Application Note

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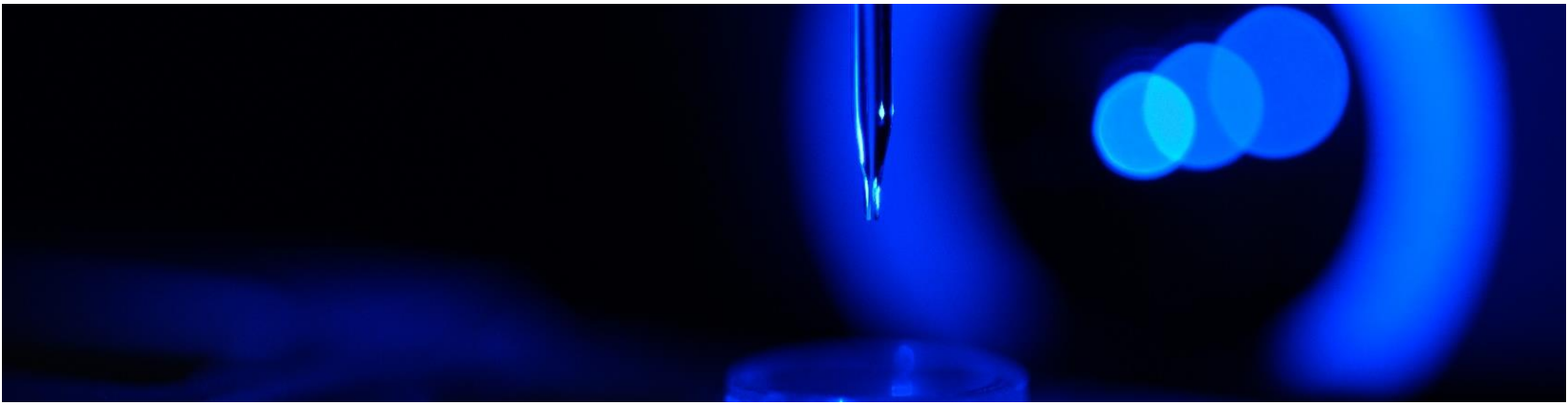

Automated single cell isolation from multiple samples

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Abstract

Most single cell dispensing technologies require user intervention to exchange sample vials or dispense cartridges between different cell samples or even when processing large volumes of the same sample is required. This manual process takes time and can introduce both contamination and human error into single cell sorting operations. The cellenONE utilizes an aspirate/dispense liquid handling technologies enabling automated sample exchange and just-in-time resuspension eliminating the need for user intervention. The Multisample Run is a method developed on the cellenONE for the isolation of single cells from multiple samples in a single automated run. Such a feature is particularly useful for cell line development when cells from different culture conditions or those with different genomic edits need to be cloned in a single day. This application note describes how the Multisample Run method is implemented in the cellenONE. The automated isolation of single cells from 8 different cell lines is demonstrated.

Introduction

This application note demonstrates how a Multisample Run enables multiple cell samples to be automatically isolated to many different wells with limited user intervention. When using this feature, the following steps are undertaken:

1. the operator loads the cellenONE with a “Source Plate” containing the different cell samples and a plate prefilled with culture media (“Target plate”) before performing the run.
2. the position of the source samples and the assigned target wells for each sample are user-defined in the software.
3. the Multisample Run is started: cells from sample 1 are loaded into the Piezo Dispensing Capillary (PDC) and dispensed into the predefined target wells.
4. Once the first sample is processed, the PDC is automatically washed, sample 2 is resuspended by the cellenONE, then aspirated and single cells are sorted into the defined wells of the target plate.
5. This process is repeated automatically until all samples are processed.

This method can be applied to any number of samples, any number of target wells, and most SBS format microwell plates and other open well devices. It can be used in combination with automated resuspension of the samples and inclusion of positive/negative control wells. Two experiments are presented for demonstration herein.

cellenONE® F1.4

The cellenONE® F1.4 (Cellenion, France) a piezo acoustic image-based cell isolation and nanoliter dispensing device, was used to generate clonal populations from multiple cell samples in a single run without manual intervention. One of the challenges presented by such an application comes from cell sedimentation and aggregation over time. A gentle and effective resuspension task named “Mix&Take” is used to ensure that aspirated samples consist of a homogeneous cell suspension. During this process, an aliquot of the sample is aspirated into the PDC and gently flushed back into the well three times to resuspend the cells (Figure 1).

