Automated and efficient isolation of single cells into low volumes for scWGA

Introduction

Tumors and tumor-derived cancer cell lines comprise a mix of diverse and heterogenous cells. It has long been established that genomic instability and altering selection pressures drive and maintain heterogeneity. To interrogate the complex genetic makeup underlying tumorigenesis, cancer progression and metastasis, it is therefore essential that researchers can analyze the genomes of individual cells, for example by next-generation sequencing or targeted PCR. A prerequisite for these analyses is whole-genome amplification (WGA), a process that can amplify genomic DNA, including the minute amounts within single cells. However, one of the main bottlenecks for efficient and successful WGA from individual cells, is the efficient isolation of single cells and their transfer into low-volume WGA reactions. Here, we demonstrate how GRIDs (Table 1, Fig. 1) and the scPicking Platform (Table 2, Fig. 2) support the isolation of individual tumor cells for subsequent single-cell WGA (scWGA).

GRIDs	
Time to make a GRID	2 - 3 minutes
Number of chambers	256
Volume per chamber	200 nl
Chamber surface area	3.2 mm^2
Optical edge effects?	No

Table 1 - Key features of GRIDs

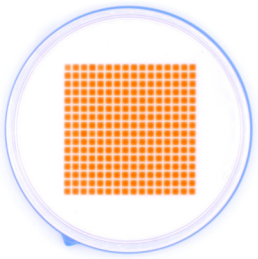

Figure 1 - A GRID containing dye (for visualisation only)

scPicking Platform

The scPicking Platform comprises a liquid handler (isoPick) and a complimentary microscopy suite (isoHub). The isoHub can optionally be equipped with our image documentation system to record individual cells as well as a fluorescence module to work with labelled cells/ fluorescent reporters if desired. The system offers complete automation of dispensing, selection and subsequent transfer of single cells into volumes of 1.5 µl to 200 µl in PCR tube strips or 96-well plates (Table 2).

Figure 2 - Components of the scPicking Platform

Materials & Methods

GRIDs (Fig. 1) were prepared using the isoPick and PL buffer (iotaSciences) according to established protocols (available through iotaSciences' Customer Portal). A single-cell suspension of K562 cells was prepared at a concentration of 7500 cells/ml in PBS, prior to plating single cells 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 transferred wirelessly to the isoPick and single K562 cells were automatically picked by the isoPick directly into PCR tubes prefilled with 3.5 µl of scWGA reagents (MALBAC, Yikon Genomics & PicoPlex, Takara Bio). As a negative control for scWGA, GRID chambers containing PBS only were also picked. For all scWGA reactions, protocols were followed according to the manufacturer's instructions and PCR was performed using a standardised protocol.

scPicking Platform 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 2 - Key features of the scPicking Platform

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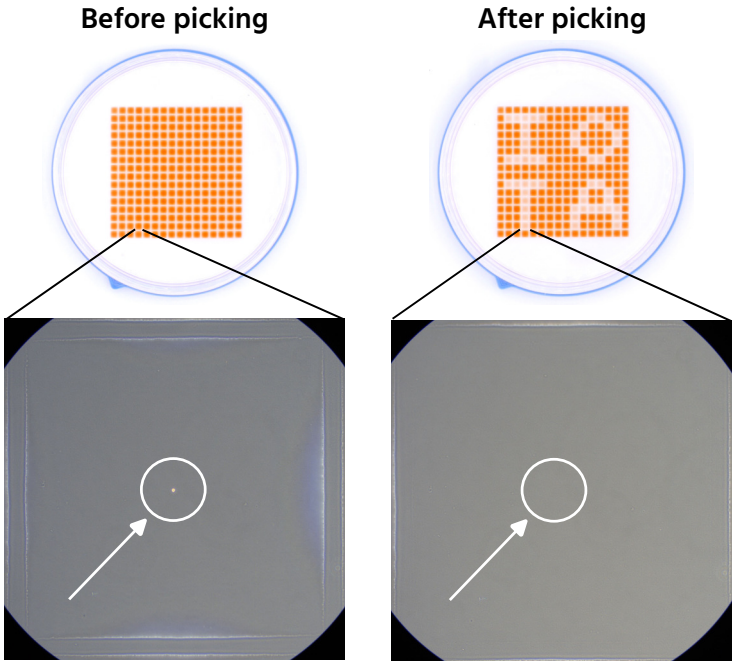

