université PARIS-SACLAY

1

« Advanced Technologies for Clinical Diagnostics and Therapeutics

Course Master 2MGB,
Teaching Unit MPAMB-2025

Pr. Christophe Sola
christophe.sola@universite-paris-saclay.fr

IAME-UMR1137, INSERM-Université de Paris

1

Table of Content

- Slides S1 to S38 : Introduction to Analytical Chemistry
- Slides S39 to S43: Brief reminder of big names
- Slides S43 to S48: Electronic industry
- Slides S48 to end:

Core course **Molecular Diagnostics**

S46-S79 : sequencing techniques
S80-S101 : PCR techniques
S102-S125 : DNA chips
S126-S128: paper-based IVD

2

Analytical Chemistry: definition

- **Analytical Chemistry** *is the study of the* ***separation****,identification, and quantification of the chemical components of natural and artificial material*

Scientific Police and Legal expertise
Foresensic Medicine, Traces analysis
Bioanalysis
Clinical Chemistry, Clinical Microbiology
Environmental analysis
Material analysis

3

How can we classify Analyses ?

-**Direct** or **Indirect** (from sample immediately to result or not)
- intermediate steps or not required ?
-**Destructive** or **Non Destructive** : recovery of the sample or Destruction of the sample at end
-**Quantitative** or **Qualitative** :detect and quantify or presence/absence. if quantitative : limit of detection, issues on sensibility/specificity
-**Classical or « Rapid »** sample handling -> growing, or PCR-based or oth.
-**Visual or Instrumental** :physico-chemical properties based
-**Preparative** or **Analytic** :purify or measure
-**Manual** or **Automatized** :technical skills?, operator, automatization
-**Supervised or non-supervised** interpretation of results

4

Clinical Chemistry (*senso lato*)

- **Clinical Chemistry** : Area of clinical pathology that is generally concerned with analysis of bodily fluids for diagnostic and therapeutic purposes.

sample

-HEMATOLOGY -BIOCHEMISTRY -IMMUNOLOGY
-MICROBIOLOGY = BACTERIOLOGY & VIROLOGY & FUNGI & PARASITES
–GENETICS-GENOMICS-TRANSCRIPT-METABOLOMICS-PROTEOMICS
TOXICOLOGY & DRUG ASSAY-PHARMACOLOGY
ANATOMO-PATHOLOGY (medical science)

5

Molecular diagnostics markets overview

- Diagnostics Companies sell **instruments** and **tests** for the analysis of the diagnosis of a disease or condition. At roughly 6%, spending on diagnostics is still a small part of overall health care expenditure, though it has a disproportionate impact as the results of testing inform roughly **75%** of health care decisions. It is therefore a high value-add activity.
- USD **10.12** Billion by 2021 from USD 6.54 Billion in 2016, at a **CAGR of 9.1%** from 2016 to 2021
- **Infectious diseases: +++ after SARS-CoV 2**
- oncology
- genetic tests
- blood screening
- other applications (cardiovascular diseases, neurological diseases, DNA fingerprinting, tissue typing, and food pathogen detection testing).

https://www.marketsandmarkets.com/Market-Reports/molecular-diagnostic-market-833.html?gclid=Cj0KCQiAhrbTBRCFARIsACY7MW14dq_HdQymJEEqfLiHBMR96FBwmKI_dYNI7ImS76nD86PNK2uwaAiejEALw_wcB

6

BASICS

Genomic Medicine era : Issues and Themas

A world full of concepts and acronyms…

NGS for Infectious Diseases Diagnostics
Mass-Spectrometry for Infectious Diseases Diagnostics

Translating Preclinical data into Rational Cancer Combination Therapies
Metabolic Microbiome
Detection and Characterization of circulating biomarkers

Translational Medicine, Theranostics, Exposome
Telemedecine, Precision Medicine, Personalized Medicine,Global Health, One Health, Environmental Health, ...

WGS: whole genome sequencing **NGS:** next generation sequencing **Metagenomics**

HRM: high resolution melting-**Applications and advance**
PCR:- Polymerase chain reaction based Qualitative, Quantitative, qPCR RT-qPCR, Digital Droplet
Gene Edition - CRISPRs
various physical principles :
SPR: surface plasmon resonance, **FRET:**Förster resonance energy transfer
SNA: PNA, LNA spherical nucleic acids, peptide nucleic acids, locked nucleic acids
Nanotech: Quantum Dots, Lab-on-Chip, .../...

7

List of immediate challenges according to WHO

WHO List of urgent global health challenges for the new decade

8

What are the main markets ?

- Key sub-markets within Molecular Diagnostics includes, among others :
- **Infectious Disease**
- **Pharmacogenomics**
- **Oncology testing**
- **Cardiology**
- **Anatomy/Pathology**
  (Formalin Fixed Paraffin Embedded material, FFPE analysis)

BASICS

9

What is « Precision Medicine »

Precision medicine : *encompasses all activities required to understand diseases at the most precise level; requires combination of genomics and genetics, basic science and medicine*

Personalized medicine : *process of delivering the medical care based on all the informations that is obtained through the scientific processus mentioned above*

involve social, business, ethics, medical, financial issues

BASICS

https://www.youtube.com/watch?v=fxttsmaglo4

10

Goal of Personalized Medicine

It is to achieve the "5 Rs":

•(1) Right patient
•(2) Right diagnosis
•(3) Right treatment
•(4) Right drug/target
•(5) Right dose/time

•*depends on accuracy of tests and prevalence of disease. In fact, positive and negative predictive values are more important for clinicians than the sensitivity and specificity of the test*

BASICS

11

What is « Precision Medicine » ? def.2

Precision medicine can also be defined as : « *a medical model that proposes the customization of healthcare, with medical decisions, practices, and/or products being tailored to the individual patient* ».

Why that ? was'nt is already the case before ?

Yes it was, but Technology now allows to target individual patients with more chances of success

BASICS

12

What is « Translational Medicine » ?

Interdisciplinary Medical research

Translational medicine is defined as : « *an interdisciplinary branch of the biomedical field supported by three main pillars:* ***benchside, bedside and community*** ».

BASICS

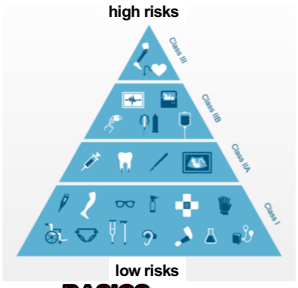
https://www.youtube.com/watch?v=BvL21QDpwcw

Medical Devices Definition and Classification

The WHO definition for a medical device

An article, instrument, apparatus or machine that is used in the prevention, diagnosis or treatment of illness or disease, or for detecting, measuring, restoring, correcting or modifying the structure or function of the body for some health purpose. Typically, the purpose of a medical device is not achieved by pharmacological, immunological or metabolic means.

www.who.int/medical_devices/definitions/en/



BASICS

What are IVDs, what is Companion Diagnostics, what is a Biomarker ?

- ***In Vitro* Diagnostics** (IVDs) *are assays that probe samples taken from a patient (urine, blood, nasal, swabs, biopsy, etc.) for molecular, genomic, epigenomic, or proteomic species to aid in the clinical diagnostics, prognostics or in treatment selection.*
- **IVD juridical status is different from RUO status and requires certification**
- Specific IVDs that are **developed in parallel with a therapeutic agent**, and are used in conjunction with one another as specified on the labeling of the drug and device, are called **Companion Diagnostics**

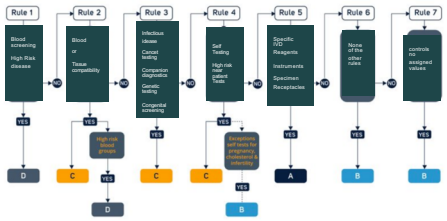
Specific analytes that IVDs probe are considered "**Biomarkers**" *if they can be objectively measured and evaluated and indicate normal or pathogenic biologic processes or pharmacologic responses to a particular therapeutic intervention*

BASICS

Class A to C of IVD regulations, based on Risk Assessment

CE

Figure 1. IVDR Classification



There will be significantly more products in Class D as compared to Annex II List A of the IVDD. The largest proportion of IVDs will fall into Class B, but there are a significant number of devices, including companion diagnostics, cancer diagnostics and those for infectious diseases falling into Class C. Classes B, C and D will require oversight by an NB who will sample more Class C devices than Class B. Only instruments and simple devices, such as wash solutions, will remain in Class A and continue to be self-declared.

https://www.raps.org/news-and-articles/news-articles/2019/9/the-essential-ivdr-and-the-challenges-it-presents

IVD and MD regulations texts EU

References

1. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02017R0745-20170505. Accessed 9 September 2019.
2. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02017R0746-20170505. Accessed 9 September 2019.
3. Kirsh D. "PIP Breast Implant Scandal: A Story That Triggered Change." 13 November 2017. Mass Device Medical Network. https://www.massdevice.com/pip-breast-implant-scandal-story-triggered-change/. Accessed 9 September 2019.
4. Principles of In Vitro Diagnostic (IVD) Medical Devices GHTF/SG1/N045:2008 Classification. http://www.imdrf.org/docs/ghtf/final/sg1/procedural-docs/ghtf-sg1-n045-2008-principles-ivd-medical-devices-classification-080219.pdf. Accessed 9 September 2019.
5. GHTF SG1(PD)/N046R3: Principles of Conformity Assessment for In Vitro Diagnostic (IVD) Medical Devices. International Medical Devices Forum (IMDF) website. http://www.imdrf.org/ghtf/ghtf-archives-sg1.asp. Accessed 9 September 2019.
6. ISO 13485 Medical Devices. International Standards Organization (ISO) website. https://www.iso.org/iso-13485-medical-devices.html. Accessed 9 September 2019.

https://health.ec.europa.eu/medical-devices-sector/new-regulations_en

History of Technologies in Analytical Chemistry

Chemistry -> Physical-Chemistry *Physical basis of chemical systems and processes*

-Physical analysis

-Chemical analysis (decomposition) pyro, hydro, electrolytic process of a sample or a mix (separation : physical), Filtration, Screening, Chemical reaction

-Spectroscopy (e.g. spectrophotometry)

-Physical techniques of separation of chemical species:

-**Chromatography** (Gel Permeation, Ion Exchange, Gaz, Liquid)

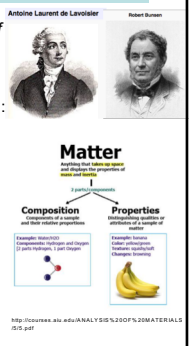
-**Electrophoresis**

-Physical techniques of transformation analysis

-*Kinetics, Statistics, electrochemistry, thermodynamics*

-**Optics**

-**Electrochemistry/Electronics**



Chromatography (Day 1903, Tswett 1906)

« A scientific method to separate chemicals in a substance or a mix, by passing it through a material such as paper »

Mobile versus **stationary** phase
Preparative versus **Analytical** (not exclusive) :
Objectives: purification or measuring
Support : Column/Surface, Paper, Thin-Layer, Gaz, High-Perf Liquid Chr.

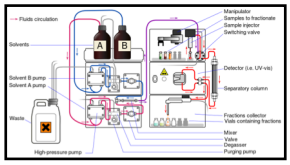
Principle of separation
1. Adsorption
2. Partition
3. Ions-Exchange
4. Exclusion-Diffusion
5. Affinity

Techniques by chromatographic bed shape
--Column, Planar (paper, thin layer)

Techniques by physical state of mobile phase
-Gaz, Liquid

Techniques by separation mechanism
- Ion exchange
- Size exclusion
- Affinity (protein A, oligodT…)

Special techniques
- Reversed-phase chromatography, Two D chromatography, Simulated moving-bed chromatography, Pyrolysis gas chromatography, Fast protein liquid chromatography (FPLC), Countercurrent chromatography



A preparative HPLC
https://fr.wikipedia.org/wiki/Chromatographie_pr%C3%A9parative

19

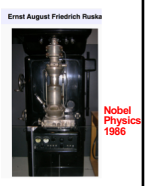
History of technologies : Optics, fluorescence

Observation

Invention of optical microscopy (XVIIth), later Electronic (1933-1965) and laser-based.
*confocal microscopy: allows studies on fixed material but also on dynamic phenomenon on cells, or on living tissus in particular thanks to GFP (**G**reen **F**luorescent **P**rotein) or similar molecules.*

microscope 1751

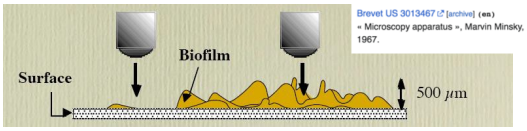
Besides, permanent evolution of techniques allow today to study **Molecular Interactions** (FRET), or molecular dynamics



20

Example : Biofilms can be analyzed by microscopical techniques

Confocal Laser Scanning Microscopy (CLSM) coupled to fluorescence



Brevet US 3013467 [archive] (en)
« Microscopy apparatus », Marvin Minsky, 1967.

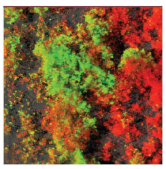


Figure 3: Mixed bacterial biofilm: the red or green fluorescent bacteria are visualized by use of CLSM. Bacteria which are present in the biofilm are *P. putida* OUS82 (Gfp labeling) and *Pseudomonas* sp. B13 (DsRed labelling) one may clearly distingusih micro-colonies of the two species (red or green) However within each micro-colony, one may also see some bacteria from the other species

M/S n° 1, vol. 19, janvier 2003

(extracted from Christine Houssin course on Biofilms, 2019)

21

Biosensor : Definition

- ***A device that detect chemicals and transform signals***

The modification of the signal is proportional to the target concentration. Techniques may be : electrochemistry, piezolectricity, conductimetry, spectrophotometry, calorimetry, etc…

ANALYTE -> BIOSENSOR -> TRANSDUCER -> SIGNAL
ANALYTE = enzyme, DNA/RNA, antigen, poluent, antibody,
BIOSENSOR = antibody, aptamer, DNA/RNA/PNA, enzyme
TRANSDUCERS = polymers, carbon nanotubes, graphen, metallic nanoparticles,
SIGNAL: electrochemical, colorimetric, conductimetric, optic, piezoelectric, spectrophotometric

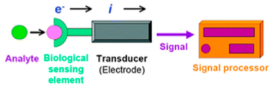


Figure 1. Scheme of a biosensor with electrochemical transducer [2]. Copyright 2010. Reproduced with permission from The Royal Society of Chemistry.
Huang et al. Sensors 2017, 17, 2375; doi:10.3390/s17102375

22

Optics & Fluorophores
Spectrophotometric techniques
(FTIR, Raman, UV/vis,…)

- **Photophysical characteristics of Fluorophores**

They are fluorescent chemical compounds that **can re-emit light upon light excitation**. Different characteristics allow to define

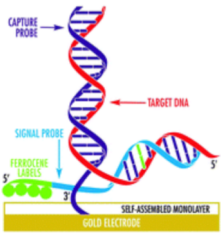
- **Excitation and Emission spectrum**. Represent the signature of the energetic structure of the fluorophore. The difference in wavelengths is called **Stokes** displacement.
- **Molar extinction coefficient** (ε). Correspond to the absorbance capacity by the fluorophore of energy provided by a photon at a given wavelength.
- **Quantum yield** (Φ). It characterize the capacity of the fluorophore to re-emit under appropriate light form, the absorbed energy. It is defined as the emission efficiency of the fluorophore.

$$\Phi = \frac{\#\ \text{photons emitted}}{\#\ \text{photons absorbed}}$$

- **Fluorescence lifetime** (τ). represent the mean duration in the excited state of the fluorophore

23

Electrons & Electric fields
Electrochemical detection



Ronkainen et al. 2010
Electrochemical biosensors

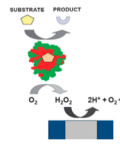


Figure 2. First-generation biosensor that depends ambient oxygen with amperometric detection [2] Copyright 2010. Reproduced with permission from The Royal Society of Chemistry.

