"Identification of micro-RNA like elements from transcriptome data of *Aspergillus Flavus*"

A PROJECT

Submitted in fulfillment of the requirements for the award of the degree of

BACHELOR OF TECHNOLOGY

IN

Biotechnology

Under the supervision of

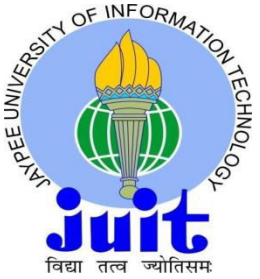
Dr. Jata Shankar

By

PawanVerma 121519

Vinayak Dev 121551

to



JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT SOLAN – 173 234

HIMACHAL PRADESH INDIA

CERTIFICATE

This is to certify that the work which is being presented in the project title **"Identification of micro-RNA like elements from transcriptome data of *Aspergillus Flavus*"** in partial fulfillment of the requirements for the award of the degree of Bachelor of technology and submitted in Biotechnology Department, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by PawanVerma (121519) and Vinayak Dev (121551) during a period from July 2016 to December 2016 under the supervision of **Dr. Jata Shankar,** Assistant Professor, Biotechnology department and **Dr. Tirath Raj Singh**, Assistant Professor, Bioinformatics department, Jaypee University of Information Technology, Waknaghat.

The above statement made is correct to the best of my knowledge.

Date: - 28/05/2016

_____	_____
Dr. Jata Shankar (Guide) Assistant Professor Department of Biotechnology Jaypee University of Information Technology	Dr. Tirath Raj Singh (Co-Guide) Assistant Professor Department of Bioinformatics Jaypee University of Information Technology

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Date: 28th May 2016

Place: JUIT, Waknaghat, *PawanVerma*

Vinayak Dev

SUMMARY

Aspergillus flavus is a sapropyhtic and opportunistic pathogenic fungus whose transcriptome was used to predict miRNA sequences. The sequences for the fungus were retrieved online and BLAST was performed to find out similar matches with already existing miRNA sequences from the reference database used (miRBase). After finding significant matches we used those sequences for prediction of our target genes. Secondary structure prediction and analysis was performed to check the integrity of our miRNA sequences.

After performing the computational task we have to take those miRNA sequences as our potential candidates to validate in wet lab using qRT-PCR. The annealing of the sequence with our primer will confirm the presence of the miRNA sequence.

_____ _____

Signature of Students
Name:PawanVerma
Vinayak Dev
Date: 28th May 2016

Signature of Supervisor
Name: Dr. Jata Shankar

Date: 28th May 2016

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Chapter1. Introduction

Aspergillus flavus is a sapropyhtic and opportunistic pathogenic fungus with a cosmopolitan distribution. It is best known for its colonisation of cereal grains, legumes, and tree nuts. Postharvest rot typically develops during harvest, storage, and/or transit.[1]

It grows by producing thread like branching filaments known as hyphae. A network of hyphae known as mycelium secretes enzymes that break down complex food sources. The resulting small molecules are absorbed by mycelium to fuel additional fungal growth.

A. flavus is unique in that it is a thermotolerant disease, so can survive at temperatures that other diseases cannot. A. flavus can contribute to the storage rots, especially when the plant material is stored at high moisture levels. *A. flavus* grows and thrives in hot and humid climates.[2]*A. flavus* has a minimum growth temperature of 12°C (54°F) and a maximum growth temperature of 48°C (118°F). Though the maximum growth temperature is around 48°C (118°F), the optimum growth temperature is 37°C (98.6°F). *A. flavus* had rapid growth at 30–55°C, slow growth at 12–15°C, and almost ceases growth at 5–8°C.[3]

Aspergillus flavus is ubiquitous in nature, capable of infecting a broad range of host and produces highly toxic metabolites called aflatoxin . Among various types of aflatoxins viz. AFB1, AFB2, AFG1 and AFG2; AFB1 hasbeen found to be most common contaminant of major crop such as corn, peanuts, cotton and other pre/post-harvested crops. Aflatoxins are genotoxic carcinogen in which AFB1 is the most toxic compound classified in 1994 by the International Agency for Research on Cancer (IARC).Regular consumption of low level of aflatoxin through contaminated food products causes aflatoxicosis which is characterized by jaundice, rapidly developing ascites, hypertension, vomiting, abdominal pain, pulmonary edema, fatty infiltration and necrosis of the liver.[11]

It is important because it produces aflatoxin as a secondary metabolite in the seeds of a number of crops both before and after harvest. Aflatoxins are toxic compounds chemically related to bisfuranocoumarin that are produced by *A. flavus* and *A. parasiticus* strains. These two aflatoxigenic species have been frequently studied due to their impact on agricultural

commodities and their devastating effects on livestock. The name “aflatoxin” comes from the genus *Aspergillus*, which is where the letter “a” in aflatoxin is derived and “fla” from the species name *flavus*. In agricultural grains the fungi *A. flavus* and *A. parasiticus* are capable of producing four major aflatoxins(AfB1, AfB2, AfG1, and AfG2). *A. flavus* typically produces only the B toxins.[4]

The effect of temperature on mycotoxin biosynthesis, the *A. flavus* profile was studied under different temperature conditions using RNA seq technology and it was predicted that non-coding RNA may play a role in trancriptional regulation. Also, most of the highly unexpressed genes were involved in aflatoxin biosynthesis. (Jiujiang Yu et. al. 2011).

MicroRNAs (miRNAs) are important regulators of eukaryotic gene expression in most biological processes. They act by guiding the RNAi-induced silencing complex (RISC) to partially complementary sequences in target mRNAs to suppress gene expression by a combination of translation inhibition and mRNA decay. The commonly accepted mechanism of miRNA targeting in animals involves an interaction between the 50-end of the miRNA called the ‘seed region’ and the 30 untranslated region (30-UTR) of the mRNA. Many target prediction algorithms are based around such a model, though increasing evidence demonstrates that targeting can also be mediated through sites other than the 30-UTR and that seed region base pairing is not always required. The power and validity of such in silico data can be therefore hindered by the simplified rules used to represent targeting interactions. Experimentation is essential to identify genuine miRNA targets, however many experimental modalities exist and their limitations need to be understood. This review summarizes and critiques the existing experimental techniques for miRNA target identification.[10]

miRNAs have been extensively characterized in plants and animals. These small RNAs of 21-25 nt originate from endogenousmiRNA genes. They are known to regulate a variety ofprocesses including growth, development and response toabiotic and biotic stresses including during host- microbe interactions. However, to the best ofour knowledge, canonical miRNAs have not been described infungi.[5]

Chapter2. Objectives

There are several small RNA molecules that are instrumental in modulating gene expression levels, out of which miRNAs have the lowest rate of evolution. miRNAs are small (19–22 nucleotides), non-coding, conserved molecules which play a major role in transcriptional and posttranscriptional regulation of gene expression. They have been implicated in growth, development, stress responses, and numerous other biological processes in eukaryotes.[6]

miRNAs have diverse biological functions. Clues about the function of miRNAs in animals have been obtained by several approaches. Several miRNAs were identified by loss- and gain-of-function genetic screens in *C. elegans* and *Drosophila.* [7]

MicroRNAs (miRNAs) control gene expression post-transcriptionally by binding to complementary sequences in target mRNAs, thereby guiding the effector proteins of RNAi-induced silencing complex (RISC) into close proximity with the mRNA. Complete complementarity between miRNA:mRNA pairs is rare in mammals, but as little as a 6 bp match with the target mRNA can be sufficient to suppress gene expression. With the exception of a handful of miRNAs reported to increase expression of a target gene, miRNAs repress gene expression by a combination of mRNA degradation and translation inhibition. They can promote mRNA degradation by either of two mechanisms: direct Argonaute2-catalysed endonucleolytic cleavage of the target, or deadenylation and exonucleolytic attack, which is the predominant mechanism for miRNA activity in mammals. Direct cleavage by Argonaute2 only occurs when there is near perfect complementarity between the miRNA and target mRNA, a situation that occurs much more frequently in plants than in mammals. For a detailed review of miRNA mechanisms of actions see Krol et al.[10]

There have been 28645 entries (Release 21) in the miRBase database. To annotate a newly identified small RNA as miRNA it must fulfill several criteria. First, miRNAs are expressed as ~22-nucleotide RNA molecules. Second, miRNAs are encoded in the genome as phylogenetically conserved hairpin structures with a low free energy. Third, miRNA expression levels are reduced in animals defective in miRNA biogenesis.[7]

