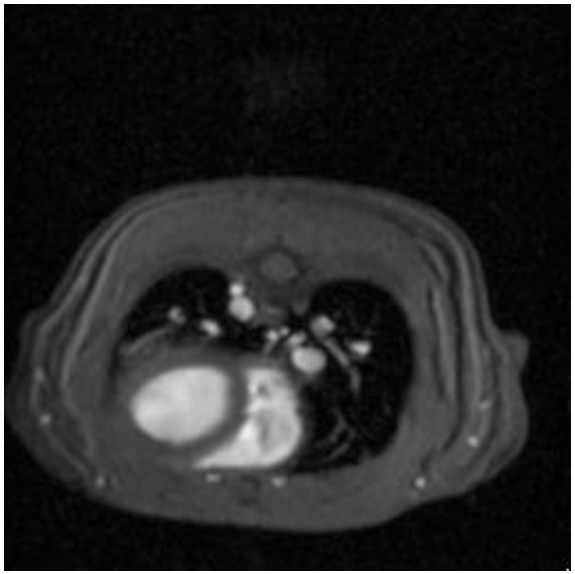
Just Do It with a MALDI!

Are Microbiologists Mutating into Chemists?

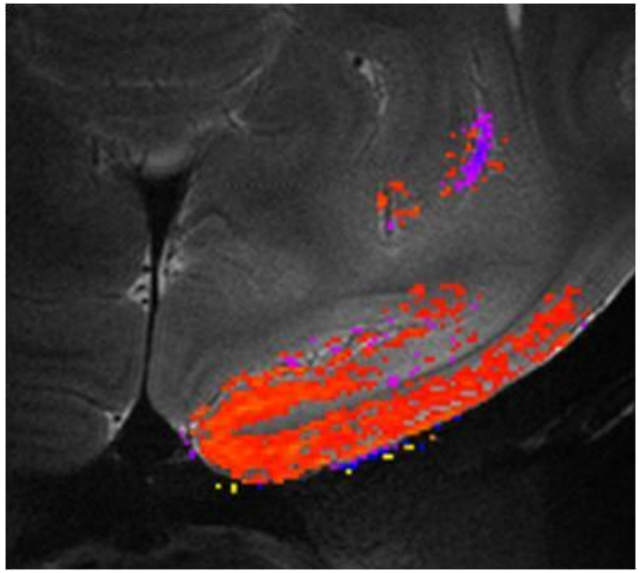
1960: Creation on Nuclear Magnetic Resonance (NMR)

Nowadays: Leader in Pre-clinical Imaging (MRI)

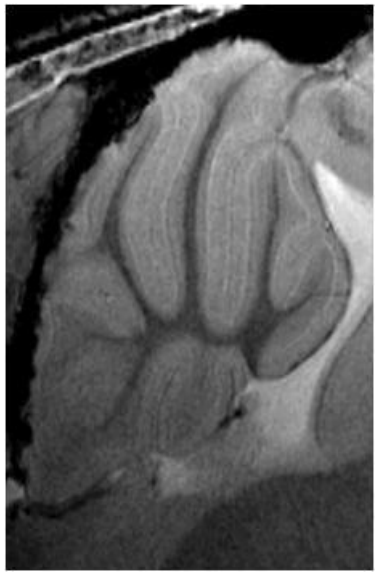
- Non-invasive molecular imaging techniques



Cardiology



Brain functions



Anatomy

Bruker Corporation
Divisions

Bruker BioSpin Group

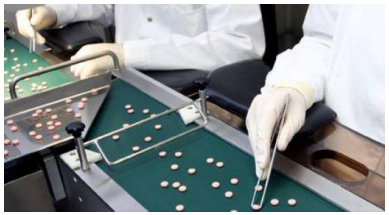
Magnetic Resonance Spectroscopy (MRS)

Preclinical Imaging (PCI)

Applied, Industrial and Clinical (AIC)

Bruker CALID Group

Mass spectrometry (Daltonics)



Infrared & Raman spectroscopy (Optics)

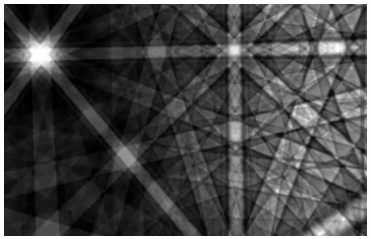
CBRNE (Detection)

CALID = Chemical, Applied Markets, Life Science, In-Vitro Diagnostics, Detection

Bruker NANO Group

Bruker Nano Surface

X-ray analysis



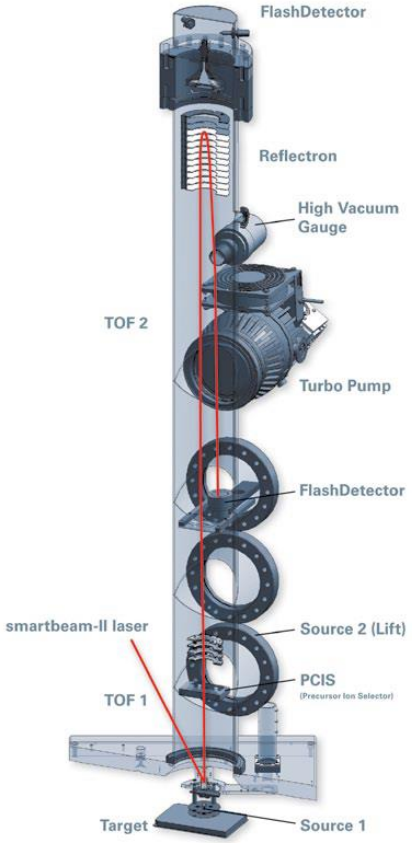
Bruker Nano Analytics

Bruker EST (BEST)

