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

AUTOMATED SINGLE-CELL CLONING OF CHO-S CELLS USING ANIMAL-COMPONENT FREE CONDITIONS IN NANOLITRE VOLUMES

INTRODUCTION

Cell line development (CLD) aims to engineer cell lines that produce recombinant proteins and antibodies with high titres, stringent quality and highest specificity. A critical yet challenging and rate limiting step as part of CLD is the generation of clonal cultures verifiably originating from a single cell. Single-cell cloning remains a tedious, lengthy and inefficient process. This is due to several reasons - i) single cells are highly sensitive and vulnerable, limiting the efficiency of outgrowth into clonal cultures (cloning efficiency); ii) common culture approaches typically require 100s of microliters for each individual cell with limited outgrowth success; iii) it is critical for regulatory submissions to provide adequate evidence, ideally image-based within a culture chamber, that a respective culture derived from a single cell only.
Chinese Hamster Ovary (CHO) cells are the main cell type utilized for CLD projects.
Here we assess the Cloning Platform for the automated cloning of single CHO cells under animal-component free (ACF) culture media conditions using only nanolitre volumes, while providing whole chamber images of single cells to verify monoclonality of derived cultures.

CLONING PLATFORM

The Cloning Platform is a modular system, comprising the isoCell and isoHub at its core, with optional add-ons, including fluorescence and an imaging system to document GRID chambers throughout the entire cloning workflow (Fig. 1). While the isoCell automates all liquid handling steps, including the formation of GRIDs, 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 (Table 1).
Both instruments communicate wirelessly with each other to synchronise and record 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	96-well plate
Verification of single-cell isolation?	yes
Tracking of clonal outgrowth?	yes
Time to generate clonal CHO cultures	~6 days

MATERIALS & METHODS

GRIDs (Fig. 2) were prepared on 60-mm suspension culture dishes utilizing PLH buffer with the isoCell according to established protocols (available through iotaSciences' Customer Portal).
PLH buffer comprises an animal-component free buffer ideally suited for GRID formation and culturing cells in suspension. A suspension of single CHO-S cells grown in CD-CHO medium (ThermoFisher) was prepared and cells were plated automatically by dispensing 200 nl into each GRID chamber using the isoCell. GRID chambers were inspected with the isoHub and those chambers containing a single cell were activated and documented via whole-chamber images at day 0. Active chambers were topped-up to 600 nl of culture medium, whereas non-activated chambers were left at 200 nl and excluded from the workflow. Cultures from single cells successfully growing out were harvested at day 8 and transferred to 96-well TC plates. Cloning efficiency denotes the number of clonal cultures at day 6 divided by the initial number of single-cell GRID chambers.

Figure 1 – *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 (both optional).*

Figure 2 – *A GRID comprising of 256 <1 µl culture chambers*

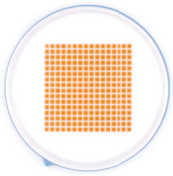
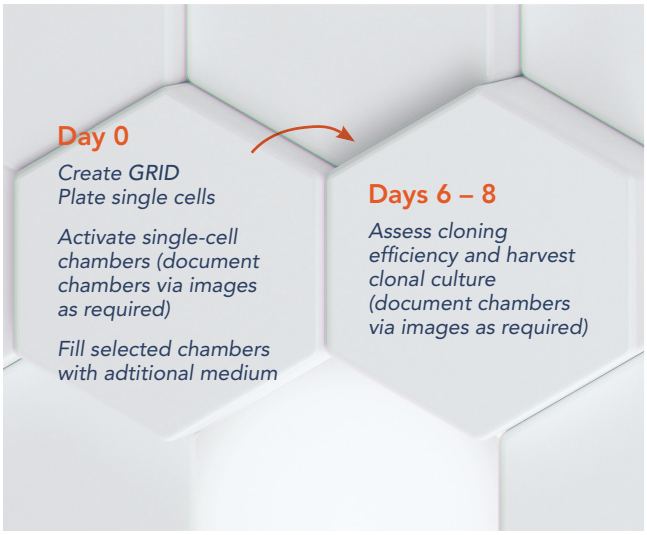


