國 立 交 通 大 學

生物資訊及系統生物研究所

博士論文

分析微小核醣核酸與目標基因間的作用關係及尋找標靶基因的應用

In Silico Analysis of microRNA-Target Interaction and Its Application for miRNA Target Identification

研 究 生：許勝達
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中華民國一百年八月

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研 究 生：許勝達　　Student：Sheng-Da Hsu

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摘要

微小核醣核酸(microRNA/miRNA)是一段長度約為 22 核苷酸的非編碼的核醣核酸分子，它們可藉由與基因的結合來調控基因的表現，研究發現微小核醣核酸調控許多的基因與細胞功能有關，例如，細胞凋零、細胞分化與細胞發育。之前的文獻認為30%或更多的人類基因受到微小核醣核酸的調控。由於微小核醣核酸在生物的重要性，所以許多相關的資料庫及工具被開發出來。本文主要是分析微小核醣核酸與其目標基因間的作用關係，並探討此關係的序列及結構特徵，根據此分析結果開發新的方法或整合系統研究微小核醣核酸的功能。我們總共分析了 1,524 個實驗驗證過的微小核醣核酸與目標基因的作用關係，並以此作為評估預測正確性的資料。通過分析已知的微小核醣酸與其目標基因的作用關係，我們可以獲得其序列與結構上的特徵。這些分析與實驗已知的資料對於尋找標靶基因工具的開發是很重要，且生物學家可以透過此結果選擇適當的預測工具。此外，還有其他因素會影響微小核醣核酸是否會與目標基因結合，例如，核醣核酸結合的蛋白質(RNA 誘導沉默複合體)、微小核醣核酸及信息核醣核酸的濃度。未來我們會將這些因素加入分析微小核醣核酸與目標基因的作用關係。

In Silico Analysis of microRNA-Target Interaction and Its Application for miRNA Target Identification

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Abstract

MicroRNAs (miRNAs) are small non-coding RNA molecules (~ 22-nt) that can bind to one or more target sites on gene transcript to negatively regulate protein expression and thus control numerous cellular mechanisms. Recent work supports miRNAs downregulate gene expression during various crucial cell processes such as apoptosis, differentiation and development. Previous research has suggested that miRNAs regulate 30% or more of the human protein-coding genes. As the important roles of miRNAs, there were multiple databases storing the miRNA-target interactions (MTIs) identified using different tools. The aim of this work is to systematically analyze the miRNA-target interactions and assess the function of miRNA by developing new methods and resources. In this study, we analyzed 1,524 experimentally verified miRNA-target interactions with strong evidence support in human and elucidate which the more accurate microRNA target prediction database is. Through analyzing the verified MTIs, we could get the overview of relative contribution of sequence and structure features in miRNA targeting. Those analyses are important for identifying putative miRNA targets and are very useful for biologist to choose the proper tool for miRNA research. There are still other factors such as RNA binding protein (RISC), the concentration of miRNA and mRNA, playing an important role in the identification of miRNA-target interaction. We need take into account these

factors in future works in order to develop the more reliable miRNA target prediction resources.

誌謝

首先，我要感謝指導教授黃憲達博士在我研究所生涯中對於我的細心指導，使得我可以在生物資訊這個領域內從無到有的學習到許多知識，也在學術研究上有顯著的進步及成長。話說當初因緣際會之下進入 microRNA 這個領域，唯哲帶著我跟立人著手建構相關的資料庫，讓我學到如何規劃設計資料庫及相關生物知識分享，透過與陽明大學鄒安平教授的合作，使我知道原來實驗與計算可以如此緊密結合。最近的兩年內由於致宏、昭昉、Sirjana、Anas、梁超、維芸、煒志、雯玲、文婷、冠州、明家很認真努力的唸 paper，並從文獻中萃取出實驗驗證過的 microRNA 目標基因、實驗方法、相關疾病，因此才可以建構如此完整的實驗已知基因資料庫。除此之外，還有一路陪著我成長的熙淵、博凱、宗夷、威霽、佳宏、緯允、豐茂、詠薰、佳融、致閔，不論是研究或生活都給予我極大的幫助。此外已經畢業的燕茹、瑞鴻、冠樺、恆嘉、定遠、家慧、伯瑋、在營、美雪、恆毅、至昶，和大家一起奮鬥的日子，是我成長的動力，實驗室內的點點滴滴更是美好的回憶。最後，我要特別感謝我的家人及正景給予我的支持，謝謝你們給予我的支持與鼓勵，才能讓我無後顧之憂的求學。能夠順利完成博士論文並取得博士學位，是大家的指導、支持、與鼓勵，誠心的謝謝大家，將這份喜悅及成果與關心我的所有人一同分享。

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ISBLAB 研究生 許勝達

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Hsu, J.B.K.*, Chiu, C.M.*, Hsu, S.D.*, Huang, W.Y., Chien, C.H., Lee, T.Y. and Huang, H.D. (2011) miRTar: an integrated system for identifying miRNA-target interactions in Human. BMC Bioinformatics, 12.

Tsai, W.C.*, Hsu, S.D.*, Hsu, C.S.*, Hsiao, M.S., Huang, H.D., Chen, S.J., Huang, R.S.Y., Huang, Y., Lai, T.C., Tsai, T.F., Chen, H.C., Hsu, M.T., Wu, J.C., Hsiao, M., Tsou, A.P. (2011) MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. (In preparation)

Lin, F.M., Hsu, S.D., Chou, C.H. and Huang, H.D. (2011) HomoloMTI: Homologous Cluster of MicroRNA Target Interactions. (In preparation)

Hsu, S.D., Chu, C.H., Tsou, A.P., Chen, S.J., Chen, H.C., Hsu, P.W., Wong, Y.H., Chen, Y.H., Chen, G.H. and Huang, H.D. (2008) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. Nucleic Acids Res, 36, D165-169.

Hsu, P.W., Lin, L.Z., Hsu, S.D., Hsu, J.B. and Huang, H.D. (2007) ViTa: prediction of host microRNAs targets on viruses. Nucleic Acids Res, 35, D381-385.

Hsu, P.W., Huang, H.D., Hsu, S.D., Lin, L.Z., Tsou, A.P., Tseng, C.P., Stadler, P.F., Washietl, S. and Hofacker, I.L. (2006) miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. Nucleic Acids Res, 34, D135-139.

* These authors contributed equally to this work

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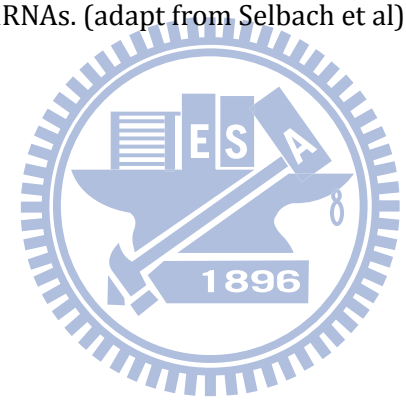

List of Tables

Chapter 1 Introduction

As small non-coding RNAs of approximately 22 nts, microRNAs (miRNAs) regulate gene expression post-transcriptionally through suppressing mRNA translation or inducing mRNA degradation by hybridizing to the 3'-untranslated regions (3'-UTR) of the mRNAs. Discovery of the first miRNA in *Caenorhabditis elegans* in 1993 (1) ushered in numerous studies on the cellular processes of these tiny regulatory RNAs for a large variety of metazoa. Thousands of miRNAs have been identified in mammalian cells over the past two decades. miRNAs play critical roles in many biological processes, including cell cycle control, cell growth and differentiation, apoptosis, and embryo development. Since their discovery, miRNAs have been found in many organisms. Until now, the miRBase (version 17) (2) contains 16,772 miRNAs which were discovered in 153 different species, the latest amount of miRNAs in miRBase is about ten times larger than the amount in 2004 (**Figure 1**). In spite of a large number of miRNAs have been identified, most of them are unknown the functions. This thesis is a compilation of the following 5 journal articles (3-6) and of unpublished data. It is consisted of the following chapters: in **Chapter 2,** the description of miRTarBase, a database which is the most updated collection of miRNA-target interactions (MTI), has accumulated 3,969 experimentally verified MTIs between 625 miRNAs and 2,433 target genes among 17 species by manually surveying pertinent literature. In **Chapter 3**, we demonstrate the miRNA target prediction system, miRTar, to enable biologists to easily identify the biological functions and regulatory relationships between a group of known/putative miRNAs and protein coding genes. **Chapter 4**, an application of using these resources or method to extend the experimentally verified miRNA-target interactions from one species to other species is presented. Finally in **Chapter 5,** presents miRNAMap a database updated to version 3, which was specifically designed to integrate 12 well-known miRNA-target interaction prediction databases and MTIs calculated by miRTar. We further compare these predicted MTIs to experimentally verified MTIs stored in miRTarBase and use cross-database predictions to improve the miRNA target identification. We close this thesis with conclusion in **Chapter 6**.

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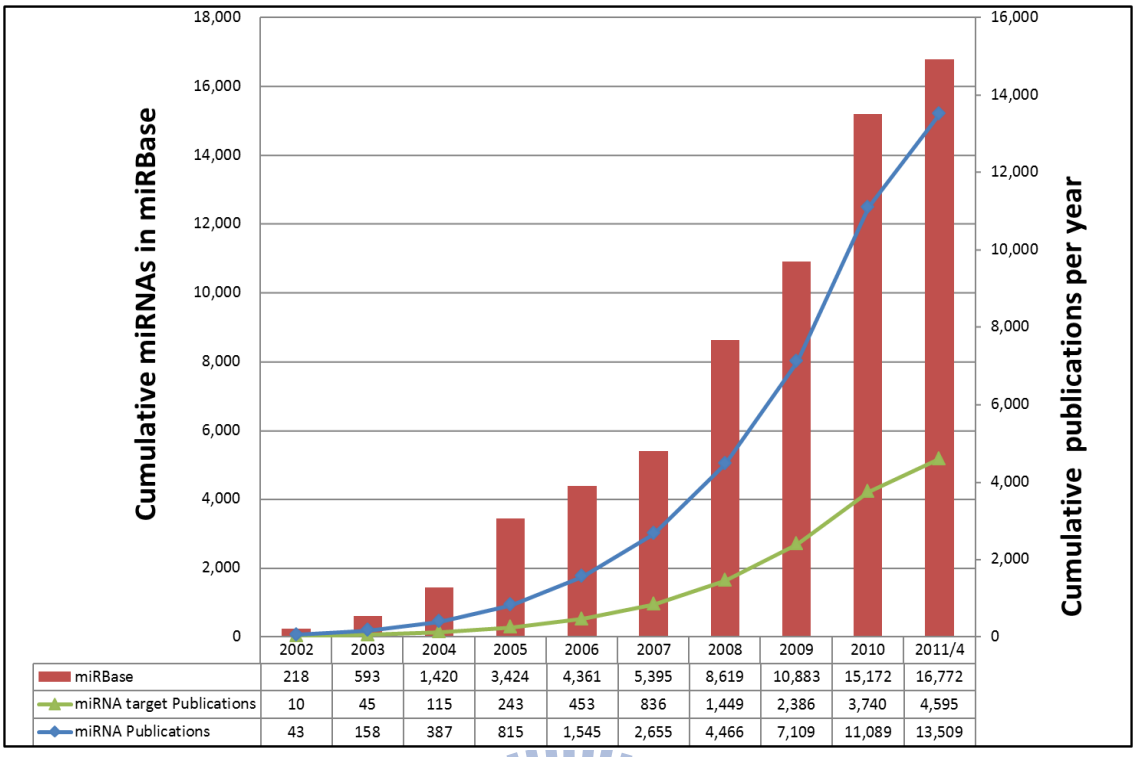


	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011/4
miRBase	218	593	1,420	3,424	4,361	5,395	8,619	10,883	15,172	16,772
miRNA target Publications	10	45	115	243	453	836	1,449	2,386	3,740	4,595
miRNA Publications	43	158	387	815	1,545	2,655	4,466	7,109	11,089	13,509

Figure 1. Growth of miRNA genes in the miRBase database and growth of the keywords with 'miRNA' and keyword with 'miRNA target' in PubMed.

1.1 Biological background

1.1.1 miRNA Biogenesis

microRNAs (miRNAs) are small non-coding RNAs of ~22 nt sequences that could regulate gene expression via hybridizing to 3' untranslated regions (3'-UTR), resulting in mRNA degradation and inhibiting mRNA translation (1,7-9). The major function of miRNAs is to repress the gene expression at post-transcriptional level (10). Recent work supports miRNAs downregulate gene expression during various crucial cell processes such as apoptosis (11-23), differentiation, development (24-47) and tumor growth (37-48).

The general biogenesis of the miRNA is shown in **Figure 2**. These microRNA (miRNA) genes are typically transcribed as primary miRNA (pri-miRNA) by RNA polymerase II (Pol II) (49,50) or RNA polymerase III (Pol III) (50,51) in the nucleus. If the miRNAs are transcribed by Pol II, the kind of primary miRNA transcripts (pri-miRNAs) contain cap structures as well as the poly(A) tails, which are the unique properties of class II gene transcripts. Then, the pri-miRNAs are processed into the

precursor of miRNAs (pre-miRNAs) by a protein complex - RNase III enzyme Drosha and DGCR8 (Pasha) (52). It's an essential process for the most miRNAs to release pre-miRNA, but a small group of miRNAs located within introns can bypass this step (53,54). **Figure S2** shows the possible biogenesis of intronic miRNAs. The pre-miRNA (~70 nts) is folded as a stem-loop (hairpin) structure which contains a short nucleotide (~17-24 nts) sequences embedded in the stem part of hairpin. The pre-miRNA is exported from the nucleus to the cytoplasm by Exportin 5. The pre-miRNA is then processed by the enzyme DICER (55-57) into a dsRNA (double strand RNA) that includes the ~22 nts mature miRNA and miRNA*. This dsRNA is further processed to the mature sequence, which becomes part of the RNA-induced silencing complex (RISC) (58-60).

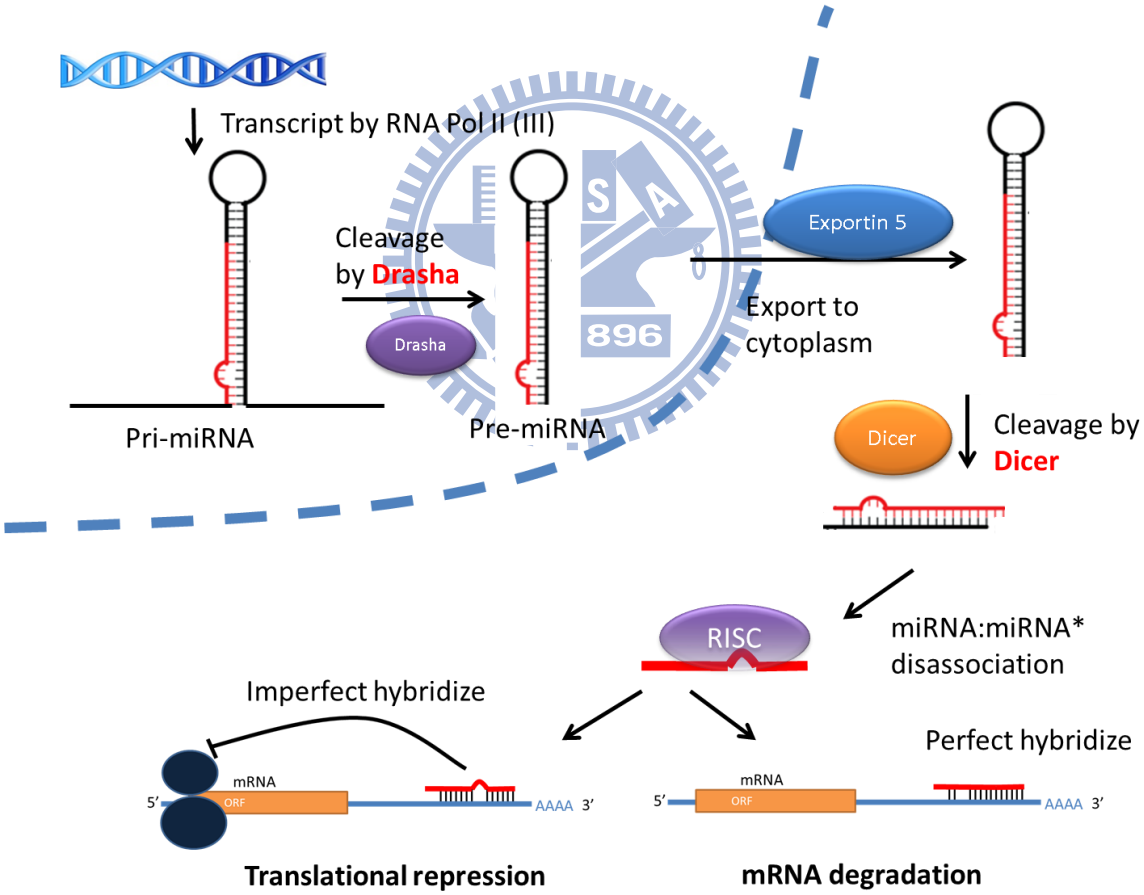


Figure 2. The biogenesis of miRNAs.

1.1.2 Intronic and exonic miRNAs

According to the miRNA locations of mRNA transcripts, four full-length pri-miRNAs have been characterized in **Figure S1** (61). **Figure S1** (a) miR-15a~16-1 cluster are intronic miRNAs which locate on non-protein-coding transcript (DLEU2 is a well-defined non-coding RNA gene) (62). **Figure S1** (b) miR-155 is located in the exon on non-protein-coding transcript (BIC). **Figure S1** (c) miR-25~93~106b cluster is embedded in the intron of MCM7 transcript. **Figure S1** (d) miR-985 was found in the last exon of CACNG8. The possible intronic miRNA biogenesis is shown in **Figure S2**.

1.1.3 Functions of miRNAs

The mature miRNA then binds to complementary sites in the mRNA target to negatively regulate gene expression through two major mechanism. **Figure 3** shows the major function of miRNAs in two mechanisms: mRNA degradation and translation repression. In plant, many miRNA target sites have perfect hybridization between miRNAs and their sites, and they cause mRNA degradation (7,63,64). However, not all the miRNAs in plants induce mRNA degradation; some of them may inhibit the mRNA translation through hybridizing to their target genes imperfectly. In animals, miRNAs are imperfectly complementary to the target mRNA which usually locates in 3'-untranslated region (3'-UTR). As shown in **Figure 3**, when miRNA-target interactions with perfect complementarity tend to result in mRNA cleavage and degradation; and miRNA-target interactions with imperfect complementarity tend to result in blocking ribosome processing and inhibiting mRNA translation.

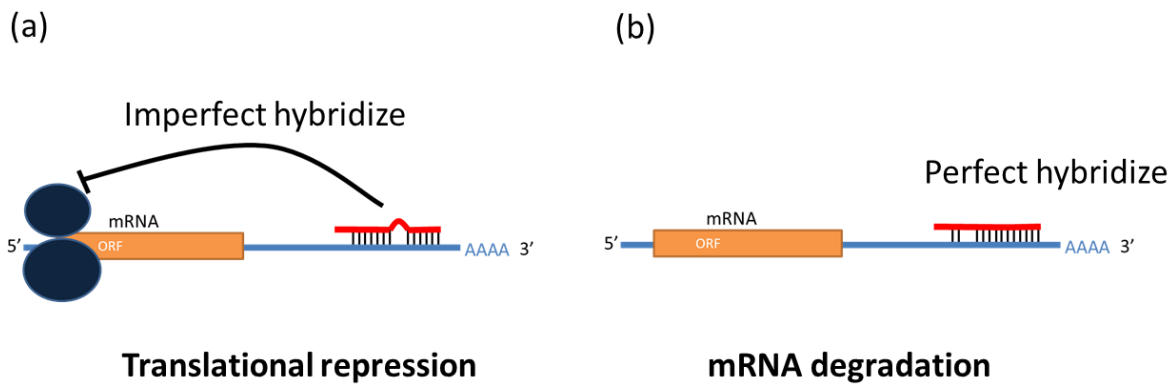


Figure 3. miRNA functions.

1.1.4 miRNA-target interactions (MTIs)

Figure 4 shows the definition of miRNA-target interaction (MTI). Each red line denotes the repressed relationship between miRNA and its target gene. As shown in this figure, miR-A repress two target genes; miR-B repress four target genes. In other words, there are six miRNA-target interactions shown in this figure.

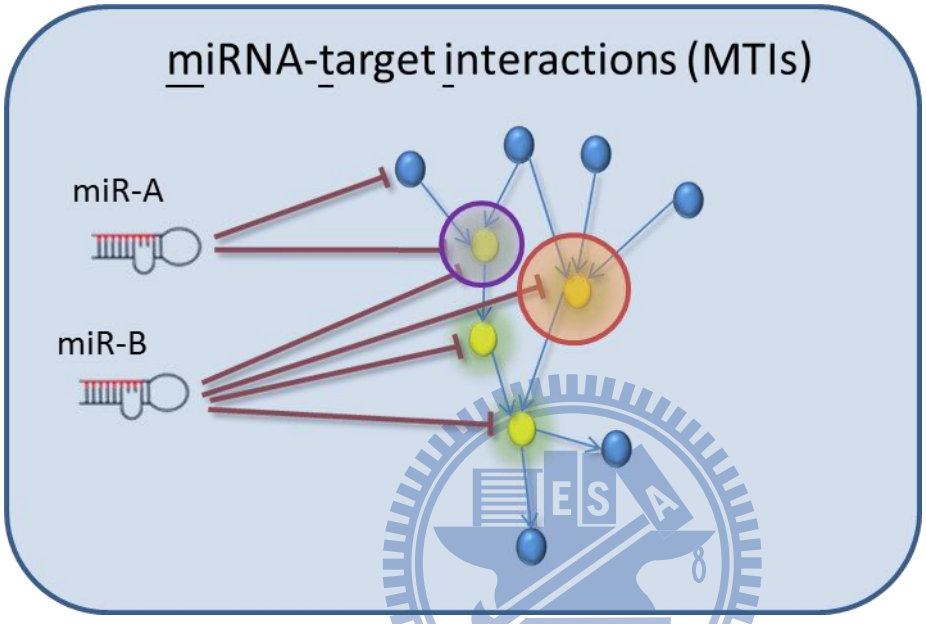


Figure 4. miRNA-target interactions (MTIs).

1.2 Principles of miRNA target prediction

1.2.1 Seed sequence complementary

There are a lot of tools or resources developed to identify the miRNA target genes. Almost all of them majorly consider this rule – perfect seed complementary to its target site. The seed region is located at nucleotides 2-7 or 2-8 of the 5' end of miRNA. The site of mRNA hybridizes to seed region called "seed match". **Figure 5** clearly shows the cartoon picture of "seed region" and "seed match". Previous works report that the perfect hybridization (Watson-Crick paring) between seed region and target site seems to be the most important rule for miRNA target prediction (65-68). Considering strictly perfect hybridization of miRNA::Target duplex dodges the highly false-positive rate in miRNA target prediction.

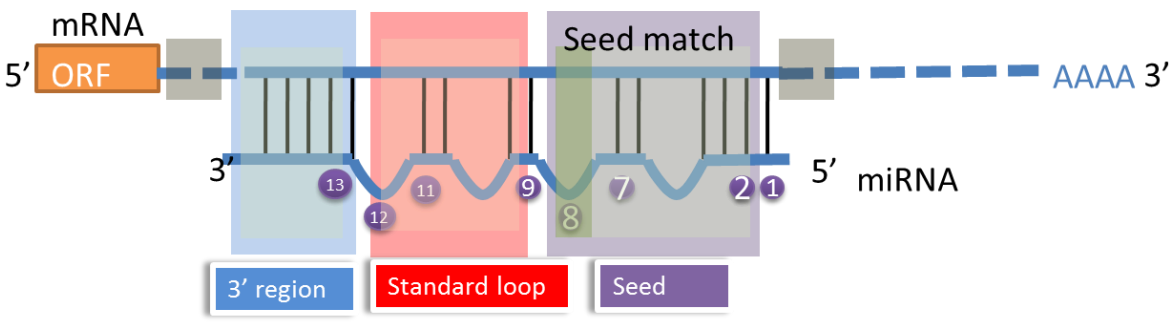


Figure 5. Seed region of miRNA:mRNA duplex.

Lewis et al discovered that conserved seed matches in vertebrates often have A anchors which maybe pair to the first position of miRNA (66). Grimson et al clearly defined the seed types of miRNA target sites – 6mer, 7mer-A1, 7mer-m8 and 8mer (67). **Figure 6** shows the seed types of miRNA target sites. 6mer site is the 6 continues perfect Watson-Crick pairs hybridize to miRNA seed region (nucleotides 2-7 of miRNA). If nucleotides 2-8 of miRNA perfectly hybridize to its target site, we call this site as 7mer-m8 (seed match + match at position 8). 7mer-A1 site means that seed match flanked A anchor which may pair to first nucleotide of miRNA or not. The 8mer site comprises the seed match flanked by both the match at position 8 and the A at position 1. It is quite reasonable that considering 8mer site could increase specificity, whereas searching for 6nt seed pairing (6mer) yields greater sensitivity.

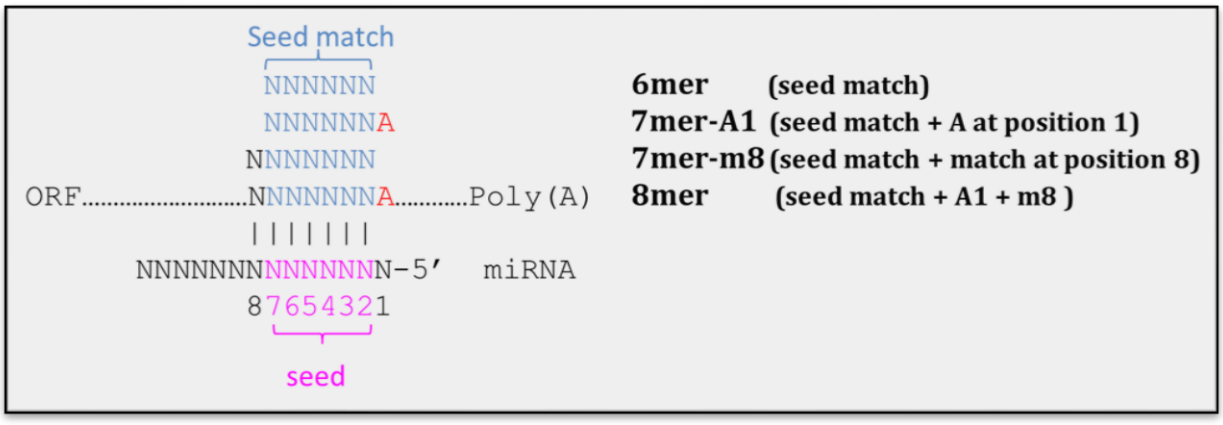


Figure 6. Type of miRNA target sites (seed type). (Defined by Grimson, A. 2007 and Bartel, D. P., 2009)

1.2.2 Conservation of miRNA targets

Conserved miRNA target is the other important rule for identifying miRNA target genes. miRNA target sites that are conserved across species are likely to be biologically significant miRNA target sites. However, the different reports sometimes took into account slightly different conservation of miRNA targets. We could conclude that three types of conserved miRNA targets as shown in **Figure 7**. **Figure S3** shows the highly conserved miR-196a target site in Hoxb8.

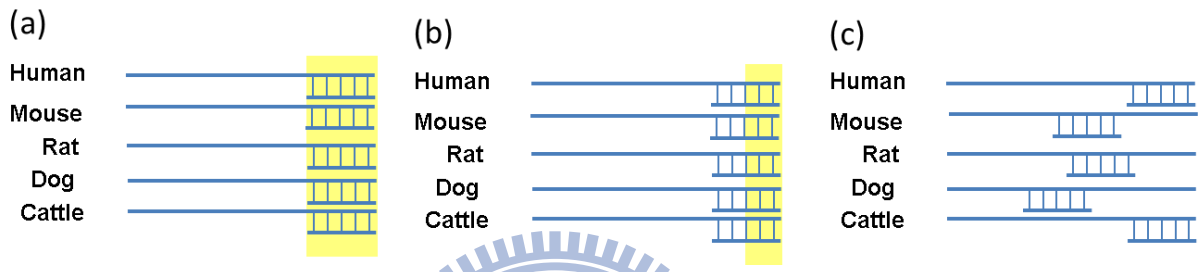


Figure 7. Three types of conserved miRNA targets.

1.2.3 Thermodynamics of miRNA:mRNA duplex

The way to measure the thermodynamics of miRNA-mRNA duplex is to calculate the free energy of miRNA:mRNA targets site. The Free Energy of the microRNA:mRNA duplex (ΔG), is often calculated with the Vienna RNA package (69) or RNAhybrid (70).

1.2.4 Site accessibility

The conventional target prediction tools consider the complementarity between the miRNA and its target sequence, the conservation of the target sites, and the kinetics and thermodynamics of miRNA::target duplex. Although these properties are important factors to determine the miRNA target sites, the sequence context surrounding miRNA target sites was reported to influence the binding affinities and the regulation of the miRNA. Harlan et al (71) hypothesized that single-strand miRNAs can only bind to stretches of free mRNA for potential target sites. Dang et al (72) postulated the target structure accessible model for miRNA target prediction and succeeded in interpreting the published data on the in vivo activity of *C. elegans* reporter genes containing

modified lin-41 3'-UTR sequences. miRNAs hybridize to the target sites, which is within more accessible regions, are with more possibility to be real, as shown in **Figure 8.**

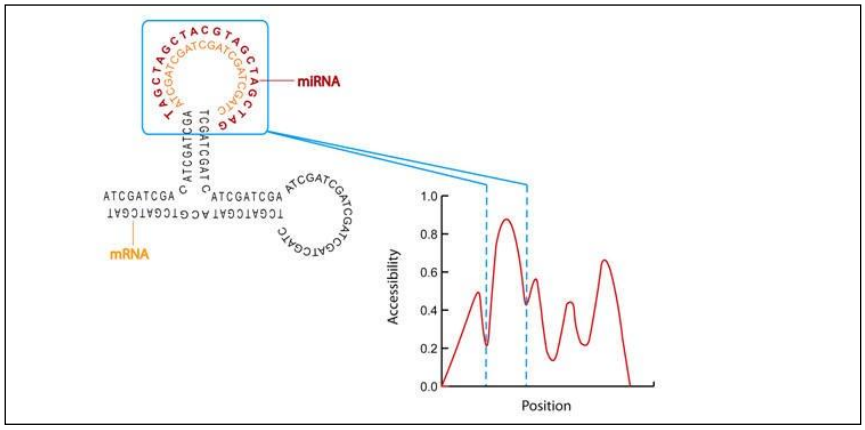


Figure 8. The accessibility of target site.

1.3 Motivation

Although there were more than one thousands human miRNA genes have been discovered, the functions of most of them are unknown. **Figure 1** shows that the exponential growth of miRNA related publications in Pubmed, it is quite urgent to extract the useful information from those articles. An up-to-date curated collection of miRNA-target interactions (MTIs) with experimental support is crucial to provide effective information for investigating miRNA functions at different conditions and in different species.

1.4 Research goals

The goal of this work is to systematically analyze the miRNA-target interactions and assess the function of miRNA by developing new methods and resources. In this dissertation, we focus on the manually curated miRNA-target interactions with experimental support, a system for miRNA-target interations prediction, identification of homologous miRNA-target interactions and incorporated miRNA-target interactions database. **Figure 9** summarizes the major aims of this dissertation and is described in the following sections.

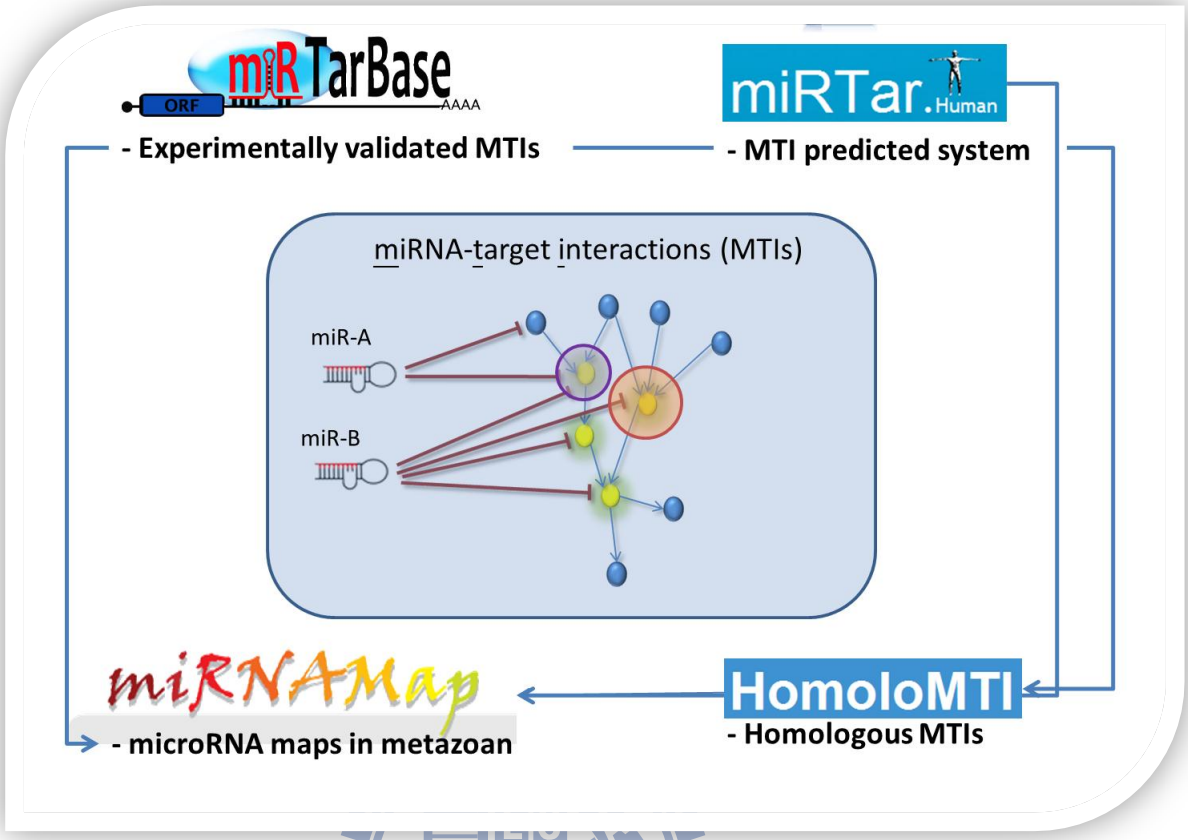


Figure 9. This thesis is consist of four parts: miRTarBase, miRTar, miRNAMap and HomoloMTI.

1.4.1 Construction of experimentally verified MTIs

First to all, we aim to develop a frequently updated database by continuously surveying research articles with the pre-screening by text-mining programs and intend to make the database become a major repository for experimentally confirmed miRNA-target interactions. Through analyzing more than seven hundreds verified MTIs with the target sequences, we could get the overview of relative contribution of sequence and structure features in miRNA targeting. The MTIs collection in the proposed database can also become a bigger amount of positive samples for the developments of computational methods to identify miRNA-target interactions.

1.4.2 Development of predicted MTIs system

Secondly, we introduces an integrated system that provides multiple analyzing functions for miRNA target identification and for the study of miRNA-target interactions, including

the regulatory relationship between one miRNA and one gene, one miRNA and multiple genes, multiple miRNAs and one gene, and multiple miRNAs and multiple genes. Besides, miRTar identifies miRNA target sites against 3'UTR, as well as the coding regions and 5'UTR. This resource provides the information concerning that miRNA-target interactions are regulated by alternative splicing. Additionally, miRTar performs a gene set enrichment analysis for miRNA-regulated gene set to decipher possible roles in biological process and pathways.

1.4.3 Identification of homologous MTIs

MicroRNA plays important roles in post-translational gene regulation among various species. Nowadays many miRNA target interaction are revealed by experiments and miRNA target site prediction tools. In order to provide more evidences for predicted miRNA target interactions and discover real miRNA target interactions, a database, HomoloMTIs, was contructed based on three databases, miRTarBase, miRBase and HomoloGene, to reveals the miRNA target interactions might be shared in homologous genes. The homologous MTIs profiles could reveal the homologous genes regulated by miRNA with at least one experimental validation in one of the homologous genes and predicted miRNA target interactions. The novel miRNA target interactions and miRNAs could be pioneer studied by HomoloMTIs. HomoloMTI aims to provide a comprehensive comparative perspective on the metazoan repertoire of miRNA-target interactions as complementary to miRTarBase, the database of experimentally verified miRNA-target interactions.

1.4.4 Integrated information of MTIs database

The main contribution of this work is the extended development to miRNAMap version 3.0. We make the focus on the investigation of miRNA-target interaction. To make miRNAMap more comprehensive, we integrate the experimentally verified MTIs, the MTIs predicted by 11 predicted miRNA target databases and more expression profiles of miRNA and protein-coding gene. A useful feature specially designed to human genome is the comparison between miRNA expression profiles and expression profiles of target genes. We also analyzed 1,524 experimentally verified miRNA-target interactions with

strong evidence support in human and elucidate which the more accurate microRNA target prediction database is. Those analyses are important for identifying putative miRNA targets and are very useful for biologist to choose the proper tool for miRNA research.

Chapter 2 miRTarBase: a database curates experimentally validated microRNA-target interactions

2.1 Introduction

As small non-coding RNAs of approximately 22 nts, microRNAs (miRNAs) regulate gene expression post-transcriptionally through suppressing mRNA translation or inducing mRNA degradation by hybridizing to the 3'-untranslated regions (3'-UTR) of the mRNAs. Discovery of the first miRNA in Caenorhabditis elegans in 1993 (1) ushered in numerous studies on the cellular processes of these tiny regulatory RNAs for a large variety of metazoa. Thousands of miRNAs have been identified in mammalian cells over the past two decades. miRNAs play critical roles in many biological processes, including cell cycle control, cell growth and differentiation, apoptosis, and embryo development.

Literature on miRNA research has recently grown exponentially (**Figure 1**). The accelerate rate of miRNA gene discovery has led to the need to elucidate the functions of these miRNAs. Additionally, more than 20 databases and computational methods have been developed for identifying candidates of miRNA-target interactions. A curated collection of up-to-date miRNA-target interactions (MTIs) with experimental support is crucial to provide effective information for investigating miRNA functions at different conditions and in different species. In this work, we propose a database, miRTarBase, which has accumulated more than three thousand miRNA-target interactions collected by manually surveying literature after a systematic text-mining process to select research articles related to functional studies of miRNAs. Generally, the collected MTIs were experimentally validated by reporter assay, western blot, or microarray experiments with overexpression or knockdown of miRNAs.

2.1.1 Experimental approaches for identifying the miRNA-target interactions

Generally, obtaining an experimentally validated miRNA-target interaction initially

involves using computational methods to identify target sites of miRNAs. These putative miRNA-target interactions are then validated by molecular experiments, including reporter assay and western blot. Reporter assay and western blot are the conventional means of confirming the interaction between miRNA and its target mRNA. Besides, Northern blot analysis, quantitative real-time PCR (qPCR), or in situ hybridization is often performed to examine the co-expression of predicted miRNA and mRNA target gene. In contrast with traditional validation, genome wide screenings approaches, including microarray experiments with overexpression or knockdown of miRNAs, stable isotope labeling with amino acids in culture (SILAC) or pulsed SILAC (pSILAC; **Figure S5**), have been developed. For instance, Selbach et al. determined the complement of all genes targeted by five miRNAs induced independently in HeLa cells using microarrays and pSILAC (73), and more than 400 miRNA-target interactions were identified.

