Genome Wide Computational Prediction of miRNAs in Kyasanur Forest Disease Virus and their Targeted Genes in Human

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RNAs are versatile biomolecules and can be coding or non-coding. Among the non-coding RNAs, miRNAs are small endogenous molecules that play important role in posttranscriptional gene regulation. miRNAs are identified in viruses too and involved in down regulation of host genes. Flavivirus family members are classified in to two groups: mosquito-borne flaviviruses (MBFV) and tick-borne flaviviruses (TBFV). Kyasanur forest disease virus (KFDV) found in India in 1957 (Karnataka) relates to TBFV. Virus has been diffuse to new areas in India and needs attention as it can cause severe hemorrhagic fever. Here in this study, we scanned the virus genome for prediction of miRNAs that can inhibit host target genes. VMir, tool was used for extraction of pre-miRNAs. A total of four miRNAs were found and submitted to ViralMir for classification in to real or pseudo. Interestingly, all four pre-miRNAs were classified as real. Eight mature miRNAs were located in pre-miRNAs by Mature Bayes. A total of 539 human target genes has been identified by using miRDB but ANGPT1 (angiopoietin 1) and TFRC (transferrin receptor) genes were screened to play role in hemorrhagic fever and neurological problems. GO analysis of target genes also supported the evidences.

Keywords: Kyasanur forest disease virus, Flavivirus, miRNA, target prediction

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Introduction

Kyasanur forest disease virus (KFDV) belongs to family *Flaviviridae*, genus *Flavivirus* [1]. KFDV was first isolated from black faced langur (Presbytis entellus) in Kyasanur forest (Karnataka, India) in March, 1957 [2]. KFDV is mainly transmitted by bite of infected ticks (Haemaphysalis spinigera) and monkeys, rodents and birds acts as reservoir of the virus; hence it is of Zoonotic origin [3]. Upon infection to a human the onset of various symptoms likes fever, frontal headache, myalgia, photophobia, severe prostration, hypotension and hepatomegaly can be commonly seen. But in some severe cases pulmonary, gastrointestinal hemorrhage and neurological manifestations occurred. The term hemorrhagic fever is commonly used for all indications and the disease is termed Kyasanur forest disease (KFD) [4, 5].

Since the first outbreak of KFD in 1957, the disease has been outbreaks many a times from 2012-2015 in its origin state Karnataka and even in new states like Kerala, Tamilnadu and Goa [6, 7]. Serological studies has been confirmed the existence of KFDV in other parts of India including parts of Kutch district, the Saurashtra region in Gujarat state, and in parts of West Bengal state, in forested regions west of Kolkata [8]. An outbreak reported in January, 2016 in Maharashtra has also confirmed 24 KFD cases in human [9]. Number of efforts has been put by the different researchers to make a successful vaccine against KFDV but till date no effective vaccine is available [10-13]. It is evident from fact that even after the administration of vaccine in a population of particular area, the cases has not been stopped [14].

The geographical location of KFDV is not just limited to India, genome analysis studies has shown that it has more than 90 % sequence identity with Alkhurma hemorrhagic fever virus (AHFV), which is isolated in 1994 from blood of hemorrhagic fever patients in Makkah, Saudi Arabia and thought to be transferred by migratory birds from India [15, 16]. Many members of Flaviviridae family such as Dengue [17], Japanese Encephalitis [18], and recently Zika virus [19] were predicted to have encoded miRNAs. It is also evident in past that viral miRNAs can down regulate host genes expression [20-22].

miRNAs are small non-coding RNAs, about 20-25 nt long [23]. In the world of non-coding RNAs, miRNAs are gaining more significance as these small RNA molecules involves in post-transcriptional gene regulation by binding to complementary sites in mRNAs [24]. Experimental

determination of miRNA is limited to its expression in specific cell type and time, so the computational approaches are frequently used for prediction of miRNAs [25].

Increase in prevalence of KFDV in India, evolutionary similarity with other viruses found outside India, no effective vaccine against it and evidences of viral miRNAs regulating host gene prompt us to analyze the KFDV genome sequence for miRNA prediction.

Materials and Methods

KFDV Genome Sequence

Complete genome sequence of KFDV strain P9605 (Genbank accession number: JF416958.1), a human isolate was obtained from NCBI (National Center for Biotechnology Information). Genome is a single stranded RNA molecule with linear topology and 10774 nucleotide base pairs.

