Increasing HD BIOP3 Seeding Efficiencies Using VIPS

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Abstract

Single cell cloning (SCC) and associated cloning efficiency (colony outgrowth) is currently regarded as an important and discrete step in stable cell line development and cell engineering. 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. We have previously demonstrated the capabilities and advantages of the VIPS platform over manual limiting dilution for several cell lines. The VIPS results in higher seeding efficiencies over manual limiting dilution whilst giving confidence in clonality thereby reducing the number of plates required for screening.

In this poster we use Horizon Discovery's HD BIOP3 GS Knockout CHO KI cell line in combination with the VIPS seeding and optimised outgrowth conditions using SAL Scientific InstiGRO CHO reagents to produce a significantly higher number of colonies per plate, thereby reducing the number of plates needing to be screened. The experiment was designed to assess the outgrowth of the HD-BIOP3 cell line by manual limiting dilution and VIPS under a variety of conditions.

Introduction

Cell line optimisation for single cell cloning is a challenging step in cell line development. The VIPS achieves high seeding efficiencies of single cells per plate which is one step in the process.

The VIPS system, is a small bench top instrument that is placed within a standard class II biological safety cabinet for sterility (figure 1) with all tubing and components that come into contact with cells being easily sterilised or replaced, in order to avoid any reliance upon expensive proprietary consumables.

Figure 1: VIPS instrument

The system dispenses a single droplet from the Cell Reservoir, which contains an agitated cell suspension, into the bottom of an empty 96 well followed by immediate imaging of the droplet. The single cell image feedback loop works by assessing these images and determining if a cell was deposited or not. If a cell was deposited, the well will be filled with media immediately. If a cell was not detected, the system will deposit another drop to the side and image again. This process will repeat up to a maximum of 16 times per well. The system uses a stack of images to assess the presence of a cell in the droplet.

The VIPS seeding results are reported in real time so that the user can see which wells have been populated (single cells, more than one cell, or no cell), and VIPS achieves the entire dispensing of a 96 well plate in approximately 10 minutes.

Achieving good outgrowth of high producing clones is also essential. Many factors impact the outgrowth of the cells following single cell cloning ranging from pre-treatment and condition of the cells, the molecule they are expressing/gene edit, the media and reagents for outgrowth amongst many others. The process of cloning itself introduces stress on the cells as they are isolated. Cell line optimisation is therefore a vital part of any CLD process to ensure good recovery of clones.

Here we demonstrate the combination of the high seeding efficiency of the VIPS in reducing the required plates for screening HD BIOP3 GS Knockout CHO KI cell line with InstiGRO CHO reagents for boosting outgrowth.

Methods and Materials

Overview of method carried out:

- Cells were passaged 24hrs prior to seeding.
- On the day of seeding cells were diluted to 9,500cells/ml in OptiCHO (Gibco).
- These cells were then used for VIPS seeding with the appropriate growth media dispensed following seeding.
- For manual LD the 9,500cells/ml in OptiCHO were further diluted to 2.5 cells/ml in the appropriate media (CD CHO (Gibco) or FortiCHO (Gibco) with conditioned media or InstiGRO CHO) for manual LD.
- 5 x 96 well plates per condition were seeded.
- The same plate type (Corning Costar 3300) was used for all conditions, the final well volume for all was 200µl.
- Cells were incubated in the same incubator at 37°C 5% CO2.
- Plates were imaged on the Cell Metric 2hrs after seeding (day 0) and on days 1, 7, and 14.
- Images were assessed by eye to confirm clonality for manual LD and VIPS.
- Results displayed are the average of the 5 plates.
- Comparison of InstiGRO CHO and InstiGRO CHO^{PLUS}.
- Seeding conditions were identical to those described in the first experiment.
- Base media was FortiCHO supplemented with InstiGRO CHO or InstiGRO CHO^{PLUS}.

Results and Discussion

Comparison of manual LD to VIPS seeding with different base medias and conditioned media vs InstiGRO CHO.

VIPS seeding of the HD-BIOP3 cell line with InstiGRO for outgrowth resulted in approximately twofold increase in clonally derived colonies per plate when compared to manual limiting dilution (Figure 2). In this case, clonal outgrowth values are taken from a whole 96-well plate to allow comparison of manual LD to VIPS seeding.

