

Article

Molecular Cloning and Recombinant Production of Growth Hormone from Japanese Quail (*Coturnix japonica*)

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Citation: Alomari, A.. Molecular Cloning and Recombinant Production of Growth Hormone from Japanese Quail (*Coturnix japonica*). American Journal of Biology and Natural Sciences 2026, 3(10), 118-126.

Received: 10th Aug 2026

Revised: 30th Aug 2026

Accepted: 20th Sep 2026

Published: 16th Oct 2026


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Abstract: Growth hormone (GH) is a crucial polypeptide hormone that controls poultry species' growth, development, metabolism, and ability to reproduce. In order to obtain the matching recombinant GH protein in a pure form for further biological research, the current study aimed to molecularly clone the growth hormone (GH) gene from Japanese quail (*Coturnix japonica*). The target GH gene was amplified via using polymerase chain reaction (PCR) after ordering the sequencing of gene from NCBI for Japanese quail. Following molecular cloning of the amplified product, the recombinant construct was confirmed using suitable molecular and sequencing techniques. The recombinant construct served as the foundation for the synthesis and purification of recombinant quail GH protein once the GH gene was successfully cloned and verified. The purified hormone will be given to quail via suitable experimental routes and dosages in future studies to examine its effects on physiological and productive traits, especially those related to growth, feed utilization, reproductive performance, and other production-related parameters. Thus, the current work presents a platform for future in vivo and in vitro studies of its functional and production-related activities and offers a molecular basis for the development of recombinant Japanese quail GH.

Keywords: Japanese Quail, *Coturnix Japonica*, Growth Hormone, GH Gene, Molecular Cloning, Recombinant Protein, Gene Expression, Protein Purification

Introduction

The Japanese quail is a member of the genus *Coturnix*, species *japonica*, family Phasianidae, and order Galliformes [1]. Because of its quick growth, early sexual maturity, good feed efficiency, and tiny body size, the Japanese quail (*Coturnix japonica*) is a popular species in poultry farming for both small-scale production and scientific study [2]. Due to their inexpensive feeding costs, excellent nutritional value, great capacity for reproduction, and financial advantages, quails are raised extensively [3]. The somatotrophs of the anterior pituitary gland in birds and other animals create growth hormone (GH), a protein hormone. Three peptidergic releasing factors—growth hormone-releasing factor (GRF) (stimulatory), thyrotropin-releasing hormone (TRH) (stimulatory), and somatostatin (SRIF) (inhibitory)—are involved in the hypothalamic regulation of GH secretion in birds [4]. The hormone IGF-1 contains a molecular structure with insulin and often performs a number of anabolic and metabolic functions [5]. Additionally, it is a necessary hormone for the optimal development of fat and bone in poultry [6]. GH typically influences the basal metabolic rate

[7] and a number of physiological processes, including growth, aging, reproduction, and egg production [8]. Both genetic and environmental factors have an impact on growth traits [9]. Due to their moderate to high heritability and genetic association with growth traits, growth traits like body weight (BW), chest width (CW), chest depth (CD), shank length (SL), average daily gain (ADG), and shank circumference (SC) have long been utilized in the selection principle of poultry breeding. Major economic parameters including quail growth and carcass were strongly correlated with the GnRH gene, one of the crucial candidate genes [3]. In the hypothalamic-pituitary-gonadal axis, GnRH is a crucial neuroendocrine regulator [10]. There were three isoforms of GnRH: GnRH-1, GnRH-2, and GnRH-3 [11].

Growth performance is a crucial characteristic for assessing various livestock species, particularly those that produce meat and birds. Growth performance is regarded as a crucial characteristic for effective poultry production since feed accounts for between 50 and 70 percent of the entire cost [12]. Without understanding the underlying biological pathways, genetic selection for a high growth rate has been routinely used in poultry production. While productivity and body weight have significantly improved, several unfavorable alterations, like metabolic muscle diseases, have emerged [13].

Materials and Methods

Location and Duration of Study

The study was carried out from February 2026, at the central lab in the College of Agriculture and Forestry, University of Mosul.

