Assurance of Monoclonality of Recombinant CHO Cell Lines Using High-Resolution Imaging

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1. INTRODUCTION

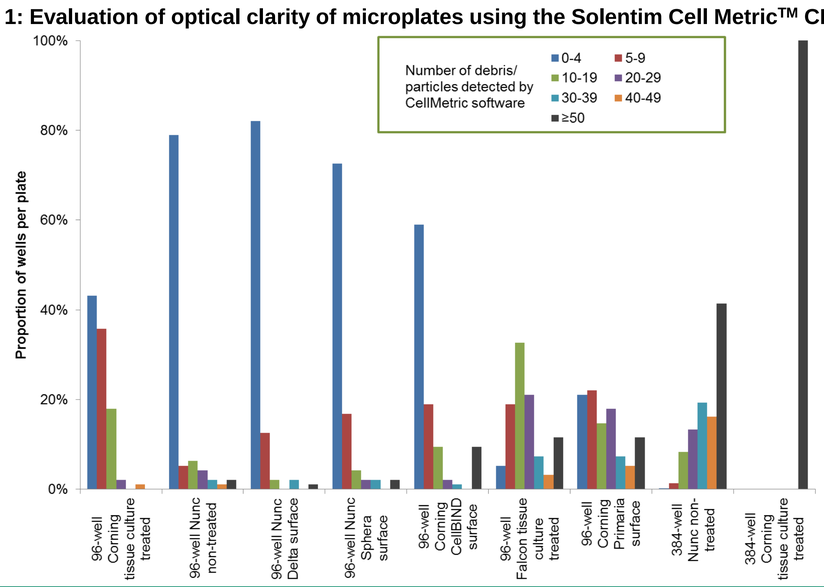
It is a regulatory requirement that a production cell line used for the manufacture of biotherapeutics should be derived from a single cell progenitor. It is therefore standard procedure to include at least one cloning step during the development of a recombinant cell line. Numerous techniques are available for single cell cloning, but regardless of the method used there should be appropriate evidence to support that the method is fit for purpose. FUJIFILM Diosynth Biotechnologies (FDB) employ a two-step cloning strategy which combines the ClonePix™ as a cloning and screening tool followed by a second cloning step using the industrially accepted method of limiting dilution cloning (LDC). This paper describes the establishment of an imaging method using the Solentim Cell Metric™ to strengthen the LDC stage of our current cloning approach by providing evidence of the single cell stage of cell line development (CLD). The Solentim Cell Metric™ uses high-resolution brightfield imaging to generate clear images across the whole well, and automatic focusing to correct for any plastic distortion across the plate or individual well.

This paper focuses on the main areas of investigation performed at FDB to integrate the Solentim Cell Metric™ into our CLD workflow; including the assessment of the suitability of a range of 96 and 384 well microplates and animal component free (ACF) medium types. The work carried out here identifies an optimum plate type and ACF cloning medium for cell growth and optical clarity. An imaging time point after cell seeding on day 0 was identified after which all cells could be visualised on the well surface.

2. OPTICAL CLARITY EVALUATION OF MICROPLATE TYPES

A range of microwell plates were assessed for optical clarity. Empty microwell plates were screened using the Cell Metric using the highest sensitivity scan setting and the instrument software was used to detect debris or imperfections in the microplates. The results from the analysis are shown in Figure 1. Proportionally, the 384-well microplates showed higher numbers of imperfections in more wells than the 96-well microplates, making them unsuitable for use in a single cell imaging method. Of the microplates assessed, 5 showed the highest proportion of wells containing low numbers of imperfections and were evaluated for their suitability further (Corning tissue-culture treated, Corning CellBIND™, Nunc™ non-treated, Nunc™ Delta™ and Nunc™ Sphera™ 96-well microplates).

