# Lab on a Chip and Microfluidics

Benoît CHARLOT

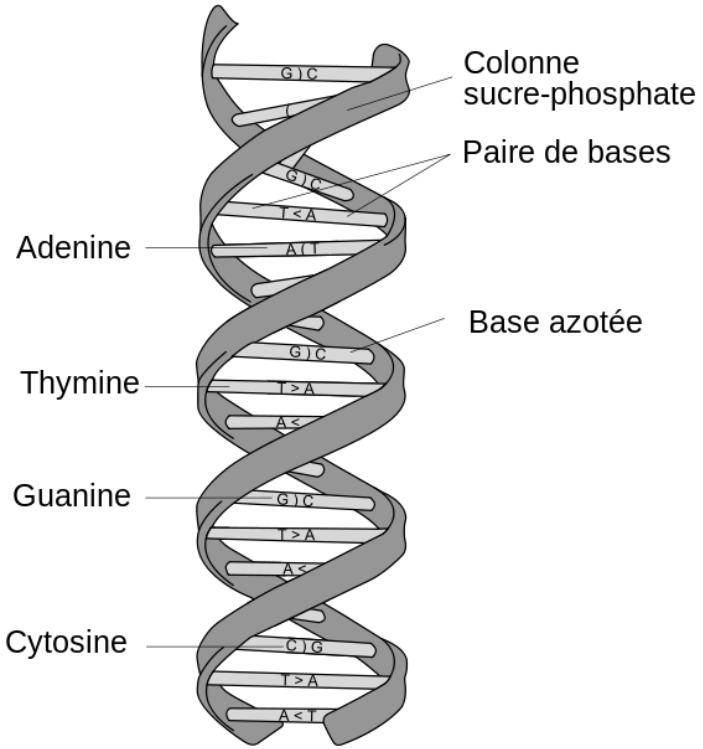
http://www.ies.univ-montp2.fr/~charlot/

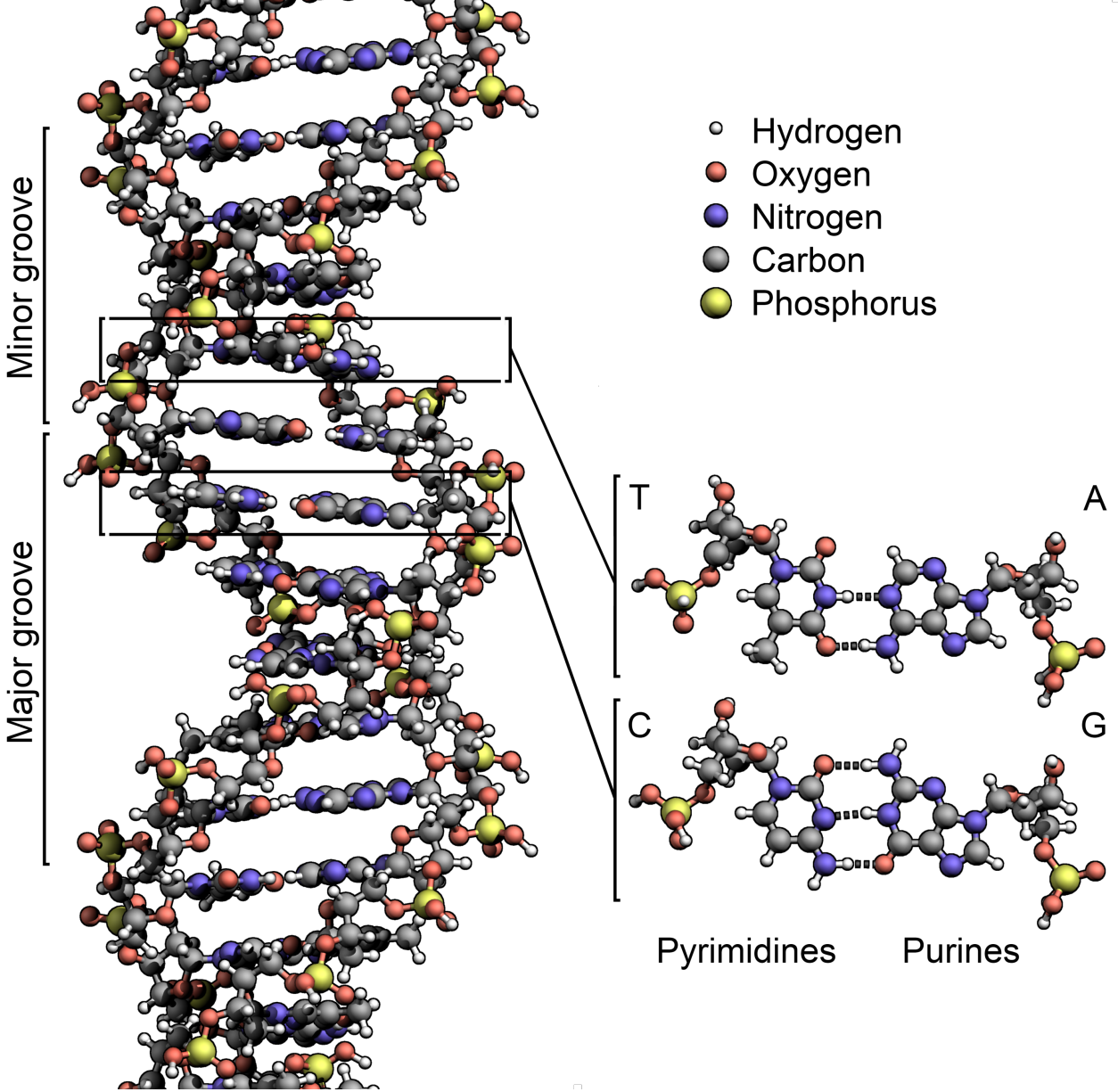

# Part VII. DNA microfluidics

# DNA

Deoxyribo Nucleic Acid

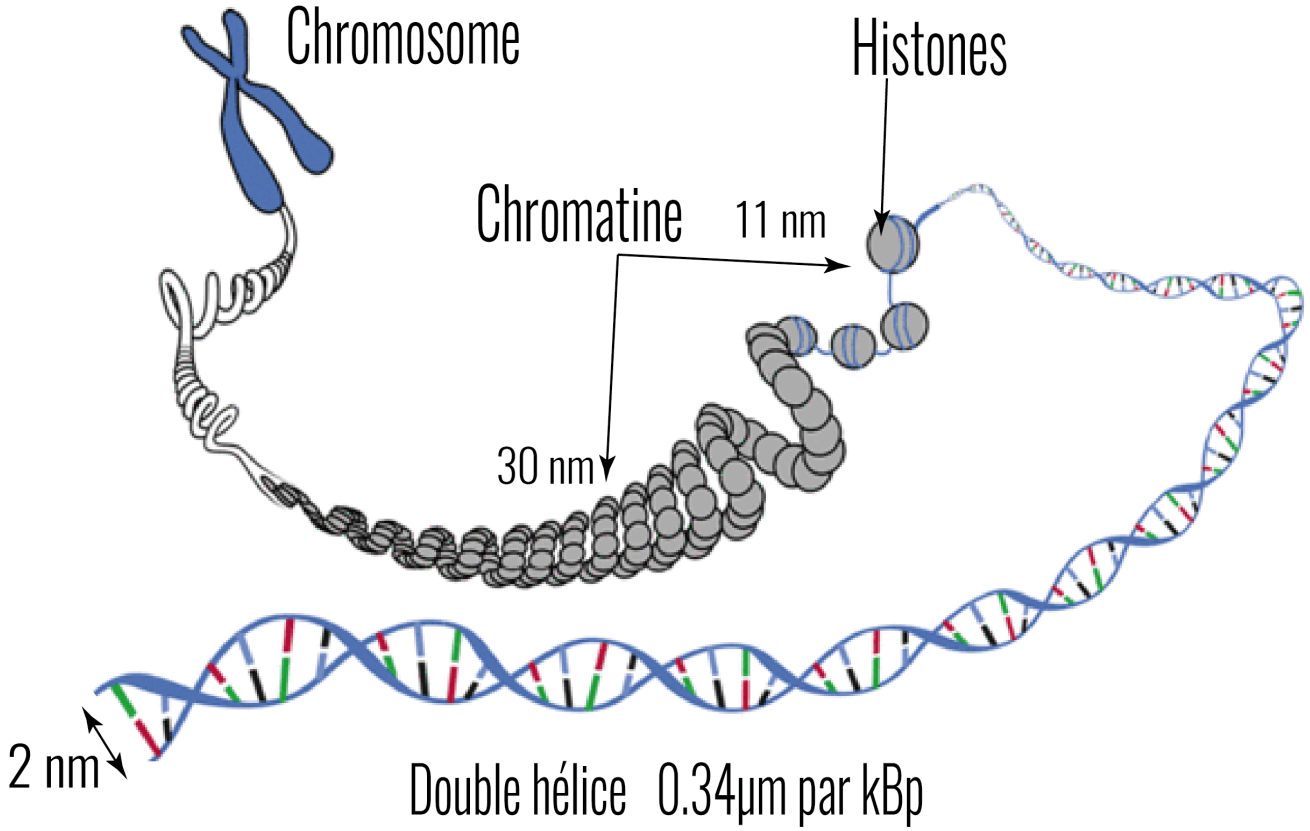
DNA molecule holds the genetic information
Two anti parallel helix
One phosphate sugar backbone
ATCG base




