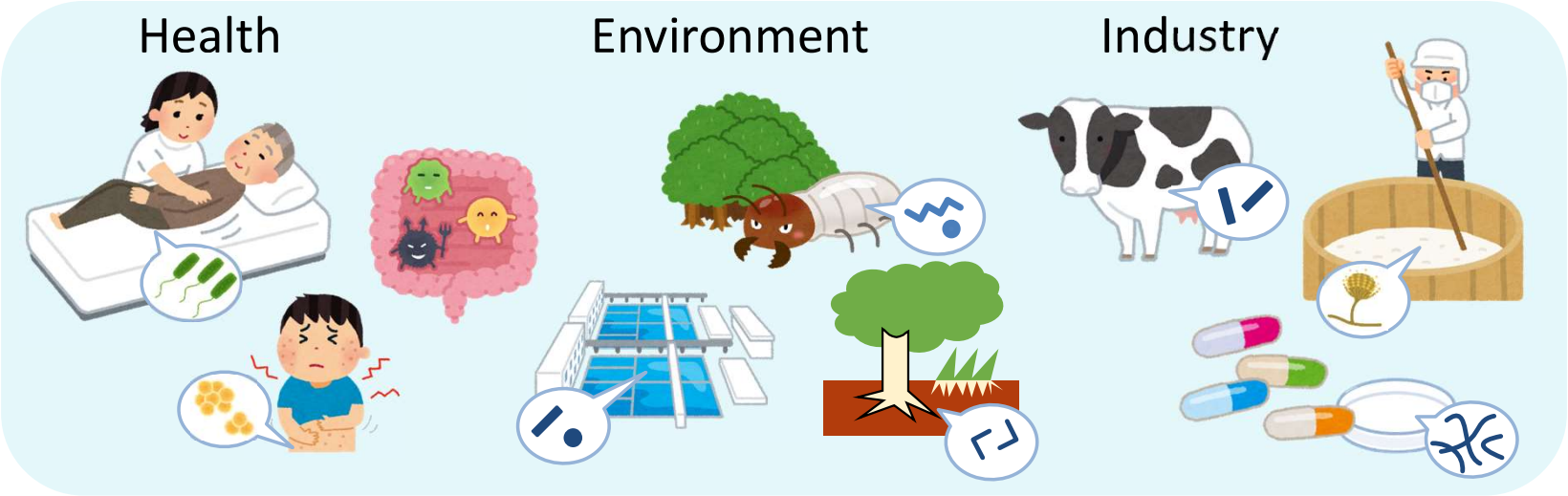
微生物用
アガロースゲル・
マイクロカプセルの開発

株式会社　東陽テクニカ
ワン・テクノロジーズ・カンパニー

青木　弘良

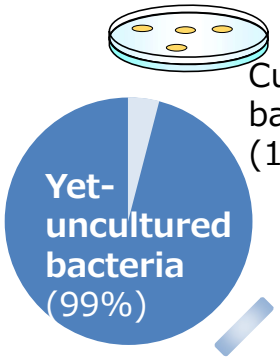
Microbiome



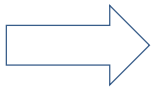
Culture

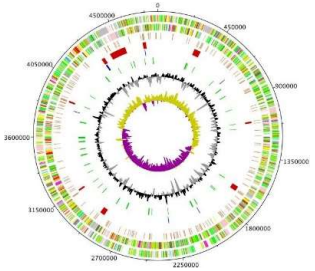
Genome Analysis

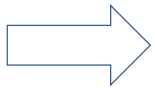
Functions

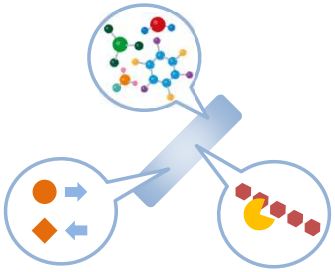


“Microbial Dark Matter”

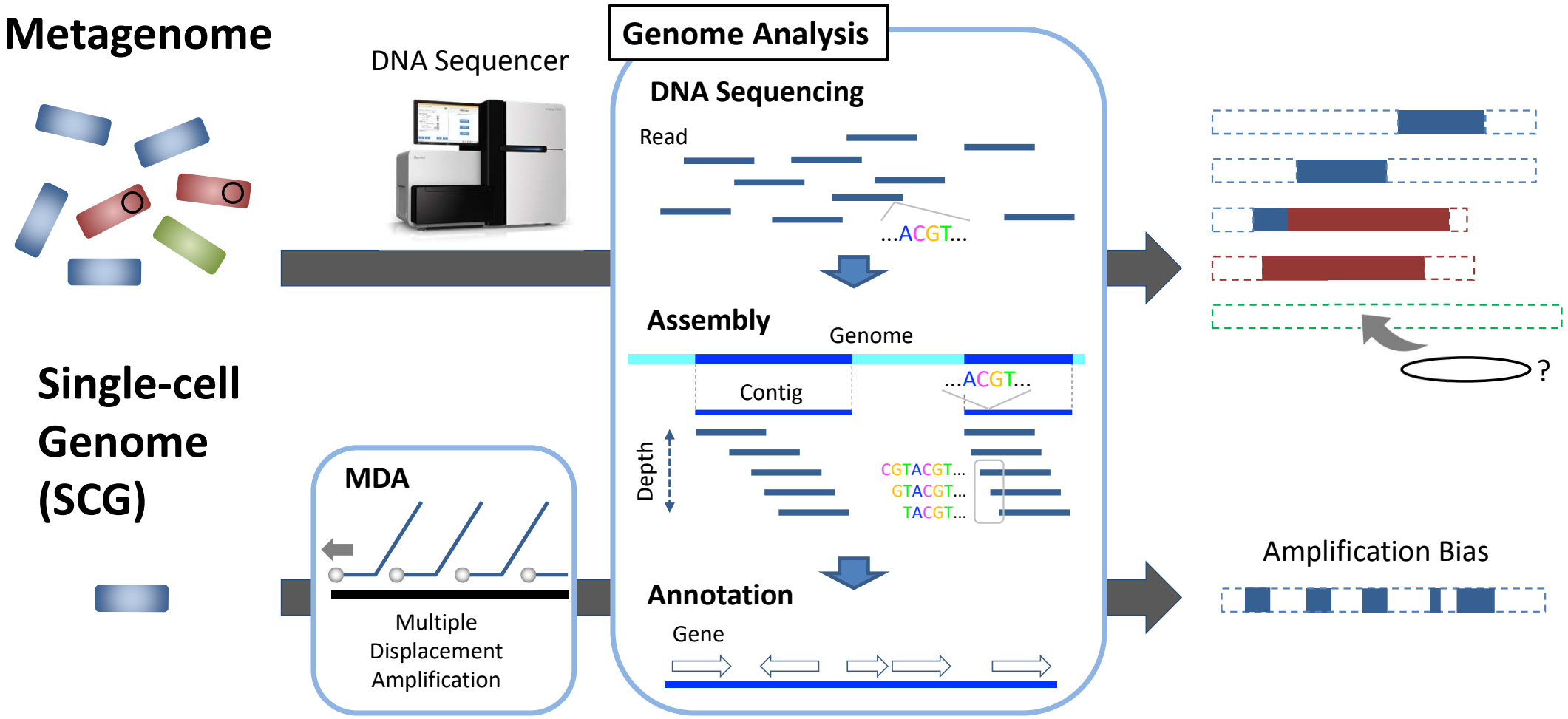




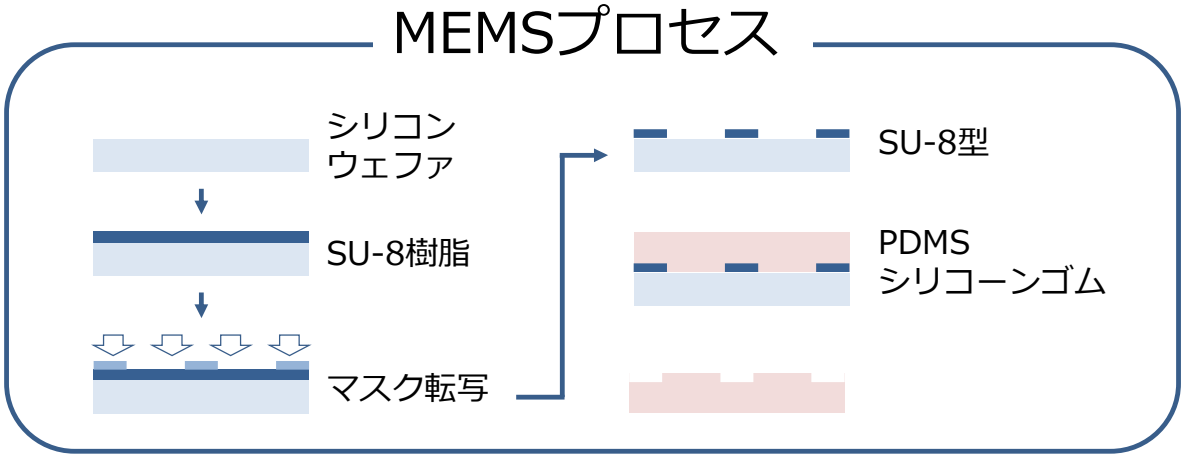




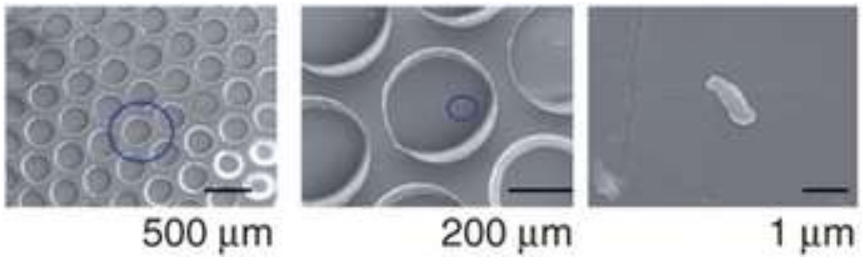
Genome Analysis of Yet-uncultured Bacteria



