

Our Second Genome Human Microbiome

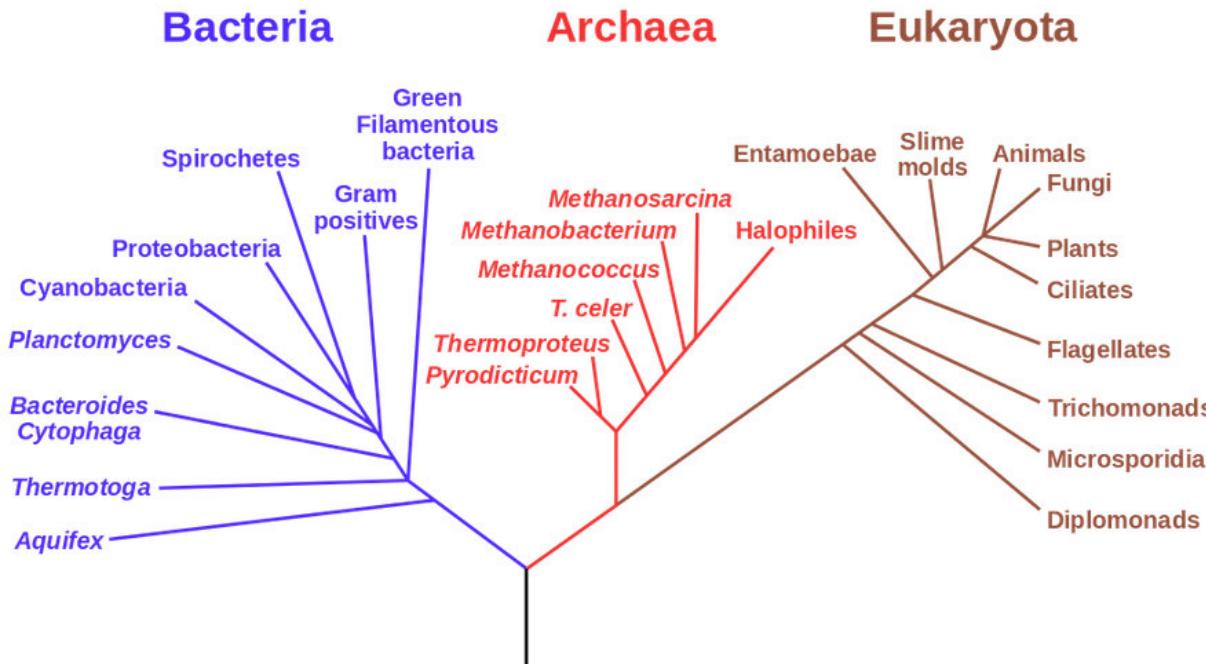
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Lecture Objectives

- I. Describe the types of micro-organisms found in the human body and give examples of each
- II. Describe the bacterial communities found normally in the gut, on the skin and in the mouth
- III. Describe the basic applications and principles of bacterial genome sequencing techniques
- IV. Appreciate how microbiology research has developed to utilise the available genome sequence data
- V. Be aware of the potential, and the limitations, of genome sequence-led research to identify novel antimicrobial strategies (therapeutics and vaccines) and other biotechnology products
- VI. Introduction to Microbiome Systems & Synthetic biology and its application in pharmaceuticals and biotechnology

Key Taxonomic Principles

Phylogenetic Tree of Life



Taxonomy: a branch of science concerned with classification, especially of organisms.

Eukaryote: an organism consisting of a cell or cells in which the genetic material is contained within a distinct nucleus, and has membrane-bound organelles.

- Animalia
- Plantae
- Protista
- Fungi

Prokaryote: single cell microorganisms that lack a distinct nucleus and membrane-bound organelles

- Bacteria
- Archaea

Life

Domain

Kingdom

Phylum

Class

Order

Family

Genus

Species

Key Definitions

Habitat: specific site of organism growth

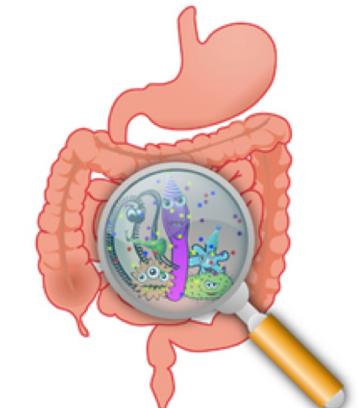
Microbial Community: the micro-organisms that are present in a given habitat

Microbiota: the total collection of micro-organisms within a microbial community

Microbiome: The microbiota and all of its associated genes

Metagenome: the total genomic DNA of all the organisms within a community

Biofilm: A physically (often temporally) structured aggregate of micro-organisms, adhered to each other and/or a defined substrate (ie. dental plaque attached to a tooth/gum margin)



Humans as “super-organisms”

“ we are an organism made up of numerous mutually interdependent smaller organisms and their genomes ”

Human

Viral

Archaea

Bacterial

Fungal



The Human Microbiome

10X

There are 10X more
microbial cells in the human body
than human cells

~ 22,000

The number of genes in the human
genome



> 10,000

The number of different species
identified to-date within the human
body

**> 10.4
million**

The number of genes in the human gut
microbiome

The Human Microbiome – why is it important?

Microbial Genes

Modulate fundamental human physiological processes

Examples: Metabolism, Energy acquisition, Immune modulation, Neurological development



Health

Specific microorganisms are protective against disease, and pathogenic bacterial species

Disease

Changes in the composition of the microbiome are associated with disease

Examples: Inflammatory Bowel Disease, Obesity, Diabetes, Rheumatoid Arthritis

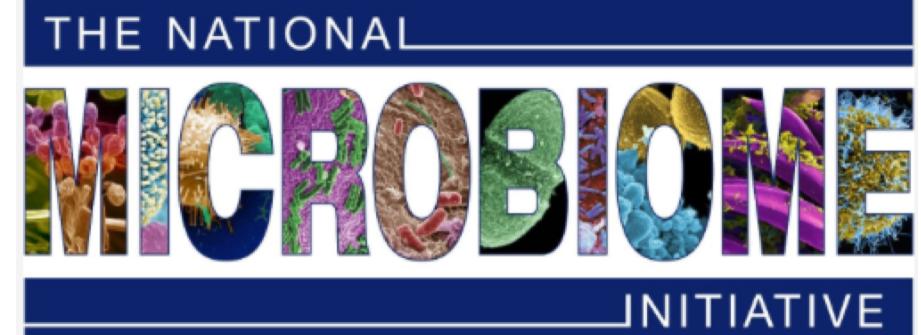
Microbiome Initiatives



NIH HUMAN
MICROBIOME
PROJECT

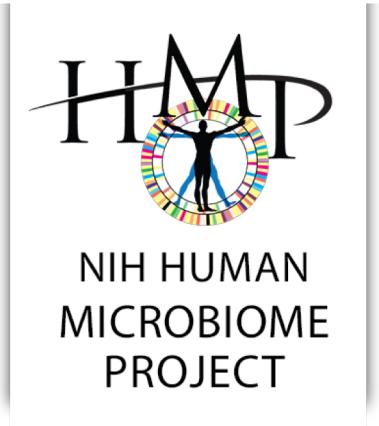


american
gut



Human Microbiome Project

- Launched in 2008 till 2013
- Funded \$115 million to International consortium
- Aims of HMP:
 - Generate resources to help study the human microbiome
 - Characterize the microbiome associated with human health and disease
 - Determine whether individuals share a core human microbiome
 - To understand whether changes in the human microbiome result in changes to human health



Five Fundamental Questions Underlying HMP

- What microbes populate the human host?
- What are these microbes doing?
- How does the host respond to these microbes?
- What are the forces that maintain equilibrium among the microbial communities?
- What are the unique characteristics of each individuals microbiome?

How do you study the Microbiome?

1. The Traditional Culture Approach

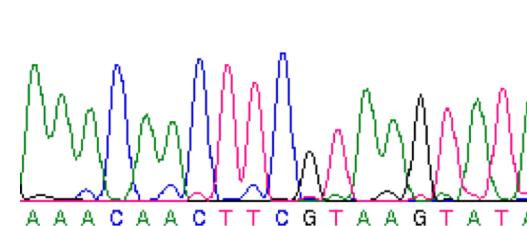
- Grow microbes directly from sample
- Requires phenotypic identification of isolates
 - Morphology / Motility
 - Biochemical
 - Antibody / Serological
 - Metabolic



FIGURE 42.4 Urease test. Tube on the left is positive (Proteus); tube on the right is negative. © The McGraw-Hill Companies/Auburn University Photographic Service

2. The New Molecular Approach

- Identifies organisms by gene sequence homology
- Extract microbial DNA from samples
- Sequence analysis of DNA
 - Targeted sequencing (16s rRNA)
 - Shotgun Metagenomics sequencing



1. Traditional Cultivation Approach

Positives

- Cheap

Negatives

- Labour intensive [> 24 hours for org ID]
- Only gross species discrimination possible
- Not many species can grow (only 50% of known oral bacteria are currently cultivable in the laboratory)
- Need to know what species to expect



2. New Molecular Approaches

Targeted 16S rRNA sequencing VERSUS shotgun metagenomic sequencing

Positives

- Tells you what is present not just what you can grow
- Can tell you what genes are present
- Higher discriminatory power for species identification



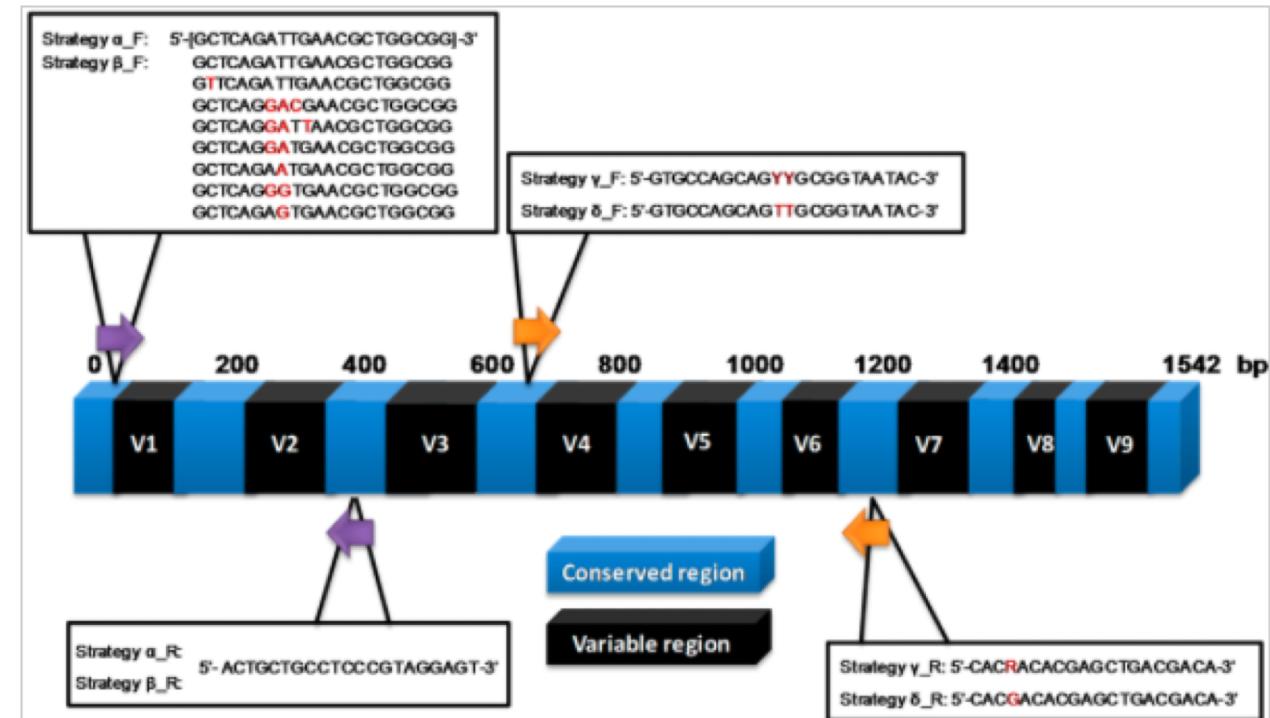
Negatives

- Sequence bias due to primer specificity
- Expensive
- Time consuming and Computational taxing

