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High-quality draft single-cell genome sequences obtained from uncultivated cells using the single-cell sequencing platform bit-MAP®

High-Quality Draft Single-Cell Genome Sequences of Two Gammaproteobacteria Strains Sampled from Soil in a Strawberry Farm" Microbiology Resource Announcement (2020)[1]

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Highlights

- ✓ High-quality draft single-cell genomes of soil microbiota in a strawberry farm were obtained using the single-cell sequencing platform bit-MAP®
- ✓ Not only bacterial but archaeal draft genomes were also obtained

Introduction

A wide variety of bacteria exist in the soil microbiome; one gram of soil is speculated to contain hundreds of millions of bacteria. Many soil bacteria produce useful substances such as antibiotics and are gaining attention as untapped biological resources. In the agricultural field, it has been reported that certain bacteria live in symbiosis with plants, providing growth-promoting effects and pathogen resistance.

Despite their prevalence in the soil environment revealed by 16S rRNA gene amplicon sequencing, little is known about the genomic diversities. Most of the soil bacteria are difficult to culture, and less than 1% of the soil bacteria have been analyzed. Shotgun metagenomics face several challenges in reconstructing individual genomes from diverse metagenomic reads, and conventional single-cell genome analysis of soil bacteria had limited success to amplify their genomes.[2]

To overcome these challenges, we employed bit-MAP® in this study to acquire high-quality single-cell genomes of soil bacteria.

Method

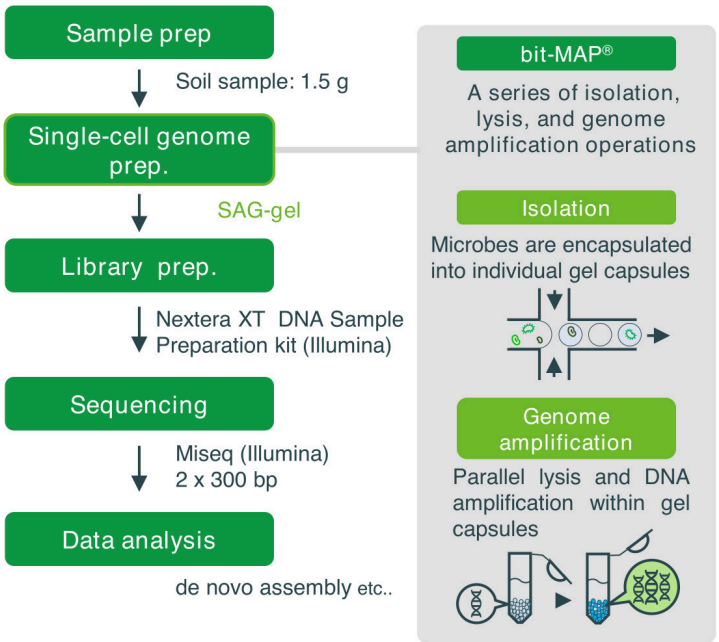

Fig.1 bit-MAP® workflow

- Soil sample preparation
  To extract the bacterial fraction, 1.5 g of the soil samples was suspended in 3 ml of Dulbecco's phosphate-buffered saline (DPBS). The mixture was vortexed and allowed to settle for 15 min and then filtered to separate the soil particles.
- Single-cell encapsulation into gel beads
  Single cells were isolated into gel beads using droplet microfluidic devices, and their genomes were following the SAG-gel method.[3] A pooled paired-end sequence library of 24 single-cell amplified genomes (SAGs) was prepared.
- Sequencing and data analysis
  The sequence reads (Ave. 263.8 Mb) were obtained using Miseq. The sequence reads were assembled using SPAdes,[4] and completeness and contamination ratio were calculated using CheckM.[5]

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Results

Ten high-quality SAGs acquired from soil microbes are shown in Table 1. Based on the operational standards for SAGs[6], we recovered 2 high-quality draft genome※1, and 17 medium-quality draft genomes※2. bit-MAP® 's report shows single-cell genome statistics, including completeness, redundancy, number of contigs, N50, GC contents, number of genes, 16S sequences, rRNAs, tRNAs, similarity, and phylogenetic annotation results by GDTB-Tk(Fig. 2).

