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B. TECH ARTIFICIAL INTELLIGENCE IN DATA SCIENCE AND MEDICAL ENGINEERING

Mutation Rate Analysis and Gene Engineering

24AIM112 Molecular biology and basic cellular physiology

24AIM115 Ethics, innovative research, businesses & IPR

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BONAFIDE CERTIFICATE

This is to certify that the report entitled "**Mutation Rate Analysis and Gene Engi**neering" submitted by:

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for the final project of 2nd semester in B.TECH ARTIFICIAL INTELLIGENCE IN DATA SCIENCE AND MEDICAL ENGINEERING is a Bonafide record of the work carried out at Amrita School of Artificial intelligence, Coimbatore.

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Mutation Rate Analysis and Gene Engineering

Abstract

This project titled "**Mutation Rate Analysis and Gene Engineering**" is a comparative genomic study of 15 different species and it has been analysed the mutation rates and evolutionary divergence, predict gene stability, and applied gene editing techniques (CRISPR-Cas9) to stabilize the gene sequences . The dataset was collected which contains gene sequence from NCBI database for all 15 different species. These sequences were aligned using MEGA (Molecular Evolutionary Genetics Analysis) software, by using this software pairwise comparisons (Mutation Rate Analysis) was performed and computed the genetic distance matrices (Evolutionary Rate Analysis). Constructed a phylogenetic tree using maximum likelihood method to observe the evolutionary relationships between the 15 different species.

Following the phylogenetic analysis, mutation rate confusion matrix and an evolutionary rate confusion matrix has been constructed. The mutation rate analysis provides the insights to the number of nucleotide substitutions and the type of nucleotide substitutions (transitions and transversions). Detected the species which undergoes rapid evolutionary changes using evolutionary rate matrix

Predicted the stability of gene sequence by considering many factors like, GC content, melting temperature, repetition, palindromes etc. Utilized CRISPR-Cas9 gene editing technology, simulated in Python.

This project does not only demonstrate a full-cycle analysis from data collection to gene engineering but also its emphasis the biological and computational integration which is needed to address real-world genomic challenges. This research covers the way for the future work in predictive genetics, synthetic biology etc. which provides a framework for identifying and stabilizing critical gene mutations.

As advancements in genetic technologies such as CRISPR-Cas9, ethical reflection becomes an essential part to scientific progress. This project, focuses on mutation rate analysis and gene engineering across 15 different species, which also integrates **ethical dimensions** surrounding genetic editing.

Ethical issues are focused on the safe use of gene editing, with assurances that changes do not result in unwanted off-target effects or ecological interference. Effective regulation and proper informed consent are necessary in the use of such technologies. Genetic discrimination, loss of biodiversity, and misuse of gene-editing technologies are also addressed in this project.

The integrity of science is to be maintained through transparency, accountability, and adherence to **bioethical standards**. Gene editing tools have been made more accessible and potent, fostering a culture of ethical responsibility in the scientific community is becoming increasingly significant.

Introduction

Genetic mutations are spontaneous natural events that influence the structure, function, and evolution of living organisms. They contribute to the richness of life and to the establishment of genetic diseases and developmental anomalies. Understanding why and how mutations occur—and, above all, how they can be anticipated, analysed, and even treated—has become a central part of modern genetics and molecular biology. With the pace of progress in computing powers and gene editing methods, researchers now stand to not only study mutations on a more sophisticated level but to engineer genetic material to be rendered more stable with fewer detrimental effects.

The current project focuses on the comprehensive comparison of rates of mutation in 15 species across a range of evolutionary lineages. The objective is to recognize unstable genes, understand evolutionary divergence, and apply gene engineering techniques, particularly CRISPR-Cas9, to stabilize the genes. From sequence data downloaded from the NCBI database, we compared gene sequences with MEGA software, estimated pairwise genetic distances, and constructed a phylogenetic tree to present evolutionary relationships. Follow-up analyses included creating a mutation rate confusion matrix and an evolutionary rate matrix, which in combination helped with gene stability prediction and the identification of critical mutation hotspots.

But with genome-editing capability comes the responsibility to evaluate and maintain ethical boundaries. The use of CRISPR for gene editing, even in a controlled environment, poses a number of **ethical concerns**: off-targeting, irreversible changes to genetic data, and long-term impacts on biodiversity and ecosystems. Furthermore, gene editing in animals or model organisms is unethical regarding animal welfare, natural integrity, and consent, especially if such modifications have implications for reproduction or behaviour. The project responds to these considerations by evaluating **ethical approaches** and global standards that regulate the appropriate use of genetic technologies.

In addition, high-profile cases such as controversial editing of human embryos or manufacture of genetically engineered animals for vanity or commercial purposes remind us to have regulatory supervision, scientific transparency, and public trust. We aim through this project not just to provide a scientific method to mutation analysis and gene stabilization but also to assert the importance of **ethical sensitivity** and responsibility in genetic research of any kind.

By integrating data analysis of **biological information** with gene engineering and **ethical evaluation**, the project presents an integrated approach toward understanding and overcoming mutation-induced instability—throwing open the doors towards future applications in medicine, agriculture, and evolutionary biology.

