Sub-Confluent Sub-Clonal Single Cell Isolation Workflow for Efficient Establishment of Clonal Cell Lines from Transfected Cells

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Abstract

Custom clonal transgenic cell lines are powerful tools in cell and molecular biology. Current methods of establishing clonal transgenic cells lines are long, arduous and can be harsh on cells. Using the cellenONE® X1 BSC, individual GFP+ cells were isolated from a pooled sample of transfected cells into a 384-well plate. After 7-10 days emerging, sub-confluent colonies were dissociated and individual GFP+ cells were either used to sub-clone the colony, or to generate single-cell DLP+ libraries using Cellenion's cellenCHIP workflow. Subsequent NGS identified the transgene insertion location and verified the clonal status of colonies from each round of sub-cloning. This process was repeated until stable, genetically homogenous cell lines emerged, a process that by traditional methods can take as long as 4 months. The high replating efficiency and low cell numbers needed for each sub-cloning step resulted in significantly faster cell line generation using the cellenONE® X1 BSC.

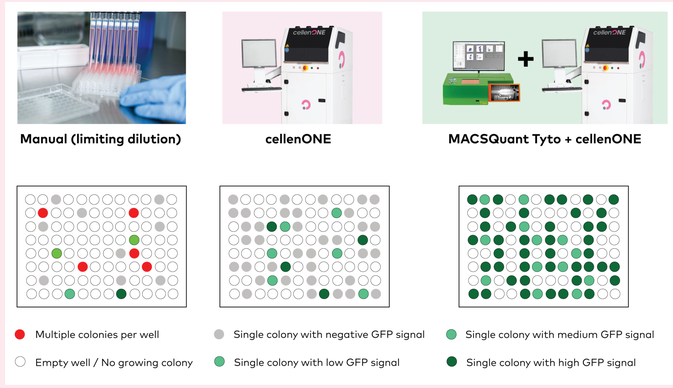
Background

A Industry Timeline for Cell Line Development

Stable Cell Line Model	Approach	Deliverable	Turnaround
Gene overexpression	Lentivirus-based	Pooled cells	9-15 weeks
		Single clone	12-20 weeks
shRNA gene knockdown	Lentivirus-based	Pooled cells	8-13 weeks
		Single clone	11-18 weeks
Tet inducible gene expression	Lentivirus-based	Pooled cells	10-16 weeks
		Single clones	13-21 weeks
Gene knockout	RNP complexes CRISPR/Cas9-based	Single clone	9-15 weeks
	Lentiviral CRISPR/Cas9-based	Single clone	10-17 weeks
Gene knockin	RNP complexes CRISPR/Cas9-based	Single clone	14-19 weeks
Point mutation	Regular plasmid CRISPR/Cas9-based	Single clone	13-19 weeks

Information provided by VectorBuilder

B



Cell line development is a tedious process that requires a great time commitment to staff and scientists and substantial funds to support the process. This commitment makes in-house production of cell lines unfeasible for most academic institutions resulting in the outsourcing of the cell line development to companies. **(A)** With dedicated facilities, the process of going from construct creation to a verified clonal line can take up to 20 weeks using standard procedures. While many parts of the process are not easily expedited, such as plasmid construction and screening, a large portion of time is dedicated to screening and growing up of colonies to establish clonal lines. **(B)** The cellenONE® seeks to address the long screening times required for clonal cell line development. The cellenONE® is an ultra-low volume image-based single cell isolating machine, capable of isolating individual cells into a 96-well plate in approx. 3 minutes. Using the cellenONE's fluorescent detection system, individual cells can be rapidly isolated based on fluorescent expression with near 100% accuracy. With the ultra-low volume dispensing technology, scientists and staff can save money on costly reagents and prevent human errors during the process. The acoustic-based isolation method is gentle on the cells and surpasses established equipment when looking at the survivability of isolated cells.