Ordering the Growth Hormone gene FOR Japanese Quail (*Coturnix japonica*)

The GH gene was designed and ordered. The designed gene and its complete sequences was obtained from the GenBank Complete Core database, as shown in the following links: [GenPept Identical Proteins Graphics](#)

CGTGACTGAT = overhang

CATATG = NdeI cut enzyme

TGA = Stop codon

GGATCC = BamHI cut enzyme

CGTGACTGATCATATGatggcgccgggcagctggtttagcccgctgctgctgacctgggtgacctgggcctgccgtggggcgcgccggcgacctttccggcgatgccgctgagcaacctgtttgcgaacgcggctgctgcgcgcgacatctgcatctgctggcgcggaacctataagaatttgaacgcacatatattccggaagatcagcgctataccaacaaaaacagccaggcgcgctttgctatagcgaaaccattccggcgccgaccggcaaatgatgcgcagcagaaaagcgatatggaactgctgcgctttagcctggctgattcagagctggctgacccgggtgcagtatctgagcaaatgtttaccaacaacctgggtgtttggcaccagcgatcgcgctgtttgaaaaactgaaagatctggaagaaggcattcaggcgctgatgcggaagtgaagatcgagcccgcgcgcccgagctgctgcgcccacctatgataaattgatattcatctgcgcaacgaagatgcgctgctgaaaactatggcctgctgagctgctttaaaaaagatctgcataaagtggaaacctatctgaaagtatgaaatgcccgctttggcgaaagcaactgccatttaaTGAGGATCCCGTGACTGAT

The corresponding primers was also ordered. The forward and reverse primers for this gene was designed based on the nucleotide sequences of the GH gene. The complete coding sequence was obtained from the GenBank NCBI database. Gene primer was ordered by the accredited American company, MacroGen.

Table 1. The sequence shows the forward and reverse primers and molecular weight of the GH genes for amplifying the gene that ordered from NCBI as whole sequence.

Gene	Primer Sequence
GH	F: ATGGCGCCGGGCAGCTGTTTAGCCC
GH	R: TTAAATGGTGCAGTTGCTTTCGCCAA

Preparation of the Gene and Primers

GH gene was reconstituted by adding 250 μ L of nuclease-free water to the gene tubes to obtain a final 100 μ M solution, according to the manufacturer's instructions. Each primer was reconstituted by adding 250 μ L of nuclease-free water to the primer tubes to obtain a final 100 μ M solution (according to the manufacturer's instructions). These primers were then diluted by adding 10 μ L of the parent solution (100 μ M) to 90 μ L of nuclease-free water to obtain a 10 μ M concentration.

Polymerase Chain Reaction

Purified gene was extracted from plasmids using PCR. Ten microliters of nuclease-free water were added to each sterile PCR tube that had been prepared for the gene samples. Three microliters of genomic DNA for the genes being utilized in each tube were then added after two microliters of the corresponding diluted starter (10 micromoles) were added to each tube. Ten microliters of Promega's Master Mix (product number: M7822), which includes buffer solution, Taq DNA polymerase, deoxynucleotides (dNTPs), magnesium chloride ($MgCl_2$), and two reference dyes (yellow and blue) to aid in subsequent electrophoresis, were then added to the reaction mixture. Standard PCR procedures were carried out. For the first five minutes of the DNA strand's denaturation, the initial or preliminary temperature was (98). After that, the primers were ligated at 95 degrees Celsius. The primer elongation process was then initiated by raising the temperature to 50 degrees Celsius. To finish the primer's elongation, one last heat cycle was carried out for five minutes at a temperature of 72. A 1% agarose gel was filled with the amplified PCR production samples. The samples were injected into the gel after a 10X Tris-Acetate-EDTA solution was added. Following transfer, the gel was inspected under a UV lamp to determine the DNA amplification findings. After cutting the 1% gel, the Wizard SV Gel and PCR Clean-Up System (LOT 0000626358) extraction kit was used to extract and purify the DNA bands.

The study's restriction enzymes consisted

Based on the gene design mentioned above, the British company New England Biolab was contacted to order the gene-cutting enzymes (see section on gene design). The gene was connected to the Pet-30a base plasmid using the T4 ligation enzyme, which is also available from New England Biolab. Every gene has a unique restriction enzyme, and these enzymes each had the following nitrogenous base sequence:

NdeI cut enzyme = CATATG

BamHI cut enzyme = GGATCC

An optimum environment must be available in order to convert the previously mentioned gene and produce a stock. Specialized bacteria of the DHa5 type, particularly a sensitive bacterium known as a Competent Cell, form this environment. The right conditions for the transformation process are provided by these bacteria.

