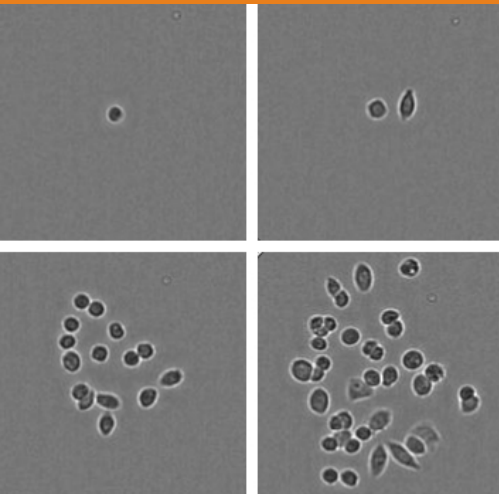


Application Note



Single cell seeding conditions for improved colony outgrowth

Optimising for HD-BIOP3 with the VIPS™ and SAL Scientific InstiGRO™ supplements

Cloning efficiency (colony outgrowth) is often overlooked in the cell line development workflow relative to the single cell isolation step. However, recovery and growth of the single cells is a well-known challenging step in the process.

Introduction

The advent of new gene editing technologies, new cell lines, supplements and automated technologies for single cell cloning are rapidly increasing the efficiency of cell line development, however, there are still numerous factors that can affect a single cells' propensity to divide and continue to divide happily into a clonal cell line after initial single cell isolation.

Determining the best conditions possible for successful single cell outgrowth can be a jigsaw puzzle of factors that include the host cell line, the transfection conditions, method, vector, selection and recovery period before seeding and even the numerous growth condition factors following seeding such as plate type, plate coatings, media types, media volumes.

In order to get the best possible results for the clonal outgrowth it is not as straightforward as selecting one factor (such as the single cell seeding method) for optimisation. Instead, many aspects require investigation to eliminate detrimental factors and enhance the beneficial ones.

All this optimisation will not be in vein as it will ultimately result in greater numbers of clones surviving single cell isolation, reducing the numbers of clones, and hence plates, needed for screening. Altogether, these reduce the time and labour spent processing the clones.

Amongst the many factors, four were considered for this experimentation as aspects key for high cloning efficiency. These are the single cell seeding method, base cell culture media, media supplements and inherent pool differences.

Materials and Methods

The HD-BIOP3 (Horizon Discovery) GS Knockout CHO K1 commercial cell line was used for all experiments. Limiting Dilutions (LD) was compared to the VIPS™ seeding (figure 1). Gibco® CD CHO and CD FortiCHO™ cell culture base media was used for outgrowth with and without conditioned media, InstiGRO™ (SAL Scientific) CHO and InstiGRO™ CHO^{PLUS}. Corning CELLBIND plates (3300) were used for all experiments. In all experiments, 200µl of the cell culture media was added to the well, plates were incubated at 37°C and monitored for outgrowth on Day 0, 1, 7 and 14 using the Cell Metric® whole well imager for clonality and confluence. All colonies (both VIPS and LD) were interrogated for single cell origin as often many colonies seen in LD plates actually derive from 2 or more cells.



