

# Collection and clonal expansion of individual cells from adherent cultures with Kuiqpick™

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## Introduction

Cell culture is a powerful tool to study cellular functions *in vitro*, providing valuable insights into their functions *in vivo*. However, a sound interpretation of results pertaining to single cells is usually impossible from cell population analyses. The heterogeneous populations result in “averaging out” of results, masking specific changes in specific cells. Therefore, single cell analysis reduces the biological noise from the heterogeneous background, providing fundamental improvements for elucidating cellular diversity and heterogeneity. In recent years, single cell analysis has appeared as a novel frontier in life and biomedical sciences, particularly for various omics studies<sup>1-4</sup> and stem cell biology<sup>5-7</sup> where understanding the functional properties of pluripotent or committed progenitors and differentiated cells within normally heterogeneous population requires the isolation of single cells for subculture and clonal expansion.

The available technologies for isolating single cells mainly consist of dilution-to-extinction, micromanipulation, laser assisted microdissection, fluorescence-activated cell sorting (FACS), microfluidics, compartmentalization of single cells, and magnetic-activated cell sorting<sup>8</sup>. However, most of these methods are unable to precisely collect single cells *in situ* from cell cultures, but rather only stochastically collect single cells or are laborious and time-consuming. The most common technology used for the collection of single cells or cell clusters from *in vitro* cultures is laser based microdissection (i.e., Arcturus, Carl Zeiss)<sup>9-11</sup>. However, these sophisticated systems are expensive and the process is time-consuming. Moreover, the cells have to be subcultured from regular culture dishes to specially designed dishes prior to collection. Under most conditions, the collection of individual cells from cultures using either system is not trivial. Therefore, there is a great demand for rapid, accurate and cost-effective technology permitting the collection of individual cells from cultures.

Kuiqpick™ from NeuroInDx is based on a capillary-based vacuum-assisted cell and tissue acquisition system (CTAS) that can accurately and rapidly microdissect subanatomical regions from fresh frozen, sucrose treated, and native live brain tissues at extremely low cost<sup>12</sup>. Under direct microscopic visualization, the tissue area of interest is positioned directly below the tip of a disposable capillary unit (DCU), and a vacuum impulse is applied to collect the desired tissue areas or individual cells into the DCU barrel. The acquired sample is transferred into a container for downstream analysis or culturing. High quality DNA, RNA, and protein extracted from Kuiqpick™ dissected samples are suitable for various downstream molecular biology studies. The accuracy is controlled by the inner diameter of DCU, and vacuum strength (V) and impulse duration (T<sub>v</sub>).

Kuiqpick™ sample acquisition does not involve chemical treatment, laser irradiation, or excessive heat, thus preserving the primary functions and activities of the dissected cells and tissues. Its advantage over existing microdissection instruments or technologies is its ability to dissect native live brain tissues with minimal effect on cellular viability, which allows their further use for primary cultures. With Kuiqpick™, the subventricular zones (SVZ) and subgranular layers (SGL) were dissected from native live embryonic and adult rat brains, and primary neurosphere cultures were successfully established using the collected tissues, indicating the minimal effect on cellular viability of Kuiqpick™ based microdissection process<sup>12</sup>.

This application note aimed at demonstrating the performance of Kuiqpick™ on adherent cell cultures determined that this is a suitable method for individual cell collection and subsequent clonal expansion. Kuiqpick™ also accurately and rapidly acquired both fluorescently labeled and non-labeled individual cells directly from standard culture dishes, and more importantly, the collected cells were suitable for subsequent recultivation.

## Materials and Methods

**Cell cultures:** Human neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells and human melanoma MDA-MB-435 cells (ATCC, Manassas, VA) were routinely cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (Life Technologies, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal calf serum containing 2 mM glutamine and 1% antibiotics (penicillin–streptomycin). Cells were maintained at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. To co-culture CHO and MDA-MB-435 cells, equal number of cells from the two cultures were mixed and seeded in a regular 6-cell culture plate (1x10<sup>5</sup> cells per well total).

**Cell collection with Kuiqpick™ from cultures:** Kuiqpick™ was used according to the manual for collecting live cells. DCU was carefully removed, and using a syringe, the collected CHO and SH-SY5Y cells were transferred onto a 12-well culture plate containing complete DMEM/F-12 culture medium. Cells were maintained as described below. Photographs were taken daily to record the growth and proliferation of cells.

