EXPLORING THE ROLE OF LONG NON-CODING RNA IN MEDITATION

A THESIS SUBMITTED TO

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

FOR THE PARTIAL FULLFILLEMENT OF THE AWARD OF THE DEGREE OF

M.Sc. LIFE SCIENCES (AYURVEDA BIOLOGY)

BY

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UNDER THE GUIDANCE OF

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INDIAN INSTITUTE OF TECHNOLOGY, JODHPUR

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THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

Private University Established in Karnataka by ACT 35 of 2013

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DECLARATION BY THE CANDIDATE

I declare that this thesis "**Exploring the Role of long non-coding RNA in Meditation**" submitted for the award of Master of Science to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Prof. Mitali Mukerji (and co-supervision of Dr.Megha.). I confirm that no part of the work reported herein has been submitted for a degree or examination at any other university. References, funding and material obtained from other sources have been duly acknowledged, and no part of this dissertation has been plagiarised.

Place: IIT Jodhpur

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CERTIFICATE FROM THESIS SUPERVISOR/S

This is to certify that the work incorporated in this thesis **“Exploring the role of long non-coding RNA”** submitted by Souravh Thakur was carried out under my/our supervision. No part of this thesis has been submitted for a degree or examination at any other university. References, help, and material obtained from other sources have been duly acknowledged. I confirm the originality of the work and that there is no plagiarism in any part of the thesis.

Name, Designation	Role	Signature, Date
Prof. Mitali Mukerji Head of the Department of Bioscience and Bioengineering, IIT Jodhpur.	Guide	Mitali Mukerji 3rd July 2022

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Summary

Studies conducted on meditation and Mind-Body intervention have demonstrated that continuous engagement in then practice have led to alteration in gene expression profile, especially in those genes that are involved in stress response, immune function, and inflammation.

Long non-coding RNA once considered as transcriptional noise, have been discovered to be involved in a number of distinct biological activities, including as the processes of development, differentiation, and illness.

There has been very little direct study done to investigate the association between lncRNAs and meditation up to this point. However, meditation has been extensively studied for its effects on gene expression and epigenetic regulation, which could directly or indirectly be influenced lncRNA activity.

The work done in the thesis explain how Lnc-RNA can be regulated during the meditative state and how regulation of these Lnc can be involved in several cellular processes.

Personal Reflection

During my Dissertation project in IIT Jodhpur, I had the opportunity to immerse myself in a challenging and rewarding journey. Upon reflection, I believe that this period has been an important phase in my academic and personal development. It provided me with valuable experiences, lessons, and a sense of accomplishment.

One of the key aspects of this research was the opportunity to work independently during my dissertation. Initially, I felt a mix of excitement and nervousness about the scope and complexity of the project. However, as time progressed, I realized that the freedom to explore and develop deep into the subject matter allowed me to develop a strong sense of autonomy and self-motivation.

Moreover, this 8-month period allowed me to develop and refine essential research skills. I acquired proficiency in data collection, analysis, and interpretation. Engaging with relevant literature and synthesizing findings helped me sharpen my critical thinking abilities and enhanced my ability to critically evaluate research studies.

Overall, the 8-month period spent on this project was transformative. It instilled in me a deep sense of accomplishment and confidence in my abilities as a researcher.

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1. INTRODUCTION

Long noncoding RNAs (lncRNA) are untranslated transcripts of size greater than 200 nucleotides, that play crucial roles in gene regulation, chromatin structure, and epigenetic processes(Deniz & Erman, 2017)lncRNA was earlier considered the junk or transcriptional noise in the eukaryotic genome as the gene regulation was equitable to central dogma (DNA-mRNA-Protein), and the function of long noncoding RNA was still not known. The continuous advancement in transcriptomics technology has revealed the importance and potential of long non-coding RNA in several gene regulation and cellular mechanisms. Statistics from databases such as Human GENCODE describes that the human genome contains about 19,928 lncRNA genes but other estimates suggest that the number can be even higher(*"Frankish A, et Al (2018) GENCODE Reference Annotation for the Human and Mouse Genomes"*, n.d.; Uszczynska-Ratajczak et al., n.d.). Long non-coding RNA are divided into five categories as per their location relative to the associated coding genes, these are namely: sense, antisense, intergenic, intronic and bidirectional lncRNAs(Mercer et al., 2009).

Among the 85% of the transcribed genome, only 20% encodes for protein-coding genes and the rest are considered non-coding. Most of the lncRNA are transcribed by RNA polymerase II, hence the lncRNA shares a similar post-transcriptional fate as that of mRNAs such as capping, splicing, and polyadenylation(Guttman et al., 2009). Despite their functional role in several regulatory processes fewer than 1% of all known functional transcripts are experimentally curated as functional lnc(de Hoon et al., 2015).

Recent development in transcriptomics has elucidated the role of long noncoding RNA in medicine and healthcare research. They have been found to target several genes which are associated with diseases such as cancer(Zhao et al., 2023)(Pourghasem et al., 2022)(Cardoso Alves et al., 2021), immune regulation(Zhang et al., 2021), cardiovascular diseases(Poulet et al., 2020), metabolic diseases, and infectious diseases(Goyal et al., 2018; Lovell & Anguera, 2022). Long noncoding RNA also plays a role in plant immunity (Huang et al., 2023). Despite recent advancements in the field of Long non coding RNA discovery, very little is known about their role in therapeutics, disease prognosis and medicine.

Long non-coding RNA has been found to exhibit high abundance within a distinct subset of sequence elements known as transposable elements, as per empirical observations. An increasing quantity of Alu-rich long non-coding RNA (LncRNA) have been experimentally characterised, demonstrating their ability to function as binding domains for DNA, RNA, or proteins within the LncRNA(Gong & Maquat, 2011).

FANTOM (Functional Annotation of the Mammalian Genome) is an international consortium establish in 2000, aiming to identify all functional elements of human genome. The recent edition of the Fantom the 6th edition is to elucidate the function of long non-coding RNA (lncRNAs) in the human genome.

The practice of modern medicine is slowly approaching towards more holistic and individualised approach through integration with traditional knowledge based medicinal systems (Ayurveda, Yoga, traditional Chinese medicine). A framework of "Integrative medicine" where multiple systems could cross-talk and complement to treat a person most effectively is being developed. Several studies have shown the effectiveness of Integrative medicine in the management of several diseases such as respiratory conditions(Yeh & Horwitz, 2017), neurological conditions(Rowin, 2019; Wells et al., 2017; Zhou et al., 2017), metabolic diseases(Dossett et al., 2017),cardiovascular diseases(Aggarwal et al., 2017),cancer(Ben-Arye et al., 2023) and pain management(Deng, 2019). "Mindfulness meditation" that includes practices of Yoga and meditation as a non-pharmacological intervention is being adopted in different parts of the world for management of many disease conditions. Several literature-based evidence has shown that this type of intervention can alleviate stress-dependent symptoms in several disease such as cancer, neurodegenerative disease, cardiovascular disease. The effects of these interventions have mostly been seen through physiological measurements and in the recent times genome-wide expression changes have also been reported. The beneficial effects of meditation have primarily been seen through modulation of inflammation and immune axis.

The role of Long non coding RNA in such traditional practices is still not clearly understood. Since lncRNA have been identified as biomarkers in a number of diseases, differential expression of these during the course of mindful meditation might not only provide objective basis of assessment of beneficial effect of these practices but also provide insights on the molecular mechanism of action. To gain insights on this, this study was carried out to understand the potential involvement of Long non coding RNA from mining and meta-analysis of omics data on "Mindfulness meditation(Koka et al., 2021)". This study will focus on the functional role of differentially expressed long non coding RNA present in meditation data with the help of some *in-silico* techniques.

The research question is to explore "Whether lncRNAs could have a potential involvement in the cellular processes that mediate the effects of meditation?"

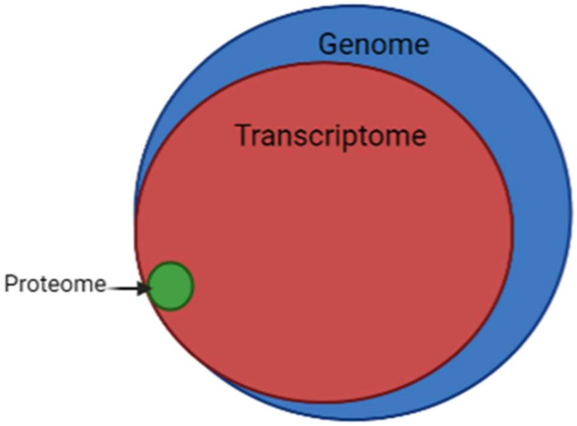


Fig1: A large proportion of genome(blue) into RNA(Red) but only few percentages are translated into Protein (Green).

