INNOVATIVE SOLUTIONS FOR SINGLE-CELL ISOLATION AND CELL-LINE ENGINEERING

STREAMLINED AND AUTOMATED GENERATION OF CLONAL hiPSC CULTURES WITH MARKET LEADING EFFICIENCIES

INTRODUCTION

Human induced pluripotent stem cells (hiPSCs), in combination with powerful gene-editing technologies, such as CRISPR-Cas9, offer unique possibilitites to generate sophisticated disease models and advance drug discovery.However, the process of generating gene-edited hiPSC lines is often tedious and time-consuming. Especially, the generation of clonal hiPSC lines from a heterogenous pool of edited cells represents a critical bottleneck for setting up an efficient pipeline. Existing methods for single-cell isolation and culture are often too harsh on cells, manually tedious and do not offer sufficient assurance of monoclonality. Here, we describe and assess the Cloning Platform for establishing clonal hiPSC lines in a streamlined and automated manner. The system utilizes special low-volume culture chambers for the isolation and culture of cells, referred to as **GRID**s.
GRIDs are uniquely suited to easily verify monoclonality visually within chambers as well as documenting single cells with whole-chamber images directly after plating.

CLONING PLATFORM

The Cloning Platform is a modular system, comprising the **isoCell** and **isoHub** at it's core, with optional add-ons, including fluorescence and an imaging system to document GRID chambers throughout the entire cloning workflow. While the **isoCell** automates all liquid handling steps, including the formation of **GRID**s, plating cells into chambers, as well as exchanging culture medium and harvesting clonal colonies, the **isoHub** automates the navigation through **GRID** chambers throughout the entire workflow and allows the selection of chambers containing a single cell. Both instruments communicate wirelessly with each other to synchronise workflow progress.

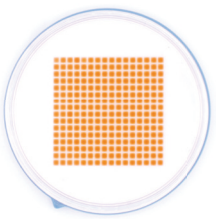
Table 1 – *Key features of the Cloning Platform*

CLONING PLATORM KEY FACTS	
Time to plate cells in GRID	2 minutes
Feeds cells in chambers?	yes
Optional heated bed?	yes
Compatible isolation formats	8-well PCR/cell culture strips
Verification of single-cell isolation?	yes
Tracking of clonal outgrowth?	yes
Time to generate clonal hiPSC cultures	~6 days

MATERIALS & METHODS

GRIDs (Fig. 1) were prepared on 60-mm dishes utilizing Laminin-521, Vitronectin-N, and iMatrix. using the isoCell according to established protocols (available through iotaSciences' Customer Portal). A single-cell suspension of hiPSCs was prepared and cells were plated automatically across the **GRID** using the **isoCell**. **GRID** chambers were inspected with the isoHub and those chambers containing a single cell (day 0) were activated. Active chambers were topped-up to 600 nl of culture medium (day 3). Culture medium was replenished on a daily basis starting from day 5 in active chambers. Colonies in active chambers were selected using the **isoHub** and automatically harvested using the **isoCell** (typically between days 6-8).

Figure 1 – *above: A GRID containing dye (for visualisation only).*
below: Overview of GRID features

