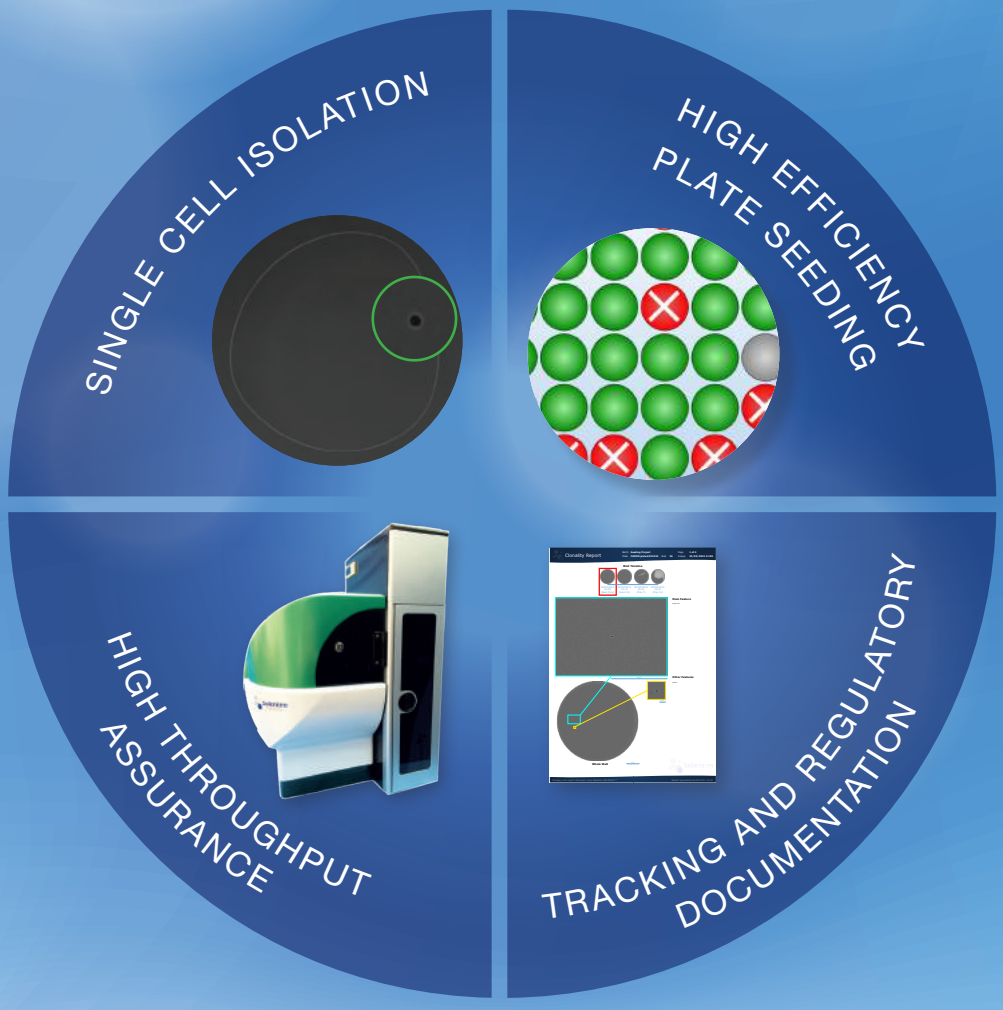
Solentim
Clarity in Cell Line Development

Clonality Assurance Efficiency

Accomplished by a suite of products designed for cell line development



The Importance of Clonality

Assessment of clonality remains a key factor in the development of any new stable cell lines for novel biopharmaceuticals or biosimilars.

The current basis for this expectation of clonality is two-fold:

- Regulatory requirements (e.g. FDA/EMA) and compliance
- Scientific considerations

Regulatory Requirements

This is the primary concern for drug developers and is governed by the FDA/EMA and other regulatory bodies.. Documentation of clonality is encouraged as part of the IND process and is mandatory by the time of BLA (Biological Licence Application).