The various miRNA target prediction programs, which use different rules of targeting, produce rather different lists of predicted targets. Differences can arise from the source of 30-UTR sequences; Targetscan uses the Ensembl database to define 30-UTRs (28), whereas miRanda uses the University of California Santa Cruz (UCSC) database. This alone manifests in major differences between prediction outcomes. Nevertheless, if the Targetscan algorithm is applied to the two separate 30-UTR databases only a 47% overlap of predicted targets is observed. Similarly if the miRanda algorithm is applied to both 30-UTR databases separately only a 65% overlap is observed. With the identification of genuine miRNA targets lacking a complete 6-mer match and the further complications of RNA structure and RNA-binding proteins affecting site accessibility, many predictions may not be bona fide targets and many genuine targets can be missed. Accordingly, the false positive rate of prediction programs has been variously calculated to be 24–70%. This underscores the requirement for experimental data to demonstrate genuine miRNA targets and miRNA function.The main focus lies in analysing the transcriptome data of *A. flavus* and to detect the role of miRNA in biosynthesis of toxin production and biology of organism. There has been no published experiments which showed identification and characterisation of miRNA in *A. flavus*.[10]

Chapter 3: Data retrieval

The transcriptome data for the fungus was downloaded via ftp from the NCBI.[8]The file downloaded contained the raw data of fungal mycelia which was grown in GMS medium for 24 hours. Raw sequence data generation was carried out by GA II, and the data was filtered and normalized using Illumina pipeline. All reads were then filtered and aligned to the annotated*Aspergillus flavus* strain NRRL3357 genome to calculate expression values for every gene in RPKM using CLC Bio Genomic Workbench with default parameters.

The downloaded file format called SRA is a "raw data" archive, and requires per-base quality scores for all submitted data. Thus, unlike GenBank and some other NCBI repositories, FASTA and other sequence-only formats are not sufficient for submission. The SRA generally prefers to obtain "container files". Container in this context means an unambiguous binary file. These are objects that contain both the data and a description or specification of the data. Examples include BAM, SFF, and PacBio HDF5 formats.

The SRA toolkit was used to convert the raw format into Fastq format which was more preferred. It contained a section of per-base quality scores and Fasta sequence. This conversion was performed using Fastq-dump command in the command line which is generally used to convert SRA to Fastq or other formats.

To convert the Fastq format to Fasta which is a sequence only format we used fastx toolkit which is a linux based program used for conversion and other sequence analysis methods. Using a simple one line command in linux i.e. Fastq_to_Fasta the output was obtained in Fasta format. This format was used in similarity search using Local BLAST program offline. The sequences from miRNA database i.e. miRBase were downloaded and BLAST+2.2.30 program was carried out on linux based system. The commands used to carry out the task are mentioned below

- makeblastdb -in database.txt -dbtypenucl -out databaseformatted.txt
- blastall -p blastn -i 37_16_AUG_SRR283858.fasta -d matura.fa -e 0.001 -o data.txt

The first command was used to create our database to be used for similarity search. The second command was used to for comparison against a miRNA database and the e-value was used as 0.001.

Chapter 4: Results and Discussions

From the list of matches obtained after performing offline BLAST, we filtered only those hits which were most significant.

The e-value in the power of 10^{-7} and 10^{-6} were used to carry out target prediction.

From the large file of miRNA database, we extracted the sequences by matching the respective miRNA ids against the database.

This was achieved using a perl script which scanned and matched miRNA ids against the miRNA database sequence.

The results were stored in a separate document which contained miRNA id on the first line and miRNA sequence on the other.

```
#!/usr/bin/perl

my $idsfile = "id-2.txt";
my $seqfile = "mature.fa";
my $files= "output.fa";
my %ids  = ();

open FILE, $idsfile;
while(<FILE>) {
  chomp;
  $ids{$_} += 1;
}
close FILE;

local $/ = "\n>";  # read by FASTA record

open FASTA, $seqfile;
open OUTFILE,">","output.fa";
while (<FASTA>) {
    chomp;
    my $seq = $_;
    my ($id) = $seq =~ /^>*(\S+)/;  # parse ID as first word in FASTA header
    if (exists($ids{$id})) {
        $seq =~ s/^>*.+\n//;  # remove FASTA header
        $seq =~ s/\n//g;  # remove endlines

    print OUTFILE "$id\n";
    print OUTFILE "$seq\n";
    print OUTFILE "\n";

    }
}
close OUTFILE;
close FASTA;
```

Fig 1: Perl script for sequence extraction

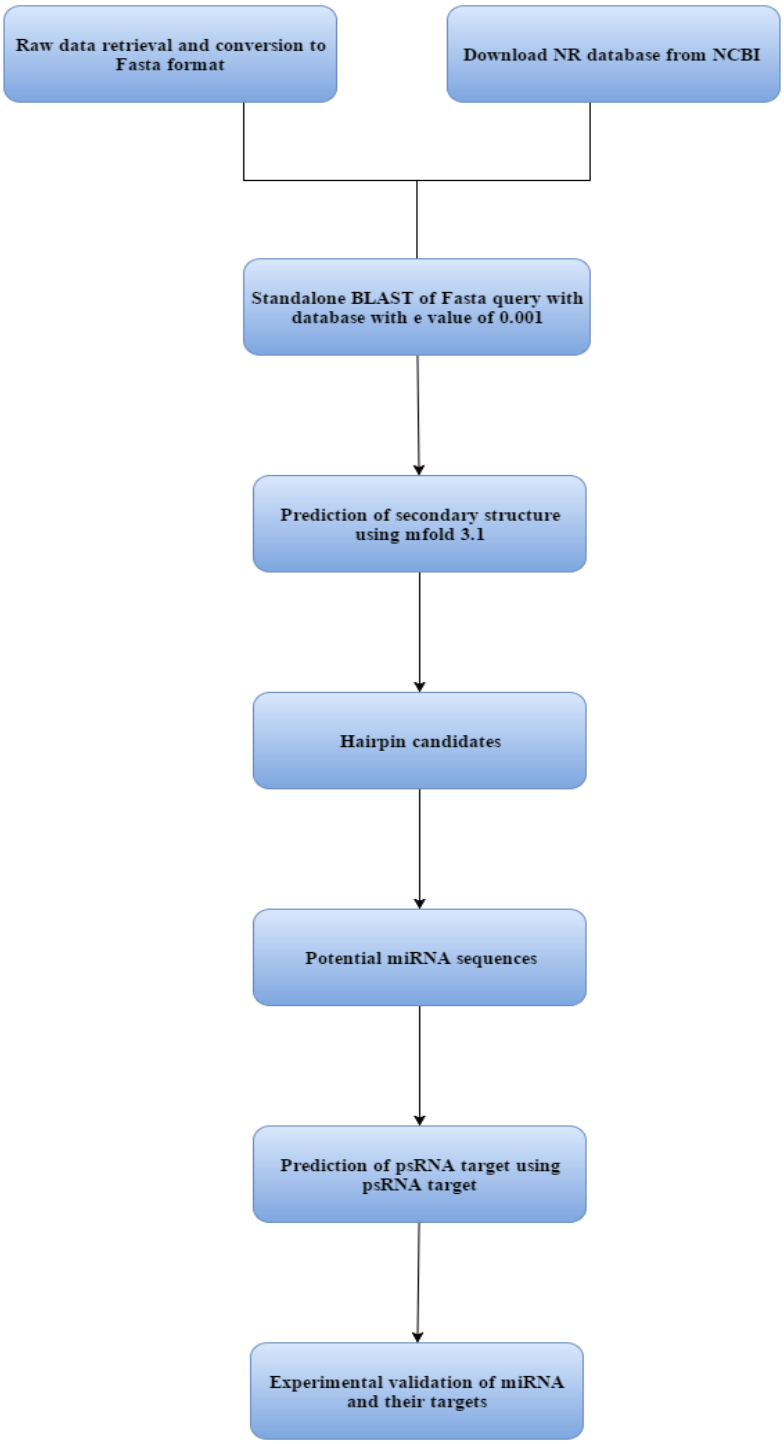

Fig 2. Flowchart showing prediction of miRNA and its targets

4.1 Target prediction using psRNA Target

The online tool for target prediction was used to predict targets for these miRNA sequences givenin Appendix 4.1.

