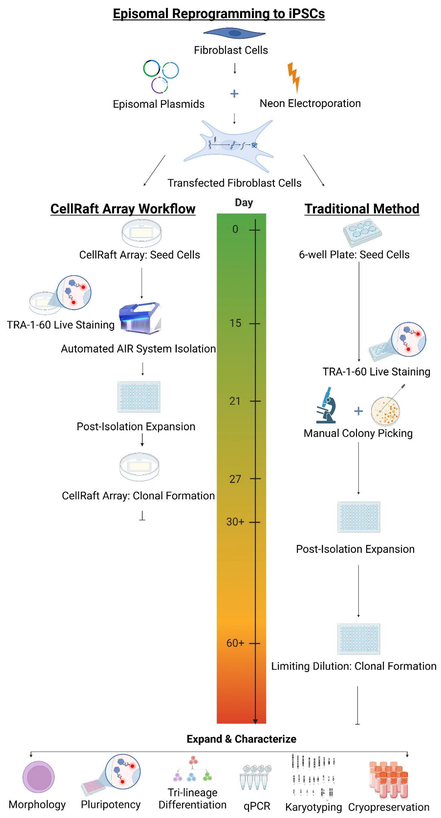
INCREASING EFFICIENCY AND CELL VIABILITY WHEN REPROGRAMMING SOMATIC CELLS TO IPSCS

Lexi Land, Anna Lane, Jessica Hartman
Cell Microsystems, Inc. Durham, NC USA

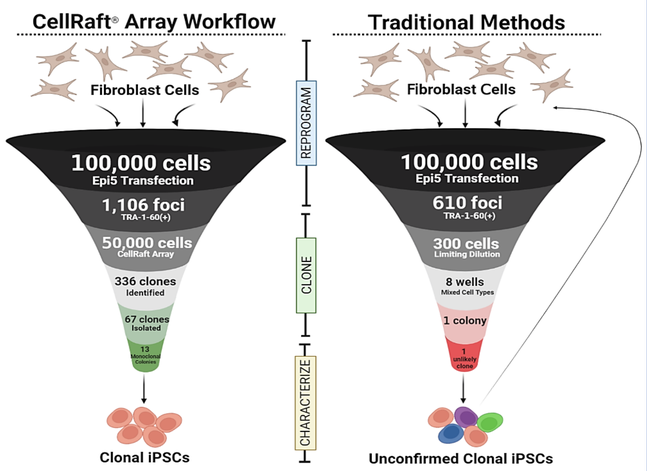
BACKGROUND

- Patient-specific induced pluripotent stem cells (iPSCs) are a valuable resource in the development of models for studying unique disease or drug responses. Donor somatic cells are reprogrammed into iPSCs then differentiated into target cells for treatment or testing purposes.
- Despite the potential of these cells, reprogramming has low efficiency (<1%), instability of pluripotency, and higher chance for mutations. Reprogramming is long, labor intensive and manual, and requires additional screening to derive a monoclonal population.
- The **CellRaft® Array** offers a more biological culture environment by providing **flask-like culture** conditions combined with single-cell segregation for tens of thousands of cells per consumable.
- The **CellRaft AIR® Technology** has the ability to accelerate reprogramming by improving efficiency, automation, and cell viability.

METHODS



CONCLUSIONS



Comparison of the number of successful iPSC monoclonal lines that were generated by the CellRaft Technology workflow (left) compared to the traditional workflow (right).

- **Reprogram, expand, or differentiate using the CellRaft Array**
- **Monitor reprogramming efficiency from D1**
- **Ensure iPSC monoclonality for downstream applications**
- **Reprogram cells up to a month faster than traditional reprogramming methods**

Take a picture to learn more

ON-ARRAY REPROGRAMMING

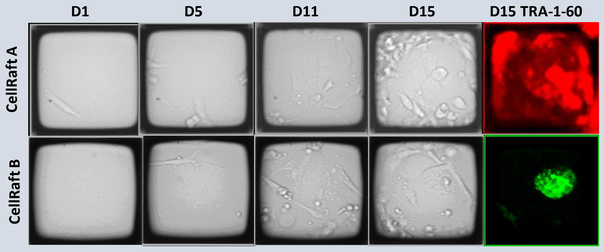


Figure 1: Representative CellRafts D1-D15 post-reprogramming. BJ Fibroblast Cells (ATCC) seeded on 200µm CellRaft Arrays post-transfection with Epi5 Episomal iPSC Reprogramming Kit (Thermo Fisher). On Day 15, CellRaft Array A was live-stained using the TRA-1-60 Alexa Fluor 594 Conjugate Kit (Thermo Fisher), and CellRaft Array B was live stained using Anti-TRA-1-60 FITC conjugate (Millipore Sigma).