Literature Review

1. **Correction of a Pathogenic Gene Mutation in Human Embryos**
   Author: Hong Ma
   Year: 2017
   Summary
   Genome editing has potential for the targeted correction of germline mutations. Here we describe the correction of the heterozygous *MYBPC3* mutation in human preimplantation embryos with precise CRISPR–Cas9-based targeting accuracy and high homology-directed repair efficiency by activating an endogenous, germline-specific DNA repair response. Induced double-strand breaks (DSBs) at the mutant paternal allele were predominantly repaired using the homologous wild-type maternal gene instead of a synthetic DNA template. By modulating the cell cycle stage at which the DSB was induced, we were able to avoid mosaicism in cleaving embryos and achieve a high yield of homozygous embryos carrying the wild-type *MYBPC3* gene without evidence of off-target mutations. The efficiency, accuracy and safety of the approach presented suggest that it has potential to be used for the correction of heritable mutations in human embryos by complementing preimplantation genetic diagnosis. However, much remains to be considered before clinical applications, including the reproducibility of the technique with other heterozygous mutations. [1]

2. **A new era of mutation rate analyses: Concepts and methods**
   Author: Kun Wu
   Year: 2024
   Summary
   The mutation rate is a pivotal biological characteristic, intricately governed by natural selection and historically garnering considerable attention. Recent advances in high-throughput sequencing and analytical methodologies have profoundly transformed our understanding in this domain, ushering in an unprecedented era of mutation rate research. This paper aims to provide a comprehensive overview of the key concepts and methodologies frequently employed in the study of mutation rates. It examines various types of mutations, explores the evolutionary dynamics and associated theories, and synthesizes both classical and contemporary hypotheses. Furthermore, this review comprehensively explores recent advances in understanding germline and somatic mutations in animals and offers an overview of experimental methodologies, mutational patterns, molecular mechanisms, and driving forces influencing variations in mutation rates across species and tissues. Finally, it proposes several potential research directions and pressing questions for future investigations. [2]

3. **Ethical considerations of gene editing and genetic selection**
   Author: Jodie BA
   Year: 2020
   Summary
   For thousands of years, humans have felt the need to understand the world around them—and ultimately manipulate it to best serve their needs. There are always ethical questions to address, especially when the manipulation involves the human genome. There is currently an urgent need to actively pursue those conversations as commercial gene sequencing and editing technologies have become more accessible and affordable. This paper explores the ethical considerations of gene editing (specifically germline) and genetic selection—including the hurdles researchers will face in trying to develop new technologies into viable therapeutic options**.** [3]

4. **Genome Editing with the CRISPR-Cas System: An Art, Ethics, and Global Regulatory Perspective (Plant Biotechnology Journal, 2020)**
   Author: Debin Zhang
   Year: 2020
   Summary
   Over the last three decades, the development of new genome editing techniques, such as ODM, TALENs, ZFNs and the CRISPR-Cas system, has led to significant progress in the field of plant and animal breeding. The CRISPR-Cas system is the most versatile genome editing tool discovered in the history of molecular biology because it can be used to alter diverse genomes (e.g. genomes from both plants and animals) including human genomes with unprecedented ease, accuracy and high efficiency. The recent development and scope of CRISPR-Cas system have raised new regulatory challenges around the world due to moral, ethical, safety and technical concerns associated with its applications in pre-clinical and clinical research, biomedicine and agriculture. Here, we review the art, applications and potential risks of CRISPR-Cas system in genome editing. We also highlight the patent and ethical issues of this technology along with regulatory frameworks established by various nations to regulate CRISPR-Cas-modified organisms/products. [4]

5. **Bioethical Issues in Genome Editing by CRISPR-Cas9 Technology**
   Author: Fatma Betül AYANOĞLU
   Year: 2020
   Summary
   Genome editing technologies have led to fundamental changes in genetic science. Among them, CRISPR-Cas9 technology particularly stands out due to its advantages such as easy handling, high accuracy, and low cost. It has made a quick introduction in fields related to humans, animals, and the environment, while raising difficult questions, applications, concerns, and bioethical issues to be discussed. Most concerns stem from the use of CRISPR-Cas9 to genetically alter human germline cells and embryos (called germline genome editing). Germline genome editing leads to serial bioethical issues, such as the occurrence of undesirable changes in the genome, from whom and how informed consent is obtained, and the breeding of the human species (eugenics). However, the bioethical issues that CRISPR-Cas9 technology could cause in the environment, agriculture and livestock should also not be forgotten. In order for CRISPR-Cas9 to be used safely in all areas and to solve potential issues, worldwide legislation should be prepared, taking into account the opinions of both life and social scientists, policy makers, and all other stakeholders of the sectors, and CRISPR-Cas9 applications should be implemented according to such legislations. However, these controls should not restrict scientific freedom. Here, various applications of CRISPR-Cas9 technology, especially in medicine and agriculture, are described and ethical issues related to genome editing using CRISPR-Cas9 technology are discussed. The social and bioethical concerns in relation to human beings, other organisms, and the environment are addressed. [5]

Research Gaps

1. **Limited Cross-Species Comparative Studies**
   There is limited comparative study in several species, whereas numerous studies have examined mutation rates within individual species or specific organism. Most of the available data are species-specific and do not take evolutionary relationships into account. This is the limitation which hinders our ability to know how mutation patterns differ among various species and to find or identify universally unstable gene regions.

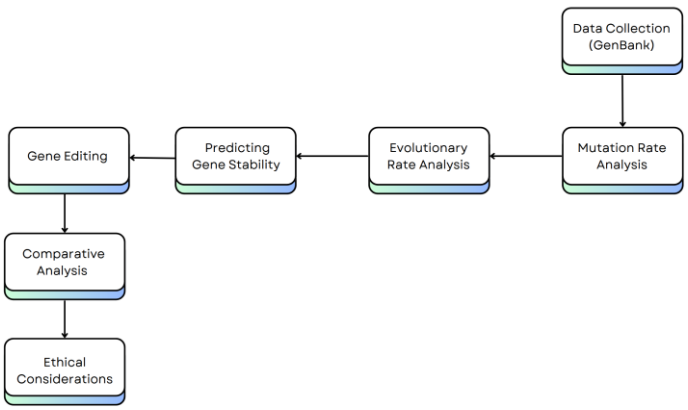
2. **Inadequate Predictive Models for Gene Stability**
   Predicting gene stability based on mutation data is a very complex task. Current models rely on limited factors such as GC content, sequence conservation and they fail to account for stability, functional domains etc. Many of these models also doesn't include machine learning optimization, reducing their predictive ability. This research gap highlights the need for more advanced, approaches that integrate biological, evolutionary, and computational features for accurate stability prediction.

