ADVANCED INSTRUMENTS

Application Note

Clonality and improved outgrowth for HEK 293 cells when developing stable producer cell lines for viral vector production in gene therapy

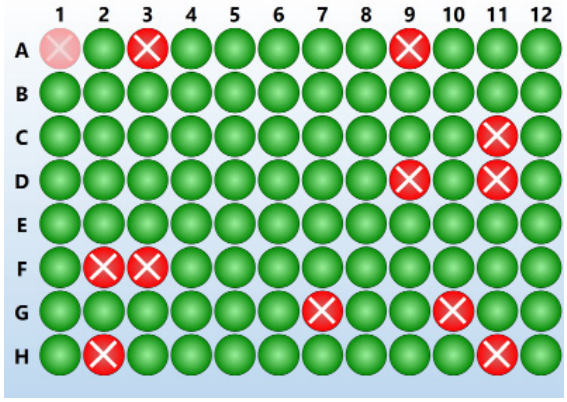
Introduction

Gene and cell therapy workflows frequently utilise HEK293/293T cells in the production of viral vectors. As was described in the 2020 FDA guidance on CMC and INDs for cell and gene therapy, both regulators and manufacturers alike are looking to standardize best practices around viral vector production workflows (1). With a keen eye on patient safety, avoiding the use of animal-derived reagents and using assurance of clonality as part of production quality control was highlighted (2).

We evaluated two technologies, both already well accepted in CHO-based cell line development workflows, for their suitability with HEK cells. These technologies were the Verified In-situ Plate Seeding (VIPS™) instrument, a high efficiency, single-cell seeder providing image-based regulatory assurance, and an animal-free cell growth supplement, InstiGRO™ HEK (SAL Scientific) designed to support early single-cell growth, both commercially available from Solentim.

Materials and Methods

The VIPS instrument was used to seed single cells from four different HEK cells lines, 293T (ECACC), HEK293 (ECACC), Exp293F™ (Thermo Fisher) and HEK293 6E (courtesy of GSK) into Corning #3596 96 well plates. As per the VIPS workflow, evidence of the existence of a single cell in each well was gathered immediately after seeding (by way of Z-stack imaging and analysis in the droplet) and by whole well imaging post-media fill at day 0. Wells were imaged on a regular basis following seeding, recording images of colony growth. Clonal outgrowth (percent of single cells growing into colonies) under different cell media conditions was evaluated. Expi293,



VIPS™ for high efficiency seeding of HEK 293 cells

293T and HEK293 cell lines were grown in base media supplemented with 30% conditioned media only, and base media plus InstiGRO HEK.

HEK293 6E cells were tested under three media conditions: Balan CD HEK 293 Irvine (Irvine Scientific) cell culture basal media plus InstiGRO HEK, media plus 25% conditioned media, and media, 25% conditioned media and ITS supplement (Stem Cell Technologies).

VIPS automated single cell seeder with image based regulatory assurance

Results

The first key point is seeding efficiency. VIPS was found to effectively identify single HEK cells both immediately in the droplet post-dispensing and in the whole well images post media fill. Together this data established a 'double lock' of assurance that can be used as evidence of clonality.

HEK293T cell in the VIPS droplet

Corresponding HEK293T cell as observed in whole well imaging at day 0

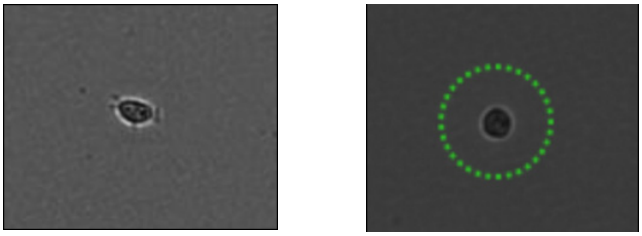


Figure 1. Imaging of HEK293T following VIPS seeding. High seeding efficiency (% of wells with a single cell) was recorded for all cell types (see table 1)

Compared to the manual limiting dilution (LD) seeding approach at 0.5 cells/well concentration where there is an expected 30% seeding efficiency, VIPS recorded between 62% and 83% seeding efficiency. On a practical level, this 2-3-fold increase over LD would result in a significant reduction in the number of plates required to meet the same experimental aims.