Huang et al. Sensors 2017, 17, 2375; doi:10.3390/s17102375

sensors — MDPI

Review
Disease-Related Detection with Electrochemical Biosensors: A Review

Huang et al. Sensors 2017, 17, 2375; doi:10.3390/s17102375

24

Genetically-based Biosensors
synthetic biology to detect tumor cells

CANCER DETECTION

Engineered bacteria detect tumor DNA

Robert M. Cooper[1], Josephine A. Wright[1], Jia Q. Ng[1], Jarrad M. Goyne[1], Nobumi Suzuki[2,3], Young K. Lee[4], Mari Ishiwata[2,3], Georgette Radford[5], Franqui J. Ryan[2,4], Shuki Kumar[6], Elaine M. Thomas[6], Laura Virbasius[7], Rob Knight[7,8,9,10], Susan L. Woods[2,3], Daniel L. Worthley[2,3], Jeff Hasty[1,2,3,11]

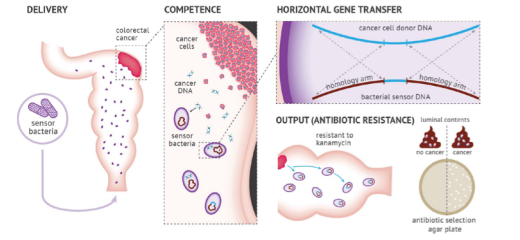


Fig. 1. Engineered bacteria to detect tumor DNA. Engineered *A. baylyi* bacteria are delivered rectally in an orthotopic mouse model of CRC. The naturally competent *A. baylyi* take up tumor DNA shed into the colorectal lumen. The tumor donor DNA is engineered with a *kanR* cassette flanked by *KRAS* homology arms. The sensor bacteria are engineered with matching *KRAS* homology arms that promote homologous recombination. Sensor bacteria that undergo HGT from tumor DNA acquire kanamycin resistance and are quantified from luminal contents by serial dilution on antibiotic selection plates.

Cooper et al., Science 381, 682–686 (2023)

25

Aptamer : definition

- DNA or RNA aptamers : ***short single-strand oligonucleotides that possess properties of MAb***
- They can be used as *bioreceptors* in *biosensors* or in *medical therapies* (*aptamer-based assays, n=2113 Hits on Jan 24th 2025*) **Systematic Evolution of Ligands by Exponential Enrichment (SELEX)**
  - **Their specificity is comparable to that of antibodies**
  - **Aptamers dissociation constants are in the range of nanomolar to picomolar**
  - **High afficity to proteins or to other molecules (toxins)**

What are their advantages ?

They are much more stable than antibodies
Hence they are suitable at high temperatures or extreme pH
They can be regenerated without loss of integrtity and selectivity
They can form loop or quadriplex structures
These structures are at the basis of their selectivity

Capture-SELEX: Selection Strategy, Aptamer Identification, and Biosensing Application *SY Lam et al. Biosensors 2022*

26

CHEMICAL REVIEWS

pubs.acs.org/CR Review

Aptamer-Based Detection of Circulating Targets for Precision Medicine

Lingling Wu, Yidi Wang, Xing Xu, Yilong Liu, Bingqian Lin, Mingxia Zhang, Jialu Zhang, Shuang Wan, Chaoyong Yang,* and Weihong Tan*

Chem. Rev. 2021, 121, 12035−12105

SELEX and **Aptamer** and **Artificial Intelligence**
25 Hits in Pubmed on Jan. 24, 2025

27

Aptamer and Covid 19 Pubmed, n=449 on Jan 24th 2025

analytical chemistry

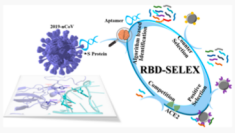
Anal Chem. 2020
pubs.acs.org/ac Article

Discovery of Aptamers Targeting the Receptor-Binding Domain of the SARS-CoV-2 Spike Glycoprotein

Yanling Song,* Jia Song, Xinyu Wei, Mengjiao Huang, Miao Sun, Lin Zhu, Bingqian Lin, Haicong Shen, Zhi Zhu, and Chaoyong Yang*

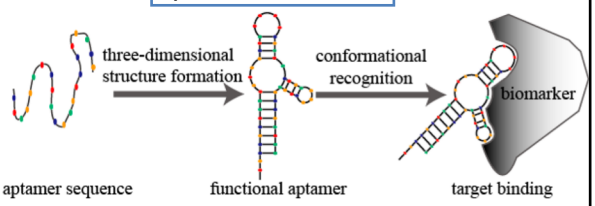
Cite This: https://dx.doi.org/10.1021/acs.analchem.0c01394 Read Online
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The World Health Organization has declared the outbreak of a novel coronavirus (SARS-CoV-2 or 2019-nCoV) as a global pandemic. However, the mechanisms behind the coronavirus infection are not yet fully understood, nor are there any targeted treatments or vaccines. In this study, we identified high-binding-affinity aptamers targeting SARS-CoV-2 RBD, using an ACE2 competition-based aptamer selection strategy and a machine learning screening algorithm. The K_d values of the optimized CoV2-RBD-1C and CoV2-RBD-4C aptamers against RBD were 5.8 nM and 19.9 nM, respectively. Simulated interaction modeling, along with competitive experiments, suggests that two aptamers may have partially identical binding sites at ACE2 on SARS-CoV-2 RBD. These aptamers present an opportunity for generating new probes for recognition of SARS-CoV-2 and could provide assistance in the diagnosis and treatment of SARS-CoV-2 while providing a new tool for in-depth study of the mechanisms behind the coronavirus infection.



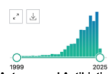
G19b

Aptamer mode of action



http://www.mdpi.com/1420-3049/20/7/11959/htm
Molecules **2015**, *20*(7), 11959-11980; doi:10.3390/molecules200711959
Review A Highlight of Recent Advances in Aptamer Technology and Its Application
Hongguang Sun and Youli Zu *

Aptamer-Based Biosensors for Antibiotic Detection Aptasensors



Aptamer Sensors for the **Detection** of **Antibiotic** Residues- A Mini-Review.
Liang G, Song L, Gao Y, Wu K, Guo R, Chen R, Zhen J, Pan L.
Toxics. 2023 Jun 7;11(6):513. doi: 10.3390/toxics11060513.
PMID: 37368613 Free PMC article. Review.

Aptamer and Antibiotic detection, n=863 hits on Jan 24th 2025

Generalization : Aptasensor Definition

n=4448 hits in Pubmed on Jan 24th 2025

- Aptasensor refers to a **sensing technology** that utilizes **Nanotechnology** and **Aptamers**, which are synthetic nucleic acid ligands with various functions. Aptasensors have been developed for applications in diagnostics, sensors, therapeutic agents, and research procedures

30

Retrospective History of some important papers

1. **"Aptamers as Biosensors" (2001)** – This paper, published in *Trends in Biotechnology*, was one of the early reviews that highlighted the potential of aptamers as biological recognition elements. While the term "aptasensor" wasn't specifically used, the paper set the stage for understanding how aptamers could be integrated into sensor technologies.
2. **"Aptamer-based Biosensors" (2004)** – This paper, published in *Analytical and Bioanalytical Chemistry*, was one of the first to describe aptamer-based sensors explicitly. It focused on the development of aptamer-based detection systems, emphasizing their specificity, stability, and ability to work in real-time biosensing applications.
3. **"Aptasensors for the Detection of Small Molecules" (2005)** – Another important paper that defined aptasensors and outlined their potential in various applications, such as detecting drugs, toxins, or environmental contaminants. It was around this time that the term "aptasensor" began to be widely used to refer to sensors that combined aptamers with different detection techniques (like electrochemical or optical sensors).
4. **"Electrochemical aptasensors" (2006)** – Published in *Biosensors and Bioelectronics*, this article was key in the advancement of aptasensor technology, focusing on electrochemical sensors that incorporated aptamers for highly sensitive detection. This helped solidify the practical application of aptasensors in analytical chemistry.

31

Kadam *et al. Applied Biological Chemistry* (2023) 66:13
https://doi.org/10.1186/s13765-023-00771-9

KSABC

REVIEW Open Access

Aptamer-based CRISPR-Cas powered diagnostics of diverse biomarkers and small molecule targets

Ulhas Sopanrao Kadam[1*], Yuhan Cho[1], Tae Yoon Park[2] and Jong Chan Hong[1,3*]

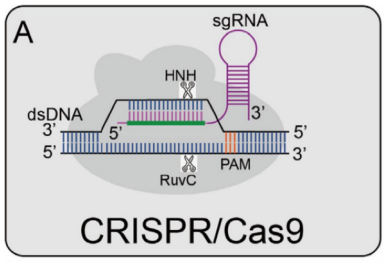
Kadam et al. 2023

Table 1: Main Characteristics of the Cas proteins used in diagnostic

Table 1 Salient features of various Cas proteins used in diagnostics

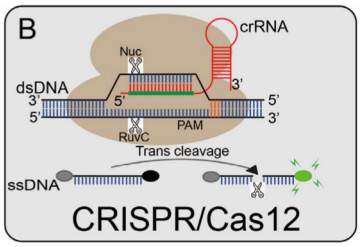
Cas Protein	Class	Target	PAM	Collateral Activity	Refs.
Cas9	Class 2	dsDNA	NGG	No	Huang et al. 2018
Cas12a	Class 2	Both (ss/dsDNA)	TTTN	Yes (ssDNA)	Li et al. 2019, Chen et al. 2018 Gootenberg et al. 2017
Cas12b	Class 2	Both (ss/dsDNA)	TTN	Yes (ssDNA)	Li et al. 2019
Cas13a	Class 2	ssRNA	–	Yes (ssRNA)	Gootenberg et al. 2017
Cas13d	Class 2	ssRNA	–	Yes (ssRNA)	Feng et al. 2022
Cas14a	Class 2	ssDNA	–	Yes (ssDNA)	Harrington et al. 2018

Kadam et al. 2023



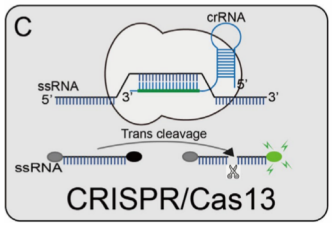
A. Cas9 can cleave the target and non-target strands of DNA; a short trinucleotide PAM is also essential for the initial DNA binding

Kadam *et al*. 2023



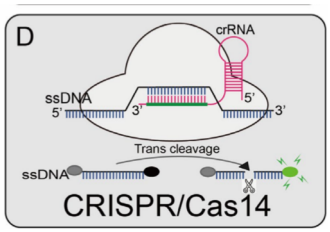
B. Cas12a can cleave dsDNA under the guidance of gRNA. The Cas12a enzyme recognizes the PAM of the original T-rich spacer and then recognizes the target sequence to generate PAM distal dsDNA breaks with staggered 5′ and 3′ ends, and Cas12 has the side chains trans-cleavage activity. At the time that the sgRNA-guided DNA is combined in Cas12, Cas12 will release a powerful, indiscriminate single-stranded DNA (ssDNA) cleavage activity

Kadam et al. 2023



C. Cas13 can activate its single-stranded RNA (ssRNA) cleavage activity by binding to crRNA, and it has a additional cleavage activity triggered by the target RNA

Kadam *et al*. 2023



D Cas14 protein is a RNA-guided nuclease and can recognize the target ssDNA without restriction sequences and cleave it, and also can non-specifically cleave the surrounding ssDNA nucleases molecule

Kadam et al. 2023

Table 2 Key representative examples of CRISPR-Cas proteins and aptamers in diagnostic assays of variety of targets

Target	Signal	CRISPR-Cas Effector	LOD	Refs.
DNA methylation	Fluorescence	Cas12b	10^{-8} nM	[6]
Extracellular vesicle	Fluorescence	Cas12a	100 particles/mL	[88]
Extracellular vesicles	Fluorescence	Cas12a	100 particles/μL	[89]
ATP	Fluorescence	Cas12a	0.39 μM	[67]
Na^+	Fluorescence	Cas12a	0.21 μM	[67]
Aflatoxin B1 (AFB1)	Biolayer interferometry (BLI)	Cas12a	0.8 ng mL − 1	[90]
Salmonella typhimurium	Electrochemical	Cas12a	20 CFU/mL	[38]
Bacillus cereus	Fluorescence/RNA Light-Up	Cas13a	10 CFU	[91]
PDGF-BB	Fluorescence	Cas12a	0.75 pM	[29]
Telomere	Fluorescence	Cas9	–	[92]
17β-estradiol	Raman sensing/LFA	Cas12a	10 pM	[93]
Thrombin	Electrochemical	Cas12a	1.26 fM	[40]
ATP and Na^+	LRET	Cas12a	~ 18 nM and ~ 0.37 μM	[68]
Prostate-specific antigen (PSA)	Colorimetric/AuNPs	Cas12a	0.030 ng/ mL	[69]
Cardiac troponin I (cTnI)	Fluorescence	Cas13d	12.6 pM	[87]

Kadam et al. 2023




Transistor: John Bardeen, Walter Brattain, and William Shockley
Information Theory: Claude Shannon
UNIX Operating System: Ken Thompson, Dennis Ritchie, and others
C Programming Language: Dennis Ritchie
Fiber Optic Communications: Charles Kao
Digital Signal Processor (DSP): Gene Franz
Charge-Coupled Device (CCD) Willard Boyle and George E. Smith

20 Nobel Prizes…

39

Why is mathematic foundation of computer sciences important ?


la Pascaline


Blaise Pascal


Alan Turing


John Presper Eckert John William Mauchly
ENIAC (1946)


Charles Ranlett Fint


Thomas J. Watson Sr.


