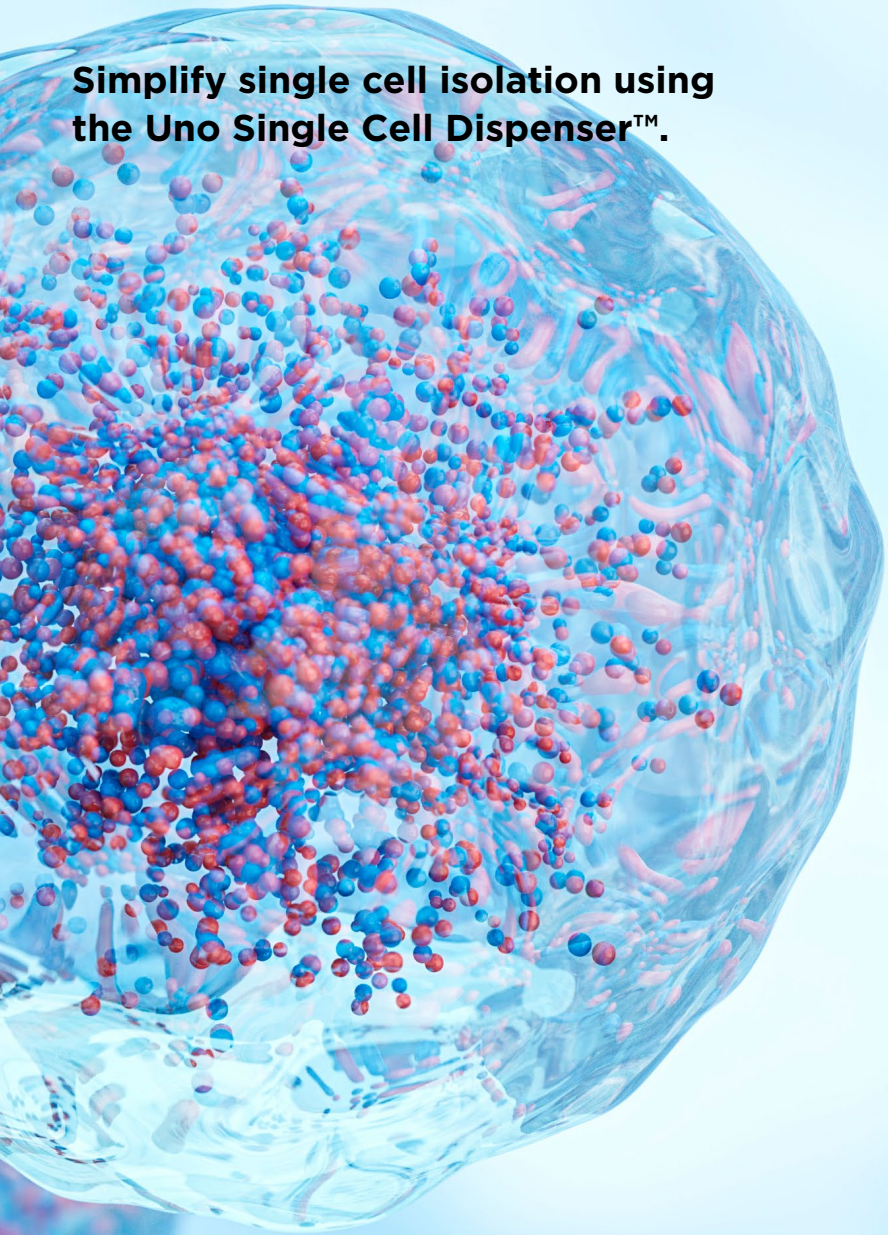
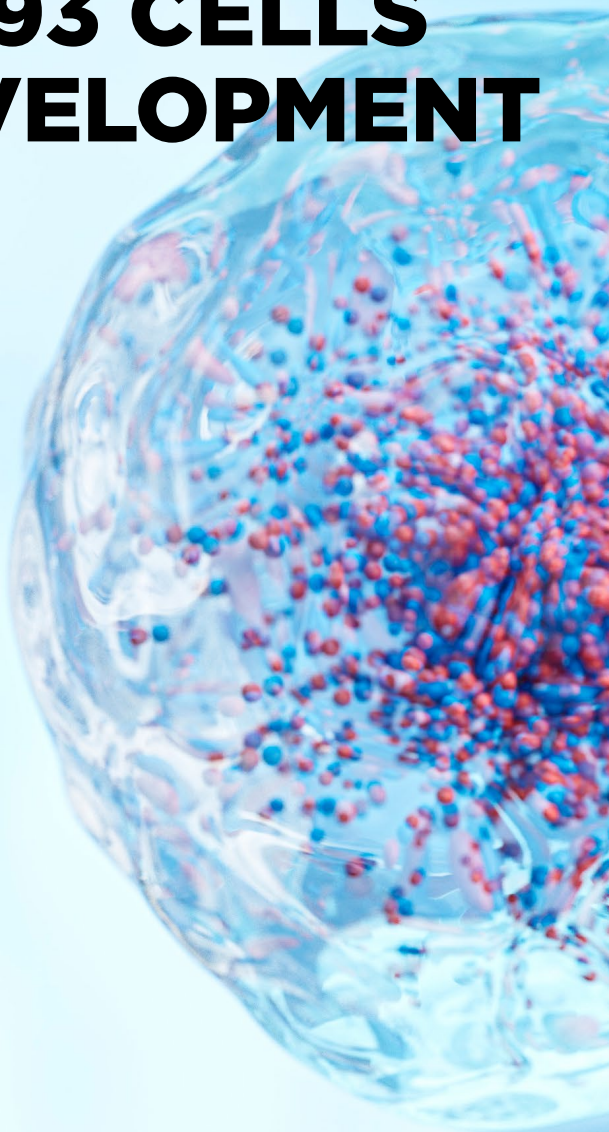
WHITE PAPER

