

Agarose gel microcapsules enable easy-to-prepare, picolitre-scale single-cell genomics, yielding near-complete genome sequences

Hiroyoshi Aoki (✉ haoki@riken.jp)

RIKEN

Yuki Masahiro

RIKEN

Michiru Shimizu

RIKEN

Yuichi Hongoh

Tokyo Institute of Technology

Moriya Ohkuma

RIKEN

Yutaka Yamagata

RIKEN

Methods Article

Keywords: agarose gel microcapsule (AGM), genome sequences

Posted Date: February 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-147972/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

A novel type of agarose gel microcapsule (AGM), consisting of an alginate picolitre sol core and an agarose gel shell, was developed to obtain high-quality single-cell amplified genomic DNA of bacteria. The AGM is easy to prepare in a stable emulsion with oil of water-equivalent density which prevents AGM aggregation, with only standard laboratory equipment. Single cells from a pure culture of *Escherichia coli*, a mock community comprising 15 strains of human gut bacteria, and a termite gut bacterial community were encapsulated within AGMs, and their genomic DNAs were obtained with massively parallel amplifications in a tube. The genome sequencing did not need second-round amplification, and showed an average genome completeness that was much higher than that obtained by the conventional amplification method in microlitre scale, regardless of the genomic guanine-cytosine contents. Our novel method using AGMs allows many researchers to perform single-cell genomics easily and effectively, and can enhance the genome analysis of yet-uncultured microorganisms.

Introduction

Microorganisms are ubiquitously distributed in diverse environments. They are often associated with other organisms, and play important roles in ecosystems. However, the majority of microbial species are difficult to culture with conventional methods and are called yet-uncultured microorganisms¹. In the past two decades, they have been extensively studied using culture-independent methods such as amplicon sequencing analysis of small subunit ribosomal RNA (SSU rRNA) genes and shotgun sequencing analysis of metagenomes. Metagenomics is a powerful tool for investigating the ecological and physiological functions of microbiota based on their encoded genetic repertoires. The recent development of the computational binning of metagenomic fragments into respective microbial taxonomic assemblies has reinforced the utility of metagenomics^{2,3}. However, computational binning based on the sequence composition, homology to database sequences, and sequence read coverage of each fragment might often fail to discriminate genomes of closely related (sub)species and to correctly sort genomic regions exhibiting distinct features from others, such as rRNA genes, prophages, and plasmids^{4,5}. Furthermore, a massive sequencing effort is required to obtain the genome sequences of minor species in the microbial community³.

Single-cell genomics, in which whole genomic DNA is amplified from physically isolated single cells⁶, has been used as an alternative or complementary method to reveal the metabolic capacity of yet-uncultured microorganisms¹. Single-cell isolation can be achieved in many cases by using technologies such as fluorescence-activated cell sorting (FACS)¹, micromanipulators⁷, microfluidic devices^{8,9}, and encapsulation in water-in-oil droplets¹⁰. Although various methods for whole genome amplification have been developed¹¹, multiple displacement amplification (MDA) using phi29 DNA polymerase with random primers is the most widely used due to its relative simplicity, reliability, and applicability⁶. However, MDA inherently accompanies extreme amplification bias among genome regions¹, which has been the major technical limitation in single-cell genomics. Additionally, the high guanine-cytosine (GC) content of

genomic DNA tends to increase the amplification bias¹. It has been reported that the MDA in a small reaction volume, for example, when using a microchannel chamber (60 nL)⁹, nano-litre microwells (12 nL)¹², and digital droplets generated by a microchannel (9.53 pL)¹³, can suppress amplification bias. However, these techniques are not available for many microbiologists, and the amount of the amplification product is often too small for the direct genome sequencing and a second-round MDA¹⁴ may be required, which ultimately enhances the amplification bias.

To accelerate study using the single-cell genomics of yet-uncultured microorganisms, it is important to develop a simple method that prepares massive small reaction vessels using commercially available reagents, disposable plasticware, and equipment. For MDA reaction vessels, droplet microchannel massively generates single-cell encapsulating water-in-oil droplets¹⁰. However, MDA reagents are sequentially introduced into the droplets in the microchannel, which complexifies the microchannel structure. Single-cell encapsulating gel beads are easily prepared by gelating cell-containing solated gel droplets¹⁴⁻¹⁶. The MDA of the single-cell genome in the bead is done by exchanging reagents through gel. However, the amount of MDA product in the gel matrix is insufficient for next generation DNA sequencing. The hollow-core hydrogel microcapsule¹⁷, which consists of a hydrogel shell and a sol core, is expected to be an efficient vessel for single-cell MDA. Many hollow-core hydrogel microcapsules have been reported to be used for cell cultivations and cell transplantations: for example, a crosslinked polyethylene glycol (PEG) gel shell and an PEG sol core¹⁸, an alginate and poly-L-lysine complex shell and an alginate sol core¹⁹, and an agarose gel shell and an alginate sacrifice core²⁰.

We here introduce a novel method for single cell genomics, using a newly developed agarose gel microcapsule (AGM) consisting of an agarose gel shell and alginate sol hollow-core, which enables single-cell isolation and picolitre-scale MDA (Fig. 1a). The fabrication process of the AGM was optimised here for easy preparation using inexpensive equipment and reagents, such as a vortex mixer, centrifuges, and commercially available reagents (Fig. 1b).

In this method, a single bacterial encapsulated cell in the picolitre-scale sol core of the AGM is subjected to MDA (designated here 'MDA-in-AGM'). Our study demonstrated that large quantities of AGMs containing single bacterial cells could be easily prepared at once, that massively parallel MDA-in-AGM in a single tube resulted in a large number of single-cell amplified genomes (SAGs) with high amplification efficiency, and that MDA-in-AGM greatly improves the genome completeness compared with conventional single-cell genomics.

Results

Preparation of AGMs containing single bacterial cells. Alginate gel cores containing bacterial cells were prepared using the emulsification/internal gelation method²¹ (Fig. 1b). Briefly, a mixture of alginate sol solution, bacterial cells, and CaCO₃ nanoparticles was emulsified with isostearyl alcohol (ISA). The resulting microdroplets were gelated with calcium ions that were released from CaCO₃ nanoparticles by

the addition of acetic acid to the water-in-oil emulsion. The concentration of bacterial cells was optimised using serial dilutions so that one alginate microdroplet contained one or no bacterial cells. The resulting alginate gel cores were washed sequentially with diethyl ether, 1-butanol, and Tris-HCl (pH 7.4) buffer.

Agarose solution (SeaPlaque, Lonza; final 2%) was added to the alginate gel cores suspended in Tris-HCl (pH 7.4) at 35 °C, and the mixture was emulsified with 0.25% (v/v) Span 80 in polyglyceryl-6 octacaprylate (PGO) at 35 °C (Fig. 1b). The use of PGO with a density of 0.997 g/mL, which is equivalent to that of water and AGMs (~ 1.0 g/mL), enabled the formation of a stable water-in-oil emulsion during the gradual gelation of agarose by cooling at 4 °C. In addition, PGO improved the AGM preparation process by preventing the aggregation of AGMs and increasing their diameter and yield (**Supplementary Fig. 1**). After PGO was removed by mixing with diethyl ether and further with 1-butanol, the alginate gel cores were solated with a one-tenth volume of 0.5 M EDTA. The solation of alginate cores in AGMs was confirmed based on the core dissolution into the buffer after dissolving the agarose shells (**Supplementary Fig. 2**). The alginate sol core is expected to provide an appropriate space for the MDA reaction, which was shown by the much greater yields of amplified DNA than those with an alginate gel core or agarose gel bead, as described below. The AGMs were washed with Tris-EDTA buffer (pH 7.4) to remove excess EDTA, and then suspended in a one-half volume of Tris-EDTA buffer and stored at 4 °C.

Escherichia coli DH5 α was used as a model microorganism. *E. coli* cells were added at a concentration of $3 \cdot 10^8$ cells mL⁻¹ alginate-CaCO₃ solution and were encapsulated in $5.64 \pm 1.72 \cdot 10^5$ AGMs (mean \pm SD) in three independent experiments, which corresponded to $12.5\% \pm 5.4\%$ of the total AGMs. Of the AGMs containing *E. coli* cells, $93.8\% \pm 8.8\%$ harboured a single cell in the core (**Supplementary Table 1**). In the three independent experiments, the diameters of the prepared AGMs were 39.6 ± 22.2 μ m, 51.4 ± 13.3 μ m and 49.3 ± 19.6 μ m, corresponding to 15.8 ± 21.2 pL, 31.5 ± 34.5 pL and 34.9 ± 56.3 pL, respectively.