Pre-miRNAs prediction

Viral genome was scanned for finding self complementary hair pin loop structures of precursor RNAs (pre-miRNAs) by VMir, an ab initio miRNAs prediction program [26]. VMir package contains two individual programs: VMir Analyzer, used for analyzing sequence and VMir Viewer, used for viewing and filtering result of analyzer. The default parameter were used for analyzer (window count: 500, conformation: linear, orientation: both). Stringent filtering was done by setting min. hairpin size: 70, min. score: 115 and min. window count: 35 in VMir viewer as previously described [26].

Identification of real pre-miRNAs

We used ViralMir, a web interface tool for identification of potential or real viral pre-miRNAs sequences. ViralMir is specially designed for viruses and is based on SVM (support vector machine). The SVM model has been trained on sequence and structural features of experimentally validated pre-miRNAs data set [27].

Secondary structure prediction and energy calculation

The Mfold web server with default parameters was used to predict the secondary structure and minimum free energy (MFE) of pre miRNAs [28].

Extraction of mature miRNAs from pre-miRNAs

For further downstream analysis, mature miRNAs were extracted from pre-miRNAs sequences using Mature Bayes- an online tool that uses Naive Bayes Classifier (NBC) taking into account sequence as well as structural information of experimental predicted miRNA precursors [29].

Target gene prediction by miRDB

miRDB, a web based server which provide custom target prediction to user to identify the target gene in human was used. All the mature miRNAs was submitted individually for identification of target genes. miRDB uses the seeding approach and scan the 3' UTR (untranslated regions) of human's gene for possible hybridization with miRNAs sequence [30].

Literature data mining and Screening of genes

NCBI Gene database was used for retrieving the list of genes (supplementary data Table 1) involved in hemorrhagic fever. Manual crosschecking was done with target genes retrieved from miRDB. Literature search was carried out for supporting the screened gene result.

GO (Gene Ontology) analysis:

Most of the miRNAs prediction works are limited to up to target prediction but now-a-days gene ontology studies were being done to gain insight in to molecular functional, biological process and cellular component of the target genes [31]. We also used PANTHER for analyzing our entire predicted target gene for GO terms [32, 33]. NCBI gene IDs of target genes in a text files were used for this analysis.

Results and Discussion

Prediction of pre-miRNAs in KFDV

An Ab-intio based program analyzes the sequence feature for making any prediction [34-36]. Genome organization studies of miRNAs have revealed that they can be present on introns of protein-coding transcriptional unit (TU), introns of non-coding TU, exons of non-coding TU and exons of coding TU [37-40]. On whatever location they may present but they tend to stabilize themselves by complementary hybridization to form hairpin loop structural features [41, 42]. Our study is also based on above mentioned concept.

After analysis of complete genome of KFDV accession number JF416958.1 by VMir we got four pre-miRNAs (Fig. 1) at stringent parameters described above in methodology. Out of four miRNAs three were found in reverse strand and one on direct strand. All the predicted miRNAs were in size range 147-185 nt. The genomic position of each pre-miRNAs varies and is shown in Table 1 along with VMir score.

Sr No.	Predicted pre-miRNA	RANK	STRAND ORIENTATION	Size pre-miRNA(nt)	Position on Genome	VMIR Score
1	KFDV-MR9	1	Reverse	185	1061 - 1245	200.6
2	KFDV-MD57	2	Direct	116	5165 - 5280	193.3
3	KFDV-MR59	3	Reverse	131	6062 - 6192	172.3
4	KFDV-MR79	4	Reverse	147	8488 - 8634	120.2

Table 1. Predicted pre-miRNAs in KFDV genome

All the four pre-miRNAs were identified as real by virus specific tool, ViralMir and their minimum free energy calculated by Mfold is shown in Table 2.

Sr No.	Predicted pre-miRNA	REAL\PSEUDO	pre-miRNA MFEs (-ΔG. kCAL/MoL)
1	KFDV-MR9	REAL	ΔG = -76.00 kcal/mol
2	KFDV-MD57	REAL	ΔG = -43.90 kcal/mol
3	KFDV-MR59	REAL	ΔG = -50.70 kcal/mol
4	KFDV-MR79	REAL	ΔG = -47.80 kcal/mol

Table 2. Classification of pre-miRNAs and MFEs

Mature miRNAs extraction

Pre-miRNAs are not functional mature miRNAs, Dicer cleaved pre-miRNAs in to small ~22 nt long mature miRNAs molecules. After processing large pre-miRNAs, two single strands one in 5' stem and other in 3' stem were obtained. One or both strands can serve as mature miRNA molecule depending on the assembly of RISC complex [43, 44]. We predicted mature miRNAs from each four pre-miRNAs both in 5' stem and 3' stem by Mature Bayes tool. A total of eight mature miRNAs were predicted and their sequence is shown in Table 3.

