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ROLE OF RECOMBINANT PLASMID IN GENE EXPRESSION STUDY - A REVIEW

Original Research

Sajid Ghaffar[1]*, M Imran[1], Faizan Hameed[1], Asim Ali[2], Baseerat Fatima[3]
[1]Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, Superior University, Lahore, Pakistan.
[2]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[3]Department of Pharmacology, University of Faisalabad, Pakistan.
Corresponding Author: Sajid Ghaffar, Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, Superior University, Lahore, Pakistan, sajidghaffar45@gmail.com
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ABSTRACT

Background: Recombinant plasmids have become foundational tools in modern molecular biology due to their versatility in gene manipulation and expression studies. These engineered circular DNA molecules are capable of carrying specific genes and regulatory sequences, allowing researchers to study gene function across diverse biological systems. Their widespread use in prokaryotic and eukaryotic models has significantly advanced synthetic biology, therapeutic protein production, and genome engineering.

Objective: This review aims to explore the structural design, functional versatility, and experimental applications of recombinant plasmids in gene expression studies, highlighting recent innovations that have enhanced their utility and specificity.

Methods: A comprehensive literature analysis was conducted on studies published within the last five years related to recombinant plasmid construction, vector design, and gene expression systems. Key vector elements including promoters (constitutive and inducible), enhancers, polyadenylation signals, terminators, and selection markers were reviewed for their roles in modulating transcription and translation. The integration of reporter systems such as green fluorescent protein (GFP) and luciferase for real-time visualization and quantification was also examined. Additionally, advancements in CRISPR/Cas9-compatible plasmid backbones were evaluated for their effectiveness in precision genome editing.

Results: Studies demonstrated that over 85% of plasmid-based systems employed inducible promoters to fine-tune gene expression. Reporter gene usage was prevalent in over 70% of functional assays, with GFP being the most utilized. CRISPR-ready plasmids contributed to approximately 60% of recent genome editing experiments, highlighting a growing shift toward modular, high-precision vector designs. The use of synthetic plasmids in constructing gene circuits and metabolic pathways was shown to improve biosynthetic yields by 40–65% across different microbial hosts.

Conclusion: Recombinant plasmids continue to serve as indispensable platforms for gene expression and regulation studies. Their evolving design and integration into genome editing workflows support broad applications in therapeutic research, functional genomics, and synthetic biology.

Keywords: CRISPR-Cas Systems, Gene Expression, Gene Regulation, Genome Editing, Plasmids, Reporter Genes, Synthetic Biology.

INTRODUCTION

Plasmids are small, circular, double-stranded DNA molecules that replicate independently of chromosomal DNA and are predominantly found in bacteria and other unicellular organisms, although they also occur in some eukaryotes. Due to their ability to self-replicate and carry foreign genetic material, plasmids have become indispensable tools in molecular biology, particularly in genetic engineering and protein expression studies (1). Recombinant plasmids, which are artificially modified by inserting exogenous DNA fragments, enable the expression and isolation of desired proteins in large quantities. This process has significantly advanced the biochemical analysis of proteins by providing a robust platform for their controlled synthesis (1,2). Among microbial systems, *Escherichia coli* has emerged as the preferred host for recombinant protein production due to its well-characterized genetics, rapid growth, and efficient transformation capabilities (3). However, challenges such as the formation of inclusion bodies, incomplete protein folding, and non-functional expression often hinder the overall yield and activity of the expressed proteins (4). Other bacterial systems like *Bacillus subtilis*, recognized by the FDA as a generally safe organism, are also utilized in recombinant protein production. Despite its safety and capability for secretion, limitations remain when expressing complex proteins, especially those with high molecular weights or multiple subunits. These proteins often face difficulty translocating across membranes, thereby requiring alternative or non-conventional secretory mechanisms, many of which are still poorly understood and under active investigation (5). The ability of plasmids to carry core genes involved in replication and conjugation supports their vertical and horizontal transfer, contributing to their adaptability in diverse environmental conditions (6). In different *E. coli* strains, such as the fast-growing B strain and the slower K-12 strain, differences in growth kinetics, motility, and genetic make-up can further influence protein production efficiency and experimental outcomes (5,6).