ISOLATE BRIGHT GFP-EXPRESSING HEK293 CELLS FOR CELL LINE DEVELOPMENT WORKFLOW.

Simplify single cell isolation using the Uno Single Cell Dispenser™.





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INTRODUCTION.

Clonal isolation of single cells is a common method in the field of cell biology, and is used to generate cell lines carrying specific genetic mutations for numerous cellular, molecular and biochemical applications. The resulting cell lines can be used to explore cellular signaling pathways, or simply to generate fluorescently-tagged cells for imaging or sorting functions. A single cell dispenser is a fast, efficient way to generate single cell clones with high fidelity and throughput. Here, we use the Uno Single Cell Dispenser to isolate single HEK293 cells expressing green fluorescent protein (GFP) to varying degrees. Cells were isolated and expanded, and clones were characterized with different levels of GFP expression, enabling the selection of HEK293 lines expressing low and high levels of GFP. Differential expression of fluorescent proteins in a population of cells is dictated by promoter methylation, but uniform expression is desirable for many cell imaging applications. The brightest GFP-expressing HEK293 cell clones were expanded for use in single cell tracking experiments, and their brightness and stability analyzed compared to a standard cell tracking dye.

METHOD.

This simple single cell cloning approach is not limited to GFP-expressing vectors, and can be used to generate stable cell lines with other genetic modifications. Clonal isolation begins by preparing a dispensing protocol, where a cell fluid is defined and applied to a microplate for dispensing. It is critical to select a cell fluid class that corresponds to the cells being isolated. For HEK293-GFP cells, the 'small' cell fluid class should be selected, to isolate 12-14 µm cells.

Workflow overview for clonal isolation of GFP-expressing HEK293 cells.
HEK293 cells expressing GFP at variable rates were isolated as single cells with the Uno Single Cell Dispenser, and expanded into colonies for 4 weeks (Figure 1).

Single cell dispensing protocol.
The 'small' cell fluid class was used to isolate 12-14 µm cells, corresponding to the average HEK293-GFP cell size (Figure 2a). This cell fluid was applied to a 24-well plate with set value dispensing of 1 cell/well (Figure 2b) to create a dispensing protocol.

Materials.
C1a Single Cell Dispensehead Cassette and cell suspension.

Assess GFP brightness and stability.
A plate reader with a 450 nm excitation filter and 535 nm emission filter was used to examine GFP expression normalized to total protein. This was compared to the GFP brightness of cells stained with calcein AM according to the manufacturers protocol.

Materials.
Lysis buffer (1 % Tween® 20/DPBS without Ca^{2+}/Mg^{2+}), Rapid Gold™ BCA kit (protein normalization), calcein AM stained cells for brightness reference, plate reader.

