



Small RNA Targets: Advances in Prediction Tools and High-Throughput Profiling

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Simple Summary: MicroRNAs (miRNAs) are a category of small RNAs (sRNAs) that have been found to regulate gene expression. Through the mediation of proteins from the Argonaute family, miRNAs target messenger RNAs (mRNAs) for destruction (cleavage or repression). Other types of sRNAs, including transfer-RNA-derived fragments (tRFs) and small interfering RNAs (siRNAs), have been indicated as potential regulators of gene expression. The complex network of RNA–RNA interactions is still under exploration, which can be assisted by the development of computational techniques. Here, we report the recent advancements in the field of bioinformatical and Machine Learning tools for the prediction of sRNA targets, and a brief overview of the development of high-throughput sequencing technologies.

Abstract: MicroRNAs (miRNAs) are an abundant class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. They are suggested to be involved in most biological processes of the cell primarily by targeting messenger RNAs (mRNAs) for cleavage or translational repression. Their binding to their target sites is mediated by the Argonaute (AGO) family of proteins. Thus, miRNA target prediction is pivotal for research and clinical applications. Moreover, transfer-RNA-derived fragments (tRFs) and other types of small RNAs have been found to be potent regulators of Ago-mediated gene expression. Their role in mRNA regulation is still to be fully elucidated, and advancements in the computational prediction of their targets are in their infancy. To shed light on these complex RNA–RNA interactions, the availability of good quality high-throughput data and reliable computational methods is of utmost importance. Even though the arsenal of computational approaches in the field has been enriched in the last decade, there is still a degree of discrepancy between the results they yield. This review offers an overview of the relevant advancements in the field of bioinformatics and machine learning and summarizes the key strategies utilized for small RNA target prediction. Furthermore, we report the recent development of high-throughput sequencing technologies, and explore the role of non-miRNA AGO driver sequences.

Keywords: miRNA target prediction; small RNA target prediction; computational biology; machine learning; high-throughput sequencing

1. Introduction

RNA-induced gene silencing, also known as RNA interference (RNAi), is a widespread, evolutionary conserved mechanism. First described in Caenorhabditis elegans [1], it takes place when double-stranded RNA (dsRNA) molecules bring about the cleavage of an mRNA molecule with which they are at least partially complementary. RNAi is essentially triggered by small RNA fragments derived from long dsRNAs. Small RNAs (sRNA), first identified in Escherichia coli in 1984 [2], act to down-regulate the expression of target genes by the means of decreased translation and/or increased mRNA turnover [3]. They are universally found in all three kingdoms of life: Archaea, Bacteria, and Eukaryotes, where



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). they adopt distinct mechanisms of RNAi. In eukaryotes, the most well-studied sRNAs are microRNAs (miRNAs, 18–26 nt) [4,5], small interfering RNAs (siRNAs, 20–27 nt) [6], and piwi-associated RNAs (piRNAs, 21–35 nt) [7]. miRNAs or siRNAs can assemble into a ribonucleoprotein complex called the RNA-induced silencing complex (RISC), composed of proteins such as RNA helicases, nucleases, and RNA-binding proteins [8]. piRNAs are found in animal germlines and their biogenesis from single-stranded RNA precursors involves primary processing by a set of proteins and the ping-pong cycle for amplification [9]. miRNAs, siRNAs, and piRNAs carry out their main functions (post-transcriptional mRNA cleavage, translational repression or decay, and transcriptional silencing) primarily by means of base-pairing with their DNA or RNA target. In eukaryotes, these RNA-silencing functions are mediated by the Argonaute family proteins, with the AGO sub-clade being associated with miRNAs and siRNAs, and the PIWI sub-clade with piRNAs [10]. For miRNAs, such interactions with their targets are classified as "canonical" when they are mediated by the "seed", a region of 6–8 nt on the 5′ end of the sRNA that forms canonical (Watson–Crick) base pairs with the target [11,12].

In addition to their fully complementary on-targets binding, siRNAs and miRNAs can bind and regulate numerous miRNA-like target sites in 3' UTRs of mRNAs using their seed sequence. These interactions with transcripts other than the intended target are called "off-target", and can involve undesirable transcript degradation and transcriptional/translational repression [13]. miRNA-like off-target effects are highly problematic in large-scale RNAi screening approaches, and many false positive hits are caused by off-target effects. Notably, since siRNAs are used widely for therapeutics as well as crop protection purposes, their miRNA-like off-target effects need to be minimized [14]. Thus, it is of major importance to understand the characteristics of the functional targets for siRNAs and enable their efficient prediction.

In plants, sRNAs are involved in reproductive transitions, for example, meiosis and gametogenesis, and regulate important epigenetic mechanisms, including genomic imprinting and paramutation. The main small-RNA classes are miRNAs, 21–22 nt long secondary siRNAs, and 24 nt long heterochromatic siRNAs (hetsiRNAs). All sRNAs in plants are modified at their 3'-end by 2'-O-methylation. This modification, nonexistent in animals, offers stability and protects sRNAs from degradation [15]. In *Arabidopsis thaliana*, 10 different AGO proteins are known to mediate the effects of several distinct types of sRNAs [16]. The movement of sRNAs in plants can be either short-range (cell-to-cell), or long-range (systemic) [17]. RNA silencing also spreads systemically over long distances in the course of days [18,19].

There is evidence that supports the existence of highly complex pathways for miRNA biogenesis and miRNA-mediated gene regulation in both animals and plants [20]. The commonalities and differences between animal and plant miRNA have been described in previous reviews in detail [20–22]. These differences can implicate the task of computational target prediction and thus should be accounted for. The summary of main commonalities and differences is shown in Table 1.

Table 1. Summary of commonalities and differences between animal and plant miRNA-mediated gene regulation.

Feature	Plants	Animals		
Size (number of nucleotides)	18–25 nt	18–25 nt		
Mechanism of target recognition	Ribonucleotide complementarity	Ribonucleotide complementarity		
Location of miRNA binding sites within target mRNAs	Predominantly in the open reading frame	Predominantly 3' untranslated region (3'UTR)		
Number of miRNA binding sites within target mRNAs	Generally single	Generally multiple		
miRNA-mRNA complementarity	Generally a perfect complementarity	Imperfect; seed sequences and variable flanking complementarity		

In bacteria, trans-acting sRNAs are a major and heterogeneous class of regulators of post-transcriptional gene expression and are often associated with chaperon proteins such as Hfq or ProQ [23,24]. They are 50–530 nt long and they regulate their mRNA targets in a (usually incomplete) base pairing-dependent manner that entails altering the mRNA translation or stability [25–28]. Even though base pairing mostly involves the 5'- and 3'-UTR regions of the target mRNA, it can also involve sites of the mRNA coding region. Bacterial sRNAs interact with their targets near the ribosomal binding site (RBS), thus repressing translation by masking the RBS or by inducing translation by making the RBS accessible [29]. Antisense sRNAs are another class of bacterial sRNAs that are cis-encoded on the opposite strand of their target gene, thus are fully complementary to their mRNA target [30]. Unlike miRNA, sRNAs may cause both up- and downregulation of their targets [31].

tRNA-derived fragments (tRFs, alternatively transfer-RNA-derived small RNAs, tsR-NAs) are an emerging class of evolutionary conserved functional non-coding RNAs found across all kingdoms of life [32–35]. They are 14–32 nt long and their biogenesis involves cleavage of tRNAs precursors or mature tRNAs at specific loci and subsequent processing [36,37]. They have been previously classified into five categories: (1) tRF-5s, derived from the 5' ends of mature tRNAs; (2) tRF-3s, from the 3' ends of mature tRNAs with 3'-CCA termini; (3) i-tRFs, derived from cleavage of mature tRNAs; (4) tRF-1s, from the 3'flanking sequences of pre-tRNAs with PolyU residues; and (5) tiRNAs, which are halves of tRNA cleaved at the anticodon [32–34,38]. The binding of tRFs to Argonaute proteins and Argonaute-mediated post-transcriptional silencing in an RNAi-like fashion have been reported only in eukaryotes [39]. Analysis of deep sequencing and AGO PAR-CLIP of sRNAs has shown that numerous reads can be mapped to tRFs, and that tRF-5s and tRF-3s can interact with target mRNAs in a fashion similar to miRNAs with 6-mers complementary to the seed [36,39–41]. It is found that in human HEK293 cells tRFs associate with AGO 1, 3, and 4, but not AGO 2, which is the main effector protein of miRNA function [39,42]. tRFs have also been indicated as cancer biomarkers [43,44].

Ribosomal RNAs (rRNAs) are the most abundant cellular RNA species that are the source of non-randomly generated fragments, namely, rRFs (ribosomal RNA Fragments). rRFs are an emerging class of regulators of gene expression. In plants, it has been shown that 5.8S rRF is involved in the cleavage of RPS13 and RPL5P mRNAs [45]. In H1299 cells, knocking down a 20-nt rRF-induced apoptosis, inhibited cell proliferation and led to a decrease in G2 phase cells [46]. In HeLa cells, overexpressing an rRF from the 5' end of 28S rRNA led to the inhibition of several ribosomal proteins [47]. In Drosophila, it was shown that rRFs exhibit age-dependent Argonaute loading, comparable to that of miRNAs and tRFs [48]. Last year, a computational meta-analysis of ribosomal RNA fragments [49] from Ago1 CLASH in human showed that guanine-rich rRFs were preferentially cut in single-stranded regions of mature rRNAs between pyrimidines and adenosine, and non-randomly paired with cellular transcripts in crosslinked chimeras. In addition, numerous identical rRFs were found in the cytoplasm and nucleus in mouse Ago2-IP.

Figure 1 illustrates the mechanisms of the RNAi pathway for the various types of sRNAs. Our review focuses on the paths that involve the RISC complex; that is, the AGO-mediated silencing of the target.

Machine learning (ML) is the field of study that enables computers to learn without being explicitly programmed [50]. ML-based methods use data in order to build models, discover statistically significant patterns and relationships, and consequently make predictions on novel data [51]. One of the drawbacks of classical ML methods is their inability to work with raw data. Instead, they require a domain expert to design a feature extractor that transforms the raw data into a suitable internal representation or feature vector from which the ML method can detect or classify patterns in the input [52]. Deep Learning (DL) is a subfield of ML that essentially encompasses a class of large artificial neural networks. DL methods are able to process raw input data by constructing simple but non-linear modules; each of them transforms the representation at one level (starting with the raw input) into a representation at a higher, slightly more abstract level. With the composition of enough such transformations, very complex functions can be learned. DL-based methods have been shown to be effective for classification tasks in domains with complex feature representation [52].





To assess any classification task, there is a need for useful metrics. The most common ones are sensitivity (also called recall), accuracy, precision, and F1 score.

$$Sensitivity (Recall) = \frac{TP}{TP + FN'}$$
(1)

$$Accuracy = \frac{TN + TP}{TP + FP + TN + FN'}$$
(2)

$$Precision = \frac{TP}{TP + FP'}$$
(3)

$$F1 \ score = 2 \frac{Precision * Recall}{Precision + Recall'}, \tag{4}$$

where TP and FP are the numbers of true positive and false positive assessments, and TN and FN are the numbers of true negative and false negative assessments, respectively. Another useful metric is the Precision–Recall Area Under the Curve, PR AUC, which is the area under the Precision–Recall curve. The Precision–Recall curve is constructed by calculating and plotting the precision against the recall at a variety of thresholds. The higher the PR AUC, the better the performance of the classifier at distinguishing the positive class from the negative class.

In this paper, we present an in-depth review on the current state of the sRNA target prediction. We discuss the basic principles of experimental methods and then we focus on computational tools. For easier navigation through the review, the various tools are grouped based on the type of sRNA they are designed for. In the case of miRNAs, we focus only on the DL methods, since they are the current state-of-the-art in the field and target interactions the majority of the previous tools have already been described in numerous reviews.

2. Materials and Methods

2.1. Experimental Identification of sRNA-Target Interactions

Elucidating the interactions of sRNAs with their targets is pivotal for diagnostics and therapeutics. Even though bioinformatic approaches are the most widely used for the exploration of miRNA targets, they produce a non-negligible number of false positives [53]. Furthermore, miRNAs might interact through "non-canonical" binding sites [54,55], or with non-coding RNAs [56]. Therefore, it is essential that the small RNA-target interactions are experimentally validated.

There are three main categories of methods for the isolation and the identification of miRNA targets: (1) gene expression profiling methods, (2) immunoprecipitation methods, and (3) pull-down methods. An extensive review of the characteristics of each strategy is presented in [57] and in [58]. Methods of category (1) rely on the core of the miRNA regulatory function; that is, the mediation of mRNA degradation or repression of mRNA translation. Overexpression or inhibition of specific miRNAs and screening the subsequent response in the expression levels of genes can indicate the mRNA targets. Luciferase reporter screening can identify direct targets for miRNAs, but is limited by the availability of 3'-UTR libraries and is low throughput. The gene of interest is fused at the 3'-UTR with a luciferase reporter gene and cotransfected with a query miRNA. Targeting is measured as the differential light emission between the target gene fused with the luciferase reporter gene and a non-targeted luciferase reporter [59–61]. Although a set of quantitative, high-throughput methods have been developed [62–64], they cannot distinguish between primary and secondary miRNA targets, and they suffer from high false positive and false negative rates.

