BIOINFORMATICS STARTER GUIDE

A BEGINNER'S COMPANION

DR NEELIMA CHITTURI

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Bioinformatics Starter Guide

From FASTQ to Biological Insight: Your First Steps into the World of Code and Biology

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Part 1: Welcome to the Digital Lab

What is Bioinformatics?

Bioinformatics is the exciting interdisciplinary field that develops methods and software tools for understanding biological data. In essence, it's the bridge between biology and code. It applies computer science, statistics, and mathematics to analyze and interpret the vast amounts of biological information generated by modern research techniques, particularly in genomics and proteomics.

Imagine you have a complex puzzle made of billions of tiny pieces. Biologists discover these pieces, but bioinformaticians build the framework and develop the algorithms to put them together, understand the patterns they form, and derive meaningful insights. It's about taking raw biological data – like the genetic code of an organism – and transforming it into actionable knowledge.

At Bruhaspathi, we empower you to navigate this bridge, providing both the conceptual understanding and the hands-on pipeline experience to go from raw data (like a FASTQ file) to a profound biological insight.

Why Now? The Data Explosion

We are living in an unprecedented era of biological data generation. Technologies like Next-Generation Sequencing (NGS) can sequence entire genomes in a matter of hours, producing terabytes of data. This massive influx of information has created an urgent need for specialists who can manage, process, and interpret it. Without bioinformatics, much of this invaluable data would remain an unreadable jumble of A's, T's, C's, and G's.

Bioinformatics is no longer a niche skill; it's a fundamental requirement for anyone working with modern biological research, drug discovery, personalized medicine, agriculture, and environmental science. It's the key to unlocking the secrets hidden within our DNA, RNA, and proteins.

What This Guide Covers

This guide is your simple checklist to start your journey into bioinformatics. It’s designed for beginners, focusing on the foundational skills that will enable you to confidently approach real-world biological data. Over the next 25 pages, we'll cover:

1.**UNIX Commands:** Learn the language of your computer's operating system, essential for handling large files and automating tasks.

2.**Python for Sequences:** Master a versatile programming language to manipulate and analyze biological sequences.

3.**Basic Statistics:** Understand how to interpret your findings and differentiate true biological signals from random noise.

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4.**Hands-On NGS Workflow:** Get a high-level overview and practical roadmap for processing real Next-Generation Sequencing data, from raw reads to biological insights.

By the end of this guide, you'll have a solid understanding of the core tools and concepts, and a clear path forward to becoming a proficient bioinformatician. Let's begin!

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Part 2: Your Essential Toolkit: The UNIX Command Line

Why the Command Line is Non-Negotiable

When dealing with gigabytes or even terabytes of biological data (think raw sequencing reads), clicking through graphical user interfaces (GUIs) becomes impractical, if not impossible. The UNIX command line (also known as the terminal, console, or shell) is your primary interface for interacting with these massive datasets and the powerful bioinformatics tools designed to process them.

It allows for:

Efficiency: Perform complex operations on many files with a single command.

Automation: Script repetitive tasks, saving immense amounts of time.

Power: Access highly optimized bioinformatics software that often only runs from the command line.

Remote Access: Work on powerful servers (e.g., cloud or institutional clusters) where GUIs are often unavailable.

This section will introduce you to the fundamental UNIX commands you'll use daily.

Your First Commands (The Checklist)

Let's get started with the essential commands you'll use to navigate and manipulate files.

ls (List): See what's in your directory.

ls: Lists contents of the current directory.

ls -l: Lists contents in a "long" format, showing details like permissions, owner, size, and modification date.

ls -a: Shows all files, including hidden ones (those starting with a .).

pwd (Print Working Directory): Know where you are.

pwd: Displays the absolute path of your current location in the file system. Always useful if you get lost!

cd (Change Directory): Move between directories.

cd [directory_name]: Moves into a specified directory.

cd ..: Moves up one directory level.

cd ~: Moves to your home directory.

cd -: Moves back to the previous directory you were in.

