université PARIS-SACLAY

2

Multiplexing, Arrays
Arrays encoding, Optics
Mass Spectrometry
Phenotypic methods, Serology, Others
Course Master 2 MGB,
Teaching Unit MPAMB-2023-2024

Pr. Christophe Sola

IAME-UMR1137, INSERM-Université de Paris

Synthesis of Evolution of Molecular Probes

With or without radioactivity ?

-radioactives probes (P^{32}, S^{35}, C^{14} H^{3})
- Cols probes (since 1984) : avidin-biotin or digoxigenin-anti digoxigenin antibodies

Before or after PCR Invention ? (K. Mullis)

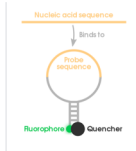
-Nick Translation (Rigby 1977) or Random Priming (Feinberg 1983)

Before or after Khorana ? (chemical synthesis)

- -democratization of chemical synthesis of DNA

Cold Probes Evolution

-*molecular beacons* and FRET. (**Tyagi 1996**)
- Hydrolysis probes : Taqman. (**Holland 1991**)
- Burst of fluorescence markers and fluorescence detection
- other systems…quantum dots, …toward nano and
- electrochemistry *Nanospheres*



The structure of a typical molecular beacon probe

Optics

What is FRET ?

Förster resonance energy transfer

- *In the process of FRET, initially a donor fluorophore absorbs the energy due to the excitation of incident light and transfer the excitation energy to a nearby chromophore, the acceptor*

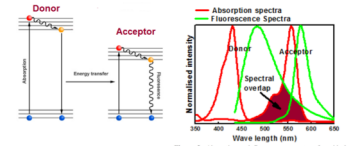


Figure 1: Jablonski diagram illustrating the FRET process.

Figure 2: Absorption and fluorescence spectra of an ideal donor-acceptor pair. Brown coloured region is the spectral overlap between the fluorescence spectrum of donor and absorption spectrum of acceptor.

Molecular Probes, Historical References

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- Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia.Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N. Science. 1985 Dec 20;230(4732):1350-4.
- Polynucleotide synthesis and the genetic code.Khorana HG, Büchi H, Ghosh H, Gupta N, Jacob TM, Kössel H, Morgan R, Narang SA, Ohtsuka E, Wells RD.Cold Spring Harb Symp Quant Biol. 1966;31:39-49
- S. Tyagi, F.R. Kramer, Molecular beacons: Probes that fluoresce upon hybridization, Nat. Biotechnol. 14 (1996) 303‡308.
- Marmé et al. 2006 : Identification of single-point mutations in mycobacterial 16S rRNA sequences by confocal single-molecule fluorescence spectroscopy Nucl. Acid. Res. 2006, 34, 13 e90.

REVIEW

doi:10.1038/nature13118

The present and future role of microfluidics in biomedical research

Eric K. Sackmann[1], Anna L. Fulton[2] & David J. Beebe[3]

13 MARCH 2014 | VOL 507 | NATURE | 181

*In 1998, Whitesides used **polydimethylsiloxane** (**PDMS**)—an optically transparent, gas- and vapour-permeable elastomer— for the fabrication of more complex microfluidic devices and helped 'soft lithography' become the most widely adopted method for fabricating microfluidic devices. It would be hard to exaggerate how important and enabling PDMS has been for microfluidics, contributing to the growth of the field in both technological development and number of publications*

photolitography
electron-beam lithography ------> *PDMS stamp, resolution : may* reach 6 nm

Unusual rheological (or flow) properties

Most widely used silicon-based organic polymer

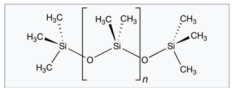
silicone oil (polymerized siloxane

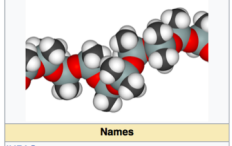
From contact lenses and medical devices to elastomers

Shampoos (as it makes hair shiny and slippery),

Food (antifoaming agent), caulking, lubricants and heat-resistant tiles

Polydimethylsiloxane





Names
IUPAC name
poly(dimethylsiloxane)

AMGEN

10/12/12

deCODE genetics

Invetech
For your most important projects

Luminex

Agilent Technologies

ALAMAR BIOSCIENCES

BIOFIRE
A BIOMÉRIEUX COMPANY

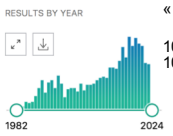
RANDOX 30
ANNIVERSARY 1982 - 2012

sequenom.

MIP= molecular inversion probes : a capture by circularization molecular technique

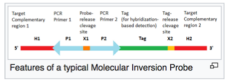
Molecular Inversion Probe (MIP) Assay is an efficient technology for large-scale Single Nucleotide Polymorphisms (SNPs) analysis. It is used for SNP discovery and genotyping. This technique produces "inverted" probes in which the information content of the SNPs is reformatted into tag sequences that could be analyzed using a universal sequence tag DNA microarrays. With this technology, multiplex analysis of more than 10,000 probes in a single tube can be done using standard laboratory equipment.

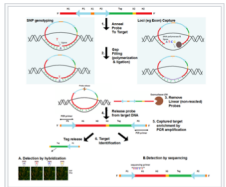
https://en.wikipedia.org/wiki/Molecular_Inversion_Probe



« MIP »

1001 papers in 2024
1053 papers in 2025

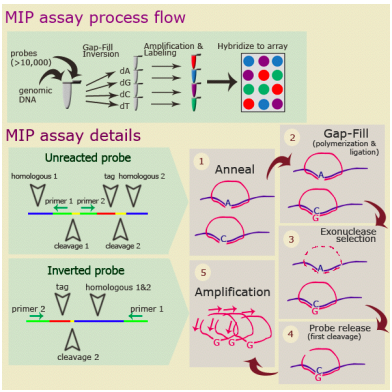

Features of a typical Molecular Inversion Probe


Procedure for genomic regions capture using Molecular Inversion Probes

MIP= molecular inversion probes

- *Molecular Inversion Probes (originally called "**padlock probes**") are **single-stranded DNA molecules** containing two regions complementary to regions in the target DNA that flank SNP in question. Each probe also contains universal primers' sequences separated by endoribonuclease recognition site and a 20-nt tag sequence. During the assay the probes undergo a unimolecular rearrangement: they are (1) circularized by filling gaps with nucleotides corresponding to the SNPs in four separate allele-specific polymerization (A, C, G, and T) and ligation reactions; (2) linearized in enzymatic reaction. As a result they become "inverted". This step is followed by PCR amplification. Further processing of the probes depends on specific assay' variation*

- padlock probe (**padlock probe rolling circle amplification (PLRCA), nuclease protection (NP) and lateral flow detection (LFA), referred to as PLAN-LFA**)



MIP= molecular inversion probes

Clin Chem. 2018 Jun;64(6):938-949. doi: 10.1373/clinchem.2017.284737. Epub 2018 Mar 16.

Ultrasensitive Detection of Chimerism by Single-Molecule Molecular Inversion Probe Capture and High-Throughput Sequencing of Copy Number Deletion Polymorphisms.

Wu D[1], Waalkes A[1], Penewit K[1], Salipante SJ[2].

