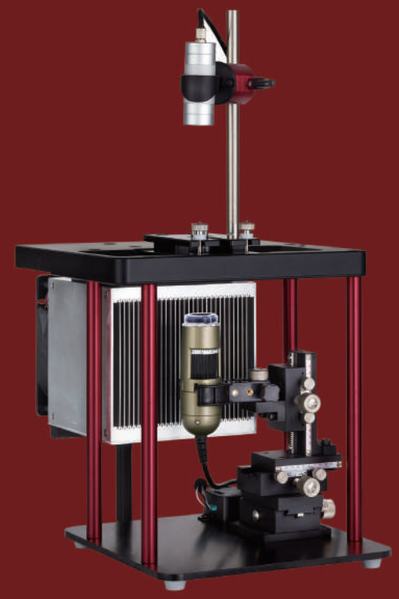
On-chip Droplet Generator

Easy generation of stable and monodisperse droplets



On-chip Biotechnologies Co., Ltd.

2-16-17 Naka-cho, Koganei-shi, Tokyo 184-0012, Japan
TEL.+81-42-385-0461 FAX.+81-42-385-0462

ONCHIP-02B-U005

On-chip Droplet Generator

Create a culture microenvironment within micro-scale aqueous droplets

On-chip Droplet Generator allows mass production of micrometer-sized water-in-oil (W/O) emulsion droplets and gel microdrops (GMDs) with a unique microfluidic chip.
The individual droplets function as miniaturized vessels for reactions such as microbial/cell cultivation and genetic analysis.
Droplet technology is now recognized as a prominent high-throughput screening method allowing single cell analysis or simultaneous assay of more than 1 million samples.

Enhance your research using On-chip Droplet Generator

- **Exploration of novel microorganisms in the environment**
  Encapsulation, cultivation and isolation of a variety of microorganisms from soil, intestinal microbiota or marine.
- **Development of highly productive strains**
  Detection and separation of enzymes and metabolites secreted in droplets.
- **Screening of antibody-producing cells**
  Development of stable and productive antibody secretion cell lines.

Features of On-chip Droplet Generator

■ Easy generation of stable droplets

On-chip Droplet Generator allows mass production of stable and monodisperse droplets by simply putting reagents in each reservoir and applying pressure.

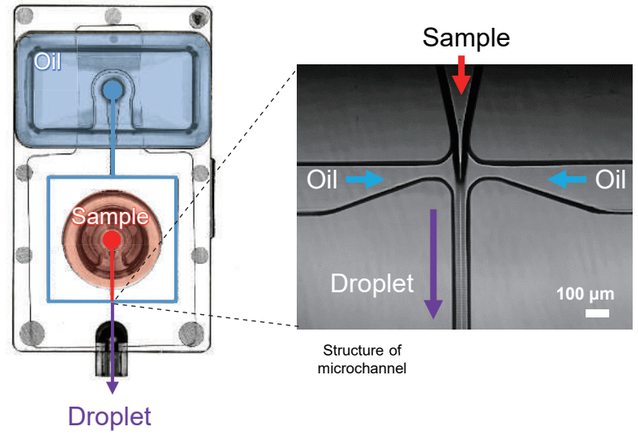
■ Easy adjustment of droplet size

Droplet size can be easily adjusted between 60 μm and 220 μm in diameter by regulating the pressure applied to the sample and oil reservoirs.

■ Real-time monitoring of droplet generation

Droplet size can be viewed and monitored real-time with the supplied monitoring device.

2D Chip-1060DG/1100DG



Structure of microchannel

Types of droplets generated

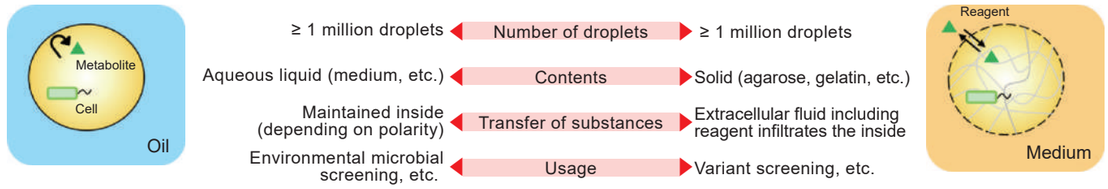
On-chip Droplet Generator can produce two types of droplets:
water-in-oil (W/O) emulsion droplets and gel microdrops (GMDs).

