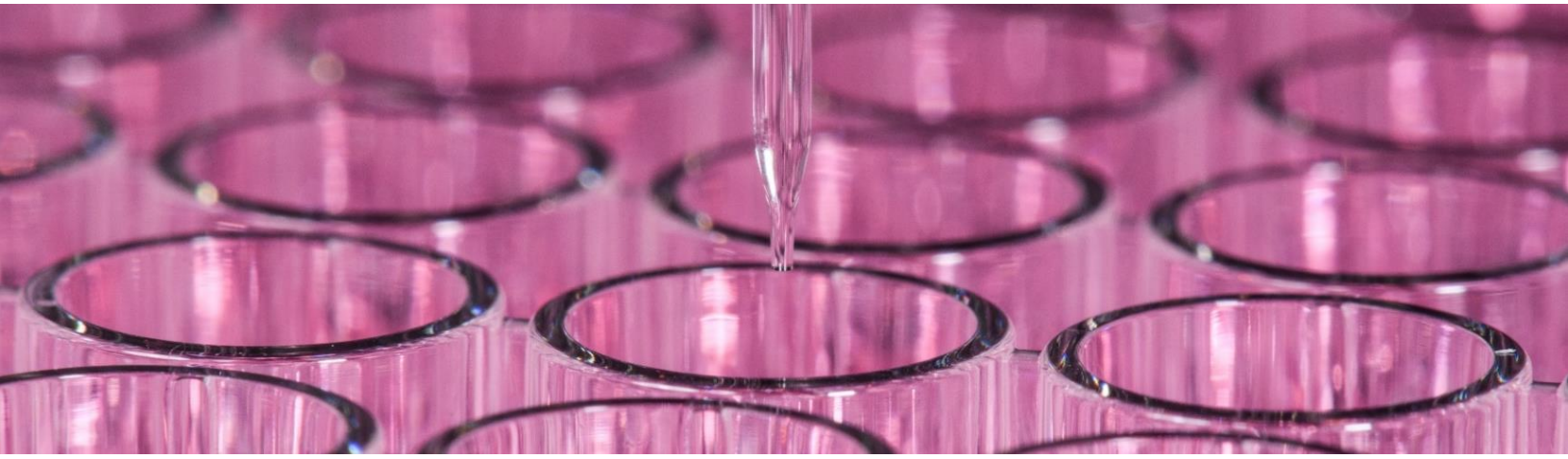
Application Note



Automated process for mother cell cloning and subsequent daughter cells isolation with the cellenONE® F1.4

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Abstract

Analyzing gene expression variability over multiple cell generations is a challenge as it requires to study daughter cells (resulting of a mother cell mitosis) at a single cell level. We have developed a two steps method to combine (1) automated and highly efficient single mother cell cloning from a cell suspension and (2) after cell division, automated dissociation, and isolation of resulting daughter cells. Both steps are achieved using one single instrument, the cellenONE® F1.4, thus improving, facilitating, and speeding up the process currently used for such experiments. We used this pipeline to successfully clone 384 chicken erythroid progenitor and then isolate 10 resulting single daughter cells in a convenient substrate for subsequent analysis.