Drug-Resistance and Population Structure of Plasmodium falciparum Across the Democratic Republic of Congo Using High-Throughput **Molecular Inversion** Probes.

Aydemir O, Janko M, Hathaway NJ, Verity R, Mwandagalirwa MK, Tshefu AK, Tessema SK, Marsh PW, Tran A, Reimonn T, Ghani AC, Ghansah A, Juliano JJ, Greenhouse BR, Emch M, Meshnick SR, Bailey JA.

J Infect Dis. 2018 Aug 14;218(6):946-955. doi: 10.1093/infdis/jiy223.

Detection of 16S rRNA and KPC Genes from Complex Matrix Utilizing a **Molecular Inversion Probe** Assay for Next-Generation Sequencing.

Stefan CP, Hall AT, Minogue TD.

Sci Rep. 2018 Feb 1;8(1):2028. doi: 10.1038/s41598-018-19501-z.

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» Absalan F, Ronaghi M. Molecular inversion probe assay. *Methods Mol Biol. 2007;396:315-30.* PMID: 18025701

» Hardenbol P et al. Multiplexed genotyping with sequence-tagged molecular inversion probes. *Nat Biotechnol. 2003 Jun;21(6):673-8. Epub 2003 May 5.* PMID: 12730666

> Acc Chem Res. 2016 Nov 15;49(11):2540-2550. doi: 10.1021/acs.accounts.6b00417. Epub 2016 Oct 24.

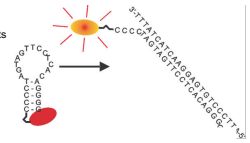
The Discovery of Rolling Circle Amplification and Rolling Circle Transcription

Michael G Mohsen [1], Eric T Kool [1]

Evolution towards spectroscopical analysis of single molecules

Allows the identification of a DNA target with cc picomolar concentration in an homogenous assay

Principle of a smart probe 1,508 results
molecular beacon, Tyagi et al. 2,580 results



> Nucleic Acids Res. 2022 Jul 22;50(13):e74. doi: 10.1093/nar/gkac242.

A molecular beacon assay for monitoring RNA splicing

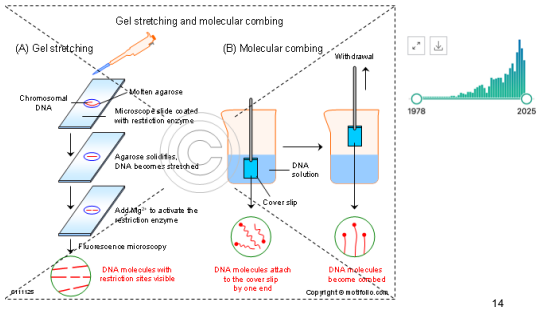
Smart probes

Technological innovations with straightforward outcomes in medicinal chemistry and chemical biology are reliant on fluorescent reporters ever more accurate and practically convenient. Numerous probes are now available, allowing for the detection of a wide panel of cellular biomarkers and biorelevant analytes. This approach has proven successful over the past years, for prognostic, diagnostic and theranostic purposes as well as intraoperative manipulations. Next generation molecular tools, known as "smart fluorescent probes", are currently being developed: these fluorogenic dyes uniquely switch on upon interaction/reaction with their targets only, thus dramatically improving the quality of the detection. In recent years, P2DA has actively contributed to these innovations via the development and applications of organic-based fluorophores (boron dipyrromethenes (BODIPY), cyanines, diketopyrrolopyrroles, porphyrins, corroles, phthalocyanines, xanthenes, etc.). Our "smart" fluorogenic molecular tools have addressed critical biological concerns, being successfully applied for imaging a wealth of cellular constituents (including enzymes (e.g., liver esterase) and alternative nucleic acid structures (e.g., DNA and RNA quadruplexes)), but also to investigate the stability of a drug in vitro through various imaging modalities (confocal, two-photon or stochastic optical reconstruction microscopies as well as Cherenkov luminescence imaging).

http://www.icmub.com/en/team/p2da-team-polyamines-porphyrins-developments-and-applications/smart-probes.html

GENOMIC VISION

Molecular combing (n=554 results on Jan 28th 2024, 591 in 2025) (Genomic Vision France)



GENOMIC VISION

Founder : A. Bensimon, 2004 a.bensimon@genomicvision.com
2 financing rounds : SGAM, Vesalius Biocapital
10 Millions € in 2006, based in Cochin Hospital
33 staff, 10 PhD, 6 Ing, 5 technicians
almost bankrupt in 2019, Saved by SANOFI and NIH in September 2019 !

TeloSizer®
La solution pour la détection précise et la mesure quantitative de la longueur des télomères.

Dystrophie musculaire
Le test de diagnostic de Genomic Vision permet une identification claire et précise des répétitions spécifiques à la Dystrophie FSH (FSHD) dans son environnement génomique.

Replication Combing Assay
Le peignage moléculaire est une approche puissante et précise permettant de suivre les caractéristiques à la fois spatiales et temporelles de la réplication de l'ADN en une seule expérience.

GV STORE
Genomic Vision lance "GV Store", son site de-commerce pour accompagner la croissance dynamique des ventes de ses activités LSR

Definitions

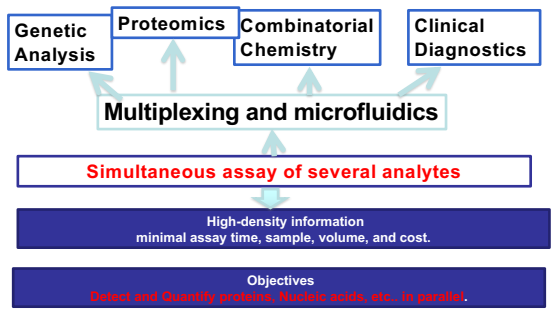
Microfluidics

ditional assays used in cell biology. Conceptually, the idea of microfluidics is that fluids can be precisely manipulated using a microscale device built with technologies first developed by the semiconductor industry and later expanded by the micro-electromechanical systems (MEMS) field. These devices, commonly referred to as miniaturized total analysis systems (μTASs)[6,7] or lab-on-a-chip (LoC) technologies, could be applied to biology research to streamline complex assay protocols; to reduce the sample volume substantially; to reduce the cost of reagents and maximize information gleaned from precious samples; to provide gains in scalability for screening applications and batch sample processing analogous to multi-well plates; and to provide the investigator with substantially more control and predictability of the spatio-temporal dynamics of the cell microenvironment.