MDA of single-cell genomes within AGMs. AGMs (in 50 μ L suspension) were washed with sterile water and collected by centrifugation. The lysis of bacterial cells and denaturation of double-stranded DNA within the AGMs were carried out at room temperature for 30 min with 50 μ L of an alkaline solution, Buffer D2 of the REPLI-g UltraFast Mini Kit (Qiagen). Denaturation was stopped by neutralisation with the addition of the same volume of Stop solution from the kit, and the supernatant was then removed by centrifugation. Reaction Buffer (93.5 μ L) containing 11 μ L of REPLI-g UltraFast DNA Polymerase from the kit was added and the resulting mixture was incubated at 30 °C for 3 h. The reaction was stopped with one-tenth volume of 0.5 M EDTA, and the AGMs were washed three times with 200 μ L of Tris-EDTA buffer. All these reagents used in MDA and the AGM preparation were irradiated with ultraviolet light to degrade any contaminating DNA prior to use.

Among $4.6 \pm 2.9 \cdot 10^5$ AGMs prepared with *E. coli* cells, $8.9\% \pm 0.8\%$ exhibited DNA amplification, which corresponded to $77.0\% \pm 27.2\%$ of the AGMs containing *E. coli* cells (n = 3) (**Supplementary Table 1**). As shown in Fig. 2, amplified DNA filled the alginate sol core, whereas AGMs with alginate gel cores that were prepared without the solation step with EDTA or agarose gel beads containing no alginate core exhibited only limited or no DNA amplification by MDA.

Quality of single-cell genome sequences compared between MDA-in-AGM and a conventional method using FACS-MDA. The quality of SAGs was evaluated and compared with that obtained by a conventional method using a combination of FACS for single-cell isolation and MDA (FACS-MDA) on a microlitre scale. AGMs containing amplified DNA that was stained with SYBR Green I were individually transferred to 0.2 mL PCR tubes with 29 μ L of sterile water under an epifluorescence microscope equipped with a TransferMan NK2 micromanipulator (Eppendorf). Amplified DNA was released to water by heating the tubes at 60 °C for 5 min, and directly used for preparation of sequencing libraries using the QIAseq FX DNA Library Kit (Qiagen). No second round of MDA was necessary to obtain the libraries suitable for genome sequencing. Paired-end sequence reads were generated on the Illumina MiSeq platform with the Reagent Kit V3 (600 cycles), and a fixed number of read pairs were randomly chosen and assembled *de novo* into contigs using SPAdes 3.13.0²² after standard quality filtering. Contigs > 1 kb were used for the subsequent analyses. Several factors describing the genome sequence quality, including completeness and contamination rate, were evaluated using CheckM²³ and QUAST²⁴, and compared between SAGs obtained using MDA-in-AGM and FACS-MDA, respectively.

The genome completeness and the total sequence length of *E. coli* SAGs obtained by MDA-in-AGM rapidly increased during the sequencing effort, and the average genome completeness reached 93.0% \pm 4.7% (n = 10) for assemblies using $9 \cdot 10^5$ read pairs, while it was 33.6% \pm 17.4% (n = 10) in FACS-MDA (Student's *t*-test, $P < 0.01$) (Fig. 3a). The genome coverage calculated by mapping unassembled reads to the reference *E. coli* DH5 α genome sequence (BOCF01000000) was 98.0% \pm 1.7% in MDA-in-AGM (n = 10), which was much higher than 52.0% \pm 13.8% in FACS-MDA (n = 10) for $9 \cdot 10^5$ read pairs ($P < 0.01$) (**Supplementary Table 2**). The number of contigs steeply decreased in MDA-in-AGM along the sequencing effort's depth probably owing to adequate assemblies of contigs, whereas it continued to increase in FACS-MDA (Fig. 3a). In addition, the amplification bias among genome regions, which is inherent to MDA, was much improved in MDA-in-AGM compared to FACS-MDA (Fig. 3b).

Single-cell genome analyses with a mock community and a natural sample. To evaluate the feasibility of MDA-in-AGM in a more realistic sample, we constructed a mock community comprising 15 cultured strains of human gut bacteria. The taxonomy and proportion of each bacterial strain are shown in **Supplementary Table 3**. SAGs from the mock community were obtained using either MDA-in-AGM or FACS-MDA in the same way as above (**Supplementary Tables 4 and 5**). The overall genome completeness of SAGs with < 5% contamination was significantly higher in MDA-in-AGM (68.0% \pm 23.3%, n = 39) than in FACS-MDA (42.4% \pm 20.5%, n = 36) for assemblies using $3 \cdot 10^5$ read pairs ($P < 0.01$) (**Supplementary Tables 6 and 7**). For example, SAGs of *Bacteroides thetaiotaomicron* and *Parabacteroides distasonis* exhibited clear differences between MDA-in-AGM and FACS-MDA in the genome completeness, total sequence length, number of contigs, and amplification bias, as seen in the *E. coli* SAGs (Fig. 3). The SAGs of most other strains also showed similar results (**Supplementary Fig. 3**). Our results indicated that MDA-in-AGM is applicable to various bacterial species and improves genome completeness in both gram-positive and gram-negative bacteria, regardless of whether there is high or low GC content in the genomes (Fig. 4).

For SAGs with > 5% contamination, which would be caused either by contaminating extracellular DNA or by multiple cells being encapsulated in a single AGM, the contigs were binned into each bacterial species using metaWRAP²⁵. As a result, 30 additional SAGs with < 5% contamination were obtained, and their average genome completeness and contamination rate were $74.1\% \pm 14.3\%$ and $1.2\% \pm 1.1\%$, respectively (**Supplementary Table 8**).

Finally, we applied MDA-in-AGM to a natural environmental sample. We used the microbiota in the gut of the termite *Reticulitermes speratus*, which comprises several hundred bacterial species from diverse phyla²⁶. The entire guts of five worker termites were removed, the gut contents were suspended, and then bacterial cells were collected by centrifugation after digesting the extracellular DNA with DNase I and the cells were washed with sterile water as described previously²⁷. The subsequent procedures were the same as those described above. We analysed 48 SAGs obtained by MDA-in-AGM and found high genome completeness ($78.6\% \pm 18.2\%$) and a low contamination rate ($1.0\% \pm 1.0\%$) (**Table 1**). The SAGs were taxonomically classified using the Genome Taxonomy Database Tool Kit (GTDB-Tk)²⁸ and were found to be affiliated with 13 bacterial classes, including known dominant groups in the termite gut, Spirochaetes, Bacteroidetes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Clostridia²⁶. In contrast, the genome completeness of SAGs obtained by FACS-MDA was only $37.7\% \pm 19.0\%$ ($n = 161$). Although a different sequencing method, i.e., a combination of the Nextera XT DNA Library Prep Kit (Illumina) and the Illumina HiSeq 2500 platform (500 cycles), was used for the latter case, the number of reads (bases) used for assembly was much larger on average ($551 \text{ Mb} \pm 64 \text{ Mb}$, $n = 161$) than in the analysis of SAGs obtained by MDA-in-AGM ($256 \text{ Mb} \pm 49 \text{ Mb}$, $n = 48$) (**Supplementary Table 9**).

In addition to the 48 SAGs, we analysed 18 SAGs of Endomicrobia with small genome sizes ($\sim 1 \text{ Mb}$)²⁹ obtained by MDA-in-AGM, and $91.2\% \pm 9.8\%$ genome completeness was obtained. Furthermore, among them, eight SAGs of phylotype Rs-D17 were compared to the complete genome sequences obtained previously^{29,30}. The genome coverages calculated by mapping reads on the two genomovars of Rs-D17 (Ri2008²⁹ and Ti2005³⁰) reached $95.7\% \pm 1.3\%$ and $96.7\% \pm 0.9\%$, respectively (**Supplementary Table 10**).

Discussion

MDA-in-AGM, the massively parallel MDAs in the picolitre-scale AGM cores, drastically improved the genome completeness of SAGs of *E. coli*, human gut bacteria in a mock community, and bacteria in the termite gut, compared to conventional microlitre scale FACS-MDA. Thus, the method is expected to contribute to revealing the metabolic capacity of yet-uncultured prokaryotes in environments.

