Computational Identification of MicroRNAs for Targeting Long and Short Segments of *Lassa Virus*

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Abstract— MicroRNAs (miRNAs), are a class of approximately 22 nucleotide long non coding RNAs which play critical role in different biological processes. The mature microRNA is usually 19–27 nucleotides long and is derived from a bigger precursor that folds into an flawed stem-loop structure which are involve in many cellular process that encompass development, proliferation, stress response, apoptosis, fat metabolism by gene regulation. Resent finding reveal that certain viruses encode their own miRNA that processed by cellular RNAi machinery. In recent research indicates that cellular microRNA can target the genetic material of invading viruses were reported. Cellular miRNA can be used in virus life cycle; either to up regulate or down regulate viral gene expression. Computational tools utilize in miRNA target prediction has been changing drastically in recently. Where many of them are available on the web and can be used by researcher and scientist without of bioinformatics. The development genomes analysis technologies recorded during the previous decade has tremendously cleared the biology of miRNA. In this study nucleotide sequences of long (23343512) and short segments (23343509) sequences of Lassa viruses; L and S segment; composing the genome of Lassa virus which naturally affect human were analyzed using VMir analyzer program (computational approach) to predict the counter human cellular miRNAs candidates targeting viral genome. The 50 nt minimum hairpin size, 90 nt maximum hairpin size and 50 minimum hairpin score were used for the filter of sequence, as well as pairing energy less than 10 kcal/mol was utilize as cutoff score. The results of RNA hybrid were categories in terms of pairing energy (minimum free energy) and hybridization pattern. Four types of hybridization patterns were obtain from RNA hybrid analysis. According to L segment analysis, 34 potent miRNA divided into three groups, subsequently, hsa-miR-608, hsa-miR-3692-5p, hsa-miR-557, hsa-miR-1273d, hsa-miR-136-5p and hsa-miR-3164 are selected as potential human cellular miRNAs. From the miRBase database 16 potential miRNA were predicted as potential miRNA targeting S segment, therefore, hsa-miR-4691-5p, hsa-miR-581 and hsa-miR-622 were selected as potential human cellular miRNAs on the basis of pairing energy. The results suggested that microRNAs from MD30, MR19 and MR30 as well as MD15 and MR1 from L segment and S segment, respectively, might be best candidate to targeting human cellular miRNAs.

Index Terms— MicroRNAs, Lassa virus, Computational tools, Gene regulation, Databases, RNAi, RNA hybrid.

1 INTRODUCTION

MICRORNA are small non coding RNAs with ~21-23 nucleotide, length have important roles in diverse biological process that encompass development, apoptosis, tumerogenesis, proliferation, stress response and fat metabolism [1, 2, 3]. Field of microRNA biology emerged with the discovery of that C. elagans lin4 gene product, a ~22 nt noncoding RNA (ncRNA), regulates the expression of lin14 by partial sequence complementarity [3, 4, 5]. The microRNA is transcribed originally in nucleus as hundred to thousand nucleotides with hairpin structure, called primary miRNA (pri-miRNA), which are generated by RNA polymerase II in all eukaryotes or by RNA polymerase III in some viruses [6, 7]. Primary miRNAs are cropped and trimmed to 60 to 100 nucleotide with a stem loop structure called precursor miRNA (pre-miRNA) that are processed in the nucleus by the RNase type III Drosa [8].

These pre miRNAs are exported to the cytoplasm by exportin 5 to be secondarily processed into miRNA duplexes by the RNase type III Dicer (Figure 1). The dicer removes the loop region of the hairpin and release the ~22 nucleotide mature miRNA duplex [9, 10] which are involved in many cellular process including post transcriptional gene silencing and inhibition of infected viral replication. It discovered those viruses that are capable to produce high level of miRNA. The resulting miRNA duplex assembles with RNA-induced silencing complex (RISC) [11, 43, 44]. The one of the miRNA strand called "passenger" is removed by a helicase activity, while the "guide" miRNA is guided to the target mRNA to either degrade or block translation. Therefore miRNA play important role in the gene regulation and expression in terms of gene silencing.