Sr. No.	MATURE miRNA	LENGTH (nt)	STEM LOCATION	Mature miRNAs SEQUENCE (5'-3')
1	KFDV-MR9-5P	22	5'	UGUCGCUGGACCCAUGGCUGGA
2	KFDV-MR9-3P	22	3'	UGUCUUGGCCGGGUUCUCCUGG
3	KFDV-MD57-5P	22	5'	GACAUGCAUCCGGGGUCAGGGA
4	KFDV-MD57-3P	22	3'	CCGGAGCUUGUGAGGCAGUGUG
5	KFDV-MR59-5P	22	5'	CUUCACCUGCUCGGGUCCAUAA
6	KFDV-MR59-5P	22	3'	UAUUGUCCAGGAGUAUCUGCGC
7	KFDV-MR79-5P	22	5'	UCUUGUAGCUUCCCCAAUAUUG
8	KFDV-MR79-3P	22	3'	CAAGAAUCACCAUAUUGGGUCU

Table 3 Mature miRNAs

Prediction of target genes in human

miRNAs target prediction can be done by experimentally or computationally. Where the experimental evidence is hard to obtained, computational predictions mainly relies on the Watson-Crick base pairing between miRNA and mRNA molecule. Most of the algorithm used seed pairing approach for predicting interaction between miRNA and "seed sites" on mRNA molecule [45-47].

To follow this approach, we also used miRDB server for prediction of target gene in human. The server uses the MirTarget algorithm, which is based on 7-mer seeding approach and custom predict miRNAs targets in human gene's 3' UTRs [48]. We successfully predicted 539 target genes with miRDB score >80, shown in supplementary data table 2. Crosschecking with NCBI gene list for hemorrhagic fever gave only two genes, ANGPT1 (angiopoietin 1) and TFRC (transferrin receptor). Both ANGPT1 and TFRC play role in blood vessel stability and neurological development respectively. PubMed ids of literature evidences of these genes were given in table 4.

Mature miRNA	Target Gene	Description	Reference PMID
KFDV-MD57-5P	ANGPT1	angiopoietin 1	20156556, 16645151
	TFRC	transferrin receptor	26339443, 19211831

Table 4 PubMed ids of literature evidence

GO analysis

Target genes were found to be involved in a number of pathways (Fig. 2) including synaptic vesicles trafficking pathway (P05734), axon guidance (P00008) that play important role in proper nervous system functioning, pathways like coagulation (P00011), plasminogen activating cascade (P00050), angiogenesis (P00005) confirmed role in blood regulation. Whereas involvement of T cell activation pathway (P00053) can be related to breach in the immune system. Biological processes (Fig. 3) involvements were also spread to immune system

(GO:0002376), response to stimulus (GO:0050896) and biological adhesion (GO:0022610) etc. Molecular functions (Fig. 4) of the targeted genes is found to be involved in transporter activity (GO:0005215), translation regulator activity (GO:0045182), binding activity (GO:0005488) and signal transducer activity (GO:0004871) etc. Cellular component analysis (Fig. 5) revealed that target gene products were the part of synapses (GO:0045202), extracellular matrix (GO:0031012) and membrane (GO:0016020) etc.

