Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0404.08**

IN SILICO IDENTIFICATION OF miRNA AND THEIR TARGET PREDICTION FROM BOLIVIAN HAEMORRHAGIC FEVER VIRUS (MACHUPO VIRUS)

Swati Srivastava[1], Ashok Kumar Saxena[2], Aayesha Farooqui[1, 3], Mahvash Ayeshah Hashmi[3], Krati Srivastava[1], Vijay Laxmi Saxena[1]*

1. National Bioinformatics Infrastructure Facility Centre of (DBT), Dept. Of Zoology (Govt. of India), D.G (PG) College Civil Lines, Kanpur.
2. Asutosh Mookerjee fellow of ISCA and Department of Zoology DAV Degree College Civil Lines, Kanpur.
3. Department of Biotechnology D.G (PG) College Civil Lines, Kanpur.

ABSTRACT: MicroRNAs are one of the most important molecules that regulate gene expression in organisms. These are short having 22~23 nucleotide which is bind untranslated region to 3' of their target mRNAs. It is important to understand to predict MiRNA target by computational approaches for getting accurate result or effect on the regulation of gene expressions. Many computational methods have been adopted for miRNA prediction by computational approaches. To understand the effect of miRNA regulation of genes. It has been focused on predicting the accurate target genes for miRNAs and considerable number of methods has been identified the method to diversify from small-scale genetic method and high-throughput biochemical processes for target sequence isolation to computational prediction methods. Inside the cell there are several features that regulate the target recognition of miRNAs. The first and the most recognized feature is the sequence specificity of miRNAs to 3' UTR of mRNAs

KEYWORDS: miRNA, SiRNA, RNAi, Target prediction, Gene expression.

Corresponding Author: Dr. Vijay Laxmi Saxena* Ph.D.
National Bioinformatics Infrastructure Facility Centre of (DBT), Dept. Of Zoology (Govt. of India), D.G (PG) College Civil Lines, Kanpur, India.
Email Address: vlaxmisaxena@gmail.com

© 2018 Life Science Informatics Publication All rights reserved
Peer review under responsibility of Life Science Informatics Publications

1. INTRODUCTION

Mature microRNAs (miRNAs) occur naturally, small non-coding RNA molecules, their length is about 21-23 nucleotide. miRNAs regulates several biological processes such as stem cell maintenance apoptosis, development timing, metabolism, cardiac skeleton muscle proliferation & host viral infection. Encoded by eukaryotic nuclear DNA in plants and animals and by viral DNA in certain viruses and this genome is based on DNA, miRNAs functions through base-pairing in association with complementary sequences within miRNA. The first miRNA was discovered in the early 1990s [1, 2]. miRNA endogenously – initiated short RNA molecules [3]. These RNA molecules play crucial rule in the gene regulation of nematodes insects and plants. [4] .Key role of miRNAs is post transcriptional regulation of gene expression [5]. Biosynthesis of miRNA has several steps, biosynthesis of miRNA starts in nucleus and generate primary transcripts (pri-miRNAs) by RNA Polymerase. This pri-miRNA get shorten into pre miRNA (miRNA precursors) by the enzyme of Drosha, which is an RNAase III like enzyme [6]. This miRNA precursor export in the cytoplasm by the function of Exportin -5 protein in cytoplasm[7] where it is cleaved by the Dicer and become mature miRNA[8] and incorporated into the RISC (RNA –Induced Silencing Complex) and makes a RISC miRNA complex is guided to target sequence in mRNA. [9,10]. The initial step of recognition of target mRNA done by RISC-miRNA complex base pairing between miRNA :mRNA involved 8 base pair of miRNA sequence binds with specific mRNA sequence which is located in 3' UTR region. These is binding inhibits translation of mRNA and degradation of mRNA.[11,12] MiRNAs are similar to the interfering RNAs (siRNAs) of interference (RNAi) pathway, except miRNAs derive from regions of RNA transcripts that fold back on themselves to form short hairpins, whereas siRNAs derives from longer regions of double-stranded RNA. MiRNAs are well conserved in both plants and animals, and are thought to be a vital and evolutionarily ancient component of gene regulation. Other than core components of the microRNA pathway are stored between plants and animals. MiRNA collected in the two kingdoms appear to have emerged independently with different primary modes of action. [1,13]. Machupo virus (MACV), which belongs to the Arenaviridae family, is the etiological. Agent of Bolivian Hemorrhagic Fever (BHF). BHF was first identified in human. Patients in Bolivia from Beni district in 1959. More than 1,000 cases of BHF, of which 180 were fatal, were reported during an outbreak from 1962 to 1964. Starting in 2006, after a long hiatus no cases reported, the number of cases steadily increased more than 200 in 2008. Bolivian haemorrhagic fever (BHF) is a potentially severe febrile illness caused by Machupo virus (family Arenaviridae). Initially headache occurs then fever, arthralgia, and myalgia. In the later stages of this illness, patients may develop hemorrhagic manifestations including subconjunctival hemorrhage, epistasis, hematemesis, melena, and hematuria, as well as neurological signs including tumour, seizures, and coma1[14]. Our objective was insilico identification of miRNA and their target prediction from Bolivian Haemorrhagic Fever

© 2018 Life Science Informatics Publication All rights reserved

virus (machupo virus).