Suppression of Amplification Bias

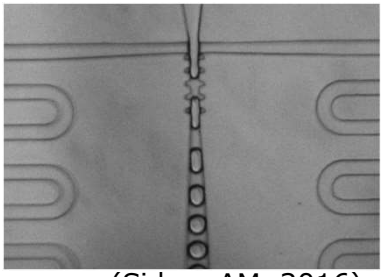


マイクロウェル

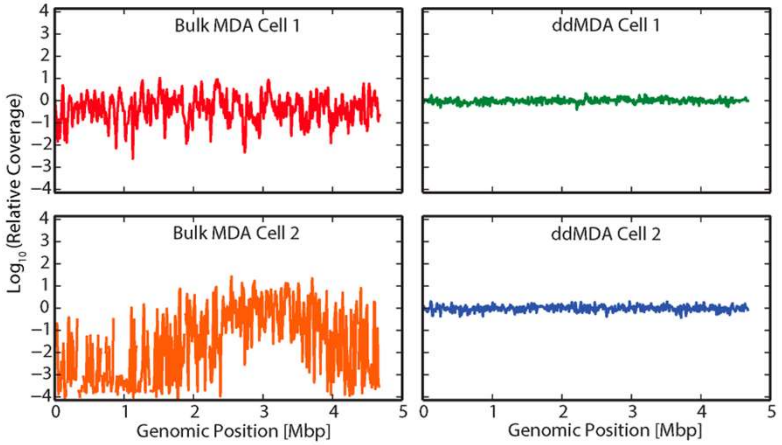


(Gole J, 2013)

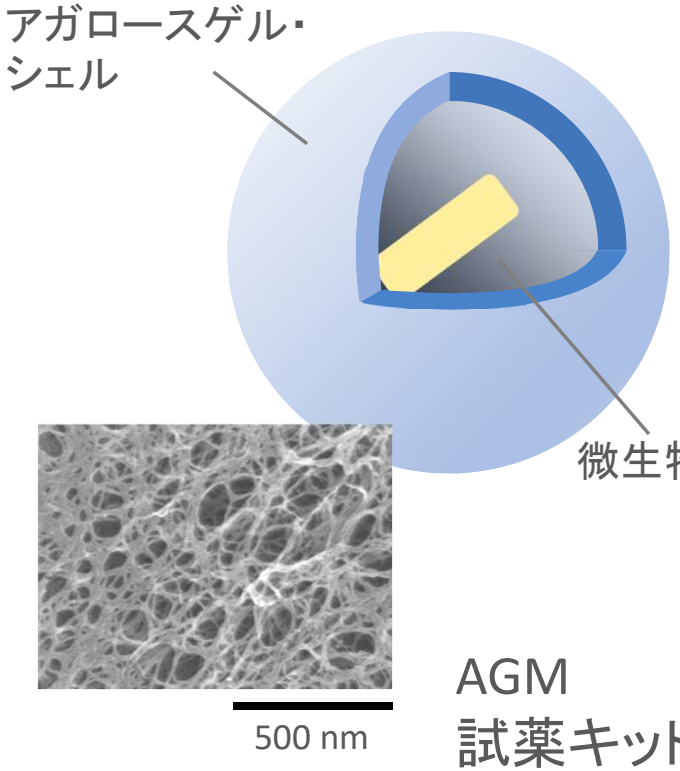
マイクロ流路
(ゲルビーズ，液滴)

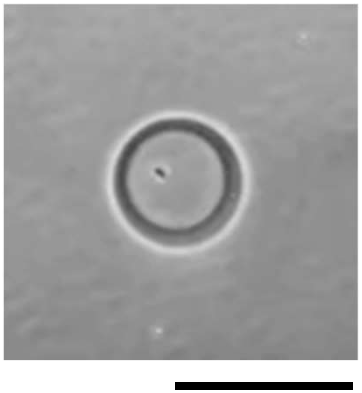


(Sidore AM, 2016)



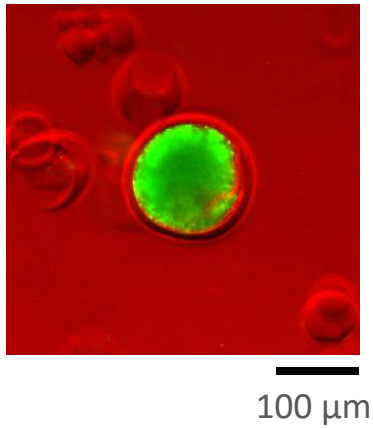
アガロースゲル･マイクロカプセル (AGM)



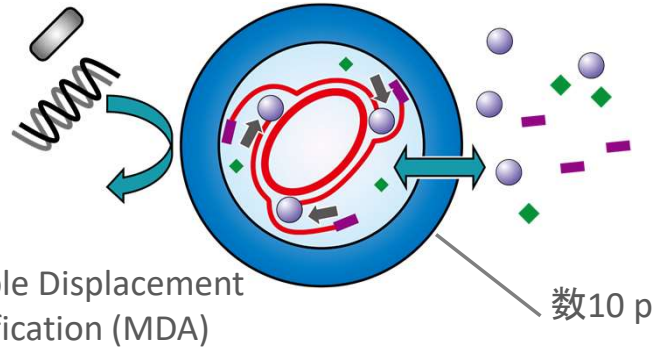


AGM
試薬キット

微生物のカプセル培養



微生物の1菌体ゲノム解析

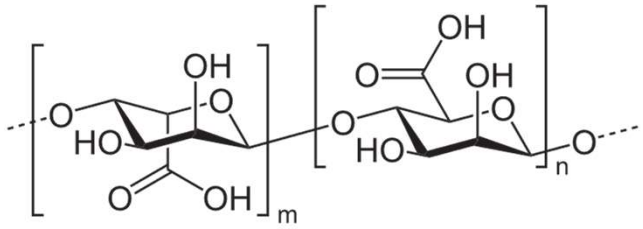


(Aoki H et. al., *Sci. Rep.*, 2022)
(特許7018685号)