Figure 1 – VIPS™ ensures high seeding efficiency

Results and Discussion

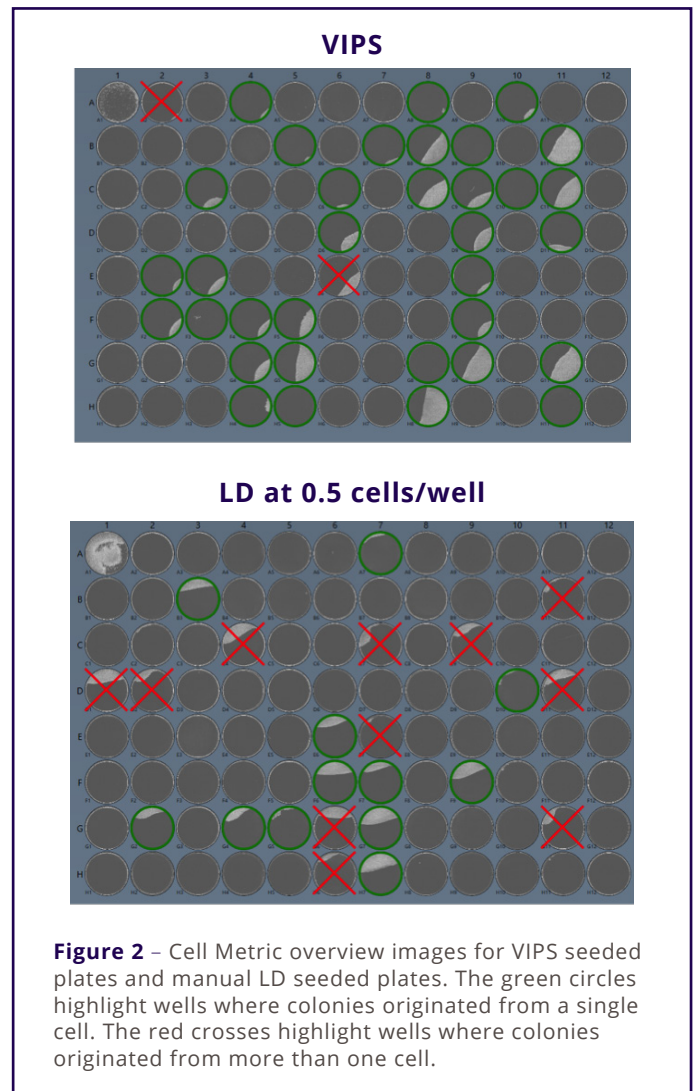
Seeding Method - LD vs VIPS

LD is assumed to be the gentlest method of single cell isolation; however, it is extremely labour intensive, inefficient, and highly variable between scientists. Another well-known practicality, highlighted by the advent of whole well imaging devices, is that, despite the chances of achieving a single cell per well and being relatively consistent with the Poisson distribution, in reality the resulting colonies are more likely to have grown from two cells or more rather than one.

These challenges were a driver for new automated seeding methods such as the VIPS. Using imaged-based cell detection, the chances of achieving a single cell per well are higher and more prevalent than limiting dilution.

With the optimal starting cell concentration, VIPS can routinely achieve 70-85% seeding efficiency, whereas LD can only achieve 30%, assuming the Poisson distribution and using 0.5cells/well concentration. Many LD users use lower concentrations - 0.2 cells/well, for greater (perceived) chance of isolating single cells, or perform multiple rounds of LD.

Figure 2 shows that the VIPS-seeded plate resulted in 33 clonal wells whereas the LD plate resulted in only 12. More importantly there were 11 further wells with colonies in the LD plate that were not clonal (shown with red crosses), without the additional assurance of imaging (both on the VIPS and Cell Metric), would historically have been assumed to be clonal.



Supplements - Conditioned Media vs InstiGRO CHO vs InstiGRO CHO^{PLUS}

We know from experience and the data shown above that some media are considered 'leaner' than others, and hence not always suitable for single cell cloning. Therefore, growth supplements used at the single cell cloning stage become vital for reducing stress on cells and aiding growth into colonies for further assessment. The addition of serum is not an option for most CLD groups, therefore historically conditioned media has provided a boost to aid single cell division, however, it can be an additional step to harvest and then validate the performance of the conditioned media because results can often be variable.

When comparing the VIPS result vs LD result (same growth conditions), it is clear to see that the VIPS is much more efficient at achieving numerous wells per plate with single cells that grow into colonies.

Base Media - CD CHO vs FortiCHO

Often commercial cell lines are provided with the optimal growth conditions; however, this may not be ideal for single cell outgrowth. It may also be the aim for cell line development groups is to keep media conditions as consistent as possible throughout the entire process from CLD to production. However, the appropriate base media may impact the cells' propensity to grow from a single cell and so should be investigated.

Figure 3 demonstrates that the clonal outgrowth result obtained with FortiCHO base media is greater than that obtained with CD CHO base media in the presence of conditioned media.

However, no differences are observed for clonal outgrowth in different base medias supplemented with InstiGRO. This implies that there is a difference between the suitability of the base media, however, by adding enough supplements to sustain single cell outgrowth, the base media-type has reduced impact.