※1 Completion >90%, Contamination <5%, Presence of the 23S, 16S, and 5S rRNA genes and at least 18 tRNAs.
※2 Completion >50%, Contamination <10%,

Table 1 Statics of single-cell genomes obtained from soil microbes

◆ High-quality genomes ◆ Extracted gene sequences ◆ Identified novel species

Quality	Comp. (%)	Cont. (%)	#Contigs	N50	GC%	#CDS	#rRNA gene			Taxonomic assignment by 16S BLAST search	Similarity (%)
							5S	16S	23S		
High	92.7	0.6	147	39,139	39.4	1,890	1	1	1	*Legionella dresdenensis* strain W03-356	88.4
High	91.5	1.1	229	41,260	37.5	1,964	1	1	1	*Aquicella siphonis* strain SGT-108	94.6
Mid	89.2	1.1	278	12,379	40.8	1,690	1	1	1	*Coxiella burnetii* strain ATCC VR-615	91.0
Mid	88.0	9.9	241	22,425	40.7	2,084	0	1	1	*Nesterenkonia jeotgali* strain JG-241	78.7
Mid	86.7	0.3	274	13,730	39.4	1,672	1	1	1	*Legionella dresdenensis* strain W03-356	88.4
Mid	86.5	1.4	120	26,444	40.8	1,673	1	0	0	-	0.0
Mid	83.0	1.9	122	24,393	41.1	1,442	0	1	1	*Nesterenkonia jeotgali* strain JG-241	78.7
Mid	80.7	0.5	341	20,258	40.9	2,209	1	1	1	*Haliscomenobacter hydrossis* strain DSM 1100	83.6
Mid	70.6	0.1	84	49,523	40.5	1,576	1	1	1	*Thermocrinis minervae* strain CR11	76.4
Mid	70.3	4.3	95	26,916	33.7	801	1	1	1	*Altererythrobacter xixiisoli* strain S36	77.4

◆ Taxonomic distribution
Gram-positive/negative bacteria and archaea

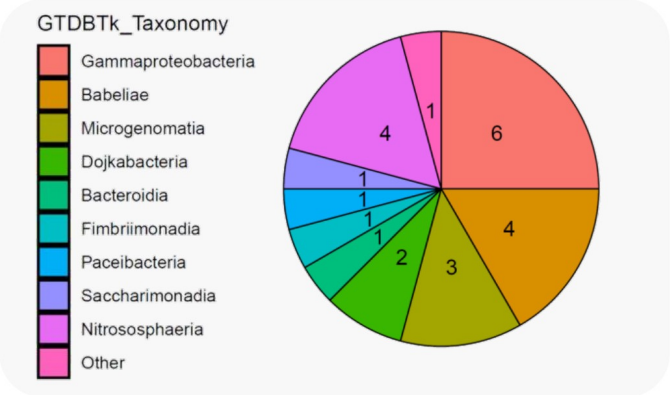

Fig. 2 phylogenetic annotation by GDTB-Tk (class)

Various samples, including environmental sample (soil, sea, river), fecal, skin, oral and so on, can be analyzed with bit-MAP®.

Conclusions

We demonstrated bit-MAP® is capable of generating high-quality draft genomes of diverse single cells from soil microbiome in a high-throughput manner. When used together with metagenomics, bit-MAP® can be to provide deeper understanding of soil bacteria, such as functional insights of individual microbes.

We believe this new single-cell sequencing platform can contribute to expand reference genomes of microbes that are otherwise difficult to obtain.

Reference

1. T. Yoda, et al., High-Quality Draft Single-Cell Genome Sequences of Two Gammaproteobacteria Strains Sampled from Soil in a Strawberry Farm. Microbiology Resource Announcement (2020)
2. C. Rinke, et al., Obtaining genomes from uncultivated environmental microorganisms using FACS–based single-cell genomics. Nature Protocols (2014)
3. R. Chijiiwa, et al., Single-cell genomics of uncultured bacteria reveals dietary fiber responders in the mouse gut microbiota. Microbiome (2019)
4. A. Bankevich, et al., SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology (2012)
5. D. Parks, et al., CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Research (2015)
6. R. Bowers, et al., Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nature Biotechnology (2017)

bitBiome, Inc.

bitBiome provides a unique single-cell, whole-genome analytical platform, called bit-MAP®, **specifically targeting microbes that can be applied to uncultured "raw" samples.**

Contact us for any inquiries on bit-MAP®: info@bitbiome.co.jp