Application Note

DEPArray™ Rare Cell Isolation Technology

Maintaining Live Cell Viability and Proliferation after Single-Cell Sorting

Introduction

There is an increasing interest in several fields such as stem cell research, cell therapy and cell based diagnostics to isolate and recover live rare cells. Being able to isolate from a mixed primary population specific cell types free of unwanted cells, which might otherwise overgrow the culture, is required to develop viable clones and expand them sufficiently for subsequent studies.

Existing methods employed today such as FACS, micromanipulation, and serial dilutions all have significant shortcomings that impact their effectiveness and reproducibility, *e.g.* harsh treatment of cells during isolation, skilled technical abilities, and labor intensiveness,.

The DEPArray™ technology enables the selection and isolation of pure live cells from heterogeneous samples, in a very gentle and automated way eliminating error-prone manual intervention and streamlining the process.

Here we show that sorting by use of the DEPArray™ system ensures excellent viability and proliferative capabilities down to the single cell, using primary cells or immortalized cell lines as a model system.

Materials and Methods

- **Cell Line and Sample Preparation**

To demonstrate cell viability and cell proliferation after processing with the DEPArray™ system different cell types were used:

- hADSC: primary human adipose derived stem cells from lipoaspirate.
- MTP-GFP: is a mouse transgenic cell line stably expressing GFP (Tissue: mammary gland, Tumor: adenocarcinoma).
- A549: hypotriploid human cell line of lung carcinoma.
- SW480: established from a primary adenocarcinoma of the colon.
- DLD-1: is a colorectal adenocarcinoma cell line.

The cells were harvested from culture flasks with 70% of maximum confluence in order to be sure to have them still in proliferative activity. The cells were detached from the flask by brief exposure to trypsin/EDTA then washed in their respective complete culture media (DMEM, William's-E, F-12K, RPMI 1640).

- **DEPArray™ Analysis and Cell Proliferation**

For each separate cell line experiment the tumor cells were suspended in their culture media and sorted by the DEPArray™ system by morphological parameters such as shape. SW40 cells were also stained with anti-EpCAM-PE antibody for detection in the PE channel with the DEPArray™ system.

To assess viability, groups of cells were recovered in a 96-well plate, adding Trypan blue and counted as dead (blue) *vs.* live (unstained) cells using an inverted microscope.

For proliferation tests, taking advantage of the multi-recovery capability of the DEPArray™ system, multiple aliquots of single cells and 5-cell batches were delivered automatically by the DEPArray™ system into a µ–Dish (Ibidi GmbH) that has a grid structure imprinted on the upper surface (in focus with the cells). Immediately after the cell recovery, the µ–Dishes were put into an incubator over night to permit adhesion. Recovered cells were periodically checked with an inverted microscope to evaluate condition and proliferation **(Fig.1)**.

Results

Typical viability of the sample to be processed, assessed with Trypan blue, was on average around 99%. Across 9 independent experiments using different cell types (test not performed on hADSC), viability on groups of recovered cells was 100% (scoring all of the 246 visible cells) **(Fig.2)**. Cloning success rate on 101 single-cells across 16 experiments ranged between 56% and 87% depending on cell type **(Fig.2)**. All recoveries of 5-cell batches show proliferation success rate of 100%, except for DLD-1 cell line (89%).

Conclusions

Cells recovered by use of the DEPArray™ system maintain viability, functional integrity and the ability to proliferate and generate clonal cultures. The clonal culture can be used for subsequent or alternative assays. Sorting for specific cells with DEPArray™ technology can be reliably used to overcome limitations of existing cell isolation techniques, making it possible to gently isolate in an automated way and proliferate rare and/or delicate cells such as stem cells, Circulating Tumor Cells, Circulating cancer Stem Cells or Circulating Endothelial Cells.

References

G Medoro et al, *RNA Analysis of pure tumor cells sorted by DEPArray™ platform from enriched blood samples,* ADAPT conference, Sep 2011

Keywords: DEPArray™; Single-cell; Cell proliferation; Cloning.

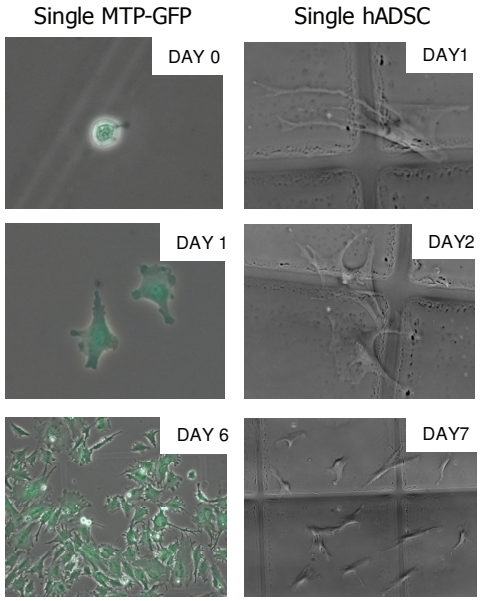

Fig.1 Single cells proliferation after DEPArray™ sorting.

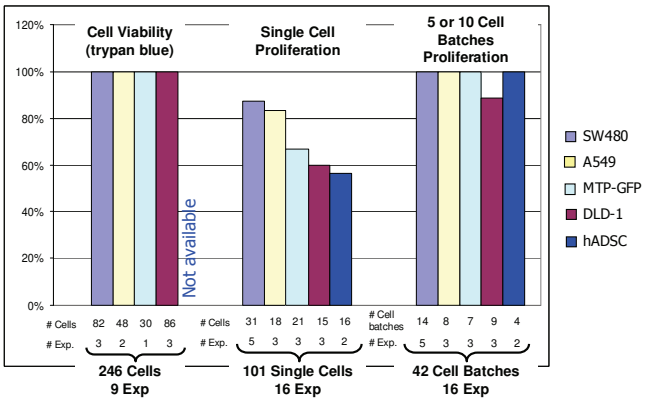

Fig.2 Results of viability and proliferation tests with different cell types

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