Just EVOTEC BIOLOGICS

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SPECIAL REPORT

Benefits of a Customized Cell Line Development Program

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Key challenges for companies developing biotherapeutics such as monoclonal antibodies (MAbs) and biosimilars are to reduce risks when selecting clones and to optimize and scale their processes efficiently. Such biopharmaceutical companies often partner with contract development and manufacturing organizations (CDMOs) to benefit from their cell line development (CLD) programs.

Having a robust CLD program in place can provide predictable performance from laboratory scale through to production bioreactors, reducing risks and enabling manufacturing at a competitive cost of goods (CoG). Using the right CLD approach can produce biotherapeutics at industry-standard or higher titers while maintaining their critical quality attributes (CQAs), thereby ensuring that they conform to current good manufacturing practice (CGMP) quality standards and regulatory requirements. At Just-Evotec Biologics, we focus on creating, selecting, and optimizing growth of stable, high-quality clones for maximum biomass and protein titers in continuous perfusion culture.

CLD at Just-Evotec Biologics is designed to be a cost- and time-efficient process that typically follows the steps pictured in Figure 1.

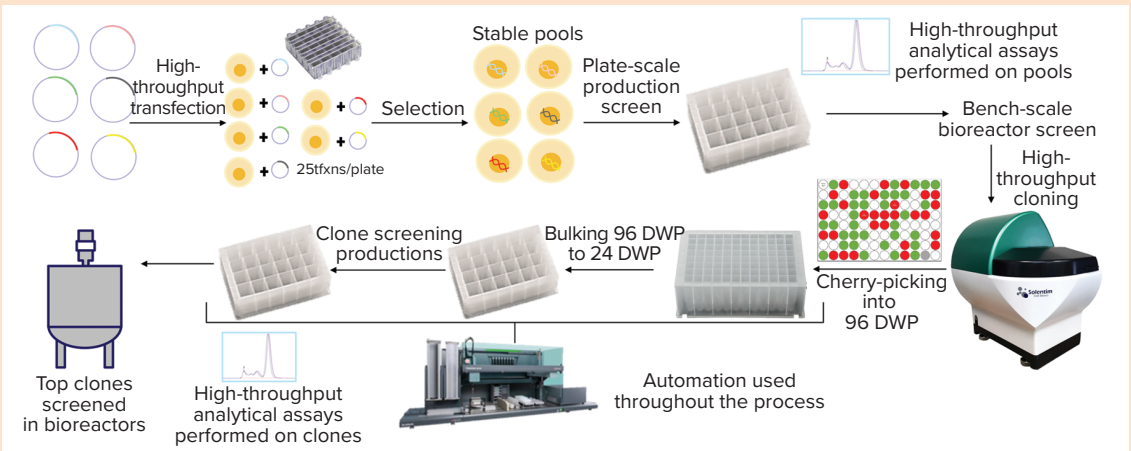
Cell Line Development and Selection

CLD begins with selection of a suitable host cell line. That choice depends on factors such as protein expression, post-translational modifications, and the type of cell culture process used (fed-batch or continuous perfusion culture, for example). Chinese hamster ovary (CHO)–based cell lines are an industry-standard workhorse because they have a proven record of safety, scalability, and productivity, making them a low-risk choice for production of MAbs and biosimilars (**1**).

At Just-Evotec Biologics, we use CHO-based host cell lines in our CLD programs. We have engineered our own CHO-based glutamine synthetase knockout (GS KO) host cell line, CL-72, because this selection system enables rapid identification of high-titer clones (**2**). Using CL-72, we have shown that by choosing the right clones for our optimized platform culture conditions, we can achieve viable cell densities (VCDs) of ~80 × 10^6 cells/mL (data not shown) and produce higher productivities than in an industry-standard CHO cell line in continuous perfusion culture (Figure 2).