"It is expected that clonal cell lines are developed as referenced by ICH Q5D and EMA/CHMP"

Rachel Novak, US FDA, Jan 2017**

Scientific Considerations

Product Quality and Consistency

"Assurance of production cell bank clonality ensures consistency of product quality and process performance throughout the lifecycle of a product" **Rashmi Rawat**, FDA, Apr 2016*

Downstream Process Impact

"To minimise the heterogeneity within the master cell bank (MCB) to allow for a consistent manufacture of a product" **Rachel Novak**, US FDA, Jan 2017**

Probability and Assurance for Clonality

Probability, defined as a forward-looking numerical calculation, is still a key factor that the regulators consider important in the assessment of clonality. To date, probabilities have mainly been based upon theoretical calculations such as Poisson distribution for limiting dilution, or extrapolation of validated control experiments for FACS seeding rates.

Most recently, the regulators have stated that "assurance" can be used in conjunction with probability to support the clonality calculation. Whole well imaging is widely accepted as the major component of assurance.

* Rashmi Rawat presentation: *Regulatory Consideration for the Biotechnology Products: Clonality of the Production Cell Bank.* April 2017, Informa Conference, Vienna. Austria.
**Rachel Novak presentation: *Regulatory Perspective on the Evaluation of Clonality of Mammalian Cell Banks.* January 2017, WCBP Conference, Washington. USA.

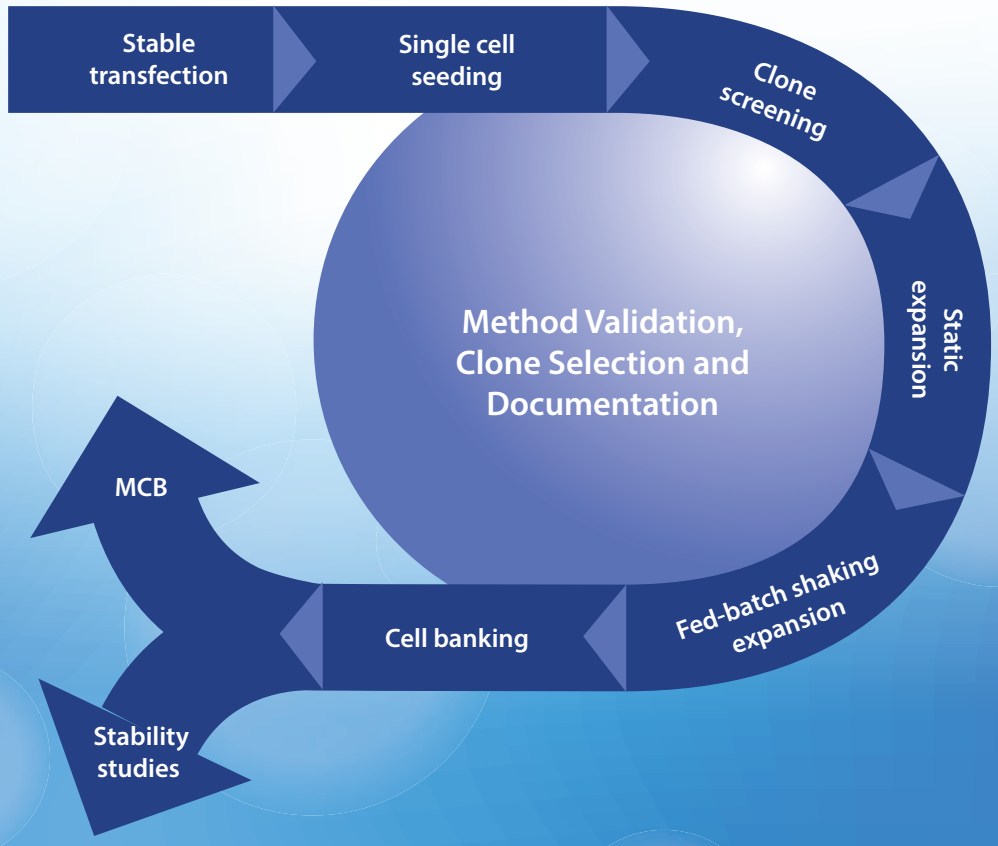
Cell Line Development Workflow

In recent years, the Cell Line Development (CLD) workflow has dramatically changed as bottlenecks are eliminated and processes improved. The advent of dedicated whole well imaging systems has eliminated the need for a second round of sub-cloning. Also, with the emergence of more targeted gene editing tools, far fewer clones need to be screened. Nowadays, it is perfectly feasible for a complete project to be fewer than 4,000 clones (approximately 10 x 384 well plates).

Cell line development labs have been required to piece together solutions themselves with different instruments. These various instruments have often originally been developed for other applications and may not be ideal for cell line development requirements. Additionally they tend to operate in isolation from other steps in the workflow.

