Proof that can travel – Documented clonality report for regulatory submission

Dr Ian Taylor*, Solentim Limited, UK. Paul Miller, Solentim Inc USA, Andrea Gough, Solentim Limited, UK
Ian.Taylor@solentim.com, Paul.Miller@solentim.com, Andrea.Gough@solentim.com
*First author

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 clonality report for regulatory submission (2). 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 can 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 (3).

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 of 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)

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 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

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”. 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


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.

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.