C297-E135

SHIMADZU
Excellence in Science

Solutions for Cell Processing

Solutions for Cell Processing

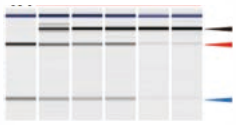
Shimadzu utilizes not only in-house technologies but also related technologies in cooperation with other companies to support researchers and companies engaged in cell research and cell production, and accelerate industrialization of regenerative medicine in order to realize our wishes for the "Well-being of Mankind and the Earth".

Cloning

A target clone strain is established through a complicated process such as gene transfer, genome editing, cell sorting, recovery, and verification of genes.

Donated Cell

Fractionating Target Cells

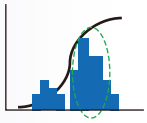


Confirming Mutations

Cell Culture

The target clone strain is raised and proliferated in a comfortable environment in a culture medium (proliferation and culture), and the cell density is maintained appropriately by subculture work.

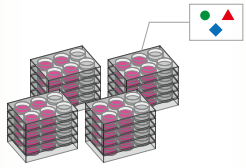
Proliferation and Culture

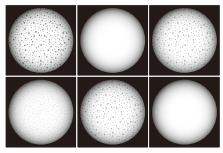


Measurement of Viable Cells

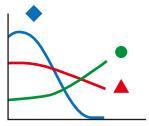
Cell Production and Non-invasive Evaluation

In the manufacturing process of cellular products, quality information is obtained to manage product status during the process.





