ADVANCED INSTRUMENTS

Application Note

High efficiency single cell cloning of iPSCs using combination of MatriClone™ and VIPS™

Figure 1. The VIPS™ (Verified In-Situ Plate Seeding)

Introduction

Induced pluripotent stem cells (iPSCs) have practically unlimited proliferation potential and a capability to differentiate into any cell type in the human body. Such is their power, they have become a major focus in nearly all disease areas and a vehicle for future therapeutic especially when combined with gene editing technologies.

As the interest in the therapeutic value of iPSCs accelerates, so does the need to establish the regulatory framework and best practice in this area. Cell line development workflows using CHO cell types are well established in the production of monoclonal antibodies. In this area, there is a widely accepted regulatory expectation to use single cell cloning. By starting from a defined source cell variability, drift and ultimately product quality is best managed. For iPSCs, it is logical to explore methods to manage quality and overcome existing process hurdles by developing robust seeding and evidence of clonality methodologies.

Limiting dilution, with a theoretical 30% seeding efficiency at 0.5 cells / well concentration has routinely been replaced in commercial laboratories by single cell seeding technologies. VIPS™ (Verified In-Situ Plate Seeding) (Figure 1) is a well characterized high efficiency single cell seeding system from Solentim. Uniquely, the instrument offers the capture of evidence of clonality both immediately after dispensing into the plate well and post media fill, the so called 'double lock' of assurance. The system additionally provides daily whole well imaging to record further evidence of clonal outgrowth.

Materials and Methods

Two different iPSC commercially available cell lines were used in the evaluation of two different cell matrices, MatriClone and in Matrigel, dispensing cells in normal media into plates pre-coated with matrix or dispensed 'in- solution' with the matrix. Percent confluence was measured after 5 days.

To compare manual limiting dilution and VIPS methods for clonal outgrowth, iPSCs were dispensed in media containing MatriClone by each method and % of verified single cells that grew into colonies at day 14.

To establish the maintenance of pluripotency of the iPSC cells when using MatriClone, immunocytochemical staining for the markers Oct4 and Nanog was used following 10 passages post-VIPS seeding.

Results

Increased outgrowth and confluency for iPSC clones using MatriClone in-solution versus MatriGel

In a comparison of 'in-solution' and 'pre-coated' methodologies with two iPSC lines, MatriClone was found to have the effect of increased average well confluency at day 5 when compared to MatriGel and to have improved 'in-solution' performance when compared to being used as 'pre-coated plates' (see Figure 2).

Average confluency at day 5 using MatriGel and MatriClone using in-solution or pre-coated methodologies fluency

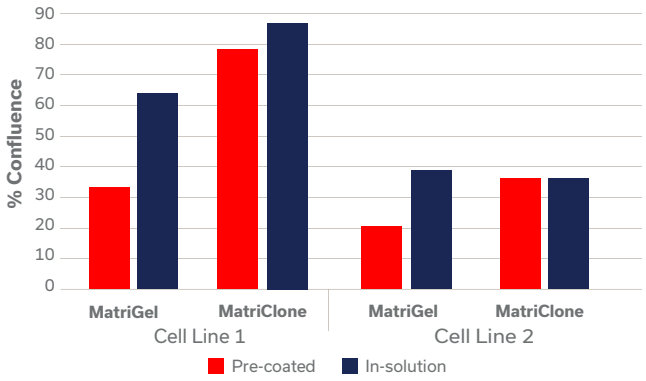

Figure 2. Average of % confluency after 5 days growth, measured from 3 wells, in two different additives either with cells seeded using limiting dilution into a pre-coated well or dispensed with matrix already in solution.

2.5 fold increase in outgrowth when using VIPS in combination with MatriClone

When using VIPS to dispense iPSC cells with MatriClone in solution, a further 2.5 fold improvements in clonal outgrowth was observed (see figures 3 and 4). This is in line with general observations from using the VIPS system vs limiting dilution from cell lines including CHO and HEK (data not shown).