Category (2) of methods is based on immunoprecipitation (IP) of RISC proteins via specific antibody, isolation, and identification of the bound mRNA [65]. miRNA targets can thus be indirectly mapped utilizing bioinformatic tools. Crosslinking and immunoprecipitation (CLIP) methods improve the capture efficiency of IP by utilizing UV irradiation to produce covalently bound AGO–miRNA and AGO–target pairs [66]. HITS-CLIP and iCLIP can identify cross-link sites with nucleotide resolution [40,67], while the development of enhanced CLIP (eCLIP) [68] significantly improved the rate of success at generating libraries with high usable read fractions. Chimeric eCLIP, a method presented earlier this year, implements a chimeric ligation step into a simplified AGO2 eCLIP and reports up to 175-fold increased yield of recovered miRNA:mRNA interactions [69]. IP and CLIP methods provide only indirect evidence for the miRNA-target interaction. This limitation is addressed by the Crosslinking, Ligation, and Sequencing of Hybrids (CLASH) method [54,70], which ligates the miRNA and its target. Overall, immunoprecipitation methods are inherently limited by the specificity of the antibody and are of low efficiency.

Lastly, pull-down methods utilize tagged miRNA as probes to directly isolate miRNAassociated targets. These methods include the use of 3'-biotinylated RNA probes to capture miRNA targets [71,72]. Since this type of probe hinders the incorporation of miRNA into RISC, the miR-CLIP method was proposed. miR-CLIP combines miR-106a mimic probe with biotin modification and photo-reactive molecule modification at middle sites [56]. The probe cross-links to target miRNA and is subsequently immunoprecipitated with AGO2 antibody. This method is not universal, has low efficiency, and is limited by the specificity of the antibody. Photoclickable miRNA function within the cells [73]. The method is based on the attachment of a biotin handle through tetrazole-alkene photoclick reaction [74] to complexes containing photoclickable miRNA.

2.2. Computational Identification of sRNA–Target Interactions

The arsenal of methods for the prediction of sRNA targets is being enriched at a fast pace, following the advancements in the experimental techniques and in computational power. Here, we present an overview of the evolution of the computational methods in tandem with important experimental landmarks. We summarize the wide range of underlying computational techniques, and we subsequently present the computational tools developed in the last decade. The tools are arranged according to the type of sRNA molecule for which the target is to be predicted. A brief explanation of the function and/or the aim is provided for each individual tool. The list of the tools, dating from 2010 till now, along with the date of publication and their repository/web interface/source code (if available) is presented in Table 2.

Table 2. Bioinformatical and Machine Learning methods for the prediction of sRNA targets. The last column indicates the availability of each method: o (open source), s (standalone), w (web service), - (not available/not functional).

	Method	Year	Repository/Web App	
tRFs t	arget prediction			
1	tRFTar [75]	2021	http://www.rnanut.net/tRFTar/ (accessed on 7 December 2022)	W
2	tRFTars [76]	2021	http://trttars.cmuzhenninglab.org:3838/tar/	0
siRNA	As off-target prediction		(accessed on 7 December 2022)	
3	ei-Fi [77]	2019	https://github.com/snowformatics/siFi21-	0
		2017	(accessed on 7 December 2022)	0
4	RIsearch2 [78]	2017	https://rth.dk/resources/risearch/ (accessed on 7 December 2022)	s
5	MIRZA-G [79]	2015	(accessed on 7 December 2022)	-
6	CWords [80]	2013	https://servers.binf.ku.dk/cwords/ (accessed on 7 December 2022), https://github.com/simras/cWords (accessed on 7 December 2022)	0, W
sRNA	s target prediction		I . , , 6	
7	sRNARFTarget [81]	2021	https://github.com/BioinformaticsLabAtMUN/sRNARFTarget (accessed on 7 December 2022)	0
8	SPOT [82]	2019	https://github.com/phdegnan/SPOT (accessed on 7 December 2022)	о
9	psRNATarget [83]	2018	https://www.zhaolab.org/psRNATarget/ (accessed on 7 December 2022)	W
10	$I_{\rm reto} DN[\Lambda, 2, 0, [94]]$	2017	http://www.bioint.uni-treiburg.de/Software/	
10	$\operatorname{IIIIaKINA} 2.0 [04]$	2017	(accessed on 7 December 2022), http://ma.informatik.uni-neiburg.de/	W
11		2014	http://cs.wellesley.edu/~btjaden/TargetRNA2/	
11	largetKINA2 [85]	2014	(accessed on 7 December 2022)	W
12	CopraRNA [86,87]	2013	http://rna.informatik.uni-freiburg.de/CopraRNA/	w
			http://rna thi univie ac at/cgi-bin/RNA predator/target search cgi	
13	RNApredator [88]	2011	(accessed on 7 December 2022)	W
14	sTarPicker [89]	2011	http://ccb.bmi.ac.cn/starpicker/ (accessed on 7 December 2022,)	-
miRN	As/isomiRs target prediction	l		
15	DMISO [90]	2022	http://hulab.uct.edu/research/projects/DMISO/ (accessed on 7 December 2022)	s
16	C. I 'D' [01]	201	https://research.nhgri.nih.gov/software/SubmiRine/index.shtml	
16	Submikine [91]	2015	(accessed on 7 December 2022)	0
miRN	As target prediction	2022		
17	largetNet [92]	2022	https://github.com/mswzeus/largetNet (accessed on 7 December 2022) https://zenodo.org/record/6360587# Yy2IV9VByV4	0
18	mintRULS [93]	2022	(accessed on 7 December 2022)	0
19	miTAR [94]	2021	https://github.com/tjgu/miTAR (accessed on 7 December 2022)	0
20	SG-LSTM-FRAME [95]	2021	https://github.com/Xshelton/SG_LSTM (accessed on 7 December 2022)	0
21	miRgo [96]	2020	(accessed on 7 December 2022,)	-
			https://www.cu-bic.ca/RPmirDIP (accessed on 7 December 2022,)	-
22	RPmirDIP [97]	2020	https://dataverse.scholarsportal.into/dataset.xhtml?persistentId=doi:	W
			https://github.com/zhengxueming/cnnMirTarget	
23	cnnMirTarget [98]	2020	(accessed on 7 December 2022)	0
24	miRTRS [99]	2020		-

Table 2. Cont.

	Method	Year	Repository/Web App	
25	miRTPred [100]	2020	http://bicresources.jcbose.ac.in/zhumur/mirtpred/	s
26	miRTMC [101]	2020	(accessed on 7 December 2022) https://github.com/hjiangcsu/miRTMC (accessed on 7 December 2022)	0, S
27	miTarDigger [102]	2020		-
28	Min3 [103]	2019	https://sourceforge.net/projects/mirt3/ (accessed on 7 December 2022)	0
29	mirTime [104]	2019	https://github.com/mirTime/mirtime (accessed on 7 December 2022)	0
30	CCmiR [105]	2018	http://hulab.uct.edu/research/projects/miRNA/CCmiR/ (accessed on 7 December 2022)	S
31	DeepMirTar [106]	2018	https://github.com/Bjoux2/DeepMirTar_SdA (accessed on 7 December 2022)	0
32	miRAW [107]	2018	https://bitbucket.org/account/user/bipous/projects/MIRAW (accessed on 7 December 2022)	0, S
33	MiTarget [108]	2018	http://rna-informatics.uga.edu/12_software.php	0
34	Tiresias [109]	2018	https://bitbucket.org/cellsandmachines/tiresias-context-specific- mirna-interactome-mapping/src/master/ (accessed on 7 December 2022)	0
35	Context-MMIA [110]	2017	http://epigenomics.snu.ac.kr/contextMMIA/ (accessed on 7 December 2022)	W
36	MicroTarget [111]	2017	https://bioinformatics.cs.vt.edu/~htorkey/microTarget (accessed on 7 December 2022)	о
37	miRBShunter [112]	2017	https://github.com/TrabucchiLab/miRBShunter	0
38	miRTar2GO [113]	2017	http://www.mirtar2go.org/ (accessed on 7 December 2022)	w
39	miRTarVis+ [114]	2017	http://hcil.snu.ac.kr/research/mirtarvisplus	W
40	miSTAR [115]	2017	http://mi-star.org/ (accessed on 7 December 2022)	W
41	chimiRic [116]	2016	https://bitbucket.org/leslielab/chimiric/src/master/	0
42	MiRTDL [117]	2016	http://nclab.hit.edu.cn/ccrm (accessed on 7 December 2022)	_
43	TargetExpress [118]	2016	http://targetexpress.ceiabreulab.org/ (accessed on 7 December 2022)	-
44	deepTarget [119]	2016	http://data.snu.ac.kr/pub/deepTarget/ (accessed on 7 December 2022)	-
45	TarPmir [120]	2016	http://hulab.ucf.edu/research/projects/miRNA/TarPmiR/	s
46	Avishkar [121]	2015	(accessed on 7 December 2022) https://bitbucket.org/cellsandmachines/avishkar/src/master/ (accessed on 7 December 2022)	0
47	MiRNALasso [122]	2015	https://nba.uth.tmc.edu/homepage/liu/miRNALasso/	s
40	···· : DT- ··. V:- [102]	2015	http://hcil.snu.ac.kr/~rati/miRTarVis/index.html	
48	mik [arvis [123]]	2015	(accessed on 7 December 2022)	S
49	largetScan v7.0 [124]	2015	https://www.targetscan.org/ (accessed on / December 2022)	W
50	MBSTAR [125]	2015	(accessed on 7 December 2022)	-
51	miRTarVis+	2017	http://hcil.snu.ac.kr/research/mirtarvisplus (accessed on 7 December 2022)	W
52	StarMir [126]	2014	https://sfold.wadsworth.org/cgi-bin/starmirtest2.pl (accessed on 7 December 2022)	W
53	mirMark [127]	2014	https://github.com/lanagarmire/MirMark (accessed on 7 December 2022)	0
54	ProMISe [128]	2014	https://bioc.ism.ac.jp/packages/3.11/bioc/html/Roleswitch.html (accessed on 7 December 2022)	о
55	TargetScore [129]	2014	http://www.bioconductor.org/packages/devel/bioc/html/ TargetScore.html (accessed on 7 December 2022)	0
56	IDA approach [130]	2013	https://academic.oup.com/bioinformatics/article/29/6/765/184183# supplementary-data (accessed on 7 December 2022)	0
57	MicroMUMMIE [131]	2013	https://ohlerlab.mdc-berlin.de/files/duke/MUMMIE/download.html (accessed on 7 December 2022)	0
58	MIRZA [132]	2013	http://www.clipz.unibas.ch/downloads/mirza/ (accessed on 7 December 2022)	-

	Method	Year	Repository/Web App	
59 60	MREdictor [133] RFMirTarget [134]	2013 2013	http://mredictor.hugef-research.org/ (accessed on 7 December 2022)	-
61	HomoTarget [135]	2013	http://lbb.ut.ac.ir/Download/LBBsoft/homoTarget/ (accessed on 7 December 2022)	-
62	CoSMic [136]	2012	https://www.weizmann.ac.il/complex/compphys/software/cosmic/ (accessed on 7 December 2022)	S
63	DIANA-microT-CDS [137]	2012	https: //dianalab.e-ce.uth.gr/html/dianauniverse/index.php?r=microT_CDS (accessed on 7 December 2022)	W
64	BcmicrO [138]	2012	http://compgenomics.utsa.edu/gene/gene_1.php (accessed on 7 December 2022)	W
65 66	mirMap [139] DIANA-microT-ANN [140]	2012 2012	https://mirmap.ezlab.org/ (accessed on 7 December 2022) http://microrna.gr/microT-ANN (accessed on 7 December 2022)	W -
67	mmPRED [141]	2012	https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164- 13-620#MOESM11 (accessed on 7 December 2022)	0
68	MTar [142]	2012		-
69	Targetprofiler [143]	2012	http://mirna.imbb.forth.gr/Targetprofiler.html (accessed on 7 December 2022)	w
70 71	PACMIT [144] miREE [145]	2011 2011	https://paccmit.epfl.ch/ (accessed on 7 December 2022)	w -
72	MultiMiTar [146]	2011	https://www.isical.ac.in/~bioinfo_miu/multimitar.htm (accessed on 7 December 2022)	-
73	ProbmiR [147]	2011	http://www.baskent.edu.tr/~hogul/probmir/ (accessed on 7 December 2022)	0
74	TargetSpy [148]	2010	http://webclu.bio.wzw.tum.de/targetspy/index.php (accessed on 7 December 2022)	s, w

Table 2. Cont.

2.2.1. Evolution of the Methods for Computational Identification of sRNA-Target Interaction

The development of computational methods for the prediction of sRNA targets followed closely the advancements in experimental techniques. The first methods for computational prediction of miRNA targets appeared in 2003, shortly after it was suggested that miRNAs are widespread and abundant in cells [149–151]. In 2009, a review article [152] highlighted the tools used for human and mouse miRNA target prediction. The sequence alignment of the miRNA seed to the 3'-UTR of candidate target genes was used as the main prediction feature in the majority of the reported methods. The most commonly used tools till then were DIANA-microT 3.0 [153], ElMMo [154], miRanda [155], miRBase [156,157], PicTar [158], PITA [159], RNA22 [160], and TargetScan 5.0 [124,161]. They use heuristic algorithms based on rule matching, following the discoveries in experimental identification of miRNA targets.

However, the complexity of sRNA:mRNA interactions is the stumbling block for the heuristic methods. The last decade was a turning point for the development of target prediction tools based on machine learning (ML) and deep learning (DL) [58,162–166] that gradually move away from describing each condition necessary to predict a functional target toward leveraging the power of data. Some tools are still focused on a smaller number of carefully curated features based on biological findings (mirMap [139], STarMir [126]), but others generate a large number of features and let the ML method pick the best ones and discover the right way to combine them to obtain the correct prediction (TargetSpy [148], miREE [145], MultiMiTar [146], RFMirTarget [134], mirMark [127], MBSTAR [125], and miRTPred [100]). sRNA target prediction can be based on thermodynamic calculations of the sRNA-putative target hybrid (sTarPicker [89], RNApredator [88], IntaRNAv2.0 [84], and TargetRNA2 [85]), or account for multiple additional features, such as seed interaction (IntaRNAv2.0 [84]), or secondary structure (TargetRNA2 [85]). Other methods implement phylogenetic conservation (CopraRNA [86,87]). The scope of the prediction can extend from

specific target site to genome-wide (sTarPicker [89], IntaRNAv2.0 [84], TargetRNA2 [85], sRNARFTarget [81], MIRZA-G [79], and RIsearch2 [78]).