【 W/O droplets 】

W/O emulsion in which water droplets are dispersed in oil

【 GMDs 】

Droplets solidified by gel are dispersed in aqueous solution



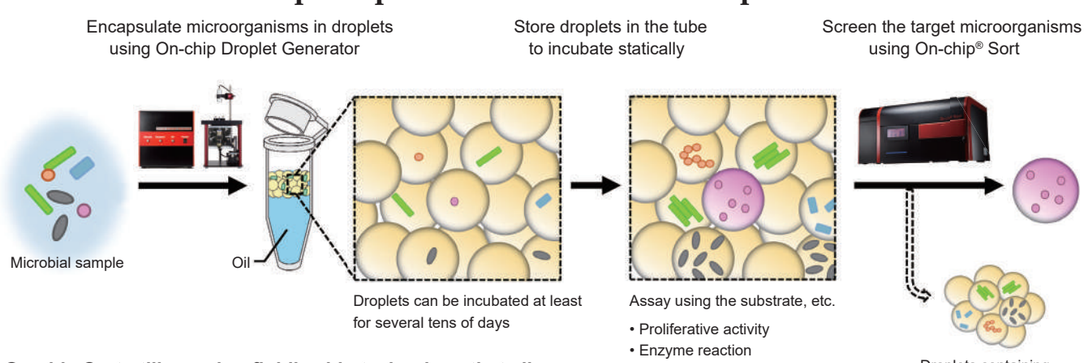
Immediately after encapsulation — After cultivation

***Escherichia coli* (*E. coli*)**

Immediately after encapsulation — After cultivation

Lung cancer-derived cell line

Utilization of On-chip Droplet Generator and On-chip® Sort



On-chip Sort utilizes microfluidic chip technology that allows oil to be used as sheath fluid, which makes the selection of W/O droplets with microorganisms possible.

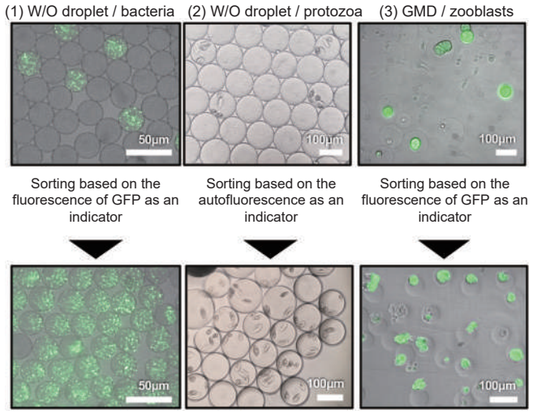
Sorting of droplets containing proliferated cells

(1) Sorting of *E. coli* encapsulated and incubated in the W/O droplets based on the fluorescence of GFP (expression within *E. coli*) as an indicator.

(2) Sorting of Tetrahymena encapsulated and incubated in the W/O droplets based on the autofluorescence as an indicator.

(3) Sorting of A549 encapsulated and incubated in the GMD based on the fluorescence of GFP as an indicator.

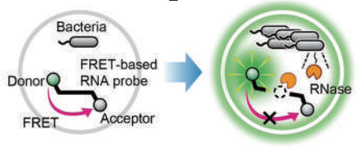
- **On-chip® Sort can be used to screen W/O droplets.**
- **On-chip® Sort can sort particles as large as 100 µm GMD.**



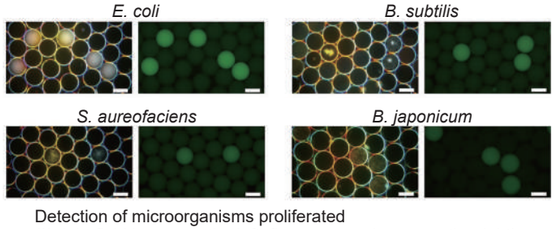
Detection of microorganisms proliferated within W/O droplets

A simple and reliable method that allows bacteria containing droplets to be isolated on On-chip® Sort has been developed. W/O droplets containing bacteria were sorted based on the fluorescence produced as a result of the interaction between the bacteria and fluorescence resonance energy transfer (FRET)-based RNA probe encapsulated inside the droplets. This method is named Fluorescent Nucleic Acid Probe in droplets for Sorting bacteria (FNAP-sort). An increase in the fluorescence intensity of *E. coli*, *Bacillus subtilis*, *Streptomyces aureofaciens* or *Bradyrhizobium japonicum* cultivated as model microorganisms were identified in the droplets in which these microorganisms proliferated. The use of this system allows screening based on the proliferative activity in the environment and other cellular events.