Reporter gene assay

Reporter gene assay is the most common experiment for verifying miRNA-target interactions. It provides the direct evidence to show the relationship between miRNA and its target gene by measuring the expression level of reporter gene. The green fluoresces protein (GFP) and luciferase are two common reporter gene using in this method.

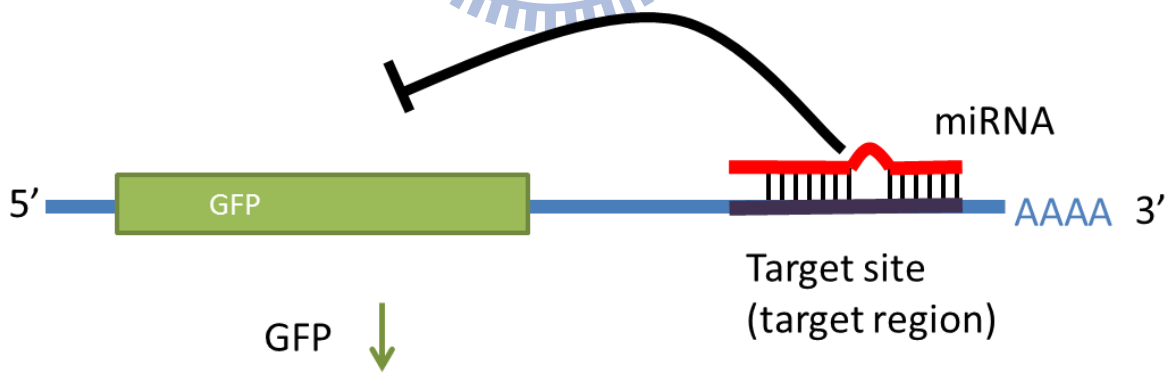


Figure 10. The concept of miRNA-target interaction validated by GFP reporter assay.

Quantitative real-time PCR (qPCR)

Quantitative real-time PCR can also be employed to quantify mRNA expression profiles when treating with over-expression miRNA or knock-down miRNA and study the potential miRNA-target interactions (MTIs).

Stable isotope labeling with amino acids in culture (SILAC) or pulsed SILAC (pSILAC)

SILAC (stable isotope labeling with amino acids in cell culture, **Figure S4** and **Figure S5**) is a technique which is a popular method for quantitative proteomics, based on mass spectrometry that detects differences in protein abundance among samples using non-radioactive isotopic labeling.

2.2 Related works

Many miRNA-related database systems have been developed in recent years to provide information on miRNAs and their target genes. miRBase (74) is the most complete repository for miRNA annotation and nomenclature. Until now, the miRBase (version 17.0) contains 16,772 miRNA entries and many more new sequences are added regularly. miRGen (75), miRGator (76), miRDB (77), microRNA.org (78) and miRNAMap (3,4) provide miRNA targets based on combinations of extensively adopted target prediction programs. Furthermore, TarBase (79), miRecords (80), and miR2Disease (81) contain experimentally validated miRNA-target interactions. TarBase is the first resource that provides experimentally verified miRNA-target interactions by surveying literature (79). miRecords collects both experimentally validated miRNA targets and computationally predicted miRNA targets (80). miR2Disease contains relationships among miRNAs, target genes and diseases in human (81). miRSel (82) utilizes a text-mining method to systematically extract miRNA-target relationships from the PubMed abstracts. Additionally, several computational methods and web-based programs were developed for computationally identifying target genes of miRNAs, e.g., miRanda (78), TargetScan (66), RNAhybrid (83), Pictar (84) and PITA (85). These tools are widely used by researcher, and these candidates of miRNA-target interactions are then confirmed experimentally.

There are 5 databases have been developed for storing experimentally verified miRNA-target interactoins (MTIs) showed in **Table 1**, including TarBase, miRecords, miR2Disease, miRSel and miRWalk.

Table 1. The related databases stored experimentally verified MTIs.

Name	Organism	Remark	Ref
TarBase	Plants, Flies, Nematodes, Virus, Vertebrates,	1. First database stored experimental verified miRNA targets.	(79)
miRecords	Nematodes, Vertebrates, Flies	1. Incorporated 11 known miRNA target prediction tools. 2. Experimentally verified miRNA targets.	(80)
miR2Disease	Human	1. miRNA deregulation in various human diseases.	(81)
miRSel	Human, mouse and rat	1. Text-mining method to extract the experimental MTIs.	(82)
miRWalk	Human, mouse and rat	1. Incorporated 8 known miRNA target prediction tools. 2. Experimental miRNA targets	(86)

TarBase

TarBase is a first database that provides information of experimentally support miRNA targets in several animal species, plants and viruses. In version 5.0 (release in October 2008), there are about 1,300 experimentally verified microRNA-target interactions stored in their database. The information of MTIs was curated from about 160 articles. Additionally, the result page of TarBase is functionally linked to other databases such as Ensembl, Hugo, UCSC Genome Browser and SwissProt (87). The TarBase 5.0 database can be queried or downloaded from http://microrna.gr/tarbase.

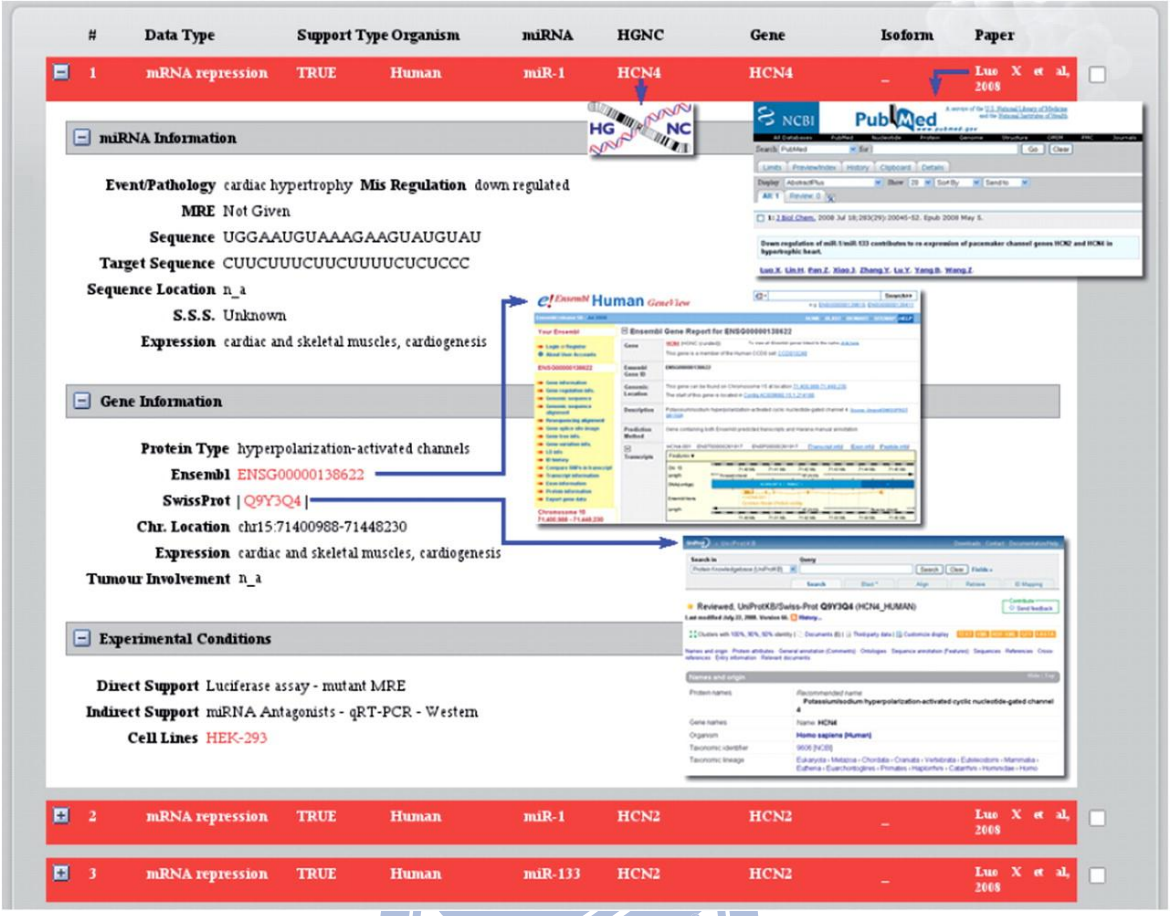


Figure 11. The result page of TarBase.

miRecords

miRecords, a resource for animal miRNA-target interactions, consists of two components including Validated Targets and Predicted Targets. The *Validated Targets* component houses about 1,600 experimentally validated miRNA targets curated from meticulous literature. The *Predicted Targets* component of miRecords integrated the pre-compiled data identified by 11 established miRNA target prediction tools. The miRecords is available at http://miRecords.umn.edu/miRecords.

Figure 12. The web interface of miRecords.

miR2Disease

miR2Disease, the other manually curated miRNA information database, provides the relationship between deregulated micorRNAs and various human diseases. It contains the brief description of microRNA::disease, the expression pattern of the miRNA, the validated method for miRNA expression, experimentally verified target genes, and the literature reference. The miR2Disease database is available at: http://www.miR2Disease.org/.

miRSel

miRsel, text-mining method for automatic extracting miRNA-target interactions from PubMed abstracts, is different from the manually curated miRNA-target interactions datbases mentioned above. It is the only one database that stores the miRNA-target interactions by extracting them from miRSel is freely available online at http://services.bio.ifi.lmu.de/mirsel.

2.3 Specific aims

During last two years, a continuously growing number of identified miRNAs and their targets, combined with their major roles in biological systems, explains why it is crucial to have an accurate, up-to-date, easily accessible and centralized information repository. In this work, we aim to develop a frequently updated database by continuously surveying research articles with the pre-screening by text-mining programs and intend to make the database become a major repository for experimentally confirmed miRNA-target interactions. The miRTarBase contains the largest amount of validated MTIs and provides the most up-to-date collection by comparing to other similar databases previously developed, such as TarBase, miRecords, and miR2Disease. Moreover, we investigated the biological features of miRNA/target duplex based on more than seven hundreds validated miRNA-target interactions in human, where the miRNA target sites of MTIs were reported in the source articles. The MTIs collection in the proposed database can also become a bigger amount of positive samples for the developments of computational methods to identify miRNA-target interactions.

2.4 Materials and methods

2.4.1 Database content

All entries in the database are collected manually that describe how a miRNA and its target genes are related with experimental support (**Figure 13**). Initially, all fields in the

PubMed database are searched based on the keywords 'microRNA targets' or 'miRNA targets', followed by downloading all full-text of these articles. Next, a text-mining system is developed to allow for screening of full-text literature that potentially describes miRNA-target interactions, as verified by various experimental methods. Each research article was carefully reviewed by at least two of our developers to extract the miRNA-target interactions, which experimentally confirmed by reporter assay, western blot, microarray experiments, pSILAC or qRT-PCR, as well as to extract other effective information, including the species of miRNAs, the species of target genes, and experimental conditions.

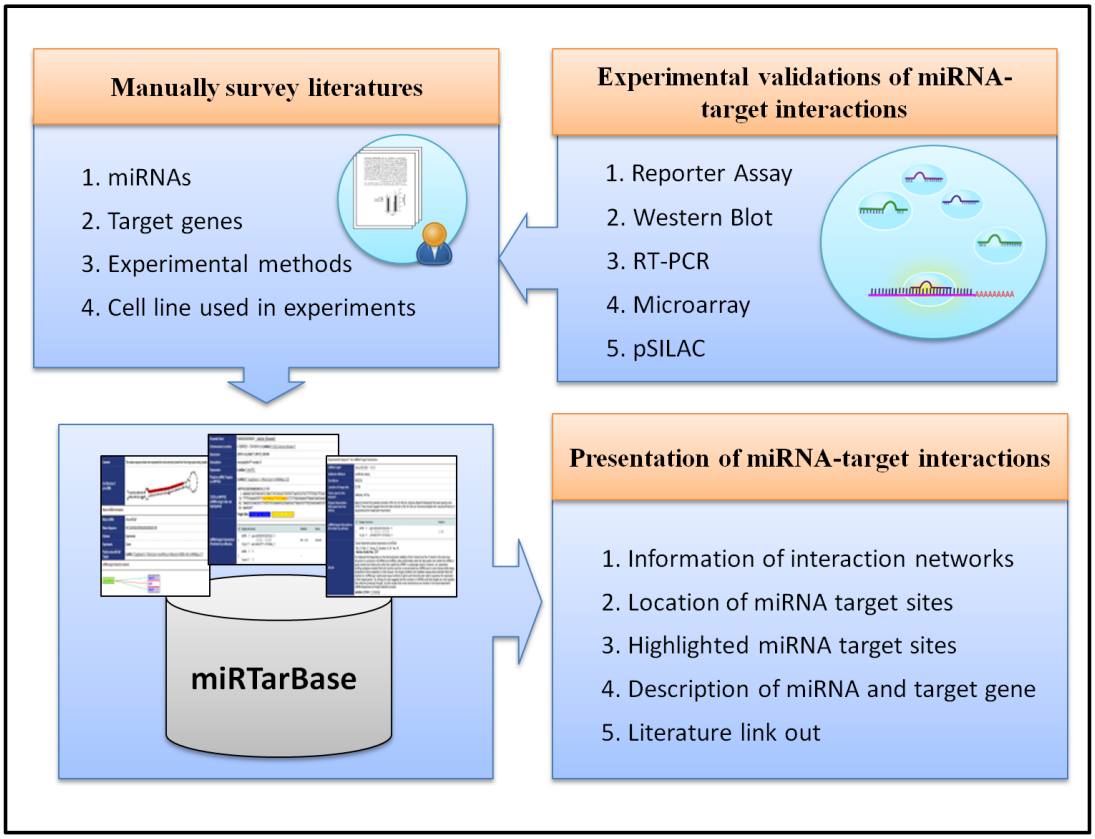


Figure 13. System flow of miRTarBase.

2.4.2 Strong experimental evidence of miRNA-target interactions

The most direct means of verifying miRNA-target interactions involves using fluorescence quantitative PCR and western blot methods to detect the mRNA expression

levels and protein expression levels at conditions of miRNA overexpression or miRNA knock-down cells. Although these methods are capable of accurately identifying miRNA target genes, other experimental methods are required to determine the location of regions targeted by miRNAs. Luciferase reporter assay is adopted conventionally. Here, we view the miRNA-target interactions with strongly support when they are validated by western blot, qPCR, or reporter assay.

2.4.3 Less strong experimental evidence of miRNA-target interactions

The high-throughput miRNA target identification methods, including pSILAC and microarray experiments, can be used to determine the genome wide changes in the mRNA expression levels or protein expression levels when the miRNA is present or not (79). Given our inability to understand whether the over-expressed miRNAs cause the changed expression patterns directly or not, these technologies only provide less strong experimental evidence for the collected miRNA-target interactions.

2.5 Results

2.5.1 Statistics

In the release 2.4 (Apr. 15, 2011) of miRTarBase, 3,969 curated miRNA-target interactions between 625 miRNAs and 2,433 target genes were collected from 1,211 articles. **Table 2** lists the number of collected miRNA-target interactions in each species. For instance, 2,819 human MTIs were collected between 269 miRNAs and 1,716 target genes with the experimental support from 906 articles, and 926 and 1,418 interactions were experimentally confirmed by western blot and reporter assay, respectively. Each human miRNA can target to five target genes in average, and **Figure 14** gives the distribution of miRNAs categorized by the number of target genes for each miRNA which are supported by reporter assay or western blot. In miRTarBase, hsa-miR-122 was recorded to have 45 target genes, which were experimentally validated by luciferase reporter assay or western blot. hsa-miR-122 is a liver-specific miRNA in human and is significantly down-regulated in liver cancers (88).

Table 2. Statistics of miRNA-target interactions collected in miRTarBase.

Species	No. of miRNA-target interactions	No. of miRNAs	No. of target genes	No. of articles collected[a]	No. of miRNA-target interactions experimentally validated by			
					Strong evidences		Less strong evidences	
					Western blot	Reporter assay	pSILAC	Microarray
Human	2,819	269	1,716	906	926	1,418	494	946
Mouse	562	138	384	210	279	414	0	192
Rat	248	99	101	52	90	58	0	170
Chicken	16	7	16	8	3	15	0	1
Cattle	4	2	4	1	0	0	0	0
Zebrafish	103	26	75	31	32	87	0	2
Fruit fly	116	38	70	32	8	115	0	11
Silkworm	2	2	1	1	0	2	0	0
African clawed frog	1	1	1	3	0	1	0	0
Nematode	31	7	26	19	1	31	0	0
Plants	61	24	35	11	10	2	0	12
Viruses	6	12	4	7	1	6	0	0
Total	3,969	625	2,433	1,281	1,350	2,149	494	1,334

[a] Articles may report various miRNA-target interactions in different species.

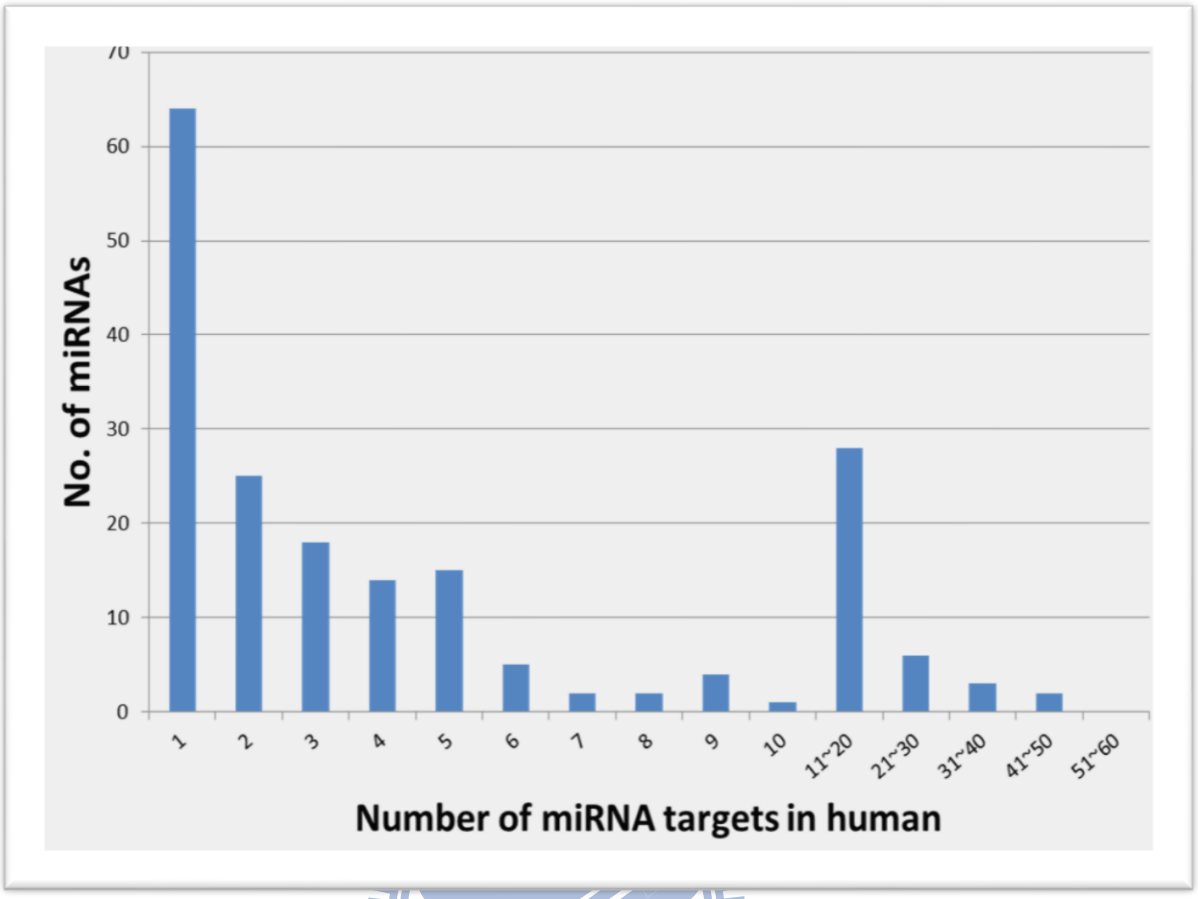


Figure 14. The distribution in number of human miRNA target genes

2.5.2 Gene enrichment and pathway analysis

Furthermore, we examined the functions of these target genes involved in human miRNA-target interactions collected in the database by performing Gene Ontology (GO) and KEGG (89) pathway enrichment annotation using the DAVID gene annotation tool (90). GO enrichment analysis indicates that the cellular process, biological regulation and metabolic process are the most significantly enriched GO terms for this selection of human target genes (**Figure 15**).

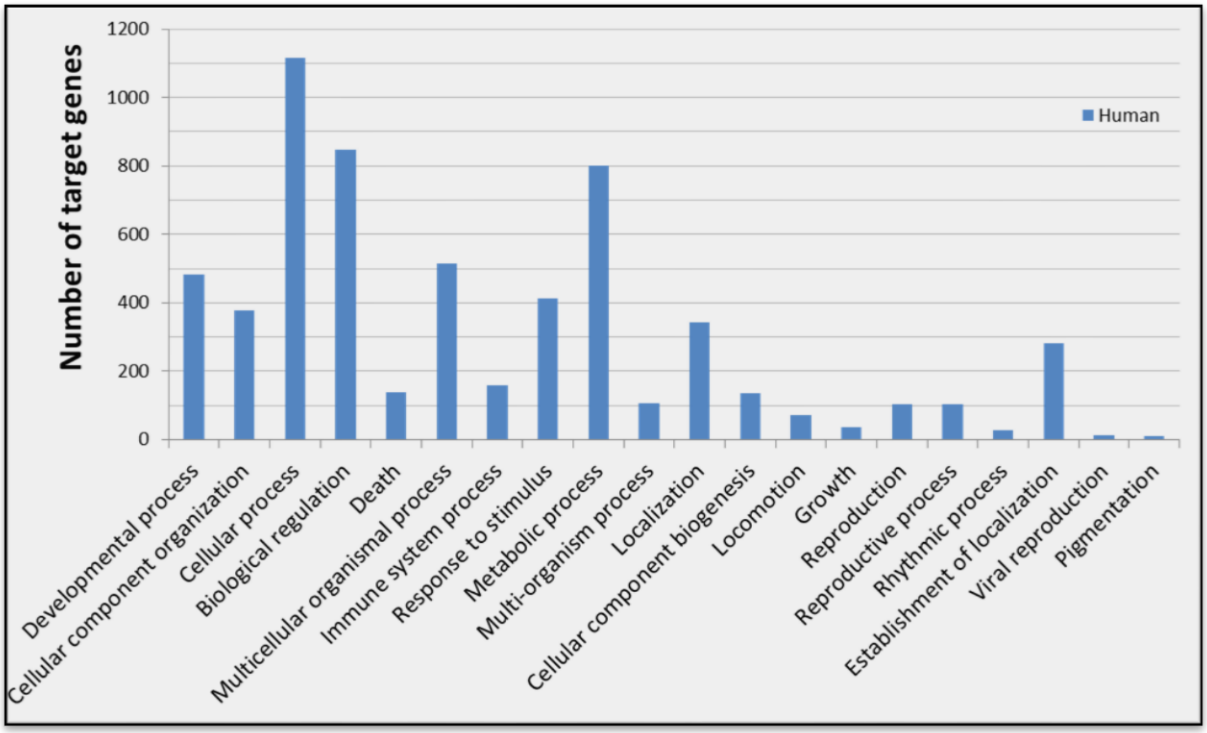


Figure 15. GO analysis of human miRNA-target interactions, as verified by reporter assay or western blot. (DAVID, grouped according to biological process of level 1)

Table 3 lists the top 20 pathways significantly enriched in these human target genes, and most of which are involved in cancer, including pancreatic cancer, colorectal cancer, prostate cancer, small cell lung cancer, bladder cancer, non-small cell lung cancer and endometrial cancer. Interestingly, above analysis provides an overview of the possible functions of human miRNAs based on this curation of miRNA-target interactions although the data should be biased due to miRNAs have attracted more attentions in cancer research recently.

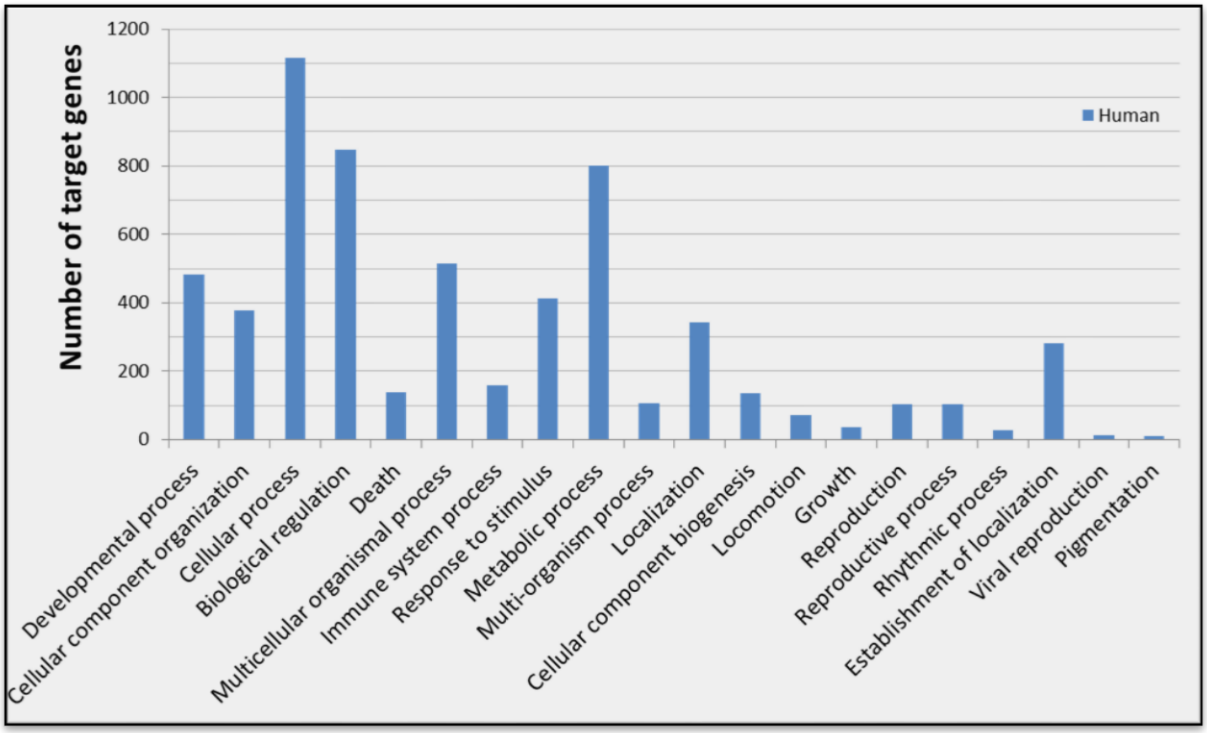


Figure 15. GO analysis of human miRNA-target interactions, as verified by reporter assay or western blot. (DAVID, grouped according to biological process of level 1)

Table 3. KEGG Pathway annotation of human miRNA-target interactions

KEGG Pathway	No. of target genes	Ratio	P-Value
Pathways in cancer	120	0.70	2.91E-32
Pancreatic cancer	42	0.25	4.30E-20
Chronic myeloid leukemia	42	0.25	3.37E-19
Colorectal cancer	40	0.23	4.11E-15
Prostate cancer	41	0.24	7.27E-15
Small cell lung cancer	38	0.22	1.69E-13
Bladder cancer	26	0.15	2.73E-13
Melanoma	32	0.19	2.20E-11
Cell cycle	44	0.26	4.30E-11
Neurotrophin signaling pathway	43	0.25	1.29E-10
Focal adhesion	58	0.34	1.91E-10
MAPK signaling pathway	70	0.41	2.07E-10
Non-small cell lung cancer	26	0.15	4.31E-10
Renal cell carcinoma	30	0.18	4.60E-10
Glioma	28	0.16	7.43E-10
Endometrial cancer	25	0.15	1.05E-09
p53 signaling pathway	29	0.17	1.10E-09
Acute myeloid leukemia	25	0.15	1.55E-08
Adherens junction	28	0.16	1.25E-07
Epithelial cell signaling in Helicobacter pylori infection	26	0.15	1.28E-07

Only 709 human miRNA-target interactions in miRTarBase have miRNA target site annotations, which can be extracted from the articles. Of these target site sequences, 9 of them only provide the sequence of seed region (< 10 nucleotides); 667 of them contain the target site sequences (10 ~ 50 nucleotides), while the others (29) provide cloned partial UTR sequences (> 50 nucleotides). Next, an attempt is made to summarize the data distributions of twelve biological features of the miRNA/target duplex in these 709 known human miRNA-target interactions, as shown in **Figure 16**. The miRNA target sites were mapped to the 3'UTR of the corresponding target gene; in addition, 70 nucleotides around the target site were extracted. Additionally, the miRNA target sites were selected when the alignment score of miRNA/target duplex is greater than 100 and the number of base-pairs within the seed region is more than 5. Notably, 1,610 miRNA target sites are obtained from the 709 miRNA-target interactions. **Figure 16** gives the histograms of various features of these miRNA-target duplex. **Figure 16** (A) and (B) show the longest consecutive matches (excluding or including wobble pairing (GU pairing) in a seed region), which is a subsequence from nucleotide 1 to 8 in the 5' end of the miRNA, respectively. More than 55% of all binding sites have more than 7 bases of consecutive pairings. The minimum free energy of the seed regions and the binding sites is also calculated, as shown in **Figure 16** (C) and (D), respectively. The mean value of the free energy of the binding site is approximately -14 kcal/mol. The free energy of most of the seeds is smaller than -6 kcal/mol. Next, analysis is performed of the number of nucleotides matches, GU matches, and mismatches in the seed regions and the target sites. **Figure 16** (E) and (J) summarize these statistics. More than 80% of all target sites have at least 6 matches in the seed region; in addition, the GU matches rarely occur in the seed region, which is smaller than 40%. The number of matches is significantly larger than the number of mismatches. GU matches in the target sites are substantially smaller than the quantity of matches and mismatches. The target site accessibility is also estimated based on the calculation of Kertesz M. et al (85). According to our results, most of the interaction energy shown in **Figure 16** (K) ranges between -10 kcal/mol and 10 kcal/mol.

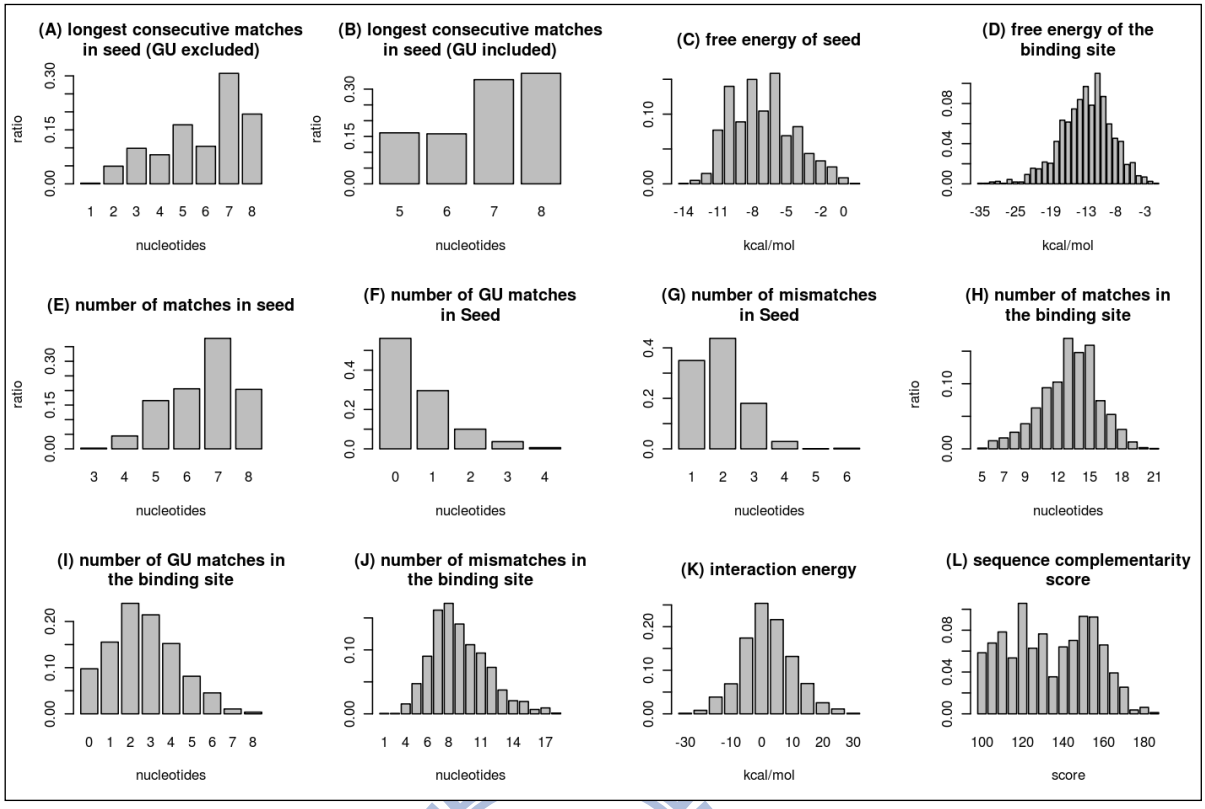


Figure 16. Histogram of various features of experimental proven miRNA-target sites.

2.5.3 Comparisons to other miRNA-target interaction databases

Comparing the other manually curated databases such as TarBase, miRecords, and miR2Disease, miRTarBase accumulates a bigger collection and a more up-to-date curation of miRNA-target interactions than other resources (**Table 4**), especially around nine hundreds research articles were collected. It also reveals that our miRTarBase has the most abundant miRNA-target interactions, even if only considering the entries supported by reporter assay or western blot experiments. Furthermore, the Venn diagrams show the intersection of articles collected in different databases (**Figure 17**). miRTarBase almost covers all the research articles collected in TarBase, miRecords and miR2Disease.

Table 4. Comparison of miRTarBase with other miRNA-target interaction databases. The final column is the number of miRNA-target interactions (MTIs) more than the largest number of records in TarBase, miRecords and miR2Disease. The miRNA-target interactions in TarBase, miRecords and miR2Disease are downloaded from their web sites.

Database names	**TarBase** (79)	**miRecords** (80)	**miR2Disease** (81)	**miRTarBase**	**Number of records added**
Last update date	2008/06	2010/05/05	2010/06/02	2011/04/15	
Support species	Metazoa x 6 Viridiplantae Viruses	Metazoa x 11 Viruses x 2	Human	Metazoa x 9 Viridiplantae x 3 Viruses x 5	
Total No. of miRNAs	223	381*	179	625	+ 244
Total No. of target genes	1028	1057*	394	2433	+1376
Total No. of articles	154	410	421*	1211	+ 790
Total No. of miRNA-target interactions (MTIs)	1264	1513*	635	3969	+2456
Supported by strong experimental evidences					
No. of MTIs validated by "Reporter assay"	305	672*	635	2149	+ 1477
No. of MTIs validated by "Western blot"	27	295*	0	1350	+ 1055
No. of MTIs validated by “Reporter assay and Western blot”	25	123*	0	1092	+ 969
No. of MTIs validated by "Reporter assay or Western blot"	307	747*	635	2407	+ 1660
Supported by less strong experimental evidences					
No. of MTIs validated by "pSILAC experiments"	455*	0	0	494	+ 39
No. of MTIs validated by "Microarray experiments"	343	380*	0	1334	+ 954

* Indicates that the largest number of records in TarBase, miRecords and miR2Disease.

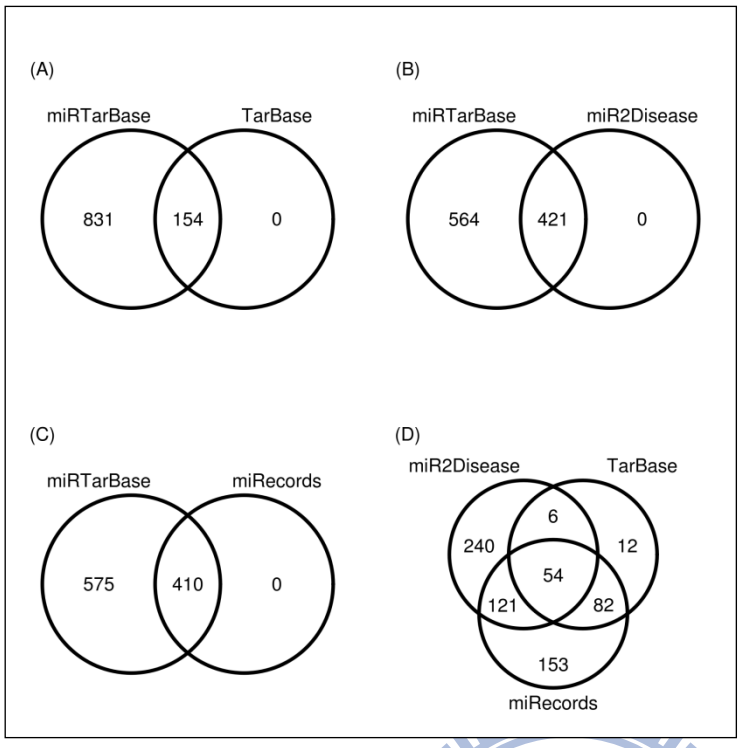


Figure 17. The overlap among articles collected in each two manually curated miRNA-target interactions databases.

Text-mining method is an alternative approach which can retrieve the information of associations between miRNAs and target genes; however, miRNA-target interaction is generally described in natural language and is not easy to be extracted correctly by only computational methods. For example, DICER1 and Drosha are very important genes which are involved in the biogenesis of miRNA, but they are usually not the target genes of a miRNA when they are discussed along with the miRNA in an article. However, text-mining methods may identify the association between a miRNA and DICER1, and incorrectly annotate the association as a miRNA-target interaction. Therefore, manually reviewing the articles potentially containing miRNA-target interactions is inevitable for extracting those experimental evidences to support a miRNA-target interaction. Here, we do not compare the contents in miRTarBase with other databases established by only text-mining methods without manual review.

2.5.4 Web interface

miRTarBase provides various query interfaces and graphical visualization pages to

facilitate the access of miRNA-target interaction data (**Figure 18**). Several search functions for retrieving miRNA-target interactions are designed, including search by miRNA accessions, search by target genes, and search by literature. Alternatively, miRTarBase provide keyword search in all fields of all data entries. We designed a result page to present a miRNA-target interaction, where each MTI was assigned a miRTarBase accession. The result page majorly comprises three main parts: miRNA information, target gene information and evidence support. Generally, web pages of the miRTarBase contain many effective quick links to several other web resources, including NCBI Entrez (91), UCSC Genome Browser (92), miRBase (74), BioGPS (93), iHOP (94) and HGNC (95). Detailed descriptions of web pages are provided below.

The 'miRNA information' page contains the characteristics of a miRNA such as accession, synonyms, descriptions, the sequence of miRNA, and links to other putative miRNA-target interaction databases. Especially, in this page all the miRNA-target interactions of the miRNA are presented as a network, which can depict the relationships between a miRNA and multiple target genes. In the 'Target Gene' page, the basic information of a target gene is provided, including gene symbol, description, genomic location, transcript sequence, and links to other resources. The information of target sites located in the transcript are carefully examined and provided on the web page. Notably, many articles only report the regulatory relationship between a miRNA and its target genes without providing the exact regions of miRNA target sites. Here, we utilized miRanda to computationally identify the potential target sites belongs to a miRNA-target interaction, which is supported by experimental evidences.