2. Literature Review

2.1 Long-noncoding RNA

In recent years, the study on long non-coding RNAs (lncRNA) has exposed the role of long non-coding RNA in gene regulation and cellular processes which had been neglected and considered as a "transcriptomic noise" throughout the transcriptomic era as they are often expressed at lower levels and are tissue specific. Recent studies on long non-coding RNA have shown that long non-coding RNA plays a very important role in several metabolic processes, diseases, immune regulation etc.

In 1980s, researchers focused on tissue specific and temporal pattern of gene expression using different differential hybridisation screen of cDNA libraries. The main focus was on the protein coding genes, an alternative approach was adopted by the researchers without taking into account the protein coding potential of RNA, resulting in the discovery of the first long non-coding RNA H19.

X-Chromosome inactivation

LncRNA XIST is one of the most extensively studied lnc RNA that plays a key role in inactivation of one of the X-chromosomes in females that is essential to compensate for gene dosage imbalance. XIST spans17 kilobases (kb), and contains several tandem repeats, the repeats present in 5`end are evolutionary conserved and are important for Xist functioning(Brown et al., 1992). Xist is expressed from the inactive X-chromosome and leads to transcriptional silencing. Xist acts as a scaffold for the chromatin modifiers such as Polycomb repressive complex 2 (PRC2), causing methylation at histone H3 lysine27 residue leading to stable and heritable silencing of genes at X-chromosome(Wutz et al., 2002).

The role of lncRNA Xist is one of the examples of how lncRNA plays a critical role in lncRNA in gene regulation.

WRAP53

P53 gene is a tumour suppressor gene that plays a critical role in development of cancer(Reedy et al., 2001). WRAP53α is an antisense transcript that regulates the expression of p53 that is encoded by WRAP53 gene. WRAP53 gene overlaps the p53 gene in as head-to-head orientation. Knockout studies of WRAP53α shows decrease in p53 RNA levels(Mahmoudi et al., 2016).

2.1.1 ORIGIN OF LNC RNA

Non coding genes can rise through several mechanism such as duplication of the genomic sequence, mutation in the protein coding gene causing loss of protein coding potential and transposable elements. Homologous non-coding genes arise through duplications of previously existing lncRNA genes.

Gene loss due to mutation can be seen in one the most studied lncRNA Xist. Xist gene is derived from ancestral lnx3gene. During the evolution of placental mammals, there was a frame shift mutation in the primordial Lnx3 gene, that resulted in loss of its protein coding ability leading to lncRNA Xist(Duret et al., 2006).

2.1.2 CODING POTENTIAL OF LNC RNA

Long non-coding sequences, as their name suggests, do not include any protein coding potential, which means that they are unable to be translated into functional protein. There are some exceptions to given statement as some long-coding RNA have seen so have open reading frame and give rise to polypeptides, but in majority of the cases such translation does not code for functional and stable polypeptides. Lnc DWORF is an exception to such cases. Lnc DWORF is a muscle specific long non-coding RNA that encodes a 34 amino acid functional polypeptide. The 34 amino acid enhances the uptake of calcium (Ca2+) by the sarcoplasmic reticulum (SR) within myocytes through the displacement of inhibitory peptide from SERCA (sarco/endoplasmic reticulum Ca2+ -ATPase).

2.1.3 Subcellular Localization of lncRNAs

The study of lncRNA function is still at its early stage. The subcellular localisation of lnc RNA is an essential factor in finding their specific function. LncRNAs have been found to engage in a wide variety of regulatory functions, according to their subcellular localization. LncRNA that are localised in the nucleus are mostly involved with epigenetic regulation, while the cytoplasmic LncRNA are involved with post-transcriptional processes. One lnc RNA can act through different mechanisms depending on their subcellular localisation. For example, the antisense lncRNA called PYCARD-AS1 supresses the expression of proapoptotic gene PYCARD by acting as a scaffold for DNA methylation enzyme such as DNMT1 and G9a, recruiting them to promotor region of PYCARD gene facilitating DNA methylation. While in the cytoplasm lncRNA PYCARD-AS1 interacts to the m-RNA of the PYCARD gene inhibiting its expression(Miao et al., 2019).

2.1.4 Lnc and Alu Content

Many experimental evidences have shown how transposable elements sequence acts as a functional domain of long non-coding RNA and can act as binding domain for DNA, RNA and protein.

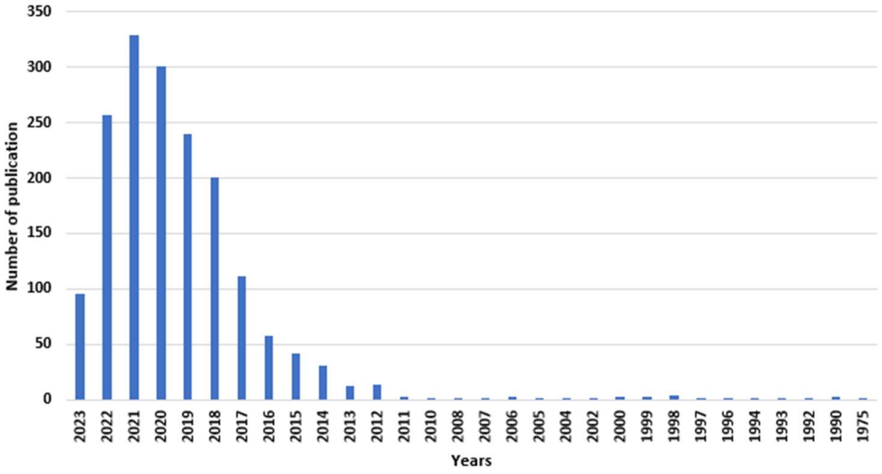


Figure2: The increase in the number of studies related to Long-noncoding RNA published from 1975 to 2023 (data obtained by searching PubMed database).

2.2 Transcriptome Profiling

Each cell in the organism contains same set of genes that are inherited from the parents, but the gene expression patterns differ within the cell types and across the cell type depending on the stage of the cell cycle and the chemical cues. In the cell, some of the genes are very sensitive and are continuously regulated while others can remain dormant for the entire life cycle of the cell. Transcription is the first step in the central dogma where DNA is converted into RNA. Transcription refers to the copying of a part of DNA (i.e., a gene) into a single stranded chain of nucleic acids, also known as RNA.

Transcriptome refers to entire set of RNA transcripts produced by the genome present in the cell. Transcriptome include not only the coding region the mRNA but also includes the non-coding part such as Long non-coding RNA, microRNA, transfer RNA, ribosomal RNA and ribozymes.

Transcriptome profiling refers to the analysis of the entire RNA transcript present in the cell. It helps us to capture the snapshot of complete transcriptome at a given point of time. Genome wide expression can be assessed using microarrays as well as next generation sequencing technologies (RNA-seq). Transcriptome profiling techniques such as RNA sequencing enables identification and quantification of different RNA transcripts and provide valuable information about gene expression.

Measuring the gene expression using the mRNA has some primary restriction since it is presumed that if the gene is transcribed, it will also be translated and there is a strong correlation between mRNA and protein synthesis but there are many other mechanisms controlling the translation of mRNA. Micro RNA (miRNA) is one of the example, miRNA is non-coding component of RNA that may cause post transcriptional alteration gene expression(Iorio et al., 2005).

2.2.1 DNA Microarray:

DNA microarray technology, which is often referred to as gene chip technology, is a sophisticated instrument that is used to investigate genome-wide patterns of gene expression as well as genetic variants. Researchers are able to do a single experiment that concurrently measures the expression levels of thousands or millions of genes thanks to this technology.

DNA microarrays consist of a solid support (usually a glass slide or silicon chip) on which thousands of short DNA sequences, called probes, are immobilized. These probes are designed to be complementary to specific genes or segments of genes. The microarray is then exposed to labelled DNA or RNA samples, and the binding of the labelled targets to the probes is detected and quantified.

2.2.2 RNA-SEQ:

RNA sequencing, also known as RNA-seq, is a high-throughput sequencing technology that is used to investigate the transcriptome. The transcriptome is the whole collection of RNA molecules that are present in a given cell or tissue at a certain point in time. RNA sequencing has brought about a major shift in the field of gene expression analysis and has emerged as an essential component of genomics research.

The fundamental concept that underlies RNA-seq is that the abundance of a particular RNA molecule in a sample is correlated with the number of sequencings reads that correspond to it. Through the process of quantifying the reads that correspond to distinct genes or transcripts, scholars can ascertain the levels of expression of individual genes, make comparisons of gene expression across various samples, detect genes that are differentially expressed, and acquire a deeper understanding of the complexity of the transcriptome.

