

Single-cell genomics for microbes

~Innovating novel findings to industry utilization~



Japan-based start-up formed
Nov. 2018



Through the world's first microbiome
single-cell genome analysis platform,
we provide:

- Analytics & insight



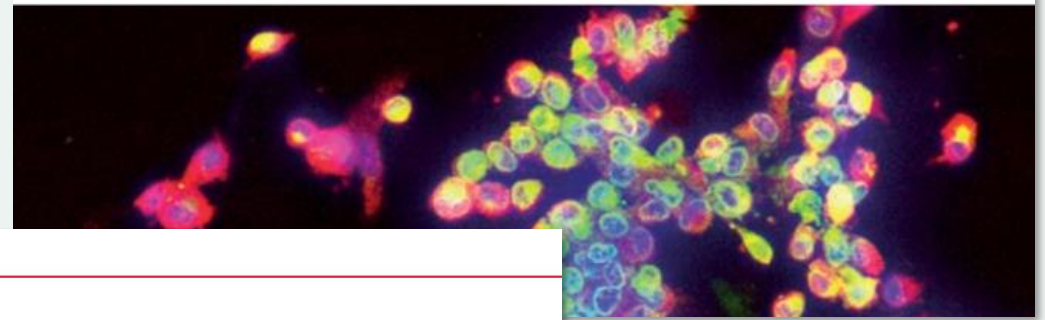
- Product & service
co-development



Illumina, Inc.

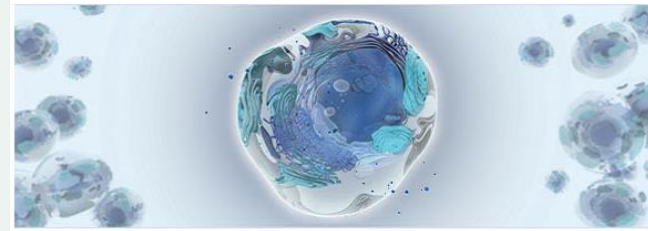
Single-Cell Research

An Overview of Recent Single-Cell Research Publications Featuring Illumina® Technology



QIAGEN

Could droplet microfluids be a game-changer
for single-cell sequencing?



**Our platform is
industry-recognized
as a potential
game-changer**

Microbiomes are untapped resources with potentially massive value



Market size of products derived from microbiomes (global)

55

\$ Billion
(2012)

and growing...

bitBiome's next-generation microbiome analysis method uses single-cell genomics and addresses the challenges of conventional methods

Conventional microbiome genome analyses

Classic isolation and cultivation



16S rRNA metagenomics



Shotgun metagenomics



Challenges

Difficulty of **establishing incubation conditions** for each microorganism, high cost of maintenance

Identifies microorganism classification and prevalence, **but unable to determine function**

Overall community genome analysis; **difficulty in precise linkage between specific gene and the microorganism**

bitBiome's technology: "bit-MAP®"



No need for cultivation



Clarifies functions from complete genome information



Single-cell genomics enable essential understanding of the gene-microorganism linkage

The bit-MAP[®] process

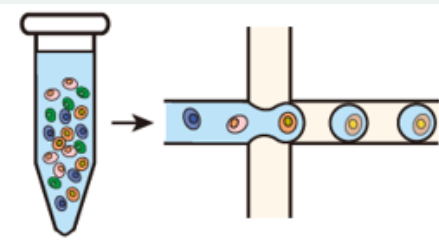
Sample acquisition & preparation

Collection & preparation of samples from skin, fecal, soil, etc.



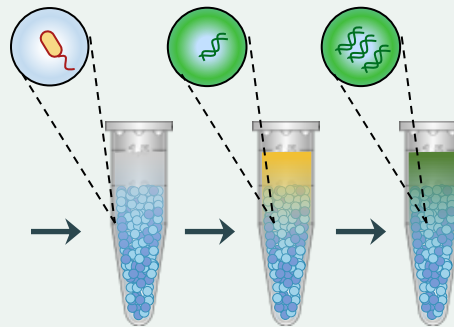
Microbial isolation

Individual microorganisms encapsulated in individual gel capsules (droplets)