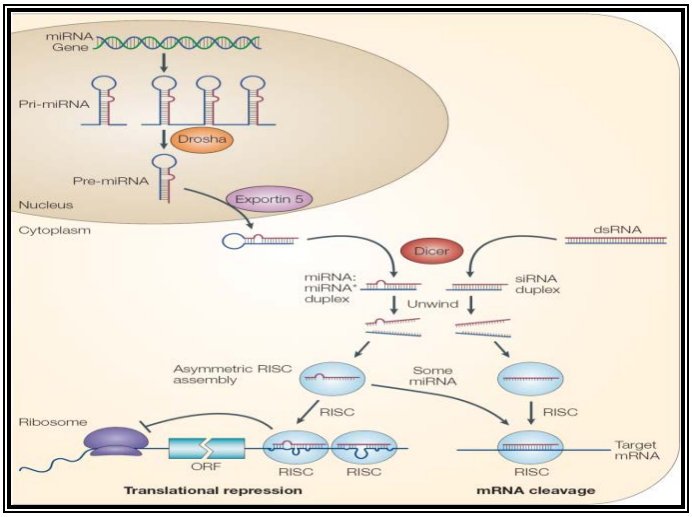

Fig. 1. Systamatic diagram of the restriction on virus replication imposed by host cell microRNA response.

Human miRNA implicated in many cellular processes such as cell proliferation, apoptosis, angiogenesis and homeostasis [13]. Many research described that miRNA play a critical role of great magnitude in regulation of virus infection and interplay between virus and host cell response [14]. Some finding showed viral genomes encoded miRNAs sequences from DNA and RNA viruses including Epistein-Barr-Virus [15], Herpies viruses [16], Simian virus 40 [17] and Human Immunodeficiency virus-1 [18]. Host miRNA can also target (up or down regulation) viral gene and involve with the replication of many incoming viruses such as vesicular stomatitis viruses [19], primate foamy virus type 1 [20], and Hepatitis C virus [21].

Lassa fever is an acute viral zoonotic illness caused by Lassa virus, a member of the Arenaviridae family and responsible for a severe hemorrhagic fever characterized by fever, sour throat, muscular pain and nausea [22]. Its first discovery in 1969 was in West Africa where it is endemic. There are estimations of 300,000 to 500,000 cases of Lassa fever annually [23-29] with a mortality rate if 15-20% for hospitalized patient. Higher mortality rates recorded 50% and 90% during epidemic and 90% were epidemics and in third month pregnancy respictively [30, 31]. Lassa virus has negative ambisense organization (two viral genes separated by an intergenic region), bisegmented, single stranded (ssRNA) genome designated the (small, ~3.4 kb) & L large, ~7.2 kb) segments [32, 33]. This study aimed at prediction of human cellular microRNA targeting both segments of the genome of Lassa virus. This might be useful to understand the host defense mechanism in term of regulating Lassa fever infection.

2 MATERIAL AND METHOD

2.1 Retrieval of sequence

Nucleotide sequences L segment (23343512) and S segment (23343509) sequence of Lassa viruses were downloaded from NCBI database (http://www.ncbi.nlm.nih.gov/) to be used during the present study.

2.2 Lassa virus miRNA Hairpins sequences prediction

The L and S segments were scanned for hairpin structured microRNA precursor by using VMir analyzer program [34, 35]. VMir is an ab initio prediction program which was designed specifically to identify pre miRNA in viral genome. For cutoff value 50nt minimum hairpin size, 90nt maximum hairpin size and 50 minimum hairpin score were used for the filter of sequence. The scanned hairpins were visualized in VMir viewer.

2.3 Human miRNAs sequences prediction

Human miRNA sequences are available in the miRBase database (http://www.mirbase.org) [36-39]. This is dependent on the average length of microRNA (~22bp), Nucleotide segments of Lassa virus were scanned with VMir program. Then every Hairpin segment (70nt) was input and circumspectly examined for nucleotide similarity with all human microRNA by using SSEARCH program in a search tool of the miRBase database (www.mirbase.org/search.shtml). According to the principal each of the input viral Hairpin segments was align with all of the microRNA in the miRBase then the highly similar were identified as target miRNA. The mature duplex microRNA consists of two stand of microRNA that is complementary to each other. The complementary strand of the target microRNA might be complementary to the input viral sequence hybridization between the viral gene fragments and complementary template of the potential miRNA was further analyzed by RNA hybrid [40]

2.4 Hybridization prediction between target miRNA and viral miRNA

Energetically most favorable hybridization between target microRNA and viral RNA was predicted by the RNA hybrid tool (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) [40]. The results of RNA hybrid were categories in terms of pairing energy (minimum free energy) and hybridization pattern Four types of hybridization patterns were obtain from RNA hybrid analysis including 5′ canonical, 3′ compensatory, 5′seed and ineffective hybridization.

