A COMBINATORIAL USE OF TITER AND TITER NORMALIZED TO CONFLUENCE AS EARLY REPORTERS ALLOWS FOR SELECTING CHINESE HAMSTER OVARY CELL CLONES WITH HIGH VOLUMETRIC PRODUCTIVITY OF ETANERCEPT

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The selection of Chinese hamster ovary (CHO) clonal cell lines with the highest production rate of recombinant glycoproteins remains a big challenge during early stages of cell line development. Different strategies using either product titer or product titer normalized to cell number are being used to assess suspension-adapted cell clones when grown statically in microtiter plates. However, no reported study so far has performed a direct head-to-head comparison of these two early reporters for predicting clone performance in suspension. Therefore, we developed a screening platform that combines enrichment of high specific productivity ($q_p$) by surface labelling-based fluorescence activated cell sorting (FACS) with high-throughput and semi-automated analysis of confluence and titer in 96-well microplates. A so-called titer-to-confluence (TTC) ratio (titer normalized to confluence) is introduced as a $q_p$ proxy for cells grown in static culture. Following screening of 850 single-cell derived clones with the established workflow, we performed an unbiased evaluation of titer- and TTC-based ranking as early reporters for growth rate, specific and volumetric productivity in suspension. Using two different suspension cultivation vessels, we observed that $q_p$ remains largely unaffected by different growth modes and vessels, despite a poor correlation between confluence and cell growth rate in suspension. Next, we observed that TTC as a $q_p$ proxy is not a precise reporter for volumetric productivity in suspension, consistent with previous reports. However, we observed that TTC can be used to identify clones with high volumetric productivity, which would not have been found if solely titer-based ranking would be used (Figure 1). In conclusion, we demonstrated that screening based on titer or TTC gives rise to similar final volumetric productivity in batch cultures (Figure 1). The two different selection criteria, however, achieved high volumetric productivity by two different means – either through high $q_p$ for clones selected with TTC-based ranking or through high integral viable cell density (IVCD) for clones selected with titer-based ranking. Therefore, a combinatorial titer- and TTC-based ranking is proposed, contributing to selection of a versatile panel of clones that can be further characterized and from which the final producer clone can be selected that best fits the production requirements.

Figure 1 – Performance of top 12 TTC-selected and top 12 titer-selected clones in shake flask batch culture.