Computational methods for the identification of sRNA-target interaction use a large variety of ML algorithms, and there seems to be no clear consensus as to which is the most suitable for this task. miREE uses a genetic algorithm to generate a set of sequences, which are then fed to a Support Vector Machine (SVM) algorithm. MultiMiTar also uses SVM but in combination with Multi-Objective Simulated Annealing (AMOSA) [167] to select biologically relevant features. chimiRic uses two SVM models, one for local and one for global context. mirMark evaluated several different algorithms on more than 700 features and Gaussian SVM and Random Forest (RF) performed the best. RF algorithm is also used by RFMirTarget, MBSTAR, and sRNARFTarget [81]. The miRTPred method uses the weighted voting ensemble approach, combining the predictions of the best-performing traditional and classical ensemble ML algorithms.

The mentioned supervised learning methods are based on labeled training samples and their success relies on extracting the effective sequence features that are capable of differentiating the positive and negative sRNA-gene association samples. The shortage of reliable negative sRNA-gene samples can be limiting their power. Unlike supervised learning methods, recommendation algorithms do not require negative samples. miRTRS [99] and miRTMC [101] predict miRNA targets based on a collaborative filtering recommendation algorithm. The biologically experimentally validated miRNA targets are used to construct a heterogeneous network and the miRNA–gene interaction that is not experimentally validated is predicted by filling out the unknown elements in the miRNA–gene interaction matrix.

There seems to be a poor agreement between the results of different algorithms, yet they achieve similar performance. Several tools are based on the integration of predictions from different algorithms (RFMirTarget [134], RPmirDIP [97], BCmicrO [138], and SPOT [82]). Authors claim that different algorithms rely on different mechanisms in making predictions, each of which has its own advantages, and it can be desirable to integrate their results. The RFMirTarget method improves the predictions produced by miRanda [155] using an additional 34 sequence-based features in an RF model. The RPmirDIP method uses the Reciprocal Perspective (RP) method [168] to refine predictions stored in the mirDIP database [169]. BCmicrO uses Bayesian Network to refine the prediction scores produced by TargetScan, miRanda, PicTar, mirTarget, PITA, and Diana-microT. SPOT is a pipeline that incorporates sTarPicker [89], TargetRNA2 [85], IntaRNA [170], and CopraRNA [86,87].

Following the ML algorithms, a variety of neural network architectures has been utilized for the task of sRNA target prediction. The utilization of artificial neural networks (ANNs) can be traced back to the year 2010 when MTar [142] used a feed-forward three-layer multi-layer perceptron (MLP) for the classification of target sites. Other early adopters of artificial neural networks were DIANA-microT-ANN [140] and HomoTarget [135]. DIANA-microT-ANN used a recurrent neural network (RNN) with two layers to combine predictions from all candidate target sites (CTSs) in the mRNA to obtain a final prediction. The year after, HomoTarget introduced the Pattern Recognition Neural Network (PRNN) for predicting miRNA targets based on manually extracted features.

The shift towards deep learning (DL) methods started around the year 2016. The main reason for this was the critique of the huge bias introduced by the manual feature crafting and selection. Even though DL methods are capable of extracting important features directly from the raw input, the first DL methods were still using handcrafted features (MiRTDL [117], DeepMirTar [106]) and neural networks were used only to make better predictions from these features. A later method, miTarDigger [102], used hand-crafted structural features together with raw sequence. Subsequent methods moved away from manual feature crafting and tried to work directly with raw data in the sequence format (deepTarget [119], miRAW [107], cnnMirTarget [98], miTAR [94], TargetNet [92], and DMISO [90]). However, miRAW used additional features (binding stability and site accessibility) in the a posteriori filtering step to improve predictions.

As mentioned before, the first common architecture in the field of sRNA target prediction is MLP, used by MTar and miRAW. Another common architecture is the convolutional neural network (CNN) [171] that is used by MiRTDL, cnnMirTarget, and other methods that combine convolutional layers with other architectures. TargetNet uses the ResNet [172] architecture that introduces residual connections on top of convolutional layers. MiTAR and DMISO use CNN followed by recurrent neural network (RNN), precisely, Long Short– Term Memory (LSTM) neural network [173] arguing that this hybrid architecture is better suited for extracting sequential and spatial features from sRNA and mRNA [98,174].

The last commonly used architecture is the autoencoder [175]. DeepTarget uses autoencoder together with RNN, DeepMirTar uses stacked denoising autoencoders, and miTarDigger adds convolutional denoising autoencoders to the stacked denoising autoencoders. The advantage of autoencoders is that they can be pre-trained in an unsupervised manner—the objective is to learn a meaningful encoding of the input sequence and then reconstruct the sequence from the encoding.

2.2.2. Description of Selected Computational Methods

Computational prediction of sRNA–target interactions is a highly active field of research that has produced tens of prediction methods during the last decade. Most tools focus on miRNA target prediction, a fraction of them predicts targets of a variety of sRNAs, and a couple of tools enable the prediction of tRF targets (tRFTars [76], tRFTar [75]). Moreover, a few methods have been developed for the prediction of siRNA off-targets, namely, MIRZA-G [79], RIsearch2 [78], and siRNA-Finder [77]. Given the fact that the vast majority of miRNA target prediction tools have already been described in several reviews published in the last two years [58,165,176], and that DL methods are the current state-of-the-art in the field, from the numerous miRNA target prediction tools we describe only those that are based on DL methods. An overview of the tools for the prediction of the target of the various types of sRNAs is presented in Table 2.

sRNA-Target Interactions

sTarPicker [89] is an ensemble classifier trained on 32 experimentally verified bacterial sRNA–mRNA repression pairs from sRNATarBase 1.0 [177,178]. Hybridization between an sRNA and an mRNA target is based on a two-step model: (1) seed matching between the sRNA and a target, and (2) elongation of the hybrid so that the duplex formed is stable. The hybridization is assessed by ΔG_{open} (free energy), ΔG_{hybrid} (computed by RNAduplex of Vienna RNA package [179]), and $\Delta\Delta G$ that indicate thermodynamic stability and site accessibility. sTarPicker picks stable seeds based on rules constructed from known seed bindings of 17 pairs, extends the binding sites by 100 nt upstream and downstream of the seed, extracts the features of the binding sites, and the ensemble classifier predicts the probability of the sRNA–target interaction. sTarPicker is no longer available online.

In the same year, RNApredator [88] became available as a web server for the prediction of bacterial sRNA targets. After an input sequence is submitted, its targets can be searched against a set of over 2155 genomes and plasmids from 11,183 bacterial species. The output contains a table of the 100 most stable duplexes predicted by the dynamic programming approach RNAplex [180], hybridization energy, and structure in dot-bracket notation. Additional features such as enrichment in Gene Ontology terms, target site accessibility, and cellular pathways can be obtained in an automatic post-processing step.

CopraRNA (Comparative Prediction Algorithm for sRNA Targets) [86,87] integrates phylogenetic information to predict sRNA targets on the genomic scale for a set of given organisms. It employs a statistical model and computes whole genome target predictions based on whole genome target screens for homologous sRNAs performed by IntaRNA [170]. The method aims to address the high false positive rate (FFP) of previous approaches relying on thermodynamic models (including RNApredator), base complementarity, or seed conservation. It combines individual *p*-values among clusters of genes predicted by IntaRNA to generate a weighted *p*-value and false discovery rate (FDR)-corrected qvalue. CopraRNA reconstructs regulatory networks upon functional enrichment (using the DAVID database [181]) and network analysis, and predicts the sRNA domains for target recognition and interaction. CopraRNA is available at the Freiburg RNA tools webserver [182], requires an input in FASTA format with its RefSeq ID, and allows for the visualization of interacting regions. The method requires the conservation of both sRNA and mRNA in a minimum of four bacterial species, which renders it unsuitable for species-specific sRNA target prediction.

TargetRNA2 [85] is a web server that identifies mRNA targets upon being given an sRNA sequence and the name of a bacterial replicon. The prediction calculates a variety of features by means of previously published methods: conservation (calculated utilizing BLASTN [183] and ClustalW2 [184]) and secondary structure (using RNAfold from Vienna RNA Package) of the sRNA. Additional features are the accessibility of regions in the mRNA secondary structure (calculated based on RNAplfold [185]) and the sRNAputative target hybridization energy (based on calculations by RNAduplex from Vienna RNA Package). TargetRNA2 produces *p*-values for predicted interactions based on the hybridization energy scores of a randomized mRNA pool. If available, the method can integrate RNA-seq data and consider co-differential gene expression. TargetRNA2 scans for sRNA–mRNA interactions around the 5'-UTR of the mRNA or proximate to the beginning of the mRNA coding sequence.

IntaRNAv2.0 [84] is the open-source reimplementation of IntaRNA [170] that favors seed interactions. It allows for user selection of energy parameters, seed constraints, and accessibility computation. The user can submit either a list of putative interacting RNA pairs to perform an all-versus-all prediction, or a single RNA so as to perform a genome-wide target screen. The method produces *p*-values based on the transformation of the energy scores calculated for all putative target binding sites with non-positive energy scores. The web server offers visualization of minimal energy profiles of interacting RNAs, thus enabling the study of alternative RNA–RNA interactions and the analysis of mutational effects.

psRNATarget [83] is a web server for the identification of target genes of plant miRNAs. The user can submit either (i) a list of sRNAs to search against preloaded target transcript libraries, (ii) candidate target transcripts to search against sRNAs from miRBase, or (iii) candidate sRNA–mRNA pairs. The procedure consists of two steps: first analysis of the sRNA–target mRNA complementary matching based on a scoring schema; and second, evaluation of the target site accessibility. It allows for customization of the scoring and search for both canonical and non-canonical targets. Mismatches in the mismatch-sensitive seed region are penalized more than the positive contribution of the complementary base pairing. The seed region (in the original version [186], defined as being in vertebrates, nucleotides 2–7 [11]) has been extended to nucleotides 2–13, allowing for up to 2 mismatches, according to the plant miRNA target recognition patterns [187]. psRNATarget is no longer available online.

In order to provide a collated and standardized result report, SPOT (sRNA Target Prediction Organizing Tool) [82] implements multiple algorithms for sRNA target prediction. SPOT is a pipeline that incorporates sTarPicker [89], TargetRNA2 [85], IntaRNA [170], and CopraRNA [86,87]. The minimal input consists of an sRNA sequence in FASTA format and the RefSeq ID of the target genome. To include CopraRNA, homologous sRNAs, and additional RefSeq IDs should be included. The interface allows for parameter setting for each method and for filtering the results. The utility and sensitivity of the pipeline were tested on two well-characterized *E. coli* sRNA models, SgrS [188] and RyhB [189]. Using more stringent parameters (stricter significance thresholds and smaller search windows upstream/downstream of start codons), or combining more than three algorithms for the prediction, decreases the FFP at the cost of sensitivity. When at least two methods converge on a prediction for those datasets, SPOT achieves sensitivity $\geq 75\%$ and FFP $\leq 50\%$.

sRNARFTarget [81] is a machine-learning-based method for transcriptome-wide sRNA target prediction. It utilizes a random forest (RF) trained on the trinucleotide frequency

difference between 745 sRNA–mRNA pairs from 37 bacterial species obtained by RNAseq [190], MAPS [191], GRIL-seq [192], RIL-seq [193], and CLASH [194]. Added information on the predicted secondary structure was not proven to improve the overall performance. It outperforms IntaRNAv2.0 in accuracy, running time, and ranking of true interacting pairs. However, CopraRNA is a more suitable option for prediction when sRNA homologs are available, as it was shown to outperform both methods in accuracy. The versatility and usability of the method on any sRNA–target pair rely on the fact that the prediction depends solely on the sequence.

miRNA-Target Interactions

cnnMirTarget [98] employs a CNN to automatically integrate the patterns in the raw sequence data, avoiding the hand-crafted selection of features. The tool predicts the target gene of miRNAs through scanning the full length of gene transcripts. cnnMirTarget is trained on a positive dataset constructed from the three sources: CLASH [54], AGO-CLIP [195], and MirTarBase [196,197], and negative data generated by pseudo combinations of miRNA and gene omitting the miRNA:mRNA pairs in MirTarBase. The trained model is evaluated on both site-level and gene-level data, the latter of which was downloaded from MirTarBase and Diana TarBase [198].

miTarDigger [102] utilizes two types of neural architectures: stacked denoising autoencoders (SDA) [199] and convolutional denoising autoencoders (CAE) [200]. Each type has its own functions: SDA is used to process sequence and CAE to process structure features. The results of two encoders are fused and then fed into the fully connected network and a logistic regression layer. Most of the existing studies have not considered the impact of upstream and downstream sequences of target sites on the prediction results and miTarDigger is exploiting this gap. miTarDigger is trained on CLASH data [54]; hence, it predicts on the site level. To be able to perform gene-level predictions, miTarDigger finds all CTSs in a given mRNA utilizing miRanda software [201].

RNNs and MLPs might be unsuitable for the task or miRNA target prediction, as they may not be able to efficiently capture the spatial and sequential features of the miRNA:target hybrid. miTAR [94] uses a combination of CNN and RNN architecture, exploiting the fact that CNNs excel in learning spatial features and RNNs discern sequential features [163,174]. The miTAR neural network is trained on site-level data obtained from miRAW [107] and DeepMirTar [106].

