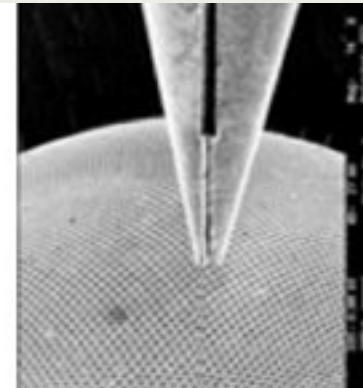
One-color detection

- Determine gene expression level
- One array per sample

Making a Spotted Arrays

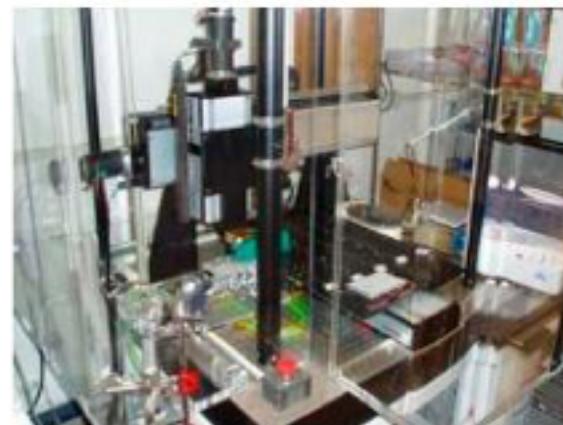
Probes

- cDNA microarrays (up to 3000 bp)
- Long-oligonucleotide spotted arrays: uniform length (20 -100 bp)



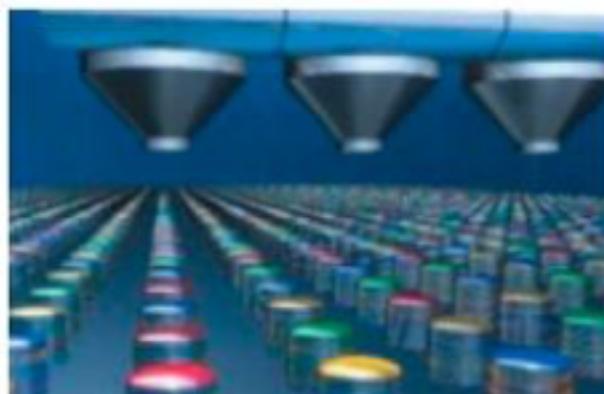
Customized spotted array

- Spotting pins draw fluid (containing DNA probes) by capillary action and form spots on the side through surface tension interaction between the surface and spotting buffer.
- “spotted” onto glass slide using robotic arms