It has been reported that the amplification bias in single-cell genomics can be suppressed by reducing the reaction volume of MDA using microfabrication techniques^{9,12,13,15,16}. Although the uniformity of the reaction volumes of the AGMs developed in this study was lower than in previously developed methods using microchannels and nanowells^{12,13,15,16}, MDA-in-AGM is much simpler and requires only

inexpensive, commercially available reagents and standard molecular biology laboratory equipment, except for a micromanipulator. The AGM alginate sol core enhanced the yield of MDA compared to that obtained by MDA in gels (Fig. 2), which is probably attributable to the enhanced diffusivity of amplified DNA in the sol core. MDA-in-AGM therefore does not require a second round of MDA, which is often necessary in other methods using microfabrication techniques^{15,16}. This direct use of the MDA product resulted in heightened genome completeness with suppressed amplification bias. Our MDA-in-AGM is applicable to both gram-positive and gram-negative bacteria and also to genomes with high GC content (Fig. 4). Additional advantages of the AGM are its physical stability and permeability for small molecules, which have made buffer exchange and physical handling much easier. To increase the uniformity and throughput of single-cell genomics using MDA-in-AGM, microchannels and molecular-barcoding³¹ may be adapted, although these approaches decrease the simplicity of the procedure

Recently, during our experiments were on going, MDA using a hollow-core hydrogel microcapsule consisting of a crosslinked PEG gel shell and a dextran sol core, a similar capsule structure to AGM, was reported³². Although amplification bias has not been evaluated, single-cell MDA in the core increases the amount of MDA product when compared with MDA in the gel bead. In addition, the shell of a hollow-core hydrogel microcapsule prevents genomic DNA leaking from the core after alkaline denaturation prior to MDA. However, near ultraviolet (UV) light (365 nm) used for photo chemical crosslink of the PEG shell damaged cells and DNA due to photooxidation³³, a polymer shell of hydrogel microcapsule for single-cell MDA is required to gelate without the UV light. We selected agarose as the AGM shell because of thermally mild reversible gelation, the commercially availability, and stabilities in MDA reagents, especially alkaline, neutralisation buffer, or EDTA.

Any methodology relying on Poisson distribution to obtain AGMs or other reaction chambers containing single bacterial cells inevitably produces a proportion of chambers containing multiple bacterial cells¹⁴, and it is difficult to completely eliminate contaminating DNA in our experimental procedures, as in other previously developed methods¹. Nevertheless, the application of a computer program for binning metagenomic fragments, such as metaWRAP²⁵, enabled us to recover a considerable number of SAGs by eliminating contaminating sequences (**Supplementary Table 8**).

In recent years, numerous studies analysing metagenome-assembled genomes from environmental samples have been reported³⁴. Although single-cell genomics has different advantages and potentially increases the quality of research in combination with metagenomics, for example, by revealing strain-level heterogeneity³⁵ and by providing information on horizontally transferred genetic components¹⁵, it is difficult for many microbiologists to perform. MDA-in-AGM is a much easier and less expensive method, and in addition, only a small amount of sample is needed. Thus, this method is also suitable for tiny specimens, such as the intestinal tracts of small insects and protist cells with endo- and ectosymbiotic bacterial communities.

Additionally, AGM potentially enables cultivation of microorganisms in its solated hollow-core, using organic solvent-free washing buffer in the preparation. Actually, a new *Dysgonomonas* species has been successfully isolated from the termite gut using the AGM cultivation method and supplying nutrients from the outside of the shell (our unpublished data). AGM thus can be also an important tool to investigate cell-to-cell interactions of symbiotic bacteria using a co-culture of host-and-donor bacteria, and their genome analyses.

Online Methods

Pipettes and plastics. AGMs were prepared with sterilised disposable plasticware: plastic-wrapped pipettes (VWR, Radnor, PA), 50-mL conical tubes (Asahi Techno Glass, Shizuoka, Japan), three-layer wrapped 1.5-mL and 2-mL microtubes (Watson, Tokyo, Japan), sterile-filtered pipette tips with standard bores (Watson) and ART-MBP pipette tips with wide bores (Thermo Fisher Scientific, Waltham, MA). Sterilised disposable glass pipettes (Thermo Fisher Scientific) were used for dispensing diethyl ether. Sterile cell strainers (100- μ m and 300- μ m nylon meshes, pluriSelect Life Science, Leipzig, Germany) were used for the filtration of the alginate gel cores and AGMs, respectively.

Reagents. Ultrapure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific) was used for all solutions prepared in-house. MDA was conducted in a biosafety cabinet while wearing sterile disposable gloves and sterile elbow covers. The surfaces of bottles and equipment were swiped with DNA Away (Thermo Fisher Scientific). Solutions were autoclaved at 121°C for 15 min and further decontaminated by UV radiation at 7.2 mW/cm² overnight (12 h) in a laminar flow cabinet.

CaCO₃ suspension and alginate solution. A suspension of uniform CaCO₃ nanoparticles (9.4% w/v, 100-nm diameter, pH 10) was provided by Shiraishi Central Laboratories (Hyogo, Japan). The fineness and uniformity of the particle size are important for the homogeneous gelation of alginate. To facilitate alginate gelation upon the addition of acetic acid, the conductivity and pH of the CaCO₃ suspension were lowered by washing the particles as follows: a suspension (10.6 mL) containing one gram of CaCO₃ particles was centrifuged at 2,000 · g for 3 min, and the pellet was re-suspended in 10 mL of water. After three washes, the resulting CaCO₃ suspension was autoclaved. The washing step decreased the conductivity and pH of the CaCO₃ suspension from 650 μ S/cm and pH 10 to 82 μ S/cm and pH 7, respectively. The CaCO₃ suspension was dispensed in screw cap tubes sealed in an aluminium bag at room temperature to prevent aggregation by freezing.

Alginate solution (5%, w/v) was prepared as follows: 2.5 g of sodium alginate (80–120 cP at 1% w/v, Wako, Osaka, Japan) was dissolved in 20 mL of water and brought to a volume of 40 mL with water in a 50-mL tube. The alginate suspension was mixed using a rotary shaker overnight until the alginate clods were completely dissolved. The alginate solution was then autoclaved and brought to a volume of 50 mL with water. Since autoclaving decreases the viscosity of alginate solution and affects the alginate core diameter, a consistent autoclave time (15 min) was used. The resulting alginate solution was stored at 4 °C. Since isostearyl alcohol (ISA; Kokyu Alcohol Kogyo Co., Ltd., Chiba, Japan) was easily removed from

the emulsion with diethyl ether or 1-butanol, the alginate was emulsified with ISA containing 3% soy bean lectin (Wako) in the following experiments.

Agarose solution. Agarose solution was prepared by dissolving SeaPlaque (final 2%, w/v, Lonza, Basel, Switzerland) in water using a microwave oven, and stored at 4 °C. To stabilise the water-in-oil emulsion containing agarose, polyglyceryl-6 octacaprylate (PGO; Nisshin Oillio, Tokyo, Japan), sorbitan monooleate (Span 80; Wako), and Tris-EDTA buffer (TE; BioUltra for molecular biology, pH 7.4, Sigma) were used as described below.

Observation of AGMs and agarose gel beads. To avoid the disruption of AGMs or agarose gel beads prepared in this study by a coverslip under microscopy, they were observed as follows. A silicone sheet with adhesive tape (20 · 500 · 0.5-mm thickness, Misumi, Tokyo, Japan) was cut into 20-mm squares, and the centres were punched with a square hole puncher (5 · 5 mm-tip, Kyoshin-elle, Tokyo, Japan). The silicone sheet was stuck on a glass slide (26 · 76 mm, Matsunami Glass Ind., Ltd., Osaka, Japan). To visualise *Escherichia coli* cells or DNA in AGMs, 50 mM Tris-HCl (pH 7.5) containing 0.5% SeaPlaque agarose, 0.1% *p*-phenylene diamine (Wako), and 10,000-fold diluted SYBR Green I (TaKaRa Bio Inc., Shiga, Japan) was prepared (designated as SYBR Green I solution). AGMs or agarose gel beads were suspended in 16 µL of SYBR Green I solution. The suspensions were individually dispensed into the holes in the silicone sheets. The holes were sealed with coverslips (18 · 18 mm, No. 1, Matsunami) and observed under an inverted fluorescence microscope (IX71, Olympus, Tokyo, Japan) equipped with a CCD camera (BU-51LN, Bitran, Saitama, Japan).