Addition of Restriction Enzymes to Uncut GH Gene

As previously mentioned, restriction enzymes were applied to the uncut genes. The restriction enzymes' method of action led to the division of the GH gene into two groups. NdeI cut and BamHI cut enzymes are responsible for cutting Pet-30a. For both groups, the incubator temperature was kept constant. After adding the restriction enzymes and their corresponding enzymes to the target gene at the same time, the incubator was set to 37°C for 30 minutes. In order to remove or denature the cutting enzymes (for the purpose of carrying out the cloning process), the tubes containing the cut target gene were then put in a water bath at 65 degrees Celsius for 15 minutes. The alkaline phosphatase (rSAP) enzyme was added to the base plasmid samples only after the plasmids Pet-30a, which belong to the EcoRI cut enzyme HindIII cut, were cut. This was done at a concentration of 5 μ L per tube with 3 μ L of Shrimp buffer solution to eliminate phosphorus from the plasmids at the 5' ends of both Pet-30a A.

Precipitation of Ethanol

Ethanol precipitation must be added to the reduced Pet-30a plasmid and the reduced genes in order to purify the DNA and eliminate potentially useful truncating enzymes, leaving only the DNA. The DNA fragments of the genes and the pure plasmid are preserved and purified.

Pet-30a plasmid's gene ligation

The NdeI and BamHI restriction enzymes were used to cut the GH gene. The Pet-30a plasmid treated with NdeI cut and BamHI -cut/SAP was then used to ligate the GH gene using the T4 ligase enzyme. 2 µL of Pet-30a and 6 µL of each gene were introduced using an insert:vector ratio of 1:3. The reaction volume was 20 µL after 3.5 µL of T4 ligation gene buffer, 1.5 µL of T4 ligation enzyme, and 7 µL of free water were added. Bacterial transformation was carried out using Competent Cells E. coli BL21 from NEB after the plasmids and the cut genes to be ligated (Pet-30a-GH) were combined for 30 minutes on ice and then exposed to a 40% water bath for 45 seconds.

PCR for colonies :

For the following genes, colony PCR primers have been established and ordered:

Forward and Reverse Primers for LH:

Forward: 5' ATGATGGCGCCGGGCAGCTGGTTT

Reverse: 5' CTTTCGTTGACGTGGTAAATTACT

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Colony PCR Reaction Medium Preparation

To identify bacteria carrying the GH gene, a number of bacterial colonies grown on Petri-dish plates were analyzed using gene sequencing primer. 10 µL of Master Mix (NEB), 2 µL of primers, 3 µL of DNA, and 10 µL of free water were used for a 25 µL PCR reaction. The expression vector regions of Pet-30a surrounding the cloned gene have been amplified using the Colony PCR technique and specific primer.

Main Induction

Competent E. Coli BL21 (DE3) cells were transfected with recombinant plasmids (pET30a-GH cut-gene) and cultured in LB media supplemented with kanamycin (50 µg/mL). 1 mM IPTG was used to increase protein expression at $OD_{600} = 0.6$, and the mixture was then incubated at 37 °C. Centrifugation was used to harvest the bacterial cells, which were then lysed in sodium phosphate buffer with protease inhibitors. Sonication and high-speed centrifugation were used to extract the soluble fraction.

HPLC Purification and GH Gene Detection

At this stage, the GH cut-gene, one of the genes being analyzed, was extracted and cloned into the pET29b bacterial expression vector. In E. Coli BL21 DE3, a strain with the T7 RNA polymerase gene under the direction of the lac factor inducer, the gene expression plasmid for each cloned gene was stimulated by adding 1 mM IPTG.

The next step involved cloning this gene in vitro and purifying the resulting enzyme. Using sodium phosphate (pH 7) as a neutralizing buffer, the proteins GH were first purified and extracted; all procedures were carried out at 4°C. Over the course of two hours, ammonium sulfate was gradually added until the desired concentration was attained. In order to wash and extract pure protein, ammonium sulfate and sodium chloride were added to the second phase solution, also known as the wash solution, to a concentration of 1 M.