Observation and Image Acquisition



Number of Days of Culture

Component Analysis in Culture Supernatants

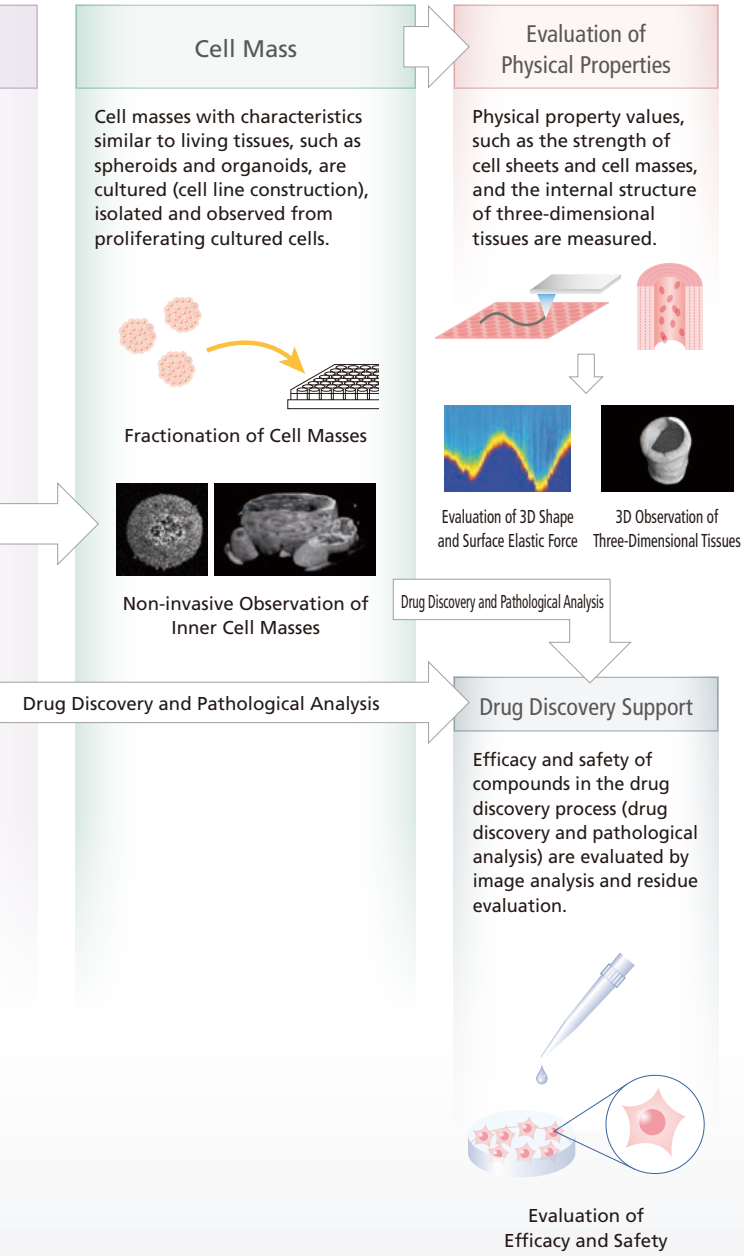
Cell Line Construction

CELL PICKER
Automated Pick and Collection Tool of Cell Colonies

C2MAP System
Cell Culture Media Analysis Platform

MultiNA
Microchip Electrophoresis System for DNA/RNA Analysis

AA-7000
Atomic Absorption Spectrophotometer



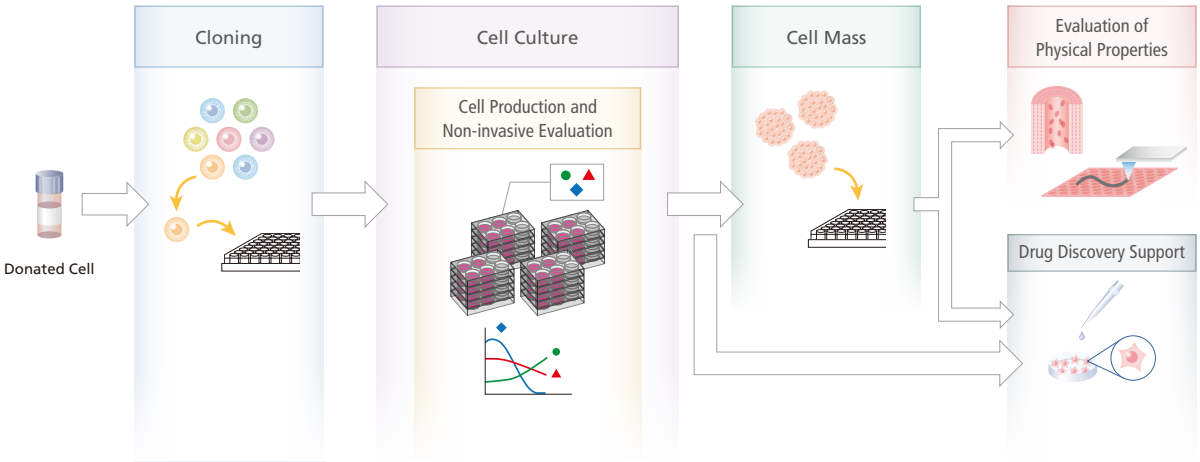
SPM-9700HT
Scanning Probe Microscope

CONTENTS

Establishment and Isolation of Target Cells

– Solutions for Cloning –

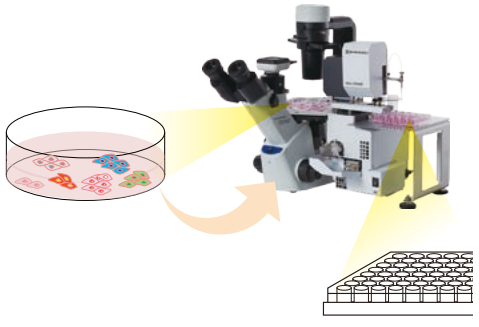
Cloning is used to detect and identify cell lines with specific DNA fragments and produce groups of cells with the same genotype (establishment of clone strains).
This section introduces systems that improve the efficiency of establishing clone strains for gene transfer and genome editing.
These products were designed to reduce costs and labor and improve work efficiency by replacing the complicated process required to establish clone strains, such as cell sorting, recovery, observation, and verification of genes, with a new method.



Automated Pick and Collection Tool of Cell Colonies

CELL PICKER

Provides the picking procedure without depending on operator expertise



Collects target colonies

Microchip Electrophoresis System for DNA/RNA Analysis

MultiNA

Enables quick and inexpensive confirmation of genetic mutations or insertions

Collecting Cell Colonies

Performing delicate work for a long time under a microscope is required.

Confirming Mutations and Genes

Continuous manual operations, such as gel creation, sampling and imaging, and obtaining highly-reproducible data are required.

Automated Pick and Collection Tool of Cell Colonies **CELL PICKER**

CELL PICKER automates the process of picking up and removing cell colonies. Automation stabilizes pipetting operations.
Software makes it easy to pick up cells while observing them.

- Enables pick-up operations equivalent to manual ones
- Records your work with simple operations
- Small and space-saving design

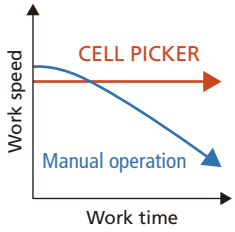
Advantages of CELL PICKER

Reduce workload and maintain work efficiency

Manual operations are thought to reduce work efficiency and speed over time.
CELL PICKER stabilizes operations to maintain efficiency.

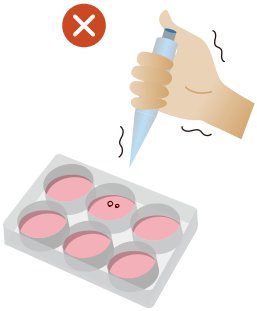
Working while looking through a microscope for an extended time leads to fatigue.

Cell observation and device operation can be performed on a tablet to reduce the burden.

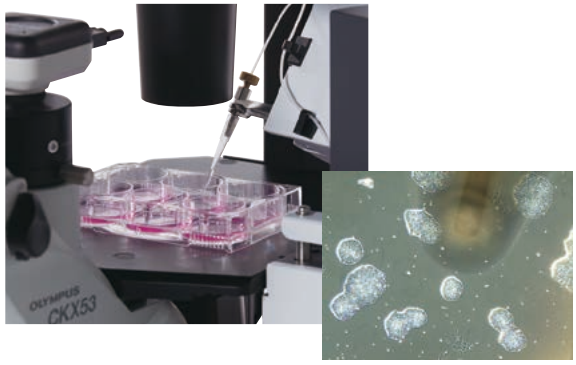


Easy operation eliminates instability

Manual operations make position adjustments of the pipette tip difficult while looking through a microscope screen. CELL PICKER allows the pipette tip to move within the microscope screen with a single click, simplifying positioning and eliminating work instability.



When a pipettor approaches cell colonies, a hand trembles and becomes unstable.



The tip of the pipette tip moves close to the colonies with a single click to eliminate instability.

Anyone Can Perform the Pickup Process with Confidence

CELL PICKER automates the cell collection, seeding, and pipette tip replacement. This stabilizes the operations of picking up and removing cell colonies, processes that typically rely on an operator's skill.

Manual Operation

Preparation

- The user searches under the microscope for the cell colonies to collect, and then marks them with a pen.

