

# Doubling the Speed of Cell Line Development in a Large Pharmaceutical Company



Tom Kelly and Angela Tuckowski from Janssen with one of their VIPS instruments

**For the past 10 years, the Cell Line Development (CLD) group at Janssen R&D (Spring House, PA) had used the same two-step process to develop the final cell cultures for manufacturing new biologic drugs. Thanks to Solentim's VIPS™ technology, they've reduced that process to single step – halving development times while still satisfying the clonality requirements of the Medicines' Regulators.**

## Introduction

Biological drugs, such as monoclonal antibodies and vaccines, have taken off since the 1990s. With the global market growing by an estimated compound annual growth rate of 10%, biologics are predicted to make up an astounding 52% of the top 100 pharma sales by 20221.

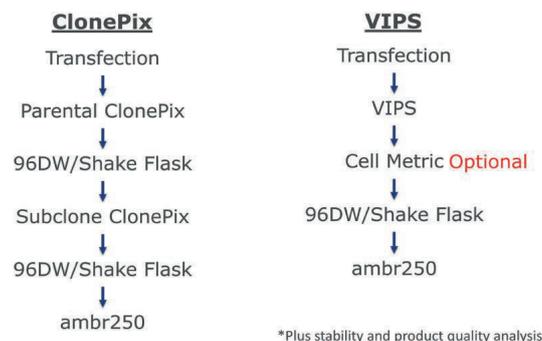
Unlike traditional small molecule drugs, biologics are manufactured inside mammalian cells with an identical genetic makeup. To ensure product quality and consistency, and to meet regulatory requirements, the CLD group at Janssen R&D must prove that their cell cultures are a clonal cell line, i.e. derived from a single cell origin.

## Cell Line Development at Janssen

For the past 10 years, the ClonePix™ technology was the cornerstone of the CLD group's process. Taking 5 months to complete a project, the four-person team began by creating multiple transfection pools totalling around 80 million Chinese Hamster Ovary (CHO) cells, of which 8 million were manually transferred to plates containing semi-solid media and screened (using fluorescence detection with Alexa Fluor™ 488 Protein-G conjugate) in the ClonePix to select the best 800-1200 antibodysecreting clones.

After leaving the 800-1200 clones to divide for a couple of days, the team scaled the colonies to 96-deep-well plates. Protein A Octet® titers were obtained from the 96-deep well plates to select the best 48 parental clones to grow in shake flasks. Fed-batch shake flask Protein A Octet titers were obtained to choose the best 20 parentals to subclone and repeat the ClonePix screening, 96-deep-well, and fed-batch shake flask process a second time, before choosing 24 cell lines to test in the group's ambr® 250 system (see Figure 1).

### ClonePix vs VIPS Process Comparison



**Figure 1** - Comparison of the old method using ClonePix and two rounds of sub-cloning versus the new VIPS approach with only one round of cloning.

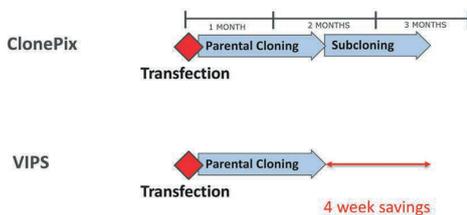
## The Problems with the ClonePix for clonality

The ClonePix screening process was repeated twice because the team couldn't demonstrate that each colony was derived from a single cell. They assumed the cells were evenly spaced, but could only be 95% certain that two cells hadn't landed close together or that when picking colonies the ClonePix picking tip had not touched another colony.

This inaccuracy was a problem for the team because the U.S. Food and Drug Administration (FDA), who license drugs for the American market, require pharmaceutical companies to show their Master Cell Bank (MCB) is clonally derived. The FDA believe this helps ensure the biologic drug is identical lot-to-lot – reducing the likelihood of unexpected side effects.

To overcome this problem, the team had to repeat the ClonePix screening to ensure there was a statistically higher chance that every cell was identical in each of the highest-producing colonies. However, this almost doubled their timelines of cell screening steps to 2.5 months (see Figure 2).

### ClonePix vs VIPS Process Timelines



\*Only showing cell screening activities



Figure 2 - Comparison of the workflow timelines for the old ClonePix method and the new VIPS approach.

## Moving to the Solentim platforms – Cell Metric CLD and the VIPS system

The CLD group decided to look for an alternative to the ClonePix. In addition to their problems ensuring clonality, the group were using expensive fluorophores (Protein-G conjugated to Alexa Fluor 488) to identify cells secreting the target protein. They also relied on expensive animal-based media (Foetal Bovine Serum (FBS)) to grow their cells.

