Optimizing the process development protocols for promoting highest viability growth of iPSCs during single-cell cloning

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Introduction

With the rapid growth and adaption of human induced pluripotent stem cells (iPSCs) in both research and Cell & Gene Therapy workflows, novel technologies are required to support more efficient methods for clonally derived cell lines. One of the main challenges for manufacturing clinical-grade iPSC therapies is deriving clonal banks and assurance of monoclonality. The goal is to unequivocally confirm that a line of cells is derived from a single clone. The Solentim portfolio of cell line development solutions by Advanced Instruments which encompasses the new VIPS® PRO single cell seeder, the Cell Metric® and STUDIUS™ platform, provide the clonal assurance necessary for documentation of monoclonality with the image-based evidence rather than probability.

The following study demonstrates superiority of iPSC single cell seeding using VIPS PRO in combination with MatriClone™ , a soluble extracellular matrix that replaces standard stem cell coating matrices, compared to the standard limited dilution (LD) technique.

Methods & Materials

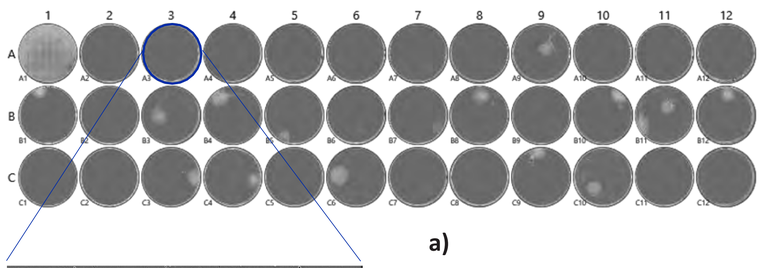
CELL CULTURE
Human iPS cell line (STBCi044-B) was cultured in a Corning® TC treated 6 well plate with MatriClone (Advanced Instruments, LLC.) at 0.25 µg/cm^2 in mTeSR™ Plus medium (STEMCELL Technologies Inc.).

SINGLE CELL SEEDING
The cell suspension for VIPS PRO seeding was added to the VIPS PRO Single-Use Seeding Kit (GMP- Certified), (9,750 cells/ml), and the LD plates were seeded at 0.5 cells/well. MatriClone (0.25 µg/cm^2) diluted in mTeSR Plus medium with 10% CloneR™ (STEMCELL Technologies Inc.) was added to cell suspension and seeding medium (VIPS PRO).
The cells were seeded into 5x 96-well plates (Corning® TC- treated).

IMAGING & STAINING
All plates were monitored using high quality whole well imaging performed between Day 0 and Day 10. Clones from VIPS PRO seeding were assessed for pluripotency using immunocytochemical staining for SSEA4, OCT4 and DAPI (Invitrogen™, A24881).

iPSCs maintain their stability and pluripotency through the seeding and clonal outgrowth processes

Analysis of whole well images collected on VIPS PRO showed that iPSCs cultured in MatriClone formed compact, multicellular colonies characterized by a distinct border which were tightly packed with a high nuclear-to-cytoplasm ratio (Fig. 4b).



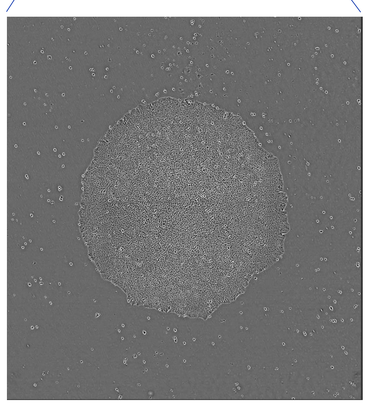


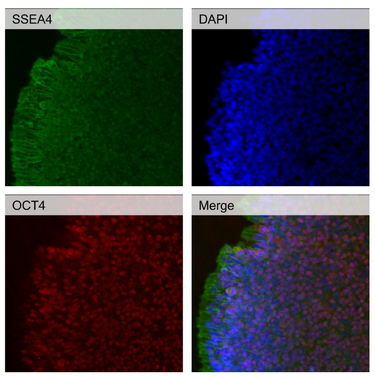
Figure 4. Whole well images of iPSCs on day 10 post-seeding in a 96-well plate.

a) Whole well images
b) Brightfield, magnified image of an iPSC colony.

Figure 5. Immunocytochemical staining with SSEA4 and OCT4 post VIPS PRO seeding.

Colonies from the 96 well plates were assessed for the pluripotency markers SSEA4 and OCT4.The results showed that seeding with VIPS PRO had no impact on the pluripotency. Figure has been contrast enhanced for the whole image.

b)



Results

Figure 1.
An innovative robust system for efficient monoclonal iPSC selection using VIPS PRO and MatriClone.

VIPS PRO and MatriClone increase seeding efficiency and clonal outgrowth of iPSCs

The combination of VIPS PRO and MatriClone resulted in significantly higher seeding efficiency (Fig. 2) and higher clonal outgrowth compared to LD methods, with approximately 50% of single seeded cells forming clonal colonies (Fig. 3).

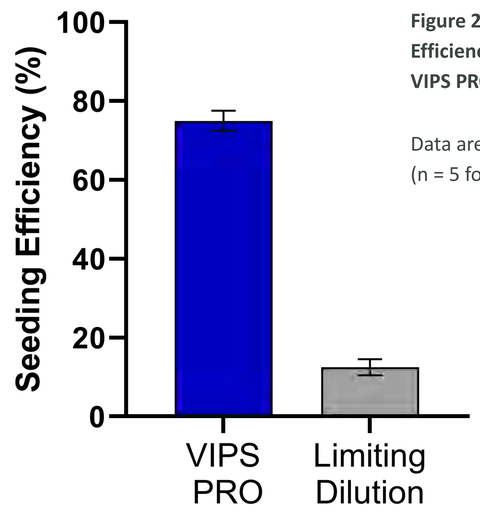


Figure 2.
Efficiency of LD (grey) and VIPS PRO seeding (blue).

Data are mean ± SEM
(n = 5 for both methods).

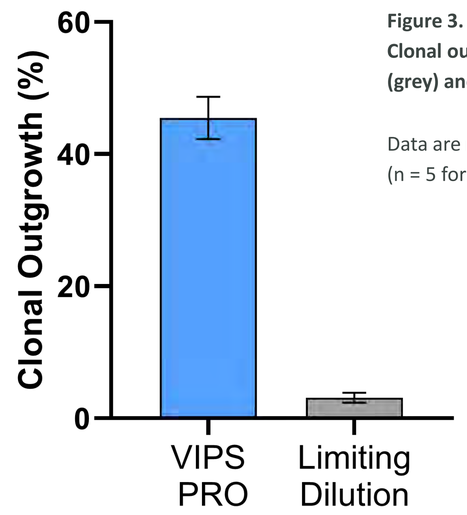


Figure 3.
Clonal outgrowth from LD (grey) and VIPS PRO (blue).

Data are mean ± SEM
(n = 5 for both methods).

Conclusion

- The combination of the VIPS PRO, MatriClone and STUDIUS provide a novel solution for human iPSCs workflow
- Gentle seeding results in high clonal outgrowth with High-quality imaging with automated data analysis and tracking in STUDIUS provide confidence with results
- VIPS PRO Single-Use Seeding Kits and 21CFR Part 11 Compliance features of STUDIUS software enable conformity with clinical manufacturing regulations

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