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Single-cell cloning of induced pluripotent stem cells using CellCelector™ and nanowell plates

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Facility for iPS Cell Therapy



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筆頭発表者のCOI開示

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演題発表に関連し、

開示すべきCOI関係にある企業等はありません。

Introduction

One of the challenges in the manufacturing of clinical-grade iPSCs is single-cell cloning, the step relevant to the standardization and automation of iPSCs 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

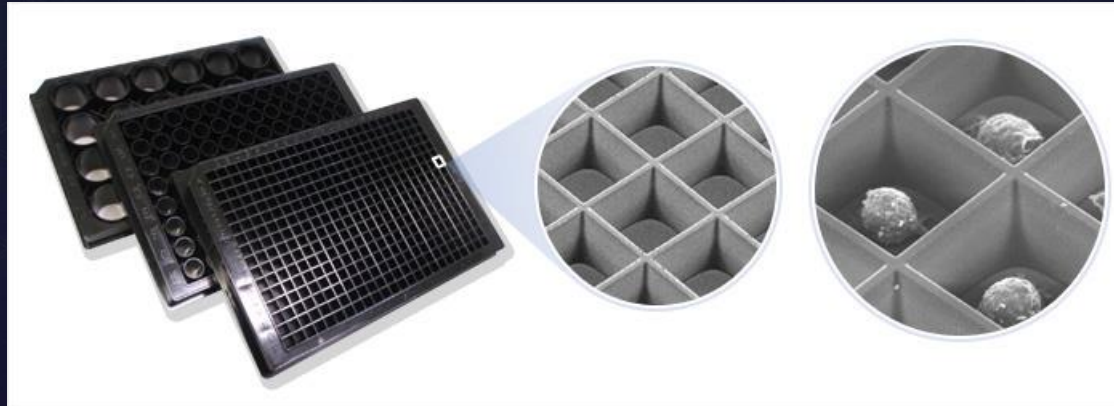
The CellCelector™ system consists of automated single-cell picking instrument, image analysis software, and nanowell plates. Cell isolation and aspiration is realized by robotics system, for imaging automated inverted microscope is used.



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 /plate

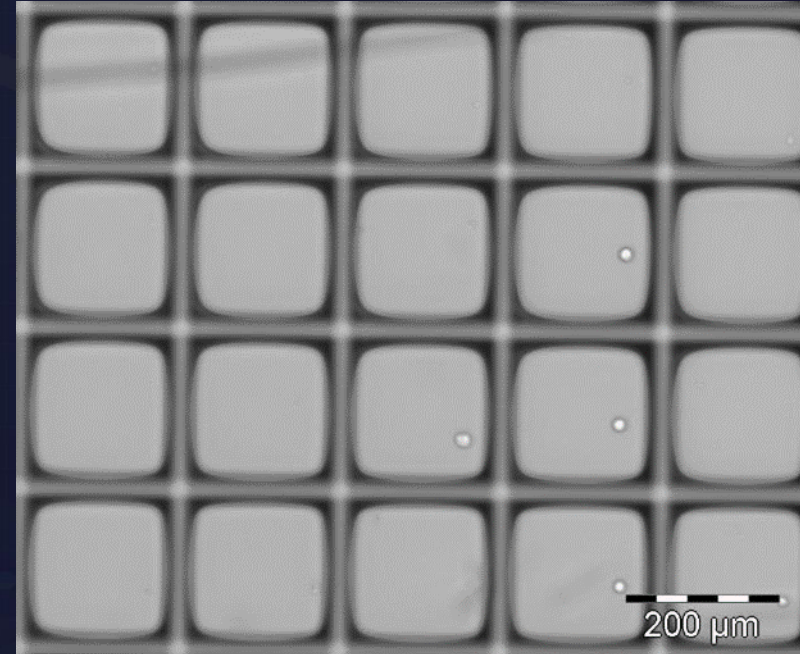


Materials and methods

- 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 cultured for 6 days, the plates scanned again, and suitable colonies transferred to 12-well plates

Single cell picking | Procedure

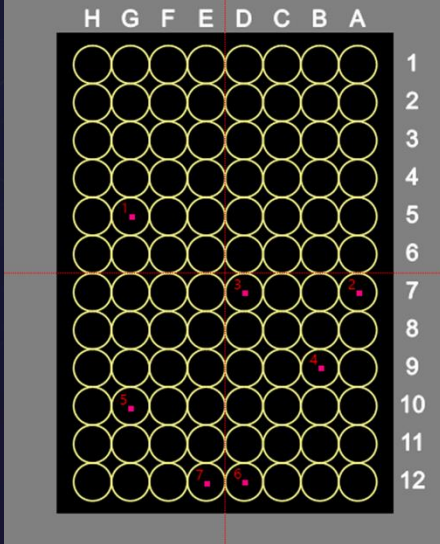
- iPS cells dissociation with Accutase® (Nacalai Tesque) and seeding on 24-well nanowell plate with density of 400 cells per well
- Nanowell scanning with CellCelector™ system and identification of nanowells containing single cells
- Automated transfer of single cells to 96-well plate
- Medium supplementation (cytoprotection) 24h
 - Y-27632 (Fujifilm) [A1-H4]
 - proprietary cocktail [A5-H8]
 - CloneR (STEMCELL Technologies) [A9-H12]
- Culture 7 days



