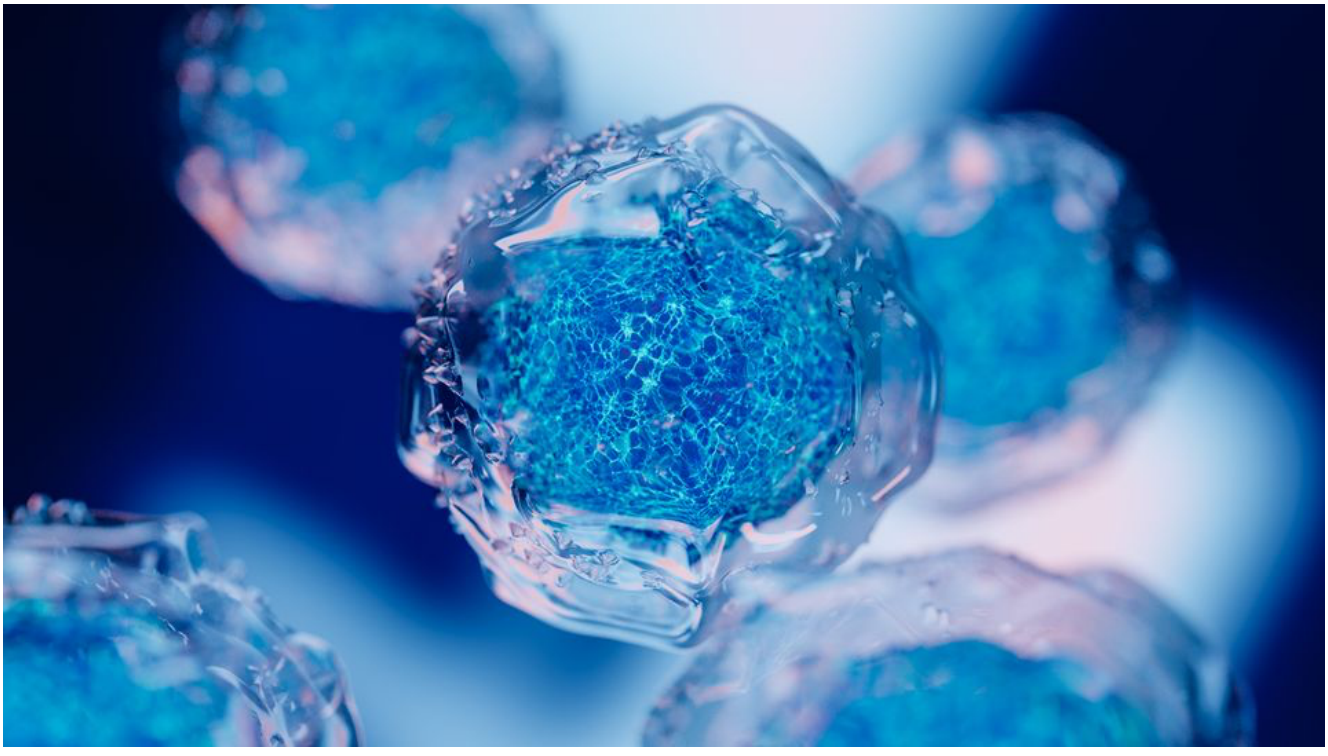
Maximizing Cell Line Productivity and Efficiency

Dr. Tabitha Bullock discusses how technological advances are improving the efficiency and productivity of cell lines.

Alison Halliday, PhD



Cell lines are invaluable tools for the biopharmaceutical industry, used to generate a range of therapeutic classes, from proteins such as antibodies to vaccines. As both the demand for biotherapeutics and pressure to reduce the cost of these treatments rise, innovative tools and approaches are being developed to optimize the cell lines used.

In this interview, Dr. Tabitha Bullock, a principal group leader at Lonza, tells us about some of the key considerations in cell line development and how advances in technology are improving the efficiency and productivity of cell lines. Tabitha also shares her views on what could be in store for the future of cell line development.

Alison Halliday (AH): Can you tell us about some of the key considerations in cell line development?

Tabith Bullock (TB): Firstly, a host cell line must be selected with the required authentication documentation in place. The transfection method must allow for optimal transfection efficiency and gene integration, for example, using Nucleofector® technology. The choice of vector is also a key consideration. Here at Lonza, we have developed a suite of GSquad™ vectors using a simple two-step process to generate a vector construct designed to maximize productivity. An appropriate selection system should be used, either antibiotic selection such as Puromycin or Zeocin or metabolic selection systems such as DHFR or GS. The speed at which cell line development can be achieved is also key. At Lonza, we have built upon many years of experience and expertise to design a

program to achieve gene to IND in less than 11 months.

AH: Why is maintaining genetic stability important? What techniques can be used to ensure this?

TB: The importance of maintaining genetic stability is primarily to ensure the quality of the recombinant protein whilst maintaining high productivity and consistent growth. Mammalian cell lines can deliver good growth whilst being adapted to varying culture conditions and withstanding genetic manipulation. This also means there can be a tendency for these cell lines to undergo genetic changes, leading to instability.