By Zephyris - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=15027555

# DNA compaction and organisation




Persistance length : **100 nm** dsDNA and **2 nm** for ssDNA

Actin : **17µm**

Microtubule : **1,4mm**

# DNA Lab on a Chip

•DNA is extracted from cell nucleus and purified
- Break cell membranes using detergent
- Remove cell debris, proteins, enzymes

DNA assays
- Detect specific fragments in fingerprint pattern-matching mode
- Sequence DNA fragment for base pair order of fragment

Analysis tools
- Chemical amplification
- Restriction digestion
- Electrophoretic separation
- Sanger sequencing process
- Hybridization
- Fluorescence visualization

Miniaturization Benefits
Reagent consumption ~ [s3]
Miniscule reaction volumes reduce reagent cost.
Heat transfer ~ [s2]
Surface phenomena
Mass transfer ~ [s2]
Reduced analysis times, with minimum assay time limited by
speed of enzyme (30-100 bp/s)
Flow is laminar
Electroosmotic flow for valveless systems ~ [s2]
Capillary flow ~ [s1]
Separation efficiency ~ [s-2]
Injection volume well-defined

# PCR Polymerase Chain Reaction

Principle :

Chain amplification of a few copies of a piece of DNA
Across several orders of magnitude ($10^9$)
Polymerase
By Thermal cycling

Invention : 1988
Development in the 90'S

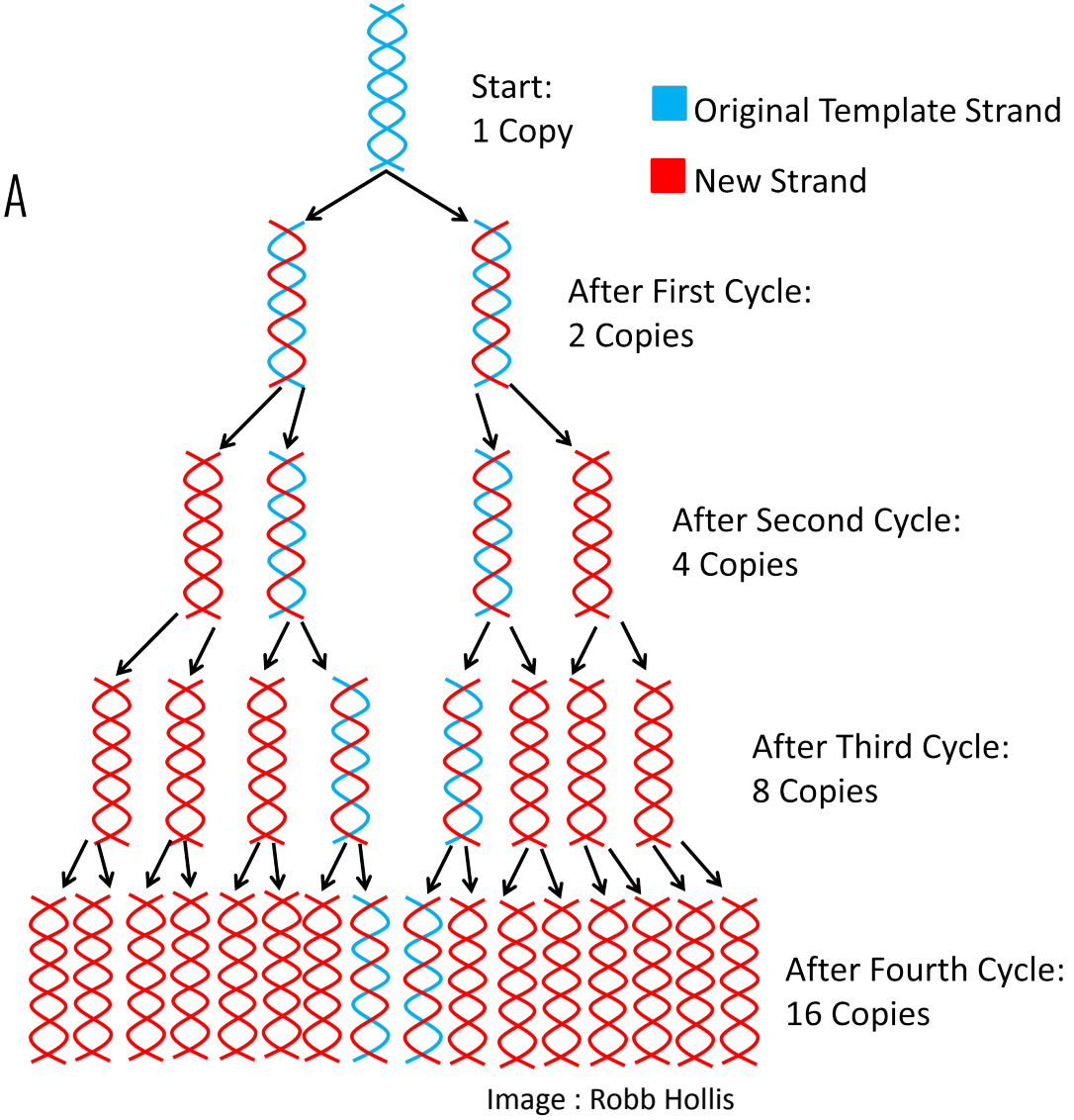


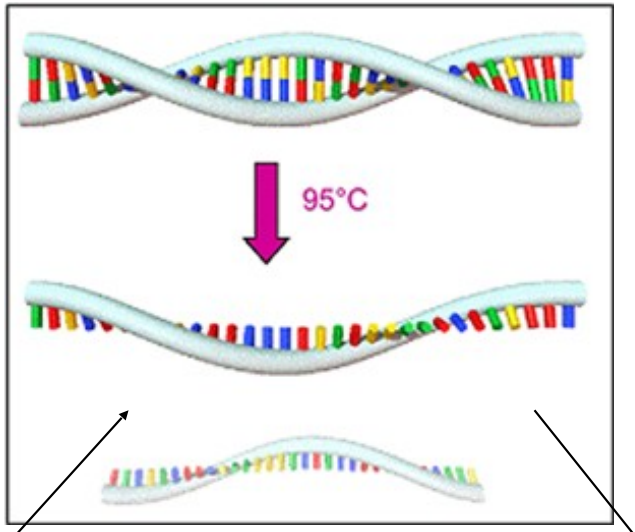
Image : Robb Hollis

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, & Erlich H (1992). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. 1986. Biotechnology (Reading, Mass.), 24, 17-27 PMID: 1422010

# PCR Polymerase Chain Reaction

Process

1. Template DNA
2. dNTPs
3. Primers
4. Buffer Solution
5. Polymerase (Taq polymerase)
6. MgCl2 Solution
7. Water

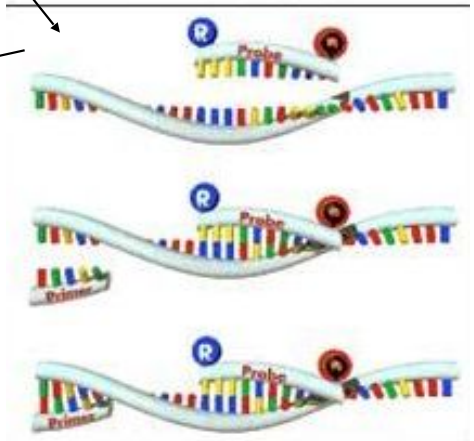



Denaturation (95°C)

