Published online 21 May 2018 *Nucleic Acids Research, 2018, Vol. 46, Web Server issue* ***W25–W29***
doi: 10.1093/nar/gky329

Freiburg RNA tools: a central online resource for RNA-focused research and teaching

Martin Raden[1], Syed M. Ali[1], Omer S. Alkhnbashi[1], Anke Busch[2], Fabrizio Costa[3], Jason A. Davis[4], Florian Eggenhofer[1], Rick Gelhausen[1], Jens Georg[5], Steffen Heyne[6], Michael Hiller[7,8], Kousik Kundu[9,10], Robert Kleinkauf[1], Steffen C. Lott[5], Mostafa M. Mohamed[1], Alexander Mattheis[1], Milad Miladi[1], Andreas S. Richter[11], Sebastian Will[12], Joachim Wolff[1], Patrick R. Wright[13] and Rolf Backofen[1,14,*]

[1]Bioinformatics, Computer Science, University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany, [2]Institute of Molecular Biology (IMB), Ackermannweg 4, 55128 Mainz, Germany, [3]Department of Computer Science, University of Exeter, Exeter EX4 4QF, UK, [4]Coreva Scientific, Kaiser-Joseph-Str 198-200, 79098 Freiburg, Germany, [5]Genetics and Experimental Bioinformatics, University of Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany, [6]Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, 79108 Freiburg, Germany, [7]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [8]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany, [9]Department of Haematology, University of Cambridge, Cambridge Biomedical Campus, Long Road, Cambridge CB2 0PT, UK, [10]Department of Human Genetics, The Wellcome Trust Sanger Institute, Hinxton Cambridge CB10 1HH, UK, [11]Genedata AG, Margarethenstrasse 38, 4053 Basel, Switzerland, [12]Theoretical Biochemistry Group, University of Vienna, Währingerstraße 17, 1090 Vienna, Austria, [13]Department of Clinical Research, Clinical Trial Unit, University of Basel Hospital, Schanzenstrasse 55, 4031 Basel, Switzerland and [14]Centre for Biological Signalling Studies (BIOSS), University of Freiburg, Schaenzlestr. 18, 79104 Freiburg, Germany

Received January 30, 2018; Revised April 3, 2018; Editorial Decision April 16, 2018; Accepted May 18, 2018

ABSTRACT

The Freiburg RNA tools webserver is a well established online resource for RNA-focused research. It provides a unified user interface and comprehensive result visualization for efficient command line tools. The webserver includes RNA-RNA interaction prediction (IntaRNA, CopraRNA, metaMIR), sRNA homology search (GLASSgo), sequence-structure alignments (LocARNA, MARNA, CARNA, ExpaRNA), CRISPR repeat classification (CRISPRmap), sequence design (antaRNA, INFO-RNA, SECISDesign), structure aberration evaluation of point mutations (RaSE), and RNA/protein-family models visualization (CMV), and other methods. Open education resources offer interactive visualizations of RNA structure and RNA-RNA interaction prediction as well as basic and advanced sequence alignment algorithms. The services are freely available at http://rna.informatik.uni-freiburg.de.

INTRODUCTION

RNA biology is an important topic in molecular biological and biomedical research. RNA function in biological systems is complex and ranges, e.g. from involvement in disease processes (1) to more recent innovations in gene-editing based on CRISPR–Cas (2,3). A wide range of bioinformatics tools have been developed to investigate the molecular properties of nucleic acids, including their sequences and interactions with other nucleic acids or proteins.

Here, we report an extensive update to the Freiburg RNA tools webserver (4), a single platform with a collection of tools for RNA analysis including RNA-RNA interaction prediction, sequence analysis (design, splicing, and polymorphisms), and CRISPR site classification. Currently, ∼2500 jobs per month are processed. Besides RNA-specific tools, the webserver offers interactive algorithm implementations for education and teaching.

In the following, a general overview of the generic webserver architecture is given followed by a summary of the featured services and their successful applications.

*To whom correspondence should be addressed. Tel: +49 761 2037461; Fax: +49 761 2037462; Email: backofen@informatik.uni-freiburg.de

METHODS AND SERVICES

General Architecture of the Webserver

The webserver is implemented via Java Server Pages (JSP) processed by an Apache Tomcat server. Available tools, general server parameters as well as static tool details (e.g. help texts, call parameters, output description) are loaded from XML-files on server startup for automated generation of server content. All user requests are processed by a central Servlet, which directs the server's activity and response. New job requests are checked for input integrity. If this check is passed, data structures and call details are generated and the job is queued for processing. Upon completion, the job results are loaded into a tool-specific container that is forwarded to the respective JSP result page, where the data is visualized. Access to specific result files is also provided by the central Servlet. The input pages are compiled from predefined JSP blocks, which enables a central maintenance of input field architecture and features.