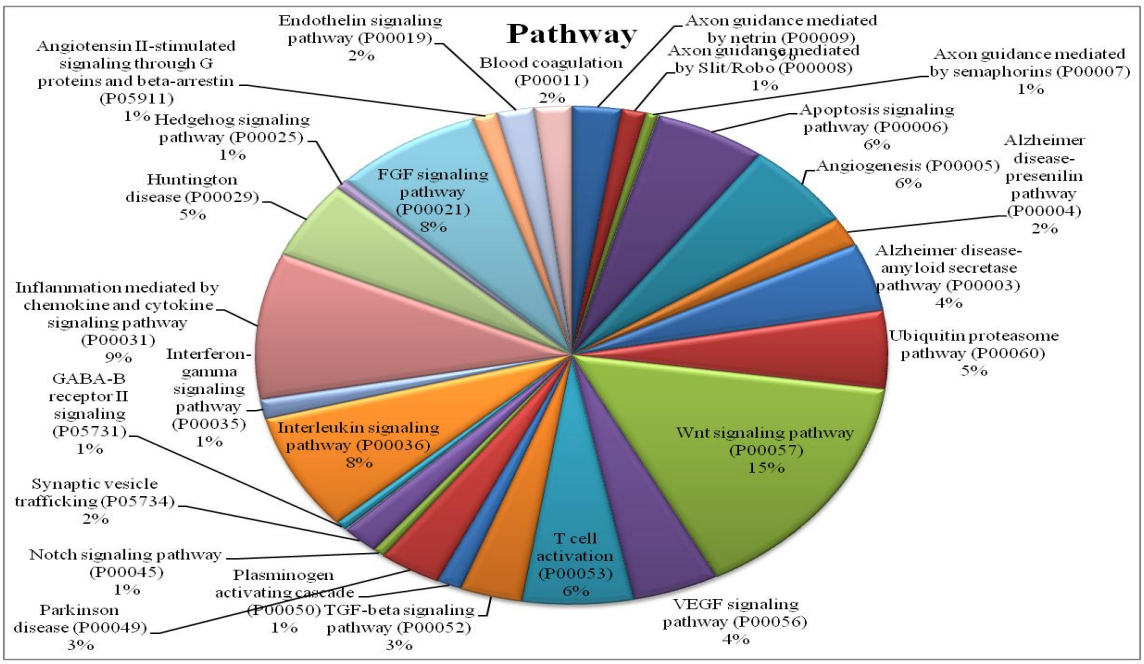


Figure 2: Pathway analysis of target genes

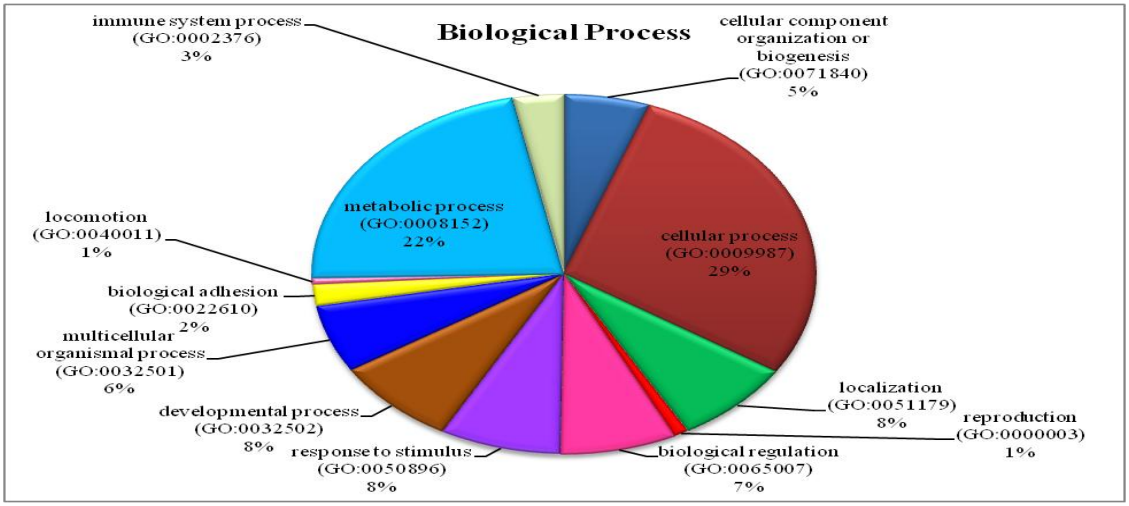


Figure: 3: Biological Process of target genes