A Shimazu 2025 HPLC system was used to purify the soluble proteins under research (~1 mL) that were introduced into the ultraring. Every stage was carried out at room temperature. The HiTrap HP column was pre-equilibrated using at least five volumes of distilled water and vacuum degassing, followed by at least five volumes of column binding buffer. A clean 15 mL ultraring was filled with GH protein (1-2 mL), one of the soluble proteins being studied. On the heparin column, the proteins were introduced into the column binding buffer. Elution Buffer was used in the column with 1 M NaCl to wash and purify the proteins along a 40-minute gradient from 0% to 100%. After the protein was purified, the column was washed with at least five column volumes of distilled water and then stored in at least five column volumes of 20% ethanol. After being purified, the proteins were preserved at -4°C by adding 30% glycerol.

Results

Findings and Conversation Regarding Gene Cloning

Initial PCR Gel: Target Gene Amplification: The results of the electrophoresis procedure are displayed in **Figure (1)**, where amplified bands of the target gene (GH gene) emerged, signifying a successful extraction of DNA.

PCR Reactions: Following the PCR reactions on the study's target genes and the electrophoresis on the agarose gel injected with the target genes, the genes' DNA bands were visible, signifying a successful extraction.

Second: Gel cutting: After the samples are injected into the gel, Figure (2) displays the final gel result. Following the transfer procedure, the gel was inspected under a UV lamp to identify the DNA amplification results. The bands containing the target gene GH were then extracted by cutting the 1% gel. The Wizard SV Gel and PCR Clean-Up System LOT 0000626358 extraction kit was then used to purify these bands in order to acquire the pure DNA bands.

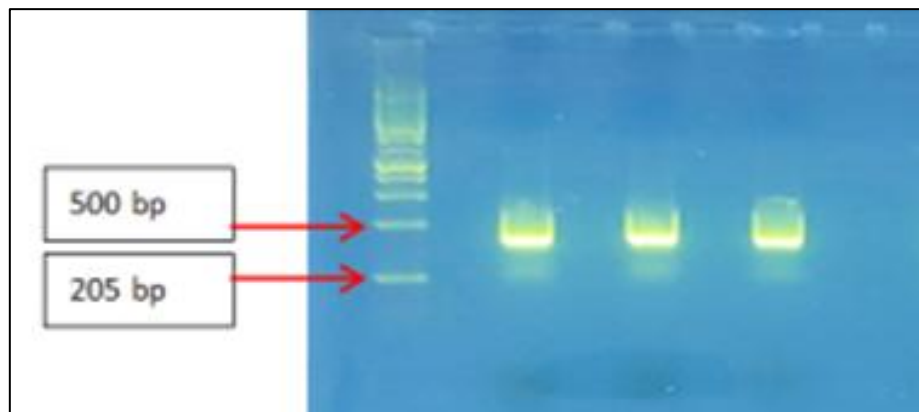


Figure 1. The DNA bands of the Growth Hormone genes



Figure 2. Gel cutting of the DNA of Growth Hormone Gene

Procedure for Cutting Plasmids

The GH gene and PET-30a gene were cut using the NdeI cut and BamHI cutting enzymes following the addition of the target gene-specific cutting enzymes, which were previously described in detail in the Materials and Methods section. After that, ethanol precipitation was used to purify the resultant DNA. This procedure purifies the cut gene (GH) and the cleaved base plasmid (PET-30a). Potentially active cutting enzymes are eliminated by ethanol precipitation, leaving only the purified plasmid and gene fragments. The uncut and shortened gene was then sequentially put into the gel after being amplified using PCR technique. Following electrophoresis, it was possible to distinguish between the uncut and truncated gene segments because they were clearly and unambiguously

visible. As seen in Figure (3), the uncut gene bands looked higher than the truncated gene bands, which were lower or smaller than the uncut bands.