2. MATERIALS AND METHODS

The genome sequence of Machupo Virus has been taken from NCBI [21]. Precursor were find out by the precursor predicting tool Mireval[15,22] & mirPARA were used to find out the precursor sequences. and then the secondary structure with optimal minimum free energy was found out with the help of different types of web servers such as Mfold web server 16 23]. RNA fold web server [17,24] for validating the energy values, miRBase [18,25] was checked, these sequences were already identified experimentally. Then we find out the optimal minimum energy value of viruses in the miRBASE (only precursors), based on multiple sequence alignment, and then find out the conserved regions by CLUSTAL W or CLUSTAL OMEGA for miRNA [19]. This miRNA predicted the target sites, and the target prediction was completed with the help of DIANA tar web server [20 , 26]. It predicted the different types of target sites.

3. RESULTS AND DISCUSSION

Precursor Analysis

miRNA was first to be discovered miRNA is a non-coding and is a component of gene regulation it is either produced from own genes or by introns. miRNA are intergenic. It is transcribed as ~70 nucleotide precursors and sub-sequently processed by dicer enzyme. The precursor analysis is totally based on insilico firstly to be finding out the precursors from machupo virus single gene sequence retreive from NCBI and then it is submitted to mireval software for prediction of precursors. (Supplementary data Table no. S1). These predicted precursor sequences then submitted to RNAfold web server for evaluating its secondary structure with its optimal minimum free energy (Supplementary data Table No. S2).

Potential miRNA Analysis

Potential miRNA can be analysing by the action of MSA (multiple sequence alignment) .these are also known as mature miRNA. Prediction of potential miRNA was completed with the help of EBI Clustal-Omega and mireval predicting software. The sequence is submitted to EBI which is retrieve from miRBases. And then MSA was done between the sequences of viruses and Machupo Virus. MSA mainly focused on conserved region. (Supplementary data Table no.S3).

Target prediction analysis

It is totally based on observation.in this procedure potential mirna predicted the different types of target sites in human gene. Then it predicted different type of target site and gene and those involved in different types of target sites with conserved region or conserved information. (Supplementary data Table no. S4). target sites Hnrnpa3, Zbtb20, Tenm1, Uggt1, Fam120c played an important role in the regulation of central nervous system and its activity in human brain and body (table 1).

© 2018 Life Science Informatics Publication All rights reserved

Table No. 1 Information of target genes

S.no.	Name of gene	Ensembl gene ID.	miTG score
1.	Hnrnpa3	ENSMUSG00000059005	0.999420361038364
2.	Zbtb20	ENSMUSG00000022708	0.999327102122872
3.	Tenm1	ENSMUSG00000016150	0.995495756295998
4.	Uggt1	ENSMUSG00000037470	0.989629388937483
5.	Fam120c	ENSMUSG00000025262	0.98613903099963

4. CONCLUSION

Finally in this study, thirteen potential miRNAs were predicted insilico and many target sites were found approx 1000 and above in many genes out of these genes including target sites Hnrnpa3, Zbtb20, Tenm1, Uggt1, Fam120c played an important role in the regulation of central nervous system and its activity in human brain and body. it is shown that if it is damaged it could lead to haemorrhagic fever and ultimately death. So, if we can control the malfunction protein at the transcription level, we will be able to save patients suffering from the attack of Machupo Virus. Since this is, a prediction based method. It has to be proved experimentally.

5. ACKNOWLEDGEMENT

I wish to thank Dr. (Mrs.) Vijay Laxmi Saxena coordinator of B.I.F.C critically examining the manuscript and giving of valuable guidance. And I also thank DBT for providing financial assistance

6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Saxena VL, Dwivedi A. In silico identification of miRNAs and their target prediction from Japanese encephalitis. Journal of Bioinformatics and Sequence Analysis. 2013 Feb 28; 5(2):25-33.
2. Betel D, Wilson M, Gabow A, Marks DS, Sander C. The microRNA. org resource: targets and expression. Nucleic acids research. 2008 Jan; 36(suppl_1):D149-53.
3. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. Genomics, proteomics & bioinformatics. 2009 Dec 31; 7(4):147-54.
4. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003 Dec 26; 115(7):787-98.
5. van der Burgt A, Fiers MW, Nap JP, van Ham RC. In silico miRNA prediction in metazoan genomes: balancing between sensitivity and specificity. BMC genomics. 2009 Dec; 10(1):204.
6. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003 Sep; 425(6956):415.