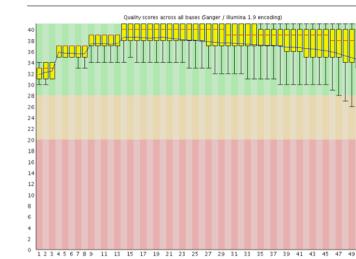
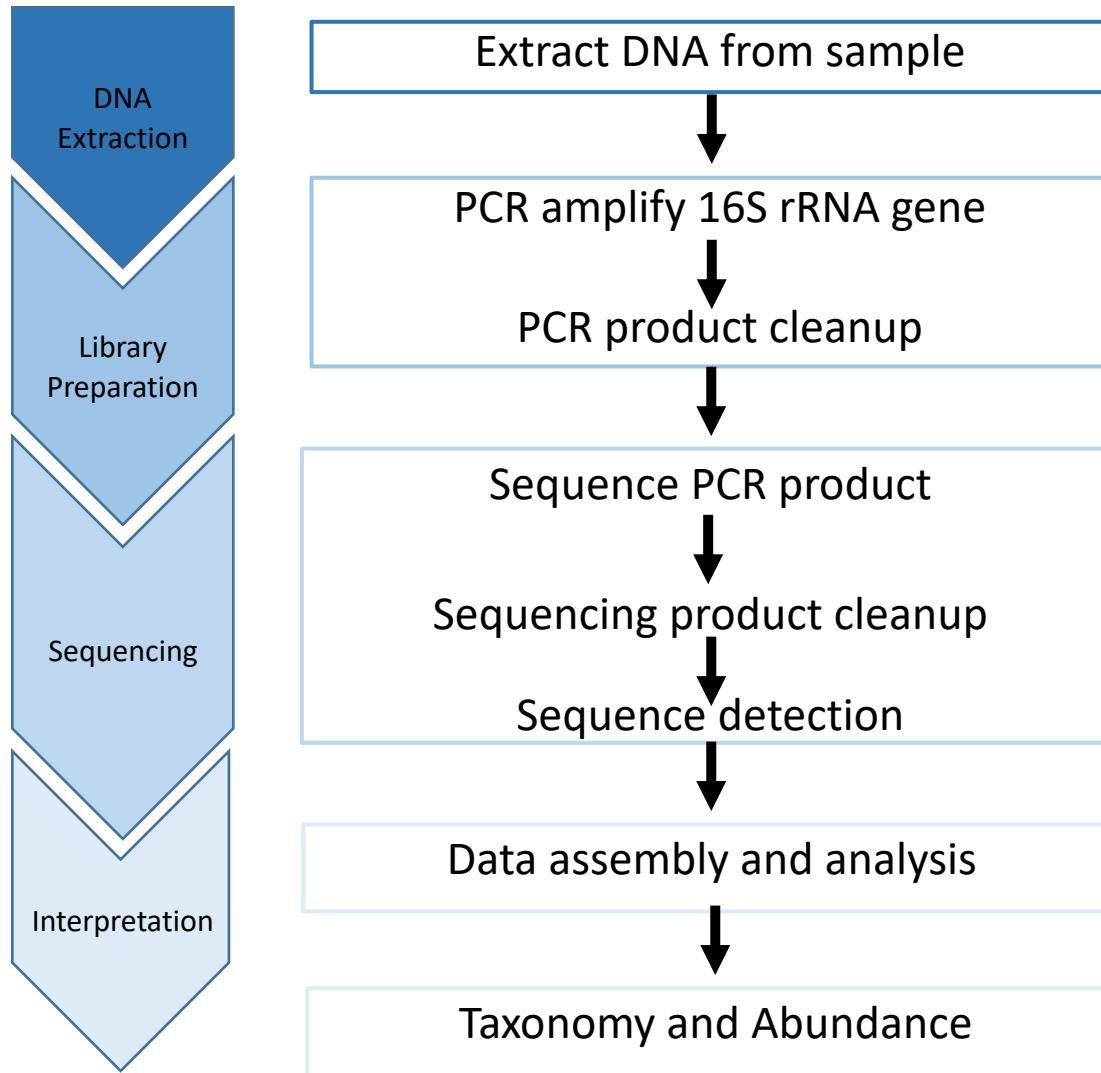


Targeting the Bacterial 16S rRNA Gene

- 16S rRNA gene and are used in reconstructing phylogenies, due to the slow rates of evolution of this region of the gene
- Found in all bacteria and archaea
- Encodes the small subunit of the ribosomal complex, necessary for protein synthesis
- “molecular clock”
 - rDNA sequence similarities correlated with ‘evolutionary relatedness’
 - Little evidence of horizontal gene transfer
- highly variable regions allow for discrimination of bacterial genera



16S rRNA Sequencing Workflow



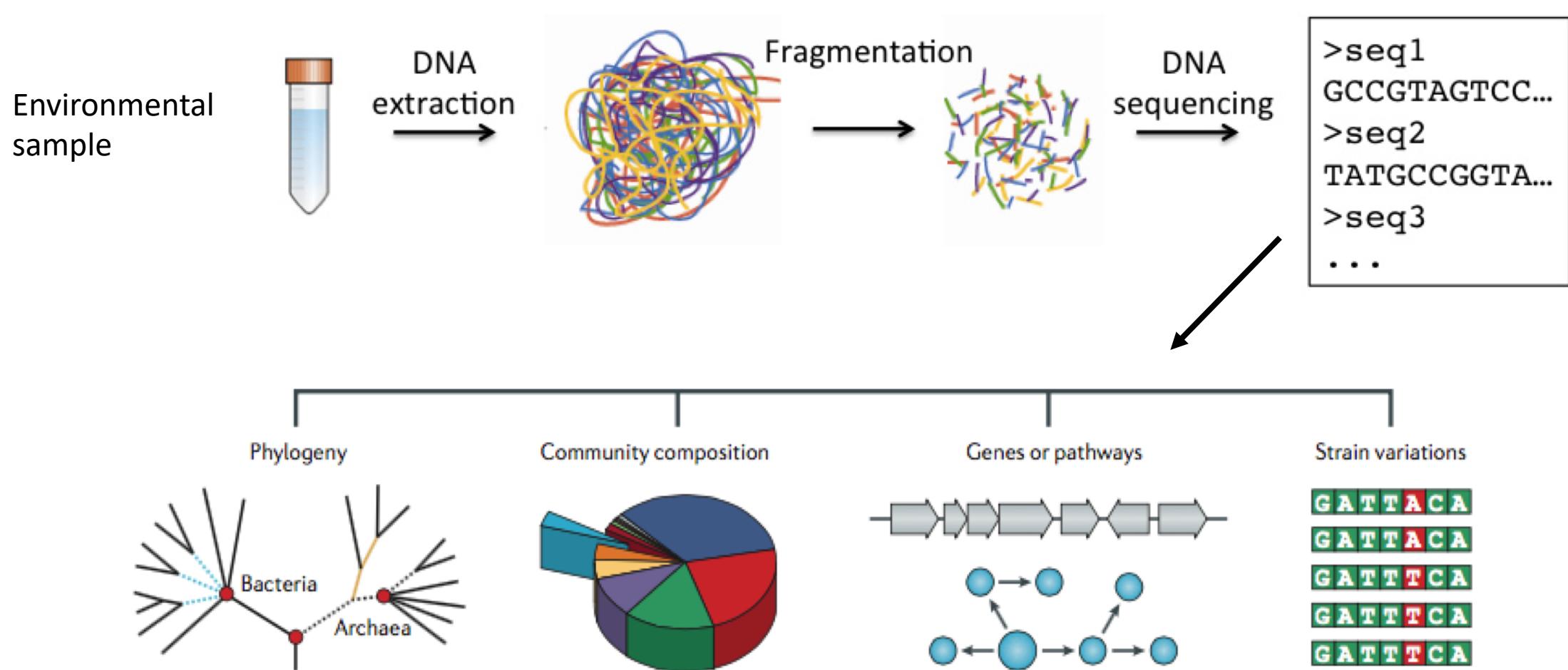
Comparison of analysis with QIIME, mothur and MG-RAST

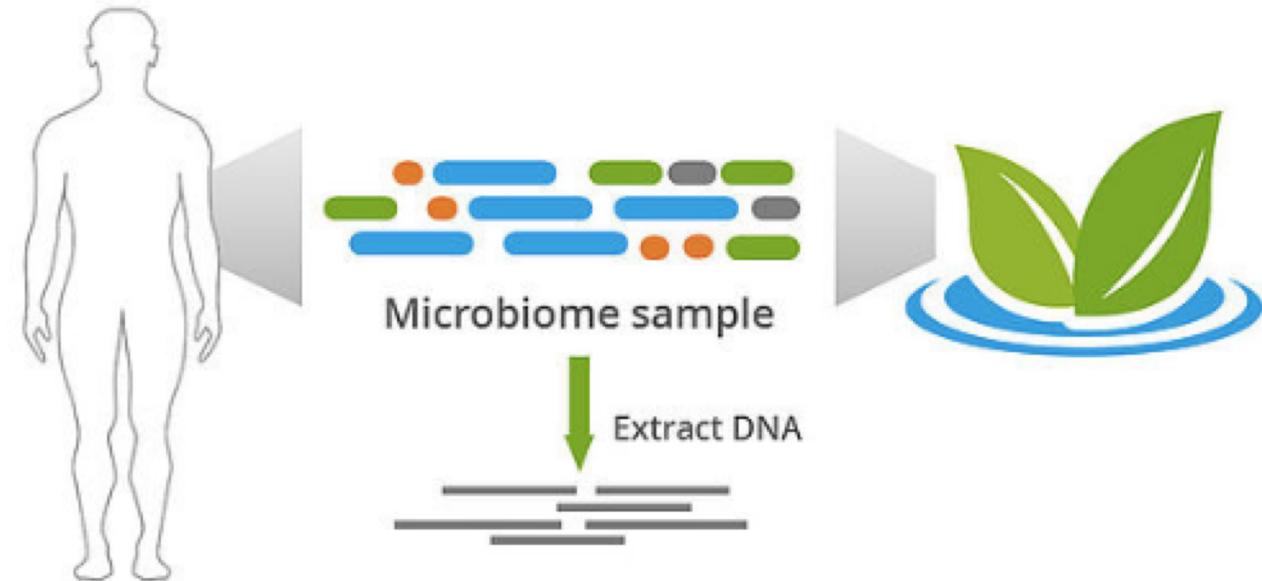
	QIIME	mothur	MG-RAST	p-value
Approximate analysis time (for the study dataset)	1 hour	10 hours	2 days	-
Number of reads uploaded	159,691	159,691	159,691	-
Number of reads post demultiplexing and QC	131,661	132,314	131,368	0.695
Number of reads assigned identity	123,909	128,064	123,022	0.0547
Number of unclassified reads at phylum level (%)	104 (0.08)	155 (0.12)	14,199 (11.54)	<0.0001*
Number of unclassified reads at genus level (%)	12,724 (10.27)	37,039 (28.92)	20,253 (16.46)	0.0814
Number of genera identified	60	50	57	-
Genus Richness (median, IQR)	10 (9-15)	8 (5-12)	9 (7-14)	<0.0001*
Effective number of genera (median, IQR)	3 (2-4)	2 (2-3)	3 (3-4)	<0.0001*

Shotgun Metagenomics

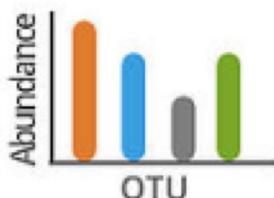
- Is the study of uncultured microbial communities, typically relying on high-throughput experimental data and bioinformatic techniques
- Sequences whole genes. (ie. Sequences all genes present in the sample not just 16S rRNA)
- Covers all kingdoms, not just Bacteria and Archaea (even human genes)
- Gives both functional and taxonomic information
- More technical and expensive

Shotgun Metagenomics Workflow



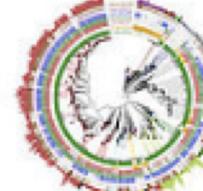


16S rRNA sequencing



Identification of species and relative frequencies

Total microbiome DNA sequencing



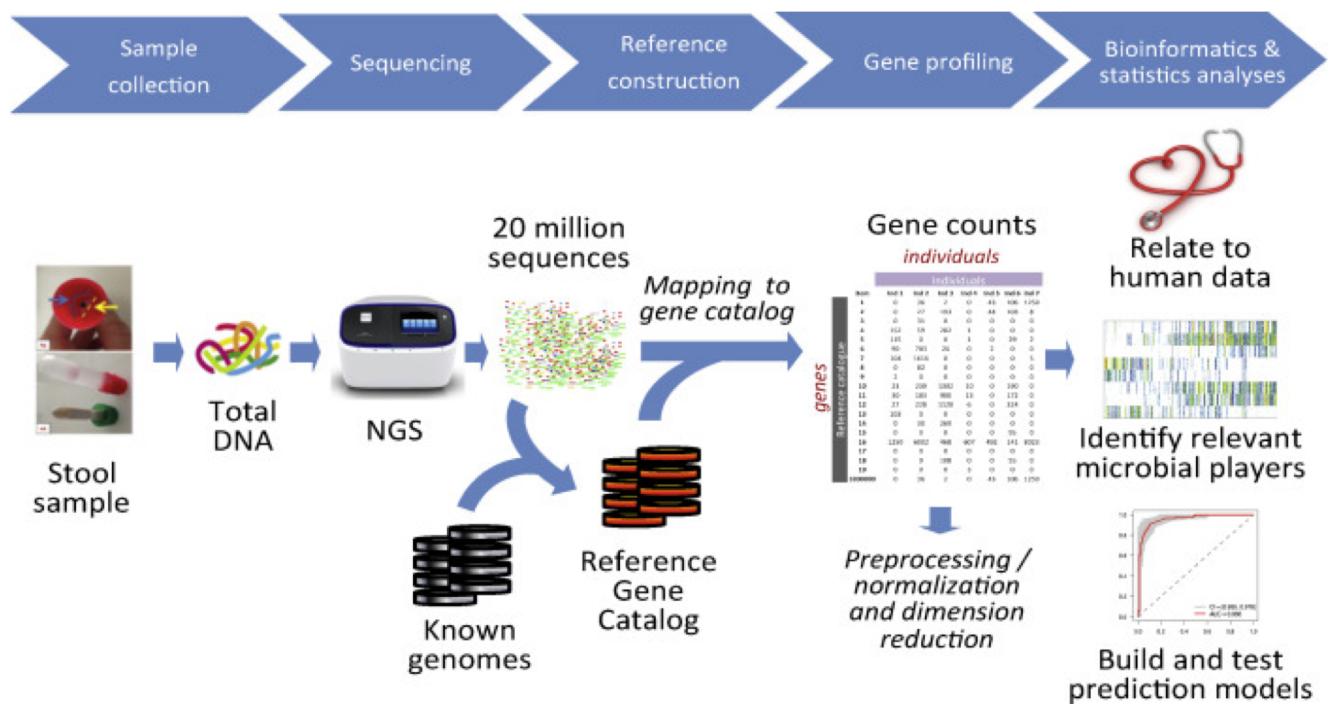
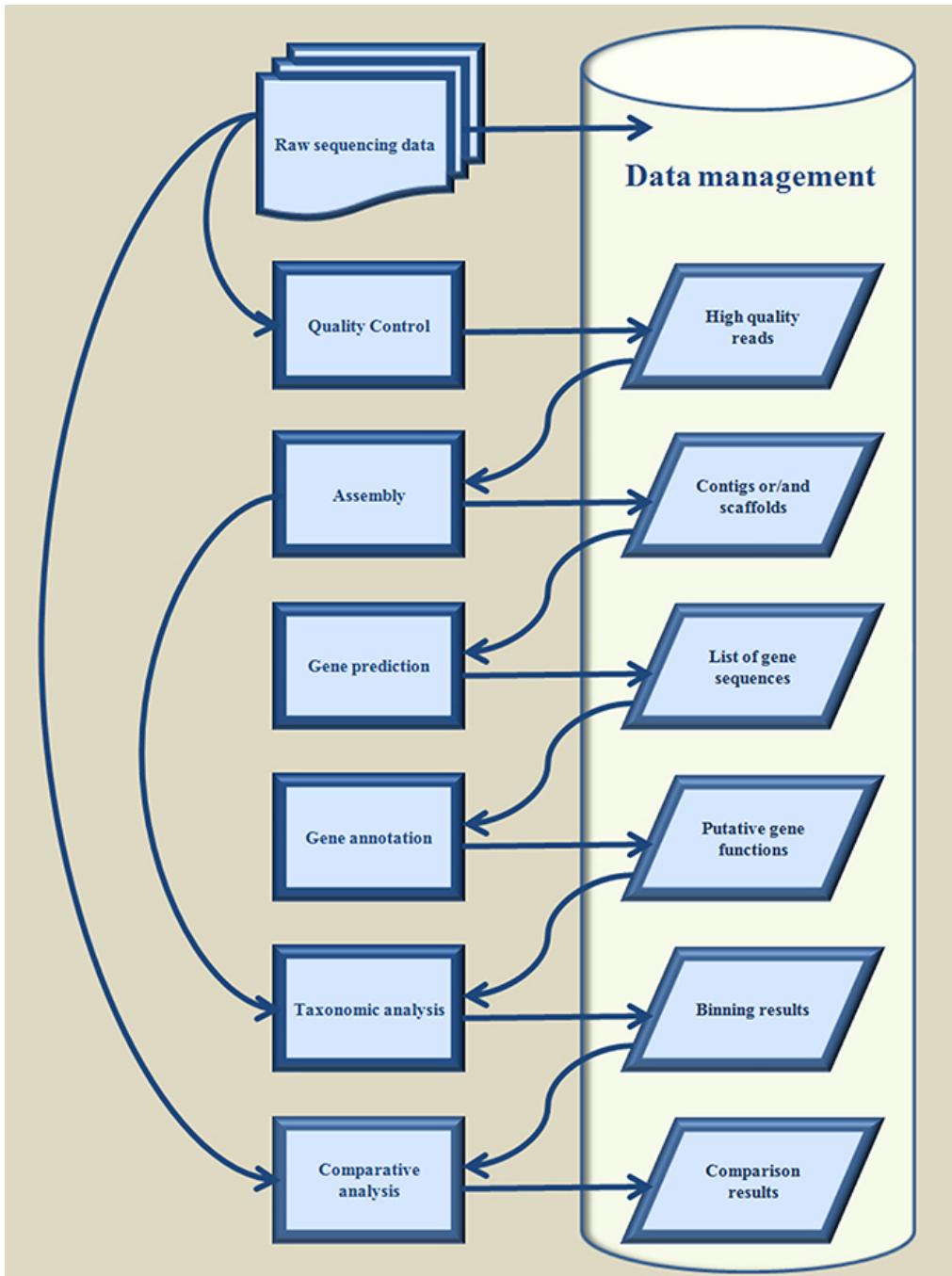
Phylogenetic view of community composition

