



Successful strategies for product optimization

Identifying the critical decision points and key stages of cell line development and process scale up is essential when designing strategies for producing successful recombinant products.

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From the selection of expression technology to clinical production, the correct balance between time and cost *versus* yield and quality is essential to maximize return on investment. Although creation of a regulatory-compliant cell line is essential for the production of clinical material, the initial choice of expression technology is often a rushed or overlooked activity that is overshadowed by financial and/or time constraints.

During recent years, there has been increasing pressure within the biopharmaceutical industry to produce high-quality products within shorter time lines, which can be achieved using robust, low-cost processes. Many recombinant proteins in the development pipeline are monoclonal antibodies, which typically require multiple doses at 10–100 times greater dosage than successful recombinant protein drugs, such as erythropoietin and human growth hormone,¹ raising concerns regarding current cell culture production capacity.²

This limited capacity has resulted in a drive to increase expression levels to >1 g/L, with some now reporting >5 g/L.² Because of the demand for greater productivity, cell line development and optimization has become a critical step and, to be effective, should be considered early in development. The parameters for optimizing a cell line may include deciding on the appropriate expression (enabling) technologies, media choice or development, clone analysis and process optimization. As with any biopharmaceutical process, the potential benefits of utilizing proprietary expression technologies need to be carefully weighed against the costs, in particular intellectual property (IP) right encumbrance, which may lead to downstream royalty stacking.

When producing a clonal cell line, the anticipated development route of molecular biology, transfection/selection and the minimum of two rounds of cloning accompanied by some scaled evaluation of each clone is often challenged by time lines, cost and the cell

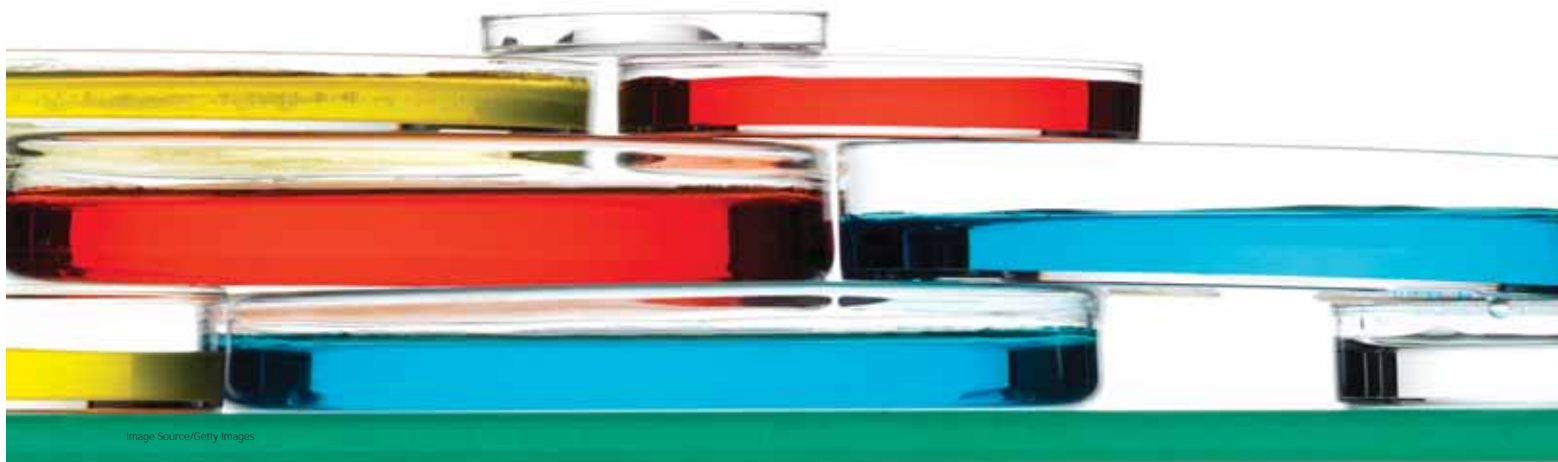


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line used. Once a suitable cell line has been established, further development and optimization of the production process can be undertaken. Different modes of cultivation and the variety of vessels available, including traditional steam-in-place bioreactors and, more recently, disposable systems, provide further decision points and areas for optimization. Demonstration of the scalability of a chosen process prior to cGMP production is essential for a rapid and cost-effective route to clinical material. Addressing the balance of all of these factors as early as possible will eliminate hurdles at later stages, thereby reaching the desired endpoint more quickly and cost effectively.

Expression technologies

This should be considered the most important decision in the development cycle of any product as an uninformed decision based on commercial requirements alone may, ultimately, have disastrous consequences on the efficacy of the target recombinants.

