Defined cGMP animal-origin-free medium supports PSC during, and promotes single-cell clonal expansion after, microfluidic sorting

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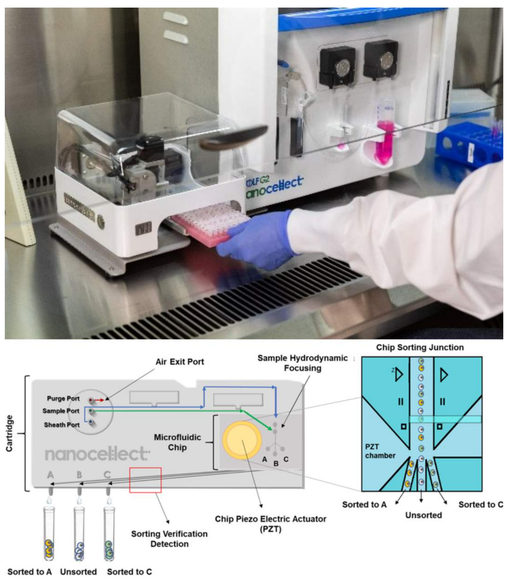
Introduction

PSC fate is modulated by environmental cues. Dissociation, followed by deposition into a single-PSC microenvironment to establish a unique clonal cell line, challenges the convention of aggregate propagation. Subsequent inefficiency of clonal expansion is a barrier to gene editing workflows for basic and translational research. Further, successful translation of stem cell-based therapies requires tracking the cell product and the components in contact with the product throughout the manufacturing process. To facilitate this, the recent launch of our cGMP-compliant, fully defined, animal-origin-free complete HiDef-B8 PSC maintenance and expansion medium kit is in high demand. HiDef-B8-cGMP is an optimized PSC maintenance medium manufactured under relevant cGMPs and was developed with traceable, animal-free raw materials. Here we describe a protocol for robust single-cell cloning using Nanocellect's WOLF G2 microfluidics-based cell sorter in combination with HiDef-B8-cGMP complete medium used as both sheath fluid, as well as PSC maintenance medium.

WOLF® G2 Microfluidic Sorter



Results

PSC maintenance and sorting

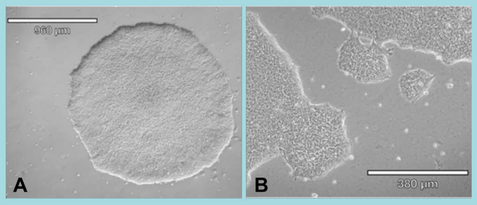


Figure 2. Morphology of PSCs cultured on cGMP-compliant CTS™ VTN-N substrate in Defined Bioscience's DMEM/F-12 formulation with L-alanyl-L-glutamine and HiDef-B8-cGMP: (A) Representative image of a healthy compact colony. (B) Higher mag representative image of colonies depicting distinct borders, well defined edges, and large nucleus:cytoplasm ratio.

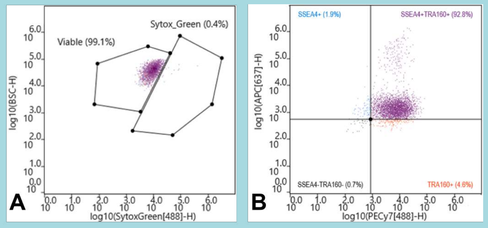


Figure 3. Single-cell suspensions of PSCs in complete HiDef-B8-cGMP medium with 1% cGMP-compliant TrypLE™ Select were gated for viability and dual expression of pluripotency markers TRA-1-60-R and SSEA-4 using WOLFViewer software: Bulk flow cytometry (after WOLF N1 single-cell deposition) demonstrates parent sorting populations with (A) 99% viability and (B) 93% dual expression.

Initial adherence and clonal outgrowth post-sort

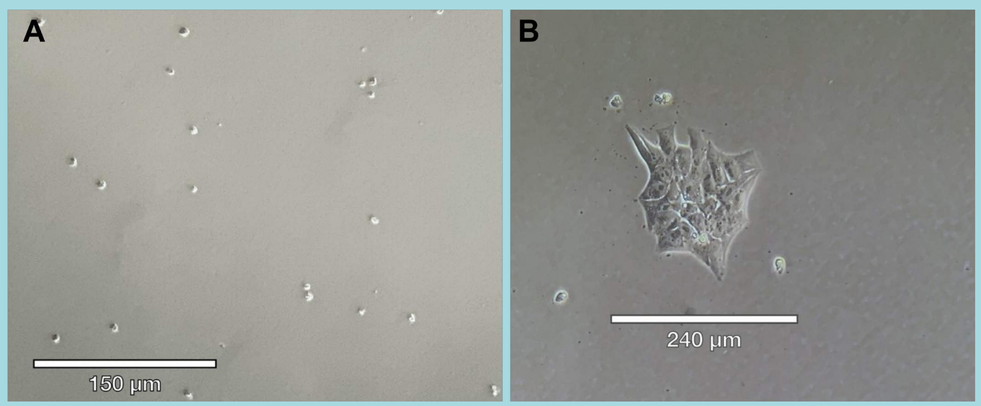


Figure 4. PSCs sorted for clonal expansion: Panel (A) is representative of a WOLF N1 'focusing well' initially plated at higher than one-cell-per-well density to aid microscopy – cell adherence shown 24 hours post-seed. Panel (B) shows archetypal PSC colony emerging (typically 16-32 cells), 5 days after seeding.

Workflow

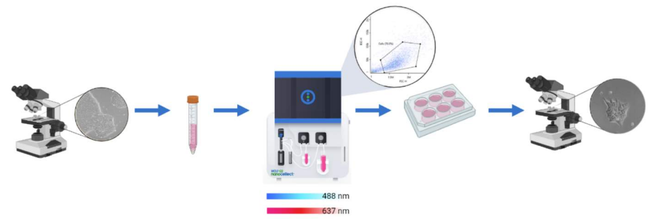


Figure 5. A simplified workflow: The experiment began with general PSC maintenance in HiDef-B8-cGMP complete medium and visual confirmation of healthy cells. A single-cell suspension was obtained using defined cGMP-compliant TrypLE Select. PSC suspension was separated into various staining cell tubes and applied to the WOLF G2. Appropriate fluorochromes were chosen to be compatible with the WOLF G2 637 nm and 488 nm LASERs. Double-stained single cell suspension is re-plated into pre-coated plates and imaged on subsequent days for cell health and growth.

HiDef-B8-cGMP Medium