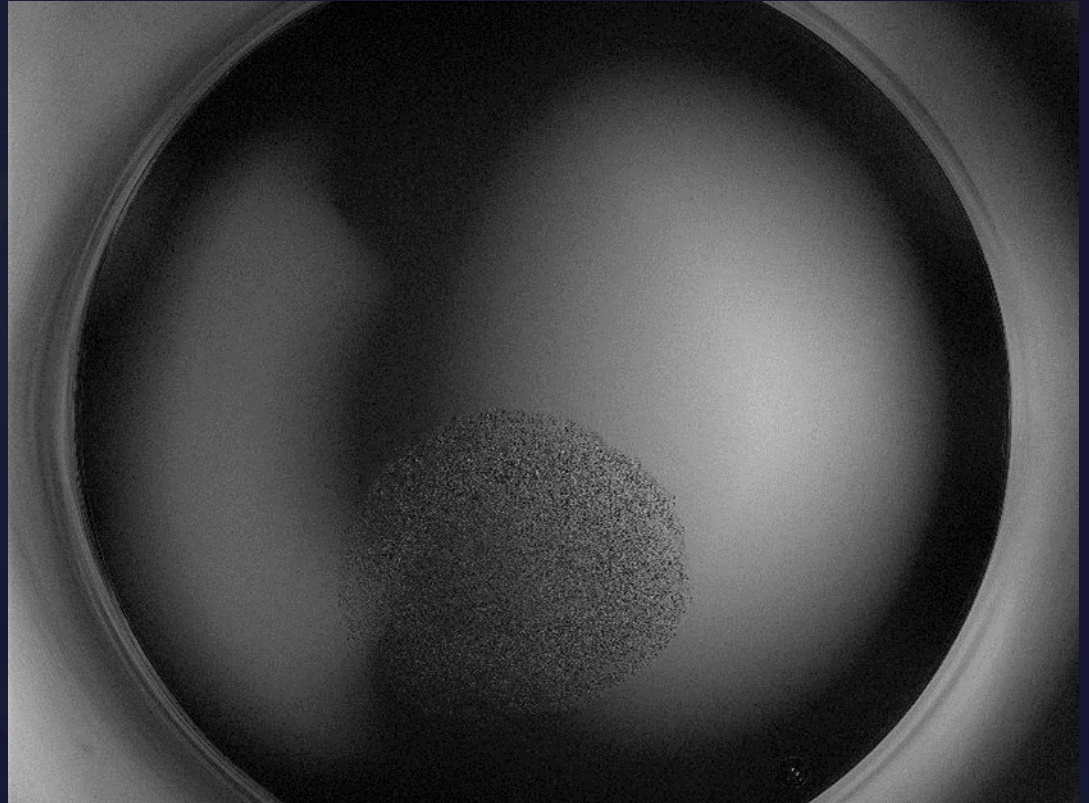
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Single cell picking | Results

- Microphotographs on day 7
- Viable iPS colonies
 - Y-27632: 0/32
 - Cocktail: 4/32
 - CloneR: 3/32

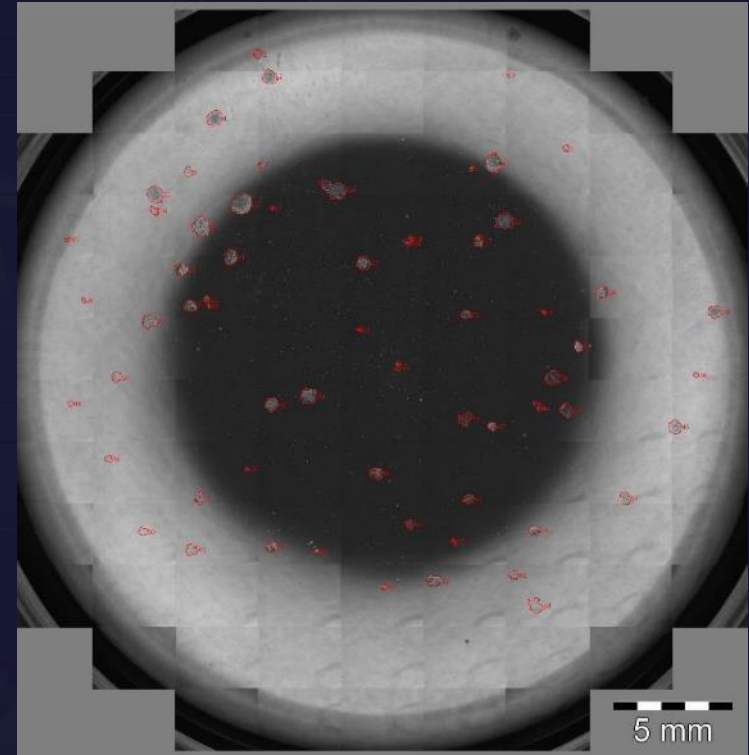


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Single cell cloning | Procedure

