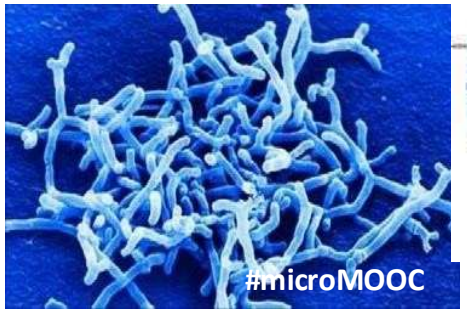
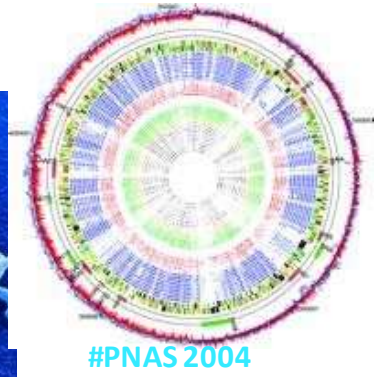
PRINCIPLES FOR SCIENTIFIC SUBSTANTIATION (cont.)

General scientific guidance for stakeholders on health claim applications

Characterisation of microorganisms





- **Species identification + strain characterisation/typing** needed, since effects are strain specific unless the contrary is demonstrated
- New **molecular tools** (multilocus sequence typing, optical mapping, whole-genome sequencing, etc.). Open list to others.
- **Several methods** often needed in combination

Experimental workflow

1. Genome Assembly

was performed using SPAdes (version 3.15.1).

Visualization via ARTEMIS tool

2. Genome Annotation

was executed using Prokka (version 1.14.5) and PGAP.

3. Comparative genomics

- Average nucleotide identity (ANI) analysis

(Pyani version 0.2.10)

- Approximately-maximum-likelihood

phylogenetic tree (FastTree 2.1)

4. Genes involved in technological and functional characteristics

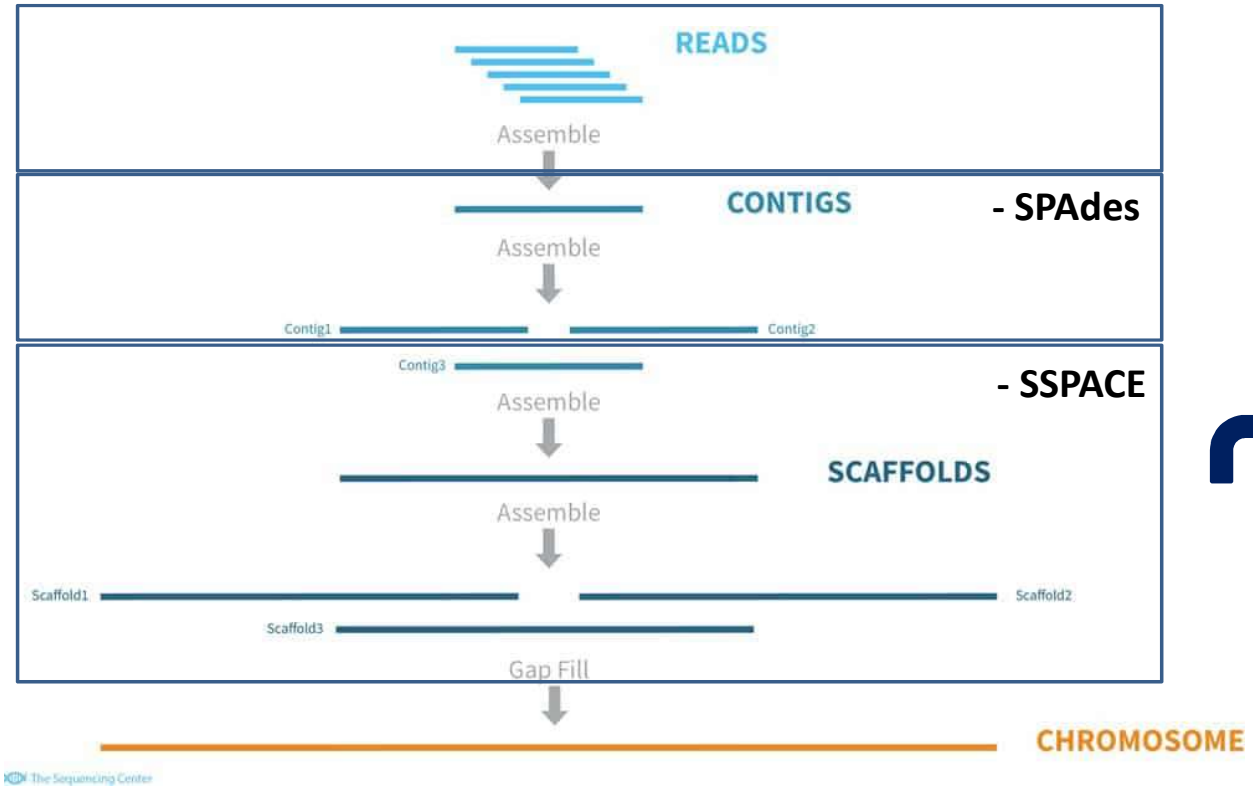
investigated using EggNOG, BlastKOALA

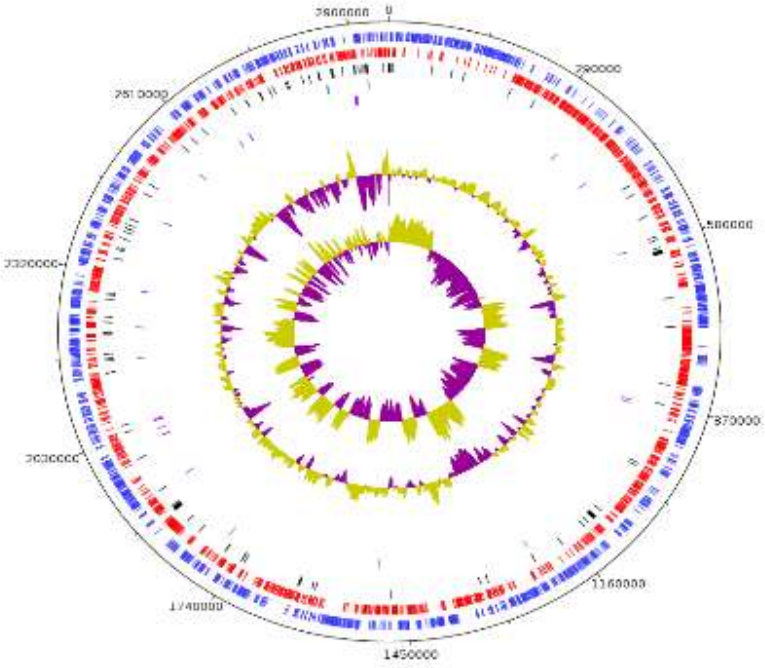
5. Safety assessment

- presence of plasmids (PlasmidFinder)
- antibiotic resistance (ResFinder 4.1)
- virulence genes (VirulenceFinder 2.0)
- pathogenicity (PathogenFinder 1.1)

Genome Assembly

De novo assembly



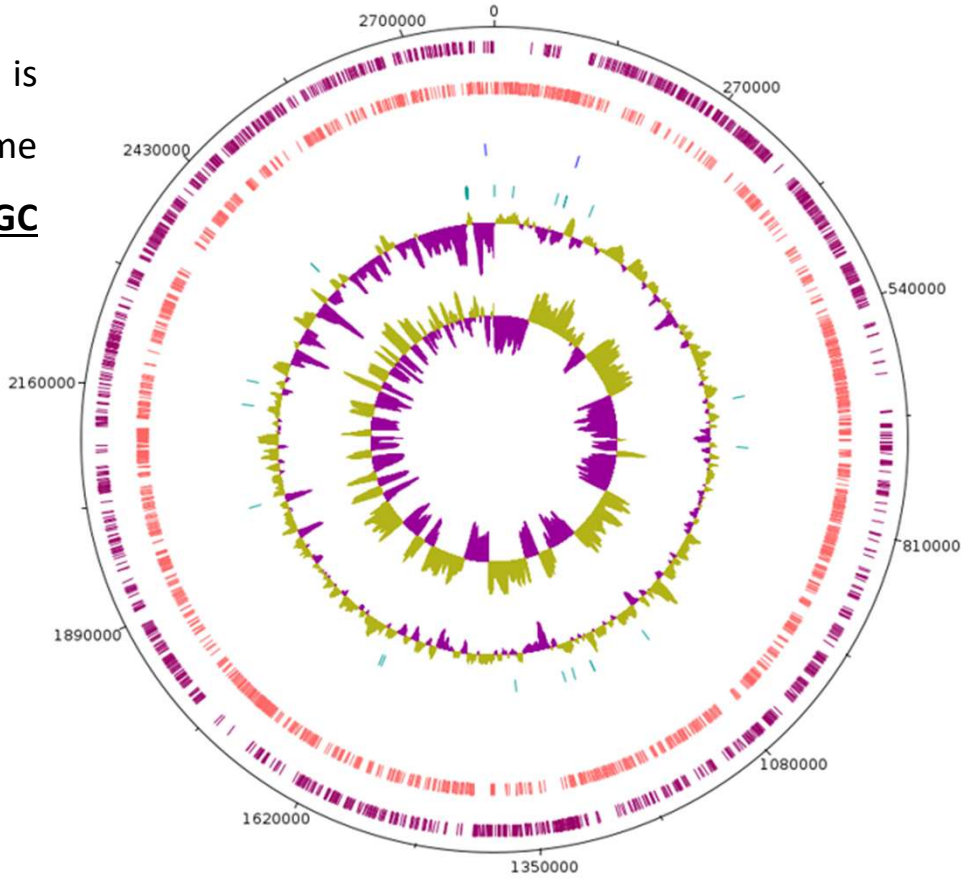


Genome feature analysis for SRX10

The genome of *Lc. paracasei* SRX10 is comprised of a circular chromosome with a total **length of 2.81 Mb** and a **GC content 46.4%**

Members of the emended *Lactobacillus* genus exhibit genomes that vary in size, ranging from 1.27 to 4.91 Mb (Duar et al., 2017)

Lc. paracasei strains possess a median genome length of 3.01 Mb and a median GC content of 46.3%



Circular chromosome map of *Lb. paracasei* SRX10

Genome characteristics	Value
Length	2,813,407 bp
GC content	46.40 %
Total genes	2,764
CDSs	2,711
rRNAs	6
tRNAs	44
ncRNAs	3
Pseudogenes	106
Contamination (%)	1.89

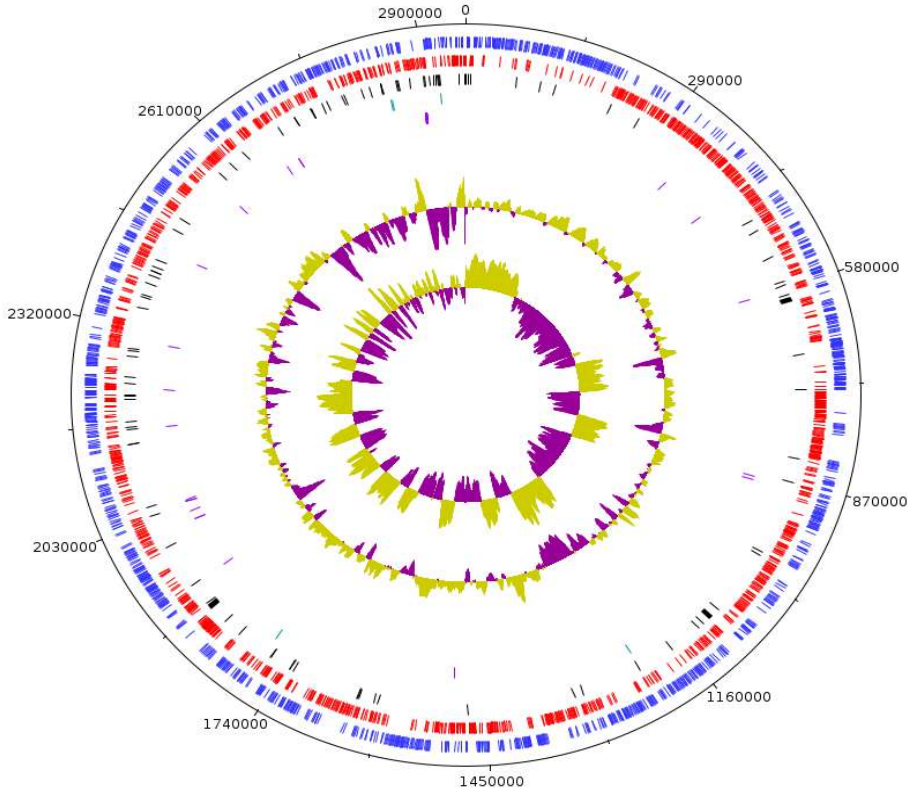
› Microorganisms. 2024 Jan 2;12(1):93. doi: 10.3390/microorganisms12010093.

Dissecting the Genetic Basis of the Technological, Functional, and Safety Characteristics of *Lacticaseibacillus paracasei* SRX10

Christina S Kamarinou [1,2], Despoina E Kiousi [1], Panagiotis Repanas [1], Anthoula A Argyri [2], Nikos G Chorianopoulos [3], Alex Galanis [1]

Genome feature analysis for SP5

Genome Characteristics	Value
Length	**2,958,982 bp**
GC content	**46.3 %**
Total genes	2,920
CDSs	**2,870**
rRNAs	5
tRNAs	42
ncRNAs	3
Pseudogenes	105
No. of CRISPR Arrays	0
Cas proteins	0
IS elements	122
Phages	
Intact	1
Incomplete	2
Questionable	2
Antibiotic resistance genes	
Perfect hits	0
Strict hits	0
Loose hits	202
Virulence genes	0
Probability of being a human pathogen	0.099
Plasmids	0

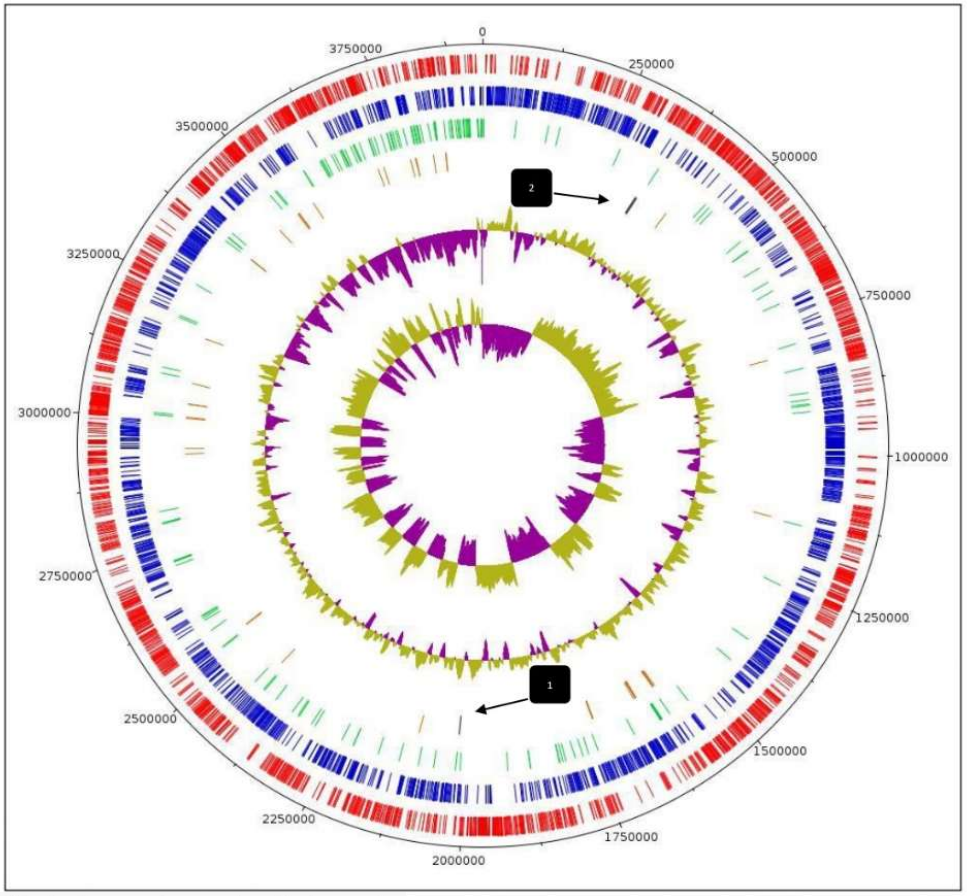


***Lc. paracasei* SP5 genome metrics are typical of the *Lc. paracasei* species**

Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Frontiers Micro 2022;13:922689.

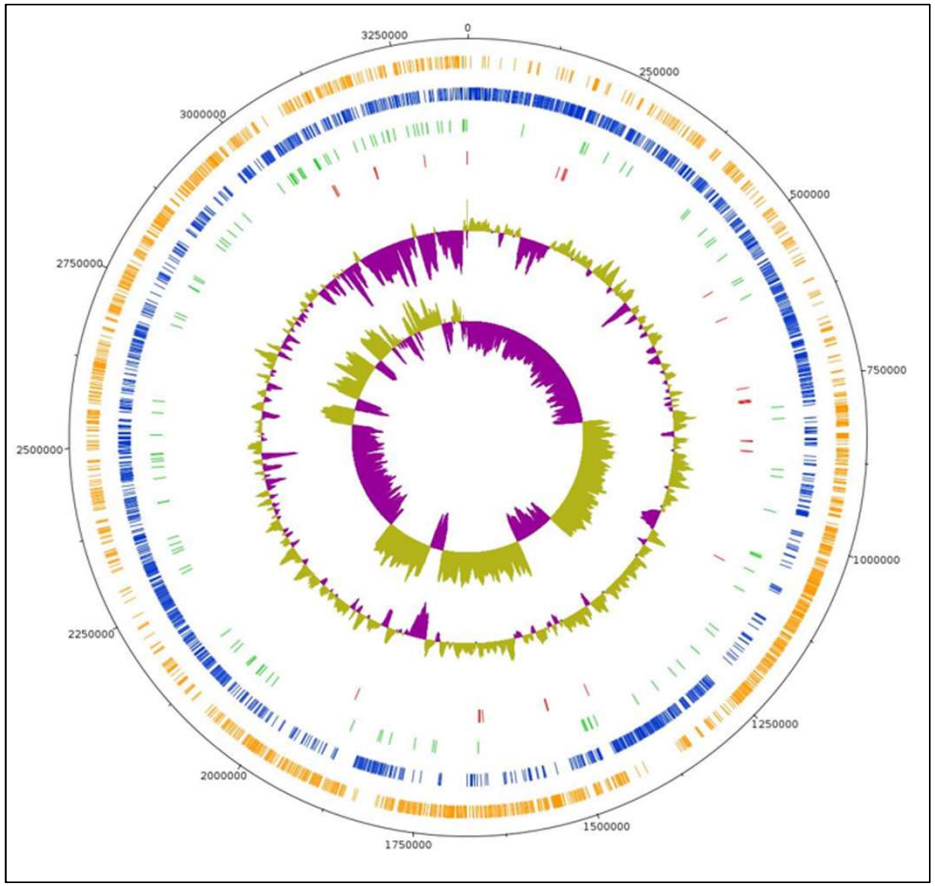
Characterisation of novel probiotic strains (Whole-genome sequencing)



Whole-Genome Sequencing, Phylogenetic and Genomic Analysis of *Lactiplantibacillus pentosus* L33, a Potential Probiotic Strain Isolated From Fermented Sausages

Odysseas Sotirios Stergiou[1†], Konstantinos Tegopoulos[1†], Despoina Eugenia Kiousi[1], Margaritis Tsifintaris[1], Aristotelis C. Papageorgiou[1], Chrysoula C. Tassou[2], Nikos Chorianopoulos[2], Petros Kolovos[1*] and Alex Galanis[1*]

Frontiers Micro 2021;12:746659.



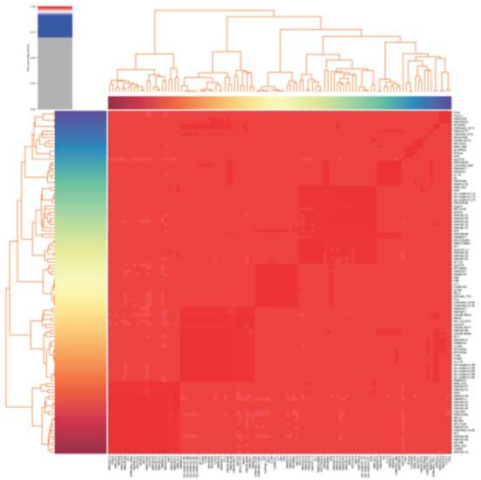
Genomic and Phylogenetic Analysis of *Lactiplantibacillus plantarum* L125, and Evaluation of Its Anti-Proliferative and Cytotoxic Activity in Cancer Cells

by Konstantinos Tegopoulos [1,†], Odysseas Sotirios Stergiou [1,†], Despoina Eugenia Kiousi [1,†], Margaritis Tsifintaris [1], Ellie Koletsou [1], Aristotelis C. Papageorgiou [1], Anthoula A. Argyri [2], Nikos Chorianopoulos [2], Alex Galanis [1,*] and Petros Kolovos [1,*]

Biomedicines 2021;9(11):1718.