The CL-72 cell line is proprietary and unavailable for out-license, but it is available exclusively as a royalty-free cell line to biopharmaceutical clients that use our JP3 Process

Figure 1: Typical cell line development program at Just-Evotec Biologics to select clones with optimum performance in continuous perfusion culture; DWP = deep-well plates, tfxn = transfection



and Product Design service. This service provides cost-effective access to high-performing CHO cell lines to smaller biopharmaceutical, startup, and academic laboratories involved in early discovery work. If clients later decide to move forward with their programs, this can easily flow into our manufacturing platform.

We also are engineering our CHO lines for special applications, from afucosylation to glycoengineering and inducible protein expression. Currently, we have engineered inducible CHO cell lines to incorporate a doxycycline-inducible expression system. We have demonstrated that our inducible host cells (CL-129 and CL-165) enable control of MAb expression and can produce hard-to-express MAbs at higher productivity than with either an industry-standard CHO line or our CHO line, CL-72 (both noninducible) (Figure 3). Developing and using well-characterized cell lines help mitigate risks by ensuring consistency of a biotherapeutic's quality. By selecting a cell line that produces a desired molecule reliably in both fed-batch and continuous perfusion culture, we can minimize batch-to-batch variability while ensuring reproducibility and continuity of supply, which is critical for regulatory compliance and patient safety.

Expression Vectors and CLD Workflow

We offer proprietary, patented transposon-based expression vectors (**3, 4**) designed for efficient transfection and high-level expression of biotherapeutic proteins in our CHO-based cell lines. Using this expression system, we have achieved expression levels of up to 60 pg of protein per cell per day for MAbs in continuous perfusion culture (Figure 2). In 2022, one client was able to file for the first Biologics License Application (BLA) for a program using this expression system. We also are optimizing vectors for expression of non-MAb molecules such as Fc-fusion, multichain bispecifics, and multiantibody complexes.

We transfect 1–32 molecule variants for a typical client project. Those variants can include antibody modifications identified using our Abacus suite of tools, alternative signal peptides, or different formats of bispecific antibodies. For multiple variants, we use a high-throughput electroporation method that allows up to 96 transfections in one run.

After transfection, cells are cultured in a selective medium that allows growth of only those cells containing the GS selectable marker along with the biotherapeutic protein gene. For fast and efficient selection, we use 24-well deep-well plates with high-

Figure 2: Comparison of MAb yields in a 20-day continuous perfusion culture using an industry-standard CHO cell line

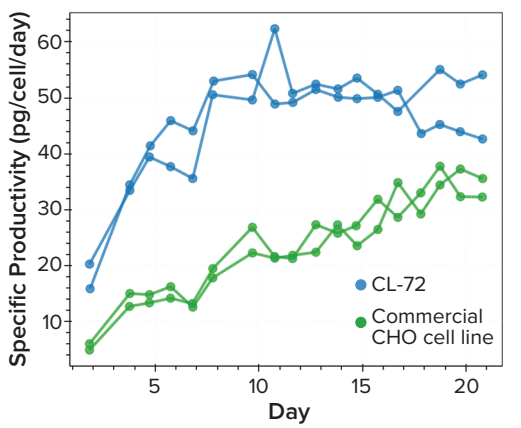
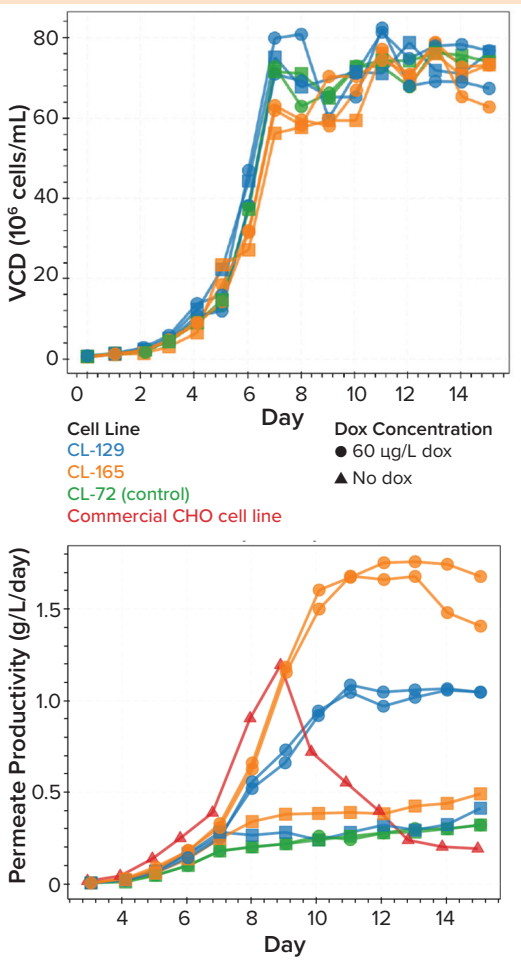