Bacterial systems are ideal because they are low cost, highly productive and rapid to use compared with mammalian systems. The absence of adventitious agents, such as viruses, that may be present in mammalian systems is also a regulatory and safety benefit. In many cases, however, bacterial production cannot yield an effective product because of the requirement for post-translational modifications, e.g. glycosylation, which are only provided by eukaryotic systems.

Yeast and insect cell production systems combine some of the benefits of microbial production with the capability for limited post-translational modifications, but some of the best production systems, such as *Pichia pastoris*, are proprietary and require licences to use.

Currently, an estimated 50% of the hundreds of biopharmaceuticals in the pipeline are developed using mammalian systems.² The principle advantage is the production of a recombinant protein that is truly 'human-like' in nature, but the production costs and challenges are greater than for microbial systems.

Another important consideration in an expression system is the choice of vectors. Table 1 details some common mammalian expression systems that can increase the level of highly productive stable clones. Ensuring

licence-free vectors from the beginning of development avoids royalty stacking and a recloning exercise later in development, which risks changes to product yield and quality.

Transfection

There are many methods for delivery of expression constructs to cell lines. Efficient delivery of plasmid DNA is variable across techniques, and consideration for the delivery method should be based on the requirement for transient, episomal or stable maintenance or integration of the expression construct.

Electroporation is a valuable and effective alternative to other physical and chemical methods. Advantages include:

- It can be used on a variety of cell lines, including prokaryotes, yeast and eukaryotes.
- A higher transfection efficiency is generally obtained compared with the use of chemical reagents.³
- Smaller quantities of DNA are required.⁴
- The method incurs lower costs compared with expensive chemical reagents.
- No expiry dates are involved.

Clone selection

There will always be demand for cell development services as the process is vital to the end product. However, selecting the final clone can be complicated and difficult because of the minimal environment in which single cells generated through limited dilution or facilitated cloning exist. The process is lengthy primarily because of many cell lines having a transfection efficiency of <1%.⁵ Most cells need to communicate with surrounding cells to enable growth, but the probability of a single cell multiplying successfully can be increased by supplementing the media with either serum or preconditioned media.⁵ The serum can easily be depleted while scaling up, but some cell clones will be lost as they cannot survive without the serum. The traditional method is to manually perform limited dilution cloning from a transfected pool. This is labour intensive as it involves initial analysis and consequent monitoring for single cell wells. Typical time lines for each round of cloning from the point of single cell seeding to evaluation of clone

productivity can be 6–12 weeks depending on the cell line and the recombinant produced. Regulatory guidelines recommend at least two rounds of dilution cloning, and the effort required for maintenance and analysis is proportional to the number of clones selected from each round.

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Many automated systems have been developed to aid cell line development because of high demand and competition. These systems assess cell growth from a single clone and isolate higher expressers for further expansion, and some can perform media optimization in parallel. Using an

Figure 1 Production process consistency demonstrated at small scale.



On the go...

- Cell line development and optimization is a critical step that should, to be effective, be considered early in development.
- The most important decision in the development cycle of a product is the choice of expression technologies; an uninformed decision based only on commercial requirements could have disastrous consequences.
- The culture medium may have dramatic effects on a production process and it is recommended that the culture choice is not changed after the production clone has been chosen.
- The question of disposable *versus* nondisposable production methods must be addressed.

Cell line development

automated system allows a higher number of clones to be analysed and removes any error associated with multiple operators. Two such systems are the ClonePix (Genetix, UK)⁶ and Cell Xpress (SAFC Biosciences, UK),⁷ both of which use fluorescence technology to detect high-expressing cells from thousands. These high-throughput screening methods can reduce the time taken to create a robust cell line from 12–18 months to 6–12 months, and provide high-throughput analysis while maintaining and selecting clones.⁵ Disadvantages include the expense and the lack of a track record, although it is envisaged that these will be overcome with the increased uptake of technologies.

Media development

Selection of the culture medium may have dramatic effects on a production process. Historically, the requirement for serum poses problems regarding lot-to-lot variability and the contaminants it contains that must be removed during downstream processing. Serum may contain transmissible spongiform encephalopathies and other

adventitious agents, which has led to a regulatory drive to avoid serum containing media for production processes. Where the use of serum cannot be avoided, it must be appropriately sourced and the associated risks assessed.

Recent times have seen a host of chemically defined media developed to support the high-cell density cultures expected during batch production and to facilitate small-scale development activities.⁸ In some cases, these media have also been developed with a preformulated feed strategy that can be applied in parallel studies to further exemplify clones in either a batch or fed batch mode.