Dehybridation of double stranded DNA

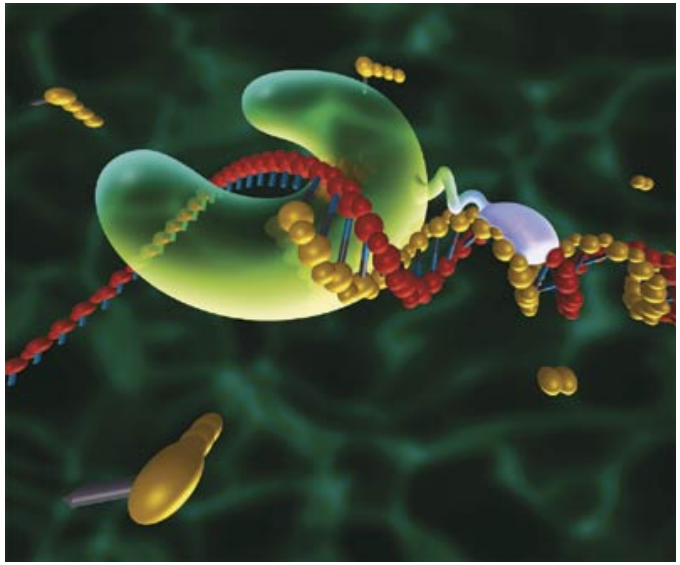
~25-35 cycles at 1-3 min/cycle
~30 cycles : 75 minutes

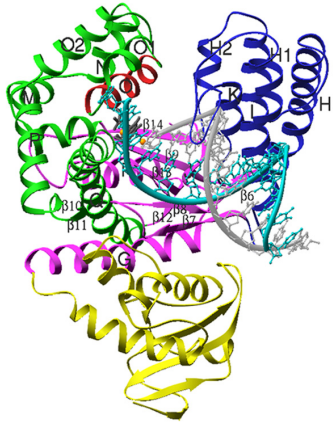
Hybridation (2)




Amorces (50°C)

Replication (72°C)





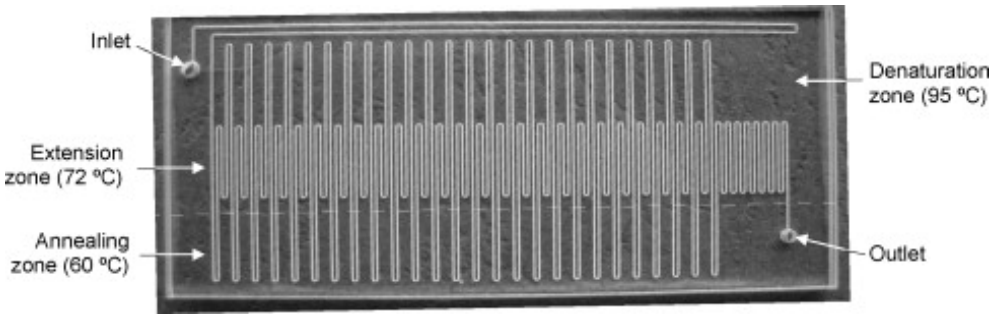
# PCR Polymerase Chain Reaction

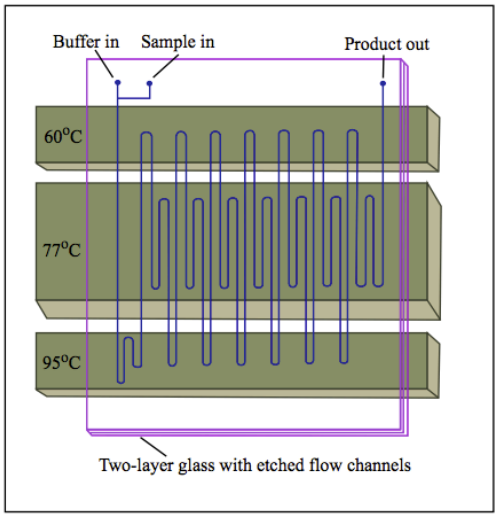
## Standard PCR equipment

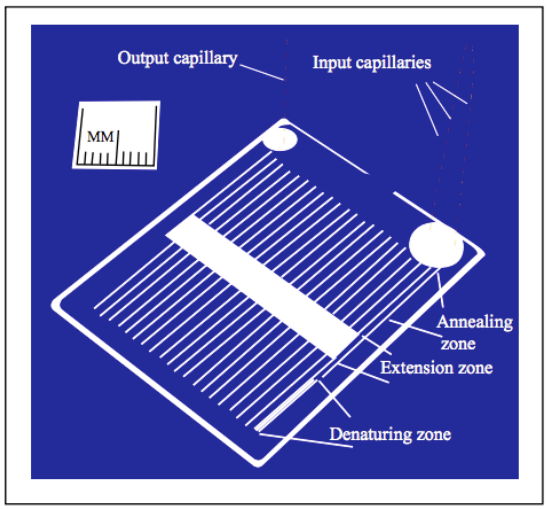
## Continuous-flow PCR on a Chip

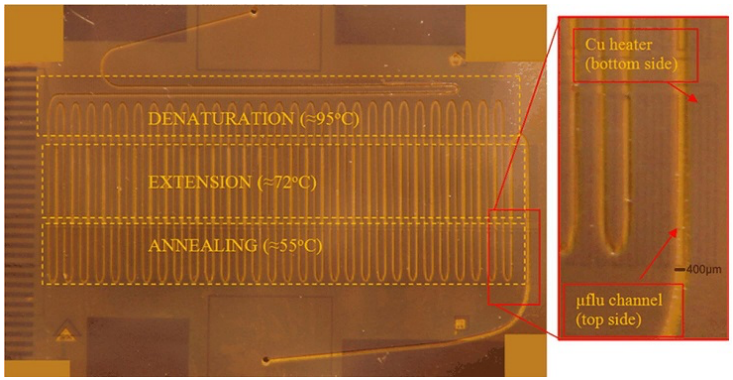
Microfluidic Channel : 40x90µm
Flow celerity
20mm/s

Thermal transition length
60µm



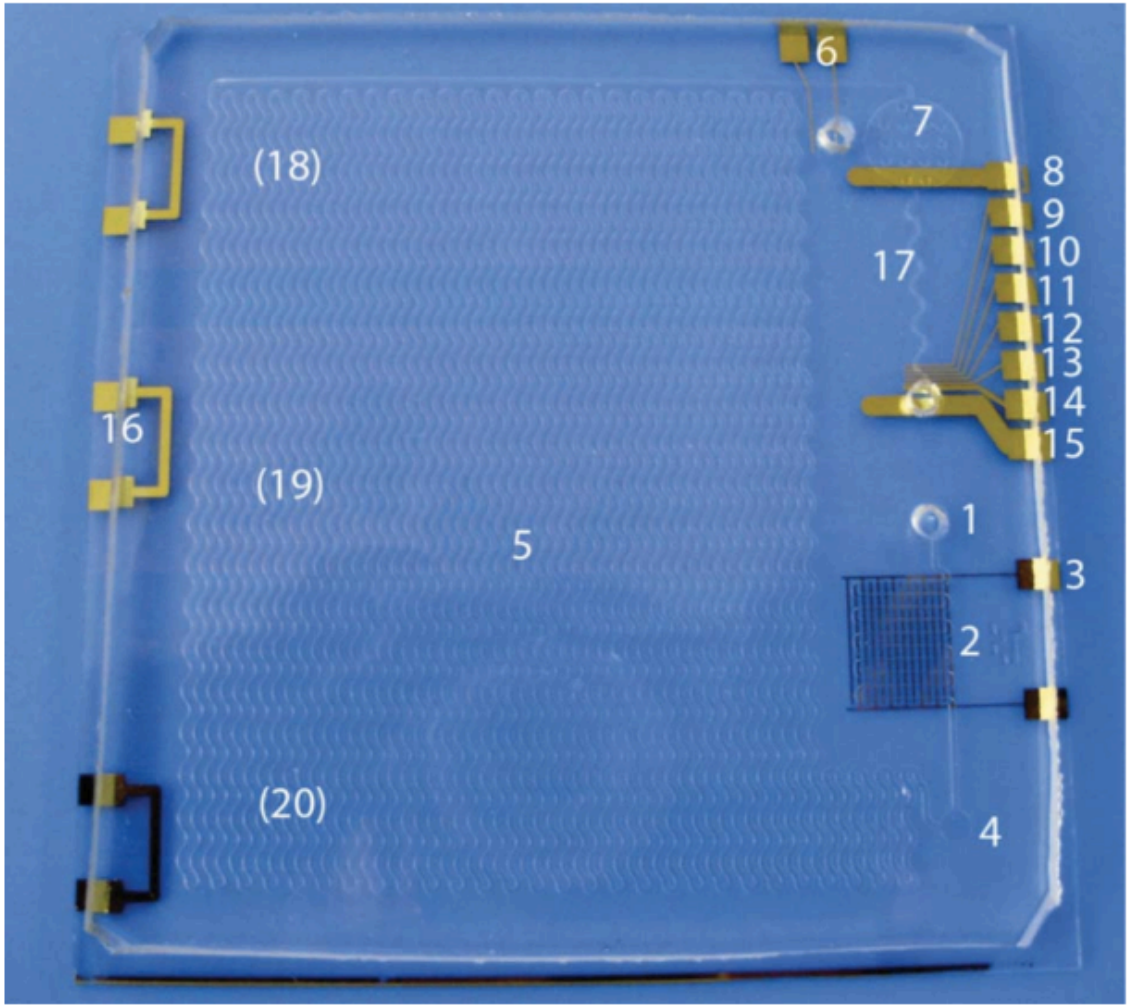




Moschou et al., Sensors & Actuators B, 2014 (39).