Predicting a functional miRNA:CTS pair from sequence only is not enough to fully capitalize the information underlying miRNA–CTS interactions. TargetNet [92] addresses this issue by proposing a novel miRNA:CTS encoding. Previous methods use one-hot encoding to convert only sequences into numerical representations. In contrast, TargetNet incorporates additional information on how the extended seed regions of a miRNA:CTS pair are aligned and form binding. The result of this encoding is a 2D matrix that is processed by a deep residual network (ResNet) with 1D convolutions.

tRF-Target Interactions

The predictive power of methods aimed at miRNA targets is poor for tRF targets [202,203]. tRFTars [76] offers the first database for predicting potential targets of tRFs in human, and is available as a web interface. It utilizes a Genetic Algorithm (GA) to select features of tRF–mRNA pairs, and Support Vector Machine (SVM) to build prediction models for tRF targets. The method utilized interacting pairs identified in AGO complexes by CLASH [54] in HEK293 cells and CLEAR-CLIP [204] in Huh-7.5 cells, and mRNA sequences from UCSC 2019 [205]. After preprocessing and filtering the data, the method was trained on 547 positive pairs (489 tRF-3 and 58 tRF-5 pairs) and 2000 negative pairs (1596 tRF-3 and 404 tRF-5 pairs). Feature assessment showed significantly different features of sequences involved in mRNA targeting and in the background. The most significant features found are minimum free folding energy (MFE), position 8 match, number of bases paired in the tRF–mRNA duplex, and length of the tRF, in agreement with previous studies [39,40]. The

trained GA-SVM models were shown to outperform the intersection of the miRNA target prediction models TargetScan [124,161] and miRanda [155].

tRFTar [75] is a publicly accessible multi-functional platform that contains 920,690 interactions between 12,102 tRFs and 5688 target genes identified by CLIP-seq in human. The authors utilized data from human 160 Ago CLIP (HITS-CLIP and PAR-CLIP), as well as annotation of 26,744 tRFs from MINTBase v2.0 [206] and genomic annotation from RefSeq [207]. After preprocessing, tRF-target interactions were predicted based on the MFE of the duplexes and simulated annealing was performed by RNAduplex [179]. Only those duplexes that met the normalized MFE threshold were retained and further validated by datasets from 6 CLASH experiments. A tRF-gene co-expression profile was constructed indicating context-dependent regulatory functions. 5'-tRFs and 3'-tRFs were found to be more likely candidates for AGO-mediated gene expression regulation, and their interaction sites tend to be preferentially distributed. The tRFTar platform allows for the custom search of interactions, genome browser, GO enrichment, and co-expressed interaction filtering.

siRNA Off-Target Interactions

MIRZA-G [79] is a suite of algorithms (currently the web server is not accessible) for the genome-wide prediction of (non-) canonical miRNA targets and siRNA off-targets. It implements the MIRZA [132] biophysical model for the prediction of the miRNA-target interaction energy. The model, trained and evaluated on data from a set of 26 experiments on humans, considers features such as nucleotide composition around putative target sites, their structural accessibility, and location within 3' UTRs. Adding evolutionary conservation as a feature improves the prediction of siRNA target sites, in accordance with previous findings [208].

RIsearch2 [78] uses a single integrated seed-and-extend framework based on suffix arrays to predict RNA–RNA interactions. Unlike its first version [209], RIsearch2 follows the two-stage strategy of the seed-and-extend paradigm. In the first step, it uses suffix arrays to locate maximal stretches of perfect complementarity (wobble pairs allowed), and in the second step extends those seed matches on either end using dynamic programming with the scoring scheme introduced in [209]. The study also presents a pipeline that predicts siRNA off-target transcripts, and the off-targeting potential for a given siRNA based on genome-wide RIsearch2 predictions combined with target site accessibilities and transcript abundance estimates. The pipeline accounts for intramolecular interactions of the targeted transcripts, and allows for user-defined seed and extension constraints. RIsearch2, originally constructed for predictions on humans, can be tuned for any other organism.

siRNA-Finder [77] is a tool for the prediction of RNAi sequences and off-target search in plants, designed for MS Windows. It utilizes the BOWTIE-based sequence similarity search for putative siRNA targets, the probability calculation of local target-site accessibility, and thermodynamics—as well as a sequence-based prediction for strand selection. It includes two pipelines with different functionalities: High Sensitivity for off-target search, and High Efficiency for RNAi-construct design.

cWords [80] is a tool based on rigorous statistical methods, designed to extract correlations of differential expression and motif occurrences. The method can assist the exploratory analysis of enriched words and degenerate motifs such as noncanonical miRNA-binding sites and RNA-binding protein binding sites by providing methods for clustering and visualization of enriched words with similar sequences. It has been demonstrated that cWords, originally designed for miRNAs, can also be used for the identification of potential siRNA off-target binding.

3. Discussion

The advancements in high-throughput experimental techniques and the increasing computational power have propelled forward the development of computational methods for the prediction of sRNA targets. A plethora of methods with a variety of features and assumptions is available for diverse types of sRNAs. The emerging field of Deep Learning

has offered current-state-of-the-art methods for sRNA target prediction; nevertheless, there are still multiple challenges to be addressed, and limitations of existing methods that still leave the field open for further development.

An important distinction to be made for the various sRNA target prediction methods presented here is the goal of the prediction. Older methods (e.g., TargetScan, microT, and others) attempted to predict targeting at the miRNA:gene level. Usually, such methods would use one model for the identification of putative target sites, and a different model that would combine such target sites into a gene-level prediction. This functionality could then be evaluated on experimental datasets coming from overexpression or knockout of specific miRNAs, and prediction of the effects on their targets. Later Deep Learning-based methods have by and large been trained on target-site level data (validated luciferase targets, CLASH, and similar). The majority of the methods consider a miRNA:gene pair to be functional when at least one functional site is present in a target gene (cnnMirTarget, miTAR, TargetNet, and DMISO), whereas some others consider additional features in the post-processing, such as binding stability and site accessibility (miRAW). We notice that many methods that are currently trained and evaluated on target-site data, use direct comparisons with older methods (commonly TargetScan) that are trained on a completely different task, namely, sRNA: gene-level prediction. These comparisons are summarized in Table 3. We would like to caution against such comparisons as non-informative for the user and the field at large. We consider it to be beneficial for subsequent target prediction programs to clarify the distinction and the goal of their program, and to benchmark against programs of the same category, on benchmarks that are relevant to the task.

Converting site-level predictions to gene-level predictions is not a trivial task. In brief, when scanning a transcriptome for candidate targets of a specific sRNA, one expects to encounter orders of magnitude more non-target sites, than bona fide target sites. Such a substantial class imbalance is another challenge that sRNA target prediction methods, and especially DL methods, have to deal with, since they perform best when classes are balanced. All of the DL methods we presented here are trained on target site-level data, often balanced in number between positive and negative samples, but the ultimate goal is to predict interactions on the gene level, which exhibits an order of magnitude class imbalance. Typically, the whole mRNA is split into smaller parts representing CTSs; however, only a fraction of them contains real target sites. A common approach involves filtering out CTSs that have a low probability of containing real target sites. Rules for filtering are based on current experimental findings about interactions between miRNA and its target: extended loose seed matching (deepTarget, TargetNet), the potential to create a stable duplex (cnnMirTarget), or both types of these constraints (miRAW), thus introducing an undesirable bias as the filtering is performed using handcrafted conditions. MiRAW claims that CTS pre-filtering is required when there are too few samples for the neural network to learn all necessary features and handle pre-filtering itself. Recently, TargetNet investigated the effect of CTS pre-filtering on the classification performance and obtained a similar F1 score with and without CTS pre-filtering, with the only difference being the computational time. At this point, the transition from the target site to target gene-level prediction is still unclear and based on heuristics. We believe that there is a need for a new method that systematically combines target site-level predictions into gene-level predictions using contemporary machine learning methods. When working with computationally predicted targets, it is important to remember that thorough experimental validation is paramount, since all current target prediction programs will produce false positive targets, as well as numerous false negative targets. Until the exact rules of small RNA targeting are fully understood, we need to treat all predictions as educated guesses until thoroughly validated.

Program	Targetnet [92]	miTAR [94]	RPmirDIP [97]	miRTMC [101]	mirTarDigger [102]	cnnMirTarget [98]	miRAW [107]
PITA [159]	0.22						0.74
miRanda [155]	0.36			0.69	0.66		
mirSVR [210]							0.41
microT-CDS [211]							0.73
miRDB [212]	0.21					0.23	0.21
mirza-G [79]							0.52
Paccmit [213]							0.41
Targetscan [124]	0.47			0.67	0.62	0.31	0.56
deepTarget [119]	0.49			0.69			
TarPmiR [120]					0.78		
metaMIR [214]						0.78	
DeepMirTar [106]	. =0	-			0.94		
miRAW [107]	0.73	0.95	0.00				0.93
mirDIP [169]			0.88	0.70			
miki ks [99]				0.70			
GNICLDA [215]				0.61		0.70	
mirTarDiggor [102]					0.06	0.79	
miRTMC [101]				0.72	0.90		
RPmirDIP [97]			0.93	0.72			
miTAR2 [94]		0.97	0.90				
TargetNet [92]	0.77	0.77					
	F1-score on balanced miRNA:mRNA target pairs (dataset from miRAW)	F1-score on miRAW dataset	Bootstrap testing PR AUC	AUC on different independent datasets. Showing dataset 1 (based on miRTarBase), as results are similar.	F1-score on target interactions vs. artificial miRNAs.	The experimentally validated positive dataset contains 7815 interactions; the negative dataset contains 281 pseudo-	F1-score using full testing dataset, constructed from various external sources

 Table 3. Comparison of computational methods for sRNA:target prediction.

Obtaining larger and more reliable experimental datasets is of utmost importance for the development of efficient computational methods that can, in turn, facilitate the experimental validation of sRNA targets. Site-level CLASH data have offered the direct identification of sRNA targets, and a recent modification of chimeric eCLIP promises the recovery of miRNA:mRNA interactions by 70-fold. Still, obtaining large-scale good quality gene-level experiments remains a challenge that can resolve the artificial assumptions for the computational prediction of sRNA targets. An additional challenge is that available datasets are quickly used by computational methods for training, making true benchmarking against never-before-seen data impossible, unless someone performs dedicated overexpression or knock-out experiments for benchmarking. We would suggest the future development and publication of dedicated benchmark datasets for both the target site level (based on new CLASH or chimeric eCLIP data) and at the target gene level (based on overexpression or knockout experiments). These benchmarks must be developed explicitly to solve the fragmentation of the field, and have exact testing sets that cannot be used for training future methods, so as to allow for continuous testing against them. In the current state of the field, meaningful benchmarking of tools is impossible unless new experiments are performed.

Finally, the field of sRNA target prediction is currently dominated by miRNA target prediction, with a small spread into tRFs and other small RNA species.

The exploration of the regulatory role of other sRNAs, including rRFs, still remains to be further elucidated, and computational methods for the prediction of their targets are yet to be developed. As was previously shown, the prediction of reliable RNA–RNA interactions can be used to infer the functional relationships of miRNAs [216]. Acquiring such interaction data can accelerate the discovery of new ncRNAs and provide insight into their involvement in regulating cellular output. We believe that exploring the potential for regulatory roles of other non-coding RNA families will be an important development in the field in the next years, as long as it is supported by high-quality experimental data and benchmarks.

To conclude, the sRNA target prediction field has seen great development in the past 5 years with the advent of Deep Learning methods and new experimental datasets such as CLASH, but no newly produced method has decisively outperformed others either in the target-site prediction level, or the target-gene prediction level. The field remains open to further developments, as the need for new and properly set up experimental benchmarks increases.

4. Conclusions

RNA interference is a widespread, evolutionary conserved mechanism that is of great significance for the fields of therapeutics and diagnostics. At its core, it is driven by small RNAs that target mRNAs for cleavage or translational repression. The recent advancements in high-throughput sequencing techniques, in tandem with the rapidly developing field of Machine Learning, have shed light on these complex RNA–RNA interactions, and have produced numerous computational methods for the prediction of sRNAs targets. However, the field of sRNA target prediction is currently dominated by miRNA target prediction, with a small spread into tRFs and other small RNA species. In this review, we document the development of ML and other computational methods for the prediction of small RNA targets, with emphasis on the non-miRNA sRNAs, and we highlight the limitations and the future prospects of the research in the field. Additionally, we provide a brief overview of the high-throughput methods utilized for the detection of RNA–RNA interactions.