They evaluated several technologies and opted initially for Solentim's proven Cell Metric® CLD imaging and analysis system. "Cell Metric had the best whole-well imaging capabilities and clonality reports," explains Tom Kelly, Scientist in the CLD group at Janssen. "And, when we demoed the instrument, we were convinced by the robustness of the system and very high image quality."

Solentim kept the group informed about forthcoming technologies, and they soon additionally purchased the Verified In-Situ Plate Seeding (or VIPS). The VIPS is designed to seed 1 cell per well in microtitre plates, by repeatedly dispensing 30 nL (nanolitre) droplets until a cell is detected.

After each droplet, the system takes 20 x z-stack images through the droplet to confirm a cell has been dispensed.

*"A lot of single cell printing or dispensing systems show a picture of the cell on the way to the plate, but not in the plate," says Kelly. "We think the fact that the VIPS images the plate itself is critical. If you take a photo on the way to the plate – it is still possible that more cells got into the well than you expected."*

In addition, Kelly adds, because VIPS dispenses nanolitre droplets, *"there's so little media centred in the well (no well edges to worry about), that it makes it easy to locate the cell – and prove to the health authorities that the cell line is clonal."*

After detection of the single cell in the droplet, growth medium is added to the well through a separate channel and the whole well DO image is also performed on the VIPS.

## The VIPS difference

By proving that one cell is in each well of the plate, VIPS removes the need for subcloning – cutting the group's timelines by 4-6 weeks per project. And to prove that, the CLD group decided to test the performance of VIPS in a study using different dilutions of cells ranging from 8,000 – 15,000 cells/mL in the cell dispensing reservoir. They used an off-the-shelf CD-CHO dispensing media for the study – rather than the expensive FBS required for ClonePix. Results were as follows:

### High Seeding Efficiency and Validation of the VIPS process:

The group found that VIPS had seeding efficiencies as high as 87%, even at the lowest cell density (Figure 3). The VIPS was accurately able to distinguish between droplets containing 1 cell or more than 1 cell when checked against whole well images on the Cell Metric (Figure 4). The VIPS correctly identified wells containing a cell 95% of the time.

### Low Incidence of Ghost wells

The incidence of ghost wells (false negatives) has been significantly reduced by VIPS – only 3 out of 4750 wells (0.06%) showed a cell on the Cell Metric, which had not been detected by the VIPS, and this was found, in all three cases, to be due to a clog in the dispensing nozzle which had caused a splashed droplet (data not shown). This compares to typical ghost well incidence of up to 10% using existing manual LD and imaging methods.

### Cell survival and expected cell outgrowth

Previously, the group employed manual limiting dilution (LD) using a multichannel pipette to load the 96-well plates. They were only able to transfer a single cell to an average of 48 wells on each plate, and only ~14 cells (~29% outgrowth rate) grew into colonies.

Using VIPS, they were able to seed and confirm single cells to most of the wells on each plate. The VIPS was gentle on cells, giving a similar outgrowth rate or cloning efficiency (31.9%), but doubled the number of colonies per plate compared to manual LD. According to Kelly, "We now can screen more clones in far fewer plates, which is great because it lessens the workload in terms of the number of plates you have to handle." (Table 1).

There was also no impact on titer distribution for the clones from VIPS versus clones from manual LD (data not shown).

Colony Outgrowth - comparison of VIPS with manual LD				
Seeding Method	#Plates	#wells	#wells with growth	Cloning Efficiency %
VIPS	10	950*	303	31.9
Manual LD	19	960	180	37.5

Notes: \*A1 is control well in each 96 well plate on VIPS

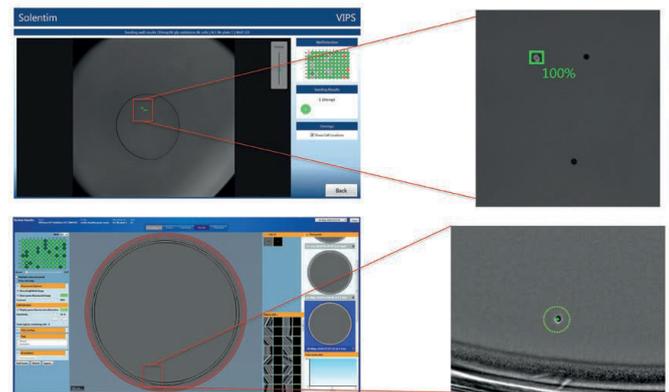
**Table 1-** Shows that the team were able to get nearly twice the number of colonies per plate at equivalent cloning efficiency to manual limiting dilutions. This also illustrates that VIPS is gentle on the cells.

