Application Note

CL-Quant Add-on Module

A method for evaluating hPSC phenotype by measuring hPSC colony numbers using "hPSC Colony Count"

< BioStation CT usage example >

Benefits

- Using the CL-Quant image analysis software with the CL-Quant Add-on Module "hPSC Colony Count" makes it possible to count colonies of human pluripotent stem cells (hPSCs) during culturing.
- To monitor forming colony numbers during hPSCs culturing is important to confirm cell phenotypic characterization. Colony forming ratio is different also in different culture conditions. hPSCs are well known to change their phenotype with genetic change after prolonged culturing and usually increase their colony forming ratio. To monitor colony numbers at every passage during culture is important and useful to confirm their cell phenotype using CL-Quant Add-on Module "hPSC Colony count".

Observation device

- BioStation CT (Nikon, MLA10000)

Software

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

CL-Quant Add-on Module

- MA-PC-4X-CO01 hPSC Colony Count (Nikon, MLS30101)

Cells

- Human induced pluripotent stem (human iPS) cell line, Tic-FX (JCRB Cell Bank, JCRB1331.01, MRC-5-derived, Substrain of Tic iPS cell (JCRB1331) adapted to feeder-free culture)

Materials

- TeSR™-E8™ Kit for hESC/hiPSC Maintenance (STEMCELL Technologies, 05990)
- Fibronectin bovine plasma (Merck, F1141)
- StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool (Thermo Fisher Scientific, 23181010)
- Cell Scraper S (Sumitomo Bakelite, MS-93100)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

Methods

Human iPS cells, Tic-FX cells which were maintained in a medium of TeSR™-E8™ on a fibronectin-coated 25cm^2 flak without feeder cells were inoculated using Ezpassage™ and cell scraper. The cell clamps were seeded into the wells of a 6-well plate coated with Fibronecti and were cultured for 3 days 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, in a BioStation CT every 12 hours from 3 days after seeding. 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 and the Add-on Module "hPSC Colony Count" to automatically measure the colony count. The masked images of the area where human iPS cell colonies were localized were confirmed on the operation screen and output in Microsoft Excel® format.

Results

The analysis results using CL-Quant with the Add-on Module "hPSC Colony Count" display the colony area of Tic-FX cells cultured in TeSR™-E8™ in random coloring. Figure 1 shows an unprocessed phase contrast image of Tic–FX and its masked image after processing using CL-Quant with the Add-on Module "hPSC Colony Count". By displaying the areas recognized as colonies in random colors, it was possible to confirm the areas of individual iPSC colonies. The colony count was obtained automatically via CL-Quant and the Add-on Module.

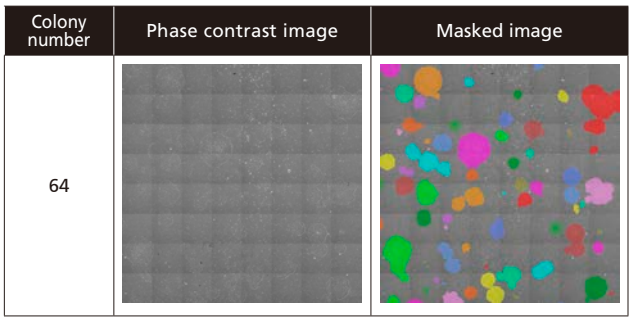

Figure 1. Phase contrast and masked images of hPSC colonies
Phase contrast image of Tic-FX human iPS cell (left) was proceeded to mask colony areas with random colors (right). Colony number was obtained by automatic calculation in CL-Quant.

Summary

- Using the combination of CL-Quant and the Add-on Module "hPSC Colony Count" makes it possible to recognize individual areas of human iPS cell colonies. The identified colonies are counted automatically and displayed on the operation screen.
- Monitoring the cell status during culturing hPSCs, and obtaining the information of the colony numbers can be used to assess the quality of hPSCs.
- The obtained numeric measured values can be output in Microsoft Excel® format.

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