In the following, the integrated services are listed according to their grouping and order within the webserver. This shows the vast extension compared to its initial version (4), which only featured IntaRNA, ExpaRNA and LocARNA. The tools' respective help pages provide detailed information about the added features and tool or interface changes.

RNA–RNA interaction prediction

IntaRNA. enables state-of-the-art, general RNA–RNA interaction prediction as recently benchmarked in (5). Its model accounts for the accessibility of the potential interaction sites combined with the requirement for an interaction seed (short consecutive interaction). A heuristic approach enables runtimes that are suited for genome-wide screens (6,7). The IntaRNA webserver (4,8) is the most popular of our offered services. Besides prediction for provided query/target sequences, it also offers genome-wide screens for prokaryotic small RNA (sRNA) targets, which was not available at the initial release in 2010 (4).

CopraRNA. (Comparative prediction algorithm for small RNA targets) is a conservation-based algorithm for the prediction of prokaryotic sRNA targets (7,9). Its comparative scheme enables a significant reduction of false positive predictions, which currently makes it the method of choice for the characterization of phylogenetically conserved sRNAs (10). Furthermore, it benefits from post-processing steps such as functional enrichment and pathway analysis, which allow to further decrease the false positive rate and aid in precisely pinpointing the physiological role of the investigated sRNAs. CopraRNA has been successfully applied in many studies with diverse bacterial species (11–18). An extended use case is provided in (19) and the Supplementary Material.

GLASSgo. (GLobal Automatic Small RNA Search go) finds homologs of sRNAs (20). It combines an iterative BLASTn (21) search with an auto-adaptive, graph-based clustering algorithm. The identified homologous sRNAs (FASTA output) are visualized in an interactive taxonomic tree. In contrast to related workflows such as the combination of RNAlien (22) and Infernal (23) or solely BLASTn, GLASSgo is fully automated, which lowers technical barriers. Furthermore, it is about two orders of magnitude faster than RNAlien+Infernal. A recent study applied GLASSgo to exhaustively detect homologs of the sRNA OxyS (24). Another detailed use case is given in (25), where GLASSgo plays a key role in the *de novo* discovery of sRNAs.

metaMIR. metaMIR (26) was developed to integrate data from the multitude of available miRNA prediction tools. Each of the algorithms is based on a set of properties to predict the likelihoods that a specific miRNA targets the messenger RNAs (mRNA). metaMIR combines these predictions with a uniquely generated collection of validated miRNA targeting results (positive and negative evidence of regulation) from genomics, proteomics, and curated databases. This not only allows scoring of the likelihood of positive targeting (i.e. miRNA downregulation of a target) but to explicitly predict non-targeting, for example to refine the list of miRNAs returned to those that target select genes while not targeting others.

Sequence-structure alignment of RNAs

LocARNA. performs simultaneous alignment and folding of multiple RNAs—a central task in the comparative analysis of non-coding RNAs with a priori unknown structures. For fast and accurate analysis, it implements the state-of-the-art light-weight alignment algorithm (27) with ensemble-based sparsification (28), which is recently improved in (29). The server supports the identification of global as well as local sequence and structure similarities. By specifying structure and alignment *anchor* constraints, users can guide the alignment with prior knowledge. In contrast to the intial release, the server also integrates LocARNA-P (30) to target even more accurate multiple alignment and for assessing local alignment quality due to alignment reliability profiles. A recent comparison with other aligners is given in (31).

MARNA. MARNA also computes multiple global RNA alignments (32). In contrast to LocARNA, MARNA relies on known or non-comparatively predicted RNA structures, thus requiring known or strongly pronounced structures. The stronger commitment to fixed structures can be advantageous for specific applications.

CARNA. CARNA complements the alignment tool LocARNA for advanced RNA alignment tasks involving pseudoknots or multiple conserved structures (33,34). Unlike LocARNA, which predicts single non-crossing structures, CARNA considers similarities due to crossing base pairs or alternative structures. Moreover, users can specify structure and anchor constraints or align (possibly pseudoknotted) known structures. CARNA's increased flexibility over LocARNA is enabled by constraint-based search, which in turn makes its run times less predictable.