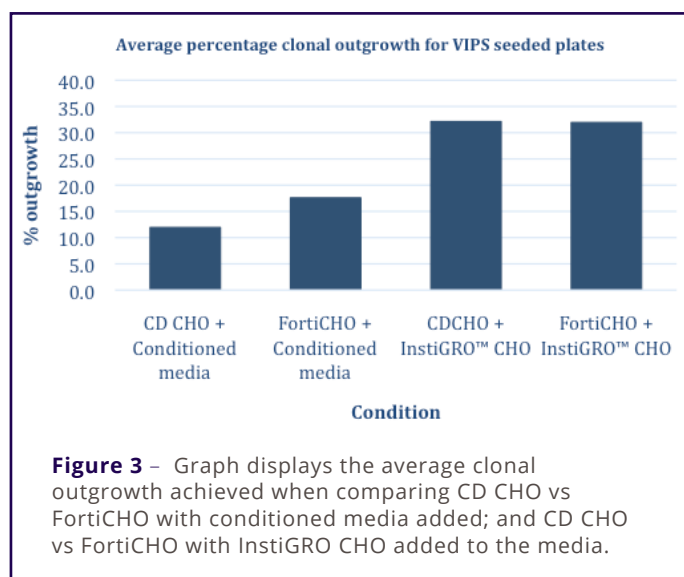


Figure 3 – Graph displays the average clonal outgrowth achieved when comparing CD CHO vs FortiCHO with conditioned media added; and CD CHO vs FortiCHO with InstiGRO CHO added to the media.

Figure 4 demonstrates that regardless of the base media, conditioned media does not perform as well as specifically optimised InstiGRO CHO.