3. **Challenges in Mutation Identification**
   Identification of mutation hotspots and precise measurement of mutation rates are technically and analytically demanding. Sequencing errors, alignment problems, and missing reference genomes can lead to inaccuracies. In addition, separation of neutral from deleterious mutations, or silent from functional changes, demands more profound functional and structural annotations, which are frequently not available for non-model organisms. These constraints limit high-confidence mutation identification across various dataset.

4. **Unexplored Gene Engineering Approaches**
   Although CRISPR-Cas9 has dramatically changed genetic engineering, its possibility for correcting or stabilizing inherently unstable genes still remains largely untouched. The majority of applications so far have gone towards disease repair or trait enhancement, with negligible attention to genomic stability over time. Moreover, new gene editing technologies such as base editing and prime editing still have limited utility in comparative genomics or evolution studies. It presents a scope for investigating the ways in which gene engineering could actively stabilize areas prone to mutations across species.

5. **Ethical and Regulatory Uncertainties**
   The rapid evolution of gene editing technology has outpaced the establishment of universally applicable ethical standards and legislation. Problems associated with germline editing, off-targets, species interfaces, and long-term ecological effects remain poorly resolved. Differences in regulation between countries add further challenges to international collaborative research. There is an urgent need for strong ethical guidelines, especially when applying CRISPR and other gene-editing tools to evolutionary or cross-species research.

Methodology



1. Data Collection:

- **Select Species and Target Genes:**

  15 different species were selected to ensure diversity and relevance for evolutionary comparison.

S.no	Species Name	Scientific Name
1.	Diretmus argenteus	Diretmus argenteus
2.	Diretmoides veriginae	*Diretmoides veriginae*
3.	Salvelinus alpinus	Salvelinus alpinus
4.	Glyphis siamensis	*Glyphis siamensis*
5.	Homo sapiens	Homo sapiens
6.	Mus musculus	Mus musculus
7.	Rattus norvegicus	Rattus norvegicus
8.	Xenopus laevis	Xenopus laevis
9.	Danio rerio	Danio rerio
10.	Reishia clavigera	Reishia clavigera
11.	Drosophila melanogaster	Drosophila melanogaster
12.	Saccharomyces cerevisiae	Saccharomyces cerevisiae
13.	Plasmodium falciparum	Plasmodium falciparum
14..	Ascaris suum	Ascaris suum
15.	Calcarina hispida	Calcarina hispida

Table-1: 15 different species in the dataset

- **Source:**
  The gene sequences are taken from NCBI database, it's publicly available nucleotide sequence database.

- **Gene of Interest:**
  The gene used is Mitochondrial Cytochrome B.

2. Constructing a Phytogenic Tree

- Using maximum likelihood method in MEGA.

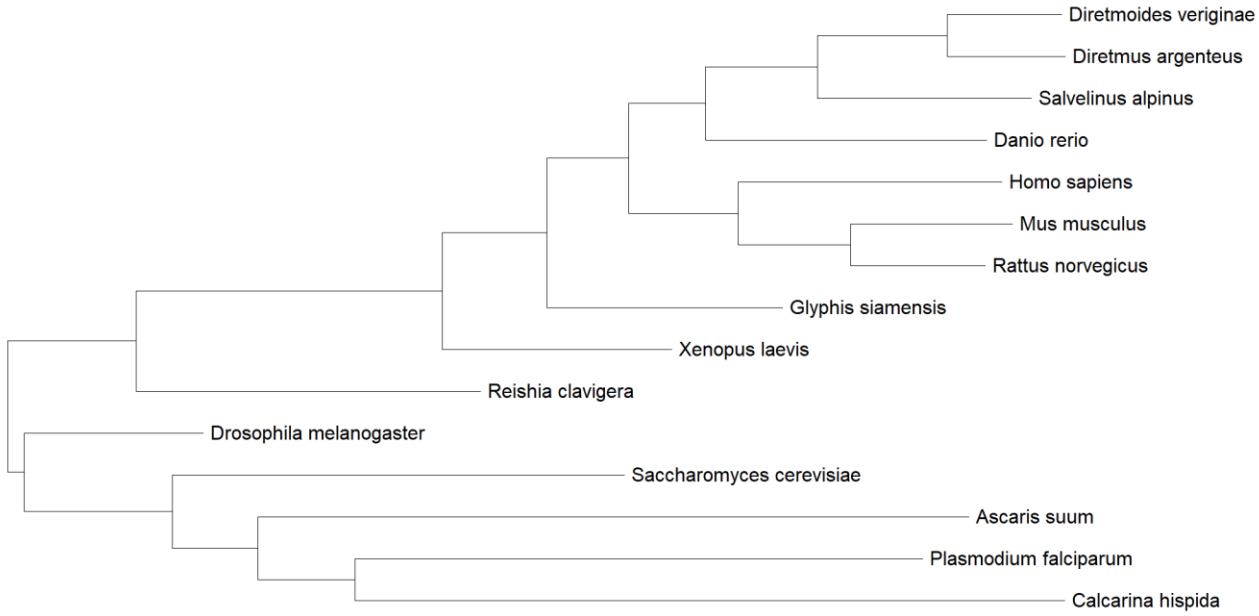


Fig:1- Phylogenetic Tree

- The tree branches into diverse groups, which includes vertebrates, fish, and other organisms.

