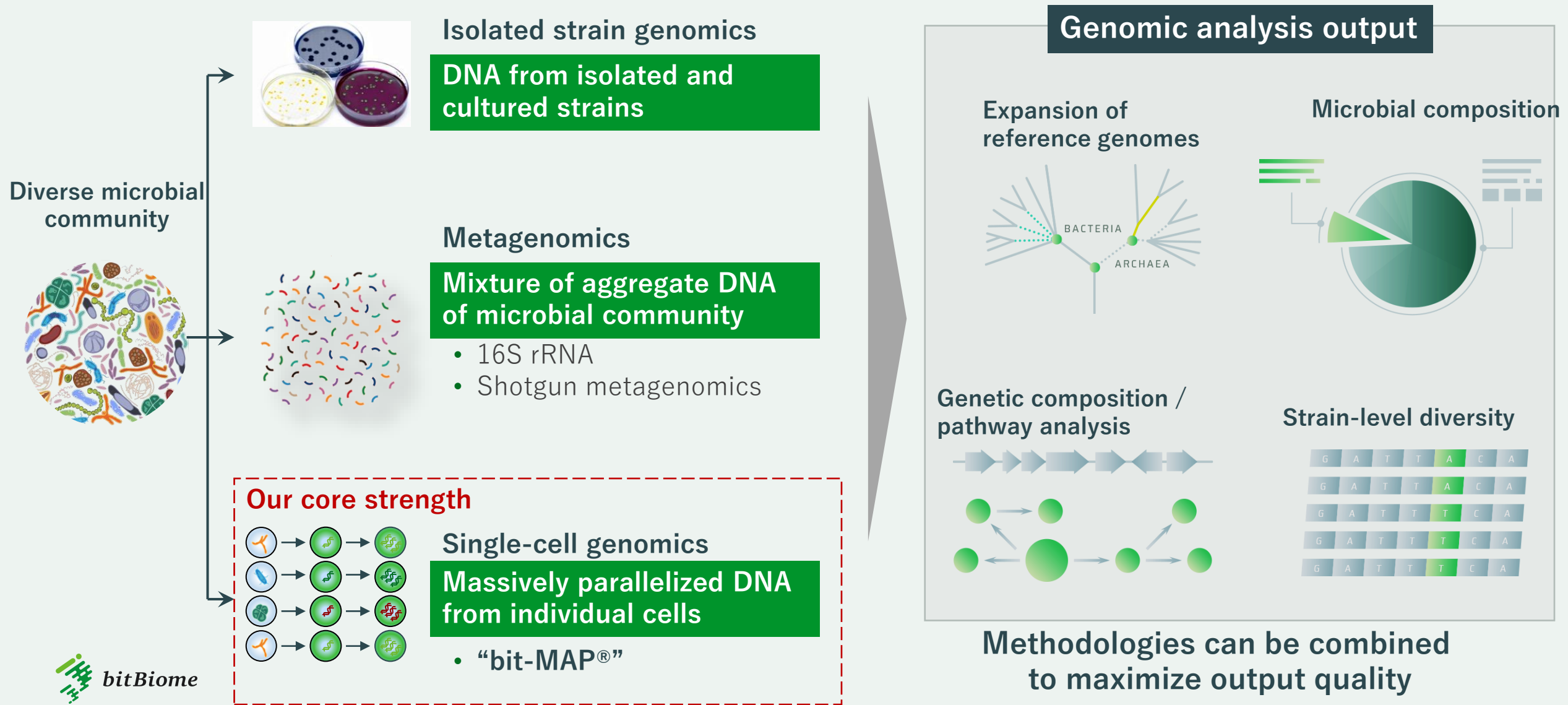


Unlock the Potential of Microbes

Single-cell microbial genomics to
bridge novel findings to industry utilization



Single-cell genomics for microbes: A novel tool for microbial genome analytics



















What we want to achieve: next-gen applications through a microbial genome catalogue combined with meta-info