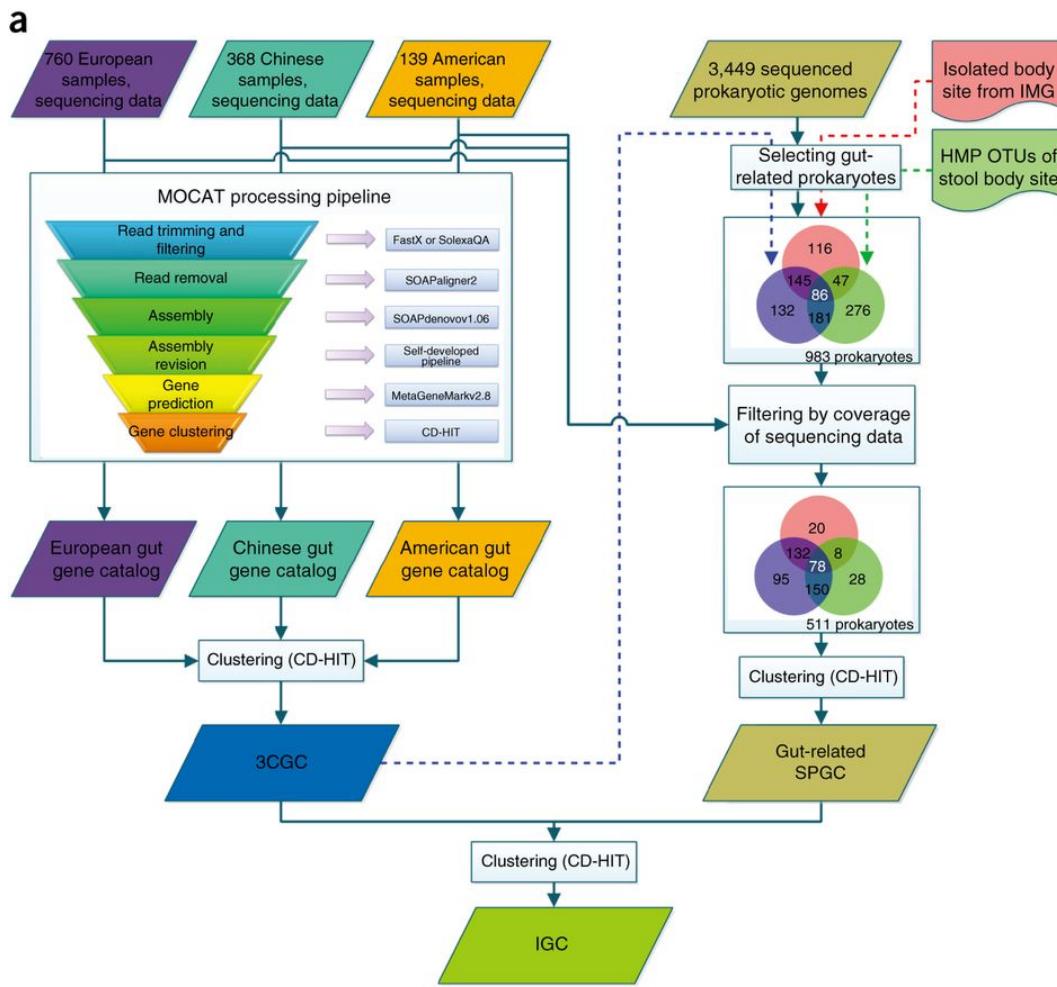
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GATCG**T**TC



Functional information

OTU = Operational Taxonomic Unit, a group of very similar 16S sequences





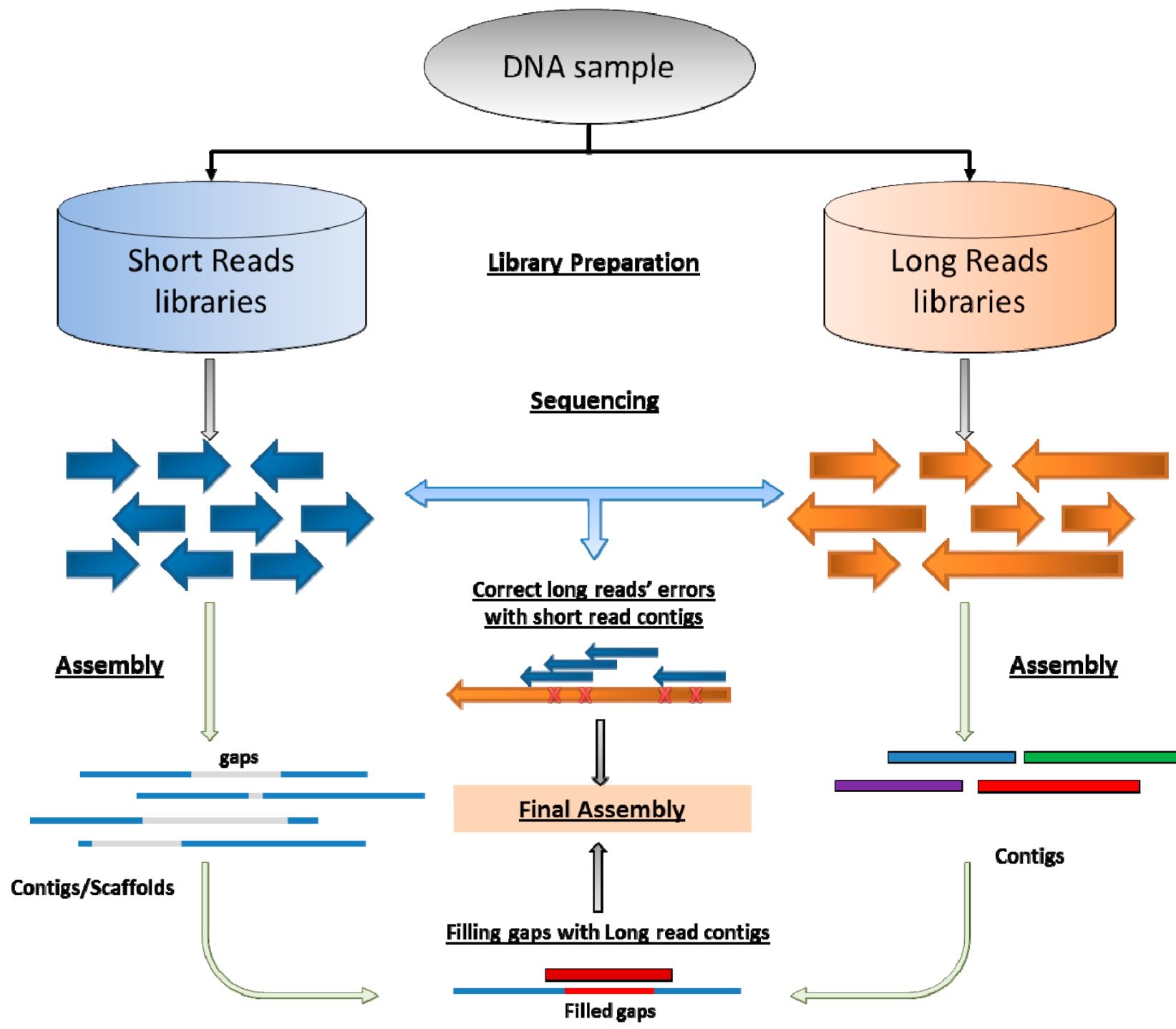
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Gene catalog	Reference	Sample size	Number of ORFs	Complete ORFs (%)	Total length (bp)	Average length (bp)	N50 (bp)	N90 (bp)	Max length	Min length
European	Current study	760	8,096,991	56.18	6,039,847,368	746	1,023	381	88,086	102
	MetaHIT 2010 study	124	3,299,822	46.26	2,323,171,095	704	909	378	23,034	102
American	Current study	139	2,681,342	55.45	1,996,356,219	745	1,005	387	40,011	102
	HMP 2012 study*	139	4,581,984	NA	2,571,088,392	561	765	285	26,109	102
Chinese	Current study**	368	3,547,396	60.05	2,750,208,618	775	1,053	405	88,230	102
3CGC	Current study	1,267	9,750,788	56.34	7,298,407,194	748	1,029	384	88,230	102
SPGC	Current study	NA	659,492	99.77	612,211,588	928	1,221	513	24,615	100
IGC	Current study	1,267***	9,879,896	57.74	7,436,156,055	753	1,035	384	88,230	100

Comparison of current common NGS platforms.

Platform	Mode	Read-Length	Reads Passing Filter per Run	Output	Run Time	Quality	Cost/Run	Instrument Price
Illumina HiSeq 2000/2500	High-Output	1 × 36–2 × 125	4 B	128 GB–1 TB	1–6 days	Q30 ≥ 80%	~\$29K	\$740K
	Rapid	1 × 36–2 × 150	600 M	18 GB–300 GB	7–60 h	Q30 ≥ 75%	~\$8K	
Illumina HiSeq X ten	X ten	2 × 150	5.3–6 B	1.6–1.8 TB	<3 days	Q30 ≥ 75%	~\$12K	\$1M*
Roche 454 FLX system	Titanium XL+	700	1 M	700 MB	23 h	99.997%	~\$6K	~\$500K
Life Technologies Ion Torrent	Proton I	200	165 M	~10 GB	2–4 h		~\$1000	\$149K
	Proton II	100	660 M	~32 GB	2–4 h			
Intelligent Biosystems (Qiagen)	MAX-Seq	2 × 55	75 M/lane	132 GB	2.5 days		~\$1200	~\$270K
	Mini-20	2 × 100	20 M/lane	80 GB			~\$150–300/sample	\$125K
PacBio RS	RS II	10–15 KB	50 K	500 MB–1 GB	4 h	>99.999%	~\$400	~\$700K
Oxford Nanopore	miniON	>200 KB	no fixed run time (~1 bp per nanosecond)				≤\$900	~\$1000

* K: thousand; M: million; B: billion; kb: kilobase; MB: millionbase; GB: gigabase; TB: terabase; h: hour.



Genome de novo assembly and post-assembly approaches

Approaches	Commonly Used Tools	Notes
Assembly Approaches		
<i>de Bruijn</i> graph	EULER, ALLPATHS, Velvet, ABySS, SOAPdenovo, <i>etc.</i>	For shorter reads (25–100 bp) assembly
Overlap-layout-consensus (OLC)	SSAKE, SHARCGS, VCAKE, Celera Assembler, Arachne, PCAP, HGAP, <i>etc.</i>	For longer reads (100–800 bp) and long reads assembly
Post-Assembly Approaches		
Contigs orientation and visualization	AlignGraph, ABACAS, CONTIGuator, Projector2, OSLay and r2cat, <i>etc.</i>	
Extending contigs and filling gaps	IMAGE, GAA program, Reconciliator, GAPFiller, Pilon <i>etc.</i>	
Reads error correction	ICORN, AutoEditor, REAPR <i>etc.</i>	
Unmapped reads Annotation	RATT, Ensembl, GARSA and SABIA, <i>etc.</i>	

So what are the important or ‘interesting’ genes?

