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Introduction

Biomedical methods based on stem cells are currently one of the most dynamic areas in life science and biomedicine especially when it comes to cellular test systems for pharmacological and toxicological screenings. The development of reprogramming somatic cells into human induced pluripotent stem cells (iPS cells) opens unique perspectives for producing human cell products in a tissue, disease and patient specific manner. To fully take advantage of the potential of this technology there is a need to produce a high number of iPS cell lines, using high throughput techniques, to standardize the available protocols and to deliver fully characterized cells.

Currently generation of hiPS cell lines require skilled personnel and are highly time consuming. Therefore a high throughput technique, which is fully automatable and capable of selecting and sorting cell clusters, is highly desirable. Union Biometrica's large particle flow cytometry technology (COPAS and BioSorter) offers promising perspectives to address these issues. Here we describe the COPAS technology for the automated sorting of human iPS cells.

Methods and Materials

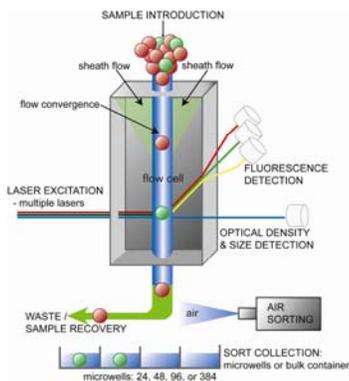


Figure 1. Sorting and dispensing mechanism for large particles. The COPAS PLUS HTS instrument (Union Biometrica, Inc) is a large particle flow cytometer which is able to analyze and sort large objects (~20-700 µm diameter) at a high rate (up to 50 events per second) on the basis of the physical characteristics of size, optical density and fluorescence signals. A gentle pneumatic sorting mechanism provides a means for dispensing sensitive objects like cell clusters to various formats.

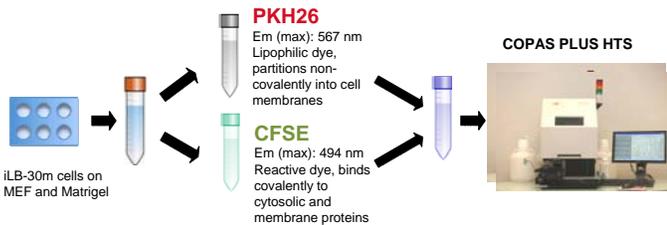


Figure 2. Experimental set-up to determine clonality of the sorted hiPS cell clusters

To determine clonality hiPS cells were cultured on MEF and Matrigel in 6-well plates. After enzymatic detachment the colonies were physically disrupted into smaller clusters and stained with the cell tracking dyes CFSE (FITC) or PKH26. Subsequently the clusters were sorted by fluorescence, cultured for several days and analyzed by fluorescence microscopy.

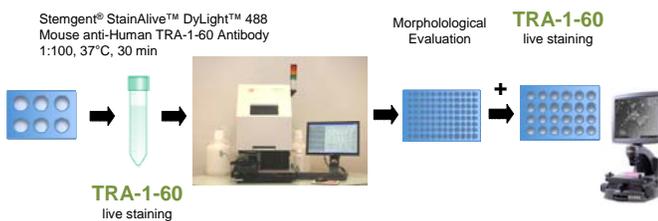


Figure 3. Experimental settings to determine pluripotency of the sorted hiPS cell clusters.

Furthermore hiPS cell colonies were detached as described above and stained with the FITC-labeled pluripotency marker TRA-1-60. Cell clusters were sorted according to size and TRA-1-60 expression and distribution in clusters (Figure 5), and single cell clusters were deposited in 96 well format for further evaluation of cell growth, morphology and expression of TRA-1-60.

Results

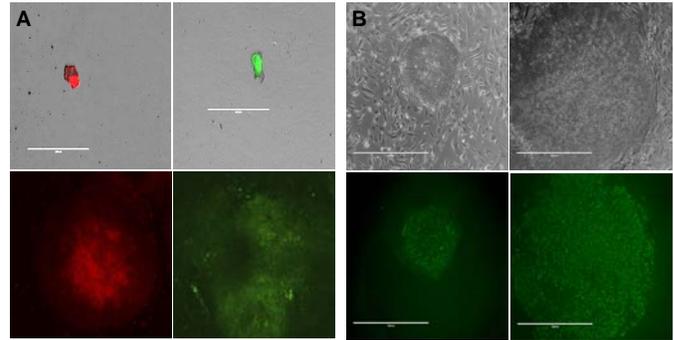


Figure 4. Colonies stained using cell tracking dyes (A). Growth and expression of TRA-1-60 of hiPS cell colonies after sorting (B).

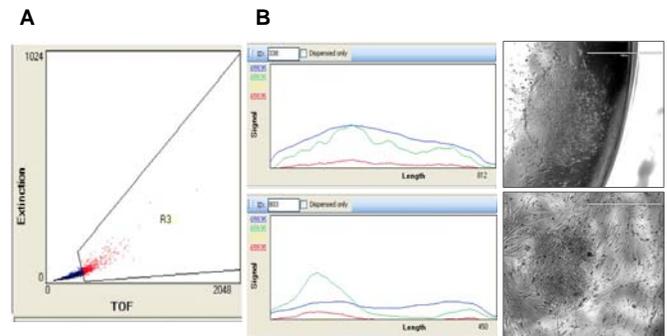


Figure 5. Dot plots from analysis of the hiPS clusters on the COPAS PLUS HTS (A). Profiler graph shows the distribution of TRA-1-60 (green) along the axis of the cell cluster and the corresponding cluster after 24h culture in 24-well format (B).

Conclusions

- The COPAS PLUS HTS can be used to analyze and sort primary human induced pluripotent stem cell colonies.
- The automated analysis and sorting process is gentle and does not influence pluripotency, morphology or viability compared to manually sorted cell clusters.
- The Profiler II allows to measure fluorescence distribution within the clusters which provides valuable information about the heterogeneity.
- Physical properties like size, optical density and fluorescence can be traced back for every dispensed cluster which allows statistical evaluation and quality control.
- The COPAS PLUS HTS provides a level of automation to the process of handling the stem cell clusters allowing for increased throughput and eliminates any biases that might be introduced by the researcher. This instrument brings the advantages of flow cytometry – statistically meaningful data, large unbiased data sets, and multi-parametric analysis – to experiments using stem cell clusters.

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