Introduction

Maintaining the undifferentiated state of iPS cells is an essential, but difficult process to control. The process requires improvement in the following three areas:

1. Feeder-free culturing: To eliminate culture variability and to reduce culturing costs.
2. Culturing: To reduce culturing costs, and to achieve consistency in the supply of iPS cells.
3. Establishment of a quantitative index: To quantify culturing status and provide a clear index for evaluation.

As a means of implementing these improvements, a system has been developed for time-lapse observation of feeder-free cultures within which iPS colonies can be automatically detected and measured. The Nikon BioStation CT cell culture observation system was used to capture time-lapse images of the entire surface of a 6-well plate (100 images with a 2x objective (4x4mm)) over a 1 week period of feeder-free culturing (using ReproFF2), from seeding to confluence. Once a culture dish is inserted and an observation schedule is set, the system automatically captures images while maintaining the observation position even with daily medium replacements, allowing the growth of each colony to be tracked with ease.

CL-Quant image analysis software was taught to detect iPS colonies, thus allowing iPS colonies to be identified within phase-contrast images. Changes within each colony were measured and plotted as a colony growth curve which could then be summarized per well, each representing a distinct culturing condition.

The system was used to generate growth curves for iPS cells cultured over a 1 week period (from seeding to passageing) while their undifferentiated state was maintained. Quantifying the rate of growth and comparing it to the undifferentiated state established a benchmark for this process.

Method

iPS cell culture and growth

- iPS cells were generated from adult human dermal fibroblasts (HDF) by retroviral transduction of four factors: OCT3/4, Sox2, KLF4 and c-Myc [1].
- Six days after transduction, the cells were harvested by trypan blue and plated onto feeder free cells using a 6-well plate. ReproFF2 medium ReproCELL was used.

Image acquisition

- Phase contrast images of a 6-well plate were acquired using BioStation CT (NIKON CORPORATION) at 37°C, 5% CO₂.
- High precision: Accurate tracing of same cells, as well as X-Y positions ±5μm for each vessel.
- Full Scan (2x) was set for acquiring full-well tiling images.

Image analysis

- Full-well tiling images were loaded into CL-Quant software (NIKON CORPORATION) and stitched to create 10,000-by-10,000 pixel composite images for analysis.
- All image analysis (colony segmentation, measurement etc.) were performed using functions built into CL-Quant software [2].

Algorithm development

Colony detection & calculation flow

Original Image

Area classification by intensity loop

Well & non-well

Center of well

Colony

Step 1) Colony detection

Colony detection 1 (whole area)

Colony detection 2 (area of well edge)

Orange = non-well region

Blue = center region

Green = colony detected in this step

Purple = center region

Step 2) Merging detection

Step 3) Calculation

Calculating area of colony region

Calculating a ratio of colony to well

Calculating an area of well region

Calculating a ratio of colony to well

Image analysis

- Manual
- CL-Quant

Comparison of results from detection using algorithm and manual detection

Manual versus auto-detection with CL-Quant calculation were made. The results were similar from center to edge of dish.

Confirmation of algorithm

NiKON has developed an algorithm that enables temporal quantitative analysis of colony proliferation, from immediately after cell seeding to confluence.

Experiment 1) Using the CL-Quant algorithm for standard cell culture

CL-Quant enables image analysis of standard 7 days iPS cell culture sequence.

Experiment 2) Comparison of cell seeding density

The results indicate that growth of colonies depends on seeding density (for 1 week cell cultivation).

Experiment 3) Drug addition

CL-Quant quantifies the influence of Tzv-addition, which affects a cell’s survival rate.

Conclusion

1. Nikon has developed an algorithm that enables quantitative analysis of colony proliferation, from immediately after cell seeding to confluence. Comparing manual and algorithm-detection, the correlation factors in full image areas have similar results 0.98 at the center of the dish, and 0.99 at the edge.
2. CL-Quant enables analysis of effects under various conditions.
   - Colony area proliferation depends on cell seeding density.
   - CL-Quant quantifies the effects of thiazovivin density.
3. Nikon has achieved non-invasive quantification of change of culture conditions of iPS cells in a feeder-free culture, based on the ratio of colony/bottom of dish area.

Reference


Acknowledgement

1. Takahata, Y., Nakada, Ch., Ishikawa, M., Nakada, C., Kiyota, Y., Watanabe, H. Nikon has developed an algorithm that enables quantitative analysis of colony proliferation, from immediately after cell seeding to confluence. Comparing manual and algorithm-detection, the correlation factors in full image areas have similar results 0.98 at the center of the dish, and 0.99 at the edge.

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