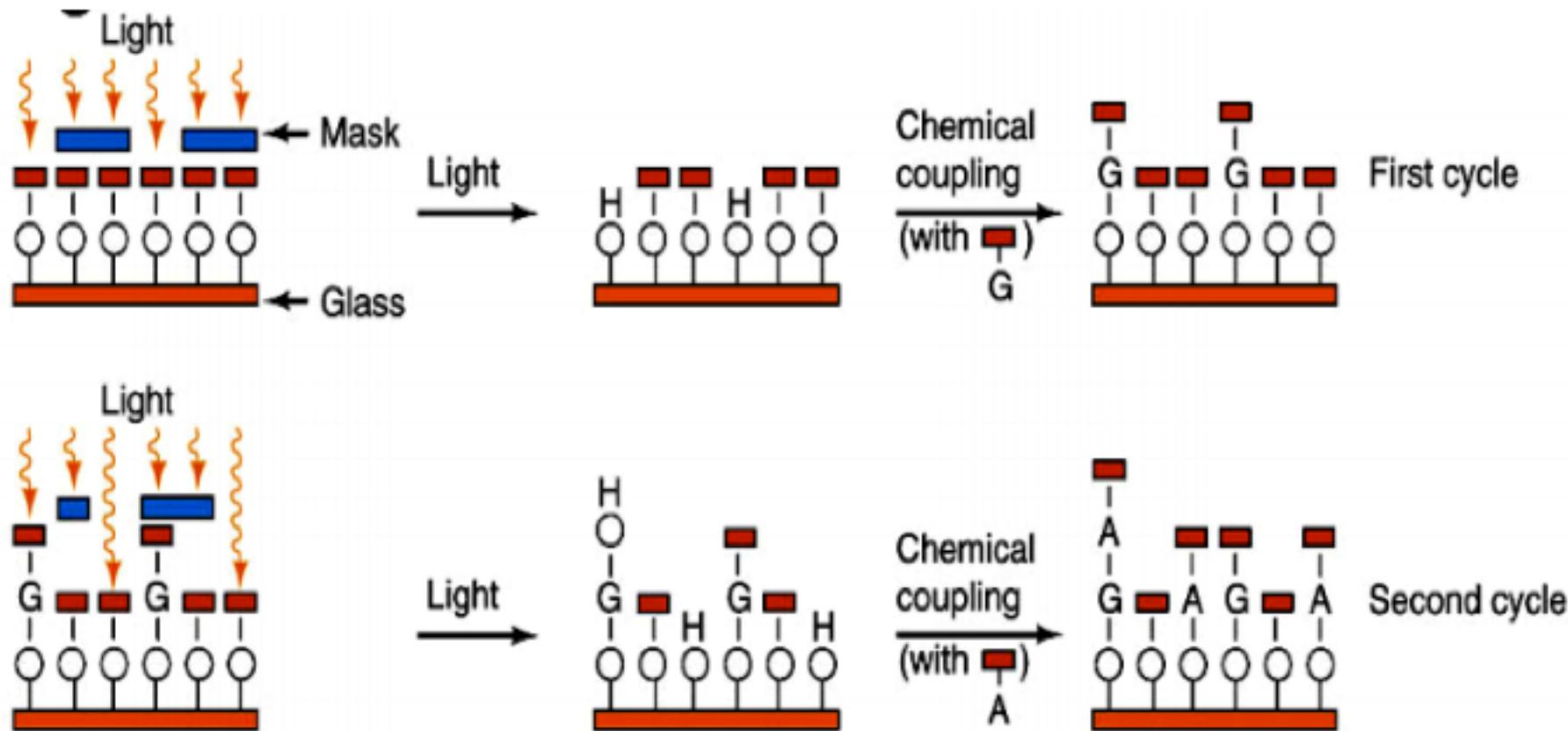


Commercial spotted arrays (Agilent)

- SurePrint Technology
- like an inkjet printer, prints sets of cDNA clones or oligonucleotides one-by-one



Masked Array Synthesis (Affymetrix)



Making a Oligonucleotide array

Probes

- 20-100 bp oligonucleotide probes

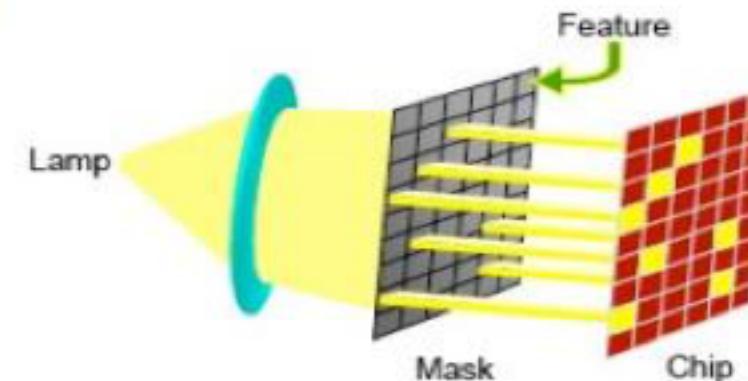
Electrochemistry - CombiMatrix Array

- Each microelectrode selectively generates chemical reagents by electrochemical reaction.
- Controls the building of DNA on a semiconductor chip by the software-controlled turning on and off of electrodes.



