Universitt des Saarlandes
Zentrum fr Bioinformatik

Bachelorarbeit

MiRNA target analysis focussing on sequence alignment

submitted by

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Declaration

I hereby confirm that this thesis is my own work and that I have documented all sources used.

Saarbrücken, September xx, 2016

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Acknoledgement

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Contents

Abstract

Small endogenous RNAs, called miRNAs, are more and more investigated because they play an important role in the regulation of gene expression. One of the many fields of miRNAs is the target prediction. This problem is very complex and there is still no peferct solution for it. Many features for a precise prediction can be incorporated like base pairing, free energy or conservation. The database miRTarBase provides data of validated miRNA target interactions. In this research these interactions are investigated with sequence alignments. MiRNA and target mRNA are aligned and the resulting binding site is further analysed, escpecially how significant the alignment scores are compared to alignments of independant sequences.

1 Introduction

miRNAs are small, non-coding RNAs that function in post-transcriptional regulation of gene expression, especially in terms of gene silencing. A miRNA consists of approximately 22 nucleotides which build a single strand RNA [Bartel, 2004]. The maturation of the miRNA is shown in figure 1.1. After transcription the miRNA folds into a hairpin structure and builds the primary miRNA [Macfarlane and Murphy, 2010]. First, this pri-miRNA is processed by Drosha which results in a precursor miRNA (pre-miRNA). This precursor is about 60 to 70 nucleotides long and contains two single stranded miRNAs. After the export into the cytoplasm another processing step is executed with Dicer to gain the actual form of a miRNA. But it is still double stranded and needs to be unwinded to be a mature miRNA [Macfarlane and Murphy, 2010]. So after processing of the initial double stranded miRNA-duplex, the guide strand will be further used in association with other molecules. In detail, the miRNA is mainly active in combination with a catalytic protein of the AGO protein family. With it, the miRNA builds an RNA-induced silencing complex [Ha and Kim, 2014]. This complex targets a mRNA mainly at its 3' untranslated region (UTR) by complementary binding to the sequence. In this case, the miRNA functions as a guide with its base pairing with the mRNA [Macfarlane and Murphy, 2010].

It can also bind at the 5' UTR or even in the coding region but these are the uncommon regions. Either way, this binding results in gene silencing either by repression of mRNA translation or degradation of the respective mRNA [Enright et al., 2003]. Which of the two pathways is chosen depends on the degree of complementarity. As figure 1.1 illustrates, if the complementarity is nearly perfect then the target mRNA is degraded otherwise if the seed regions contains mismatches the translation is only repressed. Both ways have a huge impact on the expression and the resulting genetic changes can have consequences like the initiation of cancer [Macfarlane and Murphy, 2010].

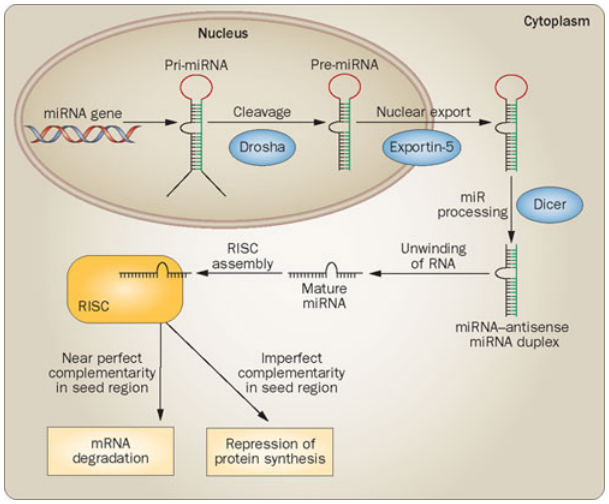


Figure 1.1: Biogenesis of miRNAs

This gene regulation by miRNAs plays an important role for many major cell functions like growth, differentiation or metabolism and is currently examined by many investigators [Ardekani and Naeini, 2010]. The importance of miRNA and their regulation mechanism can also be observed by the fact that more than 60% of all human genes have one conserved miRNA-binding site ore even more [Friedman et al., 2009]. Therefore, a disregulation of miRNAs, either up- or downregulation, may result in different human diseases, e.g. cancer or autoimmune diseases [Ardekani and Naeini, 2010]. Hence, this also leads to the assumption that miRNAs can be useful features for the diagnosis and treatment of diseases. But they follow a very special and complicated targeting and this has to be elucidated. So the first step is to understand their function and regulation mechanism. With these informa-

tion it can be possible to predict targets of miRNAs and then specifically effect these target interactions to diagnose or treat diseases.
But as already mentioned, especially this prediction of new targets of miRNAs is the main challenge in the whole field of miRNAs. There is still no perfect, reliable solution for it. There are a few tools that try to solve this problem. One of the first tools was miRanda. In their prediction algorithm they rely on three main features: sequence complementary, free energy and evolutionary conservation [Enright et al., 2003]. Another tool is miRSVR that uses bit vectors and statistical learning approaches to predict targets. In this bit vector they store information like base complementarity, UTR length and distance, AU content and conservation [Betel et al., 2010]. A third tool was developed called TargetScan. Their approach is based on seed matching and additional features like UTR positioning, AU content as well and base pairing of the 13nt to the 16nt miRNA nucleotide. They especially take the seed region into account by defining four different patterns of seed binding sites [Lewis et al., 2005].

Because the miRNA binds to the sequence of its target gene an obvious starting point can be the complementarity of the miRNA sequence to the gene sequence as almost each tool considers at first. The first idea could be that if the sequence complementarity of miRNA and target mRNA is quite high at a certain position, this could be interpreted as a new target site. With the help of sequence alignments, this regions of high complementarity can be found. In this case very high alignment scores could indicate a high probability that there is a binding site at this position. This alignment could then be used to predict completely new target sites and decide whether the considered mRNAs is targeted by a certain miRNAs. Whether this is a reliable indication or not will be discussed below. For this assumption there already exist some problems beforehand. Because it is known that the main binding happens within the seed region, other regions are not necessarily complementary and this may lower the complementarity and consequently the alignment score.

The main feature that most of the features consider first is the sequence complementarity especially at a certain seed region. In contrast to miRNAs in plants which bind nearly perfectly complementary to their targets, miRNAs in animals bind less tightly and are not perfectly complementary [**?**].

There can be regions were the nucleotides are unbound which results in complex secondary structures that are hard to predict [Rehmsmeier et al., 2004]. The main complementary region or seed region includes nucleotides from position two to eight starting from the 5' end. Figure 1.2 shows the scheme of the seed region [Peterson et al., 2014]. The irregularity of the presence of non-complementarity regions makes the reliable prediction more difficult for animals than for plants. Concentrating mainly on the seed region for a prediction will lead to many false positives. This small region of seven or eight nucleotides would be too unspecific because they can be present in the mRNA although there is no real binding site at this position. It would be recommended to take more than the seed region into account to increase the complexity and specificity of the prediction. How significant the consideration of the sequence complementarity of miRNA and mRNA sequence is, will be further analysed with validated data in this research.

In the mentioned seed region there can be different types of matching patterns: perfect matching between six nucleotides, seven nucleotides including the 8th position, seven nucleotides and an Adenine at position one or eight matching nucleotides from position one to eight and also an Adenine at position one [Lewis et al., 2005] [Brennecke et al., 2005] [Krek et al., 2005]. Figure 1.3 illustrates the different types of sites. Grimson et al. [Grimson et al., 2007] investigated the different types of sites referring to the effectiveness of the gene repression. As shown in figure 4.1, they found that the repression is the highest when an 8mer site is present and the lowest when only six nucleotides are perfectly matching.

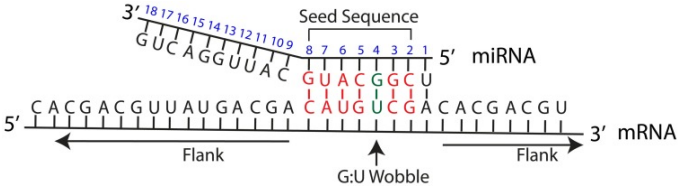


Figure 1.2: Scheme of seed matching

Staying with sequence complementarity, after a region of non complementary nucleotides towards the center of the miRNA, another matching region towards the 3' end can be observed. Aligning miRNA to target mRNA could reveal this occurance.