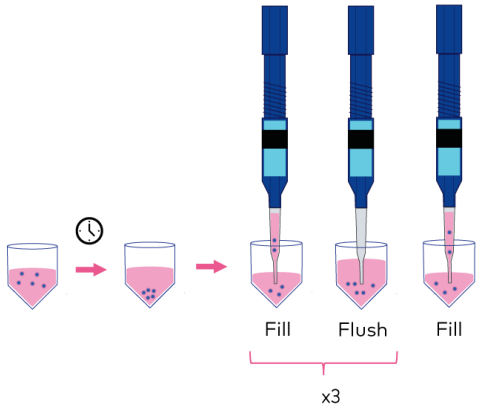

Figure 1. Mix&Take task

The Mix&Take task can be modified to work with a range of different well or vessels and also to facilitates disaggregation of clumps of cells.

Cloning 8 different cell lines in a single run

In the first experiment, 8 different cell samples loaded into a 384 well-plate were processed (1: CHO cells, 2: HEK293T cells, 3: HeLa cells, 4: A549 cells, 5: HepaRG cells, 6: Jurkat cells, 7: SKBR3 cells, 8: iPSCs cells, Figure 2). From each cell sample, a single cell was isolated into each of 48 wells in a 384 well cell culture plate. The same detection and isolation parameters were used for the 8 different cell samples.

The run was performed using the following layout:

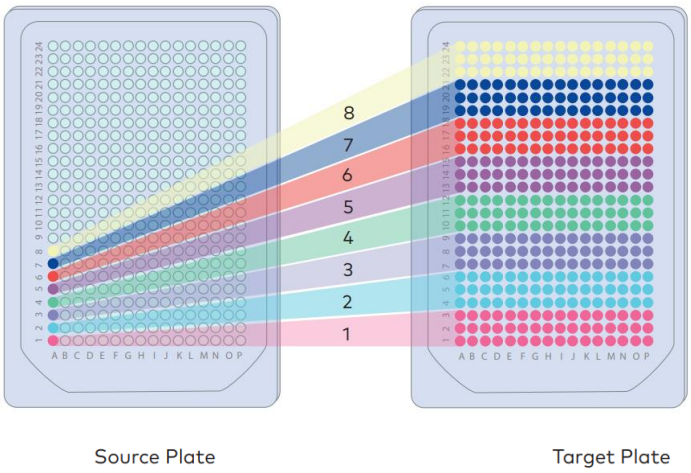

Figure 2: Source plate loaded with eight different samples and single cell dispensing in a 384 well plate1: CHO cells, 2: HEK293T cells, 3: HeLa cells, 4: A549 cells, 5: HepaRG cells, 6: Jurkat cells, 7: SKBR3 cells, 8: iPSCs cells

Since 8 different cell samples were used, 8 Mix&Take tasks and 8 single cell isolation loops were performed. This run could also be modified to accommodate as many cell samples as one would like to process. Within the run, the dispensing stability was also checked before and after each cell sample was processed.

Multisample Runs are highly flexible since one can select the target layout and the number of cell samples processed. Such an approach could also be used to dispense multiple single cells from different samples into the same target well for co-culture studies.

Experiments for outgrowth matching

In the second example, 4 cell lines (1: HeLa cells, 2: HEK293T cells, 3: CHO cells, 4: A549 cells) with different clonal outgrowth were used. In order to account for those outgrowth differences and obtain similar numbers of monoclonal colonies post cloning and culture, different number of wells in the target plate were defined for each cell lines as shown on Figure 3.
This experiment was designed with positive controls whereby specific wells contain a defined number of cells. Such a feature can be used to confirm the outgrowth rate of each sample and is particularly useful when using plate imagers that require an autofocus step for monoclonality verification. In this example, wells A1, A5, A8, A10 contained exactly 10 cells.