LIVE TRA-1-60 STAINING

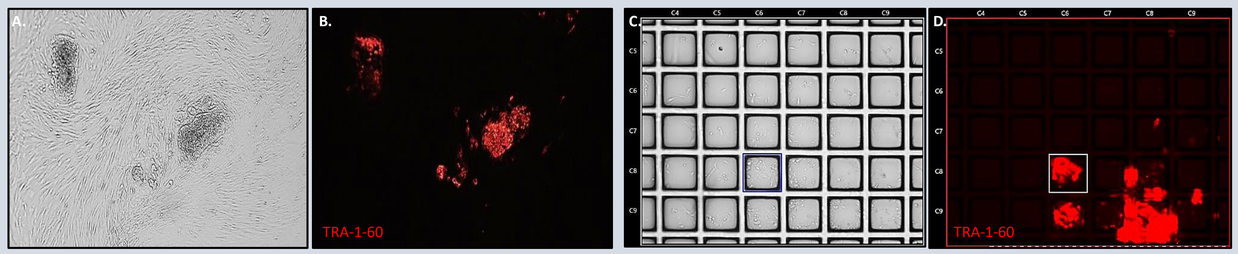


Figure 2: (A) 6-well plate containing reprogrammed BJ Cells in a lawn of non-reprogrammed BJ fibroblasts, 4X magnification. (B), same 6-well plate live-stained using TRA-1-60 Alexa Fluor 594 Conjugate Kit, 4X magnification. (C), 200µm CellRaft Array containing reprogrammed BJ Cells. (D), same 200µm CellRaft Array live-stained using TRA-1-60 Alexa Fluor 594 Conjugate Kit.

MORPHOLOGY & PLURIPOTENCY

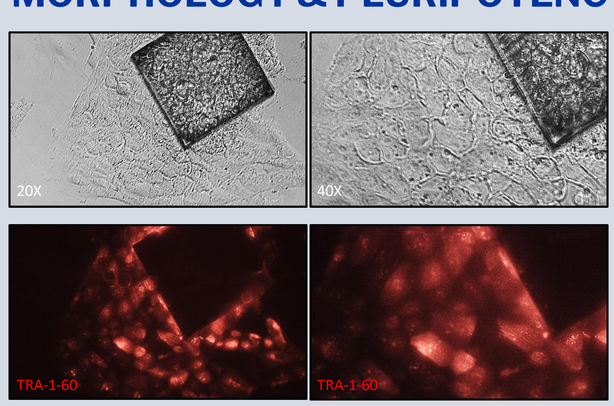
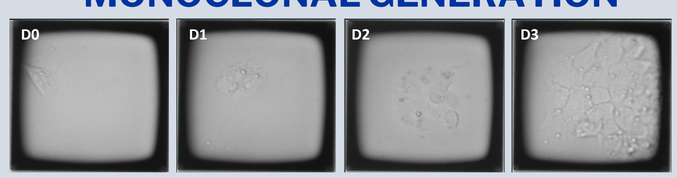


Figure 3: Top, 200µm CellRaft containing cells with iPSC-like morphology isolated into a 96-well collection plate in 20X and 40X magnification. Bottom, same 200µm CellRaft live-stained using TRA-1-60 Alexa Fluor 594 Conjugate Kit at 20X and 40X magnification.

MONOCLONAL GENERATION



D8 COLONY OUTGROWTH

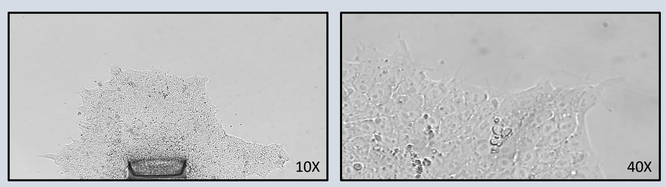


Figure 4: Top, Images of a CellRaft containing a clonal iPSC colony derived from bulk polyclonal reprogrammed cells with iPSC-like morphology (D0-D3 before being isolated into a 96-well plate). Bottom, post-isolation, the iPSCs grew off the CellRaft and a clonal colony emerged with iPSC morphology by D8.

iPSC EXPANSION

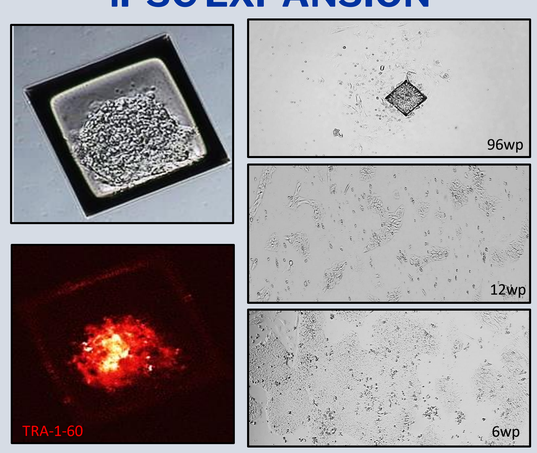


Figure 5: (Left) Outgrowth of iPSCs off 200µm CellRaft and live-staining with TRA-1-60 Alexa Fluor 594 Conjugate Kit at 20X magnification. (Right) further passage and expansion of iPSCs for downstream characterization and biobanking at 4X.

