Computational Prediction of Functional Constraints to Engineer MicroRNA for Anti-HCV Therapy

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Islamabad

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FINAL APPROVAL

It is to certify that we have read the thesis submitted by Ms. Atiya Mahmood Ahmed and it is our judgment that this research is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the award of M.S Degree in Bioinformatics

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DEDICATION

This meek exertion is dedicated to the Being who is Above and Above of all, who is The Most Exalted, The All Preserver, The Sustainer of All and The One who cures the infirmities of Human Beings, and has not left a single disease without a treatment.

DECLARATION

I hereby declare that the work presented in the thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 24:11:011

Atiya Mahmood.
(Atiya Mahmood Ahmed)

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Atiya Mahmood.
24:11:011
Atiya Mahmood Ahmed

LIST OF ABBREVIATIONS

AVI	Avian Influenza Virus.
DNA	Deoxy Ribose Nucleic Acid.
E1	Envelop glycoprotein associated with E2.
E2	Envelop glycoprotein associated with E1.
FTP	File transfer protocol.
GNU	GNU's Not UNIX.
GPL	GNU General Public License.
GT	Genotype.
GTs	Genotypes.
HA	Hemaglutanin
HBV	Hepatitis B Virus.
HCV	Hepatitis C Virus.
HIV-I	Human Immuno-deficiency Virus I.
Hsa-mir	Homo sapiens MicroRNA
ICTVdb	International Committee on Viruses's Data Base.
IRES	Internal Ribosome Entry Site.
MATLAB	Matrix Laboratory.
miRNA	MicroRNA.
mRNA	Messenger RNA
MS Excel	Micro Soft Excel.
NS	Non-Structural.
ORF	Open Reading Frame.
PB2	Polymerase Basic Protein 2.
PFV	Primate Foamy Virus.
Pre	Precursor miRNA.
miRNA	Micro RNA
RISC	RNA Induced Silencing Complex.
RNAi	RNA Interference.
RNAse-III	Ribonuclease III.

	Small Interfering RNA.
SiRNA	Single Nucleotide Polymorphism.
SNP	Single Stranded RNA.
ssRNA	United States of America.
USA	Un-translated Regions.
UTR	World Health Organization.
WHO	

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ABSTRACT

MicroRNAs are the small moieties comprising of 20-22 nucleotides, firstly reported as the gene expression controller entities at post transcriptional level but the later research proved their function as the interacting layer between the host and the viruses. This feature has been empowered to silence gene expression either in host or in the virus, because they can cope with the heterogenetic forms of different viruses, as they require only partial complementarity to target the desired gene. Hepatitis C Virus occurs in alternative genetic forms which even escape the RNAi, so the lack of effective treatment has associated high prevalence rate with it.

The present research work focuses to make out the sequential constraints that are necessary to engineer a MicroRNA that can target the genetically variant forms of Hepatitis C Virus, so that a potential and economical therapy against Hepatitis C can be developed. Well established computational techniques based upon the so far predicted rules for MicroRNA-mRNA binding, have been used to predict the human MicroRNA targets in most prevalent genotypes of the HCV. Cut-off value and consensus scoring approach has been used to identify potential MicroRNA-mRNA duplexes. Keeping in view the artificially engineered MicroRNA therapy for multiple HCV genotypes, the microRNAs were identified which targeted same gene in more than one genotypes. Hsa-mir-1202 targeted E2-Env-Protein gene in genotype 1, 2, 5 and 6, while hsa-mir-196a* targeted core protein gene in genotype 1, 2, 4 and 5 and hsa-mir-551b found to target 5`-UTR in genotype 1, 3, 4 and 5. The target sites for the respective microRNAs were recognized as significantly conserved to engineer a microRNA that can target more than 3 genotypes unanimously. The identified conserved target sequence can be further used to synthesize artificial MicroRNA that can be used as a therapeutic against Hepatitis C.

Introduction

Acute, chronic and cirrhosis are the three stipulations describing the disease conditions associated with known Hepatitis C that ultimately converts into hepato-cellular carcinoma. Hepatitis C is the disease that has prevailed as havoc to human mortality not only in a particular region or race but spans all over the world and in all the ethnicities. According to WHO there are 170 million people having chronic HCV infection all over the world of which 2-4 million belong to USA, 5-10 million from Europe, 12 million in India and other mostly do not know either they have been infected or not. As a matter of fact round about 366,000 deaths per year have been recorded due to this frightful disease. Considering the Pakistani population it is worth noting and alarming that prevalence rate of hepatitis C is never the less 3.0% which shows a startling state of human health in Pakistan (Ali *et al.*, 2009).

Taking in view the propensity of the disease all over the globe, scientists and researchers all over have put stead fast endeavours to discover a highly effective therapy against hepatitis C. Modern research has reached at the point of combination therapy to treat hepatitis C using Pegylated interferon with ribavirin a potent antiviral (Heathcote *et al.*, 2005). On the other side of the horizon gene therapeutic approaches have been tried to effectively defeat this disease. But unfortunately the virus that cause this frightening disease condition belongs to the hepaciviruses which manifest genetic reassortment during each replication cycle, so every newly formed progeny is genetically different from the parental one. This phenomenon has proven to be real stumbling block for the combination therapy and the genetic therapeutic technique, i.e., siRNA, the antivirals and siRNA become ineffective due to changed genetic makeup of the virons (Wilson *et al.*, 2005).

However the story doesn't ends here, if the virus has the ability to escape from the above mentioned therapies the nature has given the researchers a clue to identify those potential and miraculous entities that can cope up with the genetic variability of the virus and these amazing biological devices are known as miRNAs. These are the 20-22 nucleotide long RNA molecules that can effectively knockout a gene, if they are having only the partial complementarity with the target. Not only this, the ability to work on the host-virus level has built new hopes for the effective and economical treatment for hepatitis C (Scaria *et al.*, 2006).

The deep research on miRNA and their potent function has revealed that, miRNAs can be engineered artificially by observing the so far predicted rules for the hybridization of the miRNA with RNA. Bioinformaticians has not been left behind in the endeavour and they have developed such tools and algorithms which can predict the miRNA targets in the given genome, and the results predicted through these tools has got significant authentification from in-vivo results.

The present realm of the observations i.e. failure of current therapy, advent of miRNAs, their ability to function with partial complentarity, potential of miRNA engineering to design miRNA against conserved regions in genomes and the development of computational tools for miRNA target prediction, compelled to indulge in a research under the title of "Computational Identification of Functional Constraints to Engineer miRNA for Anti-HCV therapy" with the intimate objective of finding:

- ✓ Targets for Human oriented miRNAs in HCV 6 main Genotypes.
- ✓ To identify the conserved sequential constraints to aid miRNA engineered for targeting maximum number of HCV genotypes under observation.

As, amiRNAs can be synthesized in wet lab easily so the identified conserved sequences will help the scientists to design a miRNA which can at a time target more than one genotype, so, this two way designed miRNA will be having the double potential to overwhelm HCV genetically variant virons. These artificially designed miRNAs one day will over power the pharmaceutical market being the most potent and the economical health safe treatment against the world distressing disease Hepatitis C.

Literature Review

2.1 MiRNA

The recent breakthrough in biological research is the discovery of the robust RNA molecules that are known to regulate gene expression. Since many years RNA has been shown to perform its functions only in the form of coding and non-coding RNAs, as the coding RNAs are the unavoidable intermediate moieties in gene expression and the non-coding RNAs (ribosomal and transfer RNAs) are performing the necessary structural, catalytic and nucleic acid decoding functions (Ghosh *et al.*, 2007).Then in 1998 this myth was laid to ground by Andrew Fire and colleagues; when they discovered double stranded non-coding RNAs as the potential interfering agents (Fire *et al.*, 1998). Of which one was ~ 22 nucleotide in length and the other was approximately 61 nucleotide long the longer one is known to be the precursor of the shorter RNA (Lee *et al.*, 1993). This shorter RNA is now classified as a member of a vast class of small regulatory RNAs which are known as miRNAs or miRNAs (Logos-Quintana *et al.*, 2001).

Micro RNAs are small RNA molecules having length round about 22 nucleotides (Bentwich *et al.*, 2005), and act after transcription by binding to the 3`untranslated region of the messenger RNA often resulting in the silencing of the transcript (Bartel, 2004). Micro RNAs are usually encoded by genomic regions between clusters of genes or on the anti-sense strand to the genes (Lau *et al.*, 2001) and posses their own machinery for transcription and other regulatory purposes (Lau *et al.*, 2001) (Lee *et al.*, 2004).Almost 40% of miRNA genes are encoded by the intronic regions of protein and non-protein coding genes, even they are coded by the exons of genes (Cai *et* *al.*, 2004) RNA polymerase II is the transcriptional enzyme for the decoding of miRNA from the DNA (Lee *et al.*, 2004). Promoter for the miRNA is present near the DNA sequence which will encode for hair pin loop of the pre-miRNA. The miRNAs transcript is capped by a specially modified nucleotide at 5' end and polyadenylated tail at the other end (Cai *et al.*, 2004). This transcriptional product is the primary miRNA transcript thousands of nucleotide in length. If this transcript contains one or more stem loop structures in 3' untranslated region then this transcript can work dually as a pri-miRNA and mRNA (Lee *et al.*, 2004) (Cai *et al.*, 2004).

The first step of nuclear maturation of miRNA is cleavage of pri-miRNA by an endonuclease enzyme Drosha RNase III, which liberates a 60 $\simeq$ 70 nt long hair pin loops (Lee *et al.*, 2003). Drosha cuts both strands of RNA in a way which leaves a 5' phosphate and ~2 nt 3' lagging sequence (Baysuk *et al.*, 2003). Then this precursor is sent to cytoplasm by active transport with the help of an export receptor protein Exportin-5 (Lund *et al.*, 2003). The nuclear endonuclease Drosha defines the one end of the mature miRNA sequence while the other end is processed by cytoplasmic endonuclease enzyme the Dicer (Lee *et al.*, 2003). The processing by Dicer cuts the terminal base pairs and leave 5' phosphate and ~2 nt overhang at 3' end; the characteristic feature of endonuclease III, resulting into a siRNA like partially double stranded RNA comprising of a mature miRNA and a sequence of nucleotides exactly equal to the miRNA size (Bartel, 2004). This duplex release mature miRNA of 19-25 nt in length (Lee *et al.*, 2002) and a passenger strand which is denote by * and is usually degraded. But there are certain cases reported in which both strands remain

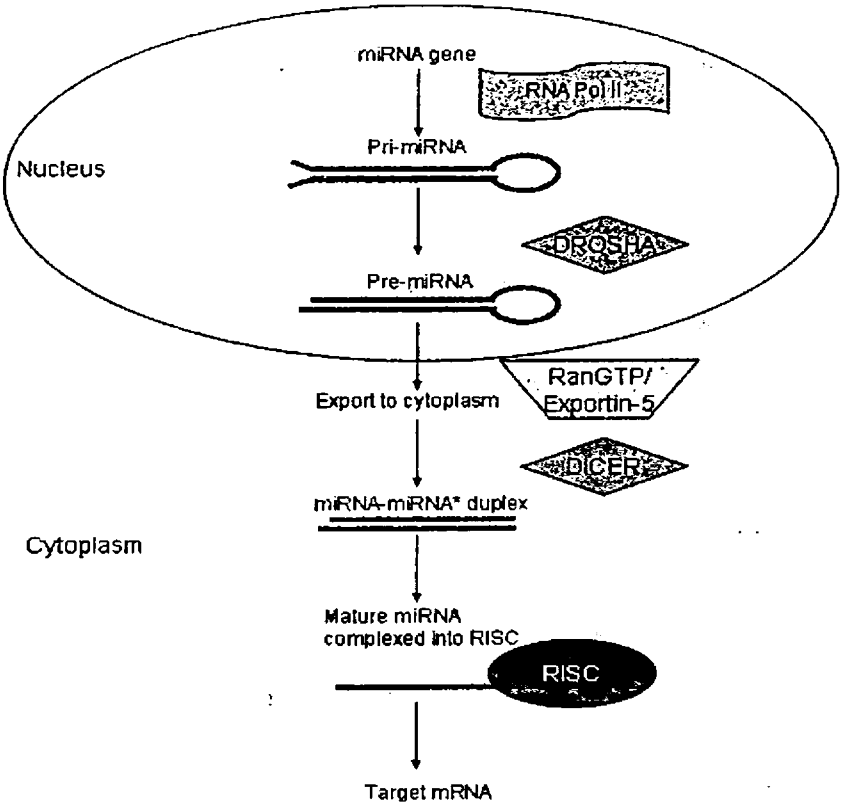

Figure 2.1 The step wise process of miRNA biogenesis

functional and target different mRNAs (Okamura *et al.*, 2008). The cleaved miRNA then enters into a protein-RNA complex known as RISC, with in which it binds to the target mRNA on the principle of base pairing, and resulting either in cleavage of the mRNA or the translational repression (Ghosh *et al.*, 2007). The complentarity between miRNA and target mRNA determines whether the transcript will be cleaved or only translationaly repressed, and single miRNA sequence can regulate many different genes (Wang *et al.*, 2005).

2.2 MiRNA Functions

MiRNAs are known to perform many regulatory functions during different cellular processes, i.e., developmental timings, cell death, haematopoiesis and patterning of nervous system (Kenneth, 2006). Tissue specific expression of many miRNAs mediates the cell lineage preservation and determination (Wienhols and Plastrik, 2005). As miRNAs perform the normal regulatory function in eukaryotic cells, their deregulation due to any reason may cause the prevalence of a disease (Jiang *et al.*, 2009). A neurological disorder has been reported to be caused by the wrong targeting of a miRNA due to a mutation in SNP located in the target site of miRNA (Ghosh *et al.*, 2007). The over or under expression of miRNAs may cause cancerous conditions in the cells, over or under concentration of miRNAs have been observed in many cancerous conditions, i.e., over expression of let-7 has been observed during in vitro studies, with anti-cancer property (Calin *et al.*, 2004). Even miRNAs are known to play an important role in B-cell chronic leukemia, and hsa-mir-15a and hsa-mir-16-1 have observed to play an oncogenic role (Calin *et al.*, 2004). MiRNAs also maintain the pluripotency of stem cells by their preferred expression in them (Chen *et al.*, 2004).Virus encoded miRNAs are known to play a supportive role in the life cycle of virus along with interacting with host genes (Nair and Zayolan, 2006). For example hsa-mir-22 supports the HCV replication inside liver tissue (Jopling *et al.*, 2005). Recently emerging small RNA mediated gene regulation data shows that host miRNAs along with their normal regulatory functions also target the foreign nucleic acids usually in the case of viruses.

2.3 Host Virus interactions

It is established from the studies of viruses having human as a host, that the way in which viral miRNAs overwhelm the cellular machinery in the same way human miRNAs have also been found to regulate the viral genes (Scaria *et al.*, 2006). It has been successfully shown that Primate Foamy Virus (PFV) accumulation with in human cells is restricted by a host encoded miRNA (Lecillier *et al.*, 2005). It was first predicted computationally and later validated experimentally that five human encoded miRNAs target the Human Immuno Deficiency Virus-I (HIV 1) including the *nef* gene (Scaria *et al.*, 2005). Human miRNA hsa-mir-507 and hsa-mir-136 are known to target the polymerase B2 (PB2) and hemaglutanin (HA) in Avian Influenza Virus (AVI), primarily predicted by the computational methods and later experimental validation (Scaria *et al.*, 2006). PFV, HIV and AVI are the viruses having RNA as a genomic material but employ different mechanisms for reverse transcription (Weiss, 1993) (The Writing Committee of WHO Consultation on Human Influenza A/H5, 2005).

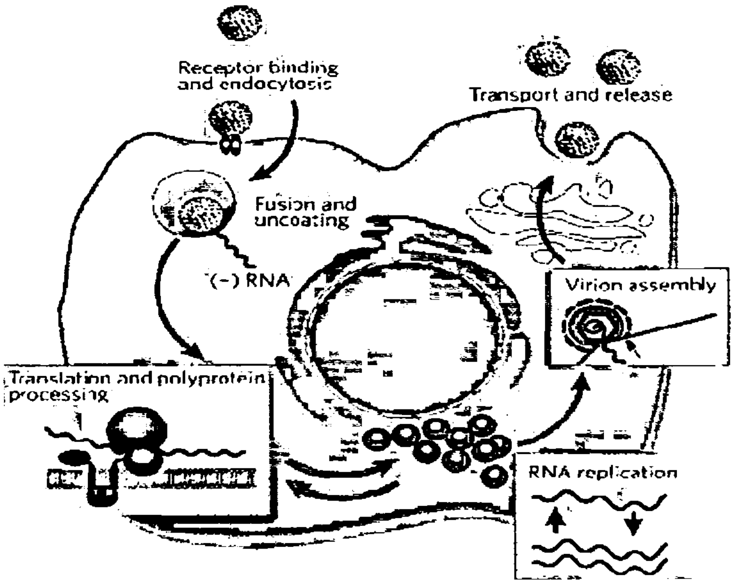

Figure 2.2 HCV Life Cycle, from cell entry to exit

2.4 Hepatitis C Virus

Hepatitis C virus (HCV) is a small ssRNA containing virus belonging to family *flaviviridae* and genus *Hepacivirus* (Simmonds, 1999), which causes chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Penin *et al.*, 2004). HCV enters human liver cells by receptor binding followed by ssRNA release into cytoplasm of infected cell. That is presumably due to low PH, seemingly this genome performs three probable functions, i.e., as a messenger RNA for translation, as a template for RNA replication and as a genomic material enclosed into the viron particles. These particles are membrane wrapped in endoplasmic reticulum and are budded off from the cell (Balterschalgar *et al.*, 2004).

HCV genome is positive and single stranded RNA approximately 10kb long and encodes only one Open Reading Frame that encodes about 3,000 amino acid long polyprotein. Processing of polypeptide occurs either simultaneously with translation or after translation by a host cell peptidase and virus encoded peptidases NS2-NS3 and NS3 into proteins E1, E2, NS2, NS3, NS4A, NS4B, NS5A and NS5B, of which first two are structural proteins contributing to viral envelop and later are non-structural proteins, i.e., regulatory proteins (Kato, 2001). A small protein p7 is formed after the peptidase mediated cleavage of E2 protein (Mizoshema *et al.*, 1994). Brief description of HCV viral genome is depicted in figure 1.3. On the basis of sequence similarity between the different isolates of HCV, it has been divided into main 6 types HCV-I, HCV-II, HCV-III, HCV-IV, HCV-V and HCV-VI, sharing 81 – 89% similarity. According to phylogenetic analysis of types, HCV-I and HCV-II are most divergent while HCV-I and HCV-4 are most related genotypes (Simmonds *et al.*, 1993).

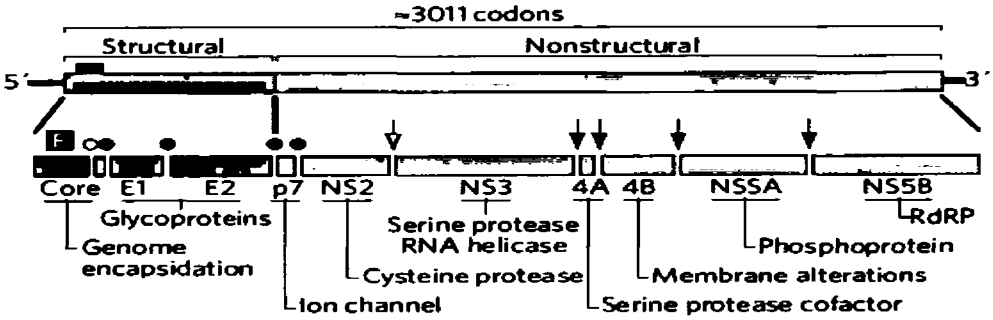

Figure 2.3 The structure of the viral genome, including the long open reading frame encoding structural and non-structural genes, and 5` and 3` NCRs. The polyprotein processing scheme is shown below. Closed circles refer to signal peptidase cleavage sites; the open circle refers to the signal peptide peptidase cleavage site

2.5 Hepatitis C

This is the liver affecting disease caused by the HCV. HCV is responsible for almost 20% cases of acute hepatitis, 70% of chronic hepatitis and 30% of the cirrhosis and liver cancer occurrence. The probable results of the HCV infection and their estimated frequencies are shown in the following figure 2.4. Majority of the patients with acute infection develop the chronic disease although the severity level varies from person to person. The rigorous stage of chronic hepatitis is development of cirrhosis either fast i.e. within 1 to 2 years or slowly within 20 to 30 years. Once cirrhosis is developed symptoms of liver disease appears, although in some patients it remains asymptotic and person dies with unknown reason (Hoofangale, 1997).