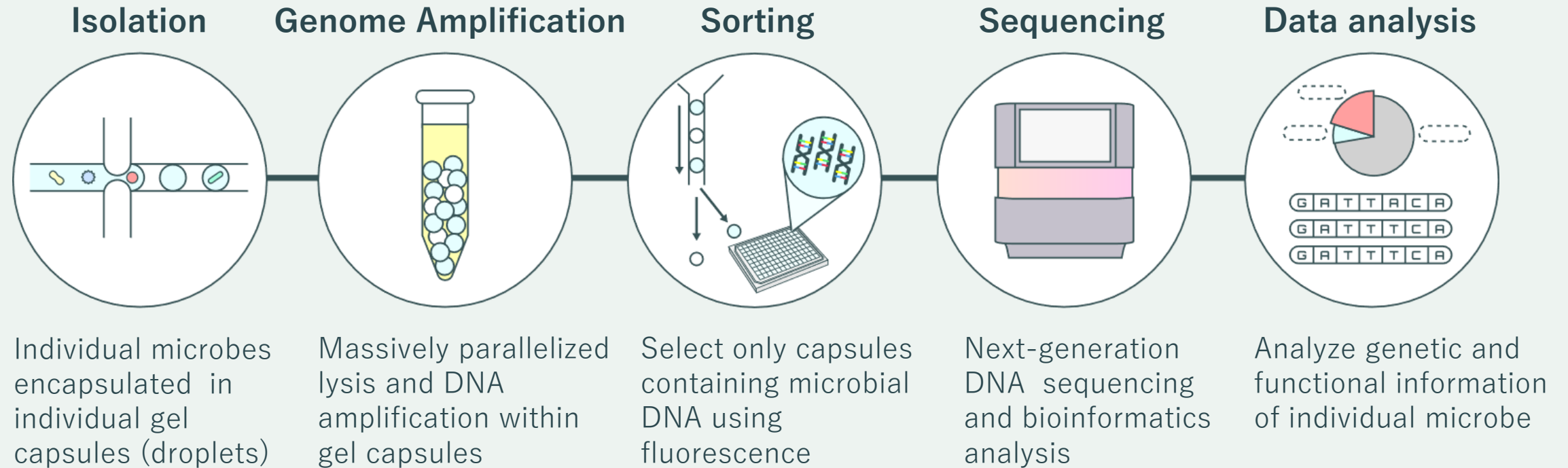
From complex microbial community..



			
Actinobacteria	Aquificales	Bacteroidetes	Chlamydiales
			
Cyanobacteria	Deinococcus-Thermus	Dictyoglomi	Firmicutes
			
Fusobacteria	Gemmatimonadetes	Planctomycetes	Proteobacteria
			
Spirochaetes	Tenericutes	Thermotogae	Verrucomicrobia

..to individual microbial genome catalogue via single-cell genomics

bit-MAP[®] bitBiome Microbiome analysis Platform



— bit-MAP[®] single-cell microbial genomics



High resolution

- Strain-level analysis (beyond species-level)



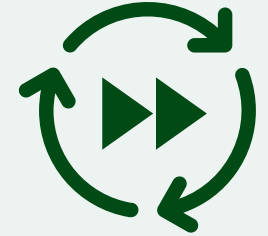
High data quality

- High genome coverage
- No contamination



Broad spectrum

- Compatible with a wide variety of microbiomes (Gram+/-)



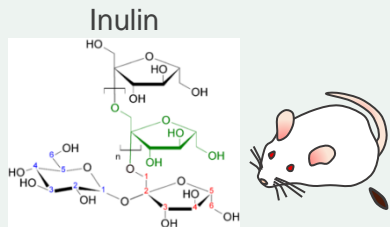
High throughput

- High throughput reduces cost

bit-MAP[®] identified species of bacteria that respond to dietary fiber

Chijiwa et al., Microbiome 2020,
<https://doi.org/10.1186/s40168-019-0779-2>

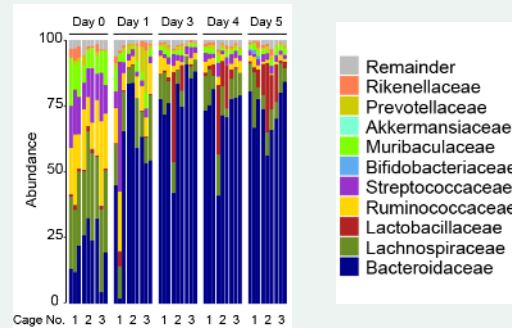
Combination of single-cell and metagenomics identified microbiome strains responding to dietary fiber in the mouse gut



Inulin-supplemented diet for 2 weeks

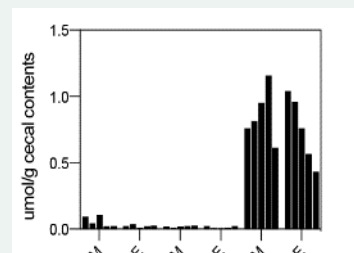
Metagenomics

Increase of *Bacteroides* sp.