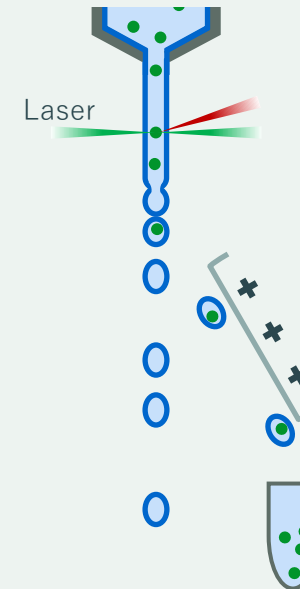
DNA amplification

Massively parallelized lysis and DNA amplification within gel capsules



Gel capsule selection

Select only capsules containing microbe DNA using fluorescence

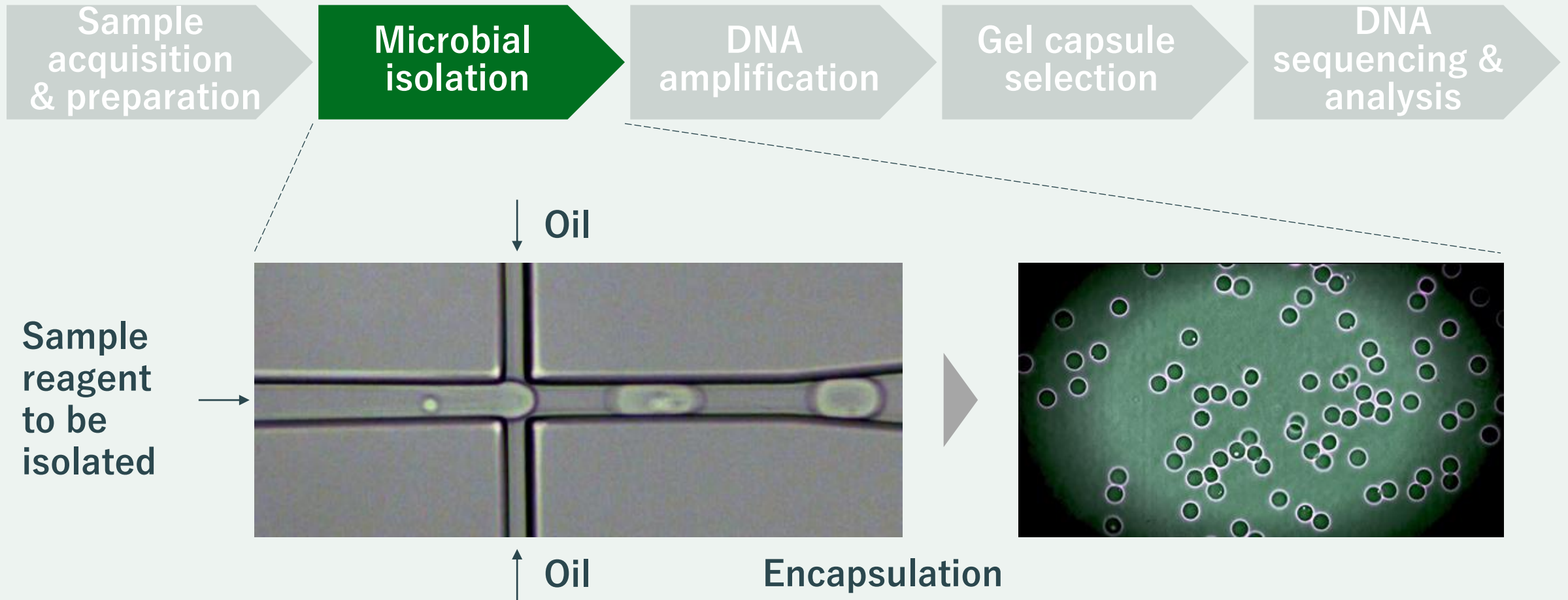


DNA sequencing & analysis

Next-generation DNA sequencing and bioinformatics analysis

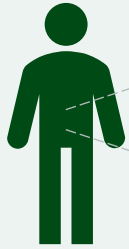


High-throughput microbiome isolation using microfluidics

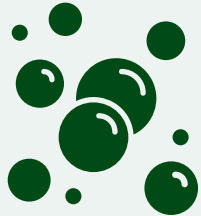


—Ref.) Basic protocol: analyze 384 single cells per sample

1 sample

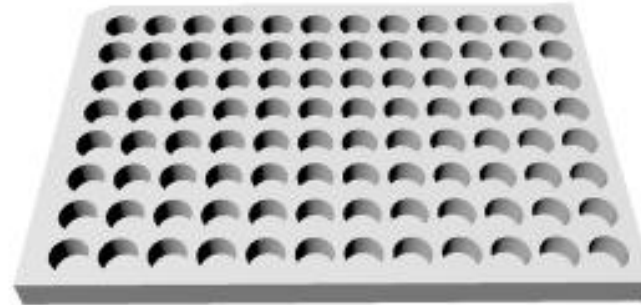


Microbial
community



Isolation
and DNA
amplification
etc.

Analyze genome of
384 individual microorganisms
(use 384 well plate)



- Each well has “an amplified DNA sample from one microorganism”, which is confirmed to be isolated.
- In some cases, some wells include the same microorganism species/strain DNA, because of a biased balance of microorganism proportion, such as dominant microorganism

Analysis report



Genome sequence data



etc.

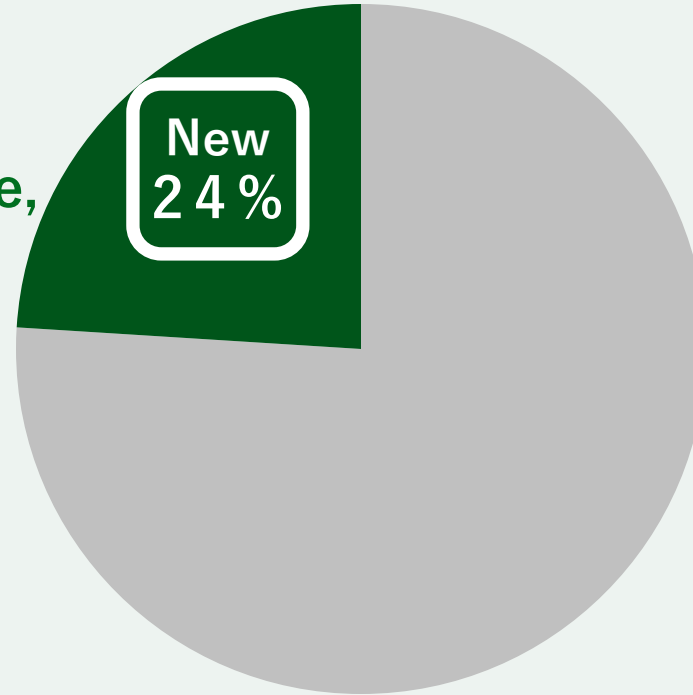
— So what?: We can explosively identify novel species

Microbiome single-cell analysis
of 3 healthy volunteers (fecal sample)¹⁾



Of 771 microbial genomes
identified, **24% (185 genome,
12 species) were new**

(No NCBI database registration)



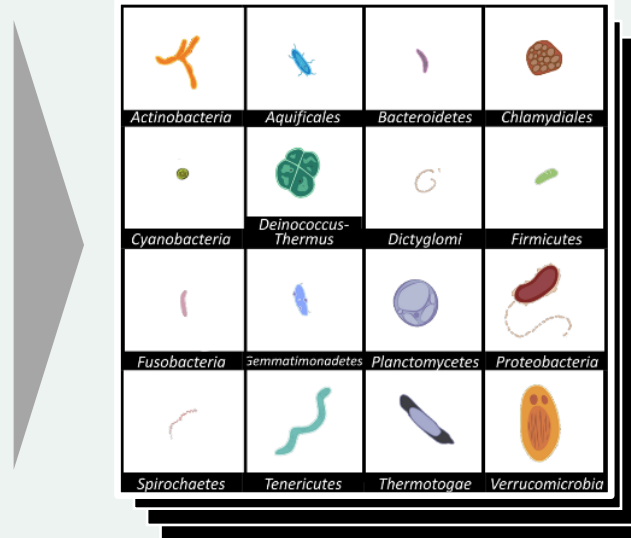
High-quality
human gut microbial genomes
registered
in the NCBI database:
425 species (617 genomes)²⁾

**We added
12 previously unknown
species (+3%) just
from sampling
3 healthy volunteers**

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



From complex
microbial community..