John Backus

40

G20

Technologies are at the heart of innovation in medical diagnostics-1


Nobel 2000
Jack Kilby
(integrated circuit 1958)


Wallace H. Coulter (1913-1998)
(coulter principle,
Industry Coulter®)


(Invention of the transistor 1947)
John Bardeen William Shockley, Walter Houser Brattain


Nobel 1948
Arne Tiselius,
(Electrophoresis 1937)


Nobel 1926
Theodor Svedberg
(Ultracentrifugation 1949)


Leonard Skeggs
(precursor of Multiplexed analysis)


Arnold Beckman (1900-2004)
(pH-meter, quartz spectrophotometer)

41

G21

Technologies are at the heart of innovation in medical diagnostics-2


Marvin Minsky (1924-2016)
(inventor of the confocal microscop,
confocal laser scanning microscopy
(CLSM)


Nobel 1984
Robert Bruce Merrifield,
(1921-2006)
Solid phase peptide synthesis


Nobel 1968,
Har Gobind Khorana,Robert Holley, Marshall Nirenberg
(The triplet and the chemical synthesis of DNA)


Nobel 1980,
W. Gilbert, F. Sanger
(DNA sequencing method)


Nobel 1993,
Kary Mullis, M.Smith
(PCR)


L. Hood
(DNA and Protein synthetizer,sequencer)


P. Nyren, M. Ronaghi
(pyrosequencing principle)

42

G22

Technologies are at the heart of innovation in medical diagnostics-3

Chad Mirkin
(dip pen nanolithography Spherical DNA)

Sir Shankar Balasubramanian
SBS sequencing

David Klenerman
SBS sequencing

Pr. Hagan Bailey
Nanopores

Dr Spike Willcocks

Dr Gordon Sanghera

(Oxford nanopore sequencing) Minion

Jennifer Doudna Emmanuelle Charpentier
CRISPR Cas9
Nobel 2020

43

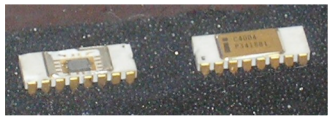
Before transistor: vacuum tube, electromechanical relay

ENIAC (1946)

Z3 (Allemagne, 1941), Colossius (UK)

Electronic Numerical Integrator Analyser and **Computer**= ENIAC

After transistor: integrated circuit (electronic chip)



with 2 300 transistors and and execution power of 90 000 computations per sec with a maximal frequency of 740 kHz, it is comprable to ENIAC, that occupied 167 m2 for a total weight of 30 tons.

Intel 4004 processor

44

G25

History of technologies : Electronics

- **Year 1948 : Transistor *trans*fer res*istor***

Semi-conductor device:

Amplifies, switches electronic signals and electric power

*« semi-conductor device with three active electrodes (**drain, source gate**), that allow to control an electric voltage on an output electrod (the **collector** for bipolar transistor and the **drain** on a field effect transistor) thanks to an entry electrode (the **basis** on a bipolar transistor and the **grid** on a field effect transistor) »*
A *« Tap water for electron » is a useful metaphore but the the **gate** doesn't precisely control the flow of electrons like a tap controls water; instead, it **modulates the conductivity of a semiconductor material** between the source and drain terminals.*

45

G26

- **Transistor** is a semiconductor device used to amplify and switch electronic signals and electrical power. It is composed of a semiconductor material with at least three terminals for connection to an external circuit. A voltage or current applied to one pair of the transistor's terminals changes the current flowing through another pair of terminals. Because the controlled (output) power can be higher than the controlling (input) power, a transistor can amplify a signal. Today, some transistors are packaged individually, but many more are found embedded in integrated circuits.
- ***The transistor is the fundamental building block of modern electronic devices, and is ubiquitous in modern electronic systems. Following its development in the early 1950s the transistor revolutionized the field of electronics, and paved the way for smaller and cheaper radios, calculators, and computers, among other things.***

https://fr.wikipedia.org/wiki/Transistor

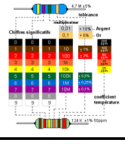
Méthode infaillible pour distinguer un transistor d'un insecte: l'insecte (à droite) a 6 pattes, alors que le transistor (à gauche) n'en a que trois.

46

Electronic components

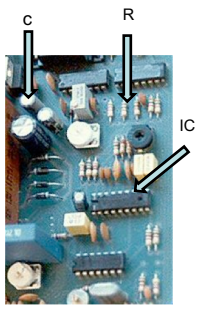
- **Definition** : *any basic **discrete device** or physical entity in an electronic system used to affect electrons or their associated fields*
- **Active Component**
  - **Allows to increase the power of a signal** (tension/voltage, courant/electric power, or both/)
  - **Ex: transistor, integrated circuit**
- **Passive Component**
  - **Does not Allow to increase the power of a signal** (in certain cases, it reduces the power, often by Joule effect
  - **Ex: resistance, condensor, bobine/coil, passive filter,power transformer, diode**

Capacitor/**conden sateur**



resistor/**resis tance**

transistor



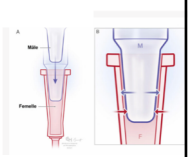
intégrated circuit

47

Mechanical components

ISO 80369-7

In 1930, Fairleigh S. Dickinson of **Becton Dickinson** , who had held the exclusive rights to market the Luer syringe in the USA since 1898, patented the “Luer-Lok”, a steel connector that attaches the needle to the syringe.



Baxter (intravenous perfusion) vacoliter®

https://www.19141918.be/soigner_perfusion.php
https://www.apsf.org/fr/article/gerer-les-raccords-luer/

48

Before the Coulter Principle (cell counter)
Malassez cell

"Use of an electric field to count, measure diluted suspensions of particles in conducting liquids".

The phrase was coined when Wallace H. Coulter was awarded US Patent #2,656,508, **Means for Counting Particles Suspended in a Fluid**.

The Coulter Principle is most commonly employed in a COULTER COUNTER®, which is an **Analytical Instrument designed for counting cells**. However, there are numerous other ways to implement the Coulter Principle.

To date, the most commercially successful application of the Coulter Principle is in **Hematology**, where it is used to obtain information about patients' blood cells. While the Coulter Principle and Coulter Counters have appeared in many thousands of peer-reviewed research papers over the past 5 decades, most people remain unfamiliar with it unless they have personally encountered it in a laboratory environment. In the1970s, this technological revolution was completed by introducing quantitative cellular cytochemistry using peroxidase, lipase, and alcian blue activity.


Malassez cell


The first cell counter

https://en.wikipedia.org/wiki/Coulter_counter#/media/File:Model_A_COULTER_COUNTER_from_Advertisement.jpg

49

Evolution of Hematological methods

- **In 1999**, the first report on the WHO classification of hemopoietic and lymphoid tissue tumors was published. It was a consensus classification using all available information (**morphology, cytochemistry, immunophenotype, genetics, and clinical features**) to define clinically significant disease entities
- **in 2022**, the International Consensus Classification and WHO 5th edition,the majority of diagnostic and prognostic subtypes are based on **molecular and next generation sequencing** tests.

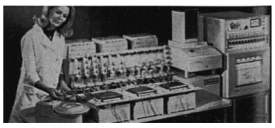
Hematological cytomorphology: Where we are, By G. Zini, 2024. DOI: 10.1111/ijlh.14330

50

Medical Diagnostic history. :Biochemistry.
Automates device in clinical chemistry : DuPont introduces the ACA, while Technicon introduces the SMA/12...

Total Proteins, Glucose, Ca^{2+}, PO_4^{3-}, creatinin, urea, bilirubin, ALAT, ASAT, ALP, Cholesterol, TG


The DuPont Automatic Clinical Analyzer in the Medium-Sized Community Hospital


Analytical ancestry: evolution of the array in analysis. Kricka LJ, Imai K, Fortina P. Clin Chem. 2010

Alexander 1974 Laboratory Medicine **Kricka *et al.* 2010**

51

G34

FACS
Flow cytometry and Microbiology recovers >22000 Articles on January 2024 on PubMed

Flow cytometry and FACS (Fluorescence Activated Cell Sorting) technology.

Technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both research and clinical practice

Allows to study: **Physical properties : size, shape**

Allows to study: **Biochemical properties : cell-cycle distribution, DNA content**

*Applications: **Immunology**, cell and mol. Biology, intra cell cytokine measurements, cell cycle, gene expression levels analysis, vaccine analysis, phagocytosis,…*

Review > Curr Protoc Cytom. 2018 Apr;84(1):e37. doi: 10.1002/cpcy.37.

Overview of Flow Cytometry and Microbiology

52

G29

RIA, EIA, ELISA…and other Tools…born in the 70s…80s… and more recently

- **RIA-EIA-ELISA (simplex)**
  Rudolf M. Lequin. Clin. Chem. 2005, 51, 2415-2418
- **Automated Pipetting Devices** Micromedics, Hamilton
- **Multichannels Pipettes** Lab Systems
- **Fully automated** Test instruments: Becton-Dickinson, Boehringer-Mannheim, Abbott, Siemens, Hitachi, Roche, Biomérieux
- **Robotics** Tecan, Perkin-Elmer, Hamilton
- **Mass-Spectrometry** Brücker, Biomerieux, ScoPE-MS
- **Genomics instruments** Illumina, Oxford Nanopore, Pacific biosciences
- **Single Cell analyzer or Single-Molecule analytic instruments.** Quanterix, ...

53

G28

Tomorrow's diagnostic : combining technologies
= Integration & miniaturization

- **1960s** : *transistor networking, miniaturization, basis of the computer science revolution thanks to power increase, fiability, cheaper and in miniaturized volumes, (integrated circuits)* **microelectronics**. **1975** : invention of **molecular biology**
- **1980s** : **MEMS= Micro-Electro-Mechanical Systems** word invented to describe *sophisticated miniaturized mechanical systems* that are built industrially, with the promess of the similar benefit to biology as was the case for integrated circuits and microelectronics in the 70-80.
- **Micromechanics**, **Microfluidics** : *science & technology of fluids processing systems with at least one dimension in the order of* **micrometer**
- **2000s** **Nanobiology, Nanomaterials** : **Lab-on-Chip,** *discovery of the new physico-chemical properties of material at nano-molecular scales* , **IT development.** (« poussière intelligente » : *smart dust (n=149 hits),* micro and nanosensors) **growth of Data-mining and Machine learning**
- **2010s : studies of single cells, studies of single molecules** (nanopores),
- **2020s : AI**

54

Microfluidics and MEMS

Microfluidics *deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale. Typically,* ***micro*** *means one of the following features*:

- **small volumes** (nL, pL, fL), **small size**
- **low energy consumption**, less reagent consumption
- **Increases throughput** through parallel processing

Low price of several modules allowing integration of analytical processes

"move, mix, control and react with fluids volumes in the micron range"

MEMS=micro-electro-mechanical systems. (C-MEMS and C-NEMS)

Carbon microelectromechanical system (C-MEMS) and carbon nanoelectromechanical system (C-NEMS)

(MEMS and antimicrobioresistance. ***n=7326*** *articles in PubMed in Jan 2025)*

55

DNA Synthesis

[16] An automated DNA synthesizer employing deoxynucleoside 3'-phosphoramidites

Suzanna J. Horvath, Joseph R. Firca, Tim Hunkapiller, Michael W. Hunkapiller, Leroy Hood

http://dx.doi.org/10.1016/0076-6879(87)54082-4, How to Cite or Link Using DOI
Permissions & Reprints

oligomaker

DNA. 1984 Oct;3(5):401-11.

Construction and evaluation of an instrument for the automated synthesis of oligodeoxyribonucleotides.

Warner BD, Warner ME, Karns GA, Ku L, Brown-Shimer S, Urdea MS.

Abstract

The details for constructing an easily used and maintained laboratory instrument capable of routine oligodeoxyribonucleotide synthesis are presented. The synthesizer consists of relatively inexpensive, commercially available components and is controlled by an Apple IIe computer. Since reagents are distributed from pressurized reservoirs through a liquid manifold by opening solenoid-activated valves, no pump is required. More than 600 oligomers containing up to 122 bases have been produced with a condensation cycle time of approximately 15 min with apparent coupling yields of 98.5%. A unique bidirectional flow reactor and controlled reagent distribution system provide for rapid mass transfer during solvent and reactant equilibrations and for long-term stability of reagent solutions. Aspects of the system should also find use in other solid-phase synthetic and analytical strategies.

56

Peptide Synthesis. (FMOC-SPPS)

[Contribution from the Rockefeller Institute, New York 21, N. Y.]

Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide[1]

By R. B. Merrifield

Received January 31, 1963

A new approach to the chemical synthesis of polypeptides was investigated. It involved the stepwise addition of protected amino acids to a growing peptide chain which was bound by a covalent bond to a solid resin particle. This provided a procedure whereby reagents and by-products were removed by filtration, and the recrystallization of intermediates was eliminated. The advantages of the new method were speed and simplicity of operation. The feasibility of the idea was demonstrated by the synthesis of the model tetrapeptide L-leucyl-L-alanylglycyl-L-valine. The peptide was identical with a sample prepared by the standard *p*-nitrophenyl ester procedure.

http://cem.com/friliberty-lite/

Beilstein J Org Chem. 2014; 10: 1197–1212.
Published online 2014 May 22. doi: 10.3762/bjoc.10.118
PMCID: PMC4077397

Automated solid-phase peptide synthesis to obtain therapeutic peptides

Veronika Mäde,[1] Sylvia Els-Heindl,[1] and Annette G Beck-Sickinger[*1]
Peter H Seeberger, Editor-in-Chief

J Pept Sci. 2016 Jan; 22(1): 4–27.
Published online 2016 Jan 20. doi: 10.1002/psc.2836

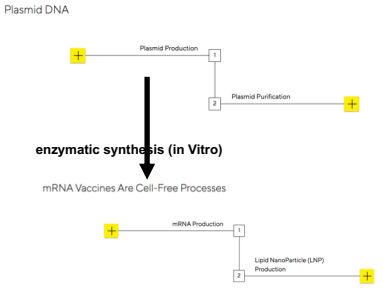
Advances in Fmoc solid-phase peptide synthesis

Raymond Behrendt,[1,*] Peter White,[2,*] and John Offer[3,*]

57

mRNA Synthesis,

vaccines, gene therapy, research



1.Chemical Modification
2.Cap Structure Modification
3. Poly(A) Tail Optimization
4.Sequence Optimization
5.In Vitro Transcription (IVT) T7, T3, SP6, RNA POL II
6. Purification Techniques

https://www.sartorius.com/download/525728/4/mrna-vaccines-biopharma-industry-white-paper-en-b-sartorius-data.pdf

58

G35

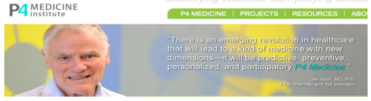
Combining sciences and Technologies and Data Analysis

Microelectronics, Molecular Biology, Genomics (NGS), Micromecanics, Microfluidics, Optics, Bioinformatics, Data Analysis

Optofluidics : Optics and Microfluidics combined to achieve new functionalities on a small chip -----> impact on **Point of Care(POC) Diagnostics.**

-omics technology (gen, trancript, proteo, metab, lipid, glyc, meta)

Bioinformatics/Statistics/Big Data/AI



predictive
participatory
preventive
personalized

59

Omics

Name	Definition	Technique
Genomics	the study of the complete set of genes within an organism	NGS
Transcriptomics	study of the complete set of RNA transcripts produced by the genome	Microarrays and RNA-Seq
Proteomics	study of the complete set of proteins within a cell, tissue, or organism	MassSpec 2D-EP, Microarrays
Metabolomics	study of the complete set of small molecules, known as metabolites, within a biological sample	NMR, MassSpec
Lipidomics	study of the complete set of lipids within a biological system.	MassSpec
Glycomics	study of the complete set of carbohydrates (glycans) within a biological system	MassSpec, Chromato Microarrays
Epigenomics	study of epigenetic modifications, such as DNA methylation and histone modifications, across the genome	ChIP, bisuflite seq.
Metagenomics	study of genetic material recovered directly from environmental samples, allowing the analysis of microbial communities	NGS

60

G37

in Medicine

***Point of care diagnostics* (POC)**

***Lab on chip* (LOC)**

***Cancer companion* diagnostic tests.**

Theranostics **: prostate-specific membrane antigen (PSMA)–targeted radioligands**

Pharmacogenetics, proteomics and biomarker profiling forms the backbone of theranostics

- ***Companion:*** *The use of molecular diagnostics for detecting variations such as mutations or amplifications of specific genes, in order to target therapies to patients who are most likely to benefit, is becoming increasingly common in anticancer drug development.*
- For example, there are already several FDA-approved diagnostics for detecting amplification of the gene encoding human epidermal growth factor receptor 2 (**HER2; also known as ERBB2**) to guide the use of **trastuzumab** (Herceptin; Genentech/Roche), a monoclonal antibody (mAb) specific for HER2, in patients with breast cancer.