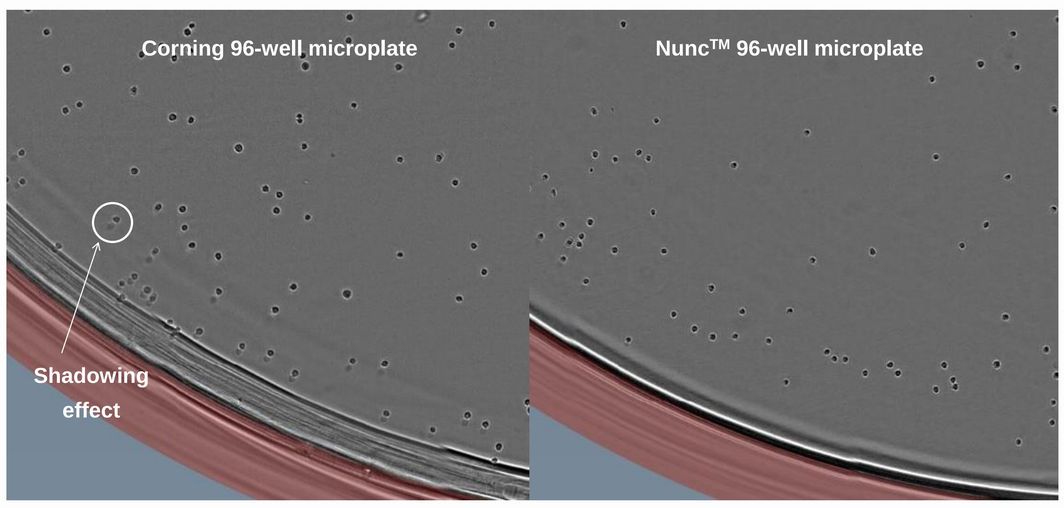
Figure 1: Evaluation of optical clarity of microplates using the Solentim Cell Metric™ CLD



3. EFFECT OF WELL VOLUME AND COMPARISON OF EDGE EFFECTS BETWEEN MICROPLATE TYPES

It is a regulatory requirement that an image of the whole well must be captured[2]. The well volume and plate type used have an effect on the illumination at the edge of the well, and cells can sometimes appear to have a "shadow" when located near the edge of the well. This effect impedes the identification of single cells at the edges of the wells. A range of working volumes were assessed and it was found that a 150 µL well volume was optimal to reduce this shadowing effect. This volume was then used to compare images from Nunc™ and Corning 96-well microplates. An example image is shown in Figure 2, the images of the cells at the well edges of the Nunc™ plates show less shadowing compared to the Corning plates. In addition, the Nunc™ plates also show a more defined well wall. Therefore the Nunc™ 96-well microplates showed less edge effects compared to the Corning 96-well microplates.

Figure 2: Comparison of images at well edges in Nunc™ and Corning 96-well microplates at a 150 µL well volume using the Solentim Cell Metric™ CLD



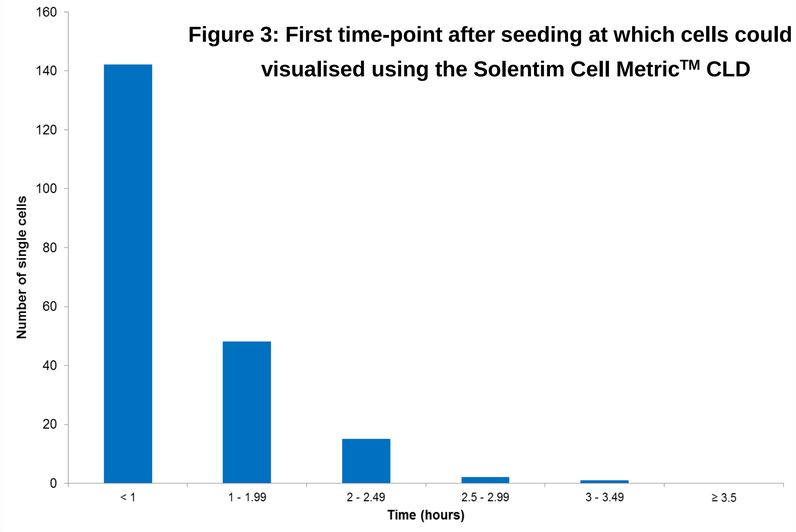
4. ASSESSMENT OF THE FIRST IMAGING TIME POINT AFTER CELL DEPOSITION

In order to demonstrate that a colony has been derived from a single cell, it is essential that all cells deposited into a well can be imaged. The time required for cells to be deposited into the focal plane of the imager after seeding was assessed.

Cells were seeded into 96-well microplates and imaged on the Cell Metric at intervals up to 5 hours. Images from the 5 hour time point were analysed and 208 wells containing single cells were identified. Subsequently, previous images were analysed to identify the first time point at which a single cell could be visualised.

The results in Figure 3 show that the majority of cells can be identified after 1 hour, and all cells can be identified within 3.5 hours. Therefore a first imaging time of 3.5 hours post multi-well plate seeding was identified.

Figure 3: First time-point after seeding at which cells could be visualised using the Solentim Cell Metric™ CLD



5. EVALUATION OF COLONY OUTGROWTH IN MICROPLATE AND ACF MEDIUM TYPES

Colony outgrowth was assessed in microplates showing good optical clarity to confirm the suitability of these microplates in the FDB LDC method. These data are shown in Figure 5. No significant differences in cell growth were observed between the microplate types. Therefore the preferred plate type (Nunc™ non-treated 96-well) was selected based upon optical clarity and the minimisation of edge effects.