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



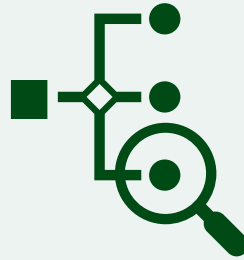
Application in
industries/academia

— Superiority of bit-MAP[®]



Extensive coverage

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



High resolution

- Strain-level analysis (beyond species-level)

Details
on the next page

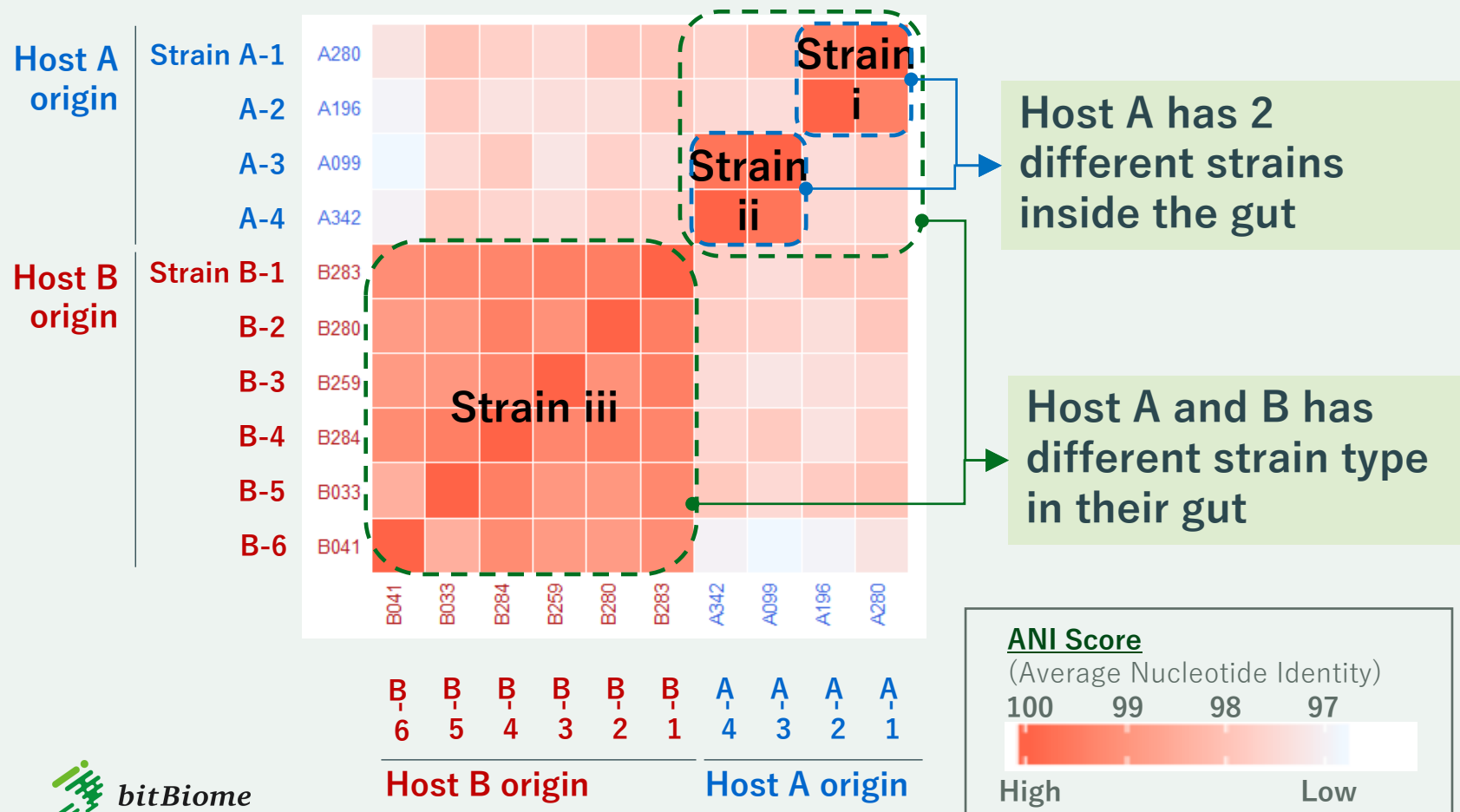


High throughput

- High throughput reduces cost at the same time

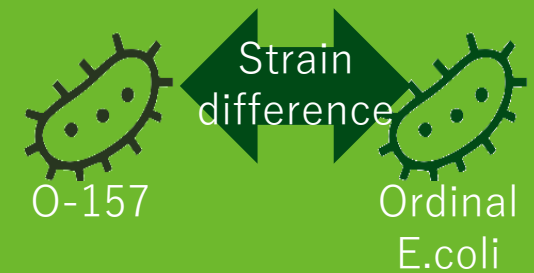
bit-MAP[®] reveals strain-level diversities in human gut microbes at single-cell resolution