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mkdir (Make Directory): Create new project folders.

mkdir [new_directory_name]: Creates a new, empty directory.

rm (Remove): Delete files and directories.

rm [file_name]: Deletes a specific file. BE CAREFUL! Deleted files are usually gone forever.

rm -r [directory_name]: Deletes a directory and all its contents recursively. Use with extreme caution!

cp (Copy): Duplicate files and directories.

cp [source_file] [destination_file]: Copies a file.

cp -r [source_directory] [destination_directory]: Copies a directory and its contents.

mv (Move): Rename or relocate files and directories.

mv [source_file] [destination_file]: Moves a file. If the destination file has a different name, it effectively renames the file.

mv [source_directory] [destination_directory]: Moves a directory.

cat (Concatenate), less, head, tail: Safely inspecting huge data files.

cat [file_name]: Displays the entire content of a file to your screen. Avoid for very large files.

less [file_name]: Allows you to view a file page by page without loading the entire file into memory. (Press 'q' to quit). Essential for large bioinformatics files!

head -n 10 [file_name]: Displays the first 10 lines of a file. (Replace 10 with any number).

tail -n 10 [file_name]: Displays the last 10 lines of a file.

grep (Global Regular Expression Print): Finding specific sequences or terms.

grep "search_term" [file_name]: Searches for lines containing "search_term" in the specified file. Case-sensitive.

grep -i "search_term" [file_name]: Case-insensitive search.

grep -c "search_term" [file_name]: Counts the number of lines matching the search term.

wc -l (Word Count - Lines): Counting reads in a file.

wc -l [file_name]: Counts the number of lines in a file. This is often used for quick checks on file size or the number of sequences in a FASTA/FASTQ.

Your Superpower: The Pipe |

The pipe (|) is one of the most powerful features of the UNIX command line. It allows you to chain commands together, sending the output of one command as the input to the next. This enables complex data manipulations with simple, modular steps.

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*Example: To count the number of sequence headers in a FASTA file (which typically start with >), you could do: "*grep ">" my_sequences.fasta | wc -l*".* This command first finds all lines starting with > and then pipes that output to wc -l, which counts those lines.

Your Turn: Command Line Exercise

1.Open your terminal. (On Windows, you can use PowerShell or install Git Bash).

2.Navigate to your home directory: cd ~

3.Create a new project directory for this guide: mkdir bioinformatics_guide

4.Move into that directory: cd bioinformatics_guide

5.Create a dummy file: echo "SEQ1\nATGCATGC\nSEQ2\nGGCCTA\nSEQ1_variant\nATGCAAGC" > dummy_sequences.txt

6.List the contents of your directory: ls -l

7.View the first few lines of the file: head -n 3 dummy_sequences.txt

8.Count how many lines contain "SEQ1": grep "SEQ1" dummy_sequences.txt | wc -l

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Part 3: Python: Your Biological Swiss Army Knife

Why Python?

Python has become the lingua franca of bioinformatics for several compelling reasons:

Readability: Its clear syntax makes it easier to learn and understand, even for those without a computer science background.

Versatility: Python can be used for everything from simple scripting to complex data analysis, web applications, and machine learning.

Rich Ecosystem: A vast collection of libraries and modules, including powerful bioinformatics-specific tools like Biopython.

Community Support: A huge, active community means plenty of resources, tutorials, and help available.

While UNIX commands are great for file manipulation, Python allows you to dive into the content of those files, perform calculations, create custom parsers, and build sophisticated analysis pipelines.

Programming Fundamentals for Biologists (The Checklist)

Here are the core Python concepts you'll need for bioinformatics:

Variables & Data Types:

Variables: Store data using meaningful names (e.g., sequence = "ATGC").

