FUJIFILM
Value from Innovation

Diosynth
biotechnologies

Delivering 'fit for purpose' biomanufacturing CHO cell lines

Authors:

Leon P. Pybus*, Nazanin Dadehbeigi*, Fay L. Saunders, Alison J. Porter

Mammalian Cell Culture R&D,
FUJIFILM Diosynth Biotechnologies, Billingham, U.K.

* Leon P. Pybus and Nazanin Dadehbeigi contributed equally to this work

Introduction

The challenge during mammalian cell line development is to identify and isolate stable, high expressing cell lines producing product with the appropriate critical product quality attributes rapidly, reproducibly and with relative ease. Obtaining a host cell line that inherently exhibits desirable biomanufacturing attributes can therefore have a significantly positive effect on the identification of recombinant cell lines with desired traits during cell line development screens. In this study, we demonstrate that it is possible to exploit intrinsic heterogeneity within host cell populations and identify host cell lines which are more "fit for purpose". 2,400 CHO-DG44 sub-populations were isolated from a parental population pre-adapted to growth in animal component-free, chemically defined media by limiting dilution cloning. One of these host cell lines 'Clone-27' displayed a number of desirable biomanufacturing attributes compared to the CHO-DG44 host (DG44-2B) currently utilised within FUJIFILM Diosynth Biotechnologies. These desirable attributes included (i) reduced doubling time during subculture; (ii) increased transient expression of a model recombinant monoclonal antibody (mAb); (iii) improved growth in semi-solid media; and (iv) increased expression of three model mAbs in transfectant mini-pools. Taken together these data reveal the potential to isolate CHO host cell variants with improved biomanufacturing attributes.

Improving the biomanufacturing phenotype of host cell populations through directed evolution

A prerequisite for the successful manufacture of a biopharmaceutical in mammalian cells is a cell line development strategy that selects a cell line with the most desirable properties from a heterogeneous transfectant population. Obtaining a cell line which inherently exhibits superior attributes for biomanufacturing would therefore be highly desirable as improving a host cell populations functional capability or removal of unsuitable variants from the host cell population would have a significant positive effect on the identification of bioproduction compatible cell lines. The ideal combination of functional phenotypes for a biomanufacturing cell line include high volumetric product yield which is a function of high specific production rate (Qp) and/or extended integral of viable cell concentration (IVC) during culture, acceptable growth in an inoculum process and generation of product with the required critical quality attributes.

Typically, during cell line development, cell line heterogeneity is managed to select biomanufacturing compatible cell lines using empirical screens at multiple evaluation stages (assessing productivity, growth, product characteristics, or a mixture of these), where the data generated is used to decrease the number of cell lines taken on to further evaluation stages.[1-3] Significant interest has been placed into targeted genetic engineering of specific cellular

processes to create new CHO cell populations with improved functionality.[4] However, the identification of suitable engineering targets has proven difficult, success had been varied and it is highly likely that the generation of an optimal cell line would require intervention at multiple targets. Furthermore, uncertainties about intellectual property and "freedom to operate" surrounding engineering targets and the methods by which they are engineered can make cell engineering an undesirable proposition.

An alternative method to develop a host cell line population which is more "fit for purpose" is the use of so-called "directed evolution" methodologies which aim to control/manage cell heterogeneity to create generically improved mammalian cell hosts.[5] These methods offer advantages compared to targeted genetic engineering as they offer the potential to simultaneously target multiple genetic/regulatory networks which underpin complex functional phenotypes relatively quickly and with little a priori knowledge. Therefore, we compared the ability of multiple directed evolution strategies to exploit phenotypic variation in CHO host cell populations and isolate sub-populations that exhibit superior attributes for biomanufacturing. We isolated host cell sub-populations that vary in a variety of functional attributes that impact biomanufacturing processes and one of these host cell lines, 'Clone-27', obtained by limiting dilution cloning, displayed a number of desirable attributes compared to our currently utilised DG44-2B host cell line.

