Namocell
a bio-techne brand

Application Note

Generating Clonal GFP Cell Lines

Introduction

Fluorescent reporter constructs are a powerful tool for cell line engineering, allowing easy identification of transfected cells. After transfection, single cells labeled with a reporter are commonly isolated for clonal expansion and generation of recombinant products. Traditionally, generation of clonal engineered cell lines entails a two step process, wherein fluorescent cells are first enriched into a pool using a conventional cell sorter, and then isolated as single clones using manual limiting dilutions. This workflow is not only time consuming, but also has limitations at each step: cells can easily be damaged in the first step from high pressure sorting (20–70 psi) and outgrowth efficiency in the second step is limited by the Poisson distribution, resulting in a relatively small number of viable clones. Namocell's single cell dispensers are user-friendly, benchtop instruments that operate at low pressure (<2 psi) to preserve the integrity and viability of sorted cells. These systems' fluorescence detection capabilities allow for easy isolation of transfected cells, yielding clonal cell lines in a single step. Here, we describe the ability of Namocell's Hana Single Cell Dispenser to isolate and dispense individual GFP-positive cells for clonal expansion.

Methods

HEK-293 cells in culture medium (DMEM supplemented with 10% FBS and 1% Pen-Strep) were transfected with a GFP expression vector. The transfected cells were cultured for 48 hours at 37 ºC (5% CO_2). Cells were then passaged 1:4 and cultured for 24 hours prior to single cell dispensing (FIGURE 1). Dissociated cells in suspension were diluted to 5,000 cells/mL in culture medium and 750 µL of the suspension was loaded into a sterile microfluidic cell cartridge (Namocell). Cells were analyzed with the FITC detection channel (533nm) as the trigger, with a lower bound of FITC=20 (FIGURE 2). During analysis and sorting, cell events were automatically indexed, such that the fluorescence of each isolated cell, along with its location in the dispensed plate, would be recorded for reference. Individual GFP-positive HEK-293 cells (with FITC>100) were dispensed (one cell per well) into a 96-well plate pre-loaded with 200 µL of culture medium per well. Isolated cells were cultured for one week and inspected for proliferation.

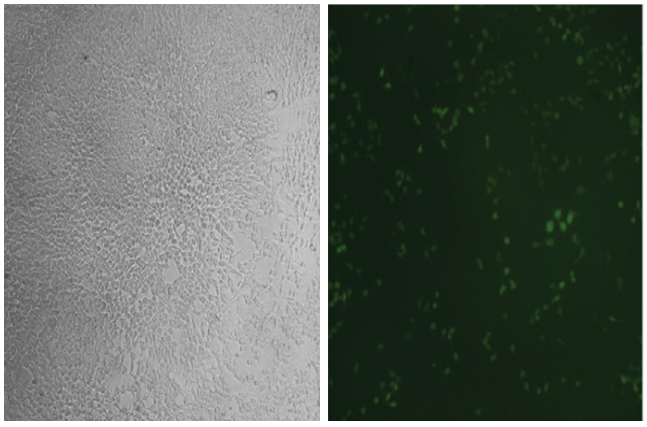

FIGURE 1. Brightfield (left) and fluorescent (right) images of a heterogeneous transfected pool of HEK- 293 cells prior to single cell dispensing.

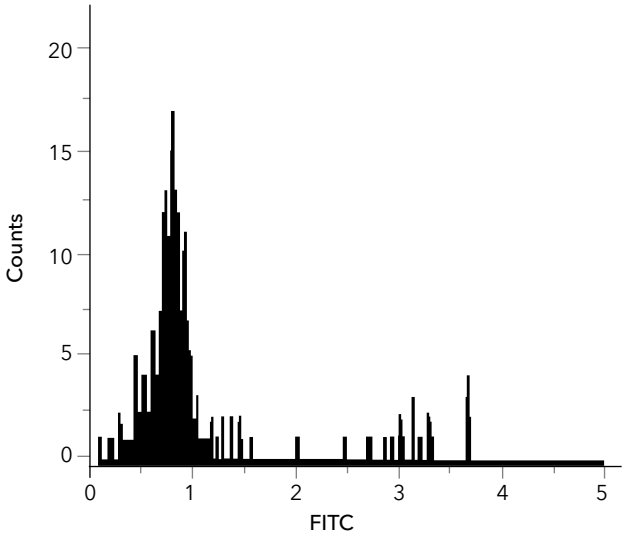

FIGURE 2. Histogram of FITC fluorescence (533nm; y-axis in log10 scale) of all HEK-293 cells analyzed from the original transfected pool.

Results

After 7 days of culture, 70% of individual cells dispensed into the 96-well plate exhibited visible proliferation. Further inspection with fluorescence microscopy confirmed clonal expansion of GFP-positive cells (FIGURE 3). Cells with the brightest GFP signal during sorting could be identified by referring to the index data generated during dispensing.

Overall, Namocell's single cell dispensers offer an easy and gentle alternative to traditional cell sorters that allow users to quickly isolate GFP-positive transfected cells from a heterogeneous pool while preserving cell viability. By directly isolating target cells as singlets, this workflow eliminates the need for clonal isolation via limiting dilutions and ensures a high rate of outgrowth among isolated clones.

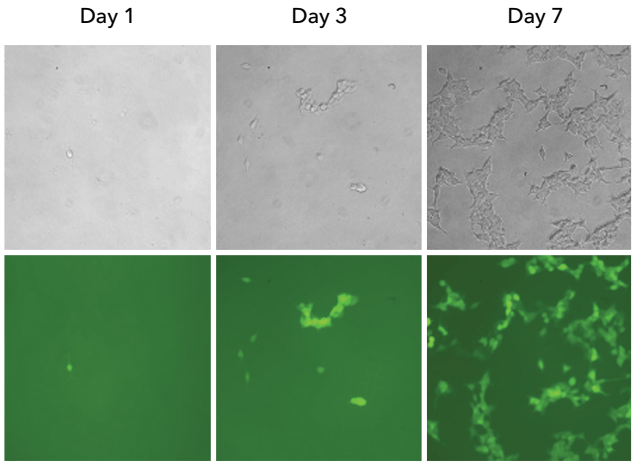

FIGURE 3. Brightfield (top panel) and fluorescent (bottom panel) images of a single GFP-positive HEK- 293 cell following isolation and clonal expansion for 7 days (20x magnification).

Bio-Techne® | R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen® Namocell™

For research use or manufacturing purposes only. Trademarks and registered trademarks are the property of their respective owners.
STRY0307523_WBU_AN_Generating-clonal-GFP-cell-lines_JA