61

***examples of* POC**

- **Rapid Blood Tests**
- **Rapid Infectious Disease Tests**
- **Pregnancy Tests**
- **Coagulation Monitoring**
- **Cardiac Markers**
- **Glucose Monitoring**
- **Hematology Tests**
- **Urine Tests**
- **Diagnostic Imaging**
- **Infectious Disease Diagnostics**

Issues in Companion Diagnostic test use

1.Patient Stratification
2.Targeted Therapies
3.Treatment Selection
4.Reducing Trial and Error
5.Risk Assessment
6.Regulatory Approval
7.Ongoing Monitoring

62

G38

Current and Emerging technologies for Clinical microbiology laboratories

1. **Nucleic Acid Sequencing Methods**
   Sanger Sequencing, NGS
2. **Molecular Methods (Nucleic Acid based)**

2.1.Single Plex Nucleic Acid tests
2.1.1 nucleic acid amplification, including PCR
2.1.2 **LAMP** and HDA technologies
2.1.3 Automation of NAATs and impact on laboratory workflow and patient care

2.2. Multiplex Nucleic Acid tests
2.2.1 Multiplex PCR and Probe Detection
2.2.2 Microarray methods
2.2.3 Impact of large multiplex panel on laboratory workflow and patient care

1. **Mass Spectrometry**
   3.1. Matrix-Assisted Laser Desorption Ionization time of flight MS
   3.2. Electrospray Ionization MS
2. **Laboratory Automation**
   4.1. Automation in Specimen inoculation
   4.2 Liquid microbiology and Total Laboratory Automation

63

G39

5. **Multiplexed serology and others (electrochemistry, …)**
6. **HPLC and mutations analysis (dHPLC)**

Main Current Issues in Medical Microbiology

1. **Rapid methods**
2. **Highly Specific and Sensitive**
3. **Automatized as much as possible (or single patient)**
4. **Multiplexed approaches (syndromic approach)**
5. **Identification of non-growing pathogens**
6. **Identification of resistance patterns**
7. **Identification of Host susceptibility factors**

64

- **Cepheid** : www.cepheid.com
- **Alere Abbott** : https://www.globalpointofcare.abbott/fr/fr/index.html
- **Diasorin** : **https://int.diasorin.com/en/licensed-technologies**
- **Qiagen :** www.qiagen.com
- **Biocartis :** www.biocartis.com
- **Biofire :** www.biofiredx.com
- **Biotechne : https://www.bio-techne.com/**
- **Oxford Nanopore:** https://nanoporetech.com/
- **My Cartis: https://www.invetechgroup.com/**
- **Biomerieux :** http://www.biomerieux.com
- **Abbott :** http://www.abbott.com/
- **Singulex :** www.singulex.com
- **Quanterix :** http://www.quanterix.com/
- **Illumina :** www.illumina.com
- **PacBio:** http://www.pacb.com/

65

1. seq

1. NA Sequencing principles

« ***single molecule sequencing*** » = 2245 articles on 2019 Jan. 23rd n=25047 on 2021 Jan. 31st, 29199 on Jan 23rd 2024, 30654 on Jan 24th 2025.

- 1. Maxam-Gilbert (chemical method)
- 2. Sanger= dideoxynucleotide, chain termination method
- 3. NGS
  - **3.1**. Rohani-Nyren = *pyrophosphate* (pyrosequencing, sequencing by synthesis)
  - **3.2**. Electronic sequencing (Ion Torrent, *H+ measurement*)
  - **3.3**. Bridge sequencing (Illumina, *sequence by synthesis*)
  - **3.4**. PacBio (*Single Molecule Real-Time,* SMRT)
  - **3.5**. Oxford Nanopore (*nanopore-based* sequencing, long read sequencing)

66

History of Sequencing Technologies companies

1981 GeneCo (Genetic Systems Company), **Foster City** (Applied Biosystems Instruments)
Andre Marion and Sam Eletr
Acquired by PE in 1993 : PE Corp and PE Biosystems Group (1998)
1994, income=1 Md US$, 6000 employees

Applera Corp-Applied Biosystems Group (NYSE: ABI) of Foster City, California, and Applera Corp-Celera Genomics Group (NYSE: CRA

1998: Formation of Solexa
2000, the Applied Biosystems name was restored
Administrator = *Jean-Luc Bélingard*, Director since 1993, CEO (PDG) of Biomérieux since 2011.
2004: Acquisition of Molecular Clustering Technology by Solexa
2005: Acquisition of Lynx Therapeutics (instrumentation company) by Solexa
in 2007, **SOLEXA was acquired by** Illumina for $600 million.
2004: PacBio foundation
2005: Oxford Nanopore Technologies was spun out from the University of Oxford
2007: Roche acquires 454

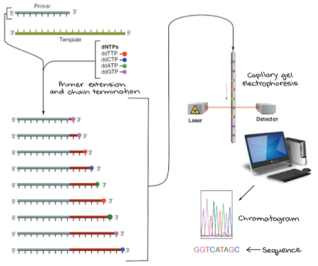
Key Market Trends 2025 (chatGPT)

- **Long-Read Sequencing**: ONT is leading in long-read sequencing, whereas Illumina is still primarily focused on short-read sequencing. The difference is key in applications like structural variation detection, de novo genome assembly, and understanding complex genomic regions.
- **Real-Time Sequencing**: ONT's ability to provide real-time sequencing data is a unique advantage, allowing for faster decision-making in clinical or field environments.
- **Cost and Accessibility**: ONT's devices like the MinION and GridION offer more affordable, portable options compared to Illumina's higher-end systems, opening up sequencing to a broader range of users, including smaller labs and non-experts.

Conclusion:
Illumina remains the dominant player in the sequencing market, especially in large-scale, short-read sequencing applications.
Oxford Nanopore has rapidly expanded its share, with a strong presence in long-read sequencing, real-time sequencing, and specialized applications.
The competition between the two is expected to intensify, with each company targeting different niches of the market—Illumina focusing on high-throughput, short-read applications and ONT leading the way for portable, real-time, and long-read sequencing.
The exact current market share for 2025 would likely show ONT's growth continuing, but Illumina's dominance in large-scale, high-throughput sequencing is expected to persist for the near future.

2. Sanger Method

Using dideoxynucleoside triphosphates (ddNTPs) as DNA elongation terminators. Sequencing reaction mixtures were divided into four parallel vessels, each containing one of four ddNTPs (ddATP, ddGTP, ddCTP, or ddTTP) along with an excess of standard dNTPs. The resulting reaction mixtures con-tained DNA fragments of different lengths representing each size fragment produced by termination following inclusion of the given ddNTP. Fragments from the four reaction mixtures could then be separated by gel or capillary electrophoresis with a resolution of one nucleotide. The ability to radioactively or fluorescently label each ddNTP enabled detection of fragments and reading of sequence data by automated sequencing instruments.



https://www.khanacademy.org/science/high-school-biology/hs-molecular-genetics/hs-biotechnology/a/dna-sequencing

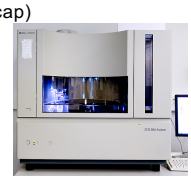
2. Sanger Method

- These advances in automation and analysis made nucleic acid sequencing a realistic option for diagnostics;
- However, several technical challenges still prevented the widespread use of Sanger sequencing in the clinical laboratory.
- **Poor quality in the first 15 to 40 bases of the sequence** and deteriorating quality of sequencing data after 700 to 900 bases limit its applicability to relatively short DNA fragments.
- Additionally, Sanger sequencing reactions are limited to **sequence analysis of a single amplicon per reaction**. This prevents the analysis of complex specimens such as sputum or abscess which contain multiple organisms

The instruments : Sanger sequencing

- PE Biosystems 373A
- ABI377
- ABI PRISM 3700 DNA Analyzer with Hitachi. 300.000 $ in 1998
- ABI3730 (48 cap) 3730 XL (96 cap)





3. NGS

- NGS. Next-generation sequencing (NGS) refers to :

high-throughput sequencing method that parallelizes the sequencing process, producing thousands or millions of sequences at once.
Intentionally broad, next-generation sequencing encompasses several different sequencing technologies that have been adapted to high-throughput, low-cost sequencing. A thorough review and comparison of these methods has been published

Liu L,Li Y,Li S,Hu N,He Y,Pong R,Lin D,Lu L,Law M.2012. Comparison of next-generation sequencing systems. J. Biomed. Biotech- nol. 2012:251364. http://dx.doi.org/10.1155/2012/251364·

1. seq

3. NGS, Performances comparison

Buchan and Ledeboer 2014

Buchan and Ledeboer

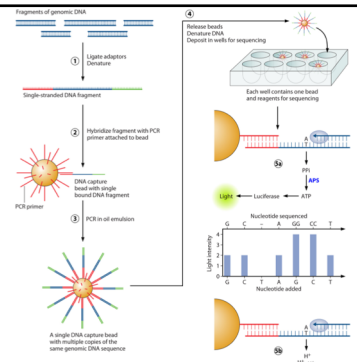
TABLE 3 Comparison of nucleic acid sequencing methods[a]

Characteristic	Ion Torrent	454 Sequencing	Sanger sequencing	SOLiD
Sequencing chemistry	Ion semiconductor sequencing	Pyrosequencing	Terminator sequencing	Ligation-based sequencing
Amplification approach	Emulsion PCR	Emulsion PCR	Liquid-phase reaction	Emulsion PCR
Mb/run	100–400	400–700	0.001 (1,000 bp)	150,000
Time/run	1.5 h	7–10 h	3 h	7–9 days
Read length (bp)	200	400	800–1,500	35 × 75
Reads/run	~1,000,000	~1,000,000	Not applicable	700,000,000–1 billion
Sequence accuracy (%)	98.4–98.9	99.51–99.96	99.999	99.94–99.99
Cost (US$) per:				
Run	~500–700	6,000–8,000	100.00	4,000
Mb	<5.00	10.00–15.00	2,400.00	0.04
Instrument	50,000	500,000	100,000	595,000

[a] Data are compiled form references 150 and 262.

What about Illumina ?

1. seq



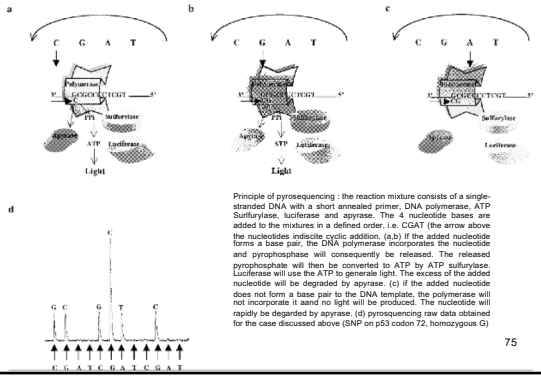
Pyrophosphate Measure (454)

H+ Measure (Ion Torrent)

1. seq

Ahmadian *et al.* 2000 — Analytical Biochemistry **280,** 103–110 (2000)
doi:10.1006/abio.2000.4493, available online at http://www.i

SINGLE-NUCLEOTIDE POLYMORPHISM ANALYSIS BY PYROSEQUENCING 105



Principle of pyrosequencing : the reaction mixture consists of a single-stranded DNA with a short annealed primer, DNA polymerase, ATP Surfurylase, luciferase and apyrase. The 4 nucleotide bases are added to the mixtures in a defined order, i.e. CGAT (the arrow above the nucleotides indiscite cyclic addition. (a,b) If the added nucleotide forms a base pair, the DNA polymerase incorporates the nucleotide and pyrophosphase will consequently be released. The released pyrophosphate will then be converted to ATP by ATP sulfurylase. Luciferase will use the ATP to generale light. The excess of the added nucleotide will be degraded by apyrase. (c) if the added nucleotide does not form a base pair to the DNA template, the polymerase will not incorporate it aand no light will be produced. The nucleotide will rapidly be degarded by apyrase. (d) pyrosquencing raw data obtained for the case discussed above (SNP on p53 codon 72, homozygous G)

1. seq

History of Pyrosequencing development

developed in **1996** by Pål Nyrén and Mostafa Ronaghi (Analytical Biochemistry 1996 and Science 1998)

« *sequencing by synthesis* » detection of pyrophosphate release on nucleotide incorporation

-first-generation pyrosequencing (1996) limited to **100 bp** reads and 30-60 Mb/run (i.e. GS 20)

-second-generation (2006) limited to **250 bps** reads and 150 Mb/run (i.e. FLX also known as GS FLX Standard)

-third-generation (2008) can read lengths more than **350 bps** long and 400 MB/run (i.e. XLR also known as GS FLX Titanium)

1. seq

- **Pyrosequencing**, licensed by 454 Life Sciences and later purchased by Roche, was the **first** next-generation sequencing method commercially marketed. Pyrosequencing employs a "sequence-by-synthesis" approach, meaning that it generates sequence data during DNA synthesis rather than analyzing nucleic acid amplicons postsynthesis as is the case with Sanger sequencing. Amplified or chromosomal target nucleic acid is fragmented, and synthetic nucleic acid adaptors are enzymatically ligated to each end of the product. One adaptor serves as an adaptor for hybridization of the nucleic acid product to a microbead, and the other serves as a sequencing primer. Following a PCR to amplify the target sequence, microbeads coated with amplicon are segregated into microwells. Each well contains all the reagents required for sequencing, including DNA polymerase, luciferase, ATP sulfurylase, and apyrase. Each of the four dNTPs is individually added and washed away from the wells in repeating cycles. When a complementary dNTP is added, it is incorporated by DNA polymerase, with the concomitant release of pyrophosphate as a by-product of DNA synthesis. ATP sulfurylase converts the released pyrophosphate to ATP, which is used to drive luciferase activity, resulting in the production of light. Sequence data are generated by monitoring the microwell reactions for **a pulse of light** following addition of each dNTP. Since each microwell contains a single microbead harboring a unique region of chromosomal DNA, parallel sequencing of hundreds of regions of the chromosome achieves high sequence coverage in a single run. Additionally, because sequencing reactions are carried out in picoliter-volume reaction wells, this technology is capable of sequencing 400 to 600 megabases of DNA per 10h run at a price per base up to 100-fold lower than that for Sanger sequencing. Pyrosequencing was initially capable of generating accurate reads of approximately 100 bases, with the limiting factor related to decreasing efficiency of apyrase in degrading unincorporated nucleotides in each successive cycle. Replacement of apyrase with thorough washing to remove unused nucleotides can extend the effective read length to approximately 400 bases. This is still a relatively short read in comparison to that with the Sanger method, but it is significantly longer than those of other NGS methods
- An extended read length can be advantageous when attempting rapid whole-genome sequencing (WGS), especially when coupled with the speed of pyrosequencing technology and sophisticated software capable of assembling short individual reads into a confluent genome sequence. The overall accuracy of the sequence data generated is 99.51% to 99.96%. A potential drawback to pyrosequencing is the inability to generate reliable sequences of homopolymers of >4 bases in length. In a study assessing the accuracy of sequences generated by pyro- sequencing, 39% of errors were attributable to homopolymer sequences.