Strings (str): Used for DNA/RNA/protein sequences, file names, etc.

```
my_sequence = "AGCT"

    len(my_sequence)# Get length

    my_sequence[0] # Get first character ('A')

    my_sequence[1:3]# Get a slice ('GC')
```

Integers (int) and Floats (float): For numbers, counts, percentages, etc.

```
read_count = 1000000

gc_content = 0.45
```

Lists (list): Ordered collections of items (e.g., a list of gene names, a list of read lengths).

```
gene_names = ["BRCA1", "TP53", "APC"]

    gene_names.append("CDH1")
```

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Dictionaries (dict): Key-value pairs, great for storing annotations or mapping (e.g., gene ID to gene name).

```
gene_annotations = {"BRCA1": "Breast Cancer Type 1 Susceptibility Protein", "TP53": "Tumor Protein P53"}

    gene_annotations["BRCA1"] # Access value
```

Reading and Writing Files:

Bioinformatics is all about file I/O. You'll constantly read data from input files and write your analysis results to output files.

Reading a file

```
with open("sequences.txt", "r") as infile:

for line in infile:

 print(line.strip()) # .strip() removes newline characters
```

Writing to a file

```
with open("output.txt", "w") as outfile:

outfile.write("My analysis results here!\n")
```

Loops (for loops): Iterating over sequences or data entries.

Crucial for processing each read in a FASTQ file or each gene in a list.

```
 dna_sequences = ["ATGC", "GCTA", "TTAA"]

 for seq in dna_sequences:

        print(f"Sequence: {seq}, Length: {len(seq)}")
```

Conditionals (if/else): Making decisions based on data.

Filtering reads based on quality, identifying sequences above a certain length, etc.

```
read_quality = 35

if read_quality >= 30:

print("High quality read.")

else:

print("Lower quality read.")
```

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Introduction to Biopython

While you can write custom code to parse FASTA or FASTQ files, Biopython is a robust and widely used library that makes these tasks much easier. It provides objects and functions for common bioinformatics operations.

Key features you'll use:

SeqIO module: *For parsing sequence files (FASTA, FASTQ, GenBank, etc.). It reads sequences as* SeqRecord objects, which conveniently store the sequence, ID, description, and quality scores.

Seq objects: Represents biological sequences with useful methods like complement, reverse_complement, and translate.

Example of using Biopython to read a FASTA file:

```
from Bio import SeqIO

# Assuming you have a file named 'example.fasta' with the content given below

# >Seq1

# ATGCATGC

# >Seq2

# GGCCTTAA


for record in SeqIO.parse("example.fasta", "fasta"):

 print(f"ID: {record.id}")

 print(f"Sequence: {record.seq}")

 print(f"Length: {len(record.seq)}")

 print(f"Reverse Complement: {record.seq.reverse_complement()}")

 print("-"20)
```

Your Turn: Python Exercise

1.Create a file named dna_data.fasta in your bioinformatics_guide directory with the following content:

```
>GeneA

ATGCGTAATGCGCGTTAGCATGC

>GeneB
```

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GGCTACGTACGTAGCTAGCTACGTACGT

>GeneC

TGCATGCATGCATGC

2.Open a Python interpreter or create a new file named gc_calculator.py:

3.Write a Python script that reads dna_data.fasta using Biopython's SeqIO and calculates the GC content for each sequence.

GC content = (Number of G's + Number of C's) / Total length of sequence

Print the sequence ID and its GC content percentage.

(Hint: You can count characters in a string like sequence_string.count('G'))

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Part 4: Basic Statistics: Finding the Signal in the Noise

Why Statistics is Crucial in Biology

Biology is inherently messy and variable. Experiments are subject to biological variation, measurement error, and environmental factors. This means that observed differences or patterns in biological data aren't always real; they could just be due to chance.

This is where statistics comes in. Bioinformatics heavily relies on statistical methods to:

Quantify Uncertainty: How confident are we in our findings?

Identify Significance: Is an observed difference between two groups likely real or just random?

Model Biological Processes: Develop mathematical descriptions of biological phenomena.

Filter Meaningful Information: Distinguish true biological signals from experimental noise.

Without a basic understanding of statistics, you risk misinterpreting your results, drawing incorrect conclusions, and ultimately undermining your biological insights.