ExpaRNA. ExpaRNA is a tool for very fast comparison of RNAs by exact local matches (35). Instead of computing

a full sequence-structure alignment, ExpaRNA efficiently computes the best arrangement of sequence-structure motifs common to two RNAs. Moreover, ExpaRNA's exact matches can be beneficially used as anchor constraints for a full sequence-structure alignment by, e.g. LocARNA. This enables the alignment of very large RNAs that could otherwise not be aligned in reasonable time. The webserver supports this approach by providing the local motifs identified by ExpaRNA as a constraint for subsequent LocARNA alignment.

CRISPR

CRISPRmap. CRISPRmap is the first automated classification of CRISPR-repeat conservation in CRISPR–Cas systems (36,37). It compiles the largest dataset of CRISPR-repeats to date and performs comprehensive, independent clustering analyses to determine conserved sequence families, potential structure motifs for endoribonucleases and evolutionary relationships. This domain-wide map provides both a quick and detailed insight into CRISPR-repeat conservation and the diversity of prokaryotic systems and also allows to reveal yet unexplored regions. Additionally, CRISPRmap was successfully applied to classify CRISPR-repeats found in metagenomic data (2,3).

Sequence design

antaRNA. antaRNA (ant-assembled RNA) enables the design of RNA sequences that will fold into a user-specified structure and comply with additional sequence and GC-content constraints (38,39). It employs ant colony foraging-strategy to optimize the sequence according to either a specific (nested or pseudoknotted) structure or a more fuzzy target structure representation. The target structure and the provided sequence constraints are interactively visualized. Both the final GC-content as well as the deviations from the different constraints are provided for each designed sequence.

INFO-RNA. INFO-RNA finds sequences that most stably fold into a target structure (40,41). This is achieved in a two-step approach by first identifying the sequence that shows the lowest energy when folded into the target structure. Subsequently, the sequence is optimized with local search to ensure that the target structure is the sequence's unique stable fold. While being fastest (42), this approach results in a high-GC bias compared to antaRNA and other tools (38).

SECISDesign. SECISDesign designs mRNA sequences that allow for the insertion of selenocystein (43,44). To this end, the mRNA has to locally fold into a SECIS element. Given an amino acid sequence and the desired selenocystein position, SECISDesign applies a constrained local search to design an mRNA that most likely forms the SECIS element and thus provides the requested protein.

Splicing

NIPU. NIPU allows to display splicing-regulatory motifs in mRNA exons and introns, and how likely these regions are single-stranded, i.e. unstructured (45). To this end, probabilities that motifs are single-stranded are computed and visualized as bar plots.

Structure aberration of point mutations

RaSE. RaSE (RNA structurAl Stability Estimator) uses the graph vectorization technique of EDeN (46,47) to compute a score that is indicative of the structural stability restraints of each single nucleotide in the input RNA sequence. The score is computed as the graph similarity between the original structure and the structure obtained by mutating individual nucleotides. Out of the three possible substitutions, the one resulting in the largest structural change is reported. The server outputs a bar chart of the scores, a graphical representation of the wild-type MFE structure annotated with distortion scores, and the most distorting mutations. Nodes are colored proportionally to the mutation effect.

Teaching

To support teaching and understanding of RNA-related algorithms, interactive Javascript-based interfaces of basic and extended methods are provided (48). These cover basic structure prediction-related tasks (e.g. optimal structure prediction, base pair/unpaired probability computation, MEA folding), RNA-RNA interaction prediction approaches (hybrid-only, co-folding, accessibility-based), common sequence alignment algorithms for pairwise (e.g. Needleman-Wunsch and Gotoh) or multiple alignments (e.g. Feng-Doolittle and t-coffee) as well as phylogenetic tree construction methods (e.g. UPGMA and Neighbor-Joining) with according visualizations. A full list is provided in the Supplementary Material.

Other integrated webservers

ModPepInt. ModPepInt (Modular Domain Peptide Interaction) predicts modular domain-mediated interactions (49). Three different methods, SH2PepInt, SH3PepInt and PDZPepInt, identify binding motifs for SH2, SH3 and PDZ domains, respectively (50–52). Predictions are based on support vector machines and non-linear models. The latter are able to capture high-order correlation between amino acids in the binding motifs. For each tool, several filtering options are available to increase prediction accuracy. Finally, a meta-server for non-expert users enables the exploration of the binding interactions of modular domains by using all three tools and providing a summary result.