2.3 Meditation

Meditation and mind-body intervention has been grown in popularity and acceptance in the health sector, in the past decade. These interventions have been shown to have various psychological benefits among healthy and clinical populations such as reduction of stress(Chiesa & Serretti, 2009), decreases pain and anxiety(Strauss et al., 2014). However underlying molecular mechanisms for the relatively well studied clinically and physiological effects is still lacking.

The very first study done to know the role of mind-body intervention on the gene expression profile was carried out in 2005 on six long-term Falun Gong Qigong practitioners and six healthy controls. In this study the gene expression profile was done on neutrophils using the microarray of 12,000 genes. It was observed that Qigong practitioners had significant number of genes regulated compared to the control which were involved in some common pathways.

It was observed that Qigong practitioners have enhanced expression of genes involved in immune functions and downregulation of genes involved in apoptosis(Li et al., 2005).

In 2008 a similar kind of study was done on the practitioners of Sudarshan Kriya. Practitioner of Sudarshan Kriya were compared to controls but only few numbers of genes were analysed. The hypothesis made here was that regular Sudarshan Kriya practice improves stress regulation, which should be reflected in Gene expression. The reverse transcriptase–polymerase chain reaction was used to analyse the level of gene expression in white blood cells that had been separated from freshly drawn blood. The gene analysed were related to oxidative stress, DNA damage, cell cycle control, aging and cell death. The results suggested that Sudarshan Kriya may enhances the immune system by upregulating the genes that inhibit cell death(Sharma et al., 2008).

A study from 2012 showed how mind-body intervention can show reversal of gene expression pattern in older adults. After the intervention the gene expression profile showed the downregulation of proinflammatory gene expression pattern which was upregulated before the intervention.

Recently a large-scale genome analysis has been carried out on more than 106 subjects in 2018 who participated in an 8 days advanced inner-engineering retreat program. The blood was drawn from the practitioners at 4 different time points. The T1 samples were collected 5 to 8 weeks before the retreat, T2 samples were collected on the day of retreat before starting the meditation method, T3 samples were collected immediately after the retreat, and T4 samples were collected 3 months after the retreat.

Differential Gene Expression analysis was done by pair-wise comparison of all 6 permutation. Differential gene expression analysis identified 1,649 genes differentially expressed in total. Out of identified 1,649 differentially expressed genes,719 were seen to be differentially expressed in T3(44%), followed by 496 differentially expressed at T4(30%). The top 10 differentially expressed genes at T3 were directly related to immune response including viral defence function. While the differentially expressed genes in T4 were involved in catalytic activity, localized to the mitochondrial membrane and translation elongation factor activity. These findings helped us to understand that meditation have immediate effect on immune cells and genes which are transient in nature followed by dynamically altered effect(Chandran et al., 021).

3. Work plan

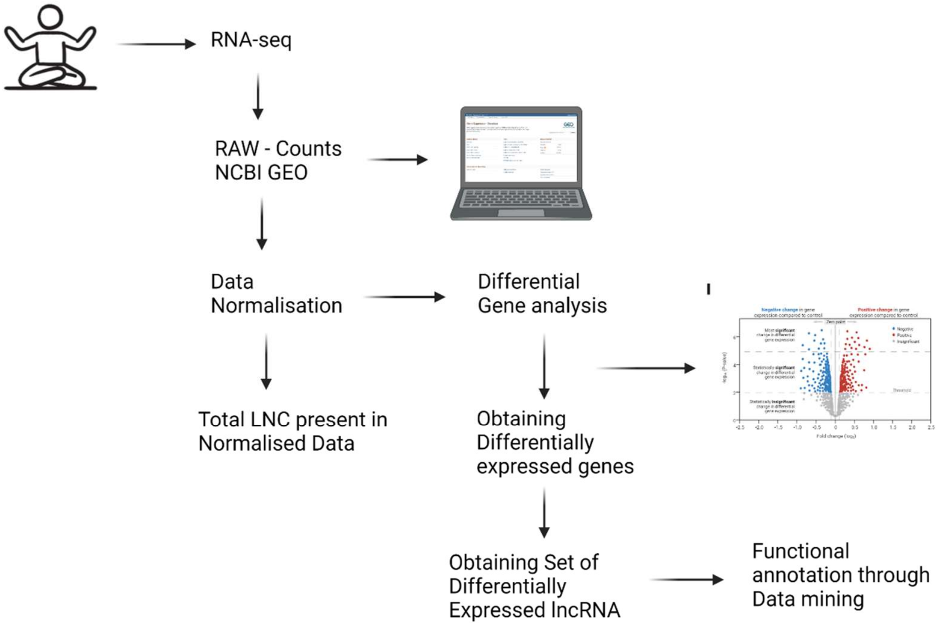


Figure3: Schematic workflow of RNA-seq data of Meditation and annotation of lncRNA

4. Material Method

4.1 Raw Data for Deseq2 Analysis

The raw data for the Deseq2 gene expression was obtained from the paper "Large-scale genomic study reveals robust activation of the immune system following advanced Inner Engineering meditation retreat". The RNA-seq data was submitted to NCBI-GEO under the accession number GSE174083. Data contained the transcriptome profile of 388 specimens obtained from 106 individuals at 4 different time points. (T1= 97, T2= 105, T3= 104, T4= 82; N= 388).

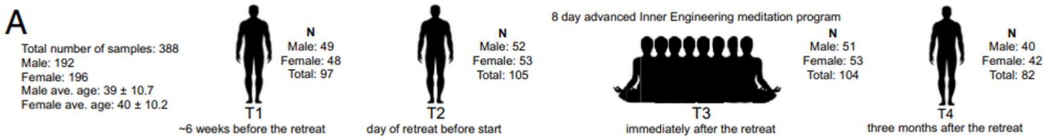
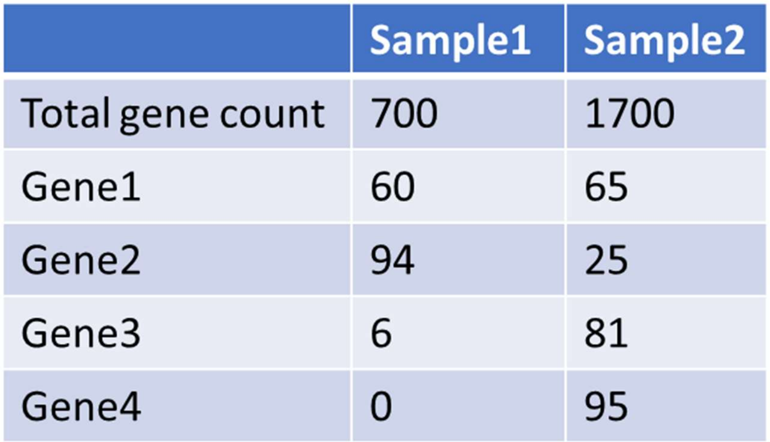


Figure4: Representing Data used for Transcriptome analysis (Figure according to Chandran et.al.)(Chandran et al., 2021).

i. Need of Data Normalisation

Prior to estimation of differentially expressed genes, there is a need for normalization of data. This ensures that the expression levels of the genes are adjusted for to account for variations in sequencing depth, library size, and other technical factors, allowing for more accurate and meaningful comparisons between samples. The following two aspects are taken into consideration.

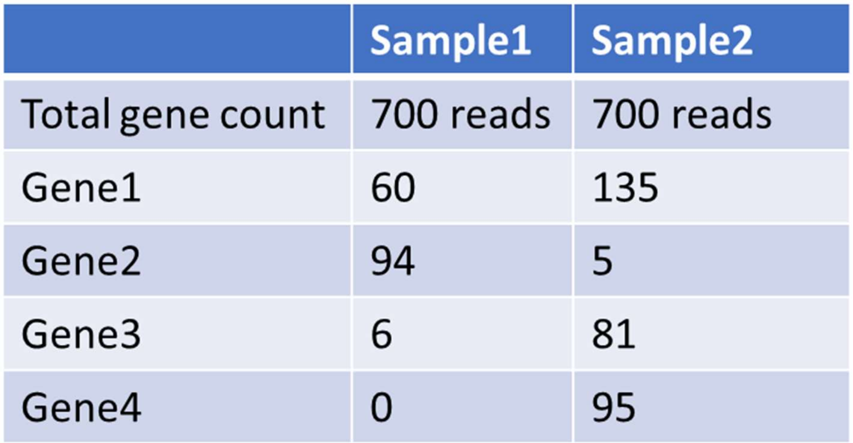
1. **Library Size:** the library size refers to the total number of sequenced reads or fragments obtained from the sample.