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References

- 1. Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by doublestranded RNA in Caenorhabditis elegans. *Nature* **1998**, *391*, 806–811. [CrossRef] [PubMed]
- Mizuno, T.; Chou, M.Y.; Inouye, M. A unique mechanism regulating gene expression: Translational inhibition by a complementary RNA transcript (micRNA). *Proc. Natl. Acad. Sci. USA* 1984, *81*, 1966–1970. [CrossRef] [PubMed]
- Lalaouna, D.; Simoneau-Roy, M.; Lafontaine, D.; Massé, E. Regulatory RNAs and target mRNA decay in prokaryotes. *Biochim. et Biophys. Acta* 2013, 1829, 742–747. [CrossRef]
- 4. Bartel, D.P. Metazoan MicroRNAs. Cell 2018, 173, 20–51. [CrossRef] [PubMed]
- 5. O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front. Endocrinol.* **2018**, *9*, 402. [CrossRef] [PubMed]
- 6. Carthew, R.W.; Sontheimer, E.J. Origins and Mechanisms of miRNAs and siRNAs. Cell 2009, 136, 642–655. [CrossRef] [PubMed]
- Ozata, D.M.; Gainetdinov, I.; Zoch, A.; O'Carroll, D.; Zamore, P.D. PIWI-interacting RNAs: Small RNAs with big functions. *Nat. Rev. Genet.* 2018, 20, 89–108. [CrossRef]
- Li, Z.; Rana, T.M. Molecular Mechanisms of RNA-Triggered Gene Silencing Machineries. Accounts Chem. Res. 2012, 45, 1122–1131. [CrossRef]
- 9. Huang, X.; Tóth, K.F.; Aravin, A.A. piRNA Biogenesis in Drosophila melanogaster. Trends Genet. 2017, 33, 882–894. [CrossRef]
- Shabalina, S.A.; Koonin, E.V. Origins and evolution of eukaryotic RNA interference. *Trends Ecol. Evol.* 2008, 23, 578–587. [CrossRef]
 Lewis, B.P.; Burge, C.B.; Bartel, D.P. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human
- Genes are MicroRNA Targets. Cell 2005, 120, 15–20. [CrossRef] [PubMed]
- 12. Bartel, D.P. MicroRNAs: Target Recognition and Regulatory Functions. Cell 2009, 136, 215–233. [CrossRef] [PubMed]
- 13. Jackson, A.L.; Burchard, J.; Schelter, J.; Chau, B.N.; Cleary, M.; Lim, L.; Linsley, P.S. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *Rna* **2006**, *12*, 1179–1187. [CrossRef]
- 14. Neumeier, J.; Meister, G. siRNA Specificity: RNAi Mechanisms and Strategies to Reduce Off-Target Effects. *Front. Plant Sci.* 2021, *11.* Available online: https://www.frontiersin.org/articles/10.3389/fpls.2020.526455 (accessed on 9 August 2022). [CrossRef]
- 15. Borges, F.; Martienssen, R.A. The expanding world of small RNAs in plants. *Nat. Rev. Mol. Cell Biol.* 2015, *16*, 727–741. [CrossRef] [PubMed]
- 16. Vaucheret, H. Plant argonautes. Trends Plant Sci. 2008, 13, 350-358. [CrossRef]
- 17. Melnyk, C.W.; Molnar, A.; Baulcombe, D. Intercellular and systemic movement of RNA silencing signals. *EMBO J.* **2011**, *30*, 3553–3563. [CrossRef]
- 18. Voinnet, O.; Vain, P.; Angell, S.; Baulcombe, D.C. Systemic Spread of Sequence-Specific Transgene RNA Degradation in Plants Is Initiated by Localized Introduction of Ectopic Promoterless DNA. *Cell* **1998**, *95*, 177–187. [CrossRef]
- Buhtz, A.; Springer, F.; Chappell, L.; Baulcombe, D.; Kehr, J. Identification and characterization of small RNAs from the phloem of Brassica napus. *Plant J.* 2007, 53, 739–749. [CrossRef]
- 20. Dexheimer, P.J.; Cochella, L. MicroRNAs: From Mechanism to Organism. Front. Cell Dev. Biol. 2020, 8, 409. [CrossRef]
- 21. Millar, A.; Waterhouse, P.M. Plant and animal microRNAs: Similarities and differences. *Funct. Integr. Genom.* 2005, *5*, 129–135. [CrossRef] [PubMed]
- 22. Axtell, M.J.; Westholm, J.; Lai, E.C. Vive la différence: Biogenesis and evolution of microRNAs in plants and animals. *Genome Biol.* **2011**, *12*, 221. [CrossRef] [PubMed]
- 23. Vogel, J.; Luisi, B.F. Hfq and its constellation of RNA. Nat. Rev. Genet. 2011, 9, 578–589. [CrossRef]
- 24. Melamed, S.; Adams, P.P.; Zhang, A.; Zhang, H.; Storz, G. RNA-RNA Interactomes of ProQ and Hfq Reveal Overlapping and Competing Roles. *Mol. Cell* **2019**, *77*, 411–425.e7. [CrossRef] [PubMed]
- Storz, G.; Vogel, J.; Wassarman, K.M. Regulation by Small RNAs in Bacteria: Expanding Frontiers. *Mol. Cell* 2011, 43, 880–891. [CrossRef]
- Papenfort, K.; Vanderpool, C.K. Target activation by regulatory RNAs in bacteria. FEMS Microbiol. Rev. 2015, 39, 362–378. [CrossRef]
- 27. Wagner, E.G.H.; Romby, P. Small RNAs in Bacteria and Archaea: Who they are, what they do, and how they do it. *Adv. Genet.* **2015**, *90*, 133–208. [CrossRef]
- Hör, J.; Matera, G.; Vogel, J.; Gottesman, S.; Storz, G. Trans-Acting Small RNAs and Their Effects on Gene Expression in *Escherichia* coli and *Salmonella enterica*. *EcoSal Plus* 2020, 9. [CrossRef]
- Babski, J.; Maier, L.-K.; Heyer, R.; Jaschinski, K.; Prasse, D.; Jäger, D.; Randau, L.; Schmitz, R.a.; Marchfelder, A.; Soppa, J. Small regulatory RNAs in Archaea. RNA Biol. 2014, 11, 484–493. [CrossRef]

- 30. Bhatt, S.; Egan, M.; Jenkins, V.; Muche, S.; El-Fenej, J. The Tip of the Iceberg: On the Roles of Regulatory Small RNAs in the Virulence of Enterohemorrhagic and Enteropathogenic Escherichia coli. *Front. Cell Infect. Microbiol.* **2016**, *6*, 105. [CrossRef]
- 31. Lease, R.A.; Belfort, M. A trans-acting RNA as a control switch in Escherichia coli: DsrA modulates function by form-ing alternative structures. *Proc. Natl. Acad. Sci. USA* 2000, 97, 9919–9924. [CrossRef] [PubMed]
- 32. Lee, Y.S.; Shibata, Y.; Malhotra, A.; Dutta, A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Genes Dev.* 2009, 23, 2639–2649. [CrossRef] [PubMed]
- 33. Haussecker, D.; Huang, Y.; Lau, A.; Parameswaran, P.; Fire, A.Z.; Kay, M.A. Human tRNA-derived small RNAs in the global regulation of RNA silencing. *RNA* **2010**, *16*, 673–695. [CrossRef] [PubMed]
- Soares, A.R.; Fernandes, N.; Reverendo, M.; Araújo, H.R.; Oliveira, J.L.; Moura, G.M.R.; Santos, M.A.S. Conserved and highly expressed tRNA derived fragments in zebrafish. *BMC Mol. Biol.* 2015, 16, 22. [CrossRef]
- Schimmel, P. The emerging complexity of the tRNA world: Mammalian tRNAs beyond protein synthesis. *Nat. Rev. Mol. Cell Biol.* 2017, 19, 45–58. [CrossRef] [PubMed]
- Kumar, P.; Anaya, J.; Mudunuri, S.B.; Dutta, A. Meta-analysis of tRNA derived RNA fragments reveals that they are evolutionarily conserved and associate with AGO proteins to recognize specific RNA targets. *BMC Biol.* 2014, 12, 78. [CrossRef]
- Chen, Q.; Zhang, X.; Shi, J.; Yan, M.; Zhou, T. Origins and evolving functionalities of tRNA-derived small RNAs. *Trends Biochem. Sci.* 2021, 46, 790–804. [CrossRef]
- Kumar, P.; Kuscu, C.; Dutta, A. Biogenesis and Function of Transfer RNA-Related Fragments (tRFs). *Trends Biochem. Sci.* 2016, 41, 679–689. [CrossRef]
- Kuscu, C.; Kumar, P.; Kiran, M.; Su, Z.; Malik, A.; Dutta, A. tRNA fragments (tRFs) guide Ago to regulate gene expression post-transcriptionally in a Dicer-independent manner. *RNA* 2018, 24, 1093–1105. [CrossRef]
- Hafner, M.; Landthaler, M.; Burger, L.; Khorshid, M.; Hausser, J.; Berninger, P.; Rothballer, A.; Ascano, M., Jr.; Jungkamp, A.-C.; Munschauer, M.; et al. Transcriptome-wide Identification of RNA-Binding Protein and MicroRNA Target Sites by PAR-CLIP. *Cell* 2010, 141, 129–141. [CrossRef]
- Burroughs, A.M.; Ando, Y.; De Hoon, M.J.L.; Tomaru, Y.; Suzuki, H.; Hayashizaki, Y.; Daub, C. Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. RNA Biol. 2011, 8, 158–177. [CrossRef] [PubMed]
- 42. Majdalani, N.; Chen, S.; Murrow, J.; John, K.S.; Gottesman, S. Regulation of RpoS by a novel small RNA: The characterization of RprA. *Mol. Microbiol.* **2004**, *39*, 1382–1394. [CrossRef] [PubMed]
- Honda, S.; Loher, P.; Shigematsu, M.; Palazzo, J.P.; Suzuki, R.; Imoto, I.; Rigoutsos, I.; Kirino, Y. Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers. *Proc. Natl. Acad. Sci. USA* 2015, *112*, E3816–E3825. [CrossRef] [PubMed]
- 44. Magee, R.G.; Telonis, A.G.; Loher, P.; Londin, E.; Rigoutsos, I. Profiles of miRNA Isoforms and tRNA Fragments in Prostate Cancer. *Sci. Rep.* **2018**, *8*, 5314. [CrossRef]
- 45. Asha, S.; Soniya, E.V. The sRNAome mining revealed existence of unique signature small RNAs derived from 5.8SrRNA from Piper nigrum and other plant lineages. *Sci. Rep.* **2017**, *7*, srep41052. [CrossRef]
- 46. Chen, Z.; Sun, Y.; Yang, X.; Wu, Z.; Guo, K.; Niu, X.; Wang, Q.; Ruan, J.; Bu, W.; Gao, S. Two featured series of rRNA-derived RNA fragments (rRFs) constitute a novel class of small RNAs. *PLoS ONE* **2017**, *12*, e0176458. [CrossRef]
- Li, S. Human 28s rRNA 5' terminal derived small RNA inhibits ribosomal protein mRNA levels. *bioRxiv* 2019. bioRxiv:618520. [CrossRef]
- 48. Guan, L. Age-Related Argonaute Loading of Ribosomal RNA Fragments. MicroRNA 2020, 9, 142–152. [CrossRef]
- 49. Guan, L.; Grigoriev, A. Computational meta-analysis of ribosomal RNA fragments: Potential targets and interaction mechanisms. *Nucleic Acids Res.* **2021**, *49*, 4085–4103. [CrossRef]
- 50. Samuel, A.L. Some Studies in Machine Learning Using the Game of Checkers. IBM J. Res. Dev. 1959, 3, 210–229. [CrossRef]
- 51. Bishop, C.M.; Nasrabadi, N.M. Bishop, Pattern Recognition and Machine Learning (Information Science and Statistics); Springer: Berlin/Heidelberg, Germany, 2006.
- 52. LeCun, Y.; Bengio, Y.; Hinton, G. Deep learning. Nature 2015, 521, 436-444. [CrossRef] [PubMed]
- Pinzón, N.; Li, B.; Martinez, L.; Sergeeva, A.; Presumey, J.; Apparailly, F.; Seitz, H. microRNA target prediction programs predict many false positives. *Genome Res.* 2016, 27, 234–245. [CrossRef] [PubMed]
- 54. Helwak, A.; Kudla, G.; Dudnakova, T.; Tollervey, D. Mapping the Human miRNA Interactome by CLASH Reveals Frequent Noncanonical Binding. *Cell* 2013, 153, 654–665. [CrossRef] [PubMed]
- Seok, H.; Ham, J.; Jang, E.-S.; Chi, S.W. MicroRNA Target Recognition: Insights from Transcriptome-Wide Non-Canonical Interactions. *Mol. Cells* 2016, *39*, 375–381. [CrossRef] [PubMed]
- Imig, J.; Brunschweiger, A.; Brümmer, A.; Guennewig, B.; Mittal, N.; Kishore, S.; Tsikrika, P.; Gerber, A.P.; Zavolan, M.; Hall, J. miR-CLIP capture of a miRNA targetome uncovers a lincRNA H19–miR-106a interaction. *Nat. Chem. Biol.* 2015, *11*, 107–114. [CrossRef] [PubMed]
- 57. Li, J.; Zhang, Y. Current experimental strategies for intracellular target identification of microRNA. ExRNA 2019, 1, 6. [CrossRef]
- 58. Riolo, G.; Cantara, S.; Marzocchi, C.; Ricci, C. miRNA Targets: From Prediction Tools to Experimental Validation. *Methods Protoc.* **2020**, *4*, 1. [CrossRef]

