Development of a Closed and Automated Culture Process Using Bag-Frozen Induced Pluripotent Stem Cell Banks

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Research and
Development Center

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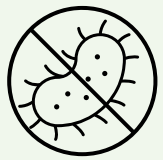
Conflict of Interest (COI)

The author has **no conflicts** of interest to disclose.

Background and Purpose

Manufacturing of regenerative medicine products requires
automated closed systems

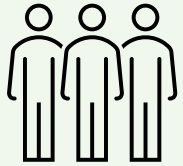




Ensuring Sterility



Consistent Quality



Process Reproducibility



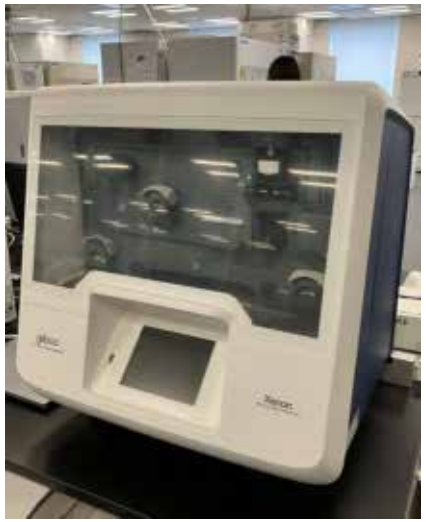
To evaluate a closed-system process from thawing of a cryopreserved iPS cell bank to the expansion and future differentiation process.

Manufacturing Workflow













Cell Thawing

・Frozen Cell Bag
・VIA Thaw

Cell Wash and Buffer Exchange

・Rotea

Electroporation

・Xenon

Cultivation

・CellSTACK
・Prodigy

Harvesting

・CellSTACK
・Prodigy

1. Cell Thawing

・Cryopreserved iPS cell bag
　：**HLA genome-edited iPS cell line** (QHJI14s04)

・Expansion culture and cryopreservation in bags were performed at Kaneka co.



<u>VIA Thaw L1000</u> (Cytiva)
→ Automated cryobag thawing system

・Thawing time: **9 min.**

・Total viable cells after thawing: **1.15 × 10⁷ cells**

・Cell viability after thawing: **88%**



2. Cell Wash and Buffer Exchange

CTS™ Rotea™ Counterflow Centrifugation System (Thermo Fisher Scientific)
→ Closed-system cell washing and concentration system

・Centrifugation at 2400 × g to form the cell bed*.

・Three-step buffer exchange while maintaining centrifugation to remove the cryoprotectant and replace it with Xenon buffer.

・Final harvest volume: 5 mL
Harvested into two syringes using a three-way stopcock.
→ **The first ~1 mL of cell suspension was used for the subsequent Xenon step**.

・Total viable cells after harvest: **5.73 × 10^6 cells**
・Cell viability: **97%**



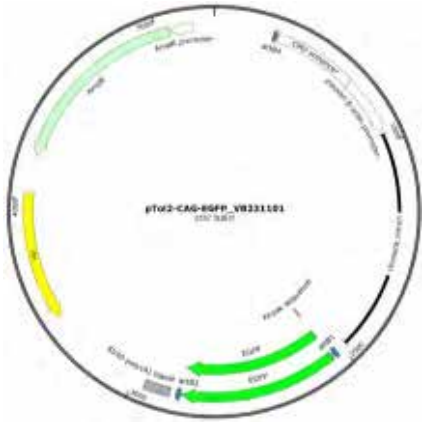
*cell bed ⋯ a concentrated layer or zone of cells formed during centrifugation

3. Electroporation

Gibco™ CTS™ Xenon™ Electroporation System (Thermo Fisher Scientific)
→ Closed-system electroporation system

・SingleShot chamber was used for small-scale electroporation.

・Transposon-CAG-EGFP: 8.3 µL (3 µg/µL)
Transposase: 6.1 µL (4.1 µg/µL)
(**Total DNA: 50 µg**)







・Total viable cells after electroporation: **3.33 × 10⁶ cells**
・Cell viability: **85%**

→ Cells seeded after electroporation
・**CellSTACK** (cell culture tray): **1.00 × 10⁶ cells**
・**6-well plate: 1.00 × 10⁵ cells/well**

4. Cultivation

<u>1-Chamber TC-treated CellSTACK</u> (Corning)
→ Large-area culture vessel for adherent cells

・Equipped with a transfer cap with tubing for closed-system connection.

・Static culture at 37°C.

<u>CliniMACS Prodigy</u> (Miltenyi Biotec)
→ Closed-system cell manufacturing system

・**Connected to CellSTACK for automated medium exchange.**
・Medium exchange was performed using the “Adherent Cell Culture” – “6. Media change” program.



↑ **CellSTACK**

5. Harvesting

CellSTACK+ Prodigy

・CellSTACK was connected to Prodigy for automated medium exchange using the “Adherent Cell Culture – 6. Media change” program.