	Sample1	Sample2
Total gene count	700	1700
Gene1	60	65
Gene2	94	25
Gene3	6	81
Gene4	0	95

Figure5: Representing RNA-Seq Data with Difference in library size

2. **Library Composition:** Library composition refers to the relative abundance or composition of different RNA molecules within a sequencing library.



	Sample1	Sample2
Total gene count	700 reads	700 reads
Gene1	60	135
Gene2	94	5
Gene3	6	81
Gene4	0	95

Figure6: Representing RNA-seq data with difference in library composition

ii. DATA Normalisation by DESEQ2

DeSeq2 is a widely utilised package in R that is used to the of conducting differential gene expression analysis. The process of normalisation is an important step in the differential gene expression analysis of RNA-Seq data as it adjusts the variation in library size and library composition that may impact the measurement of gene expression. The normalisation process helps to mitigate the influence of the influence of sequencing depth on the gene count and accounts for potential biases. The DESeq2 approach employs the negative binomial distribution to establish a statistical model for count data and to compute estimations for the size factors that are important for normalisation.

Steps in De-Seq2 Data Normalisation in R

1. Counts Matrix: Counts matrix is obtained from the RNA seq DATA. Counts matrix contain raw counts of the transcripts in each sample.

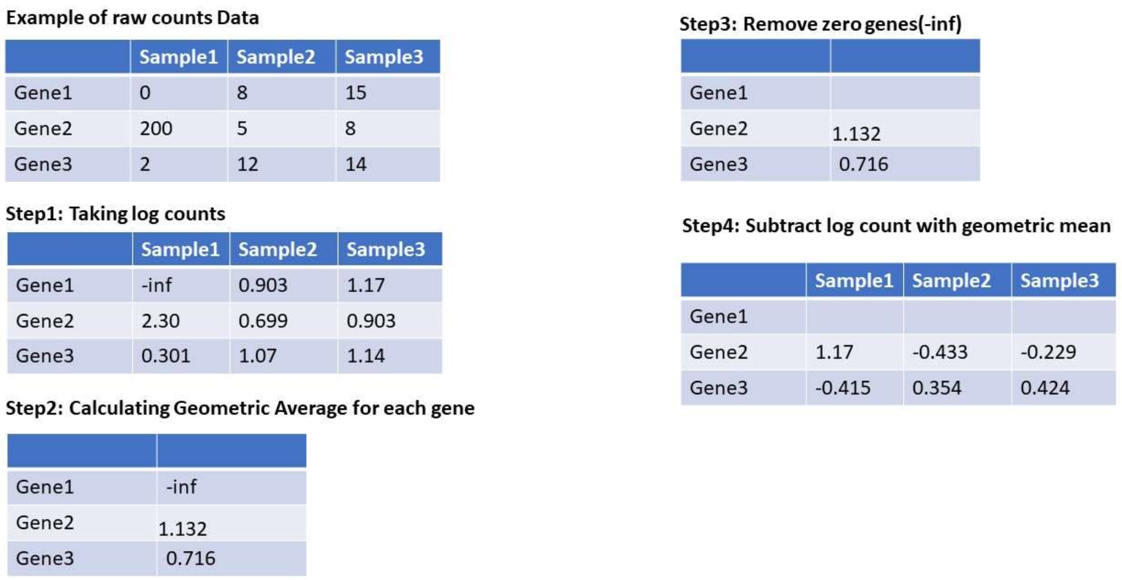
2. DeSeq data set: To create a DESeqDataSet object in R, one must utilise the raw count matrix along with relevant metadata related to each sample, such as experimental factors, treatment groups, or conditions.

3. Estimate size factors: The calculation of size factors based on library sizes can be performed by utilising the estimateSizeFactors function in DESeq2. These factors explain variations in sample-to-sample sequencing depth.

4. Normalize counts: Utilise the size factors acquired in the previous step to normalise the raw counts.

5. Differential expression analysis: DESeq2 conducts differential expression analysis by utilising statistical models, such as the negative binomial distribution, to identify genes that exhibit differential expression between groups or conditions after normalising the counts.

iii. BASIC ALGORITHM FOR DE-Seq2 Data normalisation



Example of raw counts Data

	Sample1	Sample2	Sample3
Gene1	0	8	15
Gene2	200	5	8
Gene3	2	12	14

Step1: Taking log counts

	Sample1	Sample2	Sample3
Gene1	-inf	0.903	1.17
Gene2	2.30	0.699	0.903
Gene3	0.301	1.07	1.14

Step2: Calculating Geometric Average for each gene

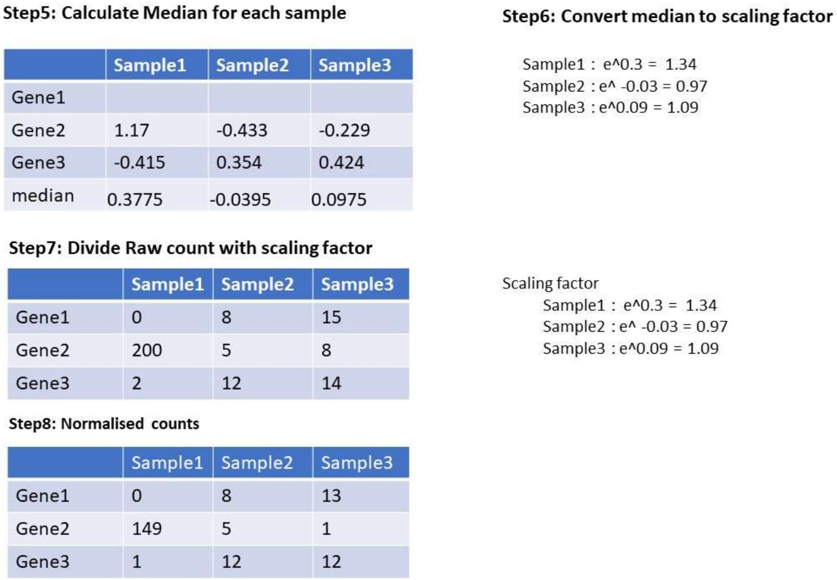
Gene1	-inf
Gene2	1.132
Gene3	0.716

Step3: Remove zero genes(-inf)

Gene1	
Gene2	1.132
Gene3	0.716

Step4: Subtract log count with geometric mean

	Sample1	Sample2	Sample3
Gene1			
Gene2	1.17	-0.433	-0.229
Gene3	-0.415	0.354	0.424



Step5: Calculate Median for each sample

	Sample1	Sample2	Sample3
Gene1			
Gene2	1.17	-0.433	-0.229
Gene3	-0.415	0.354	0.424
median	0.3775	-0.0395	0.0975

Step6: Convert median to scaling factor

Sample1 : e^0.3 = 1.34
Sample2 : e^ -0.03 = 0.97
Sample3 : e^0.09 = 1.09

Step7: Divide Raw count with scaling factor

	Sample1	Sample2	Sample3
Gene1	0	8	15
Gene2	200	5	8
Gene3	2	12	14

Scaling factor
Sample1 : e^0.3 = 1.34
Sample2 : e^ -0.03 = 0.97
Sample3 : e^0.09 = 1.09

Step8: Normalised counts

	Sample1	Sample2	Sample3
Gene1	0	8	13
Gene2	149	5	1
Gene3	1	12	12

4.2 Obtaining Alu content from LNC RNA

Alu elements are the most abundant transposable elements that are widely dispersed throughout the human genome. Many lncRNA are said to be rich in Alu elements as they can play an important role in Lnc function.

The Alu content in LncRNA was obtained from UCSC browser. UCSC browser uses Repeat Masker program, which screens DNA sequences for interspersed repeats and low complexity DNA sequences.