- ✕ Marking the back of the container while looking through the microscope is difficult.
- ✕ Capturing images one after the other is difficult.

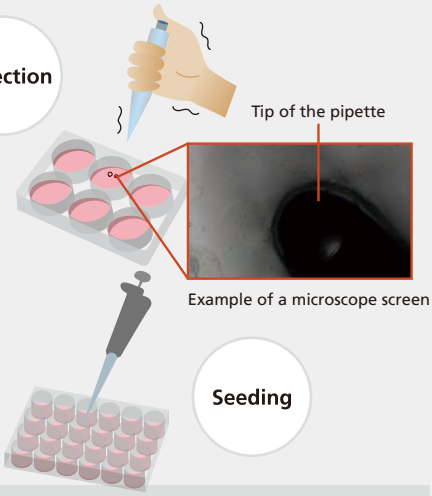
Collecting cell colonies and seeding in a new incubator

- The user operates the pipetter under the microscope, repeatedly picking up the marked colonies while replacing the pipette tip for each colony.

Observation

- ✕ Working while looking through a microscope for an extended time leads to fatigue.

Collection



- ✕ Hand tremors make position adjustments difficult, leading to instability.
- ✕ The process takes a long time to master.

Cultivation

- ✕ No record is kept of what cell colonies were collected.

CELL PICKER

Preparation

- Select the conditions (method).
- Through the software, finds the cell colonies, records them, and picks them up repeatedly.
- Cell collection, seeding, and pipette tip replacement are automated.

Collecting cell colonies and seeding in a new incubator

Stable operation

Instabilities are resolved thanks to simple pipette tip adjustment methods.

Easy operation

Procedures are no longer reliant on skill thanks to automation of the pipetter operations.

Simpler process

Burden on the operator is reduced by the monitor window display.

Cultivation

Image capture

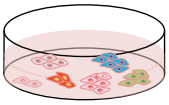
The seeded well numbers can be associated with images of the cells collected.

Cloning of Genome-Editing Cells using Cell Colony Separation Method

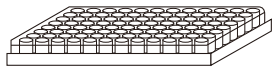
CELL PICKER can be used to pick up cell colonies after genome editing and establish target cell clones.
This section introduces an example of picking up HCT 116 cell lines, which are representative cell types used for genome editing. We confirmed that when HCT116 cells seeded in a 6-well plate were picked using CELL PICKER and cultured for six days, they were grown.

Workflow image

Genome-edited cells are seeded to form colonies.

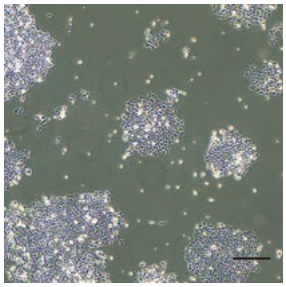


Cell colonies are picked with CELL PICKER.

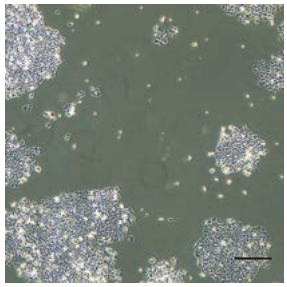


Clones are established.

Cell colonies before and after picking

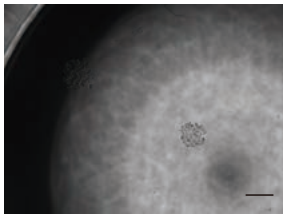


Before picking

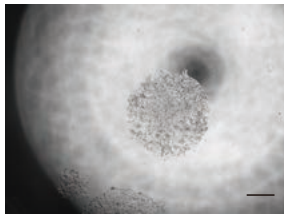


After picking

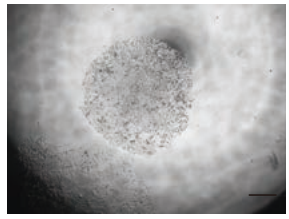
Cell growth after picking



Day1



Day4



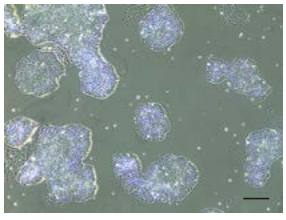
Day5

Scale bar: 200 µm

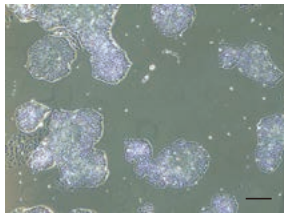
Picking at the time of establishment of iPS cell lines

CELL PICKER can be used to obtain formed colonies after reprogramming genes are transferred.
This time, CELL PICKER was used to pick colonies of iPS cell lines (cell line name: 1231A3) maintained and cultured. The cell colonies were cultured for six days after the picking operation, and fixed to have them immunostained. Expression of undifferentiated markers Oct 3/4 and Tra-1-60 was confirmed. This indicated that iPS cell colonies picked by CELL PICKER maintained undifferentiation and could be cultured continuously.