There is a growing need for a dedicated and far more integrated solution that ensures data continuity and integrity, and that delivers tangible overall workflow efficiencies.

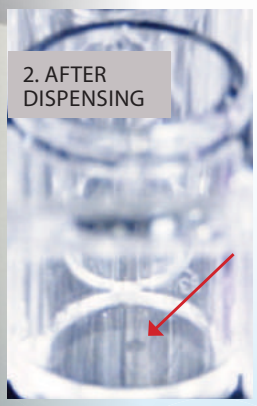
The Cell Line Development Workflow

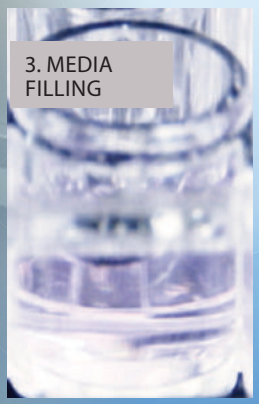


Single Cell Seeding
Introducing the VIPS™

Solentim have developed the VIPS™ (Verified *In-situ* Plate Seeding) system which combines single cell deposition with simultaneous *in-situ* image verification of a single cell in the well for high efficiency seeding of well plates.

Droplet deposited by VIPS into the dry well followed by imaging and then media addition





The VIPS™ system is a small bench top instrument, which is placed within a standard biological safety cabinet for sterility, with all components that come into contact with cell culture being interchangeable and then either sterilised or replaced with new.

VIPS uses a gentle process called SMART LD™ whereby it dispenses, from a reservoir of cells in media, a single droplet (at a dilution of 0.5 cells/droplet) into the central region of a dry well in the plate. Imaging a bright field z-stack of this droplet automatically detects the presence or absence of a single cell.

- If detection confirms the presence of a single cell per droplet, the well is automatically filled with media.
- If the detection confirms the presence of several cells per droplet, the well is excluded from further processing.
- If no cell is detected, the system will deposit another droplet and repeat the process up to several times per well.

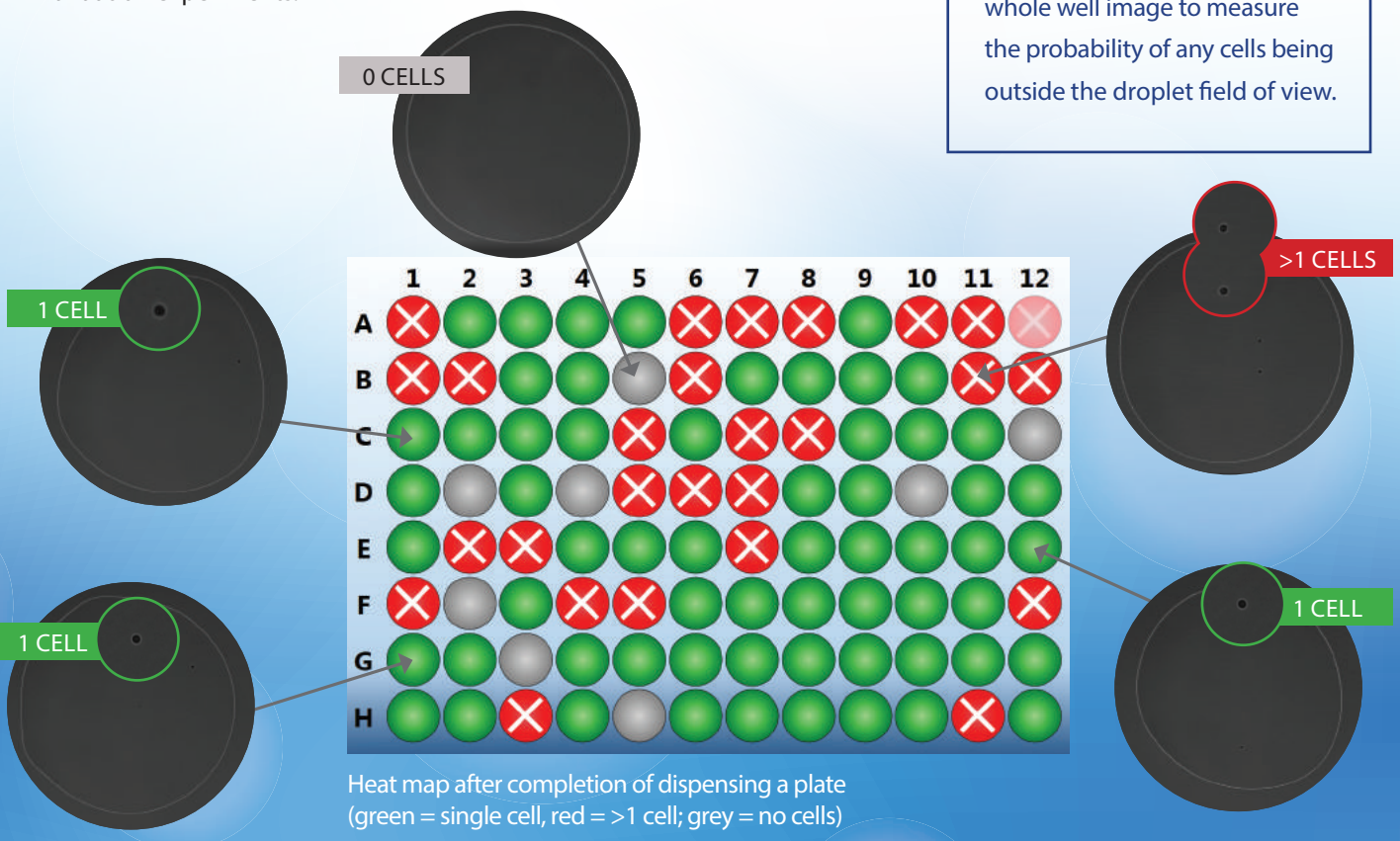
Seeding of a full 96 well plate is completed in less than 10 minutes and real time occupancy results are displayed in the form of a plate map indicating empty wells, single cells and wells with multiple cells.