Sackmann ER et al. 2014

Multiplexing

Multiplexing is a technique invented in 1891 by Emile Baudot, which consists of passing several items of information through a single transmission medium. It enables the same resource to be shared between several users. There are two main multiplexing techniques



Example of early applications in prenatal diagnostics and retinal care

1994, San Diego, California

SEQUENOM → GenomeQuébec

SEQureDx® technology is based on the pioneering work of Professor Dennis Lo, and isolates and analyzes circulating cell-free fetal nucleic acid from a maternal blood sample. The technology has particular promise for developing new, noninvasive tests for fetal gene and chromosome abnormalities such as aneuploidies - including trisomy 21

Genes included in the iPLEX ADME PGx Panel:

ABCB1	CYP2E1	SLC22A2
ABCC2	CYP3A4	SLC22A6
ABCG2	CYP3A5	SLCO1B1
COMT	DPYD	SLCO1B3
CYP1A1	GSTM1	SLCO2B1
CYP1A2	GSTP1	SULT1A1
CYP2A6	GSTT1	TPMT
CYP2B6	GSTT2	UGT1A1
CYP2C19	NAT1	UGT2B15
CYP2C8	NAT2	UGT2B17
CYP2C9	SLC15A2	UGT2B7
CYP2D6	SLC22A1	VKORC1

- Multiplex up to **40 SNPs in a single well**
- Process up to **384 samples in parallel**

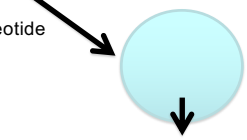
Array types

- **Planar (2D)/ Spatial (3D)**
- **Spatial (3D)**
  - ->solution kinetics, ease of assay modification, « liquid arrays »
  - ->suspension arrays (beads, particles, nano)
  - ->higher sample throughput, and better quality control batch synthesis
- **Planar (2D)/**
  - – ->ultra high-density

Principle

One material (**polymer material**) in a bi- or tri-dimensional structure

On which we may permanently coupled/graft biomolecules
-proteins
-haptens
-oligonucleotide
etc…



1 **Planar Arrays**

2. **Suspension (particle-based) Arrays**

Internal encoding allowing to specifically Identify the particle (**encoding**)

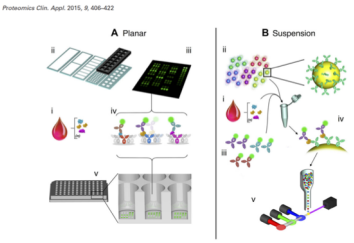
Encoding types: planar versus suspension

- **-Planar**

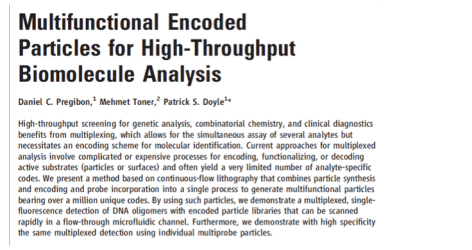
->Positions encoding

- **-Spatial**

> Many types, complex



Multifunctional Encoded Particles for High-Throughput Biomolecule Analysis
Daniel C. Pregibon *et al.*
Science **315**, 1393 (2007);
DOI: 10.1126/science.1134929



Multifunctional Encoded Particles for High-Throughput Biomolecule Analysis

Daniel C. Pregibon,[1] Mehmet Toner,[2] Patrick S. Doyle[3]*

High-throughput screening for genetic analysis, combinatorial chemistry, and clinical diagnostics benefits from multiplexing, which allows for the simultaneous assay of several analytes but necessitates an encoding scheme for molecular identification. Current approaches for multiplexed analysis involve complicated or expensive processes for encoding, functionalizing, or decoding active substrates (particles or surfaces) and often yield a very limited number of analyte-specific codes. We present a method based on continuous-flow lithography that combines particle synthesis and encoding and probe incorporation into a single process to generate multifunctional particles bearing over a million unique codes. By using such particles, we demonstrate a multiplexed, single-fluorescence detection of DNA oligomers with encoded particle libraries that can be scanned rapidly in a flow-through microfluidic channel. Furthermore, we demonstrate with high specificity the same multiplexed detection using individual multiprobe particles.

Particle Encoding schemes

- **Spectrometric** (**ref.4–11 from Pregibon et al. 2007**)
  - **-Fluorophores** : Fluorescence-encoded microbeads on Conventional flow cytometry or on fiber-optic arrays
  - **-Chromophores**
    Uniformly Colorized Beads for Multiplex Immunoassay, Zhao et al. 2006
  - **-Photonic structures**
  - **-Surface-enhanced Raman scattering (SERS)**
    Wang Y. Novel OpticalNanoprobes for Bioanalysis.Chem. Rev.2013,113, 1391–1428
- **Graphical** (**ref.12–16 from Pregibon et al 2007**),
- **Electronic** (**ref.17–19 from Pregibon et al. 2007**),
- **Physical** (**ref. 20-21 from Pregibon et al. 2007**).

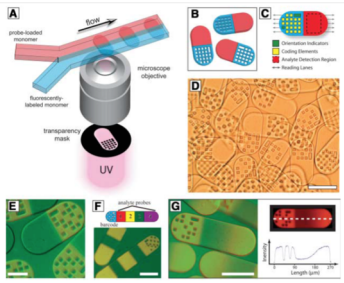


Fig. 1. (**A**) Schematic diagram of dot-coded particle synthesis showing polymerization across two adjacent laminar streams to make single-probe, half-fluorescent particles [shown in (**B**)]. (**C**) Diagrammatic representation of particle features for encoding and analyte detection. Encoding scheme shown allows the generation of 2^{20} (1,048,576) unique codes. (**D**) Differential interference contrast (DIC) image of particles generated by using the scheme shown in (**A**). (**E** to **G**) Overlap of fluorescence and DIC images of single-probe (E), multiprobe (F, bottom), and probe-gradient (G, left) encoded particles. Shown also is a schematic representation of multiprobe particles (F, top) and a plot of fluorescent intensity along the center line of a gradient particle (G, right). Scale bars indicate 100 μm in (D), (F), and (G) and 50 μm in (E).

Science. 2007 Mar 9;315(5817):1393-6.

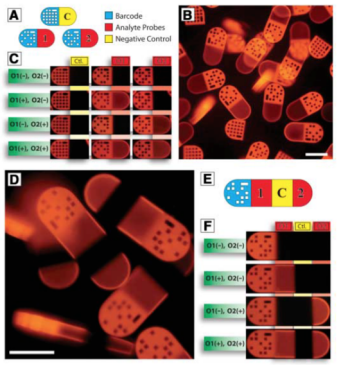


Fig. 2. Multiplexed analysis using single- (**A** to **C**) and multiprobe (**D** to **F**) encoded particles. The particles were loaded with DNA oligomer probes (O1, 5′-ATA GCA GAT CAG CAG CCA GA-3′, or O2, 5′-CAC TAT GCG CAG GTT CTC AT-3′) or no probe [negative control (C)] as shown schematically in (A) and (E). Shown are representative fluorescence images for single-probe (B) and multiprobe (D) particles after a 10-min incubation with both fluorescent-labeled targets. Fluorescence in the probe regions indicates target detection. Also shown are individual particles after incubation in solutions containing no targets, target 1 only, target 2 only, or both targets [(C) and (F)]. Scale bars, 100 μm.

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Science. 2007 Mar 9;315(5817):1393-6.