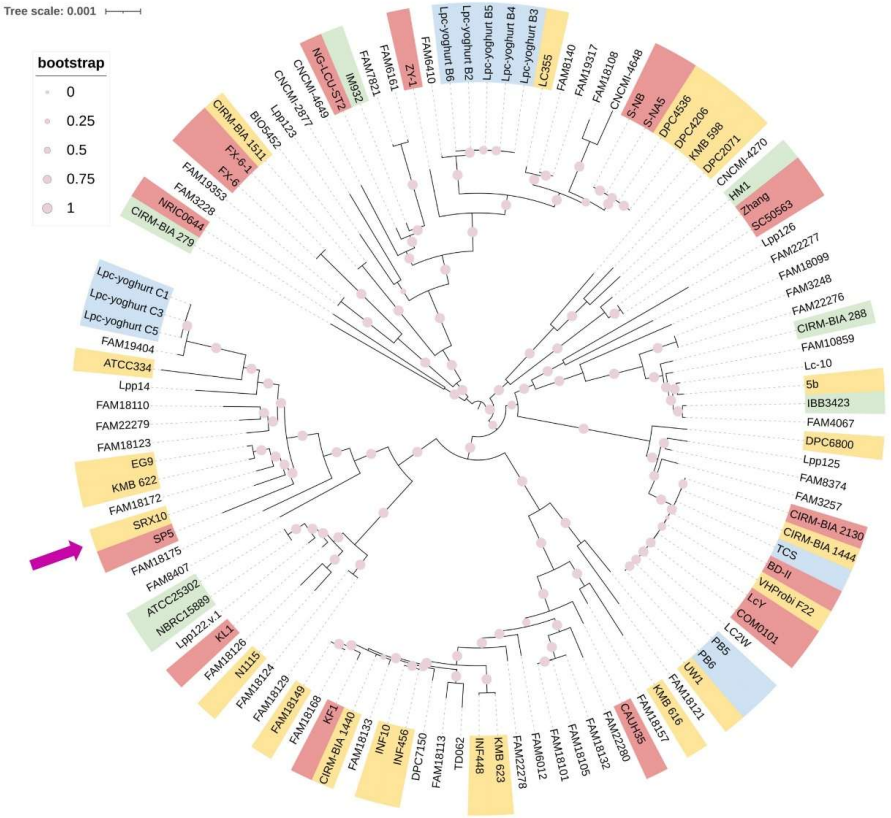
Phylogenomic and Pangenome analysis for SRX10

Strain	ANI (%) with SRX10
SRX10	100
KMB_622	99.67
EG9	99.62
FAM18172	99.58
FAM18123	99.51
FAM22279	99.50
SP5	99.43
FAM18110	99.40
Lpp14	99.34
Lpc-yoghurt_C5	99.34
Lpc-yoghurt_C1	99.31
Lpc-yoghurt_C3	99.31
FAM19404	99.26
FAM18175	99.26
ATCC334	99.23
FAM8407	99.21



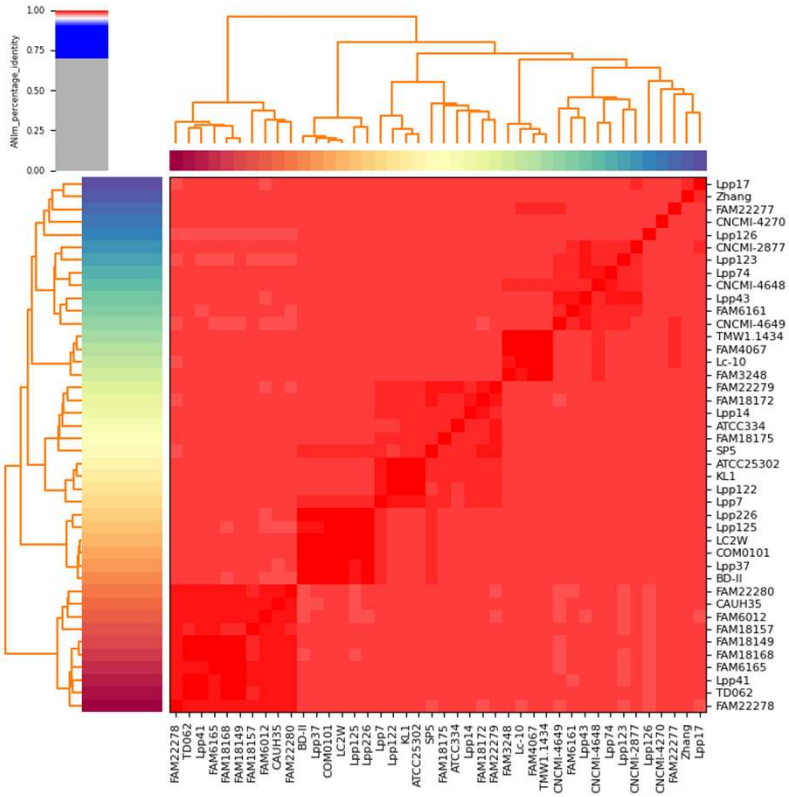
ANI of *Lc. paracasei* SRX10 derived from fermented milk products (A) ANI heatmap of all dairy isolates (B) ANI of *Lc. paracasei* SRX10 with dairy isolates

Approximately-maximum-likelihood phylogenetic tree of *Lc. paracasei* strains isolated from dairy products (pink – fermented milk products, yellow– cheese, blue- yoghurt, green- raw milk products)



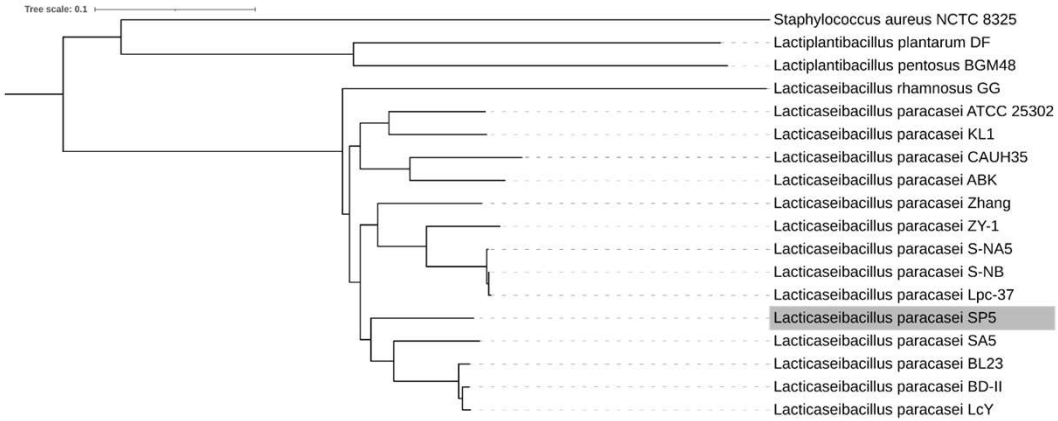
Average nucleotide identity (ANI) analysis using the python module Pyani, showed that the strain possessed **unique genomic sequences**, and further confirmed its **classification in the *Lc. paracasei* species**

Phylogenomic and Pangenome analysis for SP5



	SP5
SP5	1
FAM18172	0.994177
FAM22279	0.993258
Lpp14	0.991895
ATCC334	0.991474
FAM18175	0.990251
ATCC25302	0.990191
KL1	0.990157
LC2W	0.990041
Lpp7	0.990039
COM0101	0.989907
Lpp37	0.989872
BD-II	0.989491
Lpp226	0.989313
Lpp125	0.988772
Lpp122	0.988018
CNCMI-4270	0.987437
FAM3248	0.987297
FAM4067	0.98708
FAM22277	0.986993
TMW1.1434	0.986689
CNCMI-4648	0.986671
CNCMI-2877	0.986671
Zhang	0.986314
FAM6161	0.986299
Lpp17	0.986282
Lpp123	0.986054
Lpp43	0.986051
Lpp74	0.985998
Lc-10	0.985682
TD062	0.98547
FAM18157	0.985405
Lpp126	0.985383
FAM6165	0.985242
Lpp41	0.985202
FAM18149	0.985153
FAM18168	0.98509
CNCMI-4649	0.985044
FAM22280	0.985032
CAUH35	0.985001
FAM6012	0.984923
FAM22278	0.984349

Average Nucleotide Identity (ANI) for species identification/**strain characterization** through whole-genome similarity analysis



ANI species cut off > 96%

***Lc. paracasei* SP5 is a unique strain that belongs to the *Lc. paracasei* species**

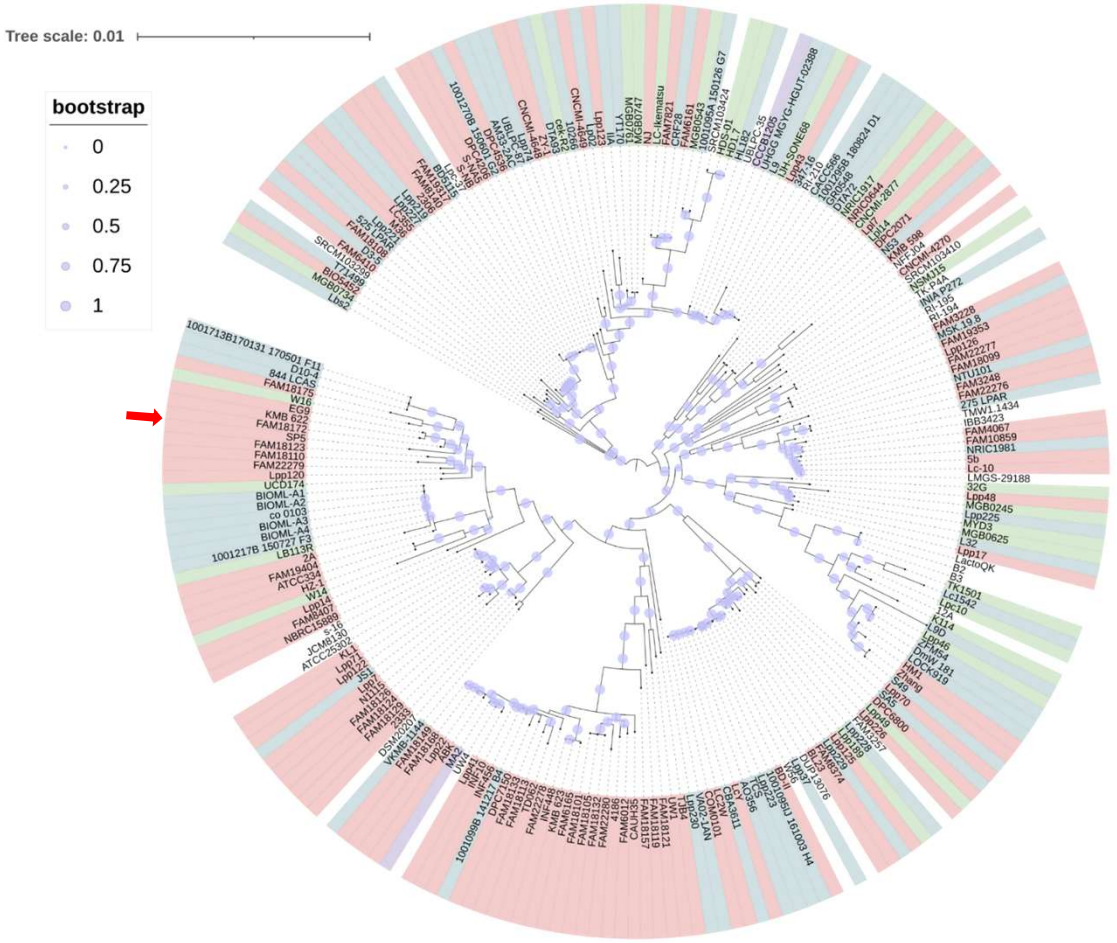
Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Despoina Eugenia Kiousi[1], Christos Efstathiou[1], Konstantinos Tegopoulos[1], Ioanna Mantzourani[2], Athanasios Alexopoulos[2], Stavros Plessas[2]*, Petros Kolovos[1], Maria Koffa[1] and Alex Galanis[1]*

Frontiers Micro 2022;13:922689.

Phylogenomic analysis for SP5

- *Lc. paracasei* strains do not cluster based on isolation source
- *Lc. paracasei* SP5 clusters with dairy strains – however it exhibits close phylogenetic relationship with human- and vegetable-derived strains



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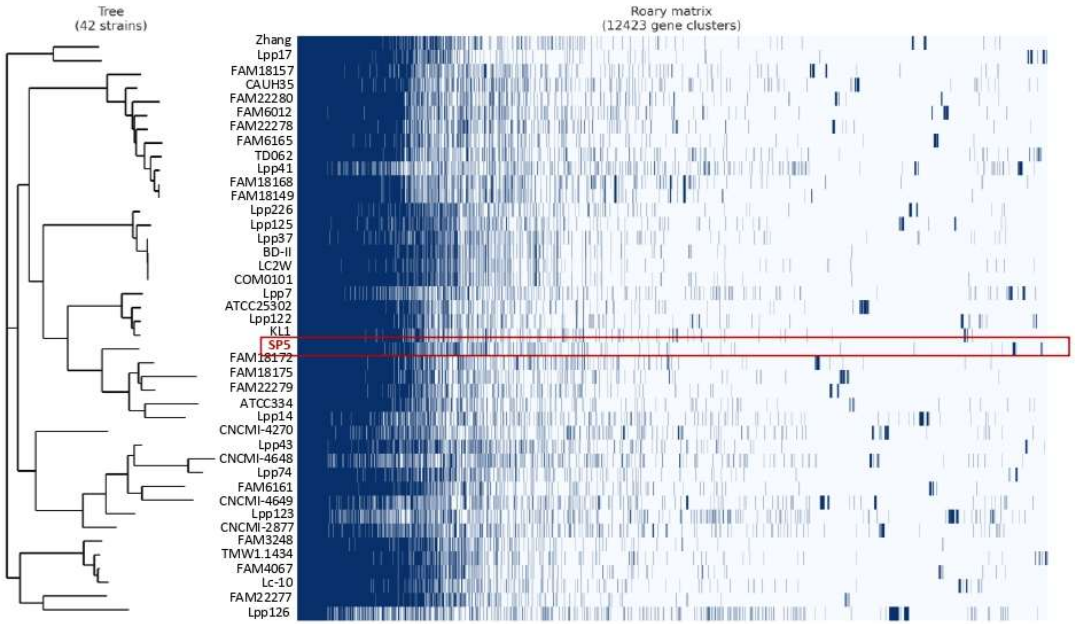
Frontiers Micro 2022;13:922689.

Pangenome analysis for SP5

Pangenome Analysis to define functional diversity and evolution, niche adaptation and host interactions



Class	SP5	Pangenome	Unique
C, Energy production and conversion	111 (4.81%)	299 (3.17%)	1 (2.3%)
D, Cell cycle control and mitosis	38 (1.62%)	134 (1.42%)	0 (0%)
E, Amino Acid metabolism and transport	184 (7.83%)	539 (5.71%)	1 (2.38%)
F, Nucleotide metabolism and transport	111 (4.72%)	237 (2.51%)	0 (0%)
G, Carbohydrate metabolism and transport	231 (9.83%)	879 (9.32%)	7 (16.67%)
H, Coenzyme metabolism	66 (2.81%)	164 (1.74%)	2 (4.76%)
I, Lipid metabolism	57 (2.43%)	134 (1.42%)	0 (0%)
J, Translation	166 (7.06%)	306 (3.24%)	0 (0%)
K, Transcription	211 (8.98%)	632 (6.70%)	6 (14.29%)
L, Replication and repair	157 (6.68%)	1521 (16.11%)	5 (11.90%)
M, Cell wall/membrane/envelop biogenesis	125 (5.32%)	598 (6.34%)	4 (9.52%)
N, Cell motility	8 (0.34%)	29 (0.31%)	2 (4.76%)
O, Post-translational modification, protein turnover, chaperone functions	52 (2.21%)	139 (1.47%)	0 (0%)
P, Inorganic ion transport and metabolism	132 (5.62%)	450 (4.77%)	1 (2.38%)
Q, Secondary Structure	26 (1.11%)	72 (0.76%)	1 (2.38%)
T, Signal Transduction	52 (2.21%)	155 (1.64%)	1 (2.38%)
U, Intracellular trafficking and secretion	45 (1.91%)	144 (1.53%)	2 (4.76%)
V, Defense mechanisms	74 (3.15%)	365 (3.87%)	1 (2.38%)
S, Function Unknown	502 (21.36%)	1614 (17.11%)	8 (19.05%)
No category, General function prediction only	269 (11.45%)	1019 (10.81%)	5 (11.9%)
Total	2350 (100%)	9430 (100%)	42 (100%)

***Lc. paracasei* SP5 unique genes are involved in carbohydrate metabolism and DNA transposition**


Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Frontiers Micro 2022;13:922689.

Genome-Wide Analysis for SRX10

Detection of genes associated with

Technological characteristics

1. **Stress tolerance responses** (cold shock, heat shock proteins and genes responsible for the resistance to osmotic shock)
2. **Metabolic pathways** and genes associated with flavor development

- *Carbohydrate metabolism (catabolism of lactose)*
- *Lipid metabolism (lipid degradation, fatty acid biosynthesis (e.g., fabD, fabH, fabZ)*
- *Amino acid metabolism (proteolytic enzymes (e.g., pepN, pepX, and pepC)*

3. **EPS production** (e.g., epsA, epsB, and epsC) were annotated in the genome of the strain

Functional characteristics

1. **Resistance to Gastrointestinal conditions** (a full cluster for the production of F0F1 ATP synthetase atpABCDEFGH)

2. **Adhesion capacity** (moonlighting proteins)

3. **Antimicrobial compounds**

4. **Vitamin synthesis** (Vitamin B1)

Genome-Wide Analysis for SP5

Table 3. Annotation of genes coded by *Lc. paracasei* SP5 that are implicated in stress response and host-microbe interactions.

Locus Tag	Gene Function	Gene	E-value
Gastrointestinal tract survival & stress response			
SP5_000699	Penicillin-binding protein	*pbpX*	0.0
SP5_001309	Penicillin-binding protein	*mrdA*	0.0
SP5_000894	Penicillin-binding protein 1A	*ponA*	0.0
SP5_001372	Penicillin-binding protein 2A	*pbp2A*	0.0
Acid tolerance			
SP5_002673	Sodium proton antiporter	*yvgP*	0.0
SP5_002530	ATP synthase subunit alpha	*atpA*	0.0
SP5_002526	ATP synthase subunit a	*atpB*	1.23e-162
SP5_002533	ATP synthase epsilon chain	*atpC*	1.88e-91
SP5_002532	ATP synthase subunit beta	*atpD*	0.0
SP5_002527	ATP synthase subunit c	*atpE*	2.57e-37
SP5_002528	ATP synthase subunit b	*atpF*	3.59e-80
SP5_002531	ATP synthase gamma chain	*atpG*	1.92e-211
SP5_002529	ATP synthase subunit delta	*atpH*	3.93e-116
SP5_000635	Decarboxylase	*yphJ*	1.07e-72
Bile salt tolerance			
SP5_002916	Linear amide C-N hydrolase, choloylglycine hydrolase family	-	2.38e-252
Extreme temperature tolerance			
SP5_001004	'Cold-shock' DNA-binding domain protein	*cspA*	3.08e-43
SP5_000711	Cold shock protein	*cspB*	4.62e-48
SP5_002482	Cold shock protein	*cspC*	6.22e-43
SP5_001854	Heat shock 40 kDa protein	*dnaJ*	9.45e-261
SP5_001853	Heat shock 70 kDa protein	*dnaK*	0.0
SP5_001695	Member of the small heat shock protein (HSP20) family	*hsp*	1.8e-99
SP5_000918	Member of the small heat shock protein (HSP20) family	*hsp1*	1.05e-111
SP5_001851	Negative regulator of class I heat shock genes (grpE- dnaK-dnaJ and groELS operons)	*hrcA*	1.6e-246
SP5_002404	Recovery of the cell from heat-induced damage, in cooperation with DnaK, DnaJ and GrpE	*clpC*	0.0
SP5_001504	Molecular chaperone	*GroEL*	0.0
SP5_001503	Co-chaperonin	*GroES*	1.7e-59
SP5_000774	Molecular chaperone	*clpB*	0.0
Cell wall formation			
SP5_000209	Cell wall formation	*murA*	9.48e-300
SP5_002250	Glycosyltransferase like family 2	*epsIIG*	3.79e-89
SP5_002585	Glycosyltransferase like family 2	*epsG*	4.18e-151
SP5_002316	Glycosyl transferases group 1	*tagE3*	0.0
SP5_002317	Glycosyl transferases group 1	*tagE2*	0.0
SP5_002642	Glycosyl transferase family 8	*arbx*	1.17e-211
SP5_001889	Glycosyl transferase family 2	*ykcC*	8.07e-233
SP5_000560	D-alanine--D-alanyl carrier protein ligase	*dltA*	0.0
SP5_000558	D-alanyl carrier protein	*dltC*	6.97e-49
SP5_000557	Involved in the D-alanylation of LTA	*dltD*	8.29e-312
SP5_000561	D-Ala-teichoic acid biosynthesis protein	*dltX*	1.04e-27
Adhesion capacity			
Putative adhesins			
SP5_000853	Fibronectin-binding protein A (Fibronectin binding domain A)	*FbpA*	0.0
SP5_002267	Putative adhesin	*yvlB*	0.0
SP5_001633	Internalin J (MucBP domain)	*inlJ*	5.78e-287
SP5_002881	LPxTG domain protein (PillinD1 domain, SpaA domain, Ig-like fold)	-	1.25e-236
SP5_002694	Hydrolase, Collagen-binding protein	*mapA*	0.0
SP5_000963	NlpC P60 family protein (SlpA domain)	*p75*	7.12e-202
SP5_002397	CHAP domain protein (SibA CHAP domains)	*p40*	4.92e-201
Moonlighting proteins			
SP5_002227	Glycosyl hydrolase (LysM domain)	-	8.06e-232
SP5_000794	Leucine-rich repeat (LRR) protein (LysM domain)	-	1.68e-104
SP5_001575	Hydrolase (LysM domain)	-	1.64e-184
SP5_000723	Phosphoglycerate mutase	*pgm6*	4.69e-159
SP5_002706	Triosephosphate isomerase	*tpiA*	2.81e-180
SP5_000756	Elongation factor Tu	*tuf*	2.74e-285
Supporting functions			
SP5_000470	Sortase family protein	*srtA*	4.31e-166
SP5_002372	Sortase family protein	*srtA*	2.1e-143
SP5_002167	Sortase family protein	*srtB*	5.2e-188
SP5_002880	Sortase family protein	-	3.16e-258
Biofilm formation			
SP5_002245	Capsular polysaccharide biosynthesis protein	*epsB*	2.52e-169
SP5_000584	S-ribosylhomocysteine lyase	*luxS*	1.69e-112
SP5_000661	Transcriptional regulatory protein DesR	*desR*	8.42e-135
SP5_001469	Catabolite control protein A	*ccpA*	3.03e-232
SP5_000097	Cell envelope-like function transcriptional attenuator common domain protein	*brpA*	4.84e-256

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Front. Micro 2022;13:922689

In silico Safety Assessment for SRX10

Genome stability

Three prophage regions were predicted in the genome of *Lc. paracasei* SRX10. **No plasmids or mobile genetic elements** were detected, while a total of 98 insertion elements were identified in the genome of the strain. Finally SRX10 **lacks functional CRISPR arrays** and does not code for Cas proteins.