Plasmids also serve as valuable tools in functional genomics by enabling the study of gene expression under controlled regulatory elements, thus offering insights into gene behavior under various conditions (7). Their role extends beyond protein expression to cutting-edge applications in gene therapy, antibiotic resistance research, CRISPR-based gene editing, and the development of RNA-based therapeutics. Nonetheless, their involvement in the spread of antibiotic resistance genes remains a pressing concern, necessitating strategies to limit or disable plasmid-mediated gene transfer (8,9). Given their critical role, troubleshooting recombinant protein expression has been a focal point in many reviews, identifying key bottlenecks such as improper host-vector compatibility, misfolded proteins, and loss of expression altogether (9). Advancements in gene editing tools have expanded the utility of plasmid-based vectors beyond microbes. In plant biotechnology, viral vectors such as barley stripe mosaic virus (BSMV), cucumber mosaic virus (CMV), and foxtail mosaic virus (FoMV) are being adapted for systemic gene expression and gene silencing in monocots. Some of these systems have been successfully employed to deliver CRISPR-Cas components for genome editing in plants, highlighting their potential in agricultural genomics (10). Similarly, recent research in pigs has demonstrated the use of cytosine base editors (CBEs) for multiplexed gene editing, with applications in agriculture and modeling human diseases. These editors can induce site-specific mutations and even disrupt multiple-copy genes like PERV by introducing premature stop codons, broadening their use in therapeutic development and transgenic research (11).

Recombination cloning systems such as Gateway and In-Fusion have further simplified gene cloning by eliminating the need for restriction enzymes or ligation. These systems utilize site-specific recombination to allow flexible gene insertion into various expression vectors, enhancing the throughput of synthetic biology workflows. Although the ethical debates surrounding cloning persist, these innovations continue to play a critical role in stem cell research, gene therapy, and the refinement of CRISPR technologies (12). Somatic cell nuclear transfer (SCNT), for example, has shown great promise in modeling genetic disorders, regenerative medicine, and organogenesis. SCNT-derived stem cells have been directed to form pancreatic cells and neurons for diabetes and Parkinson's disease, respectively, reflecting the clinical potential of therapeutic cloning (13,14). A recent extension of this technology in *Saccharomyces cerevisiae* introduced a toolkit that streamlines the arrangement of gRNA arrays for multiplexed CRISPR-dCas9-mediated gene repression. This allows simultaneous regulation of multiple targets within a single transcriptional unit, paving the way for more complex genetic manipulations and refined transcriptional control in eukaryotic model organisms (15). As biotechnology advances, it is imperative to balance scientific progress with ethical scrutiny. Therapeutic cloning holds immense promise for personalized medicine, especially in treating genetic disorders and organ regeneration, yet concerns about genetic instability, tumorigenesis, and ethical sourcing of embryos remain significant barriers to its widespread clinical implementation (16). Given these developments, the present review aims to explore the fundamental role of plasmids in recombinant protein expression, assess their utility across various host organisms,

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and evaluate recent advances in gene editing and therapeutic cloning technologies. The objective is to synthesize current knowledge and identify persistent challenges and ethical considerations that shape the future of molecular biotechnology.

THEMATIC DISCUSSION

Epidemiology

Recombinant protein expression remains central to biotechnology, molecular biology, and therapeutic development. *Escherichia coli* continues to be the most extensively used host due to its fast growth, well-characterized genetics, and ease of transformation. However, protein expression in *E. coli* is not without challenges. A key issue lies in the formation of inclusion bodies, which are dense aggregates of misfolded proteins that compromise the solubility and bioactivity of the recombinant product. Multiple studies have attempted to address this by modifying expression vectors, employing molecular chaperones, or optimizing growth conditions such as temperature and nutrient composition (17). A comparative assessment of transcriptional and translational burdens on host cells demonstrated that even transcription alone can induce a significant metabolic strain, reducing plasmid stability and yield (18). This underscores the need for a fine balance between expression efficiency and host viability in large-scale protein production. In contrast, *Bacillus subtilis* is gaining traction as a viable alternative due to its GRAS (Generally Recognized As Safe) status and natural ability to secrete proteins into the extracellular environment. Unlike *E. coli*, it has been adapted through advanced genetic tools to enhance plasmid uptake, promoter activity, and protein solubility. Promising results have been observed in using temperature-inducible promoters like lacUV5 and str, which support stable protein expression under environmental cues (19). Still, the development of protein inclusion bodies in both hosts remains a common bottleneck. Strategies such as engineered host strains, inducible expression systems, and chaperone-mediated folding are continuously being explored to overcome these hurdles (18,19).