Supercon wire and devices => NMR

BEST: Bruker Energy and Supercon Technologies

Proteomics & Metabolomics Analyses



Tissue Imaging

Top-Down Protein Sequencing

2D-Gels

TLC-MALDI

Polymer Analysis

PTM Analysis

Glycan Analysis

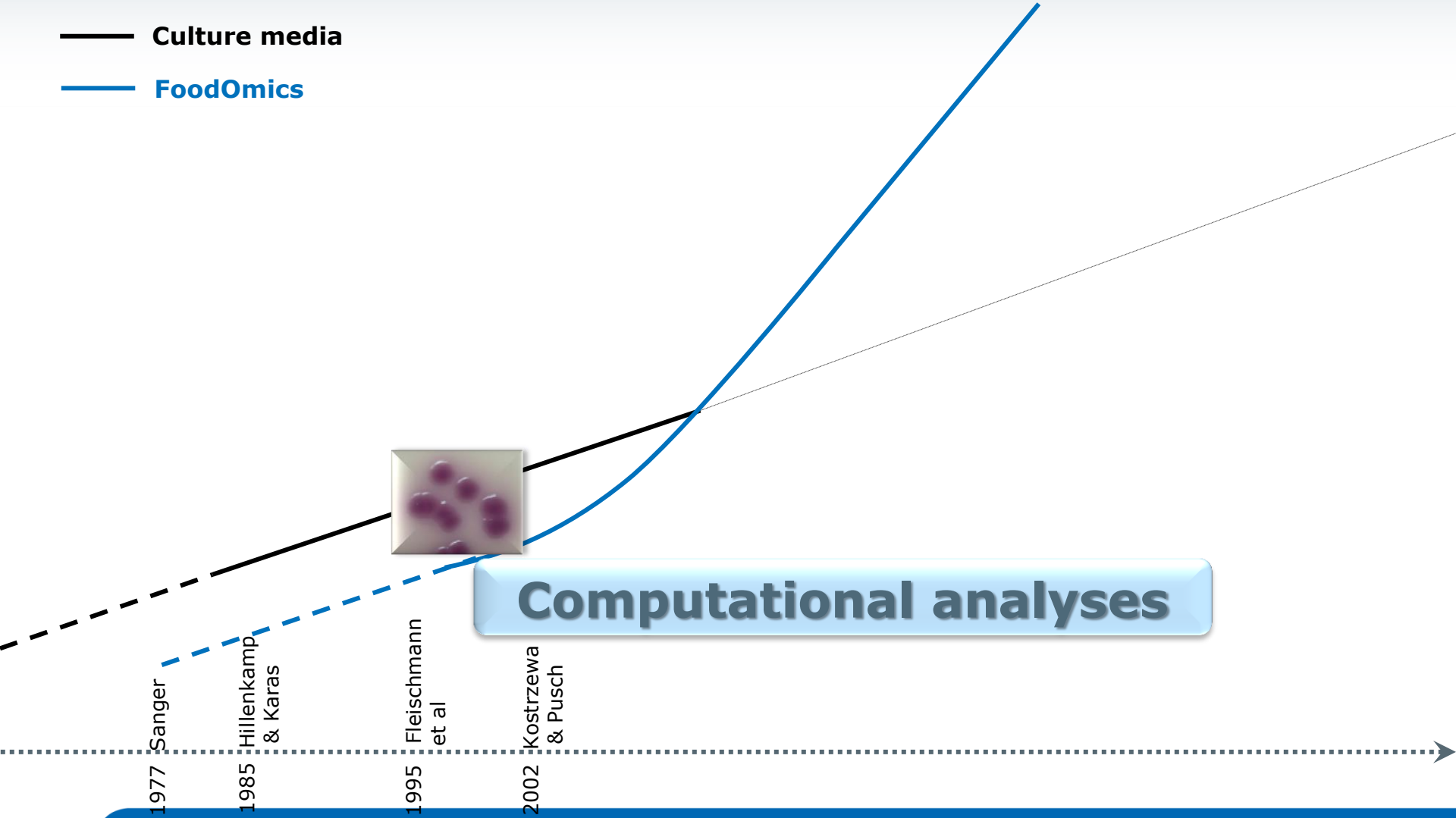
SPRi MALDI Interaction Analysis

FoodOmics

Cifuentes, 2009. J. Chromatogr. A. 1216, 7109

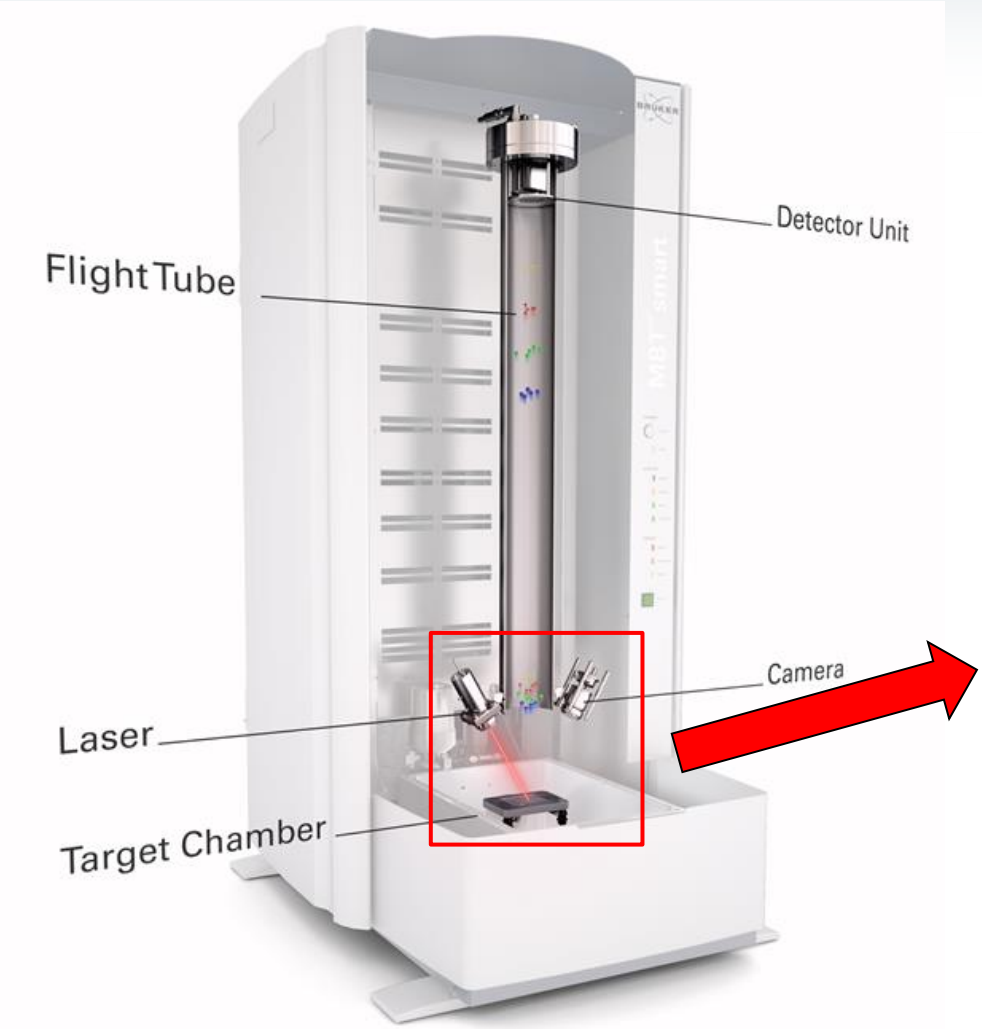
FoodOmics

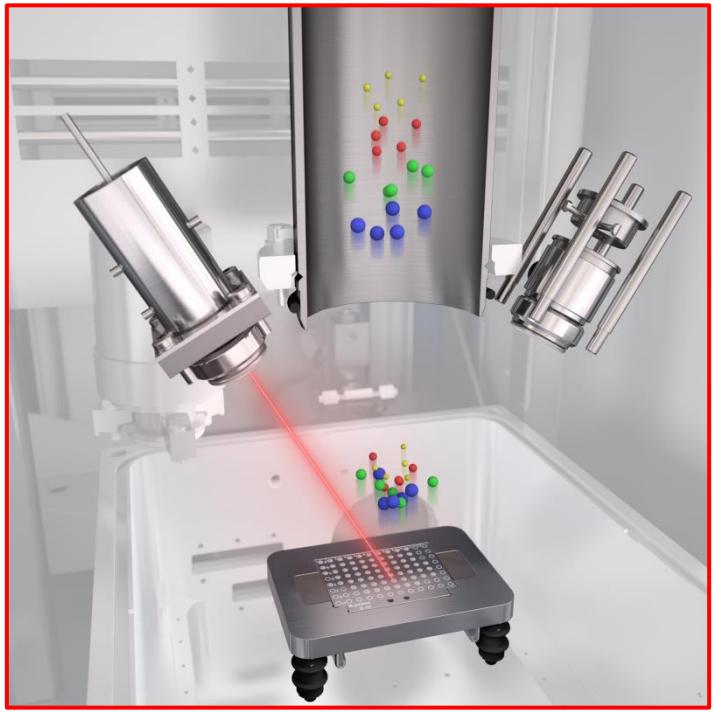
Cifuentes, 2009. J. Chromatogr. A. 1216, 7109