Virulence and antibiotic resistance

SRX10 does not possess **any virulence factors** that could negatively affect the host. **No acquired antibiotic resistance genes** were identified by RGI and ResFinder.

BLAST analysis confirmed that SRX10 **does not harbor genes encoding** the enzymes responsible for the production **of biogenic amines or genes involved in hemolysis.**

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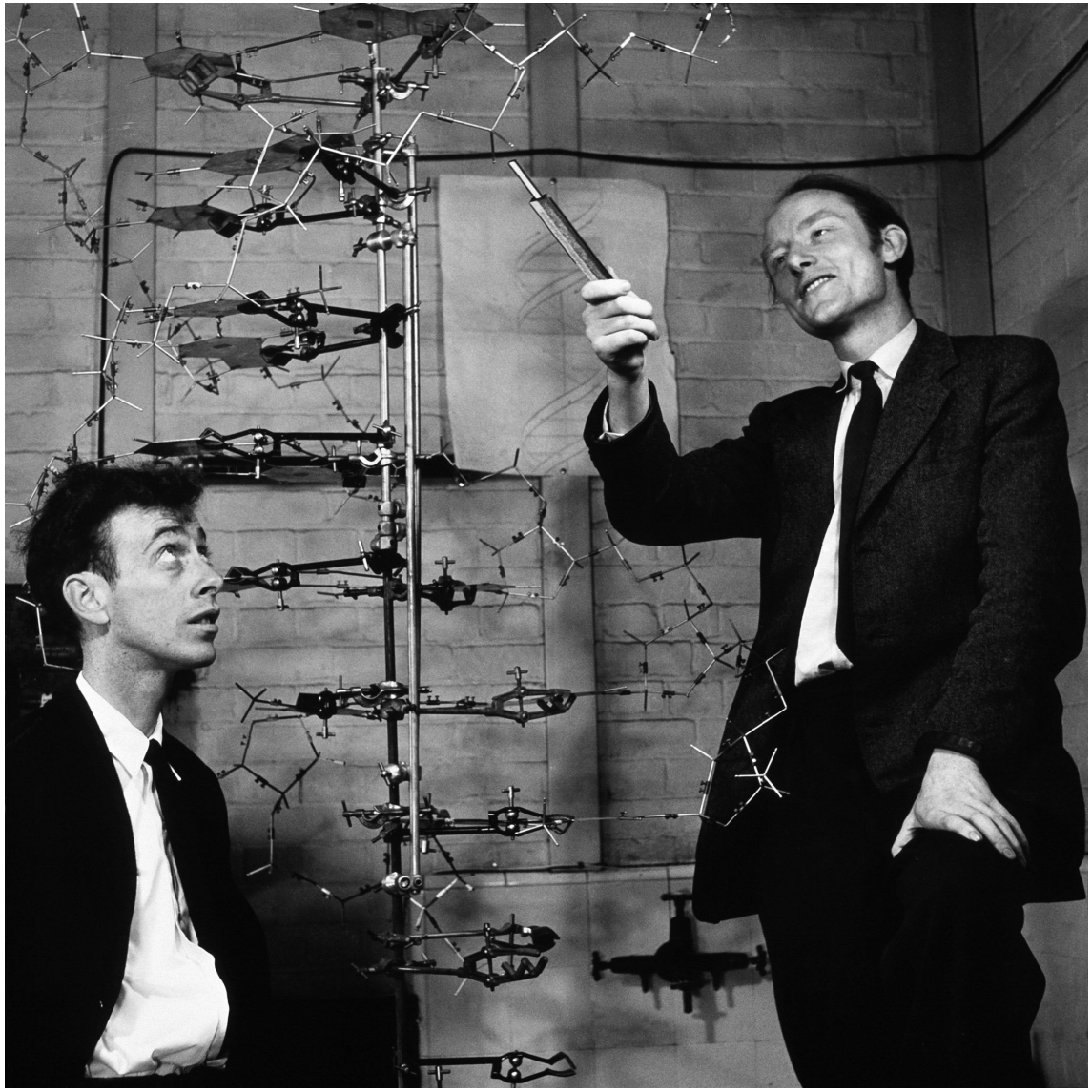
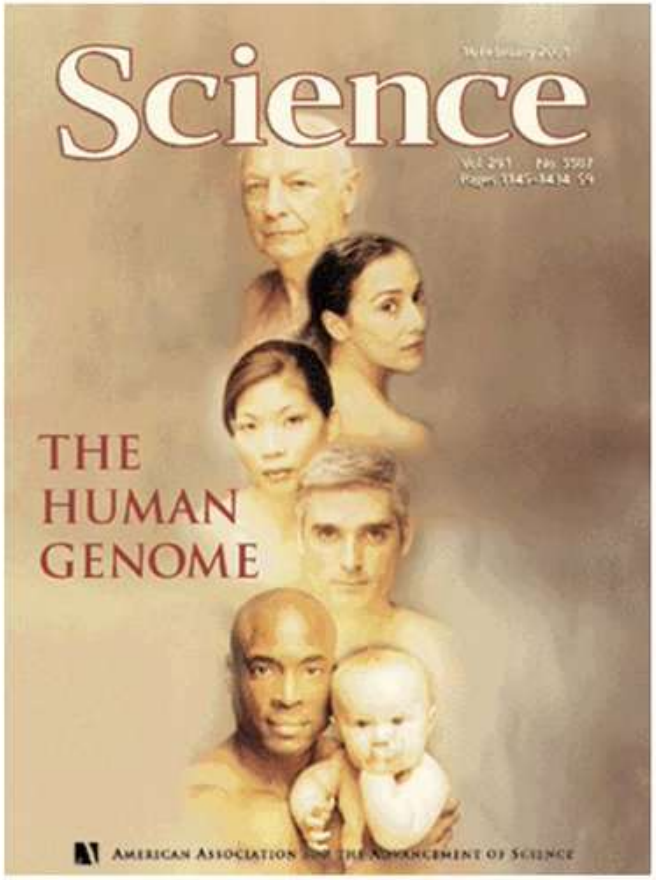
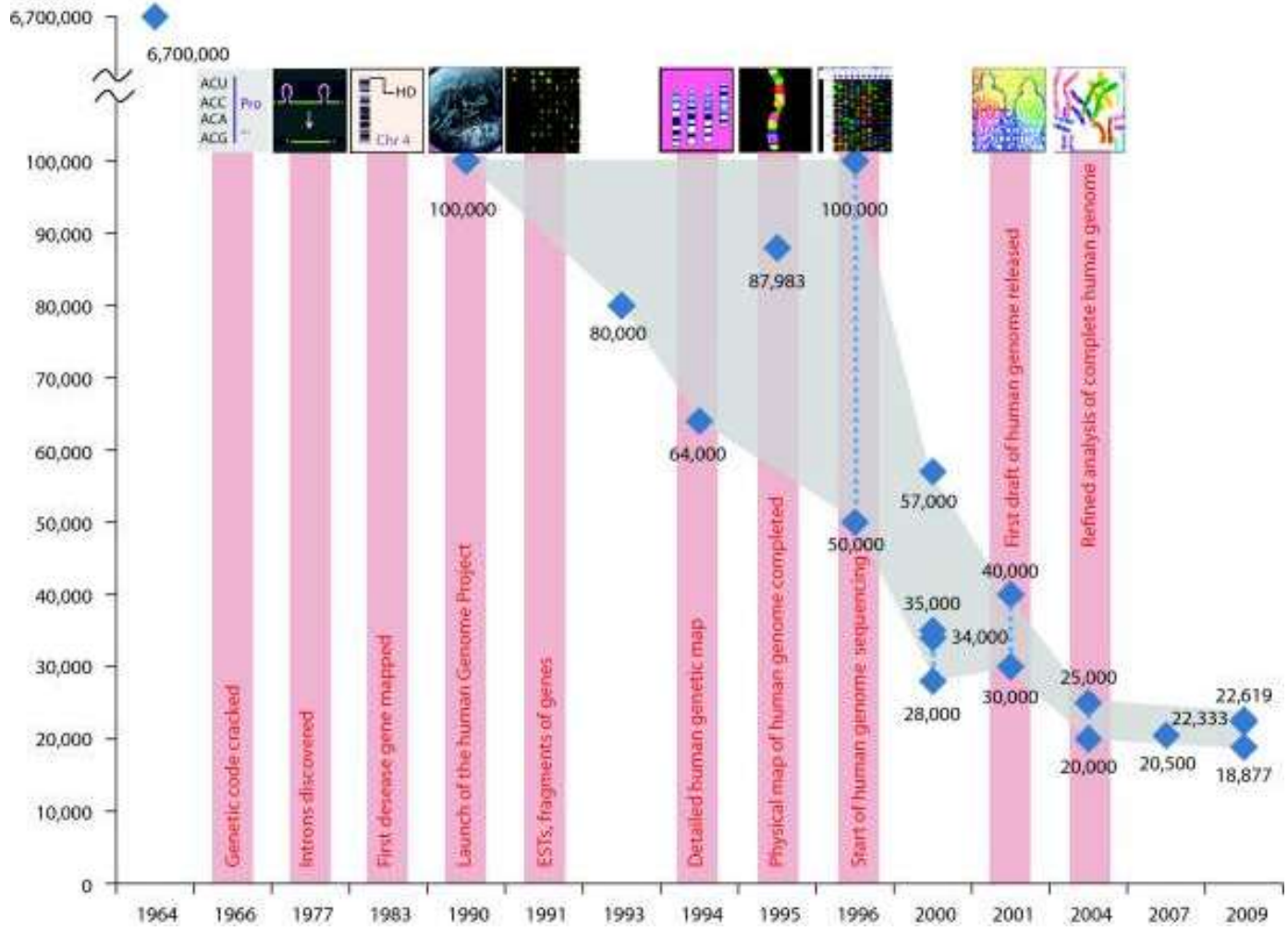


Genome sequencing

February 2001 - Publication of the first draft of the human genome

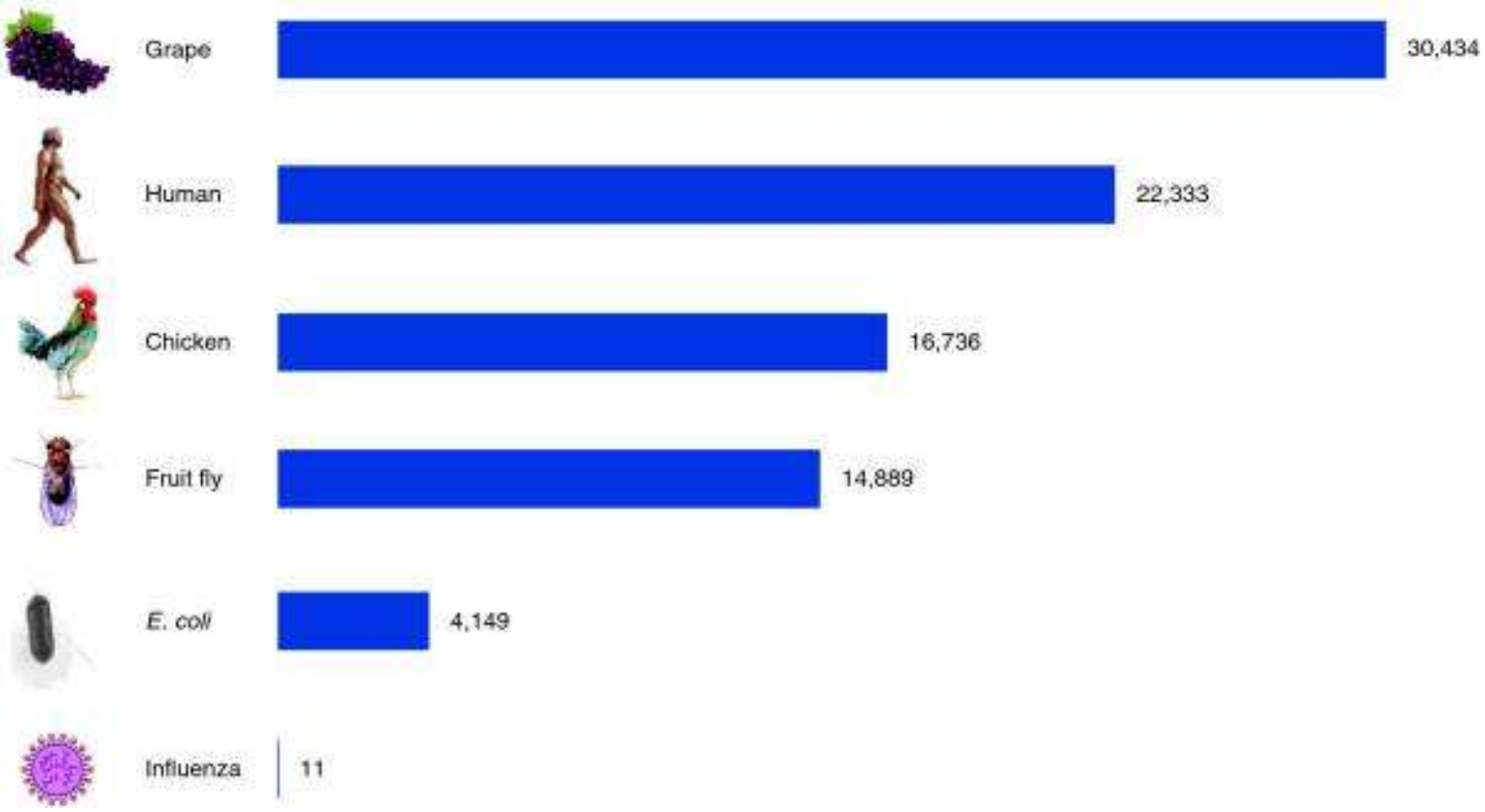


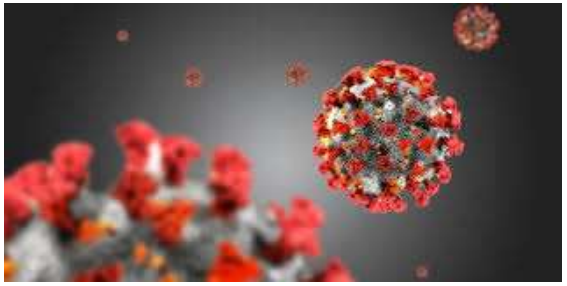


Review › Genome Biol, 11 (5), 206 2010

Between a Chicken and a Grape: Estimating the Number of Human Genes

Mihaela Pertea [1], Steven L Salzberg



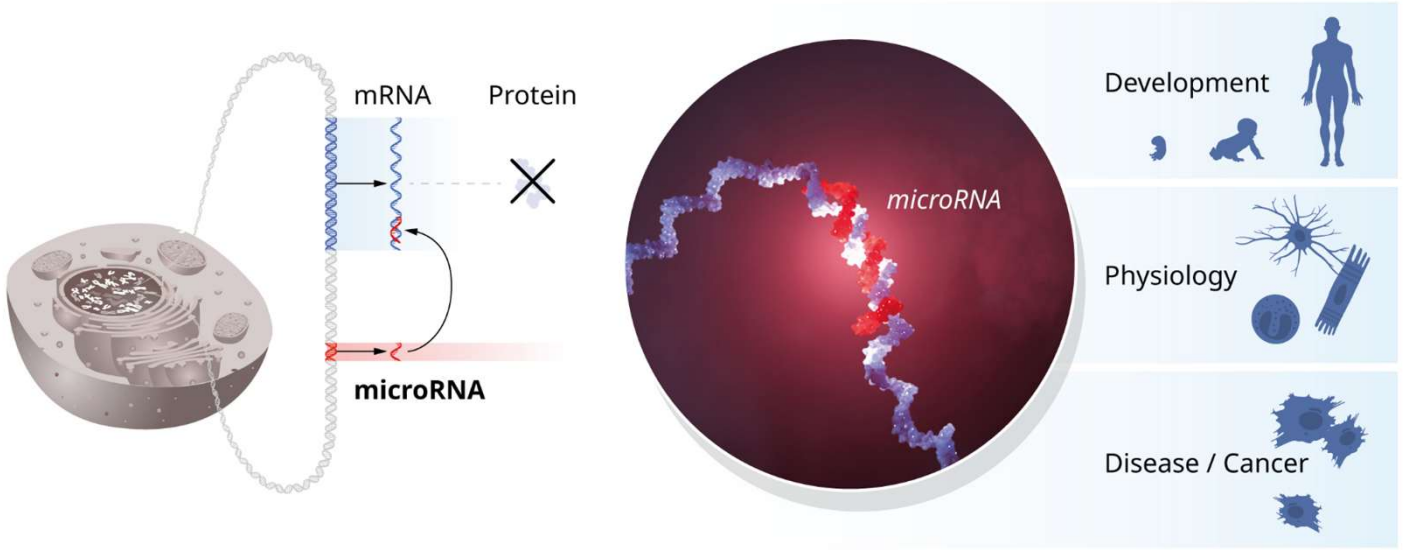


Review › Genome Biol, 11 (5), 206 2010

Between a Chicken and a Grape: Estimating the Number of Human Genes

Mihaela Pertea [1], Steven L Salzberg

The 2024 Nobel Prize in Physiology or Medicine jointly to Victor Ambros and Gary Ruvkun **for the discovery of microRNA and its role in post-transcriptional gene regulation**



© The Nobel Committee for Physiology or Medicine. Ill. Mattias Karlén

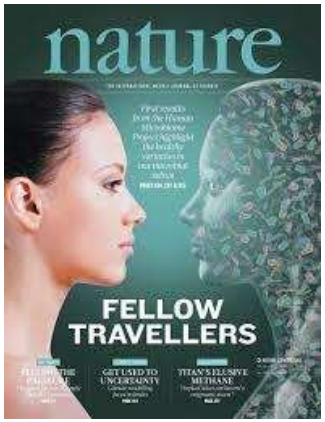
Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus *ras* gene