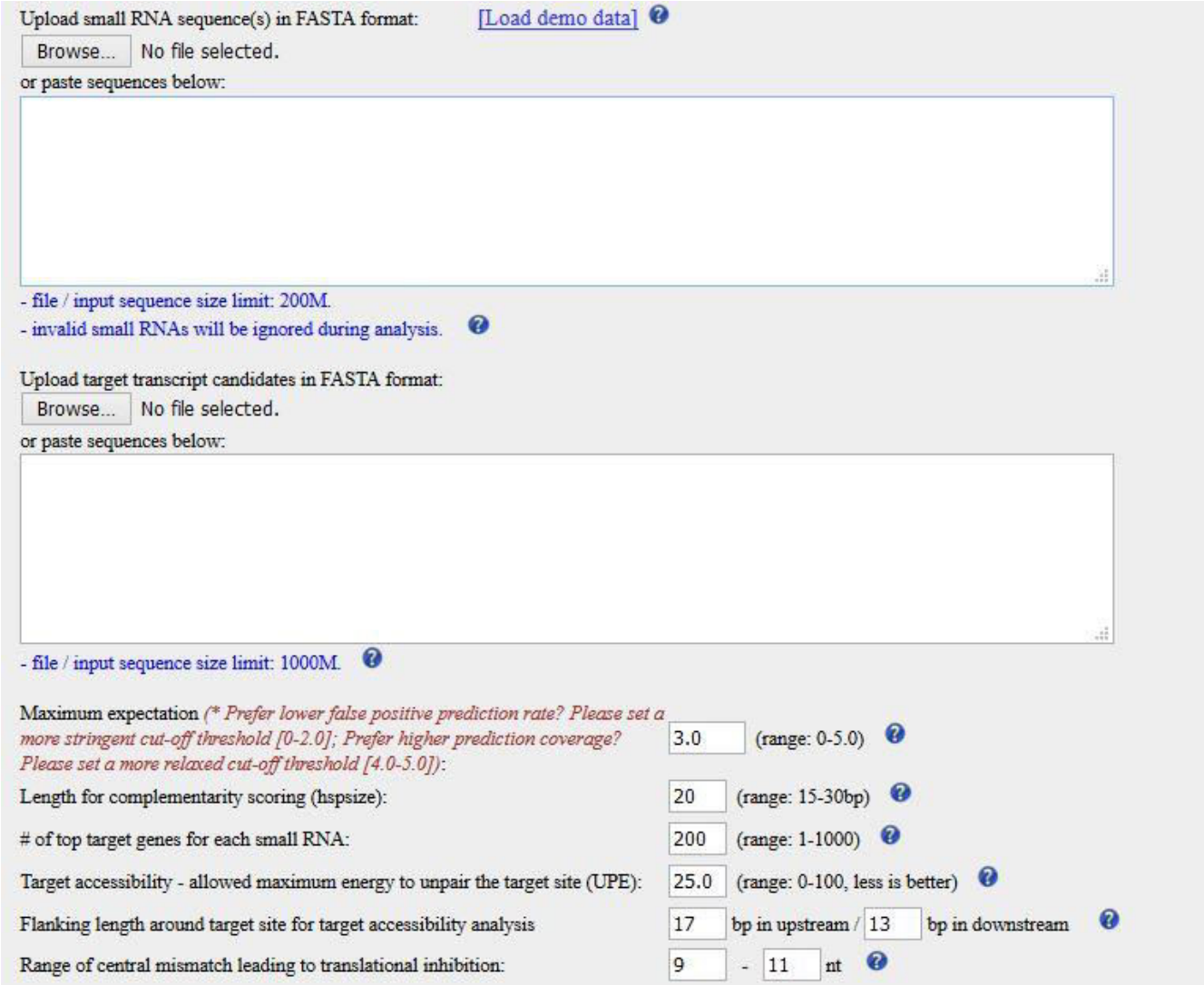

Fig 3: psRNA target server for miRNA target prediction

The sequence file was uploaded which contained the miRNA sequences as well as the reference dataset from miRBase. The parameters were set according to our requirement.

Since we were looking for more stringent results the Maximum expectation was set to 2. The remaining parameters were kept the same as shown in the above figure.

Maximum expectation:

To score the complementarity between small RNA (mainly including miRNA and ta-siRNA, *sic passim*) and their target transcript. The *maximum expectation* is the threshold of the score. A small RNA/target site pair will be discarded if its score is greater than the threshold. The default cut-off threshold is 3.0. Users are advised to set more stringent cut-off threshold [0-2.0] for lower false positive prediction or more relaxed cut-off threshold [4.0-5.0] for higher prediction coverage.

Target accessibility - maximum energy to unpair the target site (UPE):

The accessibility of mRNA target site to small RNA has been identified as one of important factors that are involved in target recognition because the secondary structure (stem *etc.*) around target site will prevent small RNA (including miRNA and ta-siRNA, *sic passim*) and mRNA target from contacting. The psRNATarget server employesRNAup to calculate target accessbility, which is represented by the energy required to open (unpair) secondary structure around target site (usually the complementary region with small RNA and up/downstream) on target mRNA. The less energy means the more possibility that small RNA is able to contact (and cleave) target mRNA.

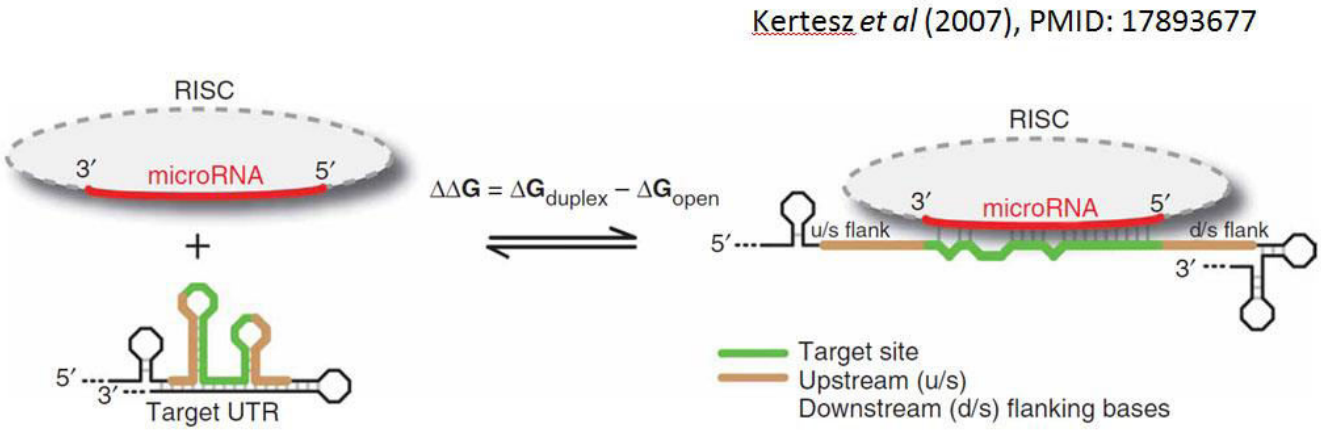

Fig 4: $\Delta\Delta G$ represents the energy that is required to open secondary structure around target site.

Flanking length around target site for target accessibility analysis:

Besides target site (complementary region with small RNA) itself, its two flanks on mRNA are also required to be opened in secondary structure for small RNA's (including miRNA and ta-siRNA, *sic passim*) binding and cleavage (see two red up-arrows in the following figure). The reason is that small RNA binds to target mRNA in the groove of RISC complex which need extra space on two sides of target site. Kertesz*et al* (2007) suggested that 17 upstream and 13 downstream nucleotides of target site should be considered in target accessibility analysis.