2.5 Criteria for selection of potent miRNA

According to the microRNA target prediction principle which requires the sufficient base pairing between the miRNA and target mRNAs that can be classified into 5′ canonical 3′compensatory, 5′ seed and in effective hybridization [41]. 5′ dominant classes of target sites can be divided into 2 subtypes: 5′ seed and 5′ canonical both indicate the effective base pairing within 2nd to 8th position from the 5′ end of miRNA. For 3′ compensatory pattern, the candidate miRNA should show half sequence from middle to 3′ end of miRNA that will perfectly match with miRNA. Pairing energy or minimum free energy (mef) indicating the stability of the hybridization. For the selection of potential miRNA the pairing energy at -10 kcal/mol was utilize as cutoff score. The miRNA targeting HIV genes with effective hybridization pattern (5′ canonical, 3′ compensatory, 5′seed) and minimum free energy less than -10 kcal/mol were selected as potential miRNA.

2.6 Prediction of secondary structure of miRNA precursor

The RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) was used to predict the secondary structure of pre-miRNA [42]. Default parameters were employed. This program to be used predicts the most stable secondary structure of both the L and S segment Hairpin sequences. The sequence applied for prediction analysis included pre-miRNA about 200 bp upstream and about 100 bp downstream flanking sequences at each end of the precursor. In all cases, folding structures with minimal free energy were depicted.

3 RESULT AND DISCUSSION

3.1 Prediction of miRNA hairpin

VMir viewer program is used to visualize the result of VMir analyzer of the programes in graphical manner with sequence length and score. The resulting graph for L and S segment are shown in Figure 2. 105 sequences with potential hairpin like structure were extracted from L segment and 41 from S segment of Lassa virus. 24 potential hairpin from L

segment and 14 from S segment were selected for further analysis.

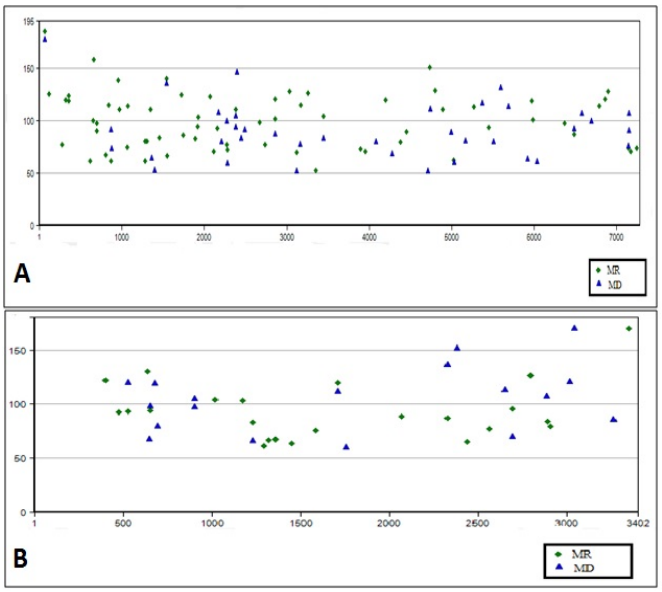

Fig. 2. Systamatic dia Graphical representation of VMir result for Long (A) Short (B) segment.

3.2 Specific miRNA targeting L segment

Strand of target miRNA was further predicted by the RNA hybrid. According to the result of pairing energy and hybridization pattern between mature human miRNA and their target viral gene. 34 miRNA were identified as potential microRNA targets in L segment of Lassa virus. Table 1 demonstrate the details of the pairing energy between cellular miRNA and their target viral gene with hybridization pattern these potent miRNA can be divided into three groups according to their hybridization pattern including 5′ canonical (21 miRNA) 5′seed (6 miRNA) and 3′ compensatory (7 miRNA). Hsa-miR-608, hsa-miR-3692-5p, hsa-miR-557, hsa-miR-1273d, hsa-miR-136-5p and hsa-miR-3164 are selected as potential human cellular miRNAs.

3.3 Specific miRNA targeting S segment

Table 2 illustrates the result of hybridization pattern and pairing energy between potential cellular microRNA and their target viral gene of S segment. From the miRBase database 16 potential miRNA were predicted as potential miRNA targeting. Here 11, 3 and 2 mircoRNA viral gene with 5′ canonical, 3′ compensatory and 5′ seed hybridization pattern found, respectively. Hsa-miR-4691-5p, hsa-miR-581 and hsa-miR-622 are selected as potential human cellular miRNAs on the basis of pairing energy.

