Efficient single-cell cloning in low-volume culture

Application Note

iotaSciences

SUMMARY

We assessed the **Cloning Platform** for single-cell cloning of mammalian cells, including iPSCs. The modular platform comprises two instruments: isoCell and isoHub. Both instruments synergistically streamline the complete single-cell cloning process and automatically share and synchronise workflow progress with each other.

Cell-culture chambers specific to the platform allow easy verification of monoclonality by visualising and identifying single cells directly after plating. Chambers containing a single cell are selected and activated by the user for all subsequent steps in the workflow.

- Increased throughput
- Scalable platform
- Reduced hands-on time
- Reproducible results
- Consistent workflows

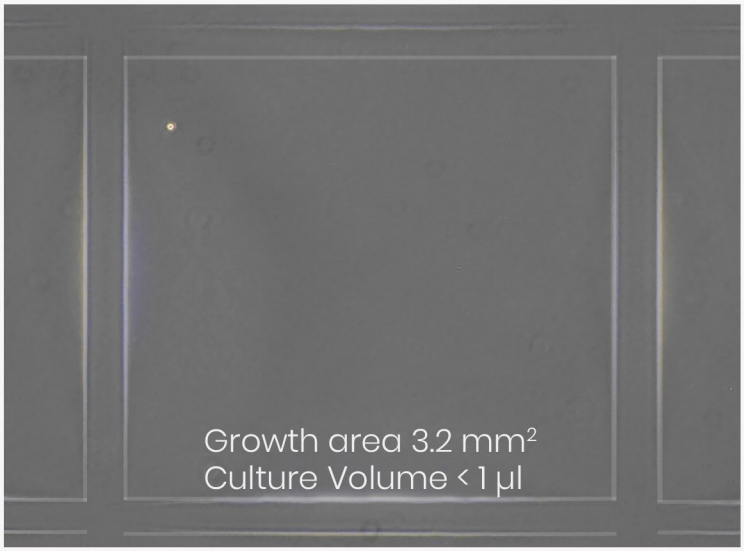
isoCell

isoHub

LOW-VOLUME CHAMBERS FOR SINGLE-CELL CLONING

The **Cloning Platform** utilizes GRIDs. A GRID consists of 256 miniature cell-culture chambers that are fabricated on polystyrene surfaces as part of the workflow. Due to the chambers small dimensions, GRIDs are uniquely suited to allow users to identify single cells directly after plating anywhere in the chamber. Hence, there is no need for cells to settle first, or spinning the plates.

The fabrication of chambers as well as all tedious pipetting steps, such as plating, feeding and extraction of cells is automated on the Cloning Platform via the isoCell. The isoHub guides the selection and activation of chambers containing a single cell, as well as the subsequent tracking of outgrowing clones.



Whole chamber view of a GRID chamber on the isoHub containing a single cell (4x objective). Grey outlines mark the boundaries of chambers. Chambers containing a single cell are identifed and selected by the user on the isoHub, which activates them for all subsequent steps in the workflow.

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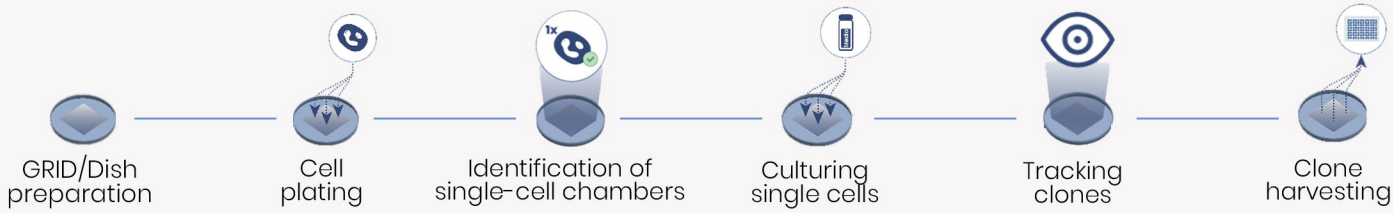
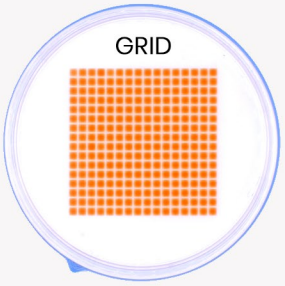
FROM SINGLE CELLS TO CLONAL CULTURES





