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Mammalian cell lines used in bioprocessing

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Abstract

A various number of expressions and host systems are used in biologics manufacturing. The most commonly preferred systems are based on bacteria, yeast, mammalian cells, insect cells, and transgenic animals. A wide range of molecules, including insulin, mAbs, vaccines, and recombinant proteins, are produced using different host systems. Because of several reasons impacting the product quality and yield, mammalian cells are utilized. Moreover, mammalian cells are generally used in virus-based vaccine manufacturing. Chinese Hamster Ovary (CHO) is the most widely used cell line for high yield stable recombinant protein production, while Human Embryonic Kidney (HEK) is favoured for transient transfection low yield protein manufacturing, viral-based vaccine and gene and cell therapy-related vector production. Other mammalian cell lines such as NSO, Sp2.0, Vero, MRC-5 and PerC.6 are also used in both recombinant protein and virus productions. Multiple modifications are carried out on industrial cell lines to make them more suitable for high yield and high-quality protein production. Thanks to these alterations, high productivity and quality levels are achieved in the biotechnology industry.

Keywords: bioprocessing, industrial cell lines, mammalian cell lines, biologics manufacturing

1. General introduction and expression systems used in bioprocessing

Biologics are gaining importance daily; even though biologics are expected to share %32 of sales, they are estimated to contribute to %50 of the Top 100 product sales by 2024 in pharma (1). Rapidly increased market demand for biologics improved expression systems and bioprocessing (2). Advancements in bioprocessing caused a remarkable increase in the yield of products, which eventually led to effective therapies against various complex and rare diseases (3). Several expression systems are used to manufacture recombinant therapeutics, including bacteria, yeast, insect cells, transgenic animals, and mammalian cells (4-7). On the other hand, gene and cell therapies are carried out mainly by using mammalian host cells (3).

Each expression system has advantages and disadvantages used for specific needs. For instance, bacteria offer large-scale manufacturing of recombinant proteins in a short process due to their fast growth rate with a doubling time of 20 minutes for *E.coli*. Simple culture conditions and low costs are required in these systems. Disadvantages of bacterial systems can be associated with accumulation of proteins in inclusion bodies, protease contamination from host proteins leading to degradation of the expressed protein, endotoxin accumulation and lack of proper post-translational modifications (8, 9). Despite these disadvantages, several molecules such as single-chain variable fragments (ScFvs), antibody fragments (Fabs), single-domain antibody (sdAb), insulin and its analogs, meningococcal vaccines, hormone analogs, pegfilgrastim and enzymes are manufactured by using *E.coli* (10).

Although bacterial expression systems are considered one of the oldest in biotech history, other expression platforms have been developed because of the drawbacks mentioned above.

Yeasts are commonly utilized as host expression systems due to their fast growth rate, similar but less than bacteria, predisposition to genetic manipulations, existing genetic information, low-cost fermentation characteristics, and capability of providing proper post-translational modifications. *Saccharomyces cerevisiae* and *Pichia pastoris* are the most widely used yeast expression systems for biologics manufacturing (11-13). Various genes associated with secretion and Golgi trafficking of proteins have been modified

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in *S.cerevisiae* to increase the productivity up to a gram per liter level (14). However, improper glycosylation, like hypermannosylation, which consequently causes faster blood clearance, is also present. This problem has been solved by removing the enzyme specific for mannosylation, called the mannosyltransferase gene (15).

Table 1. Main expression platforms used in biologics manufacturing and several examples of products manufactured by mammalian cell lines