- Boutz, D.R.; Collins, P.J.; Suresh, U.; Lu, M.; Ramírez, C.M.; Fernández-Hernando, C.; Huang, Y.; Abreu, R.D.S.; Le, S.-Y.; Shapiro, B.A.; et al. Two-tiered Approach Identifies a Network of Cancer and Liver Disease-related Genes Regulated by miR-122. *J. Biol. Chem.* 2011, 286, 18066–18078. [CrossRef]
- Wolter, J.M.; Kotagama, K.; Pierre-Bez, A.C.; Firago, M.; Mangone, M. 3'LIFE: A functional assay to detect miRNA targets in high-throughput. *Nucleic Acids Res.* 2014, 42, e132. [CrossRef]
- Carter, M.; Shieh, J. Biochemical Assays and Intracellular Signaling. In *Guide to Research Techniques in Neuroscience*, 2nd ed.; Carter, M., Shieh, J., Eds.; Academic Press: San Diego, CA, USA, 2015; pp. 311–343. [CrossRef]
- 62. Lim, L.P.; Lau, N.C.; Garrett-Engele, P.; Grimson, A.; Schelter, J.M.; Castle, J.; Bartel, D.P.; Linsley, P.S.; Johnson, J.M. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005, 433, 769–773. [CrossRef]
- 63. Baek, D.; Villén, J.; Shin, C.; Camargo, F.D.; Gygi, S.P.; Bartel, D.P. The impact of microRNAs on protein output. *Nature* **2008**, 455, 64–71. [CrossRef] [PubMed]
- 64. Guo, H.; Ingolia, N.T.; Weissman, J.S.; Bartel, D.P. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 2010, 466, 835–840. [CrossRef] [PubMed]
- Karginov, F.V.; Conaco, C.; Xuan, Z.; Schmidt, B.H.; Parker, J.S.; Mandel, G.; Hannon, G.J. A biochemical approach to identifying microRNA targets. *Proc. Natl. Acad. Sci. USA* 2007, 104, 19291–19296. [CrossRef] [PubMed]
- Chi, S.W.; Zang, J.B.; Mele, A.; Darnell, R.B. Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps. *Nature* 2009, 460, 479–486. [CrossRef]
- König, J.; Zarnack, K.; Rot, G.; Curk, T.; Kayikci, M.; Zupan, B.; Turner, D.J.; Luscombe, N.M.; Ule, J. iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nat. Struct. Mol. Biol.* 2010, 17, 909–915. [CrossRef]
- Van Nostrand, E.L.; Pratt, G.A.; Shishkin, A.A.; Gelboin-Burkhart, C.; Fang, M.Y.; Sundararaman, B.; Blue, S.M.; Nguyen, T.B.; Surka, C.; Elkins, K.; et al. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat. Methods* 2016, 13, 508–514. [CrossRef]
- Manakov, S.A.; Shishkin, A.A.; Yee, B.A.; Shen, K.A.; Cox, D.C.; Park, S.S.; Foster, H.M.; Chapman, K.B.; Yeo, G.W.; Van Nostrand, E.L. Scalable and deep profiling of mRNA targets for individual microRNAs with chimeric eCLIP. *BioRxiv* 2022. BioRxiv:2022.02.13.480296. [CrossRef]
- 70. Helwak, A.; Tollervey, D. Mapping the miRNA interactome by cross-linking ligation and sequencing of hybrids (CLASH). *Nat. Protoc.* **2014**, *9*, 711–728. [CrossRef]
- 71. Hsu, R.-J.; Yang, H.-J.; Tsai, H.-J. Labeled microRNA pull-down assay system: An experimental approach for high-throughput identification of microRNA-target mRNAs. *Nucleic Acids Res.* **2009**, *37*, e77. [CrossRef]
- Baigude, H.; Ahsanullah; Li, Z.; Zhou, Y.; Rana, T.M. miR-TRAP: A Benchtop Chemical Biology Strategy to Identify microRNA Targets. Angew. Chem. Int. Ed. 2012, 51, 5880–5883. [CrossRef]
- Li, J.; Huang, L.; Xiao, X.; Chen, Y.; Wang, X.; Zhou, Z.; Zhang, C.; Zhang, Y. Photoclickable MicroRNA for the Intracellular Target Identification of MicroRNAs. J. Am. Chem. Soc. 2016, 138, 15943–15949. [CrossRef] [PubMed]
- 74. Lim, R.K.V.; Lin, Q. Photoinducible Bioorthogonal Chemistry: A Spatiotemporally Controllable Tool to Visualize and Perturb Proteins in Live Cells. *Accounts Chem. Res.* 2011, 44, 828–839. [CrossRef] [PubMed]
- 75. Zhou, Y.; Peng, H.; Cui, Q.; Zhou, Y. tRFTar: Prediction of tRF-target gene interactions via systemic re-analysis of Argonaute CLIP-seq datasets. *Methods* 2020, *187*, 57–67. [CrossRef] [PubMed]
- 76. Xiao, Q.; Gao, P.; Huang, X.; Chen, X.; Chen, Q.; Lv, X.; Fu, Y.; Song, Y.; Wang, Z. tRFTars: Predicting the targets of tRNA-derived fragments. *J. Transl. Med.* **2021**, *19*, 88. [CrossRef] [PubMed]
- 77. Naskulwar, K.; Peña-Castillo, L. sRNARFTarget: A fast machine-learning-based approach for transcriptome-wide sRNA target prediction. *RNA Biol.* 2021, 19, 44–54. [CrossRef]
- Lück, S.; Kreszies, T.; Strickert, M.; Schweizer, P.; Kuhlmann, M.; Douchkov, D. siRNA-Finder (si-Fi) Software for RNAi-Target Design and Off-Target Prediction. *Front. Plant Sci.* 2019, 10, 1023. [CrossRef]
- 79. Alkan, F.; Wenzel, A.; Palasca, O.; Kerpedjiev, P.; Rudebeck, A.F.; Stadler, P.F.; Hofacker, I.L.; Gorodkin, J. RIsearch2: Suffix array-based large-scale prediction of RNA–RNA interactions and siRNA off-targets. *Nucleic Acids Res.* 2017, 45, e60. [CrossRef]
- 80. Gumienny, R.; Zavolan, M. Accurate transcriptome-wide prediction of microRNA targets and small interfering RNA off-targets with MIRZA-G. *Nucleic Acids Res.* 2015, 43, 1380–1391. [CrossRef]
- Rasmussen, S.H.; Jacobsen, A.; Krogh, A. cWords—Systematic microRNA regulatory motif discovery from mRNA expression data. *Silence* 2013, *4*, 2–9. [CrossRef]
- 82. King, A.M.; Vanderpool, C.; Degnan, P.H. sRNA Target Prediction Organizing Tool (SPOT) Integrates Computational and Experimental Data To Facilitate Functional Characterization of Bacterial Small RNAs. *mSphere* **2019**, *4*, e00561-18. [CrossRef]
- Dai, X.; Zhuang, Z.; Zhao, P.X. psRNATarget: A plant small RNA target analysis server (2017 release). Nucleic Acids Res. 2018, 46, W49–W54. [CrossRef] [PubMed]
- Mann, M.; Wright, P.R.; Backofen, R. IntaRNA 2.0: Enhanced and customizable prediction of RNA–RNA interactions. *Nucleic Acids Res.* 2017, 45, W435–W439. [CrossRef] [PubMed]
- 85. Kery, M.B.; Feldman, M.; Livny, J.; Tjaden, B. TargetRNA2: Identifying targets of small regulatory RNAs in bacteria. *Nucleic Acids Res.* 2014, 42, W124–W129. [CrossRef] [PubMed]
- 86. Wright, P.R.; Richter, A.S.; Papenfort, K.; Mann, M.; Vogel, J.; Hess, W.R.; Backofen, R.; Georg, J. Comparative genomics boosts target prediction for bacterial small RNAs. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3487–E3496. [CrossRef] [PubMed]

- Wright, P.R.; Georg, J.; Mann, M.; Sorescu, D.A.; Richter, A.S.; Lott, S.; Kleinkauf, R.; Hess, W.R.; Backofen, R. CopraRNA and IntaRNA: Predicting small RNA targets, networks and interaction domains. *Nucleic Acids Res.* 2014, 42, W119–W123. [CrossRef]
- Eggenhofer, F.; Tafer, H.; Stadler, P.F.; Hofacker, I.L. RNApredator: Fast accessibility-based prediction of sRNA targets. *Nucleic Acids Res.* 2011, 39, W149–W154. [CrossRef]
 Nucleic Construction of the Acids and the Acids and
- Ying, X.; Cao, Y.; Wu, J.; Liu, Q.; Cha, L.; Li, W. sTarPicker: A Method for Efficient Prediction of Bacterial sRNA Targets Based on a Two-Step Model for Hybridization. *PLoS ONE* 2011, 6, e22705. [CrossRef]
- 90. Talukder, A.; Zhang, W.; Li, X.; Hu, H. A deep learning method for miRNA/isomiR target detection. *bioRxiv* 2022. bioRxiv:2022.04.04.487002. [CrossRef]
- 91. Maxwell, E.K.; Campbell, J.D.; Spira, A.; Baxevanis, A.D. SubmiRine: Assessing variants in microRNA targets using clinical genomic data sets. *Nucleic Acids Res.* 2015, 43, 3886–3898. [CrossRef]
- Min, S.; Lee, B.; Yoon, S. TargetNet: Functional microRNA target prediction with deep neural networks. *Bioinformatics* 2021, 38, 671–677. [CrossRef]
- Shakyawar, S.; Southekal, S.; Guda, C. mintRULS: Prediction of miRNA–mRNA Target Site Interactions Using Regularized Least Square Method. *Genes* 2022, 13, 1528. [CrossRef] [PubMed]
- Gu, T.; Zhao, X.; Barbazuk, W.B.; Lee, J.-H. miTAR: A hybrid deep learning-based approach for predicting miRNA targets. BMC Bioinform. 2021, 22, 96. [CrossRef] [PubMed]
- 95. Xie, W.; Luo, J.; Pan, C.; Liu, Y. SG-LSTM-FRAME: A computational frame using sequence and geometrical information via LSTM to predict miRNA–gene associations. *Briefings Bioinform.* **2020**, *22*, 2032–2042. [CrossRef] [PubMed]
- Chu, Y.-W.; Chang, K.-P.; Chen, C.-W.; Liang, Y.-T.; Soh, Z.T.; Hsieh, L. miRgo: Integrating various off-the-shelf tools for identification of microRNA-target interactions by heterogeneous features and a novel evaluation indicator. *Sci. Rep.* 2020, 10, 1–11. [CrossRef]
- Kyrollos, D.G.; Reid, B.; Dick, K.; Green, J.R. RPmirDIP: Reciprocal Perspective improves miRNA targeting prediction. *Sci. Rep.* 2020, 10, 11770. [CrossRef]
- Zheng, X.; Chen, L.; Li, X.; Zhang, Y.; Xu, S.; Huang, X. Prediction of miRNA targets by learning from interaction sequences. *PLoS* ONE 2020, 15, e0232578. [CrossRef]
- 99. Jiang, H.; Wang, J.; Li, M.; Lan, W.; Wu, F.-X.; Pan, Y. miRTRS: A Recommendation Algorithm for Predicting miRNA Targets. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 2018, 17, 1032–1041. [CrossRef]
- Maji, R.K.; Khatua, S.; Ghosh, Z. A Supervised Ensemble Approach for Sensitive microRNA Target Prediction. IEEE/ACM Trans. Comput. Biol. Bioinform. 2020, 17, 37–46. [CrossRef]
- Jiang, H.; Yang, M.; Chen, X.; Li, M.; Li, Y.; Wang, J. miRTMC: A miRNA Target Prediction Method Based on Matrix Completion Algorithm. *IEEE J. Biomed. Health Inform.* 2020, 24, 3630–3641. [CrossRef]
- Yan, J.; Li, Y.; Zhu, M. miTarDigger: A Fusion Deep-learning Approach for Predicting Human miRNA Targets. In Proceedings of the 2020 IEEE International Conference on Bioinformatics and Biomedicine (BIBM), Seoul, Korea, 16–19 December 2020; pp. 2891–2897. [CrossRef]
- 103. Huang, T.; Huang, X.; Yao, M. Min3: Predict microRNA target gene using an improved binding-site representation method and support vector machine. *J. Bioinform. Comput. Biol.* **2019**, *17*, 1950032. [CrossRef]
- Kang, H.; Ahn, H.; Jo, K.; Oh, M.; Kim, S. mirTime: Identifying Condition-Specific Targets of MicroRNA in Time-series Transcript Data using Gaussian Process Model and Spherical Vector Clustering. *Bioinformatics* 2019, 37, 1544–1553. [CrossRef] [PubMed]
- 105. Ding, J.; Li, X.; Hu, H. CCmiR: A computational approach for competitive and cooperative microRNA binding prediction. *Bioinformatics* **2017**, *34*, 198–206. [CrossRef] [PubMed]
- 106. Wen, M.; Cong, P.; Zhang, Z.; Lu, H.; Li, T. DeepMirTar: A deep-learning approach for predicting human miRNA targets. *Bioinformatics* 2018, 34, 3781–3787. [CrossRef] [PubMed]
- 107. Pla, A.; Zhong, X.; Rayner, S. miRAW: A deep learning-based approach to predict microRNA targets by analyzing whole microRNA transcripts. *PLoS Comput. Biol.* **2018**, *14*, e1006185. [CrossRef]
- Mohebbi, M.; Ding, L.; Malmberg, R.L.; Momany, C.; Rasheed, K.; Cai, L. Accurate prediction of human miRNA targets via graph modeling of the miRNA-target duplex. *J. Bioinform. Comput. Biol.* 2018, 16, 1850013. [CrossRef]
- Koo, J.; Zhang, J.; Chaterji, S. Tiresias: Context-sensitive Approach to Decipher the Presence and Strength of MicroRNA Regulatory Interactions. *Theranostics* 2018, 8, 277–291. [CrossRef]
- Oh, M.; Rhee, S.; Moon, J.H.; Chae, H.; Lee, S.; Kang, J.; Kim, S. Literature-based condition-specific miRNA-mRNA target prediction. *PLoS ONE* 2017, 12, e0174999. [CrossRef]
- Torkey, H.; Heath, L.S.; ElHefnawi, M. MicroTarget: MicroRNA target gene prediction approach with application to breast cancer. *J. Bioinform. Comput. Biol.* 2017, 15, 1750013. [CrossRef]
- 112. Bottini, S.; Hamouda-Tekaya, N.; Tanasa, B.; Zaragosi, L.-E.; Grandjean, V.; Repetto, E.; Trabucchi, M. From benchmarking HITS-CLIP peak detection programs to a new method for identification of miRNA-binding sites from Ago2-CLIP data. *Nucleic Acids Res.* **2017**, *45*, e71. [CrossRef]
- 113. Ahadi, A.; Sablok, G.; Hutvagner, G. miRTar2GO: A novel rule-based model learning method for cell line specific microRNA target prediction that integrates Ago2 CLIP-Seq and validated microRNA-target interaction data. *Nucleic Acids Res.* **2016**, *45*, e42. [CrossRef]

