PROCESS IMPROVEMENT DELIVERED BY A HIGH EFFICIENCY, AUTOMATED SINGLE CELL CLONING SYSTEM

Andrea Gough, Solentim
Andrea.Gough@Solentim.com
Erika Parkinson, Solentim
Ian Taylor, Solentim

Key Words: Single Cell Cloning, Clonality, Regulatory, Limiting Dilution, CRISPR/Cas9

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. Even with improved targeted gene editing like CRISPR/Cas9, many hundreds of single cell clones still need to be created and analyzed.

For many laboratories, flow cytometry (FACS) or limiting dilution (LD) is the current method of choice for single cell deposition, however, each method has its drawbacks. When using FACS, very high seeding efficiency results can be achieved but often at the expense of cell survival and growth due to the harsh treatment of the cells during processing. When using LD, the reverse is the case; whereby good cell survival can be achieved but at low seeding efficiencies. These intrinsic inefficiencies often result in a combination of the two processes being implemented and used on a case by case basis; FACS for robust cell lines and LD for sensitive cell lines. In addition to these drawbacks, neither of these processes offer independent confirmation of the single cell deposition.

In this poster, we introduce a new dedicated SCC platform called Verified In-Situ Plate Seeding (or VIPS™), which combines gentle single cell deposition with concurrent *in-situ* image verification of the single cell in a well. VIPS uses a proprietary process of SMART™ LD whereby the cell droplet is imaged immediately on dispensing in the well to confirm presence or absence of a single cell.

We will present data comparing the performance of SMART LD versus standard LD with a selection of commonly used commercial cells lines: CHO-S, CHO-K1, CHOZN® (Sigma) and various other suspension and adherent cell lines. Additional data will be presented to show the process of SCC method validation using the system's on-board fluorescence. Data will show improved seeding efficiency over standard LD by up to 100% without any compromise in cell viability, as will be demonstrated by improved outgrowth results.

VIPS droplet data will form part of an enhanced Clonality Report documentation package for the regulator which tracks the colony history further back to the single cell in the dispensed droplet. Data review will be streamlined to focus on the most valuable wells which formed colonies and which were confirmed as single cells from droplet dispensing. The software will additionally generate important intra-experimental feedback metrics such as the actual incidence of "ghost" wells.