Virulence genes:

- Are genes that contribute to the pathogenicity of the organism
(ie. endotoxin genes, fimbriae genes, genes that encode cell wall proteins)

Resistance genes:

- Genes that induce antibiotic resistance (ie. *Erm* genes, *mecA* gene)

Diagnostic markers:

- Genes that aid in the rapid diagnosis of disease (ie. Hepatitis antibodies for HepC)

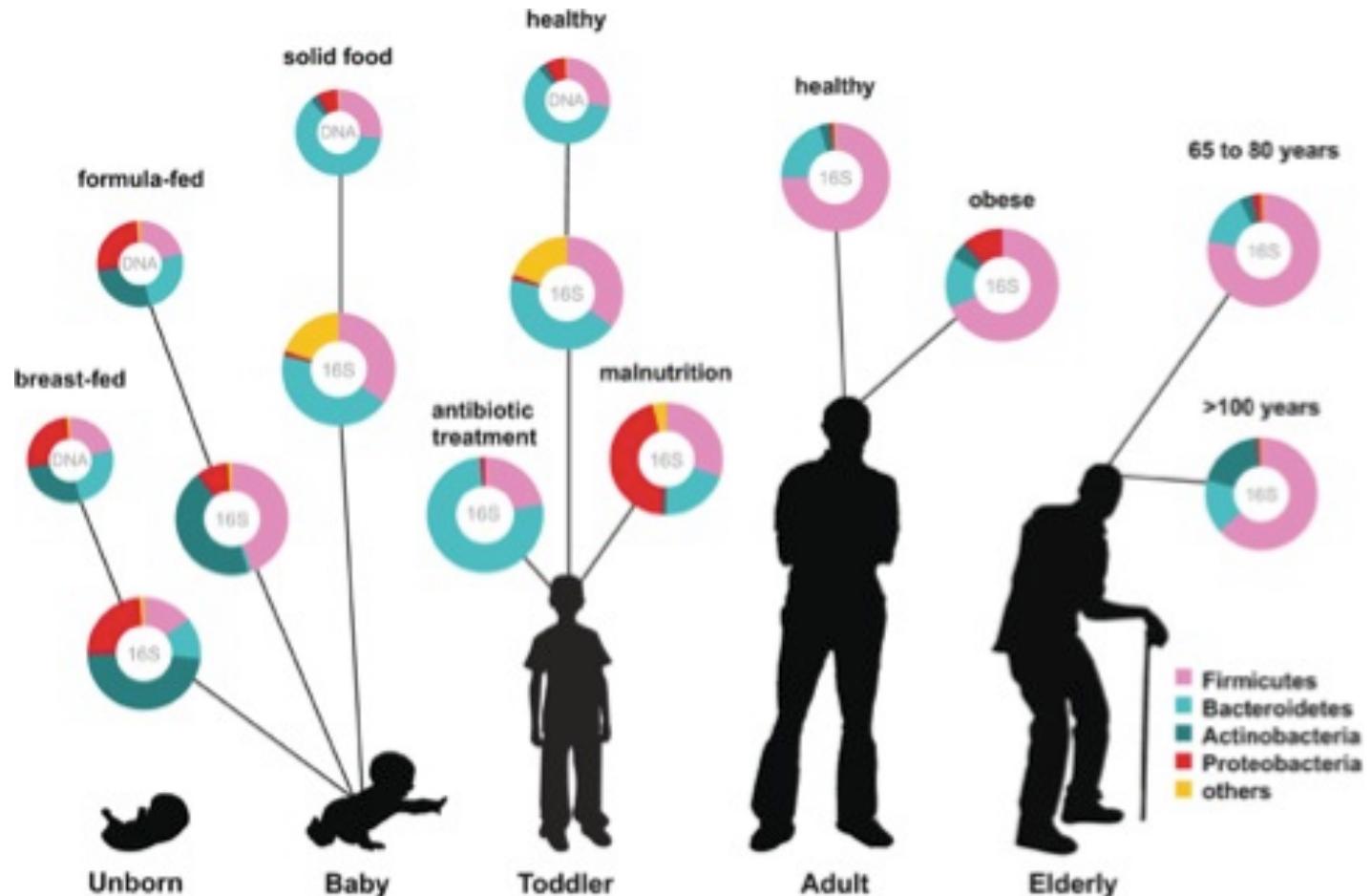
Genes for biotechnology applications:

- Novel production processes

Part 2: So who is present in our microbiome?

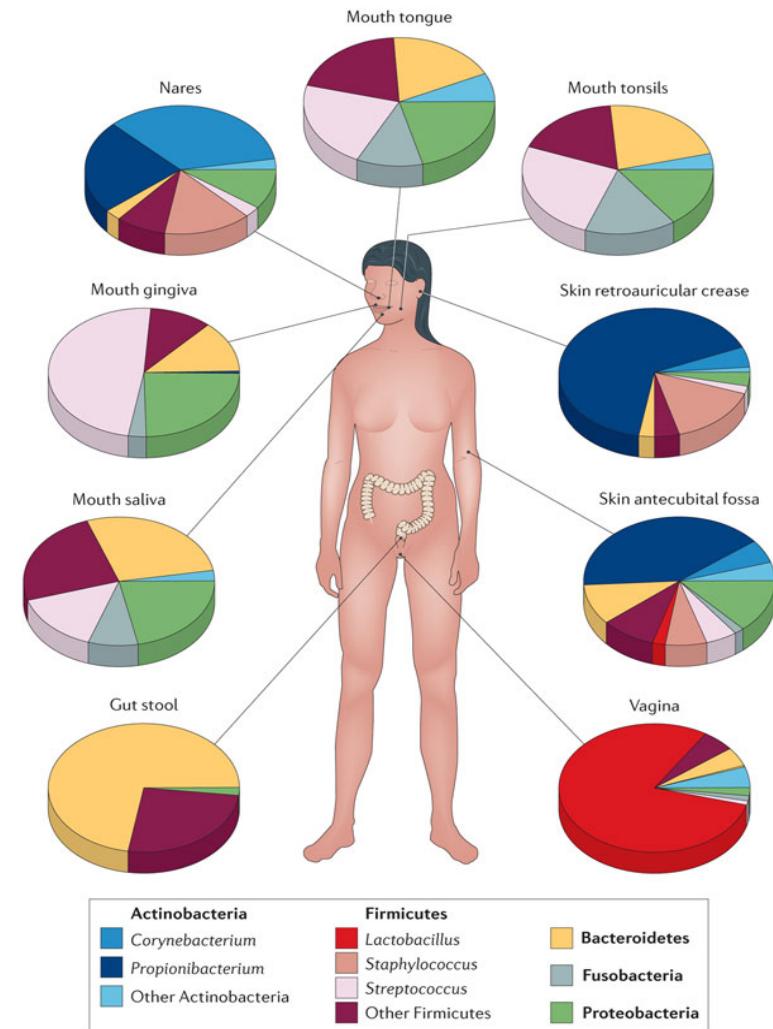
Microbiome findings so far...

- Colonisation begins at birth
- Microbiome changes over time
 - Most dramatically in the first 3 years of life
- Influenced by diet, lifestyle, environment

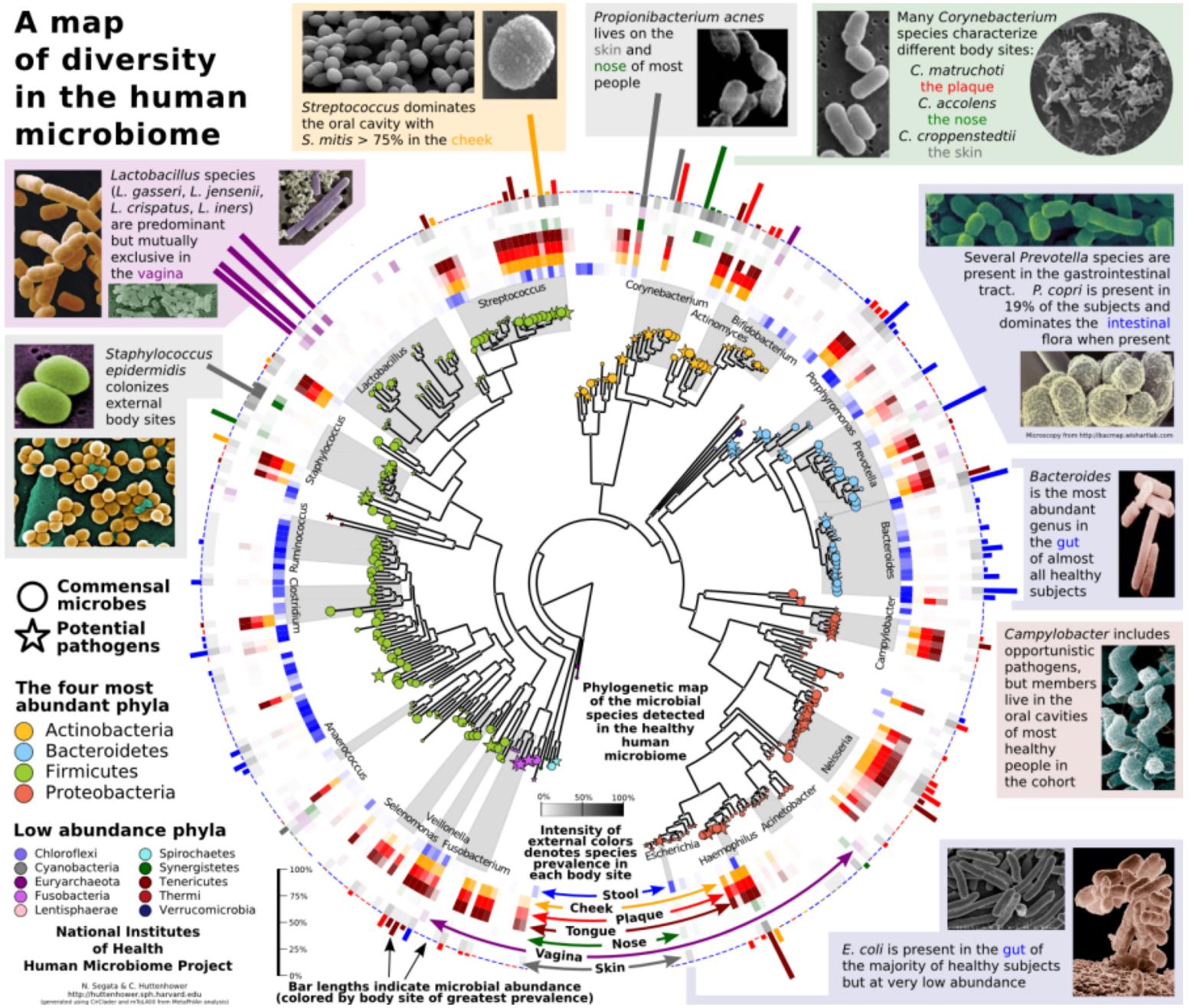


Microbiome findings so far...

- Microbiomes are characteristic of each body site
 - Body sites create different environmental habitats for bacterial growth
- The individual is the primary determinant for composition – everyone's microbiome is different
- Microbiome plays a role in disease – e.g. obesity, type II diabetes



A map of diversity in the human microbiome



Mouth:
The proportion of the "gram-positive" bacteria known as *Streptococcus mitis* in the mouths of patients with oral cancer is about half that of healthy people. Gram-positive bacteria may help detoxify tobacco smoke.



Skin:
Levels of the bacteria
Staphylococcus aureus appear to increase dramatically in the skin of children during flare-ups of eczema.

Gastrointestinal tract:
Microbes in the gut known as *Helicobacter pylori* appear to help regulate two hormones that play a role in controlling hunger and body weight.

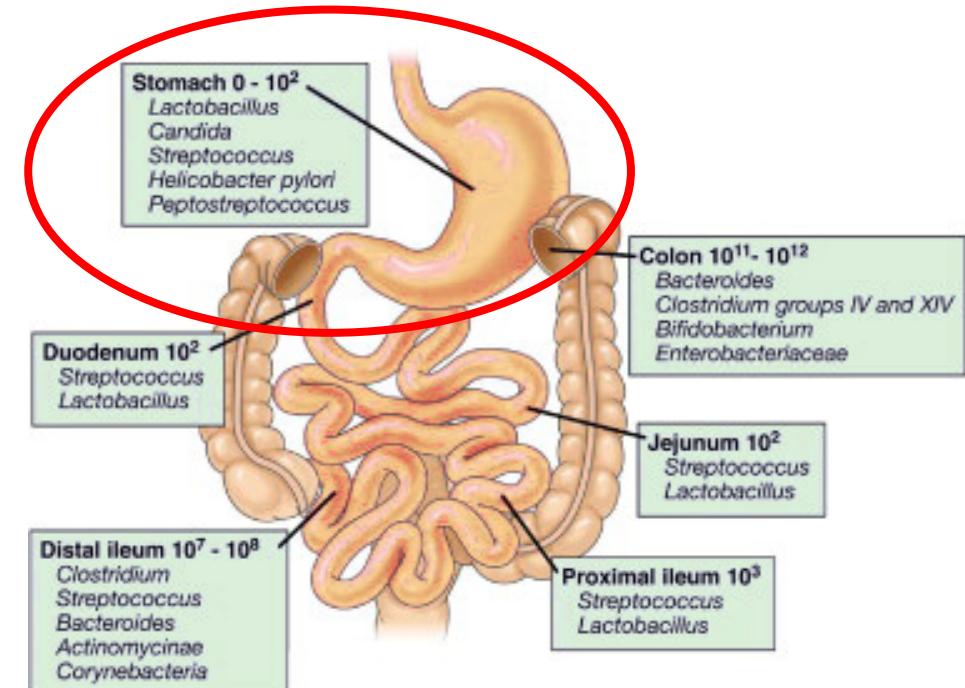
Immune system: *Bacteroides fragilis* produces a compound that can dampen the immune system's inflammatory response, which is thought to play a role in many diseases.

Urogenital tract: The bacterium *lactobacillus* appears to protect women against vaginosis.