2.6 Prevalence

It is evident from the studies that hepatitis C is one of the major reasons causing liver cirrhosis and hepato cellular carcinoma in the patients with liver related disease. It is estimated by WHO that there are 170 million people having chronic HCV infection all over the world of which 2-4 million belong to USA, 5-10 million from Europe, 12 million in India and other mostly do not know either they have been infected or not (Ali *et al.*, 2009). As, 50-80% of acute infected patients with HCV lead to chronic hepatitis. Incidence of acute HCV infection is unknown on global scale as it is asymptomatic in nature so, not well reported. Approximately 366,000 deaths have been reported to be caused by HCV all over the world. Pakistan being a dense populated country with high levels of infection and it is one of the worst distressed countries in the

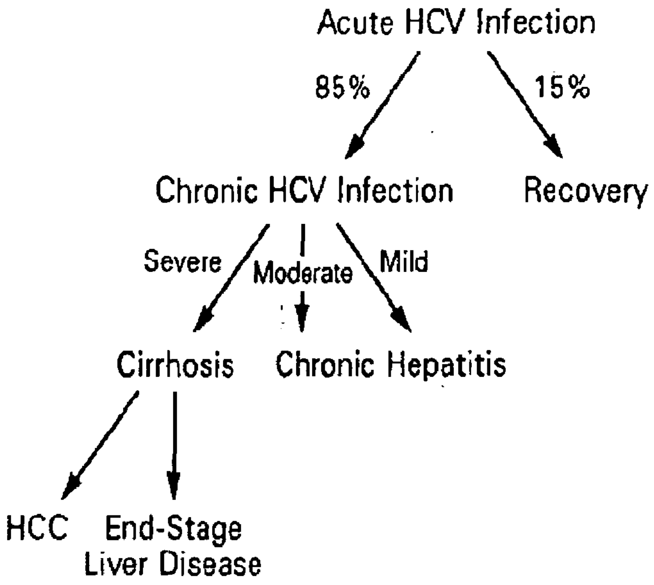

Figure2. 4 clinical spectrum and potential outcomes of the HCV infection, HCC and Chronic infection and cirrhosis

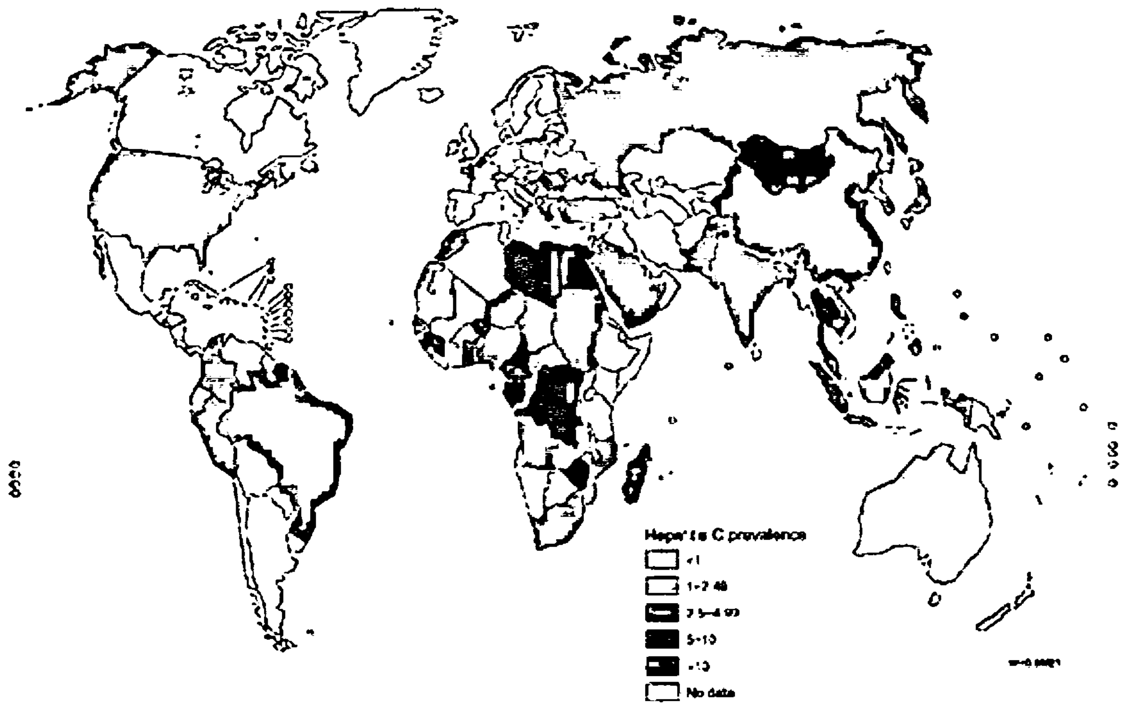

Figure2. 5 Global Prevalence of Hepatitis C

world. The seroprevelance rate in Pakistani population was found to be 3.0% that is a quite alarming figure (Ali *et al.*, 2009).

2.7 Treatment

In past few years molecular level understanding of hepatitis C virus has been greatly improved, which has increased considerably the means to treat the HCV infection. The studies have revealed that all the current treatments against hepatitis C are not suitable for all patients, approximately 50% of people having chronic infection do not respond to such therapies and after this treatment they left with very little alternative options of treatment, along with the factor that these treatments are long, not fit for all patients and they need a sturdy commitment of patient and the health care staff. The latest findings have suggested that due to virus`ability to have heterogenetic forms and the experience of antivirals against HIV-1 and HBV, combination therapy against different viral and host immune targets should be adopted in order to eradicate the drug resistant forms (Patel, 2002). So, considering this strategy interferons are used alone or in combination with ribavirin in order to treat hepatitis, but this sort of therapy is effective only in a percent of patients and this therapy is quite costly as patients belonging to developing country cannot afford it (Pan *et al.*, 2007).

Along with, many side effects have been associated with this regimen like cough, dyspnea, rash, itch, insomnia and nausea. Ribavirin is also known to accumulate inside red blood cells causing haemolytic anaemia of modest severity. The new treatment was the use of Pegylated interferon alfa-2b along with ribavirin but the side effects remained same along with increased flu like symptoms (Biscegle *et al.*, 2002). On the basis of these observations Joyce and Wilson along with other scientists suggested that RNA interference can prove a prospective therapy against HCV, but in June 2005 study from the same scientists showed that HCV replicons can break away from the RNAi designed against the NS5B coding region of viral genome, due to emergence of point mutations on the target sites (Wilson and Richardson, 2005). As, siRNA need complete complementarity with their target to perform their required activity, so they get fail when they are used against the viruses who posses ability to have heterogenetic forms (Schwab *et al.*, 2006).

2.8 MiRNAs as Antivirals

It has been previously reported that miRNAs can attest as innovative therapeutics against the viruses (Scaria *et al.*, 2006). As, it is evident from the recent research that siRNA has been failed in case of viruses having heterogenetic forms (Wilson and Richardson, 2005). By exploiting the antiviral potential of human miRNAs against different viral pathogens, it has been revealed that miRNAs can counteract PFV, AIV and HIV by targeting different genes in genome. MiRNAs have an edge over siRNA that they don't require complete complementarity for their action, so that they can handle more heterogenetic forms of a virus by captivating larger numbers of mutations (Scaria *et al.*, 2006). This urged the scientists to devise antivirals keeping in mind the enchanting aspect of miRNA function. The recent studies have shown that many computational techniques have been devised in order to identify targets for human oriented miRNAs (Min and Yoon, 2010).

2.9 Computational Prediction of Human MiRNA Targets

The very first prediction of the miRNA target was made by employing the classical approaches for genetics, but due to the long and laborious nature of the experiments computational techniques for the identification of targets have been devised. There are different tools for the miRNA target prediction in different species based upon different prediction techniques (Min and Yoon, 2010). As, it has been evident from the studies that human miRNAs can target the viral genomes (Vinod Scaria, 2006) so, many scientists has predicted targets of human oriented miRNAs in different viruses. In 2005, scientists predicted that there are five human oriented miRNAs that can target HIV-1 genes including the regulatory gene *nef* (Hariharam *et al.*, 2005). While patents to this invention are pending. Correspondingly, in 2010 the same group of scientists patented the identified targets against the hemaglutanin and polymerase genes of the Avian Influenza virus (Brahamchari *et al.*, 2010).

Methodology

MicroRNA engineering is a very emerging field, as mentioned in the previous chapter miRNAs can be used as therapeutics against viruses i.e. human miRNAs can be employed to halt the progression of virus associated disease development, by stopping the expression of certain genes that can be targeted by miRNAs. There are certain algorithmic techniques that can be used to predict targets for miRNAs in different genomes. As this goes on genomic level, so there comes the need to handle the large bulk of data in a progressive useful manner by extracting and arranging. For this purpose different scripting languages and the plotting tools can be used. In this study different dimensions of computational techniques have been suggested and effectively used in order to achieve research goals. Subsequent section defines and explains all the tools and steps that have been pursued in order to reach the set targets.

3.1 Data Retrieval

For the rationale of predicting targets different sequences have been collected by employing different online databases.

3.1.1 HCV Sequence Retrieval

'RefSeq' is a publicly available database provided by the **National Centre for Biotechnology Information** a Division of **National Library of Medicine** established in US National Institute of Health, and contains the sequences of protein, genome and transcript origin, belonging to different organisms ranging from eukaryotes to prokaryotes and viruses. The evident feature of this database is the non-redundancy. The sequences can be retrieved by employing the entrez query, Basic Local Alignment search Tool or through FTP site (Kim *et al.*, 2007). Genomic sequence of Hepatitis C Virus was retrieved by employing the query feature of database.

3.1.2 HCV Genotype Sequence Retrieval

The Sequences of five other main genotypes of HCV as, identified by **ICTVdb**, the database provided by the International Committee on Taxonomy of Viruses; which is an interactive, unanimously accessible database that provides all the information about the classification and taxonomical features of viruses (Osmond, 2003).

3.1.3 Micro RNA Sequence Retrieval

miRNA sequences for the ***Homo Sapiens*** were gathered from the **miRBase**, an online database meant for the searching and downloading of published and curated Micro RNA sequences for different organisms. The base contains the sequences of both Micro RNA precursors and the mature miRNA sequences. It provides the facility to download the required miRNA sequences by name, keyword, references or the annotation. miRNA sequences for a particular organism can be downloaded in a matter of few clicks. The database allows downloading sequence either in the unaligned fasta format or CLUSTALW alignment format (Thompson *et al.*, 2006). Total 721 miRNA mature sequences in the form of unaligned fasta format were downloaded from the database.

3.2 Target Prediction

The miRanda algorithm was used to predict the micro RNA targets for non-shuffled and shuffled sequences. This algorithm makes use of dynamic programming in order to align the miRNA with the target and generates an alignment score, during target prediction it allows G:U wobble and assigns high alignment scores to the complementary pairs present near the 5' end owing to the feature identified by the predicted miRNA-RNA duplexes. It employee the Vieena RNA Library to calculate the thermo dynamic energy of the high scoring duplexes (Enright *et al.*, 2003).

3.2.1 Procedure

As, miRanda is a LINUX based algorithm so, first of all it is configured and then installed by using the shell commands. Then two files, one containing the miRNA sequences and the other containing the target genome sequence one by one, were made to give input to the algorithm. The algorithm was used with the default parameters i.e. Gap open penalty is set to -8.0, Gap extend is set to -2.0, Score threshold is set to 50.0, Energy threshold is set to -20.0 Kcal / mol, and the scaling parameter is set to 2.0. The output file was generated containing the alignment of the query and the reference sequence, the score of the alignment, the binding energy of the duplex, the position of the miRNA binding on the genome and the length of the duplex along with some other parameters. For each genotype separate reference files were made and the algorithm was executed to predict the targets. A cut-off value for the score, was calculated in order to extract the significant hits from the obtained results, algorithm was executed again for the genotypes by taking cut-off value as score threshold.

3.3 Sequence Shuffling

An online application DNA shuffle endowed by the Sequence Manipulation Suit maintained by Bioinformatics organization, was used to shuffle the sequence by keeping its base composition constant. The sequence was input in the form of fasta formatted sequence in the chunks of 10,000 bps only, and the shuffled sequence is obtained by pressing the submit button (Stothard, 2000).

3.4 Data Parsing

The output from the target prediction algorithms was in the form of text files having thousands of line entries as records. So there was an immense need to parse the output files in order to obtain the parameter of interest. To attain this pattern extraction **gawk** scripting language is used which the GNU version of AWK is programming language (to be used as LINUX utility). **gawk** is suitable for one line programs to very complex applications with having support for the features of pattern matching to automated control of command- line argument files (Gordon , 1996). So a code was developed to extract two values from each record present in the sort of flat file database, i.e., energy and the score, while space character was taken as value separator. The parsed output was stored in a space delimited text file, which was opened by using the data import wizard of Microsoft Excel (2007) for further processing.

3.5 Data Plotting

In order to organise the output data most importantly the parsed components of output files different data plotting tools were used.

3.5.1 Using Microsoft Excel

In order to plot two data values of shuffled and non-shuffled sequences the Line-chart insert facility provided by the MS Excel (2007) was used to build a line graph of two quantities while non-shuffled scores were shown on the x-axis and the shuffled scores on the y-axis.

3.5.2 Using MATLAB 7.0

MATLAB (Matrix Laboratory) is a facility for computing the numerical data; it's a fourth generation language for programming and facilitates the user to program much easily. Due to its popularity MATLAB programming software has been developed which offers a programming interface and different tool boxes specialised for different fields (eetimes.com). MATLAB (7.0) maths tool box was used writing a code in order to generate a line graph of shuffled sequences score values versus non-shuffled sequences score values, in different colours to retain the uniqueness and to obtain the cut-off value.

3.6 Cut-off Value Determination

In order to attain a statistically significant data and to avoid the false positive results in the output, a cut-off value was derived by plotting the scores of normal sequences to the scores of shuffled sequences by using plotting tools, and the point, where all six graph line cut each other, was taken as cut-point above which all values were considered as significant.

3.7 Target Prediction Verification

In order to verify the targets predicted by miRanda, another algorithm RNAhybrid was taken up to verify the authenticity of targets. RNAhybrid is an algorithm used to predict the targets of miRNA on large genomic sequences, i.e., genomes of different organisms. It identifies the targets for the miRNAs while ignoring the intramolecular interactions either in smaller molecule or in the larger RNA molecule. It works on the principle of dynamic programming and calculates the most favourable and less favourable hits, i.e., the number can be restricted by the user or can be associated with the threshold of minimum free energy value of each duplex defined by the user (Rehmsmeier *et al.*, 2004).

3.7.1 Procedure

RNAhybrid is LINUX based algorithm and distributed under the GNU-GPL, so first of all it is configured and then installed using LINUX shell commands. As, miRNA sequences are much smaller compared to larger sequence so, prior to running RNAhybrid for target prediction, had to run RNAcalibrate in order to find out extreme value distribution parameters (i.e. shape and location parameters) for each miRNA sequence so that length normalised minimum free energies can be obtained.

3.7.1.1 Executing RNAcalibrate

This is again an open source LINUX based algorithmic module of RNAhybrid, first configured and installed using the shell commands. It took two files as input, one is query.fasta file containing the human miRNA sequences and the other is human.freq file containing the dinucleaotide frequencies of the human genome. Basically it searches a random sequence database either provided through command line or generates itself by the given sample size, length distribution parameter and dinucleaotide frequencies. Afterwards RNAcalibrate was run by using the following command:

```
. /RNAcalibrate –d 3UTR_human.freq -k 50 -l 50,30 -q query.fasta
```

This command generates 50 random sequences with lengths distributed according to the parameters of the normal distribution, i.e., mean equals to 50 and standard deviation of 30, according to the distribution parameters defined in the file 3UTR_human.freq and gives the parameters of extreme value distribution (location and shape) as output.

3.7.1.2 Taking average

As, RNAcalibrate returned the location and space parameters for all the micro RNA sequences present in the query.fasta file, so in order to run RNA hybrid I had to calculate the average of all the values by using simple mean calculation.

3.7.1.3 Parsing Genomes

As, RNAhybrid cannot take whole genomic sequence in a single batch, it only can take ~1,800 nucleotide long sequence as target, so I had to parse the genotype sequences into the lengths optimal for the input to the RNAhybrid.

3.7.1.4 Running RNAhybrid:

Finally, in order to obtain the miRNA targets and their length normalised energies following command is executed in the command line:

```
./RNAhybrid –d 1.9, 0.28 –t HCVGT11.fatsa –q query.fasta
```

This command identifies the targets for all the miRNAs present in the query.fasta file taking the sequence present in HCVGT1.fasta as target sequence, while taking given location and shape parameters to output the length normalised energies. The output file contained the aligned miRNA with target sequence along with minimum free energy and the p-value.

3.8 Genome Mapping

After predicting the targets in the genomes of different HCV genotypes, the genomic locations of those targets were identified.

1st the literature was searched for the identification of genes and their positions in the genome of HCV.

2nd the predicted targets were mapped to the genes where they were located by taking their positions, as output from RNAhybrid, the target prediction algorithm.

3.9 Sequence Alignment

The target sequences were aligned by using multiple sequence alignment tool CLUSTAL W (2.0) (ebi.ac.uk/Tools/msa/clustalw2/) whose apparent feature is incorporation of position based gap penalties (Thompson *etal.*, 1994). The alignment results were obtained by running the software with default parameters, i.e., Protein weight matrix as Gonnet, Gap open penalty as 10 and gap extend penalty as 0.20.

Results

The focal aspire of the present study was to identify the sequential patterns that are conserved in more than one genotypes of HCV, so that specific nucleotide sequence for the miRNA that can target more than one genotype can be identified.

4.1 Selected Genotypes

Six major genotypes were selected, and their gnomic sequences were obtained with the accession numbers as mentioned in the table 4.1

4.2 Human MiRNA

The ultimate objective of the research is to identify the targets of human oriented miRNAs in the genome of HCV, which takes human beings as its host. For this purpose miRBase database entries for human miRNA mature sequences were retrieved, which were found to be Seven hundred and twenty one (721) in number. Their accession numbers along with their chromosomal location were tabulated in the appendix section.

4.3 miRanda Predicted Targets

miRanda algorithm was used to predict the potential targets for the human miRNAs in six of the HCV genotypes. At first algo was executed with default parameters. This job resulted in thousands of predicted targets for each genotype. The number of predicted miRNA-mRNA duplexes is illustrated in figure 4.1.

Table 4.1 HCV six genotypes with their accession numbers and nucleotide length

Genotype	Ref Seq ID	Nucleotide Length
1	NC_004102	9,646
2	NC_009823	9,711
3	NC_009824	9,456
4	NC_009825	9,355
5	NC_009826	9,343
6	NC_009827	9,628

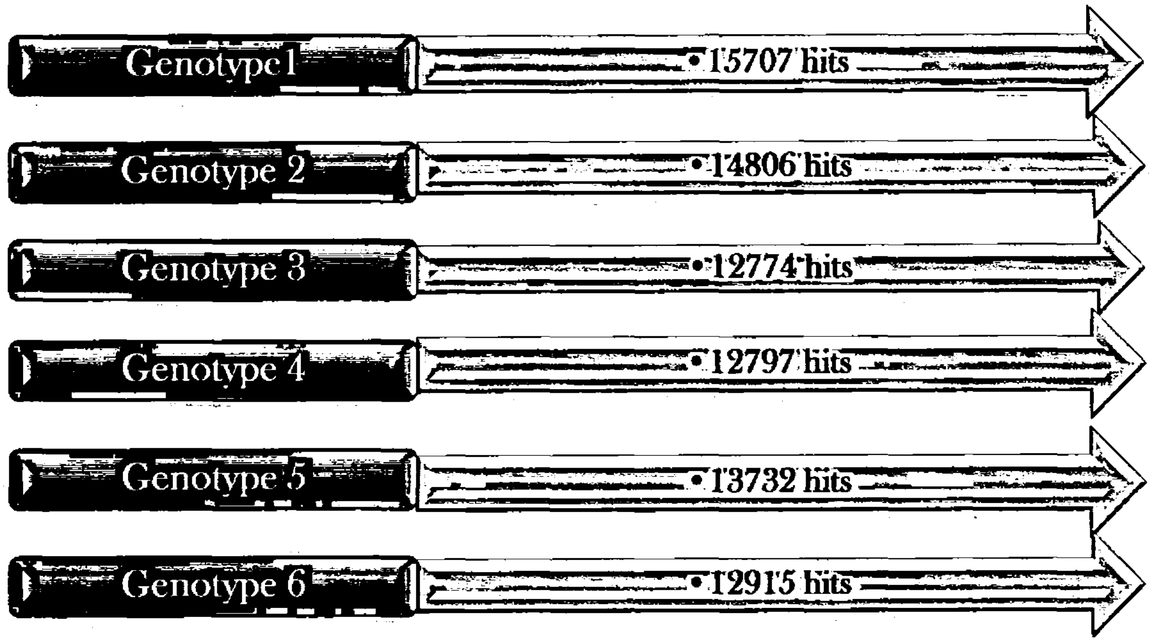

Figure 4.1 Number of identified human miRNA targets for each of the six genotypes by executing algo with default parameter

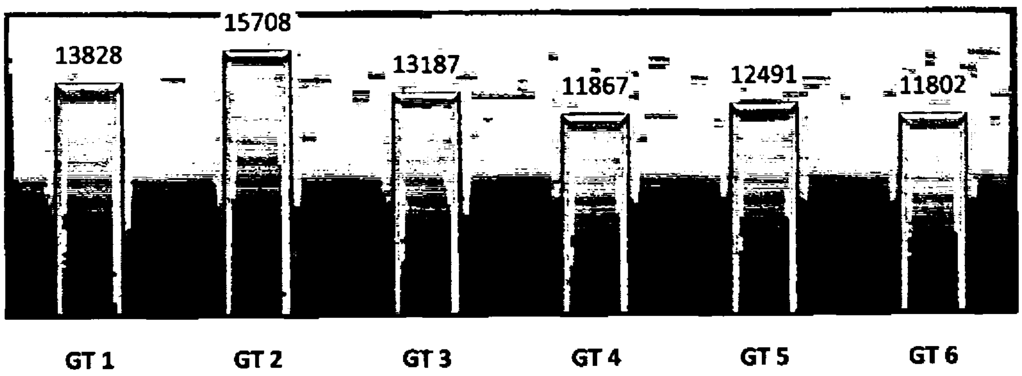

Figure 4.2 The number of miRNA-mRNA duplexes for the shuffled sequences of each of the six genotypes

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Then the genotypic sequences were shuffled by keeping their base composition constant in order to increase the stringency of results and to derive a cut-off value. After running the algorithm with default parameters, the number of targets obtained is shown in figure 4.2.

4.4 Predicted cut-off Value

To identify the most significant targets and to avoid false positive results a cut-off value is derived by plotting the miRNA-mRNA scores which were obtained from miRanda and extracted from the output file by using the gawk program. Microsoft Excel (2007) did not produced the desired plot, as the number of data points were much more than its capacity to plot, so MATLAB's plotted graph gave cut-off value to be 161evident from figure 4.3.

4.5 Cut-off applied miRanda Results

After calculating the cut-off the miRanda was again executed for the original genotype sequences in order to obtain the most significant targets for the miRNAs. The micro RNAs identified, having targets in HCV genotypes are shown in subsequent tables 4.2-4.7.