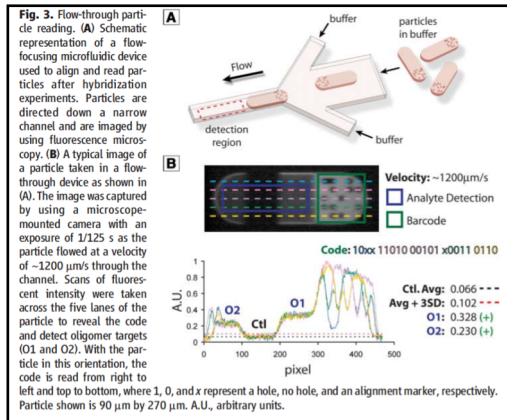

Fig. 3. Flow-through particle reading. (**A**) Schematic representation of a flow-focusing microfluidic device used to align and read particles after hybridization experiments. Particles are directed down a narrow channel and are imaged by using fluorescence microscopy. (**B**) A typical image of a particle taken in a flow-through device as shown in (A). The image was captured by using a microscope-mounted camera with an exposure of 1/125 s as the particle flowed at a velocity of ~1200 μm/s through the channel. Scans of fluorescent intensity were taken across the five lanes of the particle to reveal the code and detect oligomer targets (O1 and O2). With the particle in this orientation, the code is read from right to left and top to bottom, where 1, 0, and *x* represent a hole, no hole, and an alignment marker, respectively. Particle shown is 90 μm by 270 μm. A.U., arbitrary units.

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Science. 2007 Mar 9;315(5817):1393-6.

Sensitivity perspectives, Pregibon *et al.* 2007

(cited 809 times according to Google)

*In addition to being very reproducible, we have also shown that our system is very sensitive. With 30-min incubations, we can detect DNA oligomers comfortably at **500 attomoles** without biotin-avidin–aided signal amplification.*

Science. 2007 Mar 9;315(5817):1393-6.

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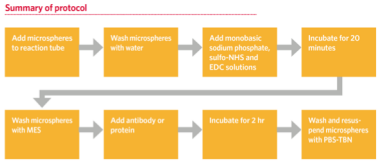
How to functionalize encoded arrays with Proteins ?

1. **Automatized Digitally-encoded Microparticles protein-coupling**

« A key feature of our method is the direct incorporation of probes into the encoded particles. This is accomplished by simply adding **acrylate modified biomolecules** into the monomer solution. After polymerization, the probes are covalently coupled to the polymer network. This process is applicable for both **oligonucleotide and protein probes**

2. **Manual Optically-encoded Microspheres protein-coupling**



The Luminex cookbook

M2 2025 MPAMB 28

How to functionalize encoded arrays with oligos ?

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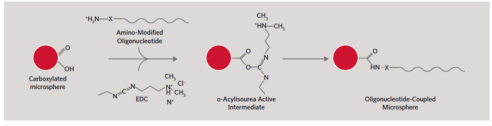
2. **Manual Optically-encoded Microspheres oligonucleotide-coupling**

Nucleic acid coupling chemistry



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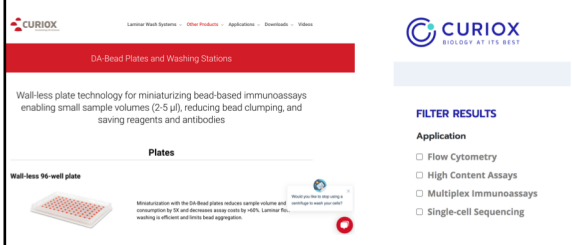
The Luminex cookbook

Decreasing Multiplex-based assay costs using the Curiox-System (e.g. tears analysis)

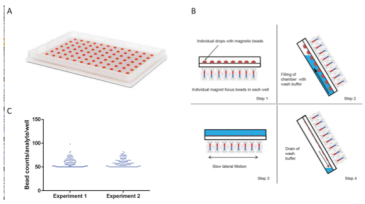
- DA-Bead (Curiox Biosystems, Singapore) is a wall-less plate defined with 96 circular wells on a **polytetrafluoroethylene (PTFE)** resin-coated polymer plastic that follows conventional microtiter plate specifications (SBS)



M2 2025 MPAMB 30

Figure 1. Overview of DropArray Technology. **A**: DA-Bead 96-well photograph. The plate follows 96-well microtiter plate standard specifications and accommodates drops of 5 µl up to 20 µl in hydrophilic well space surrounded by hydrophobic polytetrafluoroethylene (PTFE) space. **B**: Washing principle of DA-Bead with Curiox LT-MX washer. DA-Bead plate is placed in Washer LT-MX and sealed. Each drop assay is in line with an individual magnet, and focusing is performed for 30 s (Step 1). DA-Bead is rotated counter-clockwise to 120˚, and washing buffer fills the plate from the bottom to the top (Step 2). DA-Bead is returned to the horizontal position. The rinsing chamber is then returned to a horizontal position and undergoes low velocity lateral shaking for 10 s at a speed of 20 rpm which is equivalent to a lateral shear force of 70.2 mm/s (Step 3). DA-Bead is rotated counter-clockwise to 120˚, and the washing buffer is drained, producing a dry plate ready for the next reagent dispensing (Step 4). **C**: Bead count performance of experiments presented in this study on DA-Bead. Each dot represents the count for one analyte in a well. All bead counts are ≥50. Beads available per analyte in DA-Bead are reduced by 80% compared to conventional methods.

5 to 20 µl drops



Le Guezennec, Mol Vis 2015; 21:1151-1161. http://www.molvis.org/molvis/v21/1151
https://www.curiox.com/resources/publications/

Proximity Extension Assays and multiplexing in proteomics

U. Landegren *et al.* 2010

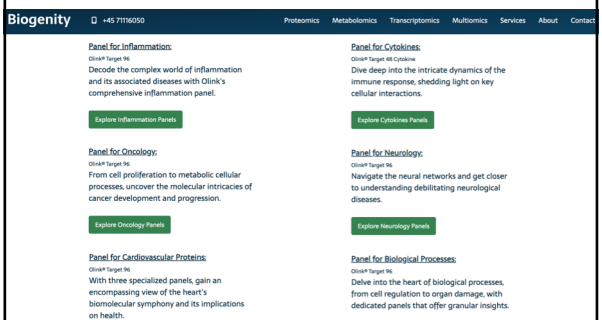
Ulf Landegren

- **Abstract:** The advent of *in vitro* DNA amplification has enabled rapid acquisition of genomic information. **We present here an analogous technique for protein detection, in which the coordinated and proximal binding of a target protein by two DNA aptamers promotes ligation of oligonucleotides linked to each aptamer affinity probe** . The ligation of two such proximity probes gives rise to an amplifiable DNA sequence that reflects the identity and amount of the target protein. This proximity ligation assay detects zeptomole (40 × 10^{-21} mol) amounts of the cytokine platelet-derived growth factor (PDGF) without washes or separations, and the mechanism can be generalized to other forms of protein analysis.