・Washed with PBS(−)

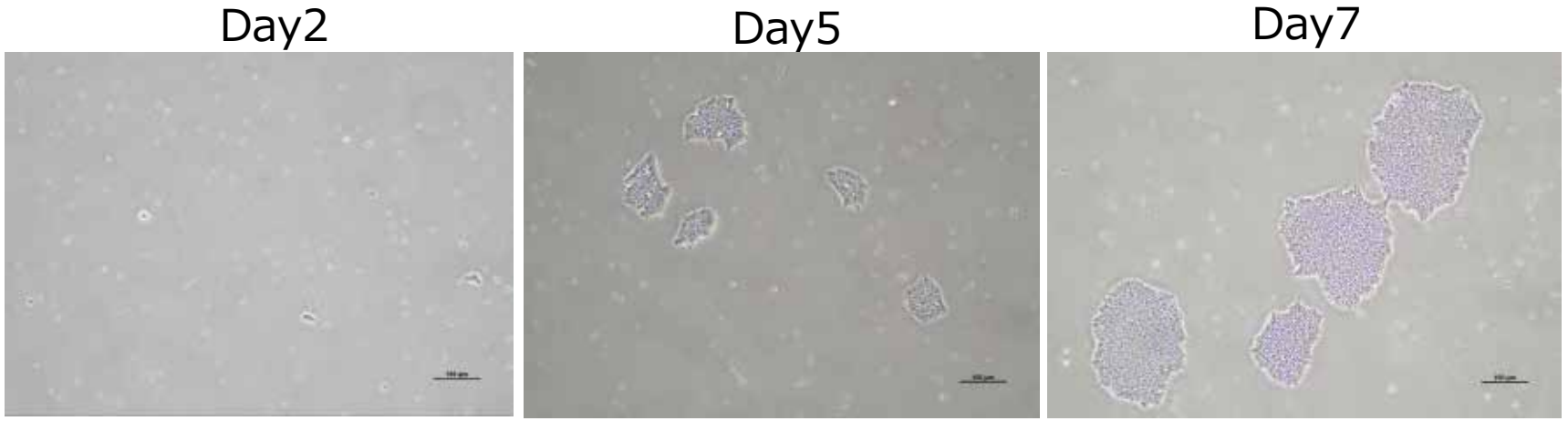
・Detached with 1× TrypLE Select (37°C, 10 min)

・Harvested into a collection bag

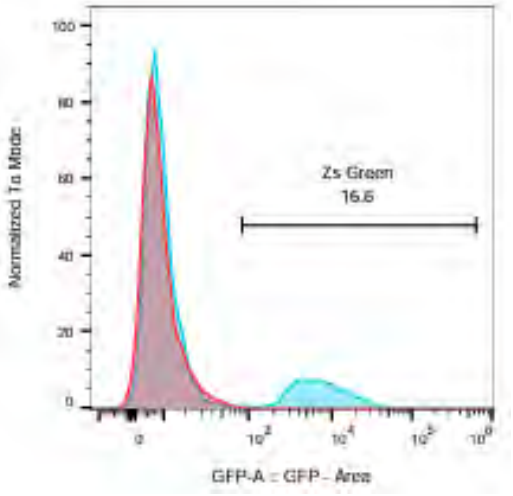
・Total viable cells after harvest: **7.82 × 10^6 cells**
・Cell viability after harvest: **99%**



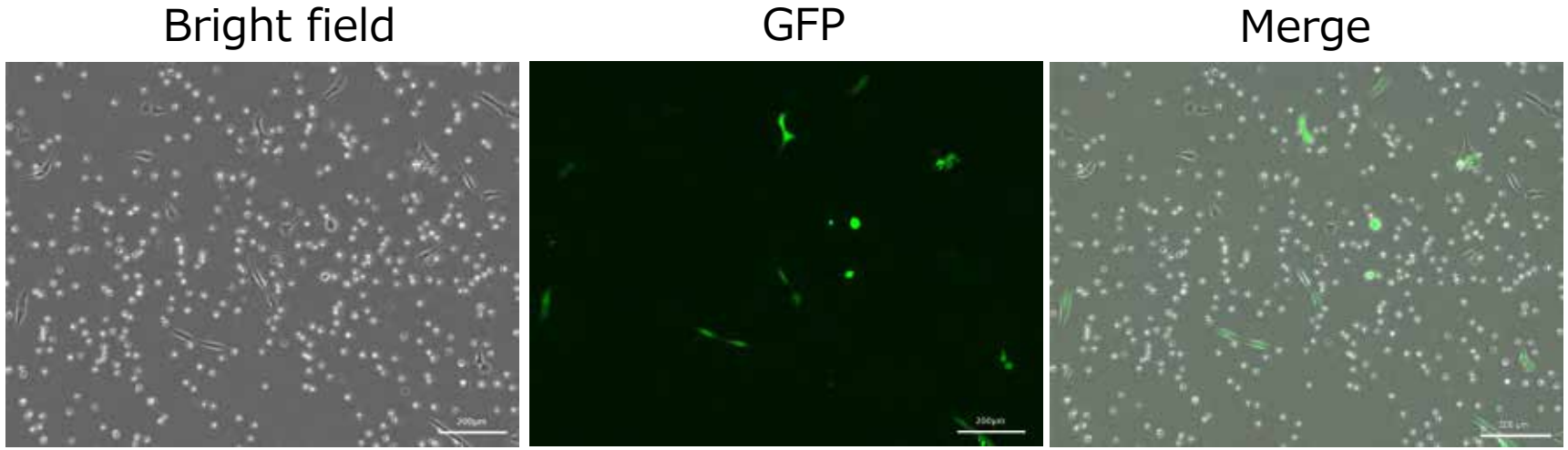
Results



↑ Culture process (CellSTACK)



↑ GFP Expression in Day 7
CellSTACK-Harvested Cells (FCM)



↑ GFP image taken approximately 24 hours after seeding (6-well plate)

Total number of live cells

About 8-fold increase

Transfection Efficiency
(24 Hours After Seeding)

42.6%

Conclusion & Future Work

Conclusion

・A mostly closed-system workflow was established from cryobag thawing to cell harvest.

・CellSTACK was shown to be applicable to closed-system iPSC culture, while cap replacement remained a challenge toward a fully closed-system workflow.

Future Work

・Optimization of the closed-system manufacturing process.

・Evaluation of the reproducibility of the closed-system workflow, including CAR gene transfer.

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