Core Concepts for Beginners (The Checklist)

Let's demystify some fundamental statistical concepts:

Descriptive Statistics: Summarizing your data.

Mean: The average value. Useful for getting a central tendency.

Median: The middle value when data is ordered. Less sensitive to outliers than the mean.

Mode: The most frequent value.

Standard Deviation: A measure of how spread out your data is from the mean. A small standard deviation means data points are close to the mean; a large one means they are more spread out.

Example: If you have a list of read lengths from a sequencing run, you'd want to know the mean length, the spread (standard deviation), and potentially the median to see if there are very short or long outliers affecting the average.

Basic Probability & P-values:

Probability: The likelihood of an event occurring.

Hypothesis Testing: The process of making inferences about populations based on sample data. You usually start with a null hypothesis (e.g., "There is no difference between group A and group B") and try to find evidence against it.

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p-value: This is one of the most misunderstood concepts! A p-value is the probability of observing data as extreme as (or more extreme than) what you measured, assuming the null hypothesis is true.

A low p-value (e.g., < 0.05) suggests that your observed data would be very unlikely if the null hypothesis were true, leading you to reject the null hypothesis and conclude there is a statistically significant difference/relationship.

A high p-value (e.g., > 0.05) suggests your data is consistent with the null hypothesis; you fail to reject the null hypothesis (you don't "accept" it, you just don't have enough evidence to say it's false).

The Importance of Data Visualization:

"A picture is worth a thousand numbers." Before doing any complex statistics, always plot your data!

Histograms: Show the distribution of a single variable (e.g., distribution of gene expression levels).

Box Plots: Compare the distribution of a variable across different groups (e.g., gene expression in diseased vs. healthy tissue).

Scatter Plots: Show relationships between two variables (e.g., correlation between two genes' expression).

Tools like matplotlib and seaborn in Python, or ggplot2 in R, are excellent for creating informative biological plots.

Your Turn: Statistics Thought Experiment

Imagine you've performed a sequencing experiment to compare gene expression in two different cell lines (Cell Line A and Cell Line B). You've quantified the expression level of a specific gene, "GeneX," in 10 samples from each cell line.

Cell Line A (GeneX expression): 10, 12, 11, 13, 10, 12, 11, 10, 13, 12

Cell Line B (GeneX expression): 25, 27, 26, 28, 25, 27, 26, 25, 28, 27

1.What descriptive statistics would you calculate for each cell line's GeneX expression? (e.g., mean, median, standard deviation). How do these immediately inform your initial impression?

2.Formulate a null hypothesis and an alternative hypothesis for comparing GeneX expression between Cell Line A and Cell Line B.

3.Without doing any calculations, what do you expect a statistical test (like a t-test) would tell you about the p-value in this scenario? Why?

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4.If you were to visualize this data, what type of plot would be most appropriate to clearly show the difference between the two cell lines?

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Part 5: The Main Quest: A Hands-On NGS Workflow

This is where all the foundational skills come together! Next-Generation Sequencing (NGS) has revolutionized biology, allowing us to read millions of DNA or RNA fragments simultaneously. But raw NGS data (often in FASTQ format) is messy and needs a standardized pipeline to extract meaningful biological insights.

Here's a generalized workflow, often seen in genomic or transcriptomic studies, from raw reads to biological interpretation. Think of this as your "infographic checklist" for a typical project.

Step 1: The Raw Data (FASTQ)

What it is: FASTQ is the standard file format for storing raw sequencing reads. Each read consists of four lines:

1.Sequence Identifier: Unique ID for the read.

2.DNA Sequence: The actual nucleotide sequence (e.g., ATGC...).

3.Plus Line: A separator, usually just a +.

4.Quality Scores: A string of ASCII characters encoding the base call quality for each nucleotide in the sequence. Higher ASCII values mean higher quality (less likely to be an error).

Why it matters: This is your starting point. You'll download these files (often gzipped for compression, ending in .fastq.gz) from public repositories like NCBI's SRA (Sequence Read Archive) or receive them directly from a sequencing facility.