Hydrogels

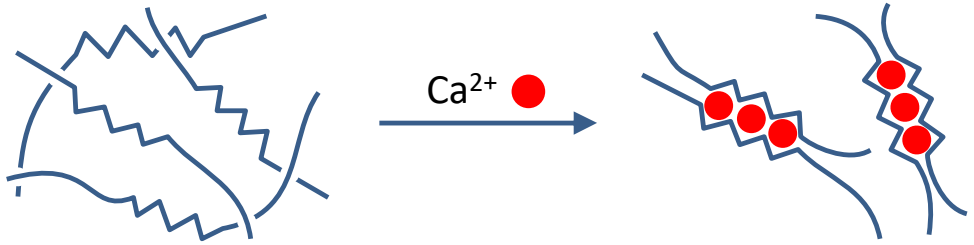
Core

Alginate



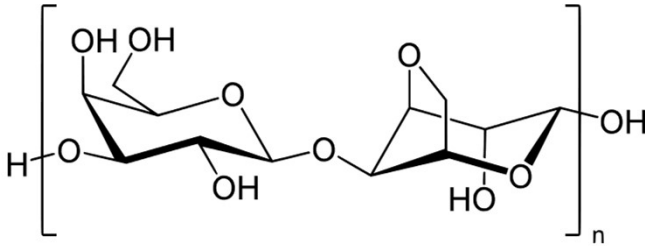
<1 min

$CaCO_3$ —Acetic acid (pH <5)→ $Ca^{2+} + H_2O + CO_2$

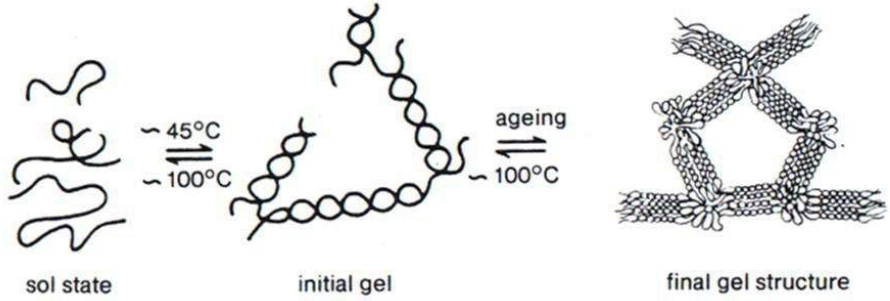


Shell

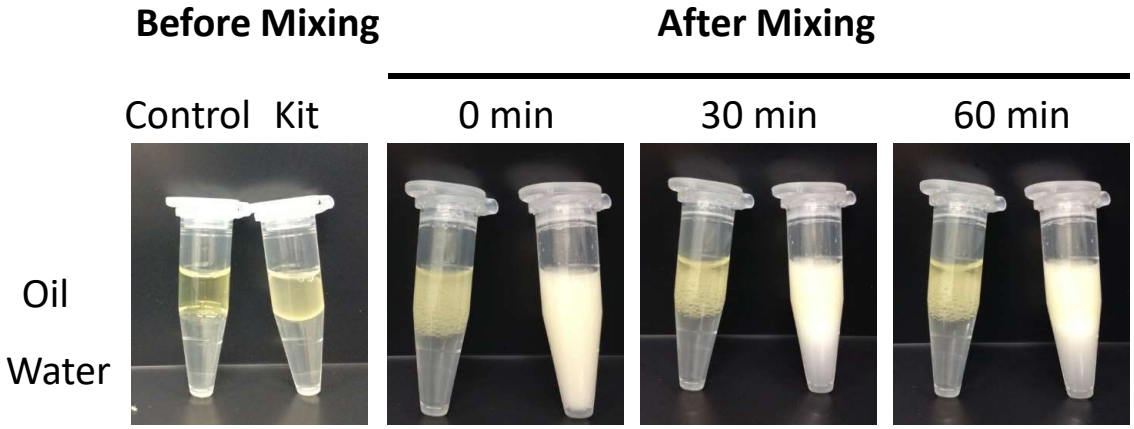
Agarose



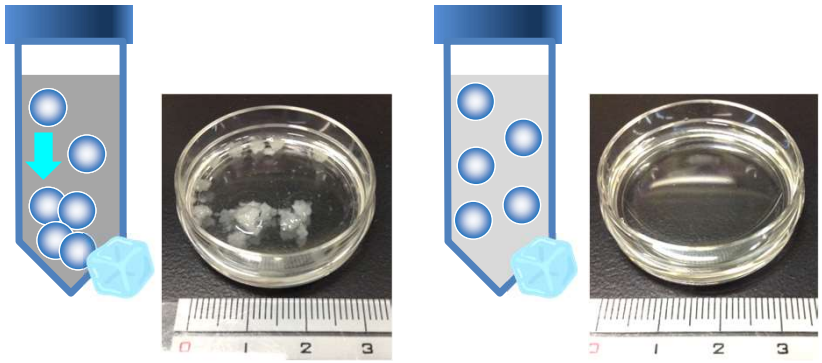
0.5–1 h

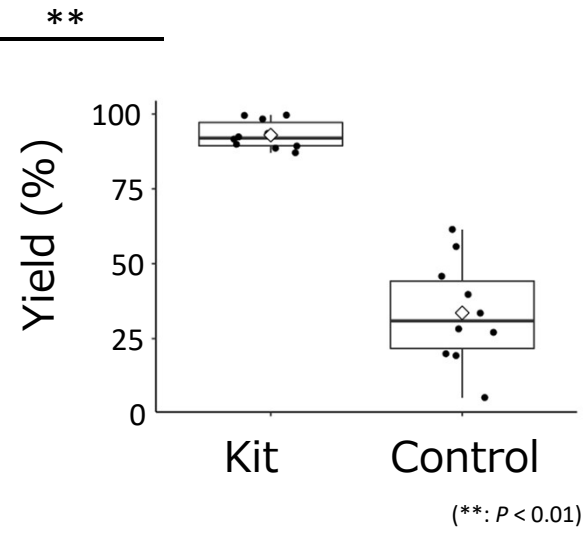


Stable Emulsion for Agarose Shell Gelation



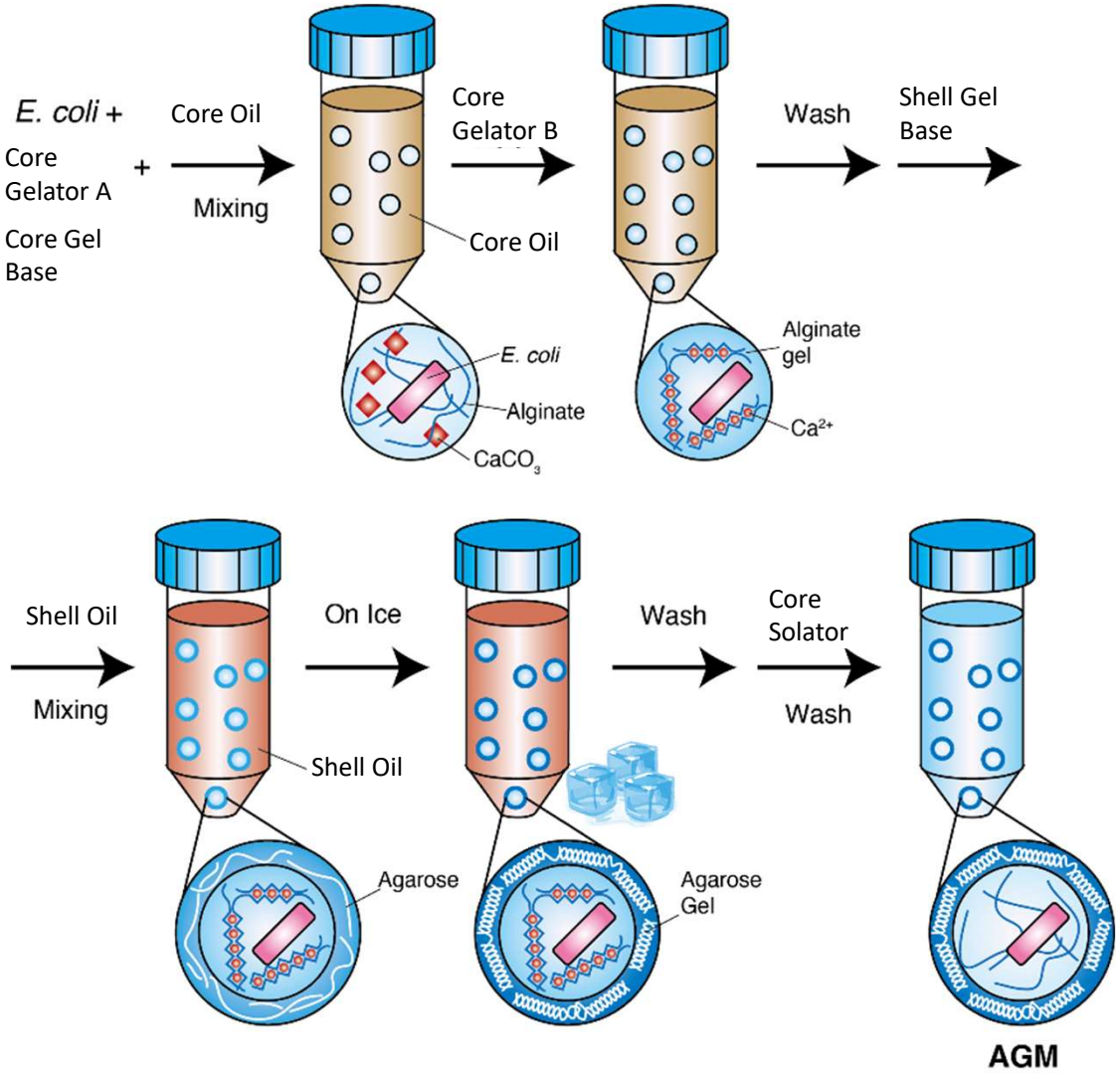
Control: Isostearyl alcohol (ISA, *d* = 0.84 g/mL)





(**: $P < 0.01$)

Agarose Gel Microcapsule



Equipment

Preparation

- Vortex Mixer
- Angle-rotor Centrifuge (15 mL and 50 mL) or Swing-rotor Centrifuge (50 mL)
- Pipette Aid
- Pipetteman
- Electric Balance

Observation

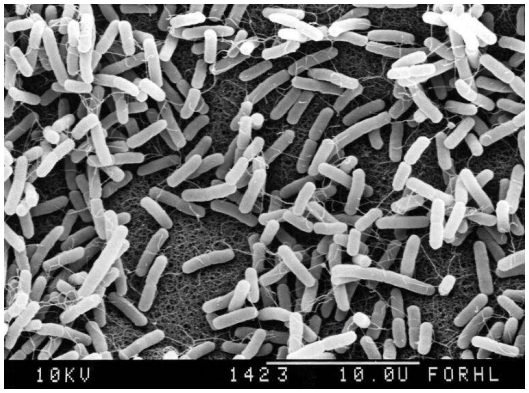
- Fluorescent Microscope

Isolation

- Micromanipulator or PIpetteman and Microscope

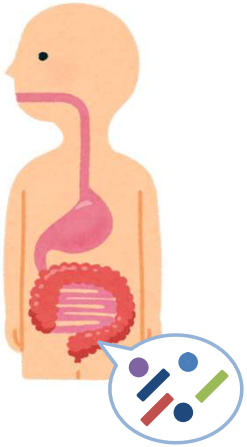
Samples

Escherichia coli



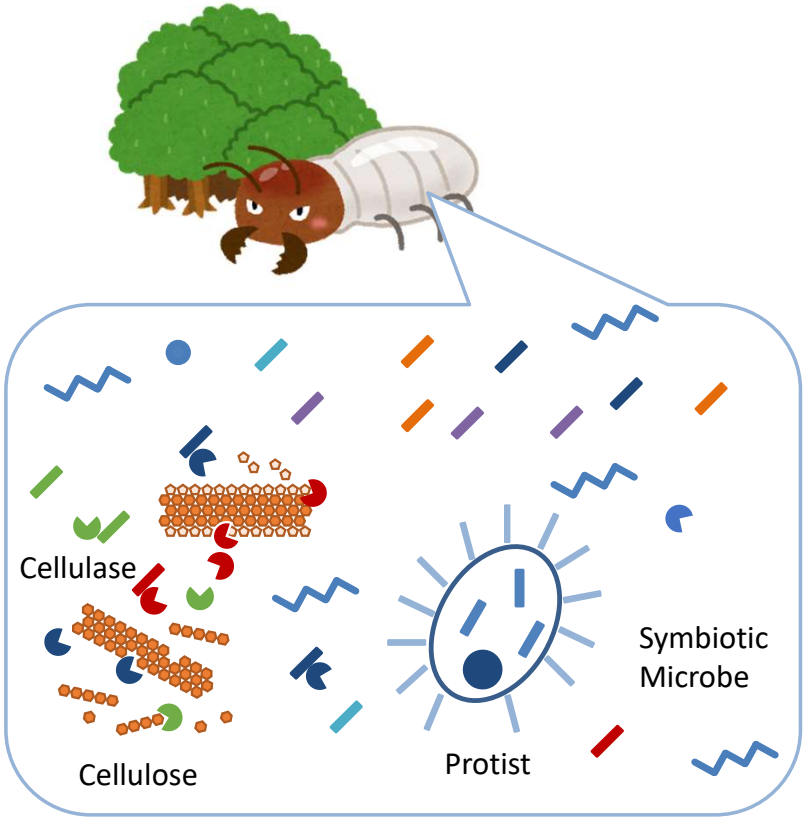
(wellcomecollection.org)