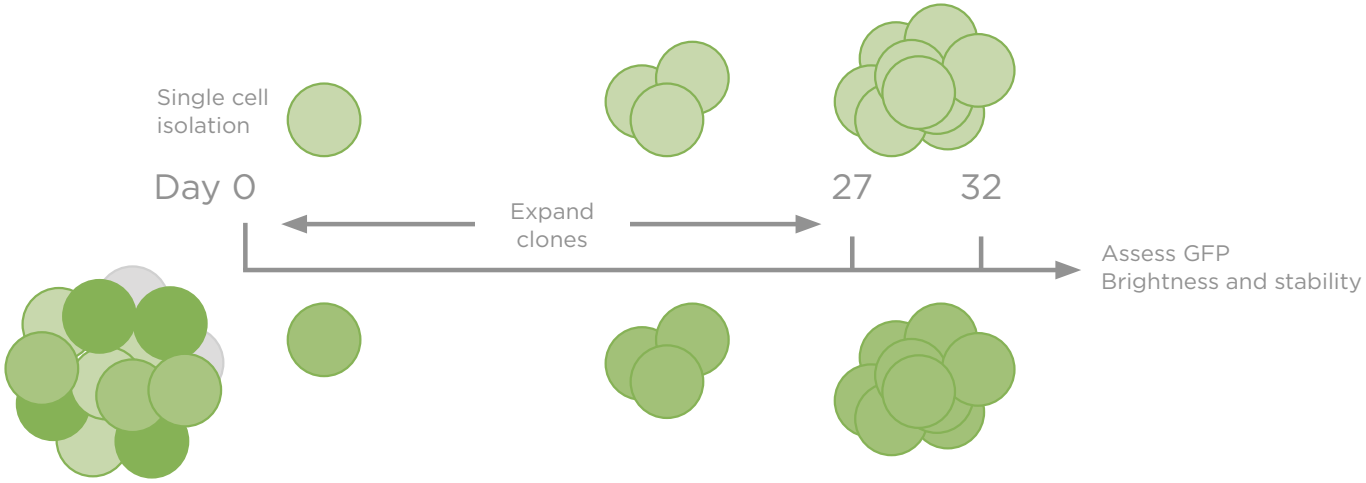
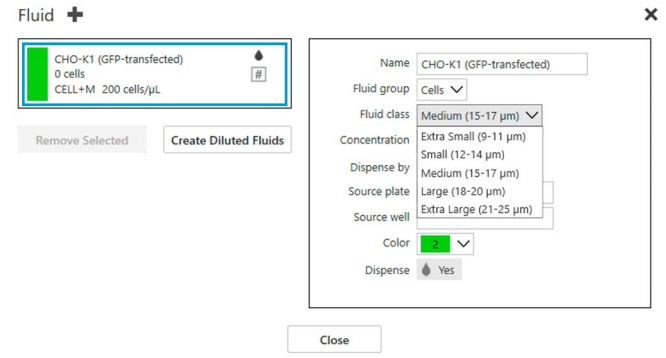


Figure 1: Timeline of single cell cloning experiment from isolation and expansion. Two HEK293 clones with different GFP expression levels are represented on top (low GFP) and bottom (High GFP).

A



B

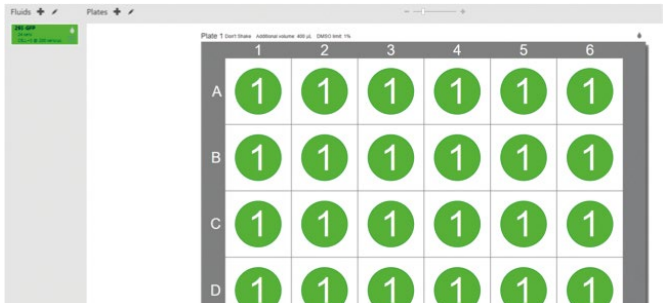


Figure 2: Selection of A) a cell fluid class (size), and B) a 24-well plate cell dispense protocol.

RESULTS.

HEK293-GFP cells with variable GFP expression rates (Figure 3, red and yellow outlined cells) dispensed with the cell dispenser were isolated with a 91 % single cell success rate (Figure 4). 10 clones were expanded into colonies, and GFP fluorescence levels were examined by microscopy (Figure 5). To quantify GFP levels across the clones, GFP fluorescence in cell lysates was analyzed on a plate reader, normalized to total protein and compared to cells stained with calcein AM (Figure 6). Clone 10 had the highest levels of GFP, approximately 7x higher than clones 1, 3, 8, and 9. Fluorescence was examined across multiple days (1 and 4 post-plating) to ensure expression stability, and also examined after cryopreservation for clone #10 and found to be stable (Figure 6).

