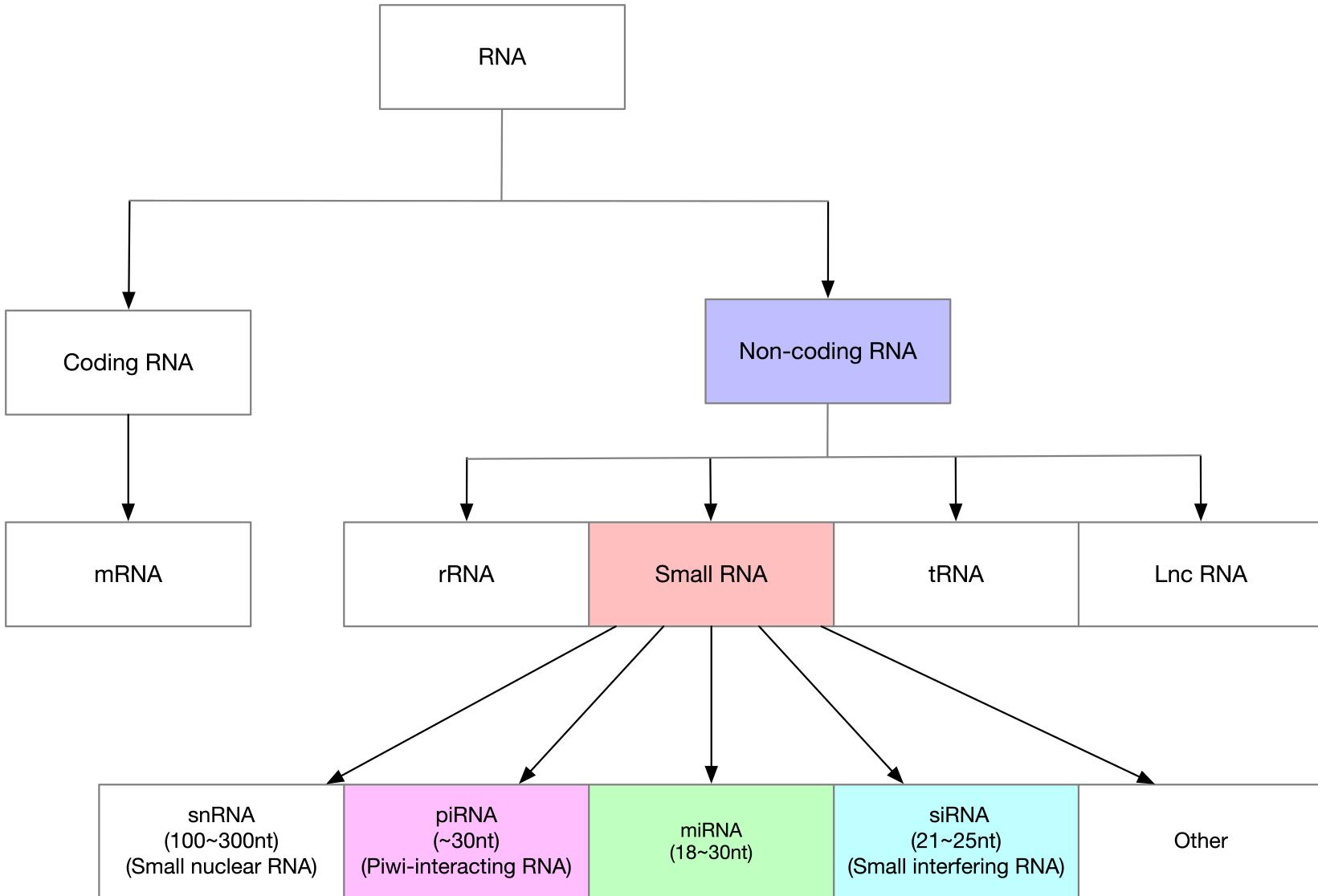


Small RNA Sequencing

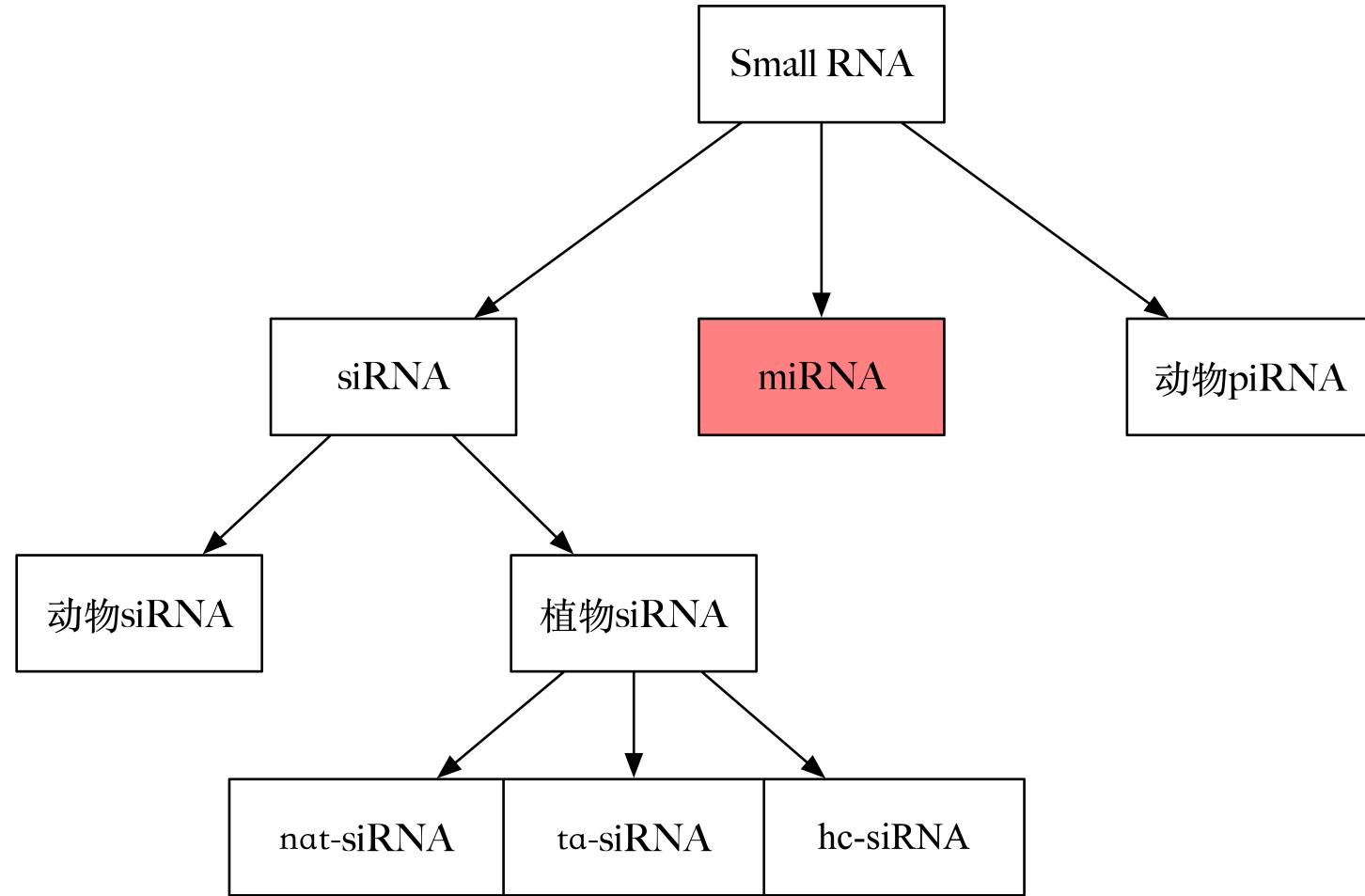


RNA分类

Small RNA 18–200 nt



Small RNA文库



miRNA概念

- 1993年microRNA (miRNA) 第一次发现在 *Caenorhabditis elegans* (秀丽隐杆线虫) 中。
- miRNA是一类内生的、进化上高度保守的、长度约21~24个核苷酸的非编码单链RNA，具有转录后调控基因表达的功能。

Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complemen
75(5):843–854

miRNA的异构体

- 一个miRNA 基因不仅仅可以形成一种成熟的miRNA，还可以形成多种在长度或序列上存在差异miRNA异构体 (isoforms of microRNA, isomiR)。
- isomiR也可以装配成RNA诱导的沉默复合物 (RNA induced silencing complex,RISC)，并能够与多核糖体结合，发挥调节作用。
- isomiR的表达非常普遍地存在，如绵羊的每一种 miRNA 几乎都具有isomiR，数量从1个到数百个不等

谢兆辉, 李学贵, 许提森. miRNA的异构体——isomiR[J]. 中国生物化学与分子生物学报, 2014.

miRNA形成

1. pri-miRNA (primary miRNA)

2. pre-miRNA(precursor microRNA)

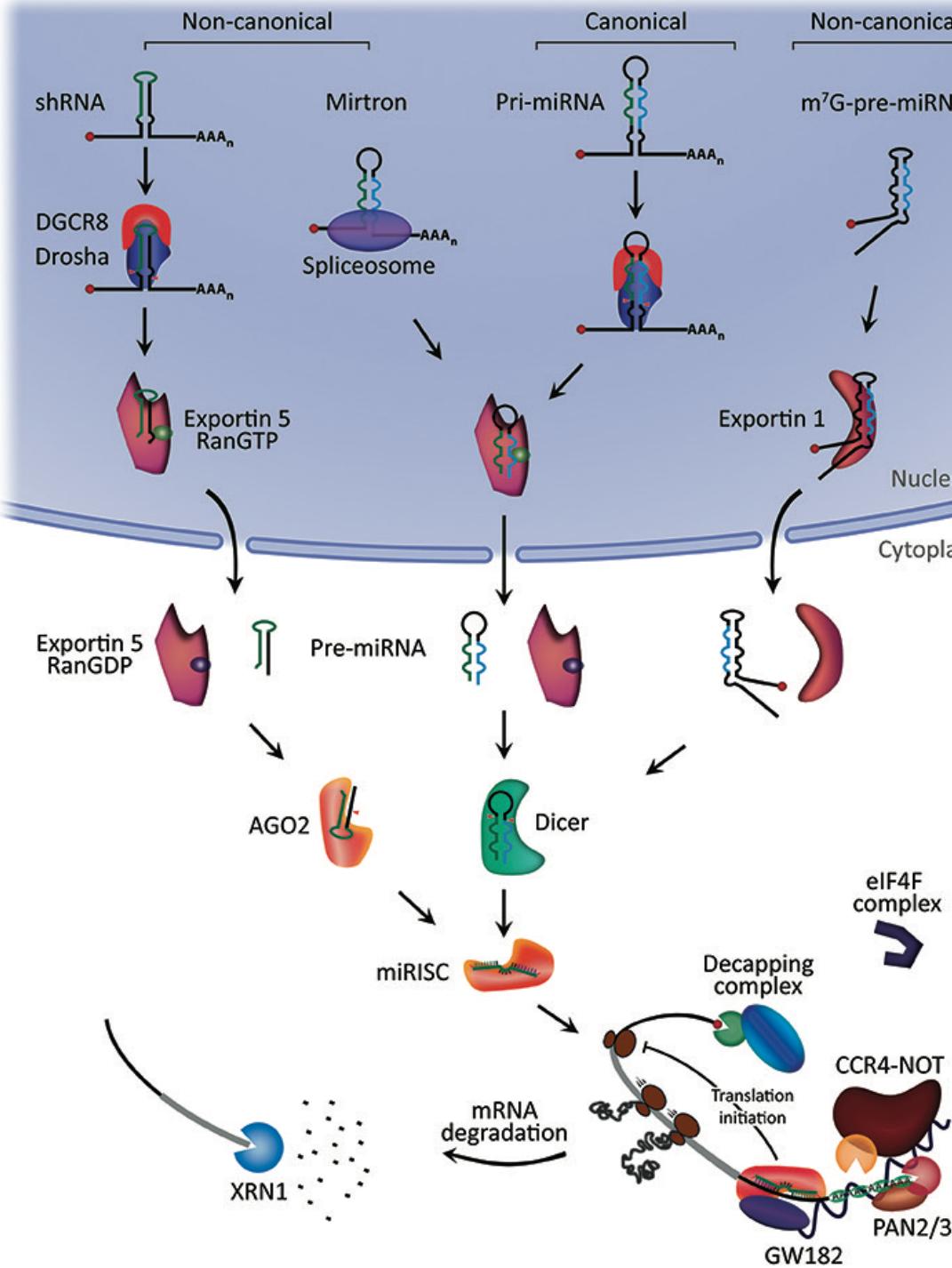
- **enzyme Drosha** cuts the pri-miRNA to a smaller 70 nucleotide stem-loop precursor

3. miRNA

- This pre-miRNA is then cleaved by **Dicer** to a ~23 nucleotide miRNA duplex.