The second key point is the significant improvements in survival and outgrowth of single HEK293 cells using the InstiGRO HEK growth supplements. Clonal outgrowth was monitored using the Solentim Cell Metric® whole well imager. Figure 2 illustrates the increased outgrowth seen with the addition of InstiGRO HEK to 293T cells post seeding with VIPS, while Figure 3 shows the percentage increase in clonal outgrowth resulting from the addition of InsitGRO HEK for all cell types.

Cell Type	Media Type	Average Seeding Efficiency from at least 3 plates at different conditions

Table 1. Average Seeding Efficiency using the VIPS and different conditions

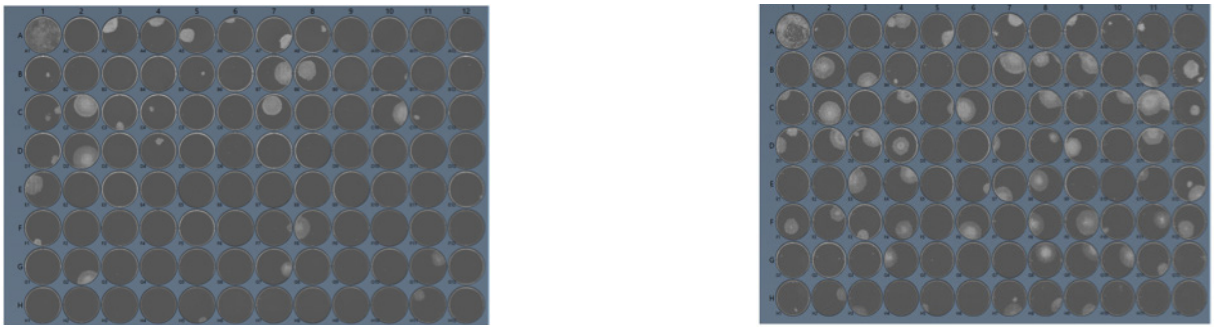


Figure 2. Clonal outgrowth images with and without InstiGRO™ HEK cell culture supplement. InstiGRO HEK had a significant positive effect on clonal outgrowth in all conditions tested, with increase in outgrowth ranging from a factor of 2.7 to 11.5

Effect of Cell Culture Supplement on Clonal Outgrowth

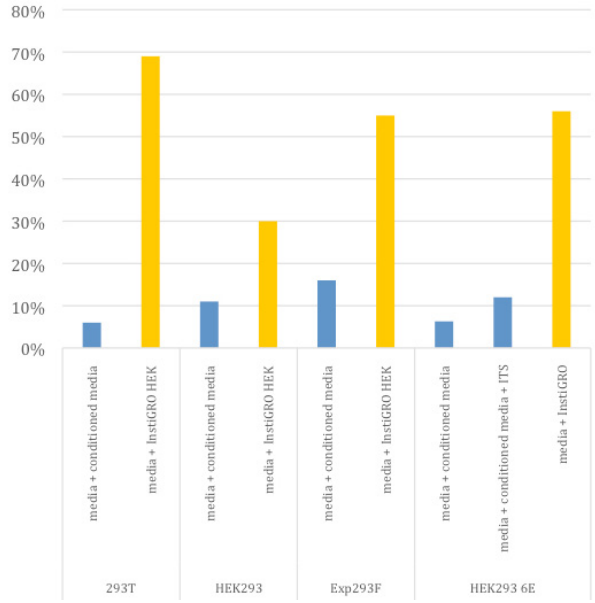


Figure 3. Comparison of clonal outgrowth with and without InstiGRO HEK cell culture supplement

Conclusion

As more gene therapy products come through clinical trials there will be an increased demand to move to stable producer cell lines for the production of viral vectors. The use of VIPS and InstiGRO HEK were found to be a strong combination for developing HEK based cell lines whilst delivering on the regulatory demands for clonally-derived Master Cell Banks.

Acknowledgements

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References

1. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)Guidance for Industry, January 2020 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapyinvestigational-new-drug
2. https://www.solentim.com/news-insights/understanding-the-latest-fda-guidance-on-investigational-newdrugs-for-cell-and-gene-therapy/

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