- **Development of a system that can easily detect microbial proliferation.**
- **Application to screening of difficult-to-cultivate microorganisms in which proliferation is slower.**



Principles of FNAP-sort: the fluorescence intensity is increased by breaking the probe with RNase produced by microorganisms



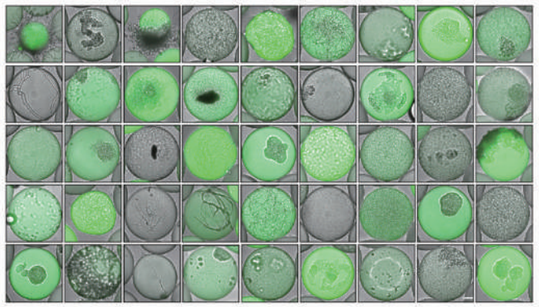
Detection of microorganisms proliferated
(*bright field image on the left, fluorescence image on the right)

Collaboration with Research Group Leader Noda, AIST, Biomedical Research Institute
Ota, Y., Saito, K. et al. PLoS ONE 14(4): e0214533.

Screening for high enzyme producing bacteria from soil

Sampled microorganisms from the soil were encapsulated and incubated with the substrate which responded to enzyme (peptidase) in W/O droplets. One million droplets were analyzed and droplets with higher fluorescence intensity (i.e., high enzymatic activity) were sorted on On-chip® Sort. Collected droplets were transferred to agar plate. For those that formed colonies,
supernatant assay was performed after re-incubation. Successful isolation of highly peptidase-producing microorganism was achieved using On-chip Droplet Generator, followed by On-chip® Sort.

- **W/O droplets allows for screening of 1 million environmental microorganisms in several hours.**
- **W/O droplets allows for screening of a variety of proliferative forms and enzyme activity.**



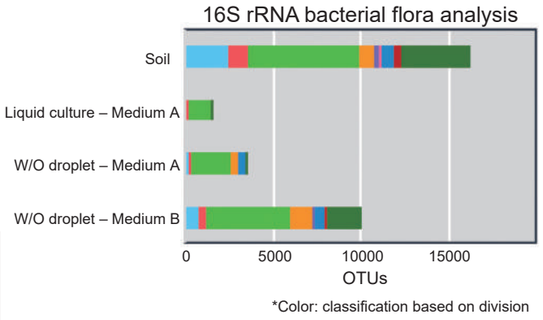
Cultivation of environmental microorganisms in W/O droplets and evaluation of the fluorescence intensity

Collaboration with Prof. Ogasawara, Nagaoka University of Technology

Cultivation within W/O droplets maintains microbial diversity

Bacteria from soil were cultivated in liquid media and in W/O droplets over 7 days. DNA was extracted and analyzed by NGS to study the bacterial flora. The bacterial flora in the droplets showed to be much higher in individual Operational Taxonomic Units (OUTs) compared to those cultivated conventionally in liquid media.

- **Cultivation using W/O droplets can possibly cultivate a wider variety of soil microorganisms than the conventional cultivation method.**
- **Putting one bacterium in one droplet allows for cultivation of a variety of microorganisms.**



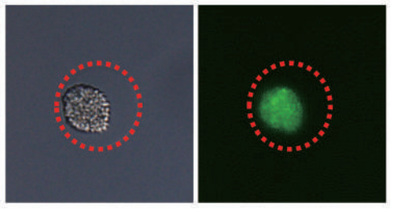
*Color: classification based on division

Collaboration with Assoc. Prof. Tashiro, Kyusyu University

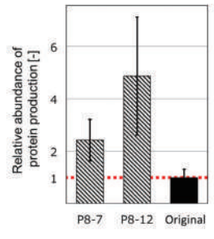
Screening of high protein producer yeast with GMD

Yeast transformant that produces secreted luciferase were UV irradiated for random mutagenesis. They were then encapsulated and cultured within GMDs. GMDs with high fluorescence intensity derived from luciferase were sorted using On-chip® Sort and transferred to agar medium. For colonies formed, the supernatant was collected and an assay was performed on the supernatant after re-incubation. As a result of the screening, strains with higher producing ability than that of the parent strain were successfully collected.