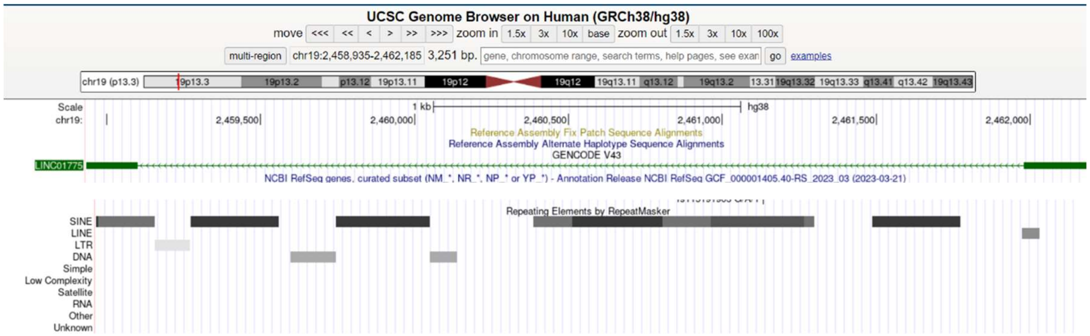


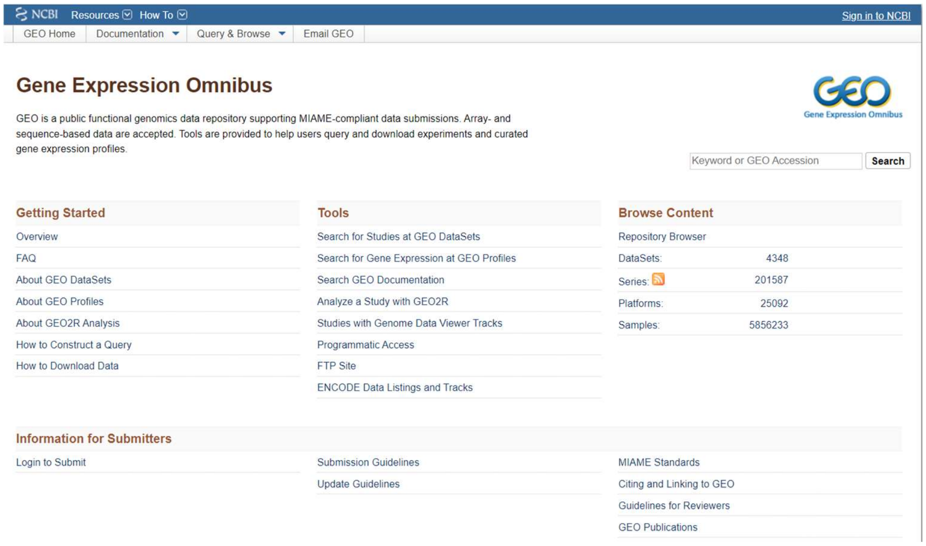
Figure7: Representing the genomic location of LINC01775 and showing the Alu repeats (SINES) using Repeat masker.

4.3 Resources for functional annotation.

4.3.1 NCBI-GEO:

NCBI-GEO: NCBI-GEO (Gene Expression omnibus) is a public repository that stores and distributes high throughput gene expression data such as RNA-seq and DNA microarray data. NCBI-Geo database was launched in 2000, as repository for high-throughput gene expression Data.

GEO provides researchers with access to a vast collection of publicly available gene expression datasets, enabling them to explore and analyse gene expression patterns across different organisms, tissues, cell types, and experimental conditions.



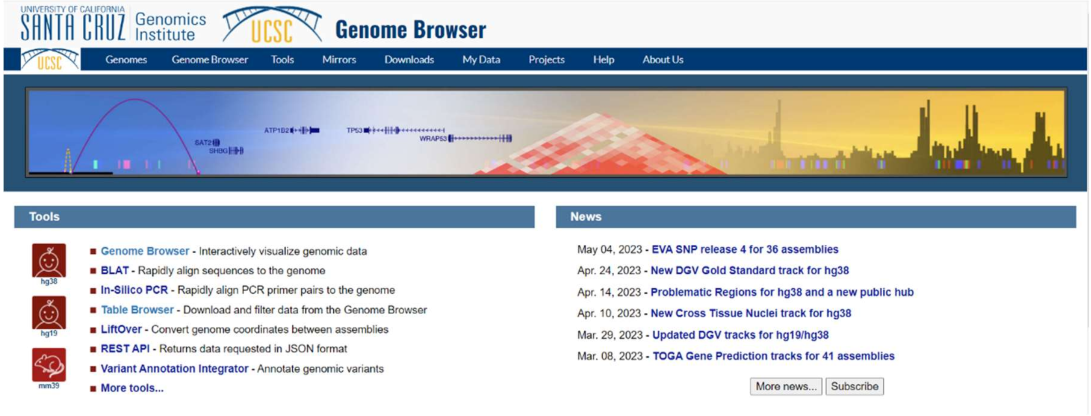
GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles.

DataSets:	4348
Series:	201587
Platforms:	25092
Samples:	5856233

Figure 8: Snapshot of homepage of NCBI-GEO

4.3.2 UCSC browser: The University of California Santa Cruz (UCSC) Genome Browser is a frequently utilised online application that facilitates the visualisation, examination, and evaluation of genomic information by researchers. The resource offers a comprehensive assortment of genome assemblies and their corresponding annotations for a diverse range of organisms, encompassing humans, animals, plants, and microbes.

The UCSC browser helps us to find the chromosomal location of lncRNAs and their location with respect to protein coding genes. The browser also contains several tools like repeat masker that helps in finding Alu content in the gene or long noncoding RNA.



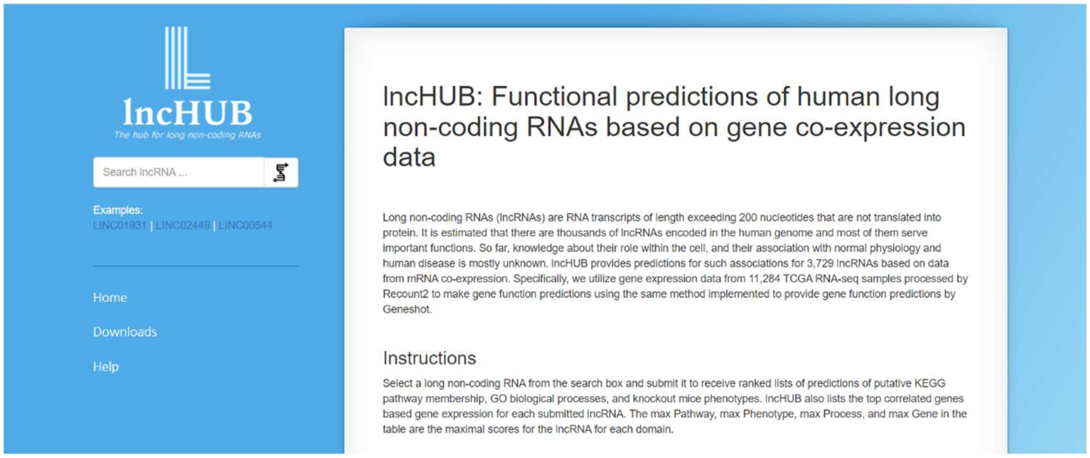
- **Genome Browser** - Interactively visualize genomic data
- **BLAT** - Rapidly align sequences to the genome
- **In-Silico PCR** - Rapidly align PCR primer pairs to the genome
- **Table Browser** - Download and filter data from the Genome Browser
- **LiftOver** - Convert genome coordinates between assemblies
- **REST API** - Returns data requested in JSON format
- **Variant Annotation Integrator** - Annotate genomic variants
- **More tools...**

May 04, 2023 - **EVA SNP release 4 for 36 assemblies**
Apr. 24, 2023 - **New DGV Gold Standard track for hg38**
Apr. 14, 2023 - **Problematic Regions for hg38 and a new public hub**
Apr. 10, 2023 - **New Cross Tissue Nuclei track for hg38**
Mar. 29, 2023 - **Updated DGV tracks for hg19/hg38**
Mar. 08, 2023 - **TOGA Gene Prediction tracks for 41 assemblies**

Figure 9: Snapshot of UCSC Browser home page

4.3.3 LncHUB:

lncHUB is a web base database developed by Mayan laboratory that provides known inferred knowledge about the 3,729 lncRNAs based on the data obtained from m-RNA co-expression. The gene expression data from 11,284 TCGA RNA-seq samples is used to make functional prediction about the lncRNA such as their involvement in biological processes.



lncHUB: Functional predictions of human long non-coding RNAs based on gene co-expression data

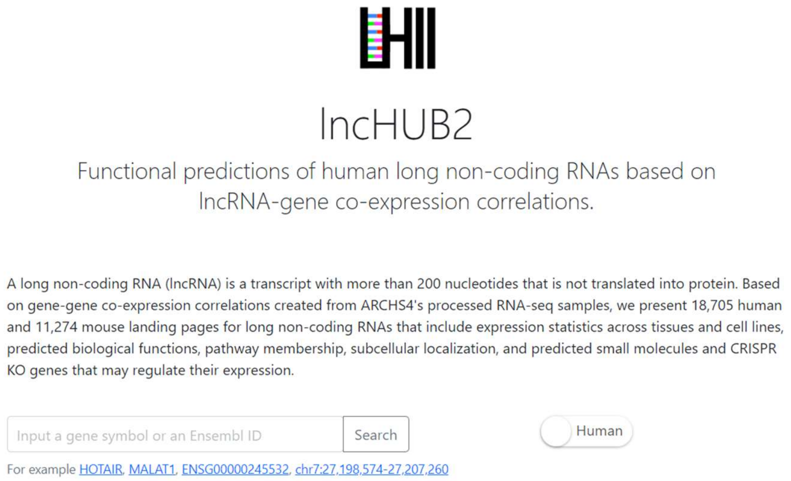
Long non-coding RNAs (lncRNAs) are RNA transcripts of length exceeding 200 nucleotides that are not translated into protein. It is estimated that there are thousands of lncRNAs encoded in the human genome and most of them serve important functions. So far, knowledge about their role within the cell, and their association with normal physiology and human disease is mostly unknown. lncHUB provides predictions for such associations for 3,729 lncRNAs based on data from mRNA co-expression. Specifically, we utilize gene expression data from 11,284 TCGA RNA-seq samples processed by Recount2 to make gene function predictions using the same method implemented to provide gene function predictions by Geneshot.