MALDI Biotyper - Basics

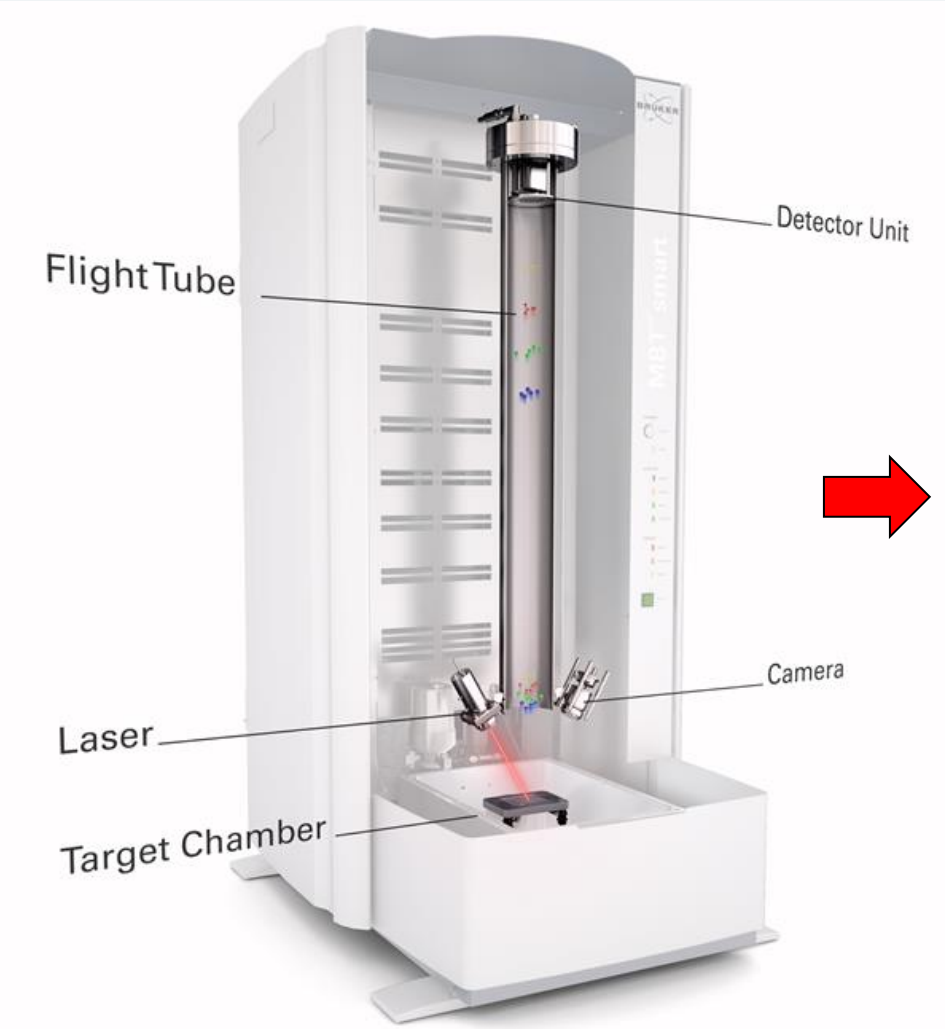
Matrix Assisted Laser Desorption / Ionization Time Of Flight Mass Spectrometry

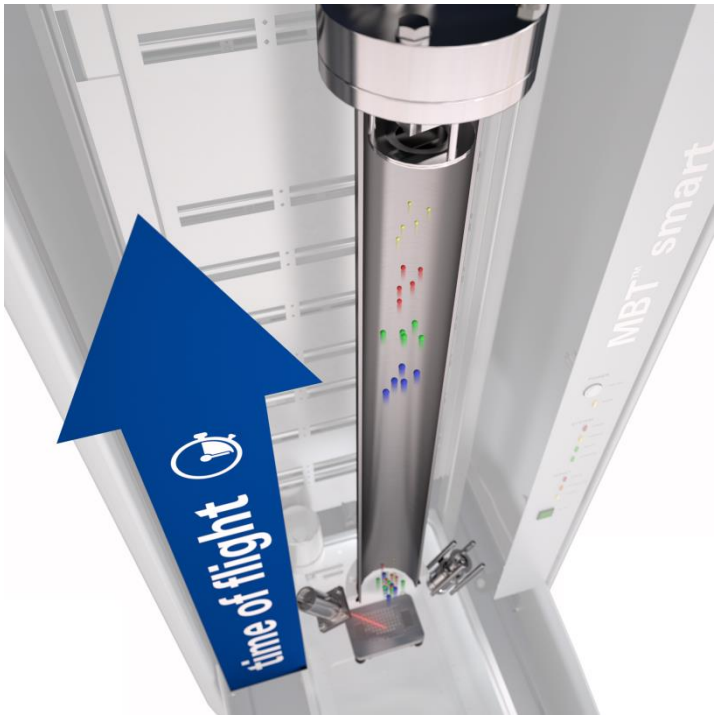




MALDI Biotyper - Basics

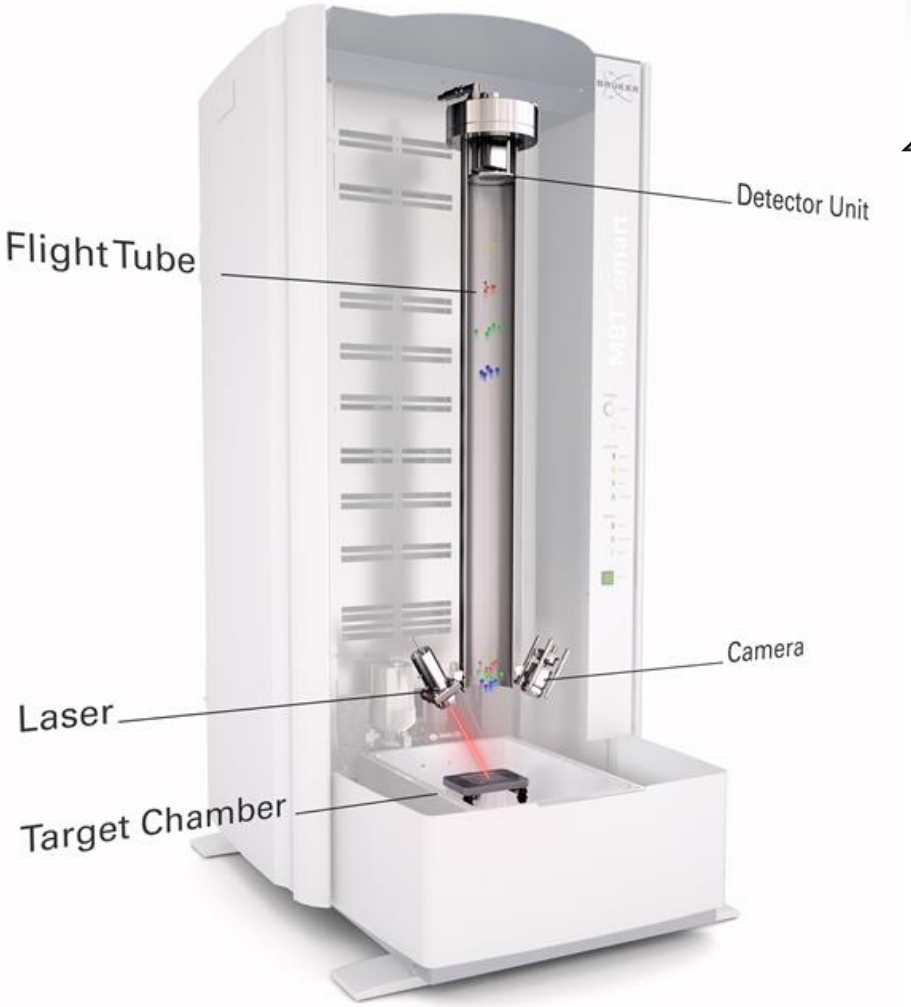
Matrix Assisted Laser Desorption / Ionization
Time Of Flight Mass Spectrometry