Step 2: Quality Control (QC)

Purpose: Raw sequencing reads are never perfect. They contain errors, adapter contamination, and low-quality bases, especially at the ends. QC assesses the overall quality of your raw data.

Common Tool: FastQC is the go-to tool. It generates HTML reports with plots and statistics for various quality metrics, including:

Per-base quality scores (how confident the sequencer was at each position).

GC content distribution.

Adapter contamination.

Sequence length distribution.

Insight: FastQC helps you identify potential problems early on, guiding subsequent processing steps. For example, if you see a sharp drop in quality at the 3' end of reads, you'll know to trim them.

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Step 3: Trimming & Filtering

Purpose: Based on the QC report, you'll remove low-quality bases, adapter sequences (short DNA fragments added during library preparation), and very short reads. This cleans up your data, ensuring that downstream analyses are based on reliable information.

Common Tools:

Trimmomatic: A popular tool for removing adapter sequences and trimming low-quality bases.

fastp: A fast, all-in-one preprocessor for FASTQ files.

Insight: This step improves the accuracy of subsequent alignment and variant calling. High-quality input leads to high-quality output!

Step 4: Alignment to a Reference Genome

Purpose: After cleaning the reads, the next step is to map (align) them to a known reference genome (e.g., the human genome, a model organism's genome). This tells you where each read originated from.

Common Tools:

BWA (Burrows-Wheeler Aligner): Widely used for aligning short-read sequences.

Bowtie2: Another popular aligner, often faster for shorter reads.

STAR: Specialized for RNA-seq reads (aligning to a genome with consideration for splicing).

Output File Format: Aligned reads are typically stored in SAM (Sequence Alignment/Map) or its compressed binary version, BAM (Binary Alignment/Map) format. BAM files are often sorted and indexed for efficient access.

Step 5: Variant Calling

Purpose: Once reads are aligned, you can identify genetic variations (differences from the reference genome) in your sample. This is where you find SNPs (Single Nucleotide Polymorphisms), insertions, and deletions.

Common Tools:

GATK (Genome Analysis Toolkit): A comprehensive suite of tools from the Broad Institute, widely considered the gold standard for variant discovery.

bcftools: A powerful set of utilities for variant calling and manipulation of VCF files.

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Output File Format: Variants are typically stored in VCF (Variant Call Format) files. A VCF file lists each identified variant, its position, reference allele, alternative allele, quality scores, and other relevant information.

Step 6: Annotation & Interpretation

Purpose: A raw list of variants (VCF file) isn't biologically meaningful on its own. This final step involves annotating the variants (determining if they fall within genes, affect protein coding, etc.) and interpreting their potential biological consequences.

Common Tools/Databases:

VEP (Variant Effect Predictor): From Ensembl, predicts the effect of variants (e.g., missense, silent, frameshift).

SnpEff/SnpEff: Similar variant annotation tools.

dbSNP, gnomAD, ClinVar: Databases to check if your identified variants are known, their frequencies in populations, and their clinical significance.

Insight: This is the crucial step where you translate raw data into biological insight. For example, identifying a novel missense variant in a disease-associated gene could lead to further research. This step takes you from FASTQ to biological insight!

Your Turn: NGS Workflow Roadmap

Imagine your goal is to find novel SNPs in a newly sequenced bacterial genome.

1.Where would you typically obtain the raw FASTQ files?

2.What would be the very first tool you'd run on these FASTQ files? What kind of issues would you be looking for?

3.After cleaning the reads, what's the next major step, and what type of file would it generate?

4.If you find a list of SNPs, what would be the key information you'd want to know about each SNP to understand its potential impact? (Think about gene location, functional change, etc.)

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Part 6: Your Learning Roadmap: Next Steps

Congratulations! You've completed your first comprehensive overview of bioinformatics essentials. This guide has provided you with the fundamental concepts and tools, from navigating the command line to understanding a full NGS workflow.