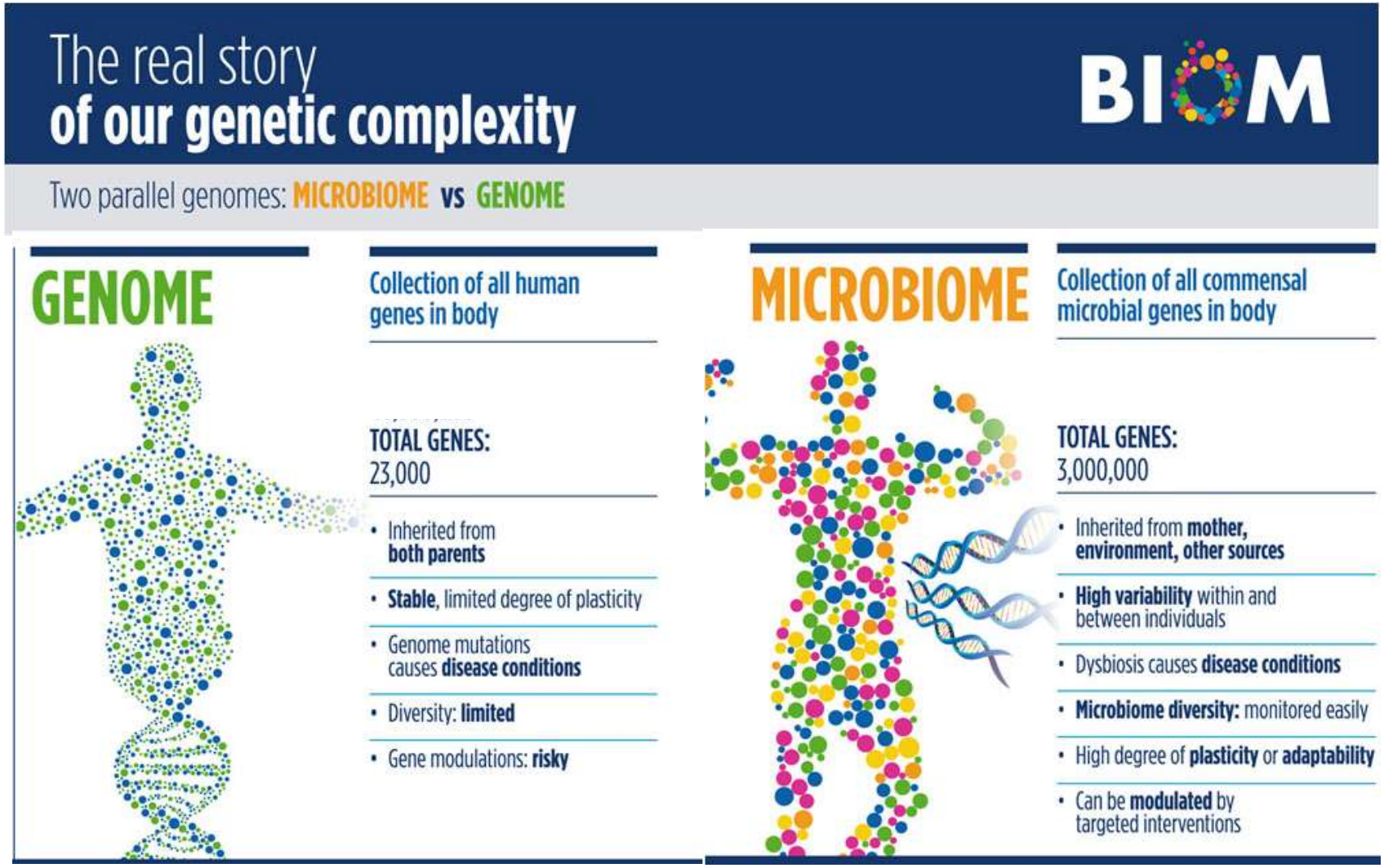
Luis F. Parada, Clifford J. Tabin, Chiaho Shih & Robert A. Weinberg

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

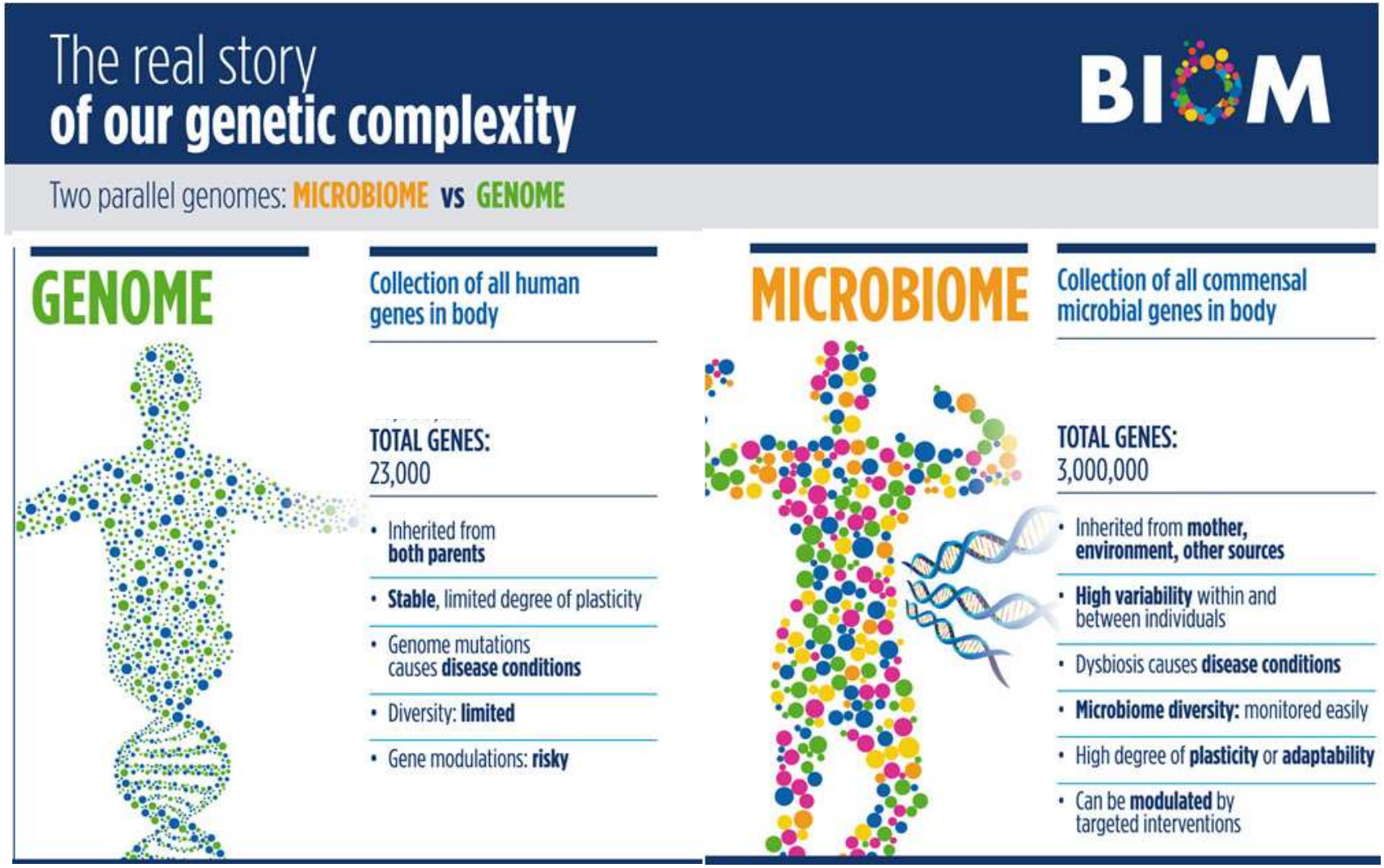
Nature Vol. 297 10 June 1982

Manipulating the human microbiome





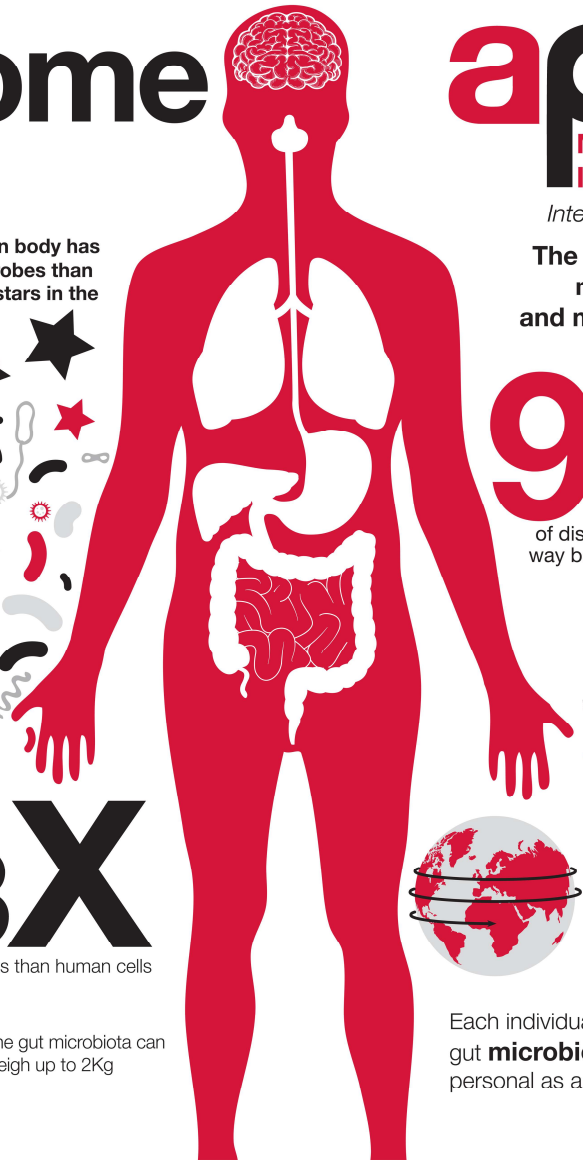
Microbiota: the ecological community of commensal and symbiotic organisms that share our body space.



Microbiome: the human microbiome was defined as the complete genome (an organism's complete set of DNA including the genes) of all microorganisms in and on the human body.

Microbiome

IN NUMBERS



100 Trillion
symbiotic microbes live in and on every person and make up the human microbiota

The human body has more microbes than there are stars in the milky way

95%
of our microbiota is located in the GI tract

150:1
The genes in your microbiome outnumber the genes in our genome by about 150 to one

The surface area of the **GI tract** is the same size as 2 tennis courts

You have 1.3X
more microbes than human cells

>10,000
Number of different microbial species that researchers have identified living in and on the human body

2kg
The gut microbiota can weigh up to 2Kg

Microbiome Ireland
Interfacing Food & Medicine

The microbiome is more medically accessible and manipulable than the human genome

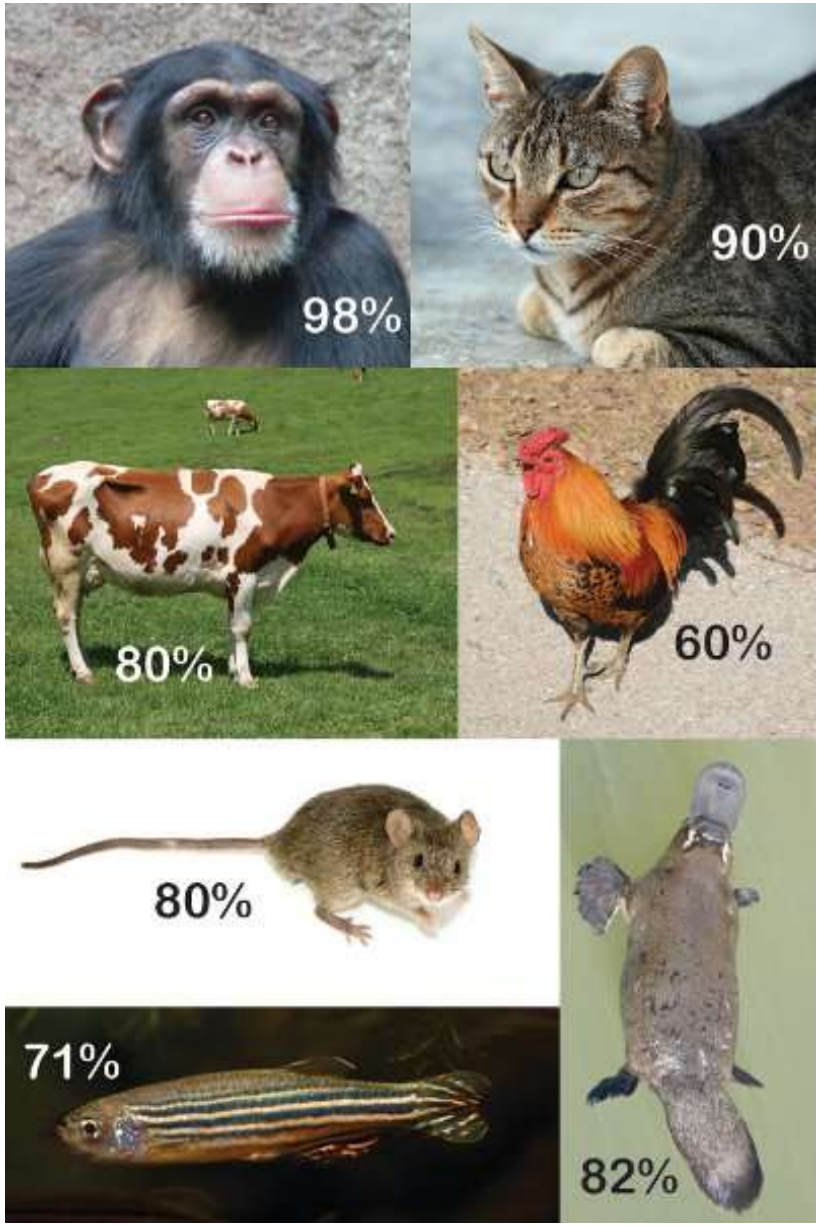
It is thought that **90%** of disease can be linked in some way back to the gut and health of the microbiome

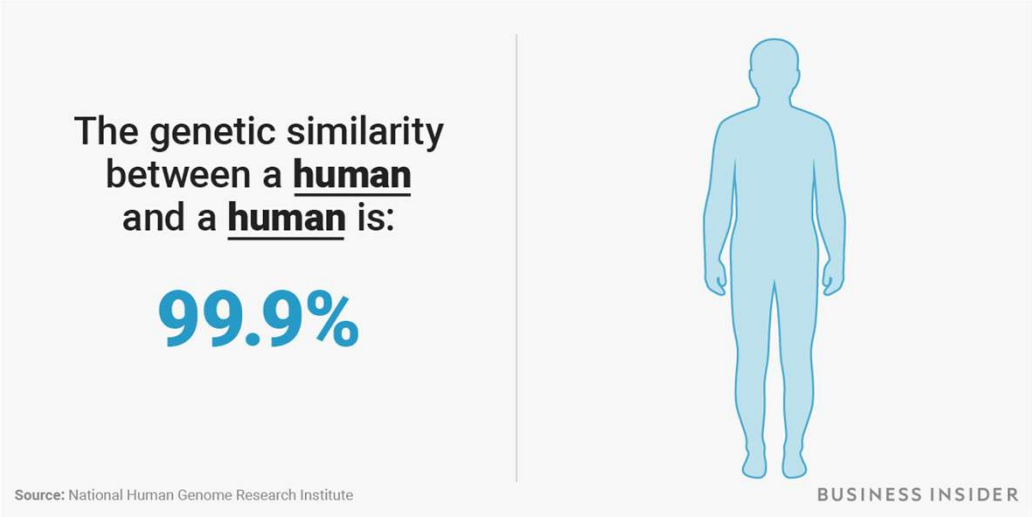
5:1
Viruses:Bacteria in the gut microbiota

2.5
The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint

https://apc.ucc.ie/





Human individuals are **99.9% identical to each other** in terms of their own genome, whereas the microbiome of each individual can be **80-90% different** from one another.

NIH Human Microbiome Project

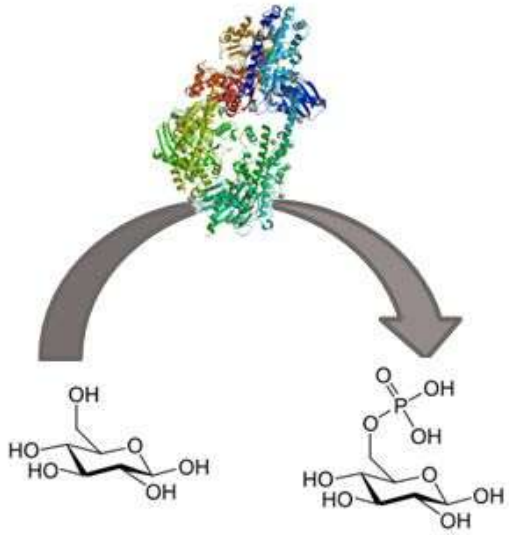
Characterization of the microbiomes of healthy human subjects at five major body sites, using 16S and metagenomic shotgun sequencing.

Enter HMP1

HMP1, (2008-2014), characterized the microbial communities from 300 healthy individuals, across different sites on the human body: nasal passages, oral cavity, skin, GI tract, and urogenital tract. 16S rRNA sequencing was performed to characterize the complexity of microbial communities at each body sites, and to begin to ask investigate whether there is a core healthy microbiome. Metagenomic whole genome shotgun sequencing provided insights into the functions and pathways present in the human microbiome.

Metagenomics is a molecular tool used to analyse DNA acquired from environmental samples, in order to study the community of microorganisms present, without the necessity of obtaining pure cultures.

The World of Omes



http://www.pdb.org/pdb/images/2nzt_bio_r_500.jpg

DNA

mRNA

Protein

Metabolites

- Genome
- Transcriptome
- Proteome
- Metabolome

Transcriptomics

- Transcriptomics, the study of RNA in any of its forms.
- The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells.

Transcriptomics

Transcriptomics is the study of the transcriptome—the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell—using high-throughput methods, such as microarray analysis. Comparison of transcriptomes allows the identification of genes that are differentially expressed in distinct cell populations, or in response to different treatments.

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Author manuscript
Cell Mol Life Sci. Author manuscript; available in PMC 2018 November 13.

Published in final edited form as:
Cell Mol Life Sci. 2015 September ; 72(18): 3425–3439. doi:10.1007/s00018-015-1934-y.

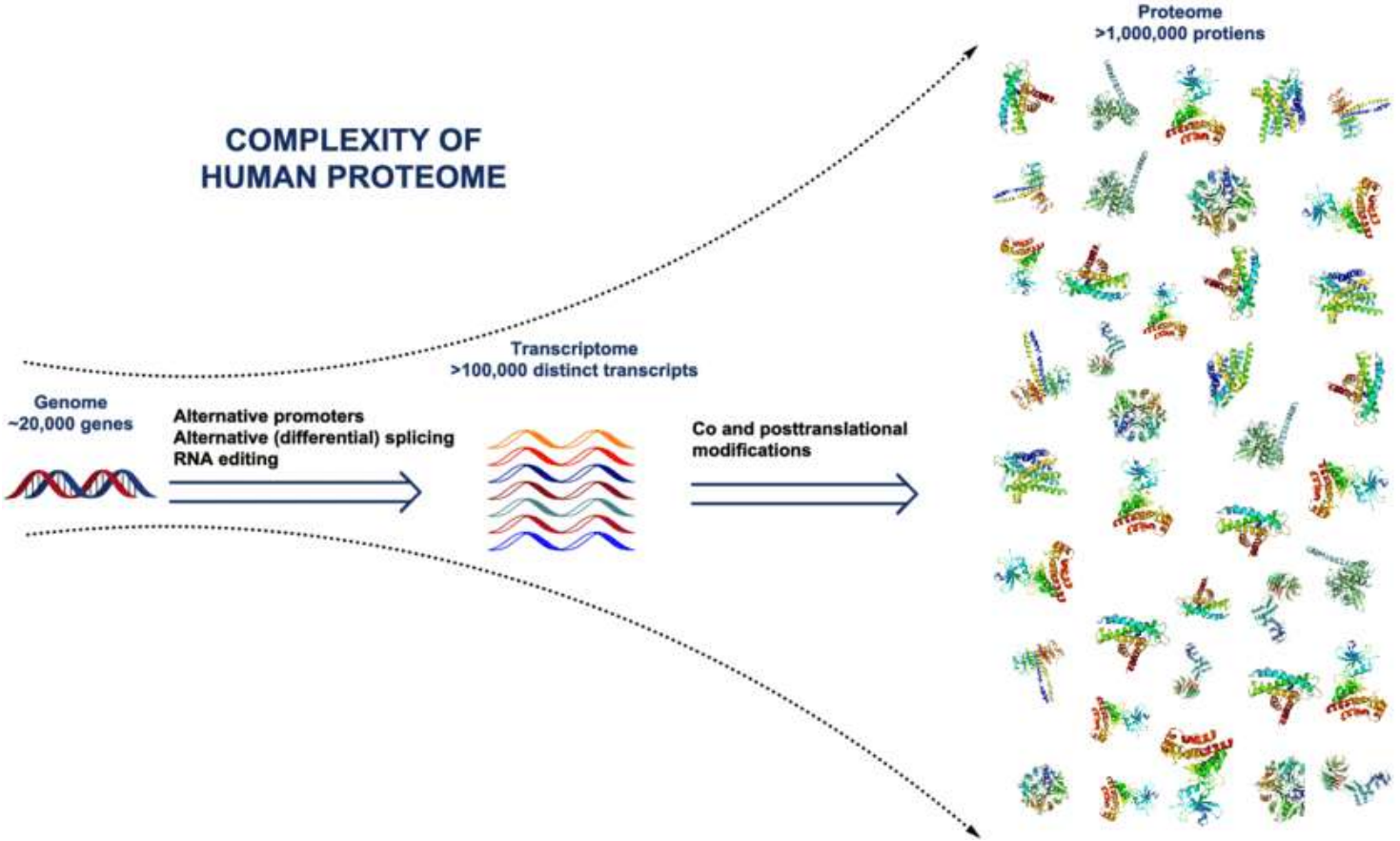
Whole transcriptome analysis with sequencing: methods, challenges and potential solutions

Zhihua Jiang[1], **Xiang Zhou**[1], **Rui Li**[1], **Jennifer J. Michal**[1], **Shuwen Zhang**[1], **Michael V. Dodson**[1], **Zhiwu Zhang**[2], and **Richard M. Harland**[3]

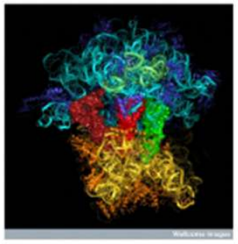
Transcriptomics

Transcriptomics is the study of the transcriptome—the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell—using high-throughput methods, such as microarray analysis. Comparison of transcriptomes allows the identification of genes that are differentially expressed in distinct cell populations, or in response to different treatments.