Figure 5: Evaluation of cell growth in a range of microplates and media types

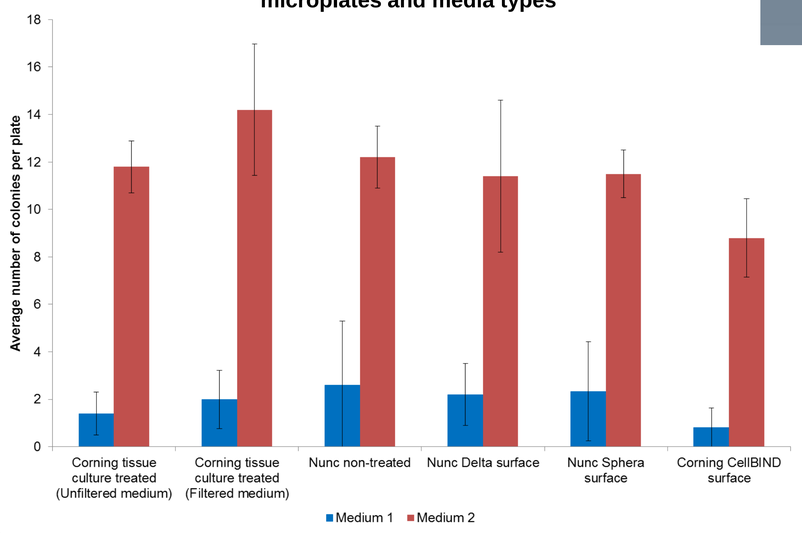
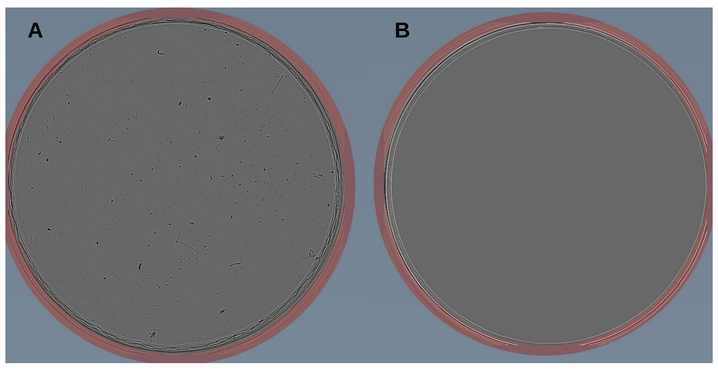


Figure 6: Image of medium 2 without filtration (A) and with filtration (B)



Imaging identified particles in the ACF medium which prevented identification of single cells. Filtration of the medium removed the particles (Figure 6), but it was unknown if removal would impact on cloning efficiency. Therefore it was necessary to evaluate cell growth in filtered and unfiltered ACF medium. Two ACF medium types were evaluated with and without filtration. Higher colony numbers were observed from cells grown in Medium 2 compared with Medium 1, and single cell cloning performance was maintained when this medium was filtered (Figure 5).

6. INTEGRATION OF HIGH RESOLUTION CELL IMAGING INTO THE APOLLO™ CLONING STRATEGY

The work described above has identified Nunc™ non-treated 96-well plates as our preferred microplate type for single cell imaging during LDC using the Solentim Cell Metric™ CLD. This was based on colony outgrowth, optical clarity and reduction of edge effects. An optimum well volume to reduce well edge effects and ACF medium type were identified. The time required for cells to be deposited into the focal plane of the imager after seeding was determined as 3.5 hours.

FDB is aware of increased interest from regulatory bodies regarding the method of cloning used and P(monoclonality) achieved during CLD. At FDB careful consideration and extensive work have been undertaken to develop the Apollo™ cloning strategy. This strategy ensures the highest quality of the cell lines with respect to monoclonality is reached. FDB employ a two-step cloning strategy which combines the ClonePix™ as a cloning and screening tool followed by a second cloning step using the industrially accepted method of LDC. The cloning strategy has been designed using cloning methods which have robust experimental and statistical evidence to support their use for generating cell lines with a high P(monoclonality). The approaches described here have assisted towards establishing an imaging method using the Solentim Cell Metric™ CLD which further strengthens the LDC stage of the current Apollo™ cloning strategy by providing evidence of the single cell stage of a colony.

7. REFERENCES

1 - Coller & Coller, 1983. Hybridoma. 2(1): 91-96.
2 - Kennett, 2014. Conference presentation 18th Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products.

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