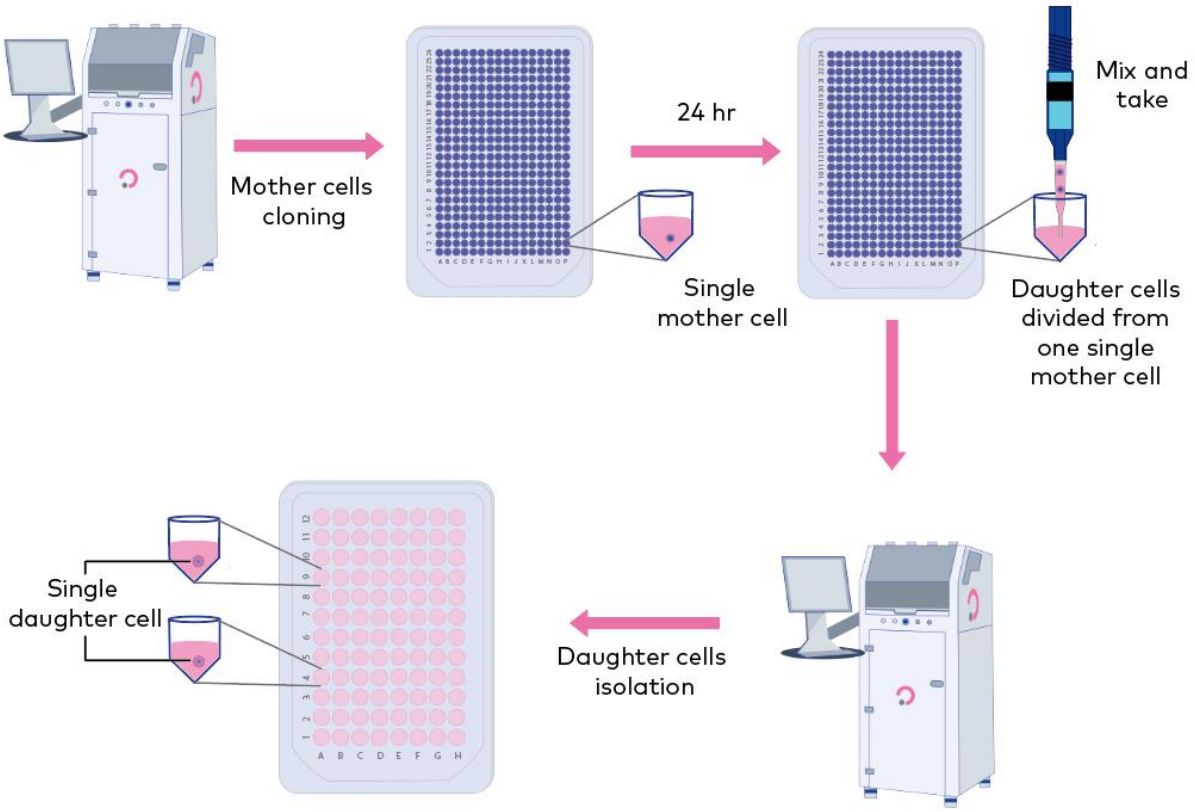

Figure 1. The two-step process for cloning mother cells, then recover and isolate resulting daughter cells

Introduction

Single cell studies have identified significant heterogeneity amongst isogenic cell populations, which has demonstrated that cells from the same lineage are not molecularly identical. This heterogeneity becomes clearly apparent at the transcriptomic level and can be characterized by gene expression variability (Richard, 2016). However, despite this mechanism being highly conserved, establishment of gene expression variability across multiple cell generations is poorly understood ((Phillips, 2019) (Kimmerling, 2016) (Shaffer, 2020)). Furthermore, it has been described that the expression of some variable genes correlates between daughter or cousin cells, irrespective of cell cycle stage, implying a gene-specific transcriptional memory over several cell generations. To investigate the extent of gene expression memory conservation between daughter cells over a single generation, we aimed to analyze the whole transcriptome using single cell RNA single sequencing (sc-RNA-Seq). Critically, daughter cells need to be isolated whilst preserving information about their relationship during the whole process. This required isolating and growing mother cells from a single population to obtain daughter cells, then retrieving each one of the two daughter cells in a minimal volume of lysis buffer, whilst avoiding pooling clones to preserve lineage information. The cellenONE® F1.4 is a multichannel fluorescence image-based single cell sorter that enables both ultra gentle automated cloning of mother cells and accurate isolation of each daughter cell for subsequent single cell transcriptomics analysis.