Cell colonies of iPS cell lines before and after picking

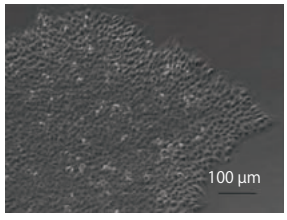


Before picking

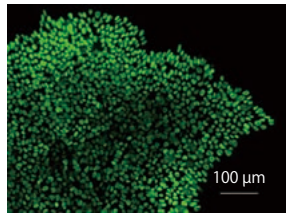


After picking

Immunostaining results



Phase-contrast microscope



Oct3/4

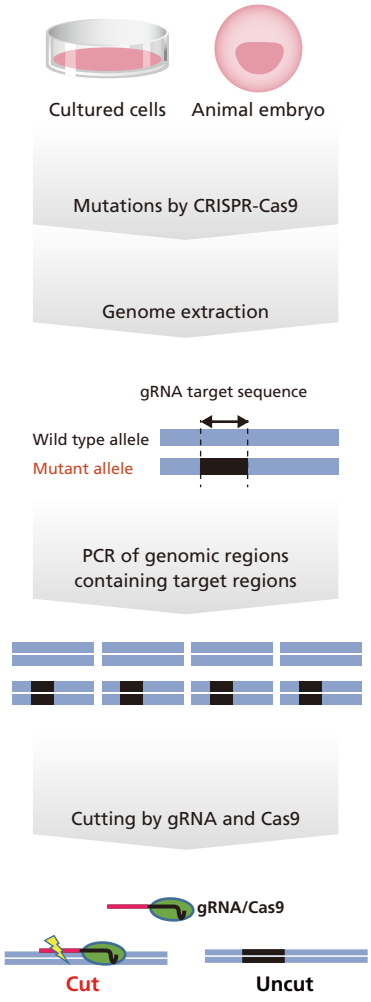
Microchip Electrophoresis System for DNA/RNA Analysis **MultiNA**

This MultiNA is an automated analytical platform designed to eliminate the frustration with Agarose Gel Electrophoresis. The presence and size of DNA and RNA samples can be checked quickly and easily.
It is perfect for genotyping various samples such as cultured cells and mice, and for detecting microbes and viruses.

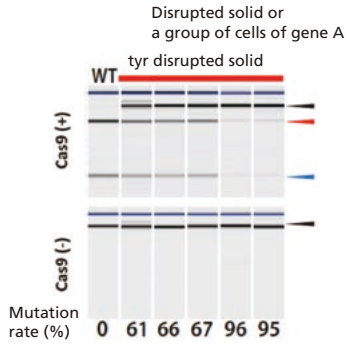
- Lower Analysis Costs
- Fully Automatic Operation for up to 120 analyses
- High-Sensitivity Detection
- High Resolution and High Reproducibility
- Pursuing Ease of Use

A Simple and Accurate Calculation Method of Mutation Rates in Genome Editing using CRISPR-Cas9

This CRISPR-Cas9 system theoretically allows for targeted analysis of all mutations transferred using gRNA and Cas9. The following figure shows the experimental workflow.

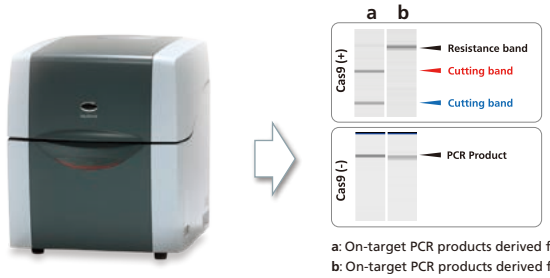


When a gRNA target region is amplified into genomes of mutant cultured cells by PCR, and PCR products are incubated with the gRNAs used for mutations and recombinant Cas9 proteins, the PCR products of wild-type alleles are cut and the PCR products of mutant alleles are not cut.



The MultiNA automatically calculates molar concentration from an electropherogram. Molarity calculations in each target band allow calculating mutation rates in a group of cells.

$$\text{Somatic mutation rate (\%)} = \left(1 - \frac{\text{Cutting band}}{\text{Resistance band} + \text{Cutting band}}\right) \times 100$$