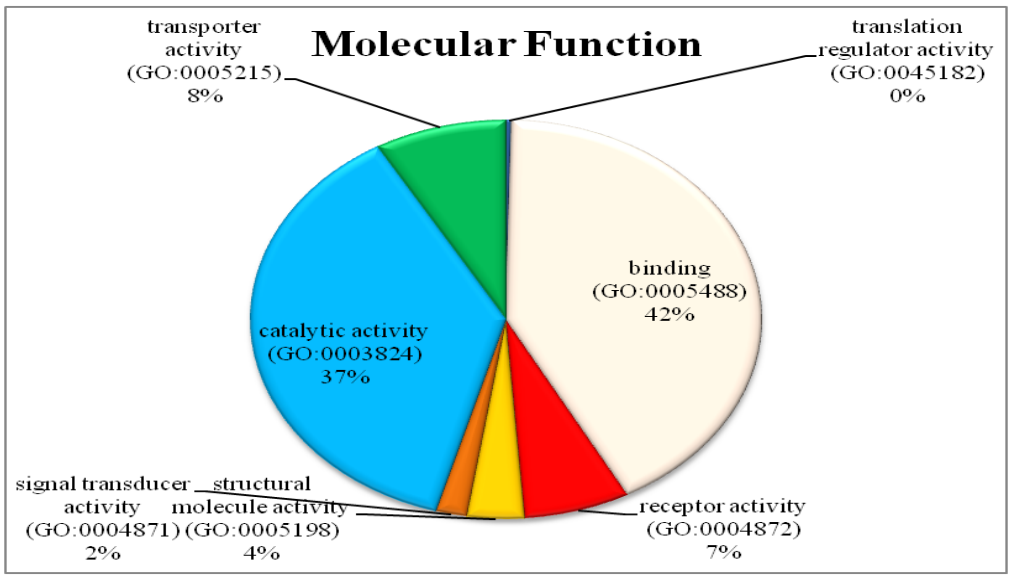


Figure 4: Molecular functional analysis of target genes

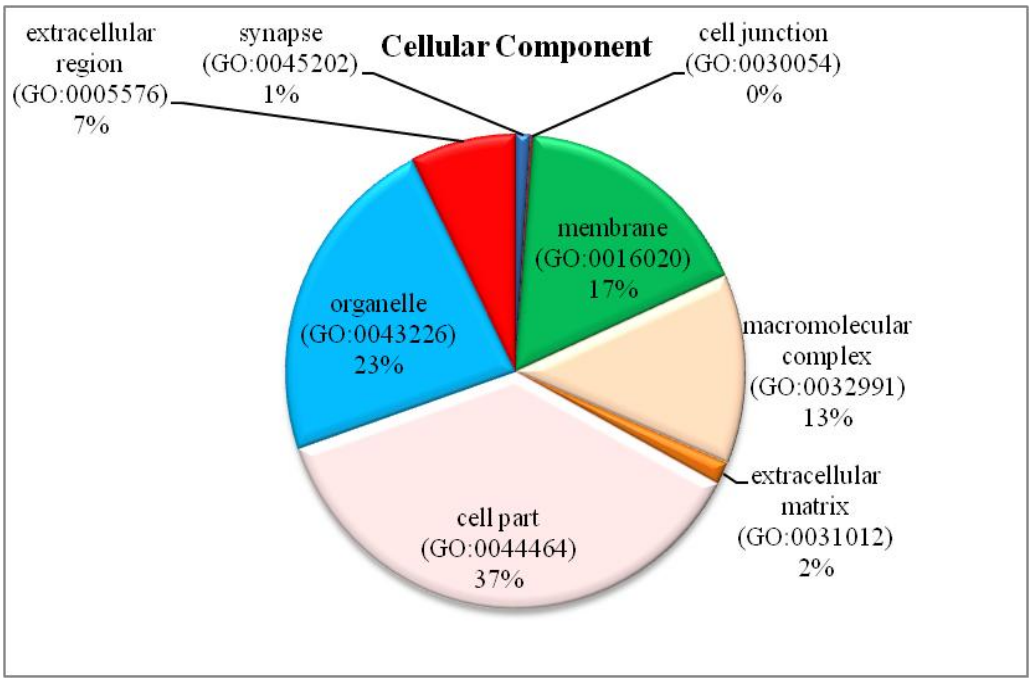
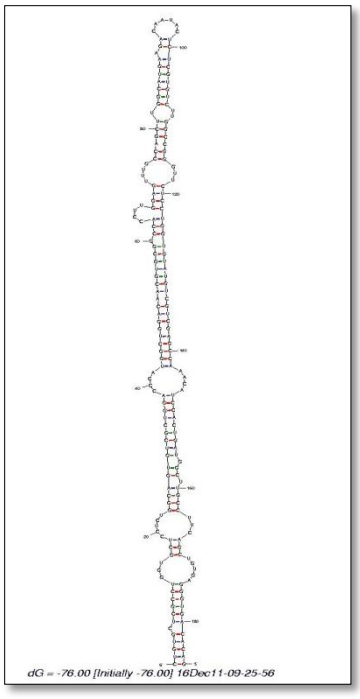