© 2018 Life Science Informatics Publication All rights reserved

7. Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004 Jan 2; 303(5654):95-8.
8. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. 2001 Jan; 409(6818):363.
9. Carrington JC, Ambros V. Role of microRNAs in plant and animal development. Science. 2003 Jul 18; 301(5631):336-8.
10. Kurihara Y, Watanabe Y. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. Proceedings of the National Academy of Sciences. 2004 Aug 24; 101(34):12753-8.
11. artel DP. MicroRNAs: target recognition and regulatory functions. cell. 2009 Jan 23; 136(2):215-33.
12. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nature reviews Molecular cell biology. 2005 May; 6(5):376.
13. Padmashree D, Swamy NR. Computational identification of putative miRNAs and their target genes in pathogenic amoeba Naegleria fowleri. Bioinformation. 2015;11(12):550.
14. Koma T, Huang C, Aronson JF, Walker AG, Miller M, Smith JN, Patterson M, Paessler S. The ectodomain of glycoprotein from the candid# 1 vaccine strain of Junín virus rendered Machupo virus partially attenuated in mice lacking IFN-αβ/γ receptor. PLoS neglected tropical diseases. 2016 Aug 31;10(8):e0004969.
15. Ritchie W, Théodule FX, Gautheret D. Mireval: a web tool for simple microRNA prediction in genome sequences. Bioinformatics. 2008 May 3;24(11):1394-6.
16. mFold web server: Michael Zuker , Nick Markham; (1955). A web tool for miRNA folding; SUNY Albany Research IT Group.
17. Tempel S, Tahi F. A fast ab-initio method for predicting miRNA precursors in genomes. Nucleic acids research. 2012 Feb 22;40(11):e80-.
18. Griffiths-Jones S. The microRNA registry. Nucleic acids research. 2004 Jan 1;32(suppl_1):D109-11.
19. Kumar S, Nei M, Dudley J, Tamura K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in bioinformatics. 2008 Jul 1;9(4):299-306.
20. Reczko M, Maragkakis M, Alexiou P, Grosse I, Hatzigeorgiou AG. Functional microRNA targets in protein coding sequences. Bioinformatics. 2012 Jan 27; 28(6):771-6.
21. (www.ncbi.nlm.nihgov)
22. (http://tagc.univmrs.fr/mireval)
23. (http://mfold.rit.albany.edu/doc/mfold-manual/)
24. (http://rna.tbi.univie.ac.at)
25. (mirbase@manchester.ac.uk)
26. (http://www.microrna.gr)
© 2018 Life Science Informatics Publication All rights reserved

SUPPLEMENTARY DATA

Table. No. S1 -: Predicted Precursor miRNA from miReval web sever

S.no	Annotated Precursor name	Precursor Sequence	M.F.E.
1.	>mir_MACV_P1	UUCCCGUGGAGCCAAGUGCCCCCCCACCAUGAACAUCCCCAAACUCGGUCCACAAGACCCCCGGGGGGCCCCAACGGGGCCCCCA	(-31.20)
2.	>mir_MACV_P2	GCCAAGUGCCCCCCCACCAUGAACAUCCCCAAACUCGGUCCACAAGACCCCCGGGGGGCCCCAACGGGGCCCCCACGGGGGAUCC	(-40.00)
3.	>mir_MACV_P3	GAACAUCCCCAAACUCGGUCCACAAGACCCCCGGGGGGCCCCAACGGGGCCCCCACGGGGGAUCCCCGGGGGUCUUGGGCUGCGU	(-53.40)
4.	>mir_MACV_P4	AAACUCGGUCCACAAGACCCCCGGGGGGCCCCAACGGGGCCCCCACGGGGGAUCCCCGGGGGUCUUGGGCUGCGUCACUCAAUGU	(-54.10)
5.	>mir_MACV_P5	CACAAGACCCCCGGGGGGCCCCAACGGGGCCCCCACGGGGGAUCCCCGGGGGUCUUGGGCUGCGUCACUCAAUGUCCUCCACUUG	(-49.50)
6.	>mir_MACV_P6	CCGGGGGGCCCCAACGGGGCCCCCACGGGGGAUCCCCGGGGGUCUUGGGCUGCGUCACUCAAUGUCCUCCACUUGGGGCGUGCUU	(-47.30)
7.	>mir_MACV_P7	GGUCUUGGGCUGCGUCACUCAAUGUCCUCCACUUGGGGCGUGCUUGCCAGGGGUCUACACAUCACACCCUUCAGUCUGAAUGACC	(-23.10)
8.	>mir_MACV_P8	UAUGUGGACAGUCUCUCACCUCCCACAUAGAAGGCACCAUCCUUCAAGACCAACGGGUCUUCUUGGUUUUCAGGUCCCGAGAGCC	(-23.70)
9.	>mir_MACV_P9	CAACGGGUCUUCUUGGUUUUCAGGUCCCGAGAGCCUGUAGGAAUAUUUGUCACUCAACUCUGGGGAAAAUCCACCUAAAUCCAAG	(-20.40)
10.	>mir_MACV_P10	UCUUGGUUUUCAGGUCCCGAGAGCCUGUAGGAAUAUUUGUCACUCAACUCUGGGGAAAAUCC	(-21.50)