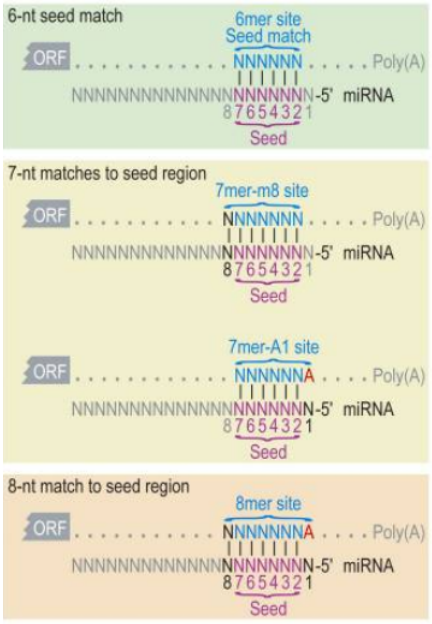


Figure 1.3: Canonical sites of seed region

To gain information about targeting and whether alignment and sequence complementarity play a big role in this process, databases provide confirmed data about miRNAs and their targets. For these miRNA analyses three main databases are useful and necessary. A collection of experimental validated miRNA-target interactions(MTIs) is essential. The database miRTarBase provides a big dataset of validated MTIs [Hsu et al., 2011]. To get information about every single miRNA for different organisms miRBase contains large datasets about the miRNAs. The most interesting information are sequences of the precursor and of all mature miRNAs [Kozomara and Griffiths-Jones, 2011]. For further analyses I will only consider miRNAs of *Homo sapiens*. The last used database is the UCSC Genome Bioinformatics Site. In their table browser you can get data files about human gene sequences and untranslated regions and their respective sequences [Karolchik et al., 2004].

2 Methods and Data

2.1 Data

2.1.1 miRTarBase

The database mirTarBase, which was released in 2010, provides by now about 7500 strong validated MTIs and 348000 weak ones from different species [Chou et al., 2016]. In this research I concentrate on humans. Different experiment types were used to validate the data, including Reporter assay, Western plot, qPCR, Microarray, NGS, pSILAC and other methods where the first three are the ones that deliver strong evidences [Hsu et al., 2011]. The strong types detect the co-epression of miRNAs and their target gene, more precisely, the mRNA expression levels when miRNA is overexpressed or knocked down. Another constraint in here, I only concentrate on the strong evidence targets. The data collection in detail provides many information about the interaction between one miRNA and its target. Interesting details are the predicted alignments of miRNA and target 3' UTR sequence by either the author of the MTI or other prediction tools like miRanda. These alignments will also play an important role in the following analyses.

From the database catalogue you can easily download the respective MTI data tables of the favoured species, in this case *Homo sapiens*. The table then contains the following information: miRTarBase ID, miRNA name, species of the miRNA, target gene symbol, target gene Entrez ID, species of the target gene, experiment type, support type, references. The interesting fields for the research are name of the miRNA, the target gene ID and the experiment type because I only concentrate on the Functional MTIs that are not weak. In fact, every single strong validated interaction was analysed.

As mentioned above, the miRTarBase provides also binding sites as alignment positions predicted by different tools. To decide whether my own found alignments are compatible with the provided ones, the miRTarBase html page of the single MTI was parsed to get the start positions of each provided alignment. These positions do not exist for every miRNA but for about 3700 of the interactions. The parsed positions can then be compared to the resulting positions by the pairwise2 module. But this will be described in detail later in the methodology.

	B	C	D	E	F	G	H	I
1	miRNA	Species (miRNA)	Target Gene	Target Gene	Species (Target)	Experiments	Support Type	References
2	hsa-miR-20a-5p	Homo sapiens	HIF1A	3091	Homo sapiens	Luciferase reporter assay//Western blot//Northern blot	Functional MTI	18632605
3	hsa-miR-20a-5p	Homo sapiens	HIF1A	3091	Homo sapiens	Luciferase reporter assay//qRT-PCR//Western blot	Functional MTI	23911400
4	hsa-miR-20a-5p	Homo sapiens	HIF1A	3091	Homo sapiens	HITS-CLIP	Functional MTI (Weak)	22473208
5	hsa-miR-146a-5p	Homo sapiens	CXCR4	7852	Homo sapiens	qRT-PCR//Luciferase reporter assay//Western blot	Functional MTI	18568019
6	hsa-miR-146a-5p	Homo sapiens	CXCR4	7852	Homo sapiens	Microarray	Functional MTI (Weak)	20375304
7	hsa-miR-122-5p	Homo sapiens	CYP7A1	1581	Homo sapiens	qRT-PCR//Luciferase reporter assay	Functional MTI	20351063
8	hsa-miR-222-3p	Homo sapiens	STAT5A	6776	Homo sapiens	qRT-PCR//Luciferase reporter assay//Western blot	Functional MTI	20489169
9	hsa-miR-222-3p	Homo sapiens	STAT5A	6776	Homo sapiens	Luciferase reporter assay	Functional MTI	24736554
10	hsa-miR-21-5p	Homo sapiens	RASGRP1	10125	Homo sapiens	qRT-PCR//Luciferase reporter assay//Western blot	Functional MTI	20483747
11	hsa-miR-21-5p	Homo sapiens	RASGRP1	10125	Homo sapiens	Microarray	Functional MTI (Weak)	18591254
12	hsa-miR-21-5p	Homo sapiens	RASGRP1	10125	Homo sapiens	HITS-CLIP	Functional MTI (Weak)	22473208
13	hsa-miR-148a-3p	Homo sapiens	DNMT1	1786	Homo sapiens	Luciferase reporter assay	Functional MTI	20146264

Figure 2.1: Extract of the miRTarBase file

2.1.2 miRBase

To get the corresponding sequence of the miRNA name, miRBase, which was already published in 2005, provides a complete dataset of all known miRNAs [Griffiths-Jones et al., 2006]. By now it contains about 35000 mature forms and 2500 of it are found in *Homo sapiens*. The table with all miRNAs includes the accession number, miRNA ID, status, sequence, accession number of first mature form, its ID, its sequence, accession number of the second mature form, its ID and its sequence. I only use miRNA id and the respective sequences to align those to the gene sequence [Kozomara and Griffiths-Jones, 2011].

	A	B	C	D	E	F	G	H	I	J
1	Accession	ID	Status	Sequence	Mature1_Acc	Mature1_ID	Mature1_Seq	Mature2_Acc	Mature2_ID	Mature2_Seq
2	MI0000001	cel-let-7	UNCHANGED	UACACUGUGGAUCCGGUGAGG	MIMAT0000001	cel-let-7-5p	UGAGGUAGUAGGUUGUAUAGUU	MIMAT0015091	cel-let-7-3p	CUAUGCAAUUUUCUACCUUACC
3	MI0000002	cel-lin-4	UNCHANGED	AUGCUUCCGGCCUGUUCCCUG	MIMAT0000002	cel-lin-4-5p	UCCCUGAGACCUCAAGUGUGA	MIMAT0015092	cel-lin-4-3p	ACACCUGGGCUCUCCGGGUACC
4	MI0000003	cel-mir-1	UNCHANGED	AAAGUGACCGUACCGAGCUGC	MIMAT0020301	cel-miR-1-5p	CAUACUUCCUUACAUGCCCAUA	MIMAT0000003	cel-miR-1-3p	UGGAAUGUAAAGAAGUAUGUA
5	MI0000004	cel-mir-2	UNCHANGED	UAAACAGUAUACAGAAAGCCA	MIMAT0020302	cel-miR-2-5p	CAUCAAAGCGGUGGUUGAUGUG	MIMAT0000004	cel-miR-2-3p	UAUCACAGCCAGCUUUGAUGUGC
6	MI0000005	cel-mir-34	UNCHANGED	CGGACAAUGCUCGAGAGGCAG	MIMAT0000005	cel-miR-34-5p	AGGCAGUGUGGUUAGCUGGUUG	MIMAT0015093	cel-miR-34-3p	ACGGCUACCUUCACUGCCACCC
7	MI0000006	cel-mir-35	UNCHANGED	UCUCGGAUCAGAUCGAGCCAU	MIMAT0020303	cel-miR-35-5p	UGCUGGUUUCUUCCACAGUGGUA	MIMAT0000006	cel-miR-35-3p	UCACCGGGUGGAAACUAGCAGU
8	MI0000007	cel-mir-36	UNCHANGED	CACCGCUGUCGGGGAACCGCG	MIMAT0020304	cel-miR-36-5p	CGCCAAUUUUCGCUUCAGUGCUA	MIMAT0000007	cel-miR-36-3p	UCACCGGGUGAAAAUUCGCAUG
9	MI0000008	cel-mir-37	UNCHANGED	UUCUAGAAACCCUUGGACCAG	MIMAT0015094	cel-miR-37-5p	UGUGGGUGUCCGUUGCGUGCUA	MIMAT0000008	cel-miR-37-3p	UCACCGGUGAACACUUGCAGU
10	MI0000009	cel-mir-38	UNCHANGED	GUGAGCCAGGUCCUGUUCCGG	MIMAT0020305	cel-miR-38-5p	UCCGGUUUUUUCCGUGGUGAUA	MIMAT0000009	cel-miR-38-3p	UCACCGGGAGAAAAACUGGAGU
11	MI0000010	cel-mir-39	UNCHANGED	UAUACCGAGAGCCCAGCUGAU	MIMAT0020306	cel-miR-39-5p	AGCUGAUUUCGUCUUGGUAAUA	MIMAT0000010	cel-miR-39-3p	UCACCGGGUGUAAAUCAGCUUG
12	MI0000011	cel-mir-40	UNCHANGED	UCCUGUCCGCACCUCAGUGGA	MIMAT0020307	cel-miR-40-5p	AGUGGAUGUAUGCCAUGAUGAUA	MIMAT0000011	cel-miR-40-3p	UCACCGGGUGUACAUCAGCUAA
13	MI0000012	cel-mir-41	UNCHANGED	GGGUCCCAGAGACCUUGGUGG	MIMAT0020770	cel-miR-41-5p	GGUGGUUUUUCUCUGCAGUGAUA	MIMAT0000012	cel-miR-41-3p	UCACCGGGUGAAAAAUCACCUA