4.6 Micro RNAs Targeting More Than One Genotype

The miRNAs that were having targets in any of the six genotypes were scanned for the identification of those miRNAs which were targeting more than one genotype. The analysis was done in a manner that miRNAs which were having target in GT1 and any of the other genotype, similarly the miRNAs having targets in GT2 and

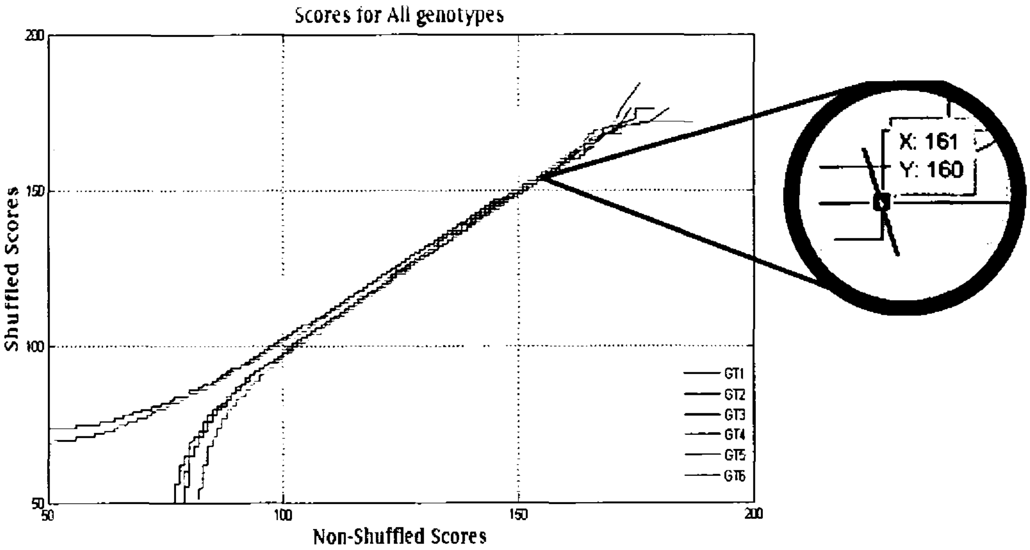

Figure 4.3 Graph depicting cut off value

other genotypes and so on so forth. Following tables 4.8- 4.13 show the miRNAs and the common genotypes they are targeting.

4.7 Target Prediction Through RNAhybrid

The miRNAs that were found to be targeting more than one genotype were tested against the six genotypes by using another algorithm RNAhybrid for the calculation of minimum free energies shown in table 4.14-4.19.

4.7 Genome Mapping

After getting the confirmed results from RNAhybrid the obtained targets were mapped into the genome of respective genotypes for identifying their actual location in the genes, constituting the HCV genome. The observed gene locations for the potentially targeting miRNAs are listed in subsequent tables 4.20-4.24 for each genotype respectively.

4.9 MiRNA Conservation

Evolutionary Relatedness of miRNAs targeting a specific gene was tested by aligning the miRNA sequences, but no significant result was obtained as evident from figure 4.4.

4.9 Target Conservation:

Finally the target sites for miRNAs targeting specific gene in maximum genotypes are aligned in order to check for conservation and to identify the sequences for artificial miRNA designing, shown in figure 4.5.

Table 4.2 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 1 of HCV

No.	Human miRNA	Scores
1	hsa-mir-194*	163
2	hsa-mir-502-3p	164
3	hsa-mir-1254	163
4	hsa-mir-1180	163
5	hsa-mir-138	162
6	hsa-mir-648	161
7	hsa-mir-615-5p	166
8	hsa-mir-1974	171
9	hsa-mir-1289	172
10	hsa-mir-2278	164
11	hsa-mir-638	162
12	hsa-mir-658	165
13	hsa-mir-298	170
14	hsa-mir-92b	163
15	hsa-mir-542-5p	162
16	hsa-mir-25*	165
17	hsa-mir-143*	172
18	hsa-mir-708	161
19	hsa-mir-145	162
20	hsa-mir-1914	171

Contd...

21	hsa-mir-484	165
22	hsa-mir-1202	164
23	hsa-mir-624	166
24	hsa-mir-1913	162
25	hsa-mir-296-5p	161
26	hsa-mir-15b	163
27	hsa-mir-885-3p	164
28	hsa-mir-936	161
29	hsa-mir-1469	164
30	hsa-mir-624*	162
31	hsa-mir-34b*	164
32	hsa-mir-1181	170
33	hsa-mir-1910	165
34	hsa-mir-487b	161
35	hsa-mir-1307	163
36	hsa-mir-518c*	165
37	hsa-let-7b	162
38	hsa-mir-92a	161
39	hsa-mir-611	176
40	hsa-mir-185	165
41	hsa-let-7c	166
42	hsa-mir-339-5p	168
43	hsa-mir-196a*	169

Contd...

44	hsa-mir-21	170
45	hsa-mir-122	165
56	hsa-mir-188-5p	166
47	hsa-mir-1265	168
48	hsa-mir-551b	163
49	hsa-mir-1183	168
50	hsa-mir-662	163
51	hsa-mir-1275	162
52	hsa-mir-671-5p	162

Table 4.3 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 2 of HCV

No.	Human miRNA	Scores
1	hsa-mir-376b	163
2	hsa-mir-637	170
3	hsa-mir-1254	162
4	hsa-mir-1180	162
5	hsa-mir-20b	164
6	hsa-mir-22	164
7	hsa-mir-1974	162
8	hsa-mir-193a-5p	161
9	hsa-mir-2278	161
10	hsa-mir-532-3p	169

Contd..

11	hsa-mir-929-2*	161
12	hsa-mir-585	164
13	hsa-mir-1471	163
14	hsa-mir-486-3p	168
15	hsa-mir-449a	162
16	hsa-mir-1226*	170
17	hsa-mir-298	162
18	hsa-mir-455-5p	161
19	hsa-mir-103	165
20	hsa-mir-615-3p	164
21	hsa-mir-92b*	162
22	hsa-mir-646	163
23	hsa-mir-1914	167
24	hsa-mir-1202	167
25	hsa-mir-15b	164
26	hsa-mir-1324	163
27	hsa-mir-1271	161
28	hsa-mir-1915*	162
29	hsa-mir-767-3p	164
30	hsa-mir-885-3p	161
31	hsa-mir-873	161
32	hsa-mir-608	182
33	hsa-mir-432	165

Contd...

34	hsa-mir-769-3p	161
35	hsa-mir-449c*	163
36	hsa-mir-942	161
37	hsa-mir-1257	170
38	hsa-mir-23b*	176
39	hsa-mir-526b	165
40	hsa-mir-149*	162
41	hsa-mir-378*	178
42	hsa-mir-339-5p	173
43	hsa-mir-1249	163
44	hsa-mir-196a	164
45	hsa-mir-345	162
56	hsa-mir-670	164
47	hsa-mir-614	176
48	hsa-mir-122	164
49	hsa-mir-1979	163
50	hsa-mir-107	165
51	hsa-mir-15a	164
52	hsa-mir-1244	171
53	hsa-mir-103-2*	161
54	hsa-mir-574-5p	162
55	hsa-mir-193b*	163
56	hsa-mir-365*	164
57	hsa-mir-497	164

Contd...

58	hsa-mir-1183	161
59	hsa-mir-499-3p	170
60	hsa-mir-662	162
61	hsa-mir-1205	163
62	hsa-mir-1225-5p	163
63	hsa-mir-1975	161

Table 4.4 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 3 of HCV

No.	Human miRNA	Score
1	hsa-mir-34c-5p	161
2	hsa-mir-1184	168
3	hsa-mir-125a-5p	161
4	hsa-mir-221	163
5	hsa-mir-483-3p	162
6	hsa-mir-204	164
7	hsa-mir-363*	161
8	hsa-mir-585	162
9	hsa-mir-1471	162
10	hsa-mir-93*	166
11	hsa-mir-769-5p	168
12	hsa-mir-1236	167
13	hsa-mir-1201	161

Contd...

14	hsa-mir-200c	163
15	hsa-mir-220c	161
16	hsa-mir-765	162
17	hsa-mir-532-5p	162
18	hsa-mir-15b	167
19	hsa-mir-29c	171
20	hsa-mir-1291	161
21	hsa-mir-1226	165
22	hsa-mir-125b	167
23	hsa-mir-220b	161
24	hsa-mir-29a	163
25	hsa-mir-940	162
26	hsa-mir-1294	172
27	hsa-mir-125a-3p	161
28	hsa-mir-331-5p	161
29	hsa-mir-762	163
30	hsa-mir-566	165
31	hsa-mir-523	164
32	hsa-mir-22*	167
33	hsa-mir-361-3p	161
34	hsa-mir-611	174
35	hsa-mir-199a-3p	162
36	hsa-mir-449c	163

Contd..

37	hsa-mir-99b	165
38	hsa-mir-424	168
39	hsa-mir-614	161
40	hsa-mir-199b-3p	162
41	hsa-mir-122	171
42	hsa-mir-377*	169
43	hsa-mir-485-3p	161
44	hsa-mir-425	176
45	hsa-mir-193b*	162
46	hsa-mir-365*	163
47	hsa-mir-497	163
48	hsa-mir-551b	163
49	hsa-mir-1183	165
50	hsa-mir-595	163
51	hsa-mir-200a*	162
52	hsa-mir-512-5p	161

Table 4.5 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 4 of HCV

No.	Human miRNAs [illegible]	Score
1	hsa-mir-132*	162
2	hsa-mir-557	163
3	hsa-mir-490-5p	166

Contd...

4	hsa-mir-105*	164
5	hsa-mir-1228*	166
6	hsa-mir-1974	168
7	hsa-mir-125a-5p	165
8	hsa-mir-668	163
9	hsa-mir-1909	166
10	hsa-mir-490-3p	162
11	hsa-mir-1226*	161
12	hsa-mir-1268	166
13	hsa-mir-298	164
14	hsa-mir-1182	171
15	hsa-mir-10a	162
16	hsa-mir-212	162
17	hsa-mir-525-5p	170
18	hsa-mir-411	165
19	hsa-mir-652	164
20	hsa-mir-489	165
21	hsa-mir-1914	162
22	hsa-mir-2116*	167
23	hsa-mir-1271	164
24	hsa-mir-939	167
25	hsa-mir-535	161
26	hsa-mir-885-3p	172
27	hsa-mir-125b	161

Contd...

28	hsa-mir-323-5p	174
29	hsa-mir-624*	164
30	hsa-mir-34b	164
31	hsa-mir-769-3p	165
32	hsa-mir-15a*	168
33	hsa-mir-29b-2*	163
34	hsa-mir-523	164
35	hsa-mir-941	161
36	hsa-mir-449b*	162
37	hsa-mir-361-3p	169
38	hsa-mir-593*	165
39	hsa-mir-760	166
40	hsa-mir-611	172
41	hsa-mir-501-5p	168
42	hsa-mir-199b-5p	171
43	hsa-mir-378*	166
44	hsa-mir-1225-5p	162
45	hsa-mir-196a*	172
46	hsa-mir-122	164
47	hsa-mir-196a	164
48	hsa-mir-632	161
49	hsa-mir-455-3p	162
50	hsa-mir-196b	171

Contd...

51	hsa-mir-767-5p	163
52	hsa-mir-551b	163
53	hsa-mir-662	166
54	hsa-mir-1293	168
55	hsa-mir-671-5p	162

Table 4.6 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 5 of HCV

No.	Human miRNA	Score
1	hsa-mir-380*	166
2	hsa-mir-1228*	166
3	hsa-mir-615-5p	161
4	hsa-mir-483-3p	162
5	hsa-let-7g*	166
6	hsa-mir-92a-2*	172
7	hsa-mir-661	167
8	hsa-mir-146b-3p	164
9	hsa-mir-130b	164
10	hsa-mir-663	165
11	hsa-mir-379	162
12	hsa-mir-329	163
13	hsa-mir-494	161
14	hsa-mir-220c	161

Contd...

15	hsa-mir-1202	161
16	hsa-let-7a-2*	179
17	hsa-mir-939	167
18	hsa-mir-1915*	163
19	hsa-mir-1291	162
20	hsa-mir-608	173
21	hsa-mir-1226	172
22	hsa-mir-1469	162
23	hsa-mir-1262	164
24	hsa-mir-624*	168
25	hsa-mir-940	167
26	hsa-mir-412	166
27	hsa-mir-634	162
28	hsa-mir-98	164
29	hsa-mir-221*	162
30	hsa-mir-378*	166
31	hsa-mir-199a-3p	165
32	hsa-mir-191*	167
33	hsa-mir-196a*	169
34	hsa-mir-199b-3p	165
35	hsa-mir-140-3p	163
36	hsa-mir-371-3p	161
37	hsa-mir-887	163

Contd...

38	hsa-mir-1197	161
39	hsa-mir-551b	163
40	hsa-mir-1183	187
41	hsa-mir-1975	162

Table 4.7 MiRNAs and their scores of target duplex, after applying cut-off value in Genotype 5 of HCV

No.	Human miRNA	Score
1	hsa-mir-637	161
2	hsa-mir-509-3-5p	162
3	hsa-mir-1254	162
4	hsa-mir-138	168
5	hsa-mir-650	165
6	hsa-mir-150*	167
7	hsa-mir-483-3p	162
8	hsa-mir-2278	166
9	hsa-mir-1909	162
10	hsa-mir-549	163
11	hsa-mir-929-2*	162
12	hsa-mir-1226*	167
13	hsa-mir-663	175
14	hsa-mir-1182	162
15	hsa-mir-329	162

Contd...

16	hsa-mir-548o	164
17	hsa-mir-1202	163
18	hsa-mir-296-5p	161
19	hsa-mir-188-3p	161
20	hsa-mir-591	165
21	hsa-mir-939	175
22	hsa-mir-873	164
23	hsa-mir-498	163
24	hsa-mir-1226	168
25	hsa-mir-2115	163
26	hsa-mir-323-5p	167
27	hsa-mir-1224-5p	161
28	hsa-mir-596	161
29	hsa-mir-503	164
30	hsa-let-7g	162
31	hsa-mir-769-3p	167
32	hsa-mir-593	168
33	hsa-mir-1910	171
34	hsa-let-7i	167
35	hsa-mir-149*	162
36	hsa-mir-1284	175
37	hsa-mir-149	179
38	hsa-mir-18a*	165

Contd...

39	hsa-mir-525-3p	161
40	hsa-mir-182	167
41	hsa-mir-485-5p	161
42	hsa-mir-601	161
43	hsa-mir-1272	168
44	hsa-mir-551b	164
45	hsa-mir-595	163
46	hsa-mir-135b*	163

Table 4.8 MiRNAs that are targeting commonly in GT1 and rest of genotypes

No.	miRNA	GT1	GT2	GT3	GT4	GT5	GT6
1	hsa-mir-1254	√	√				√
2	hsa-mir-1180	√	√				
3	hsa-mir-138	√					√
4	hsa-mir-615-5p	√				√	
5	hsa-mir1974	√	√		√		
6	hsa-mir-2278	√					√
7	hsa-mir-298	√	√		√		
No.	miRNA	GT1	GT2	GT3	GT4	GT5	GT6
8	hsa-mir-1914	√	√		√		
9	hsa-mir-1202	√	√			√	√
10	hsa-mir-15b	√	√	√			
11	hsa-mir-885-3p	√	√		√		

Contd...

12	hsa-mir-624*	√			√	√	
13	hsa-mir-1910	√					√
14	hsa-mir-611	√		√	√		
15	hsa-mir-339-5p	√	√				
16	hsa-mir-196a*	√	√		√	√	
17	hsa-mir-122	√	√	√	√		
18	hsa-mir-551b	√		√	√	√	√
19	hsa-mir-1183	√	√	√		√	

Table 4.9 MiRNAs that are targeting commonly in GT2 and rest of genotypes

NO	miRNA	GT2	GT3	GT4	GT5	GT6
1	hsa-mir-637	√				√
2	hsa-mir-92a-2*	√			√	√
3	hsa-mir-585	√	√			
4	hsa-mir-1471	√	√			
5	hsa-mir-1226*	√				
6	hsa-mir-1271	√		√		
7	hsa-mir-1915*	√			√	
8	hsa-mir-608	√			√	
9	hsa-mir-378*	√		√	√	
10	hsa-mir-193b*	√	√			
11	hsa-mir-365*	√	√			
12	hsa-mir-497	√	√			

Contd...

13	hsa-mir-662	√		√		
14	hsa-mir-1975	√			√	

Table 4.10 MiRNAs that are targeting commonly in GT3 and rest of genotypes

NO	miRNA	GT3	GT4	GT5	GT6
1	hsa-mir-220c	√		√	
2	hsa-mir-1291	√		√	
3	hsa-mir-1226	√		√	
4	hsa-mir-125b	√	√		
5	hsa-mir-125a-5p	√	√		
6	hsa-mir-483-3p	√			√
7	hsa-mir-940	√		√	
8	hsa-mir-523	√	√		
9	hsa-mir-199b-3p	√		√	
10	hsa-mir-595	√			√

Contd...

Table 4.11 MiRNAs that are targeting commonly in GT4 and rest of genotypes

NO	miRNA	GT3	GT4	GT5	GT6
1	hsa-mir-220c	√		√	
2	hsa-mir-1291	√		√	
3	hsa-mir-1226	√		√	
4	hsa-mir-125b	√	√		

5	**hsa-mir-125a-5p**	√	√		
6	**hsa-mir-483-3p**	√			√
7	**hsa-mir-940**	√		√	
8	**hsa-mir-523**	√	√		
9	**hsa-mir-199b-3p**	√		√	
10	**hsa-mir-595**	√			√

Contd...

Table 4.12 MiRNAs that are targeting commonly in GT4, GT5 and GT6

NO	miRNA	GT4	GT5	GT6
1	**hsa-mir-1228***	√	√	
2	**hsa-mir-1909**	√		√
3	**hsa-mir-1182**	√		√
4	**hsa-mir-769-3p**	√		√

Table 4.13 Showing miRNAs that are targeting commonly in GT5 and GT6

NO	miRNA	GT5	GT6
1	**hsa-mir-663**	√	√
2	**hsa-mir-329**	√	√

Table 4.14 The miRNAs and their targets predicted through RNAhybrid in GT1

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-37.7
2	Hsa-mir-1180	-35.8
3	Hsa-mir-138	-32.1
4	Hsa-mir-615-5p	-41.3
5	Hsa-mir-1974	-33.4
6	Hsa-mir-2278	-32.4
7	Hsa-mir-298	-36.8
8	Hsa-mir-1914	-35.9
9	Hsa-mir-1202	-35.7
10	Hsa-mir-15b	-26.0
11	Hsa-mir-885-5p	-30.0
12	Hsa-mir-624*	-31.1
13	Hsa-mir-1910	-32.9
14	Hsa-mir-611	-45.7
15	Hsa-mir-339-3p	-31.4
16	Hsa-mir-196a*	-29.6
17	Hsa-mir-122	-29.3
18	Hsa-mir-551b	-34.1
19	Hsa-mir-1183	-37.3
20	Hsa-mir-637	-41.6
21	Hsa-mir-92a-2*	-28.5
22	Hsa-mir-585	-

Contd...

23	Hsa-mir-1471	-36.3
24	Hsa-mir1226*	-44.7
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-42.5
27	Hsa-mir-608	-41.6
28	Hsa-mir-378*	-33.9
29	Hsa-mir-193b*	-30.9
30	Hsa-mir-365*	-33.5
31	Hsa-mir-497	-31.2
32	Hsa-mir-662	-34.9
33	Hsa-mir-1975	-37.7
34	Hsa-mir-220c	-29.6
35	Hsa-mir-1291	-34.0
36	Hsa-mir-1226	-30.7
37	Hsa-mir-125b	-28.0
38	Hsa-mir-125a-5p	-31.6
39	Hsa-mir-483-3p	-27.9
40	Hsa-mir-940	-39.8
41	Hsa-mir-523	-36.1
42	Hsa-mir-199b-3p	-27.1
43	Hsa-mir-595	-33.2
44	Hsa-mir-1228*	-34.7
45	Hsa-mir-1909	*Contd...*

46	Hsa-mir-1182	-33.2
47	Hsa-mir-769-3p	-36.9
48	Hsa-mir-663	-40.2
49	Hsa-mir-329	-28.0

Table 4.15 The miRNAs and their targets predicted through RNAhybrid in GT2

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-34.6
2	Hsa-mir-1180	-34.8
3	Hsa-mir-138	-34.2
4	Hsa-mir-615-5p	-39.3
5	Hsa-mir-1974	-28.7
6	Hsa-mir-2278	-33.0
7	Hsa-mir-298	-33.6
8	Hsa-mir-1914	-37.4
9	Hsa-mir-1202	-36.7
10	Hsa-mir-15b	-26.3
11	Hsa-mir-885-5p	-28.5
12	Hsa-mir-624*	-27.3
13	Hsa-mir-1910	-36.2
14	Hsa-mir-611	-31.5
15	Hsa-mir-339-3p	-34.1
16	Hsa-mir-196a*	-[illegible]

Contd..

17	Hsa-mir-122	-27.6
18	Hsa-mir-551b	-32.0
19	Hsa-mir-1183	-34.0
20	Hsa-mir-637	-38.0
21	Hsa-mir-92a-2*	-30.4
22	Hsa-mir-585	-30.5
23	Hsa-mir-1471	-35.4
24	Hsa-mir1226*	-40.7
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-41.3
27	Hsa-mir-608	-43.9
28	Hsa-mir-378*	-36.5
29	Hsa-mir-193b*	-30.1
30	Hsa-mir-365*	-33.2
31	Hsa-mir-497	-33.2
32	Hsa-mir-662	-31.3
33	Hsa-mir-1975	-32.5
34	Hsa-mir-220c	-30.9
35	Hsa-mir-1291	-31.9
36	Hsa-mir-1226	-30.0
37	Hsa-mir-125b	-28.4
38	Hsa-mir-125a-5p	-29.2
39	Hsa-mir-483-3p	-

Contd...