1. seq

Semiconductor sequencing, typified by the Ion Torrent system (ABI), is a similar "sequence-by synthesis" technology. Parallel sequencing reactions are carried out in 1.2 million microwells on the surface of a low-cost semiconductor chip. Each picoliter well contains template and DNA polymerase, to which each of the four nucleosides is added in sequential order, however; Ion Torrent sequencing differs from pyrosequencing in that it uses production of hydrogen as the sole marker for determining the sequence. Release of hydrogen ions following incorporation of a complementary nucleotide is detected by a **miniaturized ion sensor** integrated into each reaction well. This technology is capable of generating up to 25 Mb of sequence data in a single run with a 2h run time.
Independence from the use of multiple enzymes, sensitive optics, or modified nucleotides dramatically reduces the cost of reagents and equipment compared to those with Sanger or other NGS methods.
The reported cost of an Ion Torrent instrument is approximately US$50,000, excluding sample preparation equipment and a server for data analysis. The reported accuracy of semiconductor sequencing systems, including Ion Torrent, ranges from **98.4% to 98.9%** The major limitations of this system are that it has difficulty in enumerating long repeats (homopolymers of >6 nt in length) and has a read length of 50 to 100 nt, which is relatively a short compared to that of Sanger sequencing or pyrosequencing.

The Global next generation sequencing market was estimated to be $4,146.9 million in 2016 and is projected to reach $11,925.2 million, witnessing a CAGR of 13.61% for the forecast period from 2017 to 2024.

http://www.prnewswire.com/news-releases/global-next-generation-sequencing-market-research-report-2017---forecasts-to-2024-300541980.html

1. seq

Single Molecule Real-Time zero mode waveguide (ZMWs) Sequencing

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., . . . Turner, S. (2009). Real-time DNA sequencing from single polymerase molecules. *Science, 323*(5910), 133-138. doi: 10.1126/science.1162986

Real-Time DNA Sequencing from Single Polymerase Molecules

John Eid,* Adrian Fehr,* Jeremy Gray,* Khai Luong,* John Lyle,* Geoff Otto,* Paul Peluso,* David Rank,* Primo Baybayan, Brad Bettman, Arkadiusz Bibillo, Keith Bjornson, Bidhan Chaudhuri, Frederick Christians, Ronald Cicero, Sonya Clark, Ravindra Dalal, Alex deWinter, John Dixon, Mathieu Foquet, Alfred Gaertner, Paul Hardenbol, Cheryl Heiner, Kevin Hester, David Holden, Gregory Kearns, Xiangxu Kong, Ronald Kuse, Yves Lacroix, Steven Lin, Paul Lundquist, Congcong Ma, Patrick Marks, Mark Maxham, Devon Murphy, Insil Park, Thang Pham, Michael Phillips, Joy Roy, Robert Sebra, Gene Shen, Jon Sorenson, Austin Tomaney, Kevin Travers, Mark Trulson, John Vieceli, Jeffrey Wegener, Dawn Wu, Alicia Yang, Denis Zaccarin, Peter Zhao, Frank Zhong, Jonas Korlach,† Stephen Turner†

79

Single Molecule Real-Time zero mode waveguide (ZMWs) Sequencing. (PacBio)

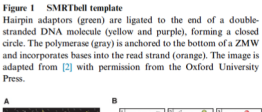


Figure 1 SMRTbell template
Hairpin adaptors (green) are ligated to the end of a double-stranded DNA molecule (yellow and purple), forming a closed circle. The polymerase (gray) is anchored to the bottom of a ZMW and incorporates bases into the read strand (orange). The image is adapted from [2] with permission from the Oxford University Press.

Figure 2 A single SMRT cell
Each SMRT cell contains 150,000 ZMWs. Approximately 35,000–75,000 of these wells produce a read in a run lasting 0.5–4 h, resulting in 0.5–1 Gb of sequence. The image is adapted with permission from Pacific Biosciences [4]. ZMW, zero-mode waveguide.

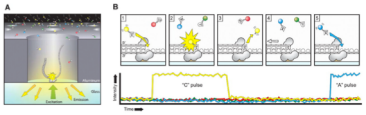


Figure 3 Sequencing via light pulses
A. A SMRTbell (gray) diffuses into a ZMW, and the adaptor binds to a polymerase immobilized at the bottom. B. Each of the four nucleotides is labeled with a different fluorescent dye (indicated in red, yellow, green, and blue, respectively for G, C, T, and A) so that they have distinct emission spectrums. As a nucleotide is held in the detection volume by the polymerase, a light pulse is produced that identifies the base. (1) A fluorescently-labeled nucleotide associates with the template in the active site of the polymerase. (2) The fluorescence output of the color corresponding to the incorporated base (yellow for base C as an example here) is elevated. (3) The dye-linker-pyrophosphate product is cleaved from the nucleotide and diffuses out of the ZMW, ending the fluorescence pulse. (4) The polymerase translocates to the next position. (5) The next nucleotide associates with the template in the active site of the polymerase, initiating the next fluorescence pulse, which corresponds to base A here. The figure is adapted from [4] with permission from The American Association for the Advancement of Science.

Rhoads et al. 2015

80

to learn more about PacBio

Genomics Proteomics Bioinformatics 13 (2015) 278–289
HOSTED BY
Genomics Proteomics Bioinformatics
www.elsevier.com/locate/gpb
www.sciencedirect.com
ELSEVIER
GPB

REVIEW

PacBio Sequencing and Its Applications

Anthony Rhoads [1,a], Kin Fai Au [1,2,*,b]

Published online 1 February 2018
Nucleic Acids Research, 2018, Vol. 46, No. 5 2159–2168
doi: 10.1093/nar/gky066

SURVEY AND SUMMARY

Single molecule real-time (SMRT) sequencing comes of age: applications and utilities for medical diagnostics

Simon Ardui[1], Adam Ameur[2,3], Joris R. Vermeesch[1] and Matthew S. Hestand[1,4,*]

[1]Department of Human Genetics, KU Leuven, Leuven 3000, Belgium, [2]Department of Immunology, Genetics and Pathology, Uppsala University, Science for Life Laboratory, Uppsala 75108, Sweden, [3]School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia and [4]Department of Clinical Genetics, VU University Medical Center, Amsterdam 1081 BT, The Netherlands

81

1. seq

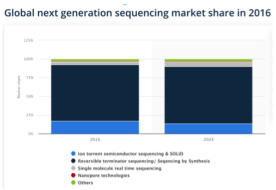
Ion Torrent presented its desktop-sized sequencer at the 2010 Advances in Genome Biology and Technology conference.

50.000 US$, run =500 US

The Ion Torrent, PacBio, Illumina instruments

PACBIO

SMRT sequencing ZMWs



NGS market CAGR = 20% between 2021-2027

https://www.statista.com/statistics/866705/next-generation-sequencing-market-share-worldwide-by-technology/

82

1. seq

Applications of bridge sequencing, pyrosequencing and semiconductor sequencing

- **Whole-genome sequencing** (WGS)
- **Amplicon sequencing**
  full sequencing of one or more genetic loci concurrently. This method is valuable when identification of multiple mutations or SNPs in a genetic locus is required to predict antimicrobial resistance. In addition to detection of multiple SNPs in a single locus, parallel sequencing offers the ability to generate sequences for multiple loci simultaneously.
- **Transcriptome sequencing**
- **Metagenomics**
  _utility of NGS to simultaneously sequence and identify multiple organisms in a single specimen. An advantage of NGS is the detection of nonculturable or fastidious organisms that may be outcompeted and overlooked in routine CF cultures. In a cohort of 66 sputum specimens from CF patients, NGS identified 122 different microbial species, compared to only 18 identified by culture
- **Epidemiological investigation of outbreaks**

83

1. seq

Ultradeep sequencing **(UDS)**

- refers to amplicon sequencing designed **to allow mutations to be detected at extremely low levels in a population**. Initial PCR amplification of a genetic region of interest followed by segregation of each amplicon into a separate reaction well allows sequencing and identification of rare sequence variants. For example, ultradeep sequencing has been successfully used to detect HIV quasispe-cies and the emergence of resistant subpopulations.
- Analysis of blood samples from HIV-infected patients using pyrosequencing identified strains with mutations in the viral reverse trans- criptase gene at levels of <0.1% of the total viral population
- **VIROLOGY**
- **ONCOLOGY**

84

1. seq

Transcriptome sequencing

- which aims to **efficiently create RNA profiles and examine the effects of mRNA transcript expression**.
- The majority of research using NGS for transcriptome analysis has involved the basic sciences; however, recent studies have utilized this method for comparison of mRNA expression in normal and malignant cell populations and for discovery of latent or cryptic viruses whose presence and expression may be associated with malignancies

85

1. seq

SOLiD

- SOLiD (**supported oligonucleotide ligation and detection**) is a method of sequencing by ligation. A target-specific sequencing primer is used to initiate sequencing by the sequential addition of octamer probes, each containing 2 specific nucleotides at the 5′ terminus followed by 6 degenerate nucleotides. Each of the 16 possible combinations of two nucleotides is represented, and oc- tamers are fluorescently labeled with one of 4 fluorophores. The 16 octamers are then grouped into 4 sets (each containing one each of the 4 fluorophores) and are added sequentially to the se- quencing reaction mixture for 7 full cycles of the 4 groups. Fluo- rescence is measured after addition of each 4-member group of probes, and the 2-base sequence is determined by the fluorophore detected. Gaps in the sequence corresponding to the 6 degenerate nucleotides in each probe are filled in by repeating the reaction using additional sequencing primers, each offset by one nucleo tide (n - 1, n - 2, etc.) from the initial primer (177). This results in short reads (26 nucleotides); however, the sequencing error rate is reduced to 0.001 because each nucleotide in the template is read twice (177). The disadvantage of this technology is turnaround time. The run time for a single sequencing reaction is 2.5 to 6 days, resulting in turnaround time for a full genome sequence of up to 2 weeks . Because of the large amount of sequence data gener- ated per run, low cost per base sequenced, and extended TAT, these platforms are currently best suited to whole-genome se- quencing projects rather than rapid identification of microorganisms or SNP polymorphisms in a clinical laboratory.

86

1. seq

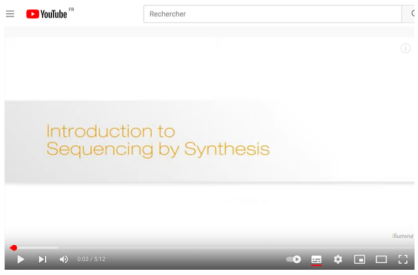
Illumina sequencing (Bridge)

- Illumina (Solexa) sequencing is based on reversible dye terminators.
- DNA molecules are first attached to primers on a slide and amplified so that local clonal colonies are formed. Four types of reversible terminator bases are added, and nonincorporated nucleotides are washed away.
- A **camera** takes images of the fluorescently labeled nucleo- tides, and then the dye along with the terminal 3′ blocker is chemically removed from the DNA, allowing the next cycle
- More recently, Illumina has begun offering full genome sequencing through it reference laboratory at a reported cost of **$4,000.00 per genome** (2014).

87

https://www.youtube.com/watch?v=fCd6B5HRaZ8

Illumina sequencing (Bridge)



88

https://www.youtube.com/watch?v=CGWZvHIi3i0

1. seq

Oxford nanopore sequencing technology

- **6407 papers** on Jan 23rd 2025

Nat Commun. 2015; 6: 10063. PMCID: PMC4703848
Published online 2015 Dec 21. doi: 10.1038/ncomms10063

Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*

Phelim Bradley,[1] N. Claire Gordon,[2] Timothy M. Walker,[2] Laura Dunn,[2] Simon Heys,[1] Bill Huang,[1] Sarah Earle,[2] Louise J. Pankhurst,[2] Luke Anson,[2] Mariateresa de Cesare,[1] Paolo Piazza,[1] Antonina A. Votintseva,[2] Tanya Golubchik,[2] Daniel J. Wilson,[1,2] David H. Wyllie,[2] Roland Diel,[3] Stefan Niemann,[4,5] Silke Feuerriegel,[4,5] Thomas A. Kohl,[4] Nazir Ismail,[6,7] Shaheed V. Omar,[6] E. Grace Smith,[8] David Buck,[1] Gil McVean,[1] A. Sarah Walker,[2,9] Tim E. A. Peto,[2,9] Derrick W. Crook,[2,9,10] and Zamin Iqbal[#,1]

« *oxford nanopore sequencing and tuberculosis* »
n=60 papers on Jan 23rd 2024, n=104 papers one year later

Genotyping resistance variants with Nanopore was highly concordant with Illumina, having zero discordant SNPs across more than 3000 SNPs and four insertions or deletions (indels), across 60 000 indels. *Hall et al. Lancet Microbes 2023*

89

1. seq

Professor of Chemical Biology, FRS
Fellow of Hertford College

Email: hagan.bayley@chem.ox.ac.uk
Tel: +44 (0)1865 285101
Fax: +44 (0)1865 285102
Office: 1st Floor, Chemical Research Laboratory, 12 Mansfield Road, Oxford OX1 3TA, UK

Biography:
Hagan Bayley received his B.A. in Chemistry from the University of Oxford. He carried out his graduate work under the supervision of Professor Jeremy Knowles at Harvard University, receiving a Ph.D. in Chemistry. His postdoctoral training was with Professor Gobind Khorana at the Massachusetts Institute of Technology. He then went on to work at Columbia University, the Worcester Foundation and Texas A&M University. He joined University of Oxford as the Professor of Chemical Biology in September 2003 and is a fellow of Hertford College.

90

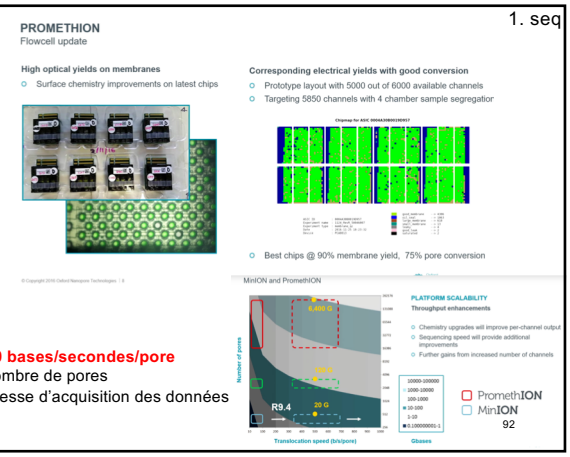
1. seq


MinION™, PromethION™ and GridION™

- **What does the platform technology consist of?**
- The MinION consists of the device and a consumable flow cell. The flow cell has a **sensor chip** containing multiple microscaffolds, each one supporting an individual nanopore sensor. A polymer membrane is formed over the surface of these microscaffolds and the modified protein nanopores are contained in these membranes. Each nanopore is a single addressable electronic channel capable of individual identification of analyte molecules. The array chip is accompanied by an Application-Specific Integrated Circuit (ASIC) that controls and measures currents during nanopore experiments.
- PromethION= 6,2 Terabases/day

91

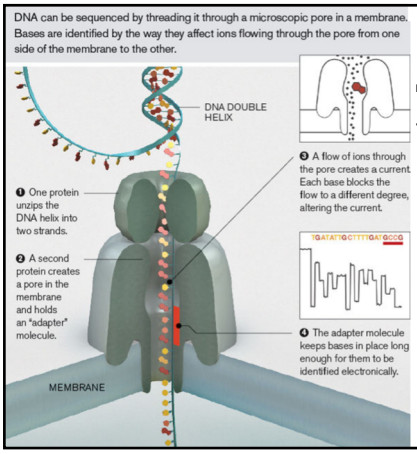
1. seq



500 bases/secondes/pore
-Nombre de pores
-Vitesse d'acquisition des données

92

1. seq



How does nanopore sensing work?