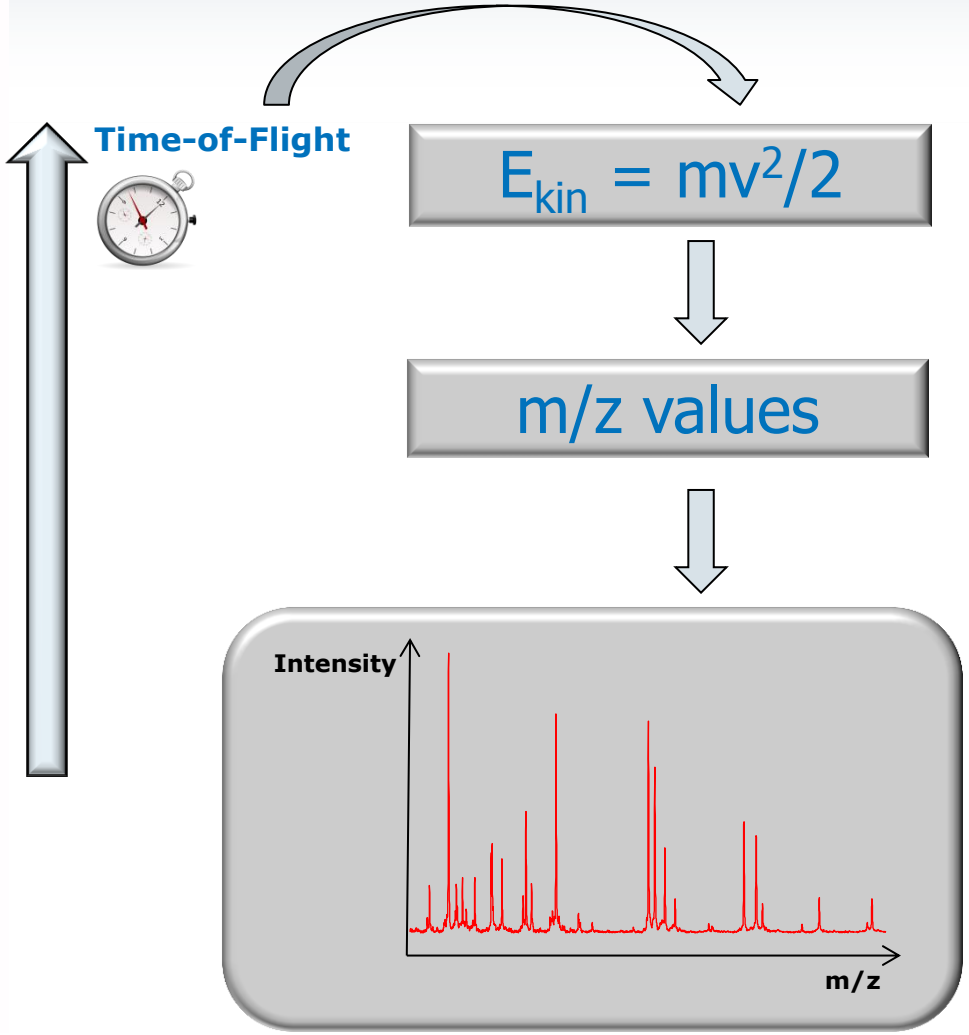




MALDI Biotyper - Basics

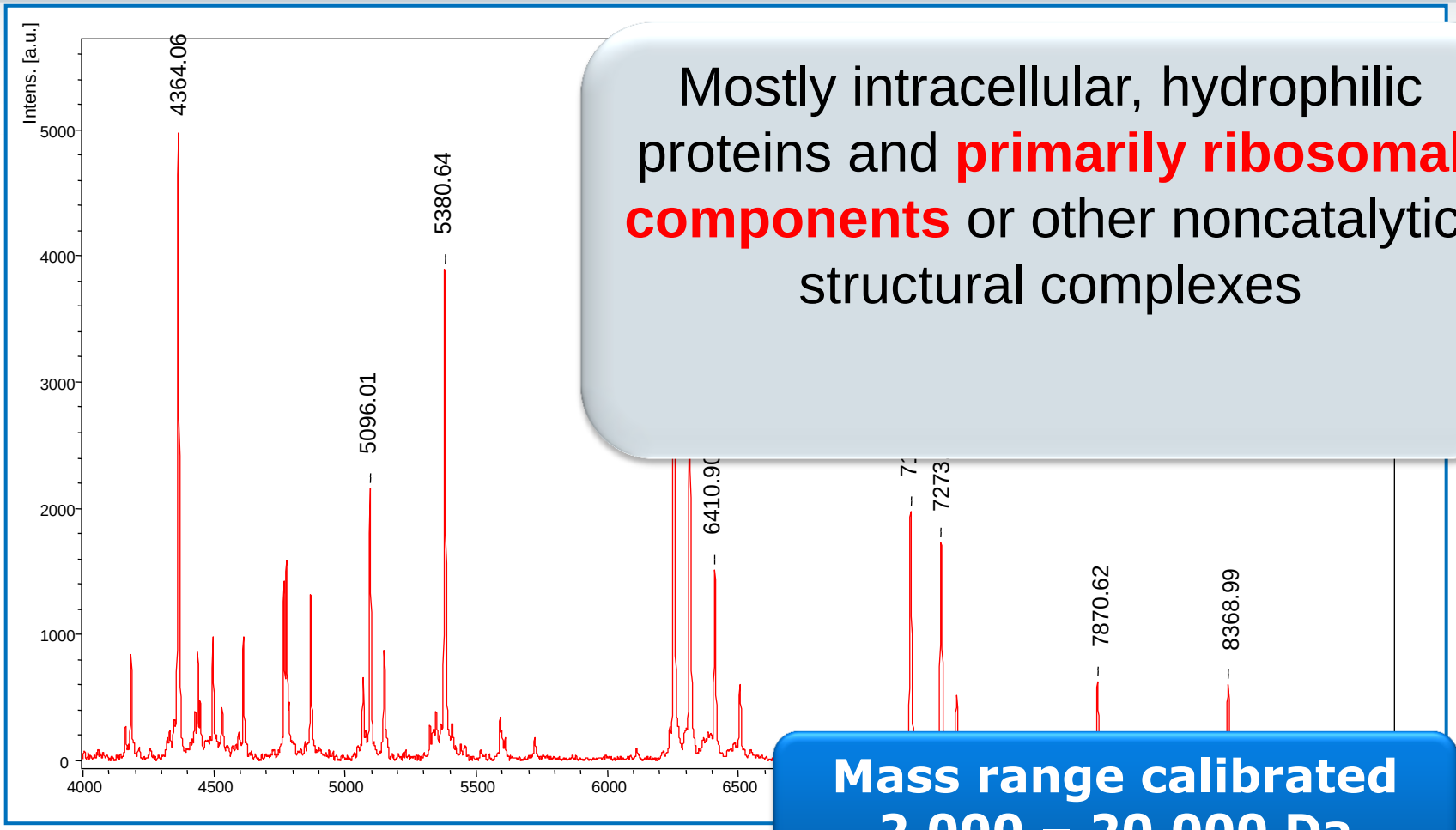
Matrix Assisted Laser Desorption / Ionization
Time Of Flight Mass Spectrometry





MALDI Biotyper - Basics

Robust identification method, as it relies on the highly abundant proteins



E. coli

Mostly intracellular, hydrophilic proteins and **primarily ribosomal components** or other noncatalytic, structural complexes

Mass range calibrated 2,000 – 20,000 Da

Routine testing

Easy and short workflow

1 room is enough

1 isolate

1 spot

1 drop

1 clic

Easy and short handling

Direct transfer

1 isolate	Approx 20 sec
48 isolates	Approx 10 min
95 isolates	Approx 18 min

Routine testing

Easy and short workflow

Spectrum acquisition

Rank	Matched Pattern	Score Value
1	Pseudomonas aeruginosa DSM 1117 DSM	2.18
2	Pseudomonas aeruginosa 8147_2 CHB	2.17
3	Pseudomonas aeruginosa ATCC 27853 THL	1.98
4	Pseudomonas aeruginosa A07_08_Pudu FLR	1.94
5	Pseudomonas aeruginosa 19955_1 CHB	1.82
6	Pseudomonas aeruginosa DSM 50071T HAM	1.78

