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# A REVIEW ON GENE CLONING AND ITS APPLICATION

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## ABSTRACT

*A contemporary demand is for protein production in adequate quantity and quality. The use of cell cultures for protein synthesis seems to be becoming more common. Expression techniques based on mammalian cells can introduce proper protein folding, post-translational modifications, and product assembly for recombinant proteins, all of which are required for complete biological activity. This review article is completely based on a literature review. In this article, the mammalian gene sequence has attracted a lot of attention. The author focused on the expression of the gene in a variety of mammalian cell lines. Potential vector methods for transferring the gene into mammalian cells included plasmid-based expression vectors, retroviral vectors, adenovirus vectors, vaccinia vectors, and baculovirus vectors. The process of transmitting genes into mammalian cells was also investigated. The uses and limits of mammalian expression systems were also addressed. The purpose of this study and the publishing of this article is to improve the understanding of researchers who are just getting started in the area of mammalian cell gene expression. The primary goal of this article, as well as its conclusion, is to make molecular techniques, expression systems, including gene expression applications in human cell lines more accessible.*

**KEYWORDS:** *Gene Cloning, Gene, Protein, Plasmid, Vector.*

## INTRODUCTION

A gene is unique piece of genetic information that is needed for the synthesis of polypeptides. In the coding sequence, you'll find the promoter, terminator, and introns. The transcription and translation of a gene are both referred to as expression. The expression of a certain gene requires the presence of a specific host. For large-scale recombinant protein synthesis, a variety of translation methods are currently available. E. coli, sought to harness insect cell translation,

yeast, as well as a number of placental mammal systems are among the techniques used[1]. Each has its own set of benefits in terms of cost, usability, including post-translational alteration profiles. The recombinant DNA technique is shown in Figure 1.

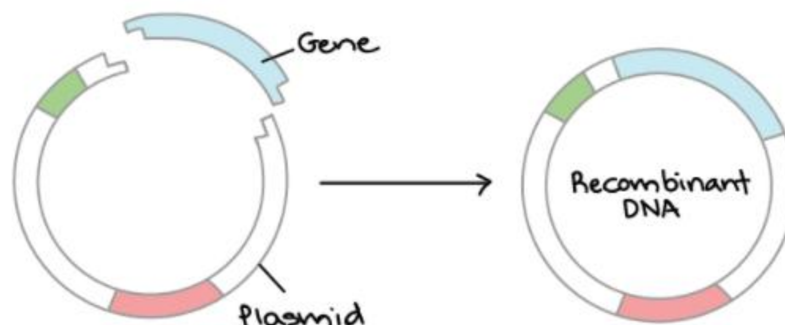


**Figure 1: The recombinant plasmid is injected into bacteria in this illustration. Bacteria that contain the plasmid are chosen and cultivated.**

E. coli, baculovirus-mediated insect cells expression, yeast, and numerous mammalian-based systems are among these expression systems. Each has its own set of benefits in terms of cost, usability, and post-translational modification profiles. Table 1 lists factors to consider when choosing an expression system for gene expression.

**TABLE 1: SHOWS FACTORS FOR SELECTING AN EXPRESSION SYSTEM**

| Sl. No | Factors effecting gene expression |
|---|---|
| 1. | Facilities of the laboratory and local expertise. |
| 2. | Type of protein to be expressed. |
| 3. | Whether the protein is toxic? |
| 4.Whether carbohydrate or other modification required? | |
| 5. | Requirement of large yield of protein. |
| 6. | How to purify protein? |
| 7. | Production cost |

In contrast to insect cells, human cells' glycosylation results in the attachment of large, complex glycans to expressed proteins, which generally prevents crystallization. However, there are techniques for changing the glycosylation. As a consequence, glycoproteins produced in mammalian cells have been crystallized effectively. Protein expression has also been studied in mammalian cells[2].This article is completely based on a literature review. The author looked at both modern and traditional techniques for doing a literature review. Search engines and data sources included Science Direct, PubMed, and Google. The literatures discussed in this article are not limited to a certain region, but rather come from all around the globe. The author tried to add to existing research on the topic. This paper included material from the preceding five years, up to and including 2012, as well as significant prior literature[3].