Materials and methods

T2EC primary cells

T2EC primary cells (chicken erythroid progenitors (Gandrillon, 1999)) were extracted from bone marrow of 19 days old SPAFAS White Leghorn chicken embryos (INRA, Tours, France). Cells were maintained in LM1 medium composed of α Minimal Essential Medium (Gibco) complemented with 10% Fetal Bovine Serum, 1% penicillin and streptomycin 10000 U/ml (Gibco), 1 mM Hepes (Sigma), 0.1 mM Beta-mercaptoethanol (Sigma), 10-3 mM Dexamethasone (Sigma), 1 ng/ml TGFβ and 5 ng/ml TGFα (Peprotech) and grown at 37 °C with 5% CO2 in an incubator. 2.105 cells were resuspended in 1 mL of media with 10% degassed PBS and conserved on ice until isolation.
T2EC primary cells are a mono-lineage differentiation system. Thus, the variability is related to gene expression and not multi-lineage choice.

cellenONE® F1.4

The cellenONE® F1.4 (Cellenion, France), a piezoacoustic image-based cell isolation and nanoliter dispensing device, was used for isolating single T2EC cells into 384 microwell plates (sciSOURCEPLATE 384 PS, SCIENION) using the following parameters: cell diameter 10-20 µm, maximum circularity 1.56; maximum elongation 1.80. The total volume used for isolation was 10µL.
T2EC primary cells were isolated at Day 0 (D0) into individual wells of a 384 well plate pre-filled with 10 µL of media. After 24 hours (24h), 10µL were aspirated from each well into the Piezo Dispensing Capillary (PDC) using a dedicated “Mix and Take” task. The cellenONE® F1.4 enabled automated aspiration, mixing and dissociation of 2 daughter cells followed by their isolation into a 96-well PCR plate (one daughter cell per well). Finally, 96-well PCR plates containing single daughter cell were frozen until required for library preparation steps prior to sequencing analyses.

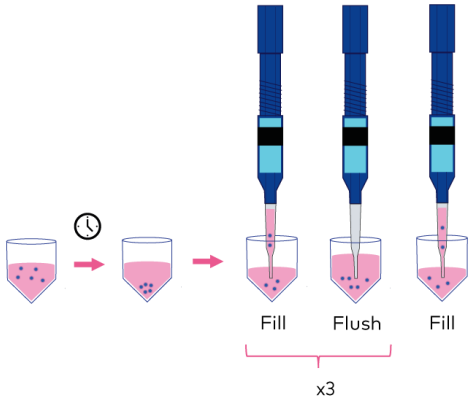

Figure 2. Mix&Take task using the cellenONE®.Daughter cells are automatically aspirated, mixed and dissociated by repetitive filling and flushing of the PDC.

