Supplementary information

Identification of RNA structures and their roles in RNA functions

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	Chemical probing								
Sequencing Platform	Structure read-out	Method	Key References	Probe	Target RNA structure scope	Biological sample type			
Short read			1	1M7	Single target	In vitro treatment			
		SHAPE-seq	In cell SHAPE-		Single target				
	RT-stalling		seq ²	1M7	(overexpressed/endogenous)	E. coli			
		DMS-seq	3	DMS	polyA ⁺ transcriptome	Yeast, cultured human cells (K562, human foreskin fibroblasts)			
			4	DMS	polyA ⁺ transcriptome	Arabidopsis seedlings			
		Structure-seq	Targeted Structure-seq ⁵	DMS	Single target	Cultured mouse cells (clonal female MEFs)			
			Structure-seq2 (Ref. ⁶)	DMS	polyA+ transcriptome	Rice shoots			
			CAP- STRUCTURE- seq ⁷	NAI	polyA ⁺ transcriptome with intact 5' cap	Arabidopsis seedlings			
		MOD-seq	8	DMS	rRNA	Yeast			
		icSHAPE	9	NAI-N3	polyA ⁺ transcriptome	Cultured mouse cells (mES)			
			10	NAI-N3	rRNA ⁻ transcriptome from cytoplasmic, nuclear and chromatin fractions	Cultured human (HEK293) and mouse cells (mES)			
		SPET-seq	11	DMS	Nascent transcriptome	E. coli			
		Keth-seq	12	N3-Kethoxal	rRNA; polyA+ transcriptome	Cultured human (HeLa) and mouse cells (mES)			
		LASER-seq	13	NAz	rRNA	Cultured human cells (K562)			
		icLASER	14	NAz-N3	polyA ⁺ transcriptome	Cultured human cells (HeLa, K562)			
		SHAPE-MaP	15	1M7	HIV genome	in vitro treatment			
	RT-MaP		16	1M7	Single target	Cultured mouse cells (TSC)			
			17	1M7	rRNA ⁻ transcriptome	E. coli			
		DMS-MaPseq	18	DMS	rRNA ⁻ transcriptome, single targets	Yeast, cultured human cells (HEK293T), Drosophila oocyte			
		DIM-2P-seq	19	DMS	3' end of polyA ⁺ transcriptome	Cultured human cells (HEK293T)			
		icSHAPE-MaP	20	NAI-N3	DICER-target small RNAs	Cultured human cells (HEK293T)			
		tRNA Structure-seq	21	DMS	tRNAs	E. coli			

Supplementary Table 1. Methods for studying RNA structures in vivo

		LASER-MaP	13	NAz	rRNA	Cultured human cells (K562)
	Lead score	Lead-seq	22	Lead(II) ions	rRNA ⁻ transcriptome	Yersinia pseudotuberculosis
Long-read	Direct	PORE-cupine	23	NAI-N3	polyA ⁺ transcriptome	Cultured human cells (H9)
	modification	nanoSHAPE	24	AcIm	pri-miRNA	In vitro
	detection	SMS-seq	25	DEPC	polyA ⁺ and polyA ⁻ transcriptome	Yeast (in vitro probed)
	RT-MaP	smStructure- seq	26	NAI	Single target	Arabidopsis thaliana seedlings
		Nano-DMS- MaP	27	DMS	HIV genome and transcriptome	Cultured human cells (HEK293T); HIV
				RNA-RNA in	teractions	
Structure rea	ad-out	Method	Key references	Crosslinker	Target RNA structure scope	Biological sample type
All mapped re	eads	RAP-RNA	28	AMT, formaldehyde, DSG	Direct RNA–RNA base-pairing and indirect RNA–RNA interactions of certain RNA of interest	Cultured mouse cells (V6.5 and pSM33 male mouse ES cells)
Chimera read	ds	PARIS	29	AMT	Transcriptome-wide direct RNA–RNA base-pairing	Cultured human (HeLa, HEK293T) and mouse (mES) cells
		SPLASH	30	Biotinylated psoralen	Transcriptome-wide direct RNA-RNA base-pairing	<i>E. coli</i> , yeast, cultured human cells (HeLa, GM12892, H1)
		LIGR-seq	31	AMT	Transcriptome-wide direct RNA-RNA base-pairing	Cultured human cells (HEK293T)
		COMRADES	32	Azide-modified psoralen	Transcriptome-wide direct RNA-RNA base-pairing	Cultured human cells (JEG-3) infected with Zika virus
		CLASH	33	UV	Spatial proximity mediated by single protein of interest	Yeast overexpressing target protein of interest
		hiCLIP	34	UV	Spatial proximity mediated by single protein of interest	Cultured human cells (Flp-In 293 T-REx) overexpressing target protein of interest
		CRIC-seq	35	Formaldehyde	Spatial proximity mediated by single protein of interest	Cultured human cells (HeLa)
		RIPPLIT	36	No crosslinking	Spatial proximity mediated by single protein of interest	Cultured human cells (HEK293) overexpressing target protein of interest
		RPL	37	No crosslinking	Transcriptome-wide spatial proximity mediated by stable base-pairing	Yeast and cultured human cells (GM12878)
		MARIO	38	Formaldehyde and ethyl glycol bis	Transcriptome-wide spatial proximity mediated by proteome	Mouse brain tissue, cultured mouse (E14 ES, MEF) and drosophila cells (S2)
		RIC-seq	39	Formaldehyde	Transcriptome-wide spatial proximity mediated by proteome	Cultured human cells (HeLa)

