Proof that can travel – Documented clonality report for regulatory submission

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Abstract

Clonality is a key element of cell line development and is an important component of a regulatory submission. Indeed for BLA, the clonality of MCB is mandatory. Historically, the regulator has insisted upon 2 rounds of cloning for developing a cell line and assurance of monoclonality based on statistical outgrowth measurements. Recent improvements in high resolution whole well imaging of cells in microplates, enables the creation of indisputable image-based evidence for the growth of a colony from a single cell. This evidence eliminates a round of sub-cloning and results in several weeks of cell line development time being saved. However, current compilation of images using 'cut and paste' into a report is laborious, error-prone and difficult to audit.

A new standardized report for evidence of clonality images is described here. The user selects wells based upon growth characteristics and dynamically interacts on-screen with the original time course images to determine which wells were originally seeded with a single cell. Single cell feature are annotated along with any other features in the well. Once analysis is complete, the user automatically generates the contemporaneous report in paper or electronic form with the click of a button. The content of the report is designed to meet the recommended guidelines from the regulator (1).

We will show an illustrative Clonality Report generated from raw data with a commercial CHO cell line.

Method

Verification that a new manufacturing cell line developed to produce a protein therapeutic is derived from a single cell (i.e. is clonal) is an absolute requirement.

Historically, using statistical methods for dilution and colony outgrowth was sufficient, but nowadays the drug regulators and the clients for upstream cell line development groups alike want to see documentary evidence of a single cell origin.

This poster illustrates how the Cell Metric™ CLD (see Figure 1) imager can identify single cells on the day of seeding (D0) before they divide, and provide a clear image of the cell and its subsequent growth into a colony for documentation.

Below and in Figure 2 is an outline a cell line development process workflow using the Cell Metric CLD system to ensure traceability of the clone:

- Transfection or fusion of cell line
- Enrichment or selective pressure applied to isolate high producers
- Single cells seeded into 96 wells plates via FACS or limiting dilution
- Cells imaged at several time points: Day 0, Day 1 (24 hours later), Day 4/5 and Day 10-14 (depending on the cell line being used)
- On the last time point (Day 10-14), based on growth data and sometimes crude titer assessments, wells are interrogated to select clones derived from a single cell. See results section for how this is achieved
- Chosen positive clones are selected for hit-picking and expansion.
- Subsequent clone growth (confluence), productivity and specificity are monitored and used to determine best clones
- Clones are scaled up for further growth/titer/stability testing and Master
- Cell Banking (MCB)

Figure 1: Cell Metric CLD is a dedicated high-resolution bench-top imaging system with integrated, heated microplate loader, specifically developed to speed up cell line development (CLD). The unique cell imaging capabilities of the Cell Metric CLD enable fast, unequivocal identification and tracking of single-cell derived clones.

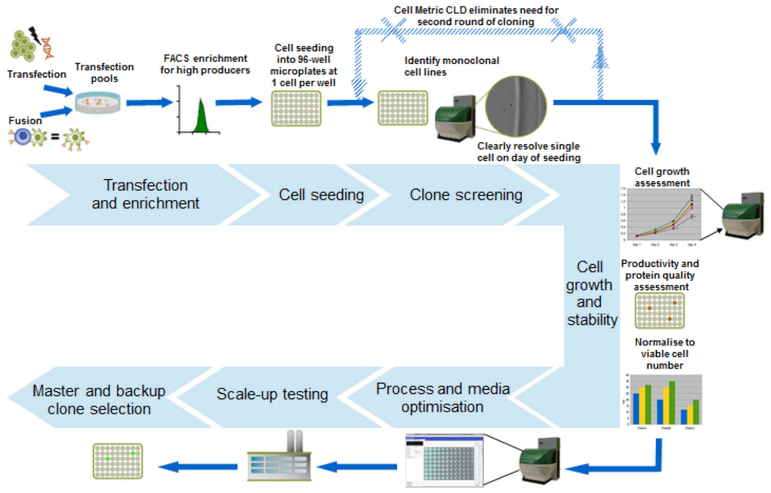

Figure 2: Overview for a typical cell line development workflow with the use of the Solentim Cell Metric CLD for verification of clonality and colony outgrowth measurements.

Results

Following imaging at set time points over the growth period, the software automatically collates the plate data for user interrogation. Each well containing growth is selected for review and the previous well images (at the earlier time points) are examined by the user to determine if each clone started as a single cell. Figure 3 below demonstrates the series of images captured by the system and how they can be reviewed to ensure clonality. To do this, the user will…

- Select a well containing cell growth; the full well image is displayed
- Click back through earlier images captured for that well
- Zoom in to the well image and review the growth loci
- Decide if the well is clonal/non-clonal and use the software to label the well appropriately

As well as high resolution and contrast imaging the instrument employs a well mapping system that occurs simultaneously during imaging to ensure each 100% of the wells are always in focus. Figure 4 shows a contour map of a 96 well plate and demonstrates that the surface height can vary dramatically which, if not accounted for, can affect cell focus. This feature is fundamental for the requirement to qualify your cloning method.