Evaluation of PGO for agarose micro droplet gelation. SeaPlaque agarose (2%, 2 mL) was mixed with PGO or ISA, each containing 0.25% Span 80 (v/v) (20 mL, 35 °C), by vortexing for 1 min in a 50-mL conical tube, and the mixtures were cooled on ice for 1 h. PGO or ISA was removed by mixing with 10 mL of diethyl ether followed by centrifugation at 4 °C for 10 min using a swing rotor (3,000 · g, 5702R, Eppendorf, Hamburg, Germany). The addition of diethyl ether was required to decrease the density of the oil phase in order to separate the PGO from the agarose beads because PGO (0.997 g/mL) and agarose beads (approximately 1.0 g/mL) have very similar density. The agarose beads were further washed with 10 mL of TE containing 0.2% Tween 20 and 10 mL of 1-butanol, suspended in 5 mL TE, and filtrated with 300-µm cell strainers. The agarose aggregates on the strainers were collected into 2-mL dishes and observed. The filtrates were brought to 20 mL (10 volumes) of TE, and the supernatants were removed by centrifugation. The agarose beads were further washed twice with TE and collected into 2-mL tubes. After the volumes of the agarose beads were calculated from their weights, the agarose beads were suspended in a one-half volume of TE. When the agarose beads were observed as described above, their diameters were measured with ImageJ (<https://imagej.nih.gov/ij/>). The yields and the diameters of the agarose beads were statically analysed with R (<https://www.r-project.org/>) and RStudio (<https://rstudio.com/>).

Preparation of *Escherichia coli* stock culture. Competent cells of *E. coli* strain DH5α were purchased from TaKaRa Bio Inc. and cultured on an LB plate (1% Bacto Tryptone, 1% NaCl, 0.5% Bacto Yeast extract, 1.5% Agar). A single colony was inoculated into LB medium (10 mL) in a 50-mL conical tube and cultured at

37°C in a rotary shaker (150 rpm) overnight. Two aliquots (2 mL) of the culture broth were inoculated into two LB (200 mL) media in 500-mL conical flasks respectively and further cultivated at 37°C overnight in the rotary shaker. Cells of *E. coli* were harvested and washed twice in PBS (20 mL) by centrifugation. The cells were re-suspended in PBS (10 mL), and their density was determined with a cell-counting chamber. The *E. coli* cells ($3.05 \cdot 10^{10}$ cell/mL) were divided into aliquots in microtubes (0.5 mL each) and stored at -80°C. The 0.5-mL aliquots of stock were further divided into microtubes (20 µL each) and stored at -80°C.

Preparation of alginate cores containing *E. coli* cells. Cells of *E. coli* were encapsulated in alginate cores by the emulsification–internal gelation method as described previously²¹ (Fig. 1b). *E. coli* cells from the stock culture (10 µL) were mixed with 990 µL of 2% sodium alginate solution containing 1% CaCO₃ and 50 mM acetate buffer (pH 7.0, 0.2-µm filtered, Sigma-Aldrich, St. Louis, MO). The mixture was emulsified with ISA containing 3% lecithin (9 mL) in a 50-mL tube by vortexing for 1 min. The emulsion was mixed with 2% acetic acid in ISA (0.1 mL each) by vortexing for 1 min with 10 repetitions. During this procedure, the pH of the mixture gradually decreased to 4.0 and the CaCO₃ nanoparticles were dissolved. The released calcium ions from CaCO₃ gelled alginate microdroplets in the emulsion. ISA was removed by mixing with diethyl ether and centrifugation, and the residual ISA was further removed from the alginate cores by mixing with 50 mM Tris-HCl buffer (pH 7.4) containing 0.2% (v/v) Tween 20 (Tris–Tween 20) (5 mL), followed by centrifugation, and then by repeating the procedure twice with 1-butanol (5 mL). The resulting alginate cores were suspended in one volume of 50 mM Tris-HCl (pH 7.4) and filtrated through a 100-µm cell strainer to remove large alginate aggregates. The filtrated alginate cores were further washed with Tris-HCl (pH 7.4) and collected in 2-mL microtubes. The alginate cores were weighed and stored at 4 °C after mixing with a one-half volume of Tris-HCl (pH 7.4). To adjust the number of *E. coli* cells in a single alginate core to less than two, 100-fold, 10-fold, and 1-fold dilutions of the *E. coli* stock were tested. The *E. coli* cells in these cores were counted, and a dilution that maximised the ratio of cores with a single *E. coli* cell was used in the following experiments.

Preparation of AGMs containing *E. coli* cells. The supernatant of the alginate core suspension (600 µL) was removed after centrifugation in a 50-mL tube. The residual alginate cores (400 µL) were incubated at 35 °C for 10 min and then mixed with the agarose solution as prepared above (2 mL, 35 °C). The mixture was emulsified with 0.25% (v/v) Span 80 in PGO (20 mL, 35 °C) by vortexing for 1 min (Fig. 1b). Then, the agarose was gelled by cooling. PGO was removed from the emulsion by washing as described above. The alginate gel core was solated by chelating calcium ions with the addition of a 1/10 volume of 0.5 M EDTA (pH 8.0, molecular biology grade, Thermo Fisher Scientific). The AGMs were then suspended in one volume of TE buffer. Large debris in the suspension was removed through a 300 µm cell strainer, and the AGMs were further washed with TE and subsequently centrifuged. The AGMs (5 µL) were suspended in SYBR Green I solution prepared as above (100 µL), and the morphology, density, diameter, and number of encapsulated *E. coli* cells were observed under microscopy. The AGMs were stored at 4 °C. For long-term storage (more than a week), the AGMs were stored in 40% ethanol at -80 °C and washed three times in 10 volumes of water before use.

Evaluation for alginate core solation in AGM. Alginate gel cores without *E. coli* were labelled with rhodamine 123 (Wako) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) (Tokyo Chemical Industry Co., Ltd., TCI, Tokyo, Japan) and *N*-hydroxysuccinimide (NHS; Wako)³⁶. Briefly, the alginate gel cores (200 µL) were washed three times with 10 gel volumes of 50 mM MES (pH 6.0). The alginate cores were mixed with 46 mM rhodamine 123 (54 µL), 0.5 M EDAC (50 µL), and 1 M NHS (50 µL), and 50 mM MES (pH 6.0) (646 µL). The mixture was left to react in the dark at room temperature with shaking for 16 h. The rhodamine 123-labelled alginate cores were washed three times with 10 volumes of 50 mM MES buffer and the cores were used for AGM preparation. AGMs containing rhodamine 123-labelled core were heated at 65 °C for 5 min in the presence of 50 mM EDTA or 50 mM CaCl₂. Residual cores were observed as mentioned above.

Effect of alginate core gelation and solation on MDA. MDA was performed using the REPLI-g UltraFast Mini Kit (Qiagen, Hilden, Germany). Aliquots (50 µL) of the AGMs containing *E. coli* cells before solation of the alginate cores (i.e., alginate gel cores) were mixed with one volume of cell lysis solutions containing 400 mM KOH with or without 10 mM EDTA, then incubated at 4 °C for 1 h. Next, they were neutralised with 100 µL of Stop buffer from the REPLI-g UltraFast Mini Kit at 4 °C for 16 h. The AGMs were washed with 1 mL of water and were brought to a volume of 50 µL with water. Reaction buffer (32 µL) containing 2 µL of phi29 DNA polymerase from the kit was added to both sets of AGMs. The reaction mixtures were incubated at 30 °C for 6 h, and the reactions were stopped with 400 µL of 10 mM EDTA. After washing three times with 0.5 mL of water, each set of AGMs was brought to a volume of 100 µL with water. AGMs without *E. coli* cells were also subjected to the same procedure as a negative control. These samples were observed for DNA amplification under fluorescence microscopy.

MDA-in-AGM. The genomic DNA of *E. coli* encapsulated within the AGMs was amplified by MDA (MDA-in-AGM). A 50 µL aliquot of AGMs was centrifuged, and the collected AGMs were washed with 200 µL water. The cell lysis and DNA denaturation were carried out with Buffer D2 (50 µL) of the REPLI-g UltraFast Mini Kit at room temperature for 30 min. After denaturation was stopped with the Stop buffer, the supernatant was removed by centrifugation. The Reaction buffer (93.5 µL) containing 11 µL phi29 DNA polymerase from the kit was added and incubated at 30 °C for 3 h. The reaction was stopped with 1/10 volume of 0.5 M EDTA, and the AGMs were washed with 200 µL of TE buffer three times. The washed AGMs were stored at 4 °C.