# PCR Polymerase Chain Reaction

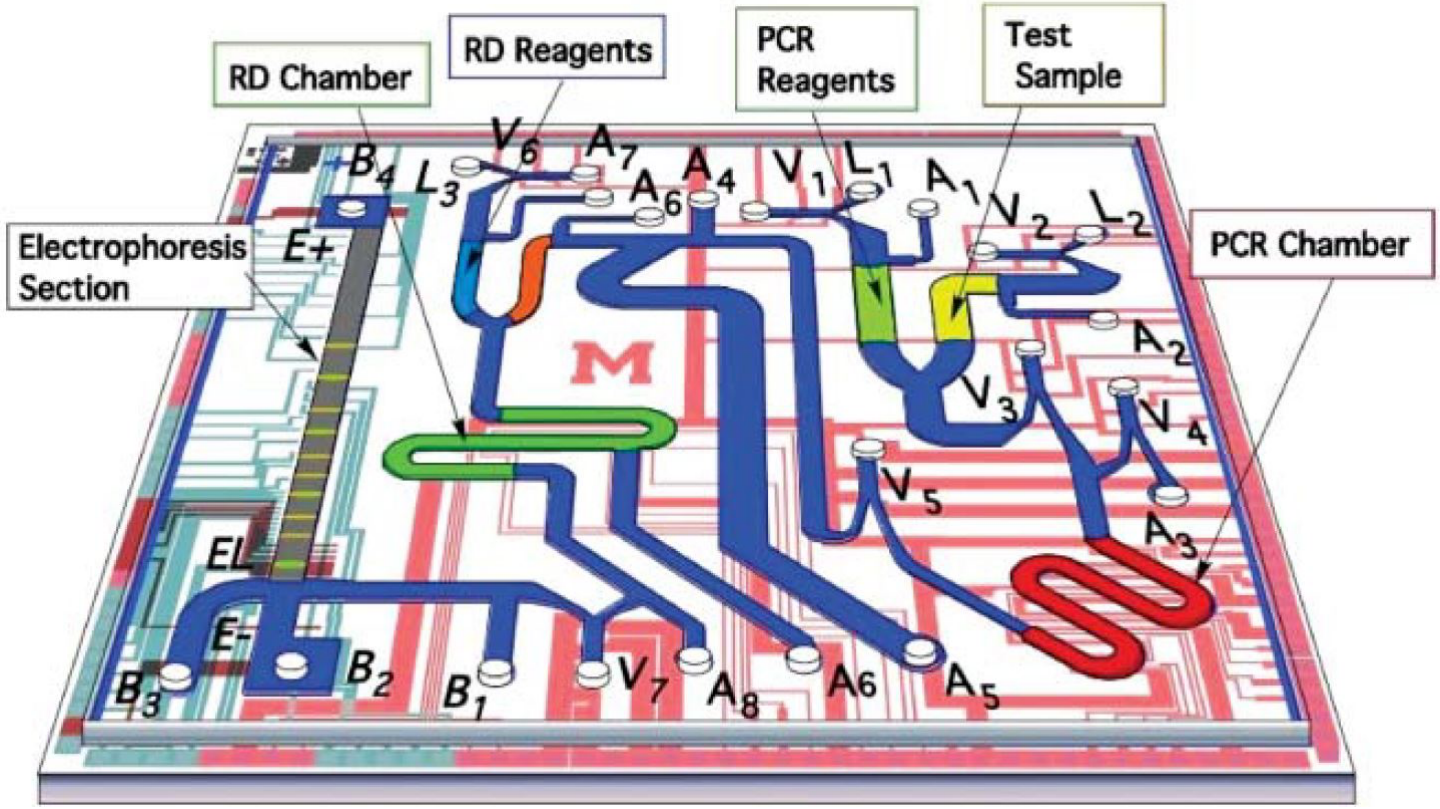
## PCR microdevice

(1) inlet reservoir for applying sample onto the microchip;
(2) Gold interdigitated microelectrodes
(3) contact pad for applying DC potential for electrochemical cell-lysis
(4) reservoir for manual extraction of cell lysate from the chip for conventional analysis
(5) microchannels for carrying out 25 cycles of PCR
(6) gold electrodes used for conductometric liquid level sensor
(7) large reservoir for collecting PCR amplicon sample
(8) and (15) gold electrodes used for applying separation voltage for CE operation
(9–11) optional decoupler electrodes
(12) reference electrode
(13) Working electrode
(14) counter electrode
(16) optional resistance temperature detector for feedback temperature
(17) Spiral CE- microchannel filled with semisolid agarose dissolved in NaOH medium for CE-AD separation of PCR amplicon;
(18–20) ITO microheaters on the back side of glass as thermocycler zones for PCR, namely: extension, annealing and denaturation.