A sub-population isolated from a CHO-DG44 host exhibits improved growth rate during subculture

Sub-populations were isolated from a parental CHO-DG44 population (DG44-C2) using a single limiting dilution step (0.5 cells/well; giving a probability of monoclonality of 0.985). Once progressed to suspension culture, cell concentration at each subculture was determined and used to calculate growth rate as previously described.[6] The data from three consecutive subcultures obtained from one of these sub-populations 'Clone-27' are shown, compared to the currently utilised FUJIFILM Diosynth Biotechnologies' expression host DG44-2B, in Figure 1. 'Clone-27' displayed an improved growth rate during subculture compared to DG44-2B. A high growth rate during subculture is a highly desirable property for a host cell line as an increase in growth rate is highly likely to remain heritable in recombinant cell lines, leading to significant reduction in cell line development timelines and the time taken to scale-up to large-scale production bioreactors.

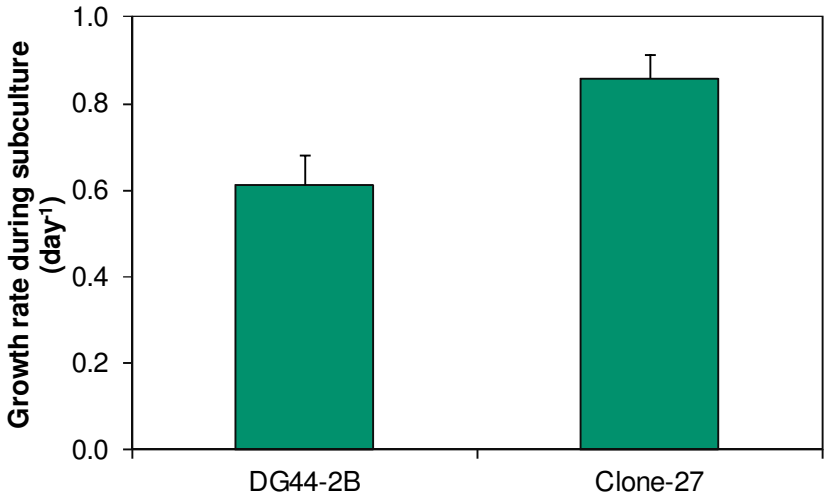

Figure 1: ***Increased mean growth rate from three consecutive subcultures in suspension conditions for 'Clone-27' compared to DG44-2B.*** *Viable cell concentration at subculture was determined using a Vi-CELL™ automated cell viability analyser (Beckman Coulter™).*

'Clone-27' exhibits an improved ability to transiently express a model mAb compared to DG44-2B

To test the relative ability of 'Clone-27' and DG44-2B to transiently produce a model mAb (mAb1) each cell line was transfected in triplicate by electroporation. These data are shown in Figure 2. 'Clone-27' displayed a greater than two-fold improvement in the transient expression of mAb1. This high-level transient production provides a rapid indication of the expression capability within the host cell population.[7]

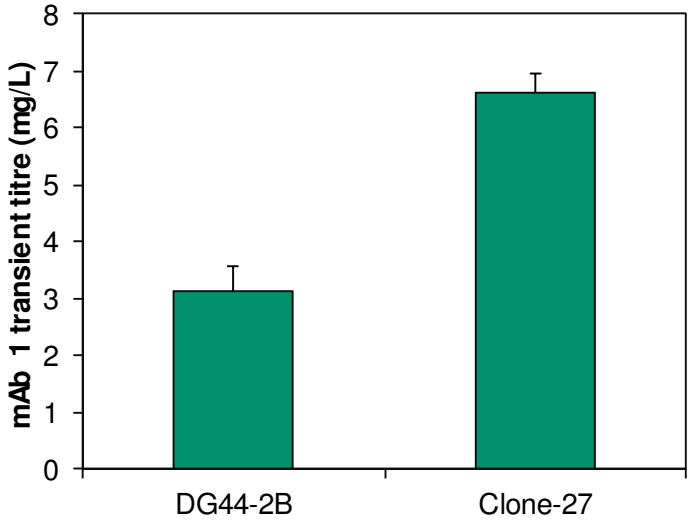

Figure 2: ***Improved mean transient expression of mAb1 with 'Clone-27' compared to the DG44-2B host.*** *All transient transfections were performed using electroporation in triplicate and titre analysed six days post-transfection. Product concentration was analysed by comparing the binding rate of the sample to protein A biosensors against a standard curve of binding rates for a known concentration of IgG using a ForteBio® Octet® QK^e*