High speed, whole well imaging can be carried out by VIPS for validation experiments.

Method Validation

It is fundamental that any cloning method can be validated. This will be done by running some representaive plates to generate statistics and to then support the probability of clonality calculation.

The VIPS has on-board capabilities to specifically enable using fluorescence based labels. Labelled cells would be dispensed in droplets and then imaged to confirm a single cell in the droplet. Importantly this is followed by a low contrast whole well image to measure the probability of any cells being outside the droplet field of view.



Heat map after completion of dispensing a plate
(green = single cell, red = >1 cell; grey = no cells)

Clone Screening
with Cell Metric CLD

Cell Metric® is a dedicated imaging system for the key step of assurance of clonality and colony outgrowth monitoring. Scanning of an entire 96 or 384 well plate is rapid with high contrast whole-well images captured automatically at single-cell resolution. Following seeding plates are imaged at Day 0 and then repeatedly over a time course of up to between 10-21 days depending on cell type.

Already used by the majority of the top 10 biopharma companies and CROs/CDMOs for biologics, Cell Metric provides the highest quality bright field images of single cells within the whole well by reliably detecting these cells even at well edges without need for any fluorescence labelling.

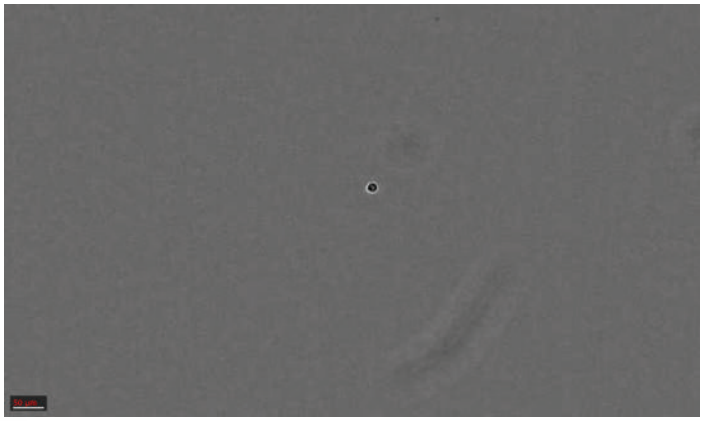
With the built-in, temperature-controlled plate loader with interchangeable cassettes of the Cell Metric CLD model, higher throughput is possible, without the need for additional robotics, to support multiple users and multiple projects.