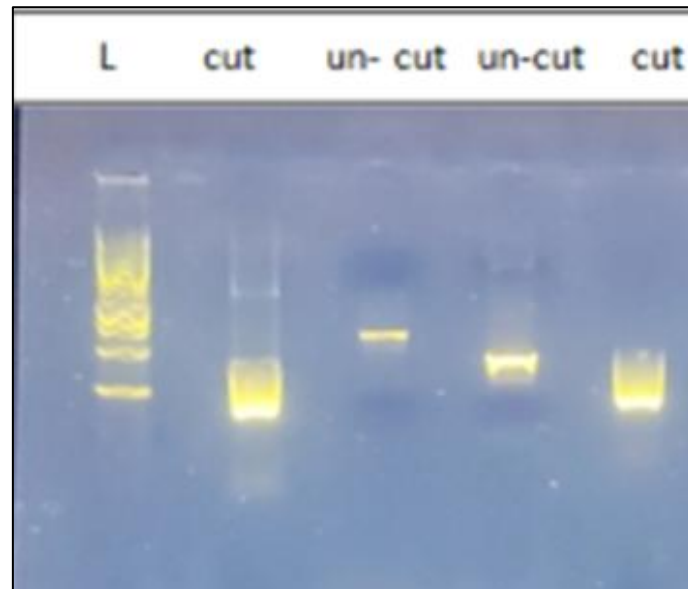


Figure 3. Gel Shows cut and un-cut of the DNA of Growth Hormone Gene

LIGAATION

The T4 ligase enzyme was used to ligate the target genes after they had been cut using restriction enzymes and their presence was confirmed by gel electrophoresis (using both the uncut and cut gene). In order to prepare the target genes for bacterial transformation using competent *E. coli* BL21 cells, this enzyme subsequently ligates them with the plasmids Pet30a.

Transformation

Single colonies were obtained after the transfer bacteria, which are DH α 5 bacteria, were added to 5 ml of each main target gene (GH), cultured by phagocytosis, followed by a spread in an LB Agar plate containing ampicillin. After that, these colonies were taken away and cultivated as showing in Figure 4.



Figure 4. BL21 bacteria that used in the cloning process for GH gene

The Colony of PCR

The gel was electrophoresed and analyzed using a spectrophotometer following the completion of the Colony PCR reaction in the DNA amplification equipment. Figure (5) showed the GH gene bundles that included the transcribed protein.

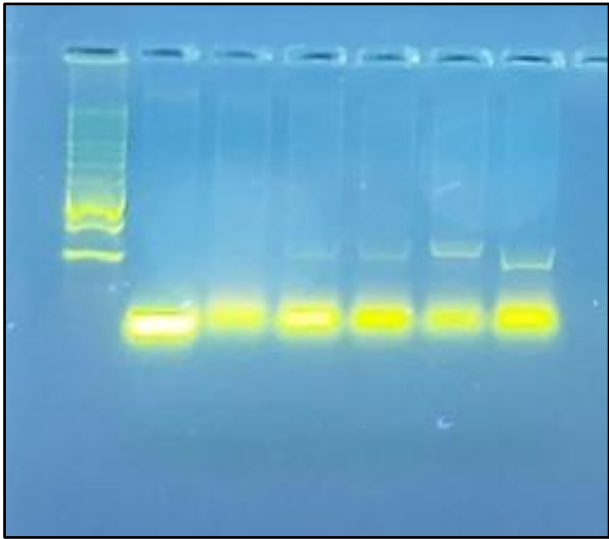


Figure 5. The GH gene band of the transcribed growth hormone protein

The Purification of Growth Hormone by Using HPLC:

The degree of purity of these proteins was determined after the HPLC procedure was carried out in the lab for the proteins cloned from the target GH gene to guarantee their purity, and after the proteins were purified at the conclusion of the laboratory work, as seen in the images below, as in Figure 6.

