### **PROLOGUE FOR THE MINI-PROJECT**

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**Title:** Cloning Strategy for Producing Human Growth Hormone (hGH)

**ABOUT THIS MINI-PROJECT**

Recombinant DNA technology allows scientists to design, manipulate, and express specific genes in host organisms to produce valuable proteins. One such application is the production of human Growth Hormone (hGH), a protein with significant therapeutic and research relevance. In this exercise, students are expected to apply their understanding of molecular cloning techniques and bioinformatics tools to design a strategy for producing hGH in a laboratory setting.

This problem challenges students to simulate a real-world scenario where they must,

1. Select an appropriate cloning method,
2. Identify and annotate the hGH gene,
3. Choose a suitable plasmid vector and expression host,
4. Use digital tools to visualize and plan the cloning construct.

The task emphasizes not just technical steps, but also rationale-based decision-making, scientific reasoning, and tool-based design. It encourages learners to experience the process of construct planning, in-silico validation, and reflection, mirroring workflows used in modern biotechnology labs.

**LEARNING OUTCOMES**

By completing this project, I was able to,

* Apply molecular cloning principles to design a gene construct for human Growth Hormone (hGH) production.
* Use bioinformatics tools to analyze DNA sequences, identify restriction sites, and plan cloning steps.
* Select and justify appropriate cloning methods, vectors, enzymes, and expression hosts based on the gene’s characteristics.
* Create and annotate a plasmid map showing successful integration of the target gene using digital tools.
* Reflect on the cloning design process, evaluate choices made, and suggest improvements based on scientific reasoning.

**PROJECT STRUCTURE**

This project guides through the process of designing a gene construct for hGH expression using molecular cloning and bioinformatics tools. Each section below outlines the expectations and deliverables.

**Phase 1: Objective and Context**

**Objective:** Establish the biological relevance and define the goal of the cloning project.

* Human Growth Hormone (hGH) and its importance in biotechnology:

70% of the teens and children in a population have a deficiency of hGH. The symptoms include short stature, delayed puberty, and poor mental development, thus affecting the quality of life.

hGH is a protein hormone produced by the pituitary gland that plays a vital role in growth, metabolism, and cell regeneration. In biotechnology, hGH is highly valuable due to its therapeutic applications in treating:

* Growth hormone deficiencies in children and adults
* Turner syndrome
* State the overall aim of your project:

The overall aim of this project is to design and plan a recombinant DNA strategy to produce human Growth Hormone (hGH) in a laboratory setting using molecular cloning techniques and bioinformatics tools. This involves: identifying and analyzing the hGH gene, designing a cloning construct, selecting suitable plasmid vectors and expression hosts, using software tools to simulate and visualize the gene integration process

**Phase 2: Cloning Strategy and Method Selection**

**Objective:** Present a justified, step-by-step cloning plan.

* Cloning method:

Restriction enzyme-based cloning was used. This method involves cutting both the gene of interest (hGH) and the plasmid vector with the specific restriction enzymes, then joining them using the ligase enzyme

* Source of the hGH Gene:

The gene sequence for human Growth Hormone (hGH) was obtained from the NCBI GenBank database. The coding region (CDS) was identified.

* Plasmid Vector Selected:

pUC19 plasmid vector was used for cloning and expression.

**Phase 3: Tool-Based Construct Design**

**Objective:** Use digital tools to plan and visualize your cloning construct.

* Bioinformatics tools used:

BioEdit: to visualize and annotate the DNA sequence of the hGH gene.

NEBcutter: to analyze restriction enzyme sites in he hGH sequence

SnapGene: to simulate the cloning process and generate the final annotated plasmid map.

**Phase 4: Your Functional Design**

**Objective:** Demonstrate how your construct will function in a biological system.

* The designed recombinant plasmid construct contains the human Growth Hormone (hGH) coding sequence under the control of prokaryotic expression elements to ensure efficient production in a bacterial host (e.g., *E. coli*). Once the plasmid is successfully transformed into the host, the molecular machinery of the cell will express the hGH protein through transcription and translation, leading to the accumulation of the recombinant protein.

