Application Note

CL-Quant Add-on Module

Evaluation of cell growth of hPSC by measuring hPSC colony coverage area using "hPSC Colony Coverage Ratio"

< BioStation CT usage example >

Benefits

- Using the CL-Quant image analysis software with the CL-Quant Add-on Module "hPSC Colony Coverage Ratio "makes it possible to measure human pluripotent stem cells (hPSCs) confluency more objectively and quantitatively than the visual inspection that has been commonly used. This helps to standardize decision criteria for good cell culture procedure.
- Cell growth is one of the important criteria to detect cell phenotypic characterization. hPSC are well known to change their phenotype with genetic change after prolonged culturing and usually increase their cell growth. To monitor cell growth daily and noninvasively is important and useful to confirm their cell phenotype during culturing using the Add-on Module "hPSC Colony Coverage Ratio".

Observation device

- BioStation CT (Nikon, MLA10000)

Software

- CL-Quant ver. 5.02 (Nikon, MLS21000)

CL-Quant Add-on Module

- MA-PC-4X-AR02 hPSC Colony Coverage Ratio (Nikon, MLS30202)

Cells

- Human induced pluripotent stem (human iPS) cell line, 253G1 (Center for iPS Cell Research and Application, Kyoto University/iPS Academia Japan), or HPS0002 (Riken BioResource Research Center)

Materials

- StemFit® AK02N (Ajinomoto, AK02N)
- iMatrix-511 (Nippi, 892011)
- Y-27632 (FUJIFILM Wako Pure Chemical, 257-00511)
- PBS, pH 7.4 (Thermo Fisher Scientific, 10010023)
- TrypLE™ Select Enzyme (1X), no phenol red (Thermo Fisher Scientific, 12563011)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

Methods

Human iPS cells, 253G1 cells maintained in an AK02N medium were dissociated into single cells using TrypLE™ Select and then seeded into a 6-well plate coated with iMatrix-511. The cells were cultured in a BioStation CT at 37˚C in a humidified atmosphere of 5% CO_2.

Phase contrast images of 8×8 fields (approx. 16.0 mm×16.0 mm) at the center of a well were automatically captured every 6 hours using a 4× objective lens, from 2 hours after cell seeding confirmed that the cells attached. Auto-focusing was adjusted on the first field of view for a well and then images were automatically taken using that auto-focusing setting within that well.

The obtained image data were analyzed using CL-Quant with the Add-on Module "hPSC Colony Coverage Ratio" to automatically calculate colony confluency value as "Ph-Mask Area Ratio". The masked images and the measured value of the area where human iPS cell colonies are localized were confirmed on the operation screen and output in Microsoft Excel® format. The output values were converted to a colony confluency (%), which was graphed over time.

Results

The analysis results using CL-Quant with the Add-on Module "hPSC Colony Coverage Ratio" demonstrated that the colony confluency of 253G1 cells cultured in AK02 in a 6-well plate reached approximately 30, 50 and 70% after 110, 122 and 134 hours respectively (Fig. 1) .

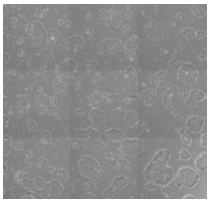
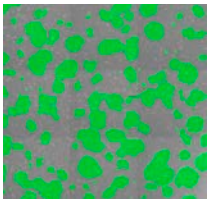
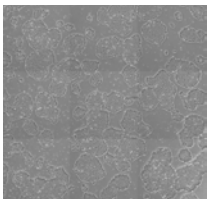
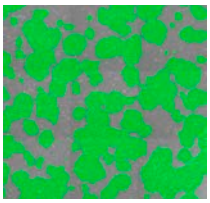
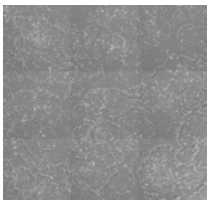
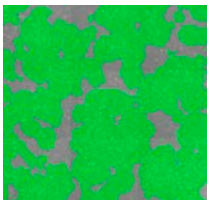
Culture time (hours)	Cell occupancy rate (%)	Phase contrast image	Masked image
110	35		
122	54		
134	73		

Figure 1. Phase contrast images and analyzed images of hPSCs
Representative phase contrast images of a 3×3 field of view (left) were proceeded to mask colonies with green (right). The numeric value of the colony confluency obtained was converted to percentage.

In addition, the time course of cell occupancy over time during the culture period is shown in Figure 2.

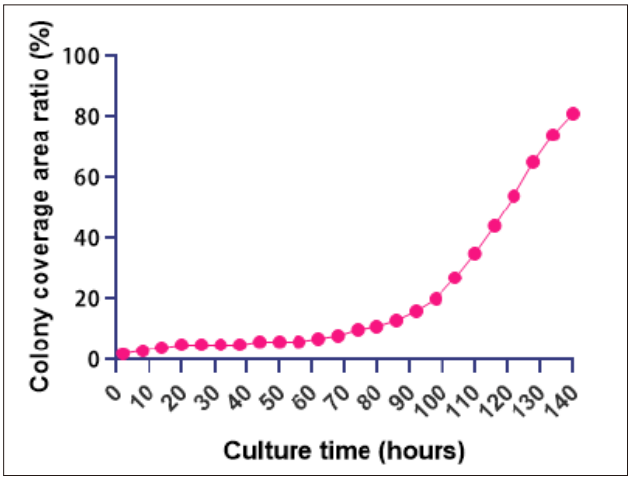


Figure 2. Time course of hPSC colony coverage area ratio
The obtained image data of 253G1 human iPS cells was analyzed by CL-Quant with the Add-on Module "hPSC Colony Confluency". The obtained value of the colony confluency was converted to a percentage and graphed over time.

Summary

- Using CL-Quant image analysis software with the Add-on Module "hPSC Colony Coverage Ratio" makes it possible to automatically measure hPSC confluency from phase contrast images.
- By referring to the masked image on the operation screen of CL-Quant, it is easily confirmed that the colonized area of hPSCs is being recognized correctly.
- Monitoring cell growth by measuring colony coverage area is useful to confirm cell phenotype.
- The Add-on Module "hPSC Colony Coverage Ratio" supports determining the proper timing for cell passage.
- The obtained numeric measured values can be output in Microsoft Excel® format.

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