The Gut Microbiome

- **Stomach:**
 - Traditionally thought to be sterile
 - Transient colonisation from food
 - *Helicobacter pylori* is a coloniser
 - Associated with gastritis and ulcers and stomach cancer
 - Up to 40% of the population colonised

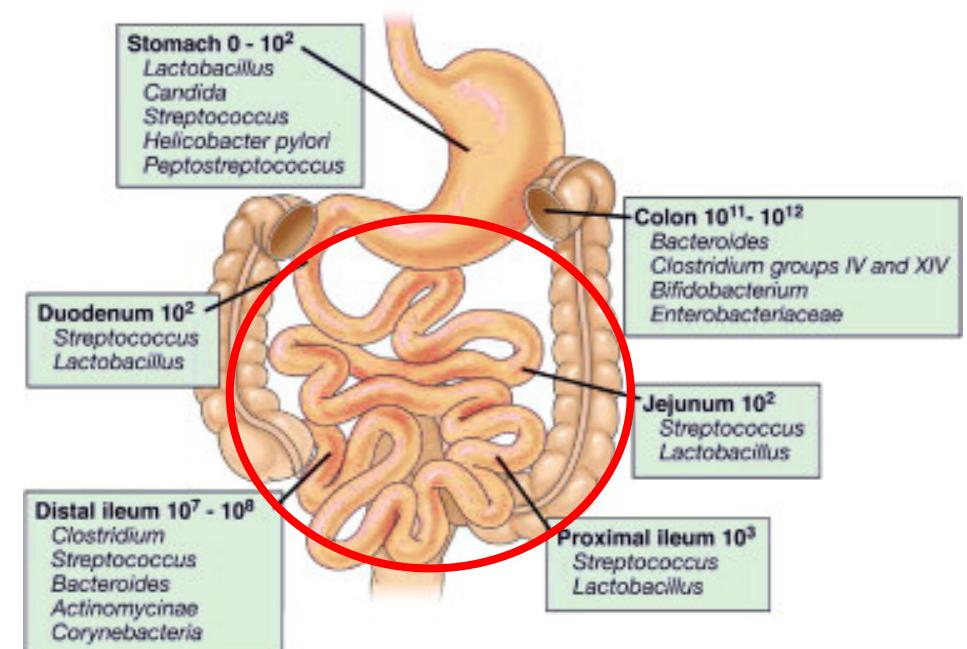
Different locations within the gut have different microbiota and microbial burdens



The Gut Microbiome

- **Small Intestine:**
 - Lower numbers of organisms
 - Number increases closer to the colon
 - Microbes found are those which have passed through stomach acid without being killed.
 - Include Streptococcus Spp. and Lactobacillus Spp. and yeasts.
 - All are aciduric (acid-tolerating)

Different locations within the gut have different microbiota and microbial burdens

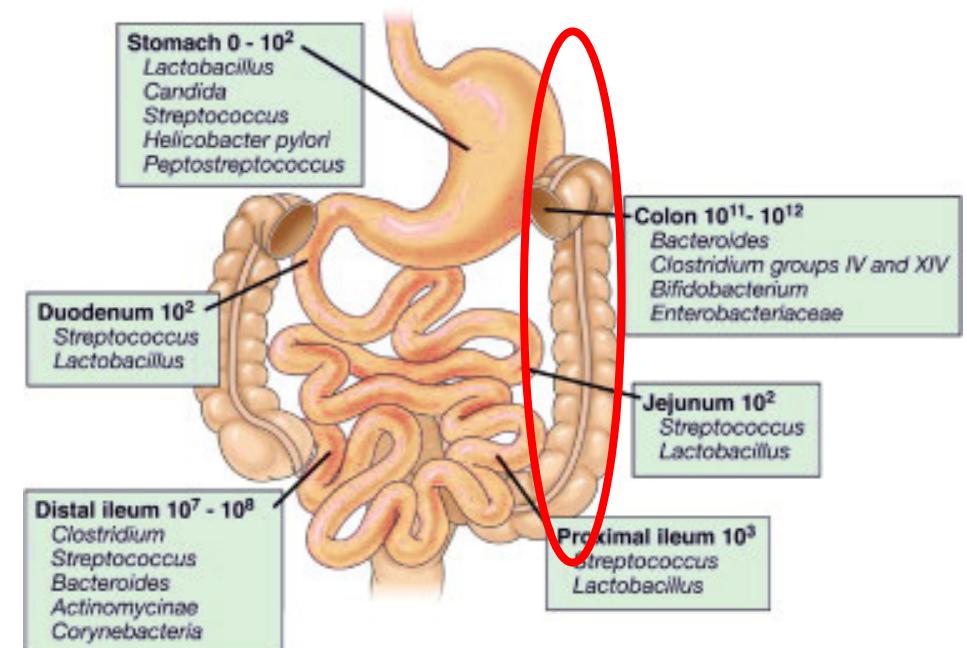


The Gut Microbiome

- **Colon/Large Intestine:**

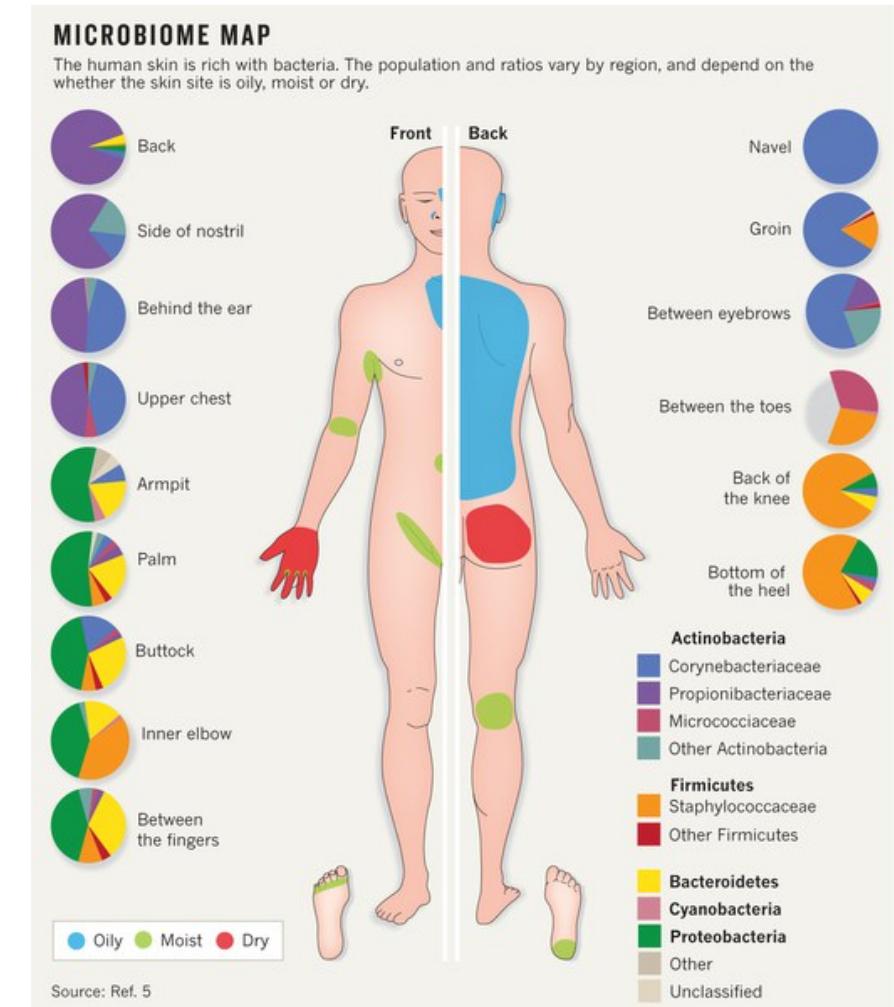
- Heavily populated with highly varied bacterial genera
- Anaerobic microbes greatly outnumber the aerobic and facultative microbes
- Heavily studied for health and disease associations

Different locations within the gut have different microbiota and microbial burdens

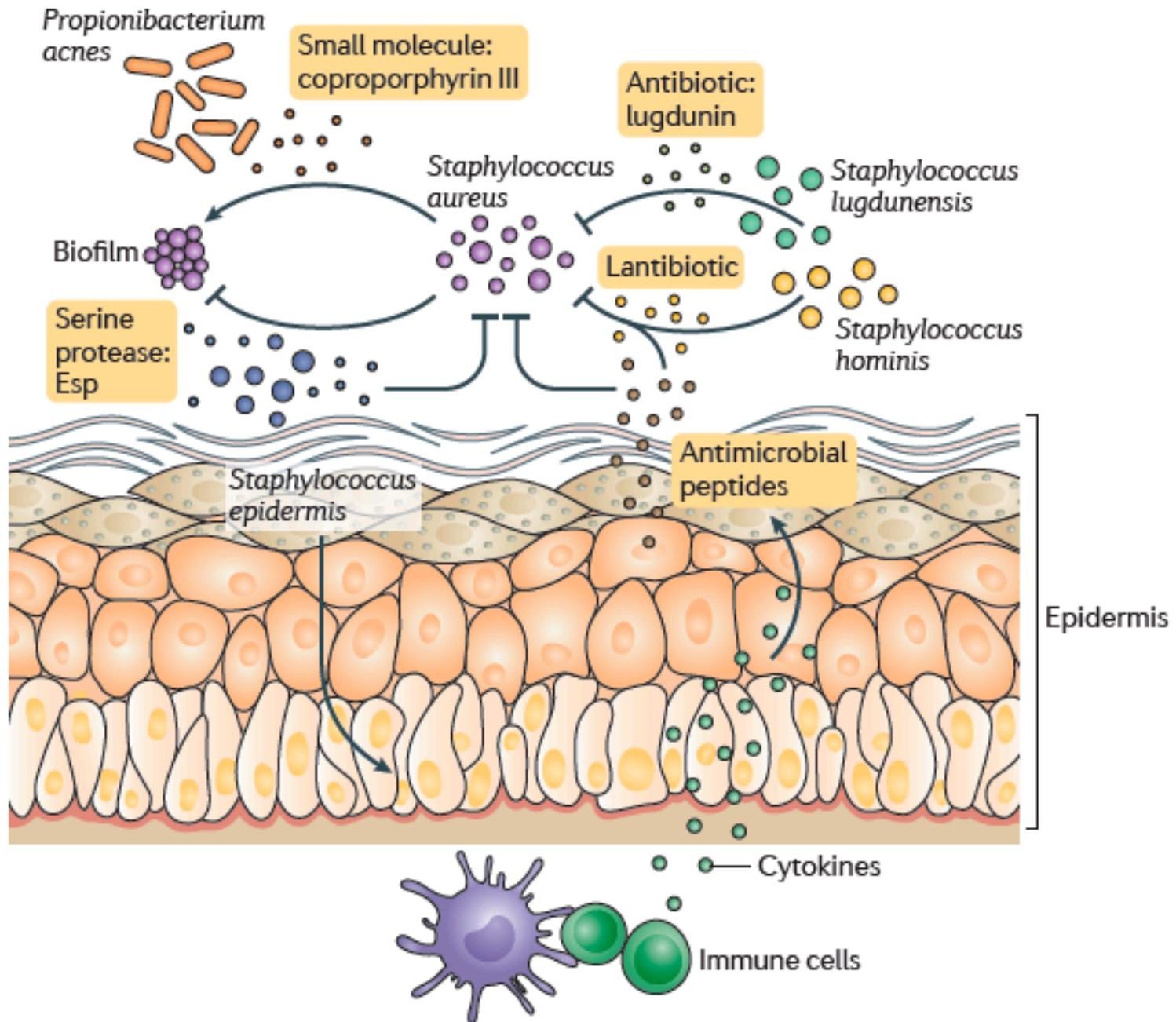


The Skin Microbiome

- Varied microbiota
 - Large variation between different sites
- Relatively low numbers of microbes on exposed areas
- Large numbers present in protected areas e.g. axilla, groin, between toes
- Principal species include those associated with skin conditions such as acne (*Propioibacterium acnes*) and dandruff (*Malassezi furfur* (yeast))

