A Closed-System Workflow for End-to-End Processing of Human Pluripotent Stem Cells from Reprogramming to Expansion

Miltenyi Biotec

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Introduction

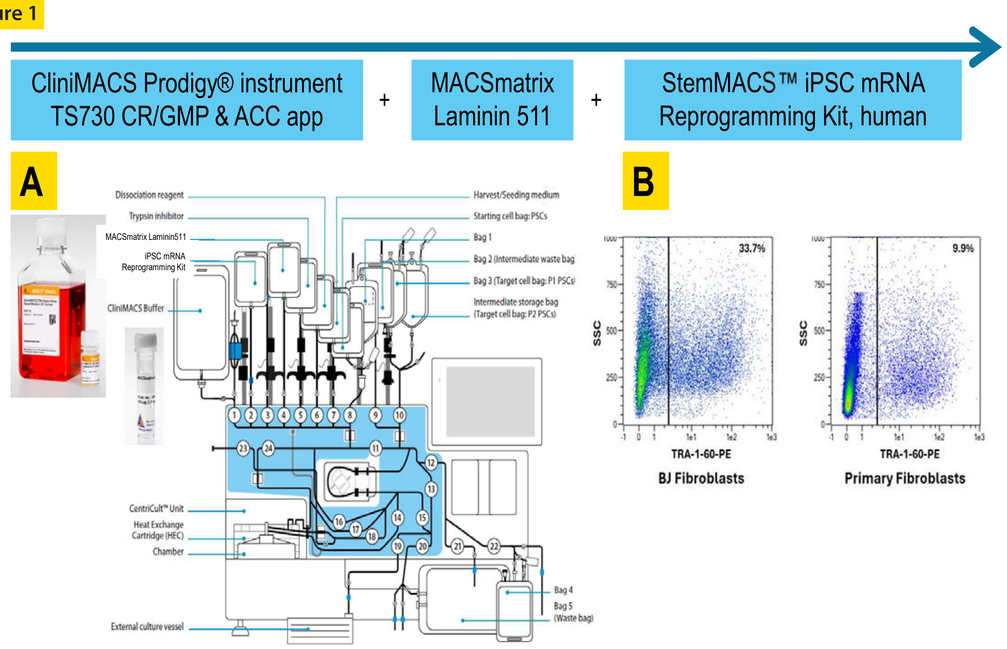
Human pluripotent stem cells (hPSCs) are a cornerstone of regenerative medicine and cell-based therapies; however, their production requires highly controlled conditions to ensure safety, reproducibility, and compliance with clinical standards. Miltenyi Biotec´s CliniMACS Prodigy® and MACSQuant® Tyto® platforms are specifically developed to enable GMP-compliant cell processing for both clinical and translational applications. These platforms offer automated, closed, and scalable workflows that minimize manual handling and reduce contamination risk. In this work, we describe a closed-system workflow for the semi-automated processing of hPSCs, encompassing somatic cell reprogramming in the cultivation chamber of the CliniMACS Prodigy, single-cell sorting using the MACSQuant Tyto and large-scale expansion utilizing the Adherent Cell Culture System on the CliniMACS Prodigy.

Results

1 Reprogramming

The workflow starts with non-integrating mRNA based reprogramming using the StemMACS™ iPSC mRNA Reprogramming Kit within the CliniMACS Prodigy cultivation chamber coated with MACSmatrix Laminin 511 (Fig.1A). Fibroblasts were seeded and transfected over five consecutive days with the StemMACS mRNA Reprogramming Cocktail containing the transcription factors POU5F1 (OCT3/4), SOX2, KLF4, MYC, NANOG and LIN28A along with the immune-modulating factor SOCS1. This approach enabled efficient reprogramming of both BJ and primary fibroblasts (Fig. 1B). Following a brief expansion phase of several days induced pluripotent stem cells (iPSCs) could be harvested for further processing. Coating, inoculation, medium exchange, and cell harvesting were all carried out using the CliniMACS Prodigy Adherent Cell Culture System application. The software allows flexible combination of the cultivation modules, making it highly adaptable to user-specific protocols.