Unsupervised Main Spectra & Updated Library

YEAR

Update

Short time-to-result, including

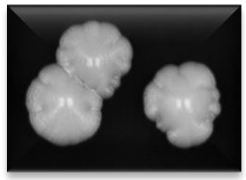
. Bacterial test standard

. Drying of matrix

. Mass spectrum acquisition

1 isolate	Approx 15 min
48 isolates	Approx 35 min
95 isolates	Approx 60 min

Bacteria, yeasts and molds

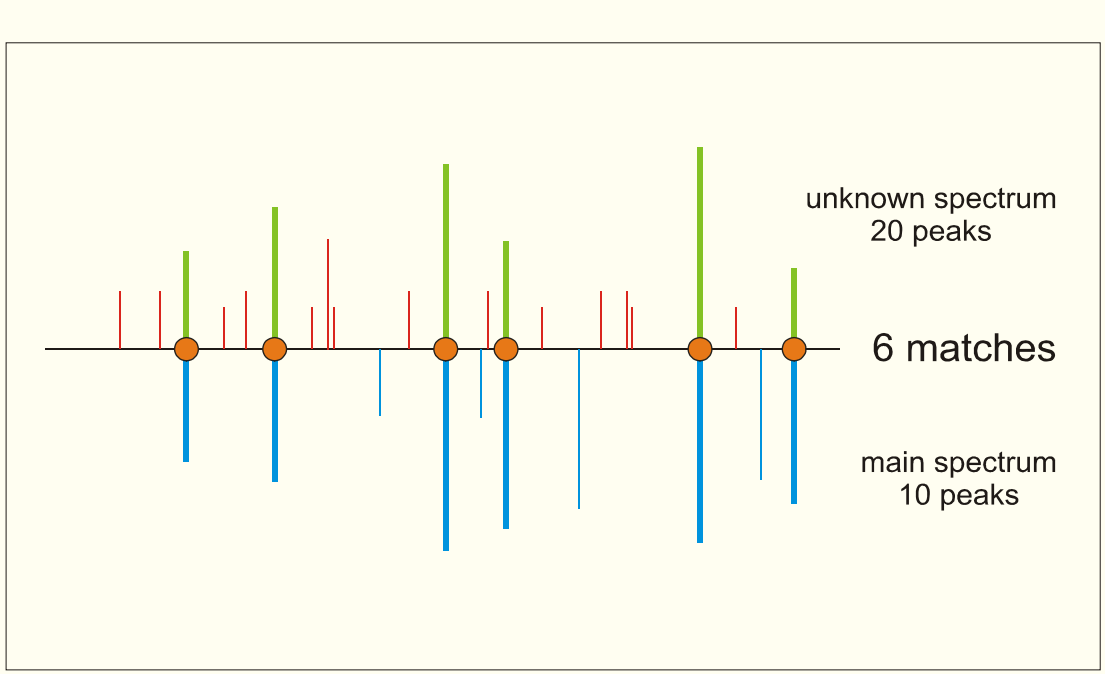


5 to 10% alternative sample preps

MALDI Biotyper - Bioinformatics

Unsupervised Main Spectra (MSP) concept

Score base pattern matching



Tested isolate is matched against each Main Spectrum (MSP) in the library

Calculation of a matching score based on:

Matches MSP to unknown

% matches of the reference spectrum (e.g. 6/10)

Matches unknown to MSP

% matches of the unknown spectrum (e.g. 6/20 = 3/10)

Correlation of intensities

value of intensity correlation

Range	Interpretation	Symbols	Color
2.000 - 3.000	High Confidence Identification	(+++)	green
1.700 - 1.999	Low Confidence Identification	(+)	yellow
0.000 - 1.699	No Organism Identification Possible	(-)	red

MALDI Biotyper - Bioinformatics
Unsupervised Main Spectra Concept

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Clostridium perfringens B 1968_NCTC 3110_BOG	2.514	1502
2 (+++)	Clostridium perfringens B 1038_NCTC 4964_BOG	2.454	1502
3 (+++)	Clostridium perfringens B 1971_ATCC 3626_BOG	2.305	1502
4 (++)	Clostridium perfringens A 1037_NCTC 8237_BOG	2.254	37763
5 (++)	Clostridium perfringens D 2150_NCTC 8346_BOG	2.253	107819
6 (++)	Clostridium perfringens C 1041_NCTC 10720_BOG	2.11	79668
7 (-)	Comamonas testosteroni DSM 50244 HAM	1.308	285
8 (-)	Listeria grayi murrei DSM 20596 DSM	1.262	1641
9 (-)	Bacillus atrophaeus DSM 675 DSM	1.163	1452
10 (-)	Clostridium beijerinckii 1072_ATCC 25752_BOG	1.136	1520

MALDI Biotyper RUO library
Updates since 2010

Clinical, environment, industrial, and veterinary and isolates provided by collaborating partners, round robin strains and strains from isolates from accredited ISO 9001:2008 certified strain collections

rDNA and relevant housekeeping genes sequencing
According to molecular taxonomy

	Spring 2010	<	Sep 2010	<	Jan 2011	<	Nov 2011	<	Aug 2012	<	Jan 2014	<	July 2015	<	April 2016
N° of MSP in total	3476		3740		3995		4110		4613		5627		5989		6903
N° of genus	315		318		368		341		364		390		410		424
N° of species	1850		1947		2012		2026		2185		2297		2371		2461
N° of total added MSP		336		319		147		515		1020		377		938	
N° of total deleted MSP		-72		-64		-32		-12		-3 (-3)		-15		-24	
N° of MSP for new genus/species entries		111		42		32		151		220		106		161	
N° of MSP for additional species entries		224		231		102		359		797		261		766	
N° of MSP replaced		1		46		13		5		3		10		11	
Further overall improvements	Continuous nomenclature updates according International Journal of Systematic and Evolutionary Microbiology (IJSEM) referenced at www.bacterio.net (LPSN = List of prokaryotic names with standing in nomenclature).														

MALDI Biotyper

RUO Reference library
Bacteria & Yeasts

Bruker MBT Compass Library (version April 2017)

7,311 MSPs
2,509 different species

Every Microorganism could be of Relevance

MALDI Biotyper library

Fungi Library

45 genus, 129 species – 366 strains

Absidia coerulea	Botrytis cinerea	Fusarium solani	Penicillium citrinum	Phoma sorghina
Acremonium strictum	Chaetomium funicola	Fusarium sporotrichoides	Penicillium commune	Rhizomucor pusillus
Alternaria alternata	Chaetomium globosum	Fusarium tabacinum	Penicillium corylophilum	Rhizopus microsporus
Arthrinium phaeospermum	Chrysosporium keratinophilum	Fusarium verticillioides	Penicillium crustosum	Rhizopus oryzae
Arthrographis_kalrae[ana] (Eremomyces_langeronii[teleo])	Cladosporium cladosporioides	Geomyces pannorum	Penicillium daleae	Rhizopus stolonifer
Aspergillus candidus	Cladosporium herbarum	Geosmithia argillaceae	Penicillium dierckxii	Scedosporium prolificans
Aspergillus clavatus	Cladosporium sp	Kerstersia gyiorum	Penicillium digitatum	Scedosporium_apiospermum[ana] Pseudallescheria_boydii[teleo]
Aspergillus flavus	Cladosporium sp	Lecythophora hoffmannii	Penicillium discolor	Schizophyllum commune
Aspergillus fumigatus	Cunninghamella elegans	Lichtheimia corymbifera	Penicillium expansum	Scopulariopsis acremonium
Aspergillus glaucus	Curvularia clavata	Microsporum canis	Penicillium funiculosum	Scopulariopsis brevicaulis
Aspergillus niger	Curvularia lunata	Microsporum cookei	Penicillium glabrum	Scopulariopsis brumptii
Aspergillus nomius	Curvularia pallescens	Microsporum equinum	Penicillium italicum	Scytalidium lignicola
Aspergillus ochraceus	Curvularia verruculosa	Microsporum fulvum	Penicillium olsonii	Sporothrix schenckii
Aspergillus oryzae	Epicoccum nigrum	Microsporum gypseum	Penicillium pseudostromaticum	Syncephalastrum racemosum
Aspergillus parasiticus	Epidermophyton floccosum	Microsporum persicolor	Penicillium purpurogenum	Thanatephorus cucumeris
Aspergillus sclerotiorum	Fennellia flavipes	Microsporum praecox	Penicillium roqueforti	Trichoderma koningii
Aspergillus sydowi	Fusarium aquaeductuum	Monilinia laxa	Penicillium rugulosum	Trichoderma longibrachiatum
Aspergillus tamarii	Fusarium cerealis	Mucor circinelloides	Penicillium sp	Trichophyton eboreum
Aspergillus terreus	Fusarium chlamydosporum	Mucor ramosissimus	Penicillium striatisporum	Trichophyton equinum
Aspergillus unguis	Fusarium culmorum	Paecilomyces farinosus	Penicillium turbatum	Trichophyton interdigitale
Aspergillus ustus	Fusarium dimerum	Paecilomyces lilacinus	Penicillium verrucosum	Trichophyton rubrum
Aspergillus versicolor	Fusarium equiseti	Paecilomyces marquandii	Phaeoacremonium sp	Trichophyton tonsurans
Aspergillus_amstelodami[ana] Eurotium_amstelodami[teleo]	Fusarium incarnatum	Paecilomyces variotii	Phialemonium sp	Trichophyton violaceum
Aspergillus_nidulans[ana] Emericella_nidulans[teleo]	Fusarium moniliforme	Penicillium brevicompactum	Phialophora bubakii	Trichophyton_mentagrophytes_var_erinacei[ana] Arthroderma benhamiae[teleo]
Aureobasidium pullulans	Fusarium oxysporum	Penicillium chrysogenum	Phoma glomerata	Trichurus sp
Beauveria bassiana	Fusarium proliferatum	Penicillium citreonigrum	Phoma herbarum	