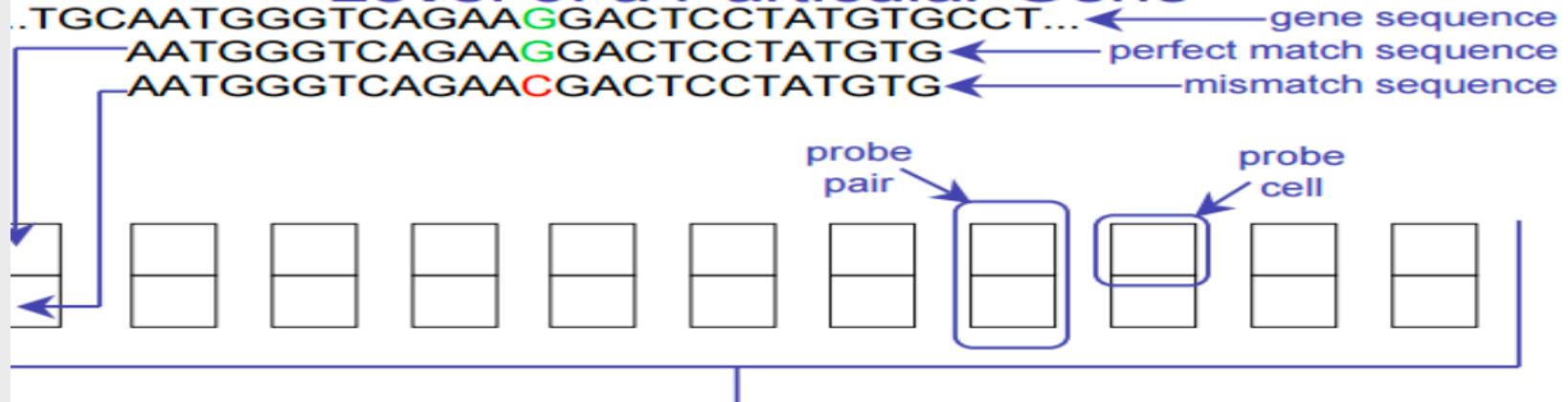
Photolithography - Affymetrix Array

- manipulates light to direct the chemical synthesis of the probes
- Mask directs the flow of U.V. light
- Uses light sensitive protecting groups.