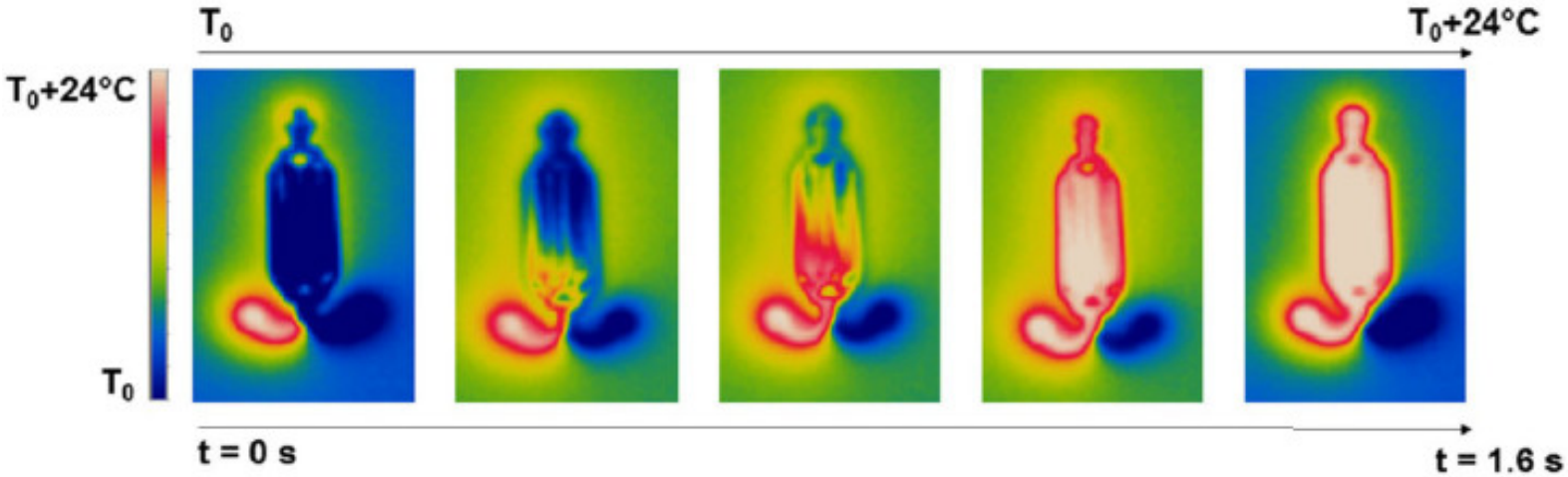

Kumar Jha et al. Lab Chip, 2012, 12, 4455–4464

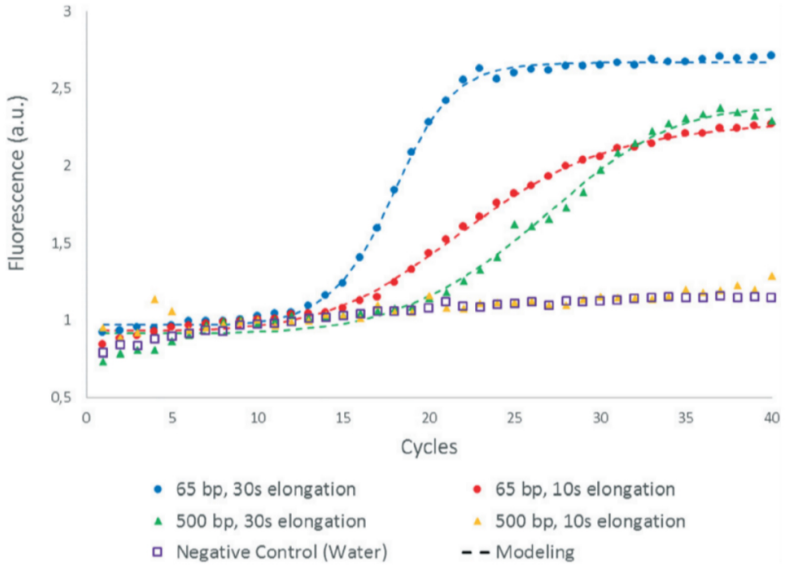
# PCR Polymerase Chain Reaction

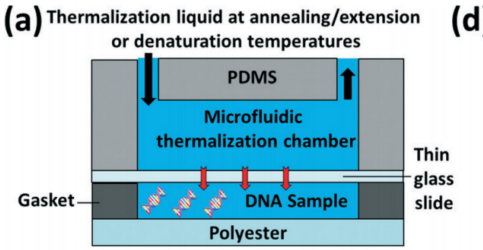


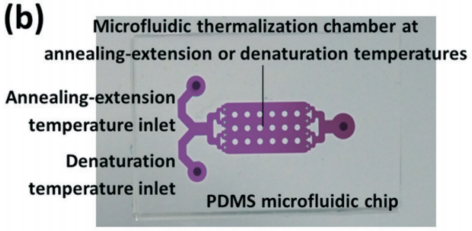

Pal et al., Lab on Chip, 2005 (7)