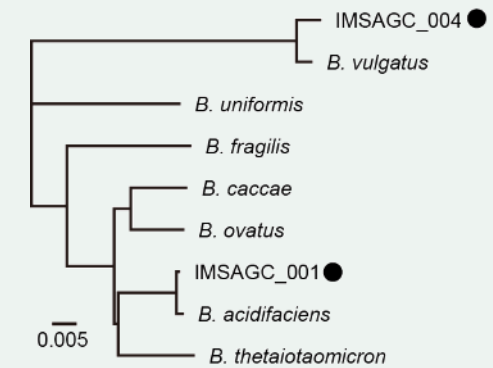
Metabolomics

Increase of succinate in cecum

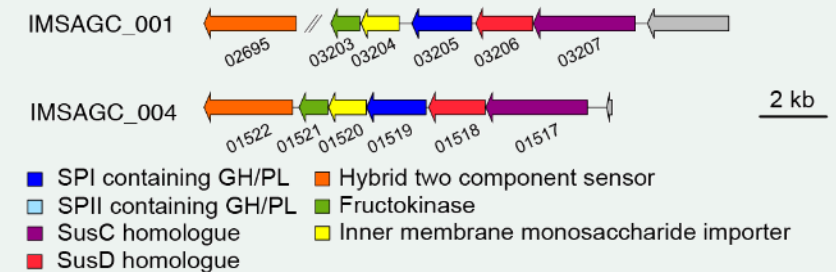


Single-cell genomics

Analysis of more than 300 single-cell genomes; identification of uncultured *Bacteroides* sp. genomes



Identification of inulin utilization gene cluster and succinate synthesis pathway from draft genomes



bit-MAP[®] provides high and medium quality draft genomes from gut microbiota at once

