Ensuring GMP Standards: A closed-system approach to expand human pluripotent stem cells

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Introduction

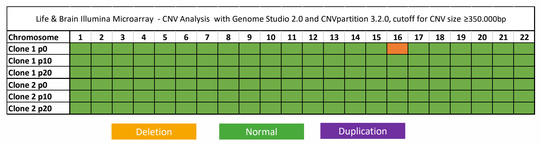
Human induced pluripotent stem cells (hiPSC) hold great promise for disease modeling, drug discovery, and clinical applications. In this regard, working with highly pluripotent, quality-controlled cell stocks during development is crucial to ensure reproducible experimental conditions. We established a workflow encompassing stable expansion of hiPSCs using a xeno-free cultivation medium in bags and assessment of pluripotency by quantitative flow cytometry using a defined marker panel. Following this workflow, hiPSCs could be stably expanded over 20 passages with persistent, high expression of pluripotency markers and almost no expression of differentiation markers. Cultured hiPSCs showed the typical morphology and retained a stable genotype. Thus, the workflow assures standardized, robust hiPSC expansion and includes characterization and quality control (QC) of the expanded cells. The flow cytometry– based QC strategy was also successfully applied for characterization of hiPSCs cultivated automatically in the closed system of the CliniMACS Prodigy®, which is of major relevance for generation of master cell banks (MCB) and working cell banks (WCB) for future clinical cell manufacturing. While using the new iPS-Brew GMP Media bag format open handling steps as well as hands-on time could be reduced significantly.

Results

1 hiPCSs can be stably expanded as single cells or cell clusters using iPS-Brew GMP medium in bags

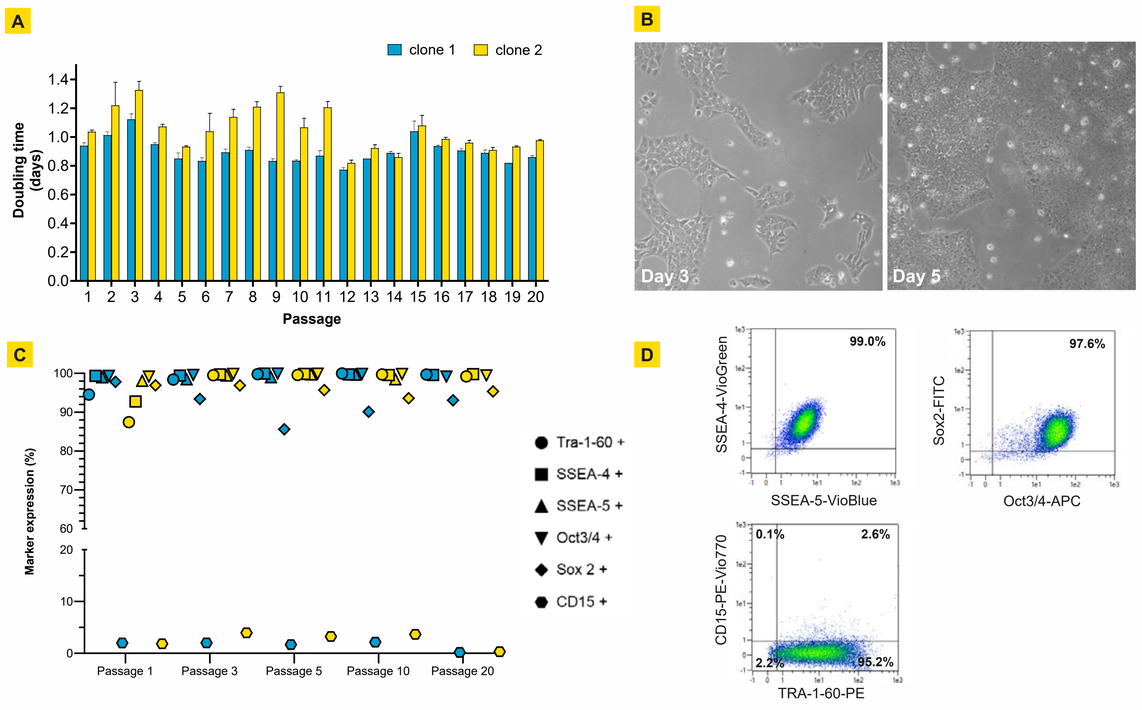
iPS-Brew GMP Medium provided in bags supports a closed way of media preparation as well as reduced amount of open-handling steps by connecting the basal media bag with supplement bags via sterile welding (Fig. 4). Two human iPSC clones were culture expanded on Laminin-511 fragment for 20 passages using complete iPS-Brew GMP Medium in bags splitted in single cells or cell clusters. Cells displayed typical morphology (Fig 1B, clone 1) and revealed a stable doubling time (Fig. 1A). Marker expression was analyzed via flow cytometry and both clones revealed high level of pluripotency markers TRA-1-60, SSEA-4, SOX-2 and OCT3/4 while lacking expression of CD15 (Fig. 1C and D).