**Collection of fluorescently labeled live cells:** CellTracker fluorescent probes (Life Technologies) were used to label live cells according to the manufacturer's protocol. Briefly, the cells were seeded on a regular 6-well culture plate. When the cells reached 50-60% confluence, the culture medium was removed and the serum-free culture medium containing 25 µM of CellTracker Green CMFDA or Blue CMAC was added, followed by incubation at 37°C for 45 minutes. The medium with probe were replaced with pre-warmed complete culture medium for 30 minutes incubation, and then the culture medium was replaced with fresh complete medium. To co-culture differently labeled cells, CHO and MDA-MB-435 cells were label with CellTracker Green CMFDA and Blue CMAC, respectively. Same number of green CHO cells and blue MDA-MB-435 cells were co-cultured on a 6-well plate as described above.

**Primary neural progenitor culture:** Live embryonic rat brain tissues (BrainBits, LLC, IL) were used for primary culture of neural progenitors. The brain tissues were coronally sectioned using Leica VT1200 vibratome generating 300 to 500 µm thick sections. The ventricular zones and subgranular layers were microdissected with Kuiqpick™. Previously established protocols for floating neural progenitor cultures (NS, neurospheres) from microdissected tissue were used<sup>12; 13</sup>. Briefly, microdissected rat tissue was dissociated in 0.05% trypsin and 1 mM EDTA followed by titration. After 15 minutes of incubation, it was triturated then incubated for another 10 minutes, then washed twice with Hank's Balanced Salt Solution (HBSS). Neural progenitor cells were plated in neurobasal media (Life Technologies) supplemented with 1% penicillin/streptomycin (Gibco), 2 µM L-Glutamine, 2 µg/ml heparin (Sigma), 2% B27 (Life Technologies),

epidermal growth factor (EGF, 10 ng/ml; Life Technologies) and fibroblast growth factor (bFGF, 20 ng/ml; Life Technologies). Primary neural progenitor cultures prior to formation of neurosphere colonies were used in the experiments.

## Results

### Collection of individual cells from cell culture dishes:

Acquisition of single cells was performed with Kuiqpick™ using DCU with internal diameters 20 to 50 µm. Chinese hamster ovary cells (CHO; **Figure 1A-B**), MDA-MB-435 human melanoma (**Figure 1C-D**) cell cultures, primary rat neuroprogenitor cultures (**Figure 1E-F**) and SH-SY5Y human neuroblastoma cells (**Figure 1G-H**) were used. Individual cells were successfully collected from regular 6-well culture plates. Depending on the confluence of the cells, on average 10 to 20 cells were collected per minute.