Proximity Extension Assay principle

- 1. **Antibody-Based Detection:** PEA uses pairs of antibodies that are specific to the target protein or biomarker. These antibodies are each conjugated to short oligonucleotides (DNA or RNA sequences).
- 2. **Proximity-Dependent Hybridization:** Each antibody in the pair binds to a distinct epitope (binding site) on the target protein.When both antibodies bind to the target protein, the two oligonucleotides attached to them come into close proximity to each other (typically < 10 nm).
- 3. **Extension Reaction:** Once the antibodies are in proximity, a DNA polymerase enzyme is used to "extend" the oligonucleotides, creating a new, longer DNA fragment. This extension occurs only if the oligonucleotides are close enough to each other (i.e., when the antibodies are bound to the target protein).
- 4. **Signal Detection:**The extended DNA product is then detected using various methods, such as quantitative PCR (qPCR), hybridization, or fluorescence-based techniques. The amount of extended DNA correlates with the amount of target protein in the sample allowing for sensitive quantification.
- 5. **High Specificity:** The proximity-dependent nature of the assay ensures high specificity, as the extension reaction only occurs when both antibodies are bound to the same protein molecule. This minimizes cross-reactivity and background noise that might arise from non-specific binding.
- 6. **Multiplexing:** PEA can be multiplexed, meaning multiple target proteins can be quantified simultaneously in the same sample by using different antibody pairs and distinct oligonucleotide sequences. This is a powerful advantage, as it allows for the analysis of complex biomarker panels in a single run.
- 7. **Sensitivity:** Because the signal amplification step (the oligonucleotide extension) is highly sensitive, PEA can detect low-abundance biomarkers in a wide range of sample types, including very small volumes

Olink® panels of multiplex protein detection



Application for Biomarker discovery

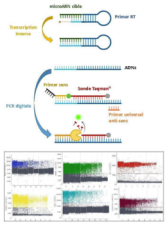
- **Proximity extension assay proteomics and renal single cell transcriptomics uncover novel urinary biomarkers for active lupus nephritis**
- Y. Li et al. J Autoimmun, 2024 Feb:143:103165. doi: 10.1016/j.jaut.2023.103165. Epub 2024 Jan 8

Lab Invest. 2018 Dec 12. doi: 10.1038/s41374-018-0143-3. [Epub ahead of print]

MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics.

Dave VP[1,2], Ngo TA[3], Pernestig AK[4], Tilevik D[4], Kant K[5], Nguyen T[1], Wolff A[5], Bang DD[1].

Multiplexing dPCR for 6 miRNA quantification



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.
Optimised protocol can be applied to different types of biological samples

Multiplex digital PCR for the simultaneous quantification of a miRNA panel.
Busato F, Ursuegui S, Deleuze JF, **Tost J.**
Anal Chim Acta. 2025 Jan 15;1335:343440.
doi: 10.1016/j.aca.2024.343440. Epub 2024 Nov 20

NULISA®. (200 Plex)

https://www.youtube.com/watch?v=9KcTXmAWCdc

nature communications

Article

https://doi.org/10.1038/s41467-023-42834-x

NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing

Received: 22 June 2023

Accepted: 23 October 2023

Published online: 09 November 2023

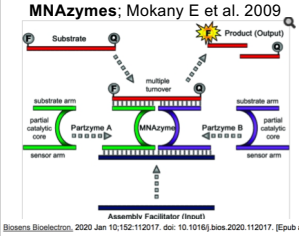
Check for updates

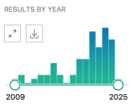
Wei Feng[1], Joanne C. Beer[1], Qinyu Hao[1], Ishara S. Ariyapala[1], Aparna Sahajan[1], Andrei Komarov[1], Katie Cha[1], Mason Moua[1], Xiaolei Qiu[1], Xiaomei Xu[1], Shweta Iyengar[1], Thu Yoshimura[1], Rajini Nagaraj[1], Li Wang[1], Ming Yu[1], Kate Engel[1], Lucas Zhen[1], Wen Xue[1], Chen-jung Lee[1], Chan Ho Park[1], Cheng Peng[1], Kaiyuan Zhang[1], Adrian Grzybowski[1], Johnnie Hahm[1], Susanne V. Schmidt[2], Alexandru Odainic[2,3], Jasper Spitzer[2], Kasun Buddika[1], Dwight Kuo[1], Lei Fang[1], Bingqing Zhang[1], Steve Chen[1], Eicke Latz[3,4], Yiyuan Yin[1], Yuling Luo[1] & Xiao-Jun Ma[1]

https://alamarbio.com/technology/nulisa-platform/

Multi-component Nucleic Acid enzymes (MNAzyme) As signal amplificators for improved sensitivity

MNAzymes; Mokany E et al. 2009





mnazyme in Pubmed
52 papers in 2024
62 papers in 2025

Biosens Bioelectron. 2020 Jan 10;152:112017. doi: 10.1016/j.bios.2020.112017. [Epub ahead of print]

DNA-only, microwell-based bioassay for multiplex nucleic acid detection with single base-pair resolution using MNAzymes.

Safdar S[1], Ven K[1], van Lent J[1], Pavie B[2], Rutten I[1], Dillen A[1], Munck S[2], Lammertyn J[3], Spasic D[1].

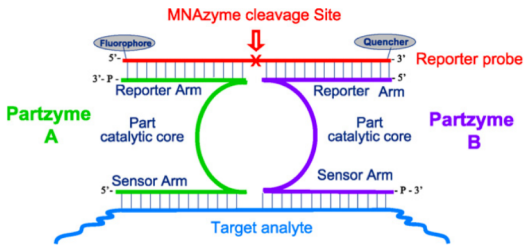


Fig. 1. Schematic representation of an MNAzyme. The MNAzyme consists of two DNA strands, called "Partzymes" bearing three distinct regions: (a) the "sensor arm" targeting one half of the desired DNA motif to be quantified, (b) one half of the "catalytic core" of the DNAzyme and (c) the "reporter arm" targeting one half of the reporter probe. The 3′ end of the Partzyme is phosphorylated to prevent the partzyme from functioning as a primer.

Nucleic Acids Research, 1998, Vol. 26, No. 22 5073–5078

Signal amplification of padlock probes by rolling circle replication

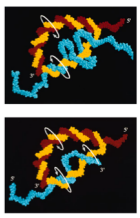
Johan Banér, Mats Nilsson, Maritha Mendel-Hartvig and Ulf Landegren*

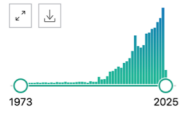
The Beijer Laboratory, Department of Genetics and Pathology, Uppsala University, Box 589, Se-751 23 Uppsala, Sweden

Received August 21, 1998; Revised and Accepted October 2, 1998

ABSTRACT

Circularizing oligonucleotide probes (padlock probes) have the potential to detect sets of gene sequences with high specificity and excellent selectivity for sequence variants, but sensitivity of detection has been limiting. By using a rolling circle replication (RCR) mechanism, circularized but not unreacted probes can yield a powerful signal amplification. We demonstrate here that in order for the reaction to proceed efficiently, the probes must be released from the topological link that forms with target molecules upon hybridization and ligation. If the target strand has a nearby free 3′ end, then the probe–target hybrids can be displaced by the polymerase used for replication. The displaced probe can then slip off the target strand and a rolling circle amplification is initiated. Alternatively, the target sequence itself can prime an RCR after its non-base paired 3′ end has been removed by exonucleolytic activity. We found the Φ29 DNA polymerase to be superior to the Klenow fragment in displacing the target DNA strand, and it maintained the polymerization reaction for at least 12 h, yielding an extension product that represents several thousand-fold the length of the padlock probe.





« **rolling circleamplification** »

1989 papers in 2024
2211papers in 2025

« **padlock probes** »
416 papers in 2025

Abbott

Alere

Covid-19

ID NOW ™ is a rapid molecular biology platform with an **isothermal detection** system for several infectious pathologies.