© 2018 Life Science Informatics Publication All rights reserved

		ACCUAAAUCCAAGGUAACAUUUC	
11.	>mir_MACV_P11	CCCGCUAUCCAUUGUUCUGCUGACCUGAAGA UCAUUCAUAAAUGGAGUCAAGUGUUCGUCAA AAAGAACUGGAUAAUUUCUCCUU	(-17.20)
12.	>mir_MACV_P12	CUCACCACUUUAGGCUUCACAGCCCAAAAAA AGUUUUGGUUGUUCAACACCAAUGGCUUUUG GGGCUCUUUAACUGUGGAAAACA	(-21.20)
13.	>mir_MACV_P13	UUUGUGAAGUUUCUGGAAAGUUUGGAUUGC AAGGUUUUAAUUAAAGUUGGCACUUCCUCCC UUUCAAGAACUCUUCUACUUGUCA	(-18.80)
14.	>mir_MACV_P14	AAUUGUGUGGGUGUUUUACACUUUACAUUGU GUAAAGCUGCAGCAACAAACUUUGUGAGCAA UGGUGUUUCUUCACCCAUGACAA	(-23.00)
15.	>mir_MACV_P15	CAAGAUCAAUUUUUGACCUGUCAACAGGGGU UCUCAAUUCCAGCAUUUGUAAUAAUGCCAGG AAAAGUGCAGGACUCAUUGUUGG	(-24.70)
16.	>mir_MACV_P16	UUUUGACCUGUCAACAGGGGUUCUCAAUUCC AGCAUUUGUAAUAAUGCCAGGAAAAGUGCAG GACUCAUUGUUGGACCCCAUUUU	(-23.00)
17.	>mir_MACV_P17	UCAACAGGGGUUCUCAAUUCCAGCAUUUGUA AUAAUGCCAGGAAAAGUGCAGGACUCAUUGU UGGACCCCAUUUUGAGUGAUCAU	(-23.40)
18.	>mir_MACV_P18	GUUGGACCCCAUUUUGAGUGAUCAUAAGAAC AUGACAAAUCACCAUUAUUCACUGCCAUCUU CAUGUCACAAAUGGCCCGUUCAA	(-16.20)
19.	>mir_MACV_P19	AGUUGGAACUAAUGCUUGUGAACUCAGUGGG CGAAUCUUCAGAAUAAAAACACAGAUUCCUU AAUGCGGCAUUGGUGAAGUAACU	(-17.30)
20.	>mir_MACV_P20	AACUCAGUGGGCGAAUCUUCAGAAUAAAAAC ACAGAUUCCUUAAUGCGGCAUUGGUGAAGUA ACUUUUAUCCAACUUGUCUGCAA	(-16.40)
21.	>mir_MACV_P21	GUUGAAUCUGGAUCUAGAUUGUACCUUCCUU UGGUUUUAAACCUCUCUGCCAUAGAGGAAAC ACAUGCUGACACUAGUUUUUCCU	(-15.90)

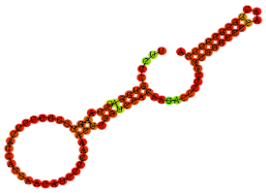
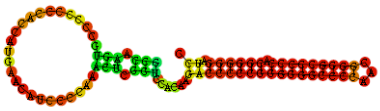
© 2018 Life Science Informatics Publication All rights reserved

22.	>mir_MACV_P22	UUGGUUUUAAACCUCUCUGCCAUAGAGGAAACACAUGCUGACACUAGUUUUUCCUUGUCAUAGGUGGAGAGGAUGUUGCCUAACU	(-25.20)
23.	>mir_MACV_P23	ACCUCUCUGCCAUAGAGGAAACACAUGCUGACACUAGUUUUUCCUUGUCAUAGGUGGAGAGGAUGUUGCCUAACUCAUCUAGUUU	(-23.50)
24.	>mir_MACV_P24	UUCCUUGUCAUAGGUGGAGAGGAUGUUGCCUAACUCAUCUAGUUUUGGUAUAACAACACUCUUGUUACUUGAUAAGUCCAAAGCA	(-19.30)
25.	>mir_MACV_P25	UAACUCAUCUAGUUUUGGUAUAACAACACUCUUGUUACUUGAUAAGUCCAAAGCAGUACUUGUUAUAUUAGGUCUAAAGGGAUUA	(-16.60)
26.	>mir_MACV_P26	CUUAGAAAAUUCUCUCUCAUUCACAAGCAAUCUUGAAGAAAAGGAUGUCUUCAUAGAAUUGAUUAGUGACAAGGAUAUUGAGGAC	(-16.00)
27.	>mir_MACV_P27	UCACAAGCAAUCUUGAAGAAAAGGAUGUCUUCAUAGAAUUGAUUAGUGACAAGGAUAUUGAGGACAAAGUCUUGAAUAACCUCAU	(-21.60)
28.	>mir_MACV_P28	AAGGAUGUCUUCAUAGAAUUGAUUAGUGACAAGGAUAUUGAGGACAAAGUCUUGAAUAACCUCAUUUGCUCAAAGCUAGACAUCU	(-23.50)
29.	>mir_MACV_P29	AACAAUUUAUGUCUACAAAUCUUAUCAGAGCCUCUUUCCACUGAAUACCUCAUAAUUGGUCUAUCUGAGACAAGAUGUUGGGCUA	(-12.90)
30.	>mir_MACV_P30	GUCUACAAAUCUUAUCAGAGCCUCUUUCCACUGAAUACCUCAUAAUUGGUCUAUCUGAGACAAGAUGUUGGGCUAUUGAAAUCAA	(-12.80)
31.	>mir_MACV_P31	UGAAGUCUGGUUGCUUUAUCAAAGUGCUUAAGUGAUGCCAUAUUUCUGGAUGUGAUCUCUCUAGAAUUUUUGAACAAUUCUUGAC	(-11.90)
32.	>mir_MACV_P32	GUUUACUCUUACAAAGGUCUCCAGCAGAAUUAGGGUUUUCCCAGUUAAUUUGAACCCAUCGGGGAUCACCUUUGGUAAAGUUGGA	(-18.50)
33.	>mir_MACV_P33	UCAGUGUUUGCCUGGCAGGCGUGUGCUUCACAGGACUCAAGUUCAACAAGACUAGAGAGCAA	(-23.70)