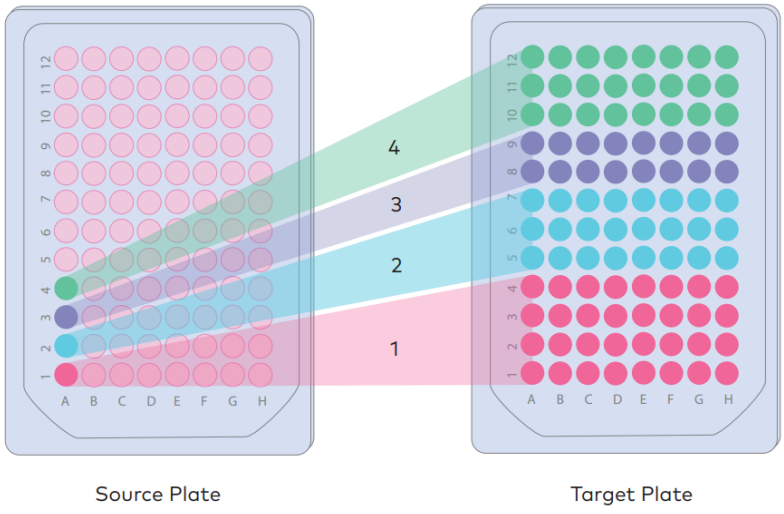

Figure 3: Source plate loaded with four different samples and single cell dispensing in a 96 well plate
1: HeLa cells, 2: HEK293T cells, 3: CHO cells, 4: A549 cells

Plates were imaged using an automated microscope at Day 0, Day 1, Day 4 and Day 7. Figure 4 below shows images recorded for a single well containing a single cell at day 1 and the resulting colony at day 7.

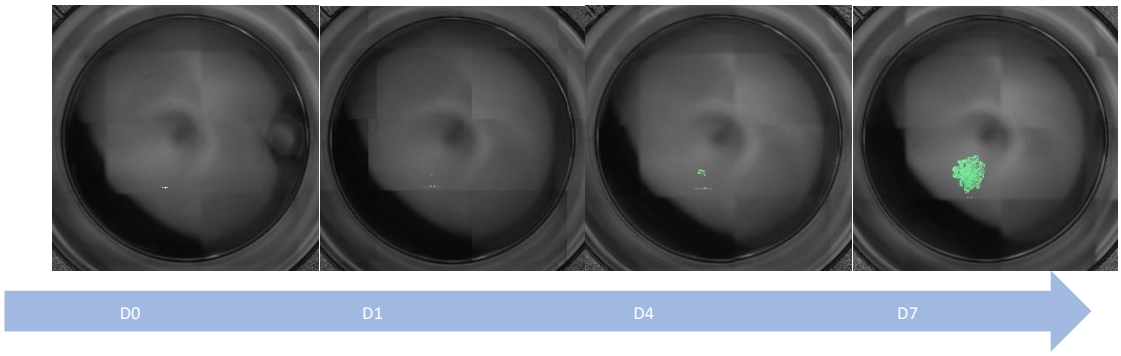

Figure 4: Clonality outgrowth

To maintain optimal viability and maximize clonal outgrowth during a Multisample Run, both the source and target plates of the cellenONE® are cooled down to 4°C.

Conclusion and future direction

The Multisample Run presented in this application note, is an automated and flexible run allowing processing of multiple cell samples. With its automated Mix&Take task designed to gently resuspend cell samples, and its automated washing protocols, the cellenONE allows complex experiments to be undertaken in a true walk-away fashion.

To further enable automation and increase throughput of cloning experiments, we have developed the cellenONE HT that allows automated loading and unloading of microwell plates using a plate shuttle. Combined with our proprietary API, this system can now be interfaced with a wide range of other lab instrumentation for completely autonomous high throughput cloning and cell line development applications (Figure 5).

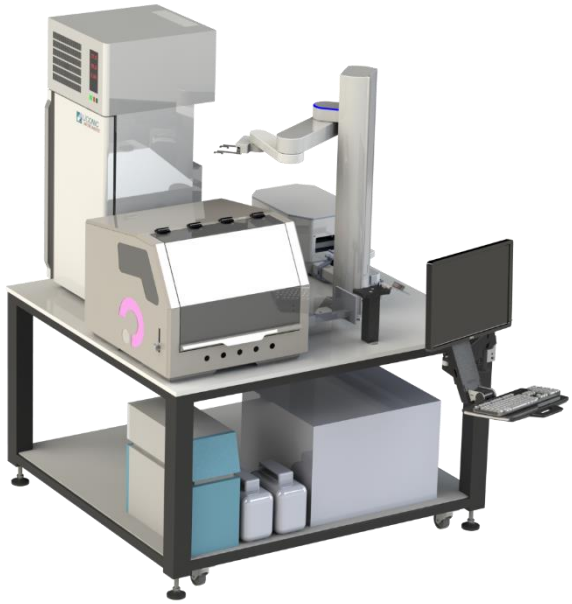

Figure 5 Representation of the cellenONE HT with associated automated devices

(e.g. robotic arm, automated incubator)

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