- A nanopore may be used to identify an analyte directly and electronically. A nanopore forms a hole in an electronically resistant membrane which is bathed in physiological solution. A voltage is applied across the membrane in which the pore is set, and the resulting ionic current through the pore is measured. When an analyte of interest passes through the pore or near its aperture, it creates a characteristic disruption in current. This disruption may be used to identify the molecule in question. This is done without the need for optical labelling or amplification of the target molecule.

93

1. seq

What are the benefits of using nanopores to sequence DNA?

- In contrast to current sequencing technologies, nanopores can measure single molecules directly, **without the need for nucleic acid amplification**, fluorescent/chemical labelling or optical instrumentation.
- The **system is scalable**
- Only with nanopores can electronic data be streamed in real-time, so that experimental analyses are performed as the experiment progresses and the user can *Run until...* their biological question is answered.
- However 2D (sense and complementary strand) remains difficult
- Industrial problems remains (see website: crowdfunding)

94

1. seq

preparing a MinION sequencing library: procedure

- 1. shearing genomic DNA using a Covaris gTUBE
- 2. an optional « PreCR » step to repair damaged DNA
- 3. End repair
- 4. dA tailing
- 5. adapter ligation
- 6. His Bead purification
- When properly ligated, a double-stranded (ds) DNA molecule has a Y shaped (Y) Adapter at one end and a hairpin (HP) adapter at the other
- Ds DNA is pulled through a pore one strand at a time starting at the 5' end of the Y, followed by The "template » strand and, ideally, the HP and Complement strand
- The information from Either Strand can be used for 1directional (1D) base calling, andintegrating the information from both strands can beused for 2directional

95

1. seq

Article http://www.human-microbiome.org

Nature **464**, 59-65 (4 March 2010) | doi:10.1038/nature08821; Received 14 August 2009; Accepted 23 December 2009

A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin[1,24], Ruiqiang Li[1,24], Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4], Chaysavanh Manichanh[5], Trine Nielsen[4], Nicolas Pons[6], Florence Levenez[6], Takuji Yamada[2], Daniel R. Mende[2], Junhua Li[1,7], Junming Xu[1], Shaochuan Li[1], Dongfang Li[1,8], Jianjun Cao[1], Bo Wang[1], Huiqing Liang[1], Huisong Zheng[1], Yinlong Xie[1,7], Julien Tap[6], Patricia Lepage[6], Marcelo Bertalan[9], Jean-Michel Batto[6], Torben Hansen[4], Denis Le Paslier[10], Allan Linneberg[11], H. Bjørn Nielsen[9], Eric Pelletier[10], Pierre Renault[6], Thomas Sicheritz-Ponten[9], Keith Turner[12], Hongmei Zhu[1], Chang Yu[1], Shengting Li[1], Min Jian[1], Yan Zhou[1], Yingrui Li[1], Xiuqing Zhang[1], Songgang Li[1], Nan Qin[1], Huanming Yang[1], Jian Wang[1], Søren Brunak[9], Joel Doré[6], Francisco Guarner[5], Karsten Kristiansen[13], Oluf Pedersen[4,14], Julian Parkhill[12], Jean Weissenbach[10], MetaHIT Consortium, Peer Bork[2], S. Dusko Ehrlich[6] & Jun Wang[1,13]

96

1. seq

New Reviews on emerging technologies for the clinical microbiology laboratory

« NGS and Clinical Microbiology Laboratory and Review » 103 Papers on Jan 2025

European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1059–1070
https://doi.org/10.1007/s10096-019-03520-3

REVIEW

Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory

Stefan A. Boers[1] · Ruud Jansen[2] · John P. Hays[1]

Received: 24 December 2018 / Accepted: 20 February 2019 / Published online: 5 March 2019

genes
MDPI

Review

Next Generation and Other Sequencing Technologies in Diagnostic Microbiology and Infectious Diseases

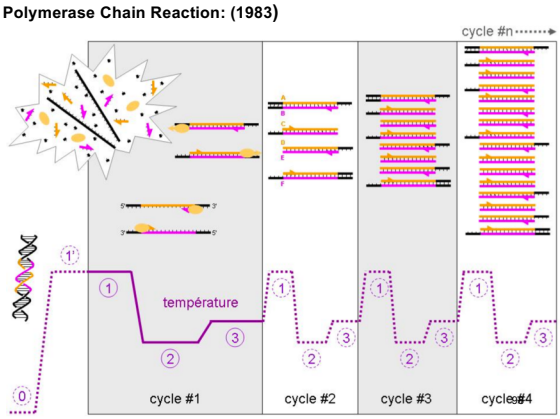
Evann E. Hilt * and Patricia Ferrieri

2022

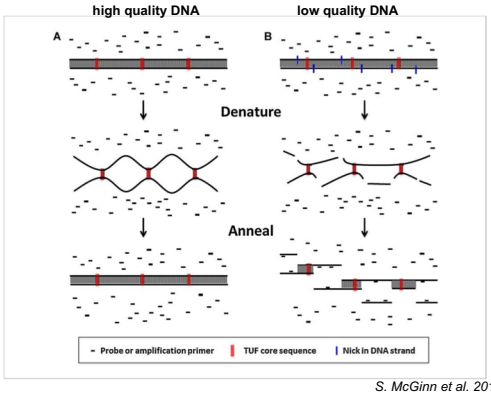
97

2.1. Single Plex Nucleic Acid tests

Polymerase Chain Reaction: (1983)



Thermodynamically Ultrafastened DNA (TUF)



S. McGinn et al. 2016

99

Alternatives to PCR (isothermal amplifications)

- 1. TMA, transcription mediated
- 2. HDA, helicase dependent
- 3. **LAMP, loop-mediated**
- 4. RPA, recombinase isothermal

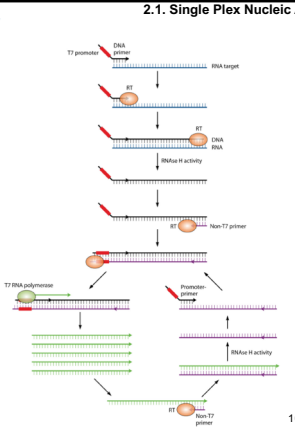
100

2.1. Single Plex Nucleic Acid tests

TMA
Transcription Mediated Isothermal Amplification

Giachetti C, Linnen JM, Kolk DP, Dockter J, Gillotte-Taylor K, Park M, Ho-Sing-Loy M, McCormick MK, Mimms LT, McDonough SH. 2002.

Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA.
J. Clin. Microbiol. 40:2408–2419



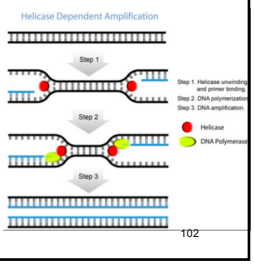
101

2.1. Single Plex Nucleic Acid tests

HDA:
Helicase Dependent Amplification

utilizes **UvrD** (DNA helicase) and **MutL** enzymes isolated from *E. coli* and single-strand binding proteins to create and maintain a single-stranded template for primer annealing and subsequent rounds of amplification.
initial heat-based denaturation required for optimal efficiency; however, reliance on a single reaction temperature without initial denaturation maintains 40% to 60% efficiency and is adequate to generate sufficient amplicon for endpoint detection assays. Like LAMP, the isothermal amplification can be carried out using simple instrumentation in the absence of electricity.
HDA has been applied to identification of *C. difficile*, *Plasmodium spp.*, and *S. aureus*. An advantage of HDA is that detection of target can be achieved by incorporation of fluorescein or digoxigenin into the amplicon, followed by capture and visualization of the amplicon as a colored line on an enzyme immunoassay (EIA) lateral-flow strip..

This maintains the ability to utilize these assays without sophisticated instrumentation but also allows the detection of multiple targets in a single reaction. A test developed to detect and differentiate herpes simplex virus 1 (HSV-1) and HSV-2 using this approach has demonstrated 100% sensitivity compared to viral culture, with a limit of detection as low as 5.5 copies per reaction. Further, this test could be performed on oral and genital cutaneous or mucocutaneous sources without the need for nucleic acid extraction and could be completed within 75 min



102

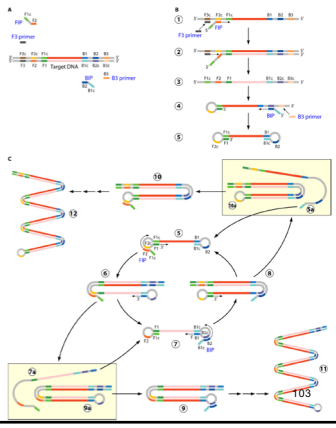
LAMP: ***Loop-Mediated Isothermal Amplification***

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T.
Nucleic Acids Res. **2000** Jun 15;28(12):E63

LAMP and diagnostics

n=12649 in 2022
n=14377 in 2024
n=15382 in 2025

This method relies on auto-cycling strand displacement DNA synthesis that is performed by a DNA polymerase with high strand displacement activity and a set of two specially designed inner and outer primers



« LAMP and Nucleic Acids Amplification Tests » **n=3986 papers in PubMed in 2024/01, n=4472 in 2025/01.**

some commercialized Applications:
SARS, Coronavirus, MTBC, Mycoplasma pneumoniae, Legionella Species, influenza type A virus, H1 pdm 2009 influenza virus, H5 influenza virus, and human papilloma virus (HPV) Mycobacterium tuberculosis, SARS-Cov2,

Tanner NA, Zhang Y, Evans TC Jr. **Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes**. Biotechniques. 2015;58(2):59–68

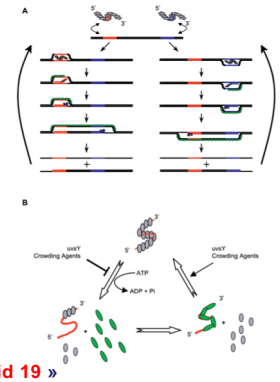
We present a novel method for visual detection of nucleic acid amplification using the inherent production of protons by DNA polymerases and a pH indicator in a weakly buffered solution. This method enables clear andrapid evaluation of amplification reactions without the need for equipment or hands-on processing

RPA: ***Recombinase Isothermal Amplification***

Olaf Piepenburg, Colin H. Williams, Derek L. Stemple, Niall A. Armes
PloS Biology. **2006** July 2006 | Volume 4 | Issue 7 | e204 Jun

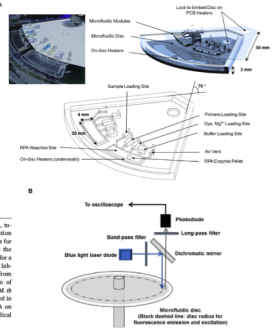
RPA and diagnostics
N=55 articles on 2018 March
13rd, 66 on 2019
Jan. 23rd

This method relies on three enztymes : a recombinase, a single-stranded DNA binding protein (SSB) and a strand-displacing polymerase
advantages: ***FAST***
WORK AT AMBIANT TEMP



« RPA and NAAT and Covid 19 »
36 Articles on Jan 30th 2022

RPA: ***Recombinase Isothermal Amplification***



Automated real-time detection of drug-resistant *Mycobacterium tuberculosis* on a lab-on-a-disc by Recombinase Polymerase Amplification

I.L.G. Law[a,1], J.F.C. Lee[b,1], H.C. Kwok[a,c], H.Y. Yeung[a], C.C.H. Leung[a], M. Hui[a], S.Y. Wu[a], H.S. Chan[b], Y.W. Kwan[a], H.P. Ho[b], S.K. Kong[a,*]

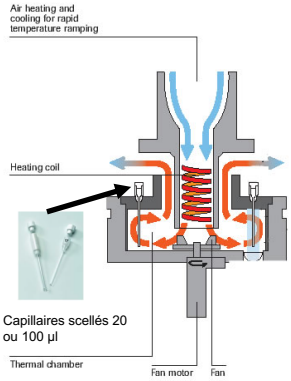
ABSTRACT

With the emergence of multi- and extensive-drug (MDR/XDR) resistant *Mycobacterium tuberculosis* (M. tb), tuberculosis (TB) persists as one of the world's leading causes of death. Recently, isothermal DNA amplification methods received much attention due to their ease of translation onto portable point-of-care (POC) devices for TB diagnosis. In this study, we aimed to devise a simple yet robust detection method for M. tb. Amongst the numerous up-and-coming isothermal techniques, Recombinase Polymerase Amplification (RPA) was chosen for a real-time detection of TB with or without MDR. In our platform, real-time RPA (RT-RPA) was integrated on a lab-on-a-disc (LOAD) with on-board power to maintain temperature for DNA amplification. Sputa collected from healthy volunteers were spiked with respective target M. tb samples for testing. A limit of detection of 10^3 colony-forming unit per milliliter in 15 min was achieved, making early detection and differentiation of M. tb strains highly feasible in extreme POC settings. Our RT-RPA LOAD platform has also been successfully applied in the differentiation of MDR-TB from *H37Ra*, an attenuated TB strain. In summary, a quantitative RT-RPA on LOAD assay with a high level of sensitivity was developed as a foundation for further developments in medical bedside and POC diagnostics.

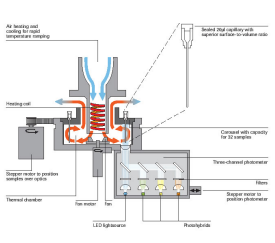
Analytical Biochemistry 544 (2018) 98–107

Real Time PCR

- *Elyse Poitras et Alain Houde (2002). La PCR en temps réel: principes et applications. Reviews in Biology and Biotechnology. 2(2):2-11*
- **Roche LightCycler 2.0 et 1.5**
- **GeneAmp5700 et Prism700 (ABI)**

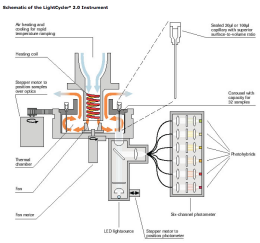


use of air for heating and cooling. ambient air is introduced into the device by a small fan and heated with a resistor. Since air has a weak heat capacity, thermal increase may reach up to 20C per second. Hence, hating and cooling can process 10 times fadter than within a traditional thermocycler.



