Identification and target prediction of small miRNA

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ABSTRACT

Aim: The aim of the study was to identify, target, and predict small miRNA. Materials and Methods: Small RNA with <50 nucleotides long non-coding RNA has various functions such as gene regulation, we took one such RNA from non-coding RNA database from NCBI and analyzed its structure and function. Quality of small RNA sequencing data of Drosophila melanogaster downloaded from NCBI was checked using next-generation sequencing, FastQC and adapters were removed using cutadapt tool. The quality check was performed before and after trimming. Results: From the data, we have obtained the miRNA which had the highest score seemed to have many regulatory functions and it is surprising to know how one miRNA could be responsible for 10 important functions. Conclusion: Earlier it was considered that the exon part was more clinically significant, but the current trend is that the miRNA which is obtained from the intron part is of equal importance and has a lot of clinical significance which could be discovered in future large scale researches.

KEY WORDS: miRNA, Next-generation sequencing, Small RNA, Gene expression, Novel miRNA

INTRODUCTION

miRNA is a practically essential class of small, non-protein coding RNA, 19–24 nucleotides long that are post-transcriptional controllers of gene expression.¹ Part of RISC (RNA-induced silencing complex), single-stranded miRNA binds specifically to mRNA targets, inhibits translation, and mediates the derogation of the targeted RNA, thus helping in gene regulation.²⁻⁶ miRNA help in translation, transcription, and often regularly explicitly enhanced in a specific tissue or during regular processes of the cell.⁷⁻¹²

It has been observed that miRNA can potentially monitor every action of cellular activity such as development, proliferation, differentiation, apoptosis, stress reaction, insulin secretion, carcinogenesis, and fat metabolism.¹³⁻¹⁵

However, the time period for this process to occur has not been estimated accurately.¹⁶

In the recent years, there is a huge interest in finding out the functions of small miRNA and its application in curing diseases and also it has been observed that abnormal miRNA activity had been associated with many diseases.¹⁷

There have been significant advancements in the small RNA library which aids in the better samples, and they show better sequencing of the outputs both quantitatively and qualitatively.¹⁸,¹⁹

Over the 10 years, the significant role of miRNA in monitoring the biological processes has increased the interest in how miRNA binds to its a receptor and uniquely interacts with miRNA response elements (MREs). Significant works have shown how MREs might compete for binding to a common miRNA.²⁰⁻²³

Analyzing the undiscovered mechanisms that characterize what functional miRNA is, therefore, compulsory to interpret biological processes and to propose new therapeutic methods. In this study, we took D. melanogaster as the model organism to identify predict and target its small miRNA and analyze its function using next-generation sequencing (NGS) and various other bioinformatic open source tools.

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MATERIALS AND METHODS

NCBI SRA
Illumina Miseq data of *D. melanogaster* were downloaded from the NCBI SRA database having an accession number SRR1287661 in FastQC format. Adapter sequences and other low-quality data from this raw data were removed using CutAdapt. Fastqc tool was used to assess the quality of the data before and after the removal. It was ensured that the data qualified the Phred 30 and above criteria.

miRNA Prediction
Trimmed reads were aligned and subjected to miRNA prediction using Mirdeep2 tool. All the basic parameters necessary for miRNA were taken into consideration. Mirbase and Miranda databases were used to search for novel and known miRNA and target identification for the predicted miRNA. Only the novel miRNA was taken up for further study, and their functions and targets were identified.

RESULTS AND DISCUSSION

Quality Check and Removal of Contamination
Quality of small RNA sequencing data of *D. melanogaster* downloaded from NCBI was checked using FastQC and adapters were removed cutadapt tool. The quality check was performed before and after trimming [Table 1 and Figure 1].

miRNA Prediction
Contamination-free sequence data were used to identify novel miRNA using miRDeep. Table 2 shows the details of the novel miRNAs identified. A total of 10 novel miRNA were identified using miRDeep. Table shows the miRDeep score of each miRNA, consensus mature, consensus star region, and consensus precursor region.

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Table 1: Quality check report

<table>
<thead>
<tr>
<th>Adapter removal</th>
<th>Before trimming</th>
<th>After trimming</th>
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<tbody>
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<td>Filename</td>
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<td>Total Sequences</td>
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<td>49636363</td>
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<td>Sequences flagged as poor quality</td>
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<tr>
<td>Sequence length</td>
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<td>18–51</td>
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<tr>
<td>% GC</td>
<td>49</td>
<td>43</td>
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</table>

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Figure 1: FastQC report (a) base quality Phred Score of the data, (b) GC content of the data after adapter removal

Figure 2: Secondary structure of novel miRNA
## Table 2: Details of novel miRNA

<table>
<thead>
<tr>
<th>Provisional Id</th>
<th>miRDeep2 score</th>
<th>Consensus mature sequence</th>
<th>Consensus star sequence</th>
<th>Consensus precursor sequence</th>
<th>Precursor coordinate</th>
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<td>uugeugcgcgeaaaccuucucuuggcucuugggagagcgcc</td>
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Novel miRNA with the highest miRDeep score was selected for predicting its secondary structure [Figure 2]. The secondary structure of miRNA has a stem and loop structure. Mature sequence always lies in the stem region of miRNA. An opposite strand of the mature strand is called a star sequence.

Target Prediction

TargetScan is a freely available tool for predicting targets of miRNA. To predict targets, seed region of novel miRNA which includes 2nd-8th nucleotide of consensus mature strand was used. Table 3 shows some of the targets identified for a single novel miRNA. Here, a single miRNA targets multiple genes with different functions such as cg, grk, robo, and sgl and also with similar functions such as Rab5 and pros.

CONCLUSION

NGS has revolutionized genomics research. Due to which more and more hidden facts about life are being unraveled. Noncoding RNA such as small RNA is not only proven to exist but also have been reported to play a key role in gene silencing and related transcriptomics. D. melanogaster is a model organism, and still several of its small RNA are yet to be predicted and assigned a putative function. In this study, small RNASeq data were analyzed for predicting novel miRNA from this species. A total of seven novel miRNA were predicted after analyzing 50061899 Illumina reads. Axon development, G-protein coupled acetylcholine receptor activity, DNA-binding transcription factor activity, border follicle cell migration axon development, cell cycle/ proliferation, epithelial cell migration, and open tracheal system are found to be the related functions of this novel miRNA.

REFERENCES


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