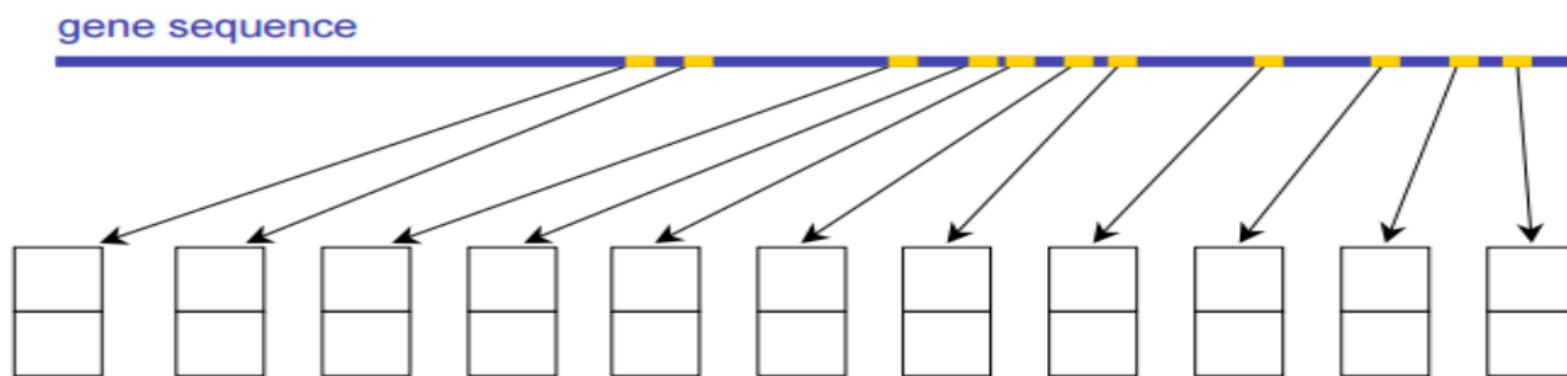


A Probe Set for Measuring Expression

Level of a Particular Gene



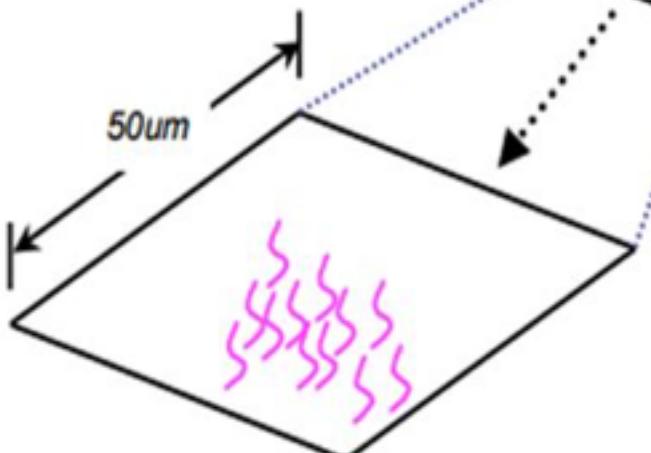
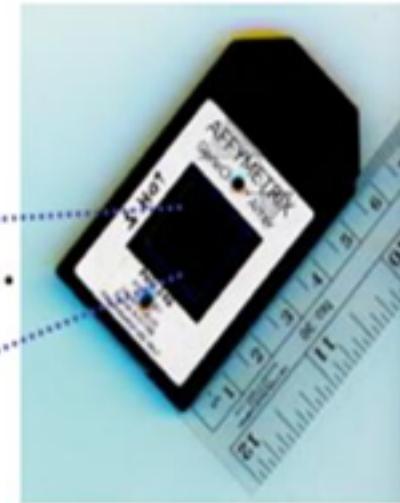
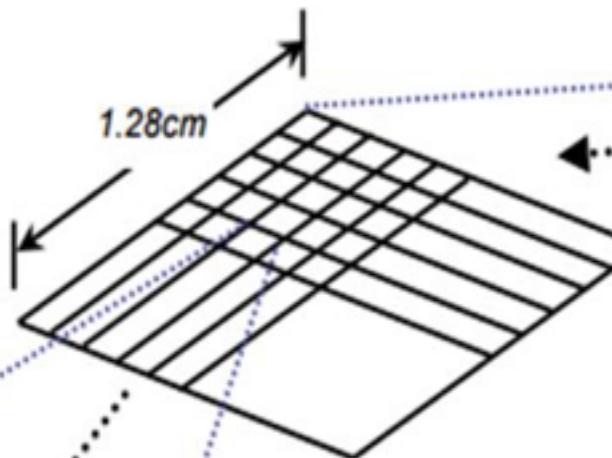
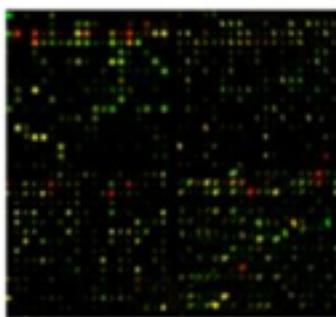
Different Probe Pairs Represent Different Parts of the Same Gene



Probes are selected to be specific to the target gene
and have good hybridization characteristics.