Mock

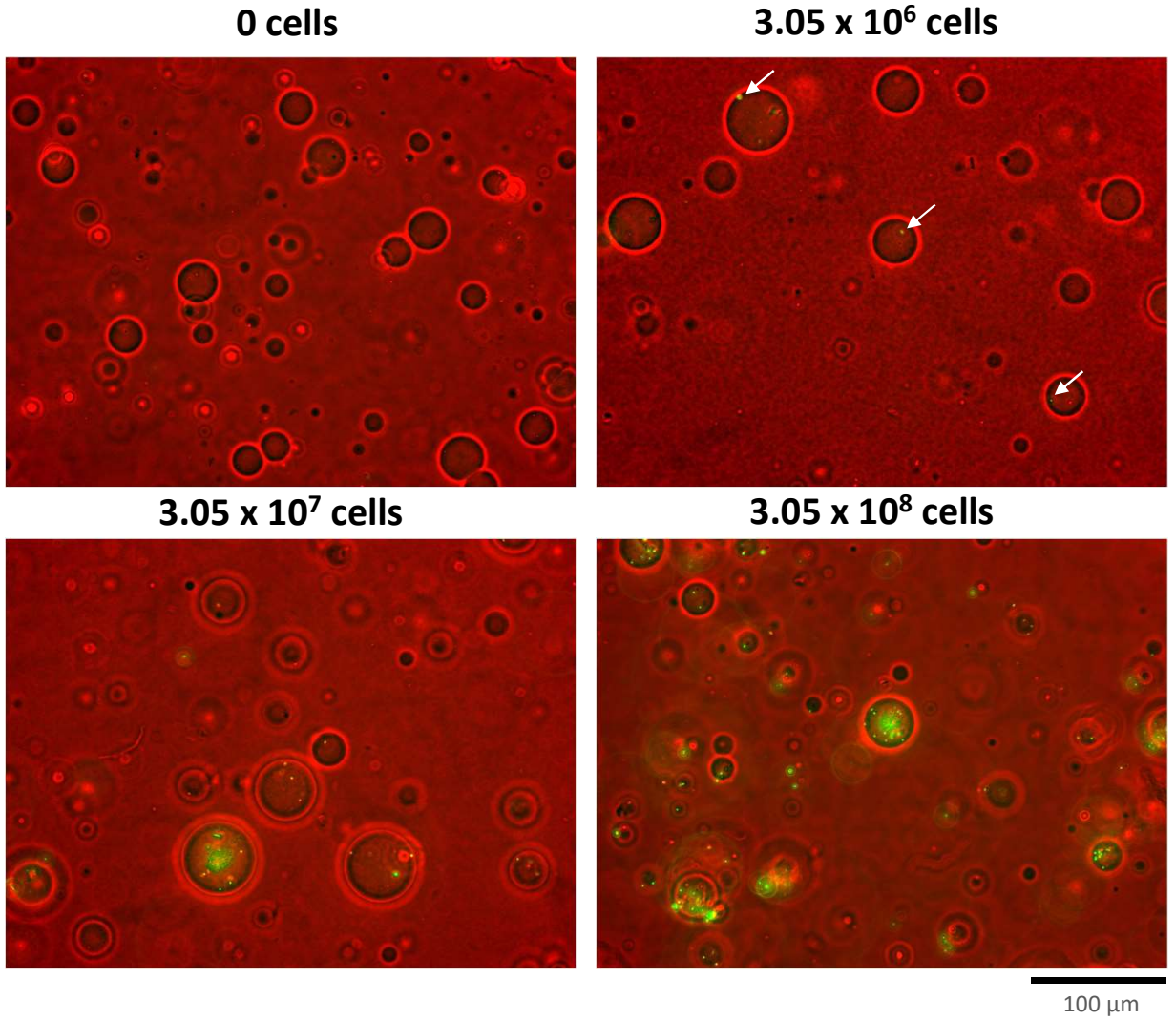


No.	Strain	No.	Strain
No. 1	*Bacteroides thetaiotaomicron*	No. 9	*Megasphaera elsdenii*
No. 2	*Blautia producta*	No. 10	*Parabacteroides distasonis*
No. 3	*Catenibacterium mitsuokai*	No. 11	*Prevotella copri*
No. 4	*Clostridium bolteae*	No. 12	*Roseburia faecis*
No. 5	*Collinsella aerofaciens*	No. 13	*Ruminococcus gnavus*
No. 6	*Faecalibacterium prausnitzii*	No. 14	*Streptococcus mutans*
No. 7	*Flavonifractor plautii*	No. 15	*Veillonella tobetsuensis*
No. 8	*Megamonas funiformis*		

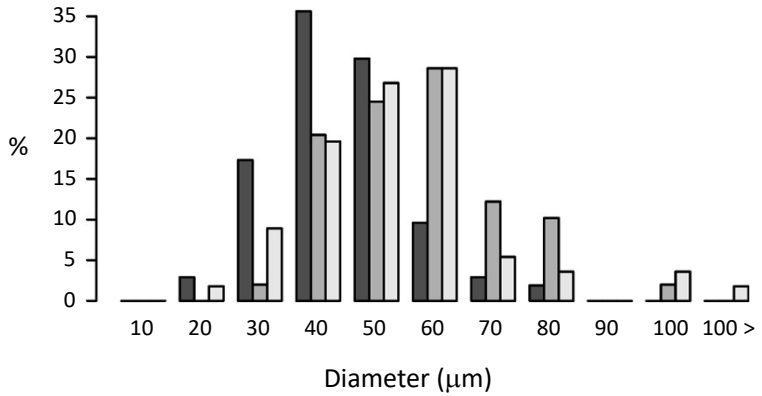
Termite Gut Microbiome

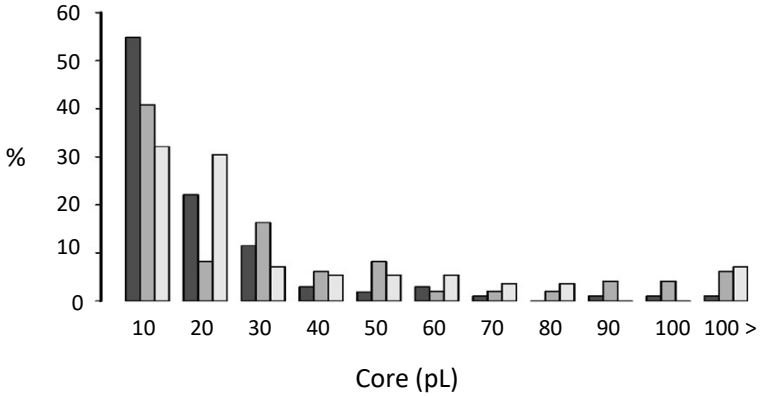


Cell Density



AGM Library

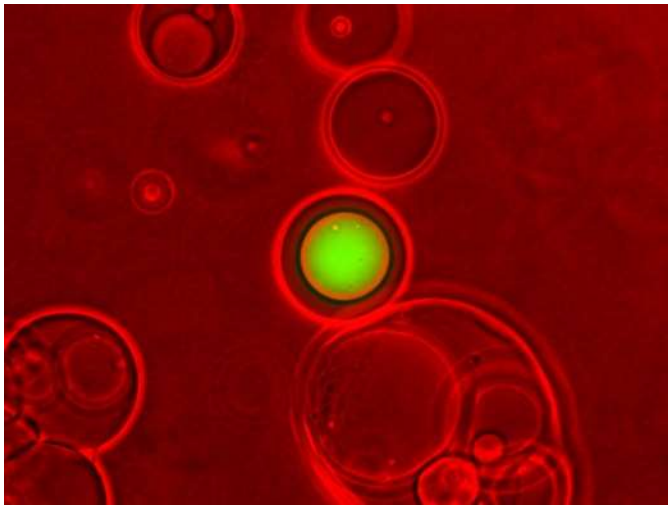




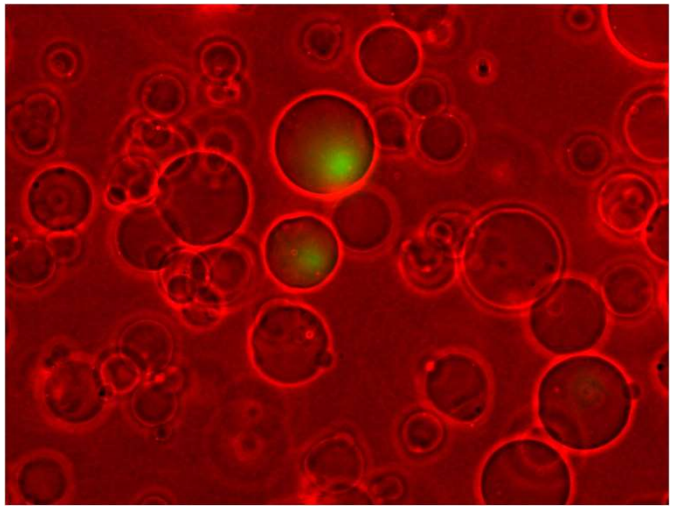
Total	53.2	±	36.8	$\times 10^5$ particle/batch
E. coli +	5.6	±	1.7	$\times 10^5$ particle/batch
	12.5	±	5.4	%
Single cell / *E. coli* +	93.8	±	8.8	%
Diameter	45.5	±	8.3	μm
Core	23.7	±	11.1	pL
Shell	7.6	±	2.7	μm

