Article | August 1, 2024

Cell Line Development Screening: The First Crucial Step For Yield And Critical Quality Attributes

The selection of representative cell lines for expressing bispecific antibodies (BsAbs) is a crucial initial stage that significantly influences overall process yield and quality. The chosen cell lines represent the basic productivity and protein quality of the BsAb, impacting the potential for subsequent process development, scalability, stability, and purity of the main product. Because the structure of a BsAb is more complex than that of antibodies, it will also encounter more production problems in manufacturing, such as low product yield, erroneous chain assembly, and high aggregate levels. Therefore, the extensive screening and evaluation of multiple BsAb-expressing cell clones at this stage are necessary and play a pivotal role. Having a suitable clone can reduce the problems that upstream and downstream processes may face. Optimizing the expression plasmid and improving the screening strategies can help us obtain the best production clone.

Expression Plasmid Design

Regulating gene expression encompasses various strategies, including codon optimization, signal peptide selection, and utilization of different host cell lines and expression vectors. Additionally, the transposon system and ubiquitous chromatin opening element (UCOE) sequence are gaining popularity in biomanufacturing applications, aiming to mitigate production instability and enhance productivity.

Among them, the most economical way to increase BsAb production is by choosing an appropriate signal peptide. Different proteins may require different signal peptides depending on their characteristics. Hence, the selection of signal peptides is crucial to increase cell productivity. The results found that the yield of BsAb in different formats can be effectively increased after replacing signal peptides (Figure 1).

Figure 1: Productivity with different signal peptides.

In addition to selecting the signal peptides, the different proportions of BsAb polypeptide chains will affect the yield of a BsAb. The gene of polypeptide chains in the different expression cassettes will affect the proportion of peptide expression. Therefore, the position of different polypeptide chains in the plasmid will also cause an effect on the yield of BsAb. In our tests, we made two different orders of polypeptide chain sequences (Figure 2a). The results found that by changing the position of the two polypeptide chain sequences on the plasmid, the yield can be significantly increased (Figure 2b), and the impurity level changed accordingly (Figure 2c).

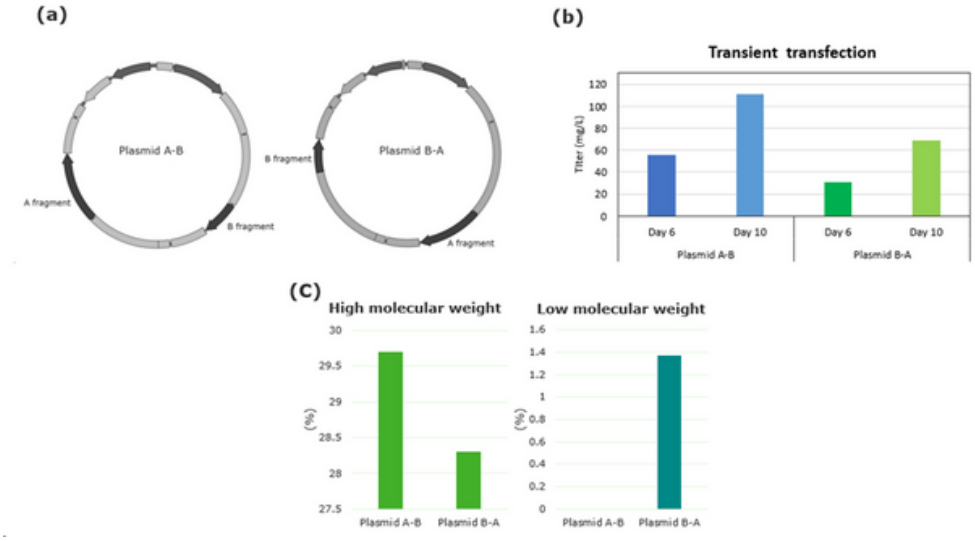

Figure 2: (a) Two different orientations of polypeptide chain sequences, (b) productivity, and (c) impurity level with different orientations of polypeptide chain sequences.