Figure 5: Cellular component analysis of target genes

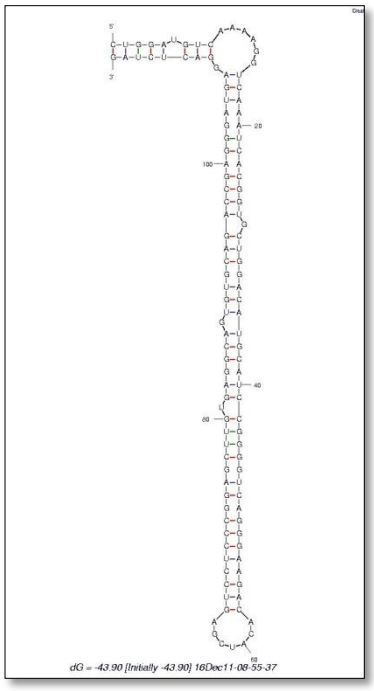
Conclusion

Ebola and Zika virus outbreak in 2014 [49] and 2015 [50] respectively posed a serious problem to world. Kyasanur forest disease virus (KFDV) in India has been outbreak time to time, caused hemorrhagic fever and neurological disturbances [51]. In our study, we have predicted eight mature miRNAs in KFDV genome and their target genes in human. Target gene analysis after data mining and literature support had screen out two genes, ANGPT1 (angiopoietin 1) and TFRC (transferrin receptor). ANGPT1 encodes a secreted glycoprotein, which plays a critical role in reciprocal interaction between endothelium and surrounding matrix, inhibits endothelial permeability and contributes to blood vessel maturation and stability. TFRC encodes a cell surface receptor necessary for cellular iron uptake. This receptor is required for erythropoiesis and neurological development. Both these target genes were predicted to have hybridized with KFDV-MD57-5P mature miRNA.

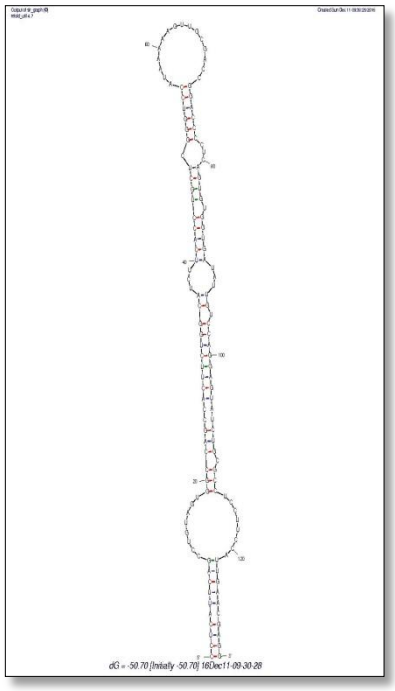
In addition, gene ontology analysis has predicted pathways related to blood coagulation, angiogenesis and axon guidance. Biological processes were related to immune system, response to stimulus and biological adhesion. Cellular processes were involved in synapses. To summarize with, the final two predicted target genes ANGPT1 and TFRC molecular function relates to Kyasanur forest disease etiology. Our work involved in-silico predictions in combination with literature mining to support for predictions results. But it needs to be still verifying by in-vitro designing of these predicted miRNAs constructs and their hybridization with the proposed target genes.



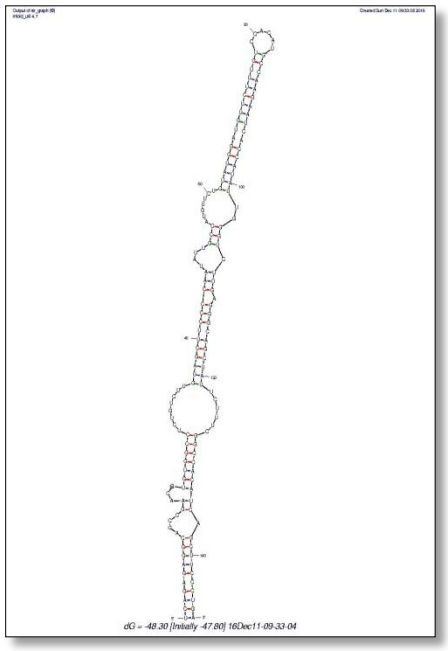
KFDV-MR-9



KFDV-MD-57



KFDV-MR-59



KFDV-MR79

Figure 1: Pre-miRNAs resulted by Mfold

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