Scheme of LightCycler® 1.5

fluorescence measure at 530, 640, and 705 nm



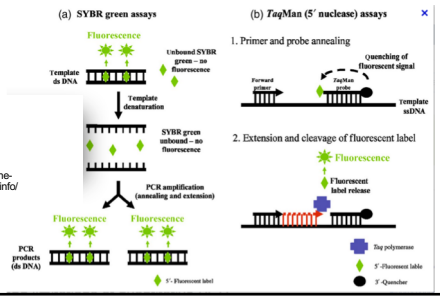
Scheme of LightCycler® 2.0

fluorescence measure at 530, **560, 610**, 640, **670 and 705** nm

N.B: SmartCycler II (Cepheid, Sunnyvale, CA) first-generation BD Max (BD, Sparks,MD)were limited to 4 channels

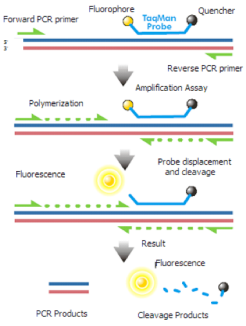
Chemical classification of Real-time PCR

- **Non-specific detection**: Real-time PCR with double-stranded DNA-binding dyes as reporters
  This method has the advantage of only needing a pair of primers to carry out the amplification, which keeps costs down; however, only one target sequence can be monitored in a tube.
- **Specific detection**: fluorescent reporter probe method



http://dyes.gene-quantification.info/

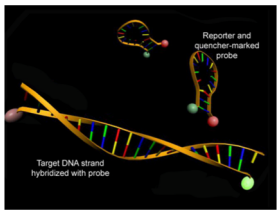
TaqMan probes



Täpp I; Malmberg L; Rennel E; Wik M; et al. (Apr 2000). "Homogeneous scoring of single-nucleotide polymorphisms: comparison of the 5'-nuclease TaqMan assay and Molecular Beacon probes". Biotechniques. 28 (4): 732–8

Molecular Beacons

25 nucleotides



Tyagi S; Kramer FR (1996). "Molecular beacons: probes that fluoresce upon hybridization". Nat. Biotechnol. 14 (3): 303–8

Nature Protocols **3**, 1101 - 1108 (2008)
Published online: 5 June 2008 | doi:10.1038/nprot.2008.73

Analyzing real-time PCR data by the comparative C_T method

Thomas D Schmittgen[1] & Kenneth J Livak[2]

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes

Jo Vandesompele, Katleen De Preter, Filip Pattyn, Bruce Poppe, Nadine Van Roy, Anne De Paepe and Frank Speleman

Genome Biology 2002 3:research0034.1 | DOI: 10.1186/gb-2002-3-7-research0034

Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method

Kenneth J. Livak* and Thomas D. Schmittgen†,[1]

**Applied Biosystems, Foster City, California 94404; and †Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, Washington 99164-6534*

METHODS 25,402–408 (2001)

SARS-CoV 2 Assays : 4 among others

SARS-COV-2 R-GENE®

Trousse de PCR en temps réel pour la détection du SARS-COV-2, agent pathogène responsable de la pandémie COVID-19

- Kit « tout inclus » : master-mix prêt à l'emploi, reverse transcriptase, contrôle positif, contrôle négatif, contrôle interne
- Contient deux PCR triplex
- Résultat en 4 à 5h
- Validée sur la majorité des plateformes d'extraction et d'amplification RT-PCR

BIOMERIEUX

QIAstat-Dx Respiratory SARS-CoV-2 Panel - Canada

QIAGEN

For multiplex syndromic testing applications

- Enables detection of the SARS-CoV-2 virus that causes COVID-19, in addition to 20 other respiratory targets
- Intuitive workflow with less than one-minute hands-on time
- All wet and dry reagents onboard and room temperature stable
- Comprehensive qualitative results available in about an hour

LUMINEX

Luminex SARS-CoV-2 RT-PCR Assay

ROCHE

Instructions For Use

LightMix® SarbecoV E-gene plus EAV control 530/660

Cat.-No. 40-0776-96 Roche SAP n° 09 164 154 001

Kit with reagents for 96 PCR reactions 20 µl for detection of WH-Human_1 genomic RNA [lyophilized]

Mixture of ModularDx kits S3-0776 Wuhan E-gene and 66-0909 EAV - **No repeat evaluation required.**

High Resolution Melting Temperature

Real-Time duplex thermodynamic dissociation tracking by Fluorescent markers

High resolution melting-curve (HRM) analysis for the diagnosis of cryptosporidiosis in humans Pangasa A, et al. Mol. Cell. Probes 2008 Oct;1: 10-5

High Resolution Melting and Covid 19

15 Pubmed results on 30th Jan 22

A High-Resolution Digital DNA Melting Platform for Robust Sequence Profiling and Enhanced Genotype Discrimination. Sinha M *et al.* 2018 SLAS technol.23:580

http://en.wikipedia.org/wiki/High_Resolution_Melt

Evolution of RT-PCR and High-Resolution Melting Temperature

Clin Chem. 2018 Nov 20. pii: clinchem.2018.296608. doi: 10.1373/clinchem.2018.296608. [Epub ahead of print]

Integrated Extreme Real-Time PCR and High-Speed Melting Analysis in 52 to 87 Seconds.

Myrick JT[1], Pryor RJ[2], Palais RA[2,3], Ison SJ[1], Sanford L[1], Dwight ZL[2], Huuskonen JJ[4], Sundberg SO[1,4], Wittwer CT[5].

Conclusions: Extreme PCR with high-speed melting can be performed in about 1 min. The integration of extreme PCR and high-speed melting shows that future molecular assays at the point of care for identification, quantification, and variant typing are feasible.

Received: 24 May 2021 | Accepted: 6 June 2021
DOI: 10.1002/jmv.27139
RESEARCH ARTICLE
JOURNAL OF MEDICAL VIROLOGY WILEY

High-resolution melting curve FRET-PCR rapidly identifies SARS-CoV-2 mutations

Subarna Barua[1] | Monirul Hoque[1] | Patrick J. Kelly[2] | Jianfa Bai[3] | Gregg Hanzlicek[3] | Lance Noll[3] | Heather Walz[3,4] | Calvin Johnson[1] | Constantinos Kyriakis[1] | Chengming Wang[1]

https://www.youtube.com/watch?v=WU3qKhIUc54

Digital PCR and Droplet Digital PCR

(n=1142 articles in PubMed in2019
n=2054 results in 2021, n=3642 in 2024)
and SARS (n=192, January 2024). (digital PCR: 9563 articles in 2025)

- **Absolute quantification of initial target by digitalization of PCR sample (partition) (nanoliters microdroplets and taqMan assay)**
- RARE VARIANT MEASUREMENT

Digital PCR as a novel technology and its potential implications for molecular diagnostics.
Clinical Chemistry 2013 59:6 Jim F. Huggett *et al.*

Application of **droplet digital PCR** to detect the pathogens of infectious diseases.
Li H, Bai R, Zhao Z, Tao L, Ma M, Ji Z, Jian M, Ding Z, Dai X, Bao F, Liu A.
Biosci Rep. 2018 Nov 15;38(6). pii: BSR20181170. doi: 10.1042/BSR20181170. Print 2018 Dec 21. **Review.**
PMID: 30341241 **Free PMC Article**

Pitfalls and Promesses of dPCR

Clinical Chemistry 61:1 79-88 (2015) Reviews

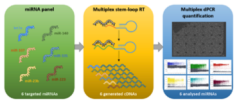
Considerations for Digital PCR as an Accurate Molecular Diagnostic Tool
Jim F. Huggett,[1,2*] Simon Cowen,[1] and Carole A. Foy[1]

Recent Applications of dPCR

Multiplex digital PCR for the simultaneous quantification of a miRNA panel
Florence Busato[1], Sylvain Ursuegui[1], Jean-François Deleuze[1*], Jorg Tost[1*]

HIGHLIGHTS
- MiRNA signatures represent promising biomarkers for clinical applications.
- First proof-of-concept of a multiplexed digital PCR assay for miRNA analysis.
- Combination of miRNA-specific stem-loop primers and dPCR with hydrolysis probes.
- Linear and reproducible quantification results for up to six miRNAs.
- Optimized protocol can be applied to different types of biological samples.

GRAPHICAL ABSTRACT



Closed Platforms

Closed-system platforms : designed to run specific assays which are cleared by regulatory agencies, including the U.S. Food and Drug Adminis- tration (FDA), the European health, safety, and environmental agency (CE-Mark) and Heath Canada.

- Examples : "*sample-to-result*" platforms
  - **GeneXpert** (Cepheid, Sunnyvale, CA),
  - **FilmArray** (BioFire, Salt Lake City, UT),
  - **Tigris** (GenProbe, San Diego, CA), and
  - **Verigene** (Nanosphere, Northbrook, IL).

- Many of these closed-platform tests can be simplified to gain designation as "moderate complexity," and as such, the end user has limited ability to modify the assay or result interpretation.

Open Platforms

Available for real-time and quantitative PCR analysis

SmartCycler (Cepheid),
ABI 7500FastDx (Applied Biosystems)
LightCycler 2.0 (Roche)
Luminex 200, **MagPix** (Luminex)

also automated or "sample-to-result" open platforms available :

BD Max (BD, Sparks, MD)
Abbott m2000 (Abbott, North Chicago, IL).

FDA-cleared molecular assays for use on these platforms may be available from the manufacturer of the **platform or another diagnostics com**pany; however, the platforms are also suitable for running **laboratory-developed tests** (LDTs) or "home brew" assays. While the menu of FDA-cleared in vitro diagnostic (IVD) molecular assays continues to expand, the ability of laboratories to develop and validate their own assays is critical to providing high-quality molecular diagnostics for novel or esoteric targets, including those involved in infectious disease. For this reason, open-system platforms will continue to have a prominent place in most clinical laboratories.

REvolutionary Approaches and Devices for Nucleic Acid analysis (READNA)

N Biotechnol. 2015 Oct 26;33(3):311-330. doi: 10.1016/j.nbt.2015.10.003. [Epub ahead of print]

New technologies for DNA analysis - a review of the READNA Project.

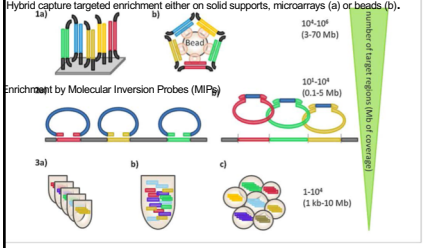
McGinn S[1], Bauer D[2], Brefort T[3], Dong L[2], El-Sagheer A[4], Elsharawy A[5], Evans G[6], Falk-Sörqvist E[7], Forster M[8], Fredriksson S[9], Freeman P[10], Freitag C[11], Fritzsche J[12], Gibson S[10], Gullberg M[8], Gut M[13], Heath S[13], Heath-Brun I[13], Heron AJ[14], Hohlbein J[6], Ke R[15], Lancaster O[10], Le Reste L[6], Maglia G[14], Marie R[16], Mauger F[1], Mertes F[17], Mignardi M[15], Moens L[7], Oostmeijer J[18], Out R[18], Pedersen JN[16], Persson F[11], Picaud V[19], Rotem D[14], Schracke N[3], Sengenes J[1], Ståhler PF[3], Stade B[8], Stoddart D[14], Teng X[18], Veal CD[10], Zahra N[10], Bayley H[14], Beier M[3], Brown T[20], Dekker C[21], Ekström B[9], Flyvbjerg H[16], Franke A[5], Guenther S[22], Kapranidis AN[6], Kaye J[23], Kristensen A[16], Lehrach H[17], Mangion J[22], Sauer S[11], Schyns E[24], Tost J[1], van Helvoort JM[18], van der Zaag PJ[25], Tegenfeldt JO[26], Brookes AJ[10], Mir K[2], Nilsson M[15], Willcocks JP[27], Gut IG[28].

1CEA - Centre National de Génotypage, 2, rue Gaston Cremieux, 91057 Evry Cedex, France
19CEA-Saclay, Bât DIGITEO 565 - Pt Courrier 192, 91191 Gif-sur-Yvette Cedex, France.
24PHOTONIS France S.A.S. Avenue Roger Roncier, 19100 Brive B.P. 520, 19106 BRIVE Cedex, France.

MultiPlex Nucleic Acid tests

Commonly used targeted enrichment techniques (McGinn et al. 2016)

Hybrid capture targeted enrichment either on solid supports, microarrays (a) or beads (b).



Overview of **Target Enrichment** Strategies.

Kozarewa I, Armisen J, Gardner AF, Slatko BE, Hendrickson CL.

Curr Protoc Mol Biol. 2015 Oct 1;112:7.21.1-7.21.23. doi: 10.1002/0471142727.mb0721s112.

121

Targeted enrichment by PCR-based approaches

Ancient and new emerging technologies for clinical microbiology laboratory

1. Nucleic Acid Sequencing Methods
   Sanger Sequencing, NGS
2. Molecular Methods (Nucleic Acid based)

   2.1. Single Plex Nucleic Acid tests
   2.1.1 nucleic acid amplification, including PCR
   2.1.2 LAMP and HDA technologies
   2.1.3 Automation of NAATs and impact on laboratory workflow and patient care

   2.2. Multiplex Nucleic Acid tests
   2.2.1 Multiplex PCR and Probe Detection
   2.2.2 Microarray methods
   2.2.3 Impact of large multiplex panel on laboratory workflow and patient care

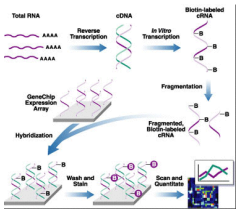
1. Mass Spectrometry
   3.1. Matrix-Assisted Laser Desorption Ionization time of flight MS
   3.2. Electrospray Ionization MS

2. Laboratory Automation
   4.1. Automation in Specimen inoculation
   4.2 Liquid microbiology and Total Laboratory Automation

122

DNA Arrays

DNA Chips History



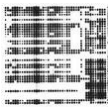
1; **Spotted arrays** (DeRisi et al., 1996)
2; ***In-situ*, Synthesized arrays** (Fodor et al., 1991)
3. **Self-assembled arrays** (Ferguson et al., 2000; Michael et al., 1998; Steemers et al., 2000; Walt, 2000)
4; **custom arrays** = *macro* membrane or microsphere-based (suspension-array; (Kaufhold et al. 1994) (Cowan et al. 2004)
5; **nanotechnology and arrays** (Gold-particle, Talo et al. 2014)

123

DNA Arrays

2 Puces/arrays

1. macroarray on planar surface » (2D) exemple: Spoligotyping (Immunetics) Kaufhold *et al.* 1994, Kamerbeek et al. 1997



2. microarray » on other surface 2D, ex: (Affymetrix®)

-poly-Lysine coated glass slides
-other surfaces, « FAST Slides » Schleicher & Schuell, 0.03-2 nL/spot

3. Microarray on particles in suspension (Luminex®) or mesoscale®
electro-chemiluminescent based immunoassays
myCartis® (end of activity?)

Meso Scale Diagnostics, LLC

Diasorin

DNA Arrays

What kind of detection of the hybridization (or antibody recognition) phenomenon ?

- **1. Optical**
  - **Radioactivity : autoradiography**
  - **Replacement of radioactivity by electrochemoluminescence**
    - **Reporters :**
      biotin/streptavidin-PER
      Digoxigenin/anti-digoxigenin-PAL
      Cy5-Cy3 cyanines molecules (and others...)
- **2 Electrochemical**
  - **Need of new materials**
  - **Need of new fluidics systems**

125

DNA Arrays

Multiplexing : Definition (parallel analysis)

In telecommunications and computer networks, **multiplexing** (sometimes contracted to **muxing**) is a method by which multiple analog or digital signals are combined into one signal over a shared medium.

