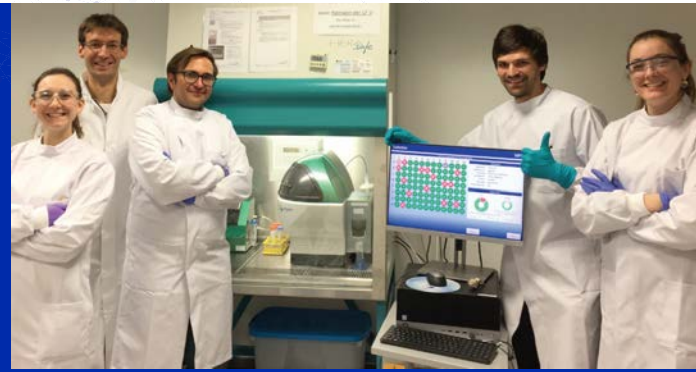
ADVANCED INSTRUMENTS

Case Study

FACS bulk enrichment in combination with the VIPS and Cell Metric delivered significant savings for a CDMO



The Celonic CLD team with their VIPS instrument. From left to right: Laura Benoit, CLD Scientist; Sébastien Lalevée, CLD Team Manager; Camille Evenou, CLD Scientist; Benjamin Renner, CLD Scientist; Camille Mathieu, DSP Process Engineer.

In early 2018, the Cell Line Development (CLD) department at Celonic AG, a European-based CDMO specializing in biologics manufacturing from mammalian cell lines, decided to optimize their workflow by acquiring new technology geared towards shortening their time lines and lightening their workload. In addition, they were searching for a gentler fluidics system for single cell isolation as the shear stress from FACS was restricting recovery and outgrowth for certain cell lines. Implementation of the VIPS™ and Cell Metric® has allowed them to shorten their CLD workflow timeline by nearly 50% and to reduce the number of plates they have to handle by up to 25-fold.

Introduction

Celonic AG is a premium biologics contract development and manufacturing organization (CDMO) specialized in mammalian cell line production. Founded in 1982, the company is split in two locations; their Basel site (Switzerland) which focuses on CLD and development activities and their Heidelberg site (Germany) which handles GMP production.

The need to optimise CLD workflow

The Cell Line Development (CLD) department at Celonic is routinely commissioned by the company's customers from all over the world to generate stable cell lines producing a wide range of proteins, from classic mAbs to bispecifics and difficult-to-express fusion proteins.

Until early 2018, this process was slow, taking around 29 weeks (from receipt of the sequence to fed-batch screening for lead clone selection) and involved up to 500 96-well plates which was logistically challenging to handle for the CLD personnel. Substantially reducing these two parameters while maintaining process quality was a primary objective of the CLD department, closely coupled with ensuring improvement for their Master Cell Bank (MCB) proof of clonal origin.

Developing a shorter workflow with reduced FTE time

Generally speaking, in order to produce a clonallyderived cell line providing high productivity for a protein of interest, using random integration methods of cloning, at least a thousand clones are screened in order to have a high probability of finding the best producing clones.
Previously, the CLD department performed an enrichment step through a mini-pool process which, although effective, was both lengthy due to the limiting dilution steps for clonality assurance, and logistically difficult to handle as it involved hundreds of 96-well plates to cover the 1000-plus target clones.
In order to shorten their workflow, significantly increase the likelihood of isolating high producers, and decrease the FTE (Full-time Equivalent) workload, the team needed to be able to enrich cells to reduce the number of clones to be screened, but also have an efficient single cell seeding method ensuring high seeding efficiency and resultant colony outgrowth. Reducing the number of plates would allow for more frequent whole well imaging and provide a more complete clone history to their customers for subsequent regulatory submissions.

The need for gentler fluidics

The department had previously acquired a FACS instrument (BD FACS Aria) in order to enable a much faster enrichment step for top-producer clones, allowing them to by-pass the lengthy minipool enrichment process entirely. Although the FACS was able to sort and enrich for the highest producing cells in bulk, the CHO-K1-derived suspension cells used at Celonic (23-CHO-S) were not able to survive the shear stress caused by using the FACS when attempting single cell sorting in 96-well plates, resulting in low cell survival and outgrowth.

"Our ability to quickly enrich our transfected population using FACS was an incomplete success for the CHO cells as it unfortunately did not enable us to single cell seed plates directly with high viability, the seeding process thus still relying on producing 100 plates through LD in order to obtain ~1000 colonies, a significant proportion of which were not clonal" explained Camille Evenou, Scientist in CLD at Celonic.

Introducing the VIPS™ and Cell Metric® had a dramatic impact

"The VIPS™ is unique, being the only single cell cloning system on the market that tracks a single cell's arrival in the actual well with high resolution (z-stack) imaging and to support the subsequent daily whole well imaging provided by the Cell Metric®" explained Evenou. The group purchased and rapidly implemented the VIPS and Cell Metric in mid-2018. The gentle, low pressure fluidics of the VIPS proved its worth very quickly for the group. Within the first week of testing, the seeding efficiency was impressive. It matched the seeding efficiency of the FACS (~80%, data not shown) without the need for any optimization. However, most impressively outgrowth showed a 2-3-fold improvement over the FACS (Figure 1).

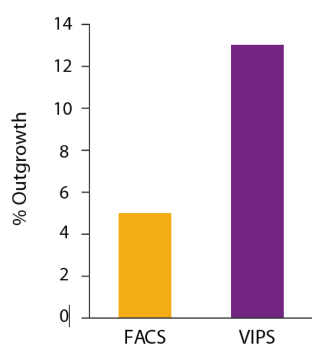


Figure 1 – Comparison of the outgrowth for the CHO cells following single cell seeding by the FACS and the VIPS using basal culture media only with no supplements; outgrowth is defined as the % of clonal wells growing into colonies.