The main aim of this research is to look at the many mammalian cell lines that are used for transfection and, eventually, gene expression. The author investigated the vectors used in

transfection in mammalian cell lines. Furthermore, the author addressed several gene transfer techniques in mammalian cells. Gene expression in mammalian cell lines has gotten a lot of attention recently. The goal of this article is to provide new researchers with information on how to express genes in mammalian cell lines to get the desired protein. Readers who are new to biotechnology and interested in mammal tissue culture or gene expression may find this article helpful.

*Requirements for Mammalian Expressing Systems:*

The degree of expression of a gene is determined by the efficiency with which it is transcribed. Transcription begins when the RNA polymerase complex interacts with the promoter regions and moves from a 5 to 3 orientation along the gene. This produces an RNA transcript, which then separates from the gene at the transcribed signal, causing the transcript to be frozen until it can be translated. For gene expression in human cells, a good cell line and appropriate vectors are required. The vectors should be used to deliver the gene of interest into the appropriate cell lines[4].

*Cellular lines:*

In the past decade, protein treatments generated from human cells have revolutionized human healthcare. Protein treatments are becoming increasingly important, prompting a search for more cost-effective and efficient cell lines capable of producing high-quality protein products. Previously, viral vaccines, diagnostic, and therapeutic proteins were produced using bioprocesses based on mammalian cells. In the development of protein therapeutics, cells serve as hosts for the production of proteins. The most often used host mammalian cells are Chinese hamster ovary (CHO) cells and mouse myeloma cells, especially NS0 and Sp2/0 cells. DUKX-X11 as well as DG44, the two most often used cell lines in bioprocessing today, are two derivatives of the CHO cell line, CHO-K1 or CHO pro-3. These two cell lines were created with reduced dihydrofolatereductase (DHFR) activity. 7-8 A number of mammalian cell lines have been utilized to produce proteins, the most prominent of which being HEK 293 (Human embryonic kidney) and CHO (Chinese hamster ovary). These cell lines may be transfected with polyethyleneimine (PEI) or calcium phosphate. The Bac Mam system, for example, uses viral-mediated transduction to produce proteins in mammalian cells. This method utilizes recombinant baculoviruses for simple mammalian cell transduction, allowing for the production of milligram quantities of protein for structural studies. COS and Vero (both green African monkey kidneys), HeLa (human cervical malignancy), and NS0 (non-small cell lung cancer) are some of the other cell lines used for structural studies (mouse myeloma). Certain cell lines, such as NS0, are more difficult to transfect. The most frequent technique of transfection is electroporation, which is used solely to create stable cell lines[5].

The signals for eukaryotic synthesis of proteins, processing, and secretion are properly and efficiently transmitted when mammalian cell expressions are used. Mammalian cells recognize it. It is important to note, however, that there are variations across species.

*Vectors:*

Self-replicating DNA molecules known as vectors are capable of carrying foreign DNA fragments. It serves as a platform for cloning genes. After the DNA of interest has been cloned into an appropriate vector, the gene may be transfected into the host for expression. Mammalian

virus-derived vectors are often used to express heterologous genes in mammalian cells. They include viruses such as Simian Viruses 40 (SV40), polyomavirus, herpesvirus, as well as papovirus. You'll need an effective promoter as well as a selection marker to create a vector. In this article, the author has explored a wide range of vectors[6].

*plasmid-based expression vectors:*

An expression vector is a vector that allows the transcription and translation of a foreign gene that is inserted into it. Plasmids are self-contained circular DNA units that reside in bacterial cells. They are naturally occurring extra - chromosomal DNA fragments that are passed down from generation to generation in an extrachromosomal state. The majority of attempts to transiently generate recombinant proteins used "conventional" expression vectors with strong viral promoters, such as SV40 or adeno-associated virus (AAV) (CMV). Since it seems to be as potent as or stronger than some viral promoters, the elongation factor (EF)-1 promotor, a non-viral promoter, has received considerable support[7].