In the "Evidences" page, the experimental information to support a miRNA-target interaction from one or multiple articles is provided by presenting the experimental validation methods, the experimental conditions, the location of target sites, computational tools used in article, partial key descriptions extracted from the article, and article abstract. Additionally, this resource also provides data submission page that allows users or researchers to submit information of miRNA-target interactions, which are not curated yet. The database provides a convenient way for users to directly suggest articles containing information about miRNA-target interaction, and then the suggested articles will be reviewed by the developer of miRTarBase.

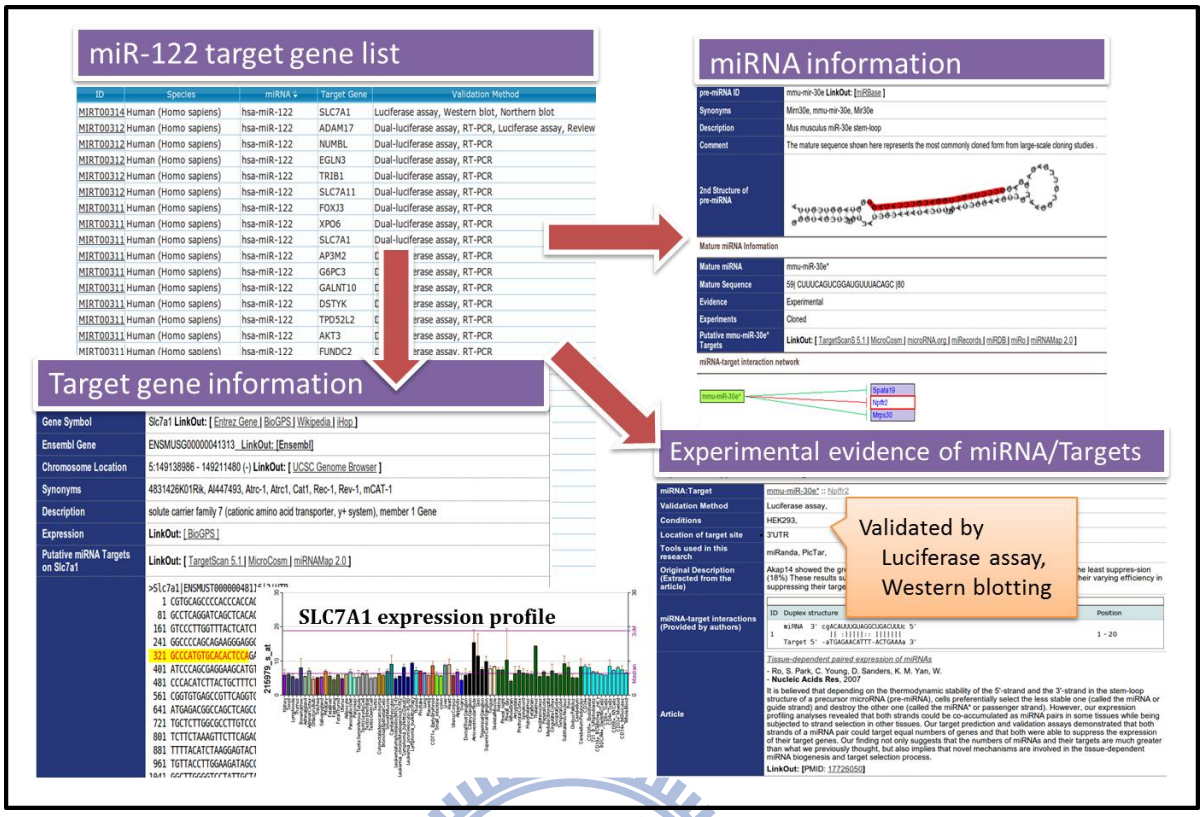


Figure 18. The miRTarBase web interface

2.6 Conclusion

This work presents a more comprehensive collection of miRNA-target interactions, which were experimentally validated. The biological features of miRNA/target duplex were observed based on largest collection of human miRNA-target interactions currently available. Various web interfaces are designed to facilitate the presentation of miRNA-target interactions. A pipeline combining text-mining and manual review was established to extract MTI information from research articles.

Application involving the proposed database is to extend the human miRNA-target interaction to mouse, rat and other mammalian genome based on evolutionary conservation of miRNA and its target sites. More probable miRNA-target interactions can be provided as the candidates for experimental confirmation. We will describe the more detail in **Chapter 4**.

According to the more high-throughput experimental technology such as high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) was introduced to validate miRNA-target interactions, we should also curate

this kind of validated data into miRTarBase.

Chapter 3 miRTar - an integrated system for identifying miRNA-target interactions in Human

3.1 Introduction

Before starting this section, we should note that this part was also done by our co-group member, *Justin, Bokai, Hsu*.

MicroRNAs (miRNAs) are small non-coding RNA molecules that are ~22 nts sequences capable of suppressing protein synthesis. Deriving from ~70–120 nts precursor transcripts that fold into stem-loop structures and thought to be highly conserved in genome evolution, miRNAs regulate 30% or more of the human protein-coding genes (65,66). Moreover, previous investigation suggested that miRNA target sites in mammalians are preferentially conserved in the mRNA sequences, especially in 3' UTR (96). Since these miRNA-regulated genes are involved in various crucial cell processes including apoptosis, differentiation and development, Gene Ontology (GO) or Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of them are helpful in understanding the biological functions of miRNA (97-100). For instance, the target genes of miR-124a such as ephrins B1, B2, and B3, ephrin receptors A2, A3, and B4, semaphorins 5A, 6A, 6C, and 6D, and plexins A3 and B2 are involved in nervous system development in the axon guidance pathway.

3.2 Related works

Our previous work, miRTarBase (101), which is the most updated collection of miRNA-target interactions (MTI), has accumulated 3,969 experimentally verified MTIs between 625 miRNAs and 2,433 target genes among 17 species by manually surveying pertinent literature. Moreover, numerous computational programs are available for identifying miRNA target sites. TargetScan (102), miRanda (103) and RNAhybrid (70) are three computational tools for determining the most energetically favored hybridization sites of small to large RNAs. PicTar (84) is capable of identifying common targets of known miRNAs. DIANA-microT (104) system utilizes experimentally derived miRNA/mRNA binding rules. miRNAMap (3,4) and miRecords (80), miRGen (75,105) and GOmir (106) provide the putative miRNA-target interactions by combining

prediction from multiple programs.

The miRU (107), MicroInspector (108), RNA22 (109), EIMMO (99), StarMir (72) and MMIA (110) are web-based tools for identifying miRNA binding sites. MicroInspector can search miRNA binding sites for a user-defined target RNA sequence that is potentially regulated by a miRNA. MicroInspector allows for variations in temperature and energies and allows the selection of various miRNA databases to identify miRNA binding sites of different strengths. The miRU tool was developed to predict plant miRNA target genes in any plant that is likely to be regulated by a user-defined miRNA. The pattern-based approach incorporated in the RNA22 program identifies putative target sites independent of miRNA target conservation and calls these sites as 'target islands'. The EIMMO considers evolutionary distance and branching when scoring the degree of miRNA target conservation. Otherwise, Dang et al. posited the target structure-accessible model for predicting miRNA targets and could also be accessed on system called StarMir. MMIA combines the inverse expression profiles of miRNA and mRNA data and then predicts the target genes by TargetScan, PicTar and PITA. Not only the aforementioned targeting of the 3' UTR of transcripts, but also the possibility of the targeting by miRNA of the coding sequence (CDS) and 5'UTR regions of the transcripts, are the subject of extensive research (66,67,109,111-120). Indeed, more than twenty miRNA target prediction tools were developed to identify potential candidates for miRNA-target interactions. However, most of them do not provide convenient functions for biologists in exploring the biological functions and regulatory relationships between miRNAs and protein coding genes. The comparisons among miRNA target prediction tools are given in **Table 5**.

Table 5. The comparisons of miRNA target prediction tools.

Features		**miRTar**	**DIANA-microT/miRPath** (121,122)	**EIMMO** (99)	**miRU** (107)	**RNAhybrid** (70)	**STarMir** (72)	**RNA22** (109)	**MMIA** (110)
Species		Human	Human and mouse	Vertebrates, nematode, fly	Plants	Human, nematodes, flies	-	Vertebrates, nematode, fly	Human
Possible relation between miRNA and gene group	* **1 to 1**	+	+	+	-	+	+	+	-
	* **1 to N**	+	1 to All genes	+	-	+	+	-	+
	* **N to 1**	+	-	+	-	+	+	-	-
	* **N to M**	+	-	+	-	+	+	-	+
	* **All to M**	+	All miRNAs to 1	+	-	-	-	-	-
	* **1 to KEGG**	+	+	-	-	-	-	-	-
miRNA targets on alternatively splicing exon		+	-	-	-	-	-	-	-
miRNA targets from mRNA		3'UTR, CDS, and 5'UTR	3'UTR	3'UTR	3'UTR, CDS, and 5'UTR	3'UTR	3'UTR, CDS, and 5'UTR	3'UTR, CDS, and 5'UTR	3'UTR
Known miRNAs		miRBase V15	-	miRBase V12	-	-	-	-	-
Accessibility of target site		+	-	-	-	-	Sfold	-	-
Conservation of target site		+	+	+	+	-	-	-	-
Expression profile of	**miRNA**	-	-	-	-	-	-	-	+
	Target	-	-	+	-	-	-	-	+

* 1 to 1 means the relation of one miRNA and one gene; 1 to N means the relation of one miRNA to multiple interesting genes; N to 1 means the relation of N miRNAs and one gene; N to M means the relation of N miRNAs and M genes; All to M means the relation of all miRNAs and M genes; 1 to KEGG means the relation of one miRNA and the genes of the selected KEGG map.

RNA alternative splicing plays important roles to regulate the gene expression in many biological processes among eukaryotic species. Recent studies have shown that more than 50% of genes undergo alternative splicing in humans (123-125). Additionally, some researchers have observed that appropriate splice variants are involved in several cellular and developmental processes, including gender determination, apoptosis, axon guidance, cell excitation and contraction (126). Relatedly, inappropriate alternative splicing causes the genetic disorders, because the expression of disease-related genes, many of which encode influential proteins in cancer biology, including those that govern cell cycle control, proliferation, differentiation, signal transduction pathways, cell death, angiogenesis, invasion, motility and metastasis, become abnormal (126-129). Moreover, generated spatio-temporal splicing variants can be divided into five classical forms, which are cassette exons, alternative 5' splice sites, alternative 3' splice sites, mutually exclusive exons and retained introns (126,130). Furthermore, the variety of combinations of cis-elements and trans-factors make understanding this mechanism difficult (126,129,130).

3.3 Specific aims

In this work, we aims to provide an integrated resource to allow biologists to elucidate miRNA-target interactions affected by the alternative splicing, thus the location of miRNA target sites may locate in the exons, which are alternatively spliced. Several previous investigations have studied the miRNA-target interactions affected by alternative splicing (111,112,116,117,119). For instance, Duursma et al. reported that human DNA methyltransferase 3b (DNMT3b) gene can be repressed by miR-148 family (116) and the miR-148 target sites are located in the DNMT3b exons, which is alternatively spliced. Furthermore, the gene set enrichment analysis (GSEA) for a group of genes, which are targeted by one or more miRNAs, can provide effective viewpoint to elucidate the miRNA functions in different biological process and pathways (131,132). Previous investigations analyzed the functions of miRNAs, mapping their putative target genes in several specific pathways (76,77,121), potentially elucidating the regulation by miRNAs of these biological pathways thereof.

This work introduces an integrated resource that provides multiple analyzing functions for miRNA target identification and for the study of miRNA-target interactions,

including the regulatory relationship between one miRNA and one gene, one miRNA and multiple genes, multiple miRNAs and one gene, and multiple miRNAs and multiple genes. Besides, miRTar identifies miRNA target sites against 3'UTR, as well as the coding regions and 5'UTR. This resource provides the information concerning that miRNA-target interactions are regulated by alternative splicing. Additionally, miRTar performs a gene set enrichment analysis for miRNA-regulated gene set to decipher possible roles in biological process and pathways.

3.4 Materials and methods

The miRTar is a web-based system that runs on an Apache web server with a Linux operating system. **Figure 19** presents in brief the intention that underlies miRTar, which is to design an analytical platform that allows researchers to focus on all possible scenarios to discuss the regulatory relationships between miRNAs and genes. After data are submitted to the system, miRTar identifies the miRNA target sites using TargetScan, miRanda, PITA, and RNAHybrid. The miRTar identifies the target sites against 3' UTR, 5' UTR and coding regions. Thus, the potential miRNA-target interactions between miRNAs and genes are constructed. For a gene set that may be regulated by single miRNA, based on gene set enrichment analysis (GSEA), a p-value is calculated to estimate the overrepresentation of genes in which the KEGG pathways, to estimate the biological function of miRNA. Additionally, miRTar can provide the information of miRNA target sites within exons, which are alternatively spliced (AS) or constitutively spliced (CS).

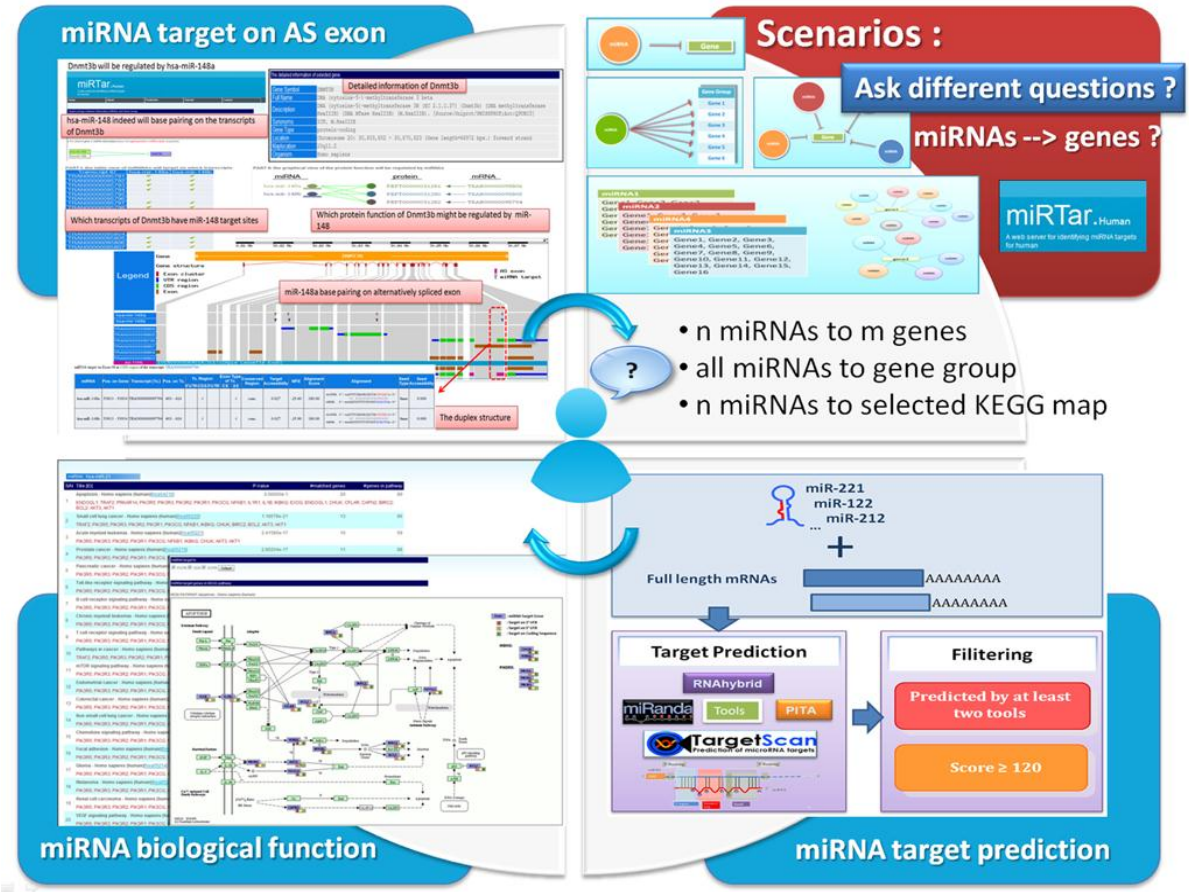


Figure 19. Concept that underlies miRTar.

3.4.1 Data collection

Figure 20 depicts the system flow of miRTar. miRTar utilizes several well-known resources, including the miRNA sequences, obtained from miRBase database Release 15 (74), gene information and relevant annotations, based on ASTD database Release 1.1 (133) and GenBank database Release 167 (134). The splice variants of transcripts are obtained from this ASTD (133), UniGene database Release 217 (135) and GenBank database (134). The biological pathways are extracted from the KEGG/PATHWAY database Release 53.0 (136). **Table 6** lists all versions and data types obtained from external data sources, and the statistics concerning the data in the proposed resource.

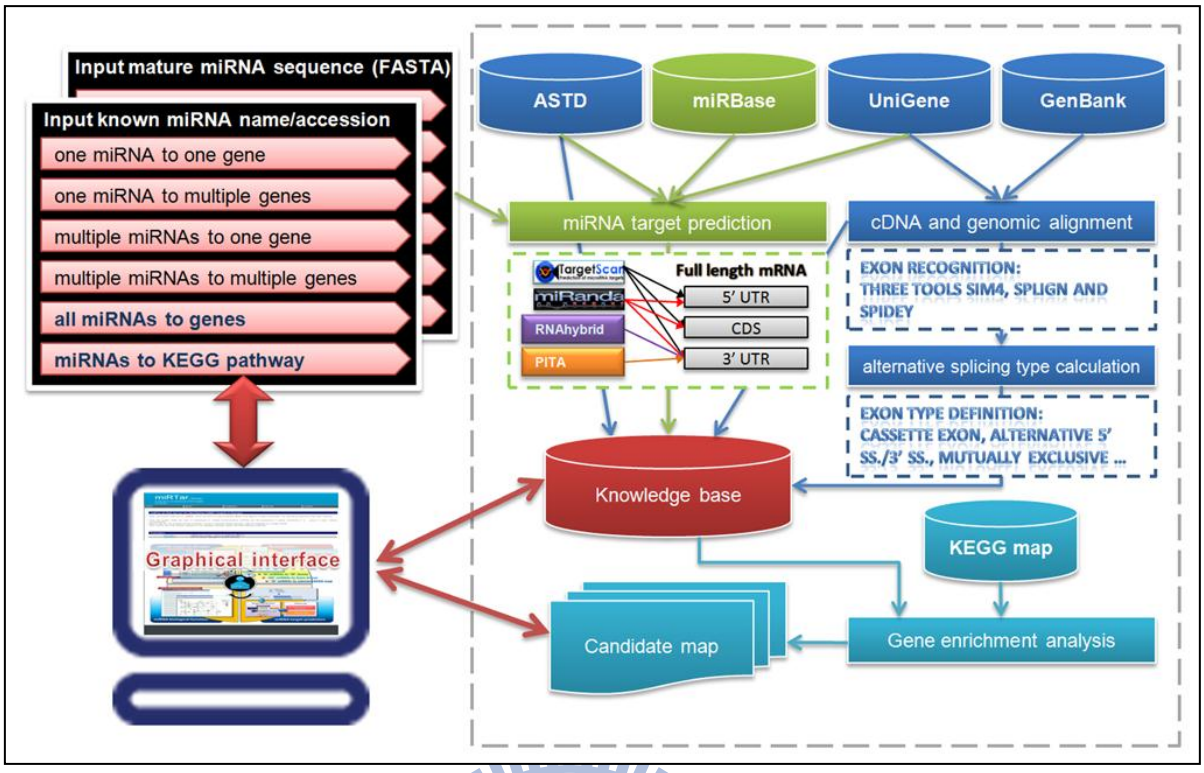


Figure 20. System flow of miRTar.

Table 6. Data statistics and data obtained from databases.

Data source	Version	Data descriptions	Data amount
miRBase (74)	V.15	MicroRNA information (name, sequences, ...)	1100
KEGG (136)	V. 53	The pathway maps	195
ASTD (133)	V. 1.1	Gene annotation	16,715
		mRNA sequences	93,467
		Protein information	34,545
		Alternative splicing events	78,165
GenBank (134)	V. 167	Gene annotation	32,123
		Genomic sequences	32,123
		Protein sequences	125,259
UniGene (135)	V. 217	mRNA sequences	137,654
		protein information (mRNA gi to protein gi)	125,259

3.4.2 Identifying miRNA target sites in human

First, TargetScanS was utilized to detect perfect Watson-Crick base pairing against all mRNA transcripts with lengths of at least six nucleotides. Four seed types, 8mer,

7mer-m8, 7mer-A1 and 6mer, which were defined clearly by the Bartel's group (65). Detecting the perfect seed region considerably reduces the number of false-positive predictions, especially for the conserved seed types (65,66,137). The latest version of miRanda (138) is also utilized to identify miRNA target sites. Notably, the terminal miRNA nucleotides the first and last two nucleotides no longer contribute to the miRanda score (139). The cutoff of minimal free energy (MFE) of the miRNA:target duplex was set to -12 kcal/mol and the cutoff of miRanda score was set to 120. Hence, miRNA targets whose MFEs are lower than -12 kcal/mol and whose score exceeds 120, are identified in the miRTar. Besides, RNAhybrid and PITA, which were developed to identify the miRNA target sites against 3'UTR, were utilized herein to identify miRNA target sites within 3'UTR. In order to reduce false positive predictions generated by multiple miRNA target prediction tools, miRTar applies several criteria concerning both their biological evolution and their structural context. These criteria are described below.

A. Target site in conserved region. Since target sites that are conserved across species are likely to be biologically functional, they are potential miRNA target sites. The UCSC PhastCons conservation score (140) is utilized to filter out the non-conserved predictions. Human data alignments were downloaded from the UCSC Genome Browser (92). The lowest bound on the PhastCons conservation score at the predicted target site in a human is set to 0.5.

B. Target site in accessible regions. Conventional target prediction tools consider the complementarity between the miRNA and its target sequence, the conservation of the target sites, and the kinetics and thermodynamics of the miRNA/target duplex. Although these properties are important in identifying miRNA target sites, the sequence context that surrounds miRNA target sites reportedly affects the binding affinities and the regulation of the miRNA. Harlan et al. (71) hypothesized that single-strand miRNAs can only bind to stretches of free mRNA for potential target sites. Dang et al. (72) posited the target structure-accessible model for predicting miRNA targets and succeeded in interpreting the published data concerning the in vivo activity of C. elegans reporter genes that contain modified lin-41 3'-UTR sequences. In this work, the RNAplfold (141) program was employed to manifest the concept of target site accessibility to reduce the number of false positive predictions. Therefore miRNAs hybridize to the target sites,

which are more likely to be real if they are in more accessible regions. RNAplfold can exactly determine the local base-pairing probabilities and the accessibilities of mRNA transcripts, which thus do not have to be computed from a Boltzmann-weighted sample of structures.

3.4.3 Exon/Intron boundary recognition

Recognition of the boundaries between exons and introns in gene transcripts has been studied for several years. Numerous technologies have been adopted to align cDNAs against genomic sequences. In this work, the cDNA sequences are obtained from UniGene and the genomic sequences are obtained from GenBank (134). Three tools are utilized to recognize these boundaries. They are SIM4 (142), splign (143), and spidey (144). The exon/intron boundaries on the transcripts were confirmed by using at least two tools. A total of around one million exons from 150,000 transcripts in about 30,000 genes were recognized.

3.4.4 Identifying different types of alternatively spliced exons

Five well-defined types of alternatively spliced exons are skipped exons, alternative 5' spliced sites, alternative 3' spliced sites, mutually exclusive exons and retained introns (130). In this work, in order to identify different exon types, the collected transcripts from UniGene were aligned pairwisely. First, the mRNA sequence was converted into a bit string of ones and zeros. Then, the logical operation (XOR, AND, OR), mentioned in SpliceInfo, is performed (145). Otherwise, alternatively spliced exons from ASTD (133) can be downloaded from the website. Of these five types of alternatively spliced exons, the cassette exon has the most ones, followed in order by the alternative 5' splice sites and the alternative 3' splice sites. Retained introns have the fewest ones (**Table 7**).

Table 7. Statistics the various types of alternative splicing exons between two different data sources.

Types of alternatively spliced exons	Data source	
	ASTD	UniGene
No. of cassette exon	34,435	9,361,222
No. of alternative 5' splice sites	6,469	1,030,325
No. of alternative 3' splice sites	3,720	913,112
No. of mutually exclusive exon	3,384	9,401
No. of intron retention	9,639	75,481

3.4.5 Alternative splicing effects to miRNA regulation

Following the prediction of miRNA target sites against all human transcripts, the alternative splicing information were considered for elucidating the miRNA-target interactions affected by alternative splicing. We utilize two data sets of alternatively spliced exons to study how alternative splicing mechanism control miRNA-target interactions. The first data set were obtained from ASTD (133) and the second data set were derived from the gene annotation in UniGene (135) and GenBank (134).

Table 8 presents the percentage of putative miRNA targets that are located on the transcripts that have been collected by miRTar. Since the average length of CDS is larger than the average length of 5' UTR and 3' UTR, generally the miRNA target sites are more probably to occur within the CDS regions than within 5' UTR and 3' UTR. Moreover, Table 5 gives the distributions of miRNA target sites within different types of alternatively spliced exons. The miRNA target sites are identified more often in cassette exons higher than in other types of alternatively spliced exons. The distribution is similar to the percentages of splicing exons given in

Table 7. Accordingly, when miRNA target sites are located in the alternatively spliced exons of a specific gene, various potential regulatory relationships between the miRNA and the gene can be further investigated. Thus, if a miRNA targets to an alternatively spliced exon, the target site can be conditionally spliced out and cannot be included in the gene transcripts. Therefore, RNA alternative splicing can cause incomplete gene suppression by a miRNA and affect miRNA regulations to diverse protein functions.

Table 8. Statistics of miRNA target site locations

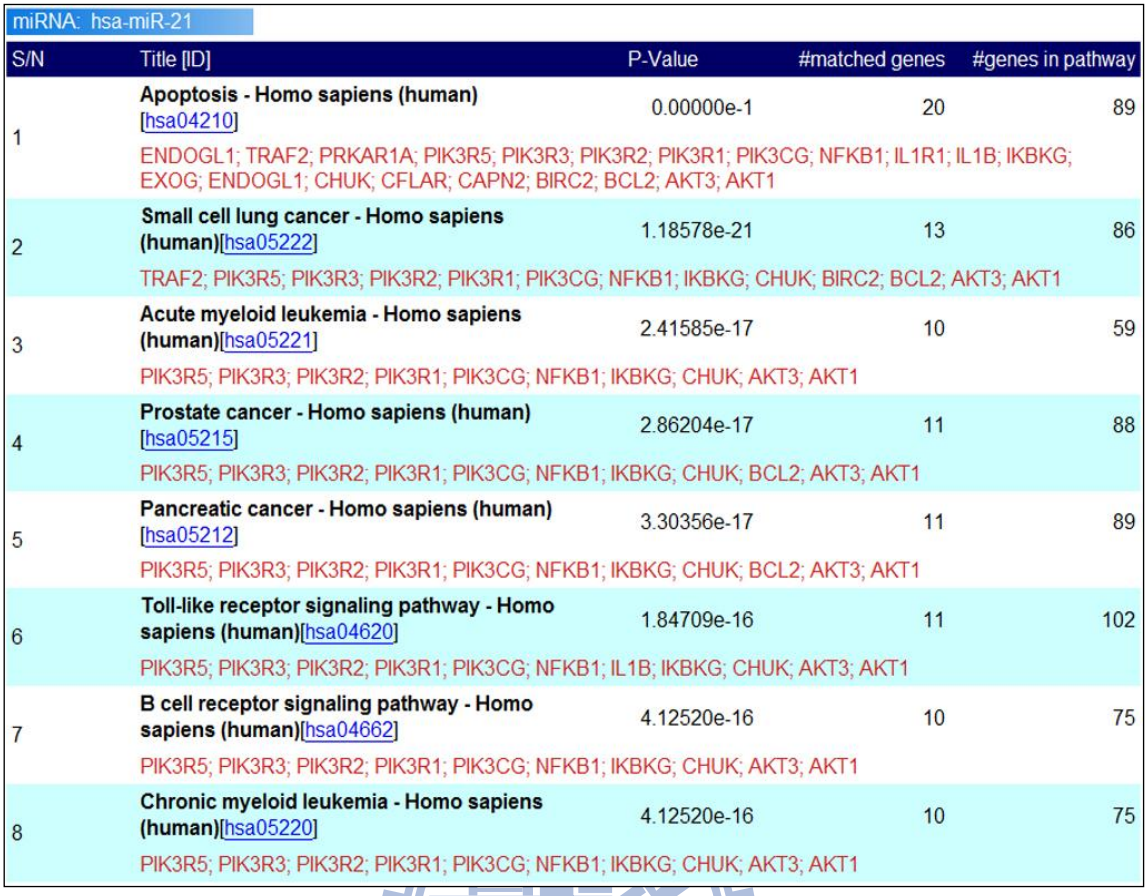
	Transcripts from ASTD	Transcripts from UniGene
*MFE	<= -12 kcal/mol	
*Score	> 120	
5'UTR	10.46 %	9.32 %
CDS	67.12 %	65.83 %
3'UTR	22.41 %	24.85 %

* miRNA target prediction parameters:
MFE: Minimum Free Energy of duplex; Score: alignment score of duplex.

3.4.6 GSEA for miRNA-regulated genes

After the prediction of miRNA targets, miRTar performs a gene set enrichment analysis (GSEA) for the miRNA-regulated genes in the KEGG pathway maps. It allows users to observe conveniently the biological pathway in which the miRNA-regulated genes participate and to determine the regulatory networks of miRNA-regulated genes.

As shown in **Figure 21**, the first step of the analysis is to determine the enrichment of specific miRNA target gene groups in various KEGG pathway maps. These maps are ranked by the number of p-values of the miRNA target genes in the biological pathway. The “Title [ID]” column provides the names of the KEGG pathway maps in which the miRNA target genes are involved; the “matched genes” column presents the number of miRNA target genes in each map; and the “gene in pathway” column presents all of the genes in each map.



miRNA: hsa-miR-21

S/N	Title [ID]	P-Value	#matched genes	#genes in pathway
1	**Apoptosis - Homo sapiens (human)** [hsa04210]	0.00000e-1	20	89
	ENDOGL1; TRAF2; PRKAR1A; PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IL1R1; IL1B; IKBKG; EXOG; ENDOGL1; CHUK; CFLAR; CAPN2; BIRC2; BCL2; AKT3; AKT1			
2	**Small cell lung cancer - Homo sapiens (human)**[hsa05222]	1.18578e-21	13	86
	TRAF2; PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BIRC2; BCL2; AKT3; AKT1			
3	**Acute myeloid leukemia - Homo sapiens (human)**[hsa05221]	2.41585e-17	10	59
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1			
4	**Prostate cancer - Homo sapiens (human)** [hsa05215]	2.86204e-17	11	88
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BCL2; AKT3; AKT1			
5	**Pancreatic cancer - Homo sapiens (human)** [hsa05212]	3.30356e-17	11	89
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BCL2; AKT3; AKT1			
6	**Toll-like receptor signaling pathway - Homo sapiens (human)**[hsa04620]	1.84709e-16	11	102
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IL1B; IKBKG; CHUK; AKT3; AKT1			
7	**B cell receptor signaling pathway - Homo sapiens (human)**[hsa04662]	4.12520e-16	10	75
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1			
8	**Chronic myeloid leukemia - Homo sapiens (human)**[hsa05220]	4.12520e-16	10	75
	PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1			

Figure 21. Analysis to identify miRNA target genes in KEGG pathway maps.

Figure 22 shows the second step of the analysis. The miRNA target genes are marked in "slate blue" in the KEGG pathway map, and the colors of traffic lights are utilized to represent the states of the miRNA target regions (3' UTR, 5' UTR and CDS). Users can focus to observe the miRNA target region of interest through changing the state in a biological pathway.

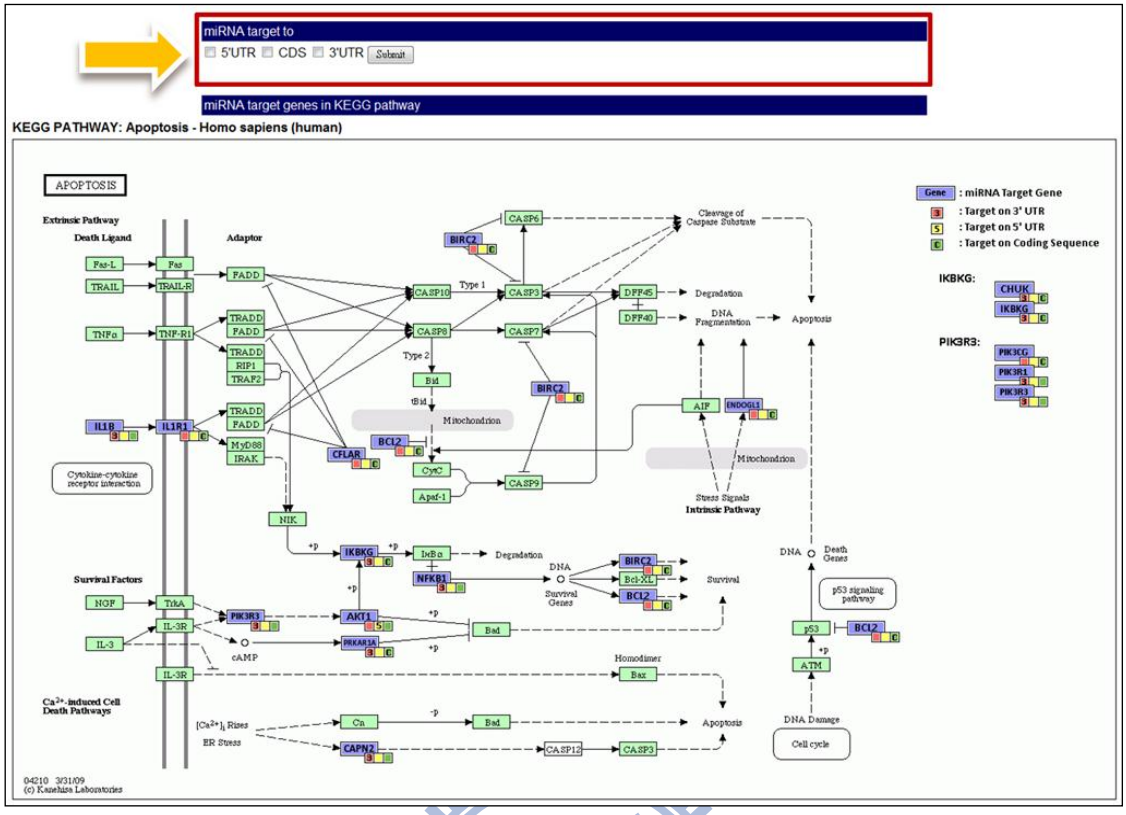


Figure 22. miRNA target genes in KEGG pathway map.

3.4.7 The approximate runtime of miRTar

Users can identify the miRNA targets on a set of groups of genes by using multiple miRNA sequences. The execution time of ten randomly selected miRNAs against the gene set (in FASTA format around 20MB file sizes) was computed on a PC server with eight CPU-cores. The miRNA target genes were predicted on average in 8.38 s for each miRNA, indicating that the proposed method can be utilized to identify the miRNA targets throughout the genome.

3.5 Utility and discussion

3.5.1 Case study of alternatively spliced target-containing exon

To demonstrate the functionality of miRTar in realizing the functional interactions between mature miRNAs and alternative pre-mRNA splicing, the miRNA (miR-148) and the protein coding gene DNA methyltransferase 3b (Dnmt3b) were considered as a case

study. Duursma et al's work (116) has shown that miR-148 can suppress Dnmt3b gene expression, targeting its protein coding region. One of its splice variants Dnmt3b3 mRNA lacks the target sites of miR-148. Additionally, the relative abundance of these splice variants results from the interactions between miRNAs and mRNA isoforms.

Upon submission of the miRNA miR-148 and Dnmt3b gene using the miRTar web interface, miR-148 target sites prediction in all of the regions (5'UTR, CDS and 3'UTR) of gene transcripts is executed. Alternatively or constitutively spliced exons on the transcripts are annotated. Subsequently, based on the tables and graphs presented on the miRTar, miR-148 targets to CDS and 3'UTR of the Dnmt3b transcripts. The region of interaction is located in the alternatively spliced exons. Consequently, parts of the Dnmt3b transcripts can splice out the exon, resisting regulation by miR-148. The complementary sequences between of miR-148 and the transcripts are similar to those found previously research (116). Hence, miRTar has the potential power to elucidate the regulatory aspect of functional interactions (in which miRNA targets alternatively spliced exons), as shown in **Figure 23**.

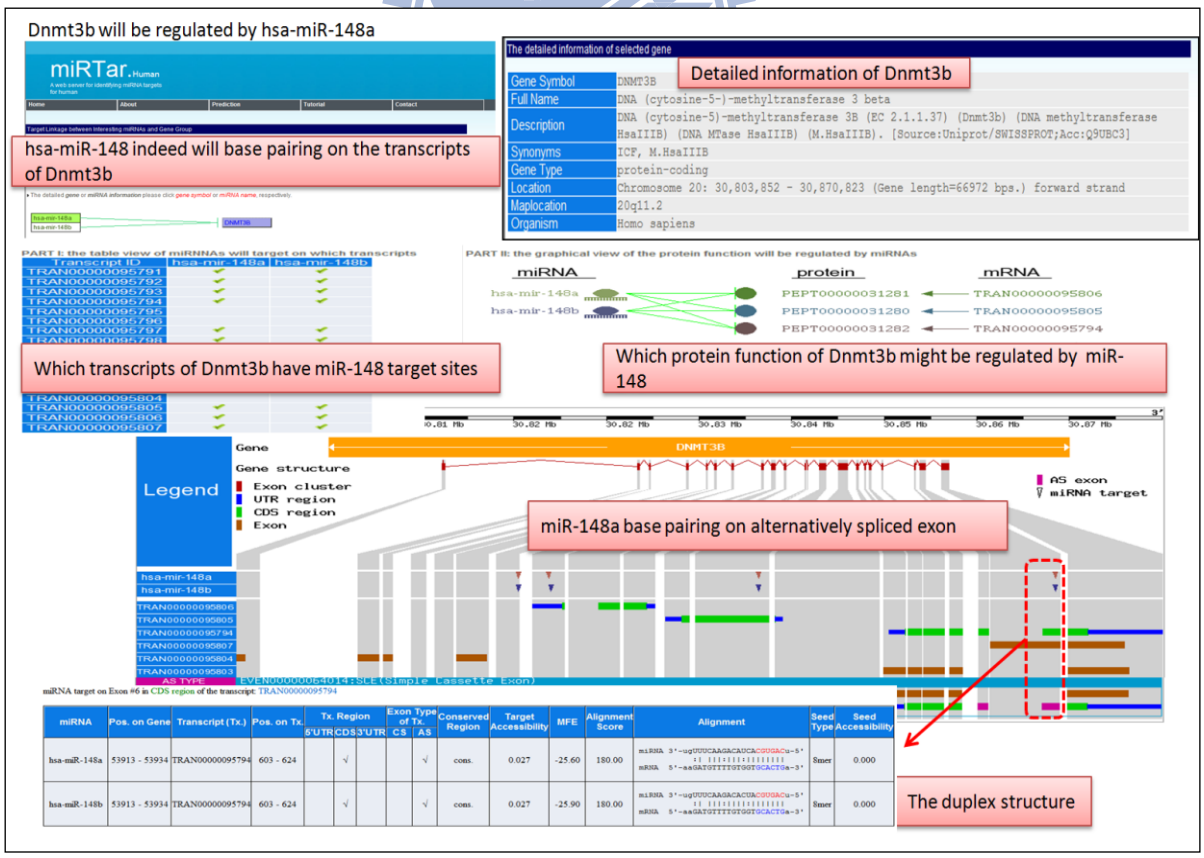


Figure 23. miR-148 targets protein coding region of DNMT3b in human.