40	Hsa-mir-940	-36.1
41	Hsa-mir-523	-31.9
42	Hsa-mir-199b-3p	-27.4
43	Hsa-mir-595	-31.5
44	Hsa-mir-1228*	-35.9
45	Hsa-mir-1909	-35.7
46	Hsa-mir-1182	-36.0
47	Hsa-mir-769-3p	-35.6
48	Hsa-mir-663	-36.4
49	Hsa-mir-329	-26.8

Table 4.16 The miRNAs and their targets predicted through RNAhybrid in GT3

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-32.7
2	Hsa-mir-1180	-34.1
3	Hsa-mir-138	-30.8
4	Hsa-mir-615-5p	-35.8
5	Hsa-mir-1974	-29.7
6	Hsa-mir-2278	-35.1
7	Hsa-mir-298	-32.1
8	Hsa-mir-1914	-33.9
9	Hsa-mir-1202	-39.0
10	Hsa-mir-15b	*Contd..*

11	Hsa-mir-885-5p	-31.4
12	Hsa-mir-624*	-30.7
13	Hsa-mir-1910	-35.8
14	Hsa-mir-611	-42.8
15	Hsa-mir-339-3p	-33.2
16	Hsa-mir-196a*	-29.4
17	Hsa-mir-122	-30.7
18	Hsa-mir-551b	-32.2
19	Hsa-mir-1183	-37.7
20	Hsa-mir-637	-38.5
21	Hsa-mir-92a-2*	-30.8
22	Hsa-mir-585	-29.2
23	Hsa-mir-1471	-34.7
24	Hsa-mir1226*	-38.0
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-39.7
27	Hsa-mir-608	-36.3
28	Hsa-mir-378*	-36.4
29	Hsa-mir-193b*	-30.6
30	Hsa-mir-365*	-34.6
31	Hsa-mir-497	-30.6
32	Hsa-mir-662	-31.8
33	Hsa-mir-1975	-

Contd...

34	Hsa-mir-220c	-31.3
35	Hsa-mir-1291	-38.0
36	Hsa-mir-1226	-32.2
37	Hsa-mir-125b	-29.6
38	Hsa-mir-125a-5p	-29.4
39	Hsa-mir-483-3p	-31.8
40	Hsa-mir-940	-36.8
41	Hsa-mir-523	-31.4
42	Hsa-mir-199b-3p	-30.0
43	Hsa-mir-595	-30.0
44	Hsa-mir-1228*	-34.9
45	Hsa-mir-1909	-37.0
46	Hsa-mir-1182	-34.4
47	Hsa-mir-769-3p	-37.0
48	Hsa-mir-663	-38.5
49	Hsa-mir-329	-25.8

Table 4.17 The miRNAs and their targets predicted through RNAhybrid in GT4

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-36.0
2	Hsa-mir-1180	36.5
3	Hsa-mir-138	-34.1
4	Hsa-mir-615-5p	[illegible]

Contd...

5	Hsa-mir-1974	-29.5
6	Hsa-mir-2278	-32.8
7	Hsa-mir-298	-36.2
8	Hsa-mir-1914	-37.4
9	Hsa-mir-1202	-33.7
10	Hsa-mir-15b	-27.0
11	Hsa-mir-885-5p	-29.5
12	Hsa-mir-624*	-27.7
13	Hsa-mir-1910	-38.8
14	Hsa-mir-611	-38.0
15	Hsa-mir-339-3p	-31.4
16	Hsa-mir-196a*	-30.5
17	Hsa-mir-122	-29.8
18	Hsa-mir-551b	-34.1
19	Hsa-mir-1183	-36.0
20	Hsa-mir-637	-36.2
21	Hsa-mir-92a-2*	-29.1
22	Hsa-mir-585	-27.1
23	Hsa-mir-1471	-35.6
24	Hsa-mir1226*	-42.1
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-36.4
27	Hsa-mir-608	[illegible]

Contd...

28	Hsa-mir-378*	-32.7
29	Hsa-mir-193b*	-31.9
30	Hsa-mir-365*	-33.2
31	Hsa-mir-497	-27.8
32	Hsa-mir-662	-31.2
33	Hsa-mir-1975	-33.2
34	Hsa-mir-220c	-32.6
35	Hsa-mir-1291	-33.7
36	Hsa-mir-1226	-31.4
37	Hsa-mir-125b	-29.2
38	Hsa-mir-125a-5p	-27.6
39	Hsa-mir-483-3p	-28.6
40	Hsa-mir-940	-36.9
41	Hsa-mir-523	-31.1
42	Hsa-mir-199b-3p	-25.5
43	Hsa-mir-595	-29.2
44	Hsa-mir-1228*	-35.1
45	Hsa-mir-1909	-35.6
46	Hsa-mir-1182	-37.1
47	Hsa-mir-769-3p	-36.2
48	Hsa-mir-663	-43.3
49	Hsa-mir-329	-25.7

Table 4.18 The miRNAs and their targets predicted through RNAhybrid in GT5

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-35.4
2	Hsa-mir-1180	-34.0
3	Hsa-mir-138	-31.4
4	Hsa-mir-615-5p	-35.9
5	Hsa-mir-1974	-32.3
6	Hsa-mir-2278	-31.0
7	Hsa-mir-298	-33.3
8	Hsa-mir-1914	-35.5
9	Hsa-mir-1202	-34.4
10	Hsa-mir-15b	-29.2
11	Hsa-mir-885-5p	-28.6
12	Hsa-mir-624*	-40.1
13	Hsa-mir-1910	-33.1
14	Hsa-mir-611	-40.7
15	Hsa-mir-339-3p	-32.0
16	Hsa-mir-196a*	-29.6
17	Hsa-mir-122	-26.4
18	Hsa-mir-551b	-34.1
19	Hsa-mir-1183	-40.2
20	Hsa-mir-637	-38.5
21	Hsa-mir-92a-2*	-36.9

Contd...

22	Hsa-mir-585	-28.8
23	Hsa-mir-1471	-37.1
24	Hsa-mir1226*	-42.0
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-37.6
27	Hsa-mir-608	-44.3
28	Hsa-mir-378*	-37.7
29	Hsa-mir-193b*	-33.5
30	Hsa-mir-365*	-32.5
31	Hsa-mir-497	-28.2
32	Hsa-mir-662	-33.0
33	Hsa-mir-1975	-37.0
34	Hsa-mir-220c	-32.0
35	Hsa-mir-1291	-33.4
36	Hsa-mir-1226	-30.7
37	Hsa-mir-125b	-29.0
38	Hsa-mir-125a-5p	-30.2
39	Hsa-mir-483-3p	-29.7
40	Hsa-mir-940	-41.3
41	Hsa-mir-523	-29.9
42	Hsa-mir-199b-3p	-27.2
43	Hsa-mir-595	-32.1
44	Hsa-mir-1228*	-40.6

Contd...

45	Hsa-mir-1909	-36.0
46	Hsa-mir-1182	-34.6
47	Hsa-mir-769-3p	-35.9
48	Hsa-mir-663	-42.3
49	Hsa-mir-329	-28.2

Table 4.19 The miRNAs and their targets predicted through RNAhybrid in GT6

NO	MiRNA	Mfe kcal/mol
1	Hsa-mir-1254	-39.3
2	Hsa-mir-1180	-37.7
3	Hsa-mir-138	-31.3
4	Hsa-mir-615-5p	-38.0
5	Hsa-mir-1974	-29.2
6	Hsa-mir-2278	-33.8
7	Hsa-mir-298	-34.0
8	Hsa-mir-1914	-35.3
9	Hsa-mir-1202	-35.2
10	Hsa-mir-15b	-26.9
11	Hsa-mir-885-5p	-28.6
12	Hsa-mir-624*	-29.2
13	Hsa-mir-1910	-36.5
14	Hsa-mir-611	-36.2
15	Hsa-mir-339-3p	-32.6

Contd...

16	Hsa-mir-196a*	-25.7
17	Hsa-mir-122	-27.8
18	Hsa-mir-551b	-32.2
19	Hsa-mir-1183	-39.2
20	Hsa-mir-637	-37.2
21	Hsa-mir-92a-2*	-33.3
22	Hsa-mir-585	-26.4
23	Hsa-mir-1471	-34.6
24	Hsa-mir1226*	-40.5
25	Hsa-mir-1271	-32.8
26	Hsa-mir-1915*	-34.2
27	Hsa-mir-608	-37.9
28	Hsa-mir-378*	-34.9
29	Hsa-mir-193b*	-32.5
30	Hsa-mir-365*	-38.3
31	Hsa-mir-497	-31.0
32	Hsa-mir-662	-34.4
33	Hsa-mir-1975	-33.6
34	Hsa-mir-220c	-34.7
35	Hsa-mir-1291	-28.9
36	Hsa-mir-1226	-37.7
37	Hsa-mir-125b	-28.6
38	Hsa-mir-125a-5p	-29.4

Contd...

39	Hsa-mir-483-3p	-28.0
40	Hsa-mir-940	-35.6
41	Hsa-mir-523	-30.0
42	Hsa-mir-199b-3p	-26.4
43	Hsa-mir-595	-29.8
44	Hsa-mir-1228*	-35.3
45	Hsa-mir-1909	-37.4
46	Hsa-mir-1182	-31.6
47	Hsa-mir-769-3p	-38.6
48	Hsa-mir-663	-42.0
49	Hsa-mir-329	-27.9

Table 4.20 The confirmed miRNA targets through RNAhybrid and their respective genomic positions, common in genotype 1 to 6 respectively. While hm stands for HomoSapians miRNA, pro for protease and env-pro for envelop protein.

No	miRNA	GT1	GT2	GT3	GT4	GT5	GT6
1	**hm-1254**	3990-4016	98-49				97-118
		NS3-Pro	5`-UTR				5`-UTR
2	**hm-1180**	3581-3601	9267-9290				
		NS3-Pro	NS5B- RDRP				
3	**hm-138**	3455-3480					887-909

Contd...

		NS3-Pro					Core-Pro
4	**hm-615-5p**	8638-8661				4685-4708	
		NS5B-RDRP				NS3-Pro	
5	**Hs-1974**	7203-7225			4685-4708		
		NS5A-Pro			NS3-Pro		
6	**hm-2278**	2061-2083					3790-3813
		E2-Env-Pro					NS3-Pro
7	**hm-298**	4501-4524	4512-4535		4437-4462		
		NS3-Pro	NS3-Pro		NS3-Pro		
8	**hm-1914**	2239-2259	2250-2270		2225-2251		
		E2-Env-Pro	E2-Env-Pro		E2-Env-Pro		
9	**hm-1202**	1788-1805	1583-1603			1522-1542	1582-1602
		E2-Env-Pro	E2-Env-Pro			E2-Env-Pro	E2-Env-Pro
10	**hm-15b**	1447-1469	7647-7668	7610-7631			
		E2-Env-Pro	NS5B-RDRP	NS5B-RDRP			
11	**hm-885-3p**	6406-6430	894-917		9139-9160		
		NS5A-Pro	C-Env/E1-		NS5B-		

Contd..

			Env		RDRP		
12	**hm-624***	4082-4103			3096-3121	4023-4044	
		NS3-Pro			NS2-Pro	NS3-Pro	
13	**hm-1910**	8512-8534					5870-5890
		NS5B-RDRP					NS4B-Pro
14	**hm-611**	8233-8256		5338-5360	1326-1349		
		NS5B-RDRP		NS4A-Pro	E1-Env-Pro		
15	**hm-339-5p**	2293-2314	802-825				
		E2-Env-Pro	C-Env-Pro				
16	**hm-196a***	433-455	430-454		369-393	371-393	
		C-Enc-Pro	C-Env-Pro		C-Env-Pro	C-Env-Pro	
17	**hm-122**	991-1013	9444-9467	8821-8841			
		E1-Env-Pro	3\`-UTR	NS5B-RDRP			
18	**hm-551b**	248-272		246-270	186-210	186-210	2133-2155
		5\`-UTR		5\`-UTR	5\`-UTR	5\`-UTR	E2-Env-Pro

Contd..

19	**hm-1183**	4927-4952	5099-5131	1052-1077		4682-4713	
		NS3-Pro	NS3-Pro	E1-Env-Pro		NS3-Pro	

Table 4.21 The confirmed miRNA targets through RNAhybrid and their respective genomic positions, common in genotype 2 to 6 respectively. While hm stands for Homo Sapians miRNA, Pro for protease and env-pro for envelop protein.

NO	miRNA	GT2	GT3	GT4	GT5	GT6
1	**hm-637**	7563-7589				2150-2175
		NS5A-Pro				E2-Env-Pro
2	**hm-92a-2***	4410-4431			4340-4361	4619-4642
		NS3-Pro			NS3-Pro	NS3-Pro
3	**hm-585**	4389-4406	3439-3458			
		NS3-Pro	NS3-Pro			
4	**hm-1471**	3769-3791	2942-2962			
		NS3-Pro	NS2-Pro			
5	**hm-1226***	2452-2481				6257-6283
		E2-Env-Pro				NS5A-Pro
6	**hm-1271**	4987-5009		4915-4936		
		NS3-Pro		NS3-Pro		
7	**hm-1915***	3110-3131			6430-6456	
		NS2-Pro			NS5A-Pro	
8	**hm-608**	878-904			7805-7832	
		Core-Pro			NS5B-RDRP	

Contd...

9	**hm-378***	4183-4203		4107-4130	4113-4133	
		NS3-Pro		NS3-Pro	NS3-Pro	
10	**hm-193b***	7282-7309	8246-8271			
		NS5A-Pro	NS5B-RDRP			
11	**hm-365***	6668-6691	1944-1967			
		NS5A-Pro	E2-Env-Pro			
12	**hm-497**	7648-7668	7611-7631			
		NS5B-RDRP	NS5B-RDRP			
13	**hm-662**	6053-6073		5980-6000		
		NS4B-Pro		NS4B-Pro		
14	**hm-1975**	3314-3336			1102-1127	
		NS2-Pro			E1-Env-Pro	

Table 4.22 The confirmed miRNA targets through RNAhybrid and their respective genomic positions, common in genotype 3 to 6 respectively. While hm stands for Homo Sapians miRNA, Pro for protease and env-pro for envelop protein.

NO	miRNA	GT3	GT4	GT5	GT6
1	**hs-220c**	6824-6845		6749-6770	
		NS5A-Protein		NS5A-Protein	
2	**hs-1291**	7955-7975		3807-3829	
		NS5B-RDRP		NS3-Protease	
3	**hs-1226**	5912-5933		4021-4042	
		NS4B-Protein		NS3-Protease	
4	**hs-125b**	1458-1480	1611-1633		

		E2-Env-Protein	E2-Env-Protein		
5	**hs-125a-5p**	1458-1480	1611-1633		
		E2-Env-Protein	E2-Env-Protein		
6	**hs-483-3p**	5471-5490			5471-5490
		*			*
7	**hs-940**	8179-8201		4367-4387	
		NS5B-RDRP		NS3-Protease	
8	**hs-523**	9022-9045	8923-8946		
		NS5B-RDRP	NS5B-RDRP		
9	**hs-199b-3p**	1876-1901		7772-7798	
		E2-Env-Protein		NS5B-RDRP	
10	**hs-595**	331-352			334-355
		*			*

Table 4.23 The confirmed miRNA targets through RNAhybrid and their respective genomic positions, common in genotype 4 to 6 respectively. While hm stands for Homo Sapians miRNA, Pro for protease and env-pro for envelop protein.

NO	miRNA	GT4	GT5	GT6
1	**hsa-mir-1228***	317-337	317-337	
		5`-UTR	5`-UTR	
2	**hsa-mir-1909**	6347-6373		3706-3727
		NS5A-Protein		NS3-Protease
3	**hsa-mir-1182**	8670-8695		5757-5778
		NS5B-RDRP		NS4B-Protein

Contd...

4	**hsa-mir-769-3p**	1281-1303		489-513
		E1-Env-Protein		Core-Protein

Table 4.24 The confirmed miRNA targets through RNAhybrid and their respective genomic positions, common in genotype 5 to 6 respectively. While hm stands for Homo Sapians miRNA, Pro for protease and env-pro for envelop protein.

NO	miRNA	GT5	GT6
1	**hsa-mir-663**	7229-7251	2028-2049
		NS5A-Protein	E2-Env-Protein
2	**hsa-mir-329**	7525-7546	6289-6313
		NS5A-Protein	NS5A-Protein

Table 4.25(a) MiRNAs that are targeting 5'UTR, Core-Protein, E1-Envelope Protein or p7-Protein in multiple genotypes

miRNA	5'UTR	Core-Protein	E1-Env-Protein	E2-Env-Protein	p7-Protein
hm-1254	GT2,GT6				
hm-1180					
hm-138		GT6			
hm-615-5p					
hm-1974					
hm-2278				GT1	
hm-298					

Contd..

hm-1914				GT1,GT2,GT4	
hm-1202				GT1,GT2,GT5, GT6	
hm-15b				GT1	
hm-885-3p		GT2	GT2		
hm-624*					
hm-1910					
hm-611			GT4		
hm-339-5p		GT2		GT1	
hm-196a*		GT1,GT2,GT4, GT5			
hm-122			GT1		
hm-551b	GT1,GT3,GT4, GT5			GT6	
hm-1183					
hm-637				GT6	
hm-92a-2*					
hm-585					
hm-1471					
hm-1226*				GT2	
hm-1271					
hm-1915*					
hm-608		GT2			
hm-378*					

Contd..

hm-193b*				
hm-365*			GT3	
hm-497				
hm-662				
hm-1975		GT5		
hm-220c				
hm-1291				
hm-1226				
hm-125b			GT3,GT4	
hm-125a-5p			GT3,GT4	
hm-483-3p				
hm-940				
hm-523				
hm-199b-3p			GT3	
hm-595				
hm-1228*	GT4,GT5			
hm-1909				
hm-1182				
hm-769-3p	GT6	GT4		

Contd..

hm-663				GT6	
hm-329					

Table 4.25(b) MiRNAs that are targeting NS3-Protease, NS4A=Protein, NS4B-Protein, NS5A-Protein, NS5B-RDRP or 3'-UTR in multiple genotypes

miRNAs	NS3-Protease	NS4A-Protein	NS4B-Protein	NS5A-Protein	NS5B-RDRP	3'-UTR
hm-1254	GT1,					
hm-1180	GT1,				GT2	
hm-138	GT1,				GT1	
hm-615-5p	GT5					
hm-1974	GT4			GT1		
hm-2278	GT6					
hm-298	GT1,GT2,GT4					
hm-1914						
hm-1202						
hm-15b					GT2,GT3	
hm-885-3p	GT1,GT6			GT1	GT5	
hm-624*	GT1,GT5					
hm-1910			GT6		GT1	
hm-611		GT3			GT1	
hm-339-5p						
hm-196a*						
hm-122					GT3	GT2

Contd...

hm-551b						
hm-1183	GT1,GT2					
hm-637				GT2		
hm-92a-2*	GT2,GT5,GT6					
hm-585	GT2,GT3					
hm-1471	GT2					
hm-1226*				GT6		
hm-1271	GT2,GT4					
hm-1915*				GT5		
hm-608					GT5	
hm-378*	GT2,GT4,GT5					
hm-193b*				GT2	GT3	
hm-365*				GT2		
hm-497					GT2,GT3	
hm-662			GT2,GT4			
hm-1975						
hm-220c				GT3,GT5		
hm-1291	GT5				GT3	
hm-1226	GT5		GT3			
hm-125b						
hm-125a-5p						

Contd...

hm-483-3p						
hm-940	GT5				GT3	
hm-523					GT3,GT4	
hm-199b-3p					GT5	
hm-595						
hm-1228*						
hm-1909	GT6			GT4		
hm-1182			GT6		GT4	
hm-769-3p						
hm-663				GT5		
hm-329				GT5,GT6		

```
(a)CLUSTAL 2.0.12 multiple sequence alignment for 3`-UTR


hsa-miR-1254       -AGCCUGGAAGCUGGAGCCUGCAGU 24
hsa-miR-1228*      GUGGGCGGGGGCAGGUGUGUG---- 21
hsa-miR-551b       GCGACCCAUACUUGGUUUCAG---- 21
                                .     .
```

```
(b)CLUSTAL 2.0.12 multiple sequence alignment for Core-Protein


hsa-miR-138        -----AGCUGGUGUUGUGAAUCAGGCCG-- 23
hsa-miR-608        ---AGGGGUGGUGUUG-GGACAGCUCCGU- 25
hsa-miR-885-3p     AGGCAGCGGGGUGUAGUGGAUA-------- 22
hsa-miR-339-5p     ----UCCCUGUCCUCCAGGAGCUCACG--- 23
hsa-miR-769-3p     -----CUGGGAUCUCCGGGGUCUUGGUU-- 23
hsa-miR-196a*      --------CGGCAACAAGAAACUGCCUGAG 22
                            .        .
```

```
(c)CLUSTAL 2.0.12 multiple sequence alignment for E1 Env protein


hsa-miR-885-3p     ---AGGCAGCGGGGUGUAGUGGAUA------- 22
hsa-miR-769-3p     -CUGGGAUCUCCGGGGUCUUGGUU-------- 23
hsa-miR-611        GCGAGGACCCCUCGGGGUCUGAC--------- 23
hsa-miR-122        ---UGGAGUGUGACAAUGGUGUUUG------- 22
hsa-miR-1975       -------CCCCCACAACCGCGCUUGACUAGCU 25
                       *
```