Methodology

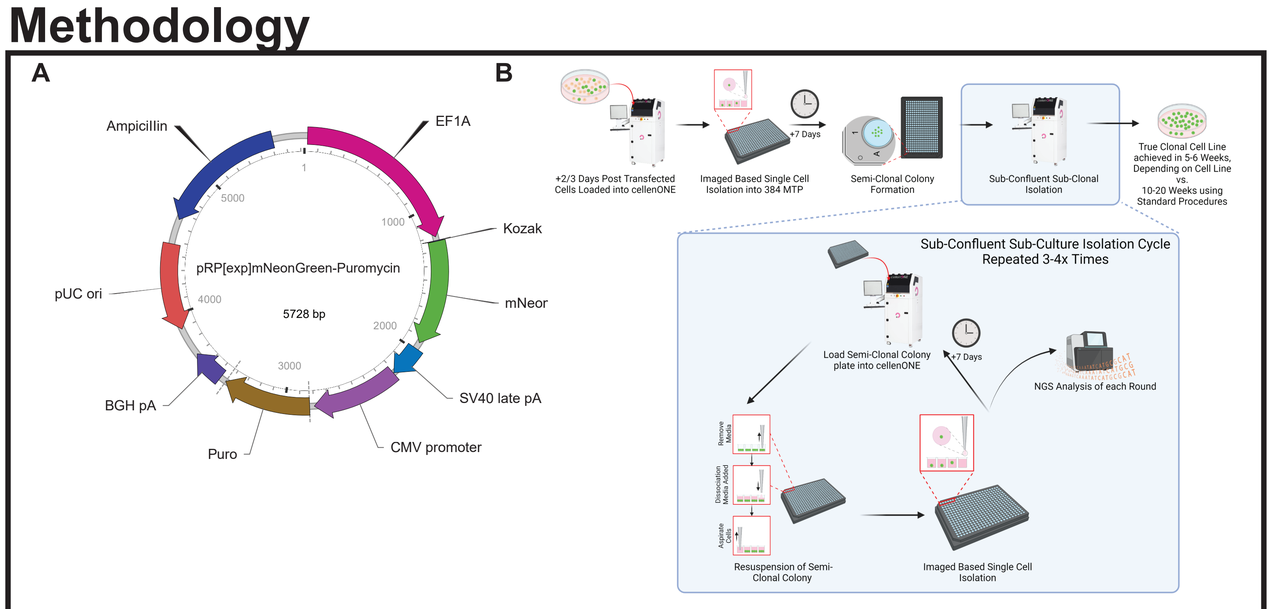


Figure 1: Sub-Confluent Sub-Clonal Single Cell Isolation Workflow Overview
Overview of the Sub-Confluent Sub-Clonal Cell Isolation workflow **(A)** A plasmid expressing mNeonGreen and a Puromycin resistance cassette was transiently transfected into HTC116 cells via Lipofectamine Treatment. Cells were cultured in McCoy's Complete media for 3 days. **(B)** mNeonGreen+ cells were isolated into individual wells of a 384-well plate using the cellenONE®. After 7 days of culturing in McCoy's Complete + 1µg/ml puromycin, small colonies ranging from 10-30 cells formed. mNeonGreen®+ colonies, spent media was removed and TrypLE® added. Cells were detached from the well and aspirated into the glass capillary. Aspirated cells were isolated into new wells and placed in an incubator for 7 to 10 days with media exchanged every 3 days. A single cell was isolated from each colony and sent for sequencing. The cycle was repeated until successful insertion could be confirmed via NGS sequencing.