- L'Yi, S.; Jung, D.; Oh, M.; Kim, B.; Freishtat, R.J.; Giri, M.; Hoffman, E.; Seo, J. miRTarVis+: Web-based interactive visual analytics tool for microRNA target predictions. *Methods* 2017, 124, 78–88. [CrossRef]
- 115. Van Peer, G.; De Paepe, A.; Stock, M.; Anckaert, J.; Volders, P.-J.; Vandesompele, J.; De Baets, B.; Waegeman, W. miSTAR: miRNA target prediction through modeling quantitative and qualitative miRNA binding site information in a stacked model structure. *Nucleic Acids Res.* 2016, 45, e51. [CrossRef] [PubMed]
- 116. Lu, Y.; Leslie, C.S. Learning to Predict miRNA-mRNA Interactions from AGO CLIP Sequencing and CLASH Data. *PLoS Comput. Biol.* **2016**, *12*, e1005026. [CrossRef] [PubMed]
- 117. Cheng, S.; Guo, M.; Wang, C.; Liu, X.; Liu, Y.; Wu, X. MiRTDL: A Deep Learning Approach for miRNA Target Prediction. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 2015, 13, 1161–1169. [CrossRef] [PubMed]
- 118. Ovando-Vázquez, C.; Lepe-Soltero, D.; Abreu-Goodger, C. Improving microRNA target prediction with gene expression profiles. BMC Genom. 2016, 17, 364. [CrossRef]
- Lee, B.; Baek, J.; Park, S.; Yoon, S. Deeptarget: End-to-end Learning Framework for microRNA Target Prediction using Deep Recurrent Neural Networks. In Proceedings of the 7th ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics, Northbrook, IL, USA, 7–10 August 2022; Available online: http://arxiv.org/abs/1603.09123 (accessed on 8 April 2022).
- 120. Ding, J.; Li, X.; Hu, H. TarPmiR: A new approach for microRNA target site prediction. *Bioinformatics* **2016**, *32*, 2768–2775. [CrossRef] [PubMed]
- 121. Ghoshal, A.; Shankar, R.; Bagchi, S.; Grama, A.; Chaterji, S. MicroRNA target prediction using thermodynamic and sequence curves. *BMC Genom.* 2015, *16*, 999. [CrossRef]
- 122. Wang, Z.; Xu, W.; Liu, Y. Integrating full spectrum of sequence features into predicting functional microRNA–mRNA interactions. *Bioinformatics* **2015**, *31*, 3529–3536. [CrossRef]
- Jung, D.; Kim, B.; Freishtat, R.J.; Giri, M.; Hoffman, E.; Seo, J. miRTarVis: An interactive visual analysis tool for microRNA-mRNA expression profile data. BMC Proc. 2015, 9, S2. [CrossRef]
- 124. Agarwal, V.; Bell, G.W.; Nam, J.-W.; Bartel, D.P. Predicting effective microRNA target sites in mammalian mRNAs. *eLife* 2015, 4, e05005. [CrossRef]
- 125. Bandyopadhyay, S.; Ghosh, D.; Mitra, R.; Zhao, Z. MBSTAR: Multiple instance learning for predicting specific functional binding sites in microRNA targets. *Sci. Rep.* 2015, *5*, 8004. [CrossRef] [PubMed]
- 126. Rennie, W.; Liu, C.; Carmack, C.S.; Wolenc, A.; Kanoria, S.; Lu, J.; Long, D.; Ding, Y. STarMir: A web server for prediction of microRNA binding sites. *Nucleic Acids Res.* 2014, 42, W114–W118. [CrossRef] [PubMed]
- 127. Menor, M.; Ching, T.; Zhu, X.; Garmire, D.; Garmire, L.X. mirMark: A site-level and UTR-level classifier for miRNA target prediction. *Genome Biol.* 2014, *15*, 500. [CrossRef] [PubMed]
- Li, Y.; Liang, C.; Wong, K.-C.; Jin, K.; Zhang, Z. Inferring probabilistic miRNA–mRNA interaction signatures in cancers: A role-switch approach. *Nucleic Acids Res.* 2014, 42, e76. [CrossRef]
- Li, Y.; Goldenberg, A.; Wong, K.-C.; Zhang, Z. A probabilistic approach to explore human miRNA targetome by integrating miRNA-overexpression data and sequence information. *Bioinformatics* 2013, 30, 621–628. [CrossRef]
- 130. Le, T.D.; Liu, L.; Tsykin, A.; Goodall, G.; Liu, B.; Sun, B.-Y.; Li, J. Inferring microRNA–mRNA causal regulatory relationships from expression data. *Bioinformatics* **2013**, *29*, 765–771. [CrossRef]
- 131. Majoros, W.H.; Lekprasert, P.; Mukherjee, N.; Skalsky, R.L.; Corcoran, D.L.; Cullen, B.R.; Ohler, U. MicroRNA target site identification by integrating sequence and binding information. *Nat. Chem. Biol.* **2013**, *10*, 630–633. [CrossRef]
- 132. Khorshid, M.; Hausser, J.; Zavolan, M.; van Nimwegen, E. A biophysical miRNA-mRNA interaction model infers canonical and noncanonical targets. *Nat. Methods* **2013**, *10*, 253–255. [CrossRef]
- Incarnato, D.; Neri, F.; Diamanti, D.; Oliviero, S. MREdictor: A two-step dynamic interaction model that accounts for mRNA accessibility and Pumilio binding accurately predicts microRNA targets. *Nucleic Acids Res.* 2013, 41, 8421–8433. [CrossRef]
- 134. Mendoza, M.R.; Da Fonseca, G.C.; Loss-Morais, G.; Alves, R.; Margis, R.; Bazzan, A.L.C. RFMirTarget: Predicting Human MicroRNA Target Genes with a Random Forest Classifier. *PLoS ONE* **2013**, *8*, e70153. [CrossRef]
- 135. Ahmadi, H.; Ahmadi, A.; Azimzadeh-Jamalkandi, S.; Shoorehdeli, M.A.; Salehzadeh-Yazdi, A.; Bidkhori, G.; Masoudi-Nejad, A. HomoTarget: A new algorithm for prediction of microRNA targets in Homo sapiens. *Genomics* 2013, 101, 94–100. [CrossRef] [PubMed]
- 136. Ben-Moshe, N.B.; Avraham, R.; Kedmi, M.; Zeisel, A.; Yitzhaky, A.; Yarden, Y.; Domany, E. Context-specific microRNA analysis: Identification of functional microRNAs and their mRNA targets. *Nucleic Acids Res.* **2012**, *40*, 10614–10627. [CrossRef] [PubMed]
- 137. Reczko, M.; Maragkakis, M.; Alexiou, P.; Grosse, I.; Hatzigeorgiou, A.G. Functional microRNA targets in protein coding sequences. *Bioinformatics* **2012**, *28*, 771–776. [CrossRef] [PubMed]
- 138. Yue, D.; Guo, M.; Chen, Y.; Huang, Y.; Yue, D.; Guo, M.; Chen, Y.; Huang, Y. A Bayesian decision fusion approach for microRNA target prediction. *BMC Genom.* 2012, 13, S13. [CrossRef]
- Vejnar, C.; Zdobnov, E.M. miRmap: Comprehensive prediction of microRNA target repression strength. *Nucleic Acids Res.* 2012, 40, 11673–11683. [CrossRef]
- 140. Reczko, M.; Maragkakis, M.; Alexiou, P.; Papadopoulos, G.L.; Hatzigeorgiou, A.G. Accurate microRNA Target Prediction Using Detailed Binding Site Accessibility and Machine Learning on Proteomics Data. *Front. Genet.* **2012**, *2*, 103. [CrossRef]

- 141. Stempor, P.A.; Cauchi, M.; Wilson, P. MMpred: Functional miRNA—mRNA interaction analyses by miRNA expression prediction. BMC Genom. 2012, 13, 620. [CrossRef]
- 142. Chandra, V.; Girijadevi, R.; Nair, A.S.; Pillai, S.S.; Pillai, R.M. MTar: A computational microRNA target prediction architecture for human transcriptome. *BMC Bioinform.* **2010**, *11*, S2. [CrossRef]
- 143. Oulas, A.; Karathanasis, N.; Louloupi, A.; Iliopoulos, I.; Kalantidis, K.; Poirazi, P. A new microRNA target prediction tool identifies a novel interaction of a putative miRNA with CCND2. *RNA Biol.* **2012**, *9*, 1196–1207. [CrossRef]
- Marín, R.M.; Vaníček, J. Efficient use of accessibility in microRNA target prediction. *Nucleic Acids Res.* 2010, 39, 19–29. [CrossRef]
 Reyes-Herrera, P.H.; Ficarra, E.; Acquaviva, A.; Macii, E. miREE: miRNA recognition elements ensemble. *BMC Bioinform.* 2011, 12, 454. [CrossRef] [PubMed]
- 146. Mitra, R.; Bandyopadhyay, S. MultiMiTar: A Novel Multi Objective Optimization based miRNA-Target Prediction Method. *PLoS* ONE **2011**, *6*, e24583. [CrossRef]
- 147. Oğul, H.; Umu, S.U.; Tuncel, Y.Y.; Akkaya, M.S. A probabilistic approach to microRNA-target binding. *Biochem. Biophys. Res. Commun.* 2011, 413, 111–115. [CrossRef] [PubMed]
- 148. Sturm, M.; Hackenberg, M.; Langenberger, D.; Frishman, D. TargetSpy: A supervised machine learning approach for microRNA target prediction. *BMC Bioinform.* 2010, *11*, 292. [CrossRef]
- Lagos-Quintana, M.; Rauhut, R.; Lendeckel, W.; Tuschl, T. Identification of Novel Genes Coding for Small Expressed RNAs. Science 2001, 294, 853–858. [CrossRef] [PubMed]
- 150. Lau, N.C.; Lim, L.P.; Weinstein, E.G.; Bartel, D.P. An Abundant Class of Tiny RNAs with Probable Regulatory Roles in *Caenorhabditis elegans. Science* **2001**, 294, 858–862. [CrossRef]
- 151. Lee, R.C.; Ambros, V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001, 294, 862–864. [CrossRef]
- 152. Alexiou, P.; Maragkakis, M.; Papadopoulos, G.L.; Reczko, M.; Hatzigeorgiou, A.G. Lost in translation: An assessment and perspective for computational microRNA target identification. *Bioinformatics* **2009**, *25*, 3049–3055. [CrossRef]
- Maragkakis, M.; Reczko, M.; Simossis, V.A.; Alexiou, P.; Papadopoulos, G.L.; Dalamagas, T.; Giannopoulos, G.; Goumas, G.I.; Koukis, E.; Kourtis, K.; et al. DIANA-microT web server: Elucidating microRNA functions through target prediction. *Nucleic Acids Res.* 2009, 37, W273–W276. [CrossRef]
- 154. Gaidatzis, D.; van Nimwegen, E.; Hausser, J.; Zavolan, M. Inference of miRNA targets using evolutionary conservation and pathway analysis. *BMC Bioinform.* **2007**, *8*, 69. [CrossRef]
- 155. Enright, A.J.; John, B.; Gaul, U.; Tuschl, T.; Sander, C.; Marks, D.S. MicroRNA targets in Drosophila. *Genome Biol.* 2003, *5*, R1. [CrossRef] [PubMed]
- 156. Griffiths-Jones, S.; Grocock, R.J.; Van Dongen, S.; Bateman, A.; Enright, A.J. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res.* 2006, 34, D140–D144. [CrossRef] [PubMed]
- Kozomara, A.; Griffiths-Jones, S. miRBase: Integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* 2010, 39, D152–D157. [CrossRef] [PubMed]
- 158. Krek, A.; Grün, D.; Poy, M.N.; Wolf, R.; Rosenberg, L.; Epstein, E.J.; MacMenamin, P.; Da Piedade, I.; Gunsalus, K.C.; Stoffel, M.; et al. Combinatorial microRNA target predictions. *Nat. Genet.* **2005**, *37*, 495–500. [CrossRef]
- 159. Kertesz, M.; Iovino, N.; Unnerstall, U.; Gaul, U.; Segal, E. The role of site accessibility in microRNA target recognition. *Nat. Genet.* **2007**, *39*, 1278–1284. [CrossRef]
- Miranda, K.C.; Huynh, T.; Tay, Y.; Ang, Y.-S.; Tam, W.-L.; Thomson, A.M.; Lim, B.; Rigoutsos, I. A Pattern-Based Method for the Identification of MicroRNA Binding Sites and Their Corresponding Heteroduplexes. *Cell* 2006, 126, 1203–1217. [CrossRef]
- 161. Friedman, R.C.; Farh, K.K.-H.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **2009**, *19*, 92–105. [CrossRef]
- 162. Libbrecht, M.W.; Noble, W.S. Machine learning applications in genetics and genomics. *Nat. Rev. Genet.* 2015, *16*, 321–332. [CrossRef]
- 163. Zhang, Z.; Zhao, Y.; Liao, X.; Shi, W.; Li, K.; Zou, Q.; Peng, S. Deep learning in omics: A survey and guideline. *Briefings Funct. Genom.* **2018**, *18*, 41–57. [CrossRef]
- 164. Koumakis, L. Deep learning models in genomics; are we there yet? Comput. Struct. Biotechnol. J. 2020, 18, 1466–1473. [CrossRef]
- 165. Quillet, A.; Anouar, Y.; Lecroq, T.; Dubessy, C. Prediction methods for microRNA targets in bilaterian animals: Toward a better understanding by biologists. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 5811–5825. [CrossRef] [PubMed]
- 166. Greener, J.G.; Kandathil, S.M.; Moffat, L.; Jones, D.T. A guide to machine learning for biologists. *Nat. Rev. Mol. Cell Biol.* 2021, 23, 40–55. [CrossRef] [PubMed]
- 167. Bandyopadhyay, S.; Saha, S.; Maulik, U.; Deb, K. A Simulated Annealing-Based Multiobjective Optimization Algorithm: AMOSA. *IEEE Trans. Evol. Comput.* **2008**, *12*, 269–283. [CrossRef]
- 168. Dick, K.; Green, J.R. Reciprocal Perspective for Improved Protein-Protein Interaction Prediction. Sci. Rep. 2018, 8, 1–12. [CrossRef]
- 169. Tokar, T.; Pastrello, C.; Rossos, A.E.M.; Abovsky, M.; Hauschild, A.-C.; Tsay, M.; Lu, R.; Jurisica, I. mirDIP 4.1—Integrative database of human microRNA target predictions. *Nucleic Acids Res.* **2017**, *46*, D360–D370. [CrossRef]
- 170. Busch, A.; Richter, A.S.; Backofen, R. IntaRNA: Efficient prediction of bacterial sRNA targets incorporating target site accessibility and seed regions. *Bioinformatics* **2008**, *24*, 2849–2856. [CrossRef]
- 171. Lecun, Y.; Bottou, L.; Bengio, Y.; Haffner, P. Gradient-based learning applied to document recognition. *Proc. IEEE* **1998**, *86*, 2278–2324. [CrossRef]