SHA	IARC		Dimeric 2'-OH acylating reagents of different lengths	Transcriptome-wide spatial proximity	Cultured human cells (HeLa, HEK293T)
KAF	ARR-seq	41	N3-kethoxal and DBCO-		Cultured human (K562, HepG2, HEK293T, virus
			modified dendrimers	Transcriptome-wide spatial proximity	infected A549) and mouse (mESCs) cells

Full names for probing and crosslinking chemicals:

1M7: 1-methyl-7-nitroisatoic anhydride

AcIm: 1-acetylimidazole

AMT: 4'-aminomethyltrioxsalen

DEPC: diethyl pyrocarbonate

DMS: dimethyl sulphate

DSG: disuccinimidyl glutarate

DBCO: dibenzocyclooctane

NAI: 2-methylnicotinic acid

NAI-N3: 2-(azidomethyl)nicotinic acid imidazole

NAz: nicotinoyl azide

Full names for chemical probing methods:

CAP-STRUCTURE-seq: 5'CAP-enriched and 3' poly(A)-enriched RNAstructure sequencing.

DIM-2P-seq: DMS-induced mutations mapped by poly(A)-primed sequencing

DMS-MaPseq: DMS mutational profiling with sequencing

DMS-seq: DMS probing with sequencing

icLASER: in vivo click selective Light Activated Structural Examination of RNA

icSHAPE: in vivo click selective 2'-hydroxyl acylation and profiling experiment

Keth-seq: N3-kethoxal probing followed by deep sequencing

LASER-MaP: light activated structural examination of RNA by mutational profiling

LASER-seq: light activated structural examination of RNA by highthroughput sequencing

Lead-seq: lead(II) ion-based structure probing with next-generation sequencing

MOD-seq: map RNA chemical modification using high-throughput sequencing

Nano-DMS-MaP: nanopore dimethylsulfate mutational profiling

nanoSHAPE: direct RNA nanopore sequencing of AcIm modified RNA

PORE-cupine: RNA structure analysis using nanopore sequencing

SHAPE-MaP: selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling

SHAPE-seq: selective 2'-hydroxyl acylation analyzed by primer extension sequencing

SMS-seq: single molecule structure sequencing smStructure-seq: single-molecule-based RNA structure sequencing SPET-seq: structural probing of elongating transcripts Full names for RNA-RNA interaction methods: CLASH: cross-linking, ligation, and sequencing of hybrids COMRADES: cross-linking of matched RNAs and deep sequencing CRIC-seq: capture RNA in situ conformation sequencing hiCLIP: hybrid and individual-nucleotide resolution ultraviolet crosslinking and immunoprecipitation KARR-seq: kethoxal-assisted RNA-RNA interaction sequencing LIGR-seq: ligation of interacting RNA and high-throughput sequencing MARIO: RNA interactome in vivo PARIS: psoralen analysis of RNA interactions and structures RAP-RNA: RNA antisense purification to identify intermolecular RNA-RNA interactions RIC-seq: RNA in situ conformation sequencing RIPPLIT: RNA immunoprecipitation and proximity ligation in tandem **RPL: RNA proximity ligation** SHARC: spatial 2'-hydroxyl acylation reversible crosslinking

SPLASH: sequencing of psoralen crosslinked, ligated, and selected hybrids

Supplementary Table 2. Representative computational tools for RNA structure studies

Function	Name	Primary algorithm	Highlights of the methods	Key references	Links
	The probabilistic model for SHAPE- seq	Maximum likelihood	Identify the true chemical-induced signals from high-throughput sequencing data.	42	
Processing RNA structure- probing Data	BUM-HMM	beta-uniform mixture hidden Markov model	Identify the true chemical-induced signals from high-throughput sequencing data with increased sensitivity.	43	https://github.com/alinaselega/BUMHM M
	dSturct	Wilcoxon signed- rank test, the	Identify differentially structured regions between groups of samples based on RNA structurome profiling data.	44	https://bioconductor.org/packages/relea se/bioc/html/dStruct.html