The combination of the expression system and expression vector needs to be carefully selected. Changing the expression vector can also help improve productivity significantly. The results show that the yields obtained using vector 1 and vector 2 in format 1 of the BsAb are similar. However, in format 2 of the BsAb, vector 2 had much more yield (Figure 3).

Figure 3: Productivity of two formats of BsAbs in two vectors.

Screening Strategies in Cell Line Development

The aim of cell line development is to identify a suitable cell line that has stability, high productivity, and desired protein quality. To enhance cell line development strategies, it is necessary to select as many candidate clones as possible and analyze the yield of these clones and the integrity of the protein produced. Recently, there have been significant improvements in the automation of single-cell cloning, such as ClonePix, Cyto-Mine, and Beacon.

In Mycenax's Beacon platform, the Beacon system can perform single-cell cloning and more than 4,000 single-cell clones will be screened in five days. Beacon will select the top 100 single-cell clones with high yields for subsequent productivity measurement. As shown in Figure 3a, the results show the yield distribution of the top 12 selected clones. The top 12 clones were then analyzed to determine their protein quality. The results show that the selected clone #9 not only has the highest yield but also has the best SE-HPLC and CE-SDS results (fig. 3b, 3c).

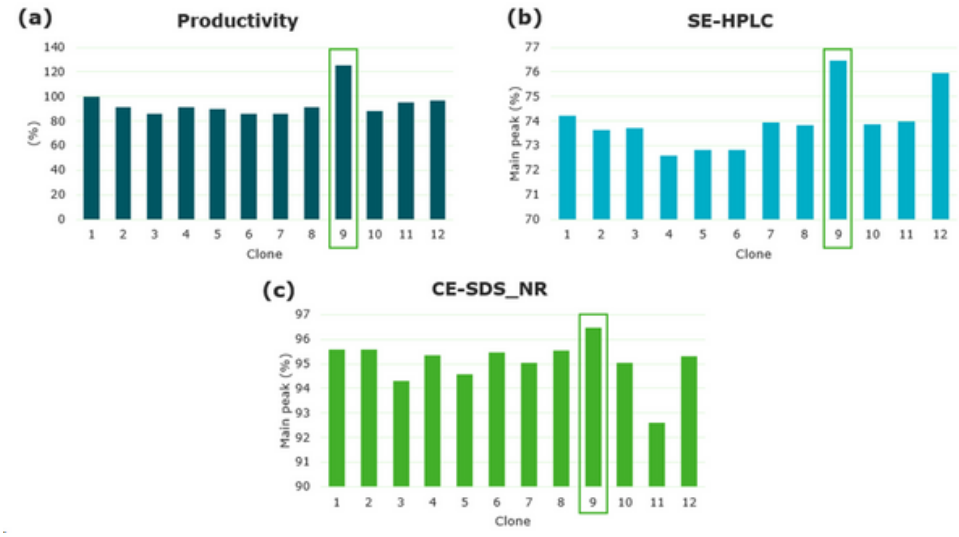

Figure 4: (a) Productivity of candidate clones, (b) SE-HPLC of candidate clones, and (c) CE-SDS of candidate clones.

Implementing Design Strategies to Build a Suitable Cell Line

The three major problems currently encountered in BsAb production are low product yield, erroneous chain assembly, and high aggregate level. For the format of single chain Fv-IgG in this case, erroneous chain assembly will not be the main problem. The problems that need to be solved are low product yield and high aggregate level. Using the design strategy of plasmids, the basic yield of BsAb can be increased and the aggregate level can be reduced before cell line development. The clone selection strategy can be used to further select production clones with high yield and the lowest aggregate level in the cell line development process. Therefore, through the above strategies, a suitable cell line can be selected for production in cell line development.

About Mycenax

Mycenax is a dedicated CDMO specializing in process development and cGMP manufacturing of biologics and biosimilars. We provide integrated DNA-to-Drug Product services for mAbs, ADCs, plasmid DNA, cell therapies, vaccines, and more through advanced platforms and PIC/S GMP-compliant facilities. Our GMP site is approved by EMA, MHRA, PMDA, Health Canada and MFDS, and has successfully passed over 70 global client audits, ensuring the highest standards of quality and compliance.