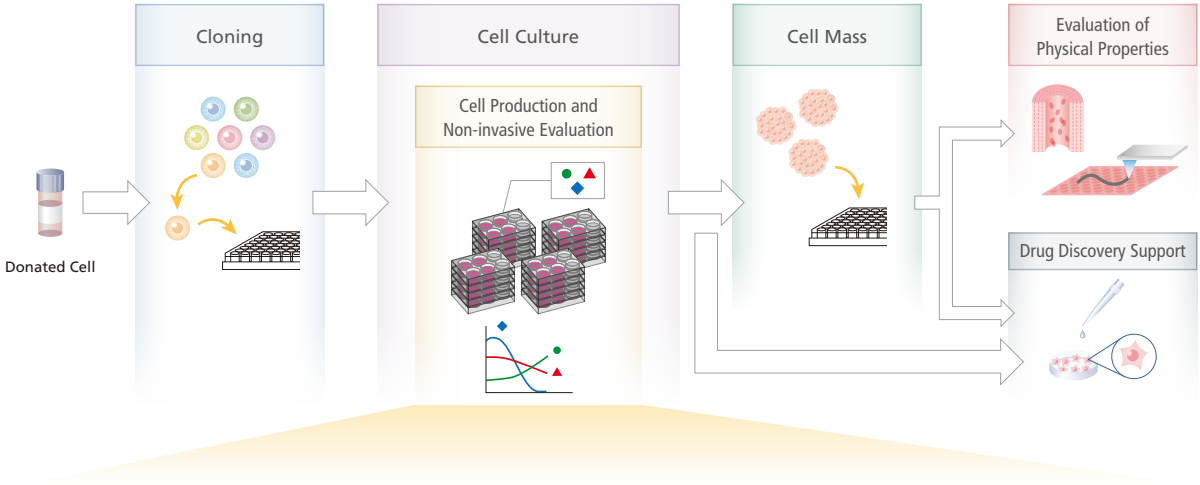


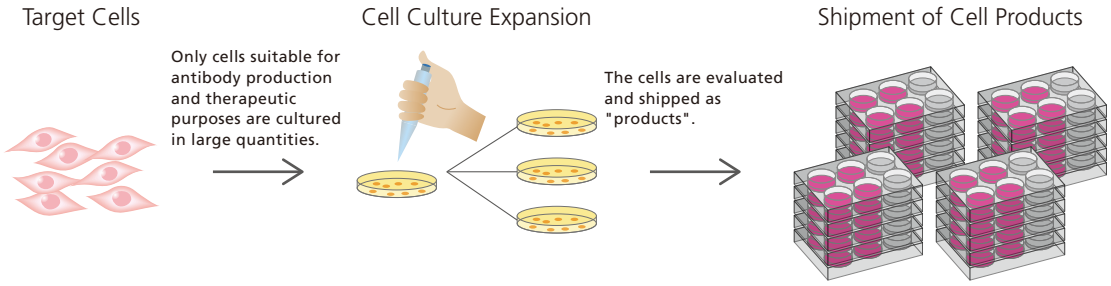
a: On-target PCR products derived from wild-type genomes
b: On-target PCR products derived from mutant genomes

Cell Culture Monitoring

– Solutions for Non-invasive Evaluation –

In order to achieve widespread implementation of regenerative medicine and industrialization of cell production, stable quality, establishment of mass production methods, and reduction of production costs are required.
In the past, various methods such as gene expression analysis and fluorescent staining were established to evaluate cell characteristics. However, these methods were so invasive to cells that they were applied as post culture evaluation. Therefore, non-invasive evaluation methods for cultured cells have been required.
One such method for culture supernatant analysis uses LC-MS/MS. It is possible to monitor component variations in culture media by adding a morphological observation from a phase contrast microscope.





Cell Culture Media Analysis Platform

C2MAP System

Non-invasive Evaluation of Media Supernatants

Everything from pretreatment to measurement in media supernatant analysis is automated and media components and secretory metabolites are measured non-invasively.

Atomic Absorption Spectrophotometer

AA-7000

Analysis of Metal Elements in Culture Media

Dilution of culture media enables measuring the concentrations of five metal elements in the culture media using atomic absorption spectrometry.

Cell Culture Media Analysis Platform **C2MAP System**

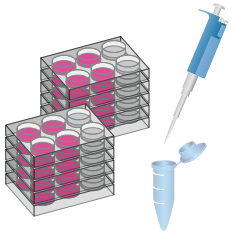
The C2MAP System is capable of analyzing up to 95 components, including medium components and secretory metabolites.
It supports monitoring of the culture process and marker search for quality control by obtaining variations in culture supernatant components.

- Automated process from pretreatment to measurement for the culture supernatant analysis
- Supports a wide range of measurement compounds and cell culture media
- Dedicated viewer software for easy verification of component variations
- Flexible system configurations

Automatic Pretreatment and Analysis by C2MAP System

- Sampling a part of a culture solution
- Cell removal

Manual operation



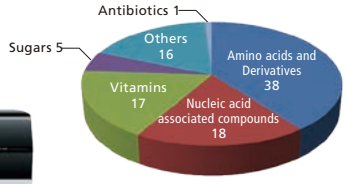
- Addition of internal standard
- Deproteinization process
- Sample delivery to the SIL-30AC

Performed by the C2MAP-2030

- Automatic sample dilution
- Dispensing and storage to the MTP
- Simultaneous analysis of 95 components by LC/MS/MS

Performed by the SIL-30AC and the LCMS-8060/8050

Breakdown of measured components



Waveform processing (Manual operation)

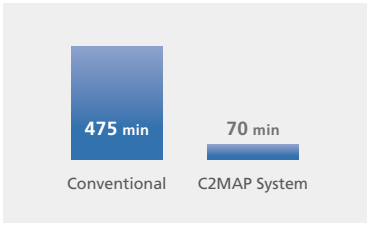
Graphing the measured components

Performed by C2MAP TRENDS

- Information on the components that cells prefer to consume or the components that are depleted during culture can be obtained.
- Can be applied as marker search for quality control of cells.

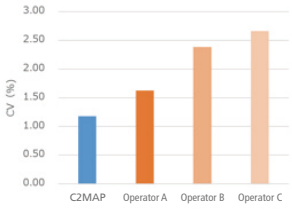
Advantages of C2MAP System

Example of Operator Labor Time Comparison



For pretreatment and analysis of 65 samples, the C2MAP System can reduce operators' labor time to approximately 1/7 compared to conventional systems.

Reproducibility*



The C2MAP System can always obtain the same quality of data without depending on the skills of the operators.