Product	Producer	Molecule Type	Host cell
Perjeta (Pertuzumab)	Roche	Monoclonal antibody (mAb)	CHO
Aimovig (erenumab)	Amgen	mAb	CHO
Fasenra (benralizumab)	AstraZeneca	mAb	CHO
Taltz (ixekizumab)	Eli Lilly	mAb	CHO
Lifmior (Etanercept)	Pfizer	Fusion recombinant protein	CHO
Truxima (rituximab)	Celltrion	mAb	CHO
Retacrit (epoetin alfa-epbx)	Eprex and Erypo	Recombinant protein	CHO
Shingrix (zoster vaccine)	GlaxoSmithKline	Recombinant Vaccine	CHO
Zessly (infliximab)	Sandoz	mAb	CHO
Herzuma (trastuzumab)	Celltrion	mAb	CHO
Adcetris (Brentuximab-Vedotin)	Takeda	Antibody-drug conjugate	CHO
Mylotarg (Gemtuzumab ozogamicin)	Pfizer	Antibody-drug conjugate	CHO
Kadycla (Trastuzumab emtansine)	Genentech (Roche)	Antibody-drug conjugate	CHO
Vihuma (simoctocog alfa)	Octapharma	Recombinant protein	HEK
Luxturna (voretigene neparvovec-rzyl), nonreplicating adeno-associated virus expressing human *RPE65* gene	Spark Therapeutics	Gene therapy	HEK
Covid-19 vaccine	Astra Zeneca	Adenoviral vaccine	HEK
Lartruvo (olaratumab)	Eli Lilly	mAb	NS0
Mylotarg (gemtuzumab ozogamicin)	Pfizer	Antibody-drug conjugate	NS0
Inflectra (infliximab-dyyb)	Hospira	mAb	Sp2/0
Erbitux (cetuximab)	Merck	mAb	Sp2/0
Rekovelle (follitropin delta)	Ferring	Recombinant protein	PER.C6
Covid-19 vaccine	Johnson and Johnson	Adenoviral vaccine	PERC.6
Gardasil (HPV vaccine)	Merck	Recombinant VLP vaccine	*S.cerevisiae*
Engerix B (HBV Vaccine)	GSK	Recombinant VLP vaccine	*S.cerevisiae*
Recombivax HB (HBV Vaccine)	Merck	Recombinant VLP vaccine	*S.cerevisiae*
Novolin R (Insulin human)	Novo Nordisk	Recombinant Insulin	*S.cerevisiae*
Lantus (Insulin Glargine)	Sanofi	Recombinant Insulin derivative	*E.coli*
Humalog (Insulin Lispro)	Eli Lilly	Recombinant Insulin derivative	*E.coli*
Covid-19 vaccine	Biontech/Pfizer	mRNA vaccine	*E.coli*
Covid-19 vaccine	Moderna	mRNA vaccine	*E.coli*
Covid-19 vaccine	Sinovac	Inactivated vaccine	Vero
Imlygic (talimogene laherparepvec), an engineered herpes simplex virus type 1 expressing GM-CSF	Amgen	Gene therapy	Vero
Cervarix (HPV Vaccine)	GSK	Recombinant VLP vaccine	Hi-5 cell line (Baculovirus)

Moreover, besides removing the hypermannosylation gene (OCH1), glycosyltransferase and glycosidase genes are also transferred to manufacture the expected glycoprotein in another platform (16). The second most striking yeast expression system is based on *Pichia pastoris*, which can secrete adequately folded and active proteins, sustaining lower protein glycosylation and reaching high cell densities (17, 18). Since N-linked glycosylations are different in higher eukaryotes, some yeasts have been genetically modified to carry out human-like N-linked glycosylation (19). One of the most significant drawbacks of the *P.pastoris* expression system is the degradation or truncation of the protein of interest, which leads to low yield and loss of functional activity. Several methods have been developed to eliminate this problem, such as adding casamino acids, yeast-based peptone, protease inhibitors, creation of protease deficient strains, reduction of temperature and pH, and usage of different carbon sources (20). Considering all these advantages and disadvantages, it can be inferred that the yeast expression systems can be characterized by medium overall cost, good folding and glycosylation, medium manufacturing time, easy handling, medium to fast growth rate, easy genomic modifications, and low-cost contamination risk (21).

Furthermore, insect expression platforms can be used for multiple recombinant products like HPV vaccine. Insect cells are grown up to the required viable cell number and then transduced with a recombinant baculovirus, including the gene of interest (22, 23). Due to the lack of glycosyltransferase

enzymes, the N-linked glycosylation pattern in the insect system is not desirable. This problem can be overcome by introducing glycosylation related genes (24). The most widely used cell line in the baculovirus expression system is Sf9 cells. Besides Sf9, S2, Sf21, Tn 368 and Hi-5 cells are used to produce recombinant proteins (25, 26). Insect expression systems are associated with proper protein folding, slow growth rate, high productivity, medium cost and manufacturing time, and very low risk of contamination (27).

