

# The RNA workbench 2.0: next generation RNA data analysis

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## ABSTRACT

RNA has become one of the major research topics in molecular biology. As a central player in key processes regulating gene expression, RNA is in the focus of many efforts to decipher the pathways that govern the transition of genetic information to a fully functional cell. As more and more researchers join this endeavour, there is a rapidly growing demand for comprehensive collections of tools that cover the diverse layers of RNA-related research. However, increasing amounts of data, from diverse types of experiments, addressing different aspects of biological questions need to be consolidated and integrated into a single framework. Only then is it possible to connect findings from e.g. RNA-Seq experiments and methods for e.g. target predictions. To address these needs, we present the RNA Workbench 2.0, an updated online resource for RNA related analysis. With the RNA Workbench we created a comprehensive set of analysis tools and workflows that enables researchers to analyze their data without the need

for sophisticated command-line skills. This update takes the established framework to the next level, providing not only a containerized infrastructure for analysis, but also a ready-to-use platform for hands-on training, analysis, data exploration, and visualization. The new framework is available at <https://rna.usegalaxy.eu>, and login is free and open to all users. The containerized version can be found at <https://github.com/bgruening/galaxy-rna-workbench>.

## INTRODUCTION

Together with the focus on RNA as regulatory key player, the number and complexity of datasets ready for analysis is steadily increasing. Although many tools for the analysis of such data exist, they are often tailored to specific experiments and not always easy to install, adapt, and run appropriately. The challenge for the individual researcher remains to chain them into useful workflows and pipelines. Often this task is further complicated, as many tools are only available for the command line, limiting their user base to computer-savvy biologists and bioinformaticians.

Although pitfalls during the installation process of tools can be circumvented with package managers like *conda*

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<https://conda.io> and its *BioConda* (1) channel, or *Docker* containers, it remains with the user to set up the appropriate computational environment. Many of these needs were already addressed with the release of the *RNA Workbench* (2). Based on the framework (3), containerized in a *Docker* instance, the workbench guarantees simple access, easy extension and flexible adaption to personal and security needs. This enables users to run sophisticated analyses that are independent of command-line knowledge while utilizing's integrated and powerful workflow manager. With the current release of the *RNA Workbench 2.0* we now additionally provide the user with a pre-configured, ready-to-use compute environment, running on dedicated hardware, available at <https://rna.usegalaxy.eu>.

The *RNA Workbench 2.0* is developed and maintained by a community consisting of experts in RNA bioinformatics and, as well as a growing number of users, and tool developers. Our commitment to keep the workbench fit for future standards and needs is one of the reasons for the release of this update. We aim to provide researchers with an up-to-date reliable and robust framework for RNA data analysis. In this release, we integrated many new RNA-related tools, and updated well established suites, such as the *ViennaRNA* (4) package, covering a broad variety of use-cases.

Currently, we provide more than 100 bioinformatics tools that are dedicated to different research areas of RNA biology including RNA structure analysis, RNA alignment, RNA annotation, RNA-protein interaction, ribosome profiling, RNA-Seq pre-processing and analysis, as well as RNA target prediction. The complete list of tools can be found at <https://rna.usegalaxy.eu> or <https://github.com/bgruening/galaxy-rna-workbench>.

Taking advantage of *Galaxy*'s powerful workflow manager allows users to easily connect single tools into computational pipelines. For common RNA related tasks we provide >25 ready-to-use workflows combining, e.g. established tools for RNA-Seq processing and analysis. For each workflow we provide a dedicated training to guide researchers through the analysis. Training is a key aspect of our effort in bringing high-quality RNA bioinformatics to researchers. Thus, each training accompanying a workflow comes with a test dataset, allowing interested users to get hands-on experience with their tools and workflows of interest. Keeping such trainings up-to-date and functional is a cooperative endeavour together with the *Galaxy* Training Network, which hosts *Galaxy* Training Material (5), a collection of tutorials developed and maintained by the worldwide *Galaxy* community. In case a user requires a novel workflow to answer a research question that is not covered by existing ones or to incorporate specific tools, we encourage users to share these workflows and if possible adequate training data and material. This directly enables all users to benefit from contributions to our community, which distributes shared knowledge and in return helps to maintain and enhance workflows and trainings where possible.