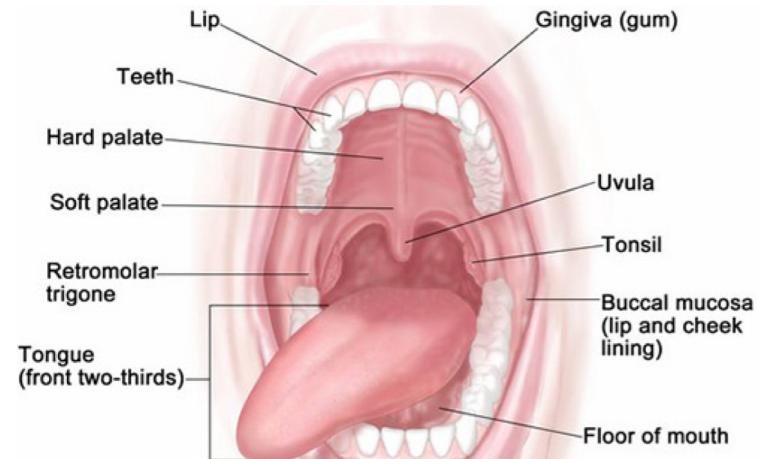


The Skin Microbiome



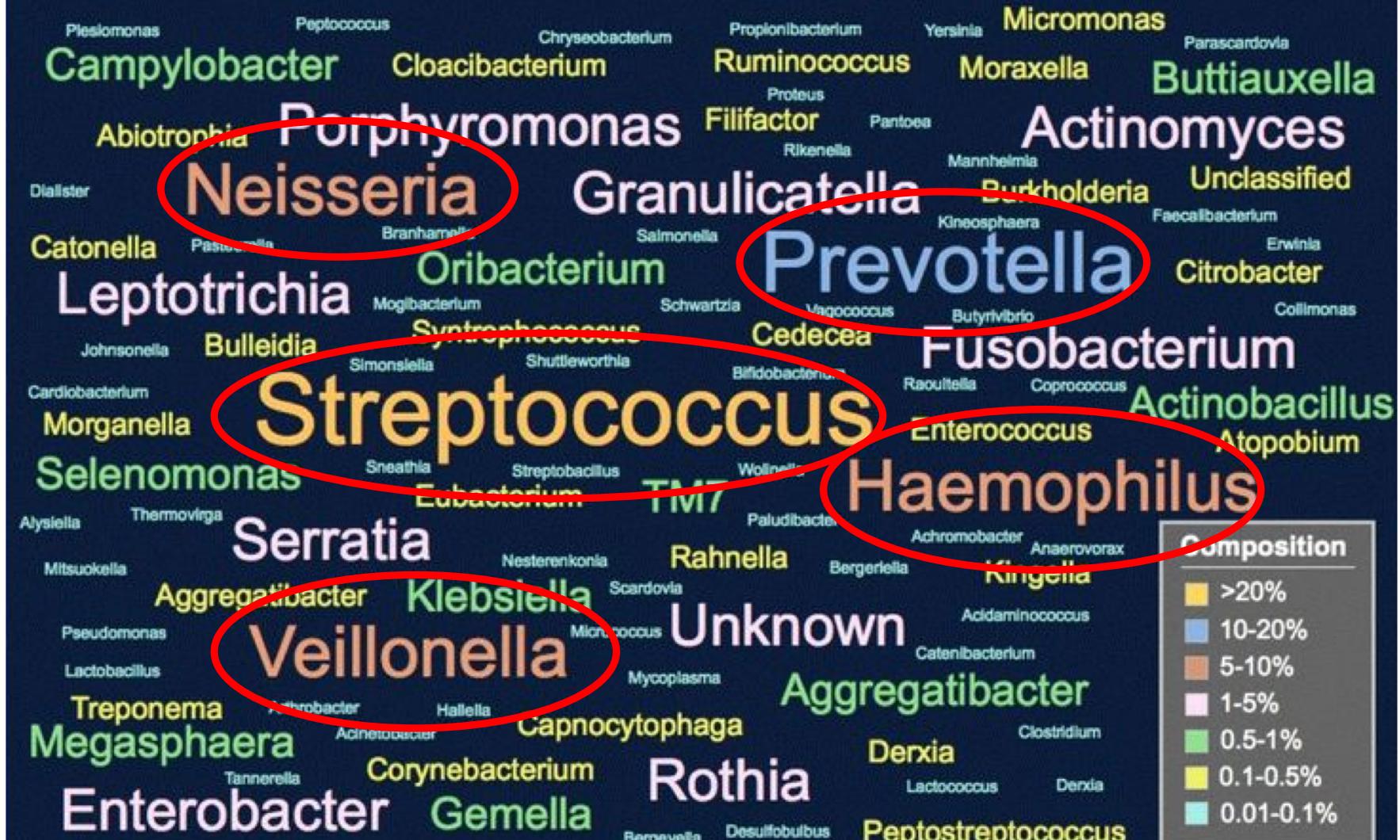
The Oral Microbiome

- High numbers of bacteria in the mouth
 - $\sim 10^8/\text{ml}$ in saliva
 - $\sim 10^9/\text{mg}$ in plaque
- Highly diverse
 - more than 700 species
- Comprised of several different microbial habitats:
 - Teeth, saliva, supragingival and subgingival plaque, tongue, buccal mucosa, hard and soft palates, tonsils
 - Saliva commonly used as a representative for the oral microbiome
- Associated with oral diseases in most individuals
- Therefore this microbiota is not in full harmony with the host.



Salivary microbiome

Source: Nasidze. 2009. *Genome Research*, 19, 636-43.



Bacteria genera commonly found in the mouth

Gram-positive

Cocci

Streptococcus
Peptostreptococcus

Bacilli

Actinomyces
Lactobacillus
Eubacterium
Propionibacterium

Gram-negative

Neisseria
Veillonella
Haemophilus

Prevotella
Fusobacterium
Porphyromonas
Spirochaetes

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Spirochaetes

Some genera associated with **healthy oral microbiota**

Bacteria genera commonly found in the mouth

Gram-positive

Cocci

Streptococcus
Peptostreptococcus

Bacilli

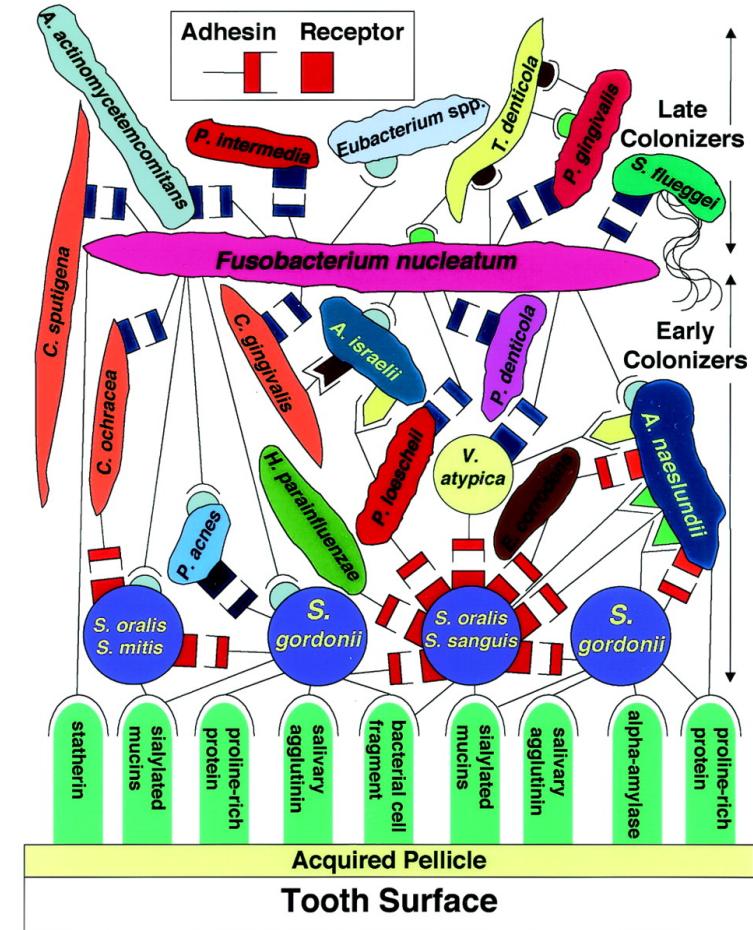
Actinomyces
Lactobacillus
Eubacterium
Propionibacterium

Some genera associated with **gingivitis**

Gram-negative

Neisseria
Veillonella
Haemophilus

Prevotella
Fusobacterium
Porphyromonas
Spirochaetes



Bacteria genera commonly found in the mouth

Gram-positive

Cocci

Streptococcus
Peptostreptococcus

Bacilli

Actinomyces
Lactobacillus
Eubacterium
Propionibacterium

Gram-negative

Neisseria
Veillonella
Haemophilus

Prevotella
Fusobacterium
Porphyromonas
Spirochaetes

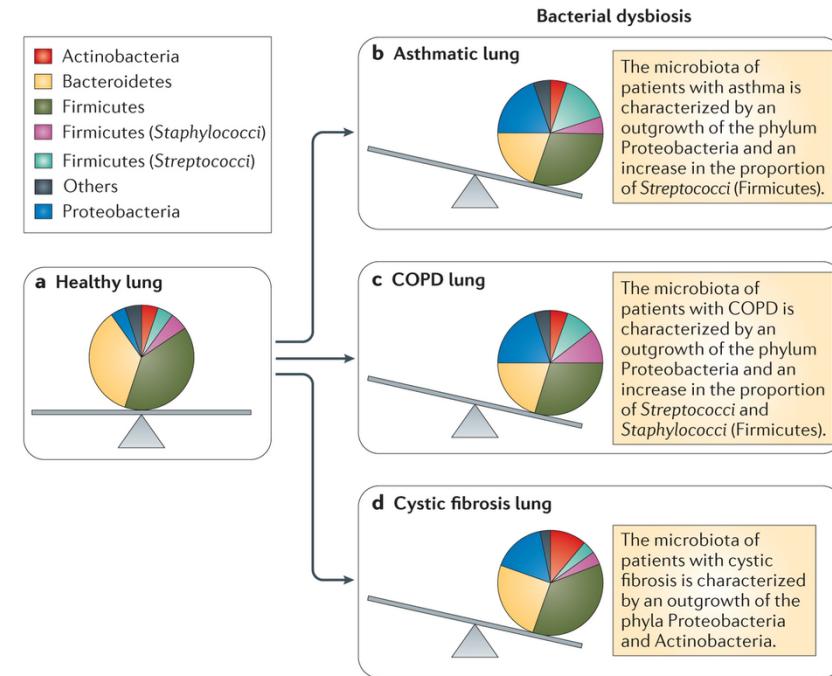


Some genera associated with **periodontitis**

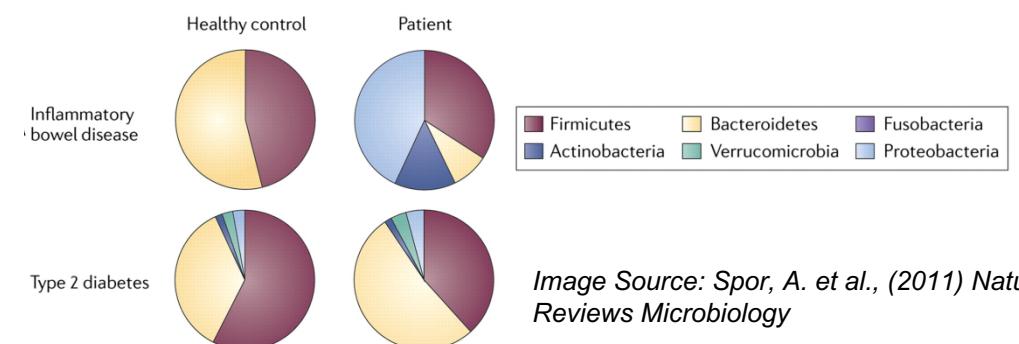
Periodontitis = inflammation of the tissue around the teeth, often causing shrinkage of the gums and loosening of the teeth.

Microbiota and Disease

- A “normal” microbiota is essential for health.
 - Required for development of gut structures and the immune system
 - Protects against colonisation with pathogens – *colonisation resistance*
 - Disruption of normal microbiota e.g. by antibiotics leads to infection by pathobionts e.g. *Candida* spp
- Non-infectious diseases have been associated with an altered microbiome.
 - e.g. obesity, Type II diabetes, ulcerative colitis, asthma, COPD
- Diseases usually associated with a loss of microbiome diversity

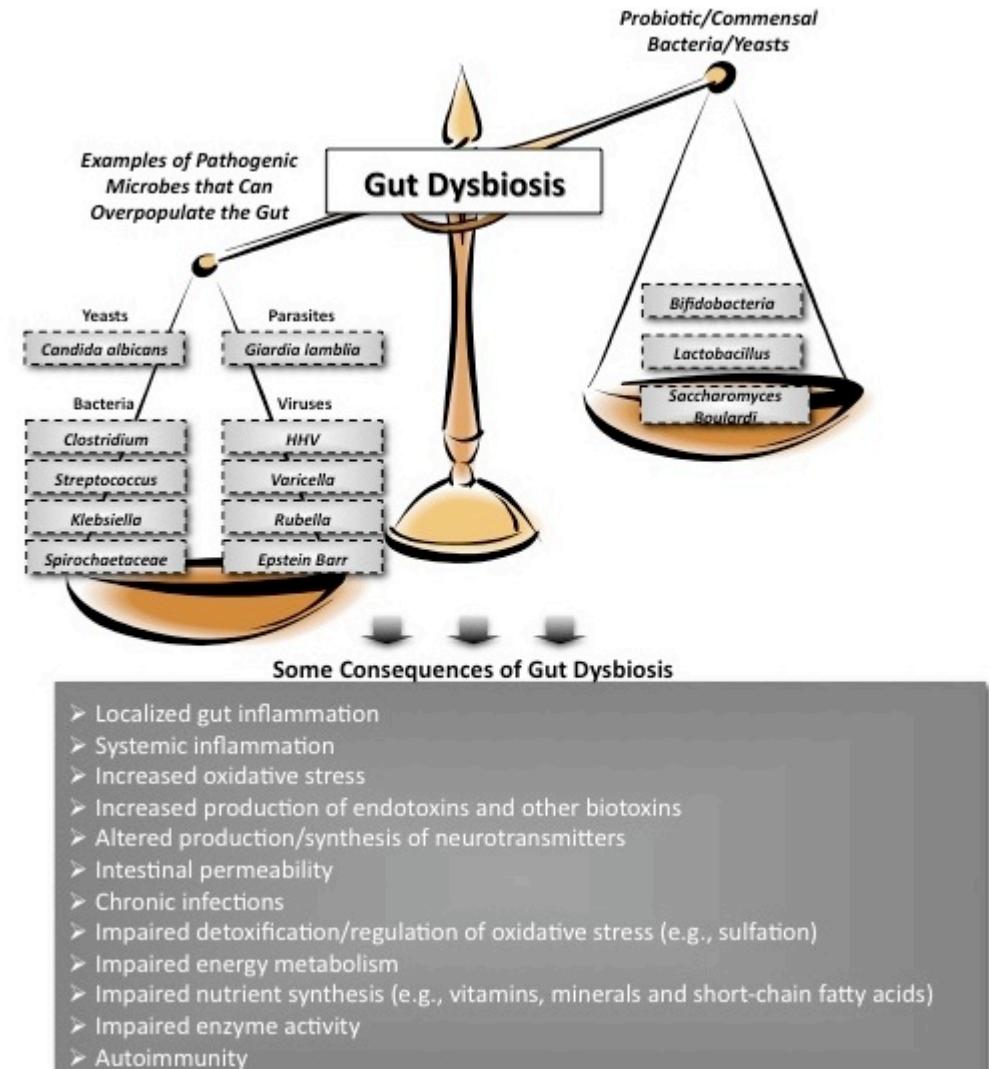


Nature Reviews | Immunology
Image Source: Marsland, B.J. and Gollwitzer E.S., (2014) *Nature Reviews Immunology* **14**, 827–835



Dysbiosis

- **Dysbiosis** – an imbalance in the host microbiota
- Can result in development of both infectious and non-infectious diseases
- Can be driven by different environmental factors
 - *(mal)nutrition*
 - *antibiotics consumption*
 - *infection*

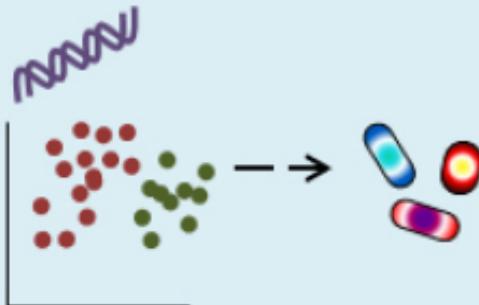


Culture-independent molecular approaches to study host–microbiome interactions

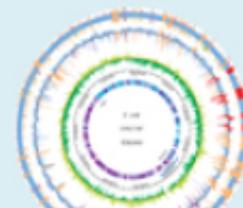
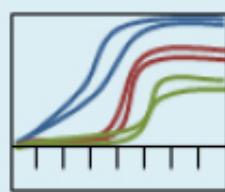
DNA-Based Approaches

Who is there?
What can they do?