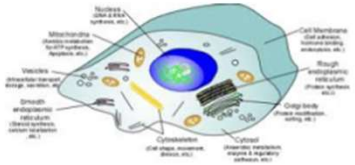
Proteomics is the analysis of the entire protein complement of a cell, tissue, or organism under a specific, defined set of conditions. In its present state, it is dependent on decades of technological and instrumental developments. These developments have included advances in mass spectrometry (MS) technology, protein fractionation techniques, bioinformatics, etc.



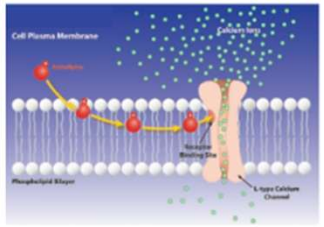
Modern quantitative proteomics is central to understanding biology



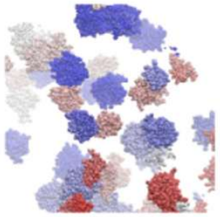
Complexes;
Stoichiometry



Spatial Localization;
micro-environment

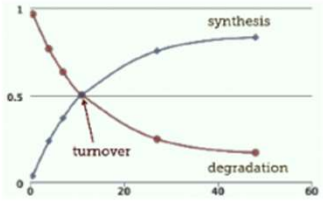


Drug MOA; targets

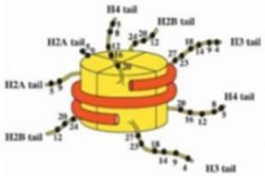


Complexes;
Dynamics

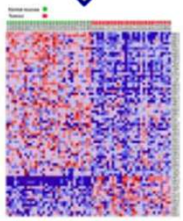
Quantitative
Proteomics
At Broad



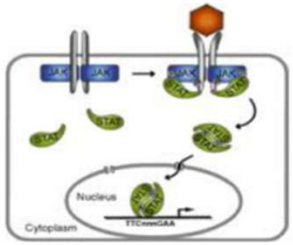
Synthesis/degradation



Epigenetics

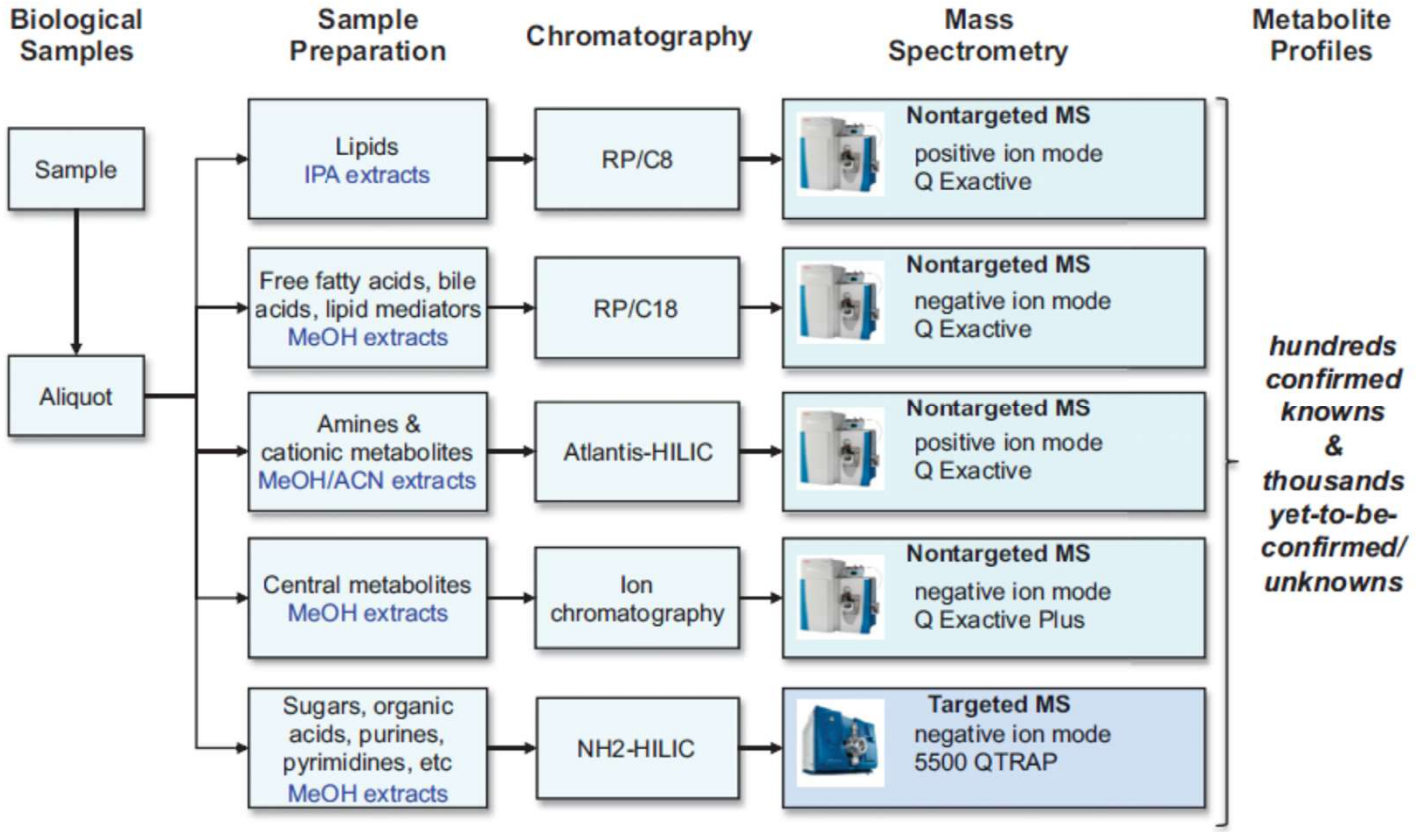


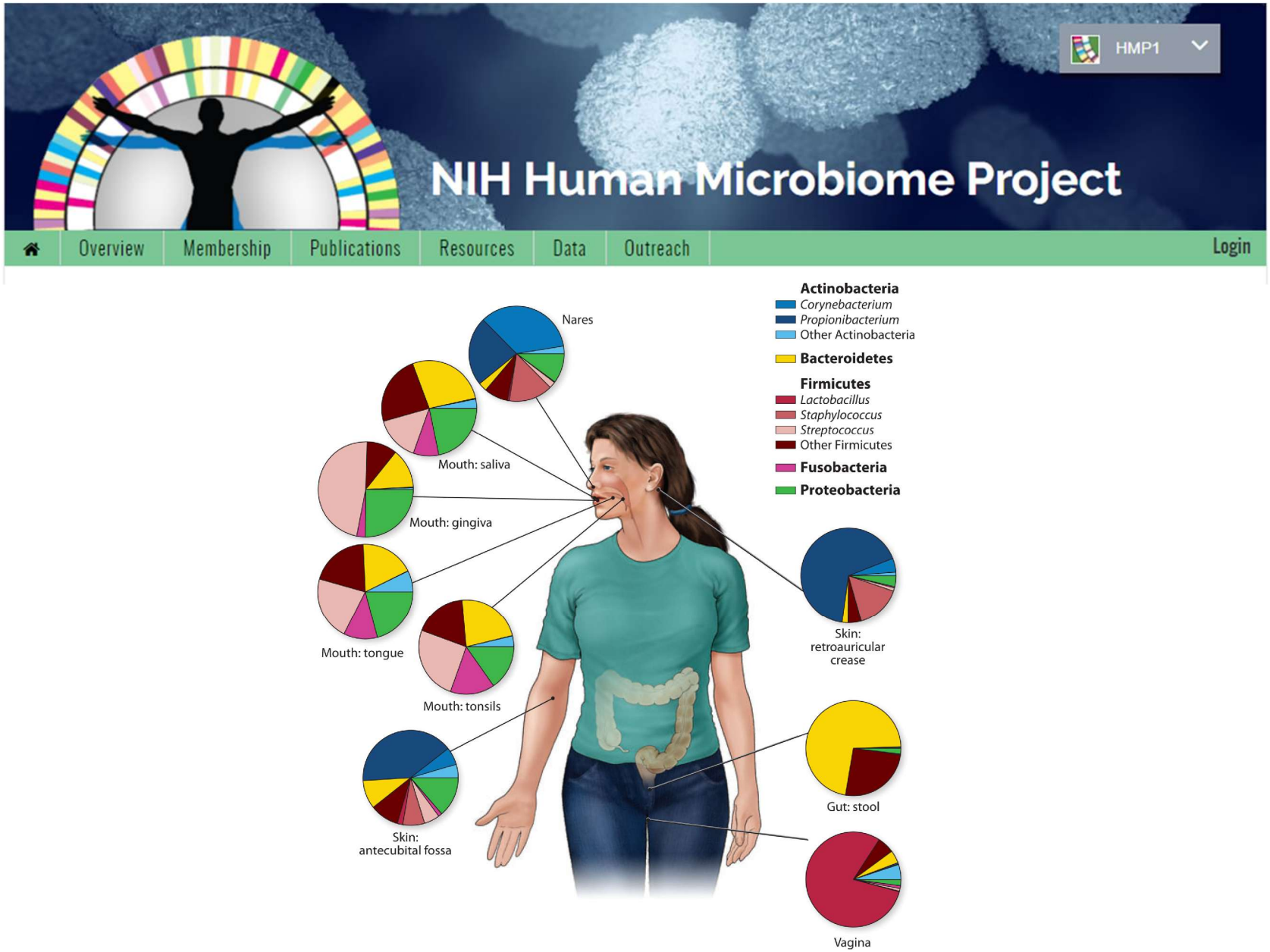
Disease Biomarkers



Signaling; Pathways

The metabolome is the complete set of metabolites within a cell, tissue or biological sample at any given time point. The metabolome is inherently very dynamic: small molecules are continuously absorbed, synthesised, degraded and interact with other molecules, both within and between biological systems, and with the environment.



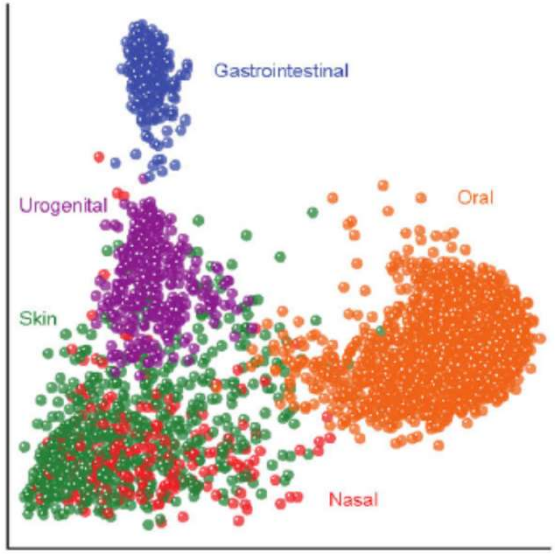

HMP1
NIH Human Microbiome Project
Overview
Membership
Publications
Resources
Data
Outreach
Login
Actinobacteria
Corynebacterium
Propionibacterium
Other Actinobacteria
Bacteroidetes
Firmicutes
Lactobacillus
Staphylococcus
Streptococcus
Other Firmicutes
Fusobacteria
Proteobacteria
Nares
Mouth: saliva
Mouth: gingiva
Mouth: tongue
Mouth: tonsils
Skin: antecubital fossa
Skin: retroauricular crease
Gut: stool
Vagina

HHS Public Access
Author manuscript
Nature. Author manuscript; available in PMC 2013 February 05.

Published in final edited form as:
Nature. ; 486(7402): 207–214. doi:10.1038/nature11234.

Structure, Function and Diversity of the Healthy Human Microbiome

The Human Microbiome Project Consortium



Diversity of the human microbiome is concordant among measures, **unique** to each individual, and strongly determined by **microbial habitat**

Microbial carriage varies between subjects down to the species and strain level

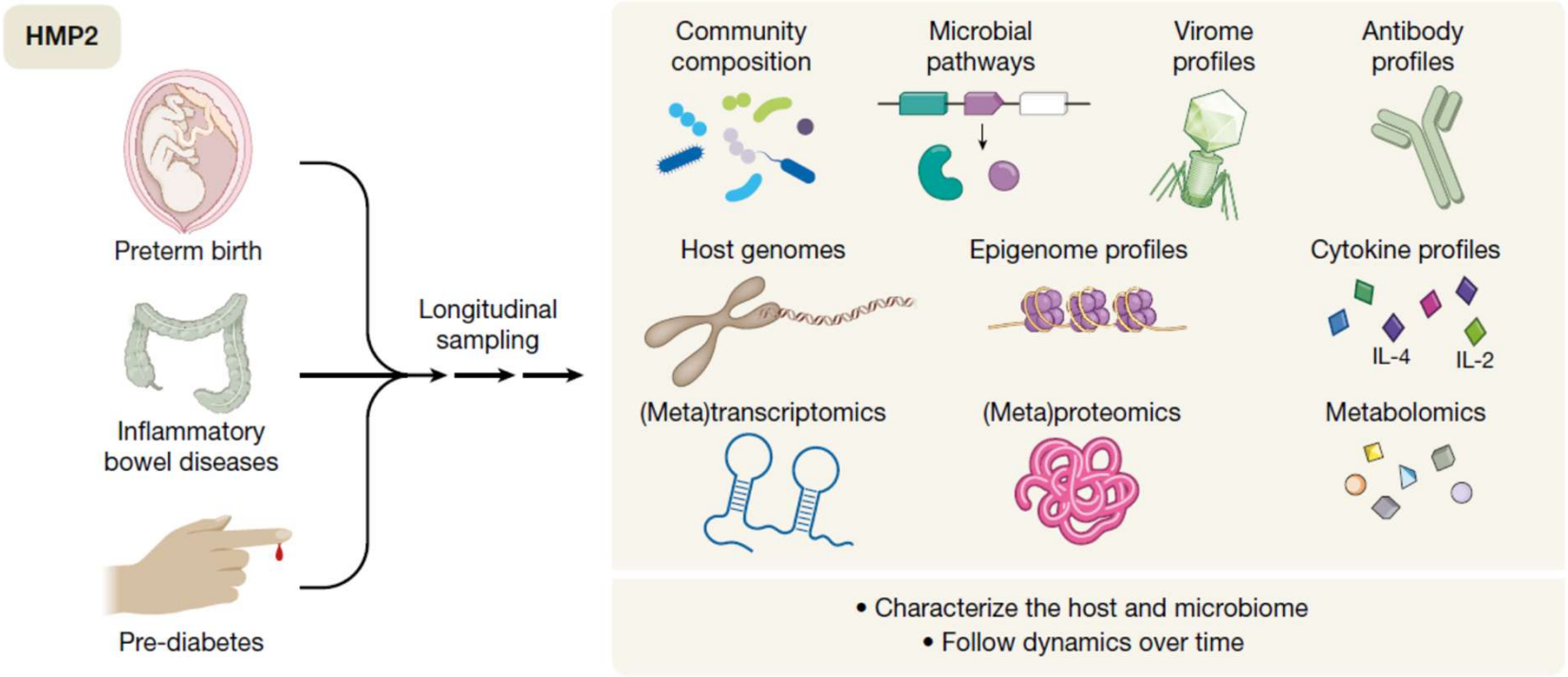
NIH Human Microbiome Project

The iHMP (2014-) will create integrated longitudinal datasets from both the microbiome and host from three different cohort studies of microbiome-associated conditions using multiple ‘omics technologies. Each of these study groups has engaged in providing new computational tools and integrative molecular perspectives on microbial activity during dysbiosis. As a result of creating these multiomic data resources, the iHMP has opened up new opportunities for data integration in the human microbiome.

Characterization of microbiome and human host from three cohorts of microbiome-associated conditions, using multiple 'omics technologies.

Enter iHMP

The second phase of the HMP, the Integrative HMP (iHMP or HMP2), was designed to explore host–microbiome interplay, including immunity, metabolism, and dynamic molecular activity, to gain a more holistic view of host–microbe interactions over time.



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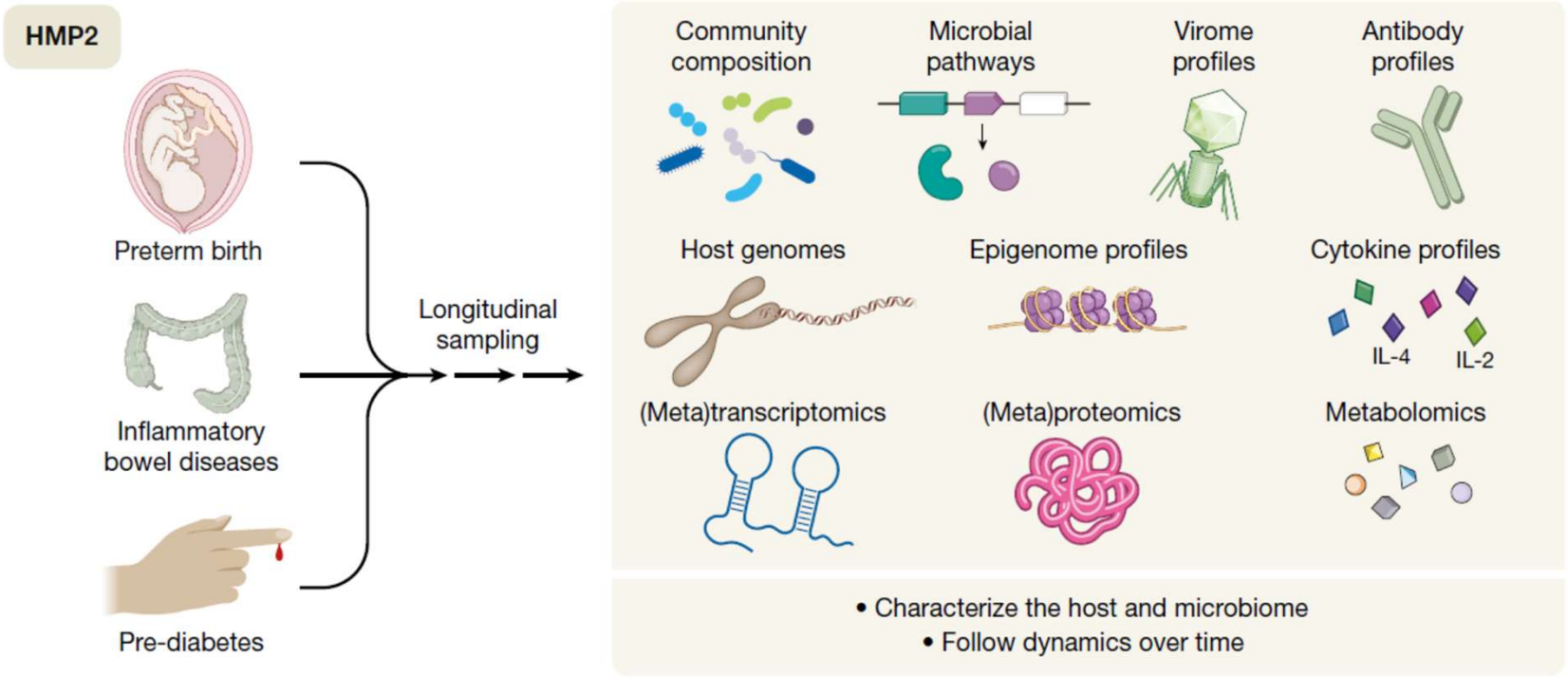

ARTICLES
https://doi.org/10.1038/s41591-019-0450-2

nature medicine

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NATURE MEDICINE | VOL 25 | JUNE 2019 | 1012–1021 | www.nature.com/naturemedicine

The vaginal microbiome and preterm birth

Approximately 15 million preterm births at less than 37 weeks of gestation occur annually worldwide. Preterm birth (PTB) remains the second most common cause of neonatal death across the globe, and the most common cause of infant mortality in middle- and high-income economies. The consequences of PTB persist from early childhood into adolescence and adulthood. In the United States, striking population differences with respect to PTB exist, with women of African ancestry having a substantially larger burden of risk. The estimated annual cost of PTB in the United States alone is over US$26.2 billion. Despite these statistics, there remains a paucity of effective strategies for predicting and preventing PTB.

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1,527 pregnancies longitudinally and involved the collection of 206,437 biospecimens for analysis of host and microbial factors (16S amplicon, metagenomic, and metatranscriptomic sequencing; cytokine profiling; metabolomics; proteomics; genomics; and microbial isolate culture). Around 600 pregnancies were analysed in depth to assess features that lead to preterm birth; this analysis identified both host (for example, cytokine) and microbial (for example, ecological and specific strain) factors.

