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

AUTOMATED AND GENTLE ISOLATION OF SINGLE CELLS FOR HIGHLY EFFICIENT CLONING OF hiPSCs

INTRODUCTION

Human induced pluripotent stem cells (hiPSCs) represent a breakthrough for the advancement of disease modelling, drug discovery and tissue engineering.
In combination with powerful gene-editing technologies such as CRISPR-Cas9, they are a promising tool to study and identify the genetic variants underlying diseases on a patient-specific basis. However, culturing hiPSCs remains tedious as the cells can be very sensitive to aberrant handling and manipulation, which in turn can result in the accumulation of cellular and genotoxic stress, resulting in undesired differentiation and loss of pluripotency. This is a particular concern when establishing single-cell derived hiPSC clones, as is required during gene-editing workflows. Common techniques are often too complex and/or harsh, resulting in poor single-cell cloning efficiencies. Additionally, they are limited in assuring monoclonality of the derived cultures. Here, we demonstrate how GRIDs and the scPicking Platform can significantly advance hiPSC gene-editing workflows, facilitating the gentle and automated isolation of single cells. Single-cell derived hiPSC cultures are obtained with market-leading cloning efficiences by following a simple and streamlined protocol.

scPICKING PLATFORM

The scPicking Platform (Fig. 1) comprises a liquid handler (isoPick) and a complimentary microscopy suite (isoHub). The isoHub can optionally be equipped with our image documentation system to document single cells, as well as a fluorescence module to work with labelled cells/fluorescent reporters if desired. The system offers powerful automation for dispensing, selection and subsequent transfer of single cells into volumes of 1.5 to 200 µl. Cells can be transferred into PCR tube strips or 96-well plates (Table 1).

scPICKING PLATORM KEY FACTS	
Time to plate cells in GRID	2 minutes
Time to pick a single cell	10 seconds
Single-cell picking efficiency	Up to 100%
Single-cell can be picked into	1,5 - 200 µl volumes
Compatible isolation formats	8-well PCR/cell culture strips; 96-well plates
Optional heated bed?	yes
Optional sample cooling?	yes
Verification of single-cell isolation?	yes

Table 1 – *Key features of the scPicking Platform*

MATERIALS & METHODS

GRIDs (Fig. 2, Table 2) were prepared using the isoPick and PL buffer (iotaSciences) according to establishe protocols (available through iotaSciences' Customer Portal). Single-cell suspensions of two independent hiPSC lines (10,000 cells/ml in PBS) were plated across the 256 chambers of a GRID, using the isoPick. GRID chambers were inspected with the isoHub to select those containing a single cell (Fig. 3).
Coordinates of selected chambers were automatically and wirelessly transferred to the isoPick. Single cells were automatically picked into individual wells of 96-well plates that have been coated with the indicated matrix and prefilled with 100 µl of CloneR2-containing culture medium prior to cell transfer (day 0).
An additional 100 µl of culture medium was added at day 3 and the medium was exchanged at day 5 (200 µl/well), followed by daily exchange from then onwards. Cloning efficiencies were determined by dividing the number of colony-containing wells by the number of transferred single cells and expressed as percentages. These were calculated between days 7-10, depending on the matrix coating.

Figure 1 –
Overview of the scPicking Platform

GRIDs	
Time to make a GRID	2 - 3 min
Number of chambers	256
Volume per chamber	200 nl
Chamber surface area	3,2 mm^2
Optical edge effects?	No

Table 2 – *Key features of GRIDs*

Figure 2 –
GRID containing dye (for visualisation only)

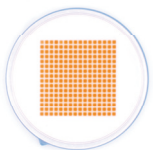
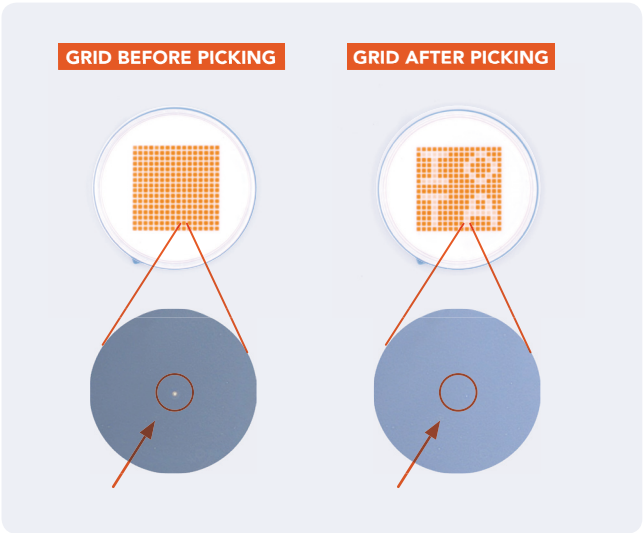