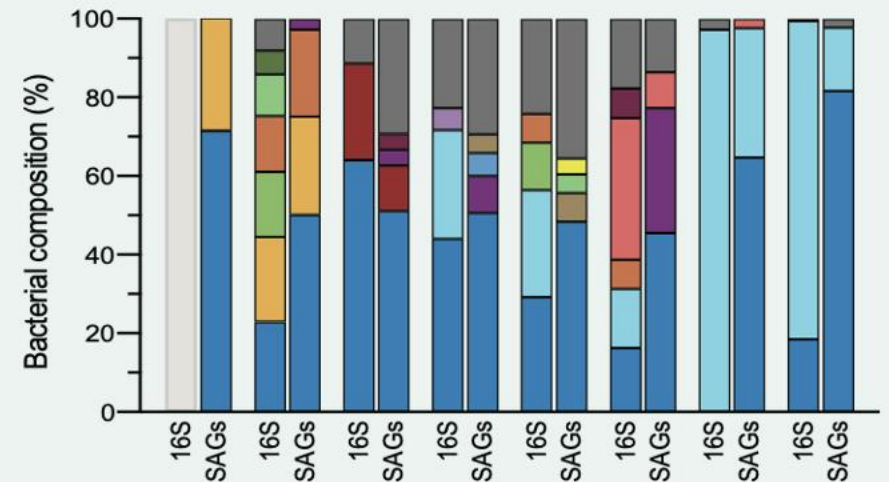
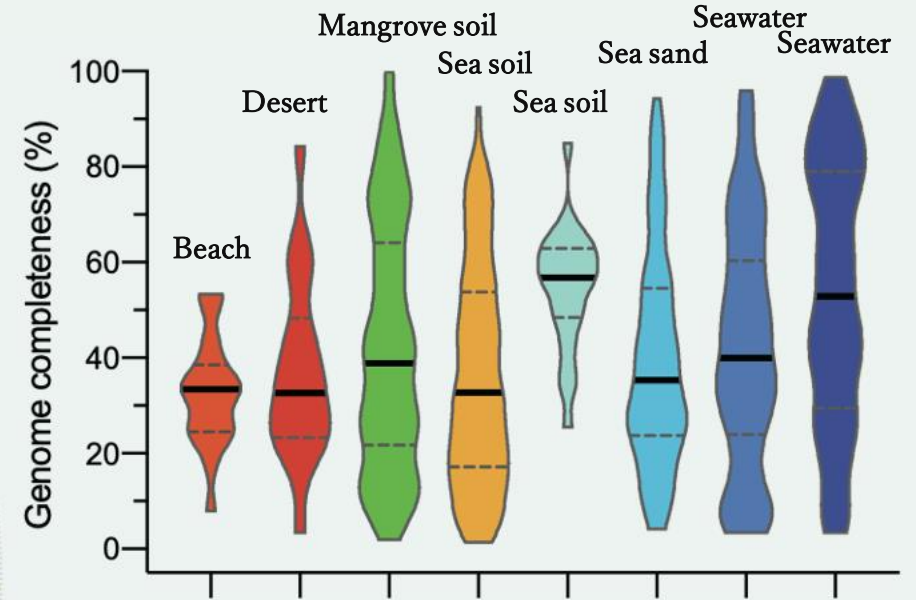
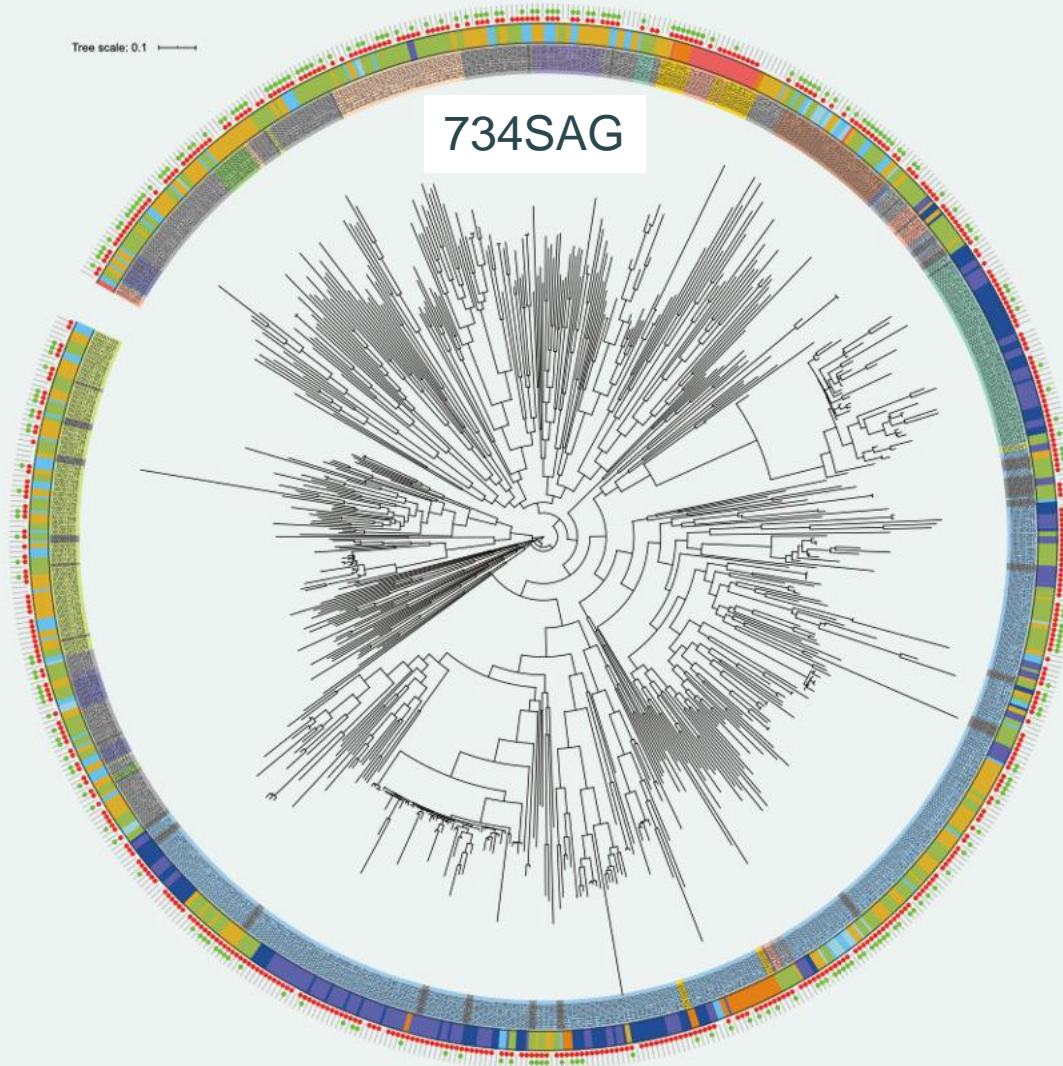
Chijiwa et al., Microbiome 2020,
<https://doi.org/10.1186/s40168-019-0779-2>