Results

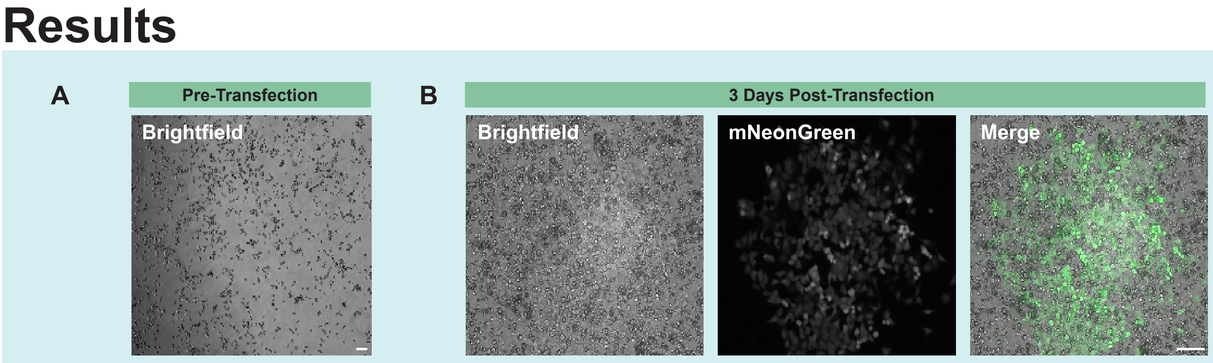


Figure 2: Pre- and Post- Transfection of HTC116 Cells With GFP-Puromycin Plasmid
Verification of transient expression of mNeonGreen®-Puromycin. **(A)** Images showing HTC116 cells prior to transfection. **(B)** Images of HTC116 cells expressing mNeonGreen-Puromycin 3 days post transient transfection. Cells were grown in a 100 mm TC dish in McCoy's complete Media (+10% FBS + 1% L-Glutamine + 1% penstrep). Images were taken on the ECHO® Revolution® microscope. Post-processing was performed using standard processes in ImageJ. Scale bars represent 100 µm.

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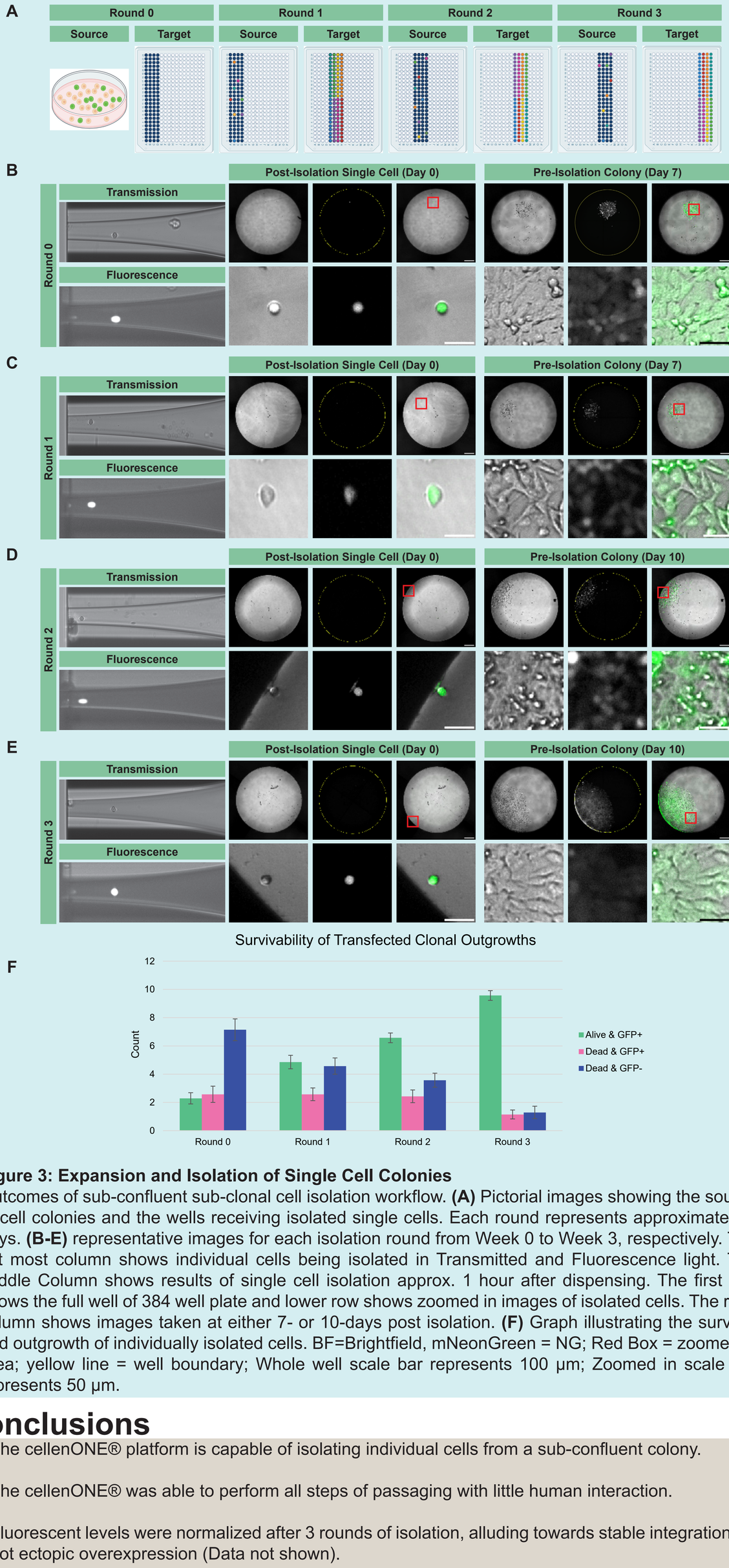