3.4 Prediction of secondary structure of miRNA precursor

RNAfold web server was used to predict the secondary structure of selected potential hairpin sequences. These potential hairpins are chosen on the basis of both VMir score and pre-

TABLE 1

Predicted potential cellular miRNAs targeting Long segment of Lassa virus.

Hairpins	VMir Score	Human miRNA	Energy (kcal/mol)	Hybridization	Pattern
MD2	92.1	hsa-miR-3945	-23.3		5′ seed
MD11	104.8	hsa-miR-3529-3p	-12.0		5′canonical
MD30	114	hsa-miR-608	-21.1		5′canonical
MR9	100.2	hsa-miR-33b-3p	-22.5		5′ seed
MR10	90.5	hsa-miR-3692-5p	-19.7		5′canonical
		hsa-miR-557	-19.6		5′canonical
		hsa-miR-1273d	-22.1		5′canonical
MR11	97.1	hsa-miR-3692-5p	-15.4		5′canonical
		hsa-miR-557	-18.4		5′canonical
		hsa-miR-1273d	-21.2		5′canonical
MR30	103.5	hsa-miR-136-5p	-14.7		5′canonical
		hsa-miR-3164	-20.9		5′canonical
MR39	120.4	hsa-miR-3668	-14.5		3′ compensatory
MR46	103.8	hsa-miR-524-5p	-16.5		3′ compensatory
MR56	113.2	hsa-miR-548t-5p	-13.4		3′ compensatory
MR58	118.6	hsa-miR-3612	-17.1		3′ compensatory
MR63	120.4	hsa-miR-4666b	-14.2		5′canonical

TABLE 2

Predicted potential cellular miRNAs targeting Short segment of Lassa virus.

Hairpins	VMir Score	Human mirna	Energy (kcal/mol)	Hybridization	Pattern
MD7	104.9	hsa-miR-31-3p	-10.2		5′canonical
MD13	113.7	hsa-miR-34a-5p	-18.3		3′ compensatory
MD15	107.5	hsa-miR-4691-5p	-23.5		5′canonical
MD17	168.8	hsa-miR-541-3p	-19.4		5′canonical
MR1	121.6	hsa-miR-581	-18.0		5′canonical
		hsa-miR-622	-23.0		3′ compensatory
MR7	102.6	hsa-miR-3160-3p	-16.1		5′canonical

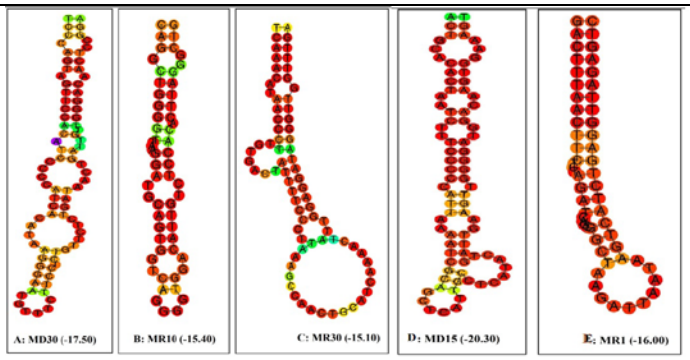

Fig 3: Predicted secondary structure of miRNA precursor for Long (A, B, C) and Short (D, E) segment.

dicted miRNA hybridization score. MD30, MR10, and MR30 were used to predict secondary structure which characterized by -17.50 kcal/mol, -15.40 kcal/mol and -15.10 kcal/mol minimum free energy respectively. The secondary structure of MD15 and MR1 hairpins are predicted which characterized by -20.30 kcal/mol and -16.00 kcal/mol minimum free energy respectively (Figure 3).

4 Conclusion

By using computational approaches we predict the candidate potential cellular miRNA targeting the L and S segments of Lassa virus. The result suggested that microRNAs from MD30, MR19 and MR30 from L segment and MD15 and MR1 from S segment might be best candidate to targeting human cellular miRNAs. It reveals that these microRNAs may have a potential for inhibition of viral replication by silencing the function of respected protein. However, further in vitro study should be performed in order to assess the inhibition influence on viral replication by the effect of selected human cellular miRNAs.

