Application Note

Cell Analysis Module

Measuring individual hPSC colony areas using "hPSC Colony Area Package"

< BioStation CT usage example >

Benefits

- Using the NIS-Elements image analysis software with Cell Analysis Module "hPSC Colony Area Package" make it possible to recognize individual human pluripotent stem cell (hPSC) colonies, and measure their area and number of colonies, as well as the average, total size and confluency of colonies.
- When hPSCs are properly dissociated at passage, they will grow as colonies of almost uniform size. If the dissociation method is inadequate, the size of the colonies varies and will affect the quality after the next passage. Also, when the cells are transformed, some colonies grow rapidly. Using this Cell Analysis Module, you can check for variations in the area of colonies from the images, and also compare to the previous passage. In this way, it is possible to evaluate the quality of hPSCs, the evaluation of dissociation process at passage, and the culture skill between workers.

Observation device

- BioStation CT (Nikon, MLA10000)

Image Analysis Software

- NIS-Elements AR ver. 5.30.02 (Nikon, MQS31000)
- NIS-A General Analysis (Nikon, MQS43110)
- NIS-A Upgrade to GA3 (Nikon, MQS43150)

Image Conversion Software

- ND2 Generator for BS-CT (Nikon)

Cell Analysis Module

- PC-AR-02 hPSC Colony Area Package (Nikon, MQS60002)

Cells

- Human induced pluripotent stem (iPS) cell line, Tic-FX (JCRB Cell Bank, JCRB1331.01, MRC-5-derived, Substrain of Tic iPS cell (JCRB1331) adapted to feeder-free culture)[1 and 2]

Materials

- TeSR™-E8™ Kit for hESC/hiPSC Maintenance (STEMCELL Technologies, 05990)
- Fibronectin from bovine plasma (SIGMA ALDRICH, F1141)
- StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool (Thermo Fisher Scientific, 23181010)
- Cell Scraper S (Sumitomo Bakelite, MS-93100)
- Corning® 25cm^2 Rectangular Canted Neck Cell Culture Flask with Vent Cap (Corning, 430639)

Methods

Human iPS cells, Tic-FX cells which were maintained and cultured in TeSR™-E8™ medium and dissociated using EZPassage™ and cell scraper, were seeded in a fibronectin-coated T-25 flask without feeder cells. The seeded cell clamps 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 the flask were automatically captured every 12 hours with a 4x objective lens, in a BioStation CT from 3 days after seeding. Auto-focusing was adjusted on the first field of view and then images were automatically taken using that autofocusing setting.

The obtained image data were converted to ND2 format image data using the ND2 Generator for BS-CT, and analyzed using NIS-Elements and the Cell Analysis Module "hPSC Colony Area Package". Then they were automatically analyzed to obtain measured values of "Number of Colonies", "Confluency (%)", "Colony area (cm^2)", "Mean of Colony area (cm^2)", and "Total of Colony area (cm^2)". In the operation screen, each human iPS cell colony was recognized in random colors and the measured values were confirmed. The obtained values can be output in CSV format, and the distribution of colony size was graphed.

Results

Figure 1 shows an unprocessed phase contrast image of Tic–FX cells and its masked image after processing using NIS-Elements with the Cell Analysis Module "hPSC Colony Area Package". By displaying the areas recognized as colonies in random colors, it was possible to confirm the areas of individual iPS cell colonies.

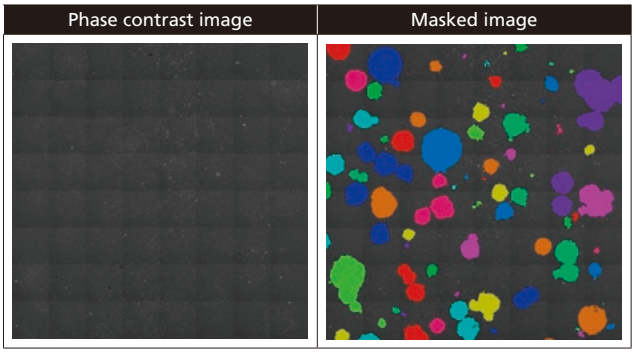

Figure 1: Phase contrast and masked images of hPSC colonies

Phase contrast image of Tic-FX human iPS cells (left) was proceeded to mask colony areas with random colors (right).

Figure 2 shows a histogram of the distribution of the areas of individual colonies, showing that there is variation in colony areas and their proportion. By using this analysis to determine the variations in colony area and number, it would be possible to evaluate cell quality, cell passage process and cell passaging skills.

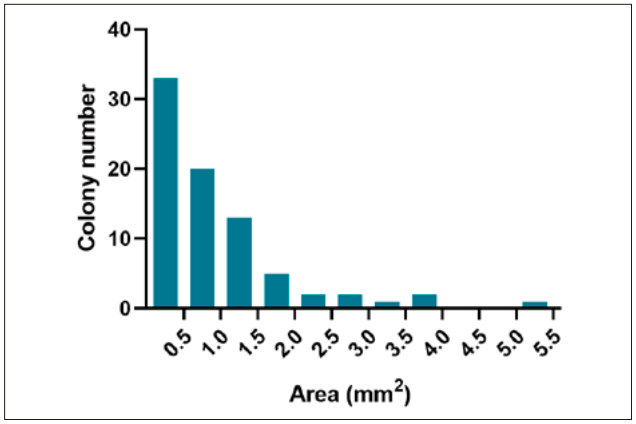

Figure 2: Distribution of hPSC colony areas

The obtained image data of Tic-FX human iPS cells were analyzed using NIS-Elements with the Cell Analysis Module "hPSC Colony Area Package". The unit of measurement for the measured colony areas was converted from cm^2 to mm^2.

Summary

- Using the combination of NIS-Elements and the Cell Analysis Module "hPSC Colony Area Package", individual hPSC colonies are identified and their areas automatically measured. The obtained values are displayed on the operation screen.
- The measured values can be output in CSV format, and used for creating graphs with software such as TIBCO Spotfire® and Microsoft Excel®.
- Monitoring the cell status during culturing of hPSCs, and obtaining the information on individual colonies, averages, and total colony area can be used to assess the quality of hPSCs and to help determine passage timing.

Reference

1. Shogo Nagata et al., Efficient reprogramming of human and mouse primary extra-embryonic cells to pluripotent stem cells, *Genes Cells*, 14: 1395-1404, 2009.
2. Mika Suga et al., A morphology-based assay platform for neuroepithelial-like cells differentiated from human pluripotent stem cells, *Int. J. Dev. Biol.*, 62(9-10):613-621, 2018.

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