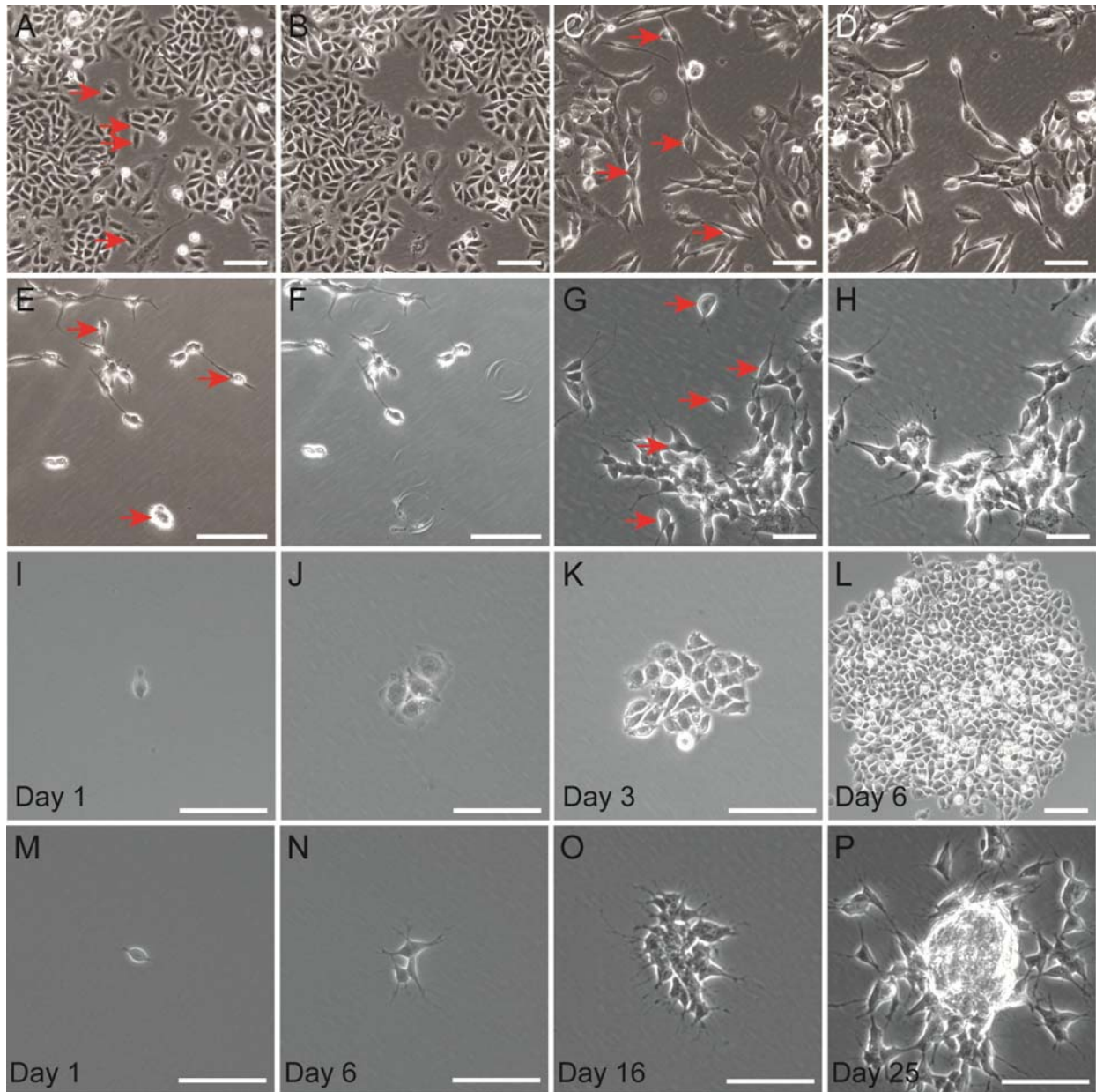
**Clonal expansion of individual cells:** Single CHO and SH-SY5Y cells were collected with Kuiqpick™ and placed into a 12-well culture plate containing complete culture medium. Clonal expansion of a single CHO (**Figure 1I-L**) and SH-SY5Y (**Figure 1M-P**) cells within 6 days and 25 days post-collection, respectively indicated that Kuiqpick™ does not affect cell viability. Clonal expansion of individual SH-SY5Y cells (n=100) gave rise to 81±4.1% colonies.

Immediately after collection with Kuiqpick™, cells were stained with 0.4% Trypan blue and the ratio of stained (dead) versus unstained (alive) cells were calculated in each case to provide estimates of survival rates immediately after the procedure. On average and depending on the cell type, 80% (SH-SY5Y) to 95% (CHO) of collected cells were alive, which was in agreement with the clonal experiments. The lowest survival rates were shown for SH-SY5Y cells and the highest were found for non neuronal cell types (e.g. CHO). V from 4.4Hg to 9.0Hg and Tv= 0.2 seconds were used. The optimal DCU ID may range from 15 to 50 µm depending on the cell type. For cells which adhere tightly to culture surface, controlled dissociation with low concentration of trypsin can be applied to facilitate lifting (not shown).

