SINGLE-CELL CLONING OF INDUCED PLURIPOTENT STEM CELLS USING CellCELECTOR™ INSTRUMENT AND NANOWELL PLATES

RAFAL P. KROL, MASAYOSHI TSUKAHARA and NAOKO TAKASU

CiRA FOUNDATION, FACILITY for iPS CELL THERAPY, KYOTO, JAPAN

INTRODUCTION

One of the challenges in the manufacturing of clinical-grade iPS cells is single-cell cloning, the step relevant to the standardization and automation of iPS cell culture protocols.

Here we report the results of our proof-of-concept study utilizing CellCelector™, high-throughput nanowell-based image-verified single cell cloning platform (HT-NIC), manufactured by ALS Automated Lab Solutions (Jena, Germany).

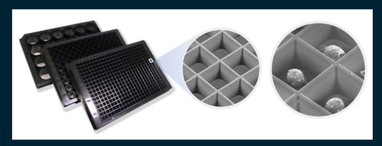
CellCELECTOR™ PLATFORM

The CellCelector™ system consists of automated single-cell picking instrument, image analysis software, and nanowell plates. Cell isolation and aspiration is realized by robotics system, for imaging automated inverted microscope is used.

NANOWELL MICROPLATES

The nanowell plates facilitate isolation and identification of single cells, verification of monoclonality, assessment of the clones' outgrowth, and fast transfer of proliferating clones.

- diameter: 200 * 200 µm
- depth: 100 µm
- 132,000 nanowells per plate



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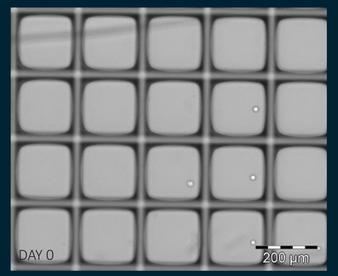
SINGLE CELL PICKING

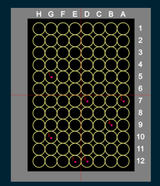
PROCEDURE

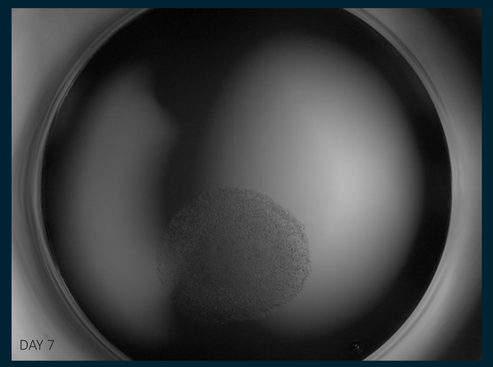
- iPS cells dissociation with Accutase® (Nacalai Tesque) and seeding on 24-well nanowell plate with density of 400 cells per well.
- Nanowell scanning with CellCelector™ system and identification of nanowells containing single cells.
- Automated transfer of single cells to 96-well plate.
- Medium supplementation (cytoprotection) for 24 hours:
  - Y-27632 (Fujifilm) [A1 - H4];
  - proprietary cocktail [A5 - H8];
  - CloneR (STEMCELL Technologies) [A9 - H12].
- Culture at 37°C in humidified atmosphere with 5% CO_2 for 7 days.



RESULTS

- Microphotographs on culture day 7.
- Viable iPSC colonies:
  - Y-27632 - 0 of 32 wells;
  - cocktail - 4 of 32 wells;
  - CloneR - 3 of 32 wells.





MATERIALS AND METHODS

CELLS

iPS cells were generated from peripheral blood mononuclear cells (ePBMC, CTL) with Human iPS Cell Generation Episomal Vector Mix (TaKaRa) and 4d-Nucleofector™ (Lonza).

CULTURE SYSTEM

Cells were cultured on iMatrix-511 substrate (Nippi) in StemFit AK03N medium (Ajinomoto).

SINGLE CELL PICKING

Single cells identified on nanowell plates were immediately transferred to 96-well plate (one cell per well).

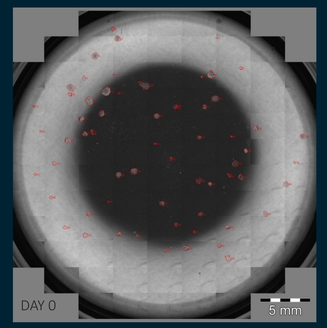
SINGLE CELL CLONING

Seeded nanowell plates were scanned to identify nanowells containing single cells, the cells were cultured for 6 days, the plates scanned again, and suitable colonies transferred to 12-well plates.

SINGLE CELL CLONING

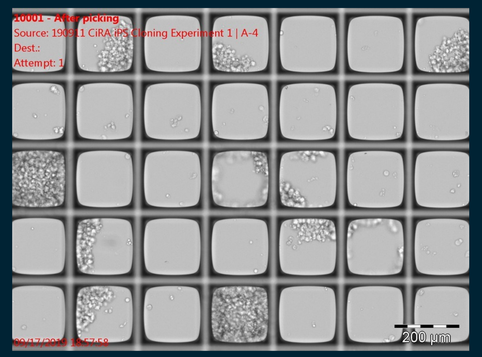
PROCEDURE

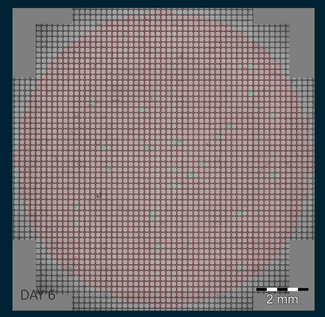
- iPS cells dissociation with Accutase® (Nacalai Tesque) and seeding on 24-well nanowell plate with density of 4,000 cells per well + 0.5 µg of iMatrix-511 (Nippi) per well.
- Medium supplementation (cytoprotection) for 24 hours:
  - Y-27632 (Fujifilm) [A2];
  - proprietary cocktail [A4];
  - CloneR (STEMCELL Technologies) [A6].
- Culture at 37°C in humidified atmosphere with 5% CO_2 for 6 days.
- Automated passage to 96-well plate:
  - substrate - iMatrix-511 (Nippi) 0.5 µg/cm^2;
  - dissociation - Accutase® (Nacalai Tesque);
  - colony picking with CellCelector™ system.



RESULTS

- Established iPSC colonies identified and successfully passaged to a 96-well plate.





CONCLUSIONS

- CellCelector™ system and nanowell microplates constitute excellent platform for generation of large number of iPS cell clones within one standard culture plate.
- High quality imaging system and image analysis software assures monoclonality of expanded iPS cells.
- Combination of CellCelector™ system and nanowell plates provides a tool for assessment of growth of iPS clones of desired characteristics for downstream applications.