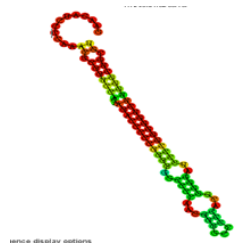
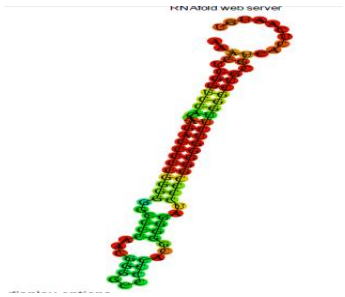
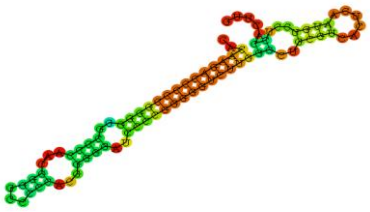
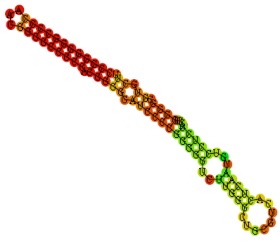
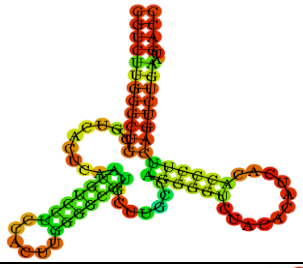
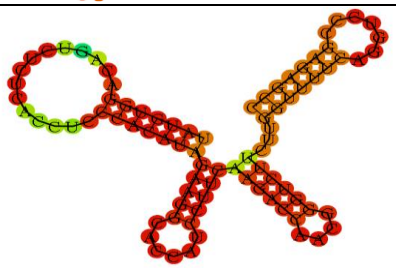
© 2018 Life Science Informatics Publication All rights reserved

		CUUGAAGCCUUCUGUGAGCAAGG	
34.	>mir_MACV_P34	GUGUGCUUCACAGGACUCAAGUUCAACAAGA CUAGAGAGCAACUUGAAGCCUUCUGUGAGCA AGGGACUGGGAUGCACCUGAGAU	(-21.70)
35.	>mir_MACV_P35	CAAGGGACUGGGAUGCACCUGAGAUAGGAAU GUGACCUUUUGAUGUCCAAACUCGCACCUCU CAGGAAUGUGCUUUCUAAUAAGC	(-22.20)
36.	>mir_MACV_P36	GUGUCGCCUAGGAUCCACUGUGCGCACUGGC CGUCGUUUUACACGCCUUCACCACAGCACAU CCAAACUUAACGACGGCCUAGGA	(-25.64)
37.	>mir_MACV_P37	GGAUCCACUGUGCGCACUGGCCGUCGUUUUA CACGCCUUCACCACAGCACAUCCAAACUUAA CGACGGCCUAGGAUCCACUGUGC	(-24.04)
38.	>mir_MACV_P38	UGCGCACUGGCCGUCGUUUUACACGCCUUCA CCACAGCACAUCCAAACUUAACGACGGCCUA GGAUCCACUGUGCG	(-21.44)

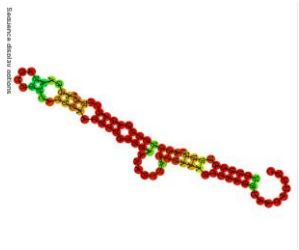
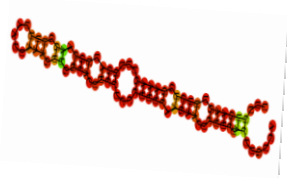
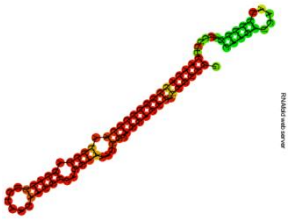
Table No. S2 - The optimal secondary structure of precursor sequence with optimal minimum energy by RNA fold web server

S.no	**Annotated precursor name**	**Secondary structure of precursor sequence**	**Optimal minimum free energy (kcal/mol)**
1.	>mir_MACV_P1		-31.40 kcal/mol
2.	>mir_MACV_P2		-39.10 kcal/mol

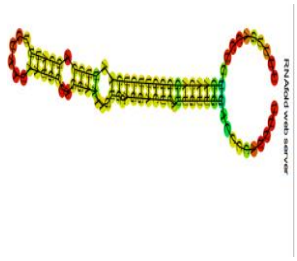
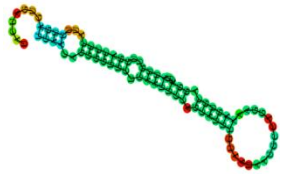
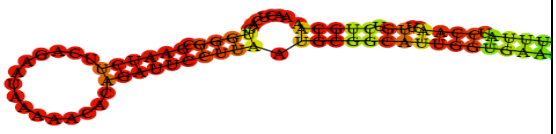
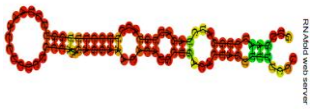
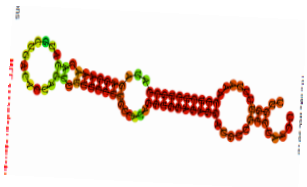
© 2018 Life Science Informatics Publication All rights reserved

3.	>mir_MACV_P3		-53.40 kcal/mol
4.	>mir_MACV_P4		-53.80 kcal/mol
5.	>mir_MACV_P5		-48.40 kcal/mol
6.	>mir_MACV_P6		-44.70 kcal/mol
7.	>mir_MACV_P7		-22.30 kcal/mol
8.	>mir_MACV_P8		-24.00 kcal/mol