Figure 3: VCD (TOP) and productivity (BOTTOM) of a hard-to-express MAb molecule in a 15-day continuous perfusion culture using doxycycline (dox)-inducible CHO cell lines (CL-129 and CL-165) compared with noninducible commercial CHO and CHO-based CL-72 cell lines; dox induction began on day 7.



Such informed decision-making enables us to **REDUCE RISK** by maximizing throughput and minimizing waste.

throughput automation based on a range of Tecan liquid handling systems. At this stage of our CLD workflow, our use of nonclonal transfection pools provides an early indication of the optimum pools to commit to for resource-intensive cloning. We screen pools for expression and product quality as described below to identify the appropriate variant pool with which to proceed.

Single-Cell Cloning

The next stage in a CLD program is to isolate clones from single cells to identify those with highest expression and appropriate product quality. We isolate our clones using the Solentim VIPS and Cell Metric platforms; these automated systems reduce risk by enabling us to track cell growth over time without manual, error-prone microscopy analysis. This provides assurance that each clone is derived from a single cell. We then use automated liquid handling with our customized Tecan systems to enable culturing of multiple clones in 96-well deep-well plates, from several hundred up to our current capacity limit of 1,000 clones.

Screening and Characterization

Clonal cell lines are screened for high protein expression and other product quality attributes (PQAs) using a number of analytical methods, most of which are built on automated platforms. Our typical workflow includes testing clones for titer and product quality by ultrahigh-performance liquid chromatography (UHPLC), size-exclusion chromatography (SEC), and capillary electrophoresis (CE). For verifying the quality of our most promising clones, we use mass spectrometry to detect sequence variants and post-translational modifications. We run the most promising clones in benchtop-scale bioreactors (1 to 3 L scale) in our 15-day platform process to determine their performance in a perfusion bioreactor. Then, using the techniques described above, we assess the biotherapeutic proteins those clones express. We evaluate data from our platforms and use the results to choose the final clone for creating the master cell bank (MCB). Such informed decision-making enables us to reduce risk by maximizing throughput and minimizing waste.

Development and Manufacturing Expertise

By using the CL-72 cell line in our CLD program, we have initiated multiple projects, created four MCBs, and performed three client manufacturing runs at 500 L scale in the past two years. We have achieved titers with two recent client antibodies of 5 g/L/d day (a titer of 5 g/L/day translates to approximately 40 g/L in a fed batch) and titers as high as 4 g/L/day with a client's Fc-fusion molecule. Additionally, using our dox-inducible platform cell lines, we can achieve as much as 1.5 g/L/day of a hard-to-express antibody molecule (Figure 3). For a more complex, poorly expressing bispecific antibody, we have achieved 1 g/L/day in 500 L manufacturing.

Robust, Sustainable Manufacturing

Biopharmaceutical companies need predictable cell line and process performance for robust and sustainable manufacturing. Partnering with a CDMO such as Just-Evotec Biologics that can provide a bespoke CLD program to deliver clones adapted for increasing productivity in continuous perfusion culture can help reduce risks as well as produce biotherapeutics with appropriate PQAs in shorter timelines.

References

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