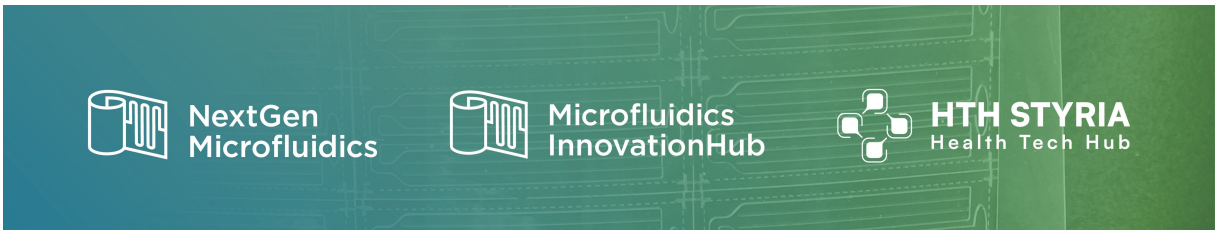

Title: *Automating organoid culture: A unique platform for stem cell and organoid generation, cultivation, and expansion*

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Organoids have revolutionized biomedical research by providing accurate models of human organs, invaluable for studying disease mechanisms, drug responses, and to advance personalized medicine. However, the manual processes involved in organoid culture are labor-intensive, prone to human error, and result in significant variability between organoids, posing a major challenge to the broader adoption of organoid technologies [1].

To address these challenges, we developed the CellXpress.ai™ Automated Cell Culture System. This integrated platform incorporates cutting-edge hardware and software technologies to automate and standardize 2D and 3D cell culture processes. From maintenance, monitoring, and incubation to imaging, analysis, and data processing, the CellXpress.ai system delivers consistent, unbiased, and biologically relevant results at scale. It supports scientists at every level of their organoid research by guiding users through iPSC workflows for differentiation into various organoids and by enabling the cultivation of complex hydrogel-based adult stem cell-derived organoid workflows.

Key features of the CellXpress.ai system include: 1. Automated cell maintenance with built-in incubation, advanced media storage (heating and cooling capabilities), liquid handling, and imaging to eliminate weekend interference. 2. Machine learning-assisted automated decision-making to optimize feeding, passaging, and annotating or ignoring wells based on real-time data analysis. 3. Minimization of human error and standardization with assisted protocol creation, tracking of protocol changes, cell monitoring, and consumables handling. 4. A unified software environment to control all hardware elements (liquid handler, incubator, imager), set up and execute workflows, and acquire and analyze data. 5. Integration of additional equipment to extend protocol capabilities.

As a proof of concept, we demonstrated the successful cultivation of iPSCs with different passaging options. Colorectal cancer organoids were maintained and expanded over multiple passages. Cardioids and midbrain organoids were successfully differentiated from the cultured iPSCs. The workflows included cell/organoid seeding, feeding, and automated passaging, including the automated use of an external centrifuge, in-line monitoring of cell/organoid growth, and external, automated endpoint analysis using a FLIPR® Penta High-Throughput Cellular Screening System.

The CellXpress.ai system enables researchers to generate high-quality organoids with greater reliability and reproducibility, addressing key bottlenecks in 3D biology

research and facilitating the wider adoption of organoids in biopharma and biotech for disease modeling, personalized medicine, drug discovery, and toxicity studies.

References

[1] Guo, L., Li, C., & Gong, W. (2024). Toward reproducible tumor organoid culture: focusing on primary liver cancer. *Frontiers in Immunology*.