Figure 3 - GRID chambers before and after single cell picking

High picking efficiencies from nanolitre volumes

In order to assess the efficiency of retrieving single tumor cells from GRID chambers (Fig. 3), we utilized K562 cells, a well-known cell line for the study of Chronic Myelogenous Leukemia. The isoPick was used to plate cells in GRIDs (n=3), followed by the subsequent automated retrieval and transfer of single cells into 96-well plates prefilled with 100 µl of culture medium. Visualisation and image-capture of single-cell chambers using the isoHub before and after single-cell picking revealed reproducible high picking efficiencies of ~98%.

Seamless scWGA of isolated single cells

scWGA workflows utilize small reaction volumes (~3-5 µl). We next utilized the isoPick to retrieve and transfer single K562 cells from GRID chambers into 8-well PCR tube strips prefilled with a low volume of scWGA reagents (3.5 µl). GRID chambers containing a single cell (sc) as well as those containing only PBS were picked (-). Purified human gDNA was included as an amplification control (+ve). Agarose gel electrophoresis of the amplified products from both applied scWGA kits showed successful and reproducible DNA amplification from each single cell, with a similar product size distribution to the amplification control (Fig. 4). No amplification was observed in all negative controls (-).

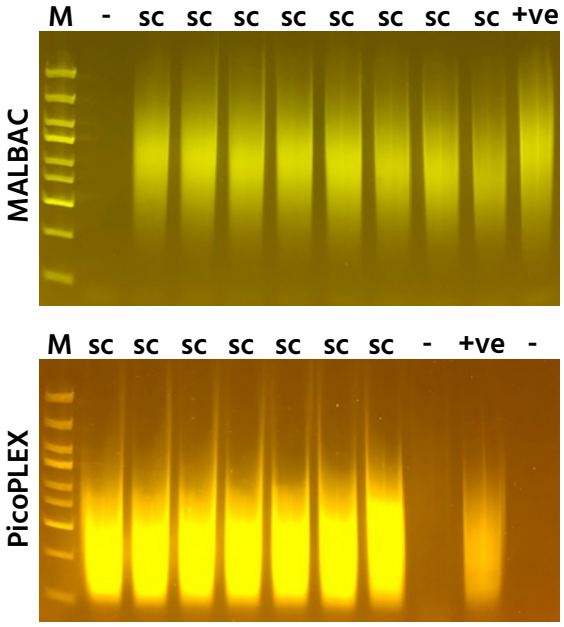

Figure 4 - scWGA from isolated single cells

Easy analysis of genes of interest

To further assess the quality of amplified DNA from isoPick-retrieved single cells, we chose to PCR amplify two genes commonly overexpressed in tumors - TP53 and GAPDH. Agarose gel electrophoresis demonstrated specific amplification of PCR products in all cases of single-cell amplified DNA (+), whereas negative controls using input from negative scWGA reactions (-) did not show any products, as expected (Fig. 5).

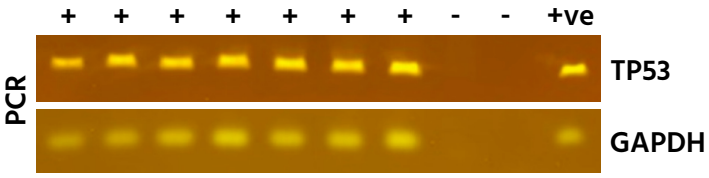

Figure 5 - PCR utilzing DNA from scWGA reactions

Conclusion

The scPicking Platform enables users to **isolate single cells** from heterogenous cell 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 choose to pick single cells from GRIDs directly into PCR-strips or 96-well plates using different volumes, including low-volumes utilized for scWGA workflows. This approach may offer an interesting solution for single-cell isolation from liquid biopsies and the investigation of circulating tumor cells (CTCs).