Results and discussion

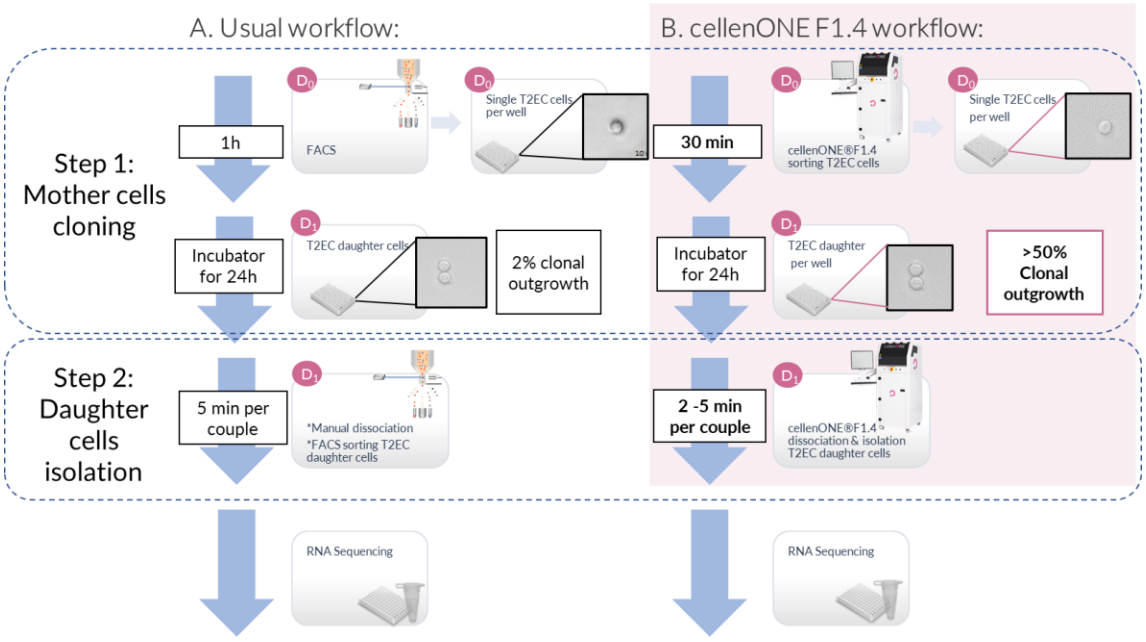

Figure 3. cellenONE® and FACS workflow comparison for daughter cell isolation. A. Limited dilution with pipette and FACS sorting workflow for cloning and omics preparations. B. Fully automated workflow with the cellenONE® for cloning and omics preparations.

<u>The cellenONE and FACS Aria II process Mother cells about the same speed, with a small higher speed for the cellenONE</u>

With the FACS Aria II (BD), T2EC primary cells stained with CFSE were isolated using the gating with the green channel parameters and allowed isolation of viable single cells positive for CFSE staining. Cells were isolated in 96 well plates in which one plate was done in 5 minutes.

With the cellenONE, the same sample was automatically isolated on a 384 Well Plate in 14 minutes using only the Transmission Channel at Room Temperature.

The cellenONE took 3.5 min to isolate 96 cells, whereas the FACS Aria II took 5 min.

<u>The cellenONE increase 25 times the Clonal outgrowth of mother cells</u>

After 24 hours of clonal outgrowth, the FACS 96 well plate was analysed in the microscope. Between 2 and 10 wells contained one viable cell, representing 2%-10% of clonal outgrowth.

After 24 hours of clonal outgrowth, the cellenONE 384 well plate was analysed in the microscope and 200 wells out of 384 wells contained two viable cells, representing 52% of clonal outgrowth.

<u>The cellenONE enabled fully automated resuspension, dissociation, and isolation of daughter cells</u>

For FACS samples, daughter cells were identified using classical microscopy. All the media of the well containing the two daughter cells was gently mixed using the pipet to allow cell dissociation and transferred into a 5ml polypropylene FACS tube with a final volume of 90ul of culture medium (FACS 50µL + 40 µL and cellenONE 10µL + 80µL). Once manually loaded in the tube, every couple took 5 minutes to isolate.

The cellenONE enables a completely automated workflow. The system is programmed to resuspend both daughter cells placed on a well from the 384 well plate with the mix and take task and then aspirates the cells into the glass capillary to isolate them by using the microscope recognition. The cellenONE aspirates 10 µL directly from every well containing two daughter cells of a 384 well plate. Each couple takes between 2 and 5 minutes to isolate.

The cellenONE speed up and improve isolation of T2EC daughter cells

After optimisation for FACS samples, each couple of daughter cells was placed in a different FACS tube and was treated independently. Each tube was loaded into the FACS sorter, 40% of the time we were able to detect the two daughter cells, and 25% of the time were able to isolate the two daughter cells.

Without optimisation with the cellenONE, we were able to detect the two sister cells 45% of the time and were able to isolate the two sister cells 45% of the time.

Conclusion and future direction

In this study, T2ECs were successfully cloned and then automatically dissociated and isolated for subsequent single-cell transcriptomic analyses. This protocol relied solely on the imaged-based single-cell isolation platform cellenONE®. The piezo-acoustic technology allowed gentle isolation of cells, as demonstrated by a drastic improvement of clonal outgrowth (52% for cellenONE compared to 2% for FACS and 20-25% for Smart Aliquotor). After 24h of culture, individual daughter cells were successfully resuspended, dissociated and isolated using a newly developed “mix and take” task prior to single-cell transcriptomic analyses, which will allow transcriptional memory studies over one cell generation.

References

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