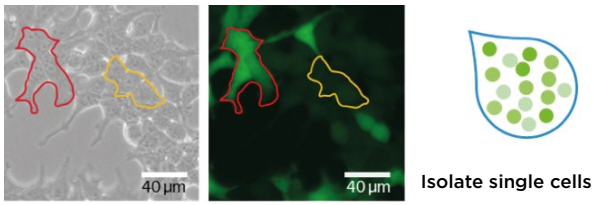


Figure 3: Image of HEK293-GFP variability.

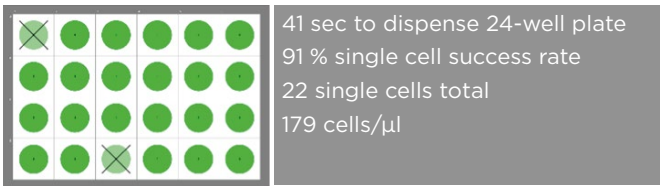


Figure 4: Single cell dispense report automatically generated from UnoControl software.

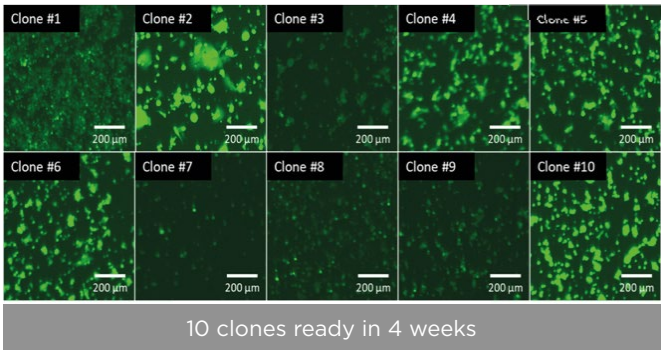


Figure 5: GFP fluorescence across 10 clones.

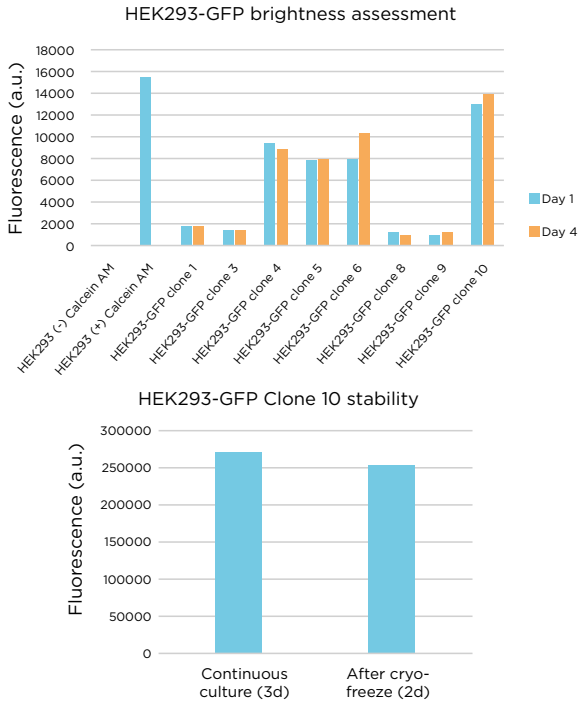


Figure 6: GFP expression comparison and stability. Clone 10 has the highest GFP expression, and will be used for cell tracking experiment.

CONCLUSION.

- HEK293 cells stably expressing GFP were generated by using Uno Single Cell Dispenser for the cell isolation step.
- Uno Single Cell Dispenser provides a high throughput system for efficiently distributing individual cells for cloning and cell line development.
- A 91 % success rate in isolating transfected HEK293 cells across a 24-well plate was achieved.
- The HEK293 cells showed stable fluorescence over time and after freeze-thawing.

Figure 7: The Uno Single Cell Dispenser is a compact, automated benchtop device engineered for simplicity. It gently and accurately dispenses single cells or reagents, delivering volumes from picoliters to microliters within minutes per 384 well plate.

ABOUT THE AUTHORS.

Dr Matthew Blake *is an Application Scientist at HP helping to develop and expand HP's microfluidic technology footprint in the Life Science research space. He received his PhD in Chemical Biology from Oregon Health & Science University where his research explored novel mechanisms of peripheral nerve regeneration.*

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