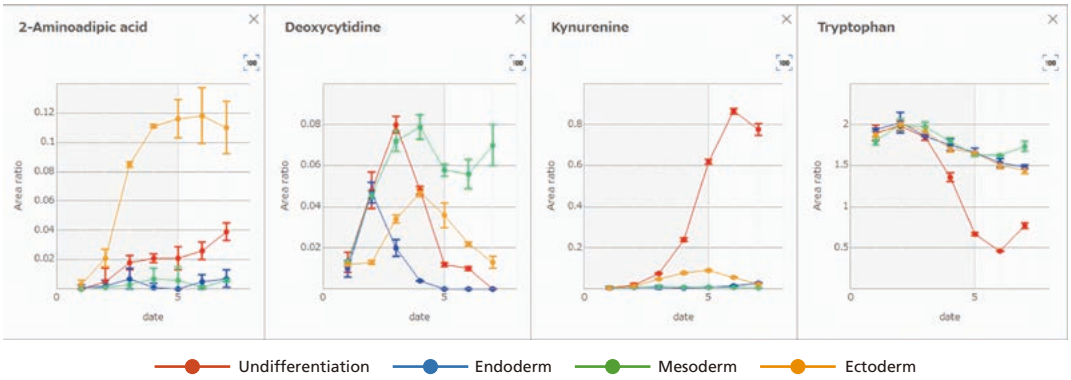
* The case when caffeine is added to culture media, and pretreatment and analysis are performed by the C2MAP-2030, Operator A, Operator B, and Operator C.

Pretreatment can be separated from LCMS.

If culture samples cannot be taken out of the culture room, sample pretreatment can be performed in the culture room and measured by LC/MS/MS in another room.

Marker Search in Culture Media Effective for Evaluation of Differentiated State of iPS Cells and Clarification of Action Mechanism

Using cell culture supernatant of undifferentiated iPS cells and human iPS cells differentiated to each germ layer as a sample, we used the C2MAP system to search for compounds in the culture supernatant that show characteristic temporal changes in each culture state. As a result, tryptophan was used for the culture supernatant of undifferentiated iPS cells, and kynurenine, a metabolite of the undifferentiated iPS cells, increased. The result also showed 2-Aminoadipic acid increased significantly in the culture supernatant of ectoderm cells.

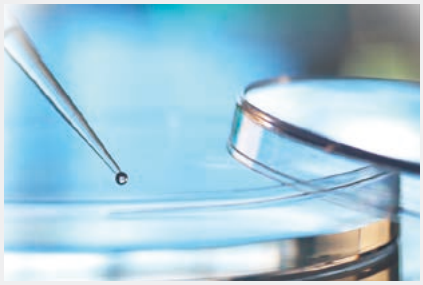


Further analysis indicated that the identified marker components were closely related to maintenance of undifferentiation and initiation of differentiation, and that this system enabled non-invasive evaluation of cells.

Reference: Takako Yamamoto, Kunitada Hatabayashi, Mao Arita, Nobuyuki Yajima, Chiemi Takenaka, Takashi Suzuki, Masatoshi Takahashi, Yasuhiro Oshima, Keisuke Hara, Kenichi Kagawa, and Shin Kawamata. Kynurenine signaling through the aryl hydrocarbon receptor maintains the undifferentiated state of human embryonic stem cells. Sci. Signal. 12, eaaw3306 (2019)

Related Products for Cell Analysis using LC/MS/MS

The combination of LCMS, various method packages, and an MRM library makes it easy to analyze components simultaneously according to the purposes.



LC/MS/MS Method Package for Cell Culture Profiling Ver.2

Simultaneous analysis of 125 components from amino acids to sugars, vitamins, and organic acids included in media components and secretory metabolites can be performed within 20 minutes per sample.

LC/MS/MS Method Package for Primary Metabolites Ver.2

Either the ion pair method (55 components), which targets important compounds from the main metabolic pathways for biological samples, or the non-ion pair method (97 components), which targets the main amino acids and organic acids, can be selected to suit the instrument environment.

Note: The C2MAP System does not include the above method packages or the MRM library (As of March 2021).

Atomic Absorption Spectrophotometer **AA-7000**

Atomic absorption spectrophotometry (AAS) is a technique in which elements are atomized at a high temperature, and the concentrations of the elements are quantified based on the fact that the light of specified wavelengths is absorbed during atomization.
Shown below are results of a simple, inexpensive measurement of metallic elements in cell culture media by AAS.

Direct Analysis of Metallic Elements in Cell Culture Media by Atomic Absorption Spectrophotometry (AAS)

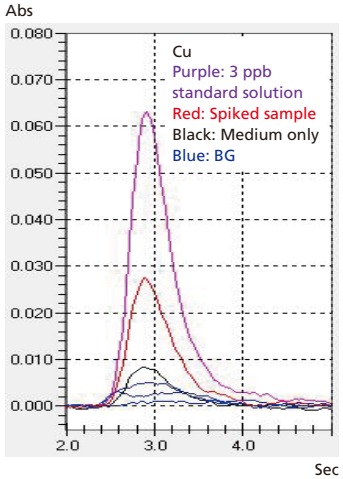
The active pharmaceutical ingredients (API) of antibody drugs are mainly produced by culturing CHO (Chinese hamster ovary) cells. In recent years, it has been reported the cellular metabolism and the primary structure of the antibodies produced in a culture medium are influenced by the nutrient composition (sugars, amino acids, etc.) and the metallic element concentration of the medium. Therefore, monitoring the concentrations of metallic elements in the culture medium is considered critical for maintaining uniform quality in antibody drugs. We measured the metallic elements in the culture media by AAS, which enables inexpensive and simple analysis of metallic elements. The instrument used in the analysis was a Shimadzu AA-7000 atomic absorption spectrophotometer with a graphite furnace atomizer*¹ and an autosampler. Each cell culture medium was diluted for the respective element to be measured, and the nitric acid concentration was adjusted to 0.5 w/v%.