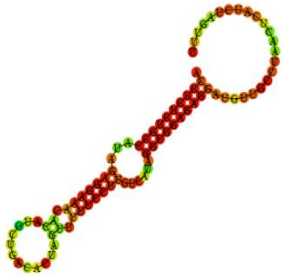
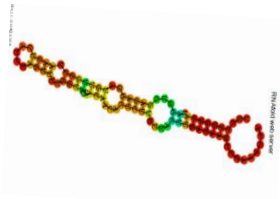
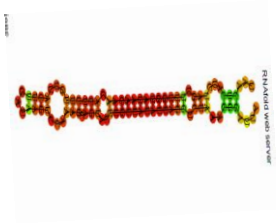
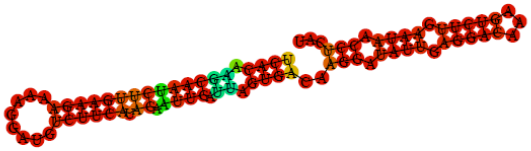
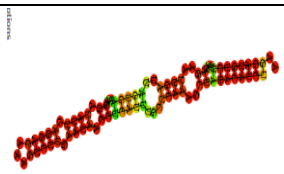
© 2018 Life Science Informatics Publication All rights reserved

9.	>mir_MACV_P9		-20.50 kcal/mol
10.	>mir_MACV_P10		-22.00 kcal/mol
11.	>mir_MACV_P11		-17.50 kcal/mol
12.	>mir_MACV_P12		-20.80 kcal/mol
13.	>mir_MACV_P13		-19.30 kcal/mol
14.	>mir_MACV_P14		-23.10 kcal/mol
15.	>mir_MACV_P15		-24.20 kcal/mol

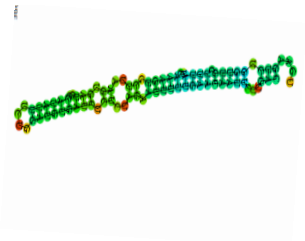
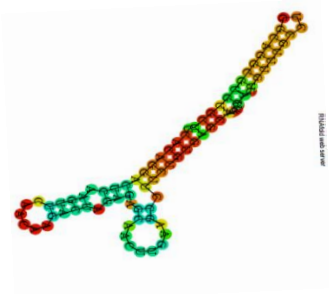
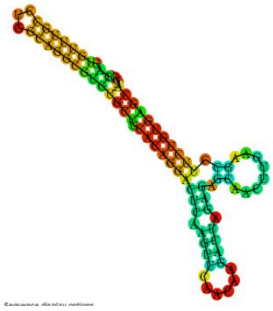
© 2018 Life Science Informatics Publication All rights reserved

16.	>mir_MACV_P16		-23.00 kcal/mol
17.	>mir_MACV_P17		-23.50 kcal/mol
18.	>mir_MACV_P18		-16.00 kcal/mol
19.	>mir_MACV_P19		-17.30 kcal/mol
20.	>mir_MACV_P20		-15.90 kcal/mol
21.	>mir_MACV_P21		-15.80 kcal/mol
22.	>mir_MACV_P22		-25.70 kcal/mol
23.	>mir_MACV_P23		-23.10 kcal/mol

© 2018 Life Science Informatics Publication All rights reserved

			
24.	>mir_MACV_P24		-19.10 kcal/mol
25.	>mir_MACV_P25		-16.20 kcal/mol
26.	>mir_MACV_P26		-15.80 kcal/mol
27.	>mir_MACV_P27		-21.70 kcal/mol
28	>mir_MACV_P28		-23.50 kcal/mol
29	>mir_MACV_P29		-13.40 kcal/mol

© 2018 Life Science Informatics Publication All rights reserved

30.	>mir_MACV_P30		-13.40 kcal/mol
31.	>mir_MACV_P31		-11.60 kcal/mol
32.	>mir_MACV_P32		-22.10 kcal/mol
33.	>mir_MACV_P33		-22.10 kcal/mol
34.	>mir_MACV_P34		-21.50 kcal/mol
35.	>mir_MACV_P35		-22.50 kcal/mol

© 2018 Life Science Informatics Publication All rights reserved

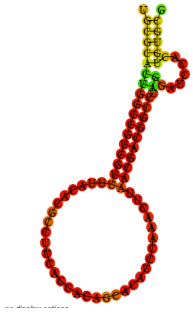
36.	>mir_MACV_P36		-26.74 kcal/mol
37.	>mir_MACV_P37		-24.54 kcal/mol
38.	>mir_MACV_P38		-21.84 kcal/mol

Table No. S3 -: The target miRNAs with their mature miRNA sequences

S.no	Name of target miRNA	mature miRNA sequence
1.	rno-miR-125b-2-3p	acaagucaggcucuugggaccu
2.	gga-miR-125b-3p	acaagucaggcucuugggaccu
3.	mmu-miR-3082	cacauggcacucaacucugcag
4.	hsa-miR-8087	gaagacuucuuggauuacagggg
5.	mmu-miR-125b-2-3p	acaagucagguucuugggaccu
6.	mmu-miR-137-5p	acggguauucuuggguggauaau
7.	rno-miR-137-5p	acggguauucuuggguggauaa
8.	mmu-miR-802-3p	acggagagucuuugucacucag
9.	mmu-miR-1966-3p	uuucugacucaacucucccuuag
10.	gga-miR-137-5p	acggguauucuuggguggauaaua
11.	dre-mi4R-137-5p	acggguauucuuggguggauaaua
12.	hsa-miR-125b-2-3p	ucacaagucaggcucuugggac
13.	dme-miR-1012-3p	Uuagucaaagauuuuccccauag