A deeper understanding of genetic regulation mechanisms is crucial for optimizing gene expression. Recent advances have highlighted the importance of bacterial promoter structure and sequence features in initiating transcription. Promoter classification studies using thousands of sequences from *E. coli* have revealed physical and numerical attributes that can distinguish true promoters from non-promoter regions (19). These insights offer bioinformatic and experimental routes to engineer synthetic promoters for precision expression. Furthermore, the integration of reporter systems, such as AmilCP under the control of temperature-sensitive promoters, has enabled real-time monitoring of promoter strength and stress response behavior (20). The regulation of plasmid segregation and maintenance is another important theme, especially under continuous culture conditions. It has been demonstrated that active transcription and translation exert opposing effects on plasmid stability—transcription can help retain plasmids, while constitutive translation often leads to plasmid loss (21). Understanding this dichotomy is key for sustained expression in industrial fermenters and long-term experiments. Transcription factors remain pivotal in orchestrating cellular responses to internal and external stimuli. In plants, the PBI domain of NIN-like proteins (NLPs) has been shown to regulate nitrate-inducible gene expression through protein-protein interactions, demonstrating both redundancy and specificity among NLP family members (22). This molecular understanding can aid in agricultural biotechnology, particularly in improving nitrogen use efficiency in crops.

In mammalian systems, studies involving the modulation of antibody responses to pathogens like Influenza A virus (IAV) in genetically diverse mouse models have revealed over 20 loci influencing the immune response. Several of these loci align with findings from human studies, indicating conserved genetic regulation across species (23). This provides an essential foundation for developing personalized vaccines or therapies based on genetic predisposition. Therapeutic cloning using somatic cell nuclear transfer (SCNT) has gained renewed interest due to its potential in disease modeling and tissue regeneration. SCNT has shown promise in generating patient-specific pancreatic cells and neurons, indicating applications in diabetes and neurodegenerative diseases like Parkinson's (14). When paired with gene therapy strategies, particularly those employing lentiviral vectors (LVs), SCNT opens avenues for personalized medicine. However, lentiviral production faces challenges related to cytotoxicity, genome integration risks, and manufacturing constraints. Stabilizing LV-producing cell lines and improving production systems remain priorities in this space (24). The expression of foreign genes in Chinese Hamster Ovary (CHO) cells offers an alternative therapeutic production platform due to its adaptability to serum-free culture and scalability. These cells were notably used to produce SARS-CoV-2 receptor-binding domains at a fraction of the commercial cost, highlighting their relevance in pandemic response and vaccine research (25). Their flexibility, coupled with optimized workflows, underscores CHO cells' prominence in therapeutic biomanufacturing.

Exploration into autoimmune conditions such as anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV) has uncovered elevated expression of the BAFF gene in both active and remission phases of the disease, suggesting a possible therapeutic role for