Table 1 Measurement Results of Cell Culture Media

		Electric thermal method (μg/L) *²			Flame method (mg/L) *³	
		Cu	Mn	Co	Fe	Zn
A	Actual concentration*	<5**	28	218	19	0.47
	Recovery rate	128%	113%	93%	100%	95%
B	Actual concentration*	<5**	<4**	467	3.4	0.73
	Recovery rate	93%	110%	106%	107%	93%
C	Actual concentration*	<5**	<4**	37.8	1.5	0.34
	Recovery rate	93%	118%	91%	112%	101%

* Value obtained by converting the measurement value to the stock solution of the cell culture medium.
** Indicates limit of quantitation (LOQ) or lower.

The concentrations of metallic elements in three types of CHO cell culture media were measured by AAS (electric thermal method, flame method). This experiment demonstrated the possibility of measuring metallic elements by AAS with simple sample preparation consisting of only dilution of the culture medium. Differences were detected in the concentrations of designated metallic elements in the CHO cell culture media obtained from each vendor. Based on these results, it is clear that AAS is a simple technique for measurement of metallic elements in culture media.

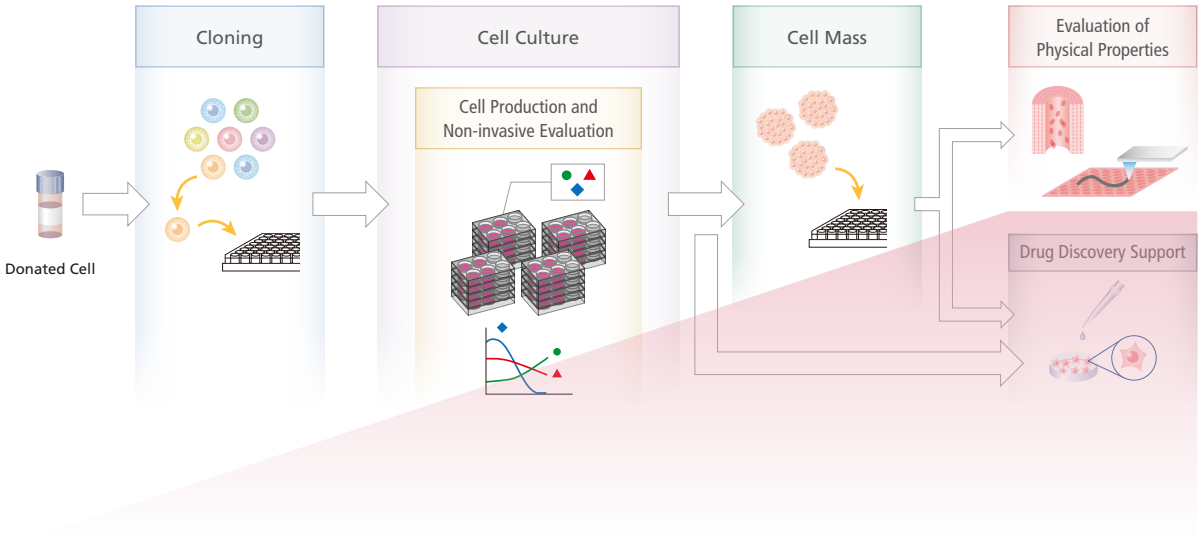


*¹ Automatic switching between the flame method and electric thermal method is possible.
*² Electric heating, in which the sample is heated by passing an electric current. Sensitivity: ppt to ppb
*³ Flame heating by the flame of a combustible gas. Sensitivity: ppb to ppm

Analysis of Cells

– Solutions for Evaluation of Physical Properties –

Physical tests and inspections of cells has been considered difficult because they exist in fluid and are delicate. However, if physical property values, such as cell elasticity and the strength of cell sheets, can be measured, they are expected to be a new evaluation index and a process parameter.
For this reason, Shimadzu proposes new solutions in the cell field utilizing scanning probe microscopes and material testing machines which have been developed for material analysis applications.



Scanning Probe Microscope

SPM-9700HT

Observation of Surface Shapes in Cells and Cell Sheets

Three-dimensional shapes and local physical properties can be observed at the nanoscale.

Micro Compression Tester

MCT™-510

Measurement of Cell Mass Intensity

The intensity of cells and cell masses can be evaluated.

Scanning Probe Microscope **SPM-9700HT**

A scanning probe microscope scans a sample surface three-dimensionally by moving the probe close to the sample surface and detecting the force between the sample and the probe and electromagnetism.

- Three-dimensional shapes and local physical properties can be observed at the nanoscale.
- Observation in air and liquid is possible.
- Observation magnification: 1,000 to 1 million times

Shape Observation and Deformation Measurement of HeLa Cells and iPS Cells in Culture Solution

Shapes and deformation of HeLa and iPS cells in a culture solution were observed and measured using the SPM.

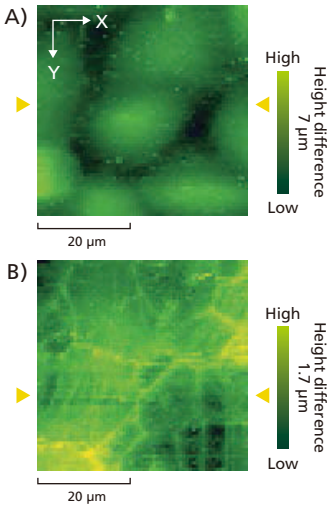


Fig. 1 Shapes of HeLa cells (A) and iPS cells (B) in culture solution
The upper part is bright and the bottom part is dark.