SAG ID	Lineage ^a	# contigs	Total length (Mb)	N50 (bp)	GC (%)	# CDS	Completeness (%) ^b	Contamination (%) ^b	SAG quality ^c
IMSAGC_001	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides acidifaciens	369	4.93	29923	43.1	4165	97.27	1.12	High
IMSAGC_002	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;28-4	709	5.08	13000	44.6	4725	93.87	1.78	Medium
IMSAGC_003	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;CAG-65	308	4.62	48295	50.7	4203	98.85	3.97	Medium
IMSAGC_004	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides_B	305	4.22	34968	43.1	3598	98.62	0.8	Medium
IMSAGC_005	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;CAG-95	317	4.23	39279	43.6	4110	96.45	1.45	Medium
IMSAGC_006	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;UBA3263;GCA_001689615.1	265	2.42	24935	50.4	2284	85.47	0.89	Medium
IMSAGC_007	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae	496	5.06	25158	47.1	4957	95.89	3.59	Medium
IMSAGC_008	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;CAG-873	380	2.66	16733	52.1	2436	78.2	1.58	Medium
IMSAGC_009	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;CAG-95	597	5.02	23340	44.4	4633	95.33	2.87	Medium
IMSAGC_010	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus johnsonii	54	1.91	104736	34.3	1789	99.22	0.78	High
IMSAGC_011	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;COE1	431	4.25	27757	37.9	3664	92.66	2.59	High
IMSAGC_012	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;Eubacterium_J	419	3.92	25561	45.0	3748	92.92	4.75	Medium
IMSAGC_013	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;14-2	884	5.14	11980	44.6	4945	85.42	0.95	Medium
IMSAGC_014	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;F0040	273	2.64	20006	47.1	2340	95.18	1.58	Medium
IMSAGC_015	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;Dorea	388	2.55	12891	45.3	2429	67.29	2.53	Medium
IMSAGC_016	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;CAG-485	385	1.94	8558	46.4	1735	56.91	0.5	Medium
IMSAGC_017	Firmicutes;Bacilli;Erysipelotrichales;Erysipelatoclostridiaceae;Erysipelatoclostridium;Erysipelatoclostridium cocleatum	299	2.69	19801	28.9	2384	95.28	0.94	Medium
IMSAGC_019	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;Dorea	311	2.42	20896	44.1	2370	62.98	0	Medium
IMSAGC_020	Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;CAG-95	756	4.13	11653	45.8	4150	57.89	2.63	Medium
IMSAGC_021	Bacteria;Firmicutes_A;Clostridia;Lachnospirales;Lachnospiraceae;Dorea	642	3.03	9299	45.8	2954	60.15	2.58	Medium
IMSAGC_027	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;CAG-1031;GCA_001689585.1	314	2.00	15566	50.5	1738	59.25	0	Medium
IMSAGC_028	Bacteroidota;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes	265	1.73	22105	52.5	1581	56.76	1.92	Medium
IMSAG_013	Firmicutes_A;Clostridia;Oscillospirales;CAG-272	229	1.72	16943	45.4	1622	59.65	0	Medium
IMSAG_025	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;CAG-485	667	2.51	6077	45.3	2558	50	7.76	Medium
IMSAG_044	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_H;Lactobacillus_H reuteri_A	200	1.82	17958	38.3	1735	86.54	0.54	Medium
IMSAG_049	Firmicutes_A;Clostridia;Lachnospirales	317	1.74	11294	39.6	1762	54.88	1.34	Medium

bit-MAP[®] revealed complex microbial diversity in various environmental samples

Nishikawa et al., bioRxiv 2020,
<https://doi.org/10.1101/2020.03.05.962001>

- Marker tophit phylum**
- ◆ Proteobacteria
 - ◆ Desulfobacterota
 - ◆ Cyanobacteriota
 - ◆ Bacteroidota
 - ◆ Patescibacteria
 - ◆ Bdellovibrionota
 - ◆ Actinobacteriota
 - ◆ Firmicutes
 - ◆ Omnitrophota
 - ◆ Planctomycetota
 - ◆ multiple_hits
 - ◆ Others
- Sampling site**
- S1 Beach
 - S2 Desert
 - S3 Mangrove soil
 - S4 Sea soil
 - S5 Sea soil
 - S6 Sea sand
 - W1 Seawater
 - W2 Seawater
- Dataset legend**
- 16S rDNA detected(>500 bp)
 - <97% identity to top hit result