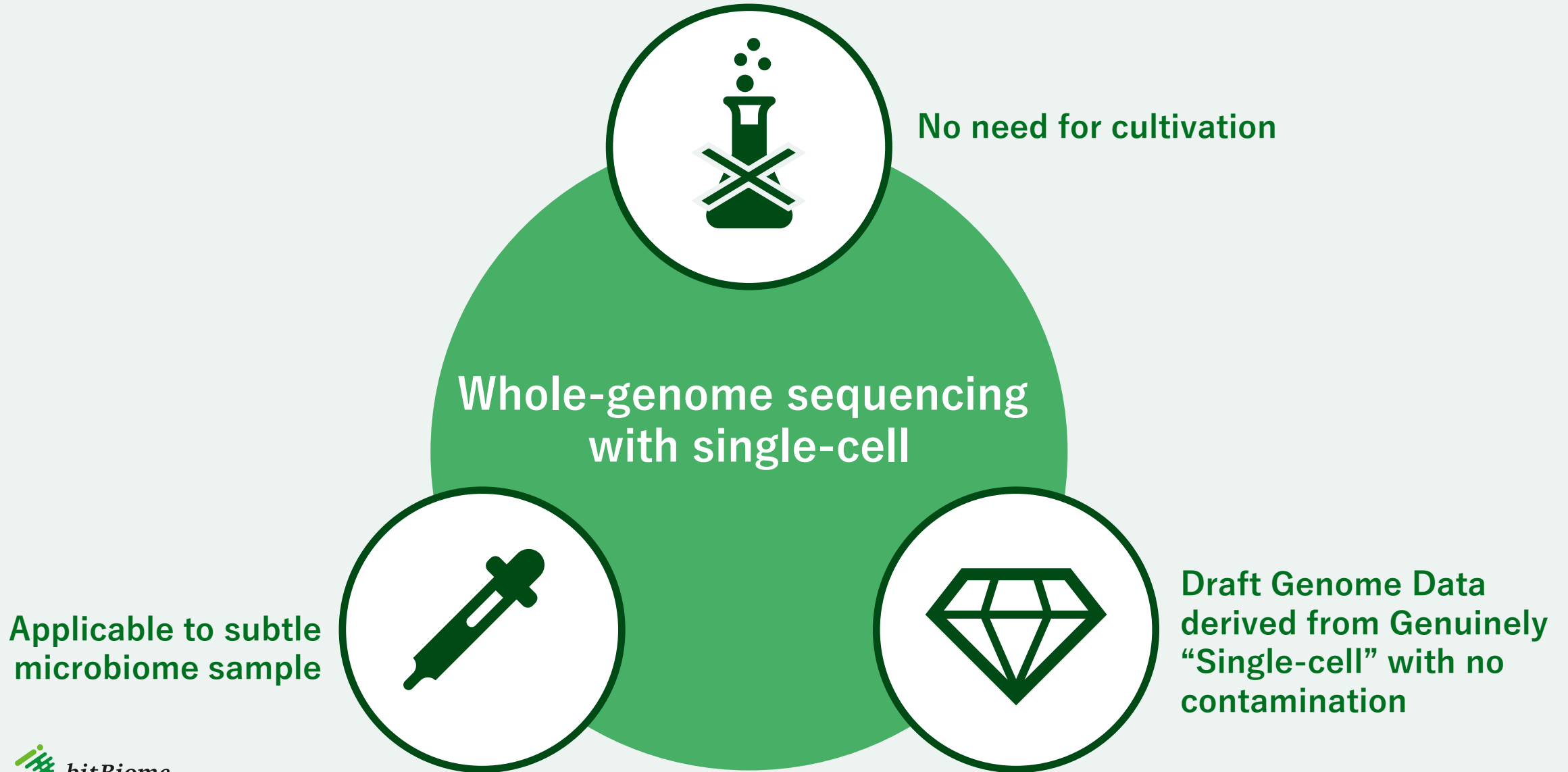
Heatmap of the species “a” strain-level comparison from different hosts (Gut microbiome, fecal sample)



<Impact of strain difference>

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





Technological competitive advantage through seven pending patents and proprietary database

Patent portfolio related to bit-MAP®

Foundational patents



Wet (Biology)



Dry (Bio-Informatics)



Application-oriented patents



Single-cell analysis

Six other pending patents
(Confidential)