3.5.2 Case study of cancer-associated gene group

Analysis of the regulatory roles of miRNA in a biological pathway is one of the main functions of the miRTar system. Many studies have demonstrated that miRNA participates in various biological processes, including development, cell differentiation, proliferation, and apoptosis. In this work, the apoptosis-related properties of miRNAs and group of genes are taken from research data (146) that have been established experimentally. To use the data as case study in KEGG pathway analysis of miRTar is carried out.

After the aforementioned data were submitted to miRTar, the results concerning the miRNAs indicated that each miRNA putatively regulates various gene groups by the predicting the target on the transcripts of the genes. Additionally, the function of these gene groups in the biological pathway is associated with apoptosis. Hence, the results shown in **Figure 21** and **Figure 22** can demonstrate that human miRNA hsa-miR-21 might be an important regulator in the apoptosis pathway when most of biological function of the target genes are involved in it. Many other miRNAs were also observed in this case study, but not shown in Figures. The results can imply that the important regulatory roles of these miRNAs in the biological pathway are consistent with previous findings. Therefore, miRTar can be utilized to elucidate the possible function of miRNA in the KEGG pathways.

3.5.3 Case study of miR-122 target analysis in mouse

We identify the miR-122 targets from 3'-UTR of up-regulated genes in miR-122 KO mouse liver by using miRTar. We also identified these up-regulated orthologous genes in mouse and human. The predictive parameters of each miRNA target prediction tool were optimized to yield a better set of miRNA targets candidate (See performance evaluation). Furthermore, we recalculated the miRNA/target duplex score using the following single-position base-pairing values. A score of +5 was assigned for G:C and A:U pairs, +2 for G:U wobble pairs, and −3 for mismatch pairs, and the gap-open and gap-elongation parameters are set to −8.0 and −2.0, respectively. The match value s(i) is multiplied by a position specific weight w(i). The position specific weights emphasize the importance of the 'seed region' generally defined as the position 2-8 of the miRNA 5'-end. Thus the

total score S for a particular alignment is $S = \sum_{i=1}^{n} w(i) \times s(i)$. Higher score indicates more stable miRNA/target duplex. Finally, 252 up-regulated orthologous genes identified by at least three target prediction tools by are selected for experimental validation and further analysis

3.5.4 Comparison with other miRNA target prediction web servers

The discovery of hundreds of miRNA genes has raised questions concerning how a specific miRNA regulates a specific gene, and what is the specific function of miRNA in a group of genes, among others. Most of miRNA target prediction tools can merely identify putative interactions between a miRNA and its targets, but they do not allow either gene set enrichment analysis for miRNA-regulated genes or the analysis of alternative splicing effects to miRNA-target interactions. Numerous analyzing scenarios, with various combinations of miRNAs and genes or KEGG maps input to miRTar can be considered to yield preliminary answers. **Table 5** lists the comparisons among miRTar and other miRNA target prediction tools or web servers. The miRTar provides the most convenient way for miRNA target prediction analysis and presents the most plentiful information for miRNA-target interactions such as KEGG pathways and alternative splicing information. Besides, miRTar integrated several external databases in advance, for instance, the sequences and annotations of miRNAs and genes were stored in the resource. It only requires inputting the accessions for miRNA and genes into miRTar instead of input the sequences of miRNAs and genes, which should be prepared by the users when using other tools or web servers.

3.6 Discussion

In this work, we aim to develop an integrated system for identifying miRNA-target interactions rather than to develop a new algorithm for identifying miRNA target genes. Further information including KEGG pathways and alternative splicing of genes were presented and analyzed. The miRTar can identify putative miRNA target sites on transcript sequences of genes under the severe constraints that have been discussed in previous studies. In miRTar, the default parameters are set based on the analysis 972 known miRNA target sites, collected in miRTarBase (101). 76% of known miRNA-target interactions can be identified under the criteria MFE<=-7 and alignment score>=125.

As given in **Table 9**, part of miRNA targets are located in the alternative splicing exon regions that means the site in the exon of one mRNA isoform is recruited but in another mRNA isofrom of the same gene is not. In this work, the proportion of this kind target sites is larger than 50% in all of the predicted sites in human. Therefore, when discussing the regulatory relationship between miRNAs and target genes, it is important to have another point of view in RNA alternative splicing. Accordingly, one of directions is the observation of miRNA base-pairing in the particular region of the gene-exon sequence that may be comprised alternatively spliced exons. This information is useful in discussing the possible regulatory relationship between RNAi and RNA alternative splicing, which has been mentioned in previous investigations (116,147). Notably, the prediction of miRNA targets in miRTar involves not only 3' UTR but also 5' UTR and CDS, implying that the protein products of one gene might also be repressed by the targeting by miRNA of the CDS and UTR.

Table 9. Statistics of miRNA target sites within different types of alternatively spliced exons.

		Data source	
		ASTD	**UniGene**
***MFE**		<= -12 kcal/mol	
***Score**		> 120	
****AS**	**cassette exon**	11.98 %	52.70 %
	alternative 5' SS.	0.76 %	6.80 %
	alternative 3' SS	0.99 %	7.55 %
	mutually exclusive	0.92 %	0.19 %
	intron retention	3.92 %	1.26 %
CS (Constitutively spliced exon)		81.43 %	31.50 %

*miRNA target prediction parameters: MFE (Minimum Free Energy of duplex); Score (alignment score of duplex).
**AS: alternatively spliced exon.

Another direction concerns the possible roles of miRNA in biological processes. Whereas several studies have identified genes that are regulated by miRNAs, the mechanisms of these miRNAs associated mechanism are less well known. Therefore, miRTar adopts the enrichment method in the KEGG pathway of the gene group to evaluate each the potential biological functions of miRNA. Moreover, when using miRTar to predict known miRNA target sites, some of them cannot be identified based on the default predictive parameters. For example, the research (148) shows one of the experimental data that hsa-let-7a can target on FOXA1, but miRTar can't detect any miRNA:target base pairing interaction in 3' UTR of gene transcripts (**Figure 24**).

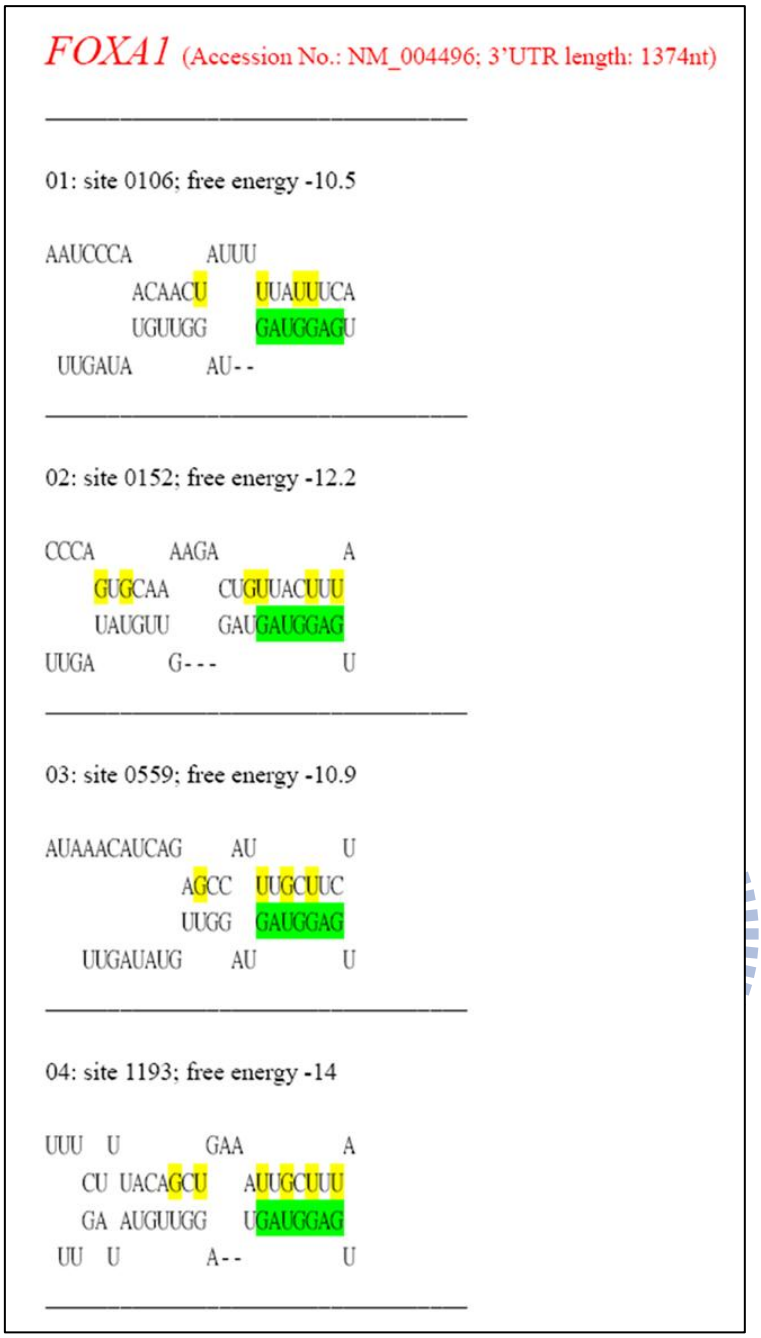


Figure 24. hsa-let-7a can target on FOXA1 (148)

3.7 Conclusion

The miRTar develops an integrated resource for deciphering miRNA-target interaction networks, and provides a broad range of analyzing scenarios for miRNA-target interactions, including one miRNA to one gene, one miRNA to multiple genes, and others, to help biologists understand the regulation between the miRNAs and target genes. By integrating several external databases and analyzing tools, miRTar can provide further

information for elucidating the miRNA regulation affected by alternative splicing. Besides, miRTar can enable biologists to easily identify the biological functions and regulatory relationships between a group of known/putative miRNAs and protein coding genes. miRTar is now available at http://miRTar.mbc.nctu.edu.tw/.

Chapter 4 Homologous cluster of miRNA target interactions

4.1 Introduction

Before starting this section, we should note that this part was also done by our co-group member, Feng-Mao Lin and Chih-Hung Chou.

MicroRNA, a small non-coding RNA sequence (18~25 nt), plays important roles in gene regulations (7). They regulate gene expressions via recognizing the binding site in three prime untranslated region (3'UTR). Currently, many miRNAs and their target genes have been identified in a wide variety of species (79,149-151). The computational and experiment studies of miRNA target sites show that the sites are usually located in the conserved 3'UTR region of the orthologous genes (96,152). Since the first miRNA is discovered from *Caenorhabditis elegans* in 1993 (153), around 15,000 mature miRNAs have been discovered and stored in miRBase (150) and around 3,500 experimental validated miRNA-target interactions have been collected in miRTarbase (149). In order to understand how miRNA suppresses gene expression, many miRNA target site prediction tools were also proposed such as TargetScan (96), RNAHybrid (70), miRanda (138), PITA(85), PicTar (84) and so on. The target site prediction tools based on sequence complementary usually provide many false positive target sites. In order to reduce the false positive sites, the miRNA target sites located at the conserved sequences among various groups of organisms have higher potential to be the functional sites (96,99).

To extent the concept of finding miRNA target sites in conserved sequences among multiple orthologous species. A database, HomoloMTIs, were presented which integrated the resources of experimental validated miRNA target sites, predicted miRNA target sites based on the homologous genes from the HomoloGene. The predicted target sites having experimental validated miRNA target sites in homologous genes were annotated to strengthen the possibility the homologous species might have similar miRNAs or miRNA target sites. This database might reveal novel target sites or miRNA among various species.

4.2 Specific aims

MicroRNA plays important roles in post-translational gene regulation among various species. Nowadays many miRNA target interaction are revealed by experiments and miRNA target site prediction tools. In order to provide more evidences for predicted miRNA target interactions and discover real miRNA target interactions, a database, HomoloMTIs, was contructed based on three databases, miRTarBase, miRBase and HomoloGene, to reveals the miRNA target interactions might be shared in homologous genes. The homologous MTIs profiles could reveal the homologous genes regulated by miRNA with at least one experimental validation in one of the homologous genes and predicted miRNA target interactions. The novel miRNA target interactions and miRNAs could be pioneer studied by HomoloMTIs. HomoloMTI aims to provide a comprehensive comparative perspective on the metazoan repertoire of miRNA-target interactions as complementary to miRTarBase, the database of experimentally verified miRNA-target interactions.

4.3 Materials and method

Three databases are required for producing homologous miRNA-target interaction (MTI) database. The experimental validated MTIs were obtained from miRTarBase release 1.0 including 3,576 MTIs. The homologous genes were obtained from HomoloGene release 64. The miRNA sequences were obtained from miRBase release 14.

Each profile of homologous MTI was constructed based on one of the experimental validated MTIs in miRTarBase. **Figure 25** illustrates the construction of the homologous MTI profiles. Each experimental validated MTI contains one miRNA and one target gene. The homologous genes of the target gene from experimental validated MTI were collected from HomoloGene. According the miRNA in experimental validated MTIs, miRNAs having identical suffix was collected. Then, the miRNA target sites of homologous genes were predicted by miRanda. The cutoff of target site prediction is the score larger than 50 and the minimum free energy smaller than -7 kcal/mol. Homologous MTI group might contain more than one validated interaction because the miRNA and target genes were studied in different species.

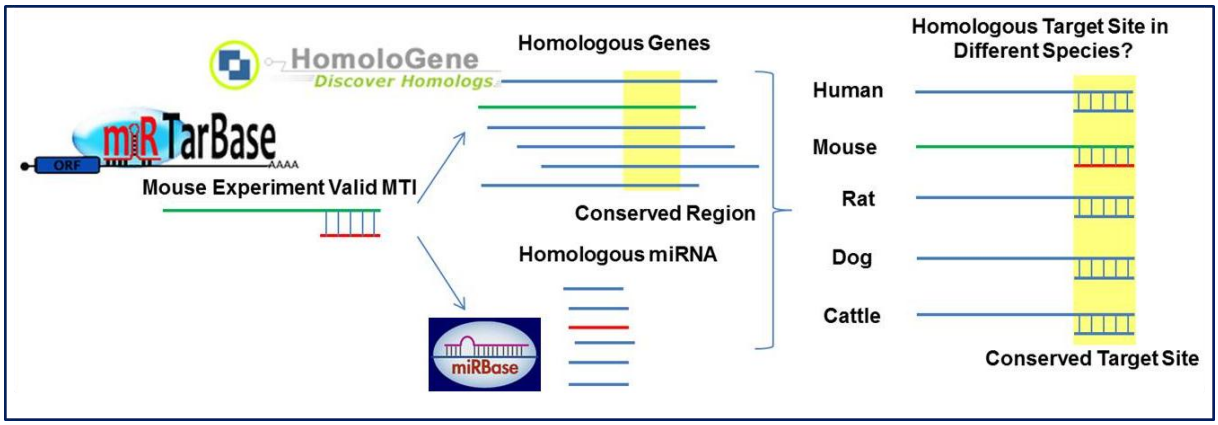


Figure 25. The construction diagram of HomoloMTIs. The database integrated three databases and predicted miRNA target sites on the homologous genes genes based on the experimental proved MTIs from miRTarBase.

4.4 Results

4.4.1 Statistics

There are 1,658 miRNA-target interactions between 430 miRNAs and 1,006 target genes validated by luciferase reporter assay AND western blot. To obtain the corresponding homologous gene pairs, we required the related metazoan genes in NCBI HomoloGene. **Table 10** lists the number of homologous MTIs in each species. The overall homologous MTI groups is 1,569 which means some groups were shared in many species. For example, human HomoloMTIs includes 229 miRNAs and 618 target genes extracted from 1,114 MTIs in miRTarBase. Although, the MTIs of Chimpanzee, Cattle and Dog cannot be found in miRTarBase, some homologous MTI groups included their homologous genes.

Table 10. Statistics of homologous miRNA-target interactions collected in HomoloMTI

Species	No. of miRNA-target interactions		No. of miRNAs in miRTarBase	No. of target genes in miRTarBase
	miRTarBase MTIs	HomoloMTI groups		
Human	1114	1411	229	618
Chimpanzee	-	1017	-	-
Mouse	301	1356	107	222
Rat	46	671	31	29
Chicken	12	647	5	12
Cattle	-	769	-	-
Dog	-	544	-	-
Zebrafish	73	657	23	54
Fruit fly	96	234	28	58
Nematode	16	78	7	13
Total	1658	1569	430	1006

HomoloMTI aims to provide a comprehensive comparative perspective on the metazoan repertoire of miRNA-target interactions as complementary to miRTarBase, the database of experimentally verified miRNA-target interactions.

4.4.2 Case Study of homologous miR-122::ALDOA

Experimentally verified MTIs included in HomoloMTI was retrieved from miRTarBase. A homologous MTI group was constructed based on the experimental validated MTI. For example, HMTI000328 shown in **Figure 26** was created from an experimental validated MTI in human and mouse. The homologous MTIs of cattle, rat, zebrafish and dog were predicted with their own miR-122 and homologous genes of ALDOA. The MTI in the species without miR-122 are predicted based on validated interactions of miR-122.

(a)

HMTI000328

Species	miRNA	Interaction	Gene Symbol
Bos taurus	bta-miR-122	Predicted	ALDOA
Mus musculus	mmu-miR-122	Validated	Aldoa
Rattus norvegicus	rno-miR-122	Predicted	Aldoa
Homo sapiens	hsa-miR-122	Validated	ALDOA
Danio rerio	dre-miR-122	Predicted	aldoaa
Danio rerio	dre-miR-122	Predicted	aldoab
Canis lupus familiaris	cfa-miR-122	Predicted	ALDOA

(a)

Homologous Region

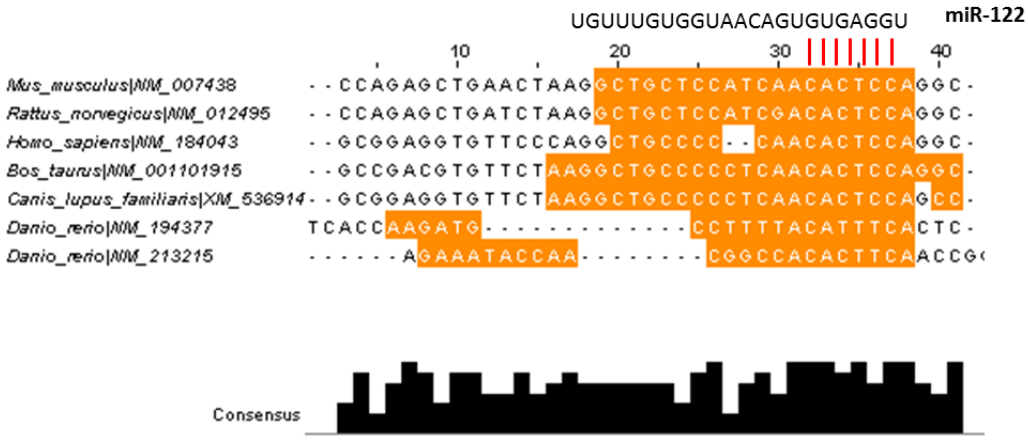


Figure 26. The homologous MTI group of miR-122 and target gene ALDOA.

Chapter 5 miRNAMap – integrated database of microRNA-target interactions

5.1 Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules, which are capable of negatively regulating gene expression by typically hybridizing to the 3'-untranslated regions (3'-UTR) of the target gene. A vast of miRNAs play important roles in time development, cell death, cell proliferation, fat metabolism, hematopoiesis and nervous system patterning in animals, and stress responses, and leaf and flower development in plants (7,154-156).

Many miRNAs and miRNA targets are discovered and experimentally verified during last few years. miRBase (2), which is the most comprehensive database to collect experimentally validated miRNAs across many genomes, provides integrated interfaces to present miRNA annotation and computational predicted miRNA targets. DIANA TarBase (157) provides experimentally validated miRNA targets in eight different species. It contains totally 750 miRNA target sites in 550 target genes. Recently, many biologists pay much more attention on the investigation of the roles that miRNAs play in biological systems. Several miRNA target prediction tools were developed previously, such as miRanda (138), TargetScan (102) and RNAhybrid (83), for determining the energetically most favorable hybridization sites of a small RNA to a large RNA. Lu et al. developed a miRNA microarray to measure the expression profiles of all known miRNA in various normal tissues and tumors (158).

In this work, we develop a resource, miRNAMap, to accumulate experimental verified microRNAs and experimental verified miRNA target genes in human, mouse, rat, and other metazoan genomes. In addition to known miRNA targets, three computational tools previously developed, such as miRanda, RNAhybrid and TargetScan, were applied for identifying miRNA targets in 3'-UTR of genes. In order to reduce the false positive prediction of miRNA targets, three criteria are supported for filtering the putative miRNA targets to retain the more probable miRNA targets. Especially, the RNA accessibilities of the identified miRNA target site were investigated and provided for an effective viewpoint for the study of miRNA/target relationship.

Furthermore, the miRNA expression profiles can provide valuable clues for

investigating the properties of miRNAs, such tissue specificity and differential expression in cancer/normal cell. Therefore, we performed the Q-PCR experiments for monitoring the expression profiles of 224 human miRNAs in eighteen major normal tissues in human. The cross-reference between the miRNA expression profiles and the expression profiles of its target genes can provide effective viewpoint to understand the regulatory functions of the miRNA. Finally, both textual and graphical web interface are redesigned and enhanced to facilitate the retrieval of data from the miRNAMap.

5.2 Related works

There are several existing resources that provide updated data regarding each of these areas of research. miRBase is the main database of experimentally validated mature miRNA sequences. The miRBase database also provides integrated interfaces to comprehensive miRNA annotation and predicted gene targets. ARGONAUTE (159) provides a larger miRNA tissue expression dataset — collected from various miRNA expression studies. DIANA TarBase (79,157) collects experimentally validated miRNA targets in eight species. **Table 11.** shows other related predicted MTI resources. The detail information about miRecords and miRWalk describe in the following section.

Table 11. The related resources of MTI.

Name	Organism	Remark	Refs
Softwares			
miRanda	Vertebrates, Flies	1. Weighted seed match	
TargetScan	Vertebrates	1. Seed match (seed type) 2. Context score	
RNAhybrid	Nematodes, Flies	1. Feasible seed region parameter 2. Calculate MFE without the linker	
MicroTar	Vertebrates	1. The first tool addressed the accessibility of mRNA.	
PITA	Vertebrates, Nematodes, Flies	1. Target accessibility feature. 2. ddG is used to rank the miRNA target. 3. 1 GU pair is allowed in seed region.	
Web server			
miRU	Plants	1. Mismatch is allowed in seed region	
MicroInspector	Plants, Nematodes, Virus, Vertebrates, Flies	1. Novel miRNA sequence input is allowed. 2. Can change the folding temperature.	
DIANA-microT	Human and mouse	1. KEGG pathway included . 2. The interactions predicted by TargetScan and PicTar are noted	
MMIA	Human	1. Incorporate PITA, TargetScan and PicTar 2. Expression profile of miRNA and mRNA	

TargetRank	Human and mouse	1. Seed match types. (6mer, 7mer-A1, 7mer-m8 and 8mer) 2. Flanking region conservation
TargetSpy	Vertebrates and fly	1. Machine learning (Multiboost)
EIMMo	Vertebrates and nematodes	1. Machine learning (Bayesian) 2. Including miRNA and target gene expression
STarMir	Nematodes and flies	1. Target accessibility feature. 2. 4 consecutive complementary nucleotides.
DataBases		
miRNAMap 2.0	Nematodes, Vertebrates, Flies	1. miRNA/mRNA expression profile. 2. Incorporated three prediction tools. 3. Criteria for filtering predicted miRNA targets.
PicTar	Vertebrates	1. Studying translational gene regulation by multiple microRNAs
Microcosm	Nematodes, Vertebrates, Flies	1. Using miRanda. 2. Exact seed match.
miRDB	Vertebrates	1. Machine learning method (SVM)
RepTar	Human and mouse	1. Machine learning method (HMM) 2. Human and mouse miRNA targets on virus
miRWalK	Human, mouse and rat	1. Incorporated 8 known miRNA target prediction tools. 2. Experimental miRNA targets

miRecords

miRecords, a resource for animal miRNA-target interactions, consists of two components including Validated Targets and Predicted Targets. The *Validated Targets* component houses about 1,600 experimentally validated miRNA targets curated from meticulous literature. The *Predicted Targets* component of miRecords integrated the pre-compiled data identified by 11 established miRNA target prediction tools. The miRecords is available at http://miRecords.umn.edu/miRecords.

miRWalk

miRWalk (http://www.ma.uni-heidelberg.de/apps/zmf/mirwalk/) is a database that provides the information of miRNAs in human, mouse and rat. It contains two major modules including 'Predicted target module' and 'Validated target module'. In predicted target module, they integrated the novel miRNA target interactions from eight established miRNA target prediction resources.

5.3 The specific aims

To facilitate the annotation of the miRNA function, it is obliged to integrate relative information. We developed an integrated database, namely miRNAMap (http://mirnamap.mbc.nctu.edu.tw/) (4), to compile the miRNA genes, miRNA targets and the regulatory relationships between the miRNAs and the miRNA target genes. miRNAMap was published in 2006, is also one of the main databases to comprehensive study miRNA. It provides a variety of search functions and graphical interface to facilitate the researchers who interested in the miRNA riles in cell regulations (**Figure 27**).

The main contribution of this work is the extended development to miRNAMap version 3.0. We make the focus on the investigation of miRNA-target interaction. To make miRNAMap more comprehensive, we integrate the experimentally verified MTIs, the MTIs predicted by 11 predicted miRNA target databases and more expression profiles of miRNA and protein-coding gene. A useful feature specially designed to human genome is the comparison between miRNA expression profiles and expression profiles of target genes.

Figure 27 Graphical web interface of miRNAMap

5.4 Improvement

Table 13 lists the major differences between the previous version and miRNAMap 3.0. In miRNAMap 2.0, we mainly focus on the collection of the known miRNAs in metazoan genomes, including 2 insects, 9 vertebrates and 1 worm. In addition to human, mouse, rat and dog, other metazoan genomes, such as chicken, fruit fly, worm, zebrafish, fugu, frog (Xenopus tropicalis), malarial mosquito and opossum, were supported in the resource. There are 2,241 known miRNAs of the 12 genomes, which were obtained from miRBase (release 9.2, May 2007). The experimentally verified miRNA targets were obtained from DIANA TarBase and by surveying literature. The numbers of experimental miRNA targets extracted from DIANA TarBase and by surveying literature are 346 and 29, respectively. The major enhancements and new features in miRNAMap 3.0 are described below. In miRNAMap 3.0, the number of miRNAs almost twice as much as previous version (**Table 12**, the miRBase release 16, August 2010). The more miRNA expression profiles are incorporated into miRNAMap 3.0, especially for miRNA expression profiles measured by next-generation sequencing technology. The other major improvement is integration of 11 external MTI prediction database.

Table 12. Numbers of mature miRNAs categorized by type of species in miRNAMap 2.0 and 3.0

Species	**Abbr.**	**Numbers of mature miRNAs**	
		miRNAMap 2.0	**miRNAMap 3.0**
human (homo sapiens)	Hsa	475	1,223
mouse (mus musculus)	Mmu	377	1,055
zebrafish (danio rerio)	Dre	337	248
rat (rattus norvegicus)	Rno	234	680
frog (xenopus tropicalis)	Xtr	177	169
chicken (gallus gallus)	Gga	149	544
worm (caenorhabditis elegans)	Cel	132	233
pufferfish (fugu rubripes)	Fru	131	109
opossum (monodelphis domestica)	Mdo	107	146
fly (drosophila melanogaster)	Dme	78	196
mosquito (anopheles gambiae)	Aga	38	65
dog (canis familiaris)	Cfa	6	289
Total		2,241	4,957

Table 13. Enhancements and new features of miRNAMap 3.0

Features	miRNAMap 1.0	miRNAMap 2.0	miRNAMap 3.0
Known miRNA	miRBase (version 6.0)	miRBase (version 9.2)	miRBase (version 17.0)
Support species	4 mammalian	2 insects, 8 vertebrates, 2 worms	2 insects, 8 vertebrates, 2 worms
Experimental miRNA-target interactions	Literature	TarBase and literature	miRTarBase
miRNA expression profiling	MIT microRNA profiling	MIT microRNA profiling, qPCR microRNA profiling	MIT microRNA profiling, qPCR microRNA profiling, NGS microRNA profiling
Expression profile of miRNA targets	-	NCBI GEO	NCBI GEO, NGS
miRNA target prediction tools and resources	miRanda	miRanda, RNAhybrid, TargetScan	12 external miRNA
Accessible region of miRNA target sites	-	Sfold	RNAplfold
Criteria for filtering predicted miRNA targets		Criterion 1: predicted by at least two tools Criterion 2: target genes contain multiple sites Criterion 3: target site is accessible	Combinatorial prediction parameters of MTI
miRNA tissue specificity	Text description	Analysis of the miRNA expression level in each tissue.	

5.5 Materials and methods

Figure 28 presents a flow chart of the generation of data in miRNAMap 3.0 database. It comprises the following three main parts: (i) integration of human miRNA-target interactions from the external predicted MTI databases; (ii) identification of other metazoan miRNA-target interactions and (iii) integration the microRNA and protein-coding gene expression profiles.

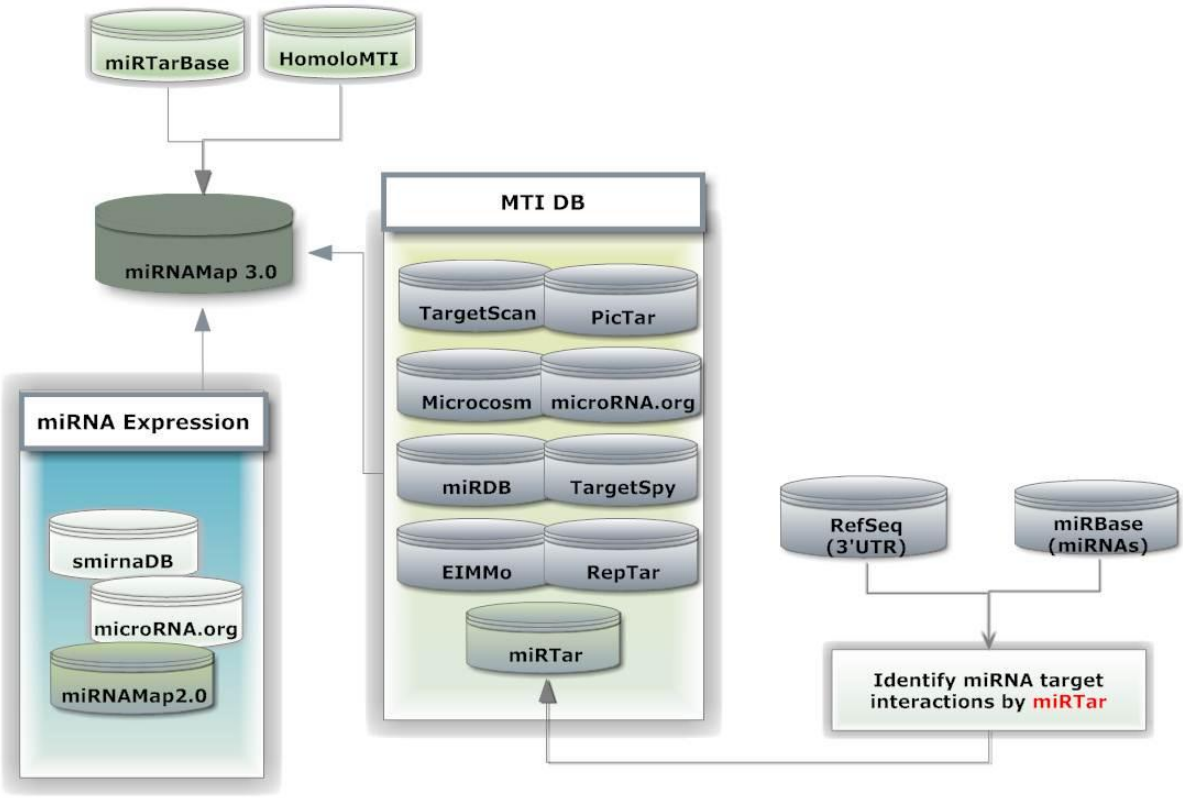


Figure 28. Data generation of miRNAMap 3.0.

5.5.1 Annotation resources of Protein-coding gene

The annotations of the protein coding genes were obtained from NCBI database, Gene Ontology (160) and HGNC gene grouping/family data (161). The conserved regions among the genomes in the database are obtained from the UCSC Genome Browser (162). Several useful tools were integrated in miRNAMap to identify miRNA functions and structures. **Table 14** shows the integrated databases and tools in miRNAMap.

Table 14. The list of the integrated external data sources in miRNAMap.

Integrated Databases	Description	Reference
Tools of identifying miRNA-target interactions		
miRanda		(138)
TargetScan		(102)
RNAhybrid		(83)
PITA		
Resources of protein-coding gene		
UCSC Genome Browser	PhastCon score to evaluated the conservation of target site.	(162)
Lu et al. 's work	Gene expression profiles of known miRNAs	(158)
Gene Ontology		(160)
Ensembl		(163)
NCBI Entrez Gene		
HGNC		(161)
Information of miRNA		
miRBase	Known miRNAs	(2,164)
Other tools used in miRNAMap		

5.5.2 Integration of external human miRNA target prediction databases

In this study, all predicted miRNA-target interactions from different databases were downloaded from the following links (**Table 15**). We could get the predicted MTIs from 11 external databases, including 24 datasets. **Table 16** lists the number of integrated miRNA-target interactions in each database. Notably, we did not integrate all these datasets into miRNAMap, because of the redundant MTIs and other factors.

Table 15. The external link of integrated miRNA target prediction databses.

Databases of predicted miRNA-target interactions		
microRNA.org	http://www.microrna.org/microrna/getDownloads.do	(78,165)
TargetScan	http://www.targetscan.org/	(67,96)
Microcosm	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/	(166)
EIMMo	http://www.mirz.unibas.ch/ElMMo3/	(99)
PicTar	http://pictar.mdc-berlin.de/	(84)
PITA	http://genie.weizmann.ac.il/pubs/mir07/index.html	(85)
RepTar	http://bioinformatics.ekmd.huji.ac.il/reptar/	(167)
miRDB	http://mirdb.org/miRDB/	(77)
microT	http://diana.cslab.ece.ntua.gr/microT/	(168)
TargetRank	http://genes.mit.edu/targetrank/	(169)
TargetSpy	http://www.targetspy.org/	(170)

Table 16. Statistics of predicted human MTIs from different prediction databases.

Integrated Databases	**Dataset**	**# of MTIs**	**# of unique miRNAs**	**# of unique targets**
TargetScan	Conserved	187,352	675	11,605
	NonConserved	1,433,350	677	17,385
microRNA.org	2010_aug_0_0※	4,314,342	851	19,747
	2010_aug_0_C◎	1,282,903	249	19,437
	2010_aug_S_0•	2,196,833	851	19,686
	2010_aug_S_C☆	732,474	249	19,192
Microcosm		338,840	685	14,705
PicTar	4way	36,905	127	6,038
	5way	11,651	93	2,358
PITA	3_15_TOP	206,974	660	10,097
	3_15_ALL#	3,893,016	660	16,796
microT		1,319,950	530	16,678
miRDB		217,612	684	14,973
EIMMo		1,770,434	692	18,230
RepTar		3,003,558	1,076	17,287
TargetRank		342882	530	14,300
TargetSpy	ALL#	10,254,029	675	17,659
	noseed_sens	2,373,282	675	17,377
	noseed_spec	645,573	675	16,461
	seed_sens	566,580	675	16,380
	seed_spec	240,491	675	14,992
miRTar	overlap2tools#	12,540,960	1,223	19,279
	overlap3tools	7,861,925	1,223	19,213
	overlap4tools	1,957,642	1,223	19,002
Total*		12,558,267	1,240	21,606

※ Non-good mirSVR score, Non-conserved miRNA
◎ Non-good mirSVR score, Conserved miRNA
• Good mirSVR score, Non-conserved miRNA
☆ Good mirSVR score, Conserved miRNA
* Denotes the number of total MTIs, total unique miRNAs and total unique targets in miRNAMap 3.0.
# TargetSpy (ALL), PITA (ALL) and miRTar (overlap2tools) are excluded from calculating the total unique MTIs, total unique miRNAs and total unique targets.

5.5.3 Identification of novel miRNA targets in metazoan

MTI prediction in miRNAMap 2.0

In the previous version, miRNAMap 2.0, we incorporated three computational tools previously developed, such as miRanda (138), TargetScan (102) and RNAhybrid (83), for identifying miRNA targets against the conserved regions of 3'-UTR of genes in 12 metazoan genomes. The conserved regions were extracted from UCSC Genome Browser Most Conserved Regions (171). The MFE threshold of the miRNA and target duplex was specified as -12 kcal/mol and the miRanda score was specified as 120. Consequently, the miRNA targets whose MFEs are smaller than -12 kcal/mol and the score exceeds 120 are identified and compiled in the miRNAMap database. The predictive parameters of TargetScan and RNAhybrid are set as default values. Each miRNA target prediction tool discovers a set of candidates of miRNA target sites. However, some of these candidates might be false positive predictions. In order to filter more probable miRNA target sites, we propose three criteria to filter out false positives and to retain better candidates for miRNA targets.

Criterion 1: target sites predicted by at least two tools. The three tools, including miRanda, RNAhybrid and TargetScan, were applied separately to identify miRNA targets against the conserved regions of 3'-UTR in all metazoan genomes. This criterion is to retain the putative miRNA targets predicted by at least two programs for a miRNA, as the illustration in **Figure 29**(a).

Criterion 2: target gene contains multiple target sites. Previous investigations suggest that one gene can contain multiple miRNA target sites bound by multiple distinct miRNAs or single miRNA, for example, six let-7 miRNA target sites were discovered in lin-4 (72,103,172), and the eye development gene seven-up (svp) is targeted by miR-33, miR-124, miR-277 and miR-312 (103). Therefore, the criterion is to retain the miRNA target sites and the corresponding gene, which contains multiple target sites, as illustrated in **Figure 29**(a).

Criterion 3: target site locates in accessible regions. The conventional target prediction tools consider the complementarity between the miRNA and its target sequence, the conservation of the target sites, and the kinetics and thermodynamics of miRNA/target duplex. Although these properties are important factors to determine the miRNA target sites, the sequence context surrounding miRNA target sites was reported

to influence the binding affinities and the regulation of the miRNA. Harlan et al (71) hypothesized that single-strand miRNAs can only bind to stretches of free mRNA for potential target sites. Dang et al (72) postulated the target structure accessible model for miRNA target prediction and succeeded in interpreting the published data on the in vivo activity of C. elegans reporter genes containing modified lin-41 3'-UTR sequences. In this work, we incorporated this idea to filter the false positive predictions, thus miRNAs hybridize to the target sites, which is within more accessible regions, are with more possibility to be real, as shown in **Figure 29**(b). The accessibility of RNA sequences are determined by Sfold (173).