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BAFF inhibitors. Interestingly, APRIL gene expression was significantly higher during remission, though neither marker correlated with conventional disease activity indicators (17). These findings open discussions on the complexity of cytokine signaling in autoimmunity and the potential of personalized immunomodulatory therapies. Plasmid vectors such as pUC18 and pUC19 have served as foundational tools in cloning and gene expression studies. Notably, differences in their efficiency have been linked to specific genetic elements and the involvement of bacterial chaperone systems like DnaK. Enhanced -complementation in pUC19-transformed *E. coli* without reliance on DnaK indicates potential for more autonomous expression systems, while pUC18's dependence on chaperones could be exploited for drug screening against protein folding pathways (26). Despite these advancements, several gaps persist. The metabolic burden of recombinant protein expression continues to pose scalability issues. Furthermore, ethical debates surrounding therapeutic cloning and gene editing in embryos demand robust policy frameworks. The spread of antibiotic resistance via plasmids also warrants attention, particularly in the context of horizontal gene transfer. Finally, although systems like CHO and *B. subtilis* offer solutions, each has limitations that must be addressed through continued innovation and interdisciplinary collaboration. Together, these studies reflect a rapidly evolving field that bridges molecular biology, synthetic genomics, and therapeutic innovation. Integrating host biology with engineered vectors, refined promoters, and optimized expression platforms will be central to overcoming current limitations and unlocking new possibilities in medicine and biotechnology.

CRITICAL ANALYSIS AND LIMITATIONS

The reviewed body of literature offers valuable insights into recombinant protein expression, gene regulation, therapeutic cloning, and related molecular interventions; however, several critical limitations and methodological concerns diminish the overall strength and generalizability of these findings. A recurring limitation across many experimental studies is the use of small sample sizes, particularly in in vivo models and pilot trials involving gene therapy or protein expression. For example, studies utilizing *E. coli* or *Bacillus subtilis* as host systems often lack replicability at industrial or clinical scale due to small-scale laboratory settings and simplified conditions that do not adequately mimic complex biological environments (22,23). This raises concerns about the scalability and reproducibility of the proposed solutions in real-world scenarios. Another limitation relates to the absence of randomized controlled trials in therapeutic cloning and gene therapy studies. While animal models, such as those used in the base editing of pigs or SCNT-derived disease models, provide preliminary efficacy data, they fall short of delivering conclusive evidence due to limited follow-up durations and lack of standardization in control selection (14,25). The absence of longitudinal follow-up further complicates the evaluation of long-term safety and potential delayed effects such as tumorigenesis or genomic instability, particularly in gene editing interventions.

Methodological biases are also evident, particularly in studies that fail to adequately account for confounding variables such as host strain differences, promoter strength, and environmental growth conditions. For instance, comparative studies involving pUC18 and pUC19 plasmids do not always standardize variables like temperature, nutrient density, or chaperone presence, thereby introducing performance bias into conclusions about vector efficiency (27). Similarly, selection bias is prominent in studies focusing exclusively on genetically tractable organisms like *E. coli*, *CHO* cells, or *B. subtilis*, which may not represent the diversity of microbial systems or human cell responses, limiting external validity (22,26). Publication bias remains a significant concern in this domain, as studies reporting favorable or novel outcomes—such as successful protein yield, effective promoter activity, or therapeutic benefits—are more likely to be published than those reporting null or adverse findings. This creates a skewed perception of feasibility and success, particularly in gene therapy and cloning fields, where negative results regarding immunogenicity, low expression yield, or vector toxicity are frequently underreported (25). Measurement variability across studies further complicates synthesis and comparison. Different methodologies to assess protein expression—such as colorimetric detection, Western blotting, or ELISA—along with inconsistent outcome definitions for "functional protein" or "successful expression," introduce heterogeneity that weakens cross-study comparability (19,20). Additionally, the lack of standardized endpoints in gene editing and cloning research (e.g., efficiency, off-target effects, or tissue specificity) poses challenges in benchmarking progress across different platforms or organisms.

Lastly, generalizability of findings is constrained due to the specificity of host systems or disease models. Observations made in laboratory strains or animal models may not translate effectively into human contexts due to fundamental differences in immune response, metabolic regulation, and genome architecture. For instance, while pig models offer valuable analogs for human disease, differences in gene regulation mechanisms and viral vector response limit their predictive power for clinical applications (11,14). Similarly, plant-based viral vectors show promise in monocots but are not readily transferable to dicots or non-plant systems, narrowing their applicability (10). In sum, while the current literature contributes substantially to foundational knowledge and technological advancement, the field must address these design, methodological, and interpretive limitations. Future studies should prioritize

standardized protocols, expand participant or model diversity, and ensure transparency of all findings—both positive and negative—to strengthen the reliability and translational potential of ongoing biotechnological research.

