

Scaling Up Pluripotent Cells: Expansion of iPSCs in a Hollow-Fiber Bioreactor

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Abstract

The number of applications for expanded induced pluripotent stem cells (iPSCs) has increased notably in recent years. iPSCs can provide the raw material required for differentiated cell types, serve as a tailored substrate for drug development and testing, or produce the therapeutic agents themselves, in the case of iPSC-derived exosomes. As the range of applications for iPSCs grows, so does the need for reliable methods for large-scale expansion of this versatile cell type. The hollow-fiber bioreactor (HFB) of the Quantum Flex™ Cell Expansion System provides such an option for the scale-up and manufacture of iPSCs for a range of applications. In this study, human iPSCs from a single donor were expanded in the small (2000 cm²) HFB of Quantum Flex. HFBs were coated with either 1 mg recombinant laminin (LN; 17 µg/mL; 0.5 µg/cm²) or 2 mg recombinant vitronectin (VN; 34 µg/mL; 1.0 µg/cm²) prior to cell seeding using a small bioreactor, device-specific protocol designed to maximize the evenness of cell distribution in that system. Regardless of coating, iPSCs were seeded at 7500 iPSCs/cm² (1.5 × 10⁶ total cells/HFB) and expanded for ~4 days in accordance with protocols as closely aligned to typical manual culture methods as possible to facilitate ease of process transfer. In that time, iPSCs expanded on LN-coated HFBs yielded a mean of 5.0 × 10⁸ iPSCs (n = 4; SD = 7.1 × 10⁷), and iPSCs expanded on VN-coated HFBs yielded a mean of 2.5 × 10⁸ iPSCs (n = 3; SD = 9.4 × 10⁶). Of those yields, an average of 12% (LN) or 10% (VN) iPSCs were lost to the system washout were lost during the system washout occurring prior to introducing 60mL Accutase solution for harvesting the cells. Mean iPSC viability upon harvest was 96.8% (LN) or 97.9% (VN). Cells recovered from the system were assayed via flow cytometry for 4 common iPSC markers (SOX2, OCT4, TRA-1-60, SSEA4), and all cells from the system harvest displayed all 4 markers at rates > 90%. Interestingly, when cells recovered from the preharvest washout were interrogated for the same markers, these cells displayed those markers at frequencies 10% to 19% (LN) or 4% to 8% (VN) lower than those seen in the cells recovered from the harvest only. This may indicate that the preharvest washout can help prune less desirable (potentially more differentiated) iPSCs prior to harvest and leave a less differentiated population for the final harvest.