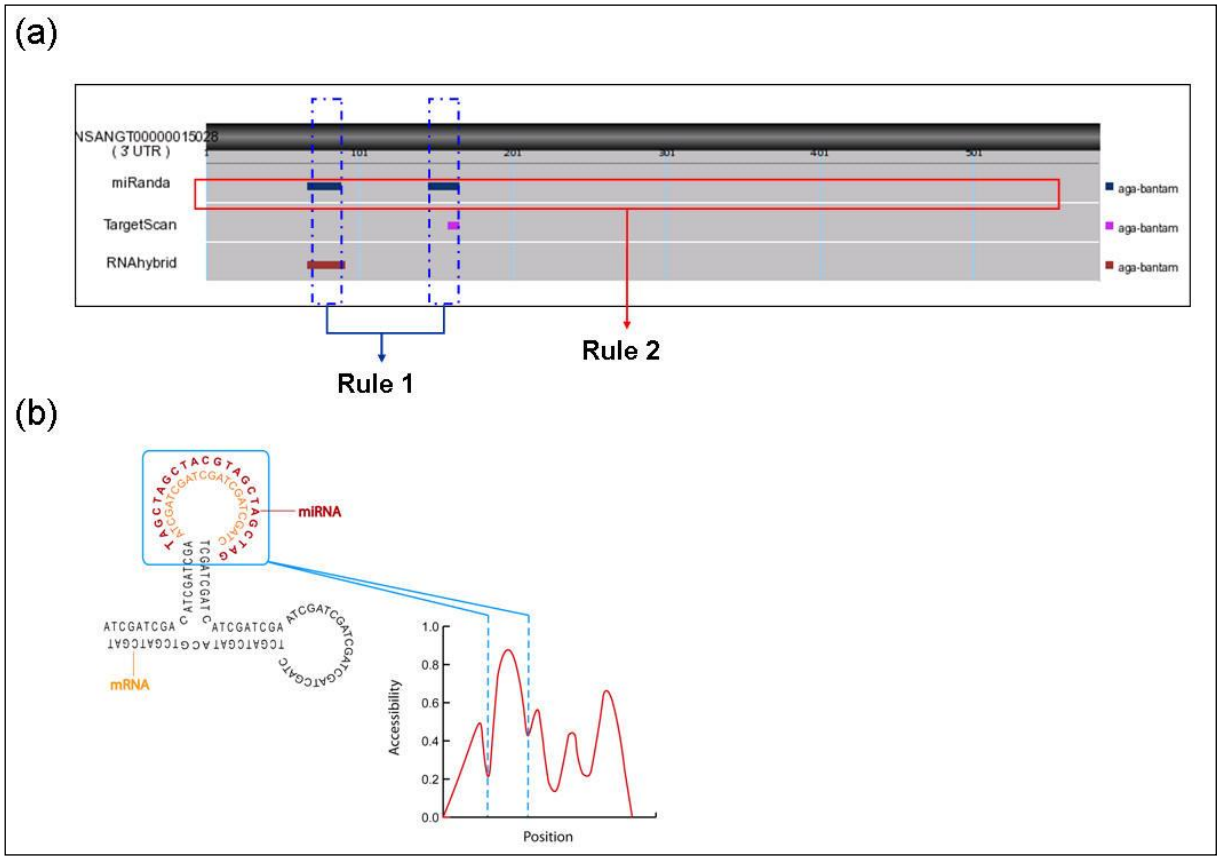


Figure 29. Criteria supported in the miRNA target search function for miRNAMap 2.0. (a) Criterion 1 is to select the potential miRNA target sites, which are predicted by at least two tools; criterion 2 is to select the target gene that contains multiple target sites; (b) RNA accessibility is incorporated into the detection of miRNA target sites.

MTI prediction in miRNAMap 3.0

In this version, we identify the miRNA-target interactions by using the previous described prediction tool, miRTar.

5.5.4 Expression profiling of microRNAs and target genes

miRNAMap 2.0

As mentioned above, the computational tools for the identification of miRNA target sites are developed based on the complementarities of miRNA and target sites, kinetics and thermodynamics of miRNA/target duplex, the combinatorial properties of miRNA target sites, and the accessibility of the target sites. Moreover, the expression profiles of miRNAs become useful for understanding the regulatory roles in complicated biological systems. In this work, we integrate two data sets of miRNA expression profiles, which were obtained by different experimental methods including quantitative polymerase chain reaction (Q-PCR) and miRNA-bead array (158).

In order to analyze the tissue-specificity of human miRNAs, we detected the expression level of 224 human miRNAs in 18 major normal tissues in human by using a real-time PCR-based 220-plex miRNA expression profiling method. The detailed experimental protocol is described in Supplementary Material. The expression levels of miRNAs are currently provided in the miRNAMap for the study of tissue-specificity of human miRNAs. Another data set was generated by Lu. et al, who applied a bead-based flow cytometric miRNA expression profiling method to present a systematic expression analysis of 217 mammalian miRNAs from 334 human samples (158). As to the expression profiles of miRNA target genes, we obtained the gene expression profiles in 79 human tissues (174), GDS596 (GEO accession), from NCBI GEO (175).

Generally, the expression of miRNA and its target gene are negatively correlated since the miRNA down-regulates its target gene. To observe this phenomenon in experimental data in human, for each miRNA target sites involved by a miRNA and a target gene, the Pearson correlation coefficients is computed from the miRNA expression profile and the target gene expression profile. There are 13 overlapped tissues between our data set of miRNA expression profiles and the GDS596 data set of expression profiles of target genes.

miRNMap 3.0

In this version, we integrate the other two miRNA expression resources to extend the miRNA expression profiles collect in miRNAMap (**Table 17**).

Table 17. The statistics of external miRNA expression profiles.

Data source	Species	miRNAs	Experimental conditions
micoRNA.org	Human	516	172
	Mouse	400	68
	Rat	356	16
smirnaDB	Human	546	171
	Mouse	420	70
	Rat	251	17
	Zebrafish	170	9
	Fly	57	11
	Worm	114	2

5.6 Results

5.6.1 miRNA target prediction database similarities

The different biological features taking into account in each external predicted miRNA-target interactions database are shown in **Table 18**, and those databases list in that table are considered in this research.

Although most databases consider the biological feature - conservation, they each handle it differently. TargetScan provides two datasets, including conserved miRNA target sites and non-conserved miRNA target sites. **Figure 30** (a) shows that TargetScan has 30% overlap between its conserved and non-conserved datasets. It means that 30% of miRNA target genes share both conserved and non-conserved miRNA target sites.

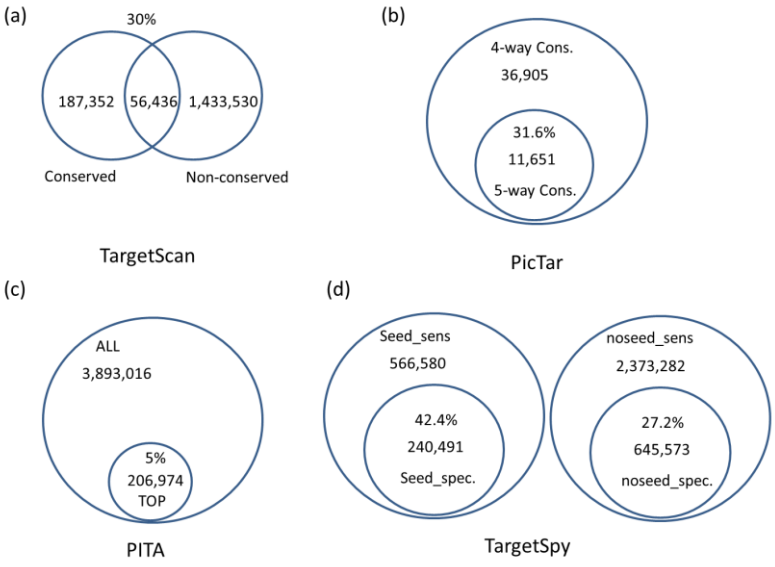


Figure 30. The overlap of different datasets provided by the same database.

Table 18. Biological features of miRNA target prediction resources. (176)

	microrNA.org	Microcosm	TargetScan	PITA	microT	PicTar	miRDB[#]	EIMMo	TargetSpy[#]	RepTar	TargetRank	miRTar
Seed match	+	+	+	+	+	+	+	+	+	+	+	+
Free energy of duplex	+	+		+	+	+	+	+	+	+		+
Conservation	+	+	+[*]		+	+	+		+	+	+	+
Site accessibility				+			+		+			+
Local AU content			+				+		+		+	
Use miRanda	+	+										+
Machine learning method							SVM	Bayesian	Multiboost	HMM		
Sequence composition of target site							+		+	+	+	
Sequence composition of flanking region									+		+	

* TargetScan (Non-conserved) did not consider the conservation of target site.
[#] miRDB and TargetSpy take the marked biological features into account when training the model.

5.6.2 Comparing miRNA target prediction databses to miRTarBase

In order to evaluate the performance of miRNA target prediction tools and our proposed method, we collected 1,524 experimentally validated miRNA-target interactions in human, including 204 non-functional MTIs and 1,320 functional MTIs. The detail list is shown in **Appendix I**. This data set is based on our manually curated MTIs with strong evidence support in miRTarBase (177). The following formulas of predictive performance are defined:

$$\text{Precision (Pre)} = \frac{TP}{TP + FP},$$

$$\text{Sensitivity (Sn)} = \frac{TP}{TP + FN},$$

$$\text{Specificity(Sp)} = \frac{TN}{TN + FP},$$

$$\text{Accuracy } (Acc) = \frac{TP + TN}{TP + FP + TN + FN},$$

$$\text{Performance (PERF)} = Sp \times Sn$$

In the equation, TN represents true negative, TP true positive, FN false negative and FP false positive. The performance of individual prediction MTI database is displayed in **Table 19**. We found that the top three in performance (PERF) are TargetScan (Conserved), microRNA.org (2010_aug_S_0) and miRTar (overlapping 4 tools) and.

Table 19. Predictive performance across miRNA-target interaction databases by using experimentally verified MTIs with strong evidence support in miRTarBase.

Integrated Databases	Dataset	Number of positive data	Number of negative data	Precision	Sensitivity	Specificity	Accuracy	PERF
TargetScan	Conserved	1,189	185	90.8%	57.2%	62.7%	57.9%	0.359
	NonConserved	1,276	197	88.9%	40.3%	67.5%	43.9%	0.272
microRNA.org	2010_aug_0_0※	123	30	82.6%	57.7%	50.0%	56.2%	0.289
	2010_aug_0_C	1,186	173	88.7%	54.0%	52.6%	53.9%	0.284
	2010_aug_S_0	121	30	84.3%	48.8%	63.3%	51.7%	0.309
	2010_aug_S_C	1,168	174	89.2%	68.8%	44.3%	65.6%	0.305
Microcosm	v5.0	1,028	163	85.8%	25.2%	73.6%	31.8%	0.185
PicTar	4way	658	102	89.0%	63.7%	49.0%	61.7%	0.312
	5way	363	47	90.6%	50.7%	59.6%	51.7%	0.302
PITA	3_15_TOP	1,156	180	89.3%	50.3%	61.1%	51.8%	0.308
	3_15_ALL	1,263	197	87.6%	88.6%	19.3%	79.2%	0.171
microT	v3	1,231	193	88.5%	76.4%	36.8%	71.0%	0.281
miRDB	v3	1,241	193	89.4%	33.8%	74.1%	39.3%	0.251
EIMMo	v4	1,252	196	88.6%	77.0%	36.7%	71.5%	0.283
RepTar	v1.2	1,308	200	87.1%	62.8%	39.0%	59.6%	0.245
TargetRank		1,172	188	90.7%	48.0%	69.2%	51.0%	0.332
TargetSpy	ALL	1,274	197	86.9%	98.7%	3.6%	85.9%	0.035
	noseed_sens	1,271	197	88.4%	52.2%	55.8%	52.7%	0.292
	noseed_spec	1,258	195	91.2%	22.2%	86.2%	30.8%	0.191
	seed_sens	1,256	195	89.3%	34.6%	73.3%	39.8%	0.253
	seed_spec	1,235	191	90.7%	15.9%	89.5%	25.7%	0.142
miRTar	overlap2tools	1,282	203	87.7%	78.8%	30.1%	72.1%	0.237
	overlap3tools	1,282	203	88.8%	69.1%	44.8%	65.8%	0.310
	overlap4tools	1,282	203	89.2%	54.4%	58.6%	54.9%	0.319

Chapter 6 Conclusion

MicroRNA plays important roles in post-translational gene regulation among various species. Nowadays many miRNA-target interactions are revealed by experimental approach and miRNA target site prediction tools. This thesis spotlights the miRNA-target interactions whether they are validated or predicted. It is composed of miRTarBase, miRTar, miRNAMap and HomoloMTI (**Figure 9**).

Firstly, we construct a more comprehensive database of miRNA-target interactions, miRTarBase, which were experimentally validated. The biological features of miRNA/target duplex were observed based on largest collection of human miRNA-target interactions currently available. Various web interfaces are designed to facilitate the presentation of miRNA-target interactions. A pipeline combining text-mining and manual review was established to extract MTI information from research articles.

Second of all, miRTar adopts various analyzing scenarios to identify putative miRNA target sites of the gene transcripts and elucidates the biological functions of miRNAs toward their targets in biological pathways. The system has three major features. First, the prediction system considers various analyzing scenarios (1 miRNA:1 gene, 1:N, N:1, N:M, all miRNAs:N genes, and N miRNAs: genes involved in a pathway) to easily identify the regulatory relationships between interesting miRNAs and their targets, in 3'UTR, 5'UTR and coding regions. Second, miRTar can analyze and highlight a group of miRNA-regulated genes that participate in particular KEGG pathways to elucidate the biological roles of miRNAs in biological pathways. Third, miRTar can provide further information for elucidating the miRNA regulation, i.e., miRNA-target interactions, affected by alternative splicing.

Thirdly, miRNAMap integrates miRNA-target interactions databases to elucidate accurate MTIs. We make the focus on the investigation of miRNA-target interaction. To make miRNAMap more comprehensive, we integrate the experimentally verified MTIs, the MTIs predicted by 11 predicted miRNA target databases and more expression profiles of miRNA and protein-coding gene. A useful feature specially designed to human genome is the comparison between miRNA expression profiles and expression profiles of target genes.

Finally, we extend the all the miRNA-target interactions in miRTarBase to other mammalian genome based on evolutionary conservation of miRNA and its target sites.

The homologous MTIs profiles could reveal the homologous genes regulated by miRNA with at least one experimental validation in one of the homologous genes and predicted miRNA target interactions. The novel miRNA target interactions could be pioneer studied by HomoloMTI. HomoloMTI aims to provide a comprehensive comparative perspective analysis on the various species of miRNA target interactions to find homologous miRNA target sites.

References

1. Wightman, B., Ha, I. and Ruvkun, G. (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell*, **75**, 855-862.
2. Griffiths-Jones, S., Grocock, R.J., van Dongen, S., Bateman, A. and Enright, A.J. (2006) miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res*, **34**, D140-144.
3. Hsu, S.D., Chu, C.H., Tsou, A.P., Chen, S.J., Chen, H.C., Hsu, P.W., Wong, Y.H., Chen, Y.H., Chen, G.H. and Huang, H.D. (2008) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. *Nucleic Acids Res*, **36**, D165-169.
4. Hsu, P.W., Huang, H.D., Hsu, S.D., Lin, L.Z., Tsou, A.P., Tseng, C.P., Stadler, P.F., Washietl, S. and Hofacker, I.L. (2006) miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. *Nucleic Acids Res*, **34**, D135-139.
5. Hsu, S.D., Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M. *et al.* (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic acids research*, **39**, D163-169.
6. Hsu, J.B., Chiu, C.M., Hsu, S.D., Huang, W.Y., Chien, C.H., Lee, T.Y. and Huang, H.D. (2011) miRTar: an integrated system for identifying miRNA-target interactions in Human. *BMC Bioinformatics*, **12**, 300.
7. Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, **116**, 281-297.
8. Ambros, V. (2001) microRNAs: tiny regulators with great potential. *Cell*, **107**, 823-826.
9. Llave, C., Xie, Z., Kasschau, K.D. and Carrington, J.C. (2002) Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science*, **297**, 2053-2056.
10. Lai, E.C. (2002) Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet*, **30**, 363-364.
11. Cimmino, A., Calin, G.A., Fabbri, M., Iorio, M.V., Ferracin, M., Shimizu, M., Wojcik, S.E., Aqeilan, R.I., Zupo, S., Dono, M. *et al.* (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A*, **102**, 13944-13949.
12. Gupta, A., Gartner, J.J., Sethupathy, P., Hatzigeorgiou, A.G. and Fraser, N.W. (2006) Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. *Nature*, **442**, 82-85.
13. Jovanovic, M. and Hengartner, M.O. (2006) miRNAs and apoptosis: RNAs to die for. *Oncogene*, **25**, 6176-6187.
14. Guo, L. and Ding, Z.H. (2007) [Advances in the studies of miRNAs and cell apoptosis]. *Sheng Li Ke Xue Jin Zhan*, **38**, 331-335.
15. Matsubara, H., Takeuchi, T., Nishikawa, E., Yanagisawa, K., Hayashita, Y., Ebi, H., Yamada, H., Suzuki, M., Nagino, M., Nimura, Y. *et al.* (2007) Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. *Oncogene*, **26**, 6099-6105.
16. Mott, J.L., Kobayashi, S., Bronk, S.F. and Gores, G.J. (2007) mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene*, **26**, 6133-6140.
17. Buscaglia, L.E. and Li, Y. (2011) Apoptosis and the target genes of microRNA-21. *Chinese journal of cancer*, **30**, 371-380.

18. Adlakha, Y.K. and Saini, N. (2011) MicroRNA-128 downregulates Bax and induces apoptosis in human embryonic kidney cells. *Cellular and molecular life sciences : CMLS*, **68**, 1415-1428.
19. Zhang, H., Cai, X., Wang, Y., Tang, H., Tong, D. and Ji, F. (2010) microRNA-143, down-regulated in osteosarcoma, promotes apoptosis and suppresses tumorigenicity by targeting Bcl-2. *Oncol Rep*, **24**, 1363-1369.
20. Yu, F., Deng, H., Yao, H., Liu, Q., Su, F. and Song, E. (2010) Mir-30 reduction maintains self-renewal and inhibits apoptosis in breast tumor-initiating cells. *Oncogene*.
21. Xu, J., Liao, X. and Wong, C. (2010) Downregulations of B-cell lymphoma 2 and myeloid cell leukemia sequence 1 by microRNA 153 induce apoptosis in a glioblastoma cell line DBTRG-05MG. *Int J Cancer*, **126**, 1029-1035.
22. Wang, K. and Li, P.F. (2010) Foxo3a regulates apoptosis by negatively targeting miR-21. *J Biol Chem*.
23. Subramanian, S. and Steer, C.J. (2010) MicroRNAs as gatekeepers of apoptosis. *J Cell Physiol*, **223**, 289-298.
24. Erson, A.E. and Petty, E.M. (2008) MicroRNAs in development and disease. *Clin Genet*, **74**, 296-306.
25. Callis, T.E., Chen, J.F. and Wang, D.Z. (2007) MicroRNAs in skeletal and cardiac muscle development. *DNA Cell Biol*, **26**, 219-225.
26. Bruneau, B.G. (2005) Developmental biology: tiny brakes for a growing heart. *Nature*, **436**, 181-182.
27. Bruneau, B.G. (2008) The developmental genetics of congenital heart disease. *Nature*, **451**, 943-948.
28. Graveley, B.R., Brooks, A.N., Carlson, J.W., Duff, M.O., Landolin, J.M., Yang, L., Artieri, C.G., van Baren, M.J., Boley, N., Booth, B.W. *et al.* (2011) The developmental transcriptome of Drosophila melanogaster. *Nature*, **471**, 473-479.
29. Hammoud, S.S., Nix, D.A., Zhang, H., Purwar, J., Carrell, D.T. and Cairns, B.R. (2009) Distinctive chromatin in human sperm packages genes for embryo development. *Nature*, **460**, 473-478.
30. Hornstein, E., Mansfield, J.H., Yekta, S., Hu, J.K., Harfe, B.D., McManus, M.T., Baskerville, S., Bartel, D.P. and Tabin, C.J. (2005) The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development. *Nature*, **438**, 671-674.
31. Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettinger, J.C., Rougvie, A.E., Horvitz, H.R. and Ruvkun, G. (2000) The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature*, **403**, 901-906.
32. Scheres, B. (2010) Developmental biology: Roots respond to an inner calling. *Nature*, **465**, 299-300.
33. Schratt, G.M., Tuebing, F., Nigh, E.A., Kane, C.G., Sabatini, M.E., Kiebler, M. and Greenberg, M.E. (2006) A brain-specific microRNA regulates dendritic spine development. *Nature*, **439**, 283-289.
34. West, J.A., Viswanathan, S.R., Yabuuchi, A., Cunniff, K., Takeuchi, A., Park, I.H., Sero, J.E., Zhu, H., Perez-Atayde, A., Frazier, A.L. *et al.* (2009) A role for Lin28 in primordial germ-cell development and germ-cell malignancy. *Nature*, **460**, 909-913.
35. Yoo, A.S., Staahl, B.T., Chen, L. and Crabtree, G.R. (2009) MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. *Nature*, **460**, 642-646.
36. Ucar, A., Vafaizadeh, V., Jarry, H., Fiedler, J., Klemmt, P.A., Thum, T., Groner, B. and

Chowdhury, K. (2010) miR-212 and miR-132 are required for epithelial stromal interactions necessary for mouse mammary gland development. *Nature genetics*.
37. Slominski, A. (2008) Cooling skin cancer: menthol inhibits melanoma growth. Focus on "TRPM8 activation suppresses cellular viability in human melanoma". *Am J Physiol Cell Physiol*, **295**, C293-295.
38. Tivnan, A., Foley, N.H., Tracey, L., Davidoff, A.M. and Stallings, R.L. (2010) MicroRNA-184-Mediated Inhibition of Tumour Growth in an Orthotopic Murine Model of Neuroblastoma. *Anticancer research*, **30**, 4391-4395.
39. Yan, L.X., Wu, Q.N., Zhang, Y., Li, Y.Y., Liao, D.Z., Hou, J.H., Fu, J., Zeng, M.S., Yun, J.P., Wu, Q.L. *et al.* (2011) Knockdown of miR-21 in human breast cancer cell lines inhibits proliferation, in vitro migration and in vivo tumor growth. *Breast cancer research : BCR*, **13**, R2.
40. Hu, G., Chen, D., Li, X., Yang, K., Wang, H. and Wu, W. (2010) miR-133b regulates the MET proto-oncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. *Cancer Biol Ther*, **10**.
41. Ma, Y., Yu, S., Zhao, W., Lu, Z. and Chen, J. (2010) miR-27a regulates the growth, colony formation and migration of pancreatic cancer cells by targeting Sprouty2. *Cancer Lett*.
42. Li, Y., Guessous, F., Zhang, Y., Dipierro, C., Kefas, B., Johnson, E., Marcinkiewicz, L., Jiang, J., Yang, Y., Schmittgen, T.D. *et al.* (2009) MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res*, **69**, 7569-7576.
43. Abdelmohsen, K., Kim, M.M., Srikantan, S., Mercken, E.M., Brennan, S.E., Wilson, G.M., Cabo, R. and Gorospe, M. (2010) miR-519 suppresses tumor growth by reducing HuR levels. *Cell Cycle*, **9**, 1354-1359.
44. Esquela-Kerscher, A., Trang, P., Wiggins, J.F., Patrawala, L., Cheng, A., Ford, L., Weidhaas, J.B., Brown, D., Bader, A.G. and Slack, F.J. (2008) The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle*, **7**, 759-764.
45. Green, D. and Karpatkin, S. (2010) Role of thrombin as a tumor growth factor. *Cell Cycle*, **9**, 656-661.
46. Slack, F. (2009) let-7 microRNA reduces tumor growth. *Cell Cycle*, **8**, 1823.
47. Braun, T. and Gautel, M. (2011) Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. *Nature reviews. Molecular cell biology*, **12**, 349-361.
48. Si, M.L., Zhu, S., Wu, H., Lu, Z., Wu, F. and Mo, Y.Y. (2007) miR-21-mediated tumor growth. *Oncogene*, **26**, 2799-2803.
49. Lee, Y., Kim, M., Han, J., Yeom, K.H., Lee, S., Baek, S.H. and Kim, V.N. (2004) MicroRNA genes are transcribed by RNA polymerase II. *EMBO J*, **23**, 4051-4060.
50. Dieci, G., Fiorino, G., Castelnuovo, M., Teichmann, M. and Pagano, A. (2007) The expanding RNA polymerase III transcriptome. *Trends Genet*, **23**, 614-622.
51. Borchert, G.M., Lanier, W. and Davidson, B.L. (2006) RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol*, **13**, 1097-1101.
52. Yeom, K.H., Lee, Y., Han, J., Suh, M.R. and Kim, V.N. (2006) Characterization of DGCR8/Pasha, the essential cofactor for Drosha in primary miRNA processing. *Nucleic Acids Res*, **34**, 4622-4629.
53. Okamura, K., Hagen, J.W., Duan, H., Tyler, D.M. and Lai, E.C. (2007) The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. *Cell*, **130**, 89-100.
54. Ying, S.Y. and Lin, S.L. (2005) Intronic microRNAs. *Biochem Biophys Res Commun*, **326**, 515-520.

55. Tijsterman, M. and Plasterk, R.H. (2004) Dicers at RISC; the mechanism of RNAi. *Cell*, **117**, 1-3.
56. Kurihara, Y. and Watanabe, Y. (2004) Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proc Natl Acad Sci U S A*, **101**, 12753-12758.
57. Melo, S.A., Ropero, S., Moutinho, C., Aaltonen, L.A., Yamamoto, H., Calin, G.A., Rossi, S., Fernandez, A.F., Carneiro, F., Oliveira, C. *et al.* (2009) A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet*, **41**, 365-370.
58. Filipowicz, W. (2005) RNAi: the nuts and bolts of the RISC machine. *Cell*, **122**, 17-20.
59. Gregory, R.I., Chendrimada, T.P., Cooch, N. and Shiekhattar, R. (2005) Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell*, **123**, 631-640.
60. Wang, H.W., Noland, C., Siridechadilok, B., Taylor, D.W., Ma, E., Felderer, K., Doudna, J.A. and Nogales, E. (2009) Structural insights into RNA processing by the human RISC-loading complex. *Nat Struct Mol Biol*, **16**, 1148-1153.
61. Kim, V.N., Han, J. and Siomi, M.C. (2009) Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*, **10**, 126-139.
62. Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K. *et al.* (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*, **99**, 15524-15529.
63. Bagga, S., Bracht, J., Hunter, S., Massirer, K., Holtz, J., Eachus, R. and Pasquinelli, A.E. (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell*, **122**, 553-563.
64. Carrington, J.C. and Ambros, V. (2003) Role of microRNAs in plant and animal development. *Science*, **301**, 336-338.
65. Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*, **136**, 215-233.
66. Lewis, B.P., Burge, C.B. and Bartel, D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, **120**, 15-20.
67. Grimson, A., Farh, K.K., Johnston, W.K., Garrett-Engele, P., Lim, L.P. and Bartel, D.P. (2007) MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell*, **27**, 91-105.
68. Ellwanger, D.C., Buttner, F.A., Mewes, H.W. and Stumpflen, V. (2011) The sufficient minimal set of miRNA seed types. *Bioinformatics*.
69. Hofacker, I.L. (2009) RNA secondary structure analysis using the Vienna RNA package. *Curr Protoc Bioinformatics*, **Chapter 12**, Unit12 12.
70. Rehmsmeier, M., Steffen, P., Hochsmann, M. and Giegerich, R. (2004) Fast and effective prediction of microRNA/target duplexes. *RNA*, **10**, 1507-1517.
71. Robins, H., Li, Y. and Padgett, R.W. (2005) Incorporating structure to predict microRNA targets. *Proc Natl Acad Sci U S A*, **102**, 4006-4009.
72. Long, D., Lee, R., Williams, P., Chan, C.Y., Ambros, V. and Ding, Y. (2007) Potent effect of target structure on microRNA function. *Nat Struct Mol Biol*, **14**, 287-294.
73. Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R. and Rajewsky, N. (2008) Widespread changes in protein synthesis induced by microRNAs. *Nature*, **455**, 58-63.
74. Griffiths-Jones, S., Saini, H.K., van Dongen, S. and Enright, A.J. (2008) miRBase:

tools for microRNA genomics. *Nucleic Acids Res*, **36**, D154-158.
75. Alexiou, P., Vergoulis, T., Gleditzsch, M., Prekas, G., Dalamagas, T., Megraw, M., Grosse, I., Sellis, T. and Hatzigeorgiou, A.G. (2010) miRGen 2.0: a database of microRNA genomic information and regulation. *Nucleic Acids Res*, **38**, D137-141.
76. Nam, S., Kim, B., Shin, S. and Lee, S. (2008) miRGator: an integrated system for functional annotation of microRNAs. *Nucleic Acids Res*, **36**, D159-164.
77. Wang, X. (2008) miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *RNA*, **14**, 1012-1017.
78. Betel, D., Wilson, M., Gabow, A., Marks, D.S. and Sander, C. (2008) The microRNA.org resource: targets and expression. *Nucleic Acids Res*, **36**, D149-153.
79. Papadopoulos, G.L., Reczko, M., Simossis, V.A., Sethupathy, P. and Hatzigeorgiou, A.G. (2009) The database of experimentally supported targets: a functional update of TarBase. *Nucleic Acids Res*, **37**, D155-158.
80. Xiao, F., Zuo, Z., Cai, G., Kang, S., Gao, X. and Li, T. (2009) miRecords: an integrated resource for microRNA-target interactions. *Nucleic Acids Res*, **37**, D105-110.
81. Jiang, Q., Wang, Y., Hao, Y., Juan, L., Teng, M., Zhang, X., Li, M., Wang, G. and Liu, Y. (2009) miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res*, **37**, D98-104.
82. Naeem, H., Kuffner, R., Csaba, G. and Zimmer, R. (2010) miRSel: Automated extraction of associations between microRNAs and genes from the biomedical literature. *BMC Bioinformatics*, **11**, 135.
83. Kruger, J. and Rehmsmeier, M. (2006) RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic Acids Res*, **34**, W451-454.
84. Krek, A., Grun, D., Poy, M.N., Wolf, R., Rosenberg, L., Epstein, E.J., MacMenamin, P., da Piedade, I., Gunsalus, K.C., Stoffel, M. *et al.* (2005) Combinatorial microRNA target predictions. *Nat Genet*, **37**, 495-500.
85. Kertesz, M., Iovino, N., Unnerstall, U., Gaul, U. and Segal, E. (2007) The role of site accessibility in microRNA target recognition. *Nat Genet*, **39**, 1278-1284.
86. Dweep, H., Sticht, C., Pandey, P. and Gretz, N. (2011) miRWalk - Database: Prediction of possible miRNA binding sites by "walking" the genes of three genomes. *Journal of biomedical informatics*.
87. Wu, C.H., Apweiler, R., Bairoch, A., Natale, D.A., Barker, W.C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R. *et al.* (2006) The Universal Protein Resource (UniProt): an expanding universe of protein information. *Nucleic Acids Res*, **34**, D187-191.
88. Tsai, W.C., Hsu, P.W., Lai, T.C., Chau, G.Y., Lin, C.W., Chen, C.M., Lin, C.D., Liao, Y.L., Wang, J.L., Chau, Y.P. *et al.* (2009) MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology*, **49**, 1571-1582.
89. Okuda, S., Yamada, T., Hamajima, M., Itoh, M., Katayama, T., Bork, P., Goto, S. and Kanehisa, M. (2008) KEGG Atlas mapping for global analysis of metabolic pathways. *Nucleic Acids Res*, **36**, W423-426.
90. Huang da, W., Sherman, B.T. and Lempicki, R.A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, **4**, 44-57.
91. Maglott, D., Ostell, J., Pruitt, K.D. and Tatusova, T. (2007) Entrez Gene: gene-centered information at NCBI. *Nucleic Acids Res*, **35**, D26-31.
92. Rhead, B., Karolchik, D., Kuhn, R.M., Hinrichs, A.S., Zweig, A.S., Fujita, P.A., Diekhans, M., Smith, K.E., Rosenbloom, K.R., Raney, B.J. *et al.* (2010) The UCSC

Genome Browser database: update 2010. *Nucleic Acids Res*, **38**, D613-619.
93. Wu, C., Orozco, C., Boyer, J., Leglise, M., Goodale, J., Batalov, S., Hodge, C.L., Haase, J., Janes, J., Huss, J.W., 3rd *et al.* (2009) BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol*, **10**, R130.
94. Fernandez, J.M., Hoffmann, R. and Valencia, A. (2007) iHOP web services. *Nucleic Acids Res*, **35**, W21-26.
95. Bruford, E.A., Lush, M.J., Wright, M.W., Sneddon, T.P., Povey, S. and Birney, E. (2008) The HGNC Database in 2008: a resource for the human genome. *Nucleic Acids Res*, **36**, D445-448.
96. Friedman, R.C., Farh, K.K., Burge, C.B. and Bartel, D.P. (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*, **19**, 92-105.
97. Lall, S., Grun, D., Krek, A., Chen, K., Wang, Y.L., Dewey, C.N., Sood, P., Colombo, T., Bray, N., Macmenamin, P. *et al.* (2006) A genome-wide map of conserved microRNA targets in C. elegans. *Curr Biol*, **16**, 460-471.
98. Stark, A., Brennecke, J., Russell, R.B. and Cohen, S.M. (2003) Identification of Drosophila MicroRNA targets. *PLoS Biol*, **1**, E60.
99. Gaidatzis, D., van Nimwegen, E., Hausser, J. and Zavolan, M. (2007) Inference of miRNA targets using evolutionary conservation and pathway analysis. *BMC Bioinformatics*, **8**, 69.
100. Grun, D., Wang, Y.L., Langenberger, D., Gunsalus, K.C. and Rajewsky, N. (2005) microRNA target predictions across seven Drosophila species and comparison to mammalian targets. *PLoS Comput Biol*, **1**, e13.
101. Hsu S.D., L.F.-M., Wei-Yun Wu, Chao Liang, Wei-Chih Huang, Wen-Ling Chan, Wen-Ting Tsai, Goun-Zhou Chen, Chia-Jung Lee, Chih-Min Chiu, Chia-Hung Chien, Ming-Chia Wu, Chi-Ying Huang, Ann-Ping Tsou and Hsien-Da Huang. (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic Acids Research*, (in press).
102. Lewis, B.P., Shih, I.H., Jones-Rhoades, M.W., Bartel, D.P. and Burge, C.B. (2003) Prediction of mammalian microRNA targets. *Cell*, **115**, 787-798.
103. Enright, A.J., John, B., Gaul, U., Tuschl, T., Sander, C. and Marks, D.S. (2003) MicroRNA targets in Drosophila. *Genome Biol*, **5**, R1.
104. Kiriakidou, M., Nelson, P.T., Kouranov, A., Fitziev, P., Bouyioukos, C., Mourelatos, Z. and Hatzigeorgiou, A. (2004) A combined computational-experimental approach predicts human microRNA targets. *Genes Dev*, **18**, 1165-1178.
105. Megraw, M., Sethupathy, P., Corda, B. and Hatzigeorgiou, A.G. (2007) miRGen: a database for the study of animal microRNA genomic organization and function. *Nucleic Acids Res*, **35**, D149-155.
106. Roubelakis, M.G., Zotos, P., Papachristoudis, G., Michalopoulos, I., Pappa, K.I., Anagnou, N.P. and Kossida, S. (2009) Human microRNA target analysis and gene ontology clustering by GOmir, a novel stand-alone application. *BMC Bioinformatics*, **10 Suppl 6**, S20.
107. Zhang, Y. (2005) miRU: an automated plant miRNA target prediction server. *Nucleic Acids Res*, **33**, W701-704.
108. Rusinov, V., Baev, V., Minkov, I.N. and Tabler, M. (2005) MicroInspector: a web tool for detection of miRNA binding sites in an RNA sequence. *Nucleic Acids Res*, **33**, W696-700.
109. Miranda, K.C., Huynh, T., Tay, Y., Ang, Y.S., Tam, W.L., Thomson, A.M., Lim, B. and Rigoutsos, I. (2006) A pattern-based method for the identification of MicroRNA

binding sites and their corresponding heteroduplexes. *Cell*, **126**, 1203-1217.
110. Nam, S., Li, M., Choi, K., Balch, C., Kim, S. and Nephew, K.P. (2009) MicroRNA and mRNA integrated analysis (MMIA): a web tool for examining biological functions of microRNA expression. *Nucleic Acids Res*, **37**, W356-362.
111. Place, R.F., Li, L.C., Pookot, D., Noonan, E.J. and Dahiya, R. (2008) MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A*, **105**, 1608-1613.
112. Easow, G., Teleman, A.A. and Cohen, S.M. (2007) Isolation of microRNA targets by miRNP immunopurification. *RNA*, **13**, 1198-1204.
113. Kloosterman, W.P., Wienholds, E., Ketting, R.F. and Plasterk, R.H. (2004) Substrate requirements for let-7 function in the developing zebrafish embryo. *Nucleic Acids Res*, **32**, 6284-6291.
114. Stark, A., Lin, M.F., Kheradpour, P., Pedersen, J.S., Parts, L., Carlson, J.W., Crosby, M.A., Rasmussen, M.D., Roy, S., Deoras, A.N. *et al.* (2007) Discovery of functional elements in 12 Drosophila genomes using evolutionary signatures. *Nature*, **450**, 219-232.
115. Nakamoto, M., Jin, P., O'Donnell, W.T. and Warren, S.T. (2005) Physiological identification of human transcripts translationally regulated by a specific microRNA. *Hum Mol Genet*, **14**, 3813-3821.
116. Duursma, A.M., Kedde, M., Schrier, M., le Sage, C. and Agami, R. (2008) miR-148 targets human DNMT3b protein coding region. *RNA*, **14**, 872-877.
117. Forman, J.J., Legesse-Miller, A. and Coller, H.A. (2008) A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. *Proc Natl Acad Sci U S A*, **105**, 14879-14884.
118. Orom, U.A., Nielsen, F.C. and Lund, A.H. (2008) MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell*, **30**, 460-471.
119. Tay, Y., Zhang, J., Thomson, A.M., Lim, B. and Rigoutsos, I. (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature*, **455**, 1124-1128.
120. Zhou, X., Duan, X., Qian, J. and Li, F. (2009) Abundant conserved microRNA target sites in the 5'-untranslated region and coding sequence. *Genetica*.
121. Papadopoulos, G.L., Alexiou, P., Maragkakis, M., Reczko, M. and Hatzigeorgiou, A.G. (2009) DIANA-mirPath: Integrating human and mouse microRNAs in pathways. *Bioinformatics*, **25**, 1991-1993.
122. Maragkakis, M., Reczko, M., Simossis, V.A., Alexiou, P., Papadopoulos, G.L., Dalamagas, T., Giannopoulos, G., Goumas, G., Koukis, E., Kourtis, K. *et al.* (2009) DIANA-microT web server: elucidating microRNA functions through target prediction. *Nucleic Acids Res*, **37**, W273-276.
123. Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P. and Burge, C.B. (2008) Alternative isoform regulation in human tissue transcriptomes. *Nature*, **456**, 470-476.
124. Chen, L. and Zheng, S. (2009) Studying alternative splicing regulatory networks through partial correlation analysis. *Genome Biol*, **10**, R3.
125. Johnson, J.M., Castle, J., Garrett-Engele, P., Kan, Z., Loerch, P.M., Armour, C.D., Santos, R., Schadt, E.E., Stoughton, R. and Shoemaker, D.D. (2003) Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science*, **302**, 2141-2144.
126. Ghigna, C., Valacca, C. and Biamonti, G. (2008) Alternative splicing and tumor progression. *Curr Genomics*, **9**, 556-570.

