A TRANSCRIPTOMICALLY GUIDED COMPUTATIONAL QUANTIFICATION OF THE SRR8671434 RNA SEQUENCE

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ABSTRACT

Ribonucleic acid (RNA) is a single strand nucleic acid responsible for genetic coding, decoding, regulation, and expression that consists of phosphate and ribose groups with several purposeful variants, notably messenger, transfer, and ribosomal RNA. RNA sequencing is a next-generation sequencing (NGS) technique capable of continuously analyzing cellular transcriptome and revealing the presence and quantity of RNA within a biological sample. The process entails reverse transcribing the extracted RNA into cDNA; subsequently, the cDNA is fragmented, enabling its input into an NGS workflow, after which adapters are added to both ends of the fragments. RNA sequencing is an expensive and time-consuming process due to its necessitation of an entire genomic library that is often difficult to analyze through traditional methods; however, applying computational methods can overcome these challenges through cutting edge data mining and informatics technologies. In this study, the SRR8671434 RNA sequence is quantified and analyzed for its ability to serve as an indicator for congenital afflictions. The findings in this paper can be applied towards preventing and curing associated diseases, as well as discerning other potential biomarkers within genetic materials.

1 Introduction

The purpose of this study is to quantitatively examine the SRR8671434 RNA sequence through data analysis and computational techniques [5].

1.1 Ribonucleic Acid

Ribonucleic acid (RNA) is a complex compound that replaces DNA as a genetic code carrier in cellular protein synthesis [3]. The structure of RNA was first described by R.W. Holley in 1965 and has since been the subject various studies exploring its function and evolution.

1.1.1 Structure

RNA is a single-stranded biopolymer made of a backbone consisting of alternating phosphate and ribose groups, each of which is attached to an adenine, uracil, cytosine, or guanine base [4]. Its three-dimensional structure allows the ribose and nitrogenous bases to be modified via cellular enzymes that attach chemical groups to the chain. These modifications lead to contortions within the RNA chain, further stabilizing its structure. RNAs are also capable of forming complexes with ribonucleoproteins (RNPs) which together serve as biological catalysts.

1.1.2 Function

Ribonucleic acid (RNA) is a single strand nucleic acid responsible for various biochemical tasks in coding, decoding, regulating, and expressing genes [1]. The central dogma of molecular biology suggest that the primary role of RNA is in converting DNA information to proteins by coding amino acids. RNA is also central in regulating cellular processes such as cell division, aging, and death.

Figure 1: Central Dogma of Biology

1.1.3 Types

Although there are numerous types of RNA in both prokaryotic and eukaryotic cells, the most main types are messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA) [5]. Although these types of RNAs are primarily responsible for carrying out biochemical reactions, they also can have complex regulatory functions in cellular processes and diseases.

mRNA carries genetic codes from DNA in the nucleus to ribosomes during protein synthesis as this is the location of protein translation in the cytoplasm [3]. rRNA encodes ribosome protein subunits which are synthesized in the nucleolus. Subsequently, they shift to the cytoplasm and read the mRNA code. The incorporation of specific amino acids in proteins is specified by the mRNA's sequence of three nitrogenous bases. The tRNA molecules transport the amino acids to the ribosomes, where they form proteins.

1.1.4 Sequencing

RNA sequencing is an emerging molecular technology that utilizes deep-sequencing techniques to examine the quantity and sequences of RNA in biological samples by analyzing the transcriptome of encoded gene expression patterns [2]. This technique is useful for detecting known or novel features within RNA sequences that can be linked to genetic diseases and disorders.

Table 1: General Statistics

Sample Name	% Assigned	M Assigned	% Dups	% GC	Length	% Failed	M Seqs	% Aligned
SRR8671434	34.0%	0.8	43.7%	42%	47 bp	30%	2.1	69.3%

2 Analysis

2.1 General Statistics

2.2 featureCounts

Subread featureCounts is a highly efficient general-purpose read summarization program that counts mapped reads for genomic features such as genes, exons, promoter, gene bodies, genomic bins and chromosomal locations.

Figure 2: featureCounts: Assignments

2.3 FastQC

FastQC is a quality control tool for high throughput sequence data, written by Simon Andrews at the Babraham Institute in Cambridge.

2.3.1 Sequence Counts

Sequence counts for each sample. Duplicate read counts are an estimate only.

2.3.2 Sequence Quality Histograms

The mean quality value across each base position in the read.

2.3.3 Per Sequence Quality Scores

The number of reads with average quality scores. Shows if a subset of reads has poor quality.

2.3.4 Per Base Sequence Content

The proportion of each base position for which each of the four normal DNA bases has been called.

2.3.5 Per Sequence GC Content

The average GC content of reads. Normal random library typically have a roughly normal distribution of GC content.