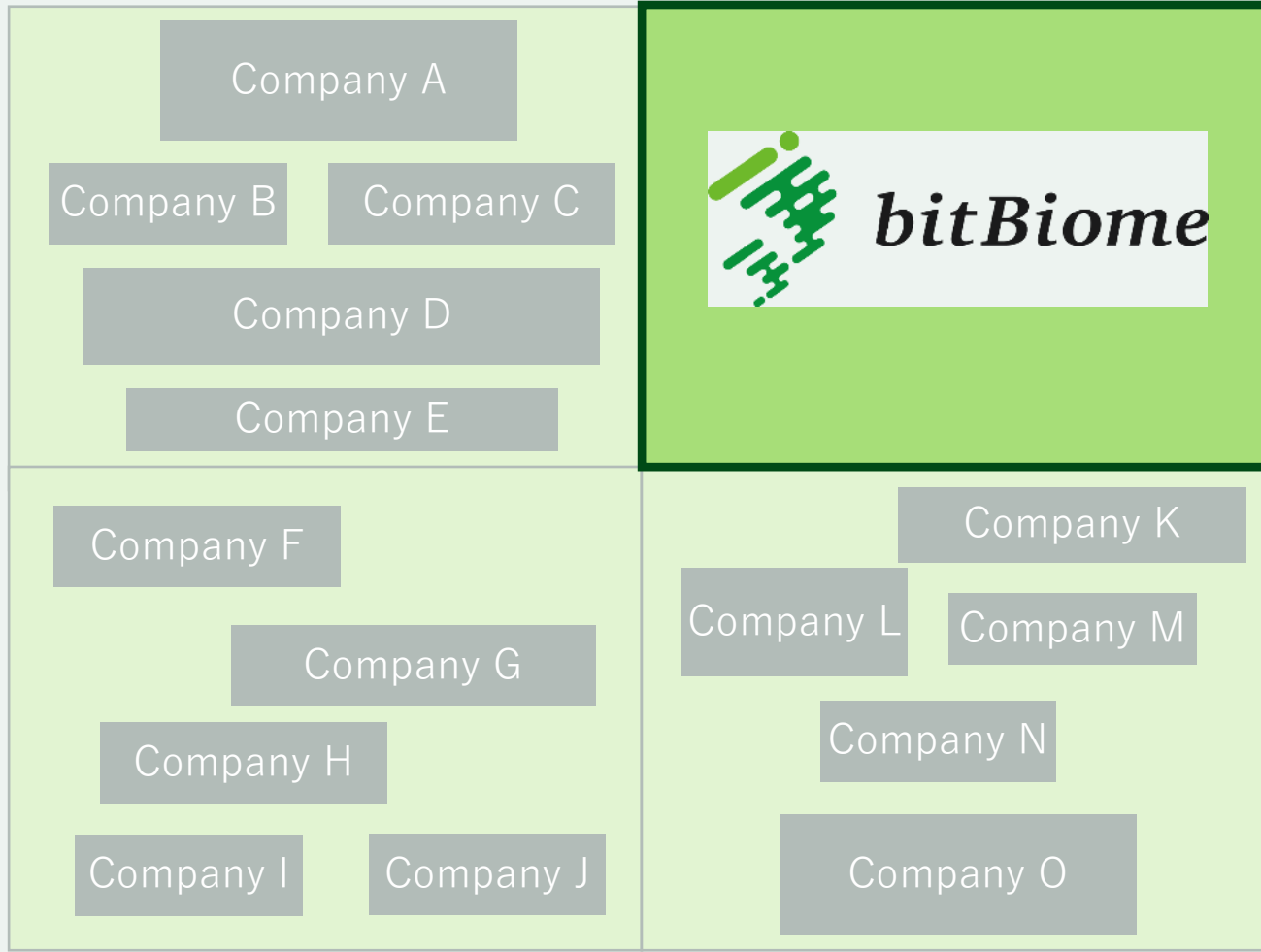


Only bitBiome can conduct single-cell genomics of microbiota

Single-cell
genomics



Meta-
genomics



Human/animal
cell



Microbiome



Technical hurdles of single-cell genomics targeting microbiomes

- Various cell types, making a uniform analysis impossible
 - Different shapes, sizes, and cell wall types
- Small cell size and small amount of DNA per cell
- Contamination risk

Individual strains play critical roles in human health

Microbiome balance is critical for health

Nature Reviews Immunology Role of the gut microbiota in immunity and inflammatory disease

Nobuhiko et al.
volume 13, pages321–335(2013)

- Community gut microbiota dysbiosis correlates with multiple immune-related diseases¹

Strain-level understanding is key

Nature

A defined commensal consortium elicits CD8 T cells and anti-cancer immunity

Takeshi et al.
volume 565, pages600–605(2019)

- Mixture of 11 strains induce CD8 T cells with synergetic effect on immune checkpoint inhibitors

Nature Microbiology

Endogenous murine microbiota member *Faecalibaculum rodentium* and its human homologue protect from intestinal tumour growth

Elena et al.
volume 5, pages511–524(2020)

- F.rodentium*, *H.biformis* strains produce SCFA for an anti-tumor activity

Cell

Host-Specific Evolutionary and Transmission Dynamics Shape the Functional Diversification of *Staphylococcus epidermidis* in Human Skin

WeiZhou et al.
Volume 180, Issue 3, 6 February 2020, Pages 454-470.e18

- S.epidermidis* - strain level diversity affects human skin microbiome

The importance of individual microbial identification led the National Cancer Center and Ministry of Agriculture to select bitBiome as a partner



国立がん研究センター
東病院
National Cancer Center Hospital

National Cancer
Center Hospital

お知らせ一覧

2019.12.13 お知らせ

MONSTAR-SCREEN付随研究、患者さんの腸内細菌叢を解析する研究「BIG BEN study」について、bitBiome株式会社と共同研究契約を締結しました



bitBiome

TECHNOLOGY SERVICE TEAM COMPANY NEWS CONTACT JP / EN

2019.11.28 PRESS

国立がん研究センターとの共同研究契約締結のお知らせ

bitBiome株式会社(代表取締役社長:大門 良仁、以下「bitBiome」)は、国立研究開発法人国立がん研究センター(理事長:中金 斉、東京都中央区 以下「国立がん研究センター」)と癌に関連する腸内細菌の解析に関する共同研究契約を2019年11月22日付で締結しましたので、お知らせ致します。

本共同研究は、SCRUM-Japan第三期プロジェクト MONSTAR-SCREENにおける付随研究として、MONSTAR-SCREENに参加された患者さんのサンプルの一部を用いて、腸内細菌叢のショットガンメタゲノム解析およびシングルセルゲノム解析を実施することで、研究にさらなる付加価値をもたらすものです。

特に腸内細菌叢のシングルセルゲノム解析は、bitBiomeによる日本発・世界初の技術的プラットフォーム(bit-MAP™)であり、がんと腸内細菌叢の関係において、これまで成し得なかった新種の菌の特定や、株レベルの菌のゲノム配列や機能の違いから臨床上的アウトカムを評価するという、世界的にも新たな試みです。

本体研究で得られる血液を用いたがん遺伝子解析(リキッドバイオプシー)と腸内細菌叢解析(16S rRNAメタゲノム)に、本共同研究の腸内細菌叢解析(ショットガンメタゲノム+シングルセルゲノム)を加えることで、様々な体細胞遺伝子変異を同時に検出可能となるため、がんの診断や治療の最適化に大きく貢献することが期待されています。



Source: 国立がん研究センター・農林水産省 web サイト、日本農業新聞 (2019年11月25日) http://www.maff.go.jp/j/press/keiei/kinyu/191105_16.html

農林水産省

Ministry of
Agriculture, Forestry

令和元年11月5日
農林水産省

プレスリリース

「令和元年度農林水産業等研究分野における大学発ベンチャー起業促進実証委託事業」公募にかかる支援対象者の決定について

支援対象者	技術シーズ名
株式会社アグロデザイン・スタジオ	硝化抑制剤のための構造ベース創薬基盤
株式会社セツロテック	受精卵エレクトロポレーション法
bitBiome株式会社	シングルセル技術を用いた微生物のゲノム解析