Single-cell MDA using a MoFlo XDP fluorescence-activated cell sorter (Beckman Coulter Inc., Brea, CA) was performed as described in previous studies as a control²⁷.

Mock community of human gut bacteria. A mock community of human gut bacteria was prepared from 15 isolates (**Supplementary Table 3**): *Blautia producta* JCM 1471, *Megasphaera elsdenii* JCM 1772, *Streptococcus mutans* JCM 5705, *Parabacteroides distasonis* JCM 5825, *Bacteroides thetaiotaomicron* JCM 5827, *Ruminococcus gnavus* JCM 6515, *Collinesella aerofaciens* JCM 10188, *Catenibacterium mitsuokai* JCM 10609, *Clostridium bolteae* JCM 12243, *Prevotella copri* JCM 13464, *Megamonas funiformis* JCM 14723, *Roseburia faecis* JCM 17581, *Veillonella tobetsuensis* JCM 17976,

Faecalibacterium prausnitzii JCM 31915, and *Flavonifractor plautii* JCM 32125. These were obtained from the Japan Collection of Microorganisms (JCM, <https://jcm.brc.riken.jp/en/>). The total genomic DNA of the mock bacterial community was extracted using the DNeasy Ultra Clean Microbial Kit (Qiagen). Amplicon sequencing of 16S rRNA genes in the mock community was performed by preparing a library with the Nextera XT Index Kit 96 indexes (Illumina, Inc, San Diego, CA) and the MiSeq platform with Reagent Kit V3 (600 cycles). The bacterial composition of the mock community was estimated on the basis of the results analysed in QIIME2³⁷.

Termite gut bacteria. Specimens of the wood-feeding termite *Reticulitermes speratus* were collected at Tsukuba in Ibaraki Prefecture, Japan. The guts of five worker termites were removed, and the gut contents were suspended in solution U, which consisted of 9.2 mM NaHCO₃, 5.1 mM trisodium citrate dihydrate, 13 mM KH₂PO₄, 37 mM NaCl, 0.75 mM CaCl₂, and 0.4 mM MgSO₄. The extracellular DNA and protist DNA were digested using DNase I (Promega, Madison, WI), and bacterial cells were collected by centrifugation and washed with water. The detailed procedure has been described previously²⁷.

Genome sequencing and bioinformatics. AGMs containing amplified DNA, detected with SYBR Green I, were transferred individually to PCR tubes, under an inverted fluorescence microscope equipped with a micromanipulator (TransferMan NK2, Eppendorf, Hamburg, Germany). Water (29 µL) was added to each PCR tube with a single AGM and incubated at 60 °C for 5 min, to release DNA by solating the agarose shell. Sequencing libraries were prepared using the QIAseq FX DNA Library Kit (Qiagen). When the sequencing libraries were constructed using adaptors with the concentration described in the manufacturer's instructions, a large number of concatenated adaptor sequences were formed. To suppress the generation of the artificial sequences, the concentration of adaptor sequences was adjusted to one-tenth of that in the manufacturer's instructions. Genome sequencing was performed on the MiSeq platform with Reagent Kit V3 (600 cycles). Sequence libraries for single cells isolated from the termite gut microbiota using FACS were prepared using the Nextera XT DNA Library Prep Kit (Illumina), and analysed on the Illumina HiSeq 2500 platform (500 cycles).

The generated short reads were trimmed using Cutadapt (<https://github.com/marcelm/cutadapt>), PRINSEQ (<http://prinseq.sourceforge.net/>), FASTX Trimmer, FASTQ Quality Trimmer (http://hannonlab.cshl.edu/fastx_toolkit/download.html), and cmpfastq_pe (http://compbio.brc.iop.kcl.ac.uk/software/download/cmpfastq_pe). The trimmed reads were assembled using SPAdes ver. 3.13.0 (k-mer: 21, 33, 55, 77, 99, and 127, options: `-sc, -careful`)²² into contigs. Only contigs > 1 kb were selected using SeqKit (<https://bioinf.shenwei.me/seqkit/>) for the subsequent analyses. The fixed number of read pairs was randomly chosen by SeqKit. Taxonomic classification of single-cell genomes from termite gut samples was conducted using the Genome Taxonomy Database Tool Kit (GTDB-Tk)²⁸. Genomic sequence data showing > 5% contamination, identified by using CheckM²³, were sequentially subjected to binning, refinement, and reassembly processes with the BINNING (including metaBAT²³⁸, MaxBin²³⁹, and CONCOCT⁴⁰), BIN_REFINEMENT, and REASSEMBLE_BINS modules of metaWRAP²⁵. For the single-cell genome analyses using *E. coli* DH5α

and the human gut bacteria, trimmed reads were mapped onto known genome sequences using Bowtie2⁴¹, and the results were visualised using IGV ver. 2.3.26 (<http://software.broadinstitute.org/software/igv/>). The genome coverage obtained by mapping reads without assembling them [total length (base) of mapped reads divided by reference genome size] was calculated using BBtools (<http://jgi.doe.gov/data-and-tools/bbtools/>). The reference genomes are listed in **Supplementary Table 3**.

Declarations

COMPETING INTERESTS STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Y. Y. and M. O. supervised the study. H. A., M. Y., Y. H., and M. O. designed the study. H. A., M. Y., and Y. H. wrote the manuscript. H. A. elucidated the AGM preparation protocol. M. Y. and M. S. performed next genome sequencings and their data analyses. All authors contributed to and edited the manuscript.

ACKNOWLEDGEMENTS

This work was supported by the RIKEN Pioneering Project "Biology of Symbiosis", JSPS KAKENHI Grants 16K07224 to H. A, and 17H01447 and 19H05679 to M. O., and Institute of Fermentation, Osaka to M. Y.

References

1. Woyke, T., Doud, D. F. R. & Schulz, F. The trajectory of microbial single-cell sequencing. *Nat. Methods* **14**, 1045–1054 (2017).
2. Wrighton, K. C. *et al.* Fermentation, Hydrogen, and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla. *Science* **337**, 1661–1665 (2012).
3. Albertsen, M. *et al.* Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. *Nat. Biotechnol.* **31**, 533–538 (2013).
4. Anantharaman, K. *et al.* Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nat. Commun.* **7**, 1–11 (2016).
5. Delmont, T. O. *et al.* Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes. *Nat. Microbiol.* **3**, 804–813 (2018).
6. Zhang, K. *et al.* Sequencing genomes from single cells by polymerase cloning. *Nat. Biotechnol.* **24**, 680–686 (2006).
7. Woyke, T. *et al.* One Bacterial Cell, One Complete Genome. *PLoS One* **5**, e10314 (2010).
8. Fu, A. Y., Spence, C., Scherer, A., Arnold, F. H. & Quake, S. R. A microfabricated fluorescence-activated cell sorter. *Nat. Biotechnol.* **17**, 1109–1111 (1999).