- **Successful collection of strains with 5-fold increase in productivity from 10^5 UV-induced variant yeast.**
- **The use of GMDs realizes the screening in a shorter time (within 2 days) and at a lower cost.**



↑Cultivation of yeast within GMD:
Yeast forms microcolonies.
Green fluorescence is from luciferase.
*Red broken line indicates the outline of GMD.



←Number of strains with high-level protein secretion:
The strain with 5 times as high producibility as that of the parent strain

Collaboration with Prof. Machida, Kanazawa Institute of Technology
Fujitani, H., et al. 2019. bioRxiv 830596; doi: https://doi.org/10.1101/830596

Specification

♦ The On-chip Droplet Generator set up contains of the main unit for supplying pressure, monitoring unit for real-time monitoring of droplets, and temperature controller for precise temperature adjustment.

Specifications		On-chip Droplet Generator Monitoring unit with temperature control
Main unit	Size	270 x 270 x 280 mm (W x D x H)
	Liquid delivery pressure	0.1 – 80.0 kPa
	Maximum liquid delivery line	3 lines (sample, oil, other reagents)
	Droplet size generated	Diameter 60 – 220 μm (can be adjusted discretionally)
	Generation rate	Approx. 100,000 droplets/3 min (at generation of 100 μm droplets)
	Regulation	Laptop PC (Windows 10)
	Power input	AC 100 – 240V, 50/60Hz
	Power consumption	1.2A typ (ACIN 100V)
Monitoring unit	Size of monitoring unit	215 x 310 x 430 mm (W x D x H)
	Accessories	Monitoring camera, Peltier unit, controller
	Temperature range	–5 – 70°C
	Ambient environment	Temperature 5 – 40°C, humidity ≤ 85%RH
	Power input	DC24V
	Power consumption	7.1A max

Product No.	Product name	Product description
60003	On-chip Droplet Generator (Monitoring unit with temperature control)	Main unit, Chip holder, Monitoring unit with temperature control

Chip & holder

♦ Stable mass production of droplets with a simple operation

Specifications

Samples applied : Aqueous solution, culture medium, gel solution, etc.

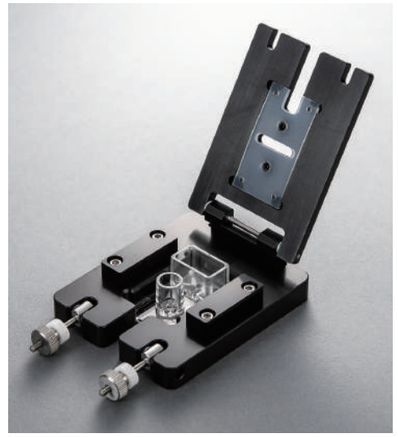
Oils applied : Fluorinated oil

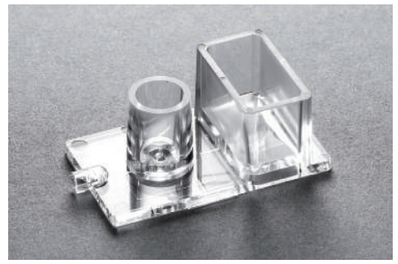
Chip material : COP

Channel size : 60 μm x 60 μm, 100 μm x 100 μm

Reservoir volume : Sample – up to 640 μL; Oil – up to 2300 μL

Droplet generation rate : > 400,000 droplets / 5 min (for 120 μm)





Product No.	Product name	Product description
63006	DG1 Chip Holder	Chip holder, Tube rack, Glass vials
1003003	2D Chip-1060DG	Droplet generation chip (60 μm channel)
1003004	2D Chip-1100DG	Droplet generation chip (100 μm channel)

Reagent

♦ Fluorinated oil is used for producing stable droplets.

Product No.	Product name	Details	Volume
2003003	On-chip FluoroSurfactant-5wtH Oil	Fluorinated oil containing 5% surfactant.	10 mL
2003004	On-chip FluoroSurfactant-0.1wtH Oil	Fluorinated oil containing 0.1% surfactant.	100 mL

For inquiries:

On-chip Biotechnologies Co., Ltd.

Phone: +81-42-385-0461 Fax: +81-42-385-0462

E-mail: info@on-chipbio.com Home page: https://www.on-chipbio.com