O'Brien J, Hayder H, Zayed Y, et al. Overview of microRNA biogenesis, mechanisms of actions, and circulation[J]. Frontiers in endocrinology, 2018, 9: 402.

Saliminejad K , Khorshid H , Fard S S , et al. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods[J]. Journal of Cellular Physiology, 2019, 234(5).



miRNA特点

结构	<ul style="list-style-type: none">● 单链小分子RNA● 前体为茎环结构● 结构具有保守性
表达	<ul style="list-style-type: none">● 时序性● 组织特异性
功能	<ul style="list-style-type: none">● 细胞生长、增殖、分化、凋亡● 个体发育、繁殖● 重大疾病
调控方式	<ul style="list-style-type: none">● 转录后调控靶基因表达● 与靶向mRNA不完全互补或完全互补都能作用● 一条miRNA调控多条mRNA● 多条miRNA同时调控一条mRNA

动植物miRNA异同

	Plants	Animals
Location within genome	Predominantly intergenic regions	Intergenic regions introns
Presence of miRNA clusters	Uncommon	Common
Number of miRNA-binding sites within target genes	Generally one	Generally multiple
Location of miRNA-binding motifs within target genes	Predominantly the open-reading frame	Predominantly the 3'-UTR
Degree of miRNA-mRNA complementarity	Perfect or near perfect	Seed region match, flexible base-pairing in flanking sequences
Length of MIRNA stem-loops	Relatively long (ranging from 100 to 900 nt, highly variable)	Relatively short (predominantly less than 100 nt, highly uniform)
Stability mediated by 3' modification	2'-O-methylated 3' end, relatively stable	Free 2'/3' hydroxyl, relatively unstable

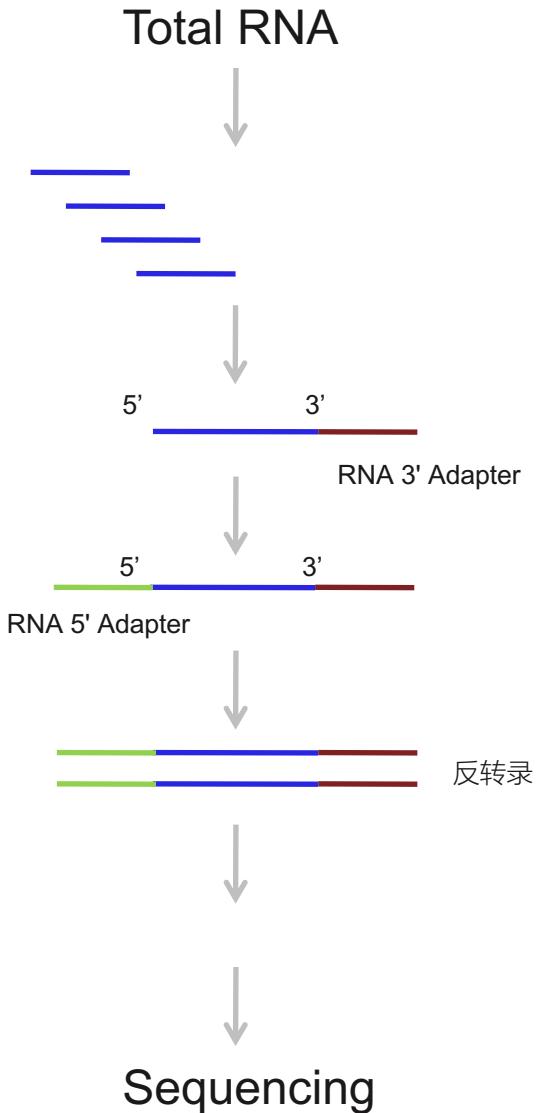
1. Millar A A, Waterhouse P M. Plant and animal microRNAs: similarities and differences[J]. *Functional & integrative genomics*, 2005, 5(3): 129-135.
2. Zhang Y, Yun Z, Gong L, et al. Comparison of miRNA evolution and function in plants and animals[J]. *Microrna*, 2018, 7(1): 4-10.