Figure 3 – *Schematic workflow overview for cloning CHO-S cells using the Cloning Platform. Creating **GRIDs**, as well as all liquid handling steps are automated via **isoCell**, whereas **GRID** navigation as well as activating and reviewing single-cell chambers is automated using the **isoHub.***



HIGH SINGLE-CELL CLONING EFFICIENCIES OF CHO-S

Following the outlined workflow (Fig. 3), several culture media were evaluated for clonal outgrowth of single cells into clonal cultures utilizing nanolitre volumes (Table 2). CHO-S cells revealed high clonal outgrowth in GRID chambers across different culture conditions (Fig.4), demonstrating that the low-volume culture approach and automated gentle handling of cells is a powerful combination, highly suitable for efficient single-cell outgrowth using animal-component free conditions. Additionally, clonal cultures transferred from GRIDs to 96-well plates showed 98-99% viability, as assessed by live-dead staining and continued to grow as expected within well-plates for further scale-up.

CONCLUSION

The Cloning Platform offers a highly streamlined, knowledge-driven and automated approach to single-cell cloning of CHO-S cells. The low-volume-culture in GRIDs and gentle handling of cells provide an optimal approach perfectly suited for cloning CHO-S with market-leading efficiencies. Furthermore, our workflow enables users to document chambers at any point in the process with whole-chamber images.

Table 2 – *Summary of culture medium and supplements for single-cell cloning of CHO-S cells in nanolitre volumes (see Figure 4)*

ABBREVIATION	DESCRIPTION	CONDITION
DMEM/F12	DMEM/F12 Medium	1
DMEM/F12+AOF	DMEM/F12 Medium + 1:40 (v/v) AOF	2
CD-CHO:DMEM/F12	CD-CHO and DMEM/F12 1:1 (v/v)	3
CD-CHO:DMEM/F12+AO	CD-CHO and DMEM/F12 1:1 (v/v) + 1:40 (v/v) AOF	4
FSE:DMEM/F12	FreeStyle™ CHO Expression Medium and DMEM/F12 1:1 (v/v)	5
FSE:DMEM/F12+AOF	FreeStyle™ CHO Expression Medium and DMEM/F12 1:1 (v/v) + 1:40 (v/v) AOF	6

Whole-chamber images of a GRID chamber containing a single CHO-S cell and documented throughout the cloning process at the indicated time points.

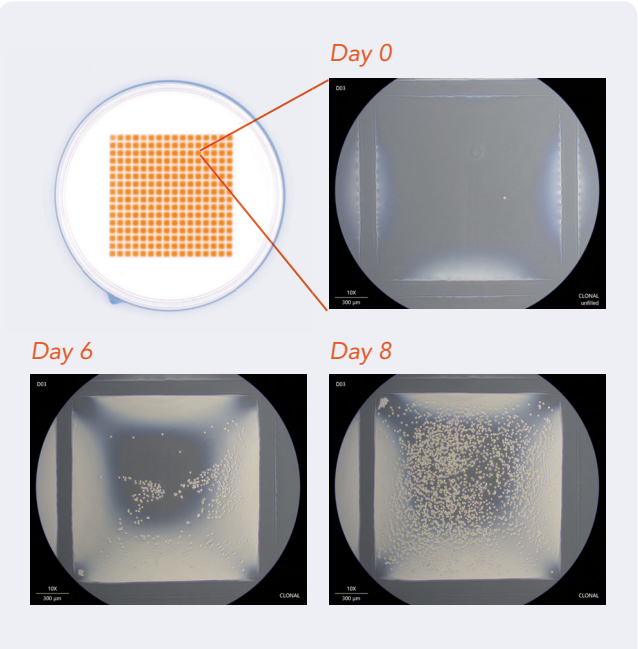


Figure 4 – *Single-cell cloning efficiencies of CHO-S cells in GRIDs. (see Table 2 for numbered culture conditions)*