(d)CLUSTAL 2.0.12 multiple sequence alignment for E2 Env protein

```
hsa-miR-339-5p      --UCCCUGUCCUCCAGGAGCUCACG--- 23
hsa-miR-365*        ----AGGGACUUUCAGGGGCAGCUGU-- 22
hsa-miR-1226*       --GUGAGGGCAUGCAGGCCUGGAUGGGG 26
hsa-miR-125b        UCCCUGAGACCCU--AACUUGUGA---- 22
hsa-miR-125a-5p     UCCCUGAGACCCUUUAACCUGUGA---- 24
hsa-miR-551b        -----GCGACCCAU--ACUUGGUUUCAG 21
hsa-miR-1914        -CCCUGUGCCCGGCCCACUUCUG----- 22
hsa-miR-663         AGGCGGGGCGCCGCGGGACCGC------ 22
hsa-miR-2278        ---GAGAGCAGUGUGUG--UUGCCUGG- 22
hsa-miR-199b-3p     ----ACAGUAGUCUGCACAUUGGUUA-- 22
hsa-miR-1202        -----GUGCCAGCUGCA-GUGGGGGAG- 21
```

(e)CLUSTAL 2.0.12 multiple sequence alignment for NS2 protein

```
hsa-miR-1915*      --------ACCUUGCCUUGCUGCCCGGGCC 22
hsa-miR-1975       CCCCCACAACCGCGCUUGACUAGCU----- 25
hsa-miR-624*       ---UAGUACCAGUACCUUGUGUUCA----- 22
hsa-miR-1471       -------GCCCGCGUGUGGAGCCAGGUGU- 22
```

(f)CLUSTAL 2.0.12 multiple sequence alignment for NS3 protease

```
hsa-miR-1254       -----AGCCUGGAAGCUGGAG-CCUGCAGU-- 24
hsa-miR-1291       ----UGGCCCUGA--CUGAAGACCAGCAGU-- 24
hsa-miR-585        ----UGGGCGUAU--CUGUAUGCUA------- 19
hsa-miR-615-5p     ----------GGGGGUCCCCGGUGCUCGGAUC 22
hsa-miR-940        ------AAGGCAGGGCCCCCGCUCCCC----- 21
hsa-miR-298        ------AGCAGAAGCAGGGAGGUUCUCCCA-- 24
hsa-miR-1909       ------CGCAGGGGCCGGGUGCUCACCG---- 22
```

```
hsa-miR-138        ------AGCUGGUGUUGUGAAUCAGGCCG--- 23
hsa-miR-1183       CACUGUAGGUGAUGGUGAGAGU-GGGCA---- 27
hsa-miR-885-3p     ------AGGCAGCGGGGUGUAGUGGAUA---- 22
hsa-miR-2278       ----GAGAGCAGUGUGUGUUGCCUGG------ 22
hsa-miR-1471       -------GCCCGCGUGUGGAGCCAGGUGU--- 22
hsa-miR-1180       -----UUUCCGGCUCGCGUGGGUGUGU----- 22
hsa-miR-378*       -----CUCCUGACUCCAGGUCCUGUGU----- 22
hsa-miR-624*       ---UAGUACCAGUACCUUGUGUUCA------- 22
hsa-miR-1226       -----UCACCAG--CCCUGUGUUCCCUAG--- 22
hsa-miR-92a-2*     ---GGGUGGGGAUUUGUUGCAUUAC------- 22
hsa-miR-1974       -----UGGUUGUAGUCCGUGCGAGAAUA---- 23
hsa-miR-1271       -------CUUGGCACCUA-GCAAGCACUCA-- 22
                                 . .
```

(g)CLUSTAL 2.0.12 multiple sequence alignment for NS4B protein

```
hsa-miR-1226       UCACCAGCCCUGUG-UUCCCUAG-- 22
hsa-miR-1910       ---CCAGUCCUGUG-CCUGCCGCCU 21
hsa-miR-662        --UCCCACGUUGUGGCCCAGCAG-- 21
hsa-miR-1182       --GAGGGUCUUGGGAGGGAUGUGAC 23
                             . .  .
```

(h)CLUSTAL 2.0.12 multiple sequence alignment for NS5A protein

```
hsa-miR-1909       ----CGCAGGG-GCCGGGUGCUCACCG---- 22
hsa-miR-663        ---AGGCGGGGCGCCGCGGGACCGC------ 22
hsa-miR-885-3p     -AGGCAGCGGGGUGUAGUGGAUA-------- 22
hsa-miR-220c       ---ACACAGGGCUGUUGUGAAGACU------ 22
hsa-miR-193b*      ------CGGGGUUUUGAGGGCGAGAUGA--- 22
hsa-miR-365*       ------AGGGACUUUCAGGGGCAGCUGU--- 22
hsa-miR-637        ---ACUGGGGGCUUUCGGGCUCUGCGU---- 24
hsa-miR-1974       ----UGG-UUGUAGUCCGUGCGAGAAUA--- 23
hsa-miR-1915*      ACCUUGCCUUGCUGCCCGGGCC--------- 22
```

```
hsa-miR-1226*      GUGAGGGCAUGCAGGCCUGGAUGGGG----- 26

hsa-miR-329        ---------AACACACCUGGUUAACCUCUUU 22
```

```
(i)CLUSTAL 2.0.12 multiple sequence alignment for NS5B RDRP


hsa-miR-1910       -------CCAGUCCUG--UGCCUGCCGCCU-------- 21

hsa-miR-1291       --------UGGCCCUGACUGAAGACCAGCAGU------ 24

hsa-miR-611        ------GCGAGGACCCCUCGGGGUCUGAC--------- 23

hsa-miR-15b        -----UAGCAGC--ACAUCAUGGUUUACA--------- 22

hsa-miR-497        -----CAGCAGC--ACACUGUGGUUUGU---------- 21

hsa-miR-122        ------UGGAGUGUG-ACAAUGGUGUUUG--------- 22

hsa-miR-199b-3p    ----ACAGUAGUCUGCACAUUGGUUA------------ 22

hsa-miR-138        -----AGCUGGUGUUGUGAAUCAGGCCG---------- 23

hsa-miR-608        ---AGGGGUGGUGUUG-GGACAGCUCCGU--------- 25

hsa-miR-885-3p     AGGCAGCGGGGUGUAGUGGAUA---------------- 22

hsa-miR-193b*      ------CGGGGUUUUGAGGGCGAGAUGA---------- 22

hsa-miR-1182       ------GAGGGUCUUGGGAGGGAUGUGAC--------- 23

hsa-miR-940        -------AAGGCAGGGCCCCCGCUCCCC---------- 21

hsa-miR-523        ---------------GAACGCGCUUCCCUAUAGAGGGU 23

hsa-miR-1180       ----UUUCCGGCUCGCGUGGGUGUGU------------ 22
```

Figure 4.4 Showing MSA of miRNAs targeting a specific gene in all genotypes

(a) MSA of Target Sequences of hsa-mir-196a

```
196aGT1         --TTGGTGGAGTTTACTTGTTGCCG

196aGT2         CGTTGGCGGAGTATACTTGTTGCCG

196aGT4         CGTTGGCGGAGTTTACTTGTTGCCG

196aGT5         --TTGGTGGAGTTTACTTGTTGCCG

                  **** ***** ************
```

(b) MSA of Target Sequences of hsa-mir-551b

```
551bGT1         TGCTAGCCGAGTAGTGTTGGGTCGC

551bGT3         CACTAGCCGAGTAGTGTTGGGTCGC

551bGT4         TGCTAGCCGAGTAGTGTTGGGTCGC

551bGT5         TGCTAGCCGAGTAGTGTTGGGTCGC

                  ***********************
```

(c)MSA of Target Sequences of hsa-mir-1202

```
1202GT1         CGCCCCTACTG---CTGGCAC

1202GT2         AACACCAATGGCAGCTGGCAC

1202GT5         AATACCAACGGGAGCTGGCAT

1202GT6         AACACCAATGGTAGCTGGCAC

                    ** *  *   ******
```

Figure 4.5 Multiple sequence alignment of the Target sites of the identified miRNAs

Discussion

The renowned discovery of human oriented miRNA targets for Influenza Virus genome, by S.K. Barahmchari and colleagues, paved a new direction and gave a new dimension to the research going on for the sake of anti-HCV therapeutic. As, hepatitis C is proving a havoc to the nations all over the world because by now, there is not any effective treatment achieved due to the phenomenal genetic re-assortment of the virons. But the breakthrough provided by the invention of human miRNA targets in AIV encouraged a quench for discovering efficient and effective therapeutics for other viruses having similar disease patterns by paving the path set down by the prior discovery. HCV is also a virus and it also manifests the alterations at genomic level in each progeny along with the fact that there is no 100% effective and economical treatment for hepatitis C. So, focusing on the methodology opted by the previous group of scientists and keeping in context the HCV and its main six genotypes along with the undulating technical factor, an endeavour has been made to spot the human oriented micro RNA targets inside HCV genome.

For this sake as a first step six main genotypes were chosen as the subject of study, limiting constraint was the genomic bulk of data and the provided time. The second research subject was the human mIRNAs, for this purpose miRBase database was consulted and 721 human miRNA sequences were fetched, because at time 721 miRNAs were discovered. miRBase was used as it is the only updated repository about human oriented miRNAs and their annotation information.

The next thing was the selection of tools to predict the potent targets in six of the genotypes. There are a number of tools that operate upon different principals and computational logics. The summary of tools and their methods is shown in table 5.1. The selection criterion was a critical step as the organism dependency along with the specificity of the predicted targets by each tool proved to be the opting criteria. The compliance with reported in vivo results and genome level target scan also modify the tool picking criterion. Ultimately miRanda and RNAhybrid were selected due to their high target specificity and in vivo accuracy of predicted targets. In first round all potential targets were identified by running the algorithmic miRanda as, the web interface was not able to run the genomic level queries. The number of miRanda predicted targets has been shown in figure 4.1. Which is a very high number, as there may be some false positives and false negative results, along with there may be some results who are not having good conservation score and some having unstable thermodynamic energies. So, In order to remove the false positive and false negative hits and to select significant hits from the predicted targets shuffled sequences were used as a second legend for the graph (figure 4.3) for cut-off value. Although there is a number of facilities to shuffle the genomic sequences but DNA Shuffle program was used due to its user friendliness and facility to input large sequence. For plotting the data values from miRanda output files have to be extracted first. As, the output files were in the form of flat file every record was there on a line and some separators were used to separate the data values present in line.

Table 5.1: listing some of the tools to predict miRNA targets along with the method, URL and reference

Tool	Prediction Method	Website	Reference
miRanda	Indulgent Seed Pairing, Minimum Free energy, Sequence conservation	miRNA.org	Enright *et al.*, 2003
PicTar	Strict Seed Pairing, Conservation, Free Energy and Probability of being Target	pictar.mdc-berlin.de	Grün *et al.*, 2005
DIANA-microT	Seed pairing, conservation, site number	diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi	Kiriakidou *et al.*, 2004
RNAhybrid	Probable miRNA-mRNA duplexes, MFE	bibiserv.techfak.uni-bielefeld.de/rnahybrid	Rehmsmeier *et al.*, 2004
PITA	Seed pairing, site accessibility, total interaction energy	genie.weizmann.ac.il/pubs/mir07/	Kertesz M *et al., 2007*

For this purpose help of the scripting languages was exploited to extract the required data fields from the output file. A number of scripting languages exist but GAWK scripting language was used for the required task completion, because it is also an open source language and can be run on LINUX easily and it was very much suiting with objective of using the script and it worked just by writing a couple of lines of code for specifying the data separator and to send the fields starting with specified identifier to a simple text file containing delimited data. Then these data files can be easily opened and saved in MS Excel (2007) for the sake of plotting data but as our data files were so huge that its data plotting utility became non-functional due to bulk of data points, so MATLAB a highly proficient programming language that posses not only the programming environment but the specialized tool kits for all the types of computations. So, the edge of mathematical tool box of MATLAB was taken in order to graphically see the score values for shuffled and non-shuffled sequences on a plot. The point where the lines for shuffled and non-shuffled sequences cut each other was taken as the cut-off value in order to extract the significant results. The similar approach for deriving cut-off values was used by V. Sacaria and colleagues for identifying highly probable targets from the output for HIV and AIV (Hariharam *et al.*, 2005).

miRanda was executed again by setting the cut-off value as score parameter As, we can`t extract more than one field from each record line so this time it was not possible to extract required values through scripting. The miRNAs which are having targets in six of HCV genotypes are shown in the tables from 4.2 -4.7 respectively.

As, the prior goal of the study was to identify such sequential constraints which can be used to design a miRNA for six stereotypes in common. So, for this purpose a visual analysis of the outputs from miRanda was done in order to identify those potential miRNAs which were having targets in more than one genotype. So this tiring analysis was done and the miRNAs which were common in GT1 and any of the other genotype, GT2 and any other remaining genotype and so on so forth for the rest of GTs were recognized. The results of this analysis have been demonstrated in tables 4.8-4.13.

Now the next stride was to reconfirm the targets identified through using another target prediction utility RNAhybrid that not only predicts the potential miRNA targets but also predicts the hybridization energies of a long and a short RNA. Along with predicting hybridization minimum free energy it also predicts P-value based upon the extreme value distribution of the length normalised energies. So, in order to calculate the parameters of evd RNAcalibrate a module of RNAhybrid was used and the resulting parameters were taken as input to the RNAhybrid. Then this algorithm was executed on the miRNAs which were having targets in more than GTs and the thermodynamic energies were noted all the energies were in favourable range i.e. -25 Kcal/Mol. The miRNAs along with respective thermodynamic energies have been shown in tables 4.14-4.19 respectively.

At this stage the miRNAs that were having very potential targets in HCV GTs were obtained, as the algorithm's output was containing only the nucleotide positions where short miRNA is hybridizing with the longer one so in order to have a very glimpse picture of the targets there was a dire need to map these targets to their actual defined gene boundaries. The potential miRNAs having targets are shown in subsequent tables from 4.20-4.24 with their gene locations. Structural, non-structural genes and 3` and 5` UTR were mapped out. Than afterwards analysis of the resultant gene targets was done to identify the miRNA who was targeting the same gene in maximum GTs out of six. Although it was a laborious manual analysis but the results were coined into the tables

4.24 (a) and (b). After scanning the results through out those three miRNAs were identified who were targeting a single gene in maximum number of GTs i.e. four. Hsa-mir-196a, hsa-mir-551b and hsa-mir-1202 were the recognized potential miRNAs. Their targeted gene and GTs are demonstrated in figure 5.1. It is made known that hsa-mir-1202 targeted E2 envelop protein in GT 1. 2. 5 and 6 and several studies reveal the important role of this specific protein in the replication cycle of HCV and along with this glycoprotein pivotally assist virus entry into the host (Dubussion, 2007). The second potent miRNA hsa-mir-196a* was targeting Core-protein in GT 1, 2, 4 and 5. Literature has manifested core protein as an RNA binding protein which forms the nucleocapsid of the virus after cleaving out from polyprotein and maturing (Santolini *et al.,* 1994). The last revealed miRNA hsa-mir-551b hybridized with 5'UTR of GT 1, 3, 4 and 5. The enormous research regarding HCV genome illustrates that this region is mostly conserved among the viral strains, and this region is involved in the replication of viral genome along with the translation of viral mRNA into protein. This region contains an internal ribosome entry site which mediates IRES-mediated translation. By virtue of being conserved this region is used to design primers for the quantitative analysis of HCV genome (Kato, 2001).

The next intricate step was to identify those sequences which are conserved among six of the GTs and can be used to design an artificial miRNA that can target more than one genotype simultaneously. So for this task two directional approach was used, one direction was to pile up those potent miRNAs which targeted same gene in different GTs and then gene by gene the conservation of miRNAs was checked by aligning them through CLUTALW as demonstrated in figure 4.4 and its subsequent parts. But as evident from the results no conservation among the miRNAs, most probable reason is that miRNAs work with partial complementarity (Hariharam *et al.*, 2005) so there are significant chances the miRNAs which are targeting the same gene may have a very little or worth ignoring conservation. So picking up the second direction of the task the target regions of the miRNAs that were targeting same gene in maximum genotypes were identified, idea behind was that target regions will have more chances to be conserved then the miRNAs. The results demonstrated through figure 4.5 successfully reveal that the target regions are having significant conservation, The most conserved target regions were of hsa-mir-551b only two nucleotide positions are variant, second miRNA targets were conserved in three chunks of sequence and the third miRNA hsa-mir-196a was having the 5` region more conserved than the other side.

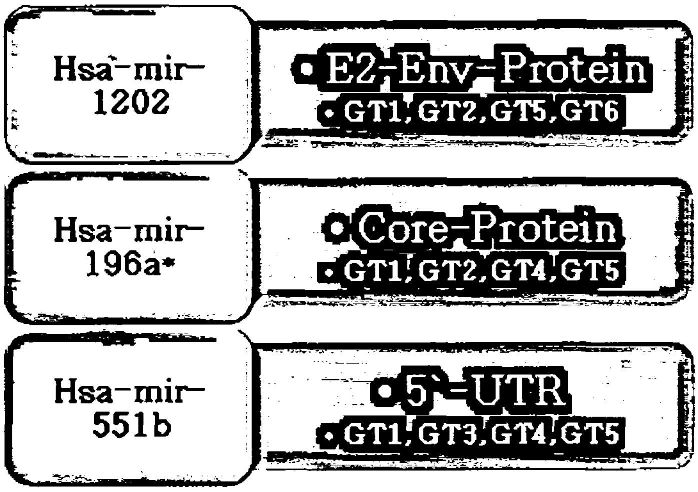

Figure 5.1: Revealing the identified miRNAs along with the genes which they are targeting and with specific genotypes mentions.

Conclusion and Future Prospects

Since their discovery to date miRNAs are proving as promising, vigilant and potent entities in different dimensions of research focusing on host-virus interactions. miRNAs have shown computationally to target certain genes potentially in order to complete knock out or to cause translational repression. Further the in-silico results have been authenticated through wet lab in case of drosophila and AIV (Enright *et al.*, 2003) (Brahamchari *et al.*, 2010). By the time the miRNA-RNA binding rules have been explored, the attempts to design artificial miRNAs were becoming somewhat fruitful (Scaria *et al.*, 2006). amiRNAs were identified to be the potential anti-viral therapeutics.

The present study was conducted to identify those conserved sequences which can be incorporated into seed region of amiRNAs in order to counter specific genes in multiple GTs. The result obtained showed successfully those sequential constraints which can be incorporated into design of amiRNAs, and these miRNAs will be potentially inhibiting more than two genotypes. The focus was identifying such sequential design parameters which can bypass the heterogenetic types of HCV strain which arise during each viron replication, as it's the most evident feature of miRNAs that they require partial complementarity for their function, so a miRNA that is already designed to already cope with four GTs, so such miRNA can target many genetic variants of HCV potentially.

As, the study focuses on the six main genotypes the same work can be extended to the other sub-types of the HCV in order to identify those sequences that can target possible maximum number of GTs. The work can be extended in wet lab by synthesizing amiRNAs and checking them in HCV infected living cell lines. And after wards synthesizing the amiRNAs in human admissible forms, this therapy will be more potent and economical than any of the present therapy for Hepatitis C treatment.

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Appendix I

No	ID	Accession	Cromosome	Start	End	Strand
1	hsa-let-7a-1	MI0000060	9	96938239	96938318	+
2	hsa-let-7a-2	MI0000061	11	122017230	122017301	-
3	hsa-let-7a-3	MI0000062	22	46508629	46508702	+
4	hsa-let-7b	MI0000063	22	46509566	46509648	+
5	hsa-let-7c	MI0000064	21	17912148	17912231	+
6	hsa-let-7d	MI0000065	9	96941116	96941202	+
7	hsa-let-7e	MI0000066	19	52196039	52196117	+
8	hsa-let-7f-1	MI0000067	9	96938629	96938715	+
9	hsa-let-7f-2	MI0000068	X	53584153	53584235	-
10	hsa-let-7g	MI0000433	3	52302294	52302377	-
11	hsa-let-7i	MI0000434	12	62997466	62997549	+
12	hsa-mir-1-1	MI0000651	20	61151513	61151583	+
13	hsa-mir-1-2	MI0000437	18	19408965	19409049	-
14	hsa-mir-7-1	MI0000263	9	86584663	86584772	-
15	hsa-mir-7-2	MI0000264	15	89155056	89155165	+
16	hsa-mir-7-3	MI0000265	19	4770682	4770791	+
17	hsa-mir-9-1	MI0000466	1	156390133	156390221	-
18	hsa-mir-9-2	MI0000467	5	87962671	87962757	-
19	hsa-mir-9-3	MI0000468	15	89911248	89911337	+
20	hsa-mir-10a	MI0000266	17	46657200	46657309	-
21	hsa-mir-10b	MI0000267	2	177015031	177015140	+
22	hsa-mir-15a	MI0000069	13	50623255	50623337	-
23	hsa-mir-15b	MI0000438	3	160122376	160122473	+
24	hsa-mir-16-1	MI0000070	13	50623109	50623197	-
25	hsa-mir-16-2	MI0000115	3	160122533	160122613	+
26	hsa-mir-17	MI0000071	13	92002859	92002942	+
27	hsa-mir-18a	MI0000072	13	92003005	92003075	+
28	hsa-mir-18b	MI0001518	X	133304071	133304141	-
29	hsa-mir-19a	MI0000073	13	92003145	92003226	+
30	hsa-mir-19b-1	MI0000074	13	92003446	92003532	+
31	hsa-mir-19b-2	MI0000075	X	133303701	133303796	-
32	hsa-mir-20a	MI0000076	13	92003319	92003389	+
33	hsa-mir-20b	MI0001519	X	133303839	133303907	-
34	hsa-mir-21	MI0000077	17	57918627	57918698	+
35	hsa-mir-22	MI0000078	17	1617197	1617281	-
36	hsa-mir-23a	MI0000079	19	13947401	13947473	-
37	hsa-mir-23b	MI0000439	9	97847490	97847586	+
38	hsa-mir-24-1	MI0000080	9	97848303	97848370	+
39	hsa-mir-24-2	MI0000081	19	13947101	13947173	-
40	hsa-mir-25	MI0000082	7	99691183	99691266	-
41	hsa-mir-26a-1	MI0000083	3	38010895	38010971	+
42	hsa-mir-26a-2	MI0000750	12	58218392	58218475	-

Contd....