Figure 2.2: Extract of the miRBase file

2.1.3 UCSC Genome Bioinformatics Site

The last required dataset is the collection of target gene sequences and their respective untranslated regions (UTRs). On the UCSC Genome Bioinformatics Site you can generate a list of all genes and their UTRs of the human genome using the Table Browser [Karolchik et al., 2004]. Used humand genome?? The list consists of a description of the gene with the transcript accession number (NM-number) and the concatenated sequences of 5'-UTR, gene and 3'-UTR.

```
>hg38_refGene_NM_001276352 range=chr1:67092176-67134971 5'pad=0 3'pad=0 strand=- repeatMasking=none
cgagtaacccgcggagccagaagagggaggaaaggagatgagatttcatc
atgttggccagcctggtctcaaactcctgacctcaagtgacccgcctgcc
tcagcctcccaaagtgctgggattacaggaatttagtgattgacaATGGC
AGAAAAAATCCTAGAGAAGTTGGATGTCCTTGATAAGCAAGCAGAGATAA
TCTTGGCCAGAAGAACAAAGATAAACAGGCTTCAGAGTGAAGGAAGAAAA
ACAACTATGGCTATACCCCTGACATTTGATTTTCAGTTGGAATTTGAAGA
...
```

Figure 2.3: Extract of the UCSC genome file

2.1.4 ID converter

For the alignment of miRNA to the corresponding gene the respective miRNA sequence and gene sequence are required. The interaction data from miRTarBase only provides the correlation between miRNA name and target gene ID. But the dataset from UCSC only delivers the NM number for the gene sequence. Therefore a conversion from gene Entrez ID to Refseq mRNA accession number is required. Biodbnet provides a conversion tool for different IDs, names and numbers [Mudunuri et al., 2009]. I entered all existing target Entrez IDs of the miRTarBase file and obtained a conversion list of IDs and their corresponding Refseq accession numbers. In my program I used this list by storing every entry in a dictionary and simply looked the particular ID up for every MTI. This is also described in the following section.

2.2 Methodology

The program for the analysis is implemented in Python. As input files the UCSC gene file, miRTarBase file and miRBase file are required in a csv format. Additionally the conversion file of gene id and nm number is needed. It can be generated as described in section 2.1.4 and given as an input file in a text format. For each data file except the MTI file an own dictionary is generated. Therefore the data files are parsed and each entry is stored into the respective dictionary that allows fast searching. For the miRNAs the dictionary is defined as following: miRNA name as key and sequence as value. The two mature forms, if two exist, are stored separately. For the gene file, the NM number is the key and the value is a list of sequences of 5' UTR, coding redion and 3' UTR. Finally, for the conversion list, the Entrez id is stored as the key and the list of NM numbers as a value. There can exist more

than one NM number for one gene Entrez id because different transcripts can be obtained by alternative splicing. Then for every MTI in the miRTarBase file the respective miRNA sequence is searched in the miRBase dictionary and combined with the gene sequence of the corresponding target in the gene dictionary. The miRTarBase file contains data of 'Functional MTI', 'Functional MTI(Weak)' and 'Non-Functional MTI'. Here I only consider the strong validated data of the Functional MTIs because they deliver strong evidence for the interaction. So if an entry in the miRTarBase file contains something different than 'Functional MTI', it is scipped.

After matching these two sequences, a local alignment is performed. An alignment is defined as the optimal positioning of the bases of one sequence, in this case the miRNA, to a region in the other sequence, the gene sequence. From the result functional or structural similarities can be obtained [Wikipedia, 2016]. In this case, the similarity can be interpreted as region where a binding site could be present because if we take the reverse complement of the miRNA sequence and align it to the mRNA sequence, this will simulate the binding. More precisely, if the alignment score is high, the sequences (mRNA and reverse complement of miRNA) are more similar, implying the actual miRNA could possibly bind at this alignment position. Because the miRNA can bind at any region in the gene, a local alignment is executed, not a global.

The Biopython library provides a module, pairwise2, for pairwise local alignments of two sequences. This tool is based on a dynamic programming algorithm. This function can be used either with default parameter or different scores and costs can be defined. The default parameters are as following: +1 for matching character, 0 for not matching ones and there are no gap penalties [Biopython, 2016]. To get a more suitable alignment, own parameters can be selected. Table 2.1 shows the different parameters I used to analyse the data. Set no. 8 is similar to the parameters they used for the tool miRanda [Enright et al., 2003]. The other parameters are just logically selected to test which influence they have on the results.

For each alignment the following steps are performed: the pairwise2 module delivers the best alignment with the highest score. Sometimes there is more than one alignment because they have the same highest score. So for each found alignment list the miRNA name, the respective NM-number and the alignment score are stored in a string. Then for each single alignment in the list the starting position is computed and added to this string. To get a

Table 2.1: Parameter sets

Parameterset	match score	mismatch score	gap open	gap extend
1	default: 1	default: 0	-4	-4
2	default: 1	default: 0	-5	-1
3	1	-2	-2	-1
4	2	-2	-5	-4
5	3	-2	-4	-4
6	5	-1	-8	-4
7	5	-2	-8	-3
8	5	-3	-8	-2
9	5	-4	-6	-4

final list of all alignments, the single strings of each MTI is written to a text file.

For the analysis of the complementarities the alignment is further examined. First both sequences are reversed because they are given from 3' to 5'. To further analyse the 5' seed region and make the plots more clearly, the sequences then start with the 5' end. Each nucleotide position of miRNA and target mRNA is compared and whenever the nucleotide is the same, a '1' is added to a list, if they are different or there is a gap, a '0' is added. This list is yielded to a big matrix for the final analysis. Going through this matrix through each column the number of '1's is computed and divided by the total number of elements in the matrix. So the result will be the ratio of complementary bases. This ratio is plotted for each position in the alignment.

To be able to analyse the data statistically a set of negative controls is required. To produce this data, 1000 miRNAs of the miRBase file were randomly assigned to a list of genes and then this non target file is processed in the same way as the file of the true target interactions. This random assigment can by chance contain true targets but generally the miRNAs are matched with genes that are not targeted by them.

For these datasets the average and standard deviation were computed with functions of LibreOffice. Then with case and control alignment scores a statistical two-tailed t-test for two samples with equal variances was performed with LibreOffice as well [King, 2013]. This function provides a p-value

as a result. The lower this p-value is, the more significant the increase of the alignment score of the true targets is. If there is an increase in the score, this would be an indication that is we only calculate the alignment score for a new miRNA and any target sequence and observe a high one, the probability that this is a true target would be high. If this is a reliable feature for the prediction will be discussed in the following.

For the analysis of the alignment starting position, the miRTarBase was parsed first. For each strong validated data in the file the respective miRBase target page was parsed and the three given starting postions were searched. For that only the MTI id was put into the url and then opened. Together with the corresponding miRNA name and target NM number the positions were stored in a text file. In the final table the found starting positions were compared to the positions given in the resulting text file above. The final number of found positions are stored in the final result table as well.