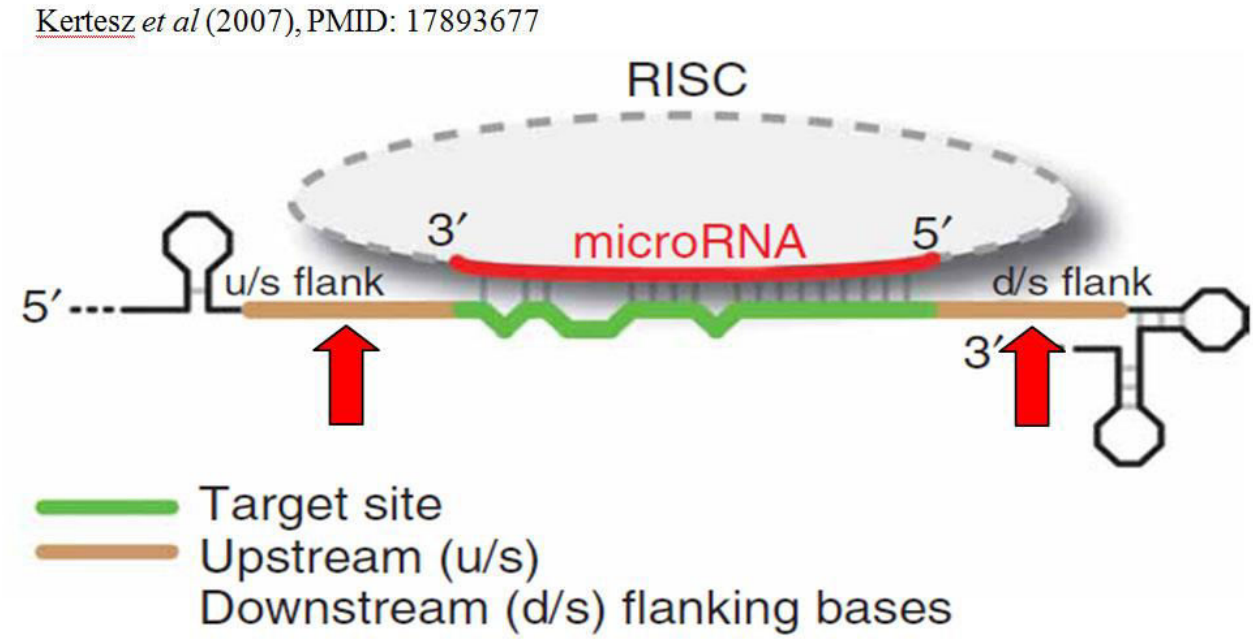

Fig 5: Flanking length around target site for target accessibility analysis

After uploading the sequences to the server , the server generated the target genes for the miRNA sequences. We had selected these six sequences as our potential candidates.

miRNA id	Target Transcript Accession	Target Gene	Target Gene Description	Target gene accession	Query coverage	E-value
hsa-miR-494-5p	37_18_Aug_SRR283858.265157	AFLA_029820	Aspergillus flavus NRRL3357 SAM domain protein, mRNA.	XM_002374451	100%	3.00E-34
mmu-miR-3971	37_18_Aug_SRR283858.131392	TEF 1 alpha	Aspergillus flavus NRRL3357 translation elongation factor EF-1 alpha subunit, putative, mRNA	XM_002380857.1	100%	1.00E-42
prd-miR-1175-5p	37_18_Aug_SRR283858.466790	AFLA_069370	Aspergillus flavus NRRL3357 phosphoglycerate kinase PgkA, putative, mRNA	XM_002380455.1	68%	2.00E-16
aca-miR-301a-5p	37_18_Aug_SRR283858.1347485	AFLA_028150	Aspergillus flavus NRRL3357 endosomal peripheral membrane protein (Mon2), putative, mRNA	XM_002374284	100.00%	3.00E-044
Mmu-miR-3971	37_18_Aug_SRR283858.6210346	AFLA_050040	Aspergillus flavus NRRL3357 DnaJ domain protein, mRNA.	XM_002383090	87.00%	4.00E-022
hsa-miR-1229-5p	37_18_Aug_SRR283858.219023	AFLA_051980	Aspergillus flavus NRRL3357 G-protein complex beta subunit CpcB, mRNA	XM_002383283	95.00%	7.00E-035

Fig 6: psRNA Target results.

Chapter 5: Secondary Structure Prediction of miRNA

The secondary structure prediction job was carried out using mfold 3.1

The RNA and DNA folding servers compute multiple foldings and dot plots for single sequences. The results include images of all computed foldings. Structures may be redrawn to produce more pleasing results.

There are currently five "bulk" servers that produce fast results for many (pairs of) sequences in a single job. Output is limited. The Nucleic Acid Quikfold server folds many sequences at once. The Zipfold server computes minimum free energies only. The Tm server computes minimum free energies together with (two-state) melting temperatures. Similarly, the 2-state hybridization server computes minimum free energies and melting temperatures for the hybridization of pairs of sequences.

It predicts the secondary structure of RNA and DNA, mainly by using thermodynamic methods. Much of his work has been on RNA structure, which is important in understanding many biological processes, including translation regulation in messenger RNA, replication of single-stranded RNA viruses, and the function of structural RNAs and RNA/protein complexes.