127. Srebrow, A. and Kornblihtt, A.R. (2006) The connection between splicing and cancer. *J Cell Sci*, **119**, 2635-2641.
128. Brinkman, B.M. (2004) Splice variants as cancer biomarkers. *Clin Biochem*, **37**, 584-594.
129. Wang, G.S. and Cooper, T.A. (2007) Splicing in disease: disruption of the splicing code and the decoding machinery. *Nat Rev Genet*, **8**, 749-761.
130. Cartegni, L., Chew, S.L. and Krainer, A.R. (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet*, **3**, 285-298.
131. Cui, Q., Yu, Z., Purisima, E.O. and Wang, E. (2006) Principles of microRNA regulation of a human cellular signaling network. *Mol Syst Biol*, **2**, 46.
132. Iliopoulos, D., Malizos, K.N., Oikonomou, P. and Tsezou, A. (2008) Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One*, **3**, e3740.
133. Koscielny, G., Le Texier, V., Gopalakrishnan, C., Kumanduri, V., Riethoven, J.J., Nardone, F., Stanley, E., Fallsehr, C., Hofmann, O., Kull, M. *et al.* (2009) ASTD: The Alternative Splicing and Transcript Diversity database. *Genomics*, **93**, 213-220.
134. Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W. (2009) GenBank. *Nucleic Acids Res*, **37**, D26-31.
135. Sayers, E.W., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., DiCuccio, M., Edgar, R., Federhen, S. *et al.* (2009) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*, **37**, D5-15.
136. Kanehisa, M., Goto, S., Furumichi, M., Tanabe, M. and Hirakawa, M. (2010) KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res*, **38**, D355-360.
137. Brennecke, J., Stark, A., Russell, R.B. and Cohen, S.M. (2005) Principles of microRNA-target recognition. *PLoS Biol*, **3**, e85.
138. John, B., Enright, A.J., Aravin, A., Tuschl, T., Sander, C. and Marks, D.S. (2004) Human MicroRNA targets. *PLoS Biol*, **2**, e363.
139. Wang, Y., Sheng, G., Juranek, S., Tuschl, T. and Patel, D.J. (2008) Structure of the guide-strand-containing argonaute silencing complex. *Nature*, **456**, 209-213.
140. Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S. *et al.* (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res*, **15**, 1034-1050.
141. Bernhart, S.H., Hofacker, I.L. and Stadler, P.F. (2006) Local RNA base pairing probabilities in large sequences. *Bioinformatics*, **22**, 614-615.
142. Florea, L., Hartzell, G., Zhang, Z., Rubin, G.M. and Miller, W. (1998) A computer program for aligning a cDNA sequence with a genomic DNA sequence. *Genome Res*, **8**, 967-974.
143. Kapustin, Y., Souvorov, A., Tatusova, T. and Lipman, D. (2008) Splign: algorithms for computing spliced alignments with identification of paralogs. *Biol Direct*, **3**, 20.
144. Wheelan, S.J., Church, D.M. and Ostell, J.M. (2001) Spidey: a tool for mRNA-to-genomic alignments. *Genome Res*, **11**, 1952-1957.
145. Huang, H.D., Horng, J.T., Lin, F.M., Chang, Y.C. and Huang, C.C. (2005) SpliceInfo: an information repository for mRNA alternative splicing in human genome. *Nucleic Acids Res*, **33**, D80-85.

146. Wang, Y. and Lee, C.G. (2009) MicroRNA and cancer--focus on apoptosis. *J Cell Mol Med*, **13**, 12-23.
147. Sengupta, S., den Boon, J.A., Chen, I.H., Newton, M.A., Stanhope, S.A., Cheng, Y.J., Chen, C.J., Hildesheim, A., Sugden, B. and Ahlquist, P. (2008) MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. *Proc Natl Acad Sci U S A*, **105**, 5874-5878.
148. Lin, Y.C., Hsieh, L.C., Kuo, M.W., Yu, J., Kuo, H.H., Lo, W.L., Lin, R.J., Yu, A.L. and Li, W.H. (2007) Human TRIM71 and its nematode homologue are targets of let-7 microRNA and its zebrafish orthologue is essential for development. *Mol Biol Evol*, **24**, 2525-2534.
149. Hsu, S.D., Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M. *et al.* (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic Acids Res*, **39**, D163-169.
150. Kozomara, A. and Griffiths-Jones, S. (2011) miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res*, **39**, D152-157.
151. Pasquinelli, A.E., Reinhart, B.J., Slack, F., Martindale, M.Q., Kuroda, M.I., Maller, B., Hayward, D.C., Ball, E.E., Degnan, B., Muller, P. *et al.* (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature*, **408**, 86-89.
152. Takane, K., Fujishima, K., Watanabe, Y., Sato, A., Saito, N., Tomita, M. and Kanai, A. (2010) Computational prediction and experimental validation of evolutionarily conserved microRNA target genes in bilaterian animals. *BMC Genomics*, **11**, 101.
153. Lee, R.C., Feinbaum, R.L. and Ambros, V. (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, **75**, 843-854.
154. Kim, V.N. and Nam, J.W. (2006) Genomics of microRNA. *Trends Genet*, **22**, 165-173.
155. Wienholds, E. and Plasterk, R.H. (2005) MicroRNA function in animal development. *FEBS Lett*, **579**, 5911-5922.
156. Jover-Gil, S., Candela, H. and Ponce, M.R. (2005) Plant microRNAs and development. *Int J Dev Biol*, **49**, 733-744.
157. Sethupathy, P., Corda, B. and Hatzigeorgiou, A.G. (2006) TarBase: A comprehensive database of experimentally supported animal microRNA targets. *RNA*, **12**, 192-197.
158. Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A. *et al.* (2005) MicroRNA expression profiles classify human cancers. *Nature*, **435**, 834-838.
159. Shahi, P., Loukianiouk, S., Bohne-Lang, A., Kenzelmann, M., Kuffer, S., Maertens, S., Eils, R., Grone, H.J., Gretz, N. and Brors, B. (2006) Argonaute--a database for gene regulation by mammalian microRNAs. *Nucleic Acids Res*, **34**, D115-118.
160. Harris, M.A., Clark, J., Ireland, A., Lomax, J., Ashburner, M., Foulger, R., Eilbeck, K., Lewis, S., Marshall, B., Mungall, C. *et al.* (2004) The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res*, **32 Database issue**, D258-261.
161. Cotton, R.G., McKusick, V. and Scriver, C.R. (1998) The HUGO Mutation Database Initiative. *Science*, **279**, 10-11.
162. Karolchik, D., Baertsch, R., Diekhans, M., Furey, T.S., Hinrichs, A., Lu, Y.T., Roskin, K.M., Schwartz, M., Sugnet, C.W., Thomas, D.J. *et al.* (2003) The UCSC Genome Browser Database. *Nucleic Acids Res*, **31**, 51-54.
163. Hubbard, T., Andrews, D., Caccamo, M., Cameron, G., Chen, Y., Clamp, M., Clarke, L., Coates, G., Cox, T., Cunningham, F. *et al.* (2005) Ensembl 2005. *Nucleic Acids Res*,

33, D447-453.
164. Griffiths-Jones, S. (2004) The microRNA Registry. *Nucleic Acids Res*, **32**, D109-111.
165. Betel, D., Koppal, A., Agius, P., Sander, C. and Leslie, C. (2010) Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*, **11**, R90.
166. Kozomara, A. and Griffiths-Jones, S. (2011) miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic acids research*, **39**, D152-157.
167. Elefant, N., Berger, A., Shein, H., Hofree, M., Margalit, H. and Altuvia, Y. (2011) RepTar: a database of predicted cellular targets of host and viral miRNAs. *Nucleic acids research*, **39**, D188-194.
168. Maragkakis, M., Vergoulis, T., Alexiou, P., Reczko, M., Plomaritou, K., Gousis, M., Kourtis, K., Koziris, N., Dalamagas, T. and Hatzigeorgiou, A.G. (2011) DIANA-microT Web server upgrade supports Fly and Worm miRNA target prediction and bibliographic miRNA to disease association. *Nucleic acids research*.
169. Nielsen, C.B., Shomron, N., Sandberg, R., Hornstein, E., Kitzman, J. and Burge, C.B. (2007) Determinants of targeting by endogenous and exogenous microRNAs and siRNAs. *RNA*, **13**, 1894-1910.
170. Sturm, M., Hackenberg, M., Langenberger, D. and Frishman, D. (2010) TargetSpy: a supervised machine learning approach for microRNA target prediction. *BMC Bioinformatics*, **11**, 292.
171. Kuhn, R.M., Karolchik, D., Zweig, A.S., Trumbower, H., Thomas, D.J., Thakkapallayil, A., Sugnet, C.W., Stanke, M., Smith, K.E., Siepel, A. *et al.* (2007) The UCSC genome browser database: update 2007. *Nucleic Acids Res*, **35**, D668-673.
172. Vella, M.C., Choi, E.Y., Lin, S.Y., Reinert, K. and Slack, F.J. (2004) The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev*, **18**, 132-137.
173. Bernhart, S.H., Muckstein, U. and Hofacker, I.L. (2011) RNA Accessibility in cubic time. *Algorithms for molecular biology : AMB*, **6**, 3.
174. Su, A.I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K.A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G. *et al.* (2004) A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci U S A*, **101**, 6062-6067.
175. Barrett, T. and Edgar, R. (2006) Gene expression omnibus: microarray data storage, submission, retrieval, and analysis. *Methods Enzymol*, **411**, 352-369.
176. Shirdel, E.A., Xie, W., Mak, T.W. and Jurisica, I. (2011) NAViGaTing the micronome--using multiple microRNA prediction databases to identify signalling pathway-associated microRNAs. *PLoS One*, **6**, e17429.
177. Hsu, S.D., Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M. *et al.* (2010) miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic acids research*.
178. Lin, S.L., Miller, J.D. and Ying, S.Y. (2006) Intronic MicroRNA (miRNA). *J Biomed Biotechnol*, **2006**, 26818.
179. Mansfield, J.H., Harfe, B.D., Nissen, R., Obenauer, J., Srineel, J., Chaudhuri, A., Farzan-Kashani, R., Zuker, M., Pasquinelli, A.E., Ruvkun, G. *et al.* (2004) MicroRNA-responsive 'sensor' transgenes uncover Hox-like and other developmentally regulated patterns of vertebrate microRNA expression. *Nat Genet*, **36**, 1079-1083.

Appendix I Supporting material

The resources that have been developed in this thesis as the majority of the published data are available online. In the following, the corresponding web links and publications are provided. The links are stable links that belong to National Chiao Tung University.

Web links

miRTarBase

miRTarBase accumulates miRNA-target interactions, which were collected by manually surveying literature after a systematic text-mining process to filter research articles related to functional studies of miRNAs. The up-to-date MTIs could be accessed through: http://mirtarbase.mbc.nctu.edu.tw

Hsu, S.D., Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M. et al. (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic acids research, 39, D163-169.

miRTar

miRTar, an integrated system for identifying novel miRNA-target interactions, enables biologists to easily identify the biological functions and regulatory relationships between a group of known/putative miRNAs and protein coding genes. miRTar is available at: http://mirtar.mbc.nctu.edu.tw

J.B.K. Hsu, C.M. Chiu, S.D. Hsu, W.Y. Huang, C.H. Chien, T.Y. Lee and H.D. Huang miRTar: an integrated system for identifying miRNA-target interactions in Human. BMC Bioinformatics, 12.

miRNAMap

miRNAMap

http://mirnamap.mbc.nctu.edu.tw

Hsu, P.W., Huang, H.D., Hsu, S.D., Lin, L.Z., Tsou, A.P., Tseng, C.P., Stadler, P.F., Washietl, S. and Hofacker, I.L. (2006) miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. Nucleic Acids Res, 34, D135-139.

Hsu, S.D., Chu, C.H., Tsou, A.P., Chen, S.J., Chen, H.C., Hsu, P.W., Wong, Y.H., Chen, Y.H., Chen, G.H. and Huang, H.D. (2008) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. Nucleic Acids Res, 36, D165-169.

HomoloMTI

HomoloMTI aims to provide the homologous cluster of miRNA-target interations with indirect experimental evidence from miRTarBase. The corresponding publication has not been submitted yet.

http://homolomti.mbc.nctu.edu.tw

Lin, F.M., Hsu, S.D., Chou, C.H. and Huang, H.D. (2011) HomoloMTI: Homologous Cluster of MicroRNA Target Interactions. (In preparation)

Appendix II Experimentally verified miRNA target interactions in human

Table A1 List of human miRNA-target interactions (MTIs) that are supported by strong experimental evidences (reporter assay or western blot)

Type	miRNA	Target gene	Validation method
	hsa-let-7a	CASP3	Reporter assay; Western blot
	hsa-let-7a	CCND2	Reporter assay; Western blot
	hsa-let-7a	DICER1	Reporter assay; Western blot
	hsa-let-7a	E2F2	Reporter assay; Western blot
	hsa-let-7a	EIF2C4	Reporter assay
	hsa-let-7a	HMGA1	Reporter assay
	hsa-let-7a	HMGA2	Western blot
	hsa-let-7a	HRAS	Reporter assay
	hsa-let-7a	IGF2	Reporter assay
	hsa-let-7a	IL6	Reporter assay; Western blot
	hsa-let-7a	ITGB3	Reporter assay; Western blot
	hsa-let-7a	KRAS	Reporter assay
	hsa-let-7a	LIN28A	Reporter assay
	hsa-let-7a	MYC	Western blot
	hsa-let-7a	NF2	Reporter assay; Western blot
	hsa-let-7a	NKIRAS2	Reporter assay; Western blot
	hsa-let-7a	NRAS	Western blot
	hsa-let-7a	PRDM1	Reporter assay
	hsa-let-7a	RAVER2	Reporter assay; Western blot
	hsa-let-7a	UHRF2	Western blot
	hsa-let-7b	CCNA2	Reporter assay
	hsa-let-7b	CCND1	Reporter assay
Functional MTI	hsa-let-7b	CCND2	Reporter assay
	hsa-let-7b	CDC25A	Reporter assay; Western blot
	hsa-let-7b	CDC34	Reporter assay; Western blot
	hsa-let-7b	CDK6	Reporter assay; Western blot
	hsa-let-7b	HMGA2	Reporter assay
	hsa-let-7b	IFNB1	Reporter assay
	hsa-let-7b	LIN28A	Reporter assay
	hsa-let-7b	LIN28B	Reporter assay
	hsa-let-7b	NR2E1	Reporter assay; Western blot
	hsa-let-7b	NRAS	Reporter assay
	hsa-let-7b	PRDM1	Reporter assay; Western blot
	hsa-let-7b	RDH10	Reporter assay
	hsa-let-7c	BCL2L1	Reporter assay; Western blot
	hsa-let-7c	HMGA2	Western blot
	hsa-let-7c	MYC	Reporter assay
	hsa-let-7c	NRAS	Western blot
	hsa-let-7c	TGFBR1	Reporter assay; Western blot
	hsa-let-7c	TRIM71	Reporter assay
	hsa-let-7d	DICER1	Reporter assay; Western blot
	hsa-let-7d	HMGA2	Reporter assay
	hsa-let-7d	SLC11A2	Reporter assay; Western blot
	hsa-let-7e	HMGA2	Reporter assay
	hsa-let-7e	SMC1A	Reporter assay

hsa-let-7f	KLK10	Reporter assay
hsa-let-7f	KLK6	Reporter assay
hsa-let-7f	PRDM1	Reporter assay; Western blot
hsa-let-7g	BCL2L1	Reporter assay; Western blot
hsa-let-7g	CDKN2A	Reporter assay; Western blot
hsa-let-7g	COL1A2	Reporter assay; Western blot
hsa-let-7g	HMGA2	Western blot
hsa-let-7g	IGF2BP1	Western blot
hsa-let-7g	KRAS	Reporter assay
hsa-let-7g	MYC	Reporter assay; Western blot
hsa-let-7i	TLR4	Reporter assay; Western blot
hsa-miR-1	ADAR	Reporter assay
hsa-miR-1	ATP6V1B2	Reporter assay
hsa-miR-1	BDNF	Reporter assay
hsa-miR-1	CALM3	Reporter assay
hsa-miR-1	CAND1	Reporter assay
hsa-miR-1	CEBPA	Reporter assay
hsa-miR-1	CNN3	Reporter assay
hsa-miR-1	FOXP1	Western blot
hsa-miR-1	G6PD	Reporter assay
hsa-miR-1	GATA4	Reporter assay
hsa-miR-1	GJA1	Reporter assay
hsa-miR-1	HCN2	Reporter assay; Western blot
hsa-miR-1	HCN4	Reporter assay; Western blot
hsa-miR-1	HDAC4	Western blot
hsa-miR-1	HSPA4	Reporter assay
hsa-miR-1	HSPD1	Reporter assay
hsa-miR-1	KCNE1	Reporter assay; Western blot
hsa-miR-1	KCNJ2	Reporter assay
hsa-miR-1	KIF2A	Reporter assay
hsa-miR-1	LARP4	Reporter assay
hsa-miR-1	MEF2A	Reporter assay
hsa-miR-1	MET	Reporter assay; Western blot
hsa-miR-1	PAX3	Reporter assay; Western blot
hsa-miR-1	PIM1	Reporter assay; Western blot
hsa-miR-1	POGK	Reporter assay
hsa-miR-1	PPP2R5A	Reporter assay; Western blot
hsa-miR-1	SOX6	Reporter assay
hsa-miR-1	TAGLN2	Reporter assay
hsa-miR-1	TWF1	Reporter assay; Western blot
hsa-miR-100	FGFR3	Reporter assay; Western blot
hsa-miR-100	MMP13	Western blot
hsa-miR-100	PLK1	Reporter assay
hsa-miR-101	APP	Reporter assay; Western blot
hsa-miR-101	ATM	Reporter assay
hsa-miR-101	ATP5B	Western blot
hsa-miR-101	ATXN1	Reporter assay; Western blot
hsa-miR-101	EED	Western blot
hsa-miR-101	EZH2	Reporter assay; Western blot
hsa-miR-101	MCL1	Reporter assay; Western blot
hsa-miR-101	MYCN	Reporter assay
hsa-miR-101	PTGS2	Reporter assay; Western blot
hsa-miR-101*	ATM	Reporter assay
hsa-miR-101*	PRKDC	Reporter assay

hsa-miR-103	CCNE1	Reporter assay; Western blot
hsa-miR-103	CDK2	Reporter assay; Western blot
hsa-miR-103	CREB1	Reporter assay; Western blot
hsa-miR-103	DICER1	Reporter assay
hsa-miR-105	TLR2	Reporter assay
hsa-miR-106a	ARID4B	Reporter assay
hsa-miR-106a	CDKN1A	Reporter assay; Western blot
hsa-miR-106a	E2F1	Western blot
hsa-miR-106a	HIPK3	Reporter assay
hsa-miR-106a	IL10	Reporter assay
hsa-miR-106a	MYLIP	Reporter assay
hsa-miR-106a	RB1	Western blot
hsa-miR-106a	RUNX1	Reporter assay; Western blot
hsa-miR-106a	VEGFA	Reporter assay
hsa-miR-106b	APP	Reporter assay; Western blot
hsa-miR-106b	CDKN1A	Reporter assay
hsa-miR-106b	E2F1	Reporter assay
hsa-miR-106b	ITCH	Reporter assay
hsa-miR-106b	KAT2B	Reporter assay; Western blot
hsa-miR-106b	VEGFA	Reporter assay
hsa-miR-107	ARNT	Reporter assay; Western blot
hsa-miR-107	BACE1	Western blot
hsa-miR-107	CDK6	Western blot
hsa-miR-107	DICER1	Reporter assay
hsa-miR-107	FBXW7	Reporter assay; Western blot
hsa-miR-107	GRN	Western blot
hsa-miR-107	HIF1A	Reporter assay; Western blot
hsa-miR-107	MYB	Reporter assay; Western blot
hsa-miR-107	NFIA	Reporter assay
hsa-miR-107	PLAG1	Reporter assay
hsa-miR-107	VEGFA	Reporter assay
hsa-miR-10a	BTRC	Reporter assay; Western blot
hsa-miR-10a	HOXA1	Reporter assay; Western blot
hsa-miR-10a	MAP3K7	Reporter assay; Western blot
hsa-miR-10a	NCOR2	Western blot
hsa-miR-10a	USF2	Reporter assay; Western blot
hsa-miR-10b	HOXD10	Reporter assay; Western blot
hsa-miR-10b	KLF4	Reporter assay; Western blot
hsa-miR-10b	NCOR2	Western blot
hsa-miR-10b	NF1	Reporter assay
hsa-miR-10b	PPARA	Reporter assay; Western blot
hsa-miR-122	AACS	Reporter assay
hsa-miR-122	ADAM10	Reporter assay; Western blot
hsa-miR-122	ADAM17	Reporter assay
hsa-miR-122	AKT3	Reporter assay
hsa-miR-122	ALDOA	Reporter assay
hsa-miR-122	ANK2	Reporter assay
hsa-miR-122	ANXA11	Reporter assay
hsa-miR-122	AP3M2	Reporter assay
hsa-miR-122	ATP1A2	Reporter assay
hsa-miR-122	BCL2L2	Reporter assay; Western blot
hsa-miR-122	CCNG1	Reporter assay
hsa-miR-122	CYP7A1	Reporter assay
hsa-miR-122	DSTYK	Reporter assay

hsa-miR-122	DUSP2	Reporter assay
hsa-miR-122	EGLN3	Reporter assay
hsa-miR-122	ENTPD4	Reporter assay
hsa-miR-122	FAM117B	Reporter assay
hsa-miR-122	FOXJ3	Reporter assay
hsa-miR-122	FOXP1	Reporter assay
hsa-miR-122	FUNDC2	Reporter assay
hsa-miR-122	G6PC3	Reporter assay
hsa-miR-122	GALNT10	Reporter assay
hsa-miR-122	GYS1	Western blot
hsa-miR-122	IGF1R	Reporter assay; Western blot
hsa-miR-122	MAPK11	Reporter assay
hsa-miR-122	MECP2	Reporter assay
hsa-miR-122	NCAM1	Reporter assay
hsa-miR-122	NFATC2IP	Reporter assay
hsa-miR-122	NUMBL	Reporter assay
hsa-miR-122	PRKAB1	Reporter assay
hsa-miR-122	RAB11FIP1	Reporter assay
hsa-miR-122	RAB6B	Reporter assay
hsa-miR-122	RAC1	Reporter assay;
hsa-miR-122	RHOA	Reporter assay
hsa-miR-122	SLC7A1	Reporter assay; Western blot
hsa-miR-122	SLC7A11	Reporter assay
hsa-miR-122	SRF	Reporter assay; Western blot
hsa-miR-122	TBX19	Reporter assay
hsa-miR-122	TPD52L2	Reporter assay
hsa-miR-122	TRIB1	Reporter assay
hsa-miR-122	UBAP2	Reporter assay
hsa-miR-122	XPO6	Reporter assay
hsa-miR-1226	MUC1	Reporter assay
hsa-miR-124	CCL2	Reporter assay
hsa-miR-124	CDK2	Reporter assay; Western blot
hsa-miR-124	CDK6	Western blot
hsa-miR-124	CEBPA	Reporter assay
hsa-miR-124	E2F6	Reporter assay; Western blot
hsa-miR-124	EFNB1	Reporter assay; Western blot
hsa-miR-124	ELK3	Reporter assay
hsa-miR-124	IQGAP1	Reporter assay; Western blot
hsa-miR-124	NFKBIZ	Reporter assay; Western blot
hsa-miR-124	NR3C1	Reporter assay
hsa-miR-124	NR3C2	Reporter assay
hsa-miR-124	RDH10	Reporter assay
hsa-miR-124	RELA	Reporter assay
hsa-miR-124	SLC16A1	Reporter assay; Western blot
hsa-miR-124	SMYD3	Reporter assay; Western blot
hsa-miR-124	VIM	Reporter assay; Western blot
hsa-miR-1258	HPSE	Reporter assay
hsa-miR-125a-5p	ARID3B	Western blot
hsa-miR-125a-5p	BAK1	Reporter assay; Western blot
hsa-miR-125a-5p	CD34	Reporter assay; Western blot
hsa-miR-125a-5p	CDKN1A	Reporter assay; Western blot
hsa-miR-125a-5p	ELAVL1	Reporter assay; Western blot
hsa-miR-125a-5p	ERBB2	Reporter assay; Western blot
hsa-miR-125a-5p	ERBB3	Reporter assay; Western blot

hsa-miR-125a-5p	KLF13	Reporter assay; Western blot
hsa-miR-125a-5p	LIN28A	Reporter assay
hsa-miR-125a-5p	NTRK3	Reporter assay; Western blot
hsa-miR-125a-5p	TP53	Reporter assay; Western blot
hsa-miR-125b	AKT1	Western blot
hsa-miR-125b	BAK1	Reporter assay; Western blot
hsa-miR-125b	BMF	Reporter assay
hsa-miR-125b	BMPR1B	Reporter assay
hsa-miR-125b	CDKN2A	Western blot
hsa-miR-125b	CYP24A1	Reporter assay; Western blot
hsa-miR-125b	E2F3	Reporter assay; Western blot
hsa-miR-125b	ERBB2	Western blot
hsa-miR-125b	ERBB3	Reporter assay; Western blot
hsa-miR-125b	GLI1	Reporter assay
hsa-miR-125b	GRIN2A	Reporter assay
hsa-miR-125b	HMGA1	Reporter assay
hsa-miR-125b	HMGA2	Reporter assay
hsa-miR-125b	IRF4	Reporter assay
hsa-miR-125b	KLF13	Western blot
hsa-miR-125b	LIN28A	Reporter assay
hsa-miR-125b	NKIRAS2	Reporter assay; Western blot
hsa-miR-125b	NTRK3	Reporter assay; Western blot
hsa-miR-125b	PRDM1	Reporter assay
hsa-miR-125b	RAF1	Western blot
hsa-miR-125b	SMO	Reporter assay; Western blot
hsa-miR-125b	TP53	Reporter assay; Western blot
hsa-miR-125b	VDR	Reporter assay; Western blot
hsa-miR-126	CRK	Reporter assay
hsa-miR-126	HOXA9	Reporter assay; Western blot
hsa-miR-126	IRS1	Reporter assay; Western blot
hsa-miR-126	PIK3R2	Reporter assay; Western blot
hsa-miR-126	PLK2	Reporter assay
hsa-miR-126	SOX2	Reporter assay; Western blot
hsa-miR-126	SPRED1	Reporter assay; Western blot
hsa-miR-126	VCAM1	Reporter assay; Western blot
hsa-miR-126	VEGFA	Reporter assay
hsa-miR-126*	SLC45A3	Reporter assay; Western blot
hsa-miR-127-3p	BCL6	Reporter assay
hsa-miR-127-3p	PRDM1	Reporter assay; Western blot
hsa-miR-127-3p	XBP1	Reporter assay
hsa-miR-128	AFF1	Reporter assay; Western blot
hsa-miR-128	BMI1	Western blot
hsa-miR-128	CDKN1B	Reporter assay; Western blot
hsa-miR-128	DCX	Reporter assay
hsa-miR-128	E2F3	Reporter assay
hsa-miR-128	EGFR	Reporter assay; Western blot
hsa-miR-128	FBXW7	Reporter assay; Western blot
hsa-miR-128	MLL	Reporter assay; Western blot
hsa-miR-128	NTRK3	Reporter assay
hsa-miR-128	RELN	Western blot
hsa-miR-128	TGFBR1	Reporter assay; Western blot
hsa-miR-1285	TP53	Reporter assay; Western blot
hsa-miR-129-5p	CAMTA1	Reporter assay
hsa-miR-129-5p	EIF2C3	Reporter assay

hsa-miR-129-5p	NOTCH1	Reporter assay
hsa-miR-1296	MCM2	Western blot
hsa-miR-130a	ATXN1	Reporter assay; Western blot
hsa-miR-130a	CSF1	Reporter assay
hsa-miR-130a	HOXA10	Reporter assay
hsa-miR-130a	HOXA5	Reporter assay; Western blot
hsa-miR-130a	KLF4	Reporter assay
hsa-miR-130a	MAFB	Reporter assay
hsa-miR-130a	MEOX2	Reporter assay; Western blot
hsa-miR-130a	TAC1	Reporter assay
hsa-miR-130b	RUNX3	Western blot
hsa-miR-130b	TP53INP1	Reporter assay; Western blot
hsa-miR-132	ARHGAP32	Reporter assay; Western blot
hsa-miR-132	CDKN1A	Reporter assay; Western blot
hsa-miR-132	SIRT1	Reporter assay; Western blot
hsa-miR-133a	CACNA1C	Reporter assay; Western blot
hsa-miR-133a	CASP9	Reporter assay
hsa-miR-133a	FSCN1	Reporter assay; Western blot
hsa-miR-133a	HCN2	Reporter assay; Western blot
hsa-miR-133a	HCN4	Reporter assay
hsa-miR-133a	KCNH2	Reporter assay; Western blot
hsa-miR-133a	KCNQ1	Reporter assay; Western blot
hsa-miR-133a	TAGLN2	Reporter assay; Western blot
hsa-miR-133b	BCL2L2	Western blot
hsa-miR-133b	IGF1R	Reporter assay
hsa-miR-133b	KCNH2	Reporter assay; Western blot
hsa-miR-133b	MCL1	Western blot
hsa-miR-133b	PITX3	Reporter assay
hsa-miR-134	ABCC1	Western blot
hsa-miR-134	VEGFA	Reporter assay
hsa-miR-135a	APC	Reporter assay
hsa-miR-135a	JAK2	Reporter assay; Western blot
hsa-miR-135a	NR3C2	Reporter assay
hsa-miR-135b	APC	Reporter assay
hsa-miR-135b	KLF4	Reporter assay
hsa-miR-135b	MAFB	Reporter assay
hsa-miR-137	CDC42	Reporter assay; Western blot
hsa-miR-137	CDK6	Reporter assay; Western blot
hsa-miR-137	CTBP1	Reporter assay; Western blot
hsa-miR-137	KDM1A	Reporter assay; Western blot
hsa-miR-138	EID1	Reporter assay; Western blot
hsa-miR-138	RHOC	Reporter assay; Western blot
hsa-miR-138	ROCK2	Reporter assay; Western blot
hsa-miR-138	TERT	Reporter assay; Western blot
hsa-miR-140-5p	HDAC4	Western blot
hsa-miR-140-5p	VEGFA	Reporter assay
hsa-miR-141	BRD3	Reporter assay; Western blot
hsa-miR-141	CLOCK	Reporter assay
hsa-miR-141	DLX5	Reporter assay
hsa-miR-141	EIF4E	Reporter assay; Western blot
hsa-miR-141	PTEN	Reporter assay; Western blot
hsa-miR-141	SFPQ	Reporter assay; Western blot
hsa-miR-141	SIP1	Reporter assay; Western blot
hsa-miR-141	TGFB2	Reporter assay

hsa-miR-141	TRAPPC2P1	Reporter assay
hsa-miR-141	UBAP1	Reporter assay; Western blot
hsa-miR-141	ZFPM2	Reporter assay; Western blot
hsa-miR-143	COL1A1	Reporter assay
hsa-miR-143	DNMT3A	Reporter assay; Western blot
hsa-miR-143	FNDC3B	Reporter assay; Western blot
hsa-miR-143	HRAS	Reporter assay
hsa-miR-143	KRAS	Western blot
hsa-miR-143	MAPK7	Western blot
hsa-miR-143	MYO6	Reporter assay; Western blot
hsa-miR-144	FGG	Reporter assay
hsa-miR-144	PLAG1	Reporter assay
hsa-miR-145	BNIP3	Reporter assay
hsa-miR-145	CBFB	Reporter assay
hsa-miR-145	CLINT1	Reporter assay
hsa-miR-145	DFFA	Reporter assay; Western blot
hsa-miR-145	FLI1	Reporter assay; Western blot
hsa-miR-145	FSCN1	Reporter assay; Western blot
hsa-miR-145	IFNB1	Reporter assay
hsa-miR-145	IGF1R	Western blot
hsa-miR-145	IRS1	Reporter assay; Western blot
hsa-miR-145	KLF4	Reporter assay; Western blot
hsa-miR-145	MUC1	Reporter assay; Western blot
hsa-miR-145	MYC	Reporter assay; Western blot
hsa-miR-145	MYO6	Reporter assay; Western blot
hsa-miR-145	PARP8	Reporter assay
hsa-miR-145	POU5F1	Reporter assay; Western blot
hsa-miR-145	PPP3CA	Reporter assay
hsa-miR-145	SOX2	Reporter assay; Western blot
hsa-miR-145	STAT1	Reporter assay; Western blot
hsa-miR-145	TIRAP	Western blot
hsa-miR-145	YES1	Reporter assay; Western blot
hsa-miR-146a	BRCA1	Reporter assay
hsa-miR-146a	BRCA2	Reporter assay
hsa-miR-146a	CCNA2	Western blot
hsa-miR-146a	CD40LG	Reporter assay; Western blot
hsa-miR-146a	CFH	Western blot
hsa-miR-146a	CXCR4	Reporter assay; Western blot
hsa-miR-146a	EGFR	Western blot
hsa-miR-146a	FADD	Reporter assay
hsa-miR-146a	FAF1	Reporter assay
hsa-miR-146a	FAS	Reporter assay; Western blot
hsa-miR-146a	IL8	Western blot
hsa-miR-146a	IRAK1	Western blot
hsa-miR-146a	IRAK2	Western blot
hsa-miR-146a	MTA2	Western blot
hsa-miR-146a	NFKB1	Reporter assay
hsa-miR-146a	PA2G4	Western blot
hsa-miR-146a	ROCK1	Western blot
hsa-miR-146a	SNAP25	Reporter assay; Western blot
hsa-miR-146a	TLR2	Reporter assay; Western blot
hsa-miR-146a	TRAF6	Western blot
hsa-miR-146b-5p	IRAK1	Reporter assay
hsa-miR-146b-5p	KIT	Western blot

hsa-miR-146b-5p	MMP16	Reporter assay; Western blot
hsa-miR-146b-5p	NFKB1	Reporter assay
hsa-miR-146b-5p	TRAF6	Reporter assay
hsa-miR-147	VEGFA	Reporter assay
hsa-miR-148a	DNMT1	Reporter assay
hsa-miR-148a	DNMT3B	Reporter assay; Western blot
hsa-miR-148a	HLA-G	Reporter assay
hsa-miR-148a	NR1I2	Reporter assay
hsa-miR-148a	RPS6KA5	Reporter assay; Western blot
hsa-miR-148a	TGIF2	Reporter assay; Western blot
hsa-miR-148b	HLA-G	Reporter assay
hsa-miR-148b*	MCL1	Reporter assay; Western blot
hsa-miR-149*	AKT1	Reporter assay; Western blot
hsa-miR-149*	E2F1	Reporter assay; Western blot
hsa-miR-150	EGR2	Reporter assay; Western blot
hsa-miR-150	MYB	Western blot
hsa-miR-150	P2RX7	Reporter assay
hsa-miR-150	VEGFA	Reporter assay
hsa-miR-151-5p	ARHGDIA	Reporter assay; Western blot
hsa-miR-152	DNMT1	Reporter assay
hsa-miR-152	HLA-G	Reporter assay
hsa-miR-153	BCL2	Reporter assay; Western blot
hsa-miR-153	FOXO1	Western blot
hsa-miR-153	MCL1	Reporter assay; Western blot
hsa-miR-153	SNCA	Reporter assay; Western blot
hsa-miR-155	AGTR1	Reporter assay
hsa-miR-155	APC	Reporter assay; Western blot
hsa-miR-155	ARID2	Reporter assay
hsa-miR-155	BACH1	Reporter assay
hsa-miR-155	CYR61	Reporter assay; Western blot
hsa-miR-155	DET1	Reporter assay
hsa-miR-155	EDN1	Reporter assay
hsa-miR-155	ETS1	Reporter assay; Western blot
hsa-miR-155	FOXO3	Reporter assay; Western blot
hsa-miR-155	HIVEP2	Reporter assay
hsa-miR-155	IFNGR1	Reporter assay
hsa-miR-155	IKBKE	Western blot
hsa-miR-155	JARID2	Reporter assay
hsa-miR-155	LDOC1	Reporter assay
hsa-miR-155	MATR3	Reporter assay
hsa-miR-155	MECP2	Reporter assay; Western blot
hsa-miR-155	MEIS1	Reporter assay; Western blot
hsa-miR-155	MLH1	Reporter assay; Western blot
hsa-miR-155	MSH2	Reporter assay; Western blot
hsa-miR-155	MSH6	Reporter assay; Western blot
hsa-miR-155	MYO10	Reporter assay; Western blot
hsa-miR-155	RHOA	Reporter assay
hsa-miR-155	RUNX2	Reporter assay; Western blot
hsa-miR-155	SMAD1	Reporter assay; Western blot
hsa-miR-155	SMAD5	Reporter assay
hsa-miR-155	SOCS1	Reporter assay; Western blot
hsa-miR-155	SPI1	Reporter assay
hsa-miR-155	TAB2	Reporter assay; Western blot
hsa-miR-155	TM6SF1	Reporter assay

hsa-miR-155	TP53INP1	Reporter assay; Western blot
hsa-miR-155	TSHZ3	Reporter assay
hsa-miR-155	ZIC3	Reporter assay
hsa-miR-155	ZNF652	Reporter assay
hsa-miR-15a	BCL2	Reporter assay
hsa-miR-15a	BMI1	Reporter assay; Western blot
hsa-miR-15a	BRCA1	Reporter assay
hsa-miR-15a	CADM1	Reporter assay
hsa-miR-15a	CCND1	Reporter assay; Western blot
hsa-miR-15a	CCND2	Reporter assay
hsa-miR-15a	CCNE1	Reporter assay; Western blot
hsa-miR-15a	CDC25A	Reporter assay
hsa-miR-15a	CHUK	Reporter assay; Western blot
hsa-miR-15a	DMTF1	Reporter assay
hsa-miR-15a	MYB	Reporter assay; Western blot
hsa-miR-15a	TSPYL2	Reporter assay
hsa-miR-15a	UCP2	Reporter assay
hsa-miR-15a	VEGFA	Reporter assay
hsa-miR-15a	WNT3A	Reporter assay
hsa-miR-15b	BCL2	Reporter assay; Western blot
hsa-miR-15b	CCNE1	Reporter assay; Western blot
hsa-miR-15b	EIF4A1	Reporter assay
hsa-miR-15b	RECK	Reporter assay; Western blot
hsa-miR-15b	VEGFA	Reporter assay
hsa-miR-16	ACVR2A	Reporter assay; Western blot
hsa-miR-16	BCL2	Reporter assay
hsa-miR-16	BMI1	Reporter assay; Western blot
hsa-miR-16	BRCA1	Reporter assay
hsa-miR-16	CADM1	Reporter assay
hsa-miR-16	CAPRIN1	Reporter assay; Western blot
hsa-miR-16	CCND1	Western blot
hsa-miR-16	CCND3	Reporter assay; Western blot
hsa-miR-16	CCNE1	Reporter assay; Western blot
hsa-miR-16	CDK6	Reporter assay; Western blot
hsa-miR-16	CHUK	Reporter assay; Western blot
hsa-miR-16	HMGA1	Reporter assay; Western blot
hsa-miR-16	MYB	Reporter assay
hsa-miR-16	PPM1D	Northern blot; Western blot
hsa-miR-16	TPPP3	Reporter assay
hsa-miR-16	WNT3A	Reporter assay
hsa-miR-16-1*	CCND1	Reporter assay; Western blot
hsa-miR-16-2*	RARB	Western blot
hsa-miR-17	APP	Reporter assay; Western blot
hsa-miR-17	BCL2	Reporter assay
hsa-miR-17	BCL2L11	Reporter assay; Western blot
hsa-miR-17	BMPR2	Reporter assay; Western blot
hsa-miR-17	CCL1	Reporter assay
hsa-miR-17	CCND1	Reporter assay; Western blot
hsa-miR-17	CDKN1A	Reporter assay; Western blot
hsa-miR-17	DNAJC27	Reporter assay
hsa-miR-17	E2F1	Western blot
hsa-miR-17	FBXO31	Reporter assay
hsa-miR-17	GPR137B	Reporter assay
hsa-miR-17	JAK1	Reporter assay; Western blot