3 Results

For each parameter set the computed alignment score for every MTI is stored in a table. Additionally the average alignment score, the standard deviation and the p-values are listed underneath. Table 3.1 shows only a summary of the table, excluding the single alignment scores of each MTI that the program provides. To draw a better comparison, the scores of the non targets are listed right under the scores of the true targets. The averages of both alignment score sets show that the scores of the true targets are in general only slightly higher than the ones of the non targets. For the lower alignment scores e.g. of set 1 -2 -2 -1, resulting from really low match scores, the difference is only 0.3, so at first sight not very significant. For the higher scores, the difference amounts to two which is an increase of about 3% for the parameter set 5 -1 -8 -4. At first site here, it is hard to say if this is significant because the scores are generally higher and then the amount of two is not much. The standard deviation of the non targets is slightly higher than for the true targets but also not very significant. Hence, the scores of both cases are equally spread from their means. The p-value sheds light on whether the increase in the alignment score is significant when considering the whole set of scores. According to the listed values of the t-test, which was computed as

Table 3.1: Table of alignment results

	3 -2 -4 -4	5 -1 -8 -4	5 -2 -8 -3
Average true targets	33.096	64.247	59.495
Average non targets	31.831	62.199	57.464
Standard deviation true targets	4.058	5.758	6.304
Standard deviation non targets	4.233	6.510	6.753
t-test p-value	5.934E-49	2.529E-61	1.040E-51

	5 -3 -8 -2	5 -4 -6 -4	-4 -4
Average true targets	56.391	55.510	13.712
Average non targets	54.269	53.294	13.362
Standard deviation true targets	6.633	6.806	1.078
Standard deviation non targets	6.876	7.01	1.311
t-test p-value	1.394E-51	1.744E-53	8.824E-50

	-5 -1	1 -2 -2 -1	2 -2 -5 -4
Average true targets	13.710	8.593	18.351
Average non targets	13.361	8.208	17.619
Standard deviation true targets	1.078	1.347	2.751
Standard deviation non targets	1.309	1.385	2.868
t-test p-value	1.131E-49	8.157E-42	3.279E-36

described in the methods, this is a significant increase in the score because they are all really low. The parameters 5 -1 -8 -4 show the most significant difference with a p-value of 2.529E-61 whereas 2 -2 -5 -4 shows the least significant increase but also here the p-value is really low with 3.279E-36. So even though the average scores are not much different, the scores of the true targets seem to be distributed in the higher scores and the ones of the non targets in the lower scores. Additionally, there are many more scores for the true targets than for the negative control. So if the average score is about two points higher for this one parameter set, for the bigger data set of true targets there have to be many more MTIs with higher alignment score to increase it by two. And taken this consideration into account, the amount of two is not that less. Hence, there must be many alignments with higher scores.

Another point that can be observed, is that the difference between the parameters is not that big. So for each set the increase is still very significant, even though they range from 10^{-36} to 10^{-61}. There is no exception that

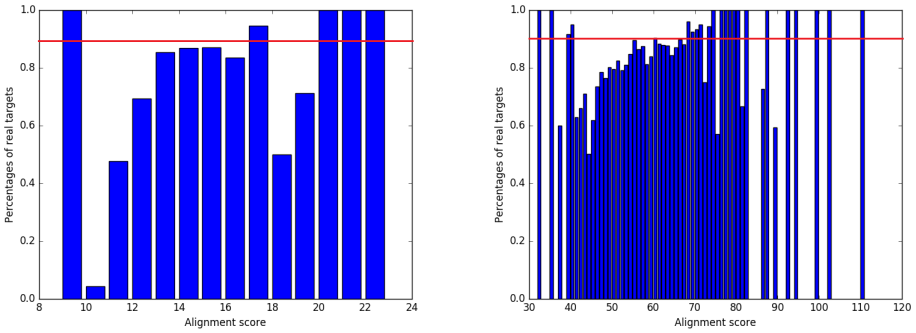


Figure 3.1: Distribution of alignment scores, parameters -4 -4 and 5 -3 -8- 2

does not show a significance in the alignment scores of the true targets. The differences in the height of the scores in general are obviously a result of the different match and mismatch scoring and penalizing gaps more or less. A certain penalty for gaps should be present because it is not very logical from a biological site if in the alignment are many gaps. This alignment as described above should simulate the binding to the target binding site. If a miRNA binds to its target it binds with some regions of complementarity as well as with some bulges but a binding with too many bulges is not common and would not function very well in terms of regulation. Therefore few gaps are sensible but many and longer ones should not be present. The parameter set with the highest and worst p-value has low match and mismatch scores compared to the gap costs. So gaps are more penalized than mismatches. This will result in binding sites with less gaps and some mismatches. The complementarity for the true targets will therefore be lower and not that specific. Analysing the non targets, which should not have binding sites, they will have in general worse binding sites where the complementarity is also not very high. Comparing them to the true targets which have also worse binding sites because of the scoring, the difference between the two groups will not be very high. And this can be seen in the worse p-value. Nevertheless it is still significant.

This increase in the alignment score can be also be observed when considering the distribution of the scores within the two groups of case and control. For two parameter sets (No. 1 and no. 8) such a distribution was plotted which is shown in figure 3.1.

threshold???

On the x-axis the different existing scores of the respective data set are shown and on the y-axis the ratio of true targets that had these scores. It can be observed that towards the higher alignment scores the ratio of true targets rises. But there are some scores that are exceptions because more non targets produced this scores than true targets, shown by lower y-values. Using this score as a prediction feature, a certain threshold needs to be determined to identify a true target. Hence, a certain ratio has to be exceeded to rank this score to be high enough to be a true target. In figure 3.1 a threshold of 90% is delineated meaning at least 90% true targets produced a certain alignment but still 10% of random non targets had this score as well. Assuming this threshold and using the alignment scores for a prediction, for the first parameter set if we have scores of nine, 17, 20, 21, 22 or 23, a mRNA would be classified as a true target. Obviously the score nine would not fit in the pattern because it is a very low alignment score and the binding site would not be very suitable because there are for sure many mismatches or gaps that lower the alignemnt score. On the other hand, alignments of miRNA and mRNA with a resulting score or 19 which belongs to the higher scores for this set, would not be classified as a true target even though the binding might be suitable. How this classification will look like depends strongly on this threshold. A lower threshold will result in a lower specificity and higher sensitivity, a higher one vice versa, so the two measurements of the performance need to be balanced and determined depending on the analysis.

For the second plot in figure 3.1 the same can be observed: some lower alignment score are above the threshold although they are not suitable and for some higher ones the probability to be a true target would be low. But in general it can be said that if the score is above 70 the likelihood that the target is a true target is quite high. In this plot is can also be seen that there are some outliers with high alignment scores. There are very few with really high scores like 100 and 110 but in between the scores were not produced, neither by true targets nor by non targets.

Summing up, assuming the score as the only prediction feature and given a certain threshold, some mRNAs are predicted right to be true targets but there are also false positives and false negatives. Given high scores of the alignment of miRNA seqeunce and mRNA sequence, a mRNA is not necessarily targeted by this miRNA. This uncertainty can be compensated by

considering many more features to combine them and get a more reliable prediction.

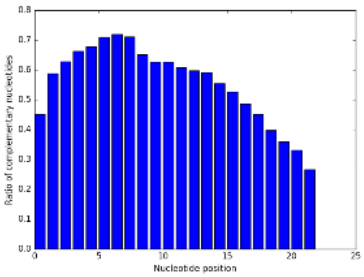
As also described in the methods, for each parameter set the complementarity of the single alignment positions of the miRNA and target gene sequence were also plotted. These plots for the different sets are shown in figure 3.2.

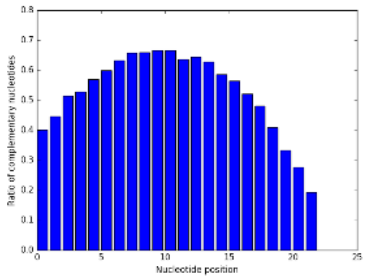
The x-axis of the plots shows the position one to 22 of the miRNA, the y-axis the ratio of bases that are complementary at this position, considering all alignments produced with these parameters. Because most of the miRNAs are only 22 nucleotides long, I will only focus on these positions because higher positions are very imprecise because of the different lengths. On the right site the non targets are shown, on the left the true targets.

