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

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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 iPSC 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-CEM™), manufactured by ALS Automated Lab Solutions (Tirau, 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 microscopes in wa.

Nanowell Microplates

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

- Diameter: 200 μm
- Depth: 100 μm
- 132,000 nanowells per plate
- 100 μm (height) ± 10 μm
- Standard of 32 wells
- μg of stem cell medium
- g/cm

Materials and Methods

Cells

iPSCs were generated from peripheral blood mononuclear cells (PBMCs, CTS+) with human iPSC Generation Episomal Vector Kit (Vector) and pHAGEcell™ (Lonza).

Culture System

Cells were cultured on Matrigel (BD Biosciences) in StemFit A100 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 4 days, the plates scanned again, and suitable colonies transferred to 96-well plates.

Results

- Established iPSC colonies identified and successfully passaged as a 96-well plate.
- Colony picking with CellCelector™ system.

Conclusions

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