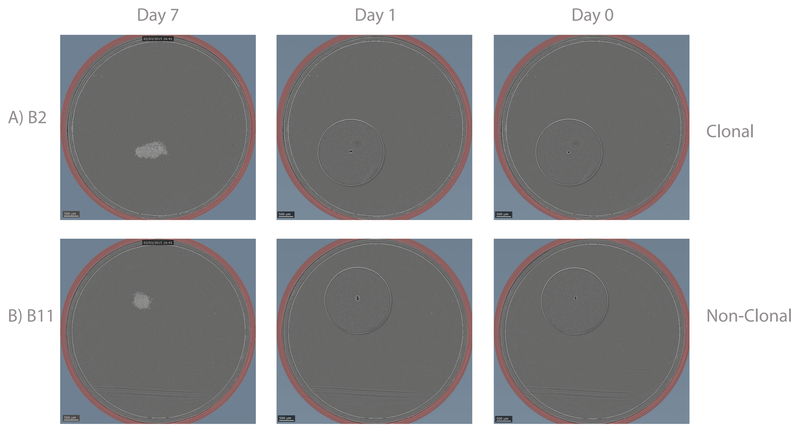

Figure 3: Time series (Day 7, 1 and 0) of whole well images interrogated for single cell derived clones. A) Displaying the images captured for well B2. B) Displaying the images captured for well B11.

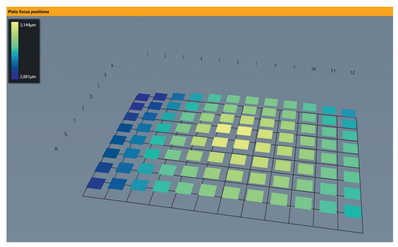

Figure 4: Focus assurance map of an entire 96 well plate.

Clonality reporting

Using the export function, the user can track and choose all/selected time points and annotate for a given well containing vital information such as:

- Single cell location
- Colony growth
- Plate debris
- Artifacts and any other features of interest

The software exports these well-specific time points as a multi-page single PDF report (see Figure 5) for individual clones which have been identified as potential high producers.

The ideal way to evaluate these wells and write the report is at the PC terminal of the Cell Metric CLD or at your desk using the remote data viewer software.

The report function provides the operator an exported option that is "proof that can travel" in the form of printed reports, electronic files and Powerpoint presentations.

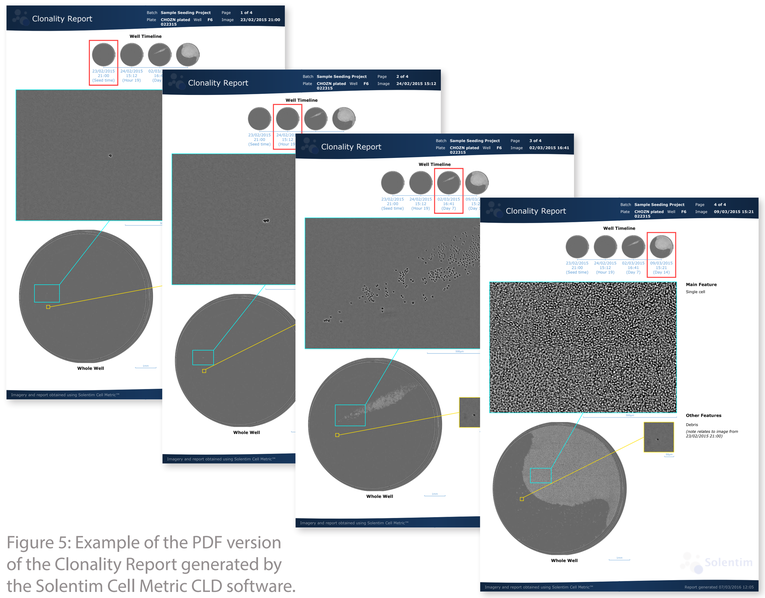

Figure 5: Example of the PDF version of the Clonality Report generated by the Solentim Cell Metric CLD software.

Conclusions

As providing proof of clonality is recommended by numerous guidance bodies (WHO and ICH) and requested by the FDA for IND submissions; *"Submit data to the IND that provides assurance that this method resulted in derivation of a single cell clone or provide information on how you will go about generating these data and the timeframe for submission of the information"*[1], typically to generate a report manually would take many hours or even days, and will vary between users. The report may not even be able to show all the specific information that the FDA have requested e.g. the single cell location within the context of the whole well image.

The clonality report was developed in collaboration with our customers and delivers the following benefits

- Simple
- Fast to generate – minutes per report
- Consistent – between different users
- Safe – gives all the information that the regulator has asked for in relation to the origin and history of the clone
- Proof – that can be sent to CMO client, cell banking departments, regulator etc.

References

1. Kennett S. "Establishing Clonal Cell Lines – a Regulatory Perspective" - FDA presentation at WCBP, Jan 30, 2014