This is just the beginning of your exciting journey. Here are some pathways you can explore to deepen your knowledge and skills:

Dive Deeper into Python: Explore advanced Python topics like functions, classes, and more complex data structures. Learn about other powerful libraries for data analysis and visualization such as pandas, numpy, and matplotlib/seaborn.

Master R for Statistical Analysis and Visualization: R is another dominant programming language in bioinformatics, particularly strong in statistics and creating publication-quality plots. Learn ggplot2 for stunning visualizations.

Explore Specific NGS Applications: This guide focused on a general genomic workflow. Research and learn about specialized pipelines for:

RNA-seq: Analyzing gene expression levels.

ChIP-seq: Identifying protein-DNA binding sites.

Metagenomics: Studying microbial communities.

Single-cell RNA-seq: Unraveling cell type heterogeneity.

Learn About Cloud Computing: As data grows, so does the need for scalable computing resources. Familiarize yourself with platforms like AWS, Google Cloud, or Azure, and how bioinformatics pipelines are deployed there.

Version Control with Git: Essential for collaborative coding and tracking changes in your scripts.

Engage with the Community: Join bioinformatics forums, attend webinars, follow experts on social media, and read relevant scientific papers.

Remember, bioinformatics is a rapidly evolving field. Continuous learning is key to staying current and making significant contributions. Keep practicing, keep building, and never stop being curious!

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Part 7: About Bruhaspathi

At Bruhaspathi, we believe in making bioinformatics accessible and actionable. We are passionate about bridging the gap between cutting-edge biological research and powerful computational tools.

Our mission is to equip the next generation of scientists, researchers, and innovators with the skills needed to tackle the biggest challenges in genomics, health, and biotechnology. We go beyond theoretical concepts, providing hands-on, project-based training that mirrors real-world bioinformatics pipelines.

Whether you're a biologist looking to enhance your computational skills, a programmer curious about biological data, or a student charting your career path, Bruhaspathi offers tailored courses and resources to help you succeed.

Ready to transform raw data into profound biological insights?

This guide is just your first step. Explore our hands-on, project-based courses at and take your bioinformatics skills to the next level.

#Bruhaspathi #Bioinformatics #NGS #LearnToCode

Our activities

Internships / Projects / Commercial collaborations

Covers all domains of bioinformatics

Short term academics

Basics of Molecular Biology

Programming: Linux, Shell Scripting, Python

Biological Databases

Sequence Alignment & Analysis

Genomics: Variant Identification, DNA Methylation Analysis, Genome Annotation

Transcriptomics: Differential Gene Expression, miRNA Profiling

Proteomics: Protein Identification & Quantification

Metagenomics: 16S rRNA, Shotgun, Antimicrobial Resistance

Mid-term learning

Comprehensive Bioinformatics Training Program (6 Months)

Long-term learning

PG diploma in bioinformatics program 1 year starting June 2026

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We have reached the end of this book, but the journey of learning continues—stay tuned for more updates