# **Executive Summary**

This project focuses on designing a recombinant DNA construct to express the human Growth Hormone (hGH) in a prokaryotic host system using digital bioinformatics tools. The purpose was to replicate the molecular cloning workflow computationally, demonstrating the theoretical and practical considerations of therapeutic protein production. We retrieved the complete coding sequence (CDS) of hGH, selected restriction enzymes BamHI and SmaI, and inserted the gene into the pUC19 plasmid vector using SnapGene. The insert replaced a portion of the lacZα gene and was placed under the control of the lac promoter. BioEdit, NEBcutter, and SnapGene were employed to simulate and validate the design. The construct is biologically functional and suitable for hGH expression in E. coli. This report documents the cloning plan, construct design, and post-cloning evaluation.

# **Phase 1: Scientific Purpose and Context**

Human Growth Hormone (hGH) is a peptide hormone essential for growth, metabolism, and cellular regeneration. Deficiency in hGH results in disorders such as dwarfism and Turner syndrome. Recombinant production of hGH allows scalable and ethical therapeutic development. By using molecular cloning to express hGH in E. coli, this project simulates the planning and construction of a recombinant plasmid construct using in-silico tools. The goal is to deepen understanding of gene cloning workflows that support biotechnological applications in medicine and research.

# **Phase 2: Cloning Strategy and Methodology**

The complete hGH gene sequence was retrieved from NCBI and evaluated for restriction sites. The vector selected was pUC19, a high-copy-number plasmid with ampicillin resistance, a lac promoter, and a multiple cloning site (MCS). The insert fragment (`fasta1.txt`) is 823 bp in length and was inserted between BamHI (sticky ends) and SmaI (blunt ends). This combination ensures one sticky-end ligation for orientation control, though the SmaI blunt end presents a risk of reverse insertion. The insert was simulated into

pUC19 using SnapGene, confirming replacement of the MCS and location downstream of the lac promoter. Transformed E. coli would be selected via ampicillin resistance, and gene expression could be induced using IPTG under the lac promoter.

# **Phase 3: Tool-Based Construct Design and Validation**

BioEdit was used to clean and verify the hGH sequence. NEBcutter helped select BamHI and SmaI, ensuring no internal cuts within the CDS. SnapGene was used to simulate digestion and ligation of the hGH insert into pUC19. The insert was placed between BamHI (3) and SmaI (723), replacing 662–669 in the vector. The plasmid map confirmed correct insert orientation and preservation of essential features: lac promoter, AmpR gene, ori, and the disruption of lacZα for blue/white screening. Translation simulation confirmed that the hGH gene remains in-frame with the promoter, validating the construct for prokaryotic expression.

# **Phase 4: Functional Design of the Construct**

The recombinant construct expresses the hGH gene under the control of the lac promoter in pUC19. The gene was inserted between BamHI and SmaI, disrupting the lacZα coding region. Upon IPTG induction in E. coli, the lac promoter drives transcription of the hGH gene. Ribosome binding and translation are facilitated by the presence of an upstream RBS and an ATG start codon. The AmpR gene provides antibiotic resistance, enabling the selection of transformed colonies. The plasmid’s ori ensures replication during cell division, allowing continuous gene expression. This construct supports all essential functions for hGH production in a prokaryotic host.

# **Phase 5: Reflection and Critical Evaluation**

This in-silico cloning simulation provided valuable experience in rational design of recombinant constructs. While BamHI offered efficient sticky-end ligation, SmaI introduced blunt-end ligation risks, potentially allowing reverse insertions. Manual orientation checks in SnapGene ensured correct directionality. Future improvements could involve using two sticky-end enzymes for higher specificity and ligation efficiency. This simulation mimicked laboratory protocols, strengthening both conceptual and practical understanding of gene cloning.