Affymetrix Microarrays

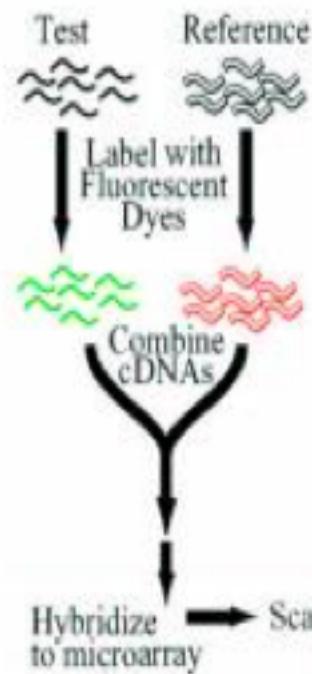
Raw image



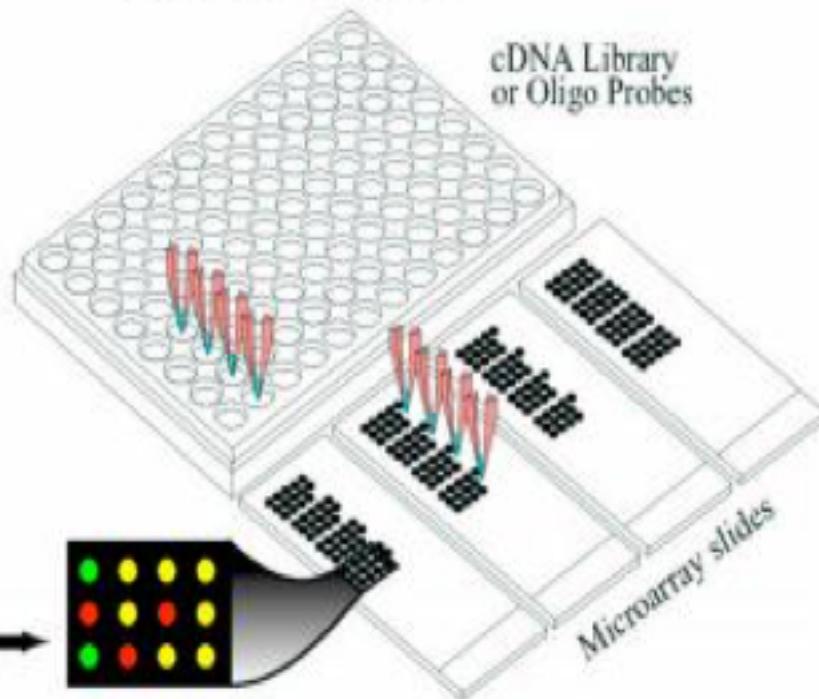
~ 10^7 oligonucleotides,
half Perfectly Match mRNA (PM),
half have one Mismatch (MM)
Raw gene expression is intensity
difference: PM - MM

Robot Spotting

Prepare Sample



Print Microarray



Applications

- Expression profiling
- Comparative genomic hybridization
- Single nucleotide polymorphism
- Sequencing



