cellenONE® as automated single cell seeding platform for hiPSC subcloning

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Introduction

Many applications using human induced pluripotent stem cells (hiPSC) depend on reliable single cell isolation methods. The widely used classical methods, i.e. limiting dilution, do not guarantee clonality. Therefore, an automated and gentle single cell seeding, combined with optimal hiPSC culture conditions, is critical for reducing time and labor while improving accuracy and reliability of hiPSC pipelines.

cellenONE® F1.4 offers a portfolio of functionalities for hiPSC workflows:
- automated cell seeding
- high survival rate of plated cells
- fluid control imaging system
- immediate clonality assessment
- cell size and morphology measurement
- fluorescence-based cell sorting

Here, we report preliminary data for hiPSC isolation, single cell seeding and clone outgrowth efficiency

1. Post-isolation parameters

Single cell isolation was performed using cellenONE® F1.4 equipped with fluorescence module.

Post-isolation analysis reports show (Fig.1A):
- uniform distribution of cell diameter (22-27 µm)
- a very small variability in circularity (~1) and elongation (~1.4)
- none PI positive isolated cells, showing an efficient exclusion of dead cells

Single cell isolation efficiency using cellenONE® F1.4 is around 94% to 100% (Fig.1B).

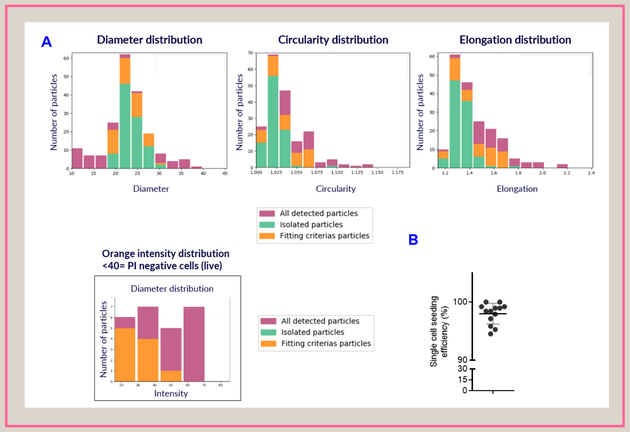

Figure 1. Single hiPSC isolation using cellenONE® F1.4
A) Representative histograms automatically generated after cellenONE® F1.4 run using the cellenONE® F1.4 report application. An example of main isolation parameters is depicted: hiPSC diameter, circularity, elongation and orange intensity for propidium iodide (PI) as cell death excluding parameter. B) Dot plot showing single cell seeding efficiency after scoring single event isolation using cellenONE® F1.4 automated acquisition during the run (n=11, mean ± SD).

2. Surface coating and anti-apoptotic molecules

To further improve cell survival and clone growth, various surface coating and anti-apoptotic molecules were tested.

The results show that:
- Geltrex and Laminin performed similarly with ~30% of wells having clonal outgrowths (Fig. 2A).
- CloneR performed the best with 42% clonal outgrowths (Fig. 2B).

Based on these findings, a combination of Laminin 521 and CloneR with StemFlex media were selected. We found that clonal expansion was more successful in 384-well plate (~43%), suggesting that smaller surfaces and lower media volumes may favor hiPSC clone growth (Fig. 2C).

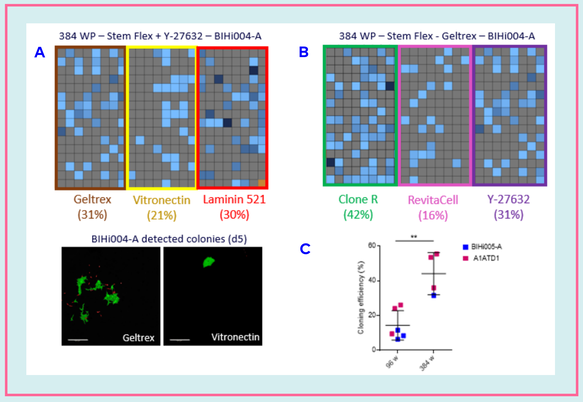

Figure 2. Cloning efficiency is affected by hiPSC culture conditions after single cell seeding with cellenONE® F1.4
A) Heatmap depicting number of wells with hiPSC clonal outgrowth (expressed below as percentage (%)= n°wells with single cell at day 0 / n° wells with positive outgrowth at day 5); dark blue indicates higher number of colonies per well, all derived from 1 single cell. The heatmap shows performance comparison between different plate coatings (left panel). Representative pictures showing in green segmented elements considered hPSC colonies for unbiased automated quantification at day 5. B) Heatmap constructed as described in panel A to compare performance of different anti-apoptotic molecules. C) Graph showing differential hiPSC outgrowth efficiency according to size well for 2 different hPSC lines. Each dot represents a technical replicate. Values are expressed as mean ± SD. Statistic analysis: non-parametric Mann-Whitney test. Scale bars= 500µm

3. Workflow across different hiPSC cell lines

Using the above culture conditions, a workflow to perform single cell seeding with cellenONE® F1.4 was defined (Fig. 3A and B). We found that there are major differences in cloning capacities of different hiPSC lines (Fig. 3C).

This variability between hiPSC highlights the importance of optimizing culture conditions and minimizing cellular stress during the single cell seeding procedure.

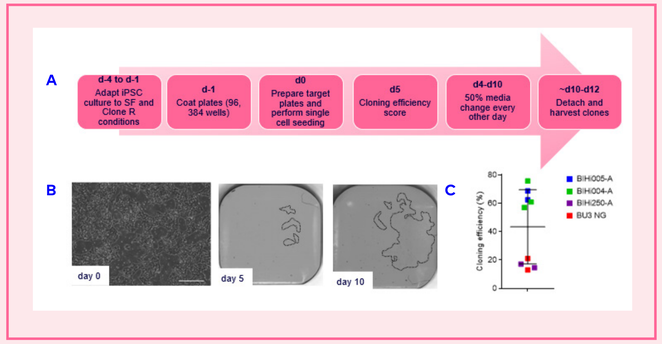

Figure 3. cellenONE® F1.4 embedded in hiPSC automated subcloning workflow
A) General Ttimeline to generate hiPSC monoclonal expansion after single cell seeding. B) Brightfield pictures show hiPSC culture before single cell dissociation at day 0 and follow up of clone growth in 384 well plate. C) Graph showing hiPSC cloning efficiency at day 5 after single cell seeding in 384 well plates. Each dot represents technical replicates. Values are expressed as mean ± SD. Statistic analysis: non-parametric Mann-Whitney test. Scale bar= 500µm.

Conclusion

Single cell isolation using cellenONE® F1.4 for hiPSC subcloning was successful thanks to:
- gentleness of dispensing
- exclusion of dead cells
- combination of specific coating (Laminin 521), media (StemFlex) and anti-apoptotic small molecule (CloneR) conditions
- the use of smaller plate formats

The results show that:
- Geltrex and Laminin performed similarly with ~30% of wells having clonal outgrowths (Fig. 2A).
- CloneR performed the best with 42% clonal outgrowths (Fig. 2B).

cellenONE® F1.4 automated platform for single cell seeding offers unmatched characteristics such as proof of clonality, gentle dispensing, high speed, possibility of fluorescence selection and potential to be embedded into a fully automated pipeline.

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