StaphyType 96

DNA Array Hybridisation Kit for *S.aureus* Resistance Gene & Pathogenicity Marker Detection

StaphyType96 Kit is designed to help

- epidemiologists, who need information about the phylogenetic relationshi of *S.aureus* strains in distinct geographic regions, about the dissemination of individual strains, or about the loss or gain of distinct genetic markers i *S. aureus* strains,
- hygienists, who want to learn, whether infections are caused by one identical or by different *S.aureus* strains, and who rapidly need to identify an evolving strain to prevent outbreaks,
- veterinary institutions, forensic medicine, food industry to survey and control for potential contaminations.

« **Abbot id now** »

86 papers in 2024
100 papers in 2025

Alere SAS
formerly Inverness Medical France SAS
7 rue Victor Hugo
92310 SEVRES
France
Tel: +33 1 46 23 63 63
Fax: +33 1 46 26 34 94
http://www.alere.fr

Laboratory evolution

Problems and Technologies that go hand in hand with miniaturization and automation in laboratories

- Aim = increase speed, movability, robustness, decrease costs of PCR conventional PCR systems
- mean= miniaturization
- Other mean= development of more multiplexed systems plus = **384 microwells**
  -> corollary: Automatization is mandatory

TECAN

PerkinElmer For the Better

Andrew Alliance

HAMILTON

Cell Elutriation

Invetech

Korus™
Elutriation. Washing. One system.

Elutriation is a process used to separate particles based on their size, density, or other properties by suspending them in a fluid and subjecting them to an upward flowing stream of the fluid. As the fluid flows upward, particles of different sizes or densities settle at different rates, allowing for separation. This technique is commonly used in various industries, including mining, environmental science, and chemical engineering, for tasks such as particle size classification and purification.

1. Centrifugal Elutriation
2. Air Elutriation:
3. Hydrocyclone Elutriation
4. Magnetic Elutriation:

Mass Spec

JJ Thomson

History of MS

CLASSIFICATION OF MICROORGANISMS BY ANALYSIS OF CHEMICAL COMPOSITION

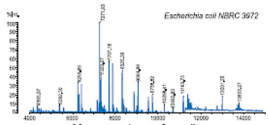
I. Feasibility of Utilizing Gas Chromatography

K. Abel, H. deSchmertzing, and J. I. Peterson[1]

Research Division, MelPar, Inc., Falls Church, Virginia

Received for publication 13 December 1962

Pyrolysis gas chromatography/Mass spectrometry GC/MS in the 1970s
Simmonds, P. G. *Appl. Microbiol.* **1970**, *20* (4), 567-572



Mass spectrum of e-coli

https://www.shimadzu.ch/microorganism-identification

Mass Spec

Actors on the market
CE Marking (conformité européenne)

- **Brucker**
- **Biomérieux**
- **Shimadzu**

BRUKER

Bruker Corporation, Fremont, CA, USA

SHIMADZU
Excellence in Science

BIOMÉRIEUX

Innovative Mass Spectrometry Systems

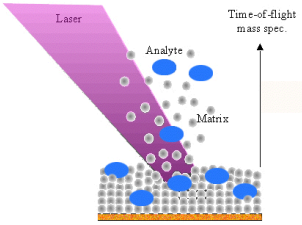
Robust, powerful and easy to implement analytical solutions for any challenge

Mass Spec

Matrix-assisted Laser induced desorption /Ionization-Time of Flight

« ***analytical technique that measures the mass-to-charge ratio of charged particles*** ».

- **MALDI-TOF**



Three highly common matrix:
(1) acide 3,5-diméthoxy-4-hydroxycinnamique, (2) acide 2-cyano-4-hydroxycinnamique (3) acide 2,5-dihydroxybenzoïque.

Koichi Tanaka

Ultra fine metal powder in glycerol (SLD)

Franz Hillenkamp

Organic matrix

Mass Spec

Nature Reviews Microbiology **8**, 74-82 (January 2010) | doi:10.1038/nrmicro2243

Review

Mass spectrometry tools for the classification and identification of bacteria

Sascha Sauer[1] & Magdalena Kliem[1] About the authors

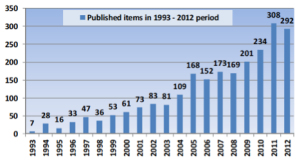
top

Mass spectrometry has become an important analytical tool in biology in the past two decades. In principle, mass spectrometry offers high-throughput, sensitive and specific analysis for many applications in microbiology, including clinical diagnostics and environmental research. Recently, several mass spectrometry methods for the classification and identification of bacteria and other microorganisms, as well as new software analysis tools, have been developed. In this Review we discuss the application range of these mass spectrometry procedures and their potential for successful transfer into microbiology laboratories.

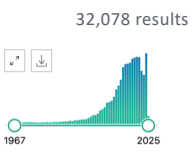
Current Trends in Microbial Diagnostics Based on Mass Spectrometry *Vladimir Havlicek, Karel Lemr and Kevin A. Schug Analytical Chemistry, Nov 2012*

Mass Spec

Published literature in MS
469.000 Articles in 2025



"mass spectral identification of bacteria"



Mass Spectrometry and Microbiology

Trend in market changes

- **Reduced interest in biochemical tests** (obsolete within the next 5 years)
- **Replaced by MS**, bioinformatics, data analysis platform
- 2 biochemical platforms : BD Phoenix, VITEK-2 Smart Carrier-50-80 papers/year
- **MS expanded to antimicrobial susceptibility testing area**

  Biochemistry = inaccurate

  Sequencing= expensive

  MS = fast, cheap, more efficient, high throughput

MALDI-TOF in microbiology

- **Bacterial typing represents a routine**
- **Fast and reliable method with reproducible and automates samples preparation protocols**
- **Cultivation and standardizes colony pick-up**
- **Mycologists can assign non fermenting bacteria**
- **30mn identification in a positive blood culture**

Main Players in Mass Spectrometry in microbiology

1. **Thermo Fisher Scientific**
2. **Agilent Technologies**
3. **Bruker Corporation**
4. **SCIEX**
5. **Waters Corporation**

.

6. **BioMérieux. VITEK® MS by BioMérieux**
7. **Shimadzu Corporation**
8. **Merck KGaA, Darmstadt, Germany**
9. **Advion (Part of Intertek)**
10. **Ion Sense**

History of the microbiological market building

- AnagnosTec (Zossen, Germany) Saramis
- Brucker Daltonics (Bremen, Germany)Biotyper

- Saramis :(Spectral ARchiving And Microbial Identification System) contained 35000 fingerprint spectra of more than 2000 bacterial, years or fugal species and 500 genera
- ->database integrated into bioMérieux VITEK-MS

(joint venture with Shimadzu who provides the Axima MALDI-TOF MS)

« SuperSpectra® »: 15-20 different isolates=1 spectrum

From various places, at different growth phases, on various growth media

VITEK®MS System
720 articles in PubMed in 2025

- CE marked 1st trim 2012
- Integrates VITEK2 AST (antibiotic susceptibility testing)
- bioMérieux Prep Station : links specimen information with each spot to the target slide using barcodes on the plated media
- 2013: launch of a new automated blod culture and incubator system (with imaging technologies)
- 2017 CE marking for Mycobacteria, Nocardia, new mold species