TRA-1-60+ COLONIES

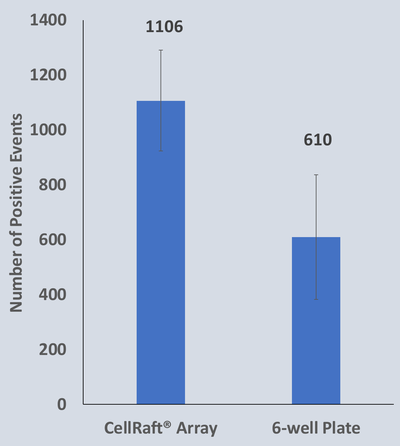


Figure 6: BJ Cells were transfected using the Epi5 Episomal iPSC Reprogramming Kit and cultured for 15 days in N2B27 reprogramming medium supplemented with fresh bFGF in a 200µm CellRaft Array or in a 6-well plate. On D15, cells were live-stained using TRA-1-60 Alexa Fluor 594 Conjugate Kit and positive events were calculated using CellRaft® Cytometry for the Array and manually counted and averaged n=3 counts for the 6-well plate. CellRaft Array n=7 and 6-well plate n=4.

TRI-LINEAGE DIFFERENTIATION

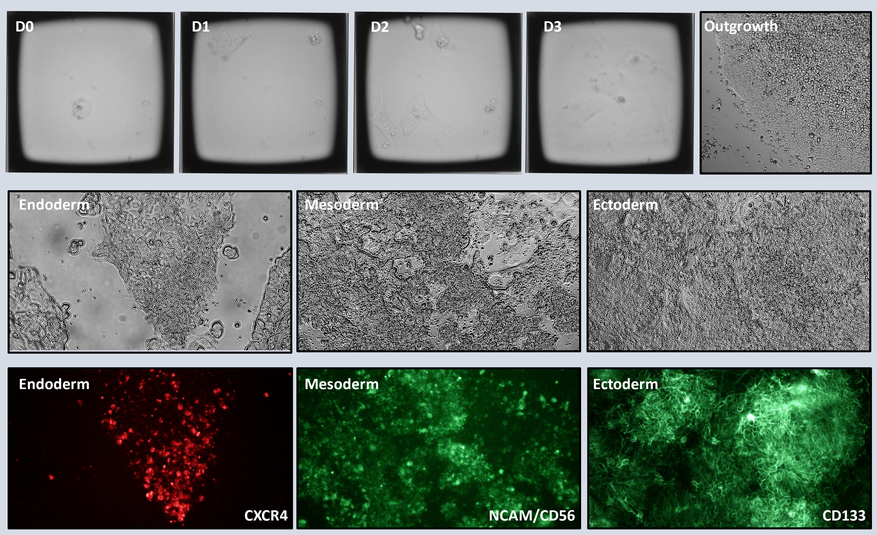


Figure 7: Top, CellRaft Array with monoclonal iPSCs isolated and expanded in a 96-well plate. Middle, iPSCs were replated for differentiation using the STEMdiff Trilineage Differentiation Kit (Stem Cell) and live-stained for Endoderm (CXCR4 Alexa Fluor 594 conjugated, Bioss Antibodies), Mesoderm (Anti-Human CD56/NCAM FITC conjugated, Stem Cell), and Ectoderm (CD133 FITC Conjugated, Novus Bio), 20X.

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GENETIC ANALYSIS

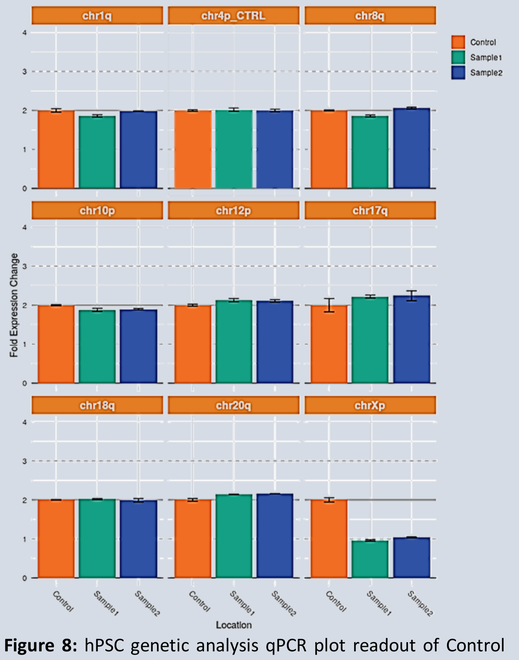

Figure 8: hPSC genetic analysis qPCR plot readout of Control (Female), Clone 1 (Male, clonal iPSC population in Figure 7), and Clone 2 (Male) using the hPSC Genetic Analysis Kit (StemCell).