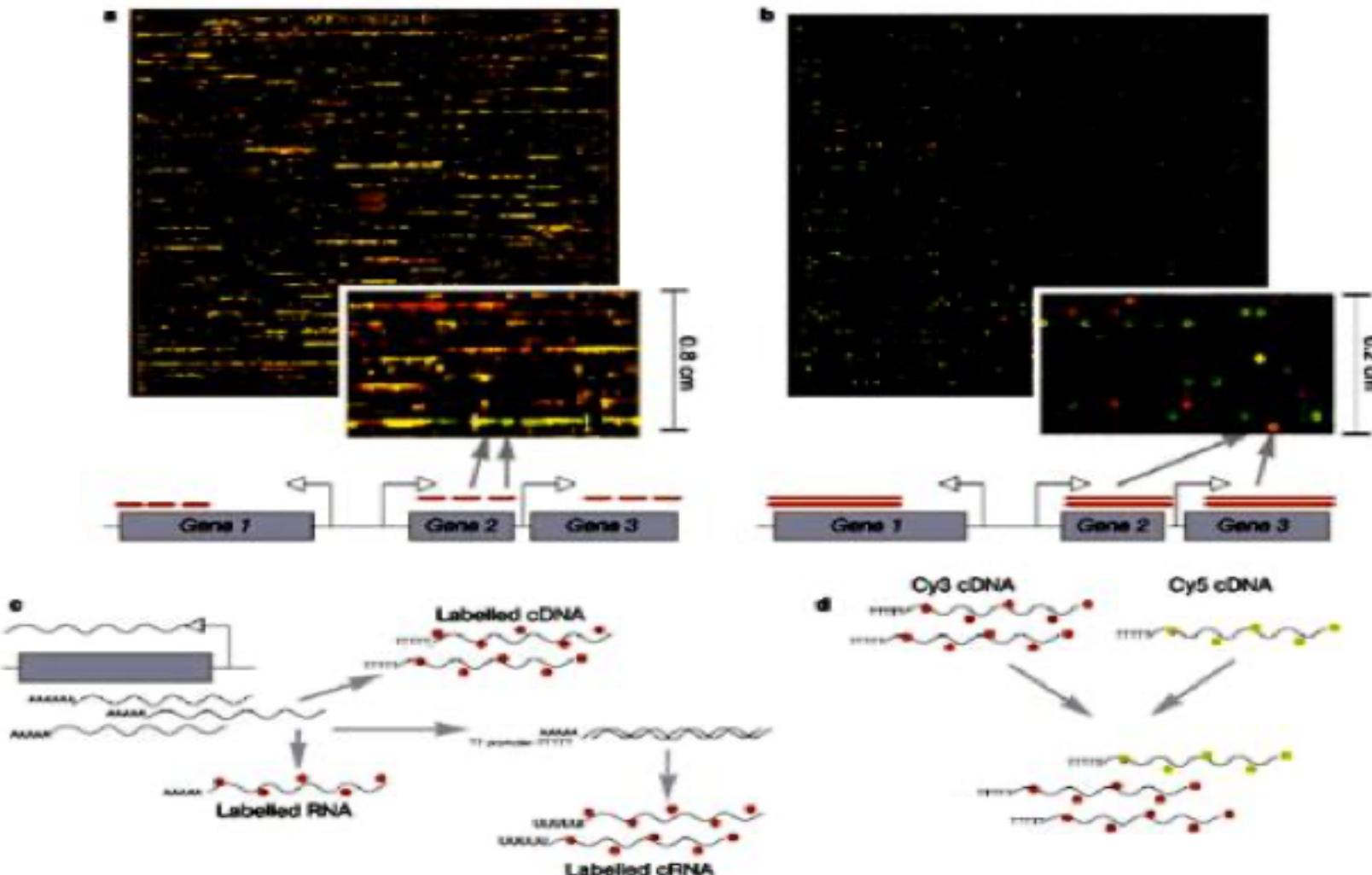
Aplikasi

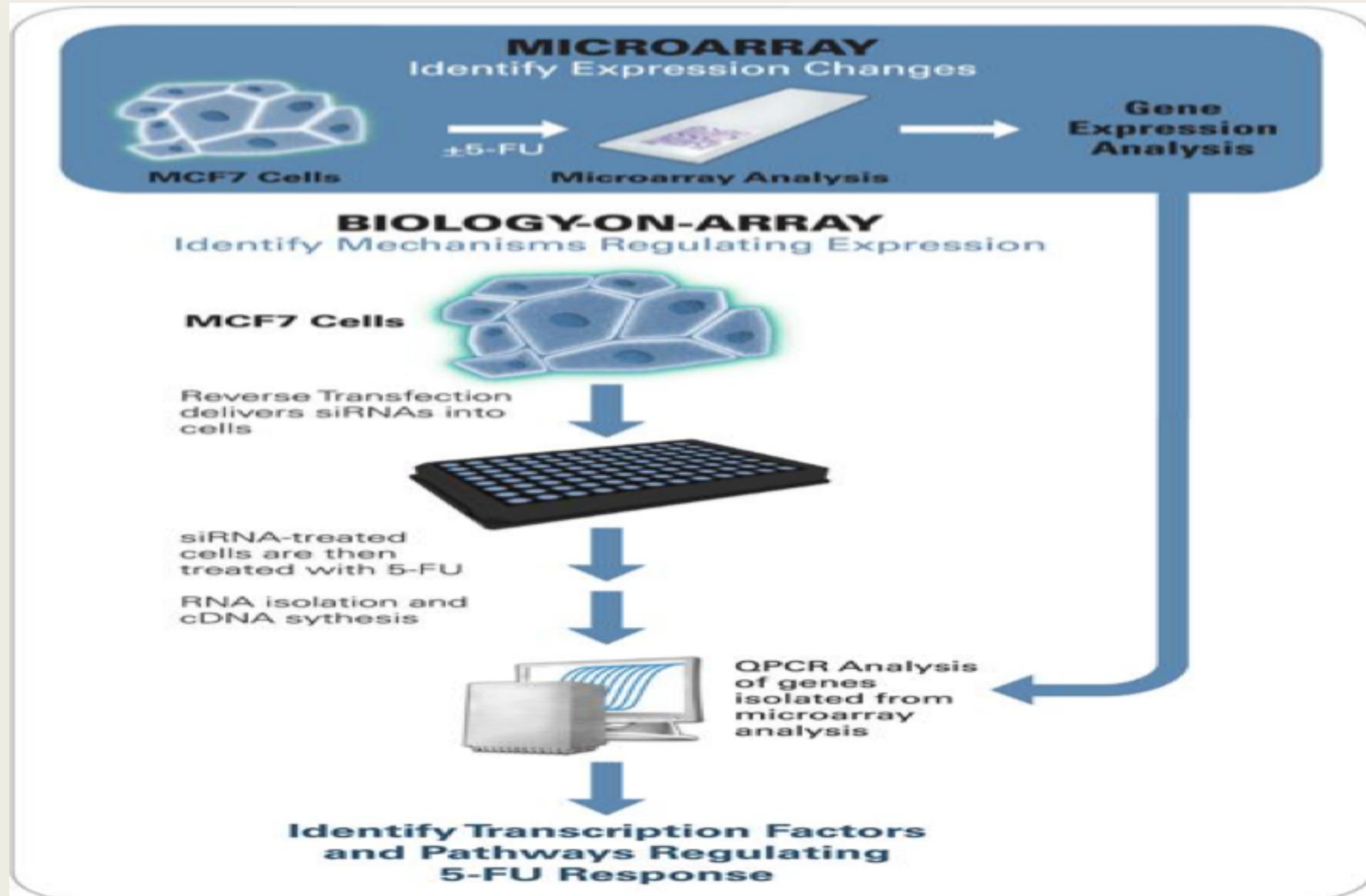
1. Gene expression profiling
2. Comparative genomic hybridization
3. GenoID
4. Chromatin immunoprecipitation on Chip
5. DamID
6. SNP detection
7. Alternative splicing detection
8. Fusion genes microarray
9. Tiling array
10. Double stranded B-DNA microarrays
11. Double stranded Z-DNA microarrays
12. Multi stranded DNA microarrays (triplex-DNA microarrays and quadruplex-DNA microarrays)