最先端技術を農業現場にー



農家の側から
働き掛け必要

大学発ベンチャー活躍

梨収穫「相棒」ロボ運人

この5年間に農業分野に参入した大学発ベンチャーの例

大学名	社名	参入する技術や商品・サービス	主な事業内容(計画を含む)	参入年
専攻大学	MIJ labo	牛乳肉の画像分析	牛乳肉の肉質自動評価システムを	2018
大学	(エムアイジェイラボ)	と肉質評価	販売中	
宇都宮大学	アイ・イート	ロボットの設計と製造	農作業支援ロボットを開発、販売中	14
東京大学	アグロデザインスタジオ	酵素の働きを利用	畜産環境改善で作物の生育を高める菌液開発	18
早稲田大学	bitBiome	細胞単位の微生物	微生物を使った農業や肥料などの	13
名古屋大学	(ビットバイオーム)	ゲノム解析	開発	17
名古屋大学	グラントグリーン	植物の成長	植物の成長を促進、収穫までの効率化	17
東京大学	セツロテック	受精卵の電気刺激	受精率向上や、生産性の高い卵を育成	17
東京大学	PLANT DATA (プラントデータ)	植物体の計測技術	完全成長計測装置を販売中	14

「アイ・イート」は、農家の側から働き掛け必要

Whereas metagenomics provides an abstract, overall picture...

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 genomics provides a complete understanding of the target.

Single-cell analysis using bit-MAP®

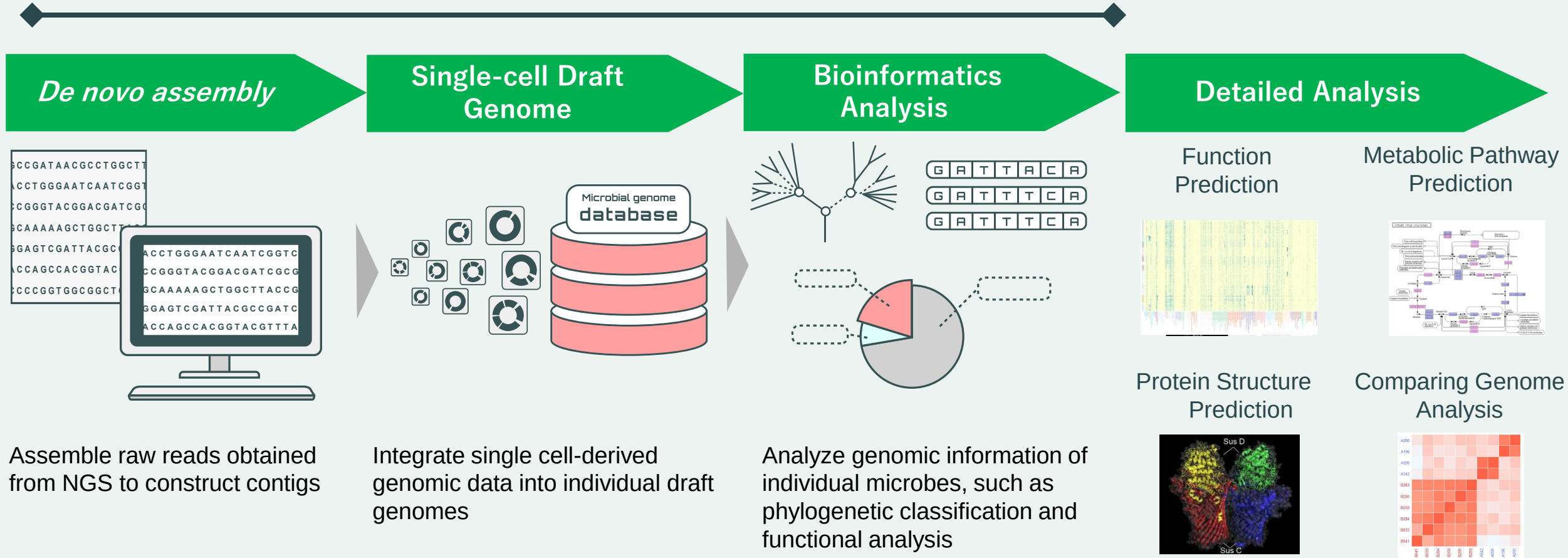
- Analyze functions from genome sequences at the strain level for individual microbes
- The original genome sequence is undoubtedly from a single cell
- Analyzes rare and/or difficult-to-cultivate species



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

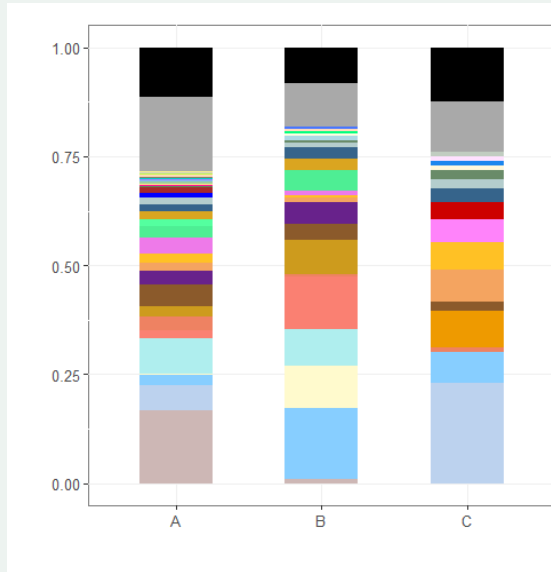
bit-MAP[®]: Bioinformatics Process

bitBiome's proprietary bioinformatics pipeline (automating general tools) enables to analyze hundreds of microbes' genome in a few hours

