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Application Note

Enrichment of High Titer CHO Cells

Introduction

Cell line generation for monoclonal biotherapeutics has traditionally been limited by the need to screen hundreds of clones from a transfected pool. Pools are typically comprised of mostly low to medium producing clones, with a relatively small proportion of high producers[1,2]. Recently, fluorescence activated cell sorting (FACS) has improved the throughput of these screens by enabling isolation of high producing clones based on fluorescently tagged surface markers. One such approach employs a vector enabling "leaky" translation of the IgG heavy chain, resulting in a subset of antibodies being tethered to an IgG transmembrane domain and thus displayed on the cell surface[3]. This creates a reliable indicator of antibody production for each individual cell, as the amount of antibody presented on the membrane will correspond directly to the total amount of antibody produced. Antibodies on the surface can be fluorescently labeled and the brightest (highest producing) cells can subsequently be isolated via FACS, expediting the generation of high titer cell lines.

One limitation of this existing approach is its reliance on a high-pressure cell sorter that often results in low cell viability, and that is not well suited to isolation of subpopulations of rare frequencies (less than 1–3%) due to high dead volume. Traditional FACS instruments operate at 20–70 psi, creating a harsh sorting environment that often destroys the isolated cells. Namocell's single cell dispensers operate at a gentle pressure of less than 2 psi, which preserves viability for clonal outgrowth following isolation. Additionally, Namocell's patented enrichment sorting mode processes up to 50,000 cells/second, enabling rapid screening of millions of cells and efficient isolation of rare events (less than 1%). In this application note, the Namocell Single Cell Dispenser was used to isolate high titer CHO cells by dispensing an enriched pool of fluorescently tagged high producers (FIGURE 1).

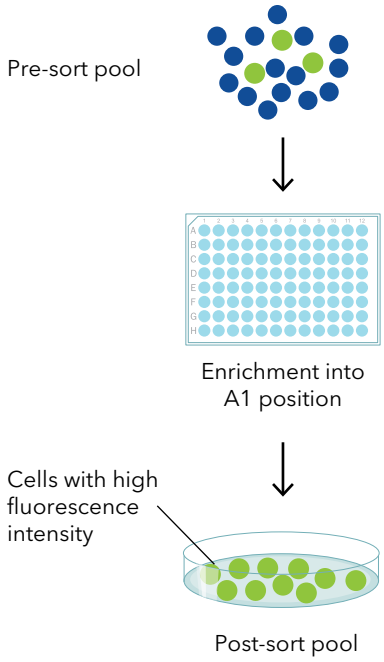

FIGURE 1. Schematic of Namo enrichment mode to isolate a rare subpopulation. The resulting post-sort pool is enriched for cells with high fluorescence intensity, along with some non-target cells.

Methods

IgG Expressing CHO Cell Culture

CHO-K1 cells were transfected with a single copy of mAbX expression vector (proprietary vector) with a leaky stop codon. A stable pool of mAbX expressing CHO cells were recovered and maintained in shake flasks using a proprietary, chemically defined culture medium.

Cell Enrichment and Measurement of Antibody Production

From the transfected pool, CHO cells at a density of 1 x 10^6 cells/mL were stained with a FITC-conjugated human anti-IgG antibody (3.3 U/mL; Molecular Devices, K8205)

at room temperature for 15 minutes. Cell sorting was performed with a Namo Single Cell Dispenser (Namocell), using its enrichment sorting mode. Cells representing the top 0.01% brightest signal in the FITC detection channel were selected and dispensed, resulting in a post-sort pool of cells enriched for the highest producers. The enriched cells were grown in a 48-well plate prior to expansion in fed-batch culture. The presort pool of cells was expanded in parallel as a control. Antibody yield from the culture supernatant of the pre-sort and post-sort pools was measured via Protein A abundance on an Octet system (Pall FortéBio, Fremont, CA) at days 4, 6, and 7.

Results

At each time point assessed, the enriched pool of post-sort CHO cells displayed higher concentrations of secreted antibodies (FIGURE 2). After 7 days of culture, a greater than 4-fold increase in antibody production was observed from the post-sort pool that had undergone selective enrichment. Consistent with previous results[3], fluorescent labeling of antibodies on the cell surface provided a reliable indicator of high producing cells, thus enabling efficient isolation of target clones. This approach provides a critical advantage in expediting the process of identifying high titer CHO cells for product development while drastically reducing time, labor, and costs. The Namo cell dispenser is an easy-to-use, gentle alternative to FACS that preserves cell viability of candidate clones and allows for rapid isolation and dispensing of rare subpopulations for cell line development workflows.

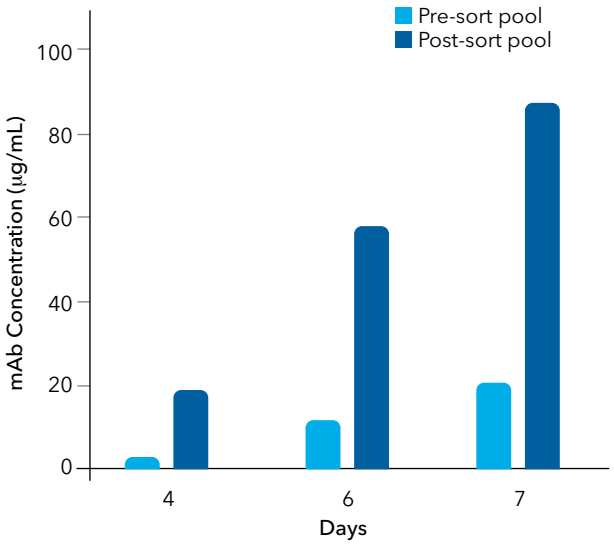

FIGURE 2. Productivity of pre-sort pool and post-sort pool analyzed from fed-batch cultures.

References

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2. Jostock, T.; *et al.* 2010. Combination of the 2A/furin technology with an animal component free cell line development platform process. *Appl. Microbiol. Biotechnol.* 87 (4), 1517-1524.
3. Lang, S.; *et al.* 2016. Surface display vectors for selective detection and isolation of high level antibody producing cells. *Biotech. Bioengr.* 113 (11), 2386-2393.