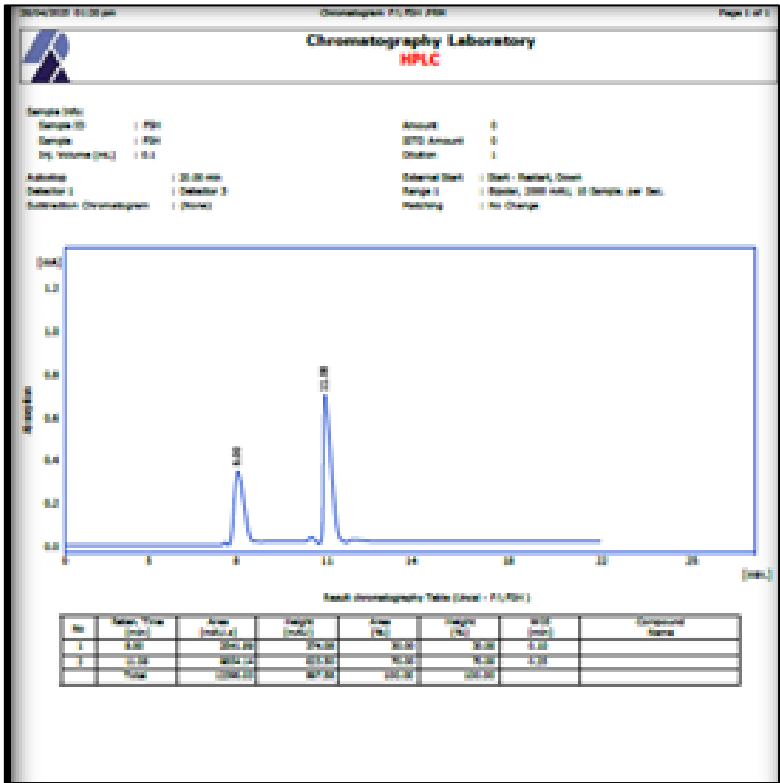


Figure 6. Shows Purification of Growth Hormone by Using HPLC

Discussion

In a study of BURNSIDE *et al.* (1991), the complementary DNA (cDNA) of the chicken growth hormone receptor was successfully isolated and excised from a chicken liver library using polymerase chain reaction (PCR). The resulting growth protein consists of 592 amino acids, in addition to a signal peptide of 16 amino acids. This study also demonstrated partial homology to growth hormone

receptors in mammals: rabbits (53%), mice (58%), and humans (50%). Despite its low degree of similarity to mammals, the receptor retained important structural features, such as the presence of 7 cysteine residues in the extracellular domain and 5 in the intracellular domain. Three transcripts of the receptor's DNA, measuring 4.7, 4.0, and 1.0 kb, were detected in the livers of 35-week-old males, and gene expression of the receptor was observed in a wide range of tissues [14].

In a different study than the current one in 2012, which used ostriches, this study provided the first technological and molecular basis for producing ostrich growth hormone using genetic engineering methods (Recombinant GH) in a bacterial fusion system, which opens up future prospects for its use in growth and physiology research, or as a biological supplement in ostrich production performance improvement programs [15].

In another study in 2022, polymorphism in the chicken growth hormone (cGH) gene was explored and investigated. The study included 50 birds from local and improved Nigerian breeds (Fulani ecotype, Noiler, FUNAAB Alpha, and Frizzled feathered), as well as the Cobb 500 breed as a reference. The studied chicken populations exhibited rich genetic variation in the cGH gene, and these discovered mutations and alleles can be utilized as molecular parameters for selecting and developing important economic and productive traits (such as growth and body weight) in future breeding programs [16].

In a study similar to the current one in terms of bird selection in 2022, it was determined how changes in the insulin-like growth factor (IGF) and its binding proteins (IGFBPs) contribute to the improved growth rates and rapid muscle mass resulting from decades of commercial genetic selection. A comparison was made between the modern Ross 308 strain (a fast-growing commercial broiler) and the traditional Athens Canadian Random Bred (an old reference line that has not undergone modern selection). The results showed that neither the gene expression of IGF in the liver nor its concentration in the blood differed between the two strains. The results also indicated that paracrine and paracrine signaling IGF within the pectoral muscle plays a crucial role in the superior muscle mass and growth of modern broilers [17].

In a very recent study in 2025, the results proved that the growth hormone (GH) gene is a prime candidate gene for developing growth traits in Japanese Pakistani quail, as mutations in the growth hormone gene of quail can be exploited as molecular markers in selection programs to improve body weights and dimensions in commercial quail breeds [18].

Conclusion

The GH gene of Japanese quail (*Coturnix japonica*) was successfully amplified using PCR, as confirmed by DNA bands observed through agarose gel electrophoresis. The gene was subsequently purified, digested with NdeI and BamHI restriction enzymes, ligated into the pET-30a plasmid, and introduced into bacterial cells for cloning and further growth hormone production.

Acknowledgment

The College of Agriculture and Forestry and the University of Mosul Presidency are acknowledged by the authors for providing the majority of the requirements needed to carry out the study.

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