MDA

AGM



Agarose Bead



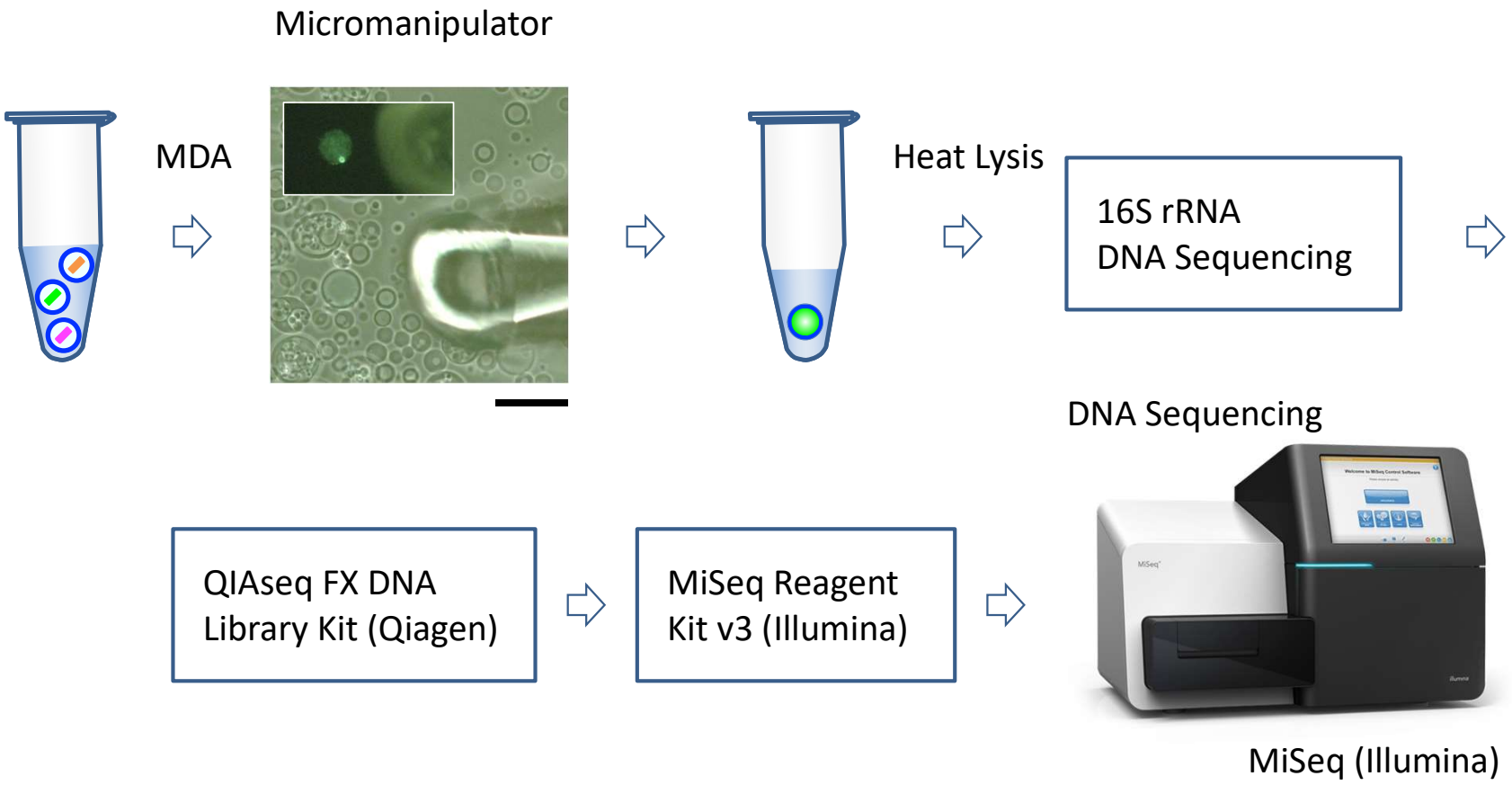
(Repli-g UltraFast Mini Kit, Qiagen)

SYBR Green I / Phase Contrast

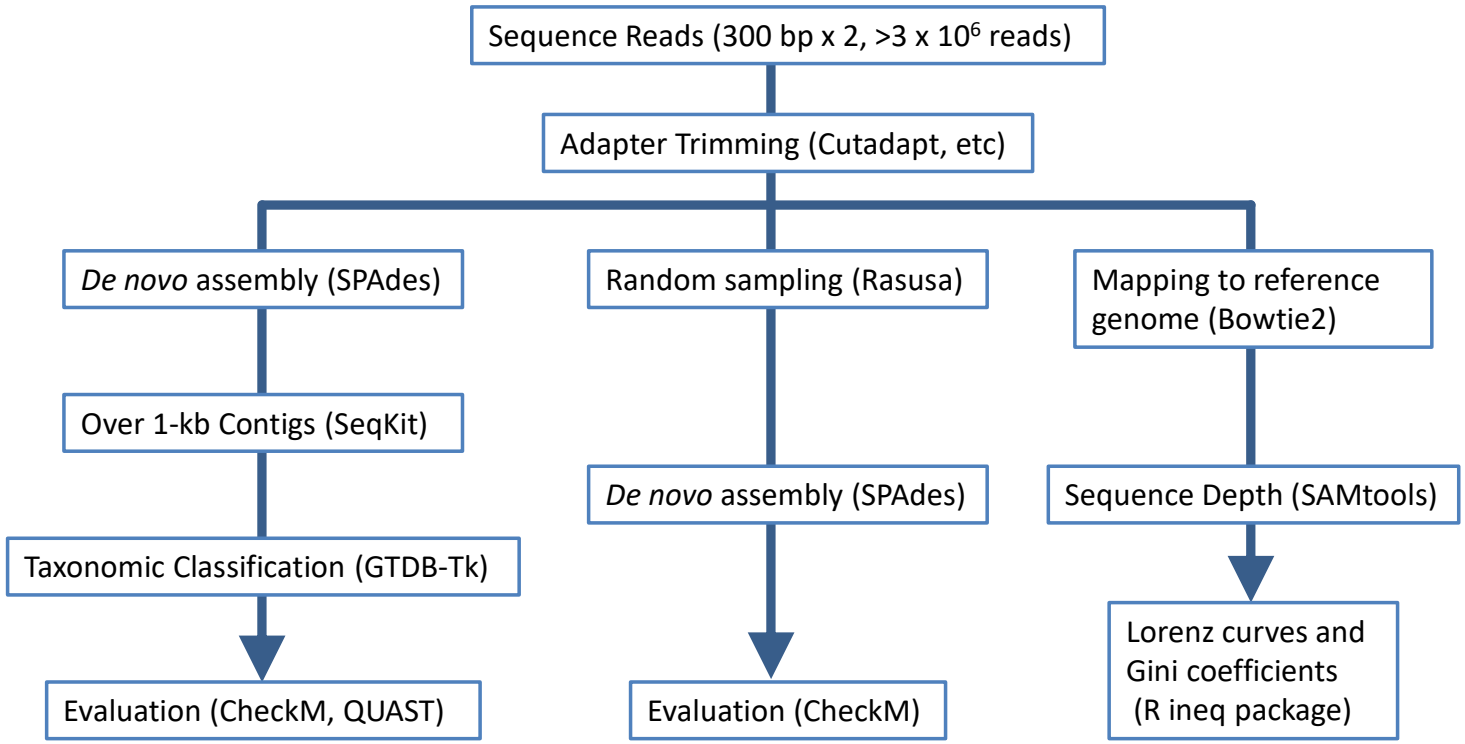
100 µm

WGA +	4.6	±	2.9	$\times 10^5$ particle/batch
	8.9	±	0.9	%
WGA + / *E. coli* +	93.8	±	8.8	%

Genome Sequencing



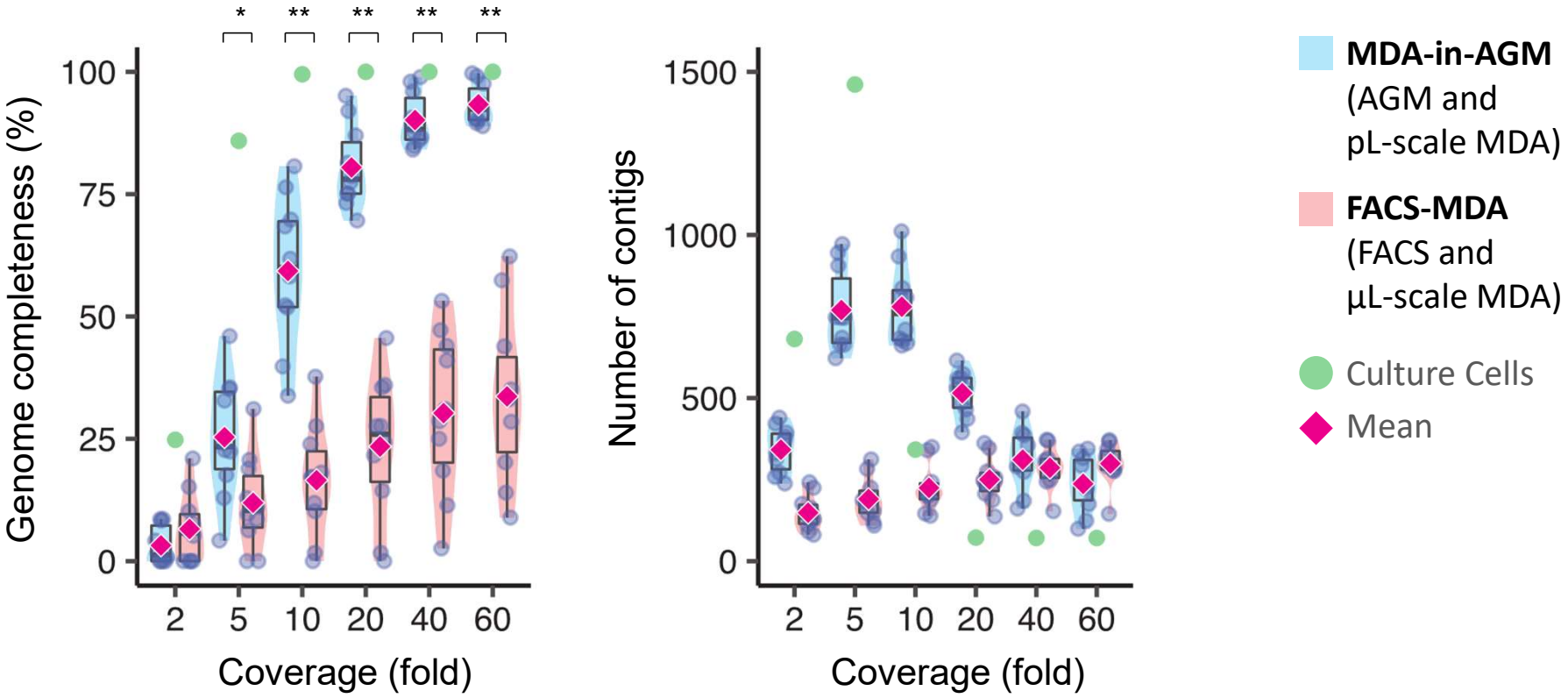
Data Analysis



Single-cell Amplified Genome (SAG)