5.1 Predicted secondary structures

1)hsa-miR-494-5p

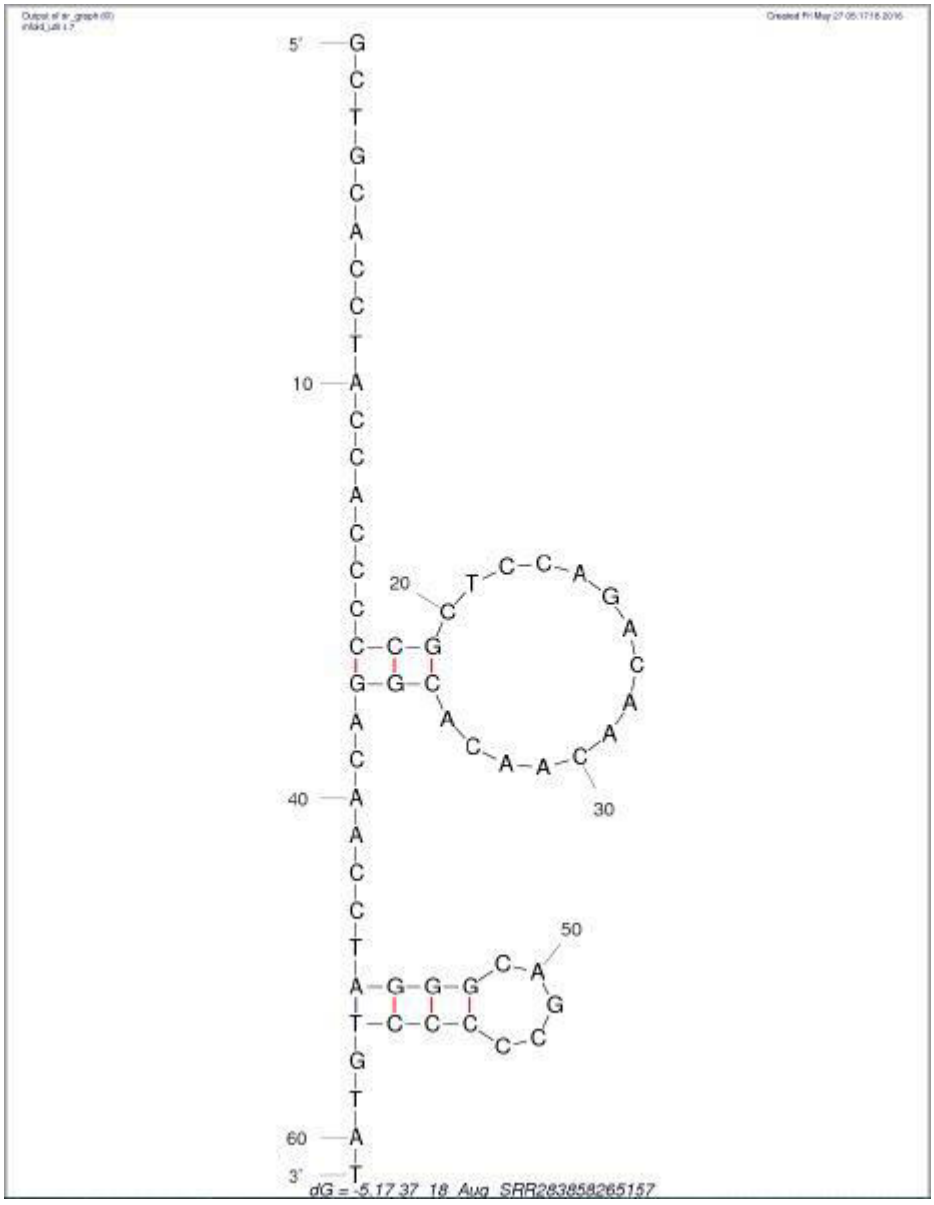

Fig 7: RNA folding form for hsa-miR-494-5p

2) mmu-miR-3971

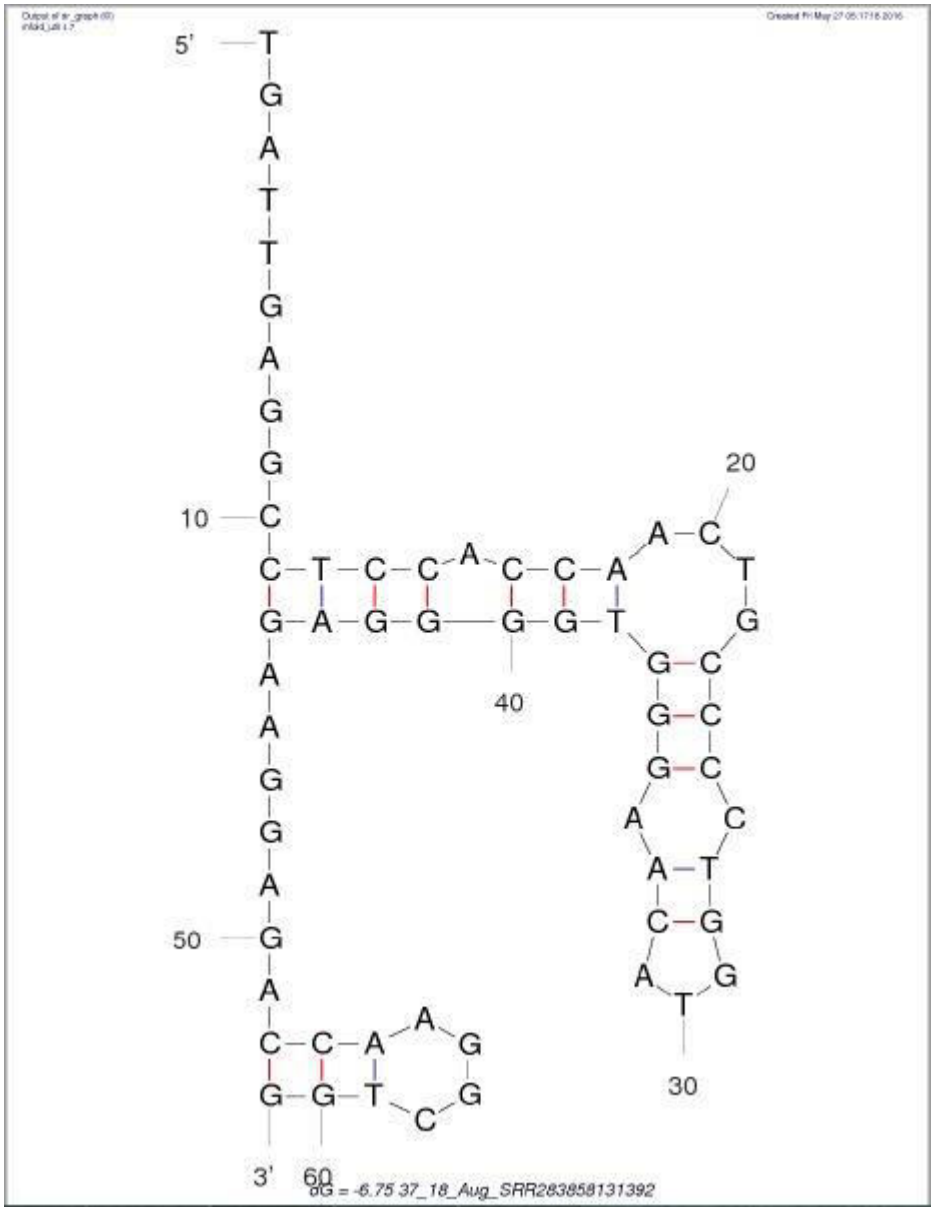

Fig 8: RNA folding form for mmu-miR-3971

3) prd-miR-1175-5p

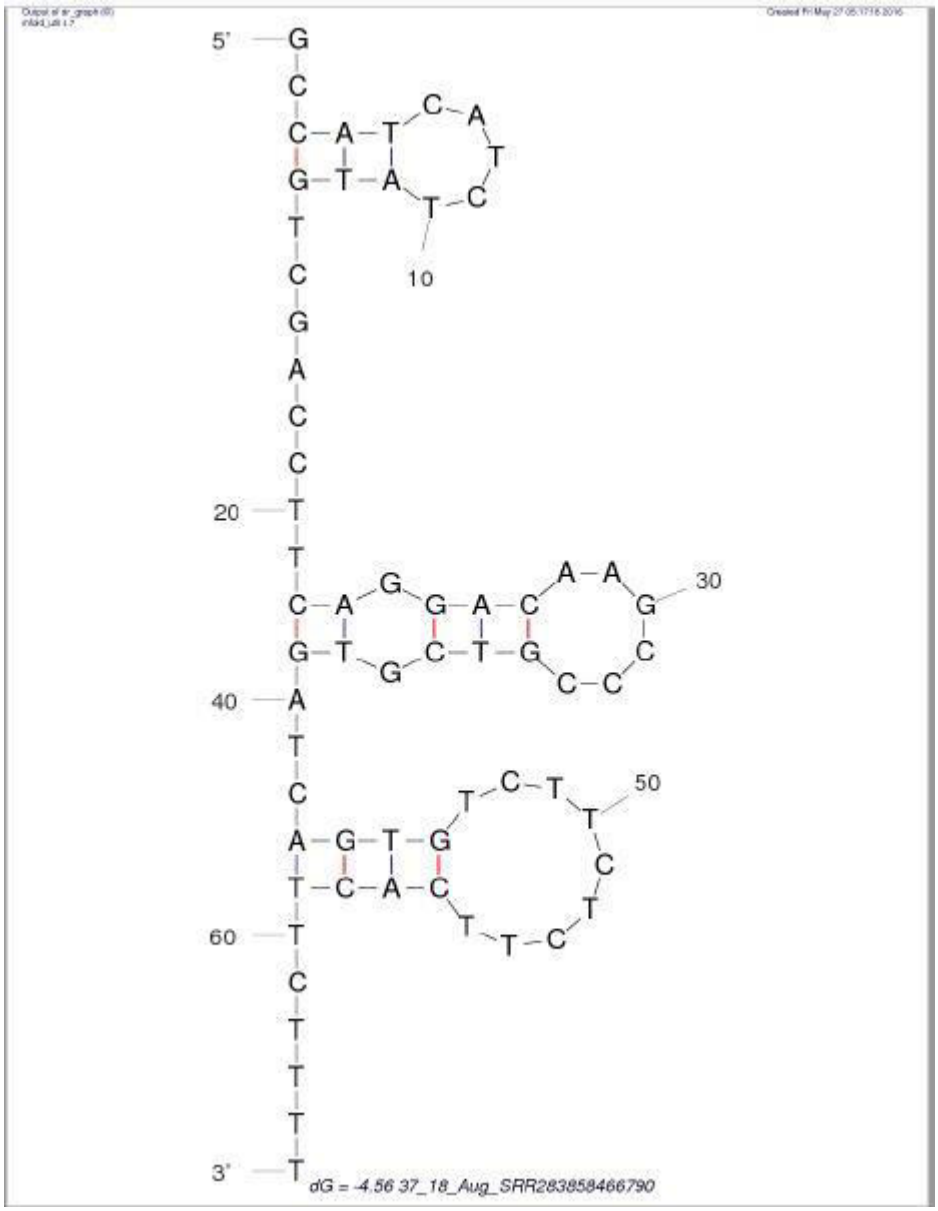

Fig 9: RNA folding form for prd-miR-1175-5p

4) aca-miR-301a-5p