16S rRNA, 18S, ITS gene sequencing



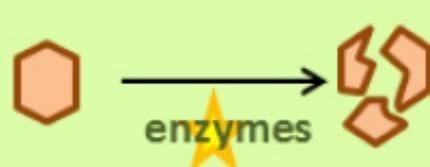
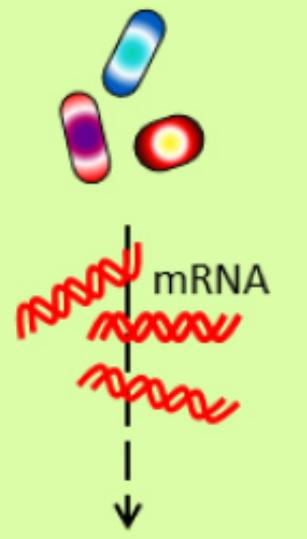
metagenomics



RNA-Based Approaches

How do they respond?
What pathways are activated?

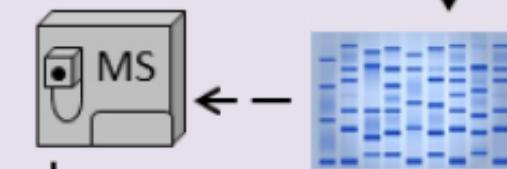
metatranscriptomics



Protein-Based Approaches

How are they interacting with the host?
What proteins are being produced?

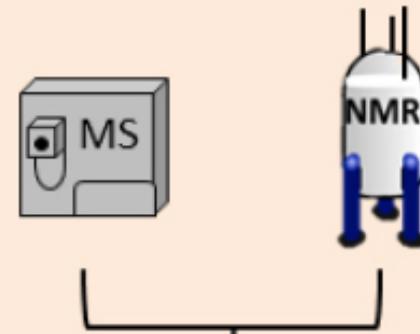
metaproteomics



Metabolite-Based Approaches

What are the chemical outcomes of their activity?

metabolomics

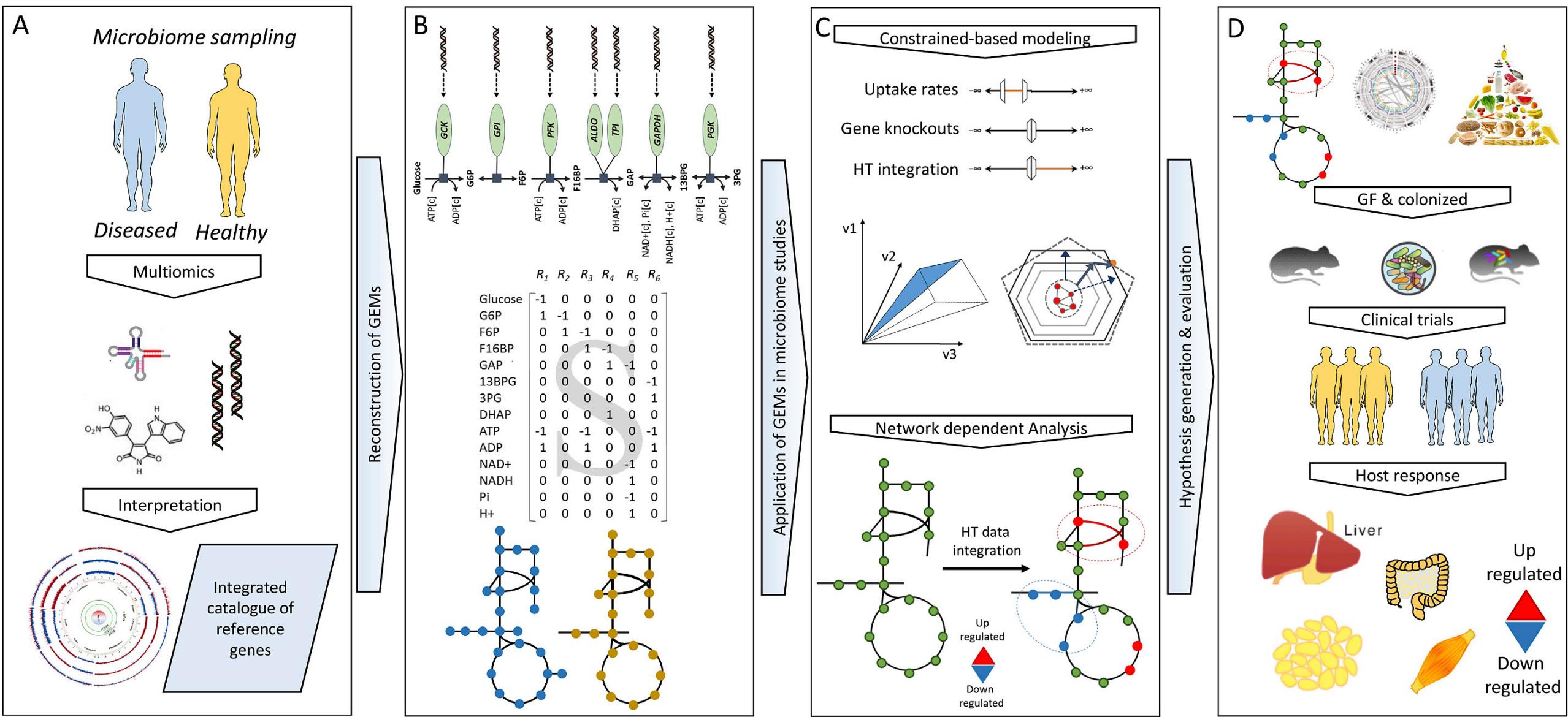


High-Throughput Approaches Used to Study Variations in the Gut Microbiota

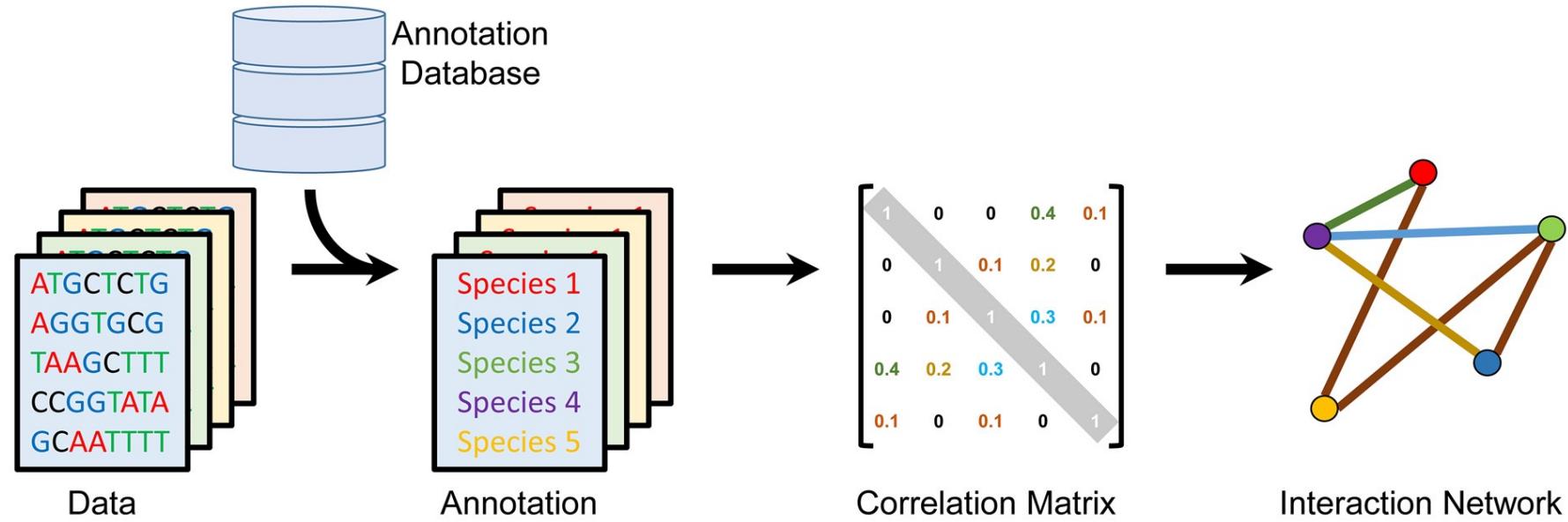
Meta-omics	Microbial Material	Outcome	Advantages	Limitations	Clinical Applications	Key Targets Identified
Phenotyping	16S rRNA amplicons generated from DNA or RNA/cDNA	Composition of total microbiota or microbiota with protein synthesis (potentially active)	Fast and cheap sequencing	<ul style="list-style-type: none"> 1. Difficulties for phylogenetic assignations at the deepest level of the taxonomic hierarchy 2. Dominance of major microbial groups mask the identification of low abundant members 3. Comparisons require amplification of same region 	<ul style="list-style-type: none"> 1. Detect imbalances in the taxonomic composition of total and active gut microbiota 2. Genera/species markers associated to diseases 	<p>Genera: <i>Prevotella</i>, <i>Ruminococcus</i>, <i>Roseburia</i>, <i>Bacteroides</i>, <i>Blautia</i>, <i>Faecalibacterium</i>, <i>Phascolarctobacterium</i>, <i>Clostridium</i>, <i>Subdoligranulum</i>, <i>Ruminococcus</i>, <i>Coprococcus</i>, <i>Enterococcus</i>, <i>Roseburia</i>, <i>Lactobacillus</i>, <i>Akkermansia</i>, <i>Clostridium</i>, <i>Butyrivibrio</i>, <i>Phascolarctobacterium</i>.</p> <p>Families: <i>Lachnospiraceae</i>, <i>Ruminococcaceae</i>, <i>Enterobacteriaceae</i></p> <p>Orders: <i>Bacteroidales</i>, <i>Enterobacteriales</i></p> <p>Class: <i>Butyrivibrio</i></p> <p>Phylum: <i>Firmicutes</i>, <i>Bacteroidetes</i></p>
Metagenomics: 1. Sequence-based metagenomics 2. Functional metagenomics	DNA	<ul style="list-style-type: none"> 1. Gene content profiling and presumptive function analysis 2. Systematic identification of diverse activities 	<ul style="list-style-type: none"> 1. No amplification bias 2. Uncovering microbial diversity 3. Finding new genes 4. High coverage and deep sequencing of total genes 5. Identification of genes with assigned functions 	<ul style="list-style-type: none"> 1. Requires high-depth coverage 2. Assembly complicated due to low coverage and high similarities 3. No information of active genes 4. Many unknown genes 5. Bioinformatics analysis required 6. Presumptive functions require experimental validations (sequence-based) 7. Limited activity-based screening protocols available 	<ul style="list-style-type: none"> 1. Microbial gene composition dysbiosis 2. Finding disease-specific genes 3. Identifying presumptive altered functions 4. Systematic identification of functions and commensal bacteria impacting host cellular functions 	<p>Genes involved in: pathogenic processes, cell wall components biosynthesis, transport, translocation, amino acid metabolism, energy processes, bile acid metabolism, dietary carbohydrate metabolism, oxidative phosphorylation, and the production of mammalian signaling molecules</p>
Metatranscriptomics	mRNA cDNA	Gene expression profiling	Reveal different gene expression from active microbiota across multiple conditions	<ul style="list-style-type: none"> 1. Instability of mRNA 2. Multiple purification steps 3. No unique protocol 4. Lack of reference databases 	<ul style="list-style-type: none"> 1. Revealing functional dysbiosis 2. Enrichment of metagenomics data by focusing on transcriptionally active genes 	<p>Genes encoding antibiotic resistance, drug metabolism, stress response pathways and <i>pks</i> genes</p>

Meta-omics	Microbial Material	Outcome	Advantages	Limitations	Clinical Applications	Key Targets Identified
Metaproteomics	Proteins	Protein expression profiling	Reveal different proteins being synthesized and expressed from active microbiota across multiple conditions	<ol style="list-style-type: none"> 1. Technologically challenging 2. No unique protocol 3. Bioinformatic analyses of protein mass or sequence is complex and time-consuming 4. Metagenome sequences needed 5. Heterogeneous stability of proteins 6. Many unknown proteins 6. Low coverage of protein landscape 	<ol style="list-style-type: none"> 1. Confirming microbial functions 2. Finding functional sequences and potential roles 3. To verify metagenomics and metatranscriptomic data 	Glycoside hydrolases; proteins involved in energy production, cellular respiration, regulation of gene expression, and proteolysis; proteins involved in pili, flagella, vitamins and short-chain fatty-acid biosynthesis, and the production of acetyl phosphate, acetyl-CoA, pyrimidine and propanediol
Metametabolomics	Metabolites	Metabolite profiling	Deeper insights into the metabolic performance of the active fraction of the microbiota at any condition	<ol style="list-style-type: none"> 1. Lack of reference databases 2. No unique protocol 3. Many unknown metabolites in databases 4. Strict identification of compounds is tedious 	<ol style="list-style-type: none"> 1. Identifying and confirming new microbiota and host metabolic alterations 2. Finding metabolic biomarkers 3. Ranking the impact diseases and interventions based on metabolic alterations 	Phytochemicals (glucosinolates, polyphenol, aglycones), N-acyl amino acids and polyamides (including arachidoyl glycine, N-stearoyl proline, N-oleoyl (iso)leucine, N-stearoyl tyrosine and N-palmitoyl threonine), short chain fatty acids, long linear and branched saturated and unsaturated fatty acids, lipids (including gluco-, glycero- and glycerophospho-lipids), bile acids, ceramides and sphingolipids, cholesterol derivatives, amino acids (i.e. tryptophan, histidine, tyrosine, and phenylalanine), metabolites implicated in porphyrin and iron metabolism (ferroxamine, protoporphyrin IX and mesoporphyrin IX), vitamins, polyols, sugars, trimethylamine-N-oxide (TMAO), carnitine, N-acetylmuramic and N-acetylneurameric acids, N-acetylglucosamine, ribose-1,5-bisphosphate, thiamine, choline, acetylputrescine, inosine, pseudouridine, hypoxanthine, creatinine, N-acetylhistamine, glyoxylic acid, succinic acid and homoserine lactone