Instructions

Select a long non-coding RNA from the search box and submit it to receive ranked lists of predictions of putative KEGG pathway membership, GO biological processes, and knockout mice phenotypes. lncHUB also lists the top correlated genes based gene expression for each submitted lncRNA. The max Pathway, max Phenotype, max Process, and max Gene in the table are the maximal scores for the lncRNA for each domain.

Figure10: Snapshot of lncHUB home page

4.3.4 LncHUB2: LncHUB2 is an upgradation to the lncHUB that provides the known and inferred knowledge about 18705 human and 11274 mouse lncRNAs. LncHUB2 produces a report that contains the secondary structure fold of the lncRNA, related publications, the most correlated coding genes, the most correlated lncRNAs, a network that visualizes the most correlated genes, predicted mouse phenotypes, predicted membership in biological processes and pathways, predicted upstream transcription factor regulators, and predicted disease associations.


lncHUB2

Functional predictions of human long non-coding RNAs based on lncRNA-gene co-expression correlations.

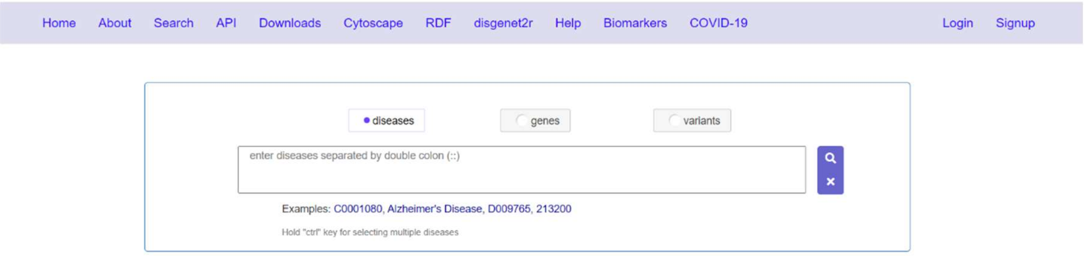
A long non-coding RNA (lncRNA) is a transcript with more than 200 nucleotides that is not translated into protein. Based on gene-gene co-expression correlations created from ARCHS4's processed RNA-seq samples, we present 18,705 human and 11,274 mouse landing pages for long non-coding RNAs that include expression statistics across tissues and cell lines, predicted biological functions, pathway membership, subcellular localization, and predicted small molecules and CRISPR KO genes that may regulate their expression.

Input a gene symbol or an Ensembl ID

For example HOTAIR, MALAT1, ENSG00000245532, chr7:27,198,574-27,207,260

Figure11: Snapshot of lncHUB2 home page

4.3.5 DisGeNET: DisGeNET is a discovery platform containing one of the largest publicly available collections of genes and variants associated to human diseases. The current version of DisGeNET contains 1,134,942 gene-disease associations (GDAs), between 21,671 genes and 30,170 diseases, disorders, traits, and clinical or abnormal human phenotypes, and 369,554 variant-disease associations (VDAs), between 194,515 variants and 14,155 diseases, traits, and phenotypes.


enter diseases separated by double colon (::)

Examples: C0001080, Alzheimer's Disease, D009765, 213200

Hold "ctrl" key for selecting multiple diseases

Figure12: Snapshot of DisGeNET home page

4.3.6 LncRNADisease 2.0: LncRNADisease 2.0 represents a revised iteration of the LncRNADisease repository, serving as an exhaustive repository for diseases that are linked to long non-coding RNA (lncRNA). The resource offers an array of lncRNA-disease associations that have been experimentally validated, accompanied by corroborating evidence and functional annotations.

The LncRNADisease v2.0 now hosts disease associated **LncRNAs** and **CircRNAs**.

- LncRNAs: 19,166
- CircRNAs: 823
- Disease: 529
- LncRNA-disease Associations: 205,959

Posted by PKU & ECNU on May 10, 2018 •

Long non-coding RNAs (lncRNAs) are an important category of non-coding RNAs (ncRNAs) which range from 200 nucleotides to multiple kilobases in length, with little or no protein-coding capacity. Circular RNAs (circRNAs) are widely expressed in diverse eukaryotic species and are characterized by covalently closed RNA loops through backsplicing events. Increasing evidence has highlighted the critical roles of lncRNAs and circRNAs in plenty of diseases development and progression. Here, we have updated the LncRNADisease database to version 2.0 by integrating comprehensive experimentally supported and predicted ncRNA-disease associations curated from manual literatures and other resources. The new developments in LncRNADisease v2.0 include (I) over 40-fold ncRNA-disease associations enhancement compare to the previous version; (II) integrating experimentally supported circRNA-disease associations; (III) providing regulatory relationship among ncRNA, mRNA and miRNA; (IV) mapping disease name to the Disease Ontology and Medical Subject Headings (MeSH); (V) providing a confidence score for each ncRNA-disease association.

Figure13: Snapshot of LncRNADisease 2.0 home page

4.4 METHODOLOGY

1. **Raw data:** RNA sequencing DATA for the given study was obtained from NCBI GEO accession number GSE 174083[ref]. The study involved 106 individuals participating in a meditation retreat at 4 different time points (T1-T4) and obtaining their transcriptome profile. T1 sample obtained 5-6 weeks before retreat, T2 samples were obtained on the day of retreat, T3 samples were obtained immediately after retreat, T4 samples were obtained after 3 months of the retreat.

2. **Data normalisation and Differential gene expression:** For the normalisation of the raw counts Deseq2 package from R was used. DeSeq2 is a popular package in R used for analysing RNA-seq data. De Seq2 algorithm estimates variance-mean from the counts data and calculates the differential expression based on negative binomial distribution.

3. **Obtaining Alu Content from Lnc RNA:** Alu content for the LncRNA was obtained from the repeat masker function in UCSC Browser.

4. **Differentially Expressed long non-coding RNA:** Differentially expressed long non coding RNA were filtered out from the list of DEG. Differentially expressed long non coding RNA were obtained by comparing 2 different time points (T2VST3 AND T2VST4).

5. **Functional Annotation of long non-coding RNA:** For the functional annotation of the long non coding RNA several data bases were used as each data base provides sone unique characteristics about the long noncoding RNA.