© 2018 Life Science Informatics Publication All rights reserved

Table No. S4 -: Target miRNA showing target sites

s.no.	Target Micro RNA	Name of a gene	UTR 3 & binding type	Position on chromosome	Binding area	Conservation information
1.	mmu-miR-1966-3p	**Hnrnpa3**	123-148 9mer	2:75665506-75665531	(Transcript)5'CAG AAC U UCCAU 3' AAGG GA GA AGUCAGAAA UUCC CU CU UCAGUCUUU (miRNA) 3' A CAAC 5'	rn5,oryCun2, panTro4, rheMac3, canFam3, bosTau7, dasNov3, loxAfr3, echTel1, monDom5, galGal4.(11)
			2987 - 3011 7mer	2:75668370-75668394	(Transcript)5'GAA AACUUUCAC U3' CUAGG AGUU GUCAGAA GAUUC UCAA CAGUCUU (miRNA) 3' CCUC CU U5'	bosTau7, loxAfr3.(2)
			3422 - 3447 6mer	2:75668805-75668830	(Transcript)5'CAU UCA CAA CA 3' UUAAG GGA AGUU CAGAAA GAUUC CCU UCAA GUCUUU (miRNA) 3' C CUCA 5'	rn5
			3979 - 3989	2:75669362-75669372	(Transcript)5'AUUUGUAGCAUAAUGAAA UUA 3' UG CAGAAA AC GUCUUU (miRNA) 3' A UCA 5'	rn5,oryCun2, hg19, panTro4, rheMac3, canFam3, bosTau7,loxAfr3. (8)

© 2018 Life Science Informatics Publication All rights reserved

			472-494. 7mer	2:75662092; 75662487-75662106; 75662494	(Transcript)5'ACACAG UAAAAUU U 3' UUGA GUUG UCAGAAA .|.| |||| ||||||| GAUU CAAC AGUCUUU (miRNA) 3' CCCUCU UC 5'	Not Conserved
2		**Zbtb20**	2223 - 2228 6mer	16:43620968-43620973	(Transcript)5'GAAAAGUAACUAUUCCAAUAACA 3' CAGAAA |||||| GUCUUU (miRNA) 3' A 5'	oryCun2, canFam3, bosTau7, loxAfr3 (4)
			2645 - 2654 7mer	16:4362130-43621399	(Transcript)5'UGACCUGUUUUAAAAAUUA CU 3' G UCAGAAA | |||||| C AGUCUUU (miRNA) 3' A UC 5'	rn5,bosTau7, loxAfr3. (3)
			3406 - 3432 6mer	16:4362211-43622177	(Transcript)5'A A CACUCC CA C3' CUGA GGA GUUGG UCAGAA ||.| ||| ||||. |||||| GAUU CCU CAACU AGUCUU (miRNA) 3' C CU C U5'	Not Conserved
			4516 - 4532 6mer	16:4362326-43623277	(Transcript)5'UCUUUCAGCAGU A AAAC 3' AGA GUU CAGAAA ||| ||| |||||| UCU CAA GUCUUU (miRNA) 3' C CUCA 5'	Not Conserved
			5023 - 5028 6mer	16:43623768-43623773	(Transcript)5'UUCAGUACCCAACAAUACCAAAC 3' CAGAAA |||||| GUCUUU (miRNA) 3' A 5'	rn5,bosTau7 (2)
			6160 - 6165 6mer	16:43624905-43624910	(Transcript)5'GGAAAGCAGACAACACACACACA 3' CAGAAA |||||| GUCUUU (miRNA) 3' A 5'	Not Conserved
			7069 - 7097 6mer	16:43625814- 43625842	(Transcript)5' U A CUUUAC CAU 3' CUG G GGAGG AG UCAGAAG ||. | ||||. || ||||||. GAU C CCUCU UC AGUCUUU (miRNA) 3' U CAAC 5'	Not Conserved

© 2018 Life Science Informatics Publication All rights reserved

			8913 - 8924 6mer	16:43627658-43627669	(Transcript)5'ACUGCCAUCAUCUGUAU CU 3' UUG UCAGAAG ||| ||||||. AAC AGUCUUU (miRNA) 3' C UC 5'	Not Conserved
			9431 - 9451 8mer	16:43628176-43628196	(Transcript)5'UCUCCCA CA CCU U3' CUG AG GGA AGUCAGAA ||. || ||| |||||||| GAU UC CCU UCAGUCUU (miRNA) 3' CUCAAC U5'	Not Conserved
3		**Tenm1**	4484 - 4504 6mer	X:42532350-42532370	(Transcript)5'UUUAUUG UU UUUUCU A C3' UA GG AG UCAGAA || || || |||||| AU CC UC AGUCUU (miRNA) 3' G UC UCUCAAC U5'	rn5,oryCun2, hg19, panTro4, rheMac3, canFam3, bosTau7, loxAfr3, monDom5, galGal4. (10)
			4300 - 4321 7mer	X:42532166-42532187	(Transcript)5'CAAACAG CCCAAAU U 3' CUAA GUU UCAGAAA |||| ||| ||||||| GAUU CAA AGUCUUU (miRNA) 3' CCCUCU CUC 5'	hg19, panTro4, canFam3, dasNov3, loxAfr3, monDom5. (6)
			4043 - 4052 9mer	X:42531909-42531918	(Transcript)5'CUUCAAGCACCAUCUCCUG 3' GAGUCAGAAA |||||||||| CUCAGUCUUU (miRNA) 3' A 5'	rn5
			3567 - 3572 6mer	X:42531433-42531438	(Transcript)5'GAGAAACAUAUUAACUACUGCUG 3' CAGAAA |||||| GUCUUU (miRNA) 3' A 5'	rn5,hg19, panTro4, rheMac3, canFam3, bosTau7, dasNov3. (7)