- iPS cells dissociation with Accutase® (Nacalai Tesque) and seeding on 24-well nanowell plate with density of 4000 cells + 0.5 μg of iMatrix-511 (Nippi) per well
- Medium supplementation (cytoprotection) 24h
 - Y-27632 (Fujifilm) [A2]
 - proprietary cocktail [A4]
 - CloneR (STEMCELL Technologies) [A6]
- Culture 7 days
- Passage to 96-well plate
 - substrate: iMatrix-511 (Nippi) 0.5 $\mu\text{g}/\text{cm}^2$
 - dissociation: Accutase® (Nacalai Tesque)
 - colony picking with CellCelector™ system

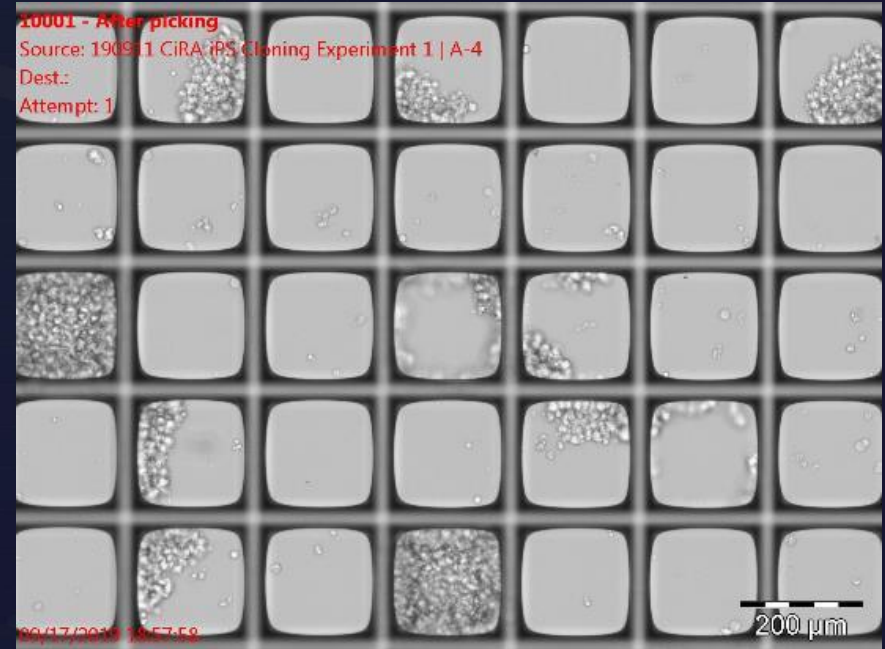
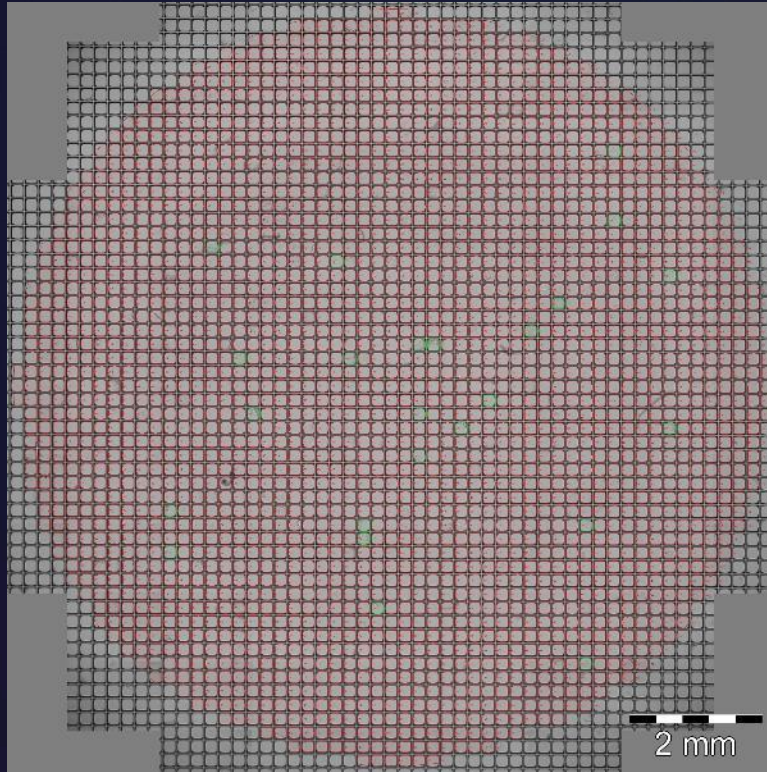


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5 mm

Single cell cloning | Results

- established iPSC colonies identified and successfully passaged to 96-well plate



Conclusions

- CellCelector™ system and nanowell plates 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 and selecting the clones of desired characteristics for downstream applications.

Acknowledgements

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The background is a dark blue gradient with a subtle grid pattern. Overlaid on this are several abstract elements: a large, thick, light blue wavy line that curves across the middle; a cluster of hexagons on the left side; and several molecular-like structures consisting of circles connected by lines, scattered across the top and right. The text "Thank You" is centered in a white, sans-serif font.

Thank You