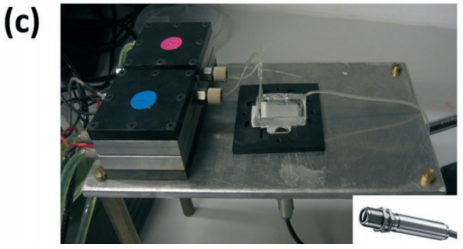
# PCR Fast PCR : FASTGENE

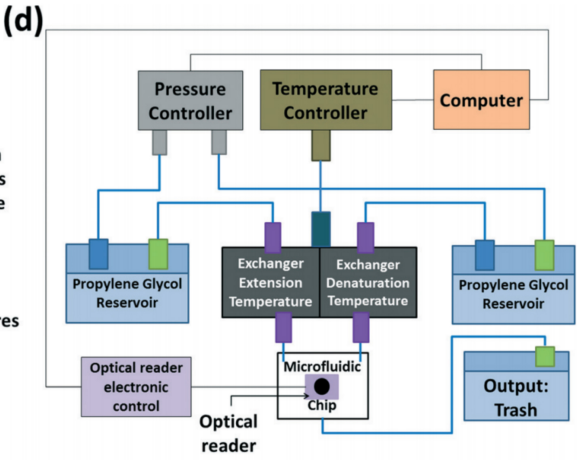


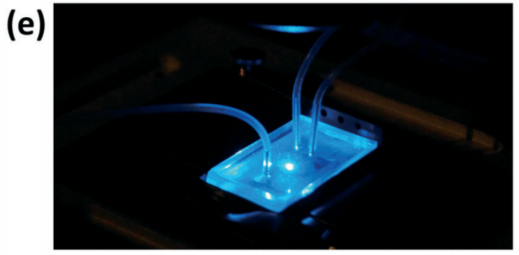



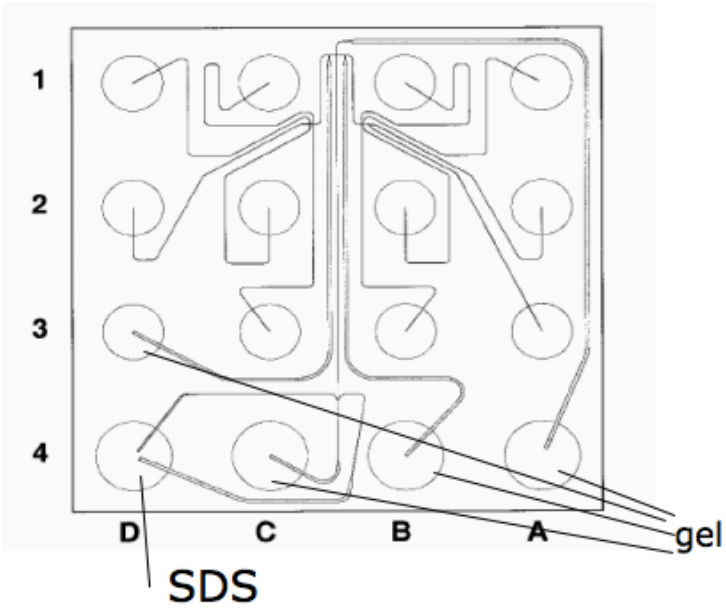



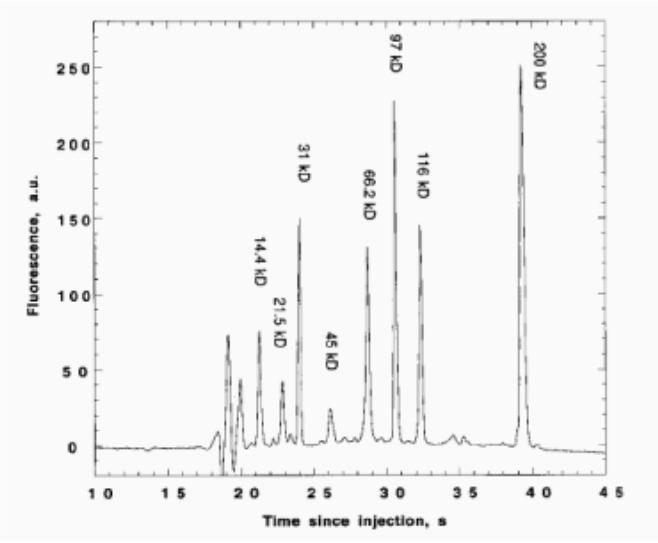



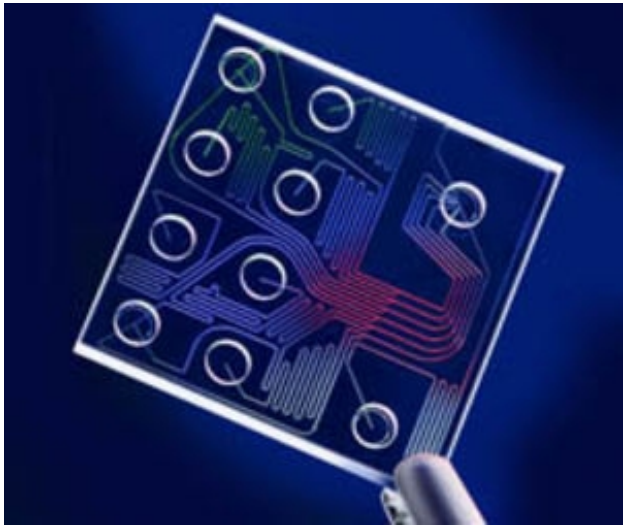



# CE Capillary Electrophoresis on chip




Dubrow et al. Anal. Chem. 2001, 73, 1207-1212

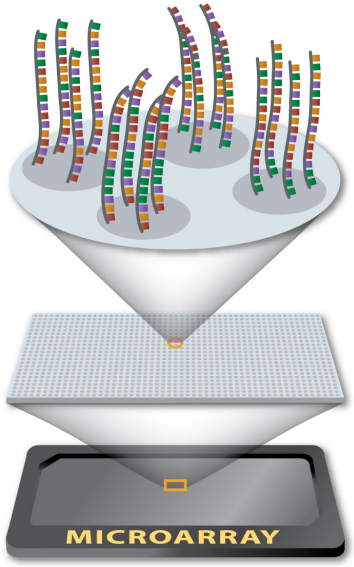


# DNA MICRO ARRAYS

A device that captures the activity pattern of thousands of genes at once

Based on **hybridation**

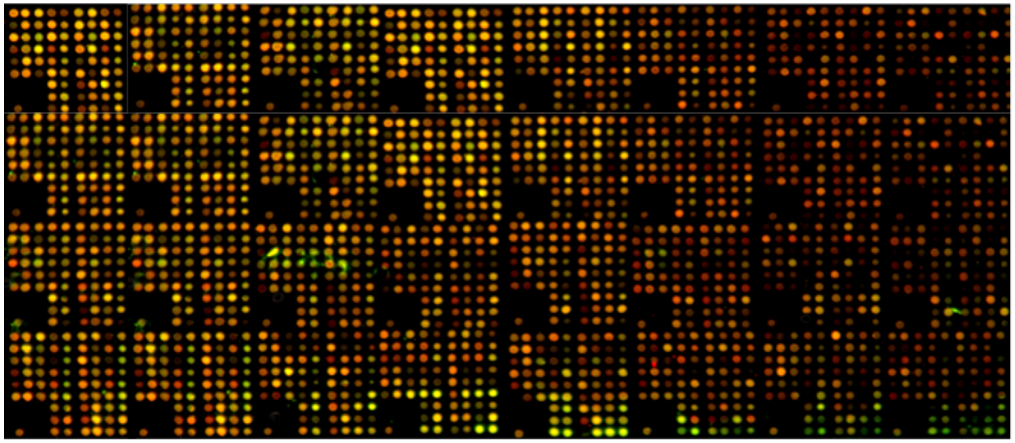
Affymetrix Genechip probe array


100 000 spots per square inch

– Each holds millions of copies of a DNA sequence from one gene
- Each sequence must be unique to a specific gene

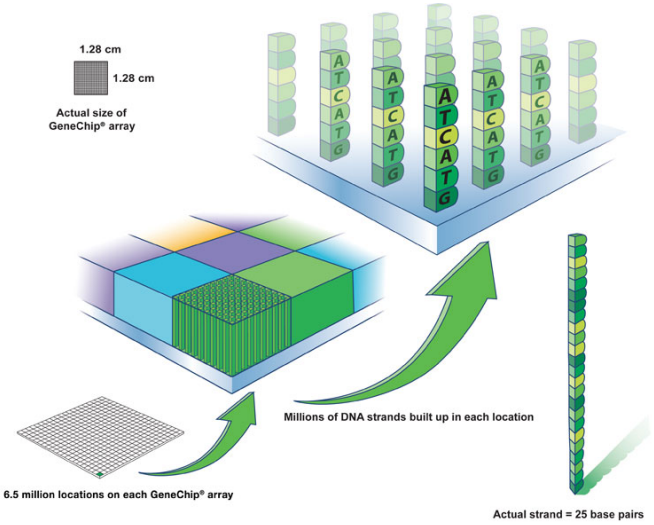
-Spotted sequence must be unique to the specific gene
-Requires careful planning,
-many genes share similar sequences

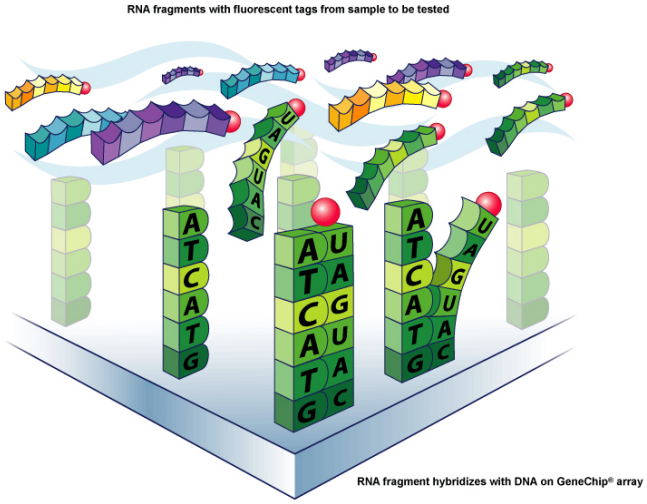


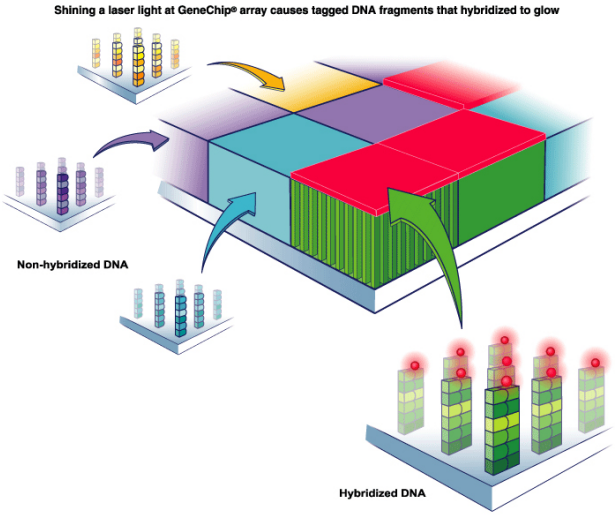
# DNA MICRO ARRAYS

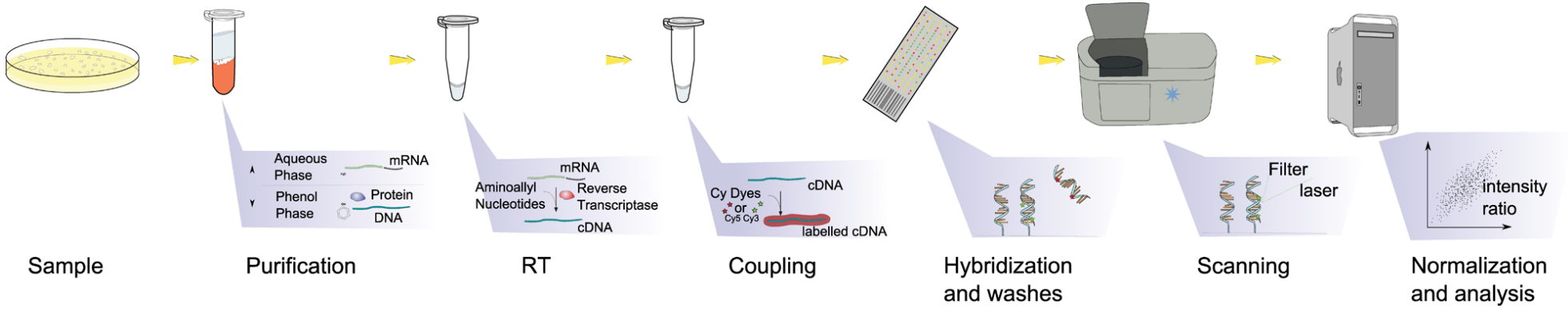
## Process flow

affymetrix
Biology for a better world




By Squidonius (talk) - Own work (Original text: I (Squidonius (talk)) created this work entirely by myself.), Public Domain, https://commons.wikimedia.org/w/index.php?curid=39423104

# DNA MICRO ARRAYS

DNA microarrays Fabrication

**Spotted microarrays,**

-Probes are oligonucleotides, cDNA or small fragments of PCR products that correspond to mRNAs.