In addition, transgenic animals are used as host expression systems as well. These animals include a gene that codes a protein integrated into their chromosome and can pass to their offspring, rendering them transferring the transgene to generations (28). The most common ways of acquiring recombinant proteins in transgenic animals are from their milk and eggs (29). Although they usually provide suitable post-translational modifications (PTMs), there are some concerns that the producers face, such as the presence of zoonotic pathogens and ethical questions about producing transgenic animals (30).

Transgenic animals are characterized by high product yield and quality, low scale-up capacity, a very high risk of contamination, and high overall cost.

Table 1 shows the list of biological products with different host and expression platforms.

2. Why are mammalian cell lines preferred in bioprocessing, and how are they processed?

Mammalian cell-based expression systems are the most dominant platform for recombinant protein and viral-based vector and vaccine production purposes. They can produce complex molecules such as mAbs, and several therapeutic proteins with high molecular weight. Compared to bacteria and yeast systems, introducing a gene of interest into the host is time-consuming and labour intensive. Also, the selection procedure while creating stable cell lines is much longer than the microbial systems.

The crucial phases during cell-line development are associated with selecting the most suitable cell line, transfection method, and the most appropriate expression vector (31). In order to select the single-cell clones to ensure monoclonality, several high throughput screening devices have currently been used. These devices can be classified as FACS (fluorescence-activated cell sorting), colony picker and single-cell printer instruments. It is also crucial to picture single-cell clones to prove monoclonality, which is extremely important in ensuring the consistent manufacturing of biological molecules (32). Several instruments are used for this purpose, some of which have cell printing ability.

When selecting the best clones after the screening, the primary factors are proteins with high expression levels, suitable post-translational modifications, stable production patterns, and critical quality attributes. Other specifications, which are taken into account during the selection processes, are viable cell density, cell viability, and easiness to scale up both in fermenter and downstream processing. So, after determining the compatibility with industrial standards and meeting the quality criteria, the best clones are selected and used for further activities (33). Generally, the cell line development procedure is followed by process optimization and, subsequently, scale-up studies. Process optimization is usually performed at lab scale, and just after scaling up to large scales, clinical and commercial batches are carried out.

Monoclonal antibodies are the most commonly used biological therapeutics on the market. Most of the mAbs present on the market are IgG class-based, consisting of two heavy and light chains connected by disulfide bridges (34). Besides disulfide bonds, glycosylation is a common and crucial post-translational modification to ensure the molecule's activity. It should be emphasized that the post-translational modifications are present in mammalian cell lines, but glycan types may differ from human-based glycosylations. As expected, human cell lines such as HEK293, HKB11, PER.C6, HeLa and CAP cells are better for getting human-like glycans (35). IgG1 molecules include a single N-linked glycan at the Asn^{297} position in both heavy chains. While synthesizing N-glycans, multiple sugar structures can be added to form different glycosylation patterns such as G0, G1, G1F, G2S, G1FS, G2FS, G2FS2, G0-GlcNAc, etc.

Glycosylation has a critical role in complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) by modifying the binding to the Fcy receptor. Both ADCC and CDC are involved in eliminating cancer cells, and particular glycans are necessary to provide therapeutic efficacy by increasing the specific interaction with various FcyR receptors and complement factor receptor C1q (36). For instance, G1F glycosylation has significantly increased the binding property of the mAb molecule to the C1q receptor, which eventually increased the complement system activity (37). Moreover, modifications associated with afucosylation have induced IgGs interaction with different FcyR receptors like FcyR IIa R131, FcyR IIa, FcyR IIb, FcyR IIIa, FcyR IIIa V158, FcyR IIIa F158 or FcyR IIIb. This strong interaction is known to boost ADCC activity (38, 39). Therefore, elevating the activity of mAbs by glycosylation modification is of great importance in the functional activity of biological molecules.

These desired glycans can be acquired either by using human cell lines such as HEK293, Per.C6, HeLa or by carrying out glycosylation engineering on most commonly used mammalian cell lines like CHO (3, 40). However, handling these human cell lines on large scales for commercial manufacturing is still in the improvement phases; hence most people in the biotech industry still prefer glycosylation modified CHO cell lines (41, 42). Besides genetic manipulations to reach the critical quality attributes in

glycosylation, media-feed screening and clone selection procedures are essential to acquire the optimal glycosylation pattern (43).