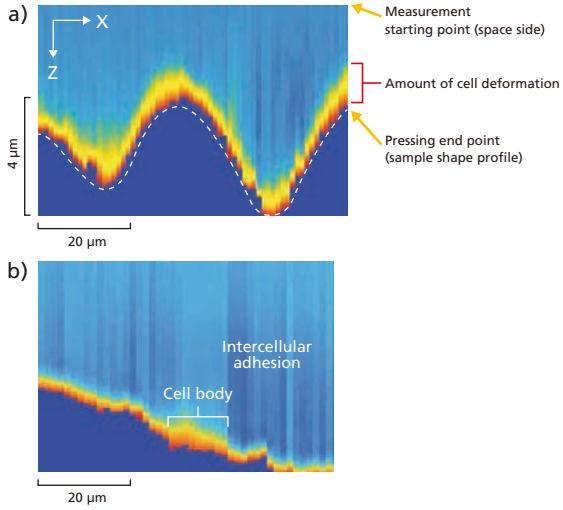


Fig. 2 ZX images (a) HeLa cells (b) iPS cells
These images show the force the probe received near the cell surface in the ZX plane.
The position where the force was detected is shown in yellow and red.
The white dotted line in (a) indicates the shape of the cells at the pressing end point.

Fig.1 shows the shapes of the HeLa cells (A) and the iPS cells (B) pressed under a constant load of 2.5 nN. Cell junctions of the HeLa cells were concave and unclear, whereas those of the iPS cells were convex and observed clearly.

In the ZX images, the positions where force was detected after the probe made contact with the sample are shown in colors from yellow to red. Because this indicates deformation of the cell by the probe, it can be understood that larger amounts of cell deformation show softer parts of the cell.
The analysis results suggested that HeLa cells have substantially uniform hardness, as there is little difference between the soft parts and hard parts. In contrast, the iPS cell body is soft and intercellular adhesion areas are hard.

As described, an SPM enables observation and measurement of cell shapes and deformation in culture solution close to inside the body.

(Specimens were provided by Prof. Hirotaka James Okano and Chikako Hara, Assistant Professor, The Jikei University School of Medicine, Division of Regenerative Medicine.)

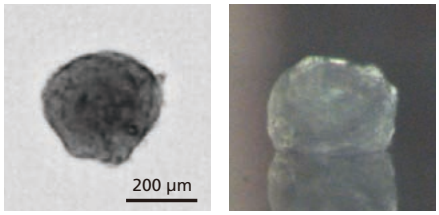
Micro Compression Tester **MCT Series**

The MCT series measures the correlation between load forces and compression displacement in real time by placing a load on a sample with a diamond platen.
It measures the minute load forces and deformation of cells and cell masses accurately and an amount of "hardness" can be obtained.

- Samples up to ø500 µm can be measured.
- Strength of test materials can be evaluated based on a sample size.
- Sample compression can be observed. (Optional)

Strength Evaluation of Cell Masses using MCT-510

Hardness evaluation of cell masses of HEK293 and two different iPS cells were performed as an example of mechanical properties evaluation of cultured tissues, which will be required in regenerative medicine. (See Table 1) The cell masses were preserved until just before measurement. (See Fig. 1-a) They were dropped on slides right before measurement and measured quickly with surrounding water removed. (See Fig. 1-b)
Fig. 2 shows the correlation between typical load force and compression displacement in each sample. The result shows a significant difference between HEK293 and the iPS cells, and the iPS cells which collapsed under a constant load were observed.
In addition, the strength of cell masses was measured based on their dimensions. (See Table 1 and Fig. 3) There was a clear difference between HEK293 and the iPS cells, as well as a significant difference between the two iPS cells. The qualitative evaluation of cell hardness indicates the possibility of identifying cell properties.



a. Upper side (in fluid) b. Side (in the air)

Fig. 1 HEK293 Cell Mass

Table 1. Average Cell Mass Diameter of Measured Samples [µm]

HEK 293	231.31
iPS cell A	243.13
iPS cell B	225.59

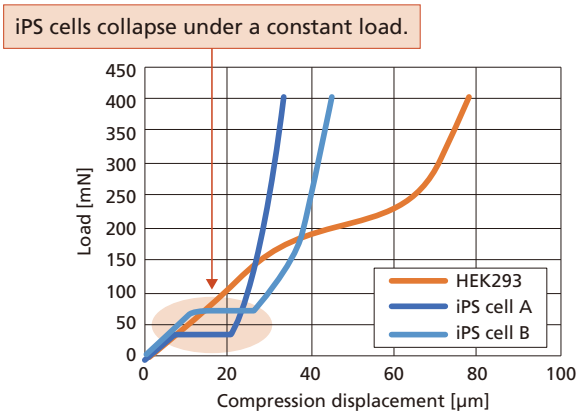


Fig. 2 Load-Displacement Diagram

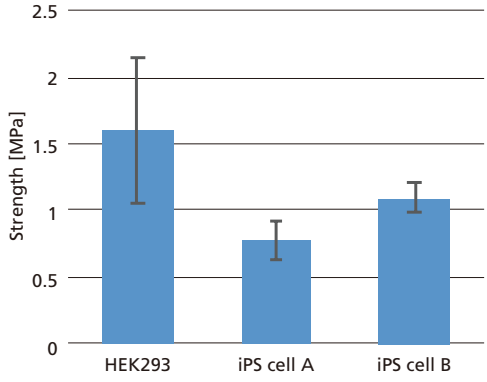


Fig. 3 Strength Measurement Result

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