9. Marcy, Y. *et al.* Nanoliter reactors improve multiple displacement amplification of genomes from single cells. *PLoS Genet.* **3**, 1702–1708 (2007).
10. Joensson, H. N. & Andersson Svahn, H. Droplet Microfluidics—A Tool for Single-Cell Analysis. *Angew. Chem. Int. Ed.* **51**, 12176–12192 (2012).
11. Zong, C., Lu, S., Chapman, A. R. & Xie, X. S. Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell. *Science* **338**, 1622–1626 (2012).
12. Gole, J. *et al.* Massively parallel polymerase cloning and genome sequencing of single cells using nanoliter microwells. *Nat. Biotechnol.* **31**, 1126–1132 (2013).
13. Sidore, A. M., Lan, F., Lim, S. W. & Abate, A. R. Enhanced sequencing coverage with digital droplet multiple displacement amplification. *Nucleic Acids Res.* **44**, e66 (2016).
14. Bigdeli, S., Dettloff, R. O., Frank, C. W., Davis, R. W. & Crosby, L. D. A simple method for encapsulating single cells in alginate microspheres allows for direct PCR and whole genome amplification. *PLoS One* **10**, e0117738 (2015).
15. Lan, F., Demaree, B., Ahmed, N. & Abate, A. R. Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding. *Nat. Biotechnol.* **35**, 640–646 (2017).
16. Chijiwa, R. *et al.* Single-cell genomics of uncultured bacteria reveals dietary fiber responders in the mouse gut microbiota. *Microbiome* **8**, 5 (2020).
17. Rabanel, J. M., Banquy, X., Zouaoui, H., Mokhtar, M. & Hildgen, P. Progress technology in microencapsulation methods for cell therapy. *Biotechnol. Prog.* **25**, 946–963 (2009).
18. Siltanen, C. *et al.* One step fabrication of hydrogel microcapsules with hollow core for assembly and cultivation of hepatocyte spheroids. *Acta Biomater.* **50**, 428–436 (2017).
19. Breguet, V., Gugerli, R., von, S. U. & Marison, I. W. CHO immobilization in alginate/poly-L-lysine microcapsules: an understanding of potential and limitations. *Cytotechnology* **53**, 81–93 (2007).
20. Sakai, S., Hashimoto, I. & Kawakami, K. Production of cell-enclosing hollow-core agarose microcapsules via jetting in water-immiscible liquid paraffin and formation of embryoid body-like spherical tissues from mouse ES cells enclosed within these microcapsules. *Biotechnol. Bioeng.* **99**, 235–243 (2008).
21. Poncelet, D. Production of alginate beads by emulsification/internal gelation. *Ann. N. Y. Acad. Sci.* **944**, 74–82 (2001).
22. Bankevich, A. *et al.* SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J. Comput. Biol.* **19**, 455–477 (2012).
23. Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. & Tyson, G. W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **25**, 1043–1055 (2015).
24. Gurevich, A., Saveliev, V., Vyahhi, N. & Tesler, G. QAST: quality assessment tool for genome assemblies. *Bioinformatics* **29**, 1072–1075 (2013).

25. Uritskiy, G. V., DiRuggiero, J. & Taylor, J. MetaWRAP-a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome* **6**, 158 (2018).
26. Hongoh, Y., Ohkuma, M. & Kudo, T. Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). *FEMS Microbiol. Ecol.* **44**, 231–242 (2003).
27. Yuki, M. *et al.* Dominant ectosymbiotic bacteria of cellulolytic protists in the termite gut also have the potential to digest lignocellulose. *Environ. Microbiol.* **17**, 4942–4953 (2015).
28. Chaumeil, P. A., Mussig, A. J., Hugenholtz, P. & Parks, D. H. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* (2019).
29. Hongoh, Y. *et al.* Complete genome of the uncultured Termite Group 1 bacteria in a single host protist cell. *Proc. Natl. Acad. Sci. USA* **105**, 5555–5560 (2008).
30. Izawa, K. *et al.* Comparison of Intracellular “Ca. Endomicrobium Trichonymphae” Genomovars Illuminates the Requirement and Decay of Defense Systems against Foreign DNA. *Genome Biol. Evol.* **8**, 3099–3107 (2016).
31. Zheng, G. X. *et al.* Massively parallel digital transcriptional profiling of single cells. *Nat. Commun.* **8**, 14049 (2017).
32. Leonaviciene, G., Leonavicius, K., Meskys, R. & Mazutis, L. Multi-step processing of single cells using semi-permeable capsules. *Lab. Chip* **20**, 4052–4062 (2020).
33. Cadet, J., Douki, T. & Ravanat, J.-L. Oxidatively Generated Damage to Cellular DNA by UVB and UVA Radiation. *Photochem. Photobiol.* **91**, 140–155 (2015).
34. Nayfach, S., Shi, Z. J., Seshadri, R., Pollard, K. S. & Kyrpides, N. C. New insights from uncultivated genomes of the global human gut microbiome. *Nature* **568**, 505–510 (2019).
35. Merino, N. *et al.* Single-Cell Genomics of Novel Actinobacteria With the Wood–Ljungdahl Pathway Discovered in a Serpentinizing System. *Front. Microbiol.* **11**, e1031 (2020).
36. Hermanson, G. T. *Bioconjugate Techniques*. (Academic Press, San Diego, 1996).
37. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
38. Kang, D. D. *et al.* MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* **7**, e7359 (2019).
39. Wu, Y.-W., Simmons, B. A. & Singer, S. W. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* **32**, 605–607 (2016).
40. Alneberg, J. *et al.* Binning metagenomic contigs by coverage and composition. *Nat. Methods* **11**, 1144–1146 (2014).
41. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).

Table

Table 1 Summary of single-cell amplified genomes (SAGs) of symbiotic bacteria in termite gut bacteria.

Taxon of bacteria	MDA-in-AGM			FACS-MDA [†]		
	Number of SAGs	Genome completeness (%)	Contamination (%)	Number of SAGs	Genome completeness (%)	Contamination (%)
Spirochaetia	15	73.5 ± 20.6	0.9 ± 1.1	35	32.5 ± 19.0	0.3 ± 0.6
Bacteroidia	8	88.2 ± 8.7	1.3 ± 0.7	51	36.1 ± 17.7	0.6 ± 1.2
Alphaproteobacteria	2	87.0 ± 1.3	0.0 ± 0.0	8	52.9 ± 19.5	0.9 ± 1.7
Betaproteobacteria	7	87.5 ± 5.9	1.2 ± 0.3	9	35.3 ± 12.6	0.9 ± 0.8
Deltaproteobacteria	3	86.4 ± 6.7	0.5 ± 0.3	3	22.8 ± 16.7	1.1 ± 0.6
Epsilonproteobacteria	2	55.0 ± 48.5	2.2 ± 3.1	2	38.8 ± 20.7	1.2 ± 0.6
Clostridia	4	70.2 ± 23.6	1.3 ± 0.4	27	36.7 ± 16.2	0.7 ± 1.1
Actinobacteria	1	51.9	0.87	3	33.5 ± 15.9	1.9 ± 1.7
Planctomycetales	1	87.1	0.1	11	42.6 ± 21.0	0.6 ± 0.8
Synergistia	2	83.9 ± 22.7	1.2 ± 1.6	4	65.5 ± 21.9	0.2 ± 0.3
Fibrobacteria	1	72.8	0.0	n. d.	n. d.	n. d.
Deferribacteres	1	72.9	2.0	n. d.	n. d.	n. d.
Candidate division SR1	1	74.1	0.0	n. d.	n. d.	n. d.
Bacilli	n. d.	n. d.	n. d.	8	47.2 ± 23.2	1.4 ± 2.0
Endomicrobia [*]	18	91.2 ± 9.8	1.5 ± 1.0	n. t.	n. t.	n. t.
Total sequenced samples	48 (66)	78.6 ± 18.2 (82.0 ± 17.2)	1.0 ± 1.0 (1.1 ± 1.0)	161	37.7 ± 19.0	0.7 ± 1.1

n. d.: not detected. n. t.: not tested.

[†] NGS libraries were made by Nextera XT and read by HiSeq.

Number of SAGs, genome completeness, and contamination including Endomicrobia are shown in parentheses.