In general it can be seen that the bars of the non targets are never higher than 70%. The true targets sometimes exceed this percentage but not very much. For some sets there is more significant difference between true targets and non targets than for others. Regarding set A, for the true target left an increase in complementarity in the seed region can be observed. Whereas the first position is blow 50%, from the second position on to the 8th the complementarity rises. On the other hand the ratio towards the end gets really low down to only 30% of complementary bases. In comparison, the maximum of complementarity of the non targets right is shifted towards the central positions, not showing the typical seed region. From then on it decreases very fast as well. Notably the plot of the true targets does not show the complementary region towards the 3' end. The same applies for plot B. Although the parameters are very different the plot for the true targets looks the same like for A. The right plot shows even more the difference between the two groups. There is again an increase in the positions eigth to 13, not fitting into any rule of targeting. Considering the other figures of the complementarities, the seed region is always recognizable, althought not very strong. The increase in the first few nucleotides amount only to a few percents. The right plots do not follow any rules. The complementarities are almost constant and drop at the end up to 30%. For the true targets the decrease at the end can also be observed but not so much. Plot H and I stay almost constant above 50%.

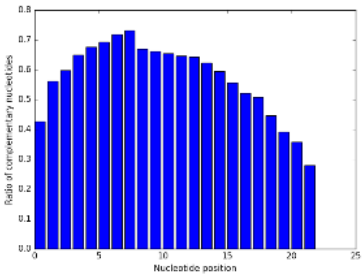
Summing up for the true targets the complementarity is almost as high as for the non targets, gaining no reliable information for the prediction. This

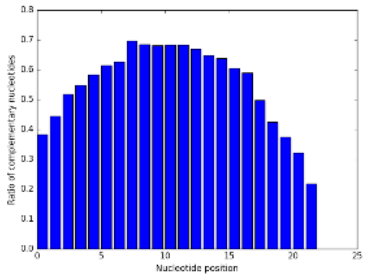
A: 1 -2 -2 -1



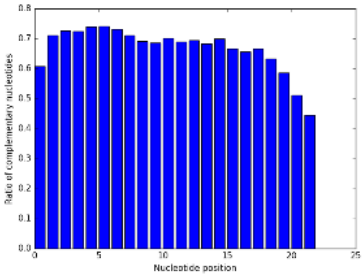


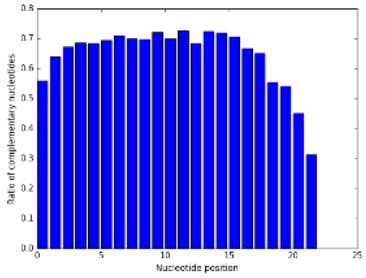
B: 2 -2 -5 -4



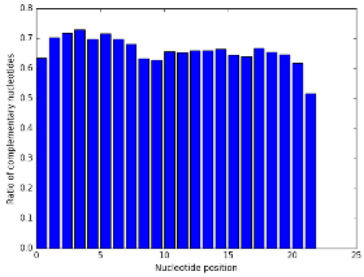


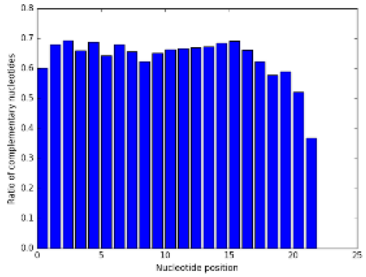
C. 3 -2 -4 -4



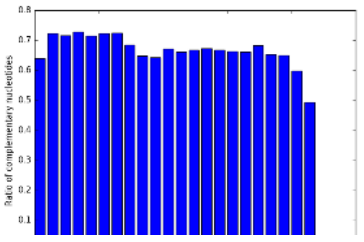


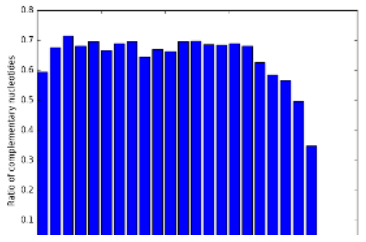
D: 5 -1 -8 -4





E: 5 -2 -8 -3





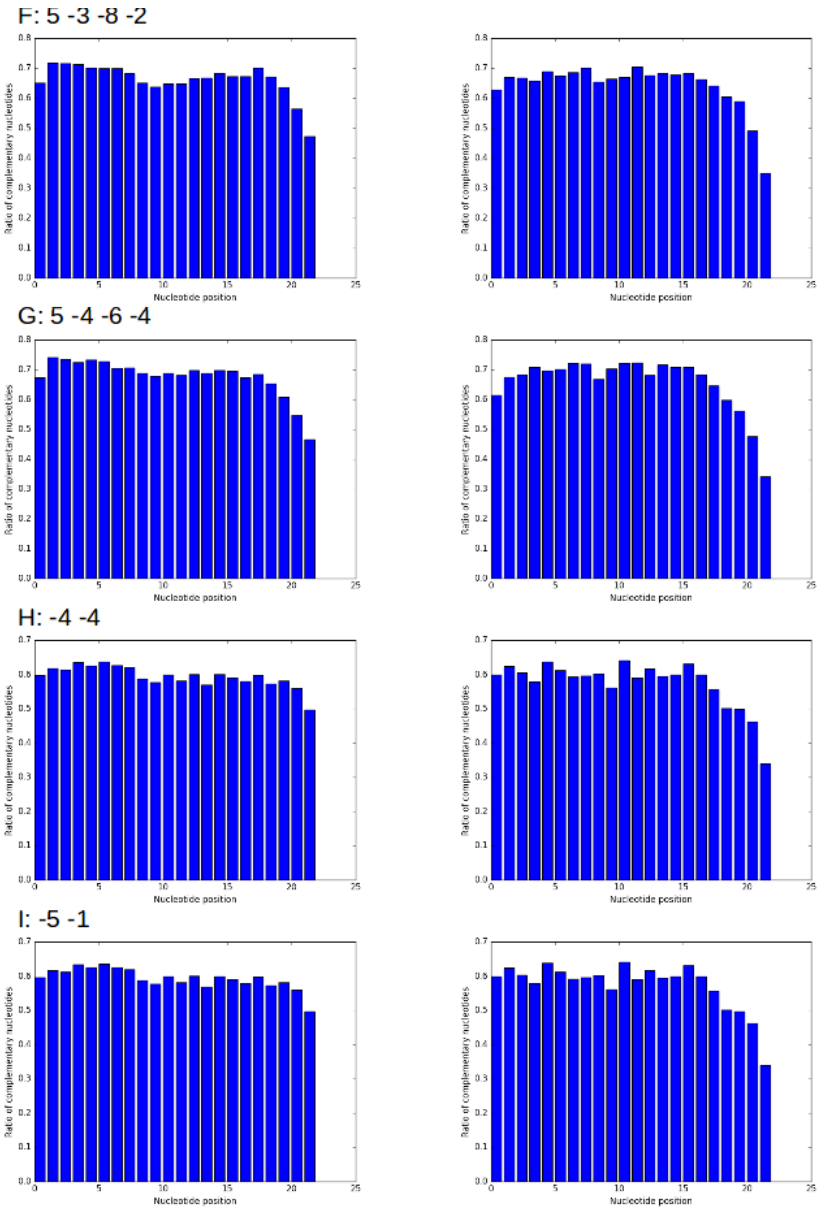


Figure 3.2: Ratios of complementarity per parameter set (A-I): targets left, non targets right

implicates that the consideration of the complementarities of the miRNA positions is not a significant and reliable prediction feature. Having only these ratios, it can not certainly be decided whether this plot shows a true targeting or not.

In figure 3.3 three plots with curves of three parameter sets illustrate the complementarities of the nucleotides in another way. In the first plot the difference in the seed region can be seen. The blue curve of the true targets is about 10% higher than the curve of the non targets. From nucleotide eight on, the red curve has its maximum and passes the blue one but the difference is not very big. From then on the curves are very similar and they both drop down to 30%. So for this parameter set only a difference in the seed region can be observed. The second plot of the parameter set 5 -4 -6 -4 shows even less differences. Again, there is a small difference in the seed region where the blue curve is a bit higher but only up to 5%. Then both curves stay high between 65 and 70% that is a contrast to the first plot. But the main point, a difference between true and non targets can not be observed. The last plot is again a bit different from the two others. The blue and the red curve alternate and are very similar in height. In here, neither seed region nor additional 3' pairing can be observed. For the two others the additional paring can no be seen at all as well. So from these plots and the complementarity of the paring nucleotides not many information can be gained. Hence, having a look at the complementarity of a new examined binding site is not significant to decide whether this position is a new found binding site and consequently the examined target is really targeted by the considered miRNA.