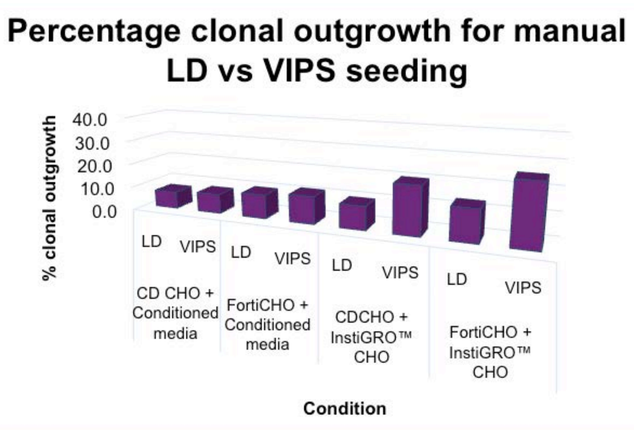

Figure 2: Comparison of clonal outgrowth following manual LD and VIPS seeding with conditioned media and InstiGRO CHO.

The VIPS reports single cell seeding, therefore outgrowth can be calculated as a percentage of the known seeded, discounting empty wells (Figure 3).

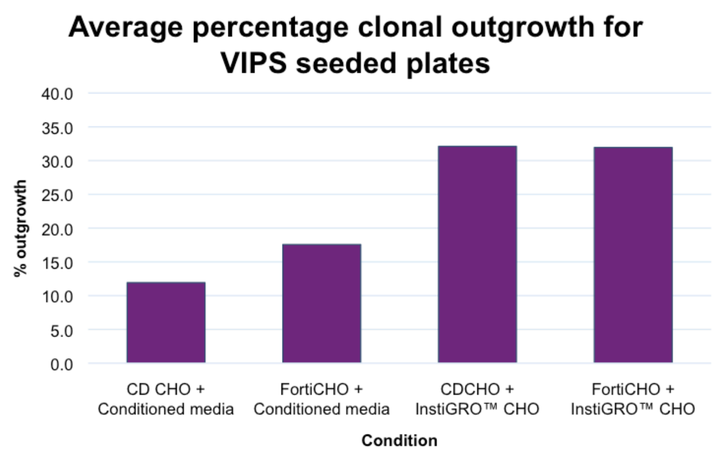

Figure 3 Comparison of clonal outgrowth when using conditioned media and InstiGRO CHO reagent with VIPS seeding.

InstiGRO CHO resulted in clonal outgrowth of 32% when compared to the used of conditioned media alone (12-17%), thereby doubling the number of clonal colonies achieved per plate. VIPS seeding also resulted in reduction in the number of colonies derived from multiple cells when compared to plates seeded manually (Figure 4).

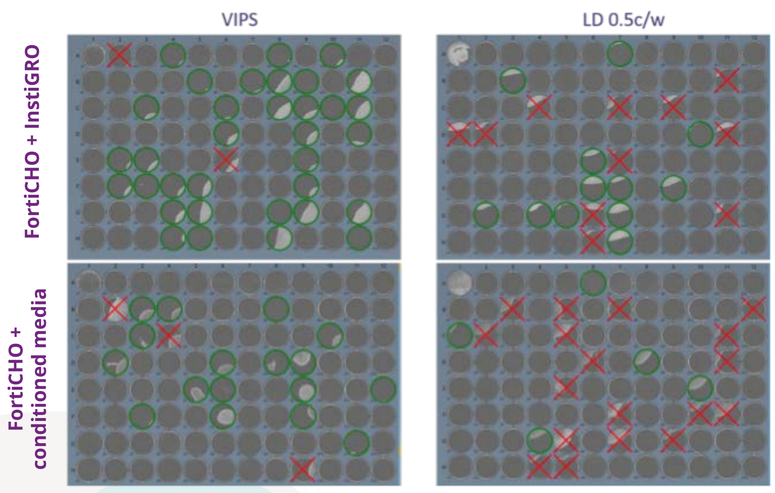

Figure 4 Cell Metric overview images for VIPS seeded plates and manual LD seeded plates. The red crosses represent wells where colonies arose from more than one cell.

The data in figure 4 demonstrates that VIPS seeding results in a higher number of clonally derived colonies per plate and that InstiGRO CHO improves outgrowth two-fold when compared to conditioned media for Horizon Discovery's HD BIOP3 GS Knockout CHO KI cell line, thereby reducing the number of plates requiring screening.

Comparison of InstiGRO CHO and InstiGRO CHOPLUS

Further optimisation of VIPS seeding for the Horizon Discovery HD BIOP3 GS Knockout CHO KI cell line was carried out comparing InstiGRO CHO to a new version of the supplement; InstiGRO CHO^{PLUS}, using FortiCHO as the base media (Figure 5).