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References

[1] V. Ambrod. "microRNAs: tiny regulators with great potential" Cell 2001, 107(7): 823-826

[2] J. C. Carrington, V. Ambros. "Role of microRNAs in plant and animal development" Science 2003, 301(5631):336-338.

[3] M. Chalfie, H. R. Horvitz, J. E. Sulston. "Mutations that lead to reiterations in the cell lineages of C. elegans" Cell 1981, 24:59–69. 2.

[4] R. C. Lee, R. L. Feinbaum, V. Ambros. "The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14" Cell 1993, 75: 843–854.

[5] B. Wightman, I. Ha, G. Ruvkun. "Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 1993, 75:855–862.

[6] O. Aparicio, N. Razquin, M. Zaratiegui, I. Narvaiza, P. Fortes. "Adenovirus virus-associated RNA is processed to functional interfering RNAs involved in virus production" J Virol, 2006. 80(3): p. 1376-84.

[7] R. F. Ketting, S. E. Fischer, E. Bernstein, T. Sijen, G. J. Hannon, R. H. Plasterk. "Dicer functions in RNA interference and in synthesis of small RNA involved in development timing in C. elegans" Gene Dev 2001, 15(20):2654-2659.

[8] J. Han, Y. Lee, K. H. Yeom, Y. K. Kim, H. Jin, V. N. Kim. "Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex" Cell, 2006. 125(5): p. 887-901.

[9] R. Yi, Y. Qin, I. G. Macara, B. R. Cullen "Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs" Genes Dev, 2003. 17(24): p. 3011-6.

[10] E. Lund, S. Güttinger, A. Calado, J. E. Dahlberg, U. Kutay. "Nuclear export of microRNA precursors" Science, 2004. 303(5654): p. 95-8.

[11] D. P. Bartel. "MicroRNAs: genomics, biogenesis, mechanism, and function" Cell 2004, 116(2):281-297.

[12] M. Chekulaeva, W. Filipowicz. "Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells" Curr Opin Cell Biol 2009, 21(3):452-460.

[13] H. W. Hwang, J. T. Mendell. "MicroRNAs in cell proliferation, cell death, and tumorigenesis" Br J Cancer 2006, 94(6):776-780.

[14] K. U. Kumar, S. P. Srivastava, and R. J. Kaufman. "Double-stranded RNA-activated protein kinase (PKR) is negatively regulated by 60S ribosomal subunit protein L18" Mol Cell Biol, 1999. 19(2): p. 1116-25.

[15] A. K. Lo, K. F. To, K. W. Lo, R. W. Lung, J. W. Hui, G. Liao, D. Hayward. "Modulation of LMP1 protein expression by EBV-encoded microRNAs" PNAS 2007, 104(41):16164-16169.

[16] S. Pfeffer, A. Sewer, M. Lagos-Quintana, R. Sheridan, C. Sander, F. A. Grässer, L. F. van Dyk, C. K. Ho. "Identification of microRNAs of the herpesvirus family" Nat Methods 2005, 2(4):269-276.

[17] C. S. Sullivan, A. T. Grundhoff, S. Tevethia, J. M. Pipas, D. Ganem. "SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells" Nature 2005, 435(7042):682-686.

[18] S. Omoto, M. Ito, Y. Tsutsumi, Y. Ichikawa, H. Okuyama, E. A. Brisibe, N. K. Saksena, Y. R. Fujii. "HIV-1 nef suppression by virally encoded microRNA" Retrovirology 2004, 1(44).

[19] M. Otsuka, Q. Jing, P. Georgel, L. New, J. Chen, J. Mols, Y. J. Kang, Z. Jiang, X. Du. "Hypersusceptibility to vesticular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR24 and miR93 expression" Immunity 2007, 27(1):123-134.

[20] C. H. Lecellier, P. Dunoyer, K. Arar, J. Lehmann-Che, S. Eyquem, C. Himber, A. Saïb, O. Voinnet. "A cellular microRNA mediates antiviral defense in human cells" Science 2005, 308(5721):557-560.

[21] C. L. Jopling, M. Yi, A. M. Lancaster, S. M. Lemon, P. Sarnow. "Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA" Science 2005, 309(5740):1577-1581.

[22] Imported Lassa fever. New Jersey: Centers for Disease Control and Prevention (CDC) 2004. MMWR Morb Mortal Wkly Rep 2004; 53(38): 894–7.

[23] J. B. McCormick. "Clinical, epidemiologic, and therapeutic aspects of Lassa fever" Med Microbiol Immunol 1986, 175:153-5.