Strategies to minimize the risk of instability include the vector design and expression system used. The choice of the promoter, number of integration sites, transfection efficiency, and sequence and codon optimization should be considered when preparing the vectors containing the genes of interest. Genetic engineering can be used to knock in/out specific genes associated with instability. Insulator elements can be utilized, such as MARs, S/MARS, cHS4 and UCOE chromatin-modifying elements, which insulate the expression cassette containing the gene of interest from surrounding genomic elements and

genome positioning effects. 3′UTR enhancers can increase mRNA stability. The use of site-directed integrations (SSI) can also be beneficial in terms of improving stability.

Single-cell cloning is a technique used to generate monoclonal cell lines, which reduces the genetic heterogeneity of a cell line. This enables selection of a more stable cell line for manufacturing.

Maintaining a consistent culture environment may also be beneficial to reduce the chance of changes that may select for genetic variants, so a well-established and robust upstream process, including media and feeds, is key.

AH: What strategies can be employed to enhance protein expression in cell lines?

TB: The selection of expression system technology can be key to maximizing cell line productivity. At the vector level, the incorporation of insulator elements and/or 3′UTR sequences can be utilized to optimize productivity as well as genetic engineering to knock in/out specific genes associated with productivity. At Lonza, the GS piggyBac® system, which uses an engineered hyperactive piggyBac® transposase enzyme to insert GS Xceed® expression

vector cargos into the host cell genome with high efficiency, has been designed to preferentially target the gene of interest to stable regions of the genome associated with highly expressed genes. The GS piggyBac® system has been shown to increase productivity for clones expressing bispecific antibodies by 45% on average as well as increasing the cell recovery time post-transfection compared to standard GS Xceed®.

With increasing interest in the production of more complex therapeutic proteins such as bispecifics, solutions such as Lonza's bYlok® technology can be employed to increase the frequency of correct HC-LC pairing, thus increasing the yield of the required protein. Through the use of metabolomics, media and feeds can be designed to maximize protein expression based on analysis of the nutrient depletion and accumulation of waste products within a culture. The entire process can also be optimized using this approach.

Cell line screening and selection strategies can be designed to maximize the chance of selecting a high-producing cell line. Lonza uses the Beacon® optofluidic system to screen thousands of clones at the single-cell stage to identify those most suitable for progression.

AH: In your opinion, what are the most important recent advances in technology in this area? How are these contributing to cell line productivity and efficiency?

TB: The development of transposon-based cell line engineering technology, such as piggyBac® has enabled the generation of high-yielding cell lines reliably and consistently as well as rapid stable pool generation. High-throughput omics technologies and gene editing have also provided a greater understanding of how these cell lines can be manipulated to produce the phenotypes of interest.

With the increase in scientific knowledge and understanding of the therapeutic proteins we are producing, developability tools allow us to screen for potential issues at an earlier stage, assessing several candidate molecules simultaneously. Protein engineering allows for improvements to the manufacturability, activity and protein structure to be made. Panels of lead candidate molecules can be expressed at a small scale for further studies at the early stage quickly and cost-effectively, contributing to shorter timelines to IND.

We have seen a rapid increase in the use of automation in all areas of cell line development over the past 5-10 years.

In early development, automation allows for the screening of large numbers of cell lines for the desired characteristics as well as screening multiple media and feed strategies. Small-scale affinity chromatography and product quality analysis allow for an early readout of product characteristics. High-throughput chromatography screening also allows the same approach for downstream processing, allowing identification of the conditions for optimum yields and reduction of aggregate and fragment levels.

AH: What are the future challenges and opportunities in cell line development? How can we accelerate cell line development timelines?

TB: In the immediate future, it is clear that demand is increasing rapidly for the development of increasingly complex recombinant proteins. Having high-throughput product-specific analytics in place to meet and accelerate timelines is key to keeping up with the demands of next-generation therapeutics whilst continuing to increase the speed to clinic. Having product characteristics analysis at an early stage and the development of a product-specific purification process using early material, enables us to create a scalable process whilst maintaining timelines. Process intensification also means that fully automated and tailored feed rate control can be used for real-time nutrient demand, through Raman PAT perfusion systems,

with the aim of increasing yield and maintaining consistent product quality.

In research and development, single-cell analysis is well established. However, it is likely that this approach will be utilized by CDMOs instead of relying on analysis of heterogeneous populations. For example, the productivity of a single cell could be measured instead of looking at the average of the population which may have an impact on stability. The relevant high-throughput screening tools also need to be established for the readout to be possible.

Omics techniques will allow us to move away from selecting cell lines from thousands of candidate clones to identifying a lead stable, productive clone early in the process. At the clone selection stage, cell lines can also be screened for process fit, so that the selection of a cell line suitable for the final manufacturing process can happen at a much earlier stage. In parallel, material from pools is already being used for early analytical, purification and formulation development, permitting acceleration of the whole cell line development timeline.

Dr. Tabitha Bullock was speaking to Alison Halliday for Technology Networks.

Dr. Tabitha Bullock is a principal group leader at Lonza.

Alison Halliday holds a PhD in molecular genetics from the University of Newcastle. As an award-winning freelance science communications specialist, she has 20+ years of experience across academia and industry.