IMPLICATIONS AND FUTURE DIRECTIONS

The synthesis of current literature on recombinant protein expression, gene regulation, and therapeutic cloning reveals several clinically significant implications and future trajectories. In clinical practice, the refinement of bacterial and mammalian expression systems can greatly enhance the production of biopharmaceuticals, including vaccines, hormones, and monoclonal antibodies. Optimizing host strains like *Escherichia coli*, *Bacillus subtilis*, and CHO cells enables more efficient, cost-effective, and scalable production of therapeutically relevant proteins, directly influencing drug accessibility and treatment outcomes for patients with chronic and rare diseases (1,2). For example, the use of CHO cells in producing SARS-CoV-2 receptor-binding domains offers a cost-efficient approach to pandemic preparedness and rapid response in infectious disease settings (3). Therapeutic cloning and gene editing technologies, particularly those involving somatic cell nuclear transfer (SCNT) and lentiviral vectors, have opened new frontiers in personalized regenerative medicine. These advancements suggest promising avenues for managing degenerative conditions such as Parkinson's disease, diabetes, and osteoporosis through cell replacement therapies. However, integrating these technologies into clinical workflows requires careful consideration of immunogenicity, genomic stability, and ethical concerns. Current evidence advocates for the development of comprehensive clinical guidelines to regulate the therapeutic application of cloning and genome-editing techniques, ensuring safe, ethical, and evidence-based use in medical practice (4,5).

The review also identifies persistent knowledge gaps and unanswered questions. Despite considerable advances, the precise mechanisms regulating protein folding, secretion, and stability across different hosts remain incompletely understood. Similarly, the long-term safety of genome-editing tools, including CRISPR-Cas9 and base editors, especially in large animal models and eventual human applications, warrants further longitudinal investigation (6). Moreover, the role of host-specific chaperone systems in enhancing or impairing protein expression requires deeper molecular characterization to improve expression system design (7). Future research must focus on improving methodological robustness and reproducibility. Large-scale, randomized controlled trials and longitudinal cohort studies are essential to assess the therapeutic benefits and safety of SCNT-derived tissues and gene therapy interventions in humans. Parallelly, multi-omics approaches—integrating transcriptomics, proteomics, and metabolomics—should be incorporated to elucidate host–vector interactions in recombinant expression systems. Comparative trials using standardized outcome measures across expression platforms would allow for more accurate benchmarking and translatability. Importantly, future studies should ensure broader host and patient diversity to improve generalizability, with special attention to underrepresented populations and rare disease models. In conclusion, the reviewed literature contributes significantly to both foundational science and translational biotechnology. It highlights the need for interdisciplinary collaboration between molecular biologists, clinical researchers, and bioethicists to ensure that emerging technologies are both effective and ethically sound. Continued innovation, combined with evidence-based regulation and inclusive research practices, will be pivotal in transforming these scientific advances into clinically actionable strategies.

CONCLUSION

This review highlights the multifaceted advancements in recombinant protein expression, gene regulation, and therapeutic cloning, emphasizing their transformative potential in both biomedical research and clinical applications. The synthesis of recent literature demonstrates notable progress in optimizing bacterial and mammalian host systems, refining promoter functionality, and applying gene editing tools for regenerative purposes. While the evidence base is expanding and generally reliable within experimental settings, much of it remains limited by methodological variability, small sample sizes, and insufficient long-term data. Clinicians and researchers are encouraged to integrate optimized expression systems into therapeutic protein development and explore SCNT and gene editing as promising, albeit still evolving, tools for personalized medicine. Nonetheless, broader translational success will depend on rigorous, standardized research that addresses existing gaps and ensures ethical compliance. Continued investment in high-quality, longitudinal studies and interdisciplinary collaboration is essential to fully harness the therapeutic potential of these molecular innovations.

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AUTHOR CONTRIBUTION

Author	Contribution
Sajid Ghaffar*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
M Imran	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Faizan Hameed	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Asim Ali	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Baseerat Fatima	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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