CPSP-tools. CPSP-tools webserver offers simplified lattice protein-related services (53,54), i.e. optimal structure prediction and sequence design with the hydrophobic-polar (HP) model (55) as well as fitting of lattice protein models for real protein structures (56). Both CPSP-tools and ModPepInt are using the generic webserver framework of the Freiburg RNA tools.

CMV. CMV (57) is a collection of tools for the visualization of Hidden Markov Models (HMMV) and RNA-family models (CMV). Moreover, CMCompare (58,59) is

used to visualize comparisons of these models (HMMCV, CMCV) and to annotate linked regions in the structural alignments (and the respective consensus secondary structure) they were constructed from. An extensive example is provided in the Supplementary Material.

DISCUSSION

The Freiburg RNA tools are an established and widely-used online resource for RNA-focused research. They provide services for a wide range of tasks via a unified interface, which eases both their usage and their maintenance. The underlying generic framework is also used by other web-services developed and hosted by our group, providing the same look-and-feel for all services. Most tools are also available via BIOCONDA (60) for local or high-throughput usage.

Both the generic webserver architecture as well as the interfaced tools are constantly extended and developed, which is reflected by the nearly monthly server version updates to enable new features or tool versions. Next planned steps are e.g. to integrate CRISPRleader (61) and CRISPR accessory proteins method (62) into CRISPRmap to provide a full annotation, characterization and classification of CRISPR–Cas systems. Also, the recently introduced extensions of LocARNA and ExpaRNA, namely SPARSE (29) and ExpaRNA-P (63), will be made available. To alleviate the cumbersome task of manual sRNA homolog retrieval for the comparative sRNA target prediction tool CopraRNA, GLASSgo will directly integrate with CopraRNA in future. Last but not least, we continuously extend the teaching section as part of our local teaching projects.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank the users of the Freiburg RNA tools for their productive feedback.

FUNDING

Bundesministerium für Bildung und Forschung [031A538A RBC, 031L0106B]; Deutsche Forschungsgemeinschaft [BA 2168/14-1, BA 2168/16-1]. Funding for open access charge: institutional funding.
Conflict of interest statement. None declared.