Methods

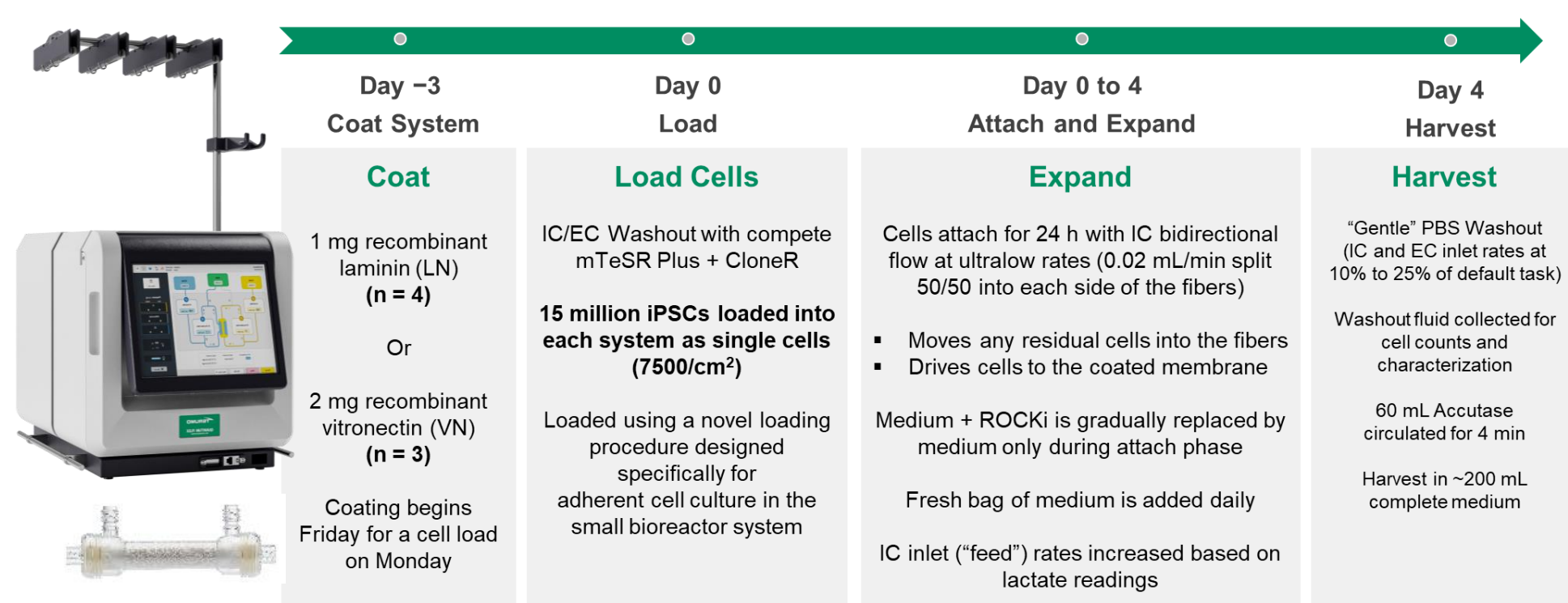


Figure 1: Methods used to expand iPSCs in the small bioreactor disposable set of the Quantum Flex hollow-fiber bioreactor system. Materials used include phosphate buffered saline (AJ30741226) from Cytiva Life Sciences, Marlborough, Massachusetts, USA; recombinant laminin 521 (LN521-05) from Biolamina (Sundbyberg, Sweden); recombinant vitronectin VT-N (A31804) from Thermo Fisher, Waltham, Massachusetts, USA; and mTeSR Plus (100-0276), CloneR (100-0691), and Accutase (#07920), all from Stemcell Technologies, Vancouver, BC.

Results

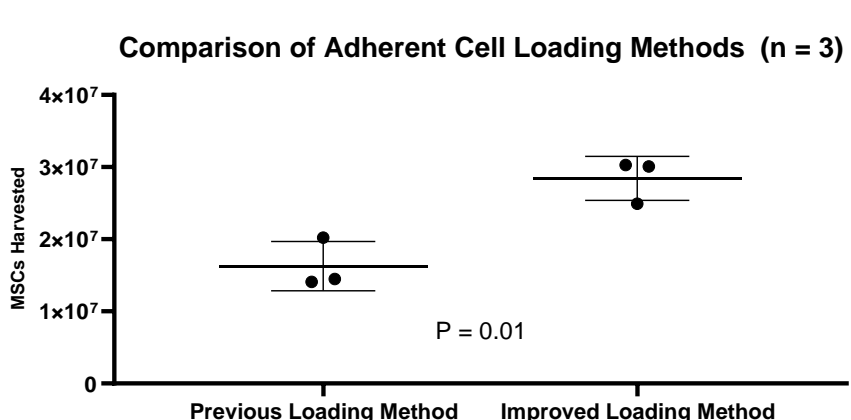


Figure 2: Obtained yield based on the method used to load the cells and spread them into the bioreactor. This graph compares the classic system "Load w/Circ" with an innovative approach to improve the distribution of the cells called "Back-and-Forth Spread" (B&F Spread). Yield data show a significant increase in harvested yield using B&F Spread.