*Vaccinia vectors are a kind of vaccination used to stop the spread of disease*:

The vaccinia virus genome is around 200,000 base pairs of double-stranded DNA that multiplies in the host cell's cytoplasm. The vaccinia virus infects cells and produces up to 5000 virus particles per cell, yielding enormous amounts of recombinant protein. Both Pasteur-Merieux29 and Immuno AG30 have successfully used the vaccinia system to produce a range of proteins on a large scale (1000 L), including HIV-1 rgp160 as well as human pro-thrombin. Retroviruses are RNA viruses that replicate via a dsDNA intermediate. The reason for employing retrovirus as a vector is that most retroviruses do not kill the host and instead produce offspring virons over an indefinite period of time. As a result, retroviral vectors may be employed to generate stable cell lines. The second point is that viral gene expression is regulated by strong promoters, which may be controlled to govern transgenic expression. Another possibility is that certain retroviruses, including such amphotropic murine leukaemia viruses strains, have a broad host range and may infect a wide range of cell types. Exogenous gene expression systems that are based on retroviral vectors have been reported as a viable alternative for producing stable and high-expressing mammalian cell lines[8].Many methods for transfecting mature osteoclasts and their precursors with lentiviruses and adenoviruses have been described. A retroviral vector was used to create the CD59 gene, which was then transfected into breast cells (MCF-7). Recombinant human factor was also made using the retroviral system.

*As vectors, baculoviruses are employed.*

Baculoviruses contain large double-stranded DNA genomes and capsids that are rod-shaped. They infect arthropods, particularly insects, in large numbers. Both the Autographacalifornica multiple nuclear polyhedrosis virus (AcMNPV) and the Bombyxmori nuclear polyhedrosis virus have been extensively used as vectors. The former is used to express proteins in insect cell lines, particularly those produced from Spodopterafrugiperda. The latter infects the silkworm in order to produce recombinant protein.

A bicistronic expression cassette has been developed and employed in a recombinant baculovirus that may be used for sustained protein synthesis in mammalian cells. The expression of a secreted protein in human cells was studied using baculovirus particles.

*The process of transferring genes is as follows:*

The method for introducing the foreign gene into mammalian cells, as well as the regulatory elements utilized to guarantee efficient mRNA expression and protein synthesis, define the vector used. There are two primary methods for introducing foreign DNA into human cells. Viral infection causes the first, whereas chemical liposomes, calcium phosphate, DEAE-de-xtran, and polybrene, as well as physical electroporation and microinjection methods, cause the second. It has been shown that calcium-phosphate, PEI, and electroporation may be used as vehicles/approaches for large-scale transient gene expression.

The bulk of commercially available DNA transfer products are offered in small quantities and are not designed for use in reactors or with large cell masses. Calcium phosphate and PEI transfer DNA by forming complexes with DNA that are subsequently taken up by cells through endocytosis when the conditions are appropriate. This author has also written on several cell transformation methods.

*Mammalian expression system application:*

The first biologic approved from a mammalian bioprocess platform was tissue plasminogen activator (tPA), which was created by Genentech Inc. in 1987. Mammalian cells now outnumber E. coli, yeast, transgenic animals, and insect cultures for biopharmaceutical products: biopharmaceuticals approved between 2006 or 2010 were made in cell cultures, in E. coli, four in yeast, three in transgenic animals, as well as two in insect cultures.

*Production of monoclonal antibodies:*

Therapeutic proteins, such as monoclonal antibodies, are now manufactured in mammalian cells for commercial purposes (mAbs). African green monkey kidney (COS) cells may be appropriate for producing modest quantities of mAbs, such as for exploratory study.They've been used to make active antibodies on a brief basis since 1987. However, since their ability to produce decreases with time, they are not the ideal cells for large-scale production processes. Actually, if large-scale production is the objective, CHO cells, which are gaining favor in the lab, are the most often used cells for this purpose. There were also cell engineering instructions for the production of mAbs. The use of heterologous promoters, enhancers, and amplifiable genetic markers may increase antibody and antibody fragment production. High amounts of chimeric antibodies and recombinant antibody fragments were generated using low copy number cell lines, much beyond those seen in parental hybridomas.