Figure 3: Expansion and Isolation of Single Cell Colonies
Outcomes of sub-confluent sub-clonal cell isolation workflow. **(A)** Pictorial images showing the source of cell colonies and the wells receiving isolated single cells. Each round represents approximately 7 days. **(B-E)** representative images for each isolation round from Week 0 to Week 3, respectively. The left most column shows individual cells being isolated in Transmitted and Fluorescence light. The middle Column shows results of single cell isolation approx. 1 hour after dispensing. The first row shows the full well of 384 well plate and lower row shows zoomed in images of isolated cells. The right column shows images taken at either 7- or 10-days post isolation. **(F)** Graph illustrating the survival and outgrowth of individually isolated cells. BF=Brightfield, mNeonGreen = NG; Red Box = zoomed in area; yellow line = well boundary; Whole well scale bar represents 100 µm; Zoomed in scale bar represents 50 µm.

Conclusions

- The cellenONE® platform is capable of isolating individual cells from a sub-confluent colony.
- The cellenONE® was able to perform all steps of passaging with little human interaction.
- Fluorescent levels were normalized after 3 rounds of isolation, alluding towards stable integration and not ectopic overexpression (Data not shown).
- With further confirmation with NGS sequencing, the Sub-confluent Sub-Clonal Isolation method could generate a clonal cell line within 4 to 5 weeks.

Future Directions

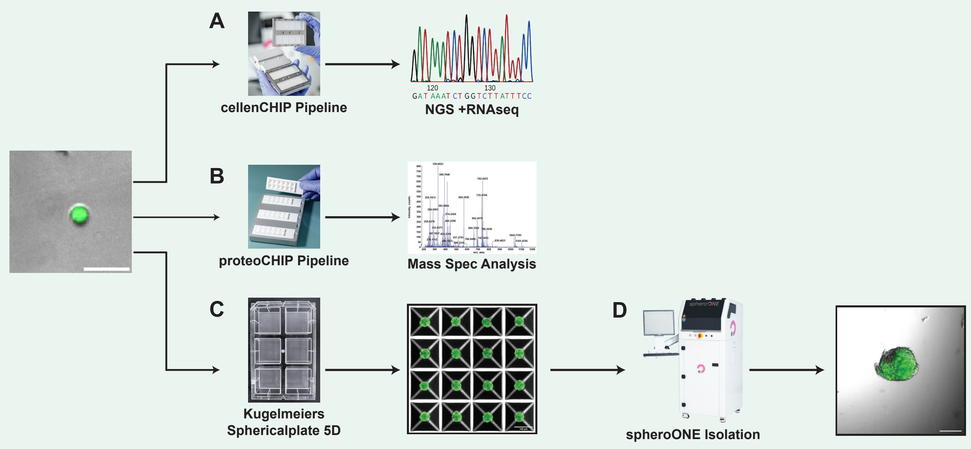

Figure 4: Downstream Applications of Sub-Confluent Sub-Clonal Isolation
Isolated Single Cells can be used for normal downstream processes in addition to Cellenion Platforms. **(A)** Isolation of single cells into the cellenCHIP Platform allows for single cell genomic and transcriptomic analysis through the cellenPIPE workflow. **(B)** Individual cells can be isolated into the proteoCHIP Platform which can be loaded directly into a mass spectrometer. **(C)** Isolated single cells can propagate and seeded into a the Sphericalplate 5D© (Kugelmeiers, Erlenbach Switzerland) to generate clonal spheroids. **(D)** Bulk spheroids can be isolated into single spheroids for drug and toxicology studies using the spheroONE. Scale bar represents 100 µm