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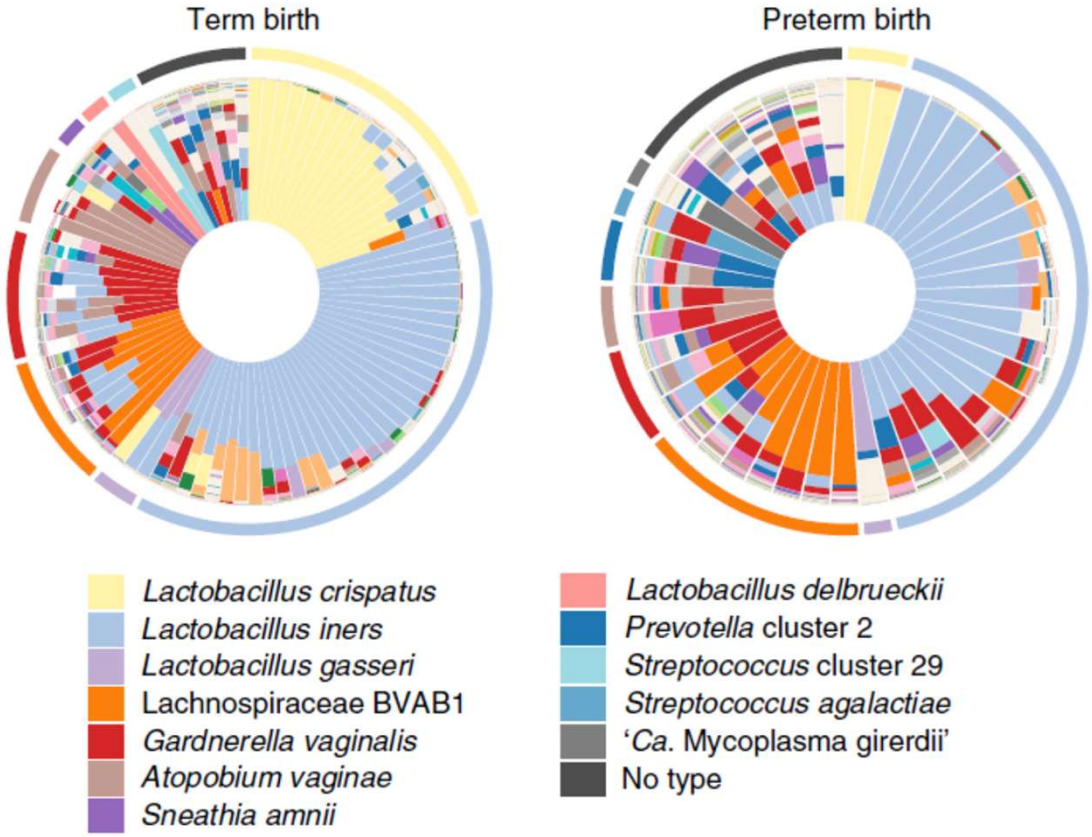

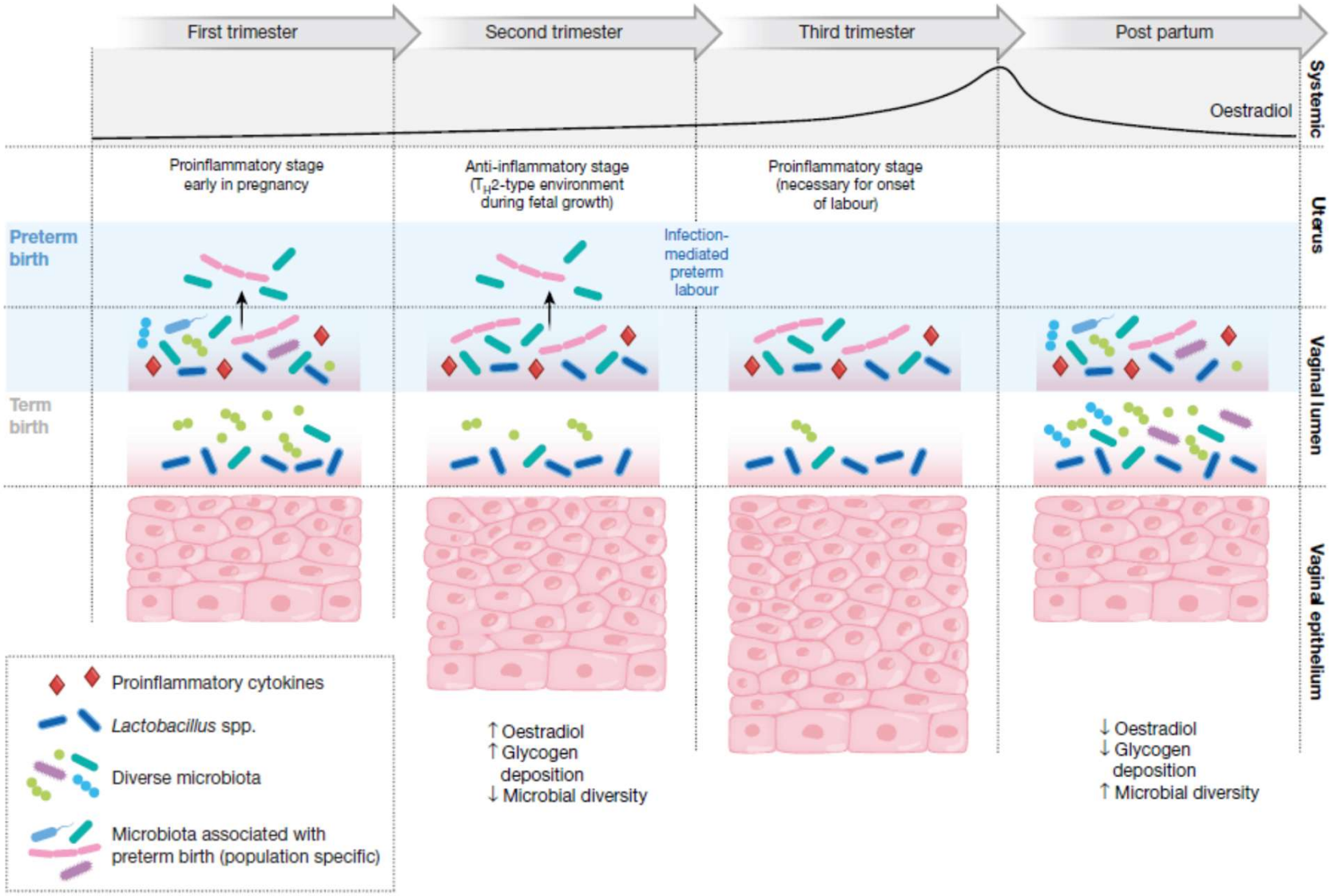
First trimester
Second trimester
Third trimester
Post partum
Systemic
Oestradiol
Uterus
Proinflammatory stage early in pregnancy
Anti-inflammatory stage (T_H2-type environment during fetal growth)
Proinflammatory stage (necessary for onset of labour)
Preterm birth
Infection-mediated preterm labour
Vaginal lumen
Term birth
Vaginal epithelium
↑ Oestradiol
↑ Glycogen deposition
↓ Microbial diversity
↓ Oestradiol
↓ Glycogen deposition
↑ Microbial diversity
Proinflammatory cytokines
Lactobacillus spp.
Diverse microbiota
Microbiota associated with preterm birth (population specific)

ARTICLES
https://doi.org/10.1038/s41591-019-0450-2

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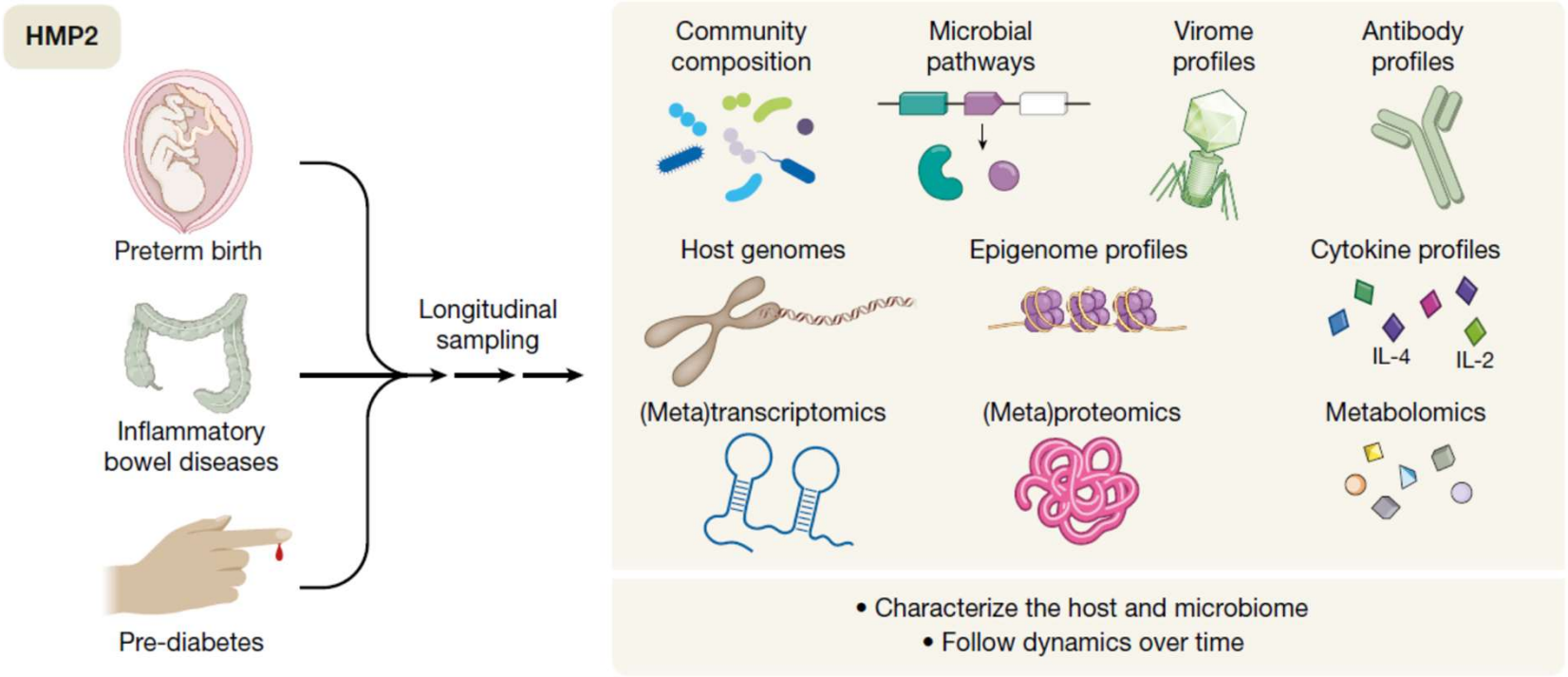
The vaginal microbiome and preterm birth

In the present study, we developed a proof-of-concept model that suggests the presence of BVAB1, *Prevotella* cluster 2, *S. amnii* and TM7-H1 early in pregnancy may be useful for prediction of risk for PTB, particularly in high-risk populations. It is possible that BVAB1, *Prevotella* cluster 2, *S. amnii* and TM7-H1, and other taxa may have roles in the causation of PTB.

Taken together, our data suggest that, coupled with other clinical and possibly genetic factors, microbiome-associated taxonomic, metabolic and immunologic **biomarkers may be useful in defining the risk of PTB**, and that this risk might be assessed early in pregnancy.

The second phase of the HMP, the Integrative HMP (iHMP or HMP2), was designed to explore host–microbiome interplay, including immunity, metabolism, and dynamic molecular activity, to gain a more holistic view of host–microbe interactions over time.



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Author manuscript

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Gut microbiome structure and metabolic activity in inflammatory bowel disease

Eric A. Franzosa[#1,2], **Alexandra Sirota-Madi**[#1], **Julian Avila-Pacheco**[1], **Nadine Fornelos**[1], **Henry J. Haiser**[3], **Stefan Reinker**[3], **Tommi Vatanen**[1], **A. Brantley Hall**[1], **Himel Mallick**[1,2], **Lauren J. McIver**[1,2], **Jenny S. Sauk**[4], **Robin G. Wilson**[4], **Betsy W. Stevens**[4], **Justin M. Scott**[1], **Kerry Pierce**[1], **Amy A. Deik**[1], **Kevin Bullock**[1], **Floris Imhann**[5,6], **Jeffrey Porter**[3], **Alexandra Zhernakova**[6], **Jingyuan Fu**[6,7], **Rinse K. Weersma**[5], **Cisca Wijmenga**[6,8], **Clary B. Clish**[1], **Hera Vlamakis**[1], **Curtis Huttenhower**[1,2], and **Ramnik J. Xavier**[1,4,9]

A

	Non-IBD Control (control)	Crohn's Disease (CD)	Ulcerative Colitis (UC)
Discovery cohort (PRISM)	34	68	53
Validation cohort (NLIBD/LLDeep)	22	20	23

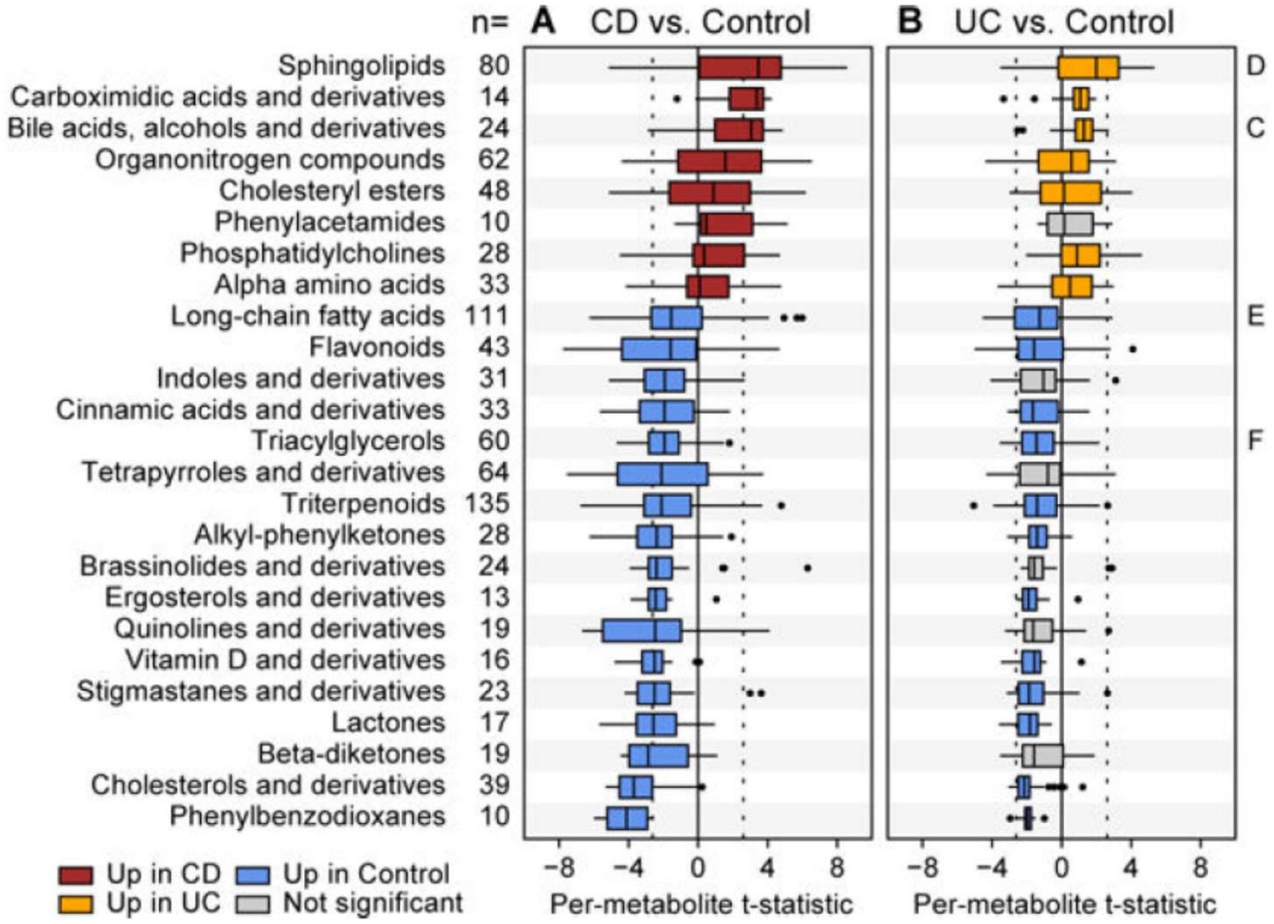
Multi'omic screening of stool samples

Metagenomic shotgun sequencing
Microbial taxa, genes, and pathways

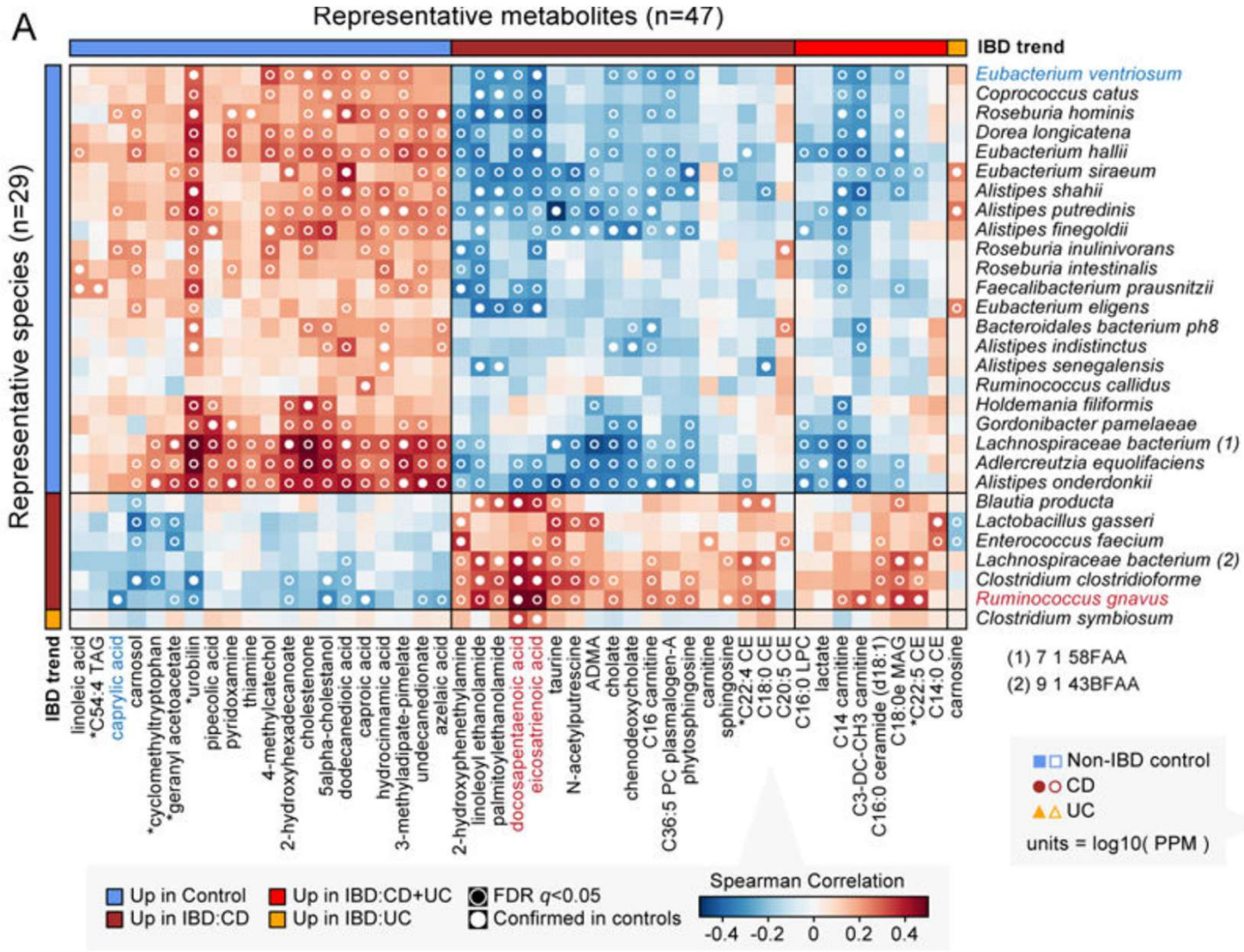
+

Four LC-MS metabolomics methods
Lipids, polar metabolites, free fatty acids, & bile acids

Metabolite enrichments in IBD versus control phenotypes



Potentially mechanistic associations between IBD-linked microbes and metabolites



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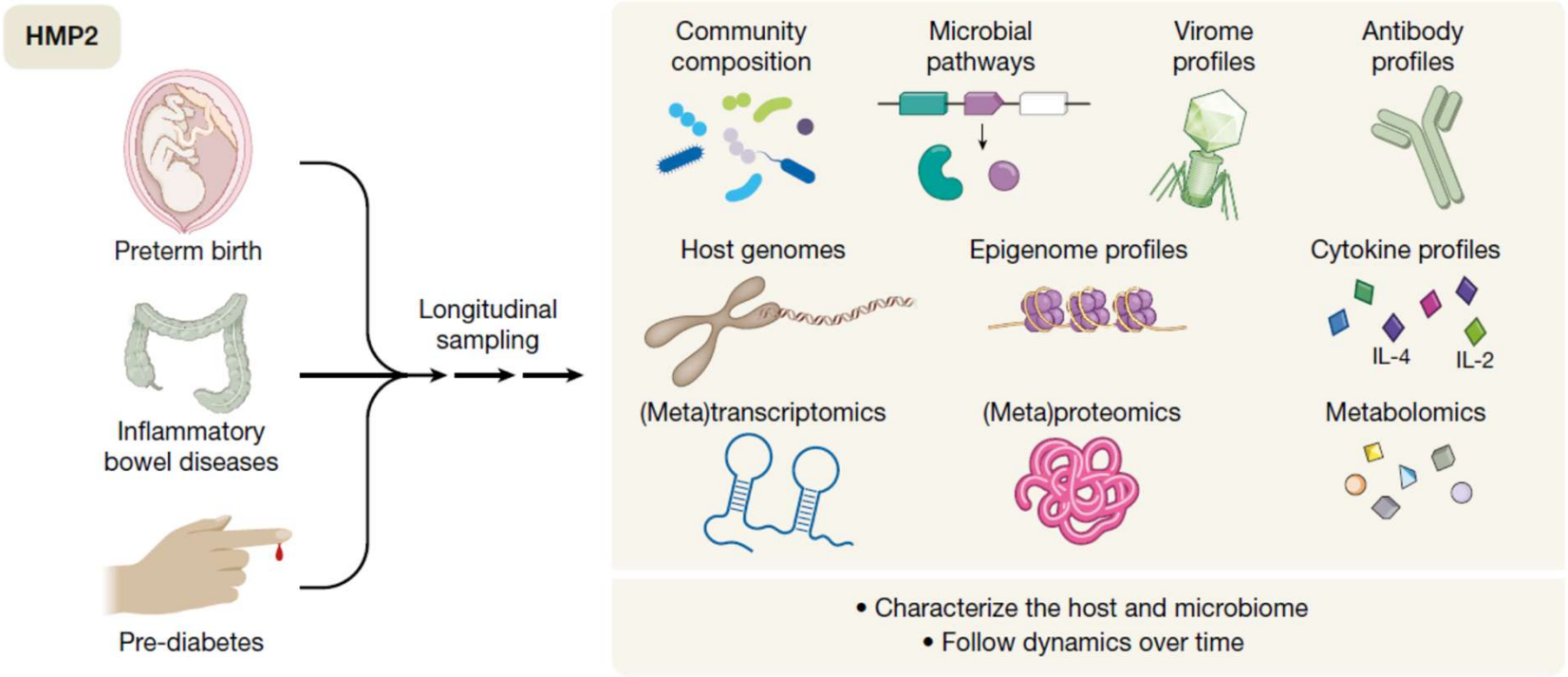


About the Study

Stanford MEDICINE | Integrated Personal Omics Profiling

Type 2 diabetes mellitus (T2D) is a significant health problem facing our nation and our study is likely to provide important insights into this disease. Close to 20 million individuals in the United States have T2D, which cost $174 billion in 2007 to treat, a figure projected to double by 2050. Currently, there are an estimated 79M prediabetics in the United States who have a lifetime risk of diabetes conversion of 50%.