*Production of urokinase:*

Urokinase is a serine protease that activates plasminogen and converts it to plasmin to break fibrin clots. Urokinase, as a consequence, is an effective anti-thromboembolic agent. The demand for urokinase has increased significantly in recent years, and current production levels have not kept up. Mammalian cell lines are increasingly used for recombinant urokinase production because they allow for post-translational alterations. CHO cells are excellent hosts for the production of recombinant urokinase because they grow so well in the bioreactor.

*Production of follicle stimulating hormone:*

Follicle-stimulating hormone is produced by the anterior pituitary's gonadotropic cells (FSH). FSH is necessary for follicular growth and development in females. Men must start pubertal spermatogenesis and sustain adult human spermatogenesis quantitatively. The structure and actions of glycoprotein hormones, as well as their therapeutic use in the treatment of infertility, have been studied using recombinant gonadotrophins. Because of their abundance, they are free of contaminating hormones, so their structure or function may be studied in great detail. Several groups have produced and reported on the effects of human recombinant (rec) FSH generated by transfected CHO cells. Recombinant rat FSH generated by CHO cells was purified and functionally characterized. The cynomolgus monkey was also used to study the cloning and expression of the gonadotropins luteinizing hormone and FSH.

*The following are examples of other mammalian expression systems:*

Using novel mammalian cell lines with reporter genes, researchers were able to detect environmental chemicals that activate endogenous aryl hydrocarbon receptors or estrogen receptors. Hemophilia B is a genetic coagulation system disease that affects one out of every 30,000 males worldwide. Recombinant human Factor IX has been used to treat hemophilia B. (rhFIX). The steady and high-level production of recombinant Factor IX in a human hepatic cell line was reported. The cytomegalovirus (CMV) promoter, for example, is used to induce high-level messenger RNA transcription. Codon optimization for the target cell type, GC/AT ratio balance, and signal sequence alteration have all been shown to improve mRNA processing as well as secretion. Gene-targeting technologies, chromatin opening elements, as well as attachment regions have all been included into vector optimization to improve end product output[9]

## DISCUSSION

A contemporary demand is for protein production in adequate quantity and quality. The use of cell cultures for protein synthesis seems to be becoming more popular. Expression techniques based on mammalian cells can introduce proper protein folding, post-translational modifications, including product assembly for recombinant proteins, all of which are required for complete biological activity. To select an optimal expression system, just the productivity, purpose, bioactivity, and physicochemical characteristics of the target protein, as well as the cost, convenience, as well as safety of the system itself, should be considered. In order to get more efficient proteins with high biological activity, new mammalian cell lines, vector systems, and gene expression methods are now required. In this paper described the cell lines, vectors, including transfection method used in mammalian expression system, which is very helpful for researchers new to animal biotechnology. In the last two decades, mammalian cell protein levels have been the most common technique of recombinant protein synthesis for therapeutic use, accounting for more than half of all biopharmaceutical products on the market, including hundreds of clinical trial candidates. The discovery and production of new cell lines, as well as the entrance of novel genetic mechanisms in expression, gene control, and gene targeting, have all made significant progress.

## CONCLUSION

Correct protein folding including post-translational alterations, which are often needed for full biological function, may be introduced via mammalian cell-based production techniques for recombinant proteins. The main conclusion reached by the author is that he has examined the most recent developments in the field of mammalian expression systems, which are significant in animal biotechnology.  In this paper described the cell lines, vectors, including transfection method used in mammalian expression system, which is very helpful for researchers new to animal biotechnology. In the last two decades, mammalian cell protein levels have been the most common technique of recombinant protein synthesis for therapeutic use, accounting for more than half of all biopharmaceutical products on the market, including hundreds of clinical trial candidates. The discovery and production of new cell lines, as well as the entrance of novel genetic mechanisms in expression, gene control, and gene targeting, have all made significant progress. Only the productivity, bioactivity, and purpose of the interest protein, including its physicochemical characteristics, as well as the cost, simplicity, and security of the system itself, may be utilized to choose an optimal expression system. In order to obtain more efficient protein with high biological activity, new human cell lines, vector methods, and gene expression methods are now required.

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