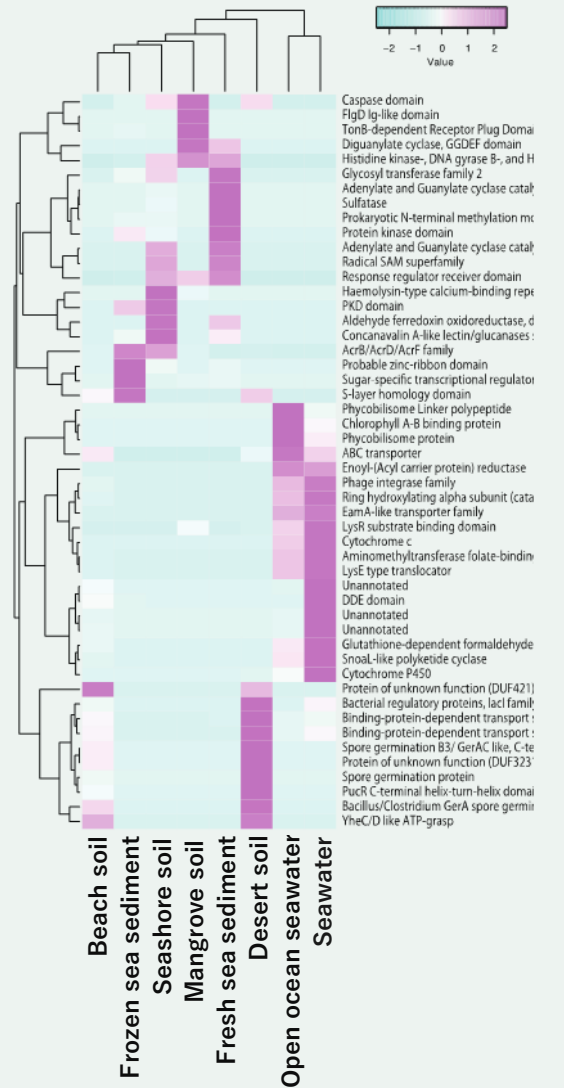


Researchers found that each genome to be almost unique and 98.7% of them were newly identified, implying the complex genetic diversities across 44 phyla.

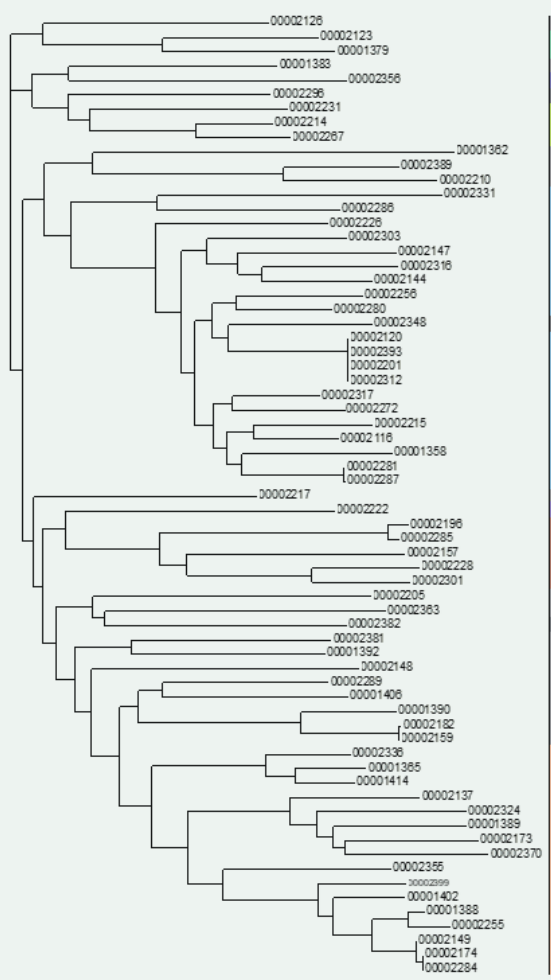
The various metabolic capabilities were found across the lineages at single-cell resolution