Figure 3: featureCounts: Sequence Counts

Figure 4: FastQC: Mean Quality Scores

2.3.6 Per Base N Content

The percentage of base calls at each position for which an N was called.

2.3.7 Sequence Length Distribution

The distribution of fragment sizes (read lengths) found.

2.3.8 Sequence Duplication Levels

The relative level of duplication found for every sequence.

2.3.9 Overrepresented Sequences

The total amount of overrepresented sequences found in each library; 1 samples had less than 1% of reads made up of overrepresented sequences.

Figure 5: FastQC: Per Sequence Quality Scores

Figure 6: Per Base Sequence Content

2.3.10 Adapter Content

The cumulative percentage count of the proportion of the library which has seen each of the adapter sequences at each position; no samples found with any adapter contamination > 0.1%.

2.4 HISAT2

HISAT2 is a fast and sensitive alignment program for mapping NGS reads (both DNA and RNA) against a reference genome or population of reference genomes.

2.5 TPM

3 Conclusion

From the results of this study, it can be concluded that the SRR8671434 RNA sequence holds significant promise in serving as a genetic predictor for various hereditary disease and carcinomas. The sequence points towards further research investigating its implications in transcriptomical technologies. Future studies should explore difference RNA sequences and assess their respective features and relations to different afflictions.

Table 2: TPM

Gene_Id	ExonLength	ExonReads	ExonTPM	IntronLength	IntronReads	IntronTPM
ENSG00000248527	681.0	1844.0	3820.5	0.0	0.0	0.0
ENSG00000240453	402.0	3.0	10.5	7202.0	0.0	0.0
ENSG00000240409	207.0	43.0	293.1	0.0	0.0	0.0
ENSG00000238009	3569.0	0.0	0.0	40703.0	1.0	0.3
ENSG00000237973	1543.0	3102.0	2836.5	0.0	0.0	0.0
ENSG00000237491	5104.0	0.0	0.0	26187.0	43.0	21.0
ENSG00000237094	5950.0	1.0	0.2	130279.0	3.0	0.3
ENSG00000230092	523.0	13.0	35.1	8760.0	0.0	0.0
ENSG00000230021	1697.0	0.0	0.0	121418.0	8243.0	869.9
ENSG00000229344	682.0	1625.0	3361.8	0.0	0.0	0.0
ENSG00000228794	7631.0	0.0	0.0	24208.0	1.0	0.5
ENSG00000227232	2073.0	152.0	103.5	13371.0	0.0	0.0
ENSG00000225972	372.0	759.0	2878.7	0.0	0.0	0.0
ENSG00000225630	1044.0	123.0	166.2	0.0	0.0	0.0
ENSG00000223972	1756.0	43.0	34.5	788.0	0.0	0.0
ENSG00000198744	547.0	893.0	2303.4	0.0	0.0	0.0
ENSG00000188976	5539.0	100.0	25.5	9567.0	0.0	0.0
ENSG00000187961	3395.0	5.0	2.1	1734.0	0.0	0.0
ENSG00000187634	3205.0	89.0	39.2	16491.0	0.0	0.0
ENSG00000187583	2833.0	1.0	0.5	6536.0	0.0	0.0

Table 3: Unique TPM

Gene_Id	ExonLength	ExonReads	ExonTPM	IntronLength	IntronReads	IntronTPM
ENSG00000248527	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000240453	9.0	3.0	679.5	7200.0	0.0	0.0
ENSG00000240409	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000238009	1821.0	0.0	0.0	38354.0	1.0	1.9
ENSG00000237973	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000237491	3172.0	0.0	0.0	18937.0	30.0	114.1
ENSG00000237094	4272.0	1.0	0.5	125189.0	3.0	1.7
ENSG00000230092	42.0	2.0	97.1	6.0	0.0	0.0
ENSG00000230021	1504.0	0.0	0.0	111070.0	1014.0	657.3
ENSG00000229344	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000228794	7631.0	0.0	0.0	24208.0	1.0	3.0
ENSG00000227232	1770.0	127.0	146.3	13371.0	0.0	0.0
ENSG00000225972	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000225630	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000223972	1706.0	0.0	0.0	788.0	0.0	0.0
ENSG00000198744	0.0	0.0	0.0	0.0	0.0	0.0
ENSG00000188976	5167.0	17.0	6.7	9567.0	0.0	0.0
ENSG00000187961	3395.0	5.0	3.0	1734.0	0.0	0.0
ENSG00000187634	2532.0	0.0	0.0	11610.0	0.0	0.0
ENSG00000187583	2679.0	1.0	0.8	6023.0	0.0	0.0

Figure 7: FastQC: Per Sequence GC Content

Figure 8: FastQC: Per Base N Content

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Figure 9: FastQC: Sequence Length Distribution

Figure 10: FastQC: Sequence Duplication Levels

Figure 11: HISAT2: SE Alignment Scores