Figure 2



Global genome structural variation analysis of hiPSCs was performed with an Infinium Global Screening Array-24 v3.0 BeadChip performed by Life and Brain. Shown are only de novo copy number variations (CNVs) larger than 350.000 bp. Clone 1 showed de novo CNVs on Chromosome 16 at passage 0 whereas those were not detected at passage 10 and 20. This might be a result of reduced selective pressure for this aberration in performed culture conditions (Fig. 2).

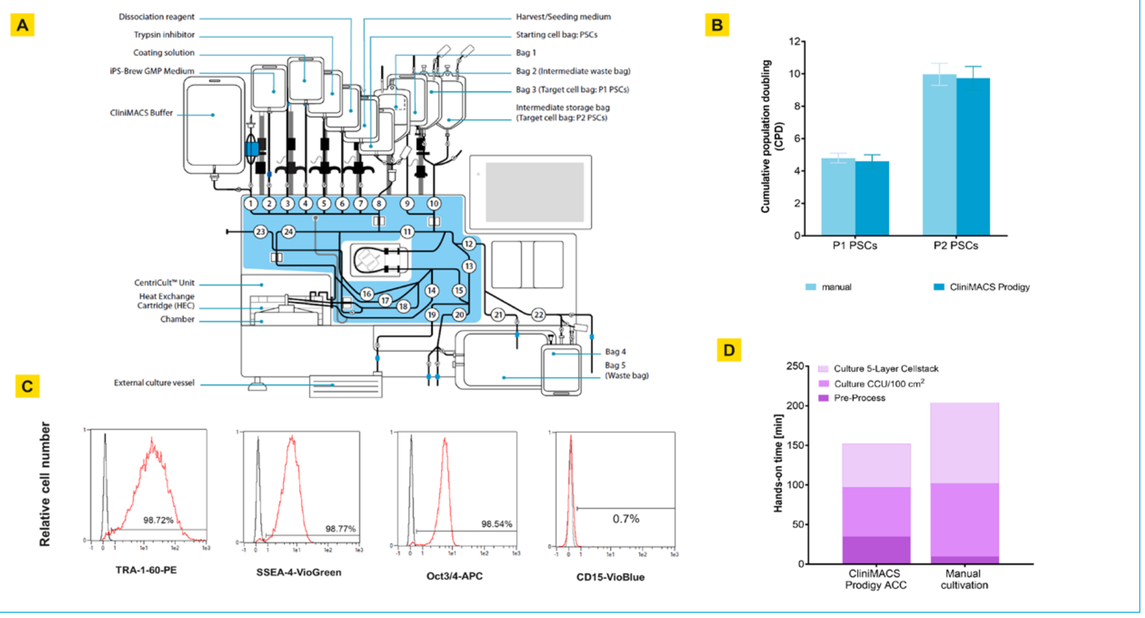
Figure 1



2 Large scale expansion of hiPSC under semi-automated and closed conditions using the CliniMACS Prodigy

Clone 1 was expanded semi-automated in the closed system of the CliniMACS Prodigy Adherent Cell Culture System (Fig. 3A). 1×10^6 cryopreserved human iPSCs were initially seeded and cultured in the CliniMACS Prodigy chamber until day 5 and further expanded in one Corning® CellSTACK® 5 Chamber from day 5 to day 10 in iPS-Brew GMP Medium (in bags) on Laminin-511 fragment. As a control, cells were cultured with manual laboratory standard using 6-well plates and T75 flasks. After 10 days of expansion, a clinically relevant number of P2 PSCs (approx. 5×10^8 cells) was harvested using the CliniMACS Prodigy Adherent Cell Culture System. The cumulative population doubling rate was comparable to the one obtained with manual laboratory standard (Fig. 3B). After expansion, a flow cytometry–based QC was applied using the PSC Analysis Cocktail Kit. iPSCs revealed high expression of pluripotency markers (TRA-1-60, SSEA-4, OCT3/4) comparable to the manually expanded control cells (Prodigy: 98–99%, control: 97-99% data not shown), with almost no expression of the differentiation marker CD15 (SSEA-1) (Prodigy: <0.1% CD15, control: <0.1% CD15 data not shown) (Fig. 3C). Interestingly, the semi-automated CliniMACS Prodigy Adherent Cell Culture Process showed about 25% reduced hands-on time compared to a comparable manual process (Fig. 3D).

Figure 3



Conclusion

We established a workflow which allows:

- efficient and stable expansion of hiPSCs using the iPS-Brew GMP Medium in bag,
- standardized quality control of the expanded cells by immunophenotyping as well as assessment of genomic stability after 20 passages,
- large scale expansion of iPSCs using the semi-automated and closed CliniMACS Prodigy Adherent Cell Culture System resulting in reduced hands-on time for generating master cell banks (MCB) and working cell banks (WCB),
- reduction of open handling steps during preparation of iPS-Brew GMP media and connection to CliniMACS Prodigy Tubing Set by introduction of large media bag format (fig 4)

Figure 4