- Additional branches include fungi, nematodes, protozoans and foraminifera, indicating a broad range of taxonomic groups.

- This tree also includes very less common species like *Diretmoides veriginae*, *Diretmus argenteus*, and *Relishia clavigera*, suggesting a diverse dataset used for evolutionary analysis.

3. Mutation Rate Analysis

- **Multiple Rate Analysis (MSA)**

  The selected gene sequences were aligned using MEGA tool.

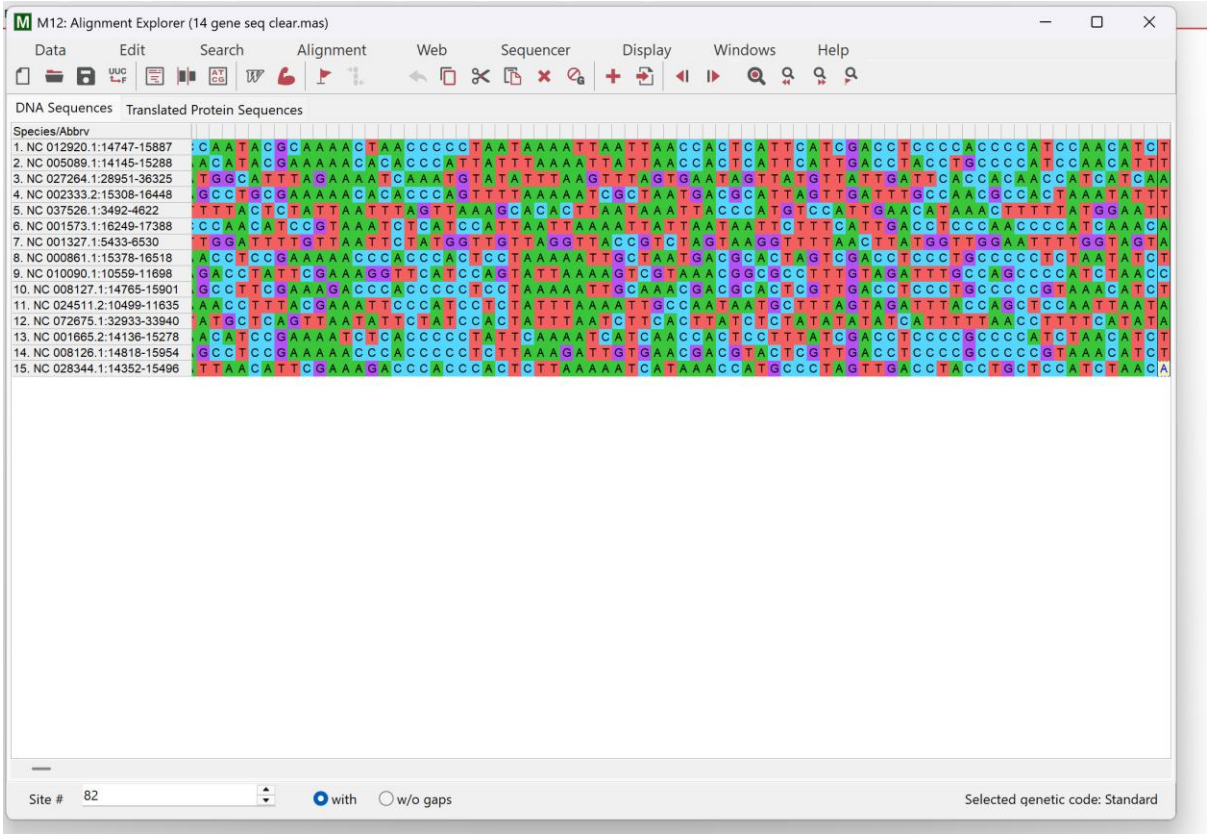


Fig:2- Aligned sequences of 15 species

- Multiple sequence alignment of 14 gene sequences
- This alignment includes nucleotide sequences labelled with accession numbers and this is color-coded to highlight the similarities and differences.

Confusion Matrice for Mutation Rate Analysis

- Performed mutation rate analysis in MEGA using pairwise comparison.