## GOALS

A main intention behind the development of the original *RNA Workbench* was the creation of an easy-to-use and

deploy environment for training and self-empowerment of biologists in RNA bioinformatics. The *RNA Workbench* was downloaded >2000 times, used for research, training courses (e.g. within de.NBI (6)), and has even been integrated into the B3Africa toolset (7). The ongoing need for such a comprehensive collection of RNA bioinformatics tools, workflows and resources led to the development of *RNA Workbench 2.0*. Although the provision of *RNA Workbench* as in a *Docker* made it easy to maintain, deploy and use, we became aware that there is additional need for an instance with freely available compute resources. Our target audience, mainly RNA biologists, requested an even more easy-to-use and ready-to-go way of accessing this collection. With the realization of the European server (<https://usegalaxy.eu>), we gained access to an infrastructure that would allow exactly that. Thus, with *RNA Workbench 2.0* we provide an updated and ready-to-use webserver, satisfying user requests and enabling even more scientists to participate in RNA research.

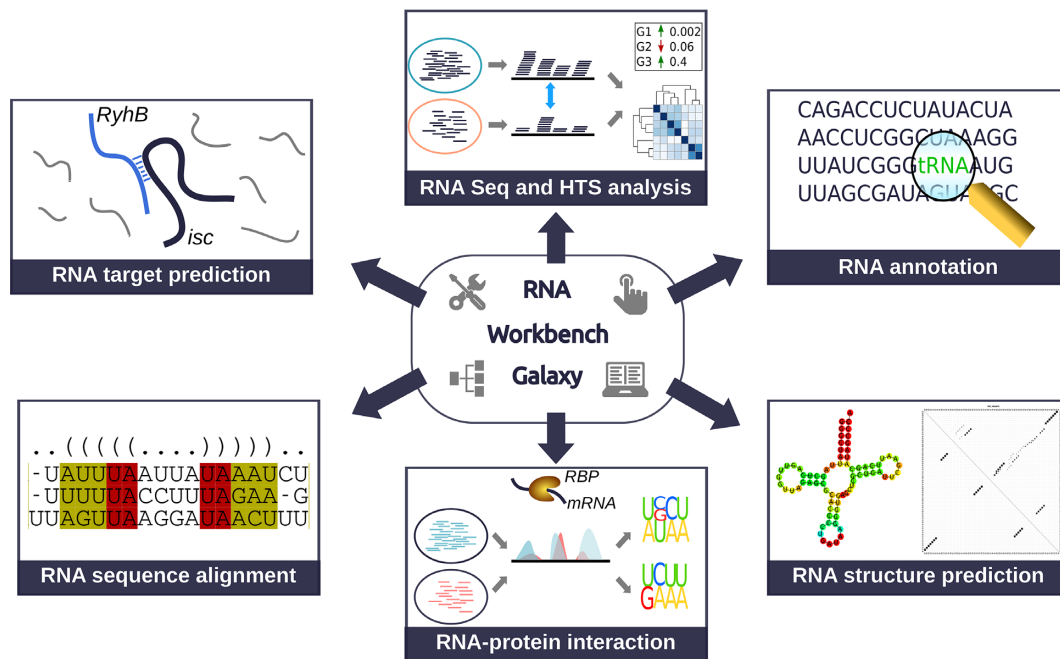
## TOOLS AND IMPROVEMENTS

In addition to providing the *RNA Workbench 2.0* as a portable *Docker* container (<https://github.com/bgruening/galaxy-rna-workbench>), users can now directly use integrated tools, workflows and tutorials at a free online instance of *Galaxy*. This makes the use of the *RNA Workbench 2.0* even easier, and allows users to train and run data analysis workflows without the need to set up hardware, software environments, or even *Docker*. New workflows and tutorials ease introduction to the environment, and guide users through analysis tasks step by step. Continuous exchange of workflows, tours and training material with the *Galaxy* Training Network ensures that the *RNA Workbench 2.0* remains a state-of-the-art training and research resource. New and updated tools and workflows are continuously integrated and made available in close cooperation between the user and developer community. This includes also updates to the underlying packages in *BioConda*. Updated tools are for example *LocaRNA* (8), *RNAz* (9), (10), *AREsite2* (11) and *Infernal* (12). In addition new tools like *edgeR* (13), *CMV* (14), *RNAlien* (15), *MultiQC* (16) and *scPipe* (17) have been added. A complete list of available tools can be found at <https://rna.usegalaxy.eu>.

Figure 1 provides an overview of tools and workflows dedicated to specific topics of RNA research in version 2 of the *RNA Workbench*.