The table in figure 3.4 shows that the average of the complementarity ratios are pretty similar comparing true targets with non-targets. Highlighted in yellow are the nucleotides of the seed region, in blue the nucleotides of the additional 3' pairing. Building the average ratios of these regions a possible increase could be observed. For the first parameter set, the true targets show a slight increase in the seed region but a decrease in the additional pairing. So the described feature from above can not be significantly observed. For the same set and the negative control, a constant average can be seen. For almost all true target an increase in the seed region and a decrease in the other region can be observed. For the non targets there are different patterns. For example, for the third parameter set both regions are increased in contrast to the standard average. For the other sets either no big difference

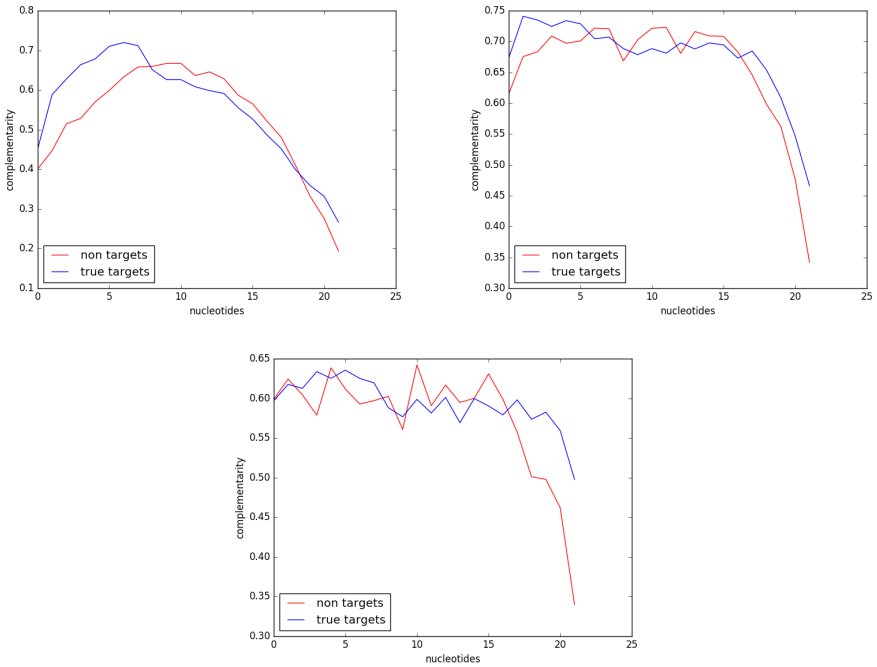


Figure 3.3: Curves of complementarity of non and true targets: 1 -2 -2 -1, 5 -4 -6 -4 and -5 -1

can be seen or both are enriched but never decreased. Going back to the plots, there the seed region in the true targets is slightly more visible than for the non targets. But all in all the differences in here are not very big and significant. Both regions can not be observed as clearly as expected.

The analysis of the alignment starting positions was not really successful and significant. Table 3.2 shows the numbers of the own predicted positions that were also given in the miRTarBase. It can be seen that two sets deliver only half the number of the other (5 -2 -8 -3 and 5 -3 -8 -2). These two sets also use very similar parameters. That shows that the alignment positions strongly depend on the selected parameters. In comparison to the given number of about 3700 provided positions in the miRTarBase the numbers of the respective own found ones are in general not very high, only about 20% were found. This can be due to the different sizes of the UTRs of the genes. In this research they are from the UCSC website whereas the miRTarBase may use another source. Therefore the sizes can be a bit different resulting in

Nucleotides	-4 -4	-5 -1	1 -2 -2 -1	2 -2 -5 -4	3 -2 -4 -4	5 -1 -8 -4	5 -2 -8 -3	5 -3 -8 -2	5 -4 -6 -4
True targets									
1	0.598	0.597	0.452	0.427	0.608	0.634	0.641	0.652	0.673
2	0.619	0.618	0.588	0.562	0.712	0.703	0.724	0.718	0.741
3	0.614	0.613	0.628	0.599	0.727	0.718	0.717	0.716	0.735
4	0.635	0.634	0.664	0.650	0.725	0.730	0.729	0.713	0.724
5	0.626	0.625	0.678	0.676	0.740	0.698	0.716	0.701	0.734
6	0.637	0.636	0.710	0.694	0.742	0.716	0.723	0.701	0.729
7	0.627	0.625	0.720	0.718	0.732	0.699	0.724	0.700	0.705
8	0.621	0.620	0.712	0.731	0.712	0.682	0.685	0.683	0.707
9	0.588	0.588	0.651	0.669	0.692	0.633	0.649	0.652	0.689
10	0.577	0.577	0.626	0.662	0.687	0.628	0.643	0.638	0.679
11	0.599	0.599	0.626	0.655	0.701	0.657	0.672	0.649	0.689
12	0.582	0.581	0.608	0.647	0.690	0.654	0.662	0.648	0.681
13	0.601	0.601	0.598	0.643	0.696	0.659	0.668	0.666	0.698
14	0.570	0.569	0.591	0.624	0.683	0.660	0.673	0.669	0.688
15	0.600	0.600	0.555	0.595	0.700	0.666	0.669	0.683	0.698
16	0.590	0.590	0.527	0.557	0.667	0.645	0.664	0.673	0.695
17	0.580	0.579	0.487	0.522	0.657	0.641	0.661	0.673	0.673
18	0.598	0.598	0.452	0.508	0.666	0.668	0.684	0.702	0.685
19	0.573	0.574	0.399	0.447	0.634	0.655	0.654	0.672	0.654
20	0.582	0.583	0.360	0.392	0.586	0.647	0.649	0.636	0.609
21	0.560	0.559	0.332	0.358	0.512	0.619	0.598	0.565	0.547
22	0.497	0.498	0.267	0.280	0.444	0.516	0.494	0.473	0.466
Average	0.594	0.594	0.556	0.573	0.669	0.660	0.668	0.663	0.677
Average 2 – 8	0.625	0.624	0.671	0.661	0.727	0.707	0.717	0.704	0.725
Average 13 – 16	0.591	0.590	0.568	0.605	0.686	0.658	0.669	0.673	0.695

Negative control									
1	0.599	0.599	0.401	0.383	0.560	0.601	0.595	0.628	0.615
2	0.625	0.624	0.447	0.445	0.639	0.679	0.676	0.671	0.676
3	0.605	0.604	0.515	0.518	0.674	0.693	0.715	0.668	0.683
4	0.579	0.579	0.529	0.549	0.686	0.660	0.681	0.658	0.709
5	0.638	0.638	0.570	0.583	0.685	0.688	0.696	0.689	0.697
6	0.612	0.612	0.599	0.614	0.695	0.643	0.666	0.675	0.701
7	0.593	0.593	0.633	0.627	0.710	0.680	0.690	0.687	0.722
8	0.597	0.597	0.658	0.697	0.701	0.657	0.697	0.701	0.721
9	0.603	0.602	0.660	0.685	0.698	0.624	0.646	0.654	0.669
10	0.561	0.561	0.667	0.682	0.722	0.651	0.671	0.665	0.703
11	0.642	0.642	0.667	0.683	0.700	0.663	0.663	0.671	0.722
12	0.590	0.591	0.637	0.683	0.727	0.667	0.697	0.705	0.723
13	0.617	0.617	0.646	0.671	0.685	0.670	0.698	0.677	0.681
14	0.595	0.595	0.629	0.649	0.726	0.674	0.687	0.684	0.716
15	0.600	0.600	0.587	0.639	0.720	0.684	0.684	0.678	0.709
16	0.631	0.631	0.565	0.606	0.707	0.692	0.691	0.684	0.709
17	0.599	0.599	0.522	0.590	0.668	0.662	0.681	0.663	0.683
18	0.558	0.557	0.481	0.498	0.652	0.623	0.626	0.642	0.646
19	0.502	0.501	0.410	0.426	0.555	0.578	0.584	0.605	0.598
20	0.498	0.498	0.333	0.376	0.542	0.589	0.567	0.590	0.562
21	0.462	0.462	0.276	0.321	0.451	0.522	0.496	0.491	0.477
22	0.340	0.340	0.194	0.219	0.314	0.369	0.349	0.350	0.342
Average	0.575	0.575	0.528	0.552	0.646	0.635	0.643	0.643	0.657
Average 2 – 8	0.607	0.607	0.564	0.576	0.684	0.671	0.689	0.679	0.701
Average 13 – 16	0.611	0.611	0.607	0.641	0.710	0.680	0.690	0.681	0.704

Figure 3.4: Table of ratios of complementarities

Table 3.2: Number of common alignment starting positions

	3 -2 -4 -4	5 -1 -8 -4	5 -2 -8 -3	5 -3 -8 -2	5 -4 -6 -4
Found number	815	804	388	411	800

	1 -2 -2 -1	2 -2 -5 -4	-4 -4	-5 -1
Found number	882	793	776	845

different defined positions, although the alignment would start at the same position. To eliminate these small disagreements, I allowed a window of 10 positions where the starting position can be. So if the provided miRTarBase position is given, the own predicted position is classified as consistent with the provided one if the position lies in a window of +5 or -5 related to the miRTarBase position.