43	hsa-mir-26b	MI0000084	2	219267369	219267445	+
44	hsa-mir-27a	MI0000085	19	13947254	13947331	-
45	hsa-mir-27b	MI0000440	9	97847727	97847823	+
46	hsa-mir-28	MI0000086	3	188406569	188406654	+
47	hsa-mir-29a	MI0000087	7	130561506	130561569	-
48	hsa-mir-29b-1	MI0000105	7	130562218	130562298	-
49	hsa-mir-29b-2	MI0000107	1	207975788	207975868	-
50	hsa-mir-29c	MI0000735	1	207975197	207975284	-
51	hsa-mir-30a	MI0000088	6	72113254	72113324	-
52	hsa-mir-30b	MI0000441	8	135812763	135812850	-
53	hsa-mir-30c-1	MI0000736	1	41222956	41223044	+
54	hsa-mir-30c-2	MI0000254	6	72086663	72086734	-
55	hsa-mir-30d	MI0000255	8	135817119	135817188	-
56	hsa-mir-30e	MI0000749	1	41220027	41220118	+
57	hsa-mir-31	MI0000089	9	21512114	21512184	-
58	hsa-mir-32	MI0000090	9	111808509	111808578	-
59	hsa-mir-33a	MI0000091	22	42296948	42297016	+
60	hsa-mir-33b	MI0003646	17	17717150	17717245	-
61	hsa-mir-34a	MI0000268	1	9211727	9211836	-
62	hsa-mir-34b	MI0000742	11	111383663	111383746	+
63	hsa-mir-34c	MI0000743	11	111384164	111384240	+
64	hsa-mir-92a-1	MI0000093	13	92003568	92003645	+
65	hsa-mir-92a-2	MI0000094	X	133303568	133303642	-
66	hsa-mir-92b	MI0003560	1	155164968	155165063	+
67	hsa-mir-93	MI0000095	7	99691391	99691470	-
68	hsa-mir-95	MI0000097	4	8007028	8007108	-
69	hsa-mir-96	MI0000098	7	129414532	129414609	-
70	hsa-mir-98	MI0000100	X	53583184	53583302	-
71	hsa-mir-99a	MI0000101	21	17911409	17911489	+
72	hsa-mir-99b	MI0000746	19	52195865	52195934	+
73	hsa-mir-100	MI0000102	11	122022937	122023016	-
74	hsa-mir-101-1	MI0000103	1	65524117	65524191	-
75	hsa-mir-101-2	MI0000739	9	4850297	4850375	+
76	hsa-mir-103-1	MI0000109	5	167987901	167987978	-
77	hsa-mir-103-1-as	MI0007261	5	167987909	167987970	+
78	hsa-mir-103-2	MI0000108	20	3898141	3898218	+
79	hsa-mir-103-2-as	MI0007262	20	3898149	3898210	-
80	hsa-mir-105-1	MI0000111	X	151560691	151560771	-
81	hsa-mir-105-2	MI0000112	X	151562884	151562964	-
82	hsa-mir-106a	MI0000113	X	133304228	133304308	-
83	hsa-mir-106b	MI0000734	7	99691616	99691697	-
84	hsa-mir-107	MI0000114	10	91352504	91352584	-
85	hsa-mir-122	MI0000442	18	56118306	56118390	+
86	hsa-mir-124-1	MI0000443	8	9760898	9760982	-

Contd....

87	hsa-mir-124-2	MI0000444	8	65291706	65291814	+
88	hsa-mir-124-3	MI0000445	20	61809852	61809938	+
89	hsa-mir-125a	MI0000469	19	52196507	52196592	+
90	hsa-mir-125b-1	MI0000446	11	121970465	121970552	-
91	hsa-mir-125b-2	MI0000470	21	17962557	17962645	+
92	hsa-mir-126	MI0000471	9	139565054	139565138	+
93	hsa-mir-127	MI0000472	14	101349316	101349412	+
94	hsa-mir-128-1	MI0000447	2	136422967	136423048	+
95	hsa-mir-128-2	MI0000727	3	35785968	35786051	+
96	hsa-mir-129-1	MI0000252	7	127847925	127847996	+
97	hsa-mir-129-2	MI0000473	11	43602944	43603033	+
98	hsa-mir-130a	MI0000448	11	57408671	57408759	+
99	hsa-mir-130b	MI0000748	22	22007593	22007674	+
100	hsa-mir-132	MI0000449	17	1953202	1953302	-
101	hsa-mir-133a-1	MI0000450	18	19405659	19405746	-
102	hsa-mir-133a-2	MI0000451	20	61162119	61162220	+
103	hsa-mir-133b	MI0000822	6	52013721	52013839	+
104	hsa-mir-134	MI0000474	14	101521024	101521096	+
105	hsa-mir-135a-1	MI0000452	3	52328235	52328324	-
106	hsa-mir-135a-2	MI0000453	12	97957590	97957689	+
107	hsa-mir-135b	MI0000810	1	205417430	205417526	-
108	hsa-mir-136	MI0000475	14	101351039	101351120	+
109	hsa-mir-137	MI0000454	1	98511626	98511727	-
110	hsa-mir-138-1	MI0000476	3	44155704	44155802	+
111	hsa-mir-138-2	MI0000455	16	56892430	56892513	+
112	hsa-mir-139	MI0000261	11	72326107	72326174	-
113	hsa-mir-140	MI0000456	16	69966984	69967083	+
114	hsa-mir-141	MI0000457	12	7073260	7073354	+
115	hsa-mir-142	MI0000458	17	56408593	56408679	-
116	hsa-mir-143	MI0000459	5	148808481	148808586	+
117	hsa-mir-144	MI0000460	17	27188551	27188636	-
118	hsa-mir-145	MI0000461	5	148810209	148810296	+
119	hsa-mir-146a	MI0000477	5	159912359	159912457	+
120	hsa-mir-146b	MI0003129	10	104196269	104196341	+
121	hsa-mir-147	MI0000262	9	123007257	123007328	-
122	hsa-mir-147b	MI0005544	15	45725248	45725327	+
123	hsa-mir-148a	MI0000253	7	25989539	25989606	-
124	hsa-mir-148b	MI0000811	12	54731000	54731098	+
125	hsa-mir-149	MI0000478	2	241395418	241395506	+
126	hsa-mir-150	MI0000479	19	50004042	50004125	-
127	hsa-mir-151	MI0000809	8	141742663	141742752	-
128	hsa-mir-152	MI0000462	17	46114527	46114613	-
129	hsa-mir-153-1	MI0000463	2	220158833	220158922	-
130	hsa-mir-153-2	MI0000464	7	157367028	157367114	-

Contd....

131	hsa-mir-154	MI0000480	14	101526092	101526175	+
132	hsa-mir-155	MI0000681	21	26946292	26946356	+
133	hsa-mir-181a-1	MI0000289	1	198828173	198828282	-
134	hsa-mir-181a-2	MI0000269	9	127454721	127454830	+
135	hsa-mir-181b-1	MI0000270	1	198828002	198828111	-
136	hsa-mir-181b-2	MI0000683	9	127455989	127456077	+
137	hsa-mir-181c	MI0000271	19	13985513	13985622	+
138	hsa-mir-181d	MI0003139	19	13985689	13985825	+
139	hsa-mir-182	MI0000272	7	129410223	129410332	-
140	hsa-mir-183	MI0000273	7	129414745	129414854	-
141	hsa-mir-184	MI0000481	15	79502130	79502213	+
142	hsa-mir-185	MI0000482	22	20020662	20020743	+
143	hsa-mir-186	MI0000483	1	71533314	71533399	-
144	hsa-mir-187	MI0000274	18	33484781	33484889	-
145	hsa-mir-188	MI0000484	X	49768109	49768194	+
146	hsa-mir-190	MI0000486	15	63116156	63116240	+
147	hsa-mir-190b	MI0005545	1	154166141	154166219	-
148	hsa-mir-191	MI0000465	3	49058051	49058142	-
149	hsa-mir-192	MI0000234	11	64658609	64658718	-
150	hsa-mir-193a	MI0000487	17	29887015	29887102	+
151	hsa-mir-193b	MI0003137	16	14397824	14397906	+
152	hsa-mir-194-1	MI0000488	1	220291499	220291583	-
153	hsa-mir-194-2	MI0000732	11	64658827	64658911	-
154	hsa-mir-195	MI0000489	17	6920934	6921020	-
155	hsa-mir-196a-1	MI0000238	17	46709852	46709921	-
156	hsa-mir-196a-2	MI0000279	12	54385522	54385631	+
157	hsa-mir-196b	MI0001150	7	27209099	27209182	-
158	hsa-mir-197	MI0000239	1	110141515	110141589	+
159	hsa-mir-198	MI0000240	3	120114515	120114576	-
160	hsa-mir-199a-1	MI0000242	19	10928102	10928172	-
161	hsa-mir-199a-2	MI0000281	1	172113675	172113784	-
162	hsa-mir-199b	MI0000282	9	131007000	131007109	-
163	hsa-mir-200a	MI0000737	1	1103243	1103332	+
164	hsa-mir-200b	MI0000342	1	1102484	1102578	+
165	hsa-mir-200c	MI0000650	12	7072862	7072929	+
166	hsa-mir-202	MI0003130	10	135061015	135061124	-
167	hsa-mir-203	MI0000283	14	104583742	104583851	+
168	hsa-mir-204	MI0000284	9	73424891	73425000	-
169	hsa-mir-205	MI0000285	1	209605478	209605587	+
170	hsa-mir-206	MI0000490	6	52009147	52009232	+
171	hsa-mir-208a	MI0000251	14	23857805	23857875	-
172	hsa-mir-208b	MI0005570	14	23887196	23887272	-
173	hsa-mir-210	MI0000286	11	568089	568198	-
174	hsa-mir-211	MI0000287	15	31357235	31357344	-

Contd....

175	hsa-mir-212	MI0000288	17	1953565	1953674	-
176	hsa-mir-214	MI0000290	1	172107938	172108047	-
177	hsa-mir-215	MI0000291	1	220291195	220291304	-
178	hsa-mir-216a	MI0000292	2	56216085	56216194	-
179	hsa-mir-216b	MI0005569	2	56227849	56227930	-
180	hsa-mir-217	MI0000293	2	56210102	56210211	-
181	hsa-mir-218-1	MI0000294	4	20529898	20530007	+
182	hsa-mir-218-2	MI0000295	5	168195151	168195260	-
183	hsa-mir-219-1	MI0000296	6	33175612	33175721	+
			HSCHR6_MHC_COX	33097238	33097347	+
			HSCHR6_MHC_DBB	33153522	33153631	+
			HSCHR6_MHC_MANN	33329535	33329644	+
			HSCHR6_MHC_MCF	33345976	33346085	+
			HSCHR6_MHC_QBL	33104442	33104551	+
184	hsa-mir-219-2	MI0000740	9	131154897	131154993	-
185	hsa-mir-220a	MI0000297	X	122695946	122696055	-
186	hsa-mir-220b	MI0005529	19	6495959	6496045	+
187	hsa-mir-220c	MI0005536	19	49063529	49063611	-
188	hsa-mir-221	MI0000298	X	45605585	45605694	-
189	hsa-mir-222	MI0000299	X	45606421	45606530	-
190	hsa-mir-223	MI0000300	X	65238712	65238821	+
191	hsa-mir-224	MI0000301	X	151127050	151127130	-
192	hsa-mir-296	MI0000747	20	57392670	57392749	-
193	hsa-mir-297	MI0005775	4	111781738	111781803	-
194	hsa-mir-298	MI0005523	20	57393281	57393368	-
195	hsa-mir-299	MI0000744	14	101490131	101490193	+
196	hsa-mir-300	MI0005525	14	101507700	101507782	+
197	hsa-mir-301a	MI0000745	17	57228497	57228582	-
198	hsa-mir-301b	MI0005568	22	22007270	22007347	+
199	hsa-mir-302a	MI0000738	4	113569339	113569407	-
200	hsa-mir-302b	MI0000772	4	113569641	113569713	-
201	hsa-mir-302c	MI0000773	4	113569519	113569586	-
202	hsa-mir-302d	MI0000774	4	113569160	113569227	-
203	hsa-mir-302e	MI0006417	11	7255997	7256068	+
204	hsa-mir-302f	MI0006418	18	27878876	27878926	+
205	hsa-mir-320a	MI0000542	8	22102475	22102556	-
206	hsa-mir-320b-1	MI0003776	1	117214371	117214449	+
207	hsa-mir-320b-2	MI0003839	1	224444706	224444843	-
208	hsa-mir-320c-1	MI0003778	18	19263471	19263558	+

209	hsa-mir-320c-2	MI0008191	18	21901650	21901699	+
210	hsa-mir-320d-1	MI0008190	13	41301964	41302011	-
211	hsa-mir-320d-2	MI0008192	X	140008337	140008384	-
212	hsa-mir-323	MI0000807	14	101492069	101492154	+
213	hsa-mir-324	MI0000813	17	7126616	7126698	-
214	hsa-mir-325	MI0000824	X	76225829	76225926	-
215	hsa-mir-326	MI0000808	11	75046136	75046230	-
216	hsa-mir-328	MI0000804	16	67236224	67236298	-
217	hsa-mir-329-1	MI0001725	14	101493122	101493201	+
218	hsa-mir-329-2	MI0001726	14	101493437	101493520	+
219	hsa-mir-330	MI0000803	19	46142252	46142345	-
220	hsa-mir-331	MI0000812	12	95702196	95702289	+
221	hsa-mir-335	MI0000816	7	130135952	130136045	+
222	hsa-mir-337	MI0000806	14	101340830	101340922	+
223	hsa-mir-338	MI0000814	17	79099683	79099749	-
224	hsa-mir-339	MI0000815	7	1062569	1062662	-
225	hsa-mir-340	MI0000802	5	179442303	179442397	-
226	hsa-mir-342	MI0000805	14	100575992	100576090	+
227	hsa-mir-345	MI0000825	14	100774196	100774293	+
228	hsa-mir-346	MI0000826	10	88024451	88024545	-
229	hsa-mir-361	MI0000760	X	85158641	85158712	-
230	hsa-mir-362	MI0000762	X	49773572	49773636	+
231	hsa-mir-363	MI0000764	X	133303408	133303482	-
232	hsa-mir-365-1	MI0000767	16	14403142	14403228	+
233	hsa-mir-365-2	MI0000769	17	29902430	29902540	+
234	hsa-mir-367	MI0000775	4	113569030	113569097	-
235	hsa-mir-369	MI0000777	14	101531935	101532004	+
236	hsa-mir-370	MI0000778	14	101377476	101377550	+
237	hsa-mir-371	MI0000779	19	54290929	54290995	+
238	hsa-mir-372	MI0000780	19	54291144	54291210	+
239	hsa-mir-373	MI0000781	19	54291959	54292027	+
240	hsa-mir-374a	MI0000782	X	73507121	73507192	-
241	hsa-mir-374b	MI0005566	X	73438382	73438453	-
242	hsa-mir-375	MI0000783	2	219866367	219866430	-
243	hsa-mir-376a-1	MI0000784	14	101507119	101507186	+
244	hsa-mir-376a-2	MI0003529	14	101506406	101506485	+
245	hsa-mir-376b	MI0002466	14	101506773	101506872	+
246	hsa-mir-376c	MI0000776	14	101506027	101506092	+
247	hsa-mir-377	MI0000785	14	101528387	101528455	+
248	hsa-mir-378	MI0000786	5	149112388	149112453	+
249	hsa-mir-379	MI0000787	14	101488403	101488469	+
250	hsa-mir-380	MI0000788	14	101491354	101491414	+
251	hsa-mir-381	MI0000789	14	101512257	101512331	+
252	hsa-mir-382	MI0000790	14	101520643	101520718	+

Contd....

253	hsa-mir-383	MI0000791	8	14710947	14711019	-
254	hsa-mir-384	MI0001145	X	76139698	76139785	-
255	hsa-mir-409	MI0001735	14	101531637	101531715	+
256	hsa-mir-410	MI0002465	14	101532249	101532328	+
257	hsa-mir-411	MI0003675	14	101489662	101489757	+
258	hsa-mir-412	MI0002464	14	101531784	101531874	+
259	hsa-mir-421	MI0003685	X	73438212	73438296	-
260	hsa-mir-422a	MI0001444	15	64163129	64163218	-
261	hsa-mir-423	MI0001445	17	28444097	28444190	+
262	hsa-mir-424	MI0001446	X	133680644	133680741	-
263	hsa-mir-425	MI0001448	3	49057581	49057667	-
264	hsa-mir-429	MI0001641	1	1104385	1104467	+
265	hsa-mir-431	MI0001721	14	101347344	101347457	+
266	hsa-mir-432	MI0003133	14	101350820	101350913	+
267	hsa-mir-433	MI0001723	14	101348223	101348315	+
268	hsa-mir-448	MI0001637	X	114058017	114058127	+
269	hsa-mir-449a	MI0001648	5	54466360	54466450	-
270	hsa-mir-449b	MI0003673	5	54466474	54466570	-
271	hsa-mir-449c	MI0003823	5	54468090	54468181	-
272	hsa-mir-450a-1	MI0001652	X	133674371	133674461	-
273	hsa-mir-450a-2	MI0003187	X	133674538	133674637	-
274	hsa-mir-450b	MI0005531	X	133674215	133674292	-
275	hsa-mir-451	MI0001729	17	27188387	27188458	-
276	hsa-mir-452	MI0001733	X	151128100	151128184	-
277	hsa-mir-453	MI0001727	14	101522527	101522606	+
278	hsa-mir-454	MI0003820	17	57215119	57215233	-
279	hsa-mir-455	MI0003513	9	116971714	116971809	+
280	hsa-mir-483	MI0002467	11	2155364	2155439	-
281	hsa-mir-484	MI0002468	16	15737151	15737229	+
282	hsa-mir-485	MI0002469	14	101521756	101521828	+
283	hsa-mir-486	MI0002470	8	41517959	41518026	-
284	hsa-mir-487a	MI0002471	14	101518783	101518862	+
285	hsa-mir-487b	MI0003530	14	101512792	101512875	+
286	hsa-mir-488	MI0003123	1	176998499	176998581	-
287	hsa-mir-489	MI0003124	7	93113248	93113331	-
288	hsa-mir-490	MI0003125	7	136587914	136588041	+
289	hsa-mir-491	MI0003126	9	20716104	20716187	+
290	hsa-mir-492	MI0003131	12	95228174	95228289	+
291	hsa-mir-493	MI0003132	14	101335397	101335485	+
292	hsa-mir-494	MI0003134	14	101495971	101496051	+
293	hsa-mir-495	MI0003135	14	101500092	101500173	+
294	hsa-mir-496	MI0003136	14	101526910	101527011	+
295	hsa-mir-497	MI0003138	17	6921230	6921341	-
296	hsa-mir-498	MI0003142	19	54177451	54177574	+

Contd....

297	hsa-mir-499	MI0003183	20	33578179	33578300	+
298	hsa-mir-500	MI0003184	X	49773039	49773122	+
299	hsa-mir-501	MI0003185	X	49774330	49774413	+
300	hsa-mir-502	MI0003186	X	49779206	49779291	+
301	hsa-mir-503	MI0003188	X	133680358	133680428	-
302	hsa-mir-504	MI0003189	X	137749872	137749954	-
303	hsa-mir-505	MI0003190	X	139006307	139006390	-
304	hsa-mir-506	MI0003193	X	146312238	146312361	-
305	hsa-mir-507	MI0003194	X	146312502	146312595	-
306	hsa-mir-508	MI0003195	X	146318431	146318545	-
307	hsa-mir-509-1	MI0003196	X	146342050	146342143	-
308	hsa-mir-509-2	MI0005530	X	146340278	146340368	-
309	hsa-mir-509-3	MI0005717	X	146341170	146341244	-
310	hsa-mir-510	MI0003197	X	146353853	146353926	-
311	hsa-mir-511-1	MI0003127	10	17887107	17887193	+
312	hsa-mir-511-2	MI0003128	10	18134036	18134122	+
313	hsa-mir-512-1	MI0003140	19	54169933	54170016	+
314	hsa-mir-512-2	MI0003141	19	54172411	54172508	+
315	hsa-mir-513a-1	MI0003191	X	146294981	146295109	-
316	hsa-mir-513a-2	MI0003192	X	146307344	146307470	-
317	hsa-mir-513b	MI0006648	X	146280562	146280645	-
318	hsa-mir-513c	MI0006649	X	146271222	146271305	-
319	hsa-mir-514-1	MI0003198	X	146360765	146360862	-
320	hsa-mir-514-2	MI0003199	X	146363461	146363548	-
321	hsa-mir-514-3	MI0003200	X	146366159	146366246	-
322	hsa-mir-515-1	MI0003144	19	54182257	54182339	+
323	hsa-mir-515-2	MI0003147	19	54188263	54188345	+
324	hsa-mir-516a-1	MI0003180	19	54259995	54260084	+
325	hsa-mir-516a-2	MI0003181	19	54264387	54264476	+
326	hsa-mir-516b-1	MI0003172	19	54240099	54240188	+
327	hsa-mir-516b-2	MI0003167	19	54228696	54228780	+
328	hsa-mir-517a	MI0003161	19	54215522	54215608	+
329	hsa-mir-517b	MI0003165	19	54224330	54224396	+
330	hsa-mir-517c	MI0003174	19	54244567	54244661	+
331	hsa-mir-518a-1	MI0003170	19	54234260	54234344	+
332	hsa-mir-518a-2	MI0003173	19	54242587	54242673	+
333	hsa-mir-518b	MI0003156	19	54205991	54206073	+
334	hsa-mir-518c	MI0003159	19	54211989	54212089	+
335	hsa-mir-518d	MI0003171	19	54238131	54238217	+
336	hsa-mir-518e	MI0003169	19	54233092	54233179	+
337	hsa-mir-518f	MI0003154	19	54203269	54203355	+
338	hsa-mir-519a-1	MI0003178	19	54255651	54255735	+
339	hsa-mir-519a-2	MI0003182	19	54265598	54265684	+
340	hsa-mir-519b	MI0003151	19	54198467	54198547	+

Contd....