Furthermore, mammalian cell lines are preferred for manufacturing molecules requiring relatively high doses, such as monoclonal antibodies. Monoclonal antibodies (mAbs) are generally administered at 1-10mg/kg doses on average, while vaccines are usually applied with much lower doses, like the Hepatitis B vaccine at 20µg/ml (44, 45). Therefore, almost a thousand times more product is required for mAb production. Because of high yield requirements, mammalian cell lines are commonly used to manufacture these therapeutic molecules. So, stably transfected CHO cells are generally utilized, and more than 3g/L titers are found favorable in the industry; also, up to 9-10 g/L yields at maximum are acquired in fed-batch cultures (46). On the other hand, up to 12-fold more yield is expected in perfusion cultures, which renders mammalian cells suitable hosts for monoclonal antibody productions (47). However, several modifications are carried out in host cells to achieve high titers in these expression platforms, which are described in detail in section 3.

Mammalian cell lines are often used to manufacture viral vectors and vaccines (48). The advent of mammalian cell cultures allowed us in vitro production of viruses and opened the path for developing a various number of vaccines (49). The most common viral vaccination forms are attenuated and inactivated vaccine forms based on mammalian cell culture passages (50). Several vaccines, such as measles, poliovirus, rubella, rabies, hepatitis A, adenoviral- based covid-19 vaccines, were developed using cell culture techniques.

On the other hand, viral vectors, which are utilized in gene therapies, viral vector vaccines and cell therapy transductions, were also developed in animal cell culture. Recent developments in mammalian cell culture technologies enabled people to improve the yields of viral vectors and allowed scale-up in suspension cultures (51). Gene therapy is a very promising technology, which will be strikingly remarkable in the near future, along with cell-based therapies. They have the potential to correct the inherited defective genes causing several disorders (52). However, highly scalable and productive gene therapy processes must achieve clinical and commercial success. Typically, adenoviruses, adeno-associated viruses, retroviruses, lentiviruses, and herpes viruses are used for gene therapy approaches and cell therapy transductions (53). Stable producer or packaging cell lines are required for viral vector formation. Generally, Human Embryonic Kidney (HEK) cells are used for this purpose (54). For instance, for AAV formation, crucial genes are required for the assembly and infectivity of the virus. These crucial genes are separated into different plasmids containing E2A, E4 and VA. The transfer plasmid, Rep/Cap, and the helper plasmid are transfected into HEK293 cells, which contain the adenovirus gene E1, to create fully infectious AAV particles. So, the E1 gene, one of the most important genes of AAV formation, is provided with HEK packaging cell lines (55). Similar features can be considered for lentiviruses and retroviruses as well.

Moreover, viral vector-based vaccines are manufactured with the help of several mammalian cell lines such as HEK, Vero, HER.96 etc. Usually, vector-based vaccines cannot replicate themselves but can deliver antigens to the immune cells in the form of nucleic acid, DNA or RNA (56). HEK cell line is commonly used to propagate adenoviral vector-based vaccines, which are replication-defective and lack E1 genes (E1A and B) (57). These viruses are the most frequently used vaccine vectors. They are easily propagated in HEK cells or Per.C6 cells with high viral titers. Eventually, transgenes are expressed, activating the immune system (58). Rather than adenoviruses, poxviruses, herpes simplex viruses, cytomegaloviruses, alphaviruses, and adeno-associated viruses are considered viral vector-based vaccine options (48).

Also, in cell therapy applications, T cells are transduced with lentiviruses, propagated by mammalian cells. The most common cell line used for lentiviral production is HEK. The viral vectors for approved cell therapies are manufactured in the HEK cell line (59, 60).

3. Genetic modifications to create industrial mammalian cell lines

Primary human cell lines were isolated from the patients for a very long time ago. Animal cells such as CHO and Sp2.0 were also isolated from their primary sources long before. However, these cell lines cannot carry out industrial production of biological molecules because several requirements are present to meet the critical quality attributes (CQA) of the respective molecule. Thus, many genetic modifications were applied to make the cells more convenient for the biopharmaceutical industry (61). Classical genetic modification techniques are applied to increase cell lines' efficiency in manufacturing.

Besides these techniques, the latest progressions in gene editing nuclease enzymes such as CRISPR/Cas systems, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) render cell modifications cheaper and more applicable (62, 63). Several cases showed the successful utilization of these enzymes (64, 65).