### Impact of input cell density on #wells with 1 cell/well

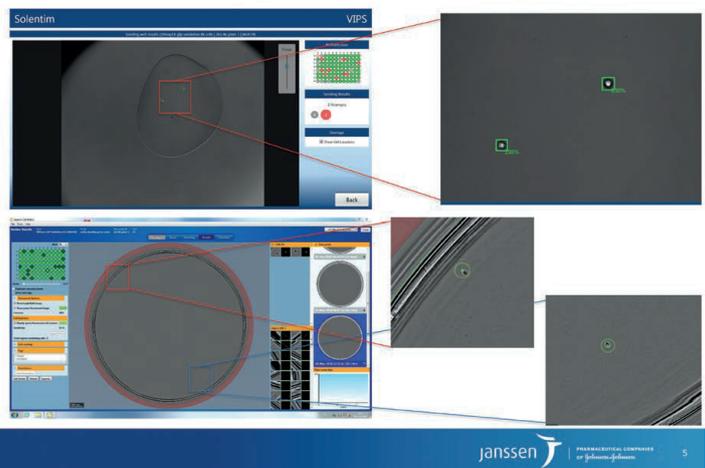


**Figure 3 -** Seeding efficiencies at different starting concentrations of cells in the cell reservoir (15,000 cells/ml versus 8,000 cells/ml); green wells = single cell, red wells = >1 cell, grey wells = no cell

### 1 cell detected by VIPS confirmed by Cell Metric



### 2 cells detected by VIPS confirmed by Cell Metric



**Figure 4 -** Comparison of VIPS result with Cell Metric result. 1 - shows the result for 1 cell detected in the VIPS droplet (upper portion) and then confirmed as 1 cell in the Cell Metric whole well image (lower portion). 2 - shows the result for 2 cells detected in the VIPS droplet (upper) and confirmed in the whole well image (lower).

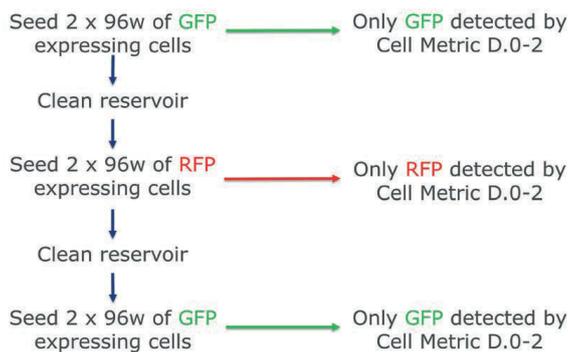
### Ease of switching between cell line projects on the VIPS and avoiding cross-contamination

The CLD group often develops cell lines for several molecules in parallel, raising the risk of cross-contamination. The group tested the VIPS' automatic cleaning and sterilization processes by seeding and imaging (Day 0 – Day 2) control plates with cells expressing Green Fluorescent Protein (GFP), then Red Fluorescent Protein (RFP), and then GFP again, with washing of the cell reservoir in between. The VIPS showed no sign of cross-contamination between cell lines. (see Figure 6).

### Workflow consistency

"The fact the VIPS is controlling for a lot of variability in media and the seeding process means we now have similar output rate per FTE scientist," commented Kelly. "In addition, the entire process is cheaper because the only additional cost is dilution media".

### Cross-contamination between projects



Purchasing multiple reservoirs also an option



**Figure 5.** Control experiments to show that when the VIPS cleaning and sterilization protocol for the cell reservoir is carried out (using the Wash Station), there is no risk of cell carryover from previous projects or cell pools.

### Future Outlook: Taking VIPS forward and further improvements in their Cell Line Development Workflow

The CLD group have purchased a second VIPS unit to increase capacity and enable groups to easily switch between the two VIPS units. They intend to use VIPS images as part of their clonality report by now including the droplet image alongside the whole well images – to prove that their cell lines are clonal. "In the long-term, we envisage just submitting the VIPS image of the droplet in the whole well for our FDA submission," Kelly explains. The group also have optimised their expression vectors such that now only the highest producing clones survive the transfection selection process. This has eliminated the need for any enrichment steps prior to single cell cloning.

Moving forward they plan to use VIPS' optional fluorescence capacity to further improve their transfection pools. "Right now, when we do transfection, the DNA randomly incorporates into the cell genome" Kelly says. The group hopes to create a 'landing pad' of DNA recognisable by recombinase so more cells will get transfected in known locations – reducing the time required. "If the landing pad is fluorescent, we can use VIPS to screen out the cells that haven't had successful recombination events."

The group currently runs 10 x 96 well plates per project. With the forthcoming application for seeding into 384 well plates on VIPS, this will further reduce the number of plates per project and eliminate any need by customers large numbers of plates or robotics in the cell line development process.

### Acknowledgements

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