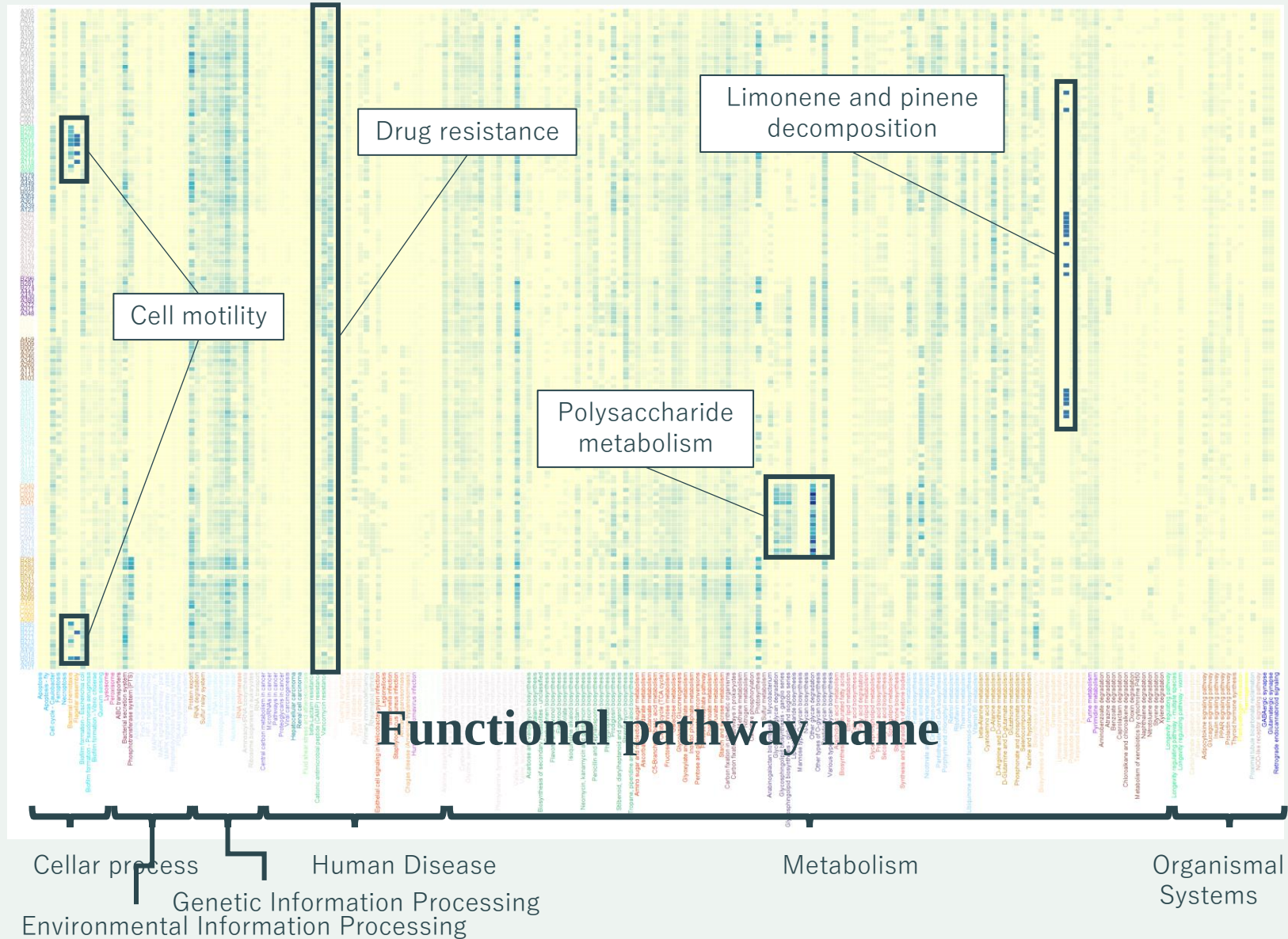


bit-MAP[®] identifies function potentials of individual microbes

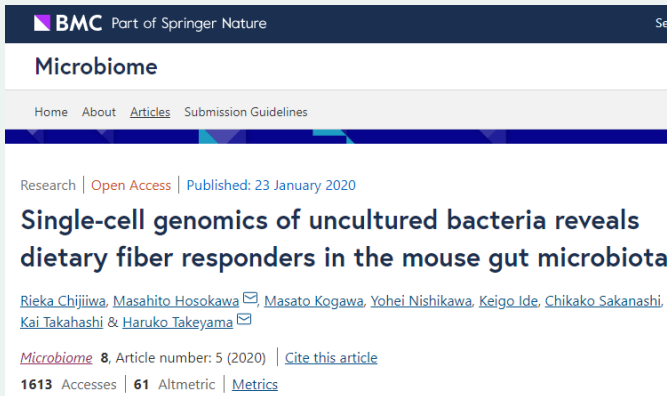
Functional species-level
microbiome catalogue
from stool samples of
healthy volunteers



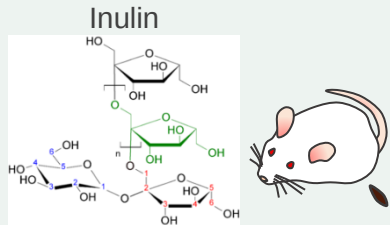
Bacterial species name



bit-MAP®'s unique capabilities were recently highlighted in *Microbiome* (Impact Factor: 11.4) (Jan. 2020; by CSO Hosokawa)



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

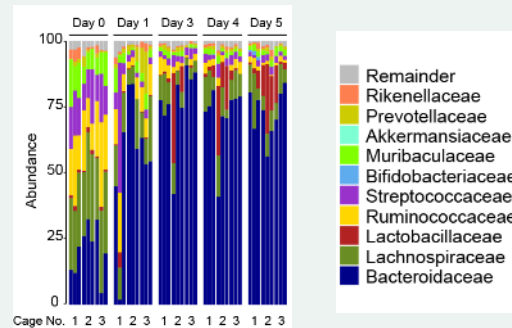


Inulin-supplemented diet for 2 weeks



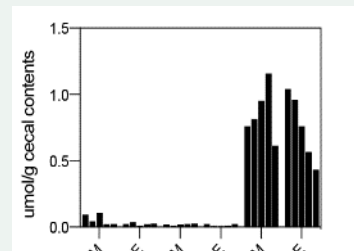
Metagenomics

Increase of *Bacteroides* sp.