- Synthesis prior to deposition on the array surface and are then "spotted" onto glass
- Contact / Non contact
- Pins or needles controlled by a robotic arm
- Easily customized

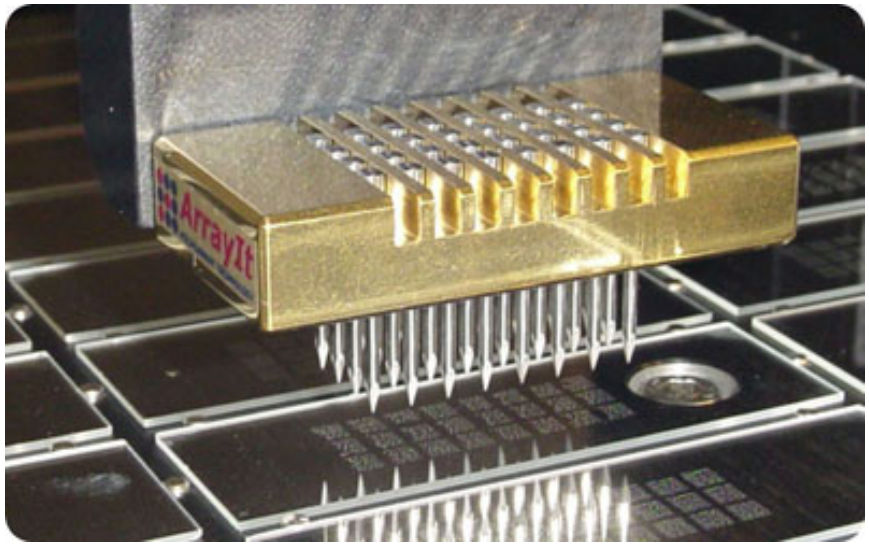
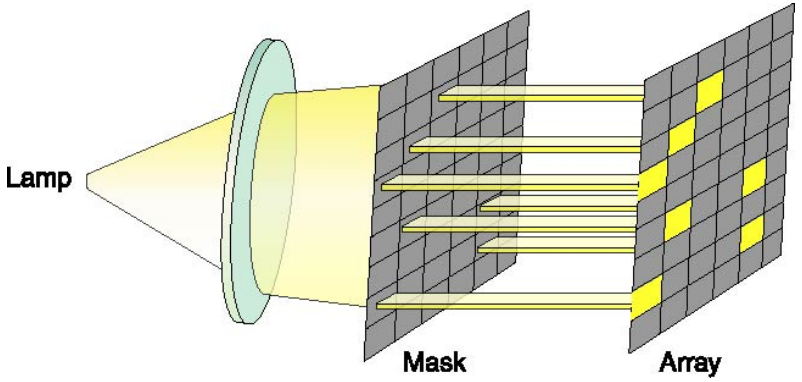



Image : Scripps Research Institute

**In situ Oligonucleotide microarrays,**

-Synthesis directly onto the array

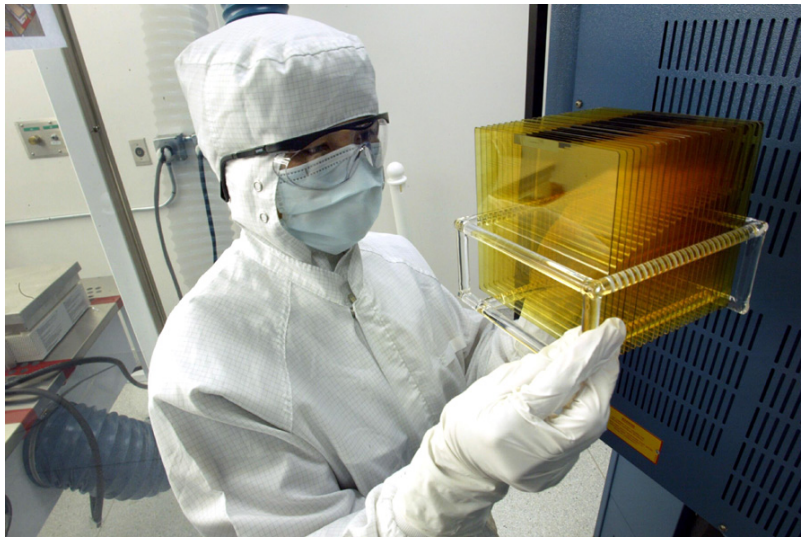
- Photolithography technique
- Shorter probes may be spotted in higher density across the array and are cheaper to

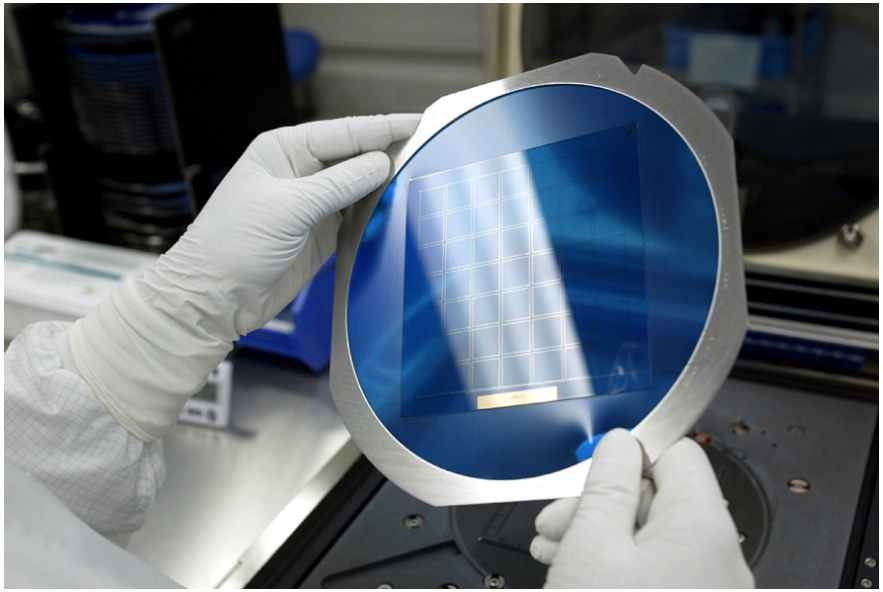


# DNA MICRO ARRAYS

## Cleanroom process

affymetrix
Biology for a better world

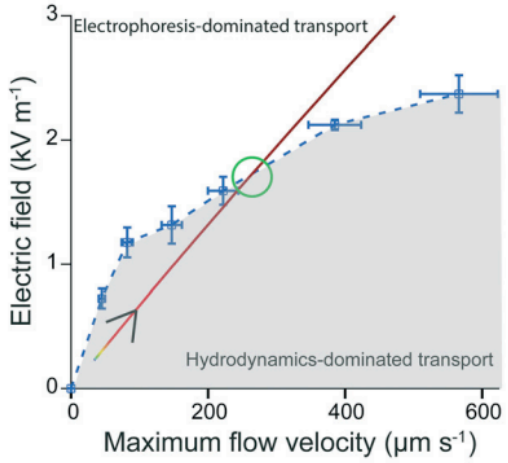


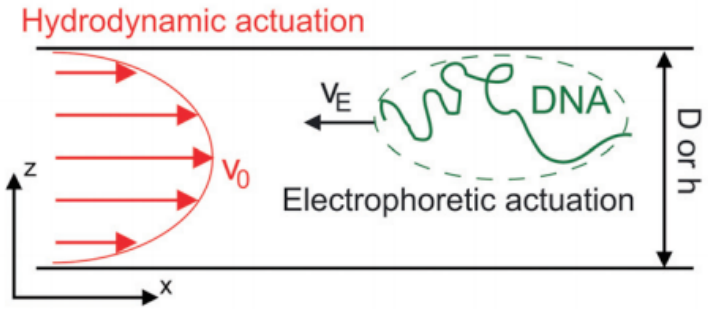


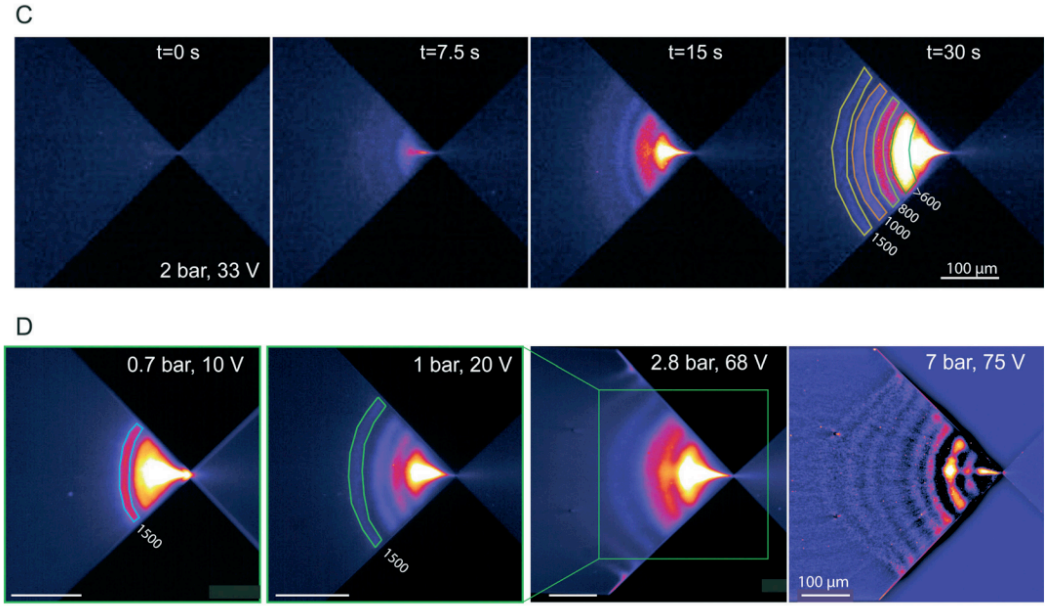
# DNA in microfluidics : Concentration

DNA separation and enrichment using electro-hydrodynamic bidirectional flows in viscoelastic liquids