miRNA 命名规则

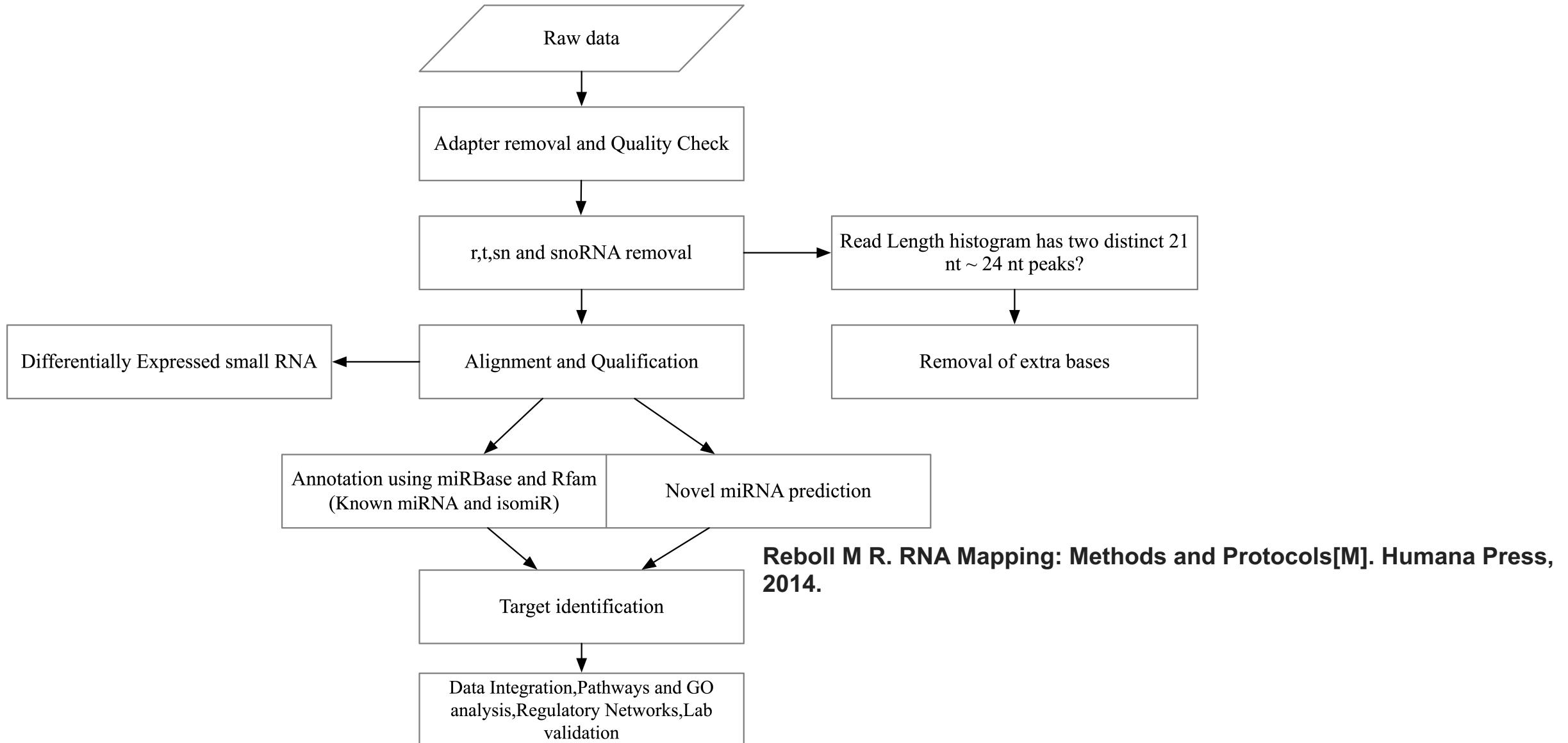
- The name of a miRNA starts with the organism name, e.g., “hsa” for Human, “mmu” for mouse.
 - **hsa-miR-34a**→Human mature miRNA
 - **hsa-mir-34a**→Small “r” indicated pre-miRNA
 - **hsa-miR-34b**→**a/b/c** at the end indicates closely **related family** of miRNA with 1 or 2 bases different.
 - **hsa-miR-34a-3p**→“3p” at end indicates miRNA originating from 3' end
 - **hsa-miR-34a-5p**→“5p” at end indicates miRNA originating from 5' end
 - **hsa-miR-34a***→“*” indicates **miRNA expression** from the opposite arm of pre-miRNA.