© 2018 Life Science Informatics Publication All rights reserved

			3405 - 3410 6mer	X:42531271 -42531276	(Transcript)5'ACUGAAUCUCUCCCAGCCAUAUG 3' CAGAAA |||||| GUCUUU (miRNA) 3' A 5'	rn5
			834- 857 6mer	X:42528700 -42528723	(Transcript)5'AAAAA CAAA AACAAAAA 3' AGAG AG CAGAAA |||| || |||||| UCUC UC GUCUUU (miRNA) 3' C AAC A 5'	rn5
			404- 430 8mer	X:42528270 -42528296	(Transcript)5'AA A CCAGCCUUUCU 3' GGAGA GU GUCAGAAA ||||| || |||||||| CCUCU CA CAGUCUUU (miRNA) 3' C ACU 5'	rn5,oryCun2, hg19, panTro4, rheMac3, canFam3, bosTau7, dasNov3, loxAfr3, echTel1.
			7360 - 7384 8mer	X:42899867 -42899891	(Transcript)5'CUG UCUUC GCAGCC C3' CUA AGU AGUCAGAA ||| ||| |||||||| GAU UCA UCAGUCUU (miRNA) 3' UCCCUC AC U5'	Not Conserved
4		**Uggt1**	4317 - 4333 8mer	1:36202797-36202813	(Transcript)5'UGGUGGUGAUG ACAUU C3' AAGG AGUCAGAA |||| |||||||| UUCC UCAGUCUU (miRNA) 3' A CUCUCAAC U5'	rn5,hg19, panTro4, rheMac3, dasNov3, monDom5, galGal4.
			3891 - 3906 7mer	1:36192402-36192417	(Transcript)5'UUUCCUGGUGUCA C C 3' UA GG AGA UCAGAAA || || ||| ||||||| AU CC UCU AGUCUUU (miRNA) 3' G U C CAACUC 5'	rn5, dasNov3, monDom5, galGal4
			3528 - 3554 6mer	1:36184363-36184389	(Transcript)5'U A UUCUGCUUAC A U3' GGGA UUGA G UCAGAA |||| |||| | |||||| CCCU AACU C AGUCUU (miRNA) 3'U CUC U5'	rn5, rheMac3, bosTau7, loxAfr3

© 2018 Life Science Informatics Publication All rights reserved

			3038 - 3044 7mer	1:36175100; 36176233-36175102;36176236	(Transcript)5'UACUAUAUGAUGCCAUUAAACA 3' UCAGAAA ||||||| AGUCUUU (miRNA) 3' C 5'	rn5,oryCun2, hg19, panTro4, rheMac3, canFam3, bosTau7, loxAfr3, echTel1, monDom5, galGal4, xenTro3.
			2612 - 2632 6mer	1:36173476-36173496	(Transcript)5'AUAUCAUC AAACAUC 3' CUGA GGGA CAGAAA ||.| |||| |||||| GAUU CCCU GUCUUU (miRNA) 3' CUCAACUCA 5'	rn5,hg19, panTro4, rheMac3, canFam3, bosTau7, dasNov3, loxAfr3, echTel1, galGal4.
			2137 - 2164 7mer	1:36163256-36163283	(Transcript)5' AUGGUAGAAUCU C3 GAGAGUUGG GUCAGAA ||||||||. ||||||| CUCUCAACU CAGUCUU (miRNA) 3'C U5	rn5,oryCun2, hg19, panTro4, rheMac3, canFam3, bosTau7, dasNov3, echTel1, monDom5, galGal4.
			1763 - 1779 6mer	1:36158188; 36159942-36158196;36159949	(Transcript)5'GCAAGAUCAUCA U A 3' AAG GA GGU UCAGAAG ||| || .|| ||||||. UUC CU UCA AGUCUUU (miRNA) 3' A C C ACUC 5'	rn5,oryCun2, hg19, panTro4, rheMac3, bosTau7, dasNov3,

© 2018 Life Science Informatics Publication All rights reserved

						loxAfr3, monDom5
			755-782 6mer	1:36144241; 36146032-36144251; 36146048	(Transcript)5'C A C CAG A GCUC 3' AG GGAGA G GA G UCAGAAG || ||||| | || | ||||||. UC CCUCU C CU C AGUCUUU (miRNA) 3'U AA 5'	rn5,loxAfr3
			405-432 6mer	1:36143891-36143918	(Transcript)5'C A C CAG A GCUC 3' AG GGAGA G GA G UCAGAAG || ||||| | || | ||||||. UC CCUCU C CU C AGUCUUU (miRNA) 3'U AA 5'	rn5.
5		**Fam 120c**	262-282 8mer	X:151469993-151470013	(Transcript)5'AAGUCCAG C UAACU 3 UGAGG AG GUCAGAAA |.||| || ||||||| AUUCC UC CAGUCUUU (miRNA) 3' G C UCAACU 5	oryCun2, hg19, panTro4, rheMac3, canFam3, dasNov3, loxAfr3
			991-1013 6mer	X:151470722-151470744	(Transcript)5'UCCAGA UUUAC AUUUCC 3' AGA UGA CAGAAA ||| ||| |||||| UCU ACU GUCUUU (miRNA) 3' C CA CA 5'	rn5,hg19, panTro4, rheMac3, canFam3, bosTau7, dasNov3, loxAfr3.

© 2018 Life Science Informatics Publication All rights reserved