REFERENCES

1. Esteller,M. Non-coding RNAs in human disease. *Nat. Rev. Genet.*, **12**, 861.
2. Gogleva,A.A., Gelfand,M.S. and Artamonova,I.I. (2014) Comparative analysis of CRISPR cassettes from the human gut metagenomic contigs. *BMC Genomics*, **15**, 202.
3. Eloe-Fadrosh,E.A., Paez-Espino,D., Jarett,J., Dunfield,P.F., Hedlund,B.P., Dekas,A.E., Grasby,S.E., Brady,A.L., Dong,H., Briggs,B.R. *et al.* (2016) Global metagenomic survey reveals a new bacterial candidate phylum in geothermal springs. *Nat. Commun.*, **7**, 10476.
4. Smith,C., Heyne,S., Richter,A.S., Will,S. and Backofen,R. (2010) Freiburg RNA Tools: a web server integrating IntaRNA, ExpaRNA and LocARNA. *Nucleic Acids Res.*, **38**(Suppl.), W373–W377.
5. Umu,S.U. and Gardner,P.P. (2017) A comprehensive benchmark of RNA-RNA interaction prediction tools for all domains of life. *Bioinformatics*, **33**, 988–996.
6. Busch,A., Richter,A.S. and Backofen,R. (2008) IntaRNA: efficient prediction of bacterial sRNA targets incorporating target site accessibility and seed regions. *Bioinformatics*, **24**, 2849–2856.
7. Wright,P.R., Georg,J., Mann,M., Sorescu,D.A., Richter,A.S., Lott,S., Kleinkauf,R., Hess,W.R. and Backofen,R. (2014) CopraRNA and IntaRNA: predicting small RNA targets, networks and interaction domains. *Nucleic Acids Res.*, **42**, W119–W123.
8. Mann,M., Wright,P.R. and Backofen,R. (2017) IntaRNA 2.0: enhanced and customizable prediction of RNA-RNA interactions. *Nucleic Acids Res.*, **45**, W435–W439.
9. Wright,P.R., Richter,A.S., Papenfort,K., Mann,M., Vogel,J., Hess,W.R., Backofen,R. and Georg,J. (2013) Comparative genomics boosts target prediction for bacterial small RNAs. *Proc. Natl. Acad. Sci. U.S.A.*, **110**, E3487–E3496.
10. Pain,A., Ott,A., Amine,H., Rochat,T., Bouloc,P. and Gautheret,D. (2015) An assessment of bacterial small RNA target prediction programs. *RNA Biol.*, **12**, 509–513.
11. Overloeper,A., Kraus,A., Gurski,R., Wright,P.R., Georg,J., Hess,W.R. and Narberhaus,F. (2014) Two separate modules of the conserved regulatory RNA AbcR1 address multiple target mRNAs in and outside of the translation initiation region. *RNA Biol.*, **11**, 624–640.
12. Robledo,M., Frage,B., Wright,P.R. and Becker,A. (2015) A stress-induced small RNA modulates alpha-rhizobial cell cycle progression. *PLoS Genet.*, **11**, e1005153.
13. Georg,J., Kostova,G., Vuorijoki,L., Sch"on,V., Kadowaki,T., Huokko,T., Baumgartner,D., Müller,M., Klähn,S., Allahverdiyeva,Y. *et al.* (2017) Acclimation of oxygenic photosynthesis to iron starvation is controlled by the sRNA IsaR1. *Curr. Biol.*, **27**, 1425–1436.
14. Georg,J., Dienst,D., Schürgers,N., Wallner,T., Kopp,D., Stazic,D., Kuchmina,E., Klähn,S., Lokstein,H., Hess,W.R. *et al.* (2014) The small regulatory RNA SyR1/PsrR1 controls photosynthetic functions in cyanobacteria. *Plant Cell*, **26**, 3661–3679.
15. Klähn,S., Schaal,C., Georg,J., Baumgartner,D., Knippen,G., Hagemann,M., Muro-Pastor,A.M. and Hess,W.R. (2015) The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7. *Proc. Natl. Acad. Sci. U.S.A.*, **112**, E6243–E6252.
16. Holmqvist,E., Wright,P.R., Li,L., Bischler,T., Barquist,L., Reinhardt,R., Backofen,R. and Vogel,J. (2016) Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking in vivo. *EMBO J.*, **35**, 991–1011.
17. Neuhaus,K., Landstorfer,R., Simon,S., Schober,S., Wright,P.R., Smith,C., Backofen,R., Wecko,R., Keim,D.A. and Scherer,S. (2017) Differentiation of ncRNAs from small mRNAs in Escherichia coli O157:H7 EDL933 (EHEC) by combined RNAseq and RIBOseq - ryhB encodes the regulatory RNA RyhB and a peptide, RyhP. *BMC Genomics*, **18**, 216.
18. Kiekens,S., Sass,A., Van Nieuwerburgh,F., Deforce,D. and Coenye,T. (2018) The small RNA ncS35 regulates growth in Burkholderia cenocepacia J2315. *mSphere*, **3**, e00579-17.
19. Wright,P.R. and Georg,J. (2018) Workflow for a computational analysis of an sRNA candidate in bacteria. In Arluison,VVC, (ed). *Methods in Molecular Biology*, Vol. **1737**, Humana Press, NY.
20. Georg,J., Lott,S.C., Schäfer,R.A., Mann,M., Backofen,R., Hess,W.R. and Voss,B. (2018) GLASSgo - automated and reliable detection of sRNA homologs from a single input sequences. *Front. Genet.*, **9**, 124.
21. Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
22. Eggenhofer,F., Hofacker,I.L., Höner,zu Siederdissen and C. (2016) RNAlien - unsupervised RNA family model construction. *Nucleic Acids Res.*, **44**, 8433–8441.
23. Nawrocki,E.P. and Eddy,S.R. (2013) Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics*, **29**, 2933–2935.
24. Barshishat,S., Elgrably-Weiss,M., Edelstein,J., Georg,J., Govindarajan,S., Haviv,M., Wright,P.R., Hess,W.R. and Altuvia,S.