The importance of media optimization

With promising initial results, the team set about tests with several different seeding media and supplements on the VIPS to optimize clonal outgrowth. The improvement was significant: without having an impact on seeding efficiency, the single cell outgrowth more than doubled (see Figure 2).

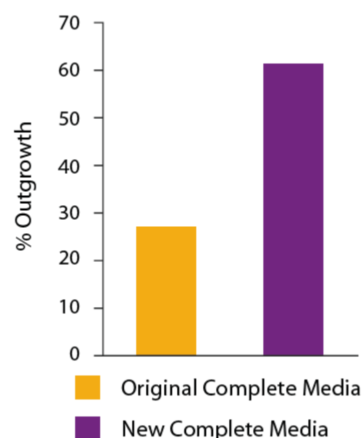


Figure 2 – Outgrowth improvements for different complete seeding media tested on the VIPS; outgrowth is defined as % clonal wells growing into colonies.

More robust proof of a clonally-derived population

When it comes to cell line development for biologics manufacturing, developing high-producing cell lines is of little value without the data to prove that the cell line is clonally-derived. Therefore, proof of a clonally-derived MCB is tantamount to the CLD workflow.

When cloning in a single step, the principal hallmark of this proof usually consists of high quality, whole-well imaging clearly showing that a single cell is present in the well and the next division steps occurring within the next 24 hours or so.

With a reduced number of seeded plates for the same target number of colonies (100 plates from limiting dilution are now down to only 20 with the VIPS), the CLD department was now able to image the freshly seeded plates more frequently (10 time points compared with 4 originally) and build a more complete time course (see Figure 3). This optimized process ensures that every division step is captured for every well which was not possible before due to the large number of plates to image. The department is now easily able to perform this process twice a day following single cell seeding, which is made even more convenient by the ability of the VIPS and Cell Metric whole well imager to communicate with each other and share data to provide the most complete history and documentation of each clone in the form of an Enhanced Clonality Report which can be provided for internal/external regulatory groups.

"The Enhanced Clonality Reports easily generated by the VIPS/Cell Metric software allows us to track both cells

and potential debris from first inception of the single cell in the well, to full-grown colonies enabling us to provide our customers with full traceability and audit trail of each cell line we produce for them with a single, well-crafted document" concludes Evenou.

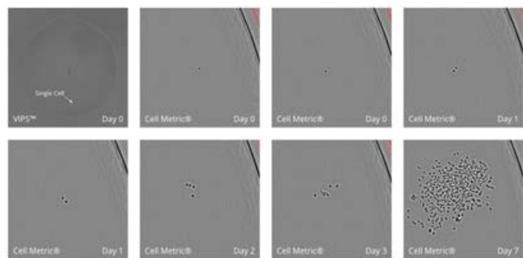


Figure 2 – Outgrowth improvements for different complete seeding media tested on the VIPS; outgrowth is defined as % clonal wells growing into colonies.

Discussion

The VIPS has shown the value for a dedicated single cell cloning system. Whilst a FACS instrument can still provide a potentially important enrichment step (especially for instances of coldcapture methods for secreted proteins), for the subsequent single cell seeding step, the high pressure and shear forces of FACS can be very detrimental to single cell survival and outgrowth. VIPS has demonstrated seeding efficiencies at least equal to the FACS, but with much higher resultant cell survival and outgrowth for these precious clones. This outgrowth has been shown to be even further enhanced using in-house media optimization in this case study, or using commercially available supplements such as InstiGRO™ (for CHO and HEK) sold by Solentim (data not shown).
The successful implementation of the VIPS and Cell Metric combination at Celonic has allowed them to quickly and consistently deliver high producing cell lines to their clients with the most complete documentation for a clonally-derived MCB population, as required by the regulatory bodies. Logistically, the new instruments have saved FTE time with a much shorter (top clones in only 13 weeks prior to the ambr step) and less onerous workflow (see summary in Figure 4), enabling Celonic to to scale out and undertake more client projects in the same timeframe without compromising the quality.

Acknowledgement

We would like to thank Camille Evenou and his colleagues at Celonic AG for putting this case study together with us.

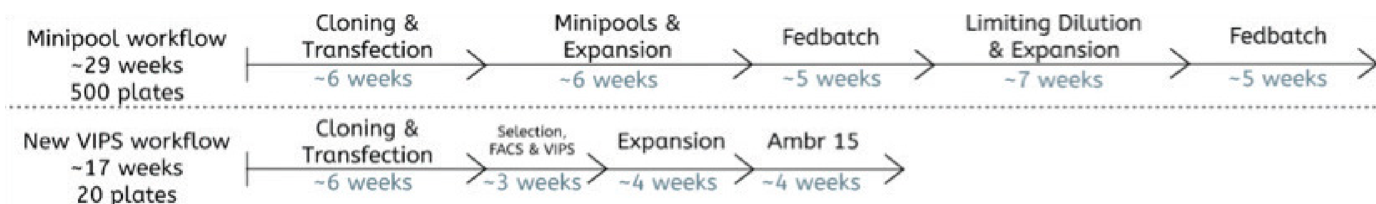


Figure 4 – Comparison of the old workflow using minipools and LD (top) versus the new workflow using FACS for enrichment and the VIPS for the single cell cloning step (bottom).

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