hsa-miR-17	MAP3K12	Reporter assay
hsa-miR-17	MAPK9	Reporter assay; Western blot
hsa-miR-17	MEF2D	Reporter assay
hsa-miR-17	MYC	Western blot
hsa-miR-17	NCOA3	Reporter assay; Western blot
hsa-miR-17	NPAT	Reporter assay
hsa-miR-17	OBFC2A	Reporter assay
hsa-miR-17	PKD2	Reporter assay; Western blot
hsa-miR-17	PTEN	Reporter assay; Western blot
hsa-miR-17	PTPRO	Reporter assay; Western blot
hsa-miR-17	RUNX1	Reporter assay
hsa-miR-17	TGFBR2	Reporter assay; Western blot
hsa-miR-17	TNFSF12	Reporter assay
hsa-miR-17	VEGFA	Reporter assay
hsa-miR-17	YES1	Reporter assay
hsa-miR-17	ZNFX1	Reporter assay
hsa-miR-17*	ICAM1	Western blot
hsa-miR-17*	VIM	Western blot
hsa-miR-181a	ATM	Reporter assay
hsa-miR-181a	BCL2	Reporter assay; Western blot
hsa-miR-181a	CDKN1B	Reporter assay; Western blot
hsa-miR-181a	CDX2	Reporter assay
hsa-miR-181a	DDIT4	Reporter assay
hsa-miR-181a	GATA6	Reporter assay
hsa-miR-181a	HIPK2	Reporter assay
hsa-miR-181a	KAT2B	Reporter assay; Western blot
hsa-miR-181a	NLK	Reporter assay
hsa-miR-181a	PLAG1	Reporter assay; Western blot
hsa-miR-181a	PROX1	Reporter assay; Western blot
hsa-miR-181a	ZNF763	Reporter assay; Western blot
hsa-miR-181b	BCL2	Reporter assay; Western blot
hsa-miR-181b	CDX2	Reporter assay
hsa-miR-181b	CYLD	Reporter assay
hsa-miR-181b	GATA6	Reporter assay
hsa-miR-181b	GRIA2	Reporter assay
hsa-miR-181b	KAT2B	Reporter assay; Western blot
hsa-miR-181b	MAP3K10	Western blot
hsa-miR-181b	NLK	Reporter assay
hsa-miR-181b	PLAG1	Reporter assay; Western blot
hsa-miR-181b	TCL1A	Reporter assay; Western blot
hsa-miR-181b	TIMP3	Reporter assay; Western blot
hsa-miR-181b	VSNL1	Reporter assay
hsa-miR-181c	BCL2	Reporter assay; Western blot
hsa-miR-181c	CDX2	Reporter assay
hsa-miR-181c	GATA6	Reporter assay
hsa-miR-181c	KRAS	Reporter assay; Western blot
hsa-miR-181c	NLK	Reporter assay
hsa-miR-181c	NOTCH4	Reporter assay; Western blot
hsa-miR-181d	BCL2	Reporter assay; Western blot
hsa-miR-182	ADCY6	Reporter assay
hsa-miR-182	CLOCK	Reporter assay
hsa-miR-182	FOXO1	Reporter assay; Western blot
hsa-miR-182	FOXO3	Reporter assay; Western blot
hsa-miR-182	MITF	Reporter assay

hsa-miR-182	RARG	Reporter assay; Western blot
hsa-miR-182	TSC22D3	Reporter assay
hsa-miR-183	BTRC	Western blot
hsa-miR-183	EZR	Reporter assay
hsa-miR-183	FOXO1	Western blot
hsa-miR-183	ITGB1	Reporter assay
hsa-miR-183	KIF2A	Reporter assay
hsa-miR-183	PDCD4	Reporter assay; Western blot
hsa-miR-184	AKT2	Reporter assay; Western blot
hsa-miR-184	NFATC2	Reporter assay; Western blot
hsa-miR-185	CDC42	Reporter assay; Western blot
hsa-miR-185	CDK6	Western blog
hsa-miR-185	RHOA	Reporter assay; Western blot
hsa-miR-185	SIX1	Reporter assay; Western blot
hsa-miR-186	FOXO1	Western blot
hsa-miR-186	P2RX7	Reporter assay
hsa-miR-18a	CTGF	Western blot
hsa-miR-18a	ESR1	Western blot
hsa-miR-18a	HSF2	Reporter assay; Western blot
hsa-miR-18a	NR3C1	Reporter assay
hsa-miR-18a	SMAD4	Reporter assay; Western blot
hsa-miR-18a	TNFSF11	Reporter assay
hsa-miR-18a*	KRAS	Reporter assay; Western blot
hsa-miR-18b	ESR1	Reporter assay
hsa-miR-191	IL1A	Reporter assay
hsa-miR-191	SOX4	Reporter assay
hsa-miR-191	TMC7	Reporter assay
hsa-miR-192	BCL2	Reporter assay
hsa-miR-192	CDC7	Reporter assay; Western blot
hsa-miR-192	CUL5	Reporter assay; Western blot
hsa-miR-192	DLG5	Reporter assay
hsa-miR-192	DTL	Reporter assay
hsa-miR-192	ERCC3	Reporter assay
hsa-miR-192	HOXA10	Reporter assay
hsa-miR-192	HRH1	Reporter assay
hsa-miR-192	KIF20B	Reporter assay
hsa-miR-192	LMNB2	Reporter assay; Western blot
hsa-miR-192	MAD2L1	Reporter assay; Western blot
hsa-miR-192	MCM10	Reporter assay
hsa-miR-192	MIS12	Reporter assay
hsa-miR-192	PIM1	Reporter assay
hsa-miR-192	PRPF38A	Reporter assay
hsa-miR-192	RACGAP1	Reporter assay
hsa-miR-192	SEPT10	Reporter assay
hsa-miR-192	SMARCB1	Reporter assay
hsa-miR-192	TRAPPC2P1	Reporter assay
hsa-miR-192	WNK1	Reporter assay; Western blot
hsa-miR-193a-3p	E2F6	Reporter assay; Western blot
hsa-miR-193a-5p	TP73	Reporter assay; Western blot
hsa-miR-193b	CCND1	Reporter assay; Western blot
hsa-miR-193b	ESR1	Reporter assay
hsa-miR-193b	ETS1	Reporter assay; Western blot
hsa-miR-193b	MCL1	Reporter assay; Western blot
hsa-miR-193b	PLAU	Reporter assay; Western blot

hsa-miR-195	BCL2	Reporter assay; Western blot
hsa-miR-195	CCL4	Western blot
hsa-miR-195	VEGFA	Reporter assay
hsa-miR-195	WEE1	Reporter assay; Western blot
hsa-miR-196a	ANXA1	Reporter assay; Western blot
hsa-miR-196a	BACH1	Reporter assay; Western blot
hsa-miR-196a	HMOX1	Reporter assay; Western blot
hsa-miR-196a	HOXA7	Western blot
hsa-miR-196a	HOXB7	Reporter assay; Western blot
hsa-miR-196a	HOXB8	Western blot
hsa-miR-196a	HOXC8	Reporter assay; Western blot
hsa-miR-196a	HOXD8	Western blot
hsa-miR-196a	KRT5	Reporter assay
hsa-miR-196a	S100A9	Reporter assay
hsa-miR-196a	SPRR2C	Reporter assay
hsa-miR-196b	HOXB8	Reporter assay
hsa-miR-196b	HOXC8	Reporter assay
hsa-miR-197	TUSC2	Reporter assay; Western blot
hsa-miR-198	CCNT1	Reporter assay
hsa-miR-199a-3p	CD44	Reporter assay; Western blot
hsa-miR-199a-3p	MAPK1	Western blot
hsa-miR-199a-3p	MET	Reporter assay; Western blot
hsa-miR-199a-5p	DDR1	Reporter assay; Western blot
hsa-miR-199a-5p	EDN1	Reporter assay
hsa-miR-199a-5p	IKBKB	Reporter assay; Western blot
hsa-miR-199a-5p	LIF	Reporter assay
hsa-miR-199b-3p	MAPK1	Reporter assay
hsa-miR-199b-3p	MET	Reporter assay
hsa-miR-199b-5p	HES1	Reporter assay; Western blot
hsa-miR-199b-5p	LAMC2	Reporter assay
hsa-miR-199b-5p	SET	Western blot
hsa-miR-19a	ATXN1	Reporter assay; Western blot
hsa-miR-19a	BCL2L11	Reporter assay
hsa-miR-19a	CCND1	Reporter assay; Western blot
hsa-miR-19a	ERBB4	Reporter assay
hsa-miR-19a	ESR1	Reporter assay; Western blot
hsa-miR-19a	HOXA5	Reporter assay
hsa-miR-19a	MECP2	Reporter assay
hsa-miR-19a	NR4A2	Reporter assay
hsa-miR-19a	PRMT5	Western blot
hsa-miR-19a	PTEN	Western blot
hsa-miR-19a	SOCS1	Reporter assay; Western blot
hsa-miR-19b	ATXN1	Reporter assay; Western blot
hsa-miR-19b	BCL2L11	Reporter assay
hsa-miR-19b	HIPK3	Reporter assay
hsa-miR-19b	MYLIP	Reporter assay
hsa-miR-19b	PTEN	Reporter assay; Western blot
hsa-miR-19b	SOCS1	Reporter assay; Western blot
hsa-miR-200a	CTNNB1	Reporter assay; Western blot
hsa-miR-200a	DLX5	Reporter assay
hsa-miR-200a	SIP1	Western blot
hsa-miR-200a	TRAPPC2P1	Reporter assay
hsa-miR-200a	WASF3	Reporter assay
hsa-miR-200a	ZEB1	Reporter assay; Western blot

hsa-miR-200a	ZEB2	Reporter assay
hsa-miR-200a	ZFPM2	Reporter assay; Western blot
hsa-miR-200b	ETS1	Reporter assay; Western blot
hsa-miR-200b	MATR3	Reporter assay
hsa-miR-200b	PTPN12	Northern blot; Western blot
hsa-miR-200b	RERE	Reporter assay
hsa-miR-200b	SIP1	Reporter assay; Western blot
hsa-miR-200b	WASF3	Reporter assay
hsa-miR-200b	ZEB1	Reporter assay
hsa-miR-200b	ZEB2	Reporter assay
hsa-miR-200b	ZFPM2	Reporter assay; Western blot
hsa-miR-200c	BMI1	Reporter assay; Western blot
hsa-miR-200c	PTPN13	Reporter assay; Western blot
hsa-miR-200c	TUBB3	Reporter assay
hsa-miR-200c	ZEB1	Reporter assay
hsa-miR-200c	ZFPM2	Reporter assay; Western blot
hsa-miR-203	ABCE1	Reporter assay; Western blot
hsa-miR-203	ABL1	Reporter assay
hsa-miR-203	BCL2L2	Reporter assay; Western blot
hsa-miR-203	CDK6	Reporter assay; Western blot
hsa-miR-203	SOCS3	Western blot
hsa-miR-203	TP63	Reporter assay
hsa-miR-204	BCL2	Reporter assay
hsa-miR-204	HOXA10	Western blot
hsa-miR-204	MEIS1	Reporter assay; Western blot
hsa-miR-204	MEIS2	Reporter assay; Western blot
hsa-miR-204	SNAI2	Reporter assay
hsa-miR-204	SPDEF	Reporter assay; Western blot
hsa-miR-204	TGFBR2	Reporter assay
hsa-miR-204	THRB	Reporter assay; Western blot
hsa-miR-205	ERBB3	Reporter assay
hsa-miR-205	IL24	Reporter assay; Western blot
hsa-miR-205	IL32	Reporter assay; Western blot
hsa-miR-205	INPPL1	Reporter assay; Western blot
hsa-miR-205	LRP1	Reporter assay; Western blot
hsa-miR-205	SIP1	Reporter assay; Western blot
hsa-miR-205	ZEB1	Reporter assay; Western blot
hsa-miR-206	ESR1	Reporter assay; Western blot
hsa-miR-206	MET	Western blot
hsa-miR-206	NOTCH3	Reporter assay; Western blot
hsa-miR-206	PAX3	Reporter assay; Western blot
hsa-miR-206	TAC1	Reporter assay
hsa-miR-208a	CDKN1A	Reporter assay; Western blot
hsa-miR-208a	ETS1	Reporter assay; Western blot
hsa-miR-208a	MED13	Reporter assay; Western blot
hsa-miR-208b	CDKN1A	Reporter assay; Western blot
hsa-miR-20a	APP	Reporter assay; Western blot
hsa-miR-20a	BCL2	Reporter assay
hsa-miR-20a	BMPR2	Reporter assay; Western blot
hsa-miR-20a	BNIP2	Western blot
hsa-miR-20a	CCND1	Reporter assay; Western blot
hsa-miR-20a	CDKN1A	Reporter assay; Western blot
hsa-miR-20a	E2F1	Western blot
hsa-miR-20a	HIF1A	Reporter assay; Western blot

hsa-miR-20a	MAP3K12	Reporter assay
hsa-miR-20a	MEF2D	Reporter assay
hsa-miR-20a	MYC	Western blot
hsa-miR-20a	RUNX1	Reporter assay; Western blot
hsa-miR-20a	TGFBR2	Reporter assay; Western blot
hsa-miR-20a	VEGFA	Reporter assay
hsa-miR-20b	ARID4B	Reporter assay
hsa-miR-20b	BAMBI	Reporter assay
hsa-miR-20b	CDKN1A	Reporter assay; Western blot
hsa-miR-20b	CRIM1	Reporter assay
hsa-miR-20b	ESR1	Western blot
hsa-miR-20b	HIF1A	Western blot
hsa-miR-20b	HIPK3	Reporter assay
hsa-miR-20b	MYLIP	Reporter assay
hsa-miR-20b	PPARG	Reporter assay
hsa-miR-20b	STAT3	Western blot
hsa-miR-20b	VEGFA	Reporter assay
hsa-miR-21	APAF1	Reporter assay; Western blot
hsa-miR-21	BASP1	Reporter assay
hsa-miR-21	BCL2	Reporter assay
hsa-miR-21	BMPR2	Reporter assay; Western blot
hsa-miR-21	BTG2	Reporter assay; Western blot
hsa-miR-21	DAXX	Reporter assay; Western blot
hsa-miR-21	DERL1	Reporter assay
hsa-miR-21	E2F1	Western blot
hsa-miR-21	ERBB2	Western blot
hsa-miR-21	FMOD	Western blot
hsa-miR-21	HNRNPK	Reporter assay
hsa-miR-21	JMY	Reporter assay
hsa-miR-21	LRRFIP1	Reporter assay; Western blot
hsa-miR-21	MARCKS	Reporter assay; Western blot
hsa-miR-21	MSH2	Reporter assay; Western blot
hsa-miR-21	MSH6	Reporter assay; Western blot
hsa-miR-21	MTAP	Western blot
hsa-miR-21	NCAPG	Reporter assay
hsa-miR-21	NFIB	Reporter assay; Western blot
hsa-miR-21	PDCD4	Reporter assay; Westren blot
hsa-miR-21	PLOD3	Reporter assay
hsa-miR-21	PPIF	Reporter assay
hsa-miR-21	PTEN	Western blot
hsa-miR-21	RASGRP1	Reporter assay; Western blot
hsa-miR-21	RECK	Reporter assay; Western blot
hsa-miR-21	REST	Reporter assay
hsa-miR-21	RHOB	Reporter assay; Western blot
hsa-miR-21	RTN4	Reporter assay
hsa-miR-21	SERPINB5	Reporter assay; Western blot
hsa-miR-21	SOX5	Western blot
hsa-miR-21	SPRY2	Reporter assay
hsa-miR-21	TGFBI	Reporter assay; Western blot
hsa-miR-21	TGFBR2	Western blot
hsa-miR-21	TGFBR3	Reporter assay
hsa-miR-21	TGIF1	Western blot
hsa-miR-21	TIAM1	Reporter assay
hsa-miR-21	TIMP3	Reporter assay

hsa-miR-21	TM9SF3	Reporter assay
hsa-miR-21	TOPORS	Reporter assay
hsa-miR-21	TP53BP2	Reporter assay
hsa-miR-21	TP63	Reporter assay; Western blot
hsa-miR-21	TPM1	Reporter assay; Western blot
hsa-miR-210	BDNF	Reporter assay; Western blot
hsa-miR-210	CPEB2	Reporter assay
hsa-miR-210	DDAH1	Reporter assay
hsa-miR-210	EFNA3	Reporter assay; Western blot
hsa-miR-210	FGFRL1	Reporter assay; Western blot
hsa-miR-210	GPD1L	Reporter assay
hsa-miR-210	HOXA1	Reporter assay
hsa-miR-210	HOXA9	Reporter assay
hsa-miR-210	ISCU	Reporter assay; Western blot
hsa-miR-210	MNT	Reporter assay; Western blot
hsa-miR-210	NCAM1	Reporter assay
hsa-miR-210	NPTX1	Reporter assay
hsa-miR-210	P4HB	Western blot
hsa-miR-210	PIM1	Reporter assay
hsa-miR-210	PTPN1	Reporter assay; Western blot
hsa-miR-210	RAD52	Reporter assay; Western blot
hsa-miR-210	TP53I11	Reporter assay
hsa-miR-210	XIST	Reporter assay
hsa-miR-212	MECP2	Reporter assay; Western blot
hsa-miR-212	PEA15	Reporter assay; Western blot
hsa-miR-212	TJP1	Western blot
hsa-miR-214	MAP2K3	Reporter assay; Western blot
hsa-miR-214	MAPK8	Reporter assay; Western blot
hsa-miR-214	POU4F2	Reporter assay; Western blot
hsa-miR-214	PTEN	Reporter assay; Western blot
hsa-miR-215	WNK1	Reporter assay; Western blot
hsa-miR-216a	PTEN	Reporter assay; Western blot
hsa-miR-217	NR4A2	Reporter assay
hsa-miR-217	PTEN	Reporter assay; Western blot
hsa-miR-217	ROBO1	Reporter assay; Western blot
hsa-miR-217	SIRT1	Reporter assay; Western blot
hsa-miR-218	IKBKB	Reporter assay; Western blot
hsa-miR-218	LAMB3	Western blot
hsa-miR-218	SP1	Reporter assay
hsa-miR-218	VOPP1	Reporter assay
hsa-miR-219-5p	CAMK2G	Reporter assay
hsa-miR-22	BMP7	Reporter assay
hsa-miR-22	ESR1	Western blot
hsa-miR-22	HDAC4	Reporter assay; Western blot
hsa-miR-22	MYCBP	Reporter assay
hsa-miR-22	PPARA	Western blot
hsa-miR-22	TFRC	Reporter assay
hsa-miR-221	BBC3	Reporter assay; Western blot
hsa-miR-221	BMF	Reporter assay; Western blot
hsa-miR-221	BNIP3	Reporter assay; Western blot
hsa-miR-221	CDKN1B	Reporter assay; Western blot
hsa-miR-221	CDKN1C	Reporter assay
hsa-miR-221	DDIT4	Reporter assay; Western blot
hsa-miR-221	ESR1	Reporter assay; Western blot

hsa-miR-221	FOS	Reporter assay; Western blot
hsa-miR-221	FOXO3	Reporter assay; Western blot
hsa-miR-221	HOXB5	Reporter assay
hsa-miR-221	ICAM1	Reporter assay; Western blot
hsa-miR-221	KIT	Reporter assay; Western blot
hsa-miR-221	NAIP	Western blot
hsa-miR-221	PTEN	Reporter assay; Western blot
hsa-miR-221	TICAM1	Reporter assay; Western blot
hsa-miR-221	TNFSF10	Western blot
hsa-miR-222	BBC3	Reporter assay; Western blot
hsa-miR-222	CDKN1B	Reporter assay; Western blot
hsa-miR-222	CDKN1C	Reporter assay; Western blot
hsa-miR-222	ESR1	Reporter assay; Western blot
hsa-miR-222	FOS	Reporter assay; Western blot
hsa-miR-222	FOXO3	Reporter assay; Western blot
hsa-miR-222	KIT	Western blot
hsa-miR-222	MMP1	Reporter assay; Western blot
hsa-miR-222	PPP2R2A	Reporter assay; Western blot
hsa-miR-222	PTEN	Reporter assay; Western blot
hsa-miR-222	SOD2	Reporter assay; Western blot
hsa-miR-222	STAT5A	Reporter assay; Western blot
hsa-miR-222	TNFSF10	Western blot
hsa-miR-223	CHUK	Reporter assay; Western blot
hsa-miR-223	E2F1	Reporter assay; Western blot
hsa-miR-223	LMO2	Reporter assay; Western blot
hsa-miR-223	MEF2C	Reporter assay
hsa-miR-223	NFIA	Reporter assay
hsa-miR-223	NFIX	Reporter assay
hsa-miR-223	RHOB	Reporter assay; Western blot
hsa-miR-223	STMN1	Reporter assay; Western blot
hsa-miR-224	AP2M1	Reporter assay
hsa-miR-224	API5	Reporter assay; Western blot
hsa-miR-224	CDC42	Reporter assay; Western blot
hsa-miR-224	CXCR4	Reporter assay; Western blot
hsa-miR-224	KLK10	Reporter assay
hsa-miR-23a	ATAT1	Reporter assay
hsa-miR-23a	CXCL12	Reporter assay
hsa-miR-23a	HES1	Reporter assay; Western blot
hsa-miR-23a	IL6R	Reporter assay; Western blot
hsa-miR-23a	POU4F2	Reporter assay
hsa-miR-23b	MET	Reporter assay; Western blot
hsa-miR-23b	PLAU	Reporter assay; Western blot
hsa-miR-23b*	PRODH	Reporter assay; Western blot
hsa-miR-24	ACVR1B	Reporter assay; Western blot
hsa-miR-24	AURKB	Reporter assay; Western blot
hsa-miR-24	BRCA1	Reporter assay; Western blot
hsa-miR-24	CCNA2	Reporter assay; Western blot
hsa-miR-24	CDK4	Reporter assay; Western blot
hsa-miR-24	CDKN2A	Western blot
hsa-miR-24	DHFR	Western blot
hsa-miR-24	E2F2	Reporter assay; Western blot
hsa-miR-24	FEN1	Reporter assay; Western blot
hsa-miR-24	MAPK14	Reporter assay
hsa-miR-24	MYC	Reporter assay; Western blot

hsa-miR-24	POLD1	Reporter assay; Western blot
hsa-miR-24	TRIB3	Western blot
hsa-miR-24-1*	SLITRK1	Reporter assay
hsa-miR-25	BCL2L11	Reporter assay; Western blot
hsa-miR-25	CDKN1C	Reporter assay; Western blot
hsa-miR-25	KAT2B	Reporter assay; Western blot
hsa-miR-25	KLF4	Western blot
hsa-miR-25	PRMT5	Western blot
hsa-miR-25	TP53	Reporter assay; Western blot
hsa-miR-26a	CCND2	Reporter assay; Western blot
hsa-miR-26a	CCNE2	Reporter assay; Western blot
hsa-miR-26a	CPEB2	Reporter assay
hsa-miR-26a	CPEB3	Reporter assay
hsa-miR-26a	CPEB4	Reporter assay
hsa-miR-26a	EZH2	Reporter assay; Western blot
hsa-miR-26a	GSK3B	Reporter assay; Western blot
hsa-miR-26a	HMGA1	Reporter assay
hsa-miR-26a	HMGA2	Reporter assay
hsa-miR-26a	IFNB1	Reporter assay
hsa-miR-26a	MAP3K2	Reporter assay; Western blot
hsa-miR-26a	PLAG1	Reporter assay
hsa-miR-26a	PTEN	Western blot
hsa-miR-26a	RB1	Reporter assay; Western blot
hsa-miR-26a	SERBP1	Reporter assay; Western blot
hsa-miR-26a	SMAD1	Western blot
hsa-miR-26a	SMAD4	Western blot
hsa-miR-26b	EPHA2	Reporter assay; Western blot
hsa-miR-26b	PTGS2	Reporter assay; Western blot
hsa-miR-27a	FOXO1	Reporter assay; Western blot
hsa-miR-27a	HIPK2	Western blot
hsa-miR-27a	MYT1	Western blot
hsa-miR-27a	PHB	Reporter assay; Western blot
hsa-miR-27a	SP1	Western blot
hsa-miR-27a	SP3	Western blot
hsa-miR-27a	SP4	Western blot
hsa-miR-27a	SPRY2	Reporter assay; Western blot
hsa-miR-27a	THRB	Reporter assay; Western blot
hsa-miR-27a	ZBTB10	Western blot
hsa-miR-27b	ADORA2B	Reporter assay
hsa-miR-27b	CYP1B1	Reporter assay; Western blot
hsa-miR-27b	MMP13	Reporter assay; Western blot
hsa-miR-27b	PPARG	Reporter assay
hsa-miR-27b	ST14	Reporter assay; Western blot
hsa-miR-28-5p	CDKN1A	Reporter assay; Western blot
hsa-miR-296-5p	HGS	Reporter assay; Western blot
hsa-miR-296-5p	WNK4	Reporter assay; Western blot
hsa-miR-298	BACE1	Reporter assay
hsa-miR-298	CDKN1A	Reporter assay; Western blot
hsa-miR-299-5p	CDKN1A	Reporter assay; Western blot
hsa-miR-299-5p	SPP1	Reporter assay
hsa-miR-29a	ADAMTS9	Reporter assay
hsa-miR-29a	BACE1	Reporter assay; Western blot
hsa-miR-29a	BCL2	Reporter assay; Western blot
hsa-miR-29a	CD276	Reporter assay; Western blot

hsa-miR-29a	CDK6	Western blot
hsa-miR-29a	COL4A1	Reporter assay; Western blot
hsa-miR-29a	COL4A2	Reporter assay; Western blot
hsa-miR-29a	CPEB3	Reporter assay
hsa-miR-29a	CPEB4	Reporter assay
hsa-miR-29a	DKK1	Reporter assay; Western blot
hsa-miR-29a	DNMT3A	Reporter assay; Western blot
hsa-miR-29a	DNMT3B	Reporter assay; Western blot
hsa-miR-29a	FGA	Reporter assay
hsa-miR-29a	FGB	Reporter assay
hsa-miR-29a	GLUL	Reporter assay
hsa-miR-29a	ITGA11	Reporter assay
hsa-miR-29a	KREMEN2	Reporter assay; Western blot
hsa-miR-29a	LPL	Reporter assay; Western blot
hsa-miR-29a	MCL1	Western blot
hsa-miR-29a	PIK3R1	Reporter assay; Western blot
hsa-miR-29a	PPM1D	Reporter assay; Western blot
hsa-miR-29a	S100B	Reporter assay
hsa-miR-29a	SFRP2	Reporter assay; Western blot
hsa-miR-29b	ADAM12	Reporter assay
hsa-miR-29b	BACE1	Reporter assay; Western blot
hsa-miR-29b	BCL2	Reporter assay; Western blot
hsa-miR-29b	CDK6	Reporter assay; Western blot
hsa-miR-29b	COL1A1	Reporter assay
hsa-miR-29b	COL3A1	Reporter assay; Western blot
hsa-miR-29b	COL4A1	Reporter assay; Western blot
hsa-miR-29b	DNAJB11	Reporter assay; Western blot
hsa-miR-29b	DNMT3A	Reporter assay; Western blot
hsa-miR-29b	DNMT3B	Reporter assay; Western blot
hsa-miR-29b	ESR1	Western blot
hsa-miR-29b	FGA	Reporter assay
hsa-miR-29b	FGB	Reporter assay
hsa-miR-29b	GRN	Reporter assay; Western blot
hsa-miR-29b	MCL1	Reporter assay; Western blot
hsa-miR-29b	MMP2	Reporter assay; Western blot
hsa-miR-29b	MMP24	Reporter assay
hsa-miR-29b	NCOA3	Western blot
hsa-miR-29b	NID1	Reporter assay
hsa-miR-29b	S100B	Reporter assay
hsa-miR-29b	SFPQ	Reporter assay; Western blot
hsa-miR-29b	SP1	Reporter assay
hsa-miR-29b	TCL1A	Reporter assay
hsa-miR-29b	TET1	Reporter assay; Western blot
hsa-miR-29c	BCL2	Reporter assay; Western blot
hsa-miR-29c	CDK6	Reporter assay; Western blot
hsa-miR-29c	COL15A1	Reporter assay
hsa-miR-29c	COL1A1	Reporter assay
hsa-miR-29c	COL1A2	Reporter assay
hsa-miR-29c	COL3A1	Reporter assay
hsa-miR-29c	COL4A1	Reporter assay
hsa-miR-29c	COL4A2	Reporter assay
hsa-miR-29c	DNMT3A	Reporter assay; Western blot
hsa-miR-29c	DNMT3B	Reporter assay; Western blot
hsa-miR-29c	FBN1	Reporter assay

hsa-miR-29c	FGA	Reporter assay
hsa-miR-29c	LAMC1	Reporter assay
hsa-miR-29c	MCL1	Reporter assay; Western blot
hsa-miR-29c	MMP24	Reporter assay
hsa-miR-29c	SPARC	Reporter assay
hsa-miR-29c	SRSF10	Reporter assay
hsa-miR-29c	TDG	Reporter assay
hsa-miR-301a	MEOX2	Reporter assay
hsa-miR-301a	NKRF	Reporter assay; Western blot
hsa-miR-302a	CCND1	Reporter assay; Western blot
hsa-miR-302a	CDK4	Reporter assay; Western blot
hsa-miR-302a	CDKN1A	Reporter assay
hsa-miR-302a	LEFTY1	Reporter assay; Western blot
hsa-miR-302a	LEFTY2	Reporter assay; Western blot
hsa-miR-302b	BMI1	Reporter assay; Western blot
hsa-miR-302b	CCND2	Reporter assay; Western blot
hsa-miR-302c	CCND1	Reporter assay; Western blot
hsa-miR-302c	ESR1	Reporter assay
hsa-miR-302d	CCND2	Reporter assay; Western blot
hsa-miR-302d	CDK2	Reporter assay; Western blot
hsa-miR-302d	ERBB4	Reporter assay
hsa-miR-302d	KLF13	Reporter assay
hsa-miR-302d	LEFTY1	Reporter assay; Western blot
hsa-miR-302d	LEFTY2	Reporter assay; Western blot
hsa-miR-302d	MBNL2	Reporter assay
hsa-miR-302d	NR4A2	Reporter assay
hsa-miR-302d	TRPS1	Reporter assay
hsa-miR-302d	VEGFA	Reporter assay
hsa-miR-30a	BDNF	Reporter assay
hsa-miR-30a	BECN1	Reporter assay
hsa-miR-30a	NOTCH1	Reporter assay
hsa-miR-30a	TNRC6A	Reporter assay
hsa-miR-30a*	CDK6	Reporter assay; Western blot
hsa-miR-30a*	CYR61	Reporter assay; Western blot
hsa-miR-30a*	SLC7A6	Reporter assay; Western blot
hsa-miR-30a*	THBS1	Reporter assay; Western blot
hsa-miR-30a*	TMEM2	Reporter assay; Western blot
hsa-miR-30a*	VEZT	Reporter assay; Western blot
hsa-miR-30c	UBE2I	Reporter assay; Western blot
hsa-miR-30d	GNAI2	Western blot
hsa-miR-30d	TP53	Reporter assay; Western blot
hsa-miR-30e	UBE2I	Reporter assay; Western blot
hsa-miR-31	CASR	Reporter assay
hsa-miR-31	DMD	Reporter assay; Western blot
hsa-miR-31	FOXP3	Reporter assay; Western blot
hsa-miR-31	FZD3	Reporter assay; Western blot
hsa-miR-31	ITGA5	Reporter assay; Western blot
hsa-miR-31	LATS2	Reporter assay
hsa-miR-31	MMP16	Reporter assay; Western blot
hsa-miR-31	MPRIP	Reporter assay
hsa-miR-31	PPP2R2A	Reporter assay
hsa-miR-31	RDX	Reporter assay; Western blot
hsa-miR-31	RHOA	Reporter assay; Western blot
hsa-miR-31	SELE	Western blot

hsa-miR-31	TIAM1	Reporter assay
hsa-miR-32	BCL2L11	Reporter assay
hsa-miR-32	KAT2B	Reporter assay; Western blot
hsa-miR-32	PRMT5	Western blot
hsa-miR-320a	HSPB6	Reporter assay
hsa-miR-320a	MCL1	Reporter assay
hsa-miR-320a	POLR3D	TaqMan miRNA assay/RT-PCR
hsa-miR-320a	TFRC	Reporter assay
hsa-miR-324-3p	CREBBP	Reporter assay
hsa-miR-324-3p	DVL2	Reporter assay
hsa-miR-324-3p	WNT9B	Reporter assay
hsa-miR-324-5p	GLI1	Reporter assay; Western blot
hsa-miR-324-5p	SMO	Reporter assay; Western blot
hsa-miR-326	GLI1	Reporter assay
hsa-miR-326	MSH3	Reporter assay; Western blot
hsa-miR-326	NOTCH1	Reporter assay; Western blot
hsa-miR-326	NOTCH2	Reporter assay; Western blot
hsa-miR-326	PKM2	Reporter assay; Western blot
hsa-miR-326	SMO	Reporter assay; Western blot
hsa-miR-328	ABCG2	Reporter assay; Western blot
hsa-miR-328	BACE1	Reporter assay
hsa-miR-330-3p	E2F1	Reporter assay; Western blot
hsa-miR-335	MERTK	Reporter assay
hsa-miR-335	PTPRN2	Reporter assay
hsa-miR-335	RB1	Reporter assay; Western blot
hsa-miR-335	SOX4	Reporter assay
hsa-miR-335	TNC	Reporter assay
hsa-miR-338-3p	DAB2IP	Western blot
hsa-miR-338-3p	MAP1A	Western blot
hsa-miR-338-3p	NOVA1	Western blot
hsa-miR-338-3p	PLA2G4B	Reporter assay
hsa-miR-338-3p	UBE2Q1	Western blot
hsa-miR-338-3p	ZNF238	Western blot
hsa-miR-33a	ABCA1	Reporter assay
hsa-miR-33a	NPC1	Reporter assay
hsa-miR-33b	ABCA1	Reporter assay
hsa-miR-33b	BCL2	Western blot
hsa-miR-340	MET	Reporter assay; Western blot
hsa-miR-345	ABCC1	Reporter assay; Western blot
hsa-miR-345	CDKN1A	Reporter assay; Western blot
hsa-miR-346	LIF	Reporter assay
hsa-miR-34a	AXIN2	Reporter assay
hsa-miR-34a	BCL2	Reporter assay
hsa-miR-34a	CCND1	Reporter assay; Western blot
hsa-miR-34a	CCNE2	Reporter assay
hsa-miR-34a	CD44	Western blot
hsa-miR-34a	CDK4	Reporter assay
hsa-miR-34a	CDK6	Reporter assay; Western blot
hsa-miR-34a	DLL1	Reporter assay
hsa-miR-34a	E2F3	Reporter assay; Western blot
hsa-miR-34a	FOXP1	Reporter assay
hsa-miR-34a	HNF4A	Reporter assay; Western blot
hsa-miR-34a	IFNB1	Reporter assay
hsa-miR-34a	JAG1	Reporter assay; Western blot

hsa-miR-34a	MAGEA12	Reporter assay; Western blot
hsa-miR-34a	MAGEA2	Reporter assay; Western blot
hsa-miR-34a	MAGEA3	Reporter assay; Western blot
hsa-miR-34a	MAGEA6	Reporter assay; Western blot
hsa-miR-34a	MAP2K1	Reporter assay; Western blot
hsa-miR-34a	MET	Reporter assay
hsa-miR-34a	MYC	Reporter assay; Western blot
hsa-miR-34a	MYCN	Reporter assay; Western blot
hsa-miR-34a	NOTCH1	Reporter assay
hsa-miR-34a	NOTCH2	Reporter assay; Western blot
hsa-miR-34a	SIRT1	Western blot
hsa-miR-34a	VAMP2	Reporter assay
hsa-miR-34a	WNT1	Reporter assay
hsa-miR-34a	YY1	Reporter assay; Western blot
hsa-miR-34b	CDK4	Western blot
hsa-miR-34b	CDK6	Reporter assay; Western blot
hsa-miR-34b	MET	Western blot
hsa-miR-34b	MYC	Reporter assay; Western blot
hsa-miR-34b*	BCL2	Reporter assay
hsa-miR-34b*	CCNE2	Reporter assay
hsa-miR-34b*	CDK4	Reporter assay
hsa-miR-34b*	CDK6	Reporter assay
hsa-miR-34b*	CREB1	Reporter assay; Western blot
hsa-miR-34b*	MET	Reporter assay
hsa-miR-34b*	MYC	Reporter assay
hsa-miR-34c-5p	BCL2	Reporter assay
hsa-miR-34c-5p	CCNE2	Reporter assay
hsa-miR-34c-5p	CDK4	Reporter assay
hsa-miR-34c-5p	E2F3	Reporter assay
hsa-miR-34c-5p	MET	Reporter assay
hsa-miR-34c-5p	MYC	Reporter assay
hsa-miR-361-5p	VEGFA	Reporter assay
hsa-miR-363	CDKN1A	Reporter assay; Western blot
hsa-miR-370	CPT1A	Reporter assay; Western blot
hsa-miR-370	MAP3K8	Reporter assay; Western blot
hsa-miR-372	CDKN1A	Reporter assay; Western blot
hsa-miR-372	ERBB4	Reporter assay
hsa-miR-372	KLF13	Reporter assay
hsa-miR-372	LATS2	Reporter assay
hsa-miR-372	MBNL2	Reporter assay
hsa-miR-372	NR4A2	Reporter assay
hsa-miR-372	TRPS1	Reporter assay
hsa-miR-372	VEGFA	Reporter assay
hsa-miR-373	CD44	Reporter assay; Western blot
hsa-miR-373	LATS2	Reporter assay; Western blot
hsa-miR-373	RABEP1	Reporter assay; Western blot
hsa-miR-373	RAD23B	Reporter assay; Western blot
hsa-miR-373	RAD52	Reporter assay; Western blot
hsa-miR-373	RECK	Reporter assay; Western blot
hsa-miR-373	TXNIP	Reporter assay; Western blot
hsa-miR-373	VEGFA	Reporter assay
hsa-miR-374a	ATM	Reporter assay
hsa-miR-374a	DICER1	Reporter assay
hsa-miR-374a	GADD45A	Reporter assay