Figures

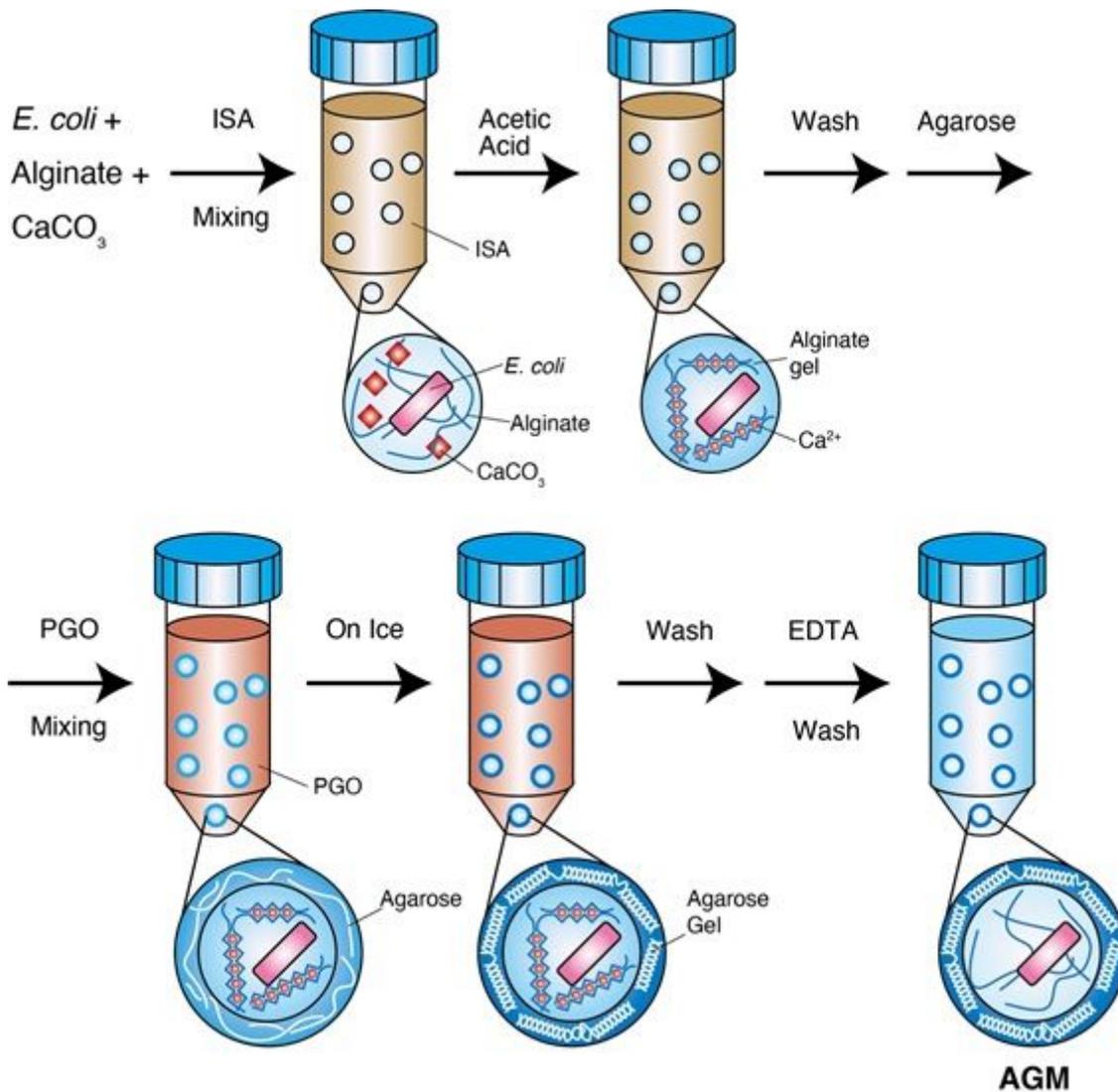


Figure 1

Schematic view of agarose gel microcapsule (AGM) and its preparation process. a. A schematic diagram of the AGM developed in this study for the single-cell isolation and multiple displacement amplification (MDA). The AGM consists of an agarose hydrogel shell and an alginate sol core. The alginate core provides a picolitre-scale reaction chamber for MDA, while the agarose shell acts both as a permeable envelope for enzymes and small molecules and as a protective wall against larger particles and molecules such as bacterial cells and genomic/amplified DNA. b. A preparation scheme for AGMs containing *Escherichia coli* cell(s). The alginate cores and agarose shells are gelled with Ca^{2+} from CaCO_3 and cooled in emulsion, respectively. PGO (polyglyceryl-6 octacaprylate) can suppress the aggregation of AGMs during agarose gelation by cooling. Finally, the alginate gel cores are solated with EDTA by chelating Ca^{2+} . ISA: isostearyl alcohol.

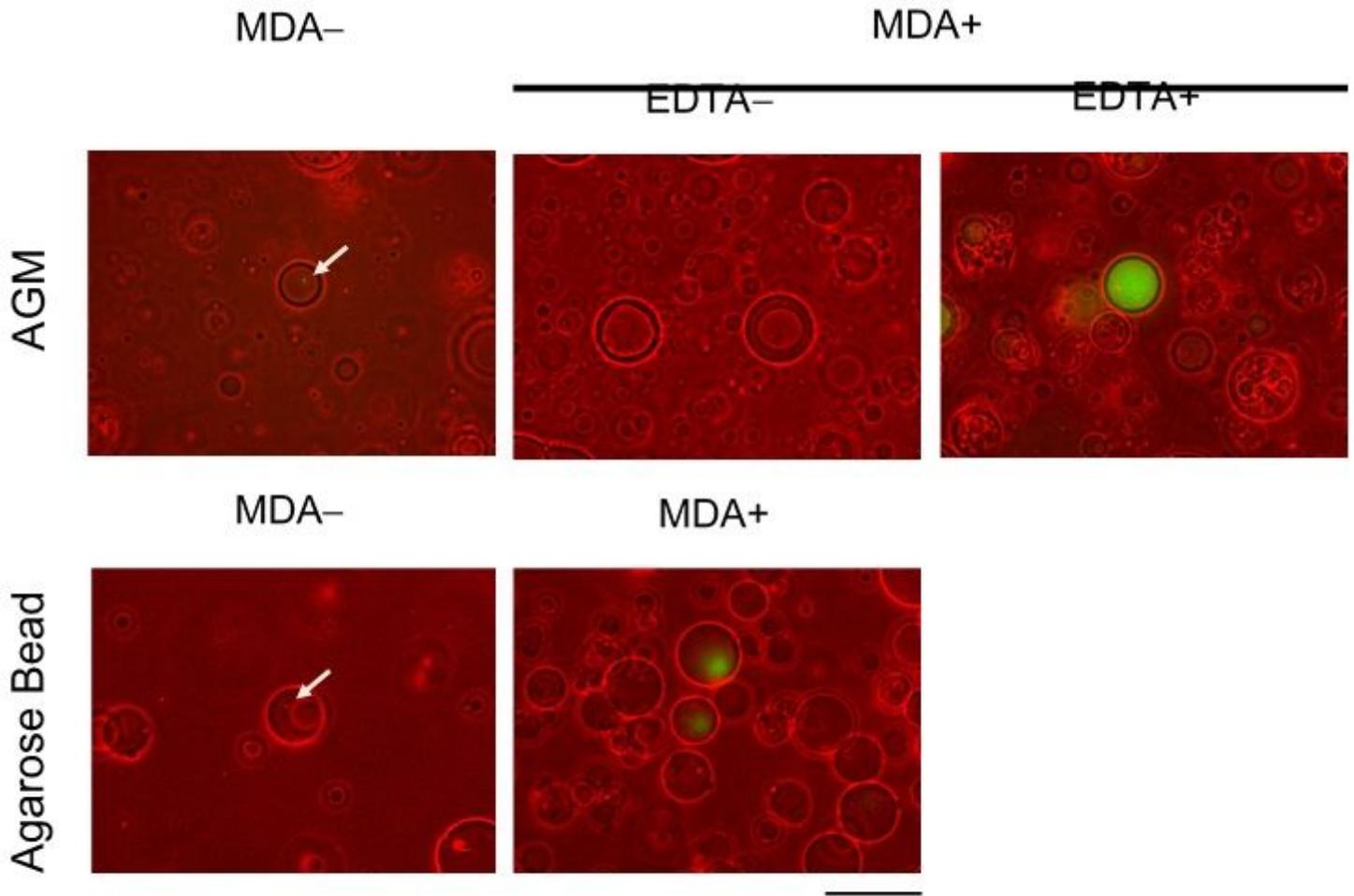


Figure 2

Yield enhancement of multiple displacement amplification (MDA) by alginate core solution. The effect of alginate core solution on the productivity of MDA within agarose gel microcapsules (AGMs) was evaluated. The genomic DNA of *Escherichia coli* encapsulated within AGMs was amplified by MDA with or without alginate solution. A phase contrast image (red) and epifluorescent image (green, SYBR Green I) are overlaid. MDA in agarose gel beads (without an alginate core) was also conducted as a control experiment. Arrows indicate *E. coli* cells stained with SYBR Green I. Bar = 100 μm .