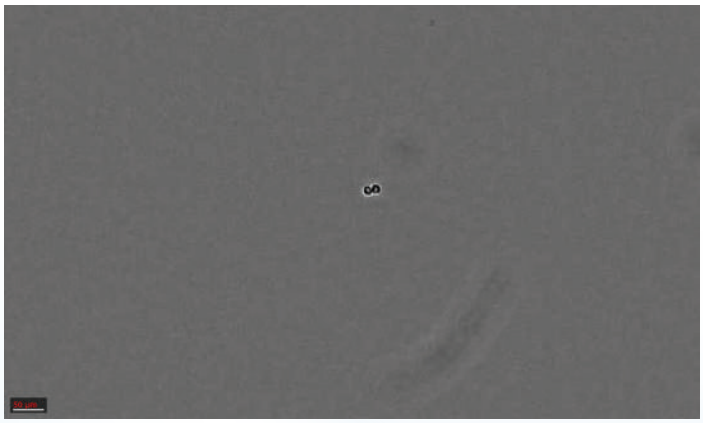
A time-stamped image library is saved for each well so that the user can toggle through the time points and track back from a colony to confirm single cell starting point.

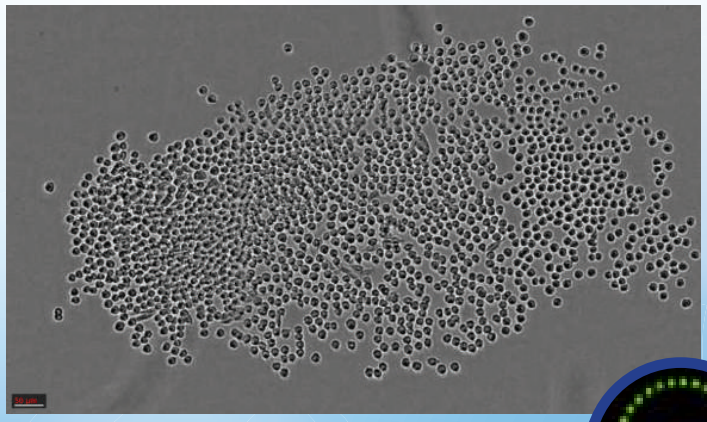
Additional capabilities are available for method validation and other dedicated cell line development applications using fluorescent dyes.

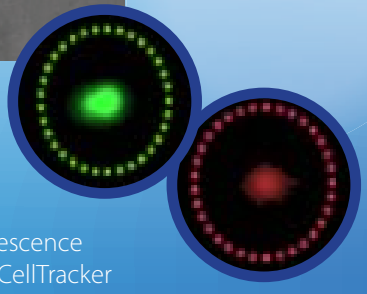
Cell Metric CLD with incubated plate loader for 10 plates.

Day 0 image and subsequent time course of whole well images. This shows a single cell at the start and subsequent doublings to form a colony outgrowth.









Example fluorescence images using CellTracker Green and CellTracker Red.

Clone Documentation

In recent years, regulators have indicated that assurance data provided by imagers should meet specific criteria and Solentim have automated this process, by way of generating the Clonality Report, to ensure no errors are made in the collation of this assurance data.

These reports, which can form part of the IND submission package, follow the valuable user-selected clonal wells from single cell to clone throughout the growth period with an image series (whole well and single cell image) in order to provide full time- and date-stamped data information for the well from single cell to colony.

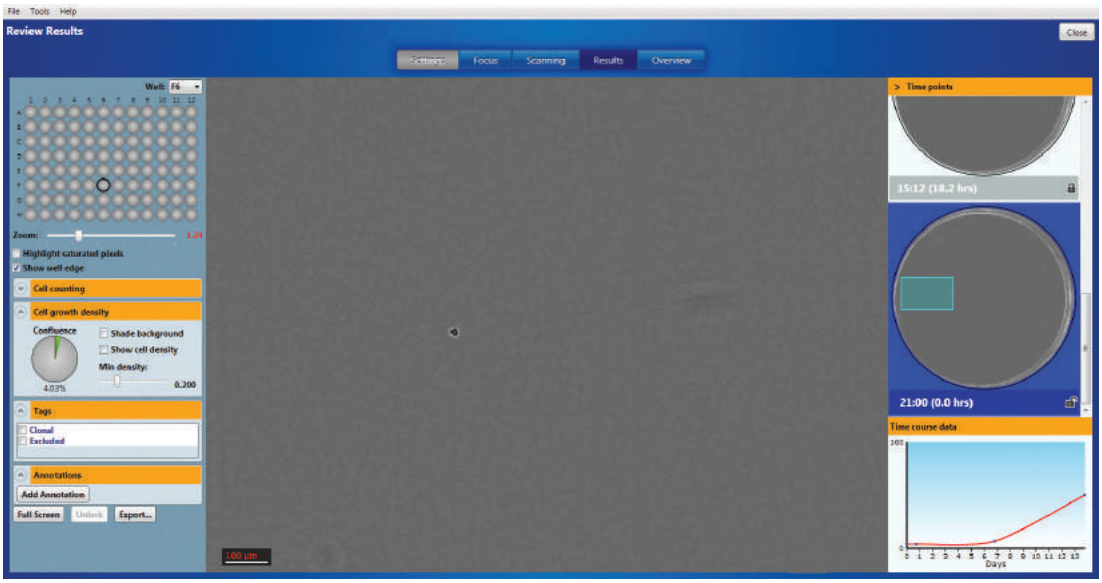
Annotation is possible of the single cell and any other features e.g. debris. The reports are generated in minutes in PDF or PowerPoint presentation formats which can be exported and shared with interested parties e.g. cell banking group, or a CMO customer.