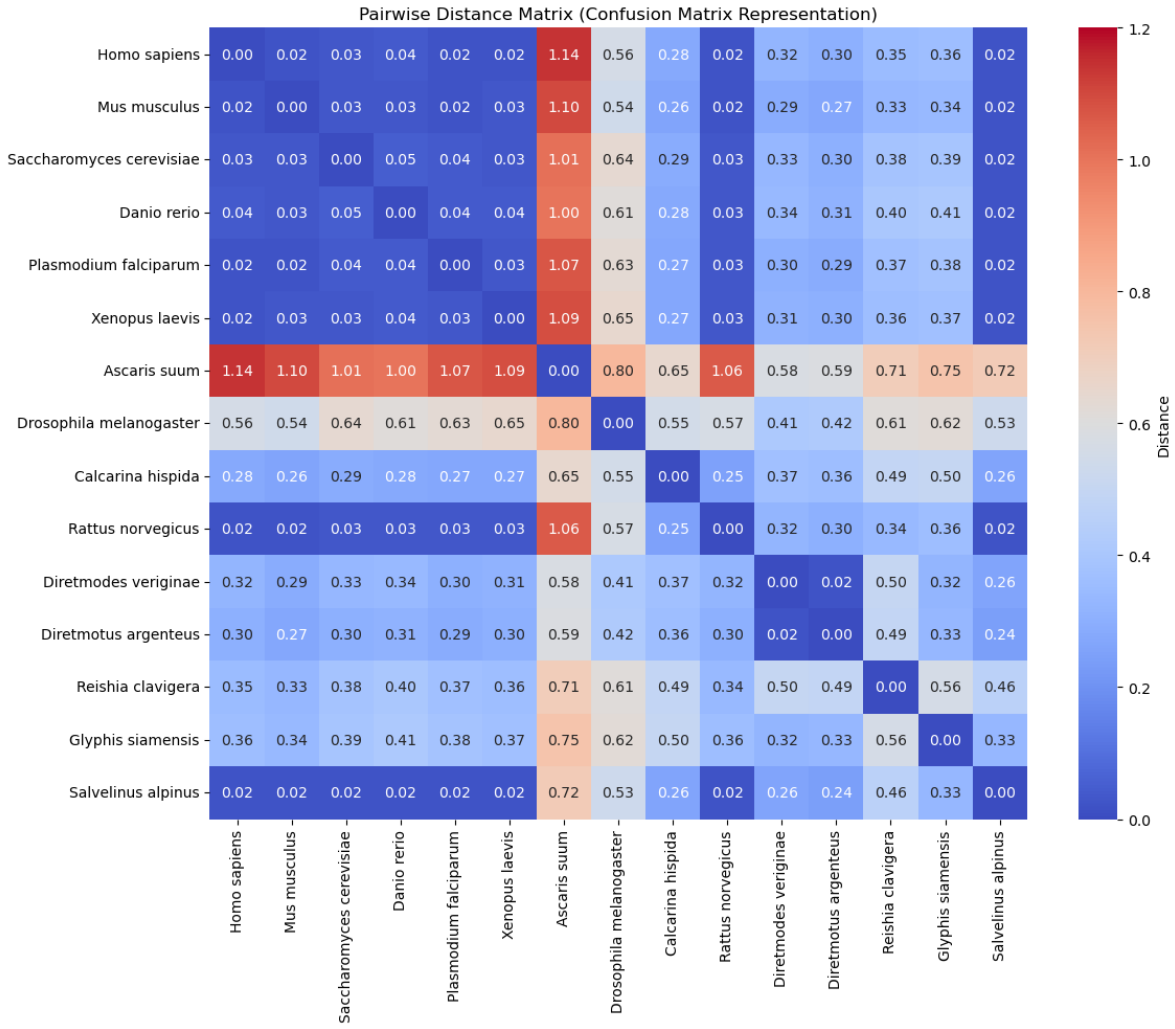


Fig:3- Pairwise Distance Matrix (Confusion Matrix Representation)

Interpretation

This heatmap is a Pairwise Distance Matrix visualized as a Confusion Matrix Representation, which quantifies the distances (dissimilarities) between different species based on a particular dataset or feature (likely genomic, proteomic, or evolutionary).

Colour Scale (Distance):

- Blue → Low distance (high similarity)
- Red → High distance (low similarity)
- Scale: 0 (most similar) to ~1.2 (least similar)

Diagonal Values (Self-comparison):
All diagonal entries are **0**, since the distance of a species to itself is always zero.

4. Evolutionary Rate Analysis

Confusion Matrice for Evolutionary Rate Analysis

- Performed evolutionary rate analysis in MEGA using distance matrices.