### Collection of morphologically distinct and/or fluorescently labeled cells:

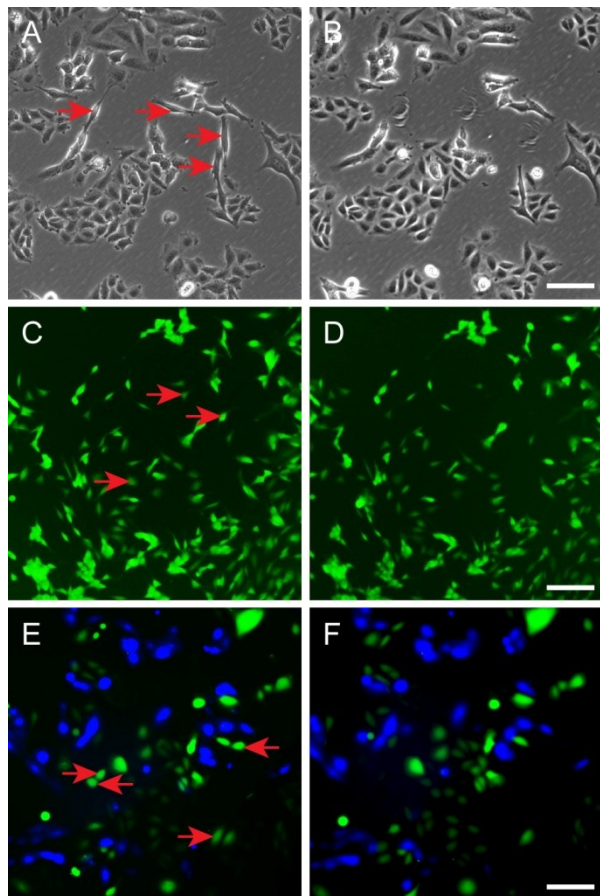
Kuiqpick™ provided a simple solution for *in situ* collection of a specific cell or cell clusters from co-cultures of various cell types with different morphologies. Individual MDA-MB-435 cells from a mixed culture of morphologically distinct CHO (gravel shape) and MDA-MB-435 (spindle-like) cells were collected (**Figure 2A and B**).

For the collection of fluorescently labeled cells, Kuiqpick™ calibration was performed under bright field<sup>12</sup>, and then individual fluorescently labeled cells were collected under dark field. Kuiqpick™ successfully collected SH-SY5Y cells stained with CellTracker Green CMFDA, a fluorescent probe that is retained in living cells through several



**FIG 1:** Representative collection of individual cells from culture dishes shows Chinese hamster ovary (CHO) cells before (A) and after (B) collection; MDA-MB-435 human melanoma cells before (C) and after (D) collection; primary rat neuroprogenitor cultures before (E) and after (F) collection; SH-SY5Y human neuroblastoma cells before (G) and after collection (H). I-L: Recultivation of a single CHO cell. M-P: Recultivation of a single SH-SY5Y cell. Parameters for the collection of CHO and MDA-MB-435 cells, DCU ID: 30 $\mu$ m, V: 8.7 Hg $^{\circ}$ , vacuum duration: 200ms; for rat primary neural progenitor cultures and for SH-SY5Y cells, DCU ID: 15 $\mu$ m, V: 6.5-13.2 Hg $^{\circ}$ , duration: 200ms. Collected cells are shown with red arrows. The scale bar is 100  $\mu$ m.

generations (**Figure 2C and D**). In addition, we co-cultured CHO and MDA-MB-435 cells labeled with CellTracker Green CMFDA and Blue CMAC, respectively, and individual green CHO cells were collected with Kuiqpick™ (**Figure 2E and F**).



**FIG 2:** Collection of individual cells from heterogeneous cultures. **A-B:** Collection of individual MDA-MB-435 cells from co-culture of MDA-MB-435 and CHO cells; **C-D:** Collection of individual fluorescent CellTracker Green CMFDA labeled SH-SY5Y cells. **E-F:** Collection of fluorescent CellTracker Green CMFDA labeled CHO cells from co-cultures of CHO cells and Blue CMAC labeled human melanoma cells MB-435. **A, C, E** – before collection, **B, D, F** – after collection. Parameters for cell collection are as identified in Fig. 1 legend. Collected cells are shown with red arrows. The scale bar is 100  $\mu$ m.

## Conclusion

Kuiqpick™ has proved to be a reliable and efficient option for precise and rapid collection of individual live cells and clonal expansion from cell cultures based on their morphology or fluorescent label. Unlike FACS that is primarily designed to acquire fluorescently labeled subpopulations from a large number of cells, Kuiqpick™ provided a simple means of accurate and rapid collection of individual fluorescently labeled live cells *in situ* from adherent cultures. The collected cells are viable and suitable for recultivation. Moreover, collected cells may be used for a variety of downstream analyses including gene expression and protein analysis.

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