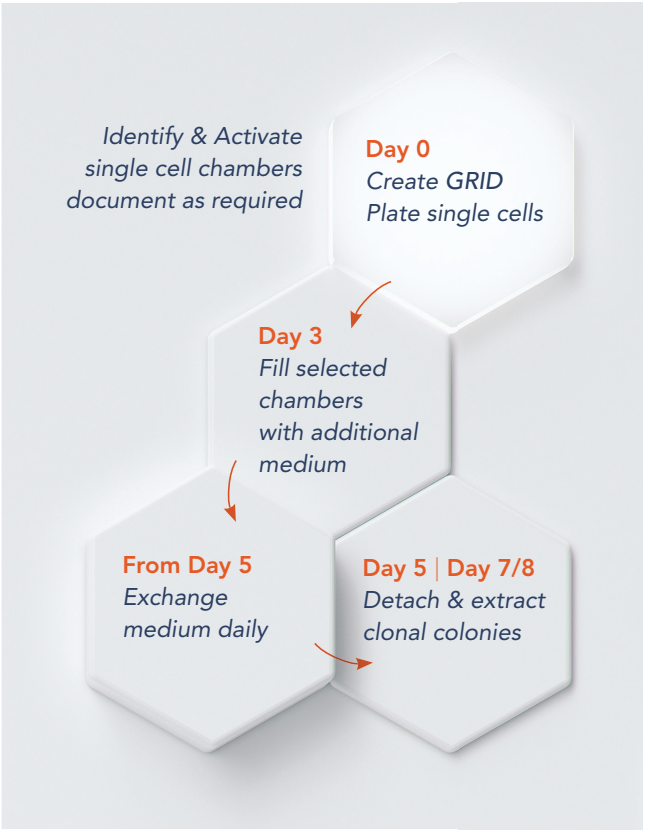


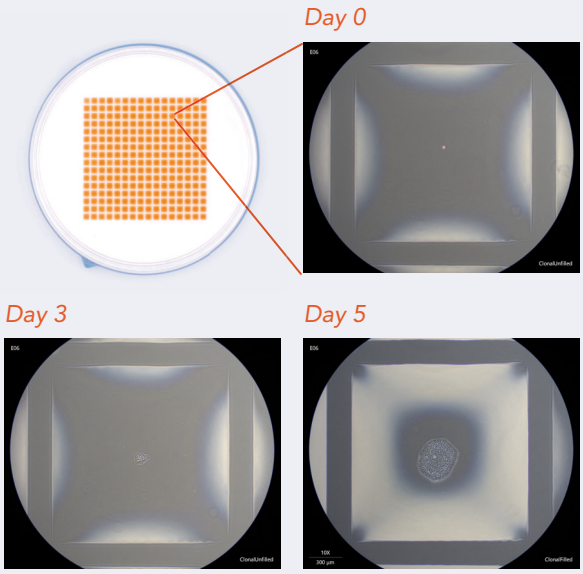
GRIDs	
Time to make a GRID	2 - 3 min
Number of chambers	256
Volume per chamber	600 nl
Chamber surface area	3,2 mm^2
Optical edge effects?	No

Figure 2 – *the Cloning Platform comprises a flexible and modular system for automating single-cell cloning workflows. It includes isoCell, isoHub, imaging app, and a 16-LED fluorescence module*

Figure 3 – *Left: Schematic workflow overview for cloning hiPSCs using the Cloning Platform. Creating **GRID**s, as well as all liquid handling steps are automated via **isoCell**, whereas **GRID** navigation as well as activating and reviewing single-cell chambers is automated using the **isoHub.***

*Right: Whole-chamber images of **GRID** chambers containing a single hiPSC cells and documented throughout the cloning process at the indicated time points.*

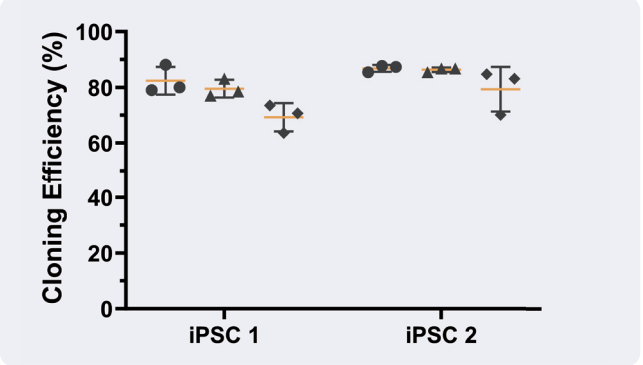




HIGH SINGLE-CELL CLONING EFFICIENCIES OF hiPSCS

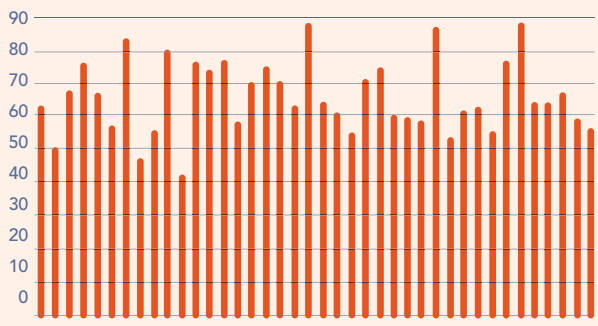
Following the outlined workflow (Fig. 3), two independent hiPSC cell lines were evaluated for single-cell clonality utilzing three distinct substrates, including VTN-N, LMN-521 and iMatrix. As shown (Fig. 4), both tested lines revealed very high clonal outgrowth in **GRID** chambers, demonstrating that the low-volume culture approach and the automated gentle handling of cells yields highly suitable culture environments for efficient single-cell outgrowth. In line with this, several additional and independent hiPSC lines have successfully been cloned as part of gene-editing workflows with high efficiency (Fig. 5)

Figure 4 – *Single-cell hiPSC cloning efficiencies in **GRID**s. Cloning efficiency denotes the percentage of single cell growing out into clonal cultures by day 5.*



● *Laminin-521 /* ▲ *Vitronectin-N /* ♦ *iMatrix*

Figure 5 – *Compilation of cloning efficiency (customer data) across multiple distinct hiPSC lines as part of gene-editing experiments. Cloning efficiency denotes the percentage of single cells growing out into clonal cultures by day 5.*



CLONING PLATFORM

The Cloning Platform offers a highly streamlined, knowledge-driven and automated approach to single-cell cloning of hiPSCs. The low-volume-culture in **GRID**s and the gentle handling of cells provide an optimal approach highly suitable for cloning hiPSCs with market-leading efficiencies.

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