Differences in the gut microbiome have been noted between diabetics and healthy individuals, and direct alteration of the microbiome in mice has been shown to lower glucose levels. To better understand the biological changes that occur T2D disease acquisition, we plan to perform a detailed analysis of the biological processes that occur in patients at risk for T2D and their microbiomes by longitudinal profiling of patients during healthy periods and periods of stress.

http://med.stanford.edu/ipop.html

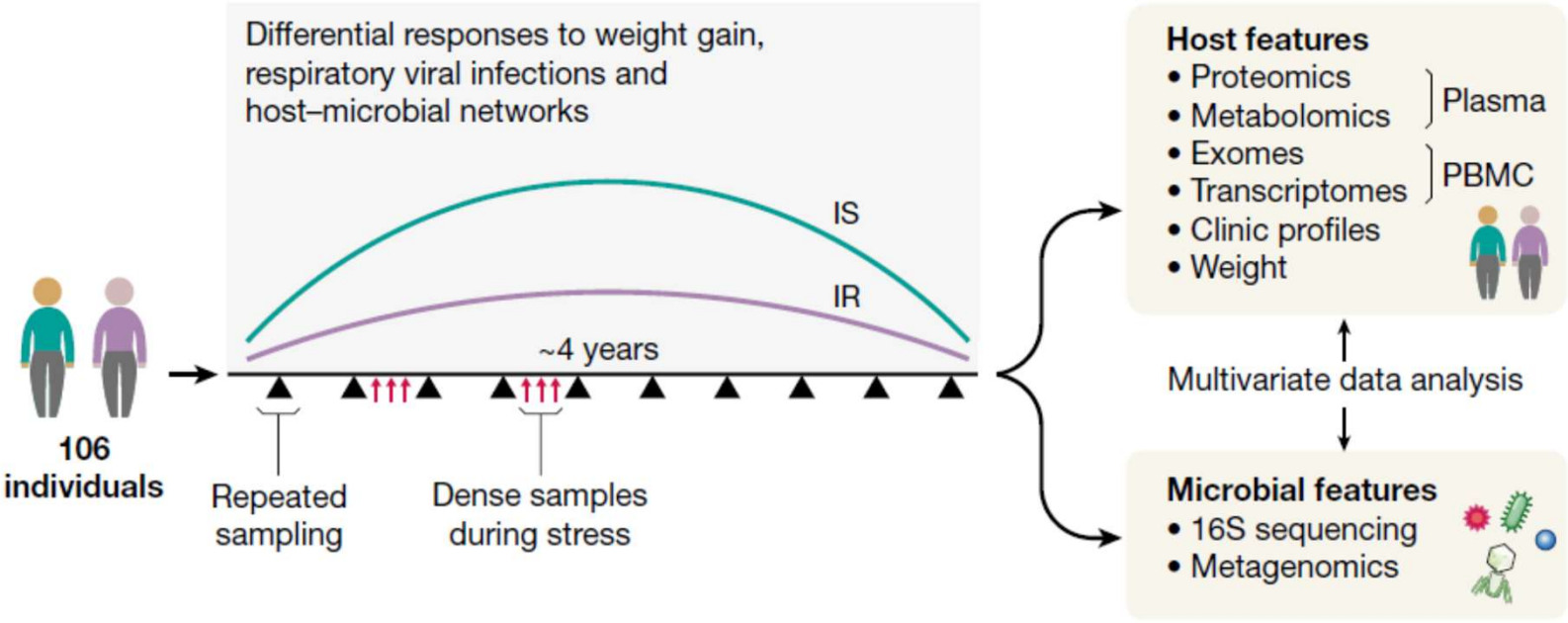
Stanford MEDICINE | Integrated Personal Omics Profiling

OPEN
https://doi.org/10.1038/s41586-019-1238-8

30 MAY 2019 | VOL 569 | NATURE | 641

Insulin sensitive (IS)
- Responsive to respiratory viral infections
- Acute phase response pathway
- Cytokine production
- Altered nasal microbial ecology

Insulin resistant (IR)
- Reduced response to respiratory viral infections
- More chronic inflammation
- Altered lipid metabloism
- ↑ *Blautia*, ↓ *Odoribacter*



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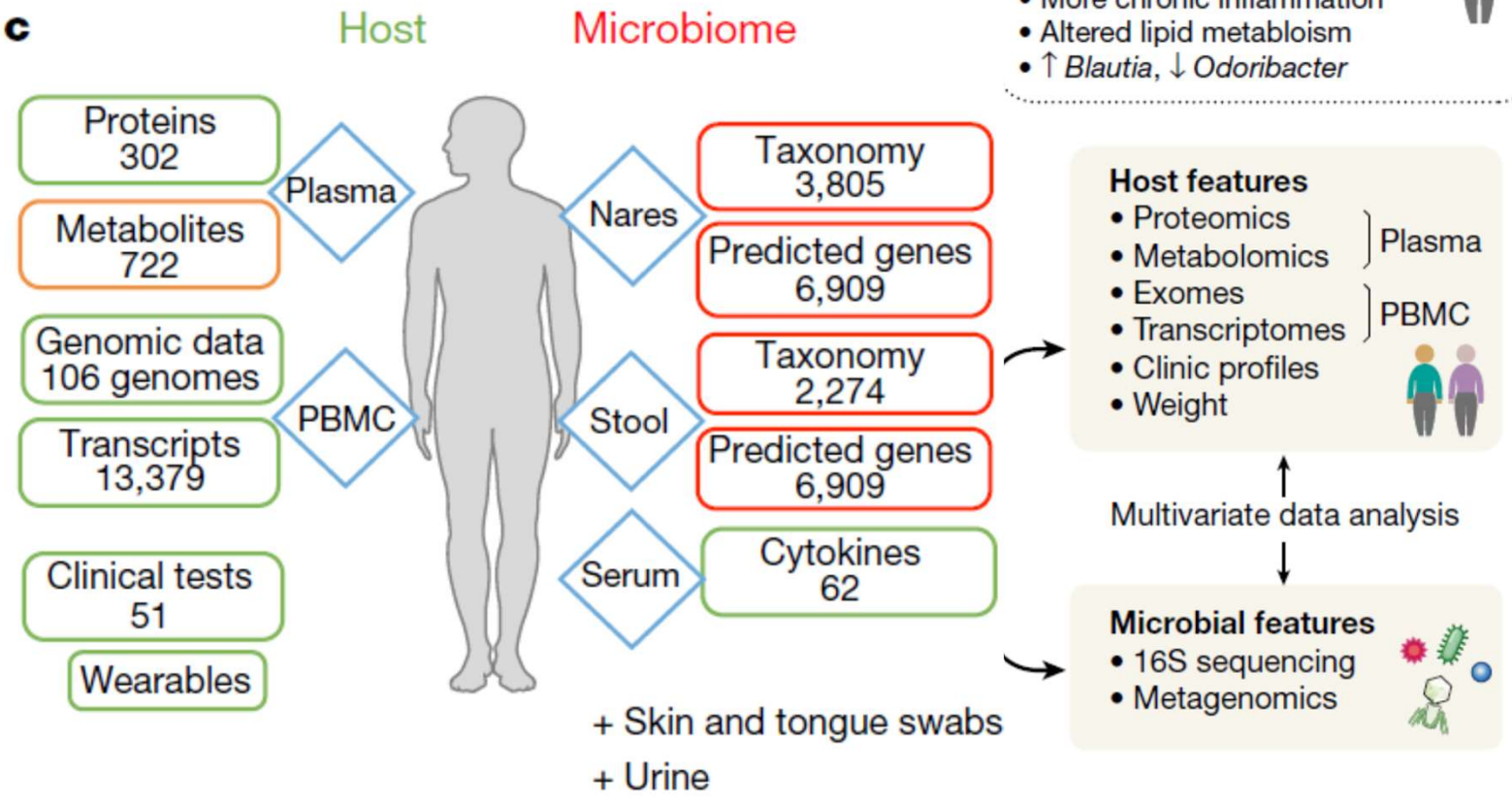

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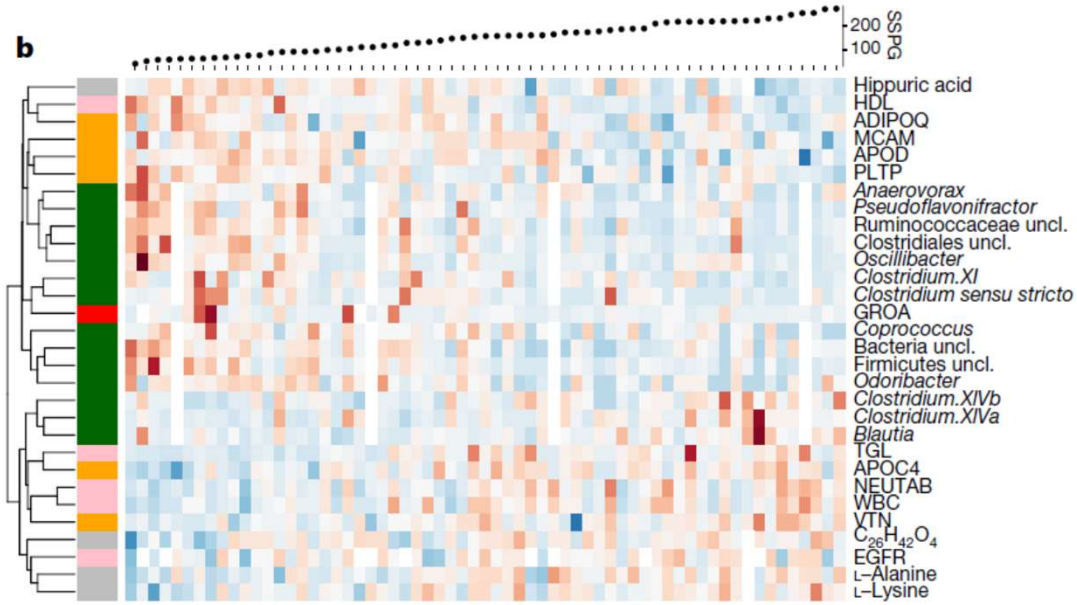
In total, 1,092 collections across all participants were profiled. For each visit, blood was assayed for host molecular 'omics profiling and two types of samples, nasal swabs and faeces, were collected for microbial profiling. Each participant's exome was sequenced once; otherwise, for each visit, 13,379 transcripts were profiled from peripheral blood mononuclear cells, 722 metabolites and 302 proteins from plasma, and 62 cytokines and growth factors from serum. In addition, thousands of gut and nasal microbial taxa and computationally predicted genes were profiled using 16S rRNA amplicons. All visits were also intensively characterized by 51 clinical laboratory tests. In addition, because of the focus on T2D, a number of glucose dysregulation tests were performed, including measurements of fasting glucose and haemoglobin A1C levels, oral glucose tolerance tests, and tests of insulin resistance.

Longitudinal multi-omics of host–microbe dynamics in prediabetes

Wenyu Zhou[1,12], M. Reza Sailani[1,12], Kévin Contrepois[1,12], Yanjiao Zhou[2,3,12], Sara Ahadi[1,12], Shana R. Leopold[2], Martin J. Zhang[4], Varsha Rao[1], Monika Avina[1], Tejaswini Mishra[1], Jethro Johnson[2], Brittany Lee-McMullen[1], Songjie Chen[1], Ahmed A. Metwally[1], Thi Dong Binh Tran[2], Hoan Nguyen[2], Xin Zhou[2], Brandon Albright[2], Bo-Young Hong[2], Lauren Petersen[2], Eddy Bautista[2], Blake Hanson[2], Lei Chen[2], Daniel Spakowicz[2], Amir Bahmani[5], Denis Salins[1], Benjamin Leopold[2], Melanie Ashland[1], Orit Dagan-Rosenfeld[1], Shannon Rego[1], Patricia Limcaoco[1], Elizabeth Colbert[6], Candice Allister[6], Dalia Perelman[6], Colleen Craig[6], Eric Wei[1,5], Hassan Chaib[1,5,7], Daniel Hornburg[1], Jessilyn Dunn[1], Liang Liang[1], Sophia Miryam Schüssler-Fiorenza Rose[8,9], Kim Kukurba[1], Brian Piening[10], Hannes Rost[11], David Tse[4], Tracey McLaughlin[6,7], Erica Sodergren[2], George M. Weinstock[2]* & Michael Snyder[1,5,7]*

Variation in healthy baseline profiles



OPEN

https://doi.org/10.1038/s41586-019-1236-x

T2D is an inflammatory disease, and several inflammation molecules are important indicators of T2D development, including IL-1RA, HSCRP, IL-6, IL-1β and TNF. In the case of participant ZNED4XZ, only IL-1RA and HSCRP increased before the onset of T2D, but not the other markers, including IL-6 and IL-1β. The development of T2D in this participant was not caused by liver or adipose inflammation at those time points.

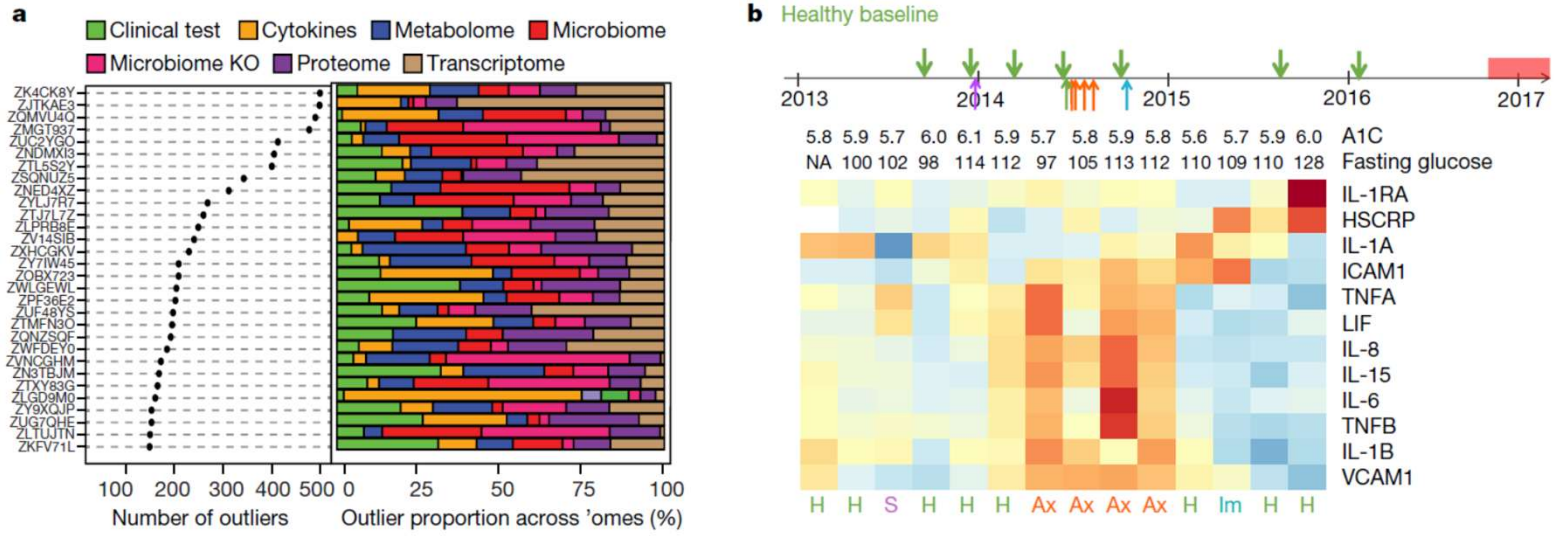
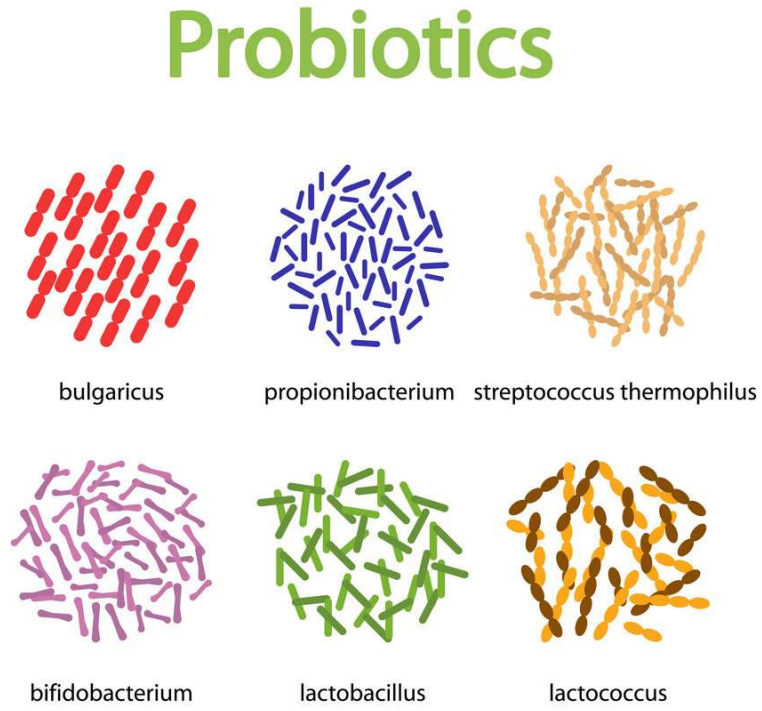


Fig. 6 | Personal features that precede the onset of T2D revealed by longitudinal tracking. a, Left, scatter plot showing the number of outliers among thousands of molecules for each participant; right, the percentage of outliers contributed by each ome. **b**, Top, collection timeline for participant ZNED4XZ in our study relative to onset of T2D, with green arrows pointing to eight healthy baselines (H), purple for one stress visit (S), red for four antibiotics visits (Ax) and blue for one immunization visit (Im). Bottom, heatmap showing levels of selected immune cytokines together with A1C (%) and fasting glucose (mg dl^{-1}) across time. Red denotes increased expression; blue represents decreased expression, with shades corresponding to levels.