As a word of caution, changing the culture media post selection of the production clone should be avoided.

Cultivation mode

The decision of format and scale up for the chosen cell line will have particular commercial implications should high final or multiple doses be required. The challenge, particularly for contract manufacturing organizations, is to (re) develop or scale current cell lines to an appropriate format.

Anchorage-dependent cells generally require large surface areas to generate sufficient biomass for the required productivity. This can be achieved by using multiple tissue culture flasks, multitray bioreactors, roller bottles, microcarrier culture and hollow fibre bioreactors.

Cell growth in suspension culture is not dependent upon 2D surface area. Significant advances in media development and their ability to support high-density cultures has resulted in increased product yields with no change in product quality, thus reducing the scales and time lines required. Suspension cells are more straightforward to cultivate than adherent cells as no enzymatic treatment is necessary, meaning less manual handling is involved and contamination risks are reduced. Suspension cells are grown as shaking cultures at small scale and in stirred tank vessels or rocking bag systems for larger-scale applications. Early consideration of the choice of cultivation mode is essential as it affects the production process and, often, the achievable yield of product.

Bioreactor choice

For anchorage-dependent and suspension cell methodologies, the question of disposable *versus* nondisposable production methods must be addressed. Table 2 highlights some of the advantages and disadvantages of these technologies. A wide range of disposable bioreactors is now available.⁹ As more data are acquired that favourably compares them with traditional bioreactors, disposable systems are becoming more and more accepted for large-scale production, and are already employed as part of process seed trains by major biological manufacturers.¹⁰

Modular disposable systems, such as roller bottles and multitray bioreactors, may be employed at manufacturing scale. A linear increase in the number of vessels is required to produce the desired quantity of product. As each vessel will contain comparable cell numbers, it is possible to predict product yield and quality from any number of vessels. As no equipment changes are necessary, scale up to a manufacturing process may be expedited by comparison with traditional bioreactor systems.¹¹

Table 1 Common expression mammalian systems.

Technology	Vendor	Technology type	Licence required
MarTech	Selexis	Chromatin remodelling	Yes
UCOE	Millipore	Chromatin remodelling	Yes
Star	Crucell	Chromatin remodelling	Yes
Ease	Amgen	Chromatin remodelling	Yes
DHFR	No IP	Amplification	No
GS System	Lonza	Amplification	Yes

Table 2 The disposables debate: a summary of advantages and disadvantages.

	Non-disposable	Disposable
Advantages	<ul style="list-style-type: none"> • Robust designs • Proven methodologies • Small to large scale production 	<ul style="list-style-type: none"> • No sterilization and cleaning validation • Reduced capital costs • Small to semi-large scale production • Flexible • Quick to implement
Disadvantages	<ul style="list-style-type: none"> • High capital costs • High maintenance • Sterilization and cleaning validation 	<ul style="list-style-type: none"> • Limited large scale production • Novel methodologies

However, the use of multiple vessels leads to increased manual handling or a demand for automation. The cost of this may be high and a large footprint for incubation is also required. Additionally, these systems offer only limited in-process monitoring and control.

More recently, the in-process monitoring and control conferred by traditional nondisposable bioreactors has been incorporated into disposable systems, including rocking bag technology and disposable stirred tank bioreactors. However, these require substantial development, optimization and proof of scale up in comparison with the modular disposable vessels, adding to the overall time and cost of development.

Further process development

Once the production clone(s) has been identified, further investigations can be made into improving cell growth and production. Cell seeding densities may result in higher or lower maximum viable cell densities that may impact the final product yield. These should, therefore, be investigated. Cell generation number may also affect productivity and stability, and must be assessed if a robust process is to be determined. Feeding strategies may also be developed to extend cell growth periods, and increase culture viability and productivity.

At these stages, statistical experimental design and high-throughput methodologies may be employed to highlight thresholds for optimization in relatively short time lines and using small amounts of starting material.¹² It is necessary for

supporting analytical tools to be available to assess any impact on product yield or quality. Changes to a process resulting from these investigations must be exemplified in representative systems, and any impacts on downstream processing highlighted and addressed as early as possible.

Process reproducibility and scalability

As part of the continued development plan, the reproducibility of the process is typically demonstrated from comparative benchtop-scale bioreactors (Figure 1). Prior to transferring a process to a cGMP production facility, it is vital to demonstrate scalability of the process using pilot-scale versions of the production equipment. These activities will identify any potential problems in advance of expensive cGMP manufacturing.

There are many factors to consider when developing a production process. One of the key criteria for successful process development is to have clear objectives for the type and scale of process that is required. The better defined the destination, the easier it is to plot the development journey. **PTE**

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