Another reason for the inconsistent positions may be, that the tools they used in miRTarBase use better and more specific alignment tools where more parameters can be defined. The tools can better be adapted to biological background like seed region and additional 3' pairing. The pairwise2 module only has 4 parameters and only delivers the alignment with the highest score and no ranking. In the database always the best three alignment positions are given.

4 Discussion

In this research the main focus was on sequence alignment of miRNA sequence and its target mRNA sequence for given validated miRNA target interactions. This alignment should simulate a binding between the two sequence. In this way, it was investigated how this approach can be used to predict new target sites for miRNAs without any other biological experiments.

The results showed that with this alignment tool I used the increase in the alignment score for true targets are significant. This means that if a certain threshold for the scoring is exceeded, with a certain error rate a considered mRNA is really targeted by this miRNA. On the other hand, with this alignment tool different binding sites were found compared to binding

sites predicted by some existing prediction tools like miRanda. So if the positions I found with the alignment tool are the right ones is questionable. The alignment strongly depends on parameters and therefore the results are that different. Nevertheless the scroes showed a significant increase for the true targets. But again in here it is questionable how certain this prediction feature is if its considered by itself. As it can be observed for the existing tools, a much better prediction can be achieved with the consideration of more prediction features which are described underneath.

A first step can be the improvement of the alignment process, especially the refinement of the alignment parameters. In some other tools like miRanda, the parameters are adapted to the binding mechanism. For example, in the present seed region the complementarity should be nearly perfect, meaning mismatches in the first few nucleotides should be highly penalized or only a certain number of mismatches should be allowed. In addition, mismatches or gaps in the central nucleotides of the miRNA can be present and are therefore not much penalized. Adapting the parameters to some targeting rules can highly improve the target prediction.

Another thing is, in general, the more data of true targets are available, the more can be learned from the mechanism and the more the prediction tools can be adapted in different ways like features and precision of features. The complexitiy of the prediction can be observed by considering the consensus of all predicted targets of the different tools. Just a really small subset of all targets are predicted by all tools. Further, comparing validated target interactions of the database with the results of all tools about 16% of all interactions are predicted by at least one tool. But there is no interaction that is predicted by all tools. That shows that there are big differences between the different tools partly because of the variable features they consider. (Quelle Keller Vorlesung 4)

Because of their potential to function in treatment and detection of diseases, miRNAs become more and more important. Therefore miRNA research must be further extended and the complex target prediction has to be improved. Many researchers have found different additional features that can be considered. As described in the introduction, the seed region is one of the most important ones. The different types of sites vary already in the effectiveness of repression. Grimson et al. investigates the different types and figure 4.1 shows their results. If one 8mer site is present, the repression effect is the highest [Grimson et al., 2007].

Another useful feature is the free energy of the binding complex. This

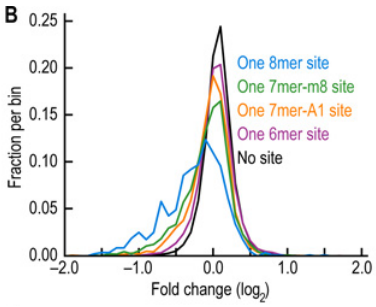


Figure 4.1: Effectiveness of different types of sites

energy determines the stability and tightness of a system, in this case the binding [Peterson et al., 2014]. If the free energy is lower than the binding between the miRNA and the mRNA sequence is tighter resulting in more evidence that the miRNA targets this mRNA. If the energy is high then the binding is not very favourable, meaning the miRNA not favours to target this mRNA at this position. Some tools like RNAhybrid [Rehmsmeier et al., 2004] rely strongly on this feature.

A completely different feature like the evolutionary conservation can be taken into account. If a sequence occurs across species, it is defined as conserved. This implicates that this part has been maintained by evolution because of a selected function [Peterson et al., 2014]. Conservation near the miRNA binding site can indicate that this part of the sequence is necessary for some mechanisms. This includes conservation of the miRNA itself as well as the conservation of the respective site of the mRNA. These conservations can be analysed with phylogenetic methods. Figure 4.4 shows how conserved the different nucleotides of a miRNA are, implicating regions like the seed region and additional 3' pairing.

Site accessibility is a feature that Kertesz et al. investigated for their target prediction. The mRNA generally folds into a secondary structure. Therefore the miRNA can not easily bind to its target because at first interactions within the mRNA have to be broken to make the target accessible. As a result miRNA will favourably bind to regions where the mRNA is more accessible. The researchers found that if the targets form highly stem structures the repression is reduced. If sites occur in open loop structures the repression is much higher. Summing up they found that site accessibility is not less important than seed matching [Kertesz et al., 2007].

As already mentioned above, in addition to the seed matching towards

the 5' end, another complementary site towards the 3' end in the miRNA is present (Figure 4.2). Grimson et al. investigated that the highest down regulation was found when the site started at position 13 and had four or five contiguous base pairings (Figure 4.3) [Grimson et al., 2007]. Considering again the conservation of the nucleotides they found that outside the seed region the contiguous nucleotides starting from nucleotide 12, 13 or 14 were the most conserved ones indicating their functional importance (Figure 4.4). Putting the information of seed region and additional base pairing together, it can be observed that if one 7mer-m8 site is present as well as a good 3' pairing that the efficacy is the highest. Even though the difference between the efficacy of the presence of one 8mer site and the one mentioned before is not very big. But the improvement of the presence of a good 3' pairing instead of a poor one is more significant (Figure 4.5). Between the two complementary areas there is usually a part of non pairing nucleotides where bulges and mismatches are found. These are important for the prevention of the AGO cleavage function [Filipowicz et al., 2008].

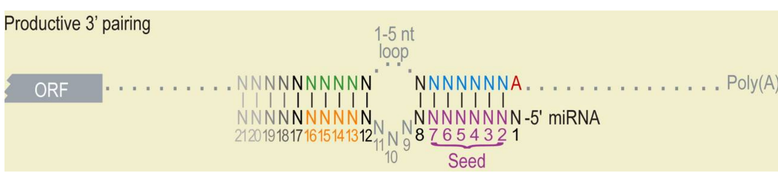


Figure 4.2: Additional 3' pairing

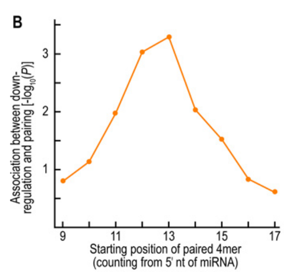


Figure 4.3: Relation between regulation and starting position of additional pairing

To get an even more reliable and precise prediction even more features can be considered. The presence of GU-wobbles is common in targeting. In this wobble positions Guanine(G) binds to Uracil(U) even though the pairing with Cytosine would be prevalent. This special pairing is thermodynamically

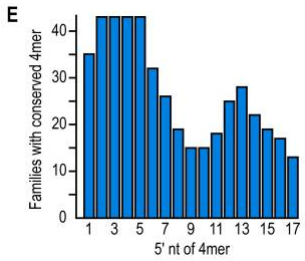


Figure 4.4: Conservation of nucleotide positions of miRNA

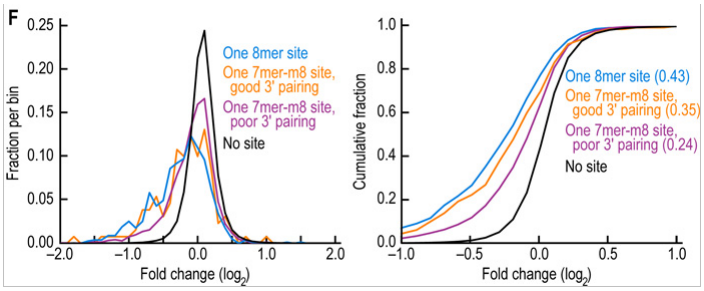


Figure 4.5: Efficacy of different combinations of seed region and additional 3' pairing

favourable and occurs therefore in many target interactions but results in lower repression of the translation [Doench and Sharp, 2004]. As mentioned before miRanda uses different scores for matches in their alignment step. They score the usual base pairing A-U and G-C with +5 and they penalize the other mismatches with a score of -3, excluding the pairing of G and U. This pairing is rewarded with at least +2 and therefore the GU wobbles are not penalized as much as other mismatches because they are very common [Enright et al., 2003].