The modifications applied to increase the efficiency of host cells are related to cell growth, maintaining cell viability, preventing cell death, promoting post-translational modifications, and enabling viral infection. These alterations are generally implemented by regulating apoptosis, performing metabolic engineering, growing cells at lower temperatures, carrying out chaperone and glyco-engineering, and modifying cell receptors to facilitate viral entry (66). Table 2 shows the genes modified during the host cell improvement process.

Table 2. Crucial genes modified to increase the yield of industrial cell lines

Pathway		Gene	Mechanism
Anti-apoptosis		mcl-1	overexpression
		30kc-6	overexpression
		bcl-2	overexpression
		bcl-w	overexpression
		bcl-xl	overexpression
		Aven	overexpression
		e1b-19k	overexpression
		Xıap	overexpression
		Crma	overexpression
Suppression of pro-apoptosis		Bax	inhibition
		Bok	inhibition
		Bak	inhibition
		Caspases	inhibition
Regulating cell cycle progression		p21	overexpression
		p27	overexpression
		CDKs	inhibition
		mTOR	overexpression
Chaperone engineering		Protein disulfide isomerase (PID)	overexpression
		BIP	inhibition
		XBP1	overexpression
		ERp57	overexpression
		C1GALT1C1	overexpression
		GRP94	overexpression
Metabolic engineering	Ammonia reduction	ornithine transcarbamylase	overexpression
		carbamoyl phosphate synthetase I	overexpression
	Lactate reduction	pyruvate carboxylase	overexpression
		lactate dehydrogenase	inhibition
		pyruvate dehydrogenase kinase (PDKs)	inhibition
Engineering cells for hypothermic growth		cold-inducible RNA binding protein (CIRP)	overexpression
Ribozyme engineering		mir-557	overexpression
		mir-1287	overexpression
		mir-30	overexpression
		mir-17	overexpression
		cgr-mir-7	overexpression
		mir-1b	overexpression
		mir-92a	overexpression

First of all, several metabolic engineering approaches have been applied to increase the yield of host cell lines. Accumulation of metabolic byproducts like ammonia and lactate is very frequent in mammalian cell cultures. These byproducts may negatively influence cell viability and product-specific yield. In order to get rid of these adverse effects of metabolites, several alterations have been implemented. For example, ammonia formation is prevented by overexpressing the glutamine-synthetase (GS) gene in CHO cells, and these cells are grown in a glutamine-free environment. This alteration provides a healthier environment for the cell and increases the viability and yield of CHO cells. Another advantage of this system is enabling people to grow the cells in a glutamine-free medium, reducing the manufacturing cost (67). Besides ammonia, lactate is a detrimental molecule for mammalian cells used in biologics production, so it is also essential to inhibit lactate production and accumulation, achieved by modifying lactate dehydrogenase pyruvate carboxylase, and pyruvate dehydrogenase kinase expressing genes (68).

Also, prevention of apoptosis is crucial in maintaining cell viability and eventually leads to elevation of productivity and extension of lifespan in antibody-expressing cells (69, 70). This is achieved by overexpressing cell survival and anti-apoptotic genes and downregulation of apoptosis-inducing genes (71). So, overexpression of anti-apoptotic survival genes like Bcl-2, Bcl-xl, Bcl-w, Mcl-1, Xiap, etc. and suppression of apoptotic genes such as Bax, Bok, Bak, Caspases (Caspase-3, 6, 7, 9), etc. increase the therapeutic protein concentration (72).

Additionally, inhibition of cell cycle progression and apoptosis is significant in elevating cell viability, viable cell density, and molecule-specific productivity (73). The cell cycle process can be disrupted by inhibiting cyclin-dependent kinases or activating CDK inhibitors. Cell cycle arrest in CHO cells caused a two to three-fold increase in productivity (74).

Generally, cell cultures are kept at 36.5 to 37 degrees Celsius. Decreasing the temperature of mammalian cell culture to 30-33 degrees Celsius has also improved biologics' productivity. These changes lower the growth of cells and extend the viability, significantly increasing the product's yield

(75). For instance, in the case of hypothermic growth, cold-related stress genes are induced and start to be expressed. Thanks to the increasing resistance to stress conditions, the cells produce more protein of interest (76).