Probiotics and the human microbiome



Pomegranate Blackberry GoodBelly Quarts

Nutrition Facts

Serving Size 8 oz.
Servings Per Package 4
Calories 110
Fat Cal 0

Amount Per Serving	%DV*	Amount Per Serving	%DV*
Total Fat 0g	**0%**	**Potassium** 110mg	**3%**
Saturated Fat 0g	**0%**	**Total Carb.** 24g	**8%**
Trans Fat 0g		Dietary Fiber 0g	**0%**
Cholesterol 0mg	**0%**	Sugars 22g	
Sodium 20mg	**1%**	**Protein** <1g	**0%**

Vitamin A 0% • Vitamin C 0% • Calcium 0% • Iron 2%

*Percent Daily Values (DV) are based on a 2,000 Calorie diet

INGREDIENTS: FILTERED WATER, ORGANIC PEAR JUICE FROM CONCENTRATE, ORGANIC BLACKBERRY JUICE FROM CONCENTRATE, ORGANIC EVAPORATED CANE JUICE, ORGANIC POMEGRANATE JUICE FROM CONCENTRATE, CONTAINS 2% OR LESS OF ORGANIC OAT FLOUR, NATURAL FLAVORS, FRUIT AND VEGETABLE JUICE (FOR COLOR), ORGANIC BARLEY MALT, CITRIC ACID, LACTOBACILLUS PLANTARUM299v. **LACTOSE-FREE, SOY-FREE, VEGAN.**

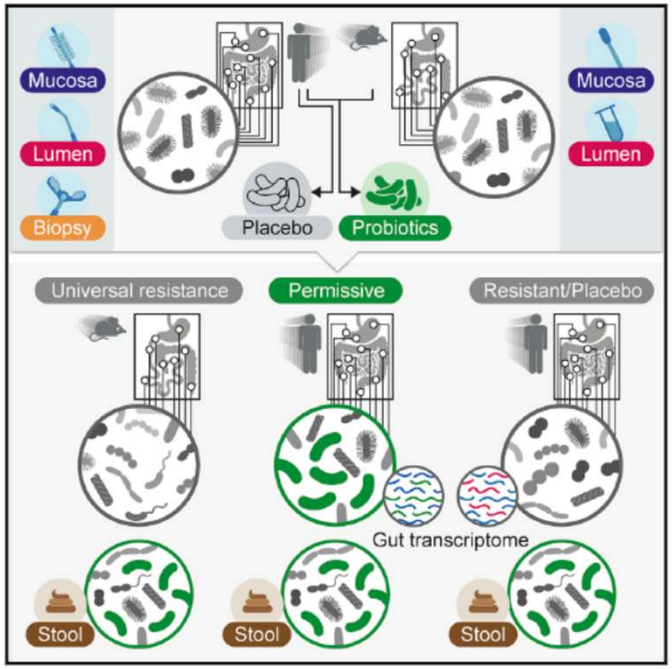
Since health benefits conferred by probiotics are strain-specific, identification to the strain level is mandatory to allow the monitoring of the presence and the levels of a specific probiotic strain in a product or in a gastrointestinal tract.

Zmora et al., 2018, Cell *174*, 1388–1405
September 6, 2018 © 2018 Elsevier Inc.
https://doi.org/10.1016/j.cell.2018.08.041

CellPress

Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features

Graphical Abstract



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Niv Zmora, Gili Zilberman-Schapira, Jotham Suez, ..., Zamir Halpern, Eran Segal, Eran Elinav

Correspondence

zamir@tlvmc.gov.il (Z.H.), eran.segal@weizmann.ac.il (E.S.), eran.elinav@weizmann.ac.il (E.E.)

In Brief

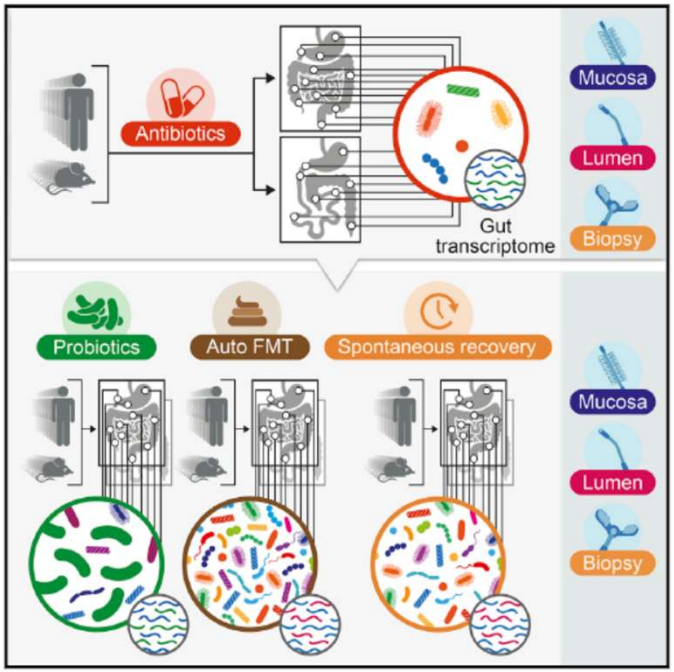
Probiotics transiently colonize the human gut mucosa in highly individualized patterns, thereby differentially impacting the indigenous microbiome and host gene-expression profile, a trait which is predictable by baseline host and microbiome features, but not by stool shedding.

Suez et al., 2018, Cell *174*, 1406–1423
September 6, 2018 © 2018 Elsevier Inc.
https://doi.org/10.1016/j.cell.2018.08.047

CellPress

Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT

Graphical Abstract



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Zamir Halpern, Eran Segal, Eran Elinav

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In Brief

Probiotics perturb rather than aid in microbiota recovery back to baseline after antibiotic treatment in humans.

FMT
Fecal
microbiome
transplantation

Prof. Peter Konturek, the President of the International Society Microbiota (ISM) from the Teaching Hospital of the University of Jena, Germany will give opening introduction during the Targeting Microbiota 2020 Congress with a talk entitled "**The present progress in the clinical application of FMT**".

The 8th International World Congress on Targeting Microbiota 2020 Congress will be held in Paris this year on October 22-23, 2020.

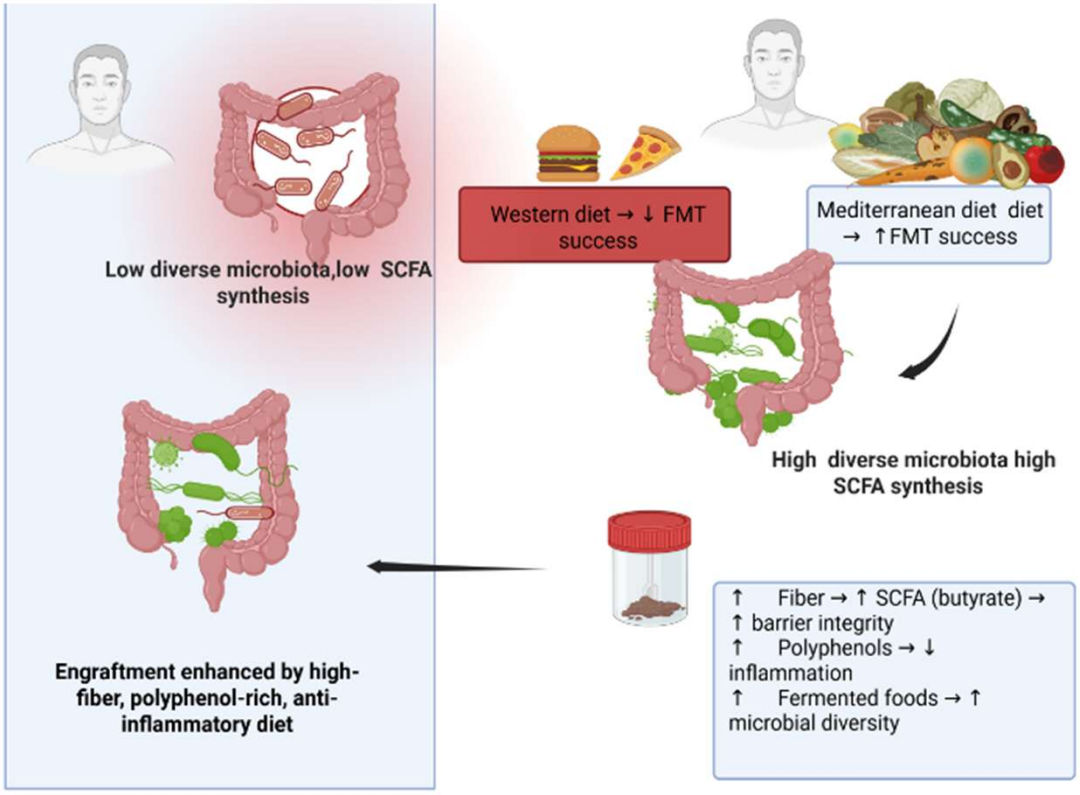
nutrients

MDPI

Review

The Impact of Diet on the Fecal Microbiota Transplantation Success in Patients with Gastrointestinal Diseases—A Literature Review

Natalia Komorniak [1], Katarzyna Gaweł [2], Anna Deskur [2], Jan Pawlus [3] and Ewa Stachowska [1,*]



Review > Nutrients. 2025 Oct 21;17(20):3314. doi: 10.3390/nu17203314.

Issue 28, 2020, Issue in Progress

Previous Article | Next Article

From the journal:
RSC Advances

Smart capsule for non-invasive sampling and studying of the gastrointestinal microbiome†

Check for updates

Jose Fernando Waimin, ‡[ab] Sina Nejati, ‡[ab] Hongjie Jiang, [bcd] Jake Qiu, [be] Jianghsan Wang, [be] Mohit S. Verma [bef] and Rahim Rahimi *[abc]

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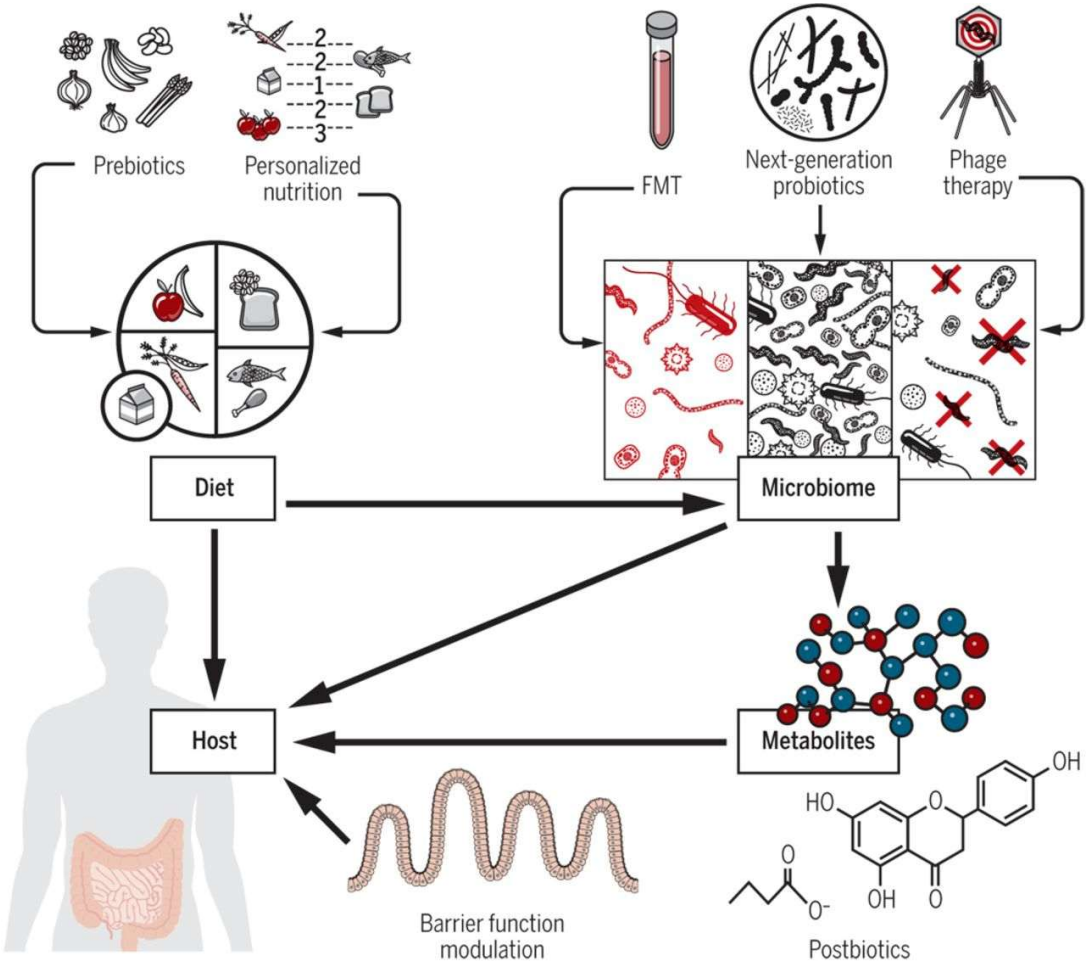
SCIENCE TRANSLATIONAL MEDICINE | FOCUS

10TH ANNIVERSARY SERIES

Transforming medicine with the microbiome

Niv Zmora[1,2,3]*, Eliran Soffer[1]*, Eran Elinav[1†]

Science Translational Medicine 2019
Vol. 11, Issue 477, eaaw1815
DOI: 10.1126/scitranslmed.aaw1815



SCIENCE TRANSLATIONAL MEDICINE | FOCUS

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Transforming medicine with the microbiome

Niv Zmora[1,2,3]*, Eliran Soffer[1]*, Eran Elinav[1†]

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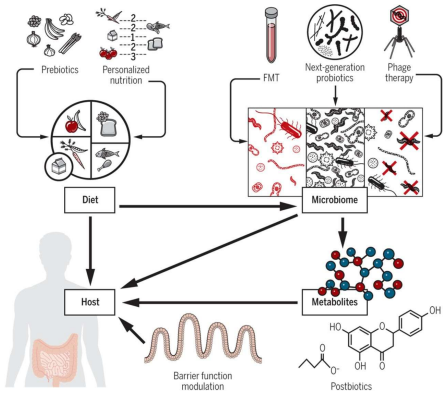


An opportunity emerges for context-specific tailoring of distinct probiotic strains to optimize gut colonization and downstream activity.

One emerging limitation of FMT is that efficacy varies between fecal donors because of unknown factors. Another concern involves the risk of transmission of communicable diseases or other microbiome-mediated traits from donor to recipient.

These challenges include the need to develop noninvasive approaches for direct sampling of the gut mucosa and technologies to enable reliable characterization of the microbiome in different regions of the gut.

We need to determine mechanisms of activities of probiotic strains in vivo, thereby enabling the prediction of alterations in the microbiome after treatment.

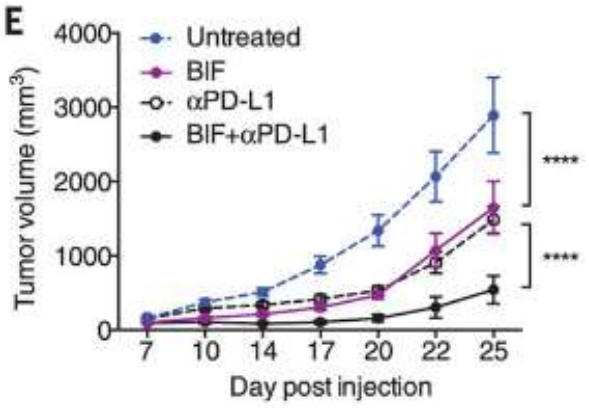
Clinical translation necessitates stringent testing, preferably in the form of randomized placebo-controlled clinical trials. In these trials, feasibility, efficacy, adverse events, and long-term safety issues need to be assessed in large cohorts to ensure that the tested interventions are used responsibly, avoiding unsubstantiated claims and contemporary hype.

Probiotics and the human microbiome

Science 2015 Nov 27;350(6264):1084-9. doi: 10.1126/science.aac4255. Epub 2015 Nov 5.

Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD–L1 efficacy

Ayelet Sivan [1], Leticia Corrales [1], Nathaniel Hubert [2], Jason B Williams [1], Keston Aquino-Michaels [3], Zachary M Earley [2], Franco W Benyamin [1], Yuk Man Lei [2], Bana Jabri [2], Maria-Luisa Alegre [2], Eugene B Chang [2], Thomas F Gajewski [4]



Direct administration of Bifidobacterium to TAC recipients with established melanoma tumors improves tumor-specific immunity and response to αPD-L1 mAb therapy

Probiotics and the human microbiome

Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients

V. GOPALAKRISHNAN, C. N. SPENCER, L. NEZI, A. REUBEN, M. C. ANDREWS, T. V. KARPINETS, P. A. PRIETO, D. VICENTE, K. HOFFMAN, [...] J. A. WARGO +61 authors Authors Info & Affiliations

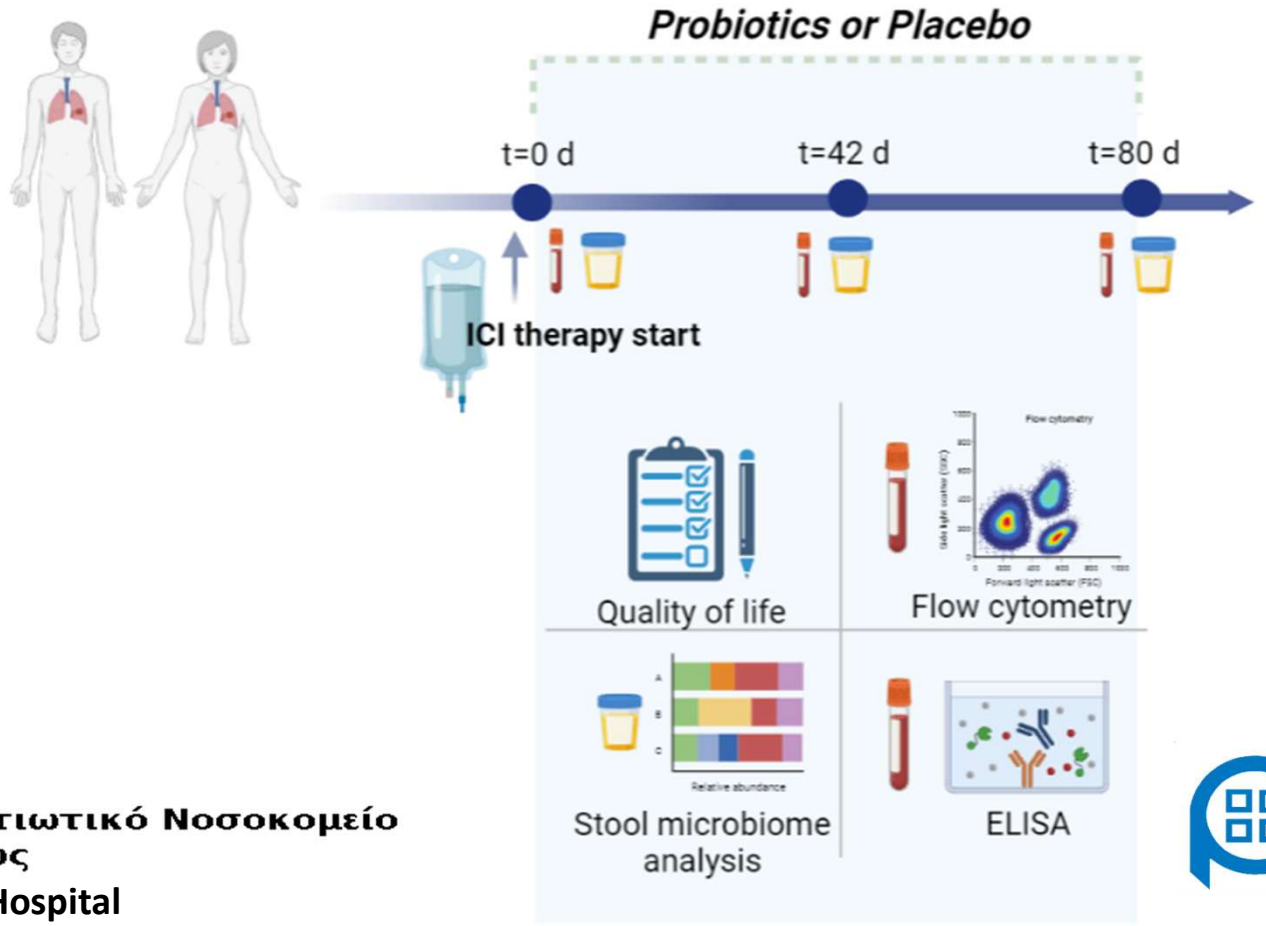
Science 2018 Jan 5;359(6371):97-103.

Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response

CHRISTINE N. SPENCER, JENNIFER L. MCQUADE, VANCHESWARAN GOPALAKRISHNAN, JOHN A. MCCULLOCH, MARIE VETIZOU, ALEXANDRIA P. COGDILL, MD A. WADUD KHAN, XIAOTAO ZHANG, MICHAEL G. WHITE, [...] JENNIFER A. WARGO +80 authors Authors Info & Affiliations

Science 2021 Dec 24;374(6575):1632-1640.

Probiotics and the human microbiome



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Γενικό Στρατιωτικό Νοσοκομείο Εκπαιδεύσεως
Army General Hospital

ΓΕΝΙΚΟ ΝΟΣΟΚΟΜΕΙΟ ΡΟΔΟΥ
"ΑΝΔΡΕΑΣ Γ. ΠΑΠΑΝΔΡΕΟΥ"
Rhodes General Hospital

Future probiotics