341	hsa-mir-519c	MI0003148	19	54189723	54189809	+
342	hsa-mir-519d	MI0003162	19	54216601	54216688	+
343	hsa-mir-519e	MI0003145	19	54183194	54183277	+
344	hsa-mir-520a	MI0003149	19	54194135	54194219	+
345	hsa-mir-520b	MI0003155	19	54204481	54204541	+
346	hsa-mir-520c	MI0003158	19	54210707	54210793	+
347	hsa-mir-520d	MI0003164	19	54223350	54223436	+
348	hsa-mir-520e	MI0003143	19	54178965	54179051	+
349	hsa-mir-520f	MI0003146	19	54185413	54185499	+
350	hsa-mir-520g	MI0003166	19	54225420	54225509	+
351	hsa-mir-520h	MI0003175	19	54245766	54245853	+
352	hsa-mir-521-1	MI0003176	19	54251890	54251976	+
353	hsa-mir-521-2	MI0003163	19	54219848	54219934	+
354	hsa-mir-522	MI0003177	19	54254465	54254551	+
355	hsa-mir-523	MI0003153	19	54201639	54201725	+
356	hsa-mir-524	MI0003160	19	54214256	54214342	+
357	hsa-mir-525	MI0003152	19	54200787	54200871	+
358	hsa-mir-526a-1	MI0003157	19	54209506	54209590	+
359	hsa-mir-526a-2	MI0003168	19	54230176	54230240	+
360	hsa-mir-526b	MI0003150	19	54197647	54197729	+
361	hsa-mir-527	MI0003179	19	54257272	54257356	+
362	hsa-mir-532	MI0003205	X	49767754	49767844	+
363	hsa-mir-539	MI0003514	14	101513658	101513735	+
364	hsa-mir-541	MI0005539	14	101530832	101530915	+
365	hsa-mir-542	MI0003686	X	133675371	133675467	-
366	hsa-mir-543	MI0005565	14	101498324	101498401	+
367	hsa-mir-544	MI0003515	14	101514995	101515085	+
368	hsa-mir-545	MI0003516	X	73506939	73507044	-
369	hsa-mir-548a-1	MI0003593	6	18572015	18572111	+
370	hsa-mir-548a-2	MI0003598	6	135560298	135560394	+
371	hsa-mir-548a-3	MI0003612	8	105496597	105496693	-
372	hsa-mir-548b	MI0003596	6	119390212	119390308	-
373	hsa-mir-548c	MI0003630	12	65016289	65016385	+
374	hsa-mir-548d-1	MI0003668	8	124360274	124360370	-
375	hsa-mir-548d-2	MI0003671	17	65467605	65467701	-
376	hsa-mir-548e	MI0006344	10	112748684	112748771	+
377	hsa-mir-548f-1	MI0006374	10	56367634	56367717	-
378	hsa-mir-548f-2	MI0006375	2	213290987	213291084	-
379	hsa-mir-548f-3	MI0006376	5	109849530	109849616	-
380	hsa-mir-548f-4	MI0006377	7	147075109	147075213	-
381	hsa-mir-548f-5	MI0006378	X	32659591	32659676	-
382	hsa-mir-548g	MI0006395	4	148265781	148265869	-
383	hsa-mir-548h-1	MI0006411	14	64561742	64561843	-
384	hsa-mir-548h-2	MI0006412	16	11400297	11400384	-

Contd....

4189809	+
4216688	+
4183277	+
4194219	+
4204541	+
4210793	+
4223436	+
4179051	+
4185499	+
4225509	+
4245853	+
4251976	+
4219934	+
4254551	+
4201725	+
4214342	+
4200871	+
4209590	+
4230240	+
4197729	+
4257356	+
9767844	+
01513735	+
01530915	+
33675467	-
01498401	+
01515085	+
3507044	-
8572111	+
35560394	+
05496693	-
19390308	-
5016385	+
24360370	-
5467701	-
12748771	+
6367717	-
13291084	-
09849616	-
47075213	-
2659676	-
48265869	-
4561843	-
1400384	-

Contd....

MI0006413	17	13446846	13446963	-
MI0006414	8	26906370	26906480	-
MI0006421	3	125509247	125509395	-
MI0006422	4	9557789	9557937	-
MI0006423	8	7946463	7946611	-
MI0006424	X	83480760	83480836	-
MI0006345	22	26951178	26951289	-
MI0006354	11	70130061	70130176	+
MI0006361	11	94199661	94199746	-
MI0006400	X	94318140	94318225	-
MI0006399	7	34980372	34980446	-
MI0006402	7	102046189	102046302	-
MI0006420	5	100152186	100152269	-
MI0010637	10	12767253	12767352	-
MI0003679	15	81134319	81134414	-
MI0003600	7	30329410	30329506	+
MI0003601	7	32772593	32772689	+
MI0003556	1	3477259	3477354	-
MI0003575	3	168269642	168269737	+
MI0003557	1	35135200	35135295	-
MI0003558	1	100746797	100746864	+
MI0003559	1	151518272	151518367	+
MI0003561	1	155316141	155316236	-
MI0003562	1	162312336	162312430	+
MI0003563	1	168344762	168344859	+
MI0003564	2	32757220	32757313	+
MI0003565	2	47604814	47604909	+
MI0003567	2	189162219	189162315	+
MI0003568	2	233037363	233037457	+
MI0003569	3	15915278	15915356	+
MI0003570	3	44903380	44903473	+
MI0003572	3	50210759	50210852	+
MI0003573	3	111831648	111831745	+
MI0003574	3	114035322	114035416	-
MI0003576	3	170824453	170824548	-
MI0003577	3	195426272	195426368	+
MI0003578	4	343946	344041	+
MI0003579	4	11370451	11370545	+
MI0003580	4	24521815	24521913	-
MI0003581	4	38869653	38869748	+
MI0003582	4	83674490	83674583	-
MI0003583	4	110409854	110409951	+
MI0003584	4	115577915	115578010	+
MI0003585	4	166307394	166307489	+

Contd....

429	hsa-mir-579	MI0003586	5	32394484	32394581	-
430	hsa-mir-580	MI0003587	5	36147994	36148090	-
431	hsa-mir-581	MI0003588	5	53247334	53247429	-
432	hsa-mir-582	MI0003589	5	58999432	58999529	-
433	hsa-mir-583	MI0003590	5	95414842	95414916	+
434	hsa-mir-584	MI0003591	5	148441876	148441972	-
435	hsa-mir-585	MI0003592	5	168690605	168690698	-
436	hsa-mir-586	MI0003594	6	45165411	45165507	-
437	hsa-mir-587	MI0003595	6	107232000	107232095	+
438	hsa-mir-588	MI0003597	6	126805777	126805859	+
439	hsa-mir-589	MI0003599	7	5535450	5535548	-
440	hsa-mir-590	MI0003602	7	73605528	73605624	+
441	hsa-mir-591	MI0003603	7	95848974	95849068	-
442	hsa-mir-592	MI0003604	7	126698142	126698238	-
443	hsa-mir-593	MI0003605	7	127721913	127722012	+
444	hsa-mir-595	MI0003607	7	158325410	158325505	-
445	hsa-mir-596	MI0003608	8	1765397	1765473	+
446	hsa-mir-597	MI0003609	8	9599182	9599278	+
447	hsa-mir-598	MI0003610	8	10892716	10892812	-
448	hsa-mir-599	MI0003611	8	100548864	100548958	-
449	hsa-mir-600	MI0003613	9	125873825	125873922	-
450	hsa-mir-601	MI0003614	9	126164804	126164882	-
451	hsa-mir-602	MI0003615	9	140732871	140732968	+
452	hsa-mir-603	MI0003616	10	24564614	24564710	+
453	hsa-mir-604	MI0003617	10	29833933	29834026	-
454	hsa-mir-605	MI0003618	10	53059333	53059415	+
455	hsa-mir-606	MI0003619	10	77312216	77312311	+
456	hsa-mir-607	MI0003620	10	98588426	98588521	-
457	hsa-mir-608	MI0003621	10	102734742	102734841	+
458	hsa-mir-609	MI0003622	10	105978547	105978641	-
459	hsa-mir-610	MI0003623	11	28078362	28078457	+
460	hsa-mir-611	MI0003624	11	61559967	61560033	-
461	hsa-mir-612	MI0003625	11	65211929	65212028	+
462	hsa-mir-613	MI0003626	12	12917583	12917677	+
463	hsa-mir-614	MI0003627	12	13068763	13068852	+
464	hsa-mir-615	MI0003628	12	54427734	54427829	+
465	hsa-mir-616	MI0003629	12	57912946	57913042	-
466	hsa-mir-617	MI0003631	12	81226312	81226408	-
467	hsa-mir-618	MI0003632	12	81329515	81329612	-
468	hsa-mir-619	MI0003633	12	109230684	109230782	-
469	hsa-mir-620	MI0003634	12	116586365	116586459	-
470	hsa-mir-621	MI0003635	13	41384902	41384997	+
471	hsa-mir-622	MI0003636	13	90883436	90883531	+
472	hsa-mir-623	MI0003637	13	100008385	100008482	+

Contd....

473	hsa-mir-624	MI0003638	14	31483852	31483948	-
474	hsa-mir-625	MI0003639	14	65937820	65937904	+
475	hsa-mir-626	MI0003640	15	41983783	41983876	+
476	hsa-mir-627	MI0003641	15	42491768	42491864	-
477	hsa-mir-628	MI0003642	15	55665138	55665232	-
478	hsa-mir-629	MI0003643	15	70371711	70371807	-
479	hsa-mir-630	MI0003644	15	72879558	72879654	+
480	hsa-mir-631	MI0003645	15	75645952	75646026	-
481	hsa-mir-632	MI0003647	17	30677128	30677221	+
482	hsa-mir-633	MI0003648	17	61021576	61021673	+
483	hsa-mir-634	MI0003649	17	64783190	64783286	+
484	hsa-mir-635	MI0003650	17	66420592	66420689	-
485	hsa-mir-636	MI0003651	17	74732532	74732630	-
486	hsa-mir-637	MI0003652	19	3961412	3961510	-
487	hsa-mir-638	MI0003653	19	10829080	10829179	+
489	hsa-mir-639	MI0003654	19	14640355	14640452	+
490	hsa-mir-640	MI0003655	19	19545872	19545967	+
491	hsa-mir-641	MI0003656	19	40788450	40788548	-
492	hsa-mir-642	MI0003657	19	46178186	46178282	+
493	hsa-mir-643	MI0003658	19	52785050	52785146	+
494	hsa-mir-644	MI0003659	20	33054130	33054223	+
495	hsa-mir-645	MI0003660	20	49202323	49202416	+
496	hsa-mir-646	MI0003661	20	58883532	58883625	+
497	hsa-mir-647	MI0003662	20	62573984	62574079	-
498	hsa-mir-648	MI0003663	22	18463634	18463727	-
499	hsa-mir-649	MI0003664	22	21388465	21388561	-
500	hsa-mir-650	MI0003665	22	23165270	23165365	+
501	hsa-mir-651	MI0003666	X	8095006	8095102	+
502	hsa-mir-652	MI0003667	X	109298557	109298654	+
503	hsa-mir-653	MI0003674	7	93112072	93112167	-
504	hsa-mir-654	MI0003676	14	101506556	101506636	+
505	hsa-mir-655	MI0003677	14	101515887	101515983	+
506	hsa-mir-656	MI0003678	14	101533061	101533138	+
507	hsa-mir-657	MI0003681	17	79099076	79099173	-
508	hsa-mir-658	MI0003682	22	38240279	38240378	-
509	hsa-mir-659	MI0003683	22	38243685	38243781	-
510	hsa-mir-660	MI0003684	X	49777849	49777945	+
511	hsa-mir-661	MI0003669	8	145019359	145019447	-
512	hsa-mir-662	MI0003670	16	820183	820277	+
513	hsa-mir-663	MI0003672	20	26188822	26188914	-
514	hsa-mir-663b	MI0006336	2	133014539	133014653	-
515	hsa-mir-664	MI0006442	1	220373880	220373961	-
516	hsa-mir-665	MI0005563	14	101341370	101341441	+
517	hsa-mir-668	MI0003761	14	101521595	101521660	+

Contd....

518	hsa-mir-670	MI0003933	11	43581206	43581303	+
519	hsa-mir-671	MI0003760	7	150935507	150935624	+
520	hsa-mir-675	MI0005416	11	2017989	2018061	-
521	hsa-mir-708	MI0005543	11	79113066	79113153	-
522	hsa-mir-711	MI0012488	3	48616335	48616410	-
523	hsa-mir-718	MI0012489	X	153285371	153285440	-
524	hsa-mir-720	MI0006654	3	164059129	164059238	+
525	hsa-mir-744	MI0005559	17	11985216	11985313	+
526	hsa-mir-758	MI0003757	14	101492357	101492444	+
527	hsa-mir-759	MI0004065	13	53384185	53384275	+
528	hsa-mir-760	MI0005567	1	94312388	94312467	+
529	hsa-mir-761	MI0003941	1	52302016	52302074	-
530	hsa-mir-762	MI0003892	16	30905224	30905306	+
531	hsa-mir-764	MI0003944	X	113873918	113874002	+
532	hsa-mir-765	MI0005116	1	156905923	156906036	-
533	hsa-mir-766	MI0003836	X	118780701	118780811	-
534	hsa-mir-767	MI0003763	X	151561893	151562001	-
535	hsa-mir-769	MI0003834	19	46522190	46522307	+
536	hsa-mir-770	Mi0005118	14	101318727	101318824	+
537	hsa-mir-802	Mi0003906	21	37093013	37093106	+
538	hsa-mir-873	MI0005564	9	28888877	28888953	-
539	hsa-mir-874	MI0005532	5	136983261	136983338	-
540	hsa-mir-875	MI0005541	8	100549014	100549089	-
541	hsa-mir-876	MI0005542	9	28863624	28863704	-
542	hsa-mir-877	MI0005561	6	30552109	30552194	+
			HSCHR6_MHC_COX	30541958	30542043	+
			HSCHR6_MHC_DBB	30542353	30542438	+
			HSCHR6_MHC_MANN	30596784	30596869	+
			HSCHR6_MHC_MCF	30630566	30630651	+
			HSCHR6_MHC_QBL	30541620	30541705	+
			HSCHR6_MHC_SSTO	30543533	30543618	+
543	hsa-mir-885	MI0005560	3	10436173	10436246	-
544	hsa-mir-886	MI0005527	5	135416177	135416297	-
545	hsa-mir-887	MI0005562	5	15935291	15935369	+
546	hsa-mir-888	MI0005537	X	145076302	145076378	-
547	hsa-mir-889	MI0005540	14	101514238	101514316	+
548	hsa-mir-890	MI0005533	X	145075793	145075869	-
549	hsa-mir-891a	MI0005524	X	145109312	145109390	-

Contd....

550	hsa-mir-891b	MI0005534	X	145082571	145082649	-
551	hsa-mir-892a	MI0005528	X	145078187	145078261	-
552	hsa-mir-892b	MI0005538	X	145078716	145078792	-
553	hsa-mir-920	MI0005712	12	24365355	24365429	+
554	hsa-mir-921	MI0005713	1	166123980	166124035	-
555	hsa-mir-922	MI0005714	3	197401367	197401447	-
556	hsa-mir-924	MI0005716	18	37202087	37202139	-
557	hsa-mir-933	MI0005755	2	176032361	176032437	-
558	hsa-mir-934	MI0005756	X	135633037	135633119	+
559	hsa-mir-935	MI0005757	19	54485561	54485651	+
560	hsa-mir-936	MI0005758	10	105807847	105807944	-
561	hsa-mir-937	MI0005759	8	144895127	144895212	-
563	hsa-mir-938	MI0005760	10	29891193	29891275	-
563	hsa-mir-939	MI0005761	8	145619364	145619445	-
564	hsa-mir-940	MI0005762	16	2321748	2321841	+
565	hsa-mir-941-1	MI0005763	20	62550794	62550882	+
566	hsa-mir-941-2	MI0005764	20	62551101	62551189	+
567	hsa-mir-941-3	MI0005765	20	62551213	62551301	+
568	hsa-mir-941-4	MI0005766				
569	hsa-mir-942	MI0005767	1	117637265	117637350	+
570	hsa-mir-943	MI0005768	4	1988111	1988204	-
571	hsa-mir-944	MI0005769	3	189547711	189547798	+
572	hsa-mir-1178	MI0006271	12	120151439	120151529	-
573	hsa-mir-1179	MI0006272	15	89151338	89151428	+
574	hsa-mir-1180	MI0006273	17	19247819	19247887	-
575	hsa-mir-1181	MI0006274	19	10514134	10514214	-
576	hsa-mir-1182	MI0006275	1	231155574	231155670	-
577	hsa-mir-1183	MI0006276	7	21510676	21510764	+
578	hsa-mir-1184	MI0006277	X X X	154115635 154612749 154687178	154115733 154612847 154687276	- - +
579	hsa-mir-1185-1	MI0003844	14	101509314	101509399	+
580	hsa-mir-1185-2	MI0003821	14	101510535	101510620	+
581	hsa-mir-1197	MI0006656	14	101491901	101491988	+
582	hsa-mir-1200	MI0006332	7	36958962	36959037	-
583	hsa-mir-1201	MI0006333	14	20794606	20794690	-
584	hsa-mir-1202	MI0006334	6	156267931	156268013	+
585	hsa-mir-1203	MI0006335	17	46233789	46233873	-
586	hsa-mir-1204	MI0006337	8	128808208	128808274	+
587	hsa-mir-1205	MI0006338	8	128972879	128972941	+
588	hsa-mir-1206	MI0006339	8	129021144	129021202	+
589	hsa-mir-1207	MI0006340	8	129061398	129061484	+
590	hsa-mir-1208	MI0006341	8	129162362	129162434	+
591	hsa-mir-1224	MI0003764	3	183959193	183959277	+

Contd....

592	hsa-mir-1225	MI0006311	16	2140196	2140285	-
593	hsa-mir-1226	MI0006313	3	47891045	47891119	+
594	hsa-mir-1227	MI0006316	19	2234061	2234148	-
595	hsa-mir-1228	MI0006318	12	57588287	57588359	+
596	hsa-mir-1229	MI0006319	5	179225278	179225346	-
597	hsa-mir-1231	MI0006321	1	201777739	201777830	+
598	hsa-mir-1233	MI0006323	15	34674270	34674351	-
			15	34820491	34820572	-
599	hsa-mir-1234	MI0006324	8	145625476	145625559	-
600	hsa-mir-1236	MI0006326	6	31924616	31924717	-
			HSCHR6_MHC_COX	31912168	31912269	-
			HSCHR6_MHC_DBB	31906802	31906903	-
			HSCHR6_MHC_MCF	32001063	32001164	-
			HSCHR6_MHC_QBL	31915008	31915109	-
			HSCHR6_MHC_SSTO	31916468	31916569	-
601	hsa-mir-1237	MI0006327	11	64136074	64136175	+
602	hsa-mir-1238	MI0006328	19	10662798	10662880	+
603	hsa-mir-1243	MI0006373	4	114028019	114028111	+
604	hsa-mir-1244	MI0006379	2	232578024	232578108	+
			5	118310281	118310365	+
			12	9392063	9392147	-
			12	12264886	12264970	+
605	hsa-mir-1245	MI0006380	2	189842818	189842887	+
606	hsa-mir-1246	MI0006381	2	177465708	177465780	-
607	hsa-mir-1247	MI0006382	14	102026624	102026759	-
608	hsa-mir-1248	MI0006383	3	186504461	186504566	+
609	hsa-mir-1249	MI0006384	22	45596835	45596900	-
610	hsa-mir-1250	MI0006385	17	79106996	79107108	-
611	hsa-mir-1251	MI0006386	12	97885687	97885756	+
612	hsa-mir-1252	MI0006434	12	79813037	79813101	+
613	hsa-mir-1253	MI0006387	17	2651372	2651476	-
614	hsa-mir-1254	MI0006388	10	70519075	70519171	+
615	hsa-mir-1255a	MI0006389	4	102251459	102251571	-
616	hsa-mir-1255b-1	MI0006435	4	36427988	36428050	-
617	hsa-mir-1255b-2	MI0006436	1	167967898	167967964	+
618	hsa-mir-1256	MI0006390	1	21314807	21314925	-
619	hsa-mir-1257	MI0006391	20	60528602	60528718	-
620	hsa-mir-1258	MI0006392	2	180725563	180725635	-
621	hsa-mir-1259	MI0006393	20	47896847	47896957	+

Contd....