‘Clone-27’ is inherently more suited to low cell density semi-solid culture than DG44-2B

The ability of a host cell line to efficaciously integrate into a cell line development process is an important prerequisite for the successful identification of biomanufactuting compatible cell lines. For example, automated screening technologies have been widely implemented into cell line development processes to screen in a more efficient, cost-effective and high-throughput manner.[1] An example of this approach is the use of automated colony pickers such as the ClonePix™ 2 (Molecular Devices™) system to isolate high-producing clones. This technology requires cells to be seeded in semi-solid media to immobilize clones and the captured secreted protein of interest can be detected as a “halo” around each colony using a fluorescently-tagged antibody. It is important to confirm that compatibility of a host cell line with such a system, realizing that optimization of growth conditions may be necessary to support low cell density cultures in semi-solid media. We therefore examined whether there exists within CHO cell host populations cells which are more intrinsically suited for low cell density culture in semi-solid media (Figure 3). Indeed, comparing colony survival after seeding in semi-solid media for ‘Clone-27’ and DG44-2B reveals that ‘Clone-27’ displays an approximate 2.5-fold increase in colony survival (Figure 3). This host cell phenotype is highly desirable allowing a higher number of recombinant cell lines to be screened during cell line development.

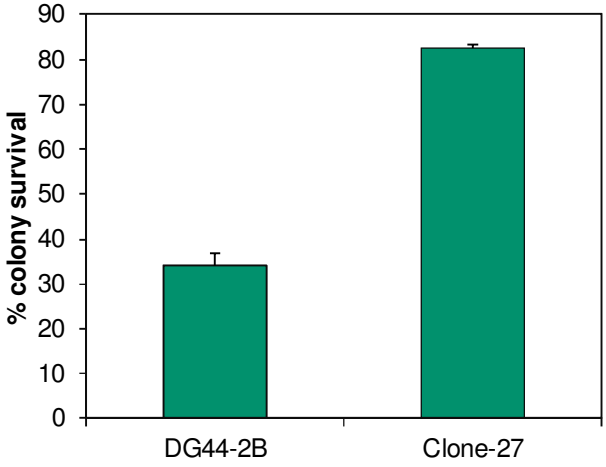

Figure 3: ***Improved mean colony survival in semi-solid media for the ‘Clone-27’ compared to the DG44-2B host.*** *Two six-well plates were seeded per cell line at a seeding density of 200 cells/well and colony survival measured after six days by image analysis using a ClonePix™ 2 (Molecular Devices™).*

Transfectant mini-pools originating from 'Clone-27' highly express multiple mAbs compared to DG44-2B

Next we compared the ability of 'Clone-27' and DG44-2B hosts to stably express a number of recombinant products. Transfectant mini-pools were generated for three mAbs (mAb 1, mAb 2, and mAb 3) by seeding transfectant pools into 96-well plates at 5000 cells/well. After three weeks, wells with the highest confluency were progressed to 24-well plates for a 10 day batch screen for titre analysis. Figure 4 shows the ability of recombinant cell lines initiating from the 'Clone-27' host to generate a number of higher expressing cell lines for multiple recombinant products compared to DG44-2B. The ability of a host cell line to express a wide range of recombinant products at a high level is especially pertinent for so called difficult-to-express products. These molecules inexplicably reach much lower bioprocess titres. The phenotypes observed by 'Clone-27' may therefore reduce the intensive cell line and process development activity that usually render such products costly or unsuitable for manufacture.[8]