MBT Library

No impact of the updates

BDD v1

Rank	Matched Pattern	Score Value
1	Pseudomonas aeruginosa ATCC 27853 THL	1.983
2	Pseudomonas aeruginosa A07_08_Pudu FLR	1.943
3	Pseudomonas aeruginosa 19955_1 CHB	1.821
4	Pseudomonas aeruginosa DSM 50071T HAM	1.78
5	Pseudomonas indica DSM 14015T HAM	1.55
6	Pseudomonas jinjuensis LMG 21316T HAM	1.49
7	Pseudomonas resinovorans LMG 2274T HAM	1.417
8	Pseudomonas citronellolis DSM 50332T HAM	1.393
9	Pseudomonas taetrolens LMG 2336T HAM	1.349
10	Pseudomonas libanensis CIP 105460T HAM	1.282

BDD v2

Rank	Matched Pattern	Score Value
1	Pseudomonas aeruginosa DSM 1117 DSM	2.188
2	Pseudomonas aeruginosa 8147_2 CHB	2.179
3	Pseudomonas aeruginosa ATCC 27853 THL	1.983
4	Pseudomonas aeruginosa A07_08_Pudu FLR	1.943
5	Pseudomonas aeruginosa 19955_1 CHB	1.821
6	Pseudomonas aeruginosa DSM 50071T HAM	1.78
7	Pseudomonas indica DSM 14015T HAM	1.55
8	Pseudomonas jinjuensis LMG 21316T HAM	1.49
9	Pseudomonas resinovorans LMG 2274T HAM	1.417
10	Pseudomonas citronellolis DSM 50332T HAM	1.393

MALDI Biotyper
Open microbiology concept
MicrobeNet by CDC

Collaboration with U.S. Centers for Disease Control and Prevention, **CDC**

Bruker Collaborates with CDC to Advance Infectious Disease Surveillance

06/16/2016

BOSTON, June 16, 2016 /PRNewswire/ -- At the American Society for Microbiology (ASM) Microbe 2016 conference, Bruker today announces the results of a successful collaboration with the Special Bacteriology Reference Laboratory at the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia to create an expanded microorganism reference library for the Bruker ***MALDI Biotyper***.

This new library, encompassing many rare and emerging pathogens, is now searchable through MicrobeNet (https://microbenet.cdc.gov). Developed by the CDC, MicrobeNet is a database curated by the pathogen subject matter experts and accessible for all public health and clinical laboratories. The ***MALDI Biotyper*** system has been rapidly adopted by microbiology laboratories worldwide as fast and affordable, and as the most comprehensive microbial identification tool available today, replacing conventional biochemical and molecular assays. The supplementary microbe knowledge base generated by the CDC not only provides expanded capabilities, but access to a wealth of other information and references for ***MALDI Biotyper*** users, from different parts of the world, in the global public health arena. It will help researchers identify a wide variety of microbial pathogens more rapidly in order to better serve public health needs.

The MBT "Open Microbiology" concept enables this initiative!

SUMMARY

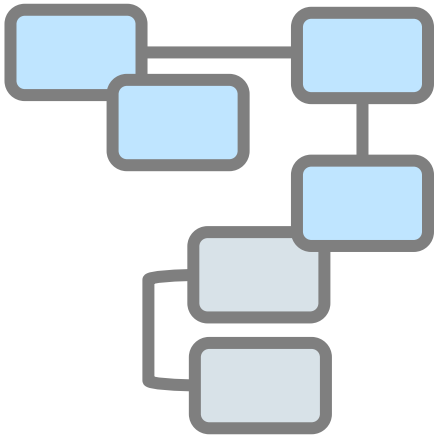
rDNA & specific gene sequencing

Mass Spectrum Profile (MSP)
20 MSPs > reference

Routine testing

For instance, NCBI website CDC MicroneNet

MBT Library
Reference Spectra
7311 MSPs/2509 species



Open system

Project creation
« In-house Library »
Inc sub-typing

Dendrograms & statistics

R&D applications

Review

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

MALDI-TOF MS identification and tracking of food spoilers and food-borne pathogens

Chris G de Koster[1] and Stanley Brul[2]

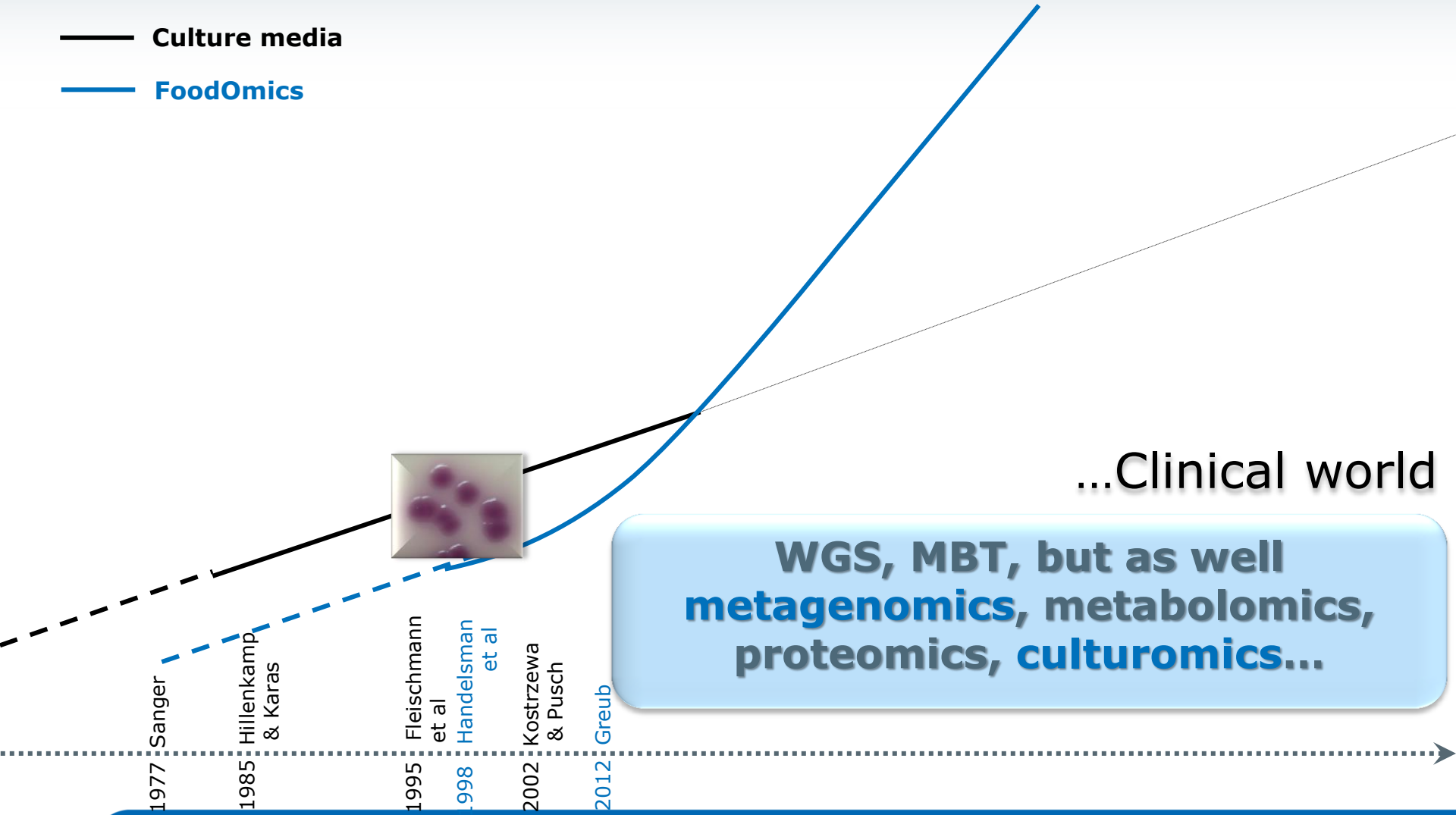
Current Opinion in Food Science 2016, **10**:1–6