Proper protein folding and post-translational modifications are crucial in sustaining the quality of molecules of interest and helping to meet the CQAs of the product. Enzymes related to folding proteins, such as chaperones and foldases, have crucial roles in maintaining the proper 3-dimensional structure of therapeutic molecules (77). Keeping proper disulfide bonding is also critical to maintaining therapeutic proteins' activity and stability. Overexpression of disulfide formation-related enzymes like protein disulfide isomerase induces disulfide bonding, which eventually leads to increased expression of mAbs (78).

Glycosylations are considered the most effective post-translational modifications of recombinant proteins in CHO cells. These structures have been shown to have a significant role in elevating the molecule's activity (79). Monoclonal antibody molecules used in therapy are generally based on IgG1 structures, containing N-linked glycosylation at Asn^{297} residue of heavy chains. Multiple glycans can be added to create various glycoforms such as G_0, G_1, G_0F, G_1S, G_2S, etc. (80). Antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are the two main mechanisms by which mAbs demonstrate their biological activity. Any modifications or changes in the glycan moieties of mAb molecules may influence these activity mechanisms. High fucosylation levels and relatively low levels of bisecting-N- acetylglucosamine (GlcNAc) are associated with monoclonal antibodies manufactured in CHO cells (81). Therefore, to increase ADCC activity, some modifications to the N-acetylglucosaminyltransferase III (GnTIII) gene were carried out for overexpression. Also, ADCC is induced by eliminating fucosylation on mAbs, which was achieved by knocking out the fucosylation related enzyme fucosyltransferase (FUT 8) in CHO cells (82).

In addition, facilitating the entry of viruses into host cell lines is also a very crucial point to be focused on. As stated above, several mammalian cells have been used for viral-based vaccine manufacturing so far. Inducing the entry of viruses into the host cells significantly increases the yield and decreases the cost of vaccine production (83). It was observed that eliminating several genes, alone or in combination, enhanced viral yields more than 20 to 50-fold in poliovirus production (84). Other cellular modifications such as adding adenovirus-related pIX gene and overexpression of tethering protein to prevent the release of endogenous retroviruses can increase the productivity of viral vaccine productions (85).

4. Conclusion: Modified mammalian cells offer new opportunities for biologics manufacturers

Several hosts and expression platforms are utilized in biologics manufacturing. Bacteria, yeasts, insects and mammalian cells are the most frequently used host systems. Different kinds of molecules like wild type insulins and their derivatives, mAbs, recombinant and traditional vaccines, and therapeutic proteins are manufactured using various host systems. For instance, some bacterial vaccines and insulins are produced with the help of bacterial expression systems, while virus-like particle (VLP) and recombinant vaccines are manufactured in yeast systems.

On the other hand, mammalian cells are commonly used to produce monoclonal antibodies, recombinant therapeutic proteins, gene therapies, viral-based inactivated and attenuated vaccines, vector-based vaccines, and vector productions for cell therapy applications. Several mammalian cell lines such as CHO, HEK, NSO, Sp2.0, Vero, MRC-5, and PerC.6 are involved in biologics manufacturing processes.

Many improvements are implemented to make these cell lines more convenient for high yield and quality protein productions. These modifications can be classified as metabolic engineering of cells, glyco and chaperone engineering of therapeutic molecules, regulation of apoptosis pathway, making improvements to keep the cells viable, and applying temperature shift to grow the cells at lower temperatures. For this purpose, several modifications were done at the genome level, and these specific changes eventually led to more productive manufacturing (86).

Moreover, the discovery of recent gene-editing tools concerning ZFNs, TALENs and CRISPR/Cas9 made the host cell modifications easier and more applicable (87). CRISPR stands out for host cell engineering for biologics manufacturing within these systems. However, it should be noted that patent restrictions should be overcome while creating an industrial cell line by using this system (88). It is crucial to target the open chromatin sites to express the gene of interest effectively. Thanks to the CRISPR system, it is possible to target the genome in desired regions that facilitates the required modifications to increase productivity. Also, open chromatin (euchromatin) region targeting mechanisms are widely used in cell line engineering approaches to boost the yield and increase stability. These mechanisms can be classified as Scaffold/matrix attached regions (S/MAR) and Ubiquitous Chromatin Opening Element (UCOE) (89, 90).

Thus, advances in cell line optimization techniques at different levels enable host cells to be used in the industrial production of biopharmaceuticals.

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