Competition between :
- Electro osmosis
- hydrodynamics

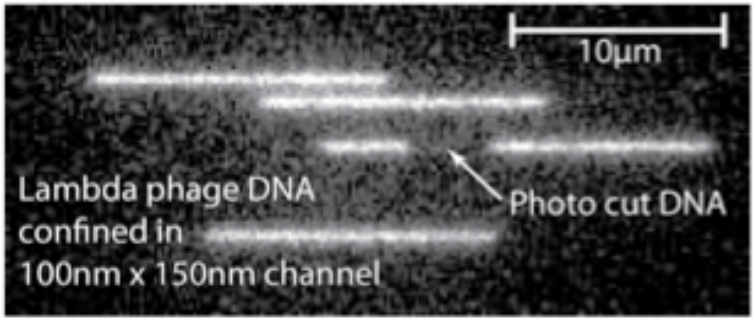




Ranchon et al. Lab Chip, 2016, 16, 1243

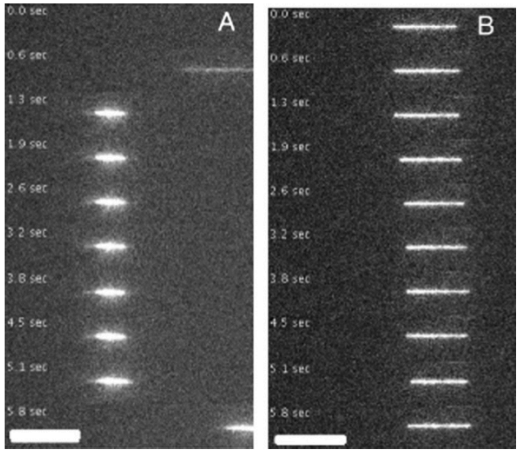
Aurélien Bancaud, LAAS

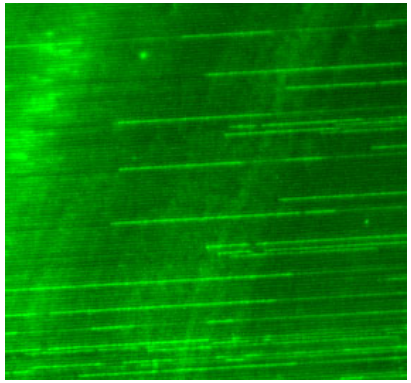
# DNA nanofluidics containment

Persistance length : **100 nm** dsDNA
and **2 nm** for ssDNA




F. Westerlund, Chalmers



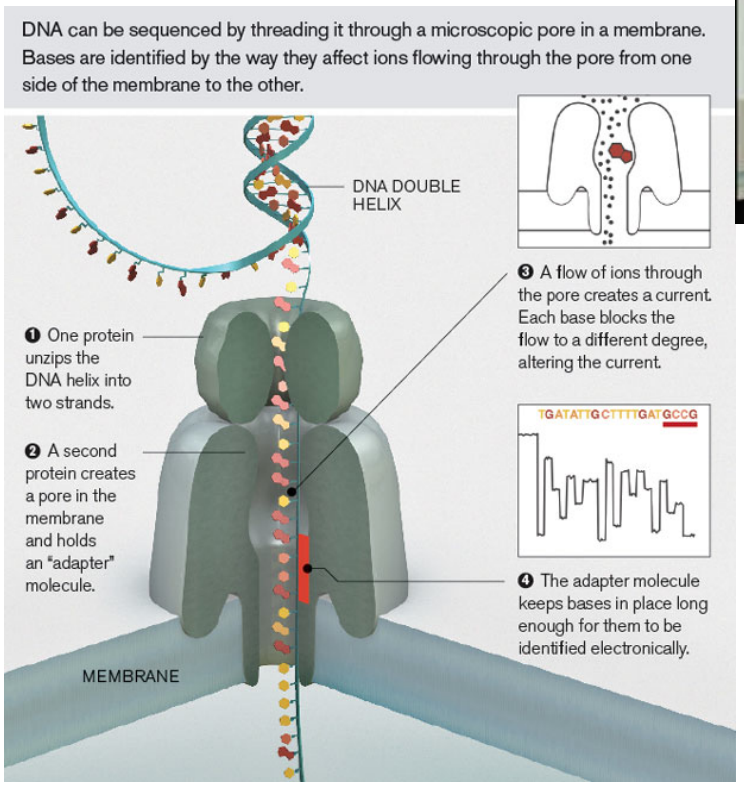



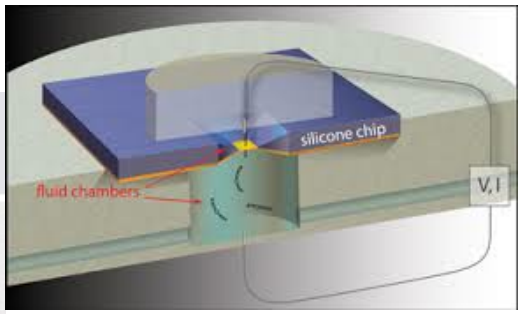
D.E.Streng; North Carolina

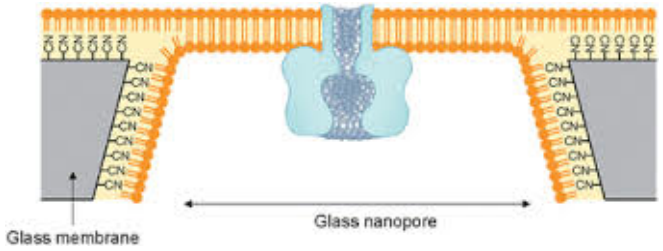
state Univ.

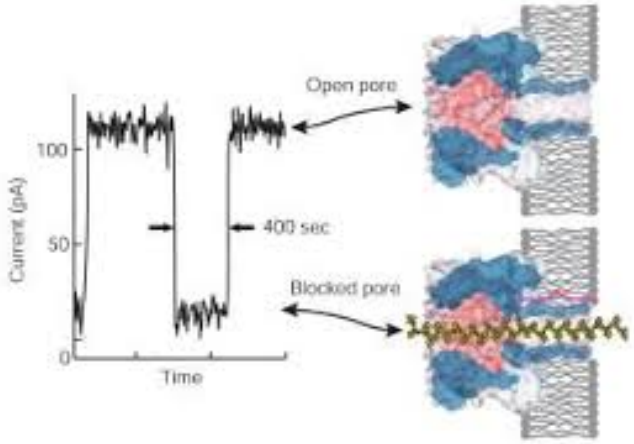
# DNA sequencing in nanopore

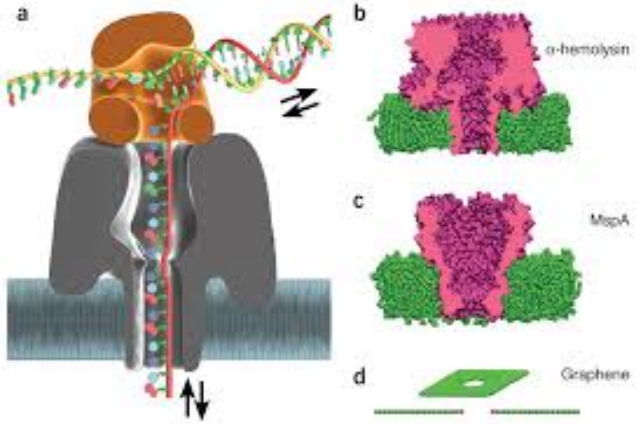
-Translocation  of DNA strands through a nanopore
-a-hemolysin inclusion in an artificial membrane
-Current measurement



# DNA sequencing in nanopore

Oxford Nanopore