This review comes from a themed issue on **Foodomics Technologies**

Edited by **Stanley Brul**

FoodOmics

Cifuentes, 2009. J. Chromatogr. A. 1216, 7109



Identification of specific biomarkers

Proteomics
Clinical Applications

PLOS ONE

RESEARCH ARTICLE

MALDI-TOF Mass Spectrometry Enables a Comprehensive and Fast Analysis of Dynamics and Qualities of Stress Responses of *Lactobacillus paracasei* subsp. *paracasei* F19

Ann-Sophie Schott[1], Jürgen Behr[2]*, Jennifer Quinn[1], Rudi F. Vogel[1]¤

1 Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising, Germany, 2 Bavarian Center for Biomolecular Mass Spectrometry, Technische Universität München, Freising, Germany

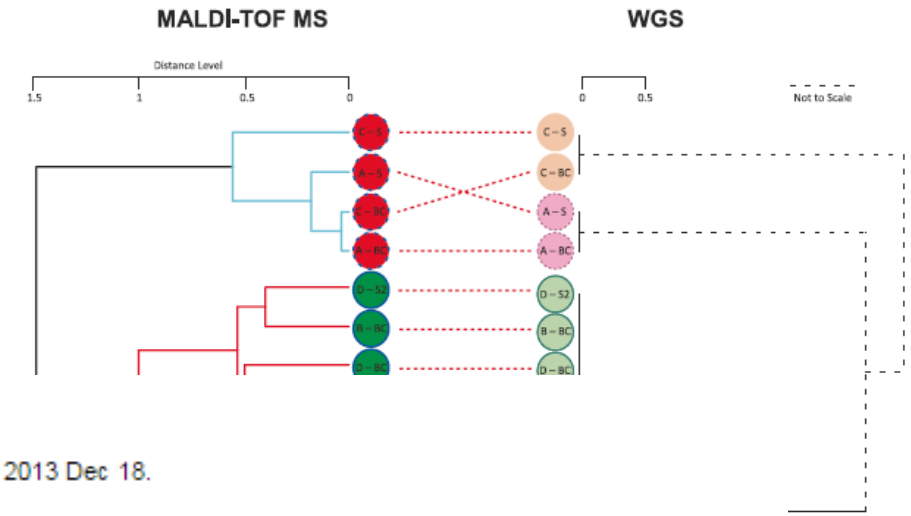
Complementarity
WGS & MALDI Biotyper

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-016-2824-4

ORIGINAL ARTICLE

MALDI-TOF MS meets WGS in a VRE outbreak investigation

S. Schlebusch[1,2] · G. R. Price[1] · R. L. Gallagher[1] · V. Horton-Szar[1] · L. D. H. Elbourne[3] · P. Griffin[2,4,5] · D. J. Venter[1,2,6] · S. O. Jensen[7,8] · S. J. Van Hal[9]



See 1 citation found using an alternative search:

J Dairy Sci. 2014 Feb;97(2):632-41. doi: 10.3168/jds.2013-7147. Epub 2013 Dec 18.

Comparison of methods for the microbiological identification and typing of Cronobacter species in infant formula.

Lu Y[1], Chen Y[2], Lu XA[2], Lv J[2], Man CX[3], Chai YL[4], Jiang YJ[5].

CulturOmics

nature microbiology

LETTERS

PUBLISHED: 7 NOVEMBER 2016 | ARTICLE NUMBER: 16203 | DOI: 10.1038/NMICROBIOL.2016.203

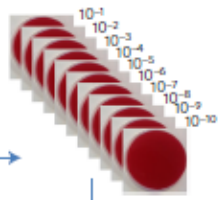
OPEN

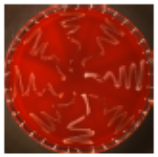
Culture of previously uncultured members of the human gut microbiota by culturomics

Jean-Christophe Lagier[1], Saber Khelaifia[1], Maryam Tidjani Alou[1], Sokhna Ndongo[1], Niokhor Dione[1], Perrine Hugon[1], Aurelia Caputo[1], Frédéric Cadoret[1], Sory Ibrahima Traore[1], El Hadji Seck[1], Gregory Dubourg[1], Guillaume Durand[1], Gaël Mourembou[1], Elodie Guilhot[1], Amadou Togo[1], Sara Bellali[1], Dipankar Bachar[1], Nadim Cassir[1], Fadi Bittar[1], Jérémy Delerce[1], Morgane Mailhe[1], Davide Ricaboni[1], Melhem Bilen[1], Nicole Prisca Makaya Dangui Nieko[1], Ndeye Mery Dia Badiane[1], Camille Valles[1], Donia Mouelhi[1], Khoudia Diop[1], Matthieu Million[1], Didier Musso[2], Jônatas Abrahão[3], Esam Ibraheem Azhar[4], Fehmida Bibi[4], Muhammad Yasir[4], Aldiouma Diallo[5], Cheikh Sokhna[5], Felix Djossou[6], Véronique Vitton[7], Catherine Robert[1], Jean Marc Rolain[1], Bernard La Scola[1], Pierre-Edouard Fournier[1], Anthony Levasseur[1] and Didier Raoult[1]*

CulturOmics

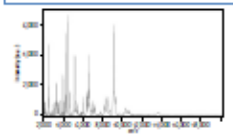
973 samples





MALDI-TOF
901,364 colonies

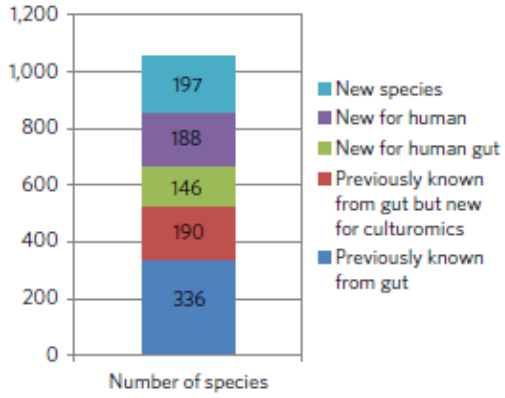
2.7 million spectra



1,258 16S rRNAs of unidentified colonies

Extension of human gut repertoire by culturomics

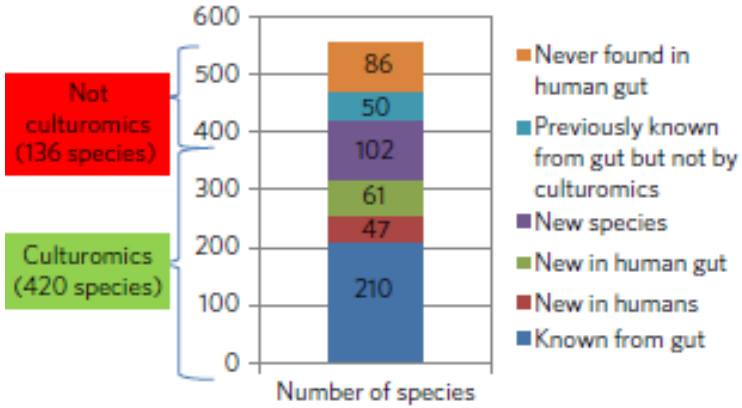
945 different prokaryotes including 2 archaea