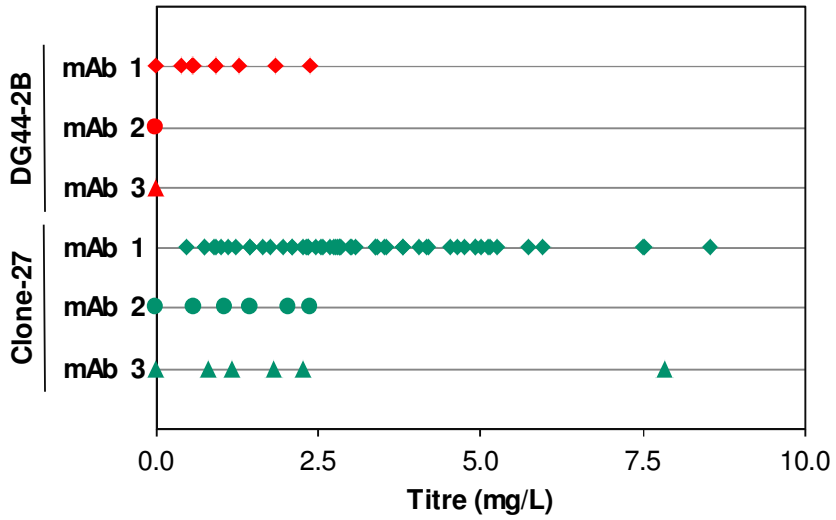

Figure 4: ***Improved expression of mAb1, mAb 2 and mAb 3 in transfectant mini-pools for 'Clone-27' compared to the DG44-2B host****. 120 mini-pools expressing mAb1 and 20 mini-pools expressing mAb2 and mAb3 were evaluated in a 24-well batch screen for 10 days before culture supernatant was analysed for mAb titre. Product concentration was analysed by comparing the binding rate of the sample to protein A biosensors against a standard curve of binding rates for a known concentration of IgG using a ForteBio® Octet® QK^e.*

Conclusion

This study reveals the potential to exploit the intrinsic variability within CHO host cell populations to obtain sub-populations that exhibit improved functional attributes that impact biomanufacturing processes. Traditionally, management of this diversity is achieved by screening of cell lines post-transfection at multiple stages for productivity, growth, product characteristics, and bioprocess performance.[1-3] Tailored genetic engineering of specific cellular processes has attracted significant interest, however, results remain disappointing. Directed evolution based strategies provide an exciting alternative and in this study we show that such approaches can be utilised to harness and control the inherent heterogeneity of CHO host cell populations.

References

1. Browne SM, Al-Rubeai M (2007) Selection methods for high-producing mammalian cell lines. *Trends Biotechnol* **25**:425-532.
2. Porter AJ, Dickson AJ, Racher AJ (2010) Strategies for selecting recombinant CHO cell lines for cGMP manufacturing: Realizing the potential in bioreactors. *Biotechnol Prog* **26**:1446-1454.
3. Porter AJ, Racher AJ, Preziosi R, Dickson AJ (2010) Strategies for selecting recombinant CHO cell lines for cGMP manufacturing: Improving the efficiency of cell line generation. *Biotechnol Prog* **26**:1455-1464.
4. Dietmair S, Nielsen LK, Timmins NE (2011) Engineering a mammalian super producer. *J Chem Technol Biotechnol* **86**: 905-914.
5. Majors BS, Chiang GG, Betenbaugh MJ (2009) Protein and genome evolution in mammalian cells for biotechnology applications. *Mol Biotechnol* **42**: 216-223.
6. Greenwood SK, Hill RB, Sun JT, Armstrong MJ, Johnson TE, Gara JP, Galloway SM (2004) Population doubling: A simple and more accurate estimation of cell growth suppression in the in vitro assay for chromosomal aberrations that reduces irrelevant positive results. *Environ Mol Mutagen* **43**: 36-44.
7. Mason M, Sweeney B, Cain K, Stephens P, Sharfstein ST (2012) Identifying bottlenecks in transient and stable production of recombinant monoclonal-antibody sequence variants in Chinese hamster ovary cells. *Biotechnol Prog* **28**: 846-855.
8. Pybus LP, Dean G, West NR, Smith A, Daramola O, Field R, Wilkinson SJ, James DC (2014) Model-directed engineering of “difficult-to-express” monoclonal antibody production by Chinese hamster ovary cells. *Biotechnol Bioeng* **111**: 372-385.

www.fujifilmdiosynth.com
Email : enquiries@fujifilmdb.com