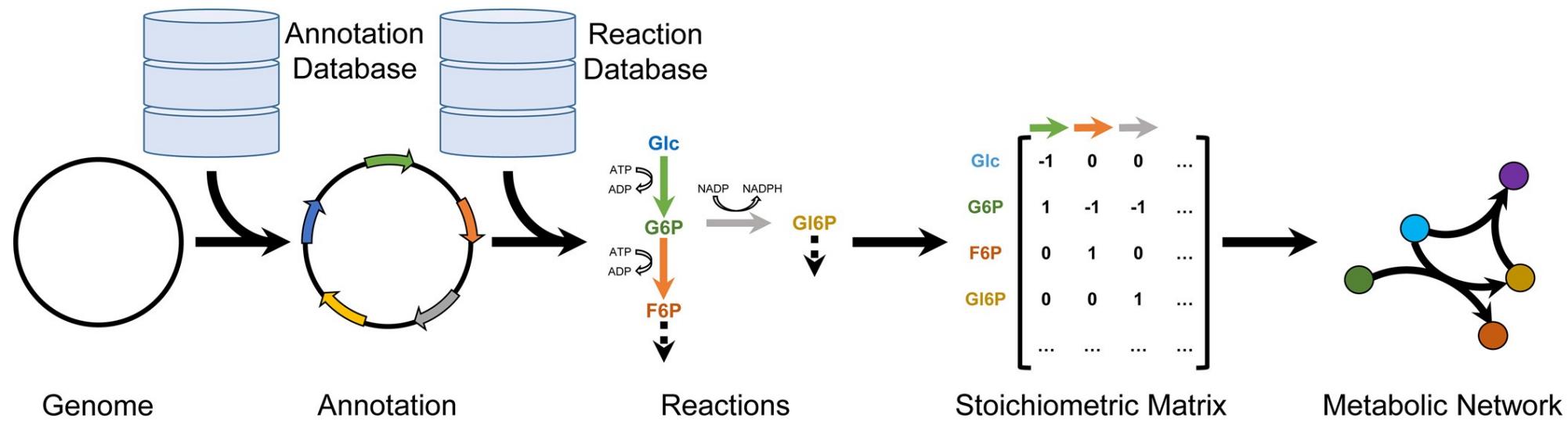
Host-Microbiome Systems Biology



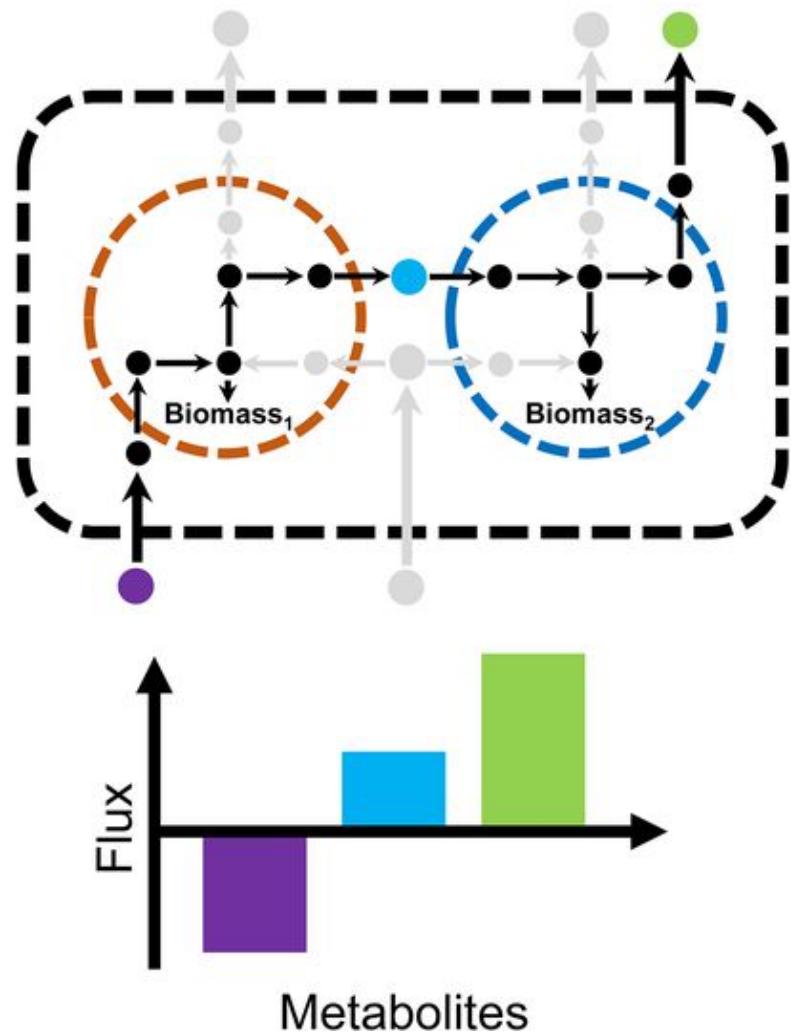
A Data-driven (top-down) networks (e.g., co-occurrence correlation network)



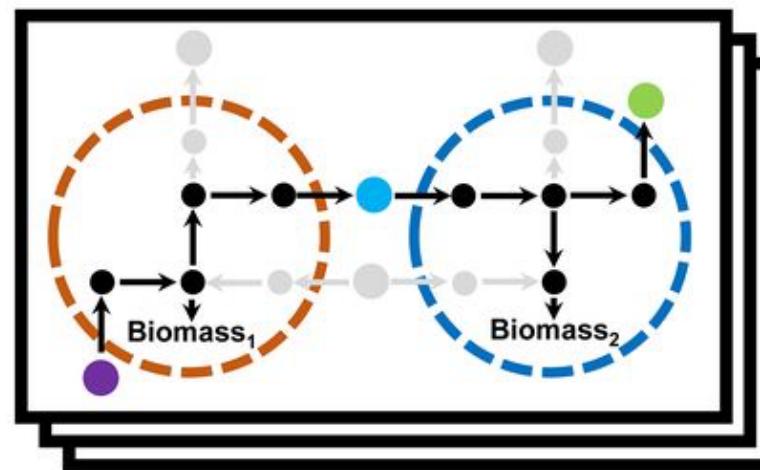
B Knowledge-driven (bottom-up) networks (e.g., genome scale metabolic reconstructions)



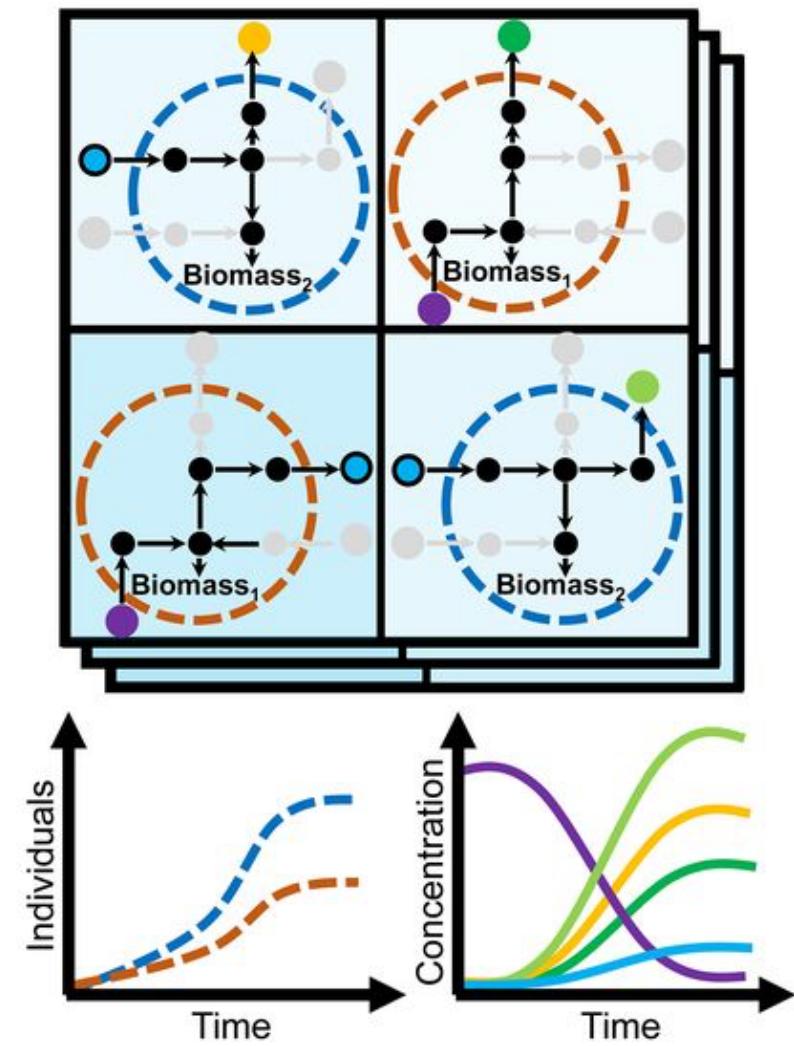
A Compartmentalized community



B Population based community

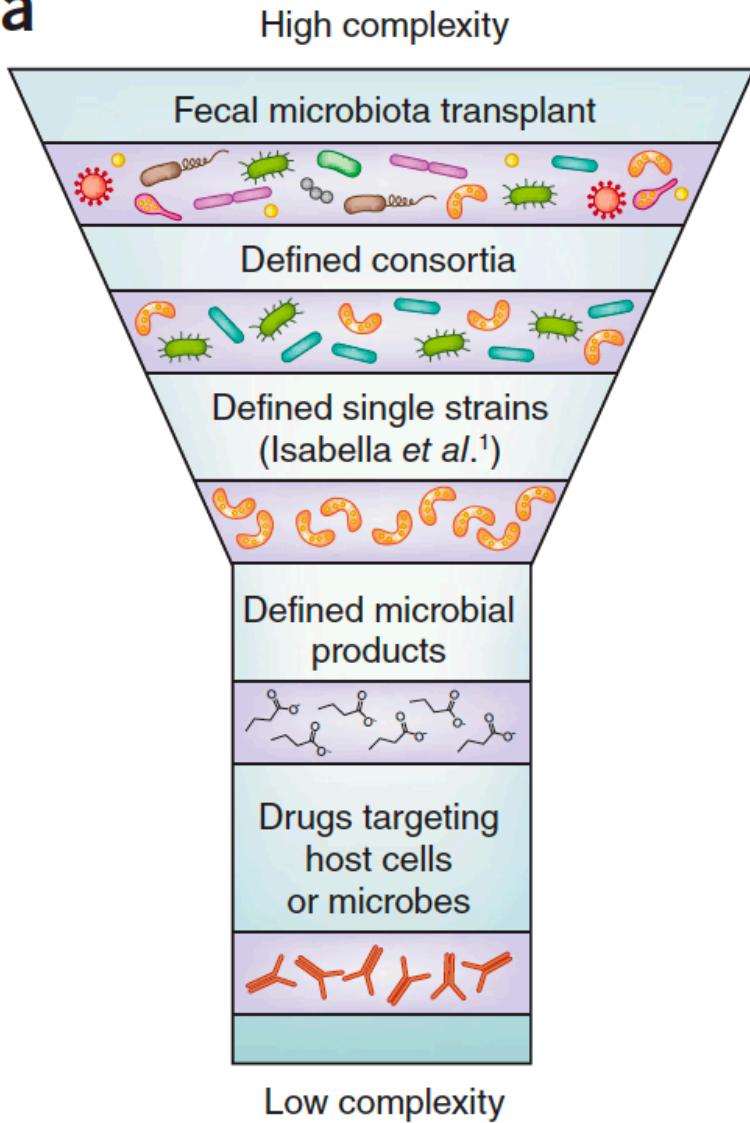


C Individual-based community



Synthetic live bacteria to treat human diseases

a



b

Synthetic bacteria	Disease	Phase
<i>E. coli</i> Nissle 1917 expressing nematode cystatin	Inflammatory bowel disease	Preclinical (pigs)
<i>E. coli</i> Nissle 1917 expressing N-acylphosphatidylethanolamines	Obesity	Preclinical (mice)
<i>L. lactis</i> expressing E7 HPV-16 antigen	Cervical cancer	Preclinical (mice)
<i>Lactobacillus jensenii</i> expressing antiviral cyanovirin	HIV	Preclinical (macaques)
<i>Lactobacillus gasseri</i> expressing glucagon-like peptide (GLP) 1	Diabetes	Preclinical (rats)
<i>L. lactis</i> expressing human trefoil factor 1	Oral mucositis	Human clinical trial (phase 1b)
<i>L. lactis</i> expressing human elafin	Inflammatory bowel disease, celiac disease	Preclinical (mice)
<i>E. coli</i> Nissle 1917 expressing phenylalanine hydroxylase	Phenylketonuria	Preclinical (mice and primates)
<i>B. ovatus</i> expressing human TGF- β 1	Inflammatory bowel disease	Preclinical (mice)