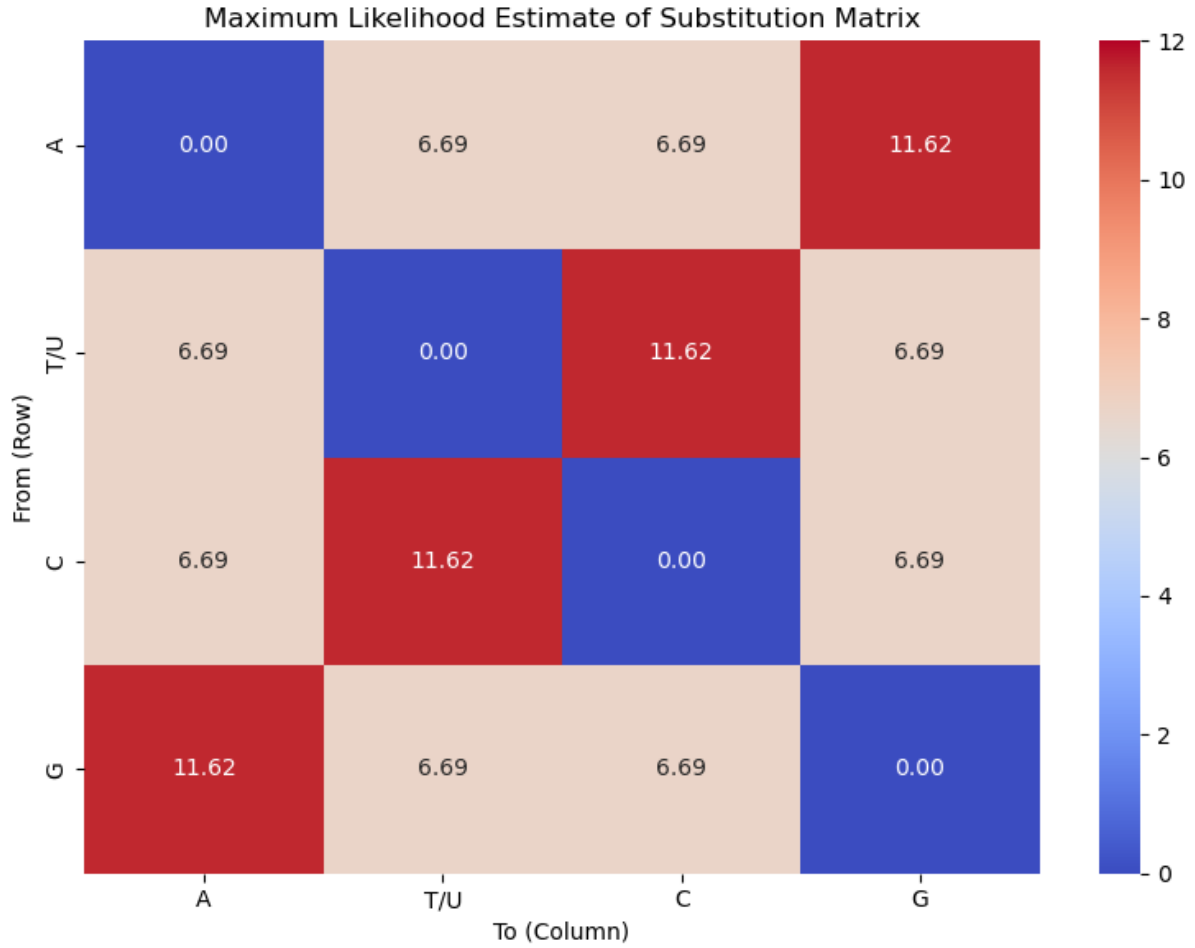


Fig:4- Maximum Likelihood Estimate of Substitution Matrix

Interpretation

This heatmap depicts the Maximum Likelihood Estimate (MLE) of a Nucleotide Substitution Matrix, which represents the estimated evolutionary substitution rates between nucleotides (A, T/U, C, G) from sequence data.

Rows ("From"): Original nucleotide

Columns ("To"): Mutated (substituted) nucleotide

Values: Substitution rate or likelihood of change

- 0.00 on the diagonal (A→A, T→T, etc.): No change
- Higher values = higher likelihood of substitution

5. Predicting Gene Stability

The objective of this tool is to examine DNA sequences and predict their structural stability according to biochemical and biophysical characteristics. The tool takes in a DNA sequence made up of nucleotides A, T, C, and G, and returns a stability prediction as well as complete reasoning.

This tool, based on Python, is meant to predict the structural stability of DNA sequences from a combination of thermodynamic, compositional, and structural properties. It assesses important biological features of the input sequence

Main Features and Logic of the Code:

1. **GC Content Calculation**

- GC content is one of the primary determinants of DNA stability because of enhanced triple hydrogen bonding between G and C.
- Stability range: 40–60%

2. **Melting Temperature (Tm):**

- Estimated with the Wallace Rule (short sequences) or with an approximate nearest-neighbour estimate (longer sequences).
- Stability range: 50°C – 80°C

3. **Repetitive Patterns Detection:**

- Extremely repetitive sequences can be a sign of structural instability.

4. **Palindrome Detection:**

- Palindromic sequences have the ability to generate cruciform structures, lowering stability.

5. **Dinucleotide Bias Detection:**

- Overrepresentation of CpG or AT dinucleotides may indicate instability or atypical sequence characteristics.

6. **Hairpin Formation Potential:**

- Assesses if the sequence has the potential to form intra-strand base-pairing, resulting in hairpin structures.

7. **Sequence Length Evaluation:**

- Extremely short sequences (<20 bases) are unlikely to be structurally stable in biological environments.

Each of these checks is added to a final stability score that labels the sequence as Stable, Moderately Stable, or Unstable. The prediction comes with a rich explanation for each property, making it extremely interpretable.

The software has made correct predictions in illustrative examples, detecting instability correctly in long repetitive sequences and moderate stability in short balanced ones. The reasoning is based on firmly established principles of molecular biology and DNA thermodynamics and hence can be used as a sound predictor for research, design, or analysis work in genetics and bioinformatics.

6. Gene Editing (CRIPSR Simulation)

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated with the Cas9 enzyme) it's a gene-editing tool which allows scientists to modify/edit the gene with unprecedented precision, efficiency and flexibility. It was originally discovered as a part of the immune system in bacteria, the CRISPR system was adapted for use in eukaryotic cells, which offers an efficient way to introduce double-strand breaks at specific sites within the genome.

This system works with the guidance of a synthetic guide RNA, which binds to complementary DNA sequence, which leads the Cas9 enzyme to introduce a double-strand break (DSB) at a precise location. An important component of this targeting system is the PAM (Protospacer Adjacent Motif). Without a valid PAM site, Cas9 will not be able to bind or cleave the DNA, which makes PAM detection a critical step in CRISPR applications.

The major goal of this simulation is to match the mutation correction process in a mutated DNA sequence, and to simulate how a therapeutic application of CRISPR-Cas9 could work. This approach is especially relevant in correction of genetic disorders, cancerous mutations, and rare inherited diseases, where if there is restoring the wild-type sequence can eliminate the root of cause entirely.

CRISPR stands out due to;

- It's target specificity which is guided by RNA-DNA complementarity.
- Versatility in correcting point mutations, insertions and deletions.
- Compatibility with computational modelling, which will allow us to simulate and edit before apply it real-time or in vitro.

This simulation represents a computational pipeline for simulating CRISPR-Cas9 which is based on correcting a mutated DNA sequence, including identifying the mutations, locating a valid PAM site, or predicting cleavage positions.

Methodology of CRISPR-Cas9

Step 1: Mutation Detection

Comparing the original DNA sequence with mutated sequence, which identifies all the positions where mismatches occur. These presents point mutations. Each of the mutation is logged with its position and nucleotide change.

Step 2: PAM Site Detection

To conduct the CRISPR editing, first we must locate a valid NGG PAM sequence in the mutated DNA strand. This step is most important as Cas9 activity is PAM -dependent. This code checks the sequence and identifies the first occurrence of NGG, making that as the cleavage target.