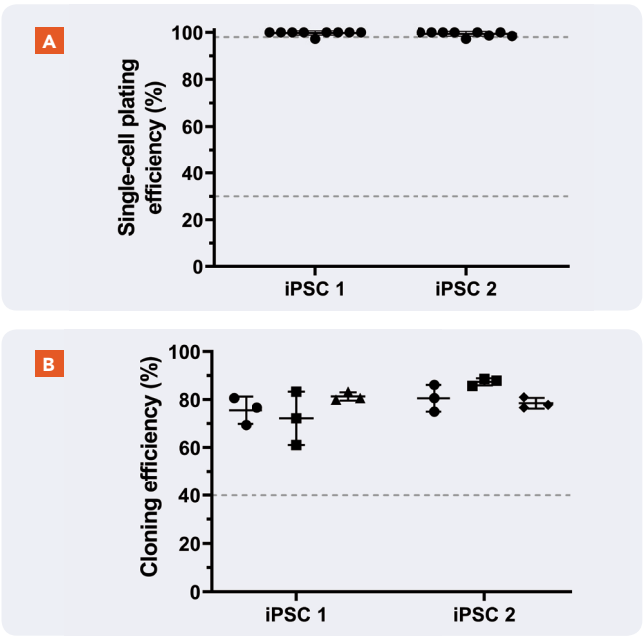
Figure 3 –
GRID chambers before and after single cell picking



MARKET-LEADING CLONING EFFICIENCIES FOR hiPSCS

Following the establishment of a high picking efficiency of hiPSCs, we next assessed the outgrowth of single hiPSCs into clonal colonies. Single hiPSCs were picked from GRIDs and transferred directly into 96-well plates. Wells were prefilled with hiPSC culture medium and matrices utilized included Laminin-521, Vitronectin-N, Synthemax and iMatrix (Laminin-511). Single-cell cloning efficiencies were assessed between days 7-10 with an average cloning efficiencies of >70%, independent of the matrix coating or hiPSC cell line used (Fig. 4B). These single-cell cloning efficiencies are considerably higher than typically achieved using other approaches, including FACS, demonstrating the ultra-gentle handling of sensitive single cells with the isoPick, assuring high cell viability and subsequent clonal outgrowth. Image-capture using the isoHub demonstrated healthy clonal outgrowth on all matrices (Fig. 5).

Figure 4 –
Plating (A) and cloning (B) efficiencies of hiPSCs

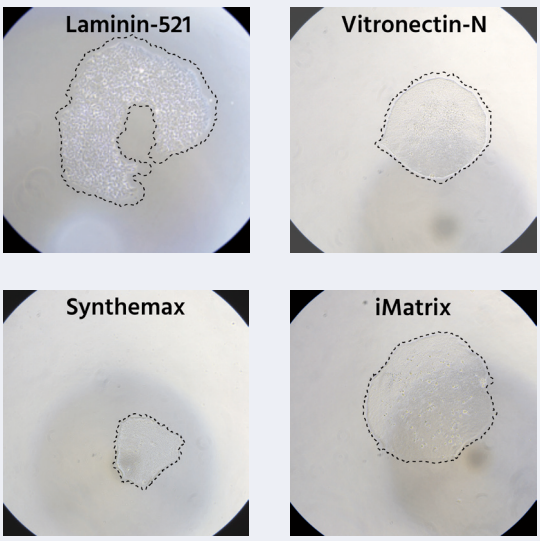


● Laminin-521 / ■ Vitronectin-N / ▲ iMatrix / ♦ Synthemax

UP TO 100% SINGLE-CELL PLATING EFFICIENCIES

To assess the efficiency of retrieving single hiPSCs in small volumes from GRID chambers, two independent hiPSC lines were tested. The isoPick was used to plate cells in PBS across all 256 chambers of a GRID (n=9), followed by the subsequent automated retrieval and transfer of single cells into 96-well plates. Visualisation and image-capture of single-cell GRID chambers using the isoHub both before and after picking with the isoPick, revealed reproducible high picking efficiencies of up to 100% (Fig. 4A). The scPicking Platform therefore delivers substantially better single-cell plating efficiencies than manual approaches and is comparable to FACS.

Figure 5 –
Single-cell derived hiPSC colonies in 96-well plates coated with various extracellular matrices. Dotted outlines represent the edge of colonies



CONCLUSION

The scPicking Platform enables users to **isolate single cells** from heterogenous populations in a **fast**, **efficient and automated** manner. Cells are automatically deposited into GRID chambers that are uniquely suited to **visualise and document** single-cell isolation. Users can then choose to pick selected single cells from GRIDs directly into 96-well plates for propagating into clonal colonies. The approach outlined here significantly out performs common alternatives for generating hiPSC clones regarding plating and cloning efficiencies, offering a streamlined and **market-leading** solution for an otherwise tedious workflow.

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