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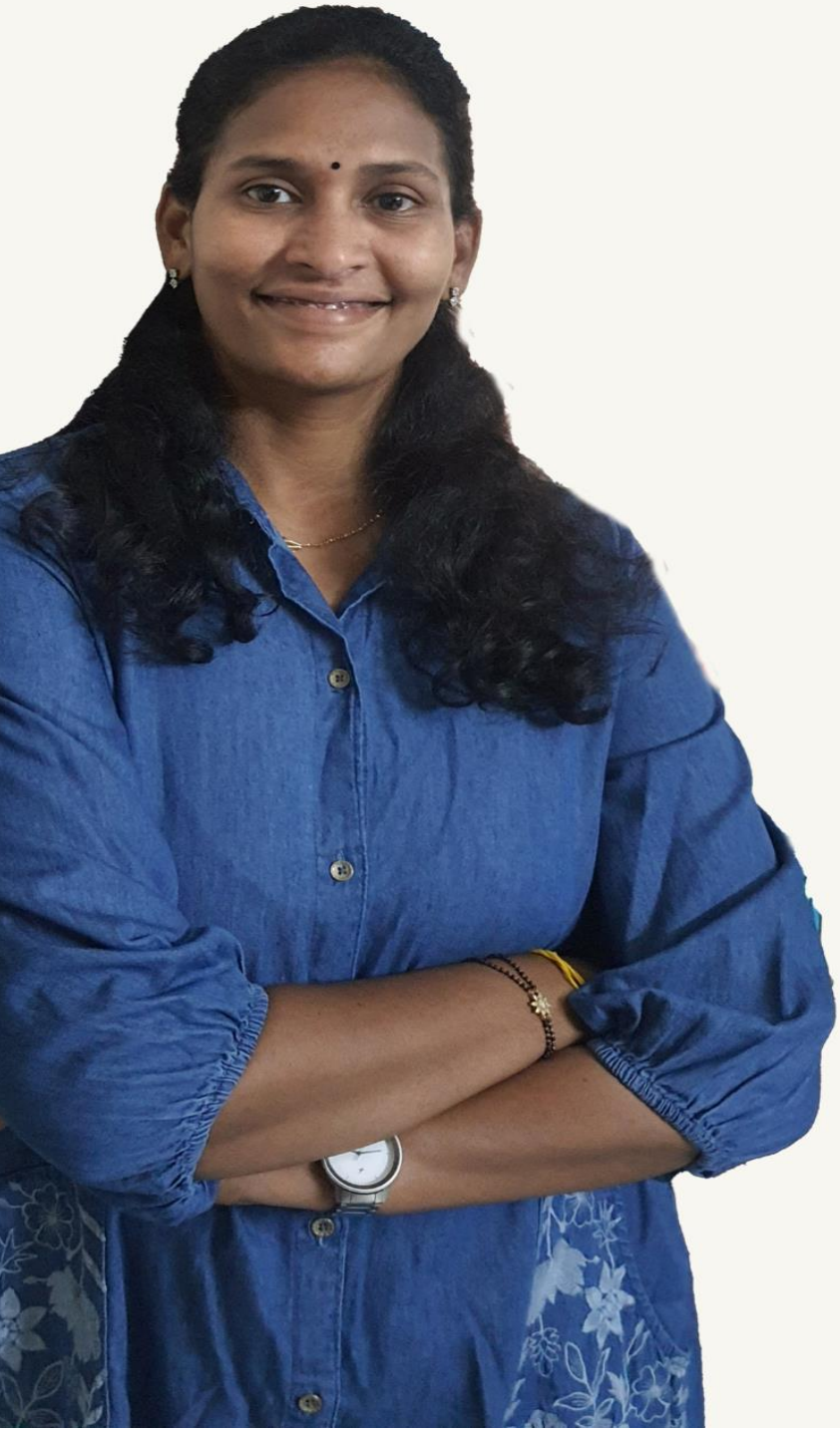
ABOUT THE AUTHOR

DRIVEN BY HER PASSION FOR TEACHING AND RESEARCH, DR. NEELIMA CHITTURI FOUNDED THE BRUHASPATHI INSTITUTE OF BIOSCIENCES. A GOLD MEDALIST FROM THE UNIVERSITY OF MADRAS, SHE BRINGS OVER 15 YEARS OF EXPERIENCE IN MOLECULAR BIOLOGY AND RELATED FIELDS. HER CAREER INCLUDES 8 YEARS DEDICATED TO RESEARCH, INCLUDING A PH.D. IN TRANSCRIPTOMICS, AND 8 YEARS IN INDUSTRY WORK. HER EXPERTISE ENCOMPASSES DEVELOPING ANALYTICAL METHODS, UTILIZING BIOINFORMATICS TOOLS, AND TRANSFORMING COMPLEX BIOLOGICAL DATA INTO VALUABLE RESEARCH AND CLINICAL OUTCOMES. COMMITTED TO BRIDGING THEORY AND PRACTICAL TRAINING, SHE ESTABLISHED THE INSTITUTE IN 2025 TO HELP STUDENTS AND PROFESSIONALS INTEGRATE BIOLOGY WITH COMPUTER SCIENCE.



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