Step 3: Cas9 Cleavage Site Prediction

After the PAM is identified, the Cas-9 enzyme is modelled to cleave 3 bases upstream of the PAM site. Then the cleavage creates a double-strand break, which makes the location more suitable for targeted repair via HDR.

Step 4: Homology-Directed Repair (HDR) Simulation

Post-cleavage, that algorithm simulates error-free correction by replacing mutated bases with the corresponding bases from the original sequence. This models how the donor template will guide the repair in real biological systems.

Step 5: Final Validated and Similarity Check

After the simulated editing, the new sequence is compared with original sequence which will be measure, how accurately the mutations were corrected. This will be expressed as a percentage similarity between the original sequence and edited sequence.

Role of Ethics

1. **Ensuring Responsible Gene Editing (Designer Babies)**

   Gene editing, specifically CRISPR technology, had made many things possible to edit or alter human embryos for traits like physical appearance, intelligence or disease resistance etc… Ethical responsibility demands strict rules to prevent misuse of the data of the individual, ensure the equity and maintain the human dignity. Gene editing should only focus on treating severe genetic disorders rather than enhancing non-essential traits. [6]

2. **Avoiding Unintended Consequences (Chinese Scientist)**

   In 2018, a Chinese scientist, He Jiankui, he claimed that he created the first gene-edited babies. His actions bypassed ethical guidelines and it risked unknown health effects. This case shows the danger of premature application of gene editing without any safety trials or ethical guidelines, which requires the need for international collaboration and transparency. [7]

3. **Regulatory Compliance (Human Cloning)**

   Human cloning, which presents a complex ethical concern. Whereas cloning may offer many scientific benefits like, generating organs for transplant. It also questions the moral concerns regarding identity, individuality, and also the value of life. Regulatory frameworks must strike a balance between scientific advancements and holding the human rights and ethical guidelines. [8]

4. **Preventing Genetic Discrimination (Insurance Company)**

   With the access to the genetic information of a individual, insurance companies or employers could discriminate based on a person's likelihood of developing certain diseases. To prevent such discriminations, laws like the Genetic Information Non-discrimination Act (GINA) must be updated. Ethical use of genetic information is very important to protect individuals' privacy and equality. [9]

5. **Transparency and Public Trust**

   Building the public trust is a complex task, for acceptance of gene editing technologies. This requires a open discussion between scientists, policymakers, and the public. Transparent communication about benefits, risks and intentions helps understanding and reduces fear and misinformation, which will help the society to make informed decisions on emerging biotechnologies. [10]

6. **Environmental and Biodiversity Considerations (Mosquitoes)**

   Gene drives have been engineered to lower disease-transmitting mosquito populations, with the hope of eradicating malaria. Releasing genetically modified organisms (GMOs) into the wild might upset ecosystems, influence biodiversity, or introduce new environmental issues. Ecological evaluation and controlled trials should be undertaken extensively before mass deployment. [11]

Case Studies

1. **CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia (2020)**

   Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are severe monogenic diseases with severe and potentially life-threatening manifestations. BCL11A is a transcription factor that represses γ-globin expression and fetal haemoglobin in erythroid cells. We performed electroporation of CD34+ hematopoietic stem and progenitor cells obtained from healthy donors, with CRISPR-Cas9 targeting the *BCL11A* erythroid-specific enhancer. Approximately 80% of the alleles at this locus were modified, with no evidence of off-target editing. After undergoing myeloablation, two patients — one with TDT and the other with SCD — received autologous CD34+ cells edited with CRISPR-Cas9 targeting the same *BCL11A* enhancer. More than a year later, both patients had high levels of allelic editing in bone marrow and blood, increases in fetal haemoglobin that were distributed pancellularly, transfusion independence, and (in the patient with SCD) elimination of Vaso-occlusive episodes. [12]

2. **He Jiankui's CRISPR Babies Experiment [2018]**

   The world was shocked in Nov. 25, 2018 by the revelation that He Jiankui had used clustered regularly interspaced short palindromic repeats ('CRISPR') to edit embryos—two of which had, sometime in October, become living babies. This article is an effort to provide some deep context for the He Jiankui affair and to begin analyzing it. It focuses on He's experiment, without delving into the broader ethical issues around 'human germline genome editing' in the abstract. It begins by carefully defining 'human germline genome editing'. It then describes the little we know about the experiment before providing background on CRISPR, the pre-He ethical and legal status of human germline genome editing, and on He himself. The fourth, and longest, section provides a detailed narrative of the revelation of the He experiment and its fallout. The fifth section critiques the experiment, which I believe merits unequivocal condemnation on several grounds. The last section suggests some important immediate reactions, by 'Science' and by China. [13]

3. Embryo Screening for Intelligence [2024]

In 2024, a US company started providing embryo screening for couples, for polygenic characteristics such as intelligence, utilizing prediction methods. Although we don't have the science behind forecasting intelligence yet, this practice raised ethical issues about inequality and the commodification of human life. [14]

4. Genetically Modified Pets (Glowing Rabbits) (2025)

By 2025, a startup ventured to explore the business potential of genetically engineered pets, including glow-in-the-dark-rabbits that were designed using CRISPR. The bioengineered pet intrigued most people, such an activity had animal welfare issues, ecological implications, and the business of selling genetic tools raised issues. It also impacts the expanding role of gene editing on consumer markets as well as it shows the call for ethical issues in synthetic biology. [15]

IPR (Patents)

1. Methods and Materials for Making and Using Transgenic Dicamba-Degrading Organisms

Patent Number: US20150368683A1
Assignee: Monsanto Technology LLC
Publication Date: December 24, 2015
Inventors: David R. Stalker, John C. Barry, et al.