Small RNA-Seq测序技术

- SE50测序 : 单端single-end 50bp
- 测序数据量: 10M reads/样本 (0.5G)



Small RNA-Seq数据分析流程



生信分析工具(一)

Name	Website	Summary
<i>Quality Control Checks and Adapter Removal</i>		
FASTQC	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/	Quality check on raw sequences in FASTQ format
cutadapt	http://cutadapt.readthedocs.io/en/stable/	remove the adapters from the reads
FASTX-Toolkit	http://hannonlab.cshl.edu/fastx_toolkit/	Reads trimming, quality filtering, adapter removal, FASTQC to FASTA conversion etc.
<i>Alignment and Quantification</i>		
Bowtie	http://bowtie-bio.sourceforge.net	Ultra-fast reads alignment
<i>Differential Expression</i>		
edgeR/DEseq2/NOISEq	http://www.r-project.org/	identify differentially expressed miRNA among two or more groups of samples

生信分析工具（二）

Name	Website	Summary
Database		
MiRBase	http://www.mirbase.org/	Database of miRNA sequences
Rfam	http://rfam.sanger.ac.uk/	Database of RNA families
MiRTarBase	http://mirtarbase.cuhk.edu.cn/php/index.php	The experimentally validated microRNA-target interactions database
Target prediction software		
TargetScan	http://www.targetscan.org/	Database of miRNA targets
miRanda	http://www.microrna.org/microrna/home.do	Predicts miRNA targets
Identify Novel small RNA		
miRDeep2	https://www.mdc-berlin.de/content/mirdeep2-documentation	Calculates known and novel miRNA. Graphical display of pre-miRNA structure.

Data Analysis & Storage

BaseSpace Small RNA App

Aligns reads against four reference databases, and outputs hits to mature miRNAs, isomiRs, and piRNAs.

miRNAs Analysis App

Allows detection of differentially expressed miRNAs between 2 conditions. Performs adapter trimming, read counting, and more.

The Broad's IGV

A genome browser developed by the Broad Institute of MIT and Harvard that displays NGS data for complex variant analysis.

Genomatix Pathway System (GePS)

Associates single gene or list of genes with annotation data for pathways, diseases, tissues, and small molecules.

iPathway Guide

Differential gene expression, drug interaction, and disease analysis.

BaseSpace Sequence Hub

The Illumina genomics computing environment for NGS data analysis and management.

BaseSpace Correlation Engine

A growing library of curated genomic data to support researchers in identifying disease mechanisms, drug targets, and biomarkers.

<https://www.illumina.com/techniques/sequencing/rna-sequencing/small-rna-seq.html>