Enright et al. and Doench et al. also found that the presence of multiple miRNA target sites results in a higher repression and destabilization of the mRNA [Enright et al., 2003] [Doench and Sharp, 2004]. Grimson et al. [Grimson et al., 2007] further investigated that the distance between two sites is also an important criterion. Generally the repression of two present site is the multiplication of the two single once because they act independently. The interesting thing now is that if the two sites are adjacent, the repression is increased and not equal to the multiplication of the single ones. The increase in repression is however not very high (Figure 4.7). To investigate the effect of cooperative miRNAs they analysed a mixture of miR-1

and miR-133 and simulated three different spacings. The results show that a spacing of four nucleotides did not show a cooperative repression but six or eight nucleotide spacing showed an increase in repression (Figure 4.6).

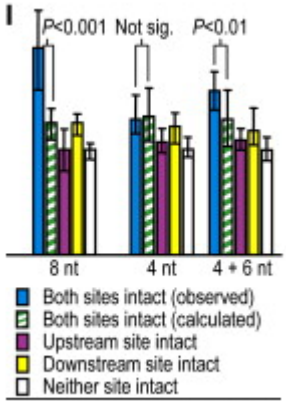


Figure 4.6: Cooperative repression with different site spacings

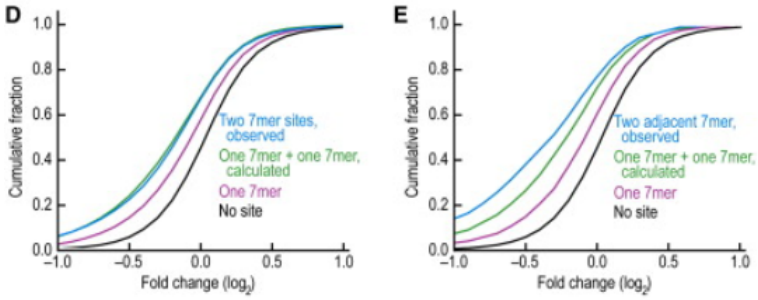


Figure 4.7: Effect of multiple sites

Another indicator is the position of the binding site relatively to the stop codon and the center of the UTR. Generally sites in the 3'UTR are investigated but Grimson et al. detected that sites in the Open reading frame (ORF) are slightly effective, sites in the 5'UTR not at all [Grimson et al., 2007].

Figure 4.8 shows the different efficacies regarding the site location. Another characteristic concerning the site locations is the distance from the stop codon. Figure 4.9 illustrates that approximately in the first 15 nucleotides the efficacy is still very low like in the ORF but afterwards it increases much.

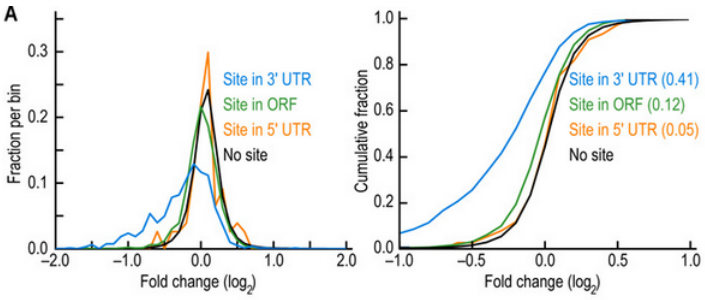


Figure 4.8: Efficacy of different site locations

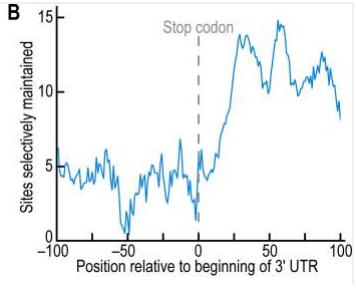


Figure 4.9: Efficacy of sites located relative to the stop codon

The sites were present at least 15 nucleotides from the stop codon and not present in the center of long UTRs but away from it.

Considering all these different common and less common features, new targets for miRNAs can be roughly reliably predicted. Figure 4.10 shows known prediction tools and the features they consider. As mentioned above the most common feature that nearly all of them consider are seed matching, conservation and free energy.

5 Conclusion

As described above there are a lot of features that can be considered and many different existing tools. They all differ from each other in the number and type of features they use and also in the weighting of the single features

FEATURES USED IN miRNA TARGET PREDICTION

Tool name	Seed match	Conservation	Free energy	Site accessibility	Target-site abundance	Machine learning	References
miRanda	X	X	X				Enright et al., 2003; John et al., 2004
miRanda-mirSVR	X	X	X	X		X	Betel et al., 2010
TargetScan	X	X					Lewis et al., 2005; Grimson et al., 2007; Friedman et al., 2009; Garcia et al., 2011
DIANA-microT-CDS	X	X	X	X	X	X	Maragkakis et al., 2009; Reczko et al., 2012; Paraskevopoulou et al., 2013
MirTarget2	X	X	X	X		X	Wang, 2008; Wang and El Naqa, 2008
RNA22-GUI	X		X				Hofacker et al., 1994; Miranda et al., 2006; Loher and Rigoutsos, 2012
TargetMiner	X	X	X	X	X	X	Bandyopadhyay and Mitra, 2009
SVMicrO	X	X	X	X	X	X	Liu et al., 2010
PITA	X	X	X	X	X		Kertesz et al., 2007
RNAhybrid	X		X		X		Rehmsmeier et al., 2004; Kruger and Rehmsmeier, 2006

Figure 4.10: Prediction tools and their features

[Peterson et al., 2014]. Concluding, each tool and feature has its advantages and strength but also its limitations which makes none of the existing tools perfect and 100% reliable.

Relying on just a few features will for sure lead to many errors, either false positives or false negatives. There are many irregularities and some features like the free energy are not always very precise [Peterson et al., 2014]. These problems have to be incorporated to lower the error rate.

Additionally not every tool is frequently updated which is a big problem and does not lead to improvements in target prediction. Some tools do not use the current data or innovations in target interactions. According to Peterson et al. the following tools are outstanding because of maintenance, newest input data and they are the easiest once to use: DIANA-microT-CDS, miRanda-mirSVR and Targetscan [Peterson et al., 2014]. All three are somehow unique but all e.g. use looser thresholds for conservation allowing less conserved regions to balance irregularities and refuse less true targets.

The early miRanda tools is also still widely used. The class of tools that use machine-learning are getting more accurate the more positive and negative target data is verified and features are found. Because of a lack of this data these tools are not significantly better than the three other tools mentioned above [Peterson et al., 2014]. The future consists of elimination of the limits of some tools and finding more useful features for the prediction.

Fujiwara and Yada [Fujiwara and Yada, 2013] followed a new approach by considering other characteristics than binding sites for the prediction,

in fact the transcriptional regulation. In their research they searched for common cis-elements in the miRNA as well as in the target gene. Compared to conventional methods their method is almost as good as the standard binding site based ones but combining the two different methods decreases the accuracy much. The advantages of this novel approach are independence of conservation of binding sites and of the amount of available training data.

Coronnello and Benos investigated another approach trying to improve the prediction power [Coronnello and Benos, 2013]. They developed a tool ComiR that considers additionally the miRNA expression levels and the combination of miRNA bindings. It also combines different scoring schemes from tools mentioned above. The innovation in this tool is the investigation of sets of miRNAs and their co-expression.

All in all the analysis of miRNAs is very complex and needs to be improved in terms of prediction.

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Figure 1.3: http://www.targetscan.org/docs/7mer.html

Figure sites: http://www.ncbi.nlm.nih.gov/pubmed/17612493

Table 4.10: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3927079/