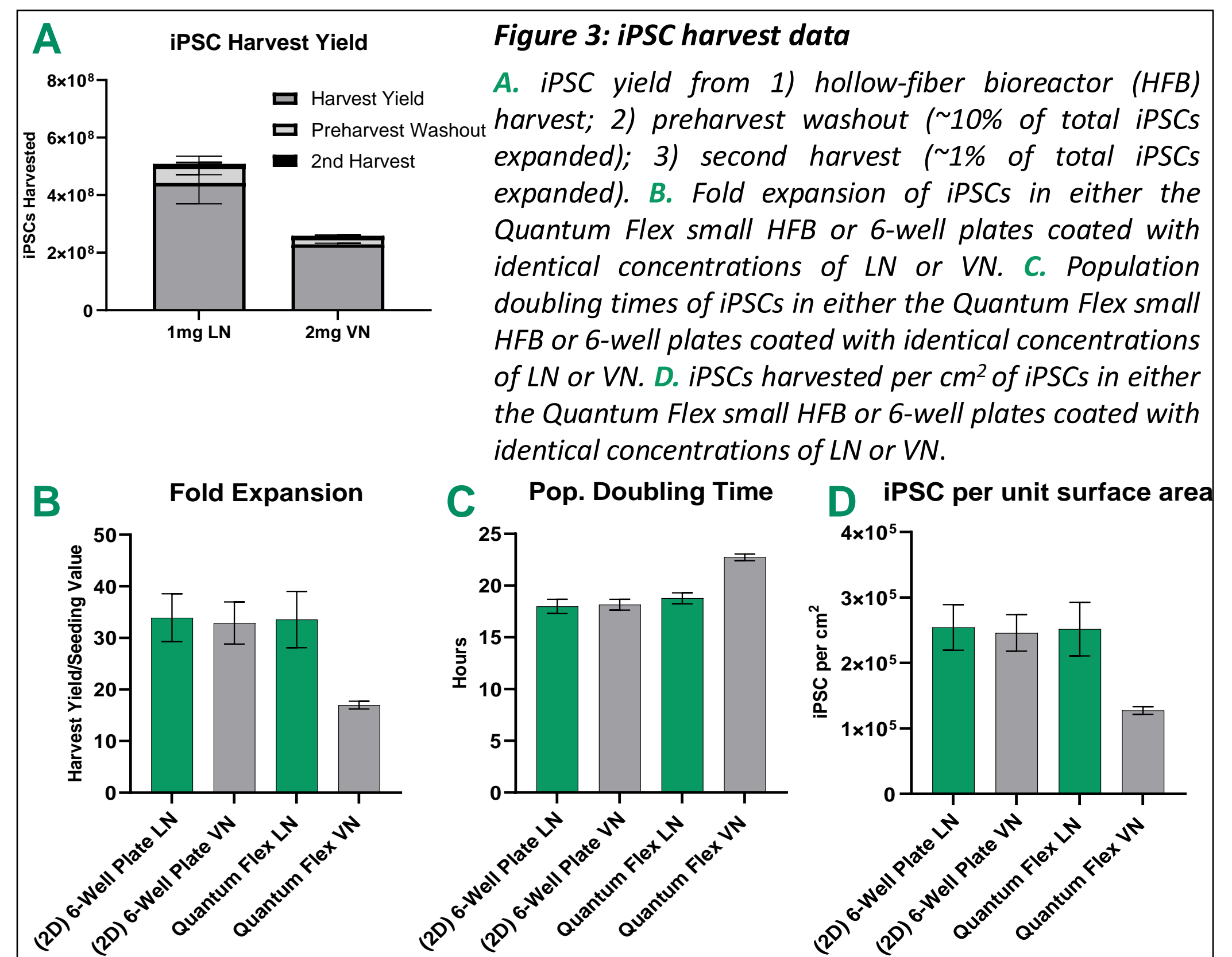


Figure 3: iPSC harvest data

A. iPSC yield from 1) hollow-fiber bioreactor (HFB) harvest; 2) preharvest washout (~10% of total iPSCs expanded); 3) second harvest (~1% of total iPSCs expanded). **B.** Fold expansion of iPSCs in either the Quantum Flex small HFB or 6-well plates coated with identical concentrations of LN or VN. **C.** Population doubling times of iPSCs in either the Quantum Flex small HFB or 6-well plates coated with identical concentrations of LN or VN. **D.** iPSCs harvested per cm² of iPSCs in either the Quantum Flex small HFB or 6-well plates coated with identical concentrations of LN or VN.