Appendix

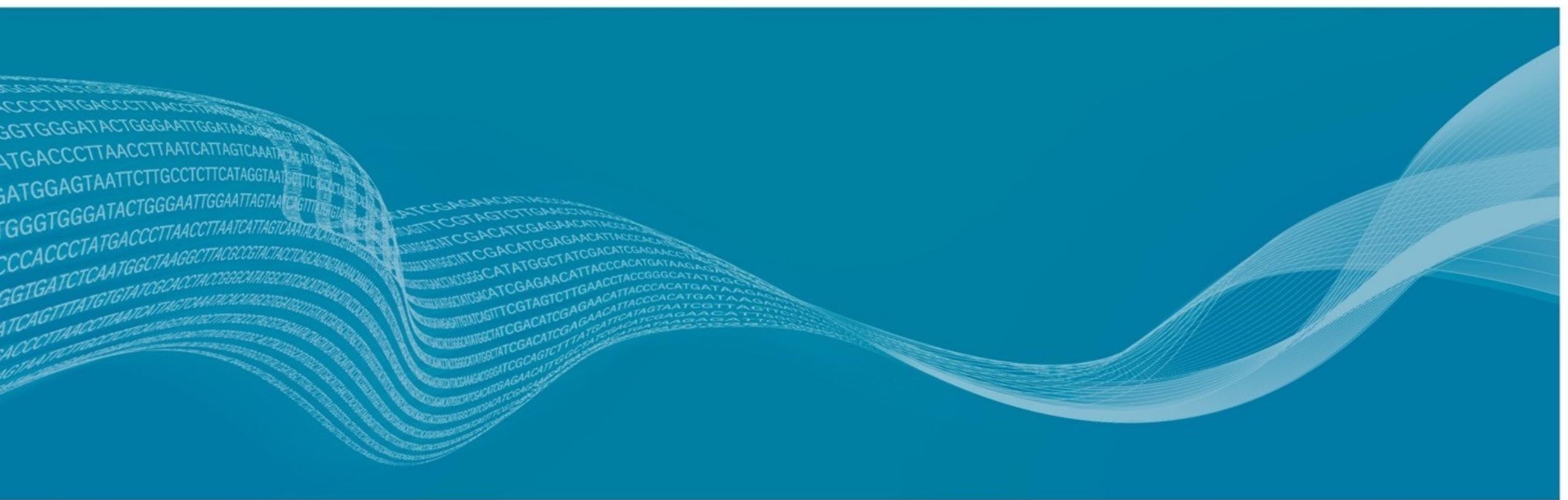


Table 1. Selected miRNA identification tools

Tool name	Organism	Algorithm category	Publication span	Last update	Current version	Platform	Link	References
MiRscan	A	EC	2003	2003	–	WB	http://genes.mit.edu/mirscan/	[53]
RNAz	A	SB, TS	2005–10	2011	v2.1	WB, SA	https://www.tbi.univie.ac.at/software/RNAz/	[82]
triplet-SVM	A, P	ML	2005	2005	–	SA	http://bioinfo.au.tsinghua.edu.cn/mirnasvm/	[83]
MiPred	A, P	ML	2007	2016	v0.1	WB	http://server.malab.cn/MiPred/	[84]
miRDeep	A	NB, SB, ML	2008–12	2016	v2.0.0.8	SA	https://www.mdc-berlin.de/8551903/en/	[55]
CID-miRNA	A	ML	2008	2008	–	WB	https://github.com/alito/CID-miRNA	[85]
UEA sRNA workbench	A, P	NB	2008–17	2018	v4.5	SA	http://srna-workbench.cmp.uea.ac.uk/mircat2/	[86]
miRanalyzer	A, P	IA, NB	2009–10	2012	v0.3	WB, SA	http://bioinfo2.ugr.es/miRanalyzer/standalone.html	[56]
MicroPC	P	EC	2009	2009	–	WB	http://www3a.biotech.or.th/micropc/	[87]
HHMMiR	A, P	ML	2009	2009	v1.2	SA	http://biodev.hgen.pitt.edu/kadriAPBC2009.html	[88]
MatureBayes	A	ML	2010	2010	–	WB, SA	http://mirna.imbb.forth.gr/MatureBayes.html	[89]
miRDeep-P	P	NB, SB, ML	2011	2011	v1.3	SA	https://sourceforge.net/projects/mirdp	[90]
miRNATfold	A, P	SB, TS	2012–16	2016	–	WB, SA	https://evryrna.ibisc.univ-evry.fr/evryrna/mirnafold/mirnafold_home	[54]
miRDeep*	A, P	NB, IA, SB	2013	2016	v37	SA	http://www.australianprostatecentre.org/search/software/mirdeep-star	[91]
miReader	A, P	NB, ML	2013	2016	–	SA	http://scbb.ihbt.res.in/2810-12/miReader.php	[92]
miRplex	A, P	NB, ML	2013	2013	v0.1	SA	https://www.uea.ac.uk/computing/mirplex	[93]
miRdentity	A	NB, TS	2014	2014	v1.0	SA	http://www.ncrnalab.dk/#mirdentity/mirdenify.php	[94]
miRPlant	P	NB, IA	2014	2017	v5.1	SA	https://sourceforge.net/projects/mirplant/	[95]
deepSOM	A, P	ML	2016	2016	v0.19	WB, SA	http://fich.unl.edu.ar/sinc/blog/web-demo/deepsom/	[96]
Mirnovo	A, P	NB, ML	2017	2018	v1.0	WB, SA	http://wwwdev.ebi.ac.uk/enright-dev/mirnovo/	[97]