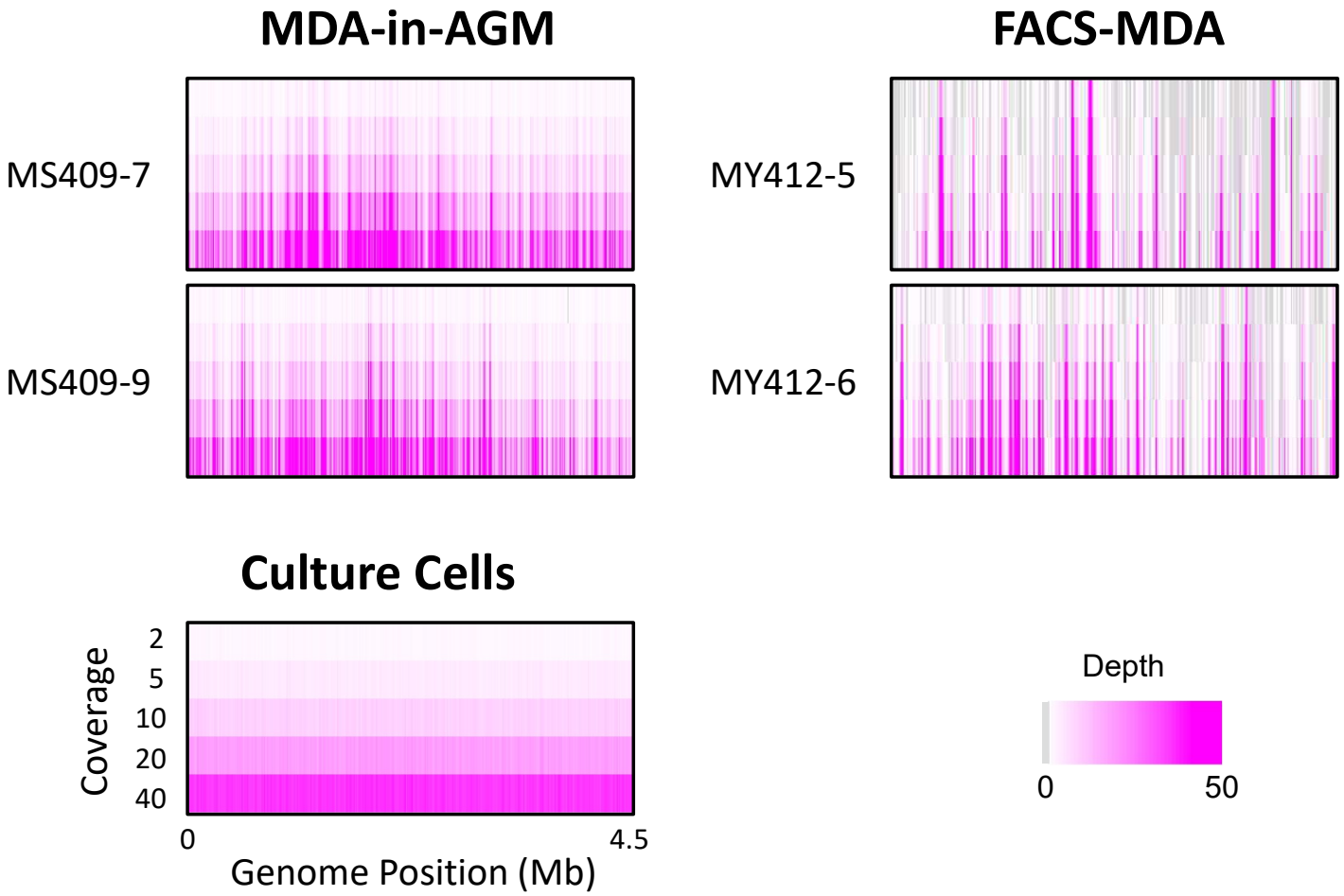
Evaluation of Sequence Read Distribution

Completeness and Contigs of *E. coli* at Different Coverages



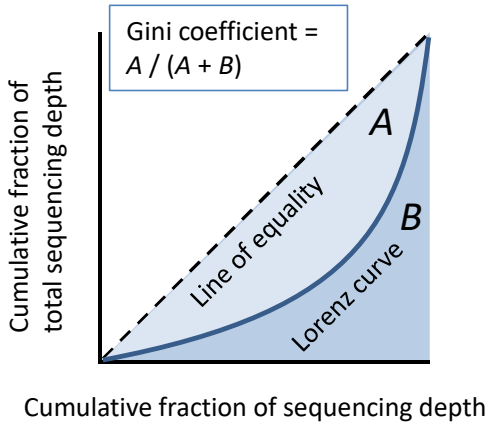
(**: $P < 0.01$. *: $P < 0.05$)

Heat Map of *E. coli*

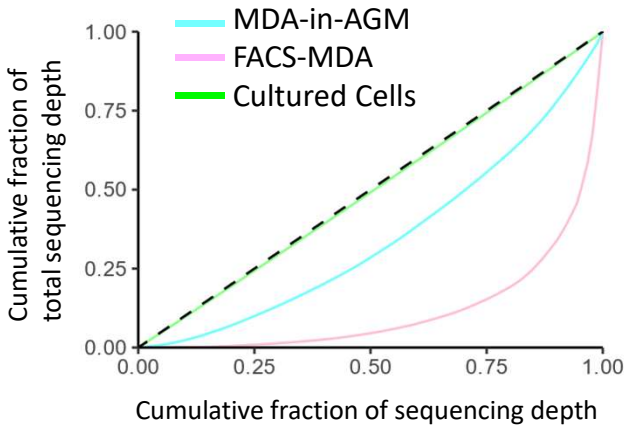


Lorenz Curve and Gini Coefficient

Lorenz Curve

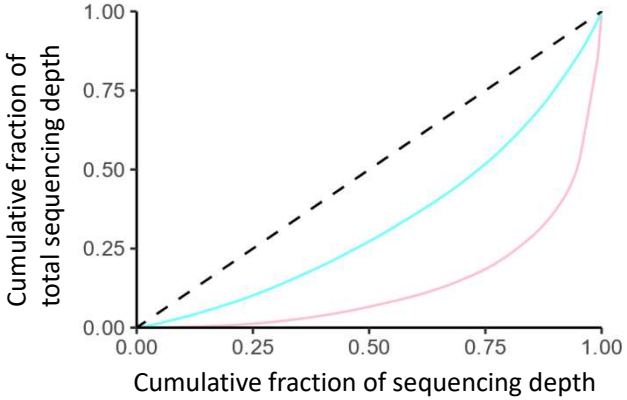


E. coli

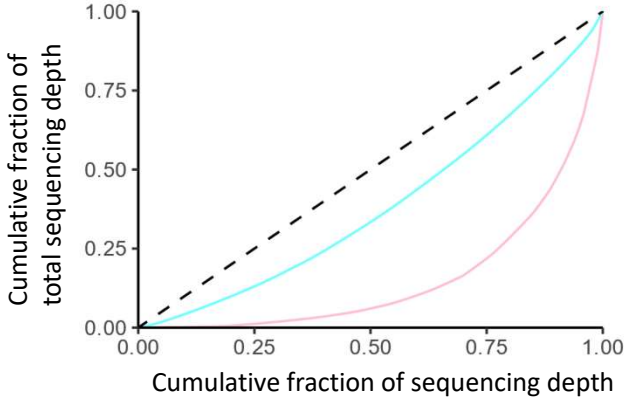


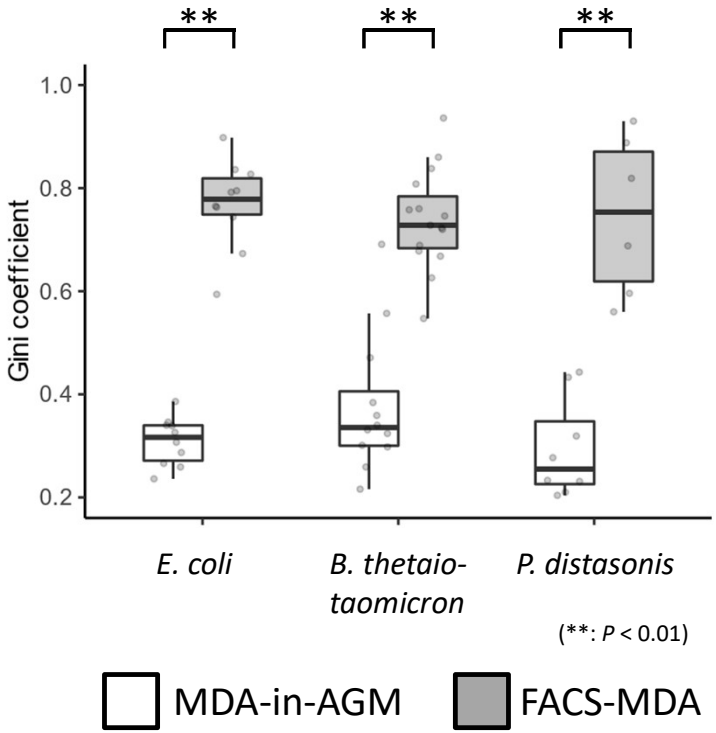
Mock

Bacteroides thetaiotaomicron



Parabacteroides distasonis





Mock

	MDA-in-AGM			FACS-MDA		
Strain	SAG	Genome completeness (%)	Contamination (%)	SAG	Genome completeness (%)	Contamination (%)
Bacteroides thetaiotaomicron	12	67.9 ± 22.0	1.9 ± 1.2	15	53.7 ± 19.6	0.8 ± 1.1
Blautia producta	9	74.5 ± 16.2	1.6 ± 0.9	3	19.1 ± 8.7	0.1 ± 0.1
Catenibacterium mitsuokai	2	97.1	0.9	n. d.	n. d.	n. d.
Clostridium bolteae	n. d.	n. d.	n. d.	2	37.9	0.0
Collinsella aerofaciens	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Faecalibacterium prausnitzii	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Flavonifractor plautii	n. d.	n. d.	n. d.	1	19.0	3.5
Megamonas funiformis	4	73.5 ± 39.6	2.0 ± 1.2	3	89.5 ± 7.7	1.2 ± 0.5
Megasphaera elsdenii	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Parabacteroides distasonis	8	84.4 ± 12.6	2.2 ± 1.3	6	44.9 ± 23.0	0.7 ± 0.7
Prevotella copri	2	88.5	1.8	4	54.0 ± 6.8	1.2 ± 1.4
Roseburia faeccis	1	28.1	1.7	n. d.	n. d.	n. d.
Ruminococcus gnavus	1	81.5	2.5	1	54.1	1.2
Streptococcus mutans	1	97.8	1.1	n. d.	n. d.	n. d.
Veillonella tobetsuensis	n. d.	n. d.	n. d.	2	75.9	0.4
Total	40			37		

