Single-Cell Cloning Automation Technologies

Overview

Independent scientists from multiple research labs and core facilities have come together to share their new protocols for improving clone isolation and expansion of human pluripotent stem cells (hPSCs). Their detailed protocols and user experiences, utilizing emerging single-cell cloning technologies, are presented in an open access technical paper published in the Wiley Online Library - Current Protocols in Stem Cell Biology e123, Volume 55, 2020.

Title: Methods for Automated Single Cell Isolation and Sub-Cloning of Human Pluripotent Stem Cells

Authors: Valeria Fernandez Vallone[1,2], Narasimha Swamy Telugu[3,4], Iris Fischer[1,2], Duncan Miller[3,4,5], Sandra Schommer[3,4], Sebastian Diecke[3,4,5] and Harald Stachelscheid[1,2].

Affiliations

[1]Charite - Universitätsmedizin Berlin, Berlin, Germany.
[2]Berlin Institute of Health (BIH), BIH Stem Cell Core Facility, Berlin, Germany.
[3]Max Delbruck Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany.
[4]BIH Stem Cell Core Facility, Berlin Institute of Health (BIH), Berlin, Germany.
[5]DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany.

Paper abstract

Advances in human pluripotent stem cell (hPSC) techniques have led them to become a widely used and powerful tool for a vast array of applications, including disease modeling, developmental studies, drug discovery and testing, and emerging cell-based therapies. hPSC workflows that require clonal expansion from single cells, such as CRISPR/Cas9-mediated genome editing, face major challenges in terms of efficiency, cost, and precision. Classical subcloning approaches depend on limiting dilution and manual colony picking, which are both time-consuming and labor-intensive, and lack a real proof of clonality. Here we describe the application of three different automated cell isolation and dispensing devices that can enhance the single-cell cloning process for hPSCs. In combination with optimized cell culture conditions, these devices offer an attractive alternative compared to manual methods. We explore various aspects of each device system and define protocols for their practical application. Following the workflow described here, single cell-derived hPSC sub-clones from each system maintain pluripotency and genetic stability.

Technologies

In their paper the authors utilized three independent technologies to improve and further automate their single-cell cloning workflows. These technologies were the **isoCell** (from iotaSciences) and two commercially available single-cell dispensers. The authors state they developed "**a workflow in which the isoCell platform is applied to enable robust, efficient, cost-effective, and easy-to-handle hPSC clone isolation and expansion**". The isoCell is the only instrument to provide automation of all liquid-handling steps including single-cell isolation, media exchange and harvesting of resultant hPSC clonal cultures in sub-microliter volumes. In contrast, both single-cell dispensers considered only performed the single-cell isolation part of the workflow.

User experience

The authors considered three instruments for use in their hPSC cloning protocols. Based on these protocols, a succinct overview of user experiences and some relevant instrument characteristics are presented in Table 4 of their paper. This table is partially reproduced below in our comparison table. The isoCell is the **only solution** that enables **in-chamber verification of monoclonality** combined with **start-to-finish liquid-handling** automation for their complete single-cell cloning workflows.

To find more information about the isoCell: www.iotasciences.com/isocell

Comparison Table

A partial reproduction of Table 4 from the paper provides a summary of the technologies considered by the authors for their single-cell cloning protocols in the hPSC field.

Aspect/Equipment	iotaSciences isoCell	Single-cell dispenser 1	Single-cell dispenser 2
User experience			
Training	Half-day	3-5 days	1 day
Handling	Easy	Difficult	Moderate
Maintenance requirements	Low	Demanding	Low
Embedded in automated platform	No	Yes	Yes
Assurance of monoclonality			
Proof of monoclonality	In-chamber verification	Before-chamber verification	Before-chamber verification
Cell culture volume	< 1 µl	60 - 100 µl	60 - 100 µl
Cost			
Hardware acquisition costs	Medium	High	High
Consumable cost	Low	Low	High

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