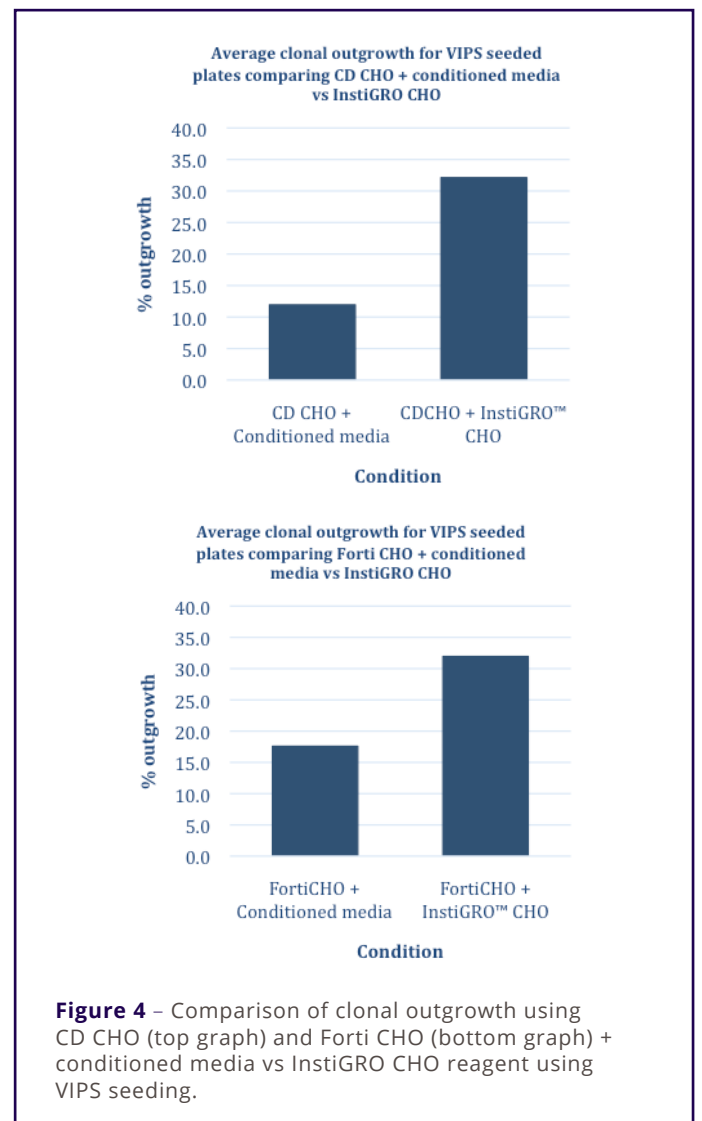
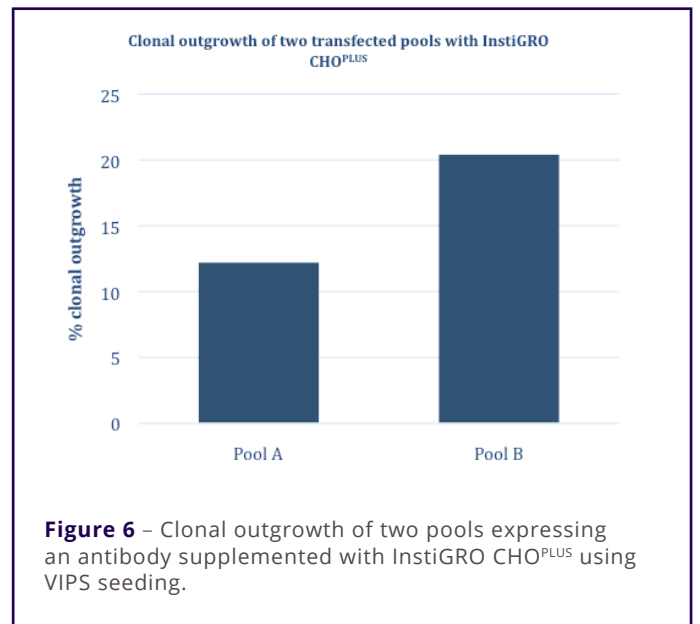
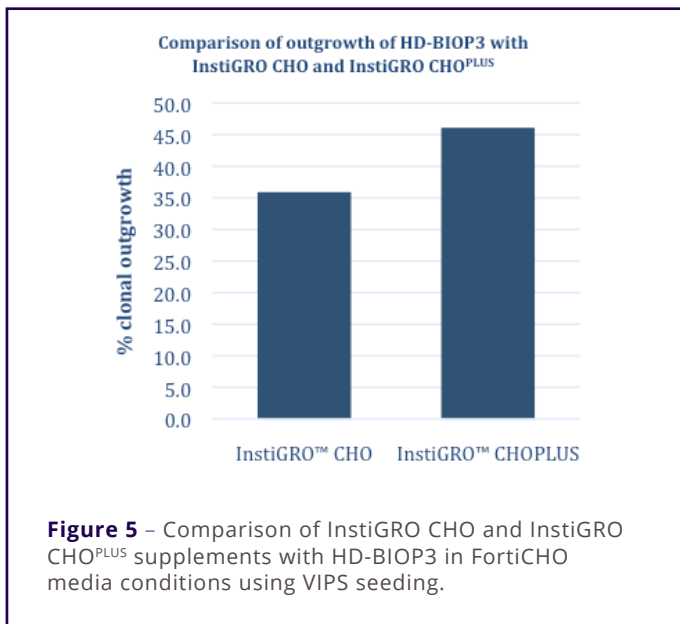


Figure 4 – Comparison of clonal outgrowth using CD CHO (top graph) and Forti CHO (bottom graph) + conditioned media vs InstiGRO CHO reagent using VIPS seeding.

For both CD CHO and FortiCHO, the addition of the supplement over conditioned media has dramatically increased the number of clonal outgrowth wells achieved.

Following on from this, further optimisation of the InstiGRO CHO product has led to the development of InstiGRO CHO^{PLUS} which again shows further improvement on the percentage of clonal outgrowth achieved in this experiment (see figure 5).



Pool Performance

Finally, after optimisation of the parent cell line to establish the best conditions to support single cell outgrowth, it is important to consider the impact of transfection and selection on outgrowth, as the same cell culture conditions may show intrinsic variation for transfected populations. Same cell culture conditions may also still show intrinsic variation. Figure 6 illustrates this, therefore optimisation should be performed on a range of pools/established expressing cell lines.

Conclusion

Data presented shows the importance of considering the numerous factors that can affect single cell outgrowth when optimising growth conditions. The seeding method and instrumentation can dramatically improve the seeding efficiency from the start, but if the conditions and environment surrounding the single cell are not optimal then subsequent outgrowth and cloning efficiency will always be a challenge to improve. The combination of the VIPS and InstiGRO CHO products have dramatically and consistently increased the percentage of clonal outgrowth achieved in these experiments. For future work, we will extend these studies for a range of different commercial cell lines from different providers.