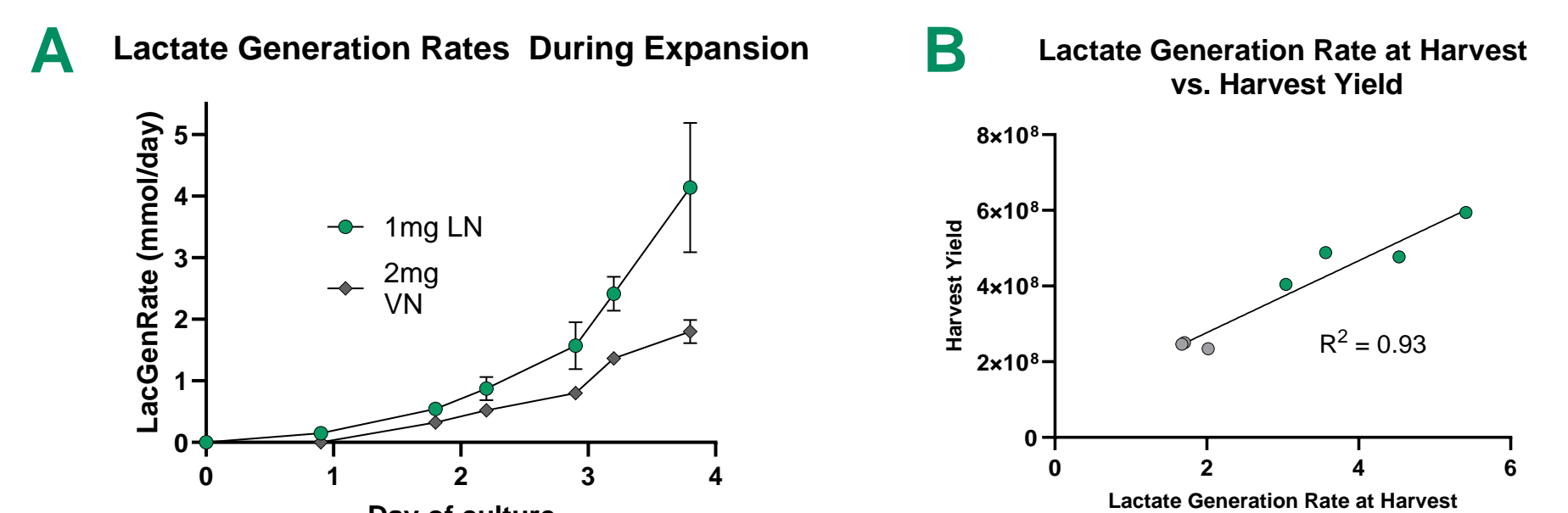


Figure 4: iPSC Expansion kinetics

A. iPSC lactate generation rates: Raw, daily lactate values taken from the bioreactor multiplied using the daily feed rate of the medium into the bioreactor. **B.** Correlation of lactate generation rate taken prior to harvest with iPSC harvest yield for small bioreactor systems coated with either LN or VN.

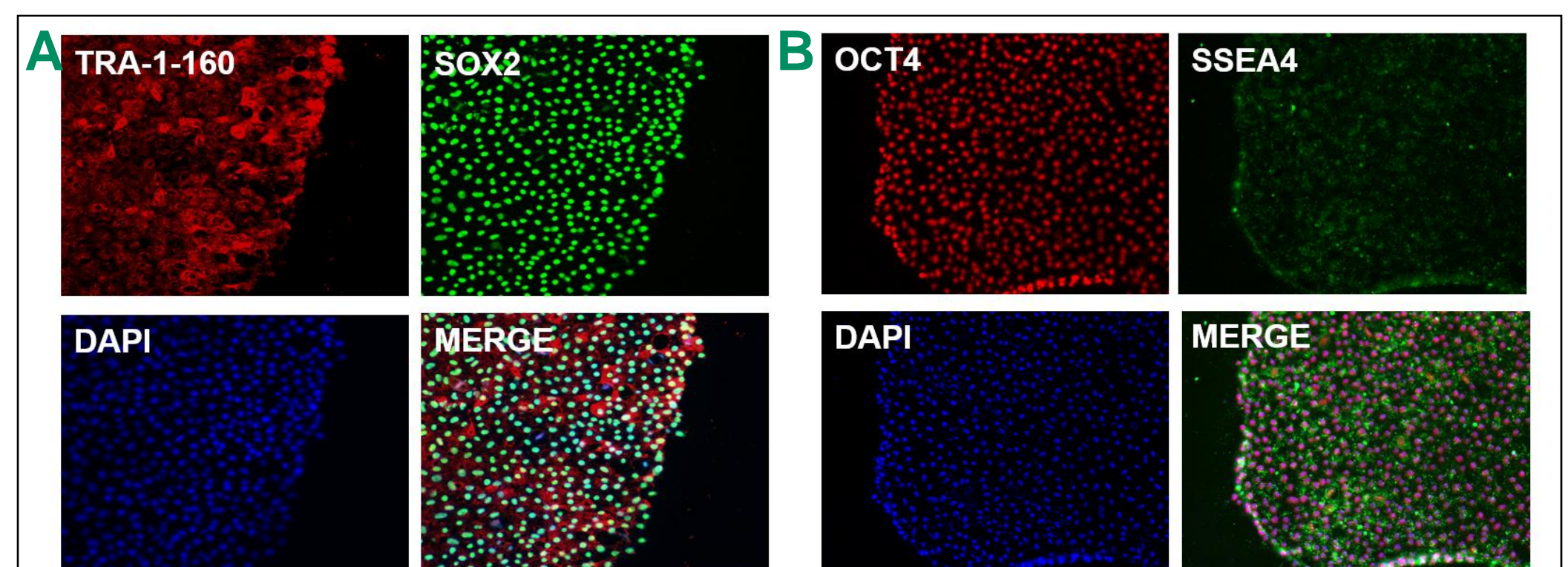


Figure 5: iPSC pluripotency

A. Fluorescent microscopy images of SOX2 and TRA-1-60 expression in LN-expanded iPSCs cultured on both LN and VN. **B.** Fluorescent microscopy images of OCT4 and SSEA4 expression in LN-expanded iPSCs cultured on both LN and VN. **C.** OCT4, SOX2, TRA-1-60, and SSEA4 expression from harvested iPSCs measured by flow cytometry. **D.** OCT4, SOX2, TRA-1-60, and SSEA4 expression from preharvest washout-collected iPSCs measured by flow cytometry (~10% of total iPSCs expanded).

Conclusion

iPSCs can be successfully expanded to between 250 million and 500 million cells in 4 days using the small bioreactor system of the Quantum Flex system using VN or LN, respectively, to coat the hollow-fiber bioreactor. Harvested iPSCs express common pluripotency markers at levels greater than ~90% as measured by flow cytometry regardless of coating.