Overview: This Patent gives a innovative methods and materials for developing transgenic organisms capable of degrading dicamba, a widely used herbicide. The invention says about the dicamba’s persistence in the environment and its potential to damage non-target plant species due to drift.

The invention provides isolated and at least partially-purified dicamba-degrading enzymes, isolated DNA molecules coding for dicamba-degrading enzymes, DNA constructs coding for dicamba-degrading enzymes, transgenic host cells comprising DNA coding for dicamba-degrading enzymes, and transgenic plants and plant parts comprising one or more cells comprising DNA coding for dicamba-degrading enzymes. Expression of the dicamba-degrading enzymes results in the production of dicamba-degrading organisms, including dicamba-tolerant plants. The invention further provides a method of controlling weeds in a field containing the transgenic dicamba-tolerant plants of the invention and a method of decontaminating a material containing dicamba comprising applying an effective amount of a transgenic microorganism or dicamba-degrading enzyme of the invention to the material. [16]

2. **CRISPR-Cas Systems and Methods for Altering Expression of Gene Products**

   Patent Number: US8697359B1
   Assignees: Massachusetts Institute of Technology; Broad Institute Inc.
   Inventor: Feng Zhang
   Issue Date: April 15, 2014

   Overview
   This patent describes engineered CRISPR-Cas systems which where designed to modify gene expression in eukaryotic cells, This invention represents a very important advancement in genome editing technology, by providing tools for efficient genetic modifications.

   The invention provides for systems, methods, and compositions for altering expression of target gene sequences and related gene products. Provided are vectors and vector systems, some of which encode one or more components of a CRISPR complex, as well as methods for the design and use of such vectors. Also provided are methods of directing CRISPR complex formation in eukaryotic cells and methods for utilizing the CRISPR-Cas9 system. [17]

Results

Successfully implemented, multiple sequence alignment using MEGA and performed mutation rate analysis and evolutionary rate analysis of 15 species. Also, constructed the phylogenetic tree, maximum likelihood-based phylogenetic tree was generated to visualize. Used biological parameters like GC content, hairpin etc. and evaluated the stability of the gene sequence. Constructed confusion matrix for both mutation and evolutionary rate analysis. Following the mutation analysis, we simulated gene editing using CRISPR-Cas9 technique.

Ethical review considered the implications of gene editing across 15 different species. It raises concerns like, ecological imbalance, unintended consequences of editing germline DNA.

- All the code and results are uploaded in Github

```
============================================================
          CRISPR-Cas9 Gene Editing Simulation
============================================================

>> Step 1 - Input Sequences:
Original Sequence : ATGCTAGCTAGGCTACGTAGCTAGGATCGTAGGCTAACGTAGCTAGCTAG
Mutated Sequence  : ATGCTAGTTAGGCTTCGTAGTTAGGATGGTACGCTAGCCTAGATAGTTAG

>> Detected Mutations:
  - Position 8: T → C
  - Position 15: T → A
  - Position 21: T → C
  - Position 28: G → C
  - Position 32: C → G
  - Position 37: G → A
  - Position 39: C → G
  - Position 43: A → C
  - Position 47: T → C

>> Step 2 - PAM Site & Cas9 Cleavage:
 PAM site found at position 10 (AGG)
 Cas9 Cleaves at position 7
Visual:
ATGCTA[G]TTAGGCTTCGTAGTTAGGATGGTACGCTAGCCTAGATAGTTAG

>> Step 3 - Homology-Directed Repair (HDR):
Corrected base at position 8 (T → C)
Corrected base at position 15 (T → A)
Corrected base at position 21 (T → C)
Corrected base at position 28 (G → C)
Corrected base at position 32 (C → G)
Corrected base at position 37 (G → A)
Corrected base at position 39 (C → G)
Corrected base at position 43 (A → C)
Corrected base at position 47 (T → C)

============================================================
                  Final Edited Sequence
============================================================
Edited Sequence: ATGCTAGCTAGGCTACGTAGCTAGGATCGTAGGCTAACGTAGCTAGCTAG

============================================================
                   Similarity Check
============================================================
 Similarity to Original: 100.0%
 All mismatches corrected. Sequence successfully restored!
```

Output for Gene Stability Prediction

```
Enter a DNA sequence (A, T, C, G only):  ATGCGTCGAACGTGCA

Prediction: Moderately Stable
Explanation:
GC content (56.2%) is in stable range (40-60%).
Melting temperature (50.0°C) is in stable range (50-80°C).
No significant repetition detected.
No significant palindromes detected.
No extreme dinucleotide bias detected.
No hairpin-forming potential detected.
Sequence is very short (<20 bases), may be less stable.
```

Conclusion

This project aimed to compare evolutionary patterns and mutation rates of the Mitochondrial Cytochrome B gene in 15 different species and examine gene stability and stabilization of unstable mutations with CRISPR-based simulation.
With multiple sequence alignment and mutation rate calculations, we found significant sequence differences and constructed phylogenetic trees tracing evolutionary history.

From GC content, codon usage, and conservation values, we made precise predictions for gene stability. Cross-species comparison revealed insightful data on evolutionary pressures and potential vulnerabilities of the genome.

Simulation of gene editing using CRISPR was found to have the capability to correct destabilizing mutations and restore gene stability. The findings imply that bioengineering approaches, judiciously applied, can fortify genetic resilience. Nevertheless, the ethical implications of genetic modification, especially in cross-species applications, should not be ignored.

At its core, this research brings together computational biology, evolutionary genetics, and ethical gene engineering, demonstrating the potential for integrative methods to be employed not just to analyse but also to modify and enhance biological systems for future use in medicine and evolutionary research.

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