(2017) OxyS small RNA induces cell cycle arrest to allow DNA damage repair. *EMBO J.*, e201797651.
25. Lott,S.C., Wolfien,M., Riege,K., Bagnacani,A., Wolkenhauer,O., Hoffmann,S. and Hess,W.R. (2017) Customized workflow development and data modularization concepts for RNA-Sequencing and metatranscriptome experiments. *J. Biotechnol.*, **261**, 85–96.
26. Davis,J.A., Saunders,S.J., Mann,M. and Backofen,R. (2017) Combinatorial ensemble miRNA target prediction of co-regulation networks with non-prediction data. *Nucleic Acids Res.*, **45**, 8745–8757.
27. Hofacker,I.L., Bernhart,S.H. and Stadler,P.F. (2004) Alignment of RNA base pairing probability matrices. *Bioinformatics*, **20**, 2222–2227.
28. Will,S., Reiche,K., Hofacker,I.L., Stadler,P.F. and Backofen,R. (2007) Inferring non-coding RNA families and classes by means of Genome-Scale Structure-Based clustering. *PLoS Comput. Biol.*, **3**, e65.
29. Will,S., Otto,C., Miladi,M., Mohl,M. and Backofen,R. (2015) SPARSE: quadratic time simultaneous alignment and folding of RNAs without sequence-based heuristics. *Bioinformatics*, **31**, 2489–2496.
30. Will,S., Joshi,T., Hofacker,I.L., Stadler,P.F. and Backofen,R. (2012) LocARNA-P: accurate boundary prediction and improved detection of structural RNAs. *RNA*, **18**, 900–914.
31. Löwes,B., Chauve,C., Ponty,Y. and Giegerich,R. (2017) The BRaliBase dent - a tale of benchmark design and interpretation. *Brief. Bioinformatics*, **18**, 306–311.
32. Siebert,S. and Backofen,R. (2005) MARNA: multiple alignment and consensus structure prediction of RNAs based on sequence structure comparisons. *Bioinformatics*, **21**, 3352–3359.
33. Dal Palù,A., Möhl,M. and Will,S. (2010) A propagator for maximum weight string alignment with arbitrary pairwise dependencies. In: Cohen,D (ed). *Principles and Practice of Constraint Programming - CP 2010. Lecture Notes in Computer Science*. Springer, Berlin, Heidelberg, Vol. **6308**, pp. 167–175.
34. Sorescu,D.A., Möhl,M., Mann,M., Backofen,R. and Will,S. (2012) CARNA - alignment of RNA structure ensembles. *Nucleic Acids Res*, **40**, W49–W53.
35. Heyne,S., Will,S., Beckstette,M. and Backofen,R. (2009) Lightweight comparison of RNAs based on exact Sequence-Structure matches. *Bioinformatics*, **25**, 2095–2102.
36. Alkhnbashi,O.S., Costa,F., Shah,S.A., Garrett,R.A., Saunders,S.J. and Backofen,R. (2014) CRISPRstrand: predicting repeat orientations to determine the crRNA-encoding strand at CRISPR loci. *Bioinformatics*, **30**, i489–i496.
37. Lange,S.J., Alkhnbashi,O.S., Rose,D., Will,S. and Backofen,R. (2013) CRISPRmap: an automated classification of repeat conservation in prokaryotic adaptive immune systems. *Nucleic Acids Res*, **41**, 8034–8044.
38. Kleinkauf,R., Mann,M. and Backofen,R. (2015) antaRNA – ant colony based RNA sequence design. *Bioinformatics*, **31**, 3114–3121.
39. Kleinkauf,R., Houwaart,T., Backofen,R. and Mann,M. (2015) antaRNA - multi-objective inverse folding of pseudoknot RNA using ant-colony optimization. *BMC Bioinformatics*, **16**, 389.
40. Busch,A. and Backofen,R. (2006) INFO-RNA–a fast approach to inverse RNA folding. *Bioinformatics*, **22**, 1823–1831.
41. Busch,A. and Backofen,R. (2007) INFO-RNA–a server for fast inverse RNA folding satisfying sequence constraints. *Nucleic Acids Res.*, **35**, W310–W313.
42. Churkin,A., Retwitzer,M.D., Reinharz,V., Ponty,Y., Waldispühl,J. and Barash,D. (2018) Design of RNAs: comparing programs for inverse RNA folding. *Brief. Bioinformatics*, **19**, 350–358.
43. Backofen,R. and Busch,A. (2004) Computational Design of New and Recombinant Selenoproteins. In: *Proceedings of the 15th Annual Symposium on Combinatorial Pattern Matching (CPM2004), Lecture Notes in Computer Science*. Springer, Berlin, Heidelberg, Vol. **3109**, pp. 270–284.