« ***Parallel detection of many biomarker types in a single tube*** »

Advantages : cost and human ressources decrease for an improved result


406 DOI 10.1002/prca.201400130 *Proteomics Clin. Appl.* 2015, *9*, 406–422

REVIEW

ELISA in the multiplex era: Potentials and pitfalls

Patrick J. Tighe, Richard R. Ryder, Ian Todd and Lucy C. Fairclough

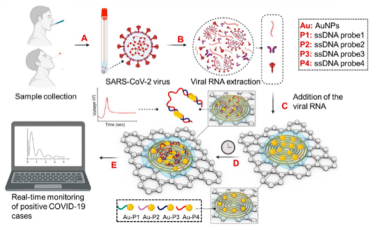
School of Life Sciences, The University of Nottingham, Nottingham, UK

126

Multiplexing : what type of encoding and what type of materials

1. **Line-Probe Assays**
   Exemple : Hain MDR-TB assays
2. **Microspheres based Assays** (suspension arrays)
   Exemple : Luminex, Austin TX (now Diasorin®)
3. **Microparticles based Assays** (encoded microparticles)
   Exemple : MyCartis, Gent, Belgium (now out of competition)
4. **Paper-based Assays**
   Exemple : Ebola assay (Magro et al. 2017)
   SARS-Cov 2 and DNA-based assays and paper-based

pubmed search: « multiplexing and paper-based IVD »-----→ 1article



Alafeef et al. 2021

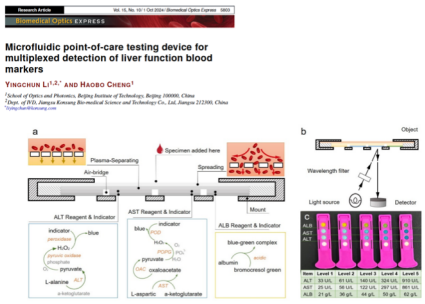


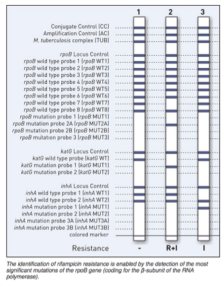
Fig. 1. Overall structure of the proposed paper-based test card. a) Schematic of the cross section of the paper-based test card, and the chemical changes of blood in the reagent and indicator layers. b) Analyzer detection principle. After the light passes through the filter, the reflected light through the color rendering region of the paper-based test card is captured by the detector. c) Color development at different concentrations with the assembled paper-based test card.

Multiplexing : what type of encoding and what type of materials ?

1. **Line-Probe Assays**
   Exemple : Hain MDR-TB assays

Hain MTB-DRplus
(Hain, Germany)

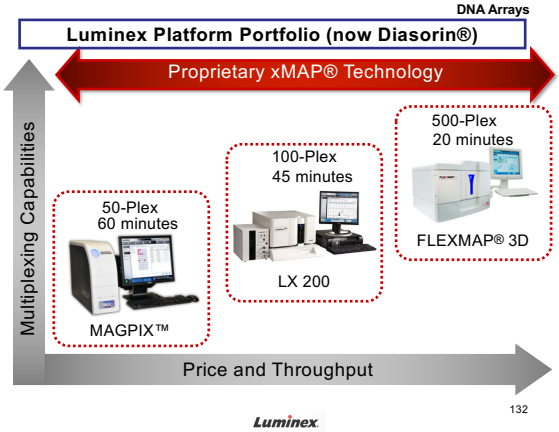
(now : Brücker, USA)



https://www.bruker.com/en/products-and-solutions/molecular-diagnostics/assays/mycobacteria/fluorotype-mtb.html?page=products/microbiology/mycobacteria/tuberculosis/fluorotype-mtb&l=en

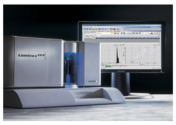
Multiplexing : what type of encoding and what type of materials ?

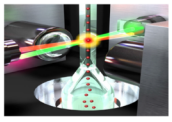
1. Line-Probe Assays
   Exemple : Hain MDR-TB assays
2. **Microspheres based Assays (suspension arrays)**



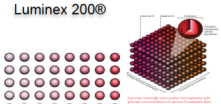
DNA Arrays

Luminex Platform Portfolio (now Diasorin®)



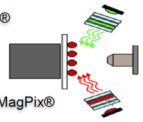


Luminex 200®



FlexMap 3D®

Differents bead Colours
MicroPlex®
MagPlex®



MagPix®

cours, 2016 Septembre 12

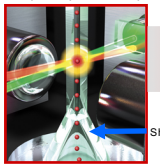
High-throughput innovative systems combining Lasers, Chemistry, Optics, Microfluidics, Bioinformatics

https://int.diasorin.com/en

DNA Arrays

Flow Cytometry and fluorescence Imaging Comparison

Flow Cytometry-Based Analysis



Interrogate label with green laser (525 nm)

Interrogate bead with red laser (635 nm)

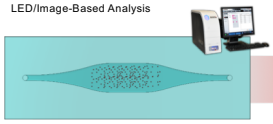
Sheath Fluid

Quantify binding events

Identify bead region based on internal dye concentrations

2500-5000 beads/target

LED/Image-Based Analysis



Beads in Chamber

Magnetic Capture

Interrogate label with green LED (525 nm)

Identify and quantify with CCD imager

Interrogate bead with red LED (635 nm)

1000 beads/target

Luminex

DNA Arrays

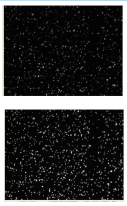
Generation of Classification and Reporter Images

LED Illumination	Capture image with CCD camera and appropriate filter	Image Analysis

Bead Classification

CL1 Filter

CL2 Filter

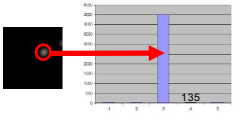


Two classification images will identify which bead set each individual bead is from – identifies assay

Assay Reporting

RP1 Filter

Reporter image quantifies assay – is your target analyte present and at what level?

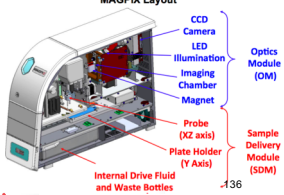


Luminex

DNA Arrays

MagPix : Light-emitting diode based imager

MAGPIX Layout



https://int.diasorin.com/en

Luminex

DNA Arrays

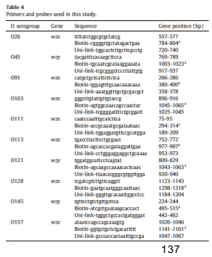
Applications: Luminex 200 and Magpix

1. Identification of 10 most clinically relevant Serogroups among STEC (shigella-toxin-enterotoxic *E.coli*).

Rapid O serogroup identification of the ten most clinically relevant STECs by Luminex microbead-based suspension array

Andrew Lin [a,*], Lam Nguyen [b], Teresa Lee [a], Laurie M. Clotilde [c], Julie A. Kase [d], Insook Son [d], J. Mark Carter [c], Carol R. Lauzon [b]

Journal of Microbiological Methods 87 (2011) 105–110



2. CRISPOL, TB-SPRINT, MLPA assays, TRIOL, TB-EFI

-L. Fabre *et al.* 2012
-I. Bergval *et al.* 2012
-S. Sengstake *et al.* 2014
-M. Gomgnimbou *et al.* 2012, 2013
Gomgnimbou *et al.* 2014
-Lin *et al.* 2015
-Klotoe et al. 2019

DNA Arrays

Multiplexing : what type of encoding and what type of materials ?

1. Line-Probe Assays
   Exemple : Hain MDR-TB assays
2. Microspheres based Assays (suspension arrays)
   Exemple : Luminex, Austin TX
3. **Microparticles based Assays (encoded microparticles)**

History or myCartis and bioCartis technology

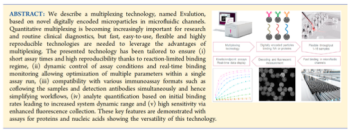
analytical chemistry

Rapid, Sensitive and Real-Time Multiplexing Platform for the Analysis of Protein and Nucleic-Acid Biomarkers

Didier Falconnet,[†,‡] Joseph She,[†] Raphaël Tornay,[†] Elisa Leimgruber,[†] David Bernasconi,[†] Lucienne Lagopoulos,[†] Philippe Renaud,[†] Nicolas Demierre,[†] and Patrick van den Bogaard[†]

[†]MyCartis, EPFL Innovation Park G, 1015 Lausanne, Switzerland
[‡]Laboratoire de Microsystèmes (LMIS4), École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

Supporting Information



Falconet *et al.* 2015

Biomérieux acquires Biofire jan. 2014

BioFire in Pubmed : 1,096 results

OPEN ACCESS Freely available online

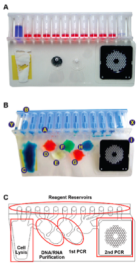
PLoS one

FilmArray, an Automated Nested Multiplex PCR System for Multi-Pathogen Detection: Development and Application to Respiratory Tract Infection

Mark A. Poritz[1¤], Anne J. Blaschke[2], Carrie L. Byington[2], Lindsay Meyers[1], Kody Nilsson[1], David E. Jones[1], Stephanie A. Thatcher[1], Thomas Robbins[1], Beth Lingenfelter[1], Elizabeth Amiott[1], Amy Herbener[2], Judy Daly[2], Steven F. Dobrowolski[1¤a], David H. -F. Teng[1¤b], Kirk M. Ririe[1]

1 Idaho Technology, Inc., Salt Lake City, Utah, United States of America, 2 Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, 3 Primary Children's Medical Center and Department of Pathology, University of Utah, Salt Lake City, Utah, United States of America

Poritz *et al.* PloS ONE 2011



Method: nested PCR
With digital PCR
As 2nd

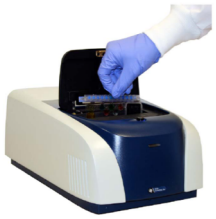


Figure 2. Schematic of second stage PCR mix entering the array. The layers of film and adhesive attaching the array to the pouch are separated to show the flow of liquid into the cells of the array (figure is not to scale). From the top the layers are: 2nd pouch film, 1st pouch film, array adhesive layer (orange), pricked cover film, array (black, with wells), and array cover film. All of the actual layers are transparent except for the array itself. Second stage PCR primers are spotted into the cells during manufacture and air-dried (Methods). Arrows show the flow of PCR master mix (without primers) entering the array through a hole cut in the 1st pouch film.
doi:10.1371/journal.pone.0026047.g002

- *Upper respiratory tract infections*
- *Identification of organisms in positive blood cultures*
- *Gastrointestinal infections*
- *Meningitis*
- *Lower respiratory tract infections*

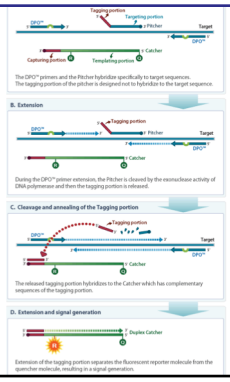
Poritz *et al.* PloS ONE 2011[141]

How to increase multiplexing 1

Seegene (Korean company)

Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene

Chun JY et al. Nucl. Acid Res, 2007



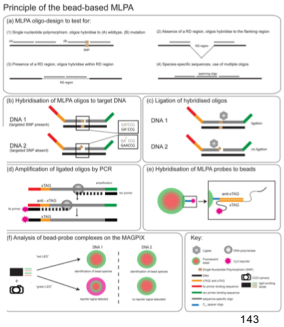
How to increase multiplexing 2

MRC (Dutch company)

Multiple Ligation Mediated Amplification system for the multiplex detection of Mtuberculosis

Bergval et al. 2012, PloS ONE
Sengstake et al. 2014 BMC Genomics

Principle of the bead-based MLPA



Microspheres-based arrays Advantages

1. Easy attachment of probes,
2. flexibility in composing a test panel,
3. improved kinetics
4. Limited costs
5. Laser-based technologies are mature
6. Ecosystem of application is broad

Microspheres-based arrays DisAdvantages

1. Multiplexing limited (100),
2. Luminex evolution towards « closed systems » like Aries®
3. Requires experience and expertise
4. Entry in technology (access) is expensive

Strategy for Multiplex analysis of Proteins in Mass Spectrometry

A **tandem mass tag** (**TMT**) is a chemical label that facilitates sample multiplexing in mass spectrometry (MS)-based quantification and identification of biological macromolecules suchas proteins, peptides and nucleic acids. (now designated as **Isobaric Tags)**

Anal Chem. 2003 Apr 15;75(8):1895-904. doi: 10.1021/ac0262560.

Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS

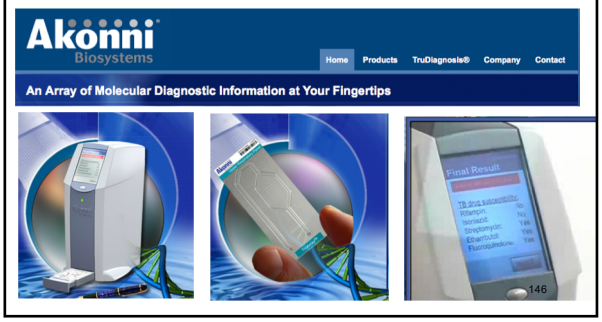
Andrew Thompson [1], Jürgen Schäfer, Karsten Kuhn, Stefan Kienle, Josef Schwarz, Günter Schmidt, Thomas Neumann, R Johnstone, A Karim A Mohammed, Christian Hamon

Petelski et al. 2021 Multiplexed single-cell proteomics using SCoPE2
Single cells are isolated by FACS or CellenONE into multiwell plates and lysed by Minimal ProteOmic sample Preparation (mPOP), and their peptides labeled by isobaric mass tags (TMT or TMTpro) for multiplexed analysis

145

Akonni Biosystems (USA) « TrueTip »

Akonni Biosystems Awarded Phase II NIH Contract to Advance its Device to Purify DNA from Sputum for Tuberculosis Testing



146

Multiplexing : what type of encoding and what type of materials ?

1. Line-Probe Assays
   Exemple : Hain MDR-TB assays
2. Microspheres based Assays (suspension arrays)
   Exemple : Luminex, Austin TX
3. Microparticles based Assays (encoded microparticles)
   Exemple : MyCartis, Gent, Belgium
4. **Paper-based Assays**

147

Paper-based IVDs

biosensors MDPI

Review

Research Progress and Future Trends of Microfluidic Paper-Based Analytical Devices in In-Vitro Diagnosis

Taiyi Zhang [†], Feng Ding [†], Yujing Yang, Gaozhen Zhao, Chuanhao Zhang, Ruiming Wang and Xiaowen Huang *

Paper-based and In vitro diagnostics
n=110 papers on Jan 27th 2025

148

Paper-based Assays

n=248 in PubMed on Jan. 23rd 2019
n=3171 in PubMed on Jan. 31st 2021
n=3724 in PubMed on Jan. 31st 2021
newest generation of lab-on-a-chip devices

MuPADS=microfluidic paper-based analytical devices (n=631, 2021. n=765, 2022 in Pubmed)

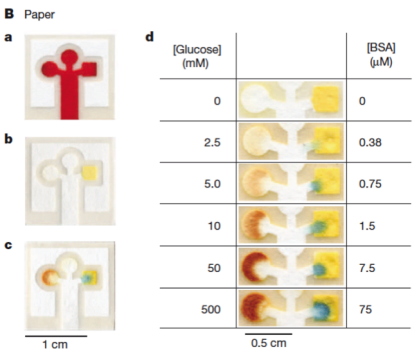
Recent Advances of Fluid Manipulation Technologies in Microfluidic Paper-Based Analytical Devices(μPADs) toward Multi-Step Assays

Kim et al. 2020

Inexpensive, portable, pump-free, and having the ability to store reagent

poor sensitivity of μPADs for the time-being

149



EK Sackmann *et al.* Nature, 2014, 507(7491):181-9150