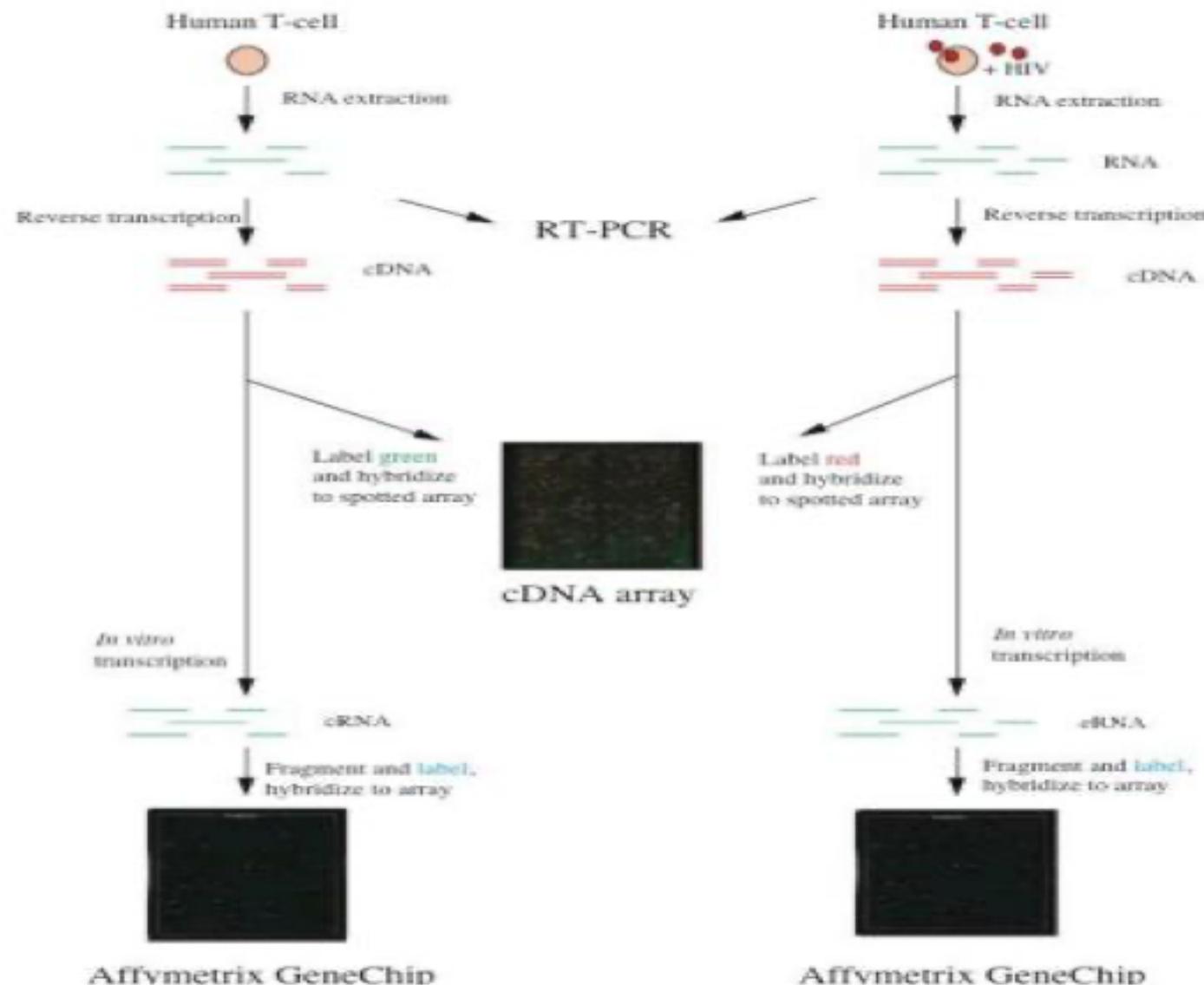
Applications of Microarray Technology



Detection of mRNA









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http://www.affymetrix.com/estore/browse/level_three_category_and_children.jsp?category=35808&categoryIdClicked=35808&expand=true&parent=35617

Aplikasi

- Uji DNA HPV (Human Papillomavirus)
- Pendeksiakan kanker dari DNA microarray ekspresi gen
- Aplikasi Microarray untuk kanker payudara
- Pola ekspresi genetik dan farmakogenomik
- DNA Profiling pada metastasis carcinoma
- Protein Profiling

Aplikasi

- <https://www.youtube.com/watch?v=AJEohQtc108>
(Produksi)
- <https://www.youtube.com/watch?v=ax0PhjmdyxM>
(Contoh penggunaan)
- <https://www.youtube.com/watch?v=AhnTT6-Jgcg>
(SNP)
- https://www.youtube.com/watch?v=Hi45_ZcN5BY
(Protein Microarray for cancer)
- <https://www.youtube.com/watch?v=luBUKLiW08>
(Protein Microarray technique)
- <https://www.youtube.com/watch?v=2c3t3tDEmsU>

Referensi

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