44. Busch,A., Will,S. and Backofen,R. (2005) SECISDesign: a server to design SECIS-elements within the coding sequence. *Bioinformatics*, **21**, 3312–3313.
45. Hiller,M., Zhang,Z., Backofen,R. and Stamm,S. (2007) Pre-mRNA secondary structures influence exon recognition. *PLoS Genet.*, **3**, e204.
46. Costa,F. and De Grave,K. (2010) Fast neighborhood subgraph pairwise distance kernel. In: *Proceedings of the 26th International Conference on Machine Learning*, Omnipress, Madison, pp. 255–262.
47. Navarin,N. and Costa,F. An efficient graph kernel method for non-coding RNA functional prediction. *Bioinformatics*, **33**, 2642–2650.
48. Raden,M., Mohamed,M.M., Ali,S.M. and Backofen,R. (2018) Interactive implementations of thermodynamics-based RNA structure and RNA-RNA interaction prediction approaches for example-driven teaching. *PLOS Comp. Biol.*, in press.
49. Kundu,K., Mann,M., Costa,F. and Backofen,R. (2014) MoDPepInt: an interactive web server for prediction of modular domain-peptide interactions. *Bioinformatics*, **30**, 2668–2669.
50. Kundu,K., Costa,F., Huber,M., Reth,M. and Backofen,R. (2013) Semi-supervised prediction of SH2-peptide interactions from imbalanced high-throughput data. *PLoS One*, **8**, e62732.
51. Kundu,K., Costa,F. and Backofen,R. (2013) A graph kernel approach for alignment-free domain-peptide interaction prediction with an application to human SH3 domains. *Bioinformatics*, **29**, i335–i343.
52. Kundu,K. and Backofen,R. (2014) Cluster based prediction of PDZ-peptide interactions. *BMC Genomics*, **15**, S5.
53. Mann,M., Will,S. and Backofen,R. (2008) CPSP-tools - exact and complete algorithms for High-throughput 3D lattice protein studies. *BMC Bioinformatics*, **9**, 230.
54. Mann,M., Smith,C., Rabbath,M., Edwards,M., Will,S. and Backofen,R. (2009) CPSP-web-tools: a server for 3D lattice protein studies. *Bioinformatics*, **25**, 676–677.
55. Mann,M. and Backofen,R. (2014) Exact methods for lattice protein models. *Bio-Algorithms Med-Syst.*, **10**, 213–225.
56. Mann,M., Saunders,R., Smith,C., Backofen,R. and Deane,C.M. (2012) Producing high-accuracy lattice models from protein atomic co-ordinates including side chains. *Adv. Bioinformatics*, **2012**, 6.
57. Eggenhofer,F., Hofacker,I.L., Backofen,R. and Höner zu Siederdissen,C. (2018) CMV - visualization for RNA and Protein family models and their comparisons. *Bioinformatics*, doi:10.1093/bioinformatics/bty158.
58. Höner,zu Siederdissen, C. and Hofacker,I.L. (2010) Discriminatory power of RNA family models. *Bioinformatics*, **26**, i453–i459.
59. Eggenhofer,F., Hofacker,I.L., Höner,zu Siederdissen and C. (2013) CMCompare webserver: comparing RNA families via covariance models. *Nucleic Acids Res.*, **41**, W499.
60. Dale,R., Grüning,B., Sjödin,A., Rowe,J., Chapman,B.A., Tomkins-Tinch,C.H., Valieris,R. and Köster,J. (2017) Bioconda: a sustainable and comprehensive software distribution for the life sciences. *bioRxiv*, **2017**, doi:10.1101/207092.
61. Alkhnbashi,O.S., Shah,S.A., Garrett,R.A., Saunders,S.J., Costa,F. and Backofen,R. (2016) Characterizing leader sequences of CRISPR loci. *Bioinformatics*, **32**, i576–i585.
62. Shah,S.A., Alkhnbashi,O.S., Behler,J., Han,W., She,Q., Hess,W.R., Garrett,R.A. and Backofen,R. (2018) Conserved accessory proteins encoded with archaeal and bacterial Type III CRISPR–Cas gene cassettes that may specifically modulate, complement or extend interference activity. *bioRxiv*, **2018**, doi:10.1101/262675.
63. Otto,C., Mohl,M., Heyne,S., Amit,M., Landau,G.M., Backofen,R. and Will,S. (2014) ExpaRNA-P: simultaneous exact pattern matching and folding of RNAs. *BMC Bioinformatics*, **15**, 6602.