		Benjamini-Hochberg procedure			
	diffBum-HMM	beta-uniform mixture hidden Markov model	Detect RNA structural changes from different samples.	45	https://github.com/marangiop/diff_BUM _HMM
	DiffScan	Nonparametric Wilcoxon test	Identify structurally variable regions at nucleotide resolution for multiple structure probing platforms.	46	https://github.com/yub18/DiffScan
	StructrueImpute	Multi-layer bidirectional long short-term memory (BiLSTM)	Recover missing signals from RNA structure probing data.	47	https://github.com/Tsinghua- gongjing/StructureImpute
	RING-MaP	Filtered correlation analysis	Measure diverse through-space RNA interaction groups corresponding to both RNA secondary and tertiary structures.	48	https://weekslab.com/software/
	DREEM	Expectation maximization (EM) algorithm	Dissect alternative RNA conformations assumed by the same RNA sequence based on the RNA structure probing data.	49	https://github.com/jyesselm/dreem
RNA conformation deconvolutio n from structure- probing data	DANCE-Map	Maximum likelihood (ML) clustering, expectation maximization (EM) algorithm	Define per-nucleotide chemical reactivity, direct base-pairing interactions, tertiary interactions, and thermodynamic populations for each state in RNA structural ensembles based on the RNA structure probing data.	50	https://github.com/MustoeLab/DanceMa pper
	DRACO	a combination of spectral clustering and fuzzy clustering	Identify an optimal number of RNA conformations without the limitation of RNA length based on the RNA structure probing data.	51	https://github.com/google/draco
	DaVinci	Stochastic context- free grammar (SCFG)	Identify RNA conformation of individual single RNA molecules.	26	https://github.com/DingLab- RNAstructure/smStructure-seq
Functional RNA structure	TEISER, pyTEISER	Context-free grammars, mutual information	Determine the motifs that are significantly informative of genome-wide measurements of RNA functions.	52,53	https://github.com/goodarzilab/pyteiser

motif prediction	SHAPEwarp	Mueen's algorithm for similarity search (MASS), dynamic time warping (DTW)	Identify both unknown and known conserved RNA structure elements based on the RNA structure chemical probing data	54	https://github.com/dincarnato/SHAPEw arp
	SPOT-RNA	Residual networks (ResNets) Bidirectional long short-term memory (BiLSTM) Fully connected neural network (FCNN)	Predict all base-pairs, regardless of if they are associated with tertiary interactions.	55	https://github.com/jaswindersingh2/SPO T-RNA
Al-based RNA secondary structure prediction	MXfold2	Bidirectional long short-term memory (BiLSTM) Convolutional neural network (CNN)	Predict RNA secondary structure with thermodynamic integration.	56	https://github.com/mxfold/mxfold2
	Ufold	Fully convolutional neural network (FCN)	Predict RNA secondary structure including pseudoknots. The performance is fast (~160 ms/sequence with up to ~1500bp).	57	https://github.com/uci-cbcl/UFold
	GCNfold	Transformer	Predict RNA secondary structure including the long-distance base pairing. The performance is fast (<0.1s).	58	https://github.com/EnbinYang/GCNfold
Al-based RNA tertiary structure prediction	ARES	Deep neural network (DNN)	Predict RNA tertiary structures with s small amount of learning data using many processing layers with each layer's outputs serving as the next layer's inputs.	59	https://github.com/wk989898/ARES- implement
	trRosettaRNA	Transformer	Predict RNA tertiary structures using 1D and 2D geometries prediction along with 3D structural energy minimization.	60	https://yanglab.qd.sdu.edu.cn/trRosetta RNA/

	DRfold	Transformer	Predict RNA tertiary structures using coarse- grained end-to-end learning or geometry-based structural optimization.	61	https://zhanggroup.org/DRfold
Al-based	iDeepS	Convolutional neural network (CNN) Bidirectional long short-term memory (BiLSTM)	Predict the RNA binding protein binding sites based on the RNA sequence and structure motifs.	62	https://github.com/xypan1232/iDeepS
RNA–Protein interaction prediction	PrismNet	Squeeze-excitation (SE) residual network	Predict RNA-protein interactions affected by single-nucleotide variants based on the RNA structure chemical probing data.	63	https://github.com/kuixu/PrismNet
	RoseTTAFoldNA	Transformer	Predict structures of protein–nucleic acid complexes without homologs. The model was trained using the dataset in the RoseTTAFold along with all RNA, protein–RNA, and protein– DNA complexes in the PDB database.	64	https://github.com/uwipd/RoseTTAFold2 NA

ARES: atomic rotationally equivariant scorer

BUM-HMM: beta-uniform mixture hidden Markov model

DANCE-Map: deconvolution and annotation of ribonucleic conformational ensemble

DaVinci: determination of the variation of the RNA structure conformation through stochastic context-free grammar

diffBum-HMM: differential beta-uniform mixture hidden Markov model

DiffScan: differential analysis of structure probing data at nucleotide resolution

DRACO: deconvolution of RNA alternative conformations

DREEM: detection of RNA folding ensembles using expectation maximization

PrismNet: protein-RNA Interaction by structure-informed modeling using deep neural NETwork

RING-MaP: RNA interaction groups by mutational profiling

TEISER, pyTEISER: pyTEISER: tool for eliciting informative structural elements in RNA; pyTEISER: pythonicTEISER

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