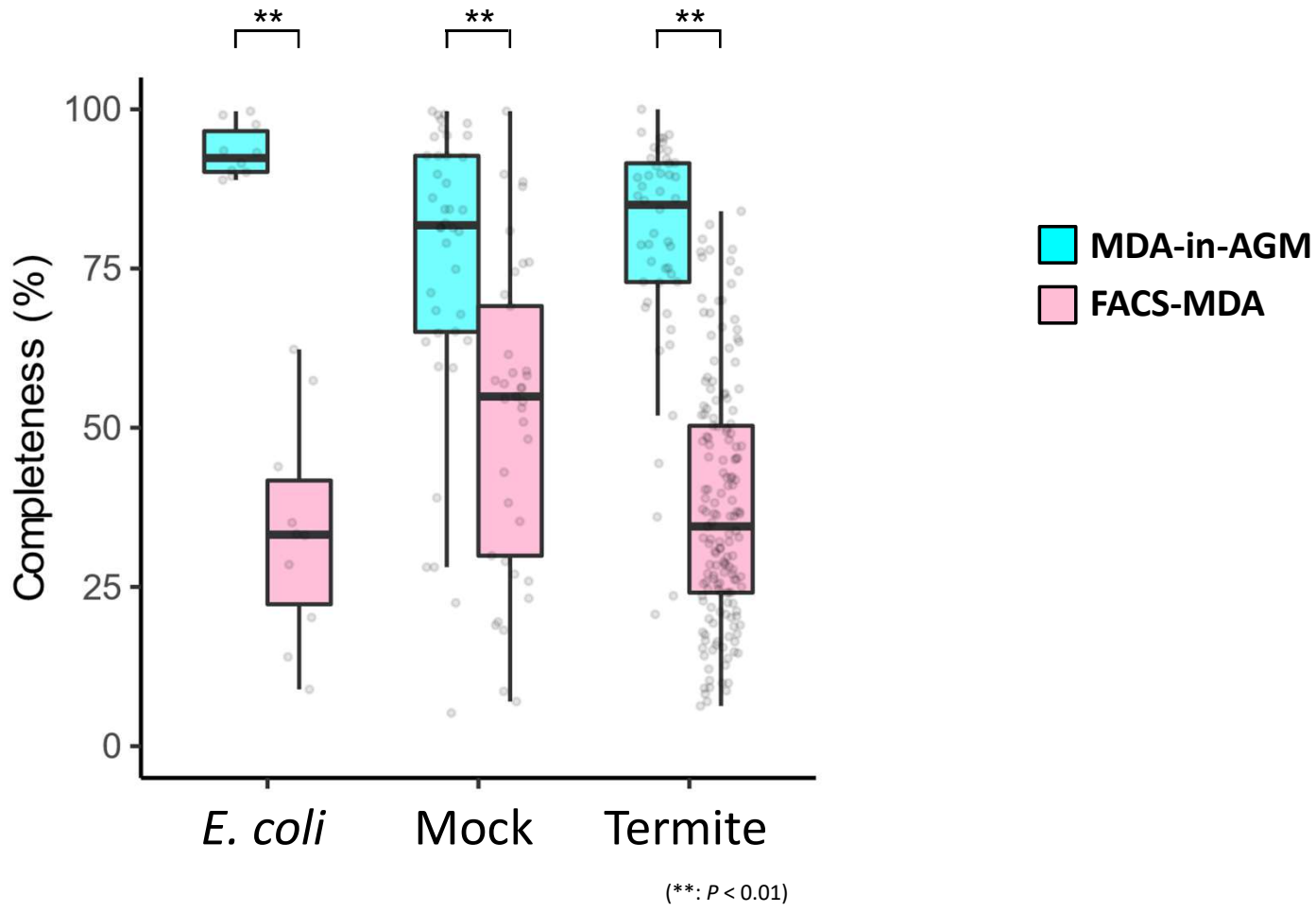
n. d.: not detected

Termite Gut Microbiome

	MDA-in-AGM			FACS-MDA†		
Taxon of bacteria	SAG	Genome completeness (%)	Contamination (%)	SAG	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	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	2.2	2	38.8	1.2
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	1.2	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	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.
†: DNA libraries were made using Nextera XT (Illumina) and read using HiSeq (Illumina).
( ): including Endomicrobia

Suppression of Amplification Bias using AGM

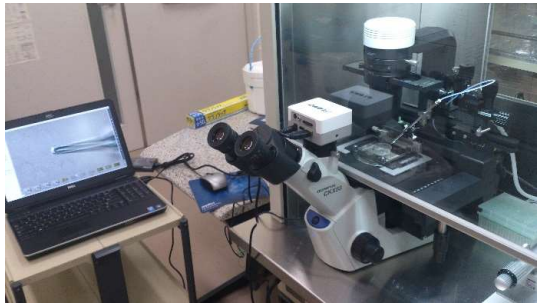


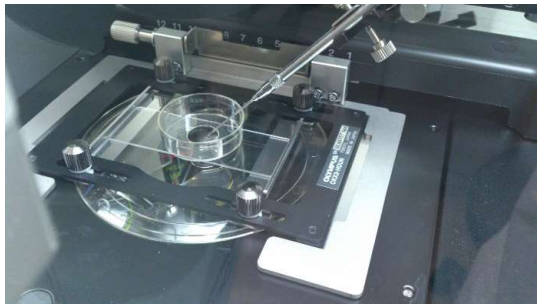
Conclusions

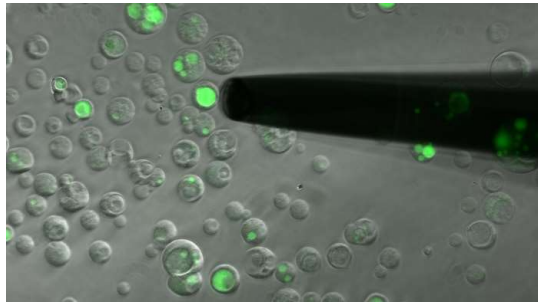
- **AGM**, a microcapsule consisting of a sol core and a hydrogel shell, was developed to **SCG** using stable shell gelation with emulsion oil of water-equivalent density.
- AGM **suppressed amplification bias** using one-cycle pL-scale MDA.
- AGM will allow many researchers easily to obtain high quality SAGs, and can accelerate genomic analysis of **yet-uncultured microorganisms**.

AGM Picker

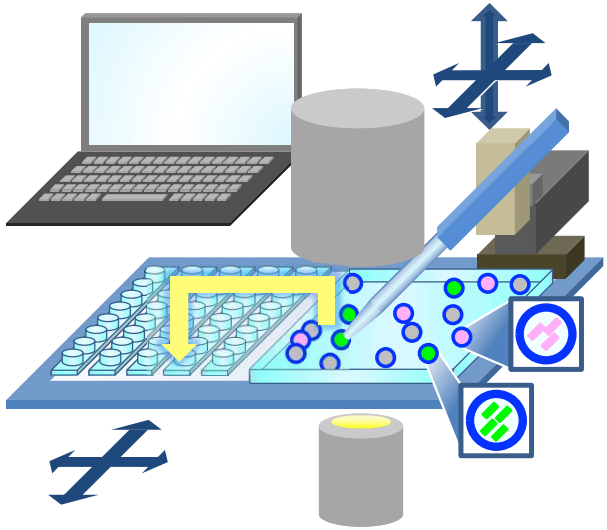
Manual Picking





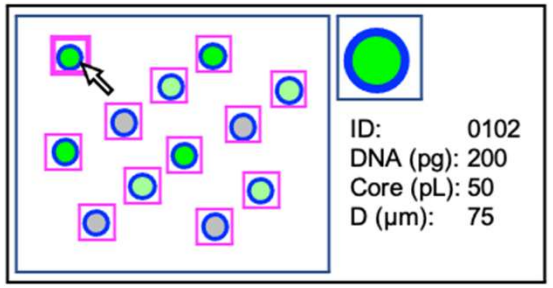


Automatic Picking



- 50—100 µm AGM Core
- Motorized Components
- Transfer Single AGM in a microtube or microplate

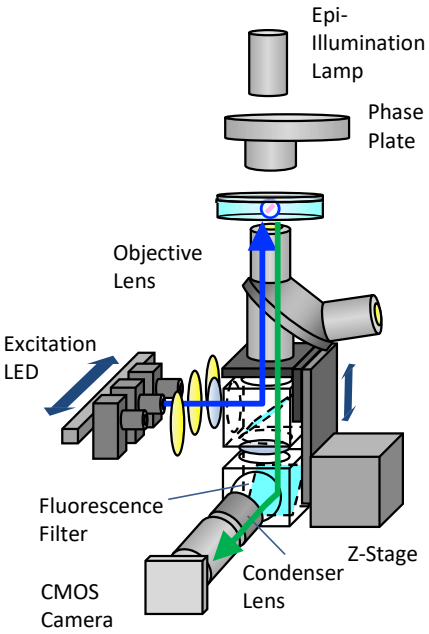
Throughput Improvement



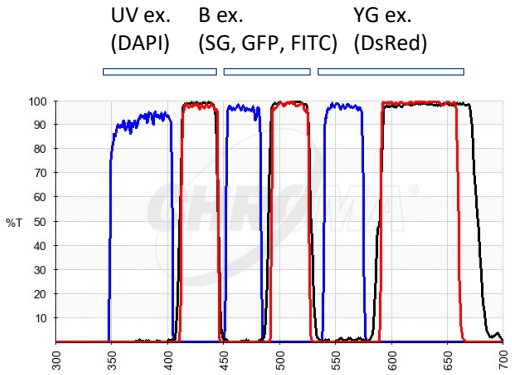
- Fluorescent Quantification

Anaerobic culture and isolation

Optics

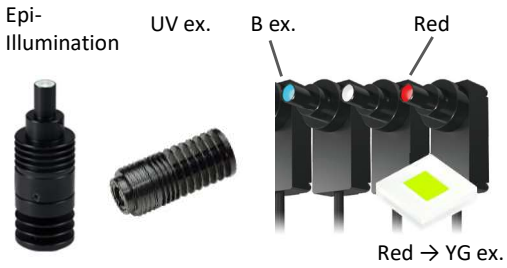


Fluorescent Filter



(Chroma Technology, Multi LED Set for CoolLED)