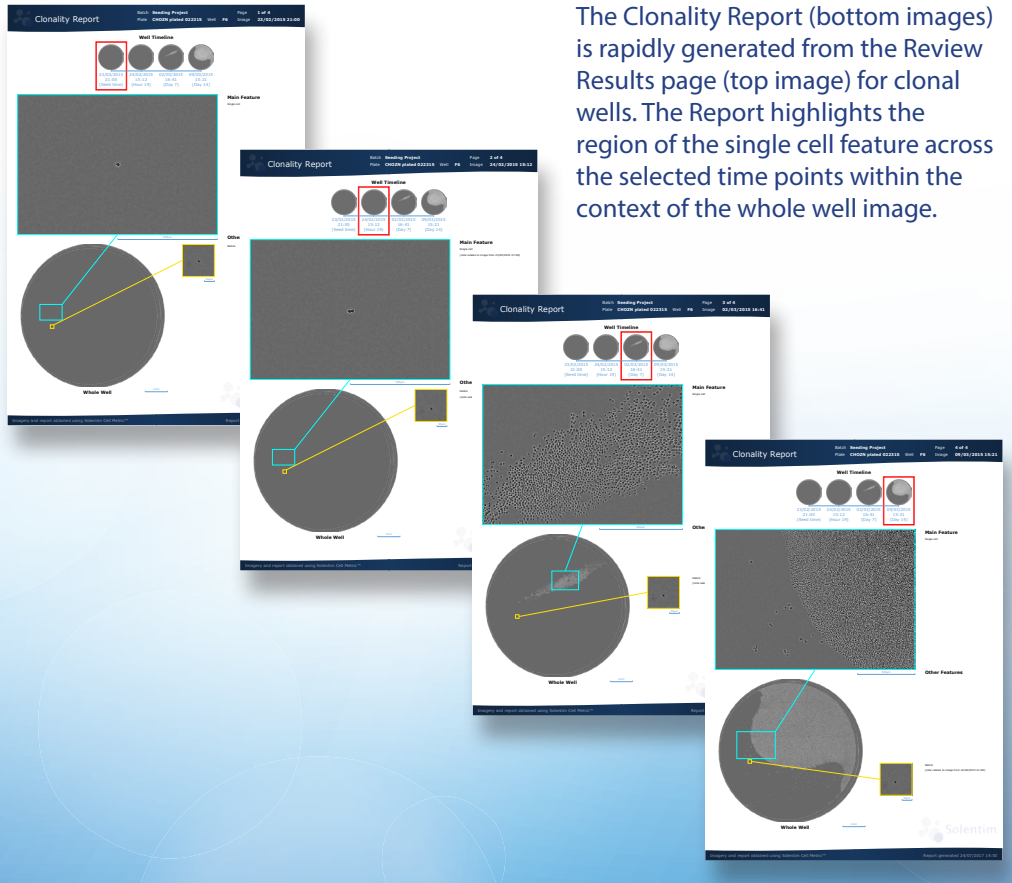
Remote data viewer software enables the user to analyse data and generate clonality reports from their office computer.

"Supporting data should include picture of a single cell and the entire well"
Dr Rashmi Rawat,
FDA presentation, Apr 2016*

The Clonality Report "meets the FDA requirements of whole well image and where single cell feature is located; allows highlighting of other features which can then be described away" **Dr Audrey Jia**, Former FDA CMC Reviewer. IBC Asia, Shanghai, May 2017

* Rashmi Rawat presentation: *Regulatory Consideration for the Biotechnology Products: Clonality of the Production Cell Bank.* April 2017, Informa Conference, Vienna. Austria.