Nishikawa et al., bioRxiv 2020,
<https://doi.org/10.1101/2020.03.05.962001>

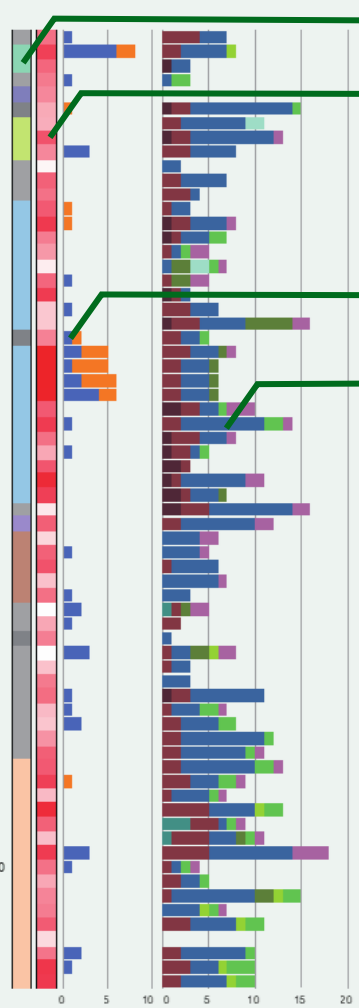
(Overview) Environmental-rich genes



(Detail) Individual cell data



Mangrove soil



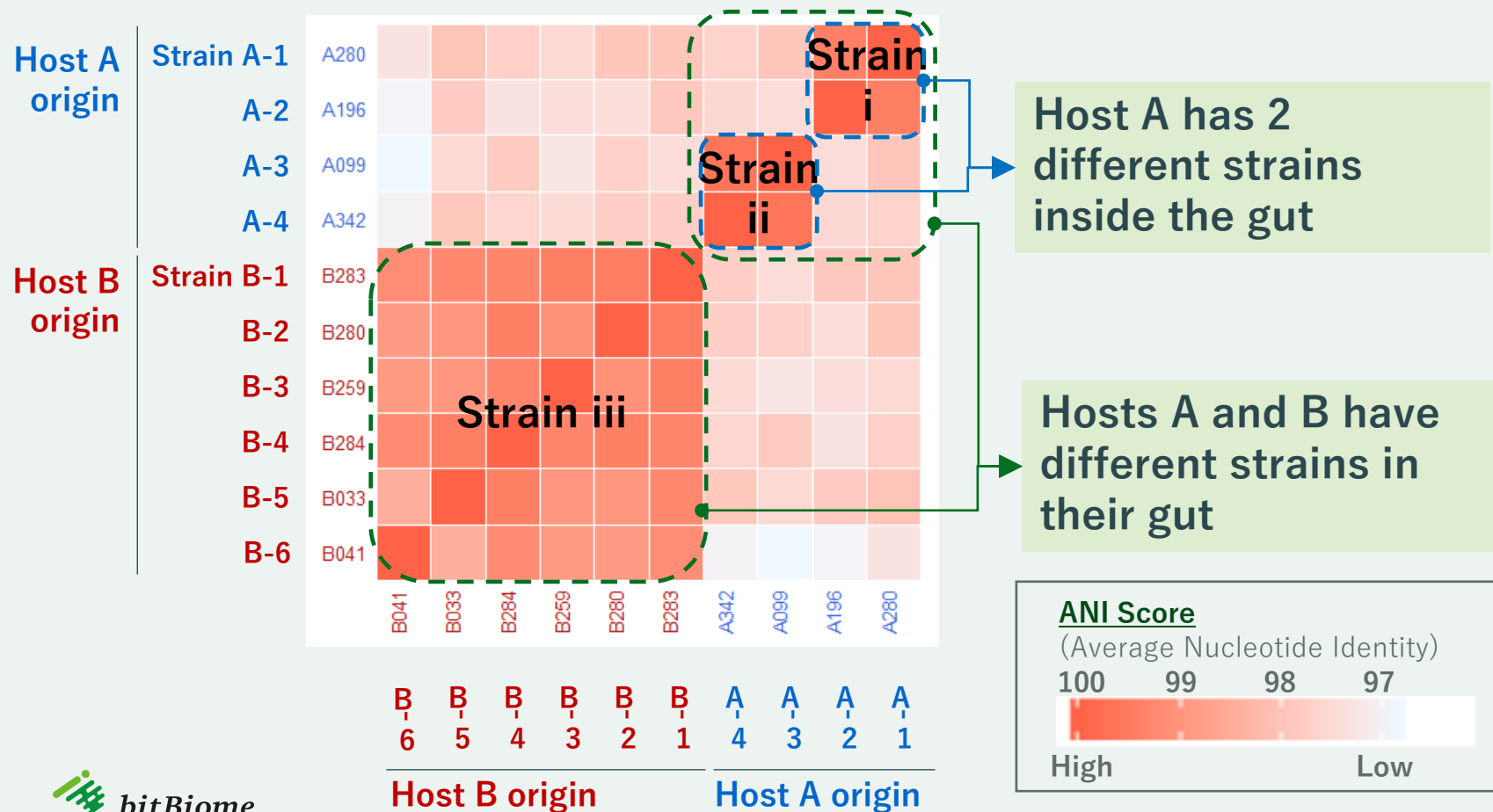
- Taxa
- Genome completeness
- Virulence factor
- Biosynthetic gene cluster