LED



High-sensitive CMOS Camera

(The Imaging Source, DBK37BUX178,
using SONY CMOS Stavis IMX178)

Objective Lens

(Olympus,
PLN10XPH)

(Olympus,
LUCPLFLN40XPH)

Optimisation of Condenser Lens using Zemax

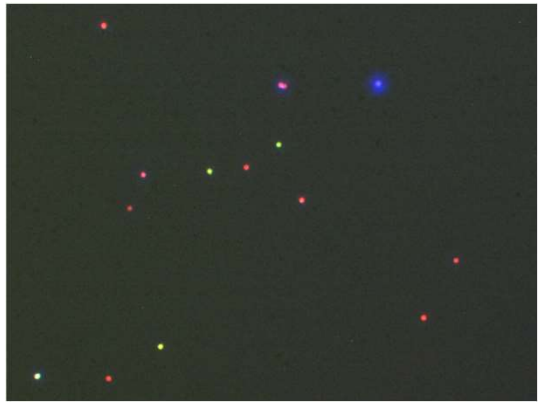
Achromatic Lens

Edmond Optics, Cat. 47-715
(d = 30 mm, WD = 75 mm)

Fix-focus Lens

Edmond Optics, Cat. 63-249
(WD = 75 mm)

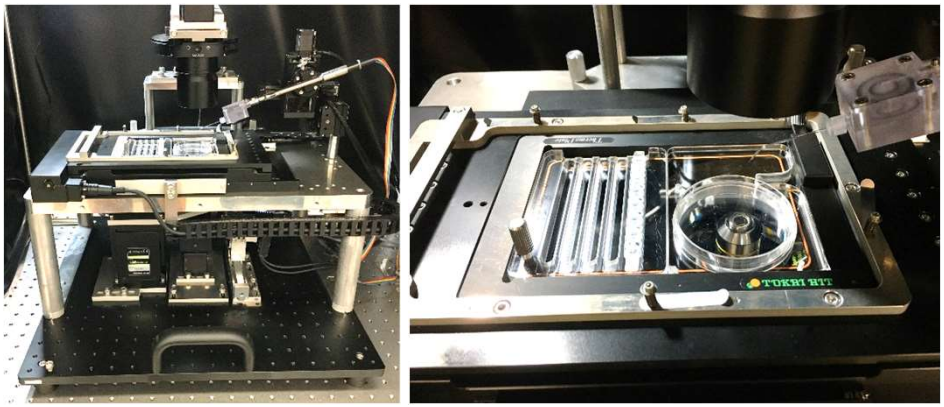
Fluorescent Beads (4 µm)

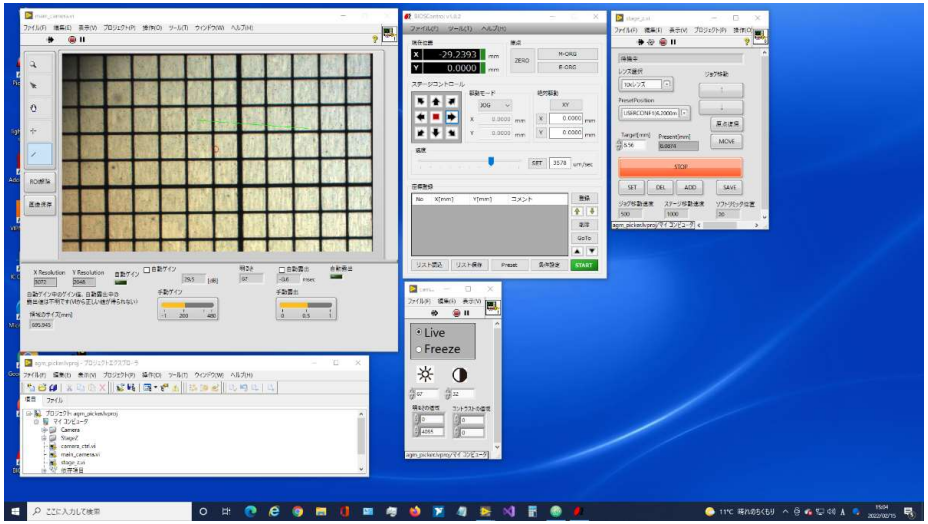


UV ex: Blue, B ex: Green, YG ex: Red

100 µm

Picking

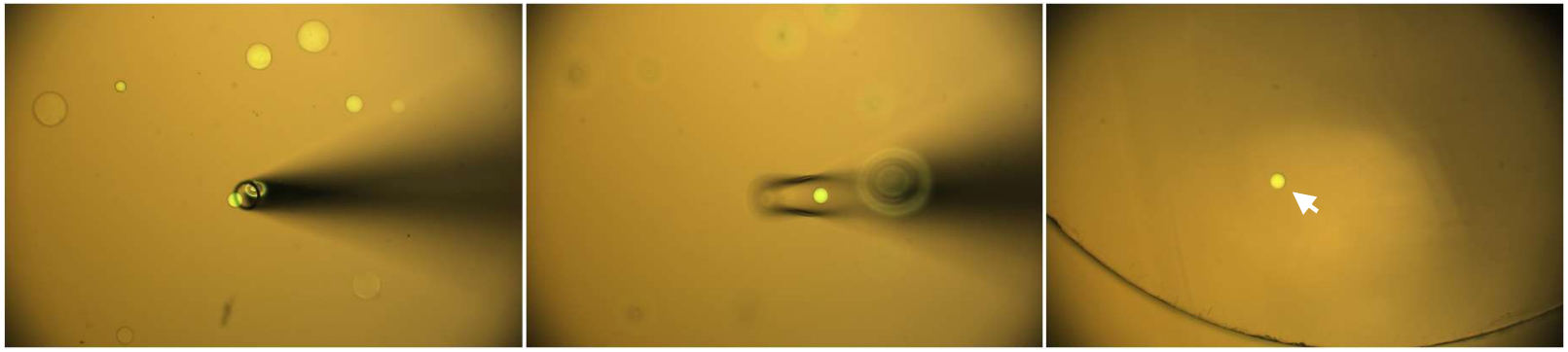




Before Picking

After Picking

Dispense



100 µm

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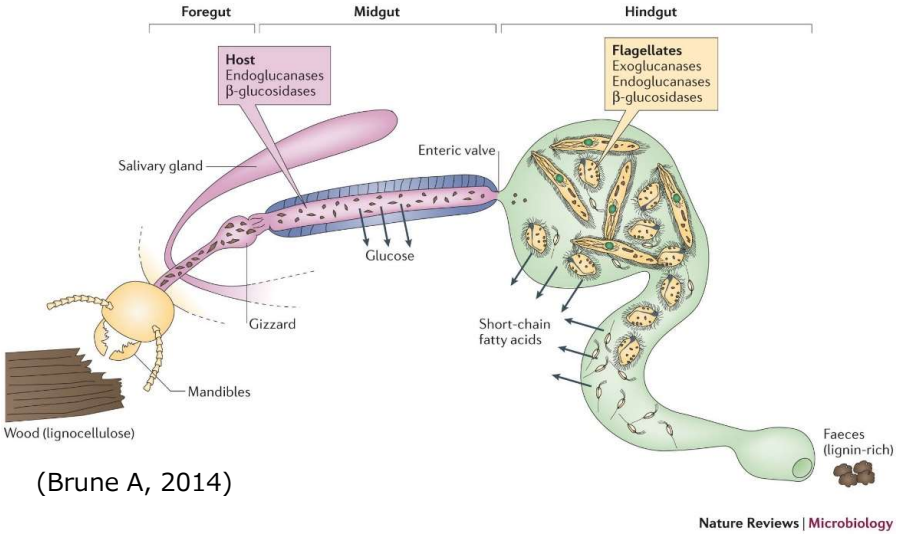
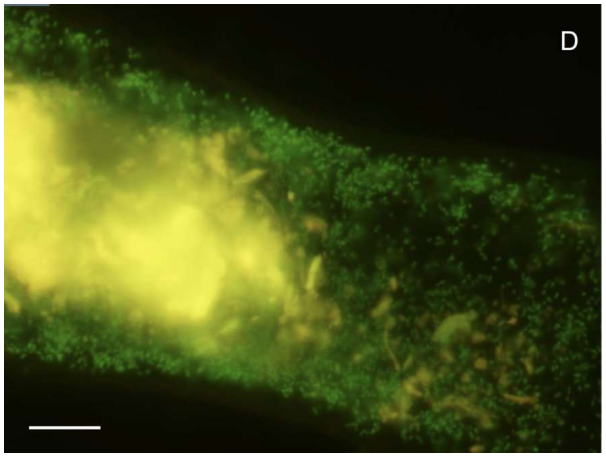
  - FY2019 Incentive Research Project
- JSPS KAKENHI
  - Grant Number 16K07224
- TOYO Corporation

Termite Gut Microbiome



(Brune A, 2014)

Yet-uncultured Bacteria



(Hongoh, 2008)

- Highly Efficient Utilization of Plant Biomass
- Complex Symbiosis between bacteria, protozoa, and host