[24] J. B. McCormick: "Epidemiology and control of Lassa fever" Current Topics in Microbiol and Immunol 1987, 134:69-78.

[25] S. P. Fisher-Hoch, J. B. McCormick "Lassa fever vaccine: A review" Expert Rev Vaccines 2004, 3:103-11.

[26] S. P. Fisher-Hoch, O. Tomori, A. Nasidi, G. I. Perez-Oronoz, Y. Fakile, L. Hutwagner, J. B. McCormick "Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice" Br Med J 1995, 311:857-9.

[27] K. Birmingham, G. Kenyon "Lassa fever is unheralded problem in West Africa" Nat Med 2001, 7(8):878.

[28] J. B. McCormick, I. J. King, P. A. Webb, K. M. Johnson, R. O'Sullivan, E. S. Smith, S. Trippel, T. C. Tong, N. Sacchi "A case-control study of the clinical diagnosis and course of Lassa fever" J Infect Dis 1987, 155(3):445-455.

[29] K. M. Johnson, J. B. McCormick, P. A. Webb, E. S. Smith, L. H. Elliot, I. J. King "Clinical virology of Lassa fever in hospitalized patients" J Infect Dis 1987, 155(3):456-64.

[30] J. B. "McCormick, P. A. Webb, J. W. Krebs, K. M. Johnson, E. S. Smith "A prospective study of the epidemiology and ecology of Lassa fever" J Infect Dis 1987, 155:437-44.

[31] J. B. McCormick, I. J. King, P. A. Webb, C. L. Scribner, R. B. Craven, K. M. Johnson, L. H. Elliott, R. Belmont-Williams "Lassa Fever. Effective therapy with ribavirin" N Engl J Med 1986, 314(1):20-6.

[32] H. Schmitz, B. Kohler, T. Laue, C. Drosten, P. J. Veldkamp, S. Günther, P.

Emmerich, H. P. Geisen, K. Fleischer, M. F. Beersma, A. Hoerauf. "Monitoring of clinical and laboratory data in two cases of imported Lassa fever" Microbes Infect 2002;4:43-50.

[33] M. J. Buchmeier, J. C. de la Torre, C. J. Peters. "Arenaviridae: the viruses and their replication." In: Knipe DM, Howley PM, editors. Fields virology, 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1791-827.

[34] C. S. Ullivan, A. Grundhoff. "Identification of Viral MicroRNAs" Methods Enzymol 2007, 427:1–23.

[35] A. Grundhoff, C. S. Sullivan, D. Ganem. "A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses" RNA 2006, 12:733–750.

[36] S. Griffiths-Jones. "The microRNA registry" Nucleic Acids Res 2004, 32(Database issue):D109-11.

[37] S. Griffiths-Jones, R. J. Grocock, A. V. Dongen, A. Bateman, A. J. Enright. "MiRBase: microRNA sequences, targets and gene nomenclature". Nucleic Acids Res 2006, 34(Database issue):D140-4.

[38] S. Griffiths-Jones, H. K. Saini, S. V. Dongen, A. J. Enright. "miRBase: tools for microRNA genomicS" Nucleic Acids Res 2008, 36(Database issue):D154-8.

[39] A. Kozomara, S. Griffiths-Jones. "MiRBase: integrating microRNA annotation and deep-sequencing data" Nucleic Acids Res 2011, 39(Database issue):D152-7.

[40] J. Kruger, M. Rehsmeier. "RNAhybrid: microRNA target prediction easy, fast and flexible" Nucleic Acids Res 2006, 34:451-454.

[41] J. Brennecke, A. Stark, R. B. Russell, S. M. Cohen. "Principles of miRNA-target recognition".

[42] A. R. Gruber, R. Lorenz, S. H. Bernhart, R. Neubo¨ck and I. L. Hofacker. "The Vienna RNA websuite" Nucleic Acids Res 2008, 36: W70 –W74.

[43] S. K. Verma, S. Yadav & J. Singh, Shraddha, A. Kumar. "Web–Based Tools and Databases for Micro-RNA Analysis: A Review" International Journal of Biological, Life Science and Engineering. 2014 Vol: 8 No: 1.

[44] S. K. Verma, Shraddha, A. Kumar. "Computational Prediction of MicroRNA for Targeting HIV-1 and HIV-2 Subtype" American Journal of Bioinformatics and Computational Biology (2013) 1: 9-22.

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