The Clonality Report (bottom images) is rapidly generated from the Review Results page (top image) for clonal wells. The Report highlights the region of the single cell feature across the selected time points within the context of the whole well image.

Integrated Workflow
Power of the VIPS - Cell Metric CLD Combination

To provide a complete integrated workflow in upstream cell line development, Solentim have directly linked the VIPS with the Cell Metric CLD from both workflow and data tracking perspectives.

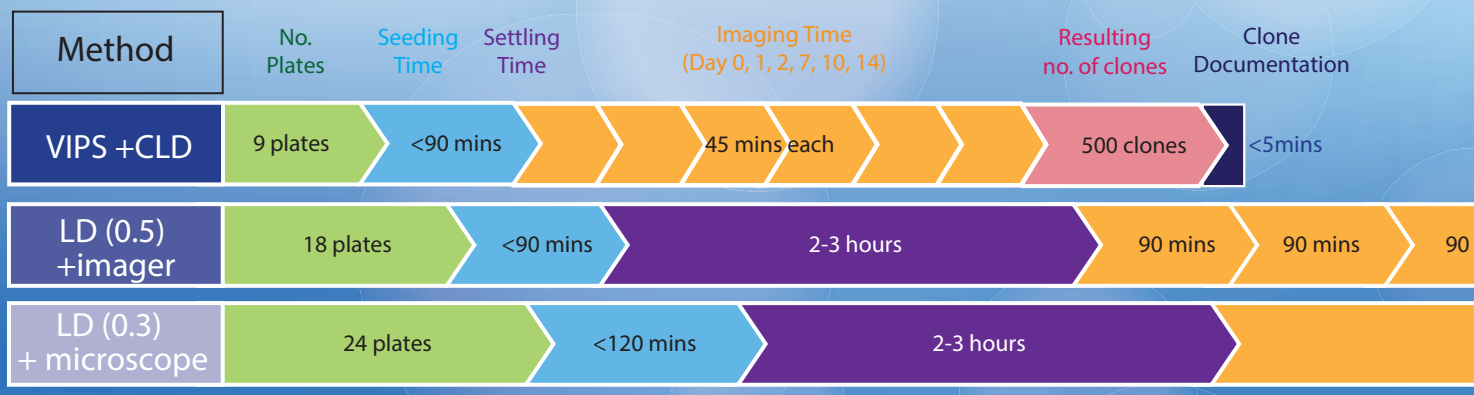
In summary, the VIPS – Cell Metric CLD Combination delivers the following two-fold solution:

- The deposition and isolation of single cells at high efficiency in well plates
- Assurance that it has been achieved every time and a clone can be tracked throughout the growth period

From a user perspective, VIPS and Cell Metric share a common software interface for ease of use. Batch information only needs to be entered once and then is carried through automatically. VIPS data will also be added to the Clonality Report for even more complete tracking and assurance.

Workflow Efficiency Improvements

The combination of VIPS and Cell Metric CLD directly impacts the cloning strategy and workflow efficiency compared with current alternatives of one round of limited dilution coupled with an imager, or traditional manual LD using statistical probabilities.



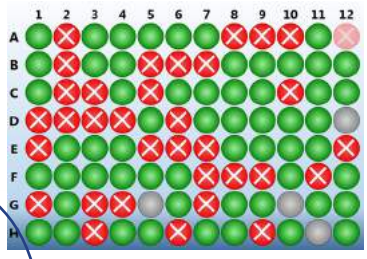
Proposed workflow improvements using the VIPS-CLD combination compared with existing approaches.

DISPENSE

High efficiency single cell seeding on the VIPS

Image of single cell in a droplet

Adds media to well



CONFIRM

Whole well image of single cell on Day 0 on the Cell Metric CLD

MONITOR

Subsequent days of imaging to monitor colony outgrowth on the Cell Metric CLD

REPORT

Review images to confirm clones Generate the Clonality Report documentation

Summary of the VIPS-Cell Metric CLD integrated workflow

mins	90 mins	90 mins	90 mins	500 clones	2-3 hours
6-8 hours (Review Time - Day 0)				500 clones	n/a

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Cell Metric Community

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