Figure 1

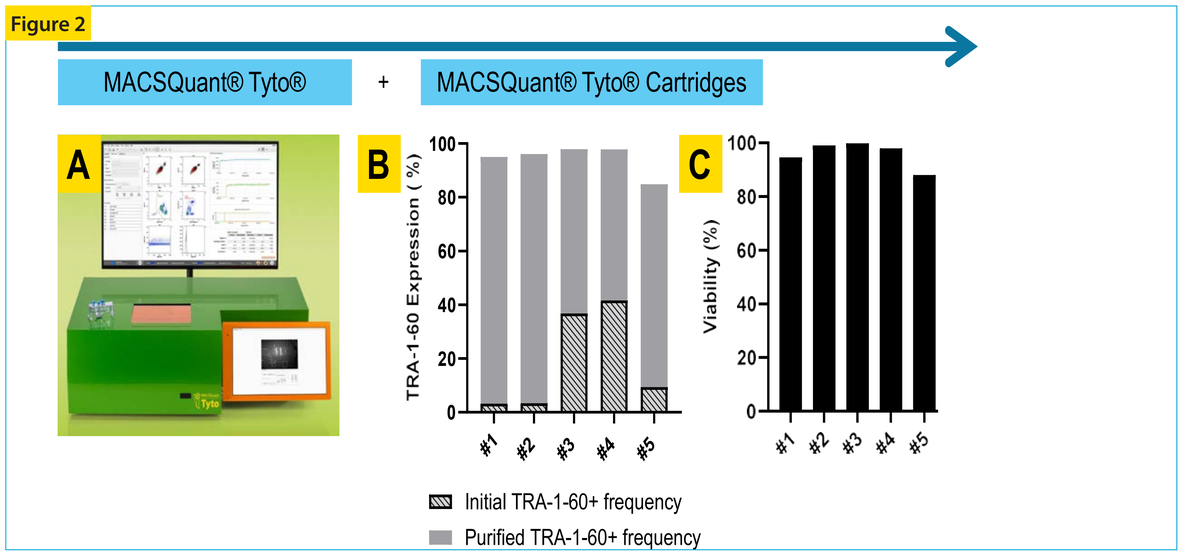


2 Cell sorting

Following reprogramming, the workflow proceeds with TRA-1-60–based positive selection of iPSC using the closed-system MACSQuant Tyto single cell sorter equipped with the MACSQuant Tyto Cartridge LC, which is specifically designed for large-cell applications. This step enables the selective isolation of fully reprogrammed cells while removing non-, partially reprogrammed, or differentiated cells (Fig. 2A). The microchip-based sorting technology allows gentle, closed, and GMP-compliant enrichment of newly generated hPSCs, supporting downstream bulk expansion or, alternatively, serving as a preparative step for single-cell deposition to establish clonal cell lines (not shown).

Here we present a series of sorting experiments of reprogrammed fibroblasts from five different donors using a manual reprogramming workflow (Fig. 2B+C). The enriched hPSCs can subsequently be transferred back into the CliniMACS Prodigy for expansion.

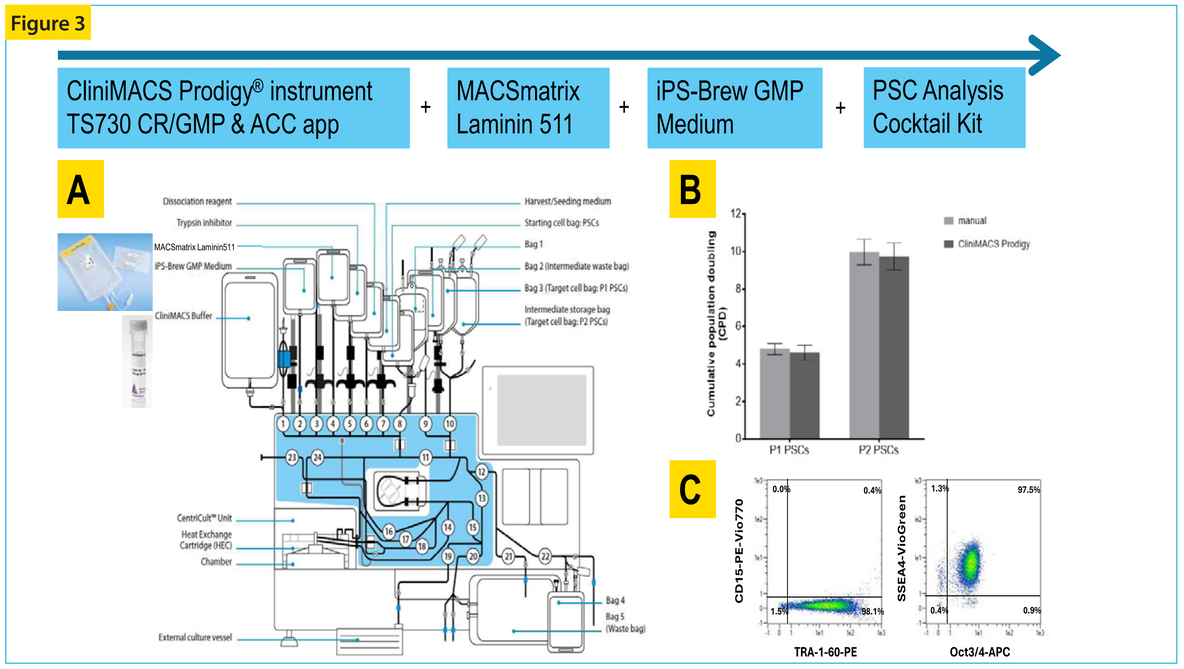
Figure 2



3 Expansion

Expansion can be initiated either from a cryopreserved iPSC sample or directly after cell sorting of the freshly reprogrammed iPSCs within the CliniMACS Prodigy cultivation chamber (cultivation area: 100 cm^2), which provides conditions comparable to a humidified incubator, or in an external cultivation vessel (ECV) connected to the CliniMACS Prodigy Tubing Set 730. Coating, inoculation, media changes and the cellular harvest was performed using the CliniMACS Prodigy Adherent Cell Culture System application (Fig. 3A). iPSCs expanded using the CliniMACS Prodigy showed proliferation rates comparable to those of manually cultured PSCs (Fig. 3B) along with a high expression of pluripotency-associated markers (TRA-1-60, Oct3/4, SSEA4) and absence of the early differentiation marker CD15. Staining was performed using the PSC Analysis Cocktail Kit (Fig.3C) in accordance with the recommendations provided by the International Society for Stem Cell Research (ISSCR).

Figure 3



Conclusion and Outlook

We demonstrate efficient reprogramming of somatic cells within a closed system with enriched and expanded hPSCs maintaining robust expression of pluripotency markers (TRA-1-60, SSEA4, OCT4, SOX2).

This streamlined workflow enhances process efficiency, reduces variability through automation, and facilitates compliance with Good Manufacturing Practice (GMP) standards. The closed-system design of the CliniMACS Prodigy tubing set and MACSQuant Tyto Cartridge LC provides a reliable platform for clinical-grade hPSC production, enabling safe, scalable applications in regenerative medicine, disease modeling, and cell therapy manufacturing.

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