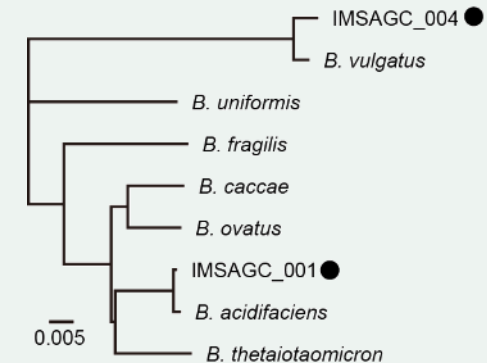
Metabolomics

Increase of succinate in cecum

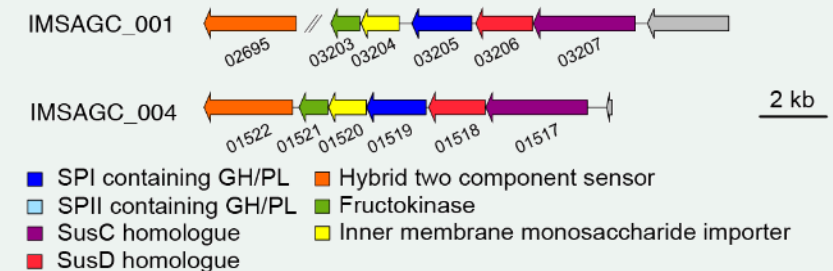


Single-cell genomics

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

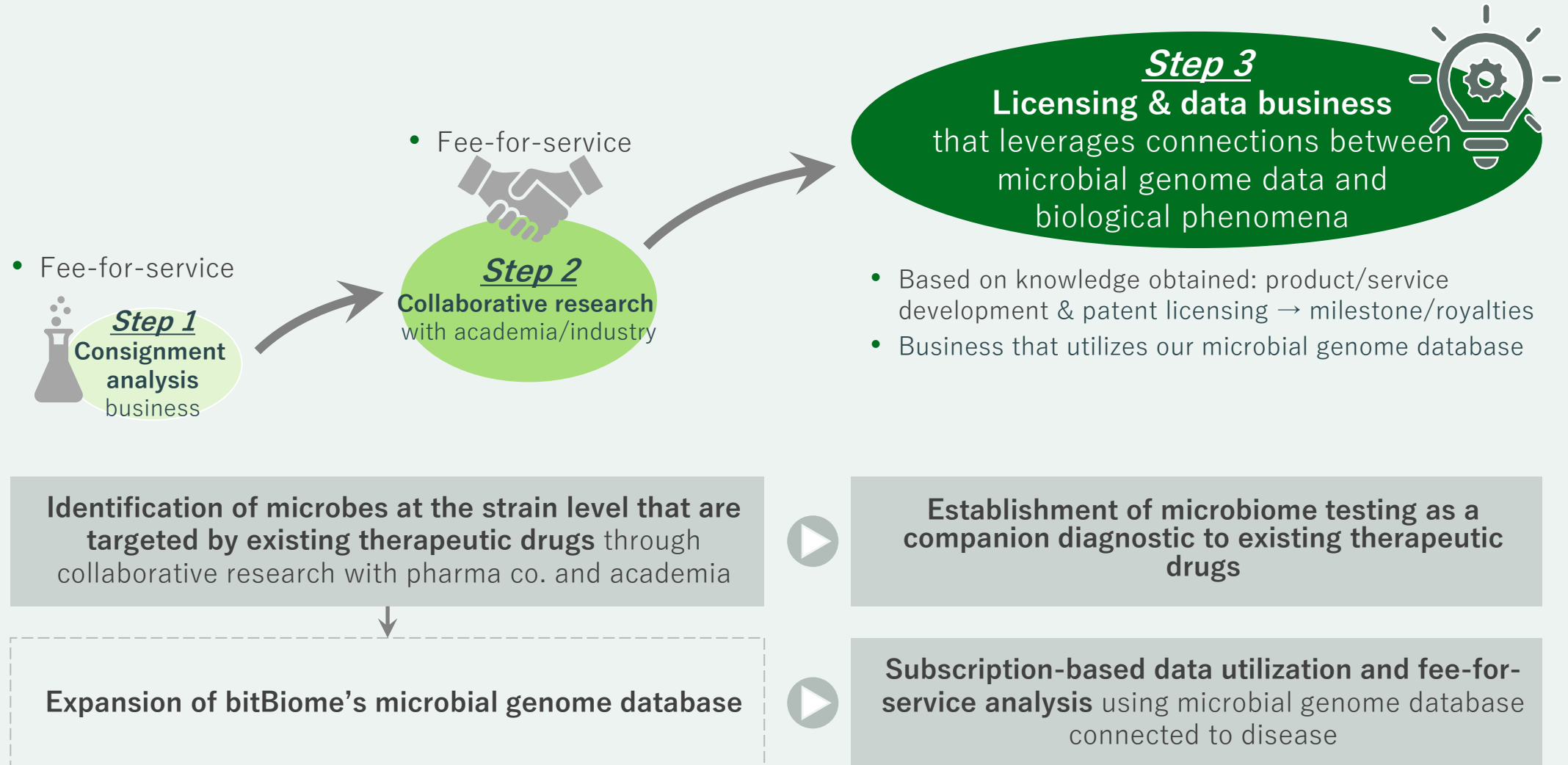


Identification of inulin utilization gene cluster and succinate synthesis pathway from draft genomes



Source: Hosokawa *et al.* Microbiome 2020 Jan 23;8(1):5.

Our business model will evolve from providing technology to a licensing and data business based on intellectual property.



bitBiome's mission

Unlock the Potential of Microbes





bitBiome