Key Products: The VITEK® MS system is a MALDI-TOF mass spectrometry platform that is widely used in clinical microbiology to rapidly identify microorganisms.
Applications: It's used extensively in **clinical settings for the identification of bacteria, yeast, fungi, and other pathogens. This is one of the most common MS platforms in routine diagnostic microbiology**

SHIMADZU Excellence in Science

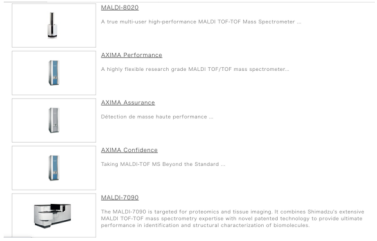
France
Analytical and Measuring Instruments

Shimadzu Corporation

- **Key Products**: Shimadzu manufactures mass spectrometers like the *LCMS-8050* and *LCMS-9030* systems, which are frequently used in both clinical and research microbiology.
- **Applications**: Shimadzu's MS technology is used in microbial proteomics, pathogen identification, and environmental microbiology

SHIMADZU
shimadzu and mass spectrometry 983 articles

- Shimadzu Axima® Idplus : saramis V4.04 contained 1394 species of 349 genera (bacteria, algae, mycota, yeasts) BioMérieux would have 25% shares (55 instruments Q3-2011 in Europe and Japan)



Brucker Corporation

- **Key Products**: Bruker is a leading player in the microbiology MS field, particularly with its *Microflex* and *UltrafleXtreme* MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) systems. These systems are known for **rapid microbial identification.**
- **Applications**: Bruker's MALDI-TOF technology is extensively used for **identifying bacterial, fungal, and viral pathogens in clinical microbiology**. Their systems are also used in research related to microbiomes, disease diagnostics, and resistance mechanisms.

MALDI Biotyper MSP

https://www.bruker.com/en/services/training/microbiology-and-diagnostics/maldi-biotyperstraining-movies.html

- **Brucke**r Microflex benchtop MALDI-TOF MAS
- Uses the « **main spectra** » concept (average peak positions and intensities in 20 replicates of a single and defined strain)
  Biotyper 3.1 : **3.1.2.0 (3,995 database entries). (Sept. 2022)**
- 350 genera and 2140 species incorporated in recent software package, continuously expanded with 50 laboratories worldwide
- 610 instruments MALDI-Biotypers worldwide. (sept 2012), 472 (Europe), 64 (Americas), 74(Asia-Pacific)
- 2013 : robotic device Kiestra Lab
- Collaboration with BD, Siemens, Copan, JMI laboratories, CDC

Systems Evaluation studies

- **Biomerieux and Brucker** ,comparable
- Importance of database version used
- Possible need for specific additional extraction steps to increase success rats
- Market is saturated, many applications or basic concepts are patent-protected

General concern

- Sample preparation methods are supposed to be standardized, however with filamentous fungi, there is considerable variability in mass fingerprints that accompany changes in cultivation conditions
- Fingerprints for prokaryotes and yeasts are largely independent of growth media, temp, oxygen supply.
- Carreful medium selection is an important parameter for analysis specificity and speed

Salmonella spp in stool
salmonella and stools and mass spectrometry n=35

- Selective selenite enrichment broth results in successful identification of *Salmonella* spp in stool samples after one day **(50% of original analysis time)**
- other purification methods (to clean and enrich) are required for investigation of microorganisms in complex matrix such as food or agricultural samples

Remaining Issues

- Beta hemolytic streptococci might pose problems at the spp level

Beta hemolytic streptococcus and mass spectrometry 31 articles

- *Streptococcus pneumoniae* : still hard to identify by MS.

streptococcus pneumoniae and mass spectrometry, *488 articles*

- *Shigella* from *E.coli* discrimination impossible

Shigella and Escherichia coli and mass spectrometry, *152 articles*

- Slow growing fungi

All problems to be solved with improvement of databases (and with AI or machine learning) 2700 Articles in 2025

Specific Markers

- **2000-20000 Da proteins**, particularly useful for microbial strain identification.
- **intra-species** variability

Mass Spectrometry-Based Escherichia coli H Antigen/Flagella Typing: Validation and Comparison with Traditional Serotyping K. Cheng et al., Clin Chem 2016 Jun;62(6):839-47.doi: 10.1373/clinchem.2015.244236

- **Specific protein signals** for *Aspergilli*
- **A 7625 Da protein specific to group B streptococci** ?
- Number of cells needed : 10000 (manual sample deposition)

Alternative Mass Spectrometical methods

- **Penicillin-Binding Protein** 2a in MRSA resistance

Sci Rep2021 Sep 15;11(1):18309. doi: 10.1038/s41598-021-97844-w. **Rapid MRSA detection via tandem mass spectrometry of the intact 80 kDa PBP2a resistance protein by JR NEil et al. 2021**

- **Panton-Valentine leucocidin** : MRSA virulence

doi: 10.1007/s10096-010-0995-y. Epub 2010 Jun 13**First outbreak of PVL-positive nonmultiresistant MRSA in a neonatal ICU in Australia: comparison of MALDI-TOF and SNP-plus-binary gene typing S. SChlebuch et al. 2010**

- **Salmonella from food, animal feed, environmental samples**
- **Virulence factor detection 2 pentyl furan in Aspergillosis**

MALDI mass spectra allows to follow the process of substrate degradation by the enzyme

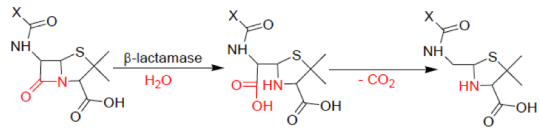


Figure 2: Beta-lactam ring degradation by lactamases

Brucker has automatized 7 penicillin, cephalosporin and carbapenem-related ATB detection

MS in Research

- **Microbial Metabolomics**
  - Untargeted LC/MS based secondary metabolomics with PCA can be used for microbail strain prioritization in drug dicovery programs and assessment of regulation of natural product production
- **Access to diseases BioMarkers**
- **Natural Products Peptidogenomics**
- **Determination of binding affinities for antimicrobial peptides to bacterial cells**

MS in Research (isobaric tags)

Budnik *et al. Genome Biology* (2018) 19:161
https://doi.org/10.1186/s13059-018-1547-5

Genome Biology

METHOD Open Access

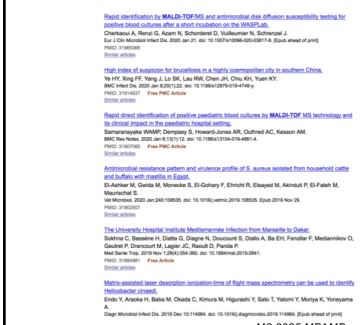
SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation

Bogdan Budnik[1*], Ezra Levy[2], Guillaume Harmange[2] and Nikolai Slavov[2,3*]

Mass Spec

MALDI-TOF and Infectious Diseases

(n=**675** in Pubmed on Jan 26th 2020, **903** results in 2021
1054 in 2022, **1,385** in Jan. 2024, **1529** in Jan.2025)



Serology

Solid-Phase Radioimmunoassay in Antibody-Coated Tubes

Abstract. *The adsorption of antibody to polymeric surfaces has been used to develop a new method of solid-phase radioimmunoassay. Incubation is performed in antibody-coated, disposable tubes that are finally washed-out with water and counted for quantitation of the bound tracer. The method is simple, rapid, inexpensive, and suitable for automation.*

K. Catt and G.W. Tregear, Science 22 dec. 1967

Serology

Hemagglutination assay
hemagglutination inhibition assay

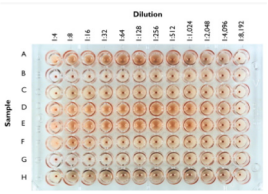
16,462 results in 2024. (1940-2020)

This assay is very reliable, when looking for the virus and not the antibodies, because it can be used in both directions. The problem of PCR is that it requires an expensive investment at start. Countries that can not bear this burden still prefer other techniques.