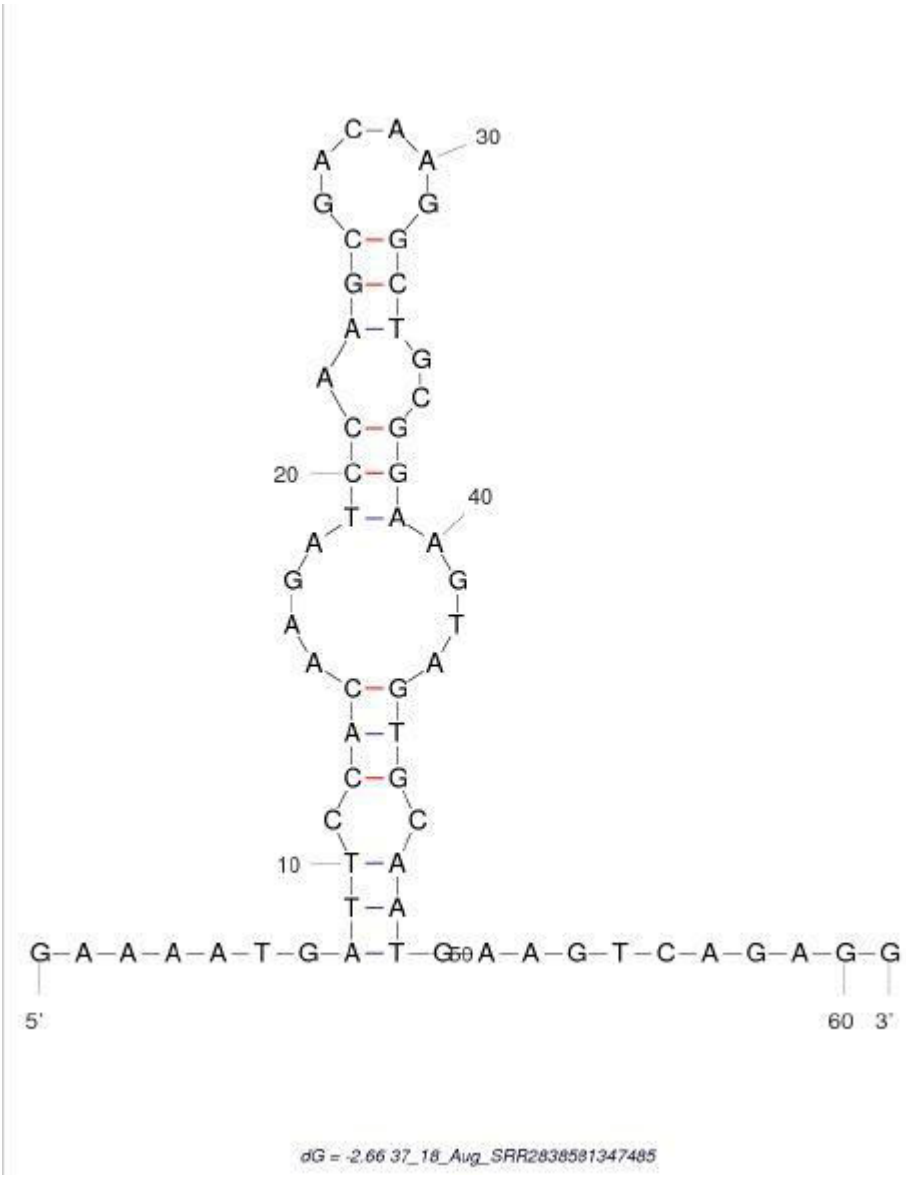

Fig 10: RNA folding form for aca-miR-301a-5p

5) Mmu-miR-3971

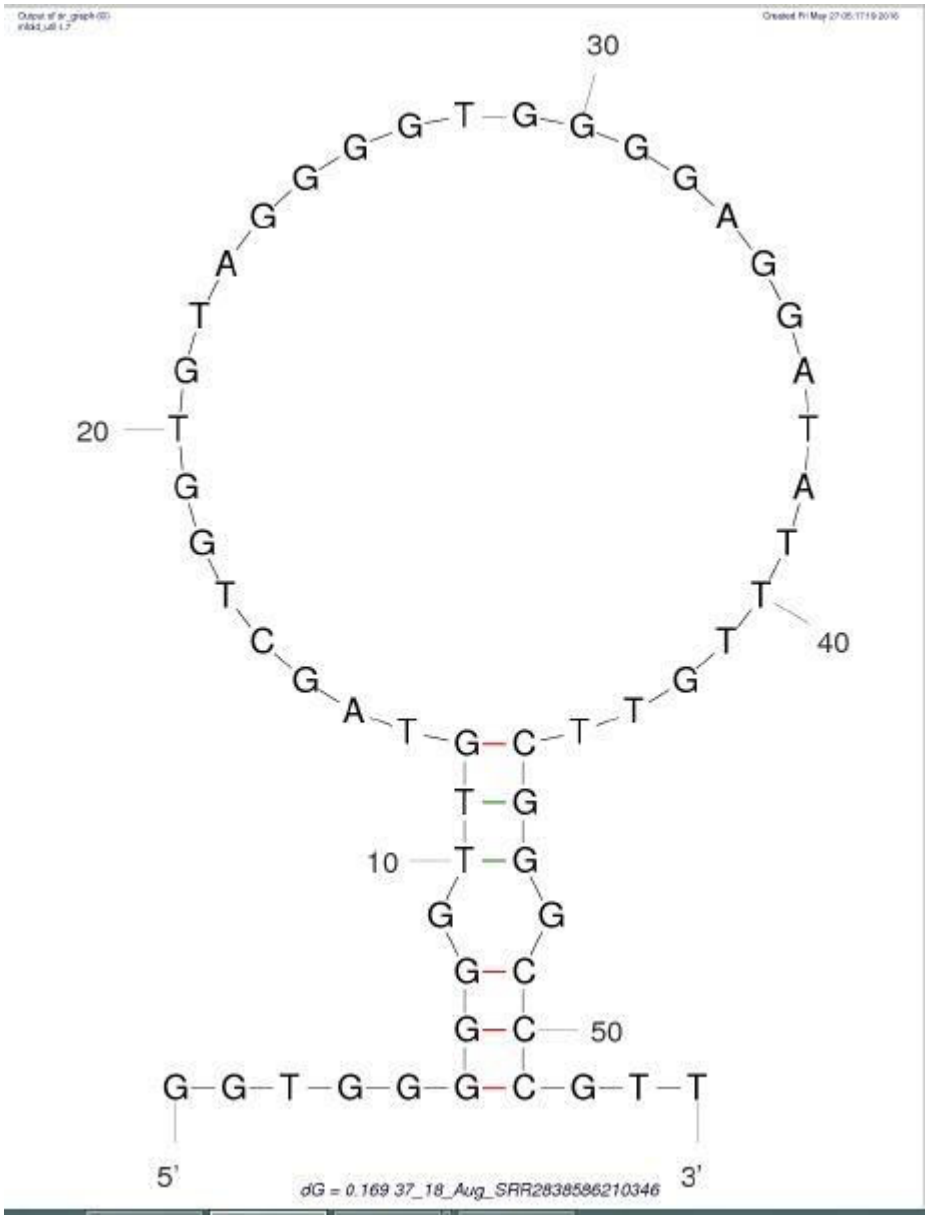

Fig 11: RNA folding form Mmu-miR-3971

6) hsa-miR-1229-5p

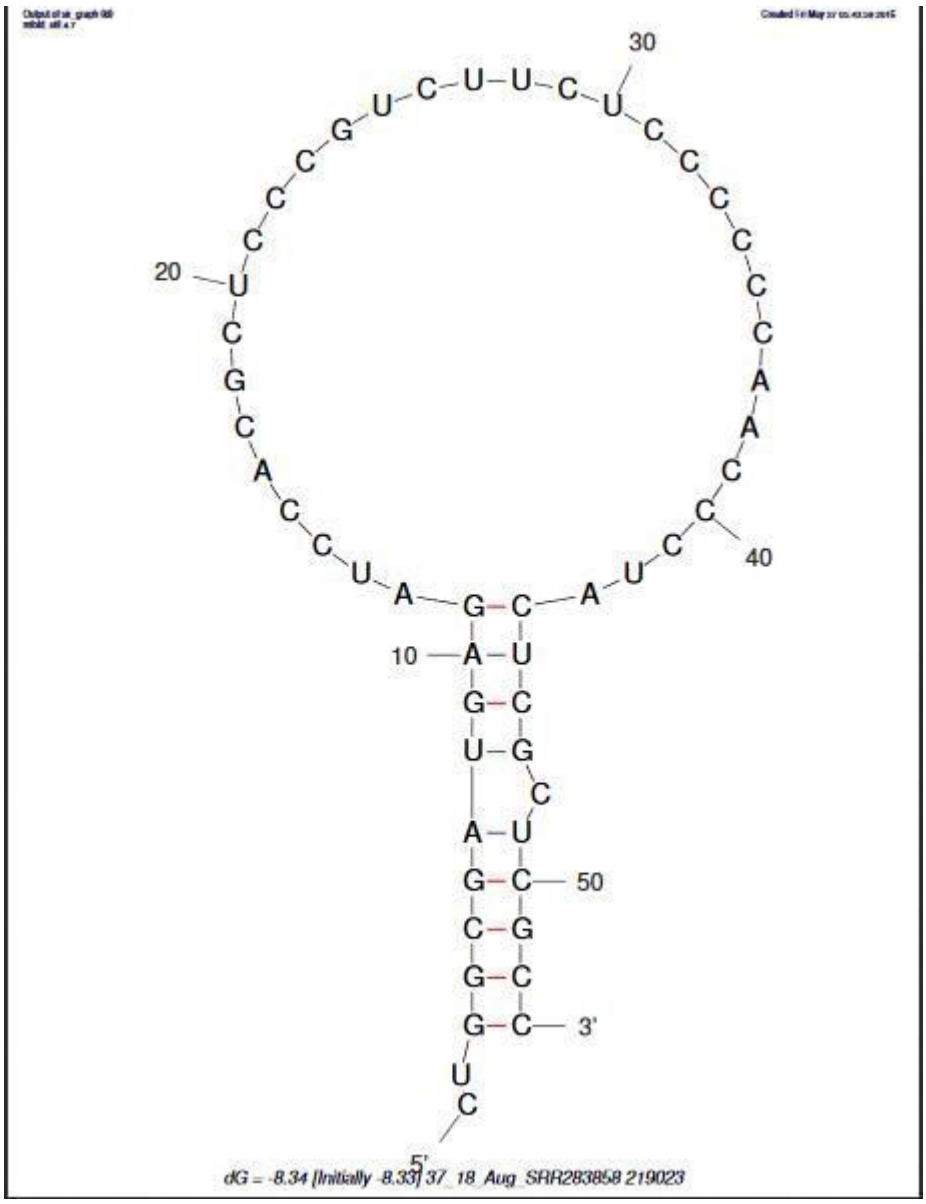

Fig 12: RNA folding form hsa-miR-1229-5p

Chapter 6: Wet Lab Work

For validation of our miRNA sequences, the culture was grown for RNA extraction.