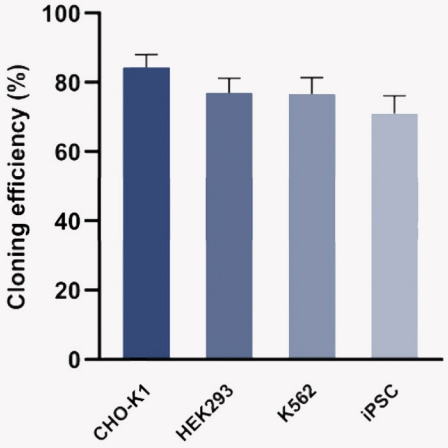
256 culture chambers on a 60-mm petri dish. Chambers were filled with dye for visibility only.

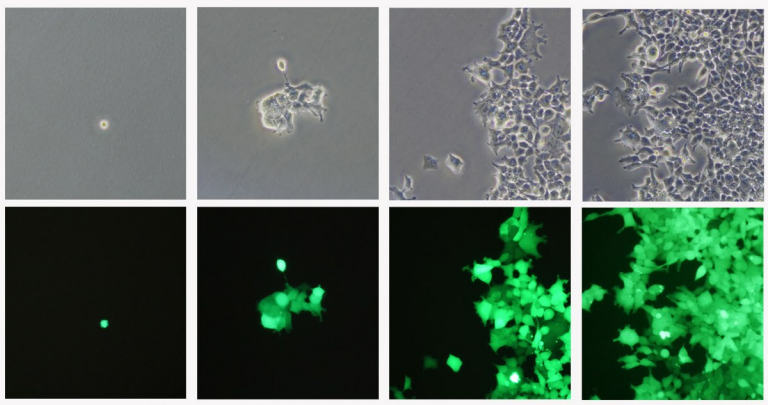
GRIDs were fabricated with the isoCell. Single-cell suspensions were adjusted to a concentration of 0.5-1x10^4 cells/ml and automatically plated with the isoCell into GRID chambers (up to 94 single-cell chambers per GRID). Single-cell chambers were identified and activated using the isoHub. Additional cell medium (600 nl) was automatically added to active chambers within the first few days of culture. With exception of iPSCs and suspension cells, all assessed cell lines were optionally fed once. iPSCs were fed every 2 days with the isoCell (exchange of 800 nl of medium in each active chamber). Clonal outgrowth was evaluated using the isoHub and selected colonies were extracted from GRIDs into PCR-tubes or flat-bottom strips for further downstream expansion or analyses.

EFFICIENT SINGLE-CELL CLONING AND TRACKING OF CLONAL CULTURES

Single-cell cloning of mammalian cells, including suspension (K562) and adherent (CHO/HEK and iPSC) was assessed on the **Cloning Platform**.

In all cases, high-cloning efficiencies were consistently and reproducibly achieved. Single cells grew out into stable clonal colonies comprising hundreds of cells within 5-14 days, depending on the respective cell line. Throughout the process, active chambers could easily be tracked for clonal outgrowth in brightfield and/or fluorescent mode, enabling quick and tailored decision making.





HEK293 cells stably expressing GFP were tracked in brightfield and fluorescent mode starting from single cell through to clonal population (20x objective).

GRID chambers are ideally suited for single-cell cloning applications due to their optical properties and high cloning efficiencies across diverse cell models, including iPSCs.

The **Cloning Platform** streamlines all aspects of the single-cell cloning process, with highly reproducible and consistent results. The level of automation offered by the platform significantly reduces hands-on time and allows users to track progress of clonal cultures conveniently anytime.