What does single-cell genomics provide?

- Bacterial functions from complex communities
- Massively parallel obtaining draft genomes
- Target cell screening

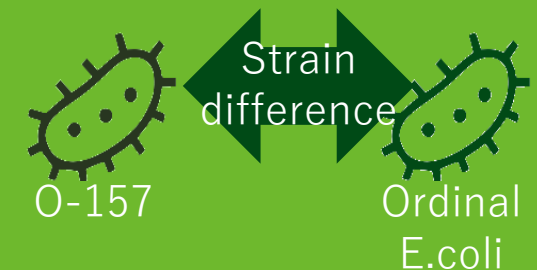
bit-MAP[®] reveals strain-level diversity at single-cell resolution

Genome identity heatmap for microbes which are identified as the same species “X” by conventional 16S rRNA genome analysis (gut microbiome, fecal sample)



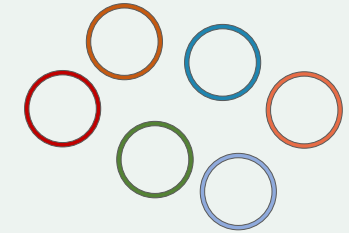
<Impact of strain difference>

O-157, toxin-producing type E. coli., is a different strain of common E. coli

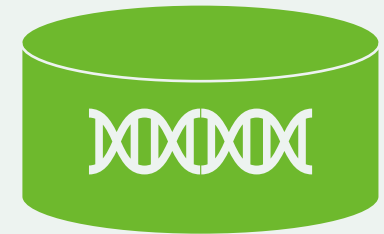


What we provide

Single-cell whole-genome sequence specific to microbes



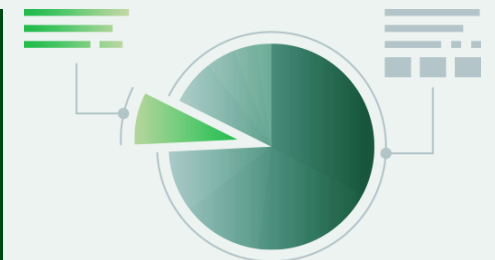
Expansion of reference genome database
Within-species diversity analysis



Functional analyses of strain/whole genome/single-nucleotide variation



Microbiota composition or quantitative analyses can be combined with metagenomics for additional insight



Whereas metagenomics provides an abstract, overall picture...

...Single-cell genomics provides a complete understanding of the target.

16S rRNA metagenomics

- Quickly identifies the members of a microbial community
- Cannot conduct functional analysis; specializes in phylogenetic analysis
- Species with a low presence are treated as “other”

Shotgun metagenomics

- Analyze composition/functions of the entire microbial community
- Comparing related species and obtaining data for rare species are difficult
- Difficult to reconstruct fragmented mixed-species genome sequences

Single-cell analysis using bit-MAP®

- Analyze functions from genome sequences at the strain level for individual microbes
- The original genome sequence is undoubtedly from a single cell
- Analyzes rare and/or difficult-to-cultivate species



bit-MAP® is not a replacement for metagenomics. Rather, bit-MAP® can be used with metagenomics to provide deeper understanding with additional value.

Our Mission

*Unlock the Potential
of Microbes*





bitBiome