The amount of solution taken has been mentioned in appendix 5.

REVIVAL OF FUNGAL CULTURE AND PREPARATION OF SLANTS FOR STORAGE

Media Used: PDA

Protocol:

- 50 ml PDA media was prepared and poured in test tubes for autoclaving at 121 °C at 15 psi for 15 minutes.
- Slants were made by pouring media into the tubes and keeping them inclined, and were allowed to cool.
- Fungal cultures maintained at 4°c were collected from the cell repository.
- Cultures were kept at room temperature to bring them to normal temperature.
- Slants of PDA were inoculated by simple streaking.
- Slants were incubated at 37°C for 72 hours.
- Slants were observed after growth.

MEDIA PREPARATION FOR RNA EXTRACTION

Media Used: PDA

Protocols:

- 250ml Potato Dextrose Broth was prepared and autoclaved at 121°C at 15 psi for 15min.

- Broth was inoculated using inoculation loop, taking inoculum from slants.
- Flasks were kept at 37°C, shaking incubation for 48 hours.
- Flasks were observed after incubation.

Fig 13: Aspergillus Flavus: media after incubation

HOMOGENIZATION OF FUNGAL CULTURE FOR RNA EXTRACTION

Material Used: Fungal culture, Liquid nitrogen.

Theory: Quick freezing of the culture with liquid nitrogen and grinding using mortar and pestle allows homogenization of cells. It is the cryogenic grinding method for the cell disruption. The liquid nitrogen is when poured on the sample and grinded using mortar and pestle, the sample shatters into pieces. Due to biohazardous nature of liquid nitrogen gloves are worn. The sample is stored in chill Eppendorf for the further experimentation.

Protocols:

- Flasks were taken out from the incubator.
- With the help of autoclaved muslin cloth, mycelium was filtered out, in the LAF hood.
- Filtered mycelium was poured in mortar and pestle.

- Liquid nitrogen was poured in the mortar.
- Using pestle cells were crushed and homogenized.
- The powdered mycelium was stored in autoclaved Eppendorf at 0°C.

RNA EXTRACTION

Reagents and chemical used:TRIzol reagent, 75% ethanol, Chloroform, Isopropanol, DEPC.

Theory: Also called as Guanidine-thiocyanate-phenol-chloroform-extraction method.The method relies on phase separation by centrifugation of a mixture of the aqueous sample and solution containing water saturated phenol and chloroform.The role of various components of RNA isolation is as follows:

- TRIzol Reagent: The TRIzol Reagent is used for isolation of total RNA from the cells. It is a mixture of guanidine thiocyanate and phenol which dissolves out the RNA on homogenization. TRIzol reagent maintains the integrity of RNA while it disrupts the cell and components. Chloroform causes proteins to become denatured and become soluble in the organic phase or interphase, while nucleic acids remain in the aqueous phase.
- Phenol and chloroform: Proteins are structured with hydrophobic residues at their core and hydrophilic residues at their surfaces. In a hydrophobic solution, the protein structure actually averts, denaturing the proteins and destroying their functionbecause it solvates the hydrophobic interiors of proteins.
- Isopropanol: In the presence of cations, high concentrations of ethanol/isopropanol exclude water atoms from the phosphate backbone of RNA molecule allowing cations to form phosphate backbone, forcing RNA molecule to precipitate out of the solution.
- DEPC: Diethyl pyrocarbonate is used to inactivate RNase enzyme in water and utensils in laboratory required for RNA isolation.

Protocols:

- (1ml/50-100mg culture) TRIzol Reagent was added in to the homogenized culture.
- Incubated for 5min at RT to permit complete dissociation of Nuclear membrane.
- Added 0.2ml of chloroform per ml of TRIzol reagent under fume hood, tube was closed.
- Tube was tapped by finger for 15sec and incubated at RT for 2-3min.
- Samples were centrifuged at 12000g for 15 min at 4°C.
- The upper aqueous phase was transferred to the fresh tube.
- Isopropyl alcohol (0.5ml per ml of TRIzol) was added.
- Incubated sample at RT for 10min.
- Centrifuged at 12000g for 10 min at 4°C.
- Supernatant was discarded.
- RNA pellet was washed with 75% alcohol.
- Centrifuged at 7500g for 5min at RT.
- Supernatant was discarded.
- The above three steps were repeated thrice.
- Pellet was obtained at the bottom.
- RNA pellet was dried at RT for 10-20min.
- For storage DEPC water was added in 1:5 and stored at -80°C.

VISUALIZATION OF RNA USING GEL ELECTROPHORESIS

Theory:RNA being negatively charged moves towards the positively charged side and is visualized by mixing gel with EtBr that intercalate in between the base pairs and make it appear as orange colored in UV light. TAE buffer here provides the free ions for the movement of RNA and agarose is a matrix, where porosity can be changed depending upon the concentration and thereby separation depending upon the size of the sample. Loading dye is used to keep track on RNA and to make it settle in the wells while loading.

- Gel was prepared using TAE buffer, 0.6 gm in 50ml of TAE buffer and heated till the agarose dissolves.
- Gel was cool down till 45-55°C and then 5 µl of EtBr was added to the gel.
- Gel was poured slowly in the casting tray with the comb on and was left for solidification.
- After solidification of gel comb was removed slowly and gel apparatus was flooded with TAE buffer till it covered the surface and the gel got dipped.
- Sample were mixed with loading dye (5:1) respectively and loaded into the wells.
- Gel was run for 60mins at 100V.

- Gel after run was slowly removed from the apparatus and visualized under Gel Doc sy.

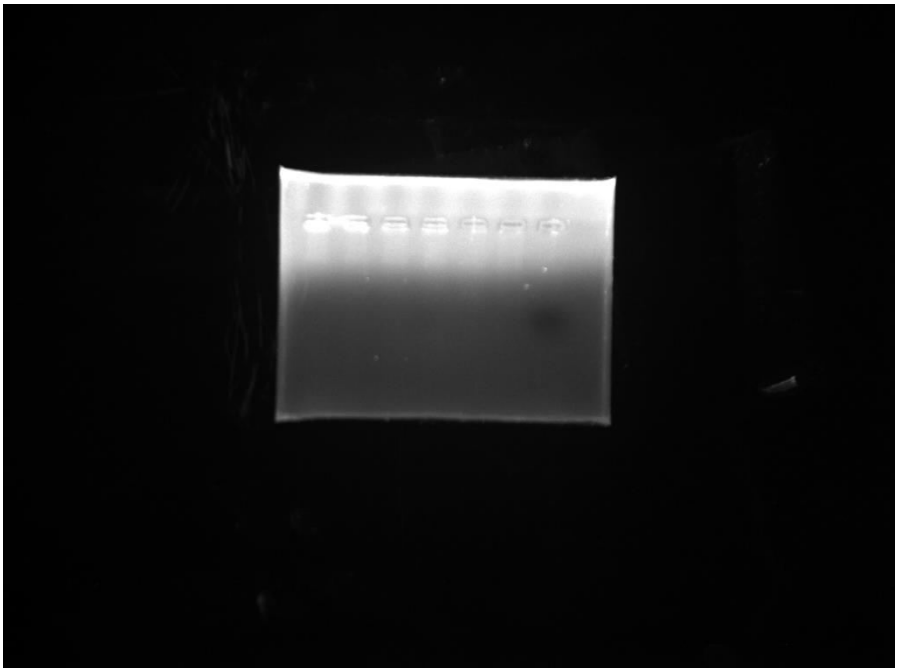

Fig 15: RNA under Gel Doc Sy

Chapter 7: Future Work and Conclusions

After proper RNA extraction and quality analysis of bands using Gel electrophoresis, the miRNA sequences will be used as forward primers for validation of presence of potential sequences. This is achieved by qRT-PCR which will check the annealing of the primer sequences with that of its downstream complimentary sequence. Successful binding will confirm the presence of the miRNA sequence.