hsa-miR-375	ELAVL4	Reporter assay; Western blot
hsa-miR-375	PLAG1	Reporter assay
hsa-miR-375	RASD1	Reporter assay
hsa-miR-375	TIMM8A	Reporter assay
hsa-miR-375	YY1AP1	Reporter assay; Western blot
hsa-miR-376a*	SLC16A1	Reporter assay
hsa-miR-376a*	SRSF11	Reporter assay
hsa-miR-376a*	TTK	Reporter assay
hsa-miR-377	PAK1	Reporter assay; Western blot
hsa-miR-377	SOD1	Reporter assay; Western blot
hsa-miR-377	SOD2	Reporter assay; Western blot
hsa-miR-378	GALNT7	Reporter assay; Western blot
hsa-miR-378	MYC	Reporter assay
hsa-miR-378	NPNT	Reporter assay; Western blot
hsa-miR-378	TOB2	Reporter assay
hsa-miR-378*	SUFU	Reporter assay; Western blot
hsa-miR-378*	TUSC2	Reporter assay; Western blot
hsa-miR-383	VEGFA	Reporter assay
hsa-miR-409-3p	FGB	Reporter assay
hsa-miR-421	ATM	Reporter assay; Western blot
hsa-miR-421	CBX7	Western blot
hsa-miR-421	RBMXL1	Western blot
hsa-miR-422a	CYP7A1	Reporter assay
hsa-miR-423-3p	CDKN1A	Reporter assay; Western blot
hsa-miR-424	ANLN	Reporter assay
hsa-miR-424	ATF6	Reporter assay
hsa-miR-424	CCND1	Reporter assay; Western blot
hsa-miR-424	CCND3	Reporter assay; Western blot
hsa-miR-424	CCNE1	Western blot
hsa-miR-424	CCNF	Reporter assay
hsa-miR-424	CDC14A	Reporter assay
hsa-miR-424	CDC25A	Reporter assay
hsa-miR-424	CDK6	Reporter assay; Western blot
hsa-miR-424	CHEK1	Reporter assay
hsa-miR-424	FGFR1	Reporter assay
hsa-miR-424	KIF23	Reporter assay
hsa-miR-424	MAP2K1	Reporter assay
hsa-miR-424	NFIA	Reporter assay; Western blot
hsa-miR-424	PLAG1	Reporter assay; Western blot
hsa-miR-424	WEE1	Reporter assay
hsa-miR-424*	LGALS3	Northern blot; Western blot
hsa-miR-429	RERE	Reporter assay
hsa-miR-429	SIP1	Reporter assay
hsa-miR-429	WASF3	Reporter assay
hsa-miR-429	ZEB2	Reporter assay
hsa-miR-429	ZFPM2	Reporter assay; Western blot
hsa-miR-433	FGF20	Reporter assay; Western blot
hsa-miR-433	GRB2	Reporter assay; Western blot
hsa-miR-448	SATB1	Reporter assay; Western blot
hsa-miR-449a	CCND1	Reporter assay
hsa-miR-449a	CDC25A	Reporter assay; Western blot
hsa-miR-449a	CDK6	Reporter assay; Western blot
hsa-miR-449a	HDAC1	Reporter assay; Western blot
hsa-miR-450a	HNRNPK	Western blot

hsa-miR-451	ABCB1	Reporter assay; Western blot
hsa-miR-451	CAB39	Reporter assay; Western blot
hsa-miR-451	MIF	Reporter assay; Western blot
hsa-miR-483-3p	BBC3	Reporter assay; Western blot
hsa-miR-483-3p	SMAD4	Reporter assay; Western blot
hsa-miR-485-3p	NFYB	Reporter assay; Western blot
hsa-miR-485-3p	NTRK3	Reporter assay
hsa-miR-488*	AR	Reporter assay; Western blot
hsa-miR-489	PTPN11	Reporter assay; Western blot
hsa-miR-491-5p	BCL2L1	Reporter assay; Western blot
hsa-miR-494	PTEN	Reporter assay; Western blot
hsa-miR-499-5p	SOX6	Reporter assay
hsa-miR-503	ANLN	Reporter assay
hsa-miR-503	ATF6	Reporter assay
hsa-miR-503	CCND1	Reporter assay; Western blot
hsa-miR-503	CCNE1	Reporter assay
hsa-miR-503	CCNE2	Reporter assay
hsa-miR-503	CCNF	Reporter assay
hsa-miR-503	CDC14A	Reporter assay
hsa-miR-503	CDC25A	Reporter assay
hsa-miR-503	CDKN1A	Reporter assay
hsa-miR-503	CHEK1	Reporter assay
hsa-miR-503	EIF2C1	Reporter assay
hsa-miR-503	WEE1	Reporter assay
hsa-miR-504	DRD1	Reporter assay
hsa-miR-504	VEGFA	Reporter assay
hsa-miR-509-3p	NTRK3	Reporter assay
hsa-miR-510	HTR3E	Reporter assay
hsa-miR-510	SPDEF	Reporter assay; Western blot
hsa-miR-512-5p	MCL1	Reporter assay; Western blot
hsa-miR-513a-5p	CD274	Reporter assay; Western blot
hsa-miR-515-3p	CDKN1A	Reporter assay; Western blot
hsa-miR-518a-5p	MCL1	Reporter assay; Western blot
hsa-miR-519a	DICER1	Reporter assay
hsa-miR-519a	ELAVL1	Western blot
hsa-miR-519a	YES1	Reporter assay
hsa-miR-519b-3p	CDKN1A	Reporter assay; Western blot
hsa-miR-519b-3p	ELAVL1	Western blot
hsa-miR-519c-3p	ABCG2	Reporter assay; Western blot
hsa-miR-519c-3p	ELAVL1	Western blot
hsa-miR-519c-3p	HIF1A	Reporter assay; Western blot
hsa-miR-519d	CDKN1A	Reporter assay; Western blot
hsa-miR-519d	PPARA	Reporter assay; Western blot
hsa-miR-519e	CDKN1A	Reporter assay; Western blot
hsa-miR-520a-3p	CDKN1A	Reporter assay; Western blot
hsa-miR-520b	CDKN1A	Reporter assay; Western blot
hsa-miR-520b	MICA	Reporter assay
hsa-miR-520c-3p	APP	Reporter assay
hsa-miR-520c-3p	CD44	Reporter assay; Western blot
hsa-miR-520g	VEGFA	Reporter assay
hsa-miR-520h	ABCG2	Reporter assay
hsa-miR-520h	CDKN1A	Reporter assay; Western blot
hsa-miR-520h	VEGFA	Reporter assay
hsa-miR-521	ERCC8	Western blot

hsa-miR-542-3p	BIRC5	Reporter assay; Western blot
hsa-miR-548d-3p	ERBB2	Reporter assay
hsa-miR-559	ERBB2	Reporter assay
hsa-miR-559	MTA1	Reporter assay; Western blot
hsa-miR-562	EYA1	Reporter assay
hsa-miR-569	SPI1	Reporter assay
hsa-miR-572	CDKN1A	Reporter assay; Western blot
hsa-miR-582-5p	MCL1	Reporter assay; Western blot
hsa-miR-584	ROCK1	Reporter assay; Western blot
hsa-miR-590-5p	TGFBR2	Reporter assay; Western blot
hsa-miR-603	TRAPPC2P1	Reporter assay
hsa-miR-605	SEC24D	Western blot
hsa-miR-625	NTRK3	Reporter assay
hsa-miR-630	BCL2	Reporter assay
hsa-miR-630	BCL2L2	Reporter assay
hsa-miR-630	YAP1	Reporter assay
hsa-miR-631	SULT1A1	Reporter assay; Western blot
hsa-miR-632	PRDX6	Western blot
hsa-miR-639	CDKN1A	Reporter assay; Western blot
hsa-miR-650	ING4	Reporter assay; Western blot
hsa-miR-654-3p	CDKN1A	Reporter assay; Western blot
hsa-miR-657	CDKN1A	Reporter assay; Western blot
hsa-miR-659	GRN	Reporter assay; Western blot
hsa-miR-661	MCL1	Reporter assay; Western blot
hsa-miR-661	MTA1	Reporter assay; Western blot
hsa-miR-663	JUNB	Reporter assay; Western blot
hsa-miR-663	JUND	Reporter assay; Western blot
hsa-miR-675	RB1	Reporter assay
hsa-miR-7	ABCC1	Reporter assay; Western blot
hsa-miR-7	EGFR	Reporter assay; Western blot
hsa-miR-7	IGF1R	Western blot
hsa-miR-7	IRS1	Western blot
hsa-miR-7	IRS2	Reporter assay; Western blot
hsa-miR-7	PAK1	Reporter assay; Western blot
hsa-miR-7	RAF1	Reporter assay; Western blot
hsa-miR-7	SLC7A5	Reporter assay; Western blot
hsa-miR-7	SNCA	Reporter assay; Western blot
hsa-miR-7	SRSF1	Reporter assay; Western blot
hsa-miR-765	NTRK3	Reporter assay
hsa-miR-802	MECP2	Reporter assay; Western blot
hsa-miR-876-3p	MCL1	Reporter assay; Western blot
hsa-miR-885-5p	CDK2	Reporter assay; Western blot
hsa-miR-885-5p	MCM5	Reporter assay; Western blot
hsa-miR-892b	MCL1	Reporter assay; Western blot
hsa-miR-9	BACE1	Reporter assay
hsa-miR-9	CDH1	Western blot
hsa-miR-9	FOXO1	Western blot
hsa-miR-9	MMP13	Reporter assay
hsa-miR-9	NFKB1	Reporter assay; Western blot
hsa-miR-9	NR2E1	Reporter assay; Western blot
hsa-miR-9	NTRK3	Reporter assay; Western blot
hsa-miR-9	ONECUT2	Reporter assay; Western blot
hsa-miR-9	PRDM1	Reporter assay
hsa-miR-9	RAB34	Reporter assay; Western blot

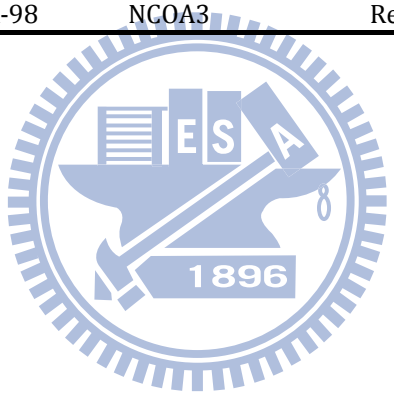
	hsa-miR-9	REST	Reporter assay; Western blot
	hsa-miR-9*	RCOR1	Reporter assay; Western blot
	hsa-miR-92a	CPEB2	Reporter assay
	hsa-miR-92a	ESR2	Reporter assay
	hsa-miR-92a	HIPK3	Reporter assay
	hsa-miR-92a	ITGA5	Reporter assay; Western blot
	hsa-miR-92a	MYLIP	Reporter assay
	hsa-miR-92a	TP63	Reporter assay; Western blot
	hsa-miR-92b	CDKN1C	Reporter assay; Western blot
	hsa-miR-92b	PRMT5	Western blot
	hsa-miR-92b	SLC15A1	Reporter assay; Western blot
	hsa-miR-93	CDKN1A	Reporter assay; Western blot
	hsa-miR-93	E2F1	Reporter assay; Western blot
	hsa-miR-93	ITGB8	Reporter assay; Western blot
	hsa-miR-93	KAT2B	Reporter assay; Western blot
	hsa-miR-93	TP53INP1	Reporter assay; Western blot
	hsa-miR-93	TUSC2	Reporter assay; Western blot
	hsa-miR-93	VEGFA	Reporter assay
	hsa-miR-96	ADCY6	Reporter assay
	hsa-miR-96	CDKN1A	Reporter assay; Western blot
	hsa-miR-96	FOXO1	Reporter assay; Western blot
	hsa-miR-96	FOXO3	Reporter assay; Western blot
	hsa-miR-96	HTR1B	Reporter assay
	hsa-miR-96	KRAS	Western blot
	hsa-miR-96	MITF	Reporter assay
	hsa-miR-96	PRMT5	Western blot
	hsa-miR-98	E2F2	Western blot
	hsa-miR-98	MYC	Reporter assay
	hsa-miR-98	SOCS4	Reporter assay; Western blot
	hsa-miR-98	TUSC2	Reporter assay; Western blot
	hsa-miR-99a	FGFR3	Reporter assay; Western blot
	hsa-miR-99a	RAVER2	Reporter assay; Western blot
	hsa-miR-99b	RAVER2	Reporter assay; Western blot
Non-Functional MTI	hsa-let-7a	APP	Reporter assay
	hsa-let-7a	CASP8	Western blot
	hsa-let-7a	CASP9	Western blot
	hsa-let-7a	E2F1	Western blot
	hsa-let-7a	FOXA1	Reporter assay
	hsa-let-7a	NR1I2	Reporter assay
	hsa-let-7a	VDR	Reporter assay
	hsa-let-7b	ACTG1	Reporter assay
	hsa-let-7b	RPIA	Reporter assay
	hsa-let-7c	DICER1	Western blot
	hsa-let-7d	APP	Reporter assay
	hsa-let-7e	EIF3J	Reporter assay
	hsa-miR-1	NETO2	Reporter assay
	hsa-miR-1	PGM2	Reporter assay
	hsa-miR-1	SERP1	Reporter assay
	hsa-miR-1	SRXN1	Reporter assay
	hsa-miR-1	TWF2	Reporter assay; Western blot
	hsa-miR-101	ARID1A	Western blot
	hsa-miR-101	FBN2	Western blot
	hsa-miR-101	FOS	Western blot

hsa-miR-101	SUZ12	Western blot
hsa-miR-103	GPD1	Reporter assay
hsa-miR-106a	APP	Reporter assay
hsa-miR-124	BACE1	Reporter assay
hsa-miR-124	PEA15	Western blot
hsa-miR-125a-5p	VEGFA	Reporter assay
hsa-miR-126	CCNE2	Reporter assay
hsa-miR-126	RGS3	Reporter assay
hsa-miR-126	SLC45A3	Reporter assay; Western blot
hsa-miR-126	TOM1	Reporter assay
hsa-miR-126	TWF1	Reporter assay; Western blot
hsa-miR-126	TWF2	Reporter assay; Western blot
hsa-miR-130a	APP	Reporter assay
hsa-miR-137	E2F6	Reporter assay; Western blot
hsa-miR-137	NCOA2	Reporter assay; Western blot
hsa-miR-138	ARHGEF3	Reporter assay; Western blot
hsa-miR-138	SLC45A3	Reporter assay; Western blot
hsa-miR-141	KLF5	Reporter assay
hsa-miR-141	STK3	Reporter assay
hsa-miR-141	ZEB1	Reporter assay
hsa-miR-141	ZEB2	Reporter assay
hsa-miR-144	FGA	Reporter assay
hsa-miR-144	FGB	Reporter assay
hsa-miR-145	CDKN1A	Reporter assay; Western blot
hsa-miR-145	HOXA9	Reporter assay; Western blot
hsa-miR-145	TMOD3	Reporter assay
hsa-miR-146a	CDKN1A	Reporter assay
hsa-miR-146b-5p	CDKN1A	Reporter assay
hsa-miR-155	FGF7	Reporter assay
hsa-miR-155	FLI1	Western blot
hsa-miR-155	NFATC2IP	Reporter assay
hsa-miR-155	PHF17	Reporter assay
hsa-miR-15a	APP	Reporter assay
hsa-miR-15a	BACE1	Reporter assay
hsa-miR-15a	TMEM184B	Reporter assay
hsa-miR-15b	CCND1	Western blot
hsa-miR-16	CCNT2	Reporter assay
hsa-miR-16	VEGFA	Reporter assay
hsa-miR-17	SMAD4	Reporter assay
hsa-miR-181c	NOTCH2	Reporter assay; Western blot
hsa-miR-182	CDKN1A	Reporter assay; Western blot
hsa-miR-184	INPPL1	Reporter assay; Western blot
hsa-miR-188-5p	UBE2I	Western blot
hsa-miR-18a	NCOA3	Western blot
hsa-miR-18a	PTEN	Reporter assay; Western blot
hsa-miR-18a	TGFBR2	Reporter assay
hsa-miR-190	CDKN1B	Reporter assay
hsa-miR-192	CDKN1B	Reporter assay
hsa-miR-193a-3p	MCL1	Reporter assay; Western blot
hsa-miR-193a-3p	PTK2	Reporter assay; Western blot
hsa-miR-196a	CDKN1B	Reporter assay
hsa-miR-198	NTRK3	Reporter assay
hsa-miR-199a-3p	AKT1	Western blot
hsa-miR-199a-3p	MAPK14	Western blot

hsa-miR-199a-3p	MAPK8	Western blot
hsa-miR-199a-3p	MAPK9	Western blot
hsa-miR-19a	BMPR2	Reporter assay; Western blot
hsa-miR-19a	KAT2B	Reporter assay; Western blot
hsa-miR-19a	SMAD4	Reporter assay
hsa-miR-19a	TGFBR2	Reporter assay
hsa-miR-19b	ARID4B	Reporter assay
hsa-miR-19b	BACE1	Reporter assay
hsa-miR-19b	BMPR2	Reporter assay; Western blot
hsa-miR-19b	ESR1	Western blot
hsa-miR-19b	KAT2B	Reporter assay; Western blot
hsa-miR-19b	NCOA3	Western blot
hsa-miR-200c	UBE2I	Western blot
hsa-miR-200c	ZEB2	Reporter assay
hsa-miR-204	SNAI1	Reporter assay
hsa-miR-204	TGFBR1	Reporter assay
hsa-miR-205	DDX5	Reporter assay
hsa-miR-205	MED1	Reporter assay; Western blot
hsa-miR-205	SIGMAR1	Reporter assay
hsa-miR-205	VEGFA	Reporter assay
hsa-miR-20a	NRAS	Western blot
hsa-miR-20a	PTEN	Reporter assay; Western blot
hsa-miR-21	NCOA3	Reporter assay
hsa-miR-21	RASA1	Reporter assay; Western blot
hsa-miR-210	MRE11A	Western blot
hsa-miR-210	XPA	Western blot
hsa-miR-216a	SIRT1	Reporter assay; Western blot
hsa-miR-217	EZH2	Reporter assay; Western blot
hsa-miR-217	TRAPPC2P1	Reporter assay
hsa-miR-218	ACTN1	Reporter assay
hsa-miR-218	BIRC6	Reporter assay
hsa-miR-218	CDKN1B	Reporter assay
hsa-miR-218	EBP	Western blot
hsa-miR-218	EFNA1	Western blot
hsa-miR-218	MBNL2	Western blot
hsa-miR-218	MRPS27	Western blot
hsa-miR-218	NUP93	Western blot
hsa-miR-218	STAM2	Reporter assay
hsa-miR-224	FOSB	Reporter assay
hsa-miR-224	NCOA6	Reporter assay
hsa-miR-224	NIT1	Reporter assay
hsa-miR-24	CDKN1B	Reporter assay
hsa-miR-24	HNF4A	Reporter assay; Western blot
hsa-miR-24	MLEC	Reporter assay
hsa-miR-26a	CDC6	Western blot
hsa-miR-26a	CDK8	Western blot
hsa-miR-26a	CTGF	Western blot
hsa-miR-26a	MYC	Western blot
hsa-miR-26a	STRADB	Western blot
hsa-miR-27b	TRAPPC2P1	Reporter assay
hsa-miR-29a	FGG	Reporter assay
hsa-miR-29a	IMPDH1	Reporter assay
hsa-miR-29a	RAN	Reporter assay
hsa-miR-29b	DNMT1	Reporter assay

hsa-miR-29b	FGG	Reporter assay
hsa-miR-29b	VEGFA	Reporter assay
hsa-miR-29c	FGB	Reporter assay
hsa-miR-29c	FGG	Reporter assay
hsa-miR-29c	GAPDH	Reporter assay
hsa-miR-301b	DNMT1	Reporter assay
hsa-miR-31	ARPC5	Reporter assay
hsa-miR-31	CXCL12	Reporter assay
hsa-miR-31	ETS1	Reporter assay
hsa-miR-31	HOXC13	Reporter assay
hsa-miR-31	JAZF1	Reporter assay
hsa-miR-31	KLF13	Reporter assay
hsa-miR-31	NFAT5	Reporter assay
hsa-miR-31	NUMB	Reporter assay
hsa-miR-31	RET	Reporter assay
hsa-miR-31	YY1	Reporter assay
hsa-miR-320a	TAC1	Reporter assay
hsa-miR-328	CD44	Reporter assay
hsa-miR-330-3p	NTRK3	Reporter assay
hsa-miR-330-3p	VEGFA	Reporter assay
hsa-miR-335	ARPC5L	Reporter assay
hsa-miR-335	RASA1	Reporter assay
hsa-miR-335	UBE2F	Reporter assay
hsa-miR-338-5p	LRP1	Reporter assay; Western blot
hsa-miR-345	NTRK3	Reporter assay
hsa-miR-346	BTK	Reporter assay
hsa-miR-346	IL18	Reporter assay; Western blot
hsa-miR-34a	CCND3	Western blot
hsa-miR-34a	CDC25A	Western blot
hsa-miR-34a	MAP3K9	Reporter assay; Western blot
hsa-miR-34a	MYB	Reporter assay; Western blot
hsa-miR-34a	PEA15	Western blot
hsa-miR-34a	VEGFA	Reporter assay
hsa-miR-34b	VEGFA	Reporter assay
hsa-miR-370	HMGA2	Reporter assay
hsa-miR-373	MRE11A	Western blot
hsa-miR-373	XPA	Western blot
hsa-miR-376a*	PRPS1	Reporter assay
hsa-miR-376a*	SNX19	Reporter assay
hsa-miR-376a*	ZNF513	Reporter assay
hsa-miR-377	PPM1A	Reporter assay; Western blot
hsa-miR-378	VEGFA	Reporter assay
hsa-miR-384	NTRK3	Reporter assay
hsa-miR-409-3p	FGA	Reporter assay
hsa-miR-409-3p	FGG	Reporter assay
hsa-miR-422a	CYP8B1	Reporter assay
hsa-miR-424	ITPR1	Reporter assay
hsa-miR-424	PIAS1	Reporter assay
hsa-miR-429	ZEB1	Reporter assay
hsa-miR-449a	HDAC8	Western blot
hsa-miR-491-5p	CHD4	Reporter assay
hsa-miR-491-5p	TAF10	Reporter assay
hsa-miR-520h	SMAD6	Reporter assay
hsa-miR-532-5p	TRAPPC2P1	Reporter assay

hsa-miR-545	LRP1	Reporter assay; Western blot
hsa-miR-559	MTA2	Western blot
hsa-miR-559	VCL	Western blot
hsa-miR-562	MET	Reporter assay
hsa-miR-562	PSEN1	Reporter assay
hsa-miR-612	TP53	Reporter assay; Western blot
hsa-miR-617	NTRK3	Reporter assay
hsa-miR-626	SLC7A5	Reporter assay; Western blot
hsa-miR-661	MTA2	Western blot
hsa-miR-661	VCL	Western blot
hsa-miR-769-5p	TRAPPC2P1	Reporter assay
hsa-miR-9	BCL6	Reporter assay
hsa-miR-9	ETS1	Reporter assay
hsa-miR-9	POU2F2	Reporter assay
hsa-miR-92a	ARID4B	Reporter assay
hsa-miR-92a	BMPR2	Reporter assay; Western blot
hsa-miR-92a	KAT2B	Reporter assay; Western blot
hsa-miR-92a	TGFBR2	Reporter assay
hsa-miR-93	MAPK9	Reporter assay
hsa-miR-942	CDKN1A	Reporter assay; Western blot
hsa-miR-98	E2F1	Reporter assay
hsa-miR-98	NCOA3	Reporter assay



Appendix III List of abbreviations

Table A2. List of abbreviations

Symbol	Meaning
miRNA	microRNA
MTI	microRNA-target interaction
RISC	RNA-induced silencing complex
3’-UTR	3' untranslated region
5’-UTR	5' untranslated region
CDS	Coding sequence
AS exon	Alternatively spliced exon
CS exon	Constitutively spliced exon
GSEA	Gene set enrichment analysis
WB	Western blot
SILAC	Stable isotope labeling with amino acids in culture
pSILAC	Pulsed SILAC
Pol II	RNA Polymerase II
GFP	Green fluoresce protein
TU	Transcript unit
dsRNA	Double strand RNA

Appendix IV Supplementary figures

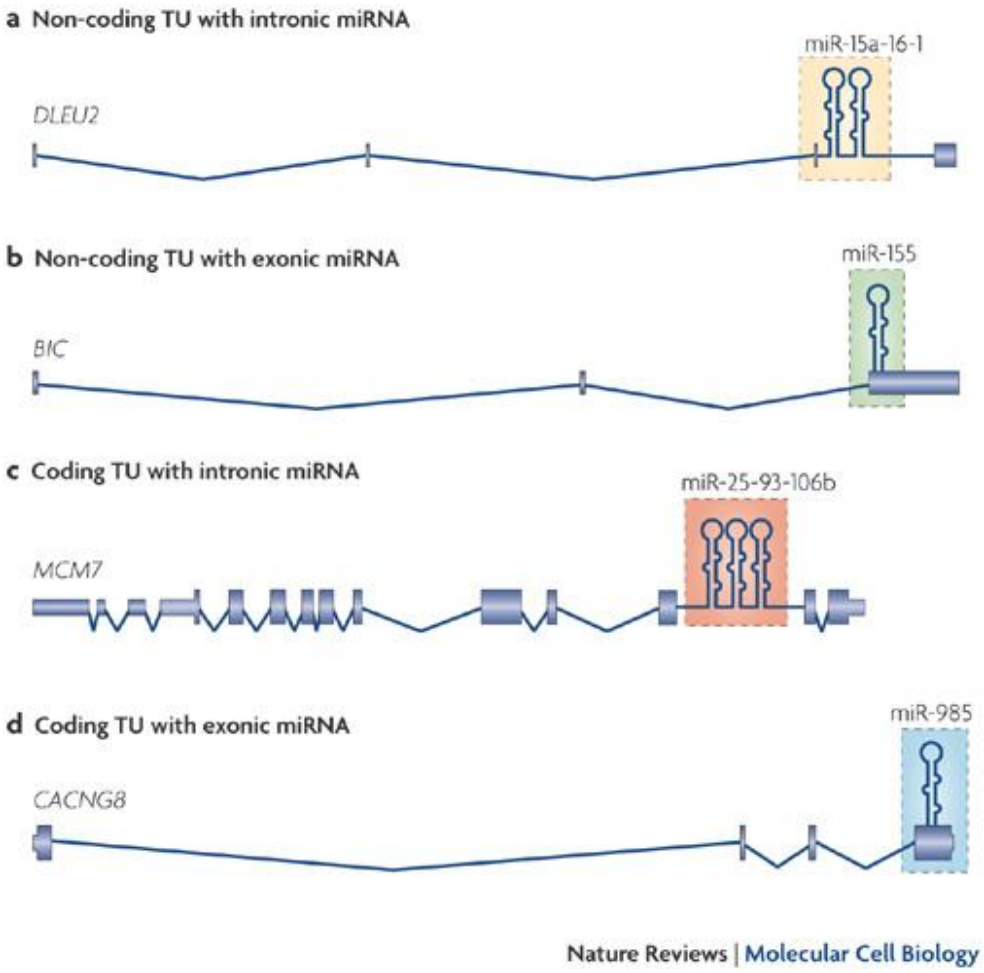


Figure S1. The four classes of miRNAs which are categorized by their genomic locations relative to the known genes (adapt from Kim, V. N. 2009)(61).

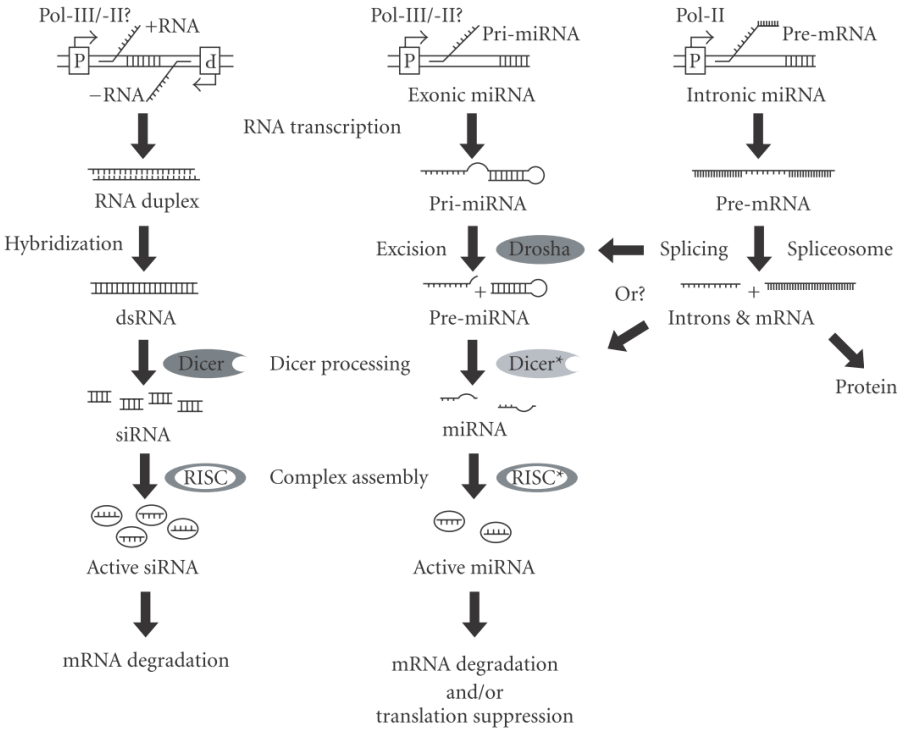


Figure S2. Different mechanism of miRNA and siRNA biogenesis. (adapt from Lin, S.L et al 2006) (178)

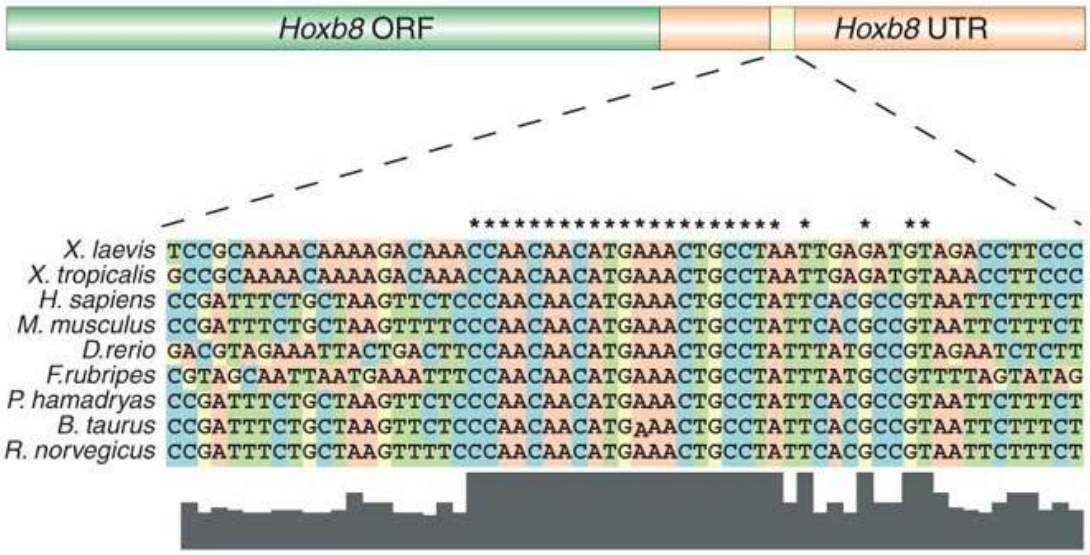


Figure S3. The miR-196a target site is conserved in Hoxb8. (adapt from Mansfield, J.H. et al, 2004) (179)

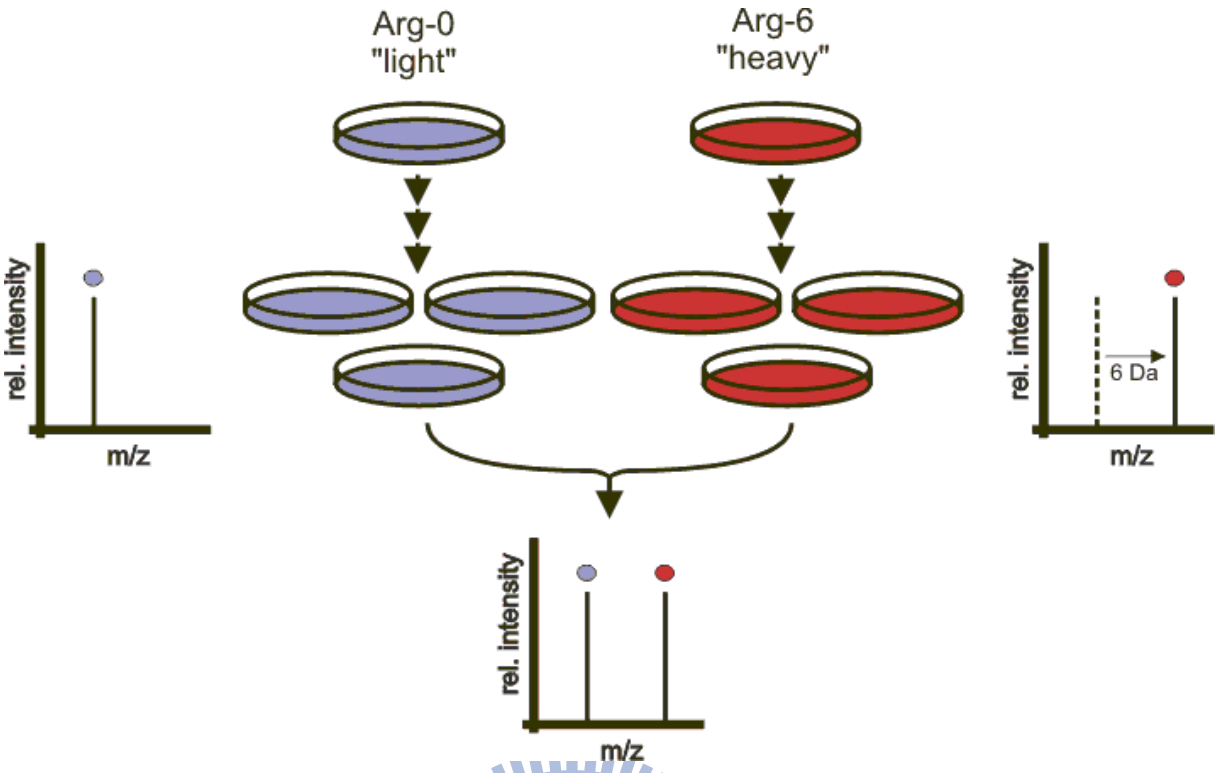


Figure S4. The principle of SILAC.

(http://en.wikipedia.org/wiki/Stable_isotope_labeling_by_amino_acids_in_cell_culture)

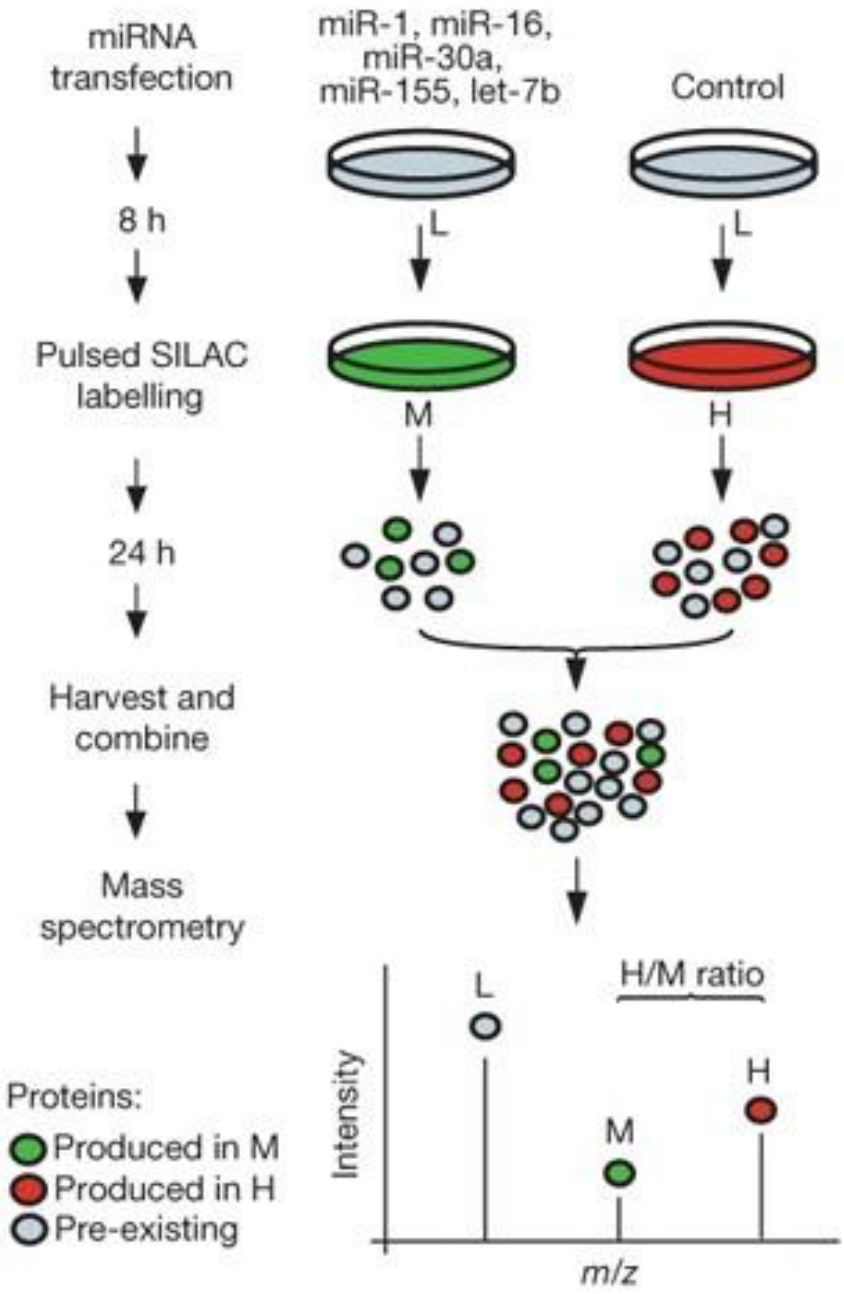


Figure S5. Matthias Selbach et al developed pSILAC to detect widespread changes in protein synthesis induced by miRNAs. (adapt from Selbach et al) (73)

Appendix V Curriculum vitae

Education and Training

1999-2004 Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

2004-2006 Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan.

2006-2011 Ph.D., Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsinchu, Taiwan.

Publication lists

1. **Hsu, S.D.**, Lin, F.M., Wu, W.Y., Liang, C., Huang, W.C., Chan, W.L., Tsai, W.T., Chen, G.Z., Lee, C.J., Chiu, C.M., Chien, C. H., Wu, M. C., Huang, C. Y., Tsou, A. P., Huang, H. D. (2011) miRTarBase: a database curates experimentally validated microRNA-target interactions. ***Nucleic acids research***, 39, D163-169. (IF=7.836, Rank=30/286, BIOCHEMISTRY & MOLECULAR BIOLOGY) (citations number = 3)
2. Hsu, J.B.K.*, Chiu, C.M.*, **Hsu, S.D.***, Huang, W.Y., Chien, C.H., Lee, T.Y. and Huang, H.D. (2011) miRTar: an integrated system for identifying miRNA-target interactions in Human. ***BMC Bioinformatics***, 12. (IF=3.028, Rank=4/37, MATHEMATICAL & COMPUTATIONAL BIOLOGY) (citations number = 0)
3. **Hsu, S.D.**, Chu, C.H., Tsou, A.P., Chen, S.J., Chen, H.C., Hsu, P.W., Wong, Y.H., Chen, Y.H., Chen, G.H. and Huang, H.D. (2008) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. ***Nucleic Acids Res***, 36, D165-169. (IF=7.836, Rank=30/286, BIOCHEMISTRY & MOLECULAR BIOLOGY) (citations number = 43)
4. Hsu, P.W., Lin, L.Z., **Hsu, S.D.**, Hsu, J.B. and Huang, H.D. (2007) ViTa: prediction of host microRNAs targets on viruses. ***Nucleic Acids Res***, 35, D381-385. (IF=7.836, Rank=30/286, BIOCHEMISTRY & MOLECULAR BIOLOGY) (citations number = 17)
5. Hsu, P.W., Huang, H.D., **Hsu, S.D.**, Lin, L.Z., Tsou, A.P., Tseng, C.P., Stadler, P.F., Washietl, S. and Hofacker, I.L. (2006) miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. ***Nucleic Acids Res***, 34, D135-139. (IF=7.836, Rank=30/286, BIOCHEMISTRY & MOLECULAR BIOLOGY) (citations number = 72)