622	hsa-mir-1260	MI0006394	14	77732561	77732633	+
623	hsa-mir-1261	MI0006396	11	90602289	90602370	-
624	hsa-mir-1262	MI0006397	1	68649201	68649293	-
625	hsa-mir-1263	MI0006398	3	163889259	163889344	+
626	hsa-mir-1264	MI0003758	X	113887130	113887198	-
627	hsa-mir-1265	MI0006401	10	14478575	14478660	+
628	hsa-mir-1266	MI0006403	15	52569314	52569397	-
629	hsa-mir-1267	MI0006404	13	108183519	108183596	-
630	hsa-mir-1268	MI0006405	15	22513229	22513280	-
631	hsa-mir-1269	MI0006406	4	67142542	67142646	+
632	hsa-mir-1270	MI0006407	19 19	20510081 20579240	20510163 20579322	- -
633	hsa-mir-1271	MI0003814	5	175794949	175795034	+
634	hsa-mir-1272	MI0006408	15	65054586	65054714	-
635	hsa-mir-1273	MI0006409	8	101036210	101036312	-
636	hsa-mir-1274a	MI0006410	5	41475734	41475804	+
637	hsa-mir-1274b	MI0006427	19	58024375	58024441	-
638	hsa-mir-1275	MI0006415	6	33967749	33967828	-
639	hsa-mir-1276	MI0006416	15	86313727	86313809	-
640	hsa-mir-1277	MI0006419	X	117520357	117520434	+
641	hsa-mir-1278	MI0006425	1	193105633	193105713	+
642	hsa-mir-1279	MI0006426	12	69666937	69666998	-
643	hsa-mir-1280	MI0006437	3	128081008	128081101	+
644	hsa-mir-1281	MI0006428	22	41488517	41488570	+
645	hsa-mir-1282	MI0006429	15	44085857	44085957	-
646	hsa-mir-1283-1	MI0003832	19	54191735	54191821	+
647	hsa-mir-1283-2	MI0006430	19	54261486	54261572	+
648	hsa-mir-1284	MI0006431	3	71591121	71591240	-
649	hsa-mir-1285-1	MI0006346	7	91833329	91833412	-
650	hsa-mir-1285-2	MI0006347	2	70480050	70480137	-
651	hsa-mir-1286	MI0006348	22	20236657	20236734	-
652	hsa-mir-1287	MI0006349	10	100154975	100155064	-
653	hsa-mir-1288	MI0006432	17	16185328	16185402	+
654	hsa-mir-1289-1	MI0006350	20	34041776	34041919	-
655	hsa-mir-1289-2	MI0006351	5	132763288	132763398	-
656	hsa-mir-1290	MI0006352	1	19223565	19223642	-
657	hsa-mir-1291	MI0006353	12	49048227	49048313	-
658	hsa-mir-1292	MI0006433	20	2633423	2633488	+
659	hsa-mir-1293	MI0006355	12	50627925	50627995	-
660	hsa-mir-1294	MI0006356	5	153726666	153726807	+
661	hsa-mir-1295	MI0006357	1	171070869	171070947	-
662	hsa-mir-1296	MI0003780	10	65132717	65132808	-
663	hsa-mir-1297	MI0006358	13	54886107	54886183	-
664	hsa-mir-1298	MI0003938	X	113949650	113949761	+

Contd....

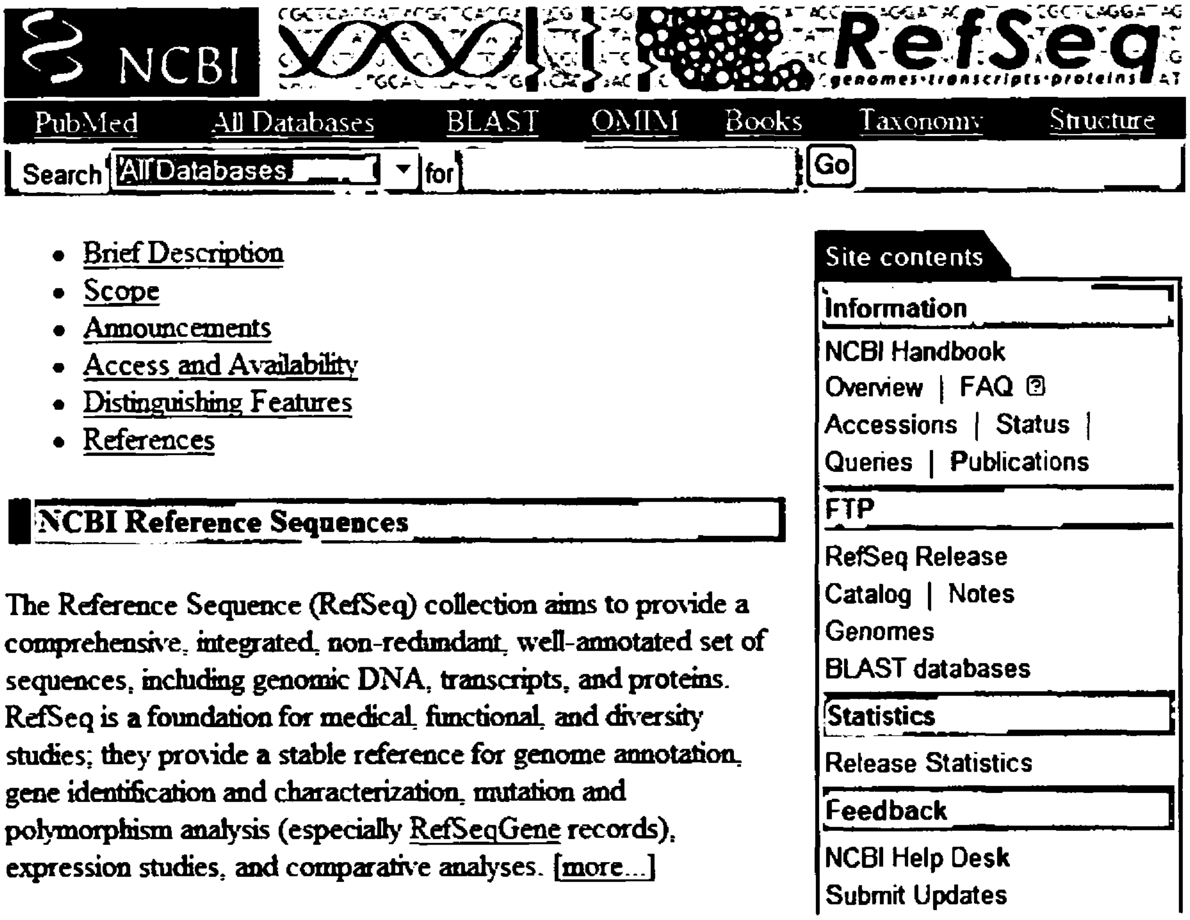
665	hsa-mir-1299	MI0006359	9	69002239	69002321	-
666	hsa-mir-1301	MI0003815	2	25551509	25551590	-
667	hsa-mir-1302-1	MI0006362	12	113132839	113132981	-
668	hsa-mir-1302-2	MI0006363	1	30366	30503	+
			9	30144	30281	+
			15	102500662	102500799	-
			19	71973	72110	+
669	hsa-mir-1302-3	MI0006364	2	114340536	114340673	-
670	hsa-mir-1302-4	MI0006365	2	208133999	208134148	-
671	hsa-mir-1302-5	MI0006366	20	49231173	49231322	-
672	hsa-mir-1302-6	MI0006367	7	18166843	18166932	-
673	hsa-mir-1302-7	MI0006368	8	142867603	142867674	-
674	hsa-mir-1302-8	MI0006369	9	100125836	100125963	-
675	hsa-mir-1303	MI0006370	5	154065336	154065421	+
676	hsa-mir-1304	MI0006371	11	93466840	93466930	-
677	hsa-mir-1305	MI0006372	4	183090446	183090531	+
678	hsa-mir-1306	MI0006443	22	20073581	20073665	+
679	hsa-mir-1307	MI0006444	10	105154010	105154158	-
680	hsa-mir-1308	MI0006441	X	22080259	22080312	-
681	hsa-mir-1321	MI0006652	X	85090785	85090863	+
682	hsa-mir-1322	MI0006653	8	10682883	10682953	-
683	hsa-mir-1323	MI0003786	19	54175222	54175294	+
684	hsa-mir-1324	MI0006657	3	75679914	75680009	+
685	hsa-mir-1468	MI0003782	X	63005882	63005967	-
686	hsa-mir-1469	MI0007074	15	96876490	96876536	+
687	hsa-mir-1470	MI0007075	19	15560359	15560419	+
688	hsa-mir-1471	MI0007076	2	232756952	232757008	-
689	hsa-mir-1537	MI0007258	1	236016300	236016360	-
690	hsa-mir-1538	MI0007259	16	69599711	69599771	-
691	hsa-mir-1539	MI0007260	18	47013743	47013792	+
692	hsa-mir-1825	MI0008193	20	30825598	30825650	+
693	hsa-mir-1826	MI0008194	16	33965508	33965592	+
694	hsa-mir-1827	MI0008195	12	100583662	100583727	+
695	hsa-mir-1908	MI0008329	11	61582633	61582712	-
696	hsa-mir-1909	MI0008330	19	1816158	1816237	-
697	hsa-mir-1910	MI0008331	16	85775227	85775306	-
698	hsa-mir-1911	MI0008332	X	113997744	113997823	+
699	hsa-mir-1912	MI0008333	X	113886019	113886098	+
700	hsa-mir-1913	MI0008334	6	166922842	166922921	-
701	hsa-mir-1914	MI0008335	20	62572818	62572897	-
702	hsa-mir-1915	MI0008336	10	21785491	21785570	-
703	hsa-mir-1972	MI0009982	16	15104178	15104254	-
			16	70064249	70064325	+
704	hsa-mir-1973	MI0009983	4	117220881	117220924	+

Contd....

705	hsa-mir-1974	MI0009984	5	93905172	93905241	-
			MT	14675	14744	-
706	hsa-mir-1975	MI0009985	7	148638580	148638654	+
707	hsa-mir-1976	MI0009986	1	26881033	26881084	+
708	hsa-mir-1977	MI0009987	1	566187	566265	-
			MT	5638	5716	-
709	hsa-mir-1978	MI0009988	2	149639365	149639417	-
			MT	622	674	+
710	hsa-mir-1979	MI0009989	4	166321814	166321889	-
711	hsa-mir-2052	MI0010486	8	75617928	75617982	+
712	hsa-mir-2053	MI0010487	8	113655722	113655812	+
713	hsa-mir-2054	MI0010488	4	126428414	126428462	+
714	hsa-mir-2110	MI0010629	10	115933864	115933938	-
715	hsa-mir-2113	MI0003939	6	98472407	98472497	+
716	hsa-mir-2114	MI0010633	X	149396239	149396318	+
717	hsa-mir-2115	MI0010634	3	48357850	48357949	-
718	hsa-mir-2116	MI0010635	15	59463382	59463461	-
719	hsa-mir-2117	MI0010636	17	41522174	41522253	+
720	hsa-mir-2276	MI0011282	13	24736555	24736643	+
721	hsa-mir-2277	MI0011284	5	92956402	92956494	-

Contd....

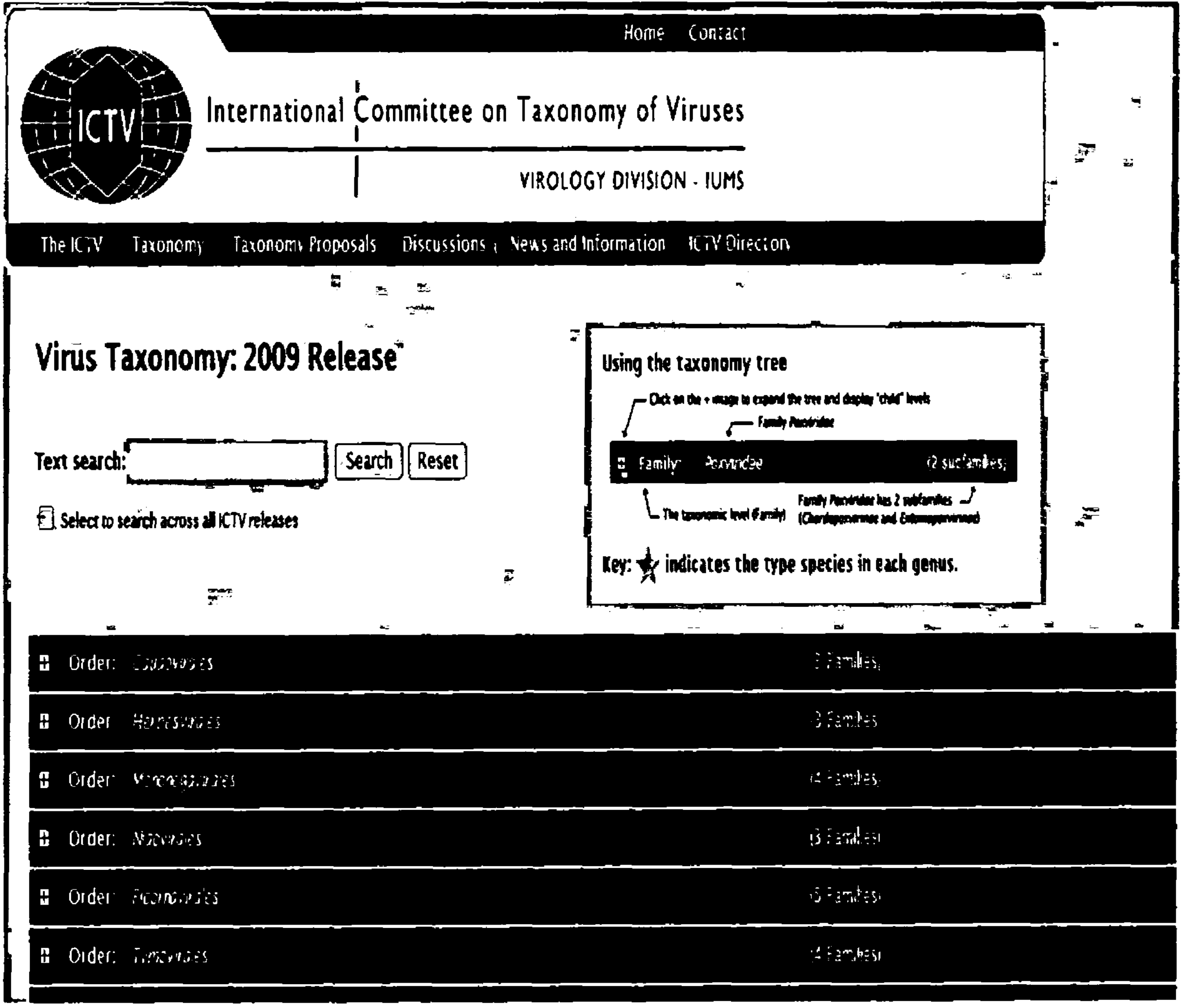
Appendix II



NCBI Reference Sequences

The Reference Sequence (RefSeq) collection aims to provide a comprehensive, integrated, non-redundant, well-annotated set of sequences, including genomic DNA, transcripts, and proteins. RefSeq is a foundation for medical, functional, and diversity studies; they provide a stable reference for genome annotation, gene identification and characterization, mutation and polymorphism analysis (especially RefSeqGene records), expression studies, and comparative analyses. [more...]

Appendix II



Virus Taxonomy: 2009 Release

Text search: ☐ Search Reset

☐ Select to search across all ICTV releases

Using the taxonomy tree

Click on the + image to expand the tree and display "child" levels

Family Poxviridae

Family: *Poxviridae* (2 subfamilies)

The taxonomic level (Family)

Family Poxviridae has 2 subfamilies (Chordopoxvirinae and Entomopoxvirinae)

Key: ★ indicates the type species in each genus.

Order: *Caudovirales* (3 Families)

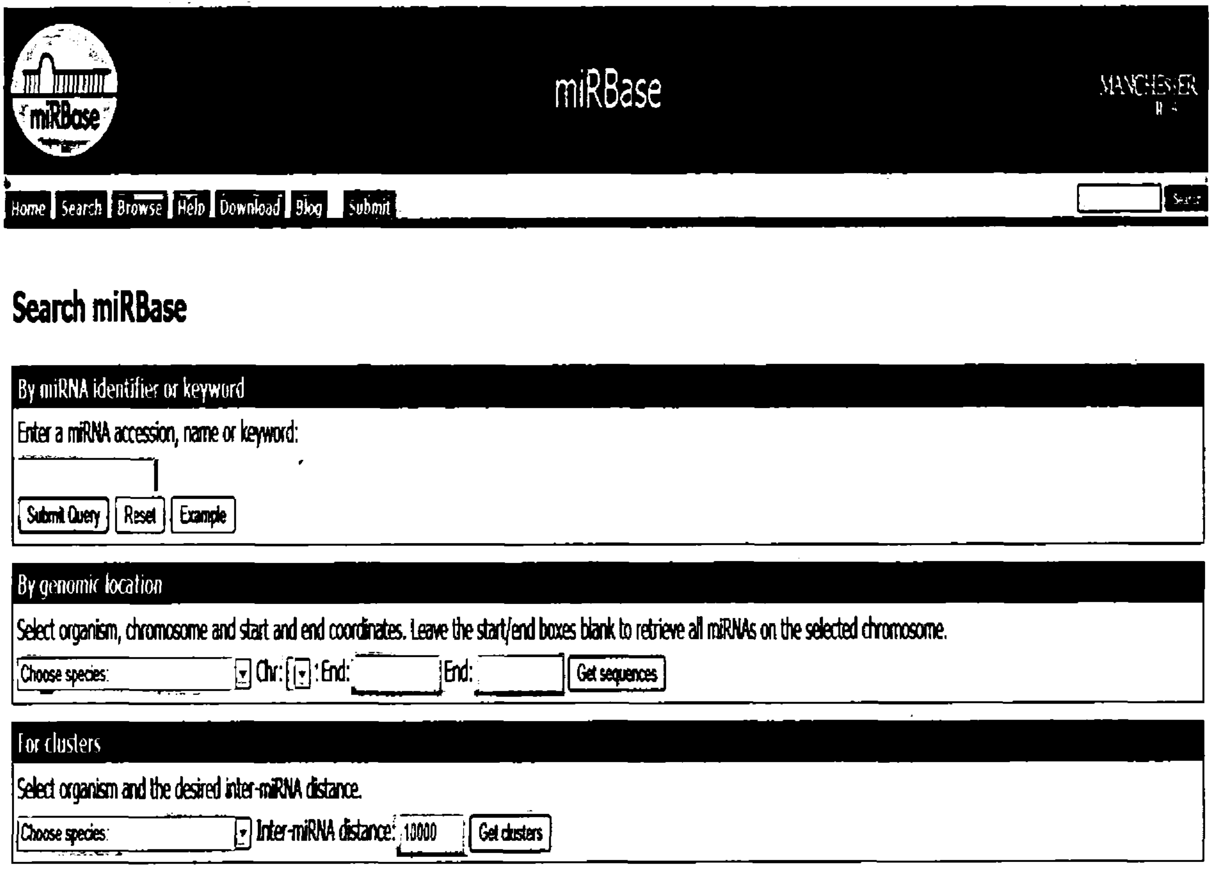
Order: *Herpesvirales* (3 Families)

Order: *Mononegavirales* (4 Families)

Order: *Nidovirales* (3 Families)

Order: *Picornavirales* (5 Families)

Order: *Tymovirales* (4 Families)



Search miRBase

By miRNA identifier or keyword

Enter a miRNA accession, name or keyword:

By genomic location

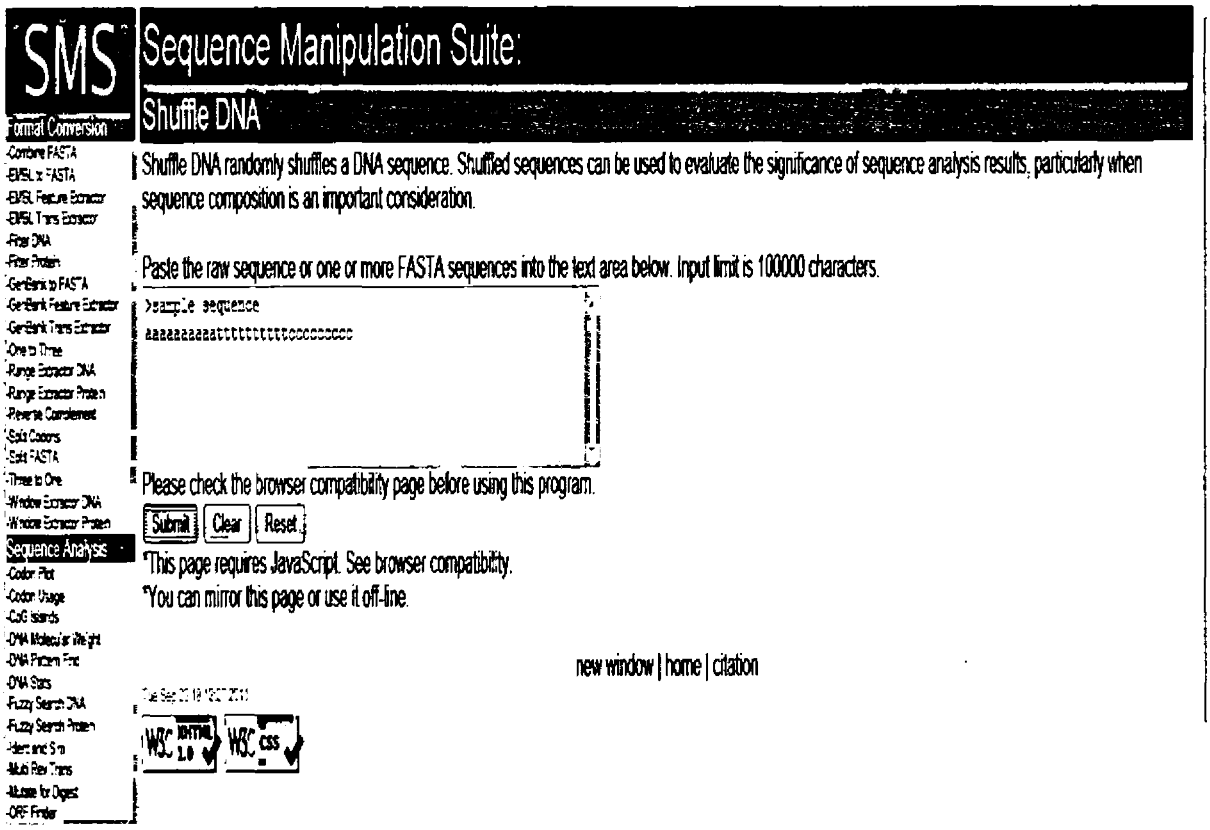
Select organism, chromosome and start and end coordinates. Leave the start/end boxes blank to retrieve all miRNAs on the selected chromosome.

Choose species: Chr: End: End:

For clusters

Select organism and the desired inter-miRNA distance.

Choose species: Inter-miRNA distance: 10000



Shuffle DNA

Shuffle DNA randomly shuffles a DNA sequence. Shuffled sequences can be used to evaluate the significance of sequence analysis results, particularly when sequence composition is an important consideration.

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100000 characters.

```
>sample sequence
aaaaaaaaaattttttttttcccccccccc
```

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