The property designated as « **hemagglutination** », is at the basis of a rapid assay to evaluate the levels of **influenza viruses** in a sample. To perform the assay , a series of virus dilution is prepared, mixed with a predetermined amount of red blood cells, and added to the wells of a plastic well .

Red blood cells that are not linked by the influenza virus **sink to the bottom of a well and form a "button".**

The red blood cells that are attached to viral particles form **a lattice that encapsulates the cells** . The test can be performed within 30 minutes, and is therefore a quick indicator of the relative amount of virus particle amounts



Hemagglutination Inhibition Assay. Spackman E, Sitaras I.Methods Mol Biol. 2020;2123:11-28. doi: 10.1007/978-1-0716-0346-8_2

Serology

Multiplexed serology

PubMed, n=77 on Jan. 26th 2020
n=1110 results on Jan 28th 2024
n=1201 results on Jan 24th 2025

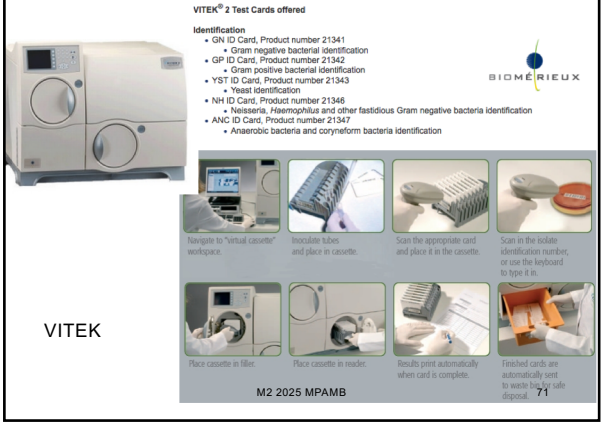
A Multiplexed Serologic Test for Diagnosis of Lyme Disease for Point-of-Care Use.
Arumugam S, Nayak S, Williams T, di Santa Maria FS, Guedes MS, Chaves RC, Linder V, Marques AR, Horn EJ, Wong SJ, Sia SK, Gomes-Solecki M.
J Clin Microbiol. 2019 Nov 22;57(12). pii: e01142-19. doi: 10.1128/JCM.01142-19. Print 2019 Dec

> Emerg Microbes Infect. 2020 Dec;9(1):1965-1973. doi: 10.1080/22221751.2020.1813636.

Accurate serology for SARS-CoV-2 and common human coronaviruses using a multiplex approach

Sophie van Tol [1], Ramona Mögling [1], Wentao Li [2], Gert-Jan Godeke [1], Arno Swart [1],

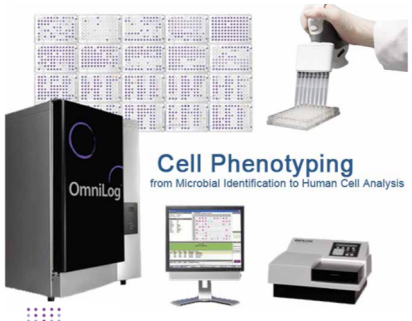
Phenotypic ID

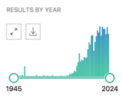


VITEK

Phenotypic ID

Global **phenotypic** characterization of bacteria.
Bochner BR.
FEMS Microbiol Rev. 2009 Jan;33(1):191-205. doi: 10.1111/j.1574-6976.2008.00149.x. Epub 2008 Nov 27.
PMID: 19054113 Free PMC article. Review.





Biolog in Pubmed
1860 articles in 2024
1951 articles in 2025

SPR

Biacore

34,231 results

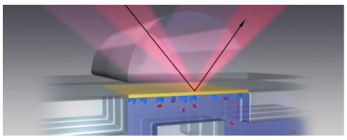
SPR Surface Plasmon Resonance

Studying biomolecular interactions in real-time without the need for labeling

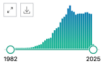
Ann N Y Acad Sci. 2007 Mar;1098:335-44.

SPR imaging-based salivary diagnostics system for the detection of small molecule analytes.

Fu E, Chinowsky T, Nelson K, Johnston K, Edwards T, Helton K, Grow M, Miller JW, Yager P



1.**Drug Discovery and Development:**
2.**Biologics Development:** In the deve
3.**Biosensor Development:** SPR-based
4.**Protein-Protein Interactions:** SPR e
5.**Immunoassays:** SPR-based immuno
6.**Kinetic Analysis:** SPR allows for the
7.**Quality Control:** In biomanufacturin
8.**Biomolecular Interaction Studies:** S



SPR

I2BC

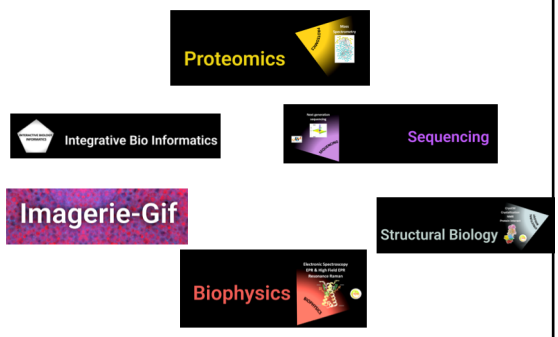
Home > Facilities > Protein-protein interaction

Surface Plasmon Resonance

The **SPR** technology allows real-time detection and monitoring of biomolecular binding events. In a SPR experiment, one of the interacting molecules (the ligand) is bound to the biosensor surface (sensor chip), whereas the other (the analyte) is delivered to the surface in a continuous buffer flow through a microfluidic system. Binding to the immobilized molecule is followed by **surface plasmon resonance** (SPR), which detects mass changes at the sensor surface. Recording SPR signal variation as a function of time (sensorgram) for several analyte concentrations allows to determine the association and dissociation rate constants, and to derive the affinity constant. This technology also allows to measure the concentration of functional molecules.

This technology is used to characterize molecular interactions involving small molecules, proteins, polysaccharides, lipids and nucleic acids. Sequential injections of several interactants allow the mapping of interaction domains and the determination of the composition and of the mechanism of assembly of multiprotein complexes.

I2BC has all facilities



Take Home message

- **Rapidly evolving industry**
- **Sovereignty becoming more and more important due to income and turnover of this industry** (and crisis...)
- **Tendancy to size increase and concentration, altough ecosystems of technologies looks as a chaos**
- **Some leading countries, France hardly on the race...**
- **Research must be integrated : technology, materials, Artificial intelligence**
- **France choose Quantum computer industry, looks as quite too late for french biotechnology companies abandoned for the profit of agro-industrial business.**