- 172. He, K.; Zhang, X.; Ren, S.; Sun, J. Deep residual learning for image recognition. In Proceedings of the 2016 IEEE Conference on Computer Vision and Pattern Recognition (CVPR), Las Vegas, NV, USA, 27–30 June 2016; pp. 770–778. [CrossRef]
- 173. Hochreiter, S.; Schmidhuber, J. Long short-term memory. Neural Comput. 1997, 9, 1735–1780. [CrossRef]
- 174. Zou, J.; Huss, M.; Abid, A.; Mohammadi, P.; Torkamani, A.; Telenti, A. A primer on deep learning in genomics. *Nat. Genet.* 2018, 51, 12–18. [CrossRef]
- 175. Rumelhart, D.E.; McClelland, J.L. Learning Internal Representations by Error Propagation. In *Parallel Distributed Processing: Explorations in the Microstructure of Cognition: Foundations*; MIT Press: Cambridge, MA, USA, 1987; pp. 318–362. Available online: https://ieeexplore.ieee.org/document/6302929 (accessed on 2 October 2022).
- 176. Kern, F.; Backes, C.; Hirsch, P.; Fehlmann, T.; Hart, M.; Meese, E.; Keller, A. What's the target: Understanding two decades of *in silico* microRNA-target prediction. *Briefings Bioinform.* **2019**, *21*, 1999–2010. [CrossRef]
- 177. Cao, Y.; Wu, J.; Liu, Q.; Zhao, Y.; Ying, X.; Cha, L.; Wang, L.; Li, W. sRNATarBase: A comprehensive database of bacterial sRNA targets verified by experiments. RNA 2010, 16, 2051–2057. [CrossRef] [PubMed]
- Wang, J.; Liu, T.; Zhao, B.; Lu, Q.; Wang, Z.; Cao, Y.; Li, W. sRNATarBase 3.0: An updated database for sRNA-target interactions in bacteria. *Nucleic Acids Res.* 2015, 44, D248–D253. [CrossRef] [PubMed]
- 179. Lorenz, R.; Bernhart, S.H.; Honer Zu Siederdissen, C.; Tafer, H.; Flamm, C.; Stadler, P.F.; Hofacker, I.L. ViennaRNA Package 2.0. *Algorithms Mol. Biol.* **2011**, *6*, 26. [CrossRef] [PubMed]
- Tafer, H.; Hofacker, I.L. RNAplex: A fast tool for RNA–RNA interaction search. *Bioinformatics* 2008, 24, 2657–2663. [CrossRef]
 [PubMed]
- 181. Huang, D.W.; Sherman, B.T.; Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **2009**, *4*, 44–57. [CrossRef] [PubMed]
- 182. Raden, M.; Ali, S.M.; Alkhnbashi, O.S.; Busch, A.; Costa, F.; Davis, J.A.; Eggenhofer, F.; Gelhausen, R.; Georg, J.; Heyne, S.; et al. Freiburg RNA tools: A central online resource for RNA-focused research and teaching. *Nucleic Acids Res.* 2018, 46, W25–W29. [CrossRef]
- Johnson, M.; Zaretskaya, I.; Raytselis, Y.; Merezhuk, Y.; McGinnis, S.; Madden, T.L. NCBI BLAST: A better web interface. *Nucleic Acids Res.* 2008, 36, W5–W9. [CrossRef]
- 184. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [CrossRef]
- 185. Bernhart, S.H.; Hofacker, I.L.; Stadler, P.F. Local RNA base pairing probabilities in large sequences. *Bioinformatics* 2005, 22, 614–615. [CrossRef]
- 186. Axtell, M.J. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 2013, 64, 137–159. [CrossRef]
- 187. Bobrovskyy, M.; Vanderpool, C.K. The small RNA SgrS: Roles in metabolism and pathogenesis of enteric bacteria. *Front. Cell Infect. Microbiol.* **2014**, *4*, 61. [CrossRef]
- 188. Salvail, H.; Massé, E. Regulating iron storage and metabolism with RNA: An overview of posttranscriptional controls of intracellular iron homeostasis. *Wiley Interdiscip. Rev. RNA* 2011, *3*, 26–36. [CrossRef] [PubMed]
- Massé, E.; Vanderpool, C.K.; Gottesman, S. Effect of RyhB Small RNA on Global Iron Use in Escherichia Coli. J Bacteriol 2005, 187, 6962–6971. [CrossRef] [PubMed]
- Wang, Z.; Gerstein, M.; Snyder, M. RNA-Seq: A revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 2009, 10, 57–63. [CrossRef]
 [PubMed]
- Lalaouna, D.; Carrier, M.-C.; Semsey, S.; Brouard, J.-S.; Wang, J.; Wade, J.T.; Massé, E. A 3' External Transcribed Spacer in a tRNA Transcript Acts as a Sponge for Small RNAs to Prevent Transcriptional Noise. *Mol. Cell* 2015, *58*, 393–405. [CrossRef]
- 192. Han, K.; Tjaden, B.; Lory, S. GRIL-seq provides a method for identifying direct targets of bacterial small regulatory RNA by in vivo proximity ligation. *Nat. Microbiol.* **2016**, *2*, 16239. [CrossRef] [PubMed]
- 193. Melamed, S.; Peer, A.; Faigenbaum-Romm, R.; Gatt, Y.E.; Reiss, N.; Bar, A.; Altuvia, Y.; Argaman, L.; Margalit, H. Global Mapping of Small RNA-Target Interactions in Bacteria. *Mol. Cell* **2016**, *63*, 884–897. [CrossRef]
- 194. Waters, S.A.; McAteer, S.P.; Kudla, G.; Pang, I.; Deshpande, N.P.; Amos, T.G.; Leong, K.W.; Wilkins, M.R.; Strugnell, R.; Gally, D.L.; et al. SmallRNAinteractome of pathogenic *E. coli* revealed through crosslinking of RNase E. *EMBO J.* 2016, 36, 374–387. [CrossRef]
- 195. Grosswendt, S.; Filipchyk, A.; Manzano, M.; Klironomos, F.; Schilling, M.; Herzog, M.; Gottwein, E.; Rajewsky, N. Unambiguous Identification of miRNA:Target Site Interactions by Different Types of Ligation Reactions. *Mol. Cell* **2014**, *54*, 1042–1054. [CrossRef]
- Chou, C.-H.; Chang, N.-W.; Shrestha, S.; Hsu, S.-D.; Lin, Y.-L.; Lee, W.-H.; Yang, C.-D.; Hong, H.-C.; Wei, T.-Y.; Tu, S.-J.; et al. miRTarBase 2016: Updates to the experimentally validated miRNA-target interactions database. *Nucleic Acids Res.* 2015, 44, D239–D247. [CrossRef]
- 197. Huang, H.-Y.; Lin, Y.-C.; Cui, S.; Huang, Y.; Tang, Y.; Xu, J.; Bao, J.; Li, Y.; Wen, J.; Zuo, H.; et al. miRTarBase update 2022: An informative resource for experimentally validated miRNA-target interactions. *Nucleic Acids Res.* 2021, 50, D222–D230. [CrossRef] [PubMed]
- 198. Karagkouni, D.; Paraskevopoulou, M.D.; Chatzopoulos, S.; Vlachos, I.S.; Tastsoglou, S.; Kanellos, I.; Papadimitriou, D.; Kavakiotis, I.; Maniou, S.; Skoufos, G.; et al. DIANA-TarBase v8: A decade-long collection of experimentally supported miRNA–gene interactions. *Nucleic Acids Res.* 2017, 46, D239–D245. [CrossRef] [PubMed]

- Vincent, P.; LaRochelle, H.; Bengio, Y.; Manzagol, P.-A. Extracting and composing robust features with denoising autoencoders. In Proceedings of the 25th International Conference on Machine Learning, Montreal, QC, Canada, 11–15 April 2016; pp. 1096–1103.
- 200. Thiam, P.; Kestler, H.; Schwenker, F. Multimodal Deep Denoising Convolutional Autoencoders for Pain Intensity Classification based on Physiological Signals. In Proceedings of the 9th International Conference on Pattern Recognition Applications and Methods, Prague, Czech Republic, 19–21 February 2020; pp. 289–296. Available online: https://www.scitepress.org/Link.aspx? doi=10.5220/0008896102890296 (accessed on 8 August 2022). [CrossRef]
- 201. John, B.; Enright, A.; Aravin, A.A.; Tuschl, T.; Sander, C.; Marks, D.S. Human MicroRNA Targets. *PLoS Biol.* 2004, 2, e363. [CrossRef] [PubMed]
- 202. Maute, R.L.; Schneider, C.; Sumazin, P.; Holmes, A.; Califano, A.; Basso, K.; Dalla-Favera, R. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. *Proc. Natl. Acad. Sci. USA* 2013, 110, 1404–1409. [CrossRef] [PubMed]
- Zhang, M.; Li, F.; Wang, J.; He, W.; Li, Y.; Li, H.; Wei, Z.; Cao, Y. tRNA-derived fragment tRF-03357 promotes cell proliferation, migration and invasion in high-grade serous ovarian cancer. *OncoTargets Ther.* 2019, 12, 6371–6383. [CrossRef]
- 204. Moore, M.J.; Scheel, T.K.H.; Luna, J.M.; Park, C.Y.; Fak, J.J.; Nishiuchi, E.; Rice, C.M.; Darnell, R.B. miRNA-target chimeras reveal miRNA 3'-end pairing as a major determinant of Argonaute target specificity. *Nat. Commun.* 2015, 6, 8864. [CrossRef]
- 205. Haeussler, M.; Zweig, A.S.; Tyner, C.; Speir, M.L.; Rosenbloom, K.R.; Raney, B.J.; Lee, C.M.; Lee, B.T.; Hinrichs, A.; Gonzalez, J.N.; et al. The UCSC Genome Browser database: 2019 update. *Nucleic Acids Res.* **2018**, 47, D853–D858. [CrossRef]
- 206. Pliatsika, V.; Loher, P.; Magee, R.; Telonis, A.; Londin, E.; Shigematsu, M.; Kirino, Y.; Rigoutsos, I. MINTbase v2.0: A comprehensive database for tRNA-derived fragments that includes nuclear and mitochondrial fragments from all The Cancer Genome Atlas projects. *Nucleic Acids Res.* 2017, 46, D152–D159. [CrossRef]
- Pruitt, K.D.; Tatusova, T.; Brown, G.R.; Maglott, D.R. NCBI Reference Sequences (RefSeq): Current status, new features and genome annotation policy. *Nucleic Acids Res.* 2011, 40, D130–D135. [CrossRef]
- 208. Schultz, N.; Marenstein, D.R.; De Angelis, D.A.; Wang, W.-Q.; Nelander, S.; Jacobsen, A.; Marks, D.S.; Massagué, J.; Sander, C. Off-target effects dominate a large-scale RNAi screen for modulators of the TGF-β pathway and reveal microRNA regulation of TGFBR. *Silence* 2011, 2, 3–20. [CrossRef]
- Wenzel, A.; Akbaşli, E.; Gorodkin, J. RIsearch: Fast RNA–RNA interaction search using a simplified nearest-neighbor energy model. *Bioinformatics* 2012, 28, 2738–2746. [CrossRef] [PubMed]
- 210. Betel, D.; Koppal, A.; Agius, P.; Sander, C.; Leslie, C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol.* 2010, 11, R90. [CrossRef] [PubMed]
- 211. Paraskevopoulou, M.D.; Georgakilas, G.; Kostoulas, N.; Vlachos, I.S.; Vergoulis, T.; Reczko, M.; Filippidis, C.; Dalamagas, T.; Hatzigeorgiou, A.G. DIANA-microT web server v5.0: Service integration into miRNA functional analysis workflows. *Nucleic Acids Res.* 2013, 41, W169–W173. [CrossRef]
- Wong, N.; Wang, X. miRDB: An online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res.* 2014, 43, D146–D152. [CrossRef] [PubMed]
- Šulc, M.; Marín, R.M.; Robins, H.S.; Vaníček, J. PACCMIT/PACCMIT-CDS: Identifying microRNA targets in 3' UTRs and coding sequences. Nucleic Acids Res. 2015, 43, W474–W479. [CrossRef]
- Davis, J.A.; Saunders, S.; Mann, M.; Backofen, R. Combinatorial ensemble miRNA target prediction of co-regulation networks with non-prediction data. *Nucleic Acids Res.* 2017, 45, 8745–8757. [CrossRef] [PubMed]
- Lu, C.; Yang, M.; Li, M.; Li, Y.; Wu, F.-X.; Wang, J. Predicting Human lncRNA-Disease Associations Based on Geometric Matrix Completion. *IEEE J. Biomed. Health Inform.* 2019, 24, 2420–2429. [CrossRef]
- Mørk, S.; Pletscher-Frankild, S.; Caro, A.P.; Gorodkin, J.; Jensen, L.J. Protein-driven inference of miRNA-disease associations. *Bioinformatics* 2013, 30, 392–397. [CrossRef]