Note: Algorithm category: Structure-based (SB), evolutionary conservation (EC), machine learning (ML), thermodynamic stability (TS), integrated approach (IA), NGS-based (NB); Organism: Animal (A), plant (P); Platform: Stand-alone (SA), Web-based (WB).

Liang C , Liisa H , Wang C , et al.
Trends in the development of miRNA bioinformatics tools[J]. *Briefings in Bioinformatics*, 2018(5):5.

Table 2. Selected miRNA target prediction tools

Tool name	Organism	Algorithm category	Publication span	Last update	Current version	Platform	Link	References
miRanda	A	SM, CH, ML, CM	2003–10	2010	v3.3a	WB, SA	http://34.236.212.39/microrna/home.do	[62]
RNAhybrid	A	SM, CH	2004–06	2006	v2.1.2	WB, SA	https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid	[65]
TargetScan	A	SM, EC, CM	2005–15	2018	v7.2	WB, SA	http://www.targetscan.org	[63]
PicTar	A	CM, EC	2005–06	2007	–	WB	http://pictar.mdc-berlin.de/	[64]
TargetFinder	P	CM	2005–10	2015	v1.7	SA	https://github.com/carringtonlab/TargetFinder	[104]
TarBase	A, P	MC, IA	2006–17	2017	v8	WB	http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8	[105]
RNA22	A	CM, CH	2006–12	2015	v2.0	WB	https://cm.jefferson.edu/rna22/	[106]
GenMiR++	A, P	EX, ML	2007	2007	–	SA	http://www.psi.toronto.edu/genmir/	[107]
PolymiRTS	A	IA, SM, PE	2007–14	2014	v3.0	WB	http://compbio.uthsc.edu/miRSNP/	[108]
miRDB	A	ML	2008–16	2016	v5.0	WB	http://www.mirdb.org	[109]
miRGator	A	IA, EX	2008–13	2013	v3.0	WB	http://mrgator.kobic.re.kr/	[110]
miRecords	A	MC	2009	2013	v4	WB	http://c1.accurascience.com/miRecords/	[67]
mirSOM	A	ML, SM, CM	2011	2011	–	WB	https://bioinformatics.uef.fi/mirsom/	[111]
miRWalk	A	IA, TM	2011–15	2018	v3.0	WB	http://mirwalk.umm.uni-heidelberg.de/	[112]
mirDIP	A	IA	2011–17	2018	v4.1	WB	http://ophid.utoronto.ca/mirDIP/	[113]
miRTarBase	A, P	MC	2011–18	2017	v7.0	WB	http://mirtarbase.mbc.nctu.edu.tw	[114]
psRNATarget	P	SM, CM	2011–18	2018	v2	WB	http://plantgrn.noble.org/psRNATarget/	[115]
miRTarCLIP	A	IB	2013	2013	v1.0.1	WB, SA	http://mirtarclip.mbc.nctu.edu.tw/	[116]
MiRTDL	A	ML	2016	2016	–	WB, SA	http://nclab.hit.edu.cn/CCRM/	[117]
miRBShunter	A	IB	2017	2017	v0.2	SA	https://github.com/TrabucchiLab/miRBShunter	[59]
miRTar2GO	A	IB, CH, SM	2017	2017	–	WB	http://www.mirtar2go.org	[61]

Note: Algorithm category: Seed matching (SM), complement matching (CM), compensatory hybridization (CH), evolutionary conservation (EC), machine learning (ML), Immunoprecipitation-Methods based (IB), expression correlation (EX), text mining (TM), manually curated (MC), integrated approach (IA), polymorphism effects (PE); Organism: Animal (A), plant (P); Platform: Stand-alone (SA), Web-based (WB).

Liang C , Liisa H , Wang C , et al.
Trends in the development of miRNA
bioinformatics tools[J]. Briefings in
Bioinformatics, 2018(5):5.