This is done using NCode miRNA First-Strand cDNA Synthesis and qRT-PCR Kits.

Following isolation of total RNA, all the miRNAs in thesample are polyadenlyated using poly A polymerase andATP. Following polyadenylation, SuperScriptR III RT and aspecially-designed Universal RT Primer are used tosynthesize cDNA from the tailed miRNA population.The first-strand cDNA is ready for analysis in qPCR usingSYBRR Green or SYBRR GreenER™ detection reagents, theUniversal qPCR Primer provided in the kit, and a forwardprimer designed by the user that targets the specific miRNA sequence of interest.

Workflow Diagram:

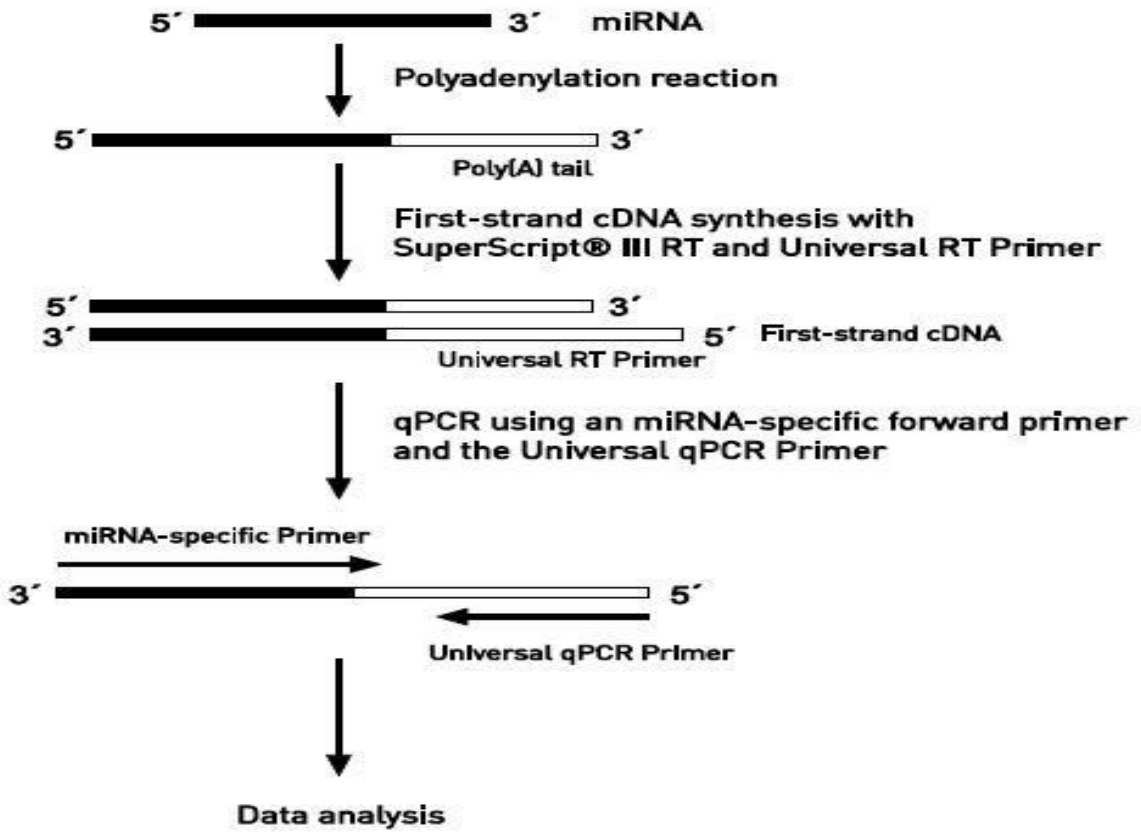

Fig 14: qRT-PCR workflow

The forward primer in qPCR is specific for the miRNA sequence of interest. As a starting point, we recommend ordering a DNAoligo that is **identical to the entire mature miRNA sequence**.

For example, note the following primer design for themiRNA hsa-miR-124a:

miRNA sequenceuuaaggcacgcggugaaugcca

Primer sequencettaaggcacgcggtgaatgcca

In most cases, using an oligo that is identical to the entiremature miRNA is optimal. In some cases, truncating theprimer sequence may be necessary

Conclusion:

MicroRNAs (miRNAs) are a recently discovered class of small, ~19–23-nucleotide non-coding RNA molecules. They are cleaved from hairpin precursors and are believed play an important role in translation regulation of target mRNAs by binding to partially complementary sites in the 3´untranslated regions (UTRs) of the message.

Several groups have hypothesized that there may be up to 20,000 non-coding RNAs that contribute to eukaryotic complexity.

Though hundreds of miRNAs have been discovered, little is known about their cellular function. They have been implicated in regulation of developmental timing and patternformation (Lagos-Quintana *et al.*, 2001), restriction ofdifferentiation potential (Nakahara &Carthew, 2004),regulation of insulin secretion (Stark *et al.*, 2003), and genomicrearrangements (John *et al.*, 2004).Several unique physical attributes of miRNAs—includingtheir small size, lack of poly-adenylated tails, and tendency tobind their mRNA targets with imperfect sequencehomology—have made them elusive and challenging tostudy. In addition, strong conservation between miRNAfamily members means that any detection technology mustbe able to distinguish between ~22-base sequences thatdiffermby only 1–2 nucleotides. Recent advances in microarray andqPCR detection have enabled the use of these technologies formiRNA screening.

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Appendix

Appendix 1: miRNA Sequences taken for target prediction using psRNA target.

>mmu-miR-150-5p
UCUCCCAACCCUUGUACCAGUG

>hsa-miR-150-5p
UCUCCCAACCCUUGUACCAGUG

>rno-miR-150-5p
UCUCCCAACCCUUGUACCAGUG

>dre-miR-150
UCUCCCAAUCCUUGUACCAGUG

>hsa-miR-494-5p
AGGUUGUCCGUGUUGUCUUCUCU

>xtr-miR-150
UCUCCCAACCCUUGUACCAGAG

>bta-miR-150
UCUCCCAACCCUUGUACCAGUGU

>tae-miR1134
CAACAACAACAAGAAGAAGAAGAU

>oan-miR-150-5p
UCUCCCAACCCUUGUACCAGAG

>mml-miR-150-5p
UCUCCCAACCCUUGUACCAGUG

>mml-miR-494-5p
AGGUUGUCCGUGUUGUCUUCUCU

>cfa-miR-150
UCUCCCAACCCUUGUACCAGUG

>ptr-miR-150
UCUCCCAACCCUUGUACCAGUG

>mdo-miR-150-5p
UCUCCCAACCCUUGUACCAGAGU

>eca-miR-150
UCUCCCAACCCUUGUACCAGUG

>ppy-miR-150
UCUCCCAACCCUUGUACCAGUG

>mmu-miR-3971
CUCCCCACCCCUGUACCAGUGA

>aca-miR-150-5p
UCUCCCAACCCUUGUACCAGUG

>aca-miR-301a-5p
GCUCUGACUUCAUUGCACUACU

>ggo-miR-150
UCUCCCAACCCUUGUACCAGUG

>sac-miR-150
UCUCCCAACCCUUGUACCAGUG

>ipu-miR-150
UCUCCCAAUCCUUGUACCAGUG

>oar-miR-150
UCUCCCAACCCUUGUACCAGUG

>prd-miR-1175-5p
AAGUGAAGAGAAGAAUCUGACU

Appendix 2:

1) **TAE**

For each litre of solution:

242 g **Tris Base** (MW=121.1)

57.1 mL **Glacial Acetic Acid**

10 mL 0.5 M **EDTA**

Mixed Tris with stir bar to dissolve in about 600 mL of ddH2O.

Added the **EDTA and Acetic Acid**, pH to 8.0.

Brought final volume to 1 L with ddH2O.

Stored at room temperature.

2) **PDB media composition**: Potato dextrose Broth composition

~1000 **water**

200 **potatoes**

(sliced washed unpeeled)

20 **dextrose**

Agarose is 1.2%

BIODATA

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