5. Results

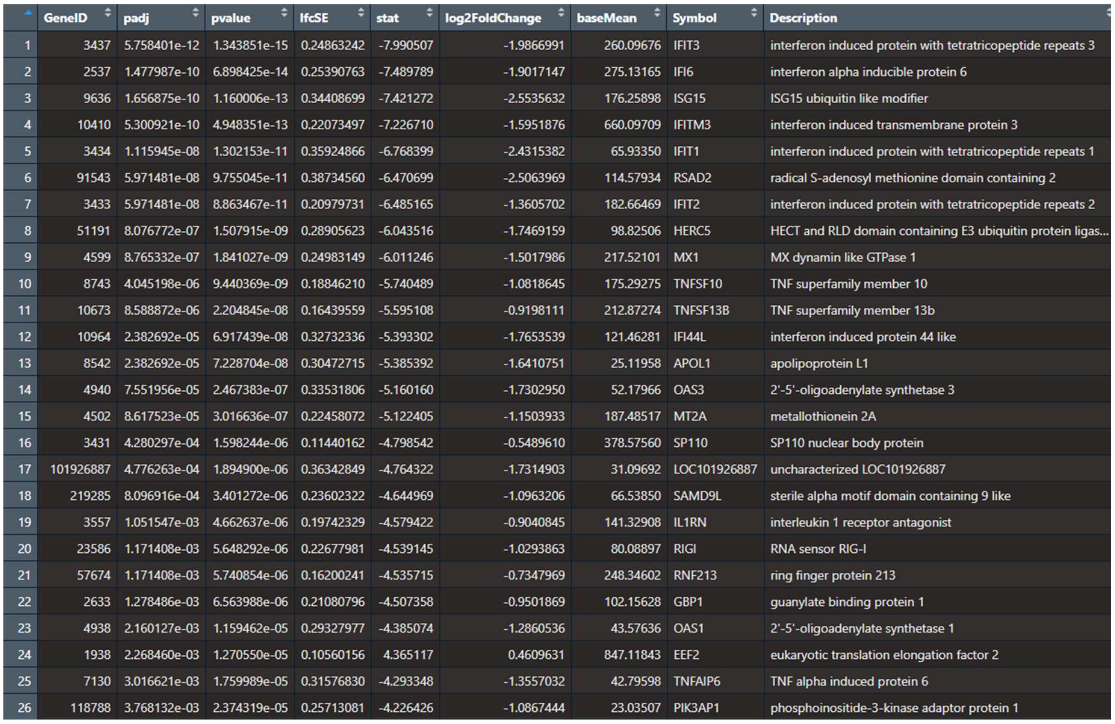
a. **Differential gene analysis:** The differential gene expression analysis was performed on the dataset comprising of samples at different timepoints (T2 vs T3 and T2 vs T4). The obtained data set contained transcriptome profile of 106 individuals at 4 different time points (T1 = 97, T2 = 105, T3 = 104, T4 = 82; N = 388).

Analysis of DeSeq2 results takes into account the magnitude of log2Fold change, significance of p-value and the degree of stringency applied (adjusted p-value).

Log2fold change: It indicates the magnitude of change in gene expression profile between two time points.

p-value: The p-value represents the statistical significance of the observed difference in gene expression between conditions.

Adjusted p-values: it takes into account the multiple testing problem and correct for the possibility of false positives. Adjusted p-values (also known as corrected p-values) take into account the multiple testing problem and correct for the possibility of false positives



	GeneID	padj	pvalue	lfcSE	stat	log2FoldChange	baseMean	Symbol	Description
1	3437	5.758401e-12	1.343851e-15	0.24863242	-7.990507	-1.9866991	260.09676	IFIT3	interferon induced protein with tetratricopeptide repeats 3
2	2537	1.477987e-10	6.898425e-14	0.25390763	-7.489789	-1.9017147	275.13165	IFI6	interferon alpha inducible protein 6
3	9636	1.656875e-10	1.160006e-13	0.34408699	-7.421272	-2.5535632	176.25898	ISG15	ISG15 ubiquitin like modifier
4	10410	5.300921e-10	4.948351e-13	0.22073497	-7.226710	-1.5951876	660.09709	IFITM3	interferon induced transmembrane protein 3
5	3434	1.115945e-08	1.302153e-11	0.35924866	-6.768399	-2.4315382	65.93350	IFIT1	interferon induced protein with tetratricopeptide repeats 1
6	91543	5.971481e-08	9.755045e-11	0.38734560	-6.470699	-2.5063969	114.57934	RSAD2	radical S-adenosyl methionine domain containing 2
7	3433	5.971481e-08	8.863467e-11	0.20979731	-6.485165	-1.3605702	182.66469	IFIT2	interferon induced protein with tetratricopeptide repeats 2
8	51191	8.076772e-07	1.507915e-09	0.28905623	-6.043516	-1.7469159	98.82506	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligas...
9	4599	8.765332e-07	1.841027e-09	0.24983149	-6.011246	-1.5017986	217.52101	MX1	MX dynamin like GTPase 1
10	8743	4.045198e-06	9.440369e-09	0.18846210	-5.740489	-1.0818645	175.29275	TNFSF10	TNF superfamily member 10
11	10673	8.588872e-06	2.204845e-08	0.16439559	-5.595108	-0.9198111	212.87274	TNFSF13B	TNF superfamily member 13b
12	10964	2.382692e-05	6.917439e-08	0.32732336	-5.393302	-1.7653539	121.46281	IFI44L	interferon induced protein 44 like
13	8542	2.382692e-05	7.228704e-08	0.30472715	-5.385392	-1.6410751	25.11958	APOL1	apolipoprotein L1
14	4940	7.551956e-05	2.467383e-07	0.33531806	-5.160160	-1.7302950	52.17966	OAS3	2'-5'-oligoadenylate synthetase 3
15	4502	8.617523e-05	3.016636e-07	0.22458072	-5.122405	-1.1503933	187.48517	MT2A	metallothionein 2A
16	3431	4.280297e-04	1.598244e-06	0.11440162	-4.798542	-0.5489610	378.57560	SP110	SP110 nuclear body protein
17	101926887	4.776263e-04	1.894900e-06	0.36342849	-4.764322	-1.7314903	31.09692	LOC101926887	uncharacterized LOC101926887
18	219285	8.096916e-04	3.401272e-06	0.23602322	-4.644969	-1.0963206	66.53850	SAMD9L	sterile alpha motif domain containing 9 like
19	3557	1.051547e-03	4.662637e-06	0.19742329	-4.579422	-0.9040845	141.32908	IL1RN	interleukin 1 receptor antagonist
20	23586	1.171408e-03	5.648292e-06	0.22677981	-4.539145	-1.0293863	80.08897	RIGI	RNA sensor RIG-I
21	57674	1.171408e-03	5.740854e-06	0.16200241	-4.535715	-0.7347969	248.34602	RNF213	ring finger protein 213
22	2633	1.278486e-03	6.563988e-06	0.21080796	-4.507358	-0.9501869	102.15628	GBP1	guanylate binding protein 1
23	4938	2.160127e-03	1.159462e-05	0.29327977	-4.385074	-1.2860536	43.57636	OAS1	2'-5'-oligoadenylate synthetase 1
24	1938	2.268460e-03	1.270550e-05	0.10560156	4.365117	0.4609631	847.11843	EEF2	eukaryotic translation elongation factor 2
25	7130	3.016621e-03	1.759989e-05	0.31576830	-4.293348	-1.3557032	42.79598	TNFAIP6	TNF alpha induced protein 6
26	118788	3.768132e-03	2.374319e-05	0.25713081	-4.226426	-1.0867444	23.03507	PIK3AP1	phosphoinositide-3-kinase adaptor protein 1

Figure14: Result of Deseq2 analysis (T2 vs T3)

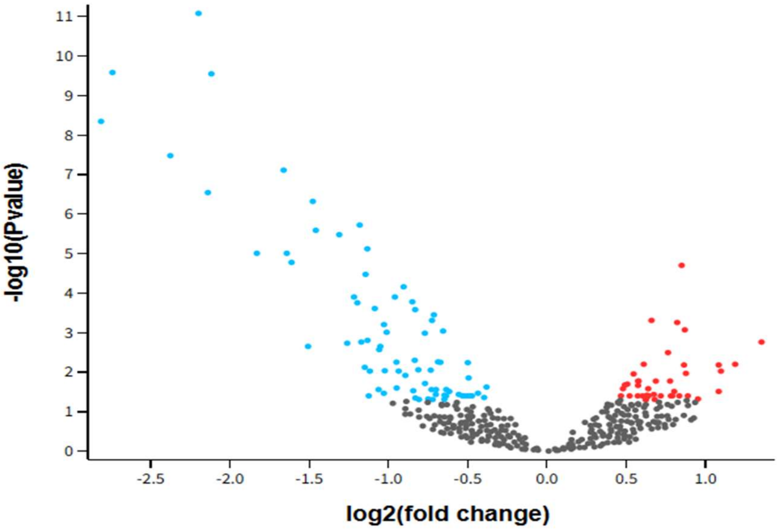


Figure15: Volcano plot representing differentially regulated gene (Blue down regulated regulate and red upregulated.)

b. **Filtration of Differentially Expressed Lnc RNA:** From the differentially expressed genens obtained from the Deseq2 results Differentially expressed Long non-coding RNA were filtered out for 2 different time points.