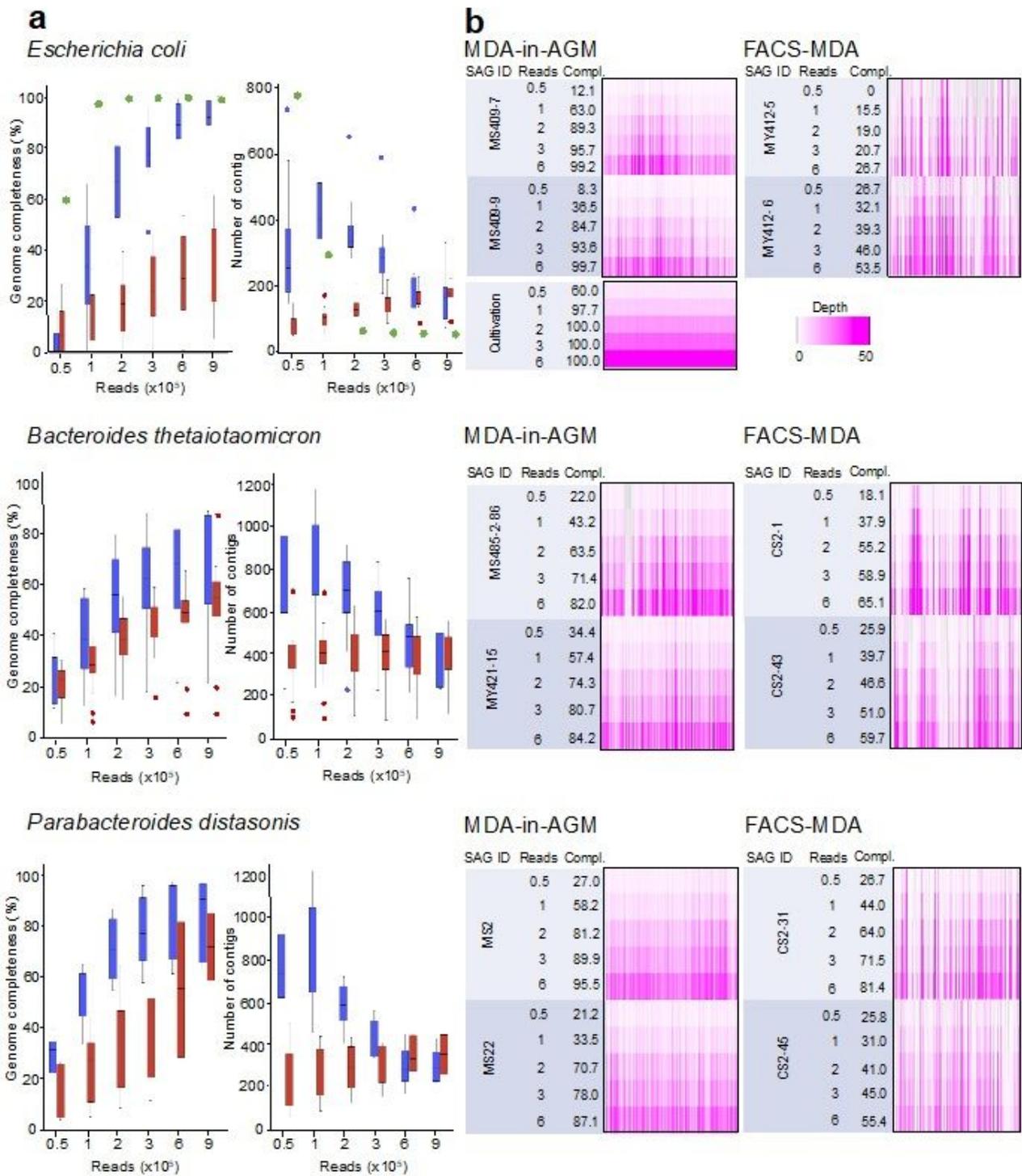


Figure 3

Comparison of genome completeness and amplification bias of single-cell genomes obtained by MDA-in-AGM and FACS-MDA. a. Completeness and number of contigs of SAGs during the sequencing process. Box plots (blue: MDA-in-AGM; red: FACS-MDA) are shown for *Escherichia coli* and two bacterial species as examples from the mock community of human gut bacteria (Supplementary Tables 2, 4, and 5). In the *E. coli* graphs, the results for DNA prepared from cultured *E. coli* cells without MDA are also shown

(green). b. The number of sequence reads mapped against genome regions is shown as an indicator of amplification bias caused during MDA. The number of reads (x 10⁵) used for de novo assembly (shown as 'Reads') and genome completeness (%) ('Compl.') estimated using CheckM23 are shown.

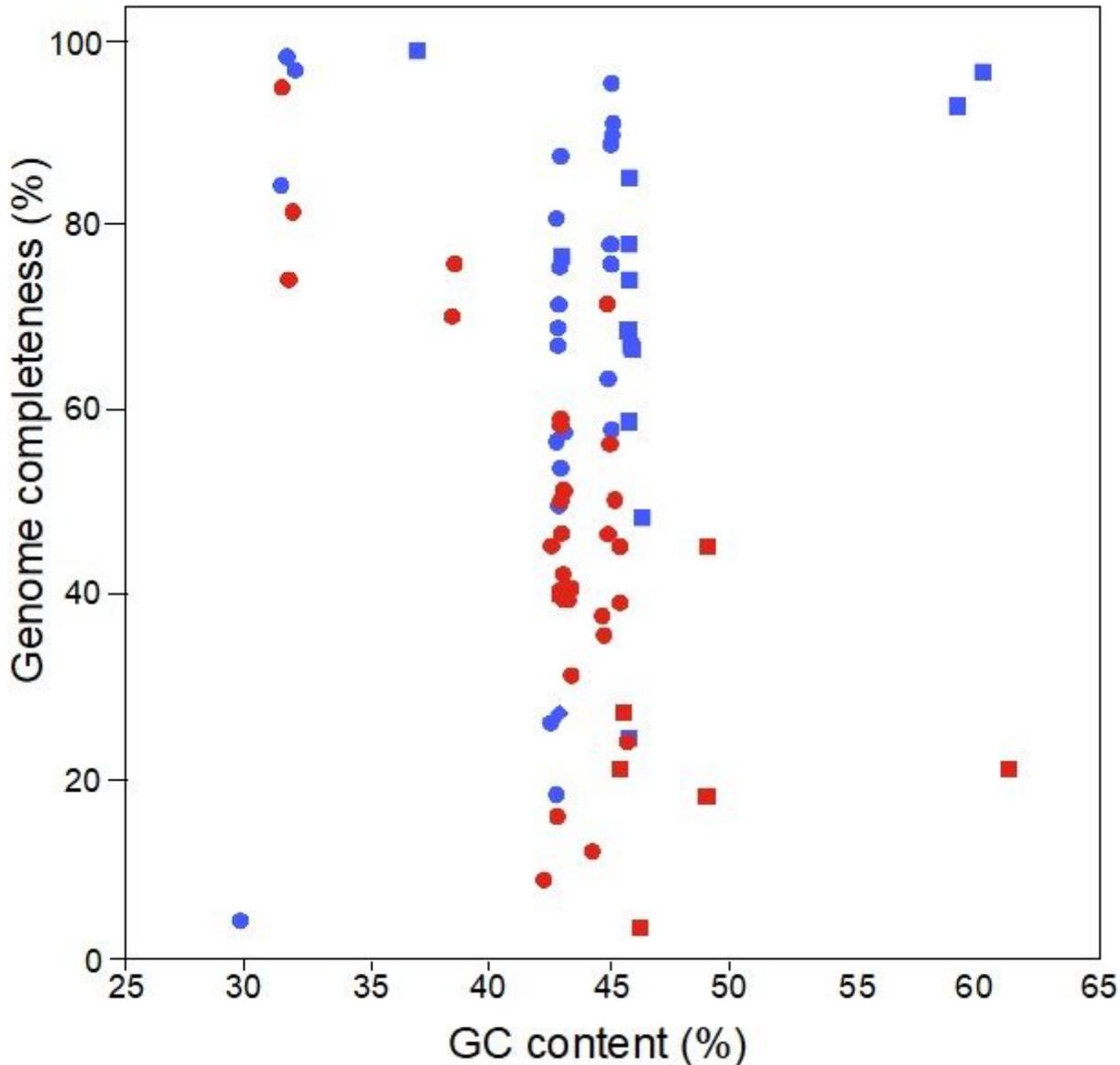


Figure 4

Relationships between genome completeness and GC contents of single-cell genomes in the mock community of human gut bacteria. Results were obtained by assembling randomly chosen 3 x 10⁵ read pairs for each single-cell amplified genome. MS485-1-51 of MDA-in-AGM and CS1-94 of FACS-MDA were assembled using all read pairs. The bacterial species comprising the mock community and detailed results are shown in Supplementary Tables 3 and 4, respectively. Blue and red indicate the MDA-in-AGM and the FACS-MDA, respectively. Squares, circles, and rhombuses indicate gram-positive, gram-negative, and gram-variable bacteria, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SingleCellMDAinAGMSITables.zip](#)
- [SupplementaryInformation.pdf](#)