Comparison of 84 samples analysed by metagenomics and culturomics

(4) Among the 200 16S rRNAs of the new species: 102 recovered 827 times (average 9.8 per stool)

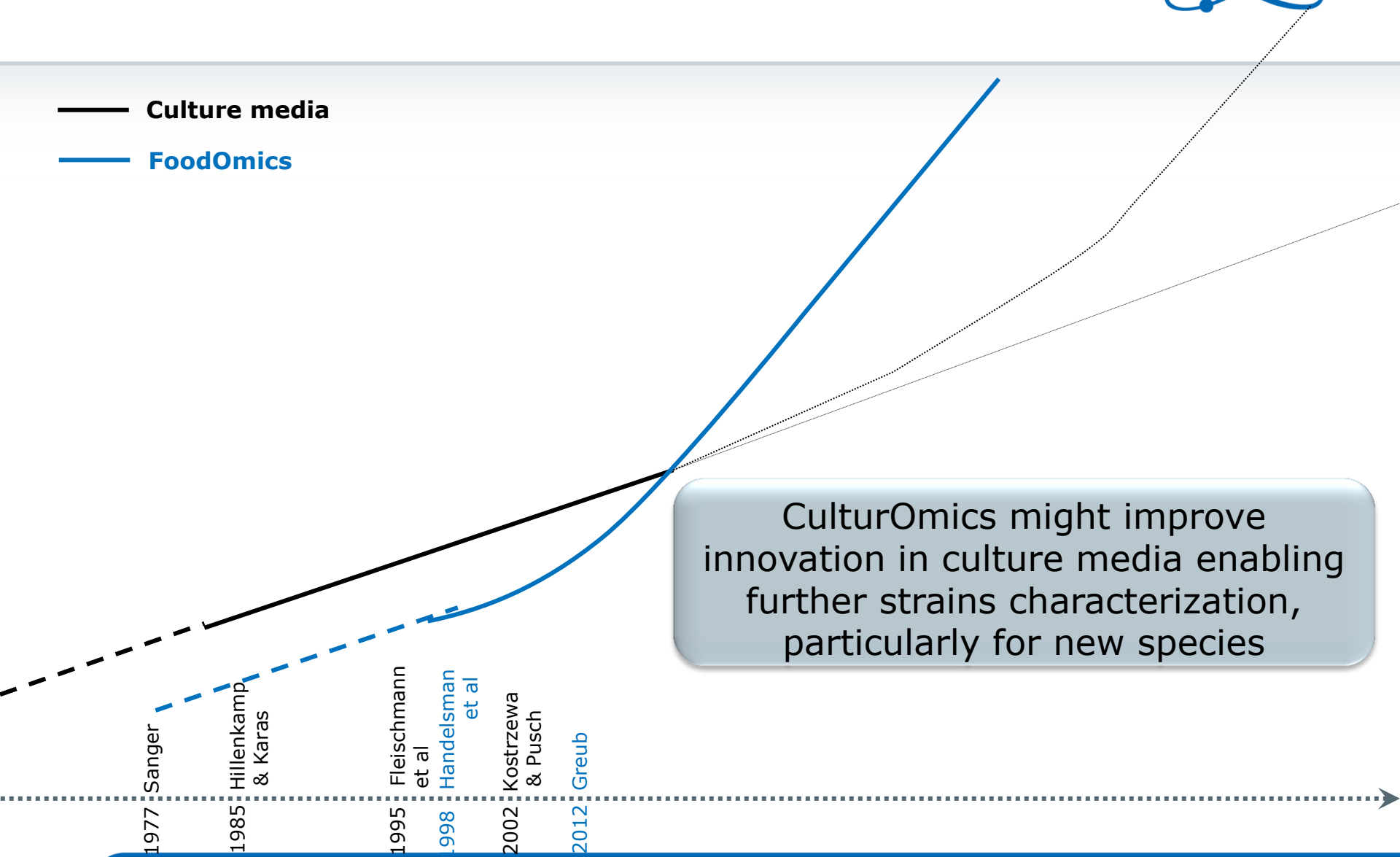
(5) Analysis of the species with a cut off of 20 reads = 4,158 OTU and 556 species



Food microbiomes, e.g fermentation and ripening microbiomes, biofilms, etc…

FoodOmics

Cifuentes, 2009. J. Chromatogr. A. 1216, 7109



CulturOmics might improve innovation in culture media enabling further strains characterization, particularly for new species

Are Microbiologists Mutating into Chemists?

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

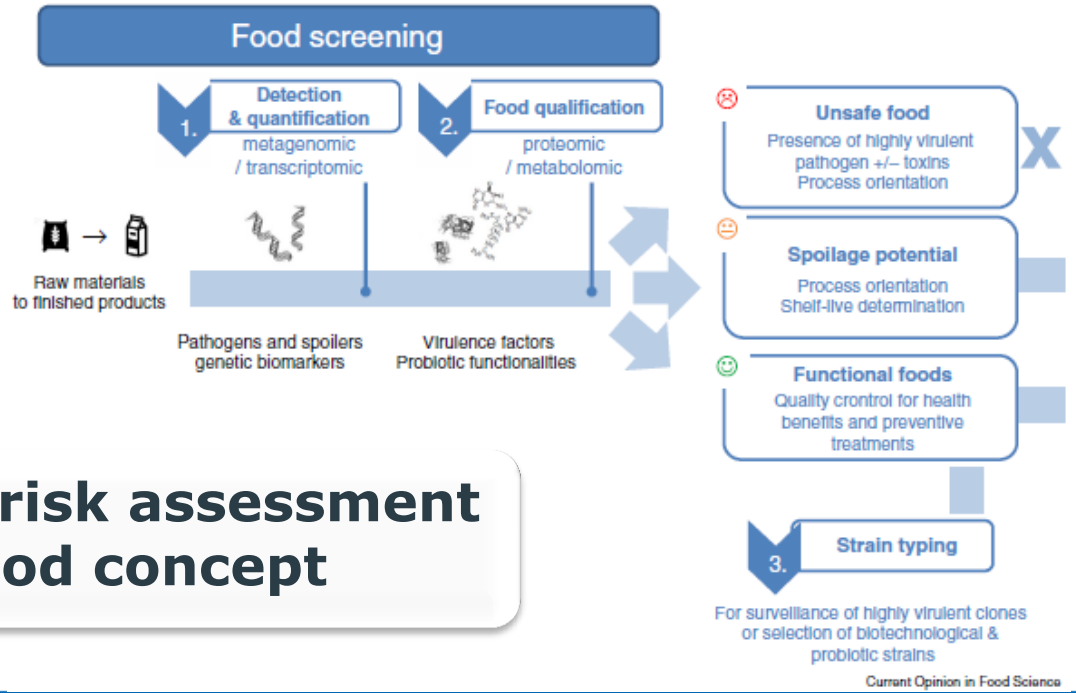
A typical day working in a laboratory in 2050: are microbiologists becoming chemists and serene workers?

Daniele Sohier, Armelle Riou and Florence Postollec

Current Opinion in Food Science 2016, 10:1–6

This review comes from a themed issue on Foodomics Technologies

Edited by Stanley Brul



1. Improve microbial risk assessment
2. Develop pharma-food concept

Thank you, and see you in