Clonal Outgrowth of iPSCs using different seeding mechanisms

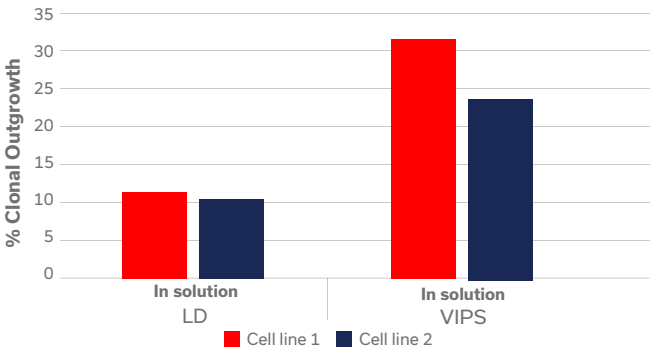

Figure 3. % clonal outgrowth from 96 well plates seeded manually at 0.5 cells/well versus using the VIPS with MatriClone in solution

Example plate overviews from VIPS software

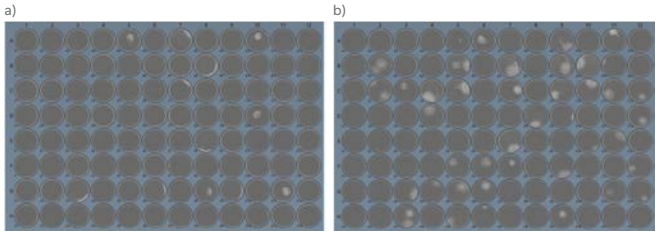

Figure 4. Image captured using VIPS instrument at day 14 as an example of enhanced colonies from a) LD seeded plates and b) VIPS seeded plates

Ensuring Maintenance of Pluripotency

Both in the passaging study and post VIPS seeding, iPSCs cells showed positive and strong staining for both Oct4 and Nanog demonstrating maintenance of pluripotency (see Figure 5).

Stability of iPSCs was found to be maintained throughout the VIPS/MatriClone workflow by way of karyotyping / G-banding assessment (data to be published).

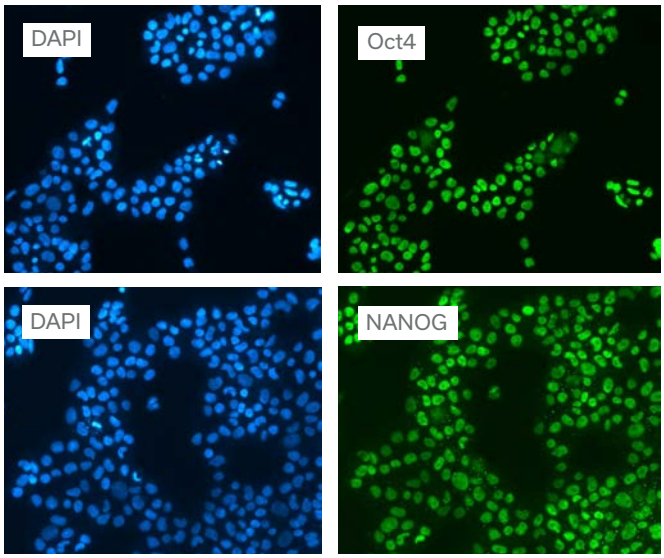

Figure 5. Immunohistochemical staining of cells for Oct4 and Nanog of cell line 1 following 10 passages with MatriClone used in solution

Conclusion

As the applications for iPSCs grow, there will be increasing needs for standardisation in the generation of clonal iPSC Master Cell Banks. This will be true for gene edited cell lines in research through to the generation of iPSC therapeutic cell lines. VIPS is a well-established commercial platform for generation and documentation of clonal cells lines for CHO and HEK.

MatriClone is a novel 'animal-free' matrix designed to support iPSCs in their early growth and was found inthis study, especially when used 'in-solution' in conjunction with VIPS, to be an effective turnkey solution for generating significantly more viable iPSC clones per plate along with image documentation for their clonal derivation.

Product Code	Product Name	Description

Products utilize Matrixome technology developed by Professor Kiyotoshi Sekiguchi
This product is not available for sale in Japan, Taiwan and Korea

References

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Two Technology Way/ Norwood, Massachusetts 02062, USA

800-225-4034 | 781-320-9000 | www.aicompanies.com

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