T2vsT3:

Ensemble Id	base Mean	log2FoldChange	lfcSE	stat	P value	P adj	lnc name
ENSG00000233975.2	174.5217539	1.851244726	0.221627845	8.3529429	6.66E-17	4.08E-13	LINC02574
ENSG00000246223.10	33.44347751	-0.808535825	0.233686378	-3.459919	0.00054	0.073955	LINC01550
ENSG00000234277.3	6.562572496	0.677255825	0.244764353	2.7669708	0.005658	0.238284	LINC01641
ENSG00000260388.2	3.195381159	-1.147291232	0.416476228	-2.754758	0.005874	0.241598	LINC00562
ENSG00000204650.14	8.144042176	-0.974388454	0.358669531	-2.716675	0.006594	0.253804	LINC02210

Table1: List of differentially expressed lncRNA at T2 vs T3

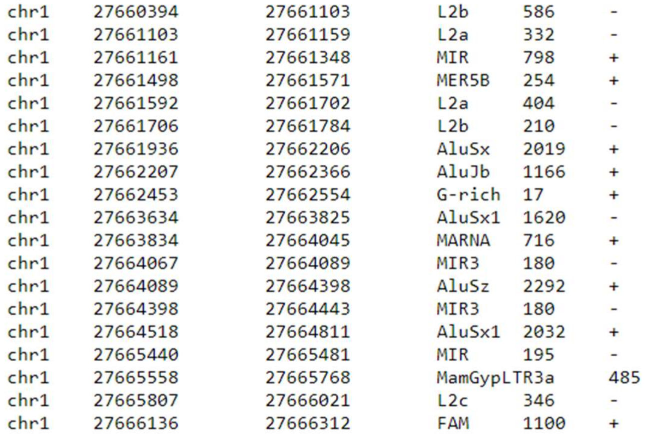
T2vsT4:

Ensemble id	base Mean	log2FoldChange	lfcSE	stat	P value	P adj	Lnc names
ENSG00000204054.14	36.34939	0.5798396887	0.2215073698	2.617699309	0.008852477396	0.5140518744	LINC00963
ENSG00000225226.2	0.87574	2.416433258	0.9198318287	2.627038098	0.008613169001	0.5069320891	LINC01795
ENSG00000233559.2	35.75638	0.7526160857	0.2153412555	3.494992559	0.000474074971	0.1456739121	LINC00513
ENSG00000233730.2	52.63710	-0.5166061089	0.1878125757	2.750647059	0.005947768796	0.4219544492	LINC01765
ENSG00000264247.2	11.33081	-0.9711936313	0.3706491904	2.620250243	0.008786526481	0.5135399183	LINC00909
ENSG00000271856.1	9.60658	-1.294436342	0.4115028679	-3.145631399	0.001657287302	0.2554292376	LINC01215

ENSG00000272888.8	45.70877	-0.7435953776	0.1997348514	-3.722912513	0.0001969377772	0.1101179487	LINC01578

Table1: List of differentially expressed lncRNA at T3 vs T4

c. **Lnc and Alu-content:** Alu content for the Differentially expressed long non-coding RNA was obtained using repeat masker from the UCSC database.



```
chr1    27660394        27661103        L2b     586     -
chr1    27661103        27661159        L2a     332     -
chr1    27661161        27661348        MIR     798     +
chr1    27661498        27661571        MER5B   254     +
chr1    27661592        27661702        L2a     404     -
chr1    27661706        27661784        L2b     210     -
chr1    27661936        27662206        AluSx   2019    +
chr1    27662207        27662366        AluJb   1166    +
chr1    27662453        27662554        G-rich  17      +
chr1    27663634        27663825        AluSx1  1620    -
chr1    27663834        27664045        MARNA   716     +
chr1    27664067        27664089        MIR3    180     -
chr1    27664089        27664398        AluSz   2292    +
chr1    27664398        27664443        MIR3    180     -
chr1    27664518        27664811        AluSx1  2032    +
chr1    27665440        27665481        MIR     195     -
chr1    27665558        27665768        MamGypLTR3a     485
chr1    27665807        27666021        L2c     346     -
chr1    27666136        27666312        FAM     1100    +
```

Figure16: Results obtained from UCSC browser for Alu content in linc02574

Alu Ratio = Total base pair of Alu/ Total length of LncRNA

d. **Functional annotation of lncRNA:** With the help of data mining the lnc were annotated using data from different databases like ncpath for lnc-gene interaction, DisGnet2 and LncRNADisease 2.0 database for disease prediction and lnchub and lnchub2 for biological pathways and processes prediction.

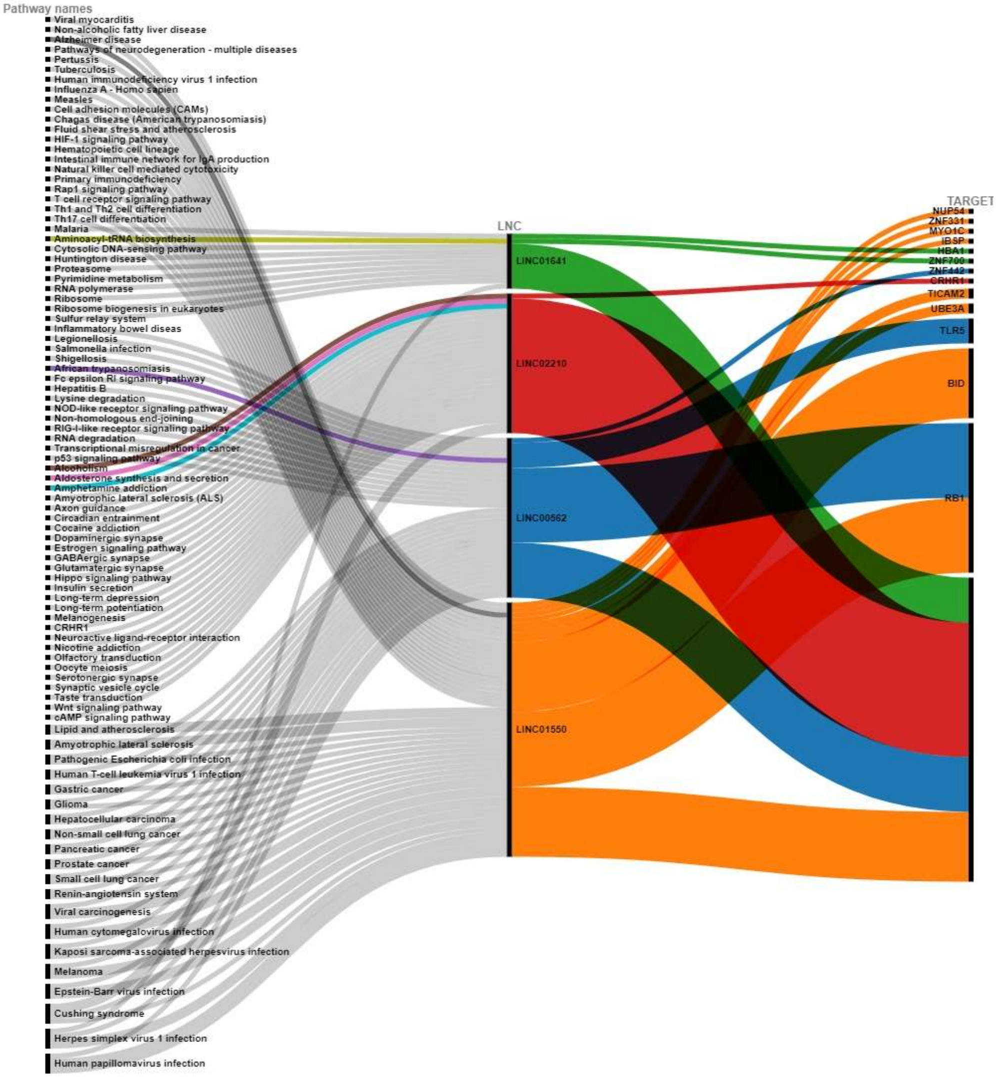


Figure15: Representing relation between pathways, long non-coding RNA and target genes

6. Discussion:

Long non-coding RNA have shown to play an important role in diverse biological processes, encompassing development, differentiation, and pathological mechanisms. The study on long non-coding RNA (lncRNA) have remain an active field of exploration for past few decades due to their potential application in human health and welfare.

In contrast, meditation is a contemplative technique that as been mentioned in ayurvedic literature such as "*Hatha Yoga Pradīpikā",* and has been practiced for centuries for physical and mental benefits.

At present direct there are no direct research exploring the involvement of lncRNA with meditation, while number of publications have supported the change in gene expression pattern in response to meditation.

This change in gene expression pattern could be directly or indirectly related to lncRNA regulation as both lncRNA and meditation are seen to be involved in biological process related to stress and inflammation. However, more research is needed to explore this relationship and uncover the specific mechanisms involved.

In the present study, we tried to explore the role of long non-coding RNA in meditative state through *in-silico* analysis. This study in a preliminary investigation and cannot give conclusive evidence for the role of long non coding RNA in meditation but can provide an initial frame work for lnc-RNA analysis.

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