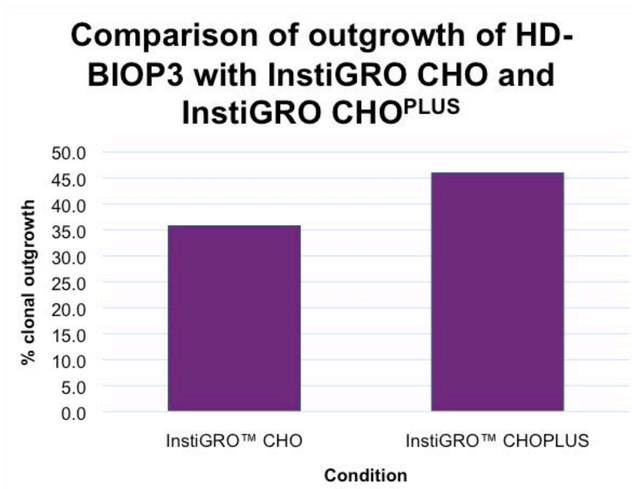

Figure 5 Comparison of InstiGRO CHO and InstiGRO CHO^{PLUS} reagents with Horizon Discovery's HD BIOP3 GS Knockout CHO KI cell line.

InstiGRO CHO^{PLUS} resulted in a 10% increase in clonal outgrowth when compared to InstiGRO CHO, resulting in 46% of single seeded wells forming colonies. In order to test the conditions optimised on the parent cell line, the performance of InstiGRO CHO^{PLUS} was then assessed with two pools expressing a relevant molecule (Figure 6).

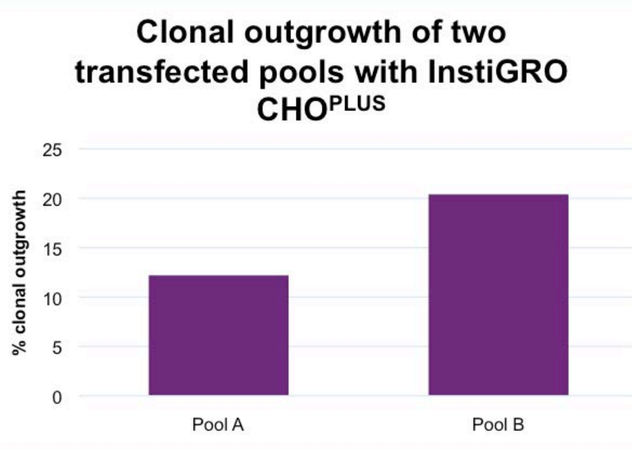

Figure 6 Clonal outgrowth of two pools expressing a molecule supplemented with InstiGRO CHOPLUS.

As Figure 6 demonstrates InstiGRO CHO^{PLUS} supplementation results in between 12 and 21% clonal outgrowth of pools expressing a relevant molecule. The results also demonstrate the impact of expression of a molecule on clonal outgrowth and highlights the importance of optimising conditions in order to achieve as high a possible clonal outgrowth. It also demonstrates the performance difference between pools expressing the same molecule and derived from the same parent cell line.

Figure 7 demonstrates an example of one of the clones from seeding (Day 0) through to day 7 growth, as well as an example of VIPS identifying a well will multiple cells seeded within a drop (7d)

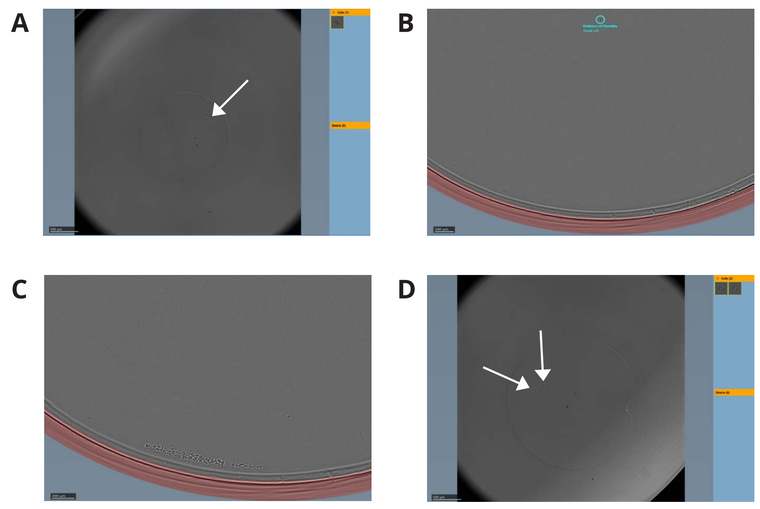

Figure 7: A-C are an example of a single seeded cell forming a colony, a is the VIPS seeding image, B is the day 0 image on the Cell Metric and C is the day 7 image. D is an example of a well seeded with multiple cells identified by VIPS.

Conclusion

Overall, our data show that the combination of the VIPS for high seeding efficiency and the improved outgrowth conditions using the cloning supplement with Horizon Discovery's HD BIOP3 GS Knockout CHO KI cell line is a powerful platform for cell line development. The increased number of single cells seeded per plate and the improved outgrowth using InstiGRO CHO and InstiGRO CHO^{PLUS} enables the number of plates required for screening to be significantly reduced.