## TRAINING

A key aspect behind the development of the original and now updated *RNA Workbench* was to provide an accessible platform, easing the process of gaining expertise in and applying bioinformatics. To this end, considerable effort went into extensive documentation and a large set of training material, empowering beginners and non-bioinformaticians to use, adapt, and apply workflows based on their needs and standards. The recently published *Galaxy* Training Material provides users with a collection of hands-on training material and data on many top-



**Figure 1.** Overview of RNA research topics, dedicated tools and example workflows in *RNA Workbench 2.0*. RNA target prediction enables to analyze potential interaction partners of RNA molecules. Included annotation tools allow the discovery of homologous sequences in genomes. The secondary structure of input RNA sequences can be predicted and visualized or for example used to create sequence-structure alignments. High-throughput and RNA sequencing data analysis can be performed with available tools and results directly intersected with *e.g.* databases for RNA-protein interactions.

ics of (not exclusively high-throughput sequencing (HTS)-related) life-science research. This collection is constantly improved and extended in an international community effort, including de.NBI, ELIXIR and EMBL. We tightly integrate *Galaxy* Training Material into the *RNA Workbench 2.0*, exchanging workflows and training material on various RNA related topics. As an example, for RNA-Seq data analyses we provide training instances as specific introduction to the topic. These consist of self-explanatory presentation slides, hands-on training documentation and a *Galaxy* Interactive Tour guiding through the analysis workflow with all required input files ready-to-use, hosted by *Zenodo*.

## WORKFLOWS

One of the strengths of the framework is that users can easily create, customize and share their workflows with other users of the same or other instances. A workflow is not only a chain of tools applied to a fixed dataset, *Galaxy* workflows also save tool versions, required data formats and other metadata ensuring a maximum of reproducibility. The built-in graphical workflow editor facilitates repurposing or adaptation of workflows.

A set of >25 workflows dedicated to specific analysis goals is included in *RNA Workbench 2.0*. We provide for example a set of workflows for the analysis of non-coding RNA and cover a range of analysis tasks, from structure conservation and coding potential of homologous RNAs, based on *Locarna* (8) and *RNAz* (9), as well as automatic construction of RNA family models, based on *RNAlien* (14). The workbench features workflows for processing, analyzing and visualizing data from RNA-Seq,

CLIP-Seq, RNA folding, network analysis, sRNA-Seq, RNA family model construction and more.

Datasets for analysis can be imported from a local source, from dedicated databases or via link, easing the integration of data from different sources. Training datasets can be imported directly from *Zenodo*.

## TOURS

Another training aspect is provided via *Galaxy* Interactive Tours. These guide users through an entire analysis in an interactive and explorative way. In contrast to training videos, a *Galaxy* Interactive Tour can be easily created, updated and improved to guide the *Galaxy* user step-by-step, *e.g.* through a whole HTS analysis starting from uploading the data to using complex analysis tools. The *RNA workbench* currently integrates more than 15 *Galaxy* Interactive Tours. These range from general tours introducing new users to the *Galaxy* interface and its usage, with RNA-seq example datasets, to specialized tours, *e.g.* illustrating secondary structure prediction of RNA molecules using parts of the *ViennaRNA* package.

## INPUTS AND OUTPUTS

Users of the *RNA Workbench 2.0* have access to a diverse set of *Galaxy* implemented data formats and format conversion tools. Common formats for sequence and/or structure information are readily accepted as input, generic data can be imported and converted to fit tool specifications, guaranteeing reproducibility and interoperability. Output data follows the same principle, defined by the analysis tool, but can be converted to a range of standard and



specific formats, including plots and figures. For the latter, the *RNA Workbench 2.0* contains tools for visualizations of RNA-Seq related data (e.g. *mQC* (18), *Mul-tiQC* (16), *sRNAPipe* (19)), RNA structure datasets, such as dot-bracket strings RNA 2D or 3D structures or RNA family models and alignments (*cmv* (15)).

## COMMUNITY CONTRIBUTIONS

The *RNA Workbench 2.0* is hosted on *GitHub* (<https://github.com/bgruening/galaxy-rna-workbench>) and users are welcome to suggest new tools, workflows and tours to be made available through *GitHub* and the workbench *Docker* container. Tools should be published to the *Galaxy* Tool Shed (20) via <https://github.com/bgruening/galaxytools> followed by a pull request at *GitHub*. After passing continuous integration tests and approval after manual review, new tools will be integrated into the *RNA Workbench*. More information about tool development can be found on the *Galaxy* community page. Workflows can easily be contributed by running them at <https://rna.usegalaxy.eu> and sharing them, ideally accompanied by test datasets and a shared history of the workflow run. A pull request adding them to the workflow folder of <https://github.com/bgruening/galaxy-rna-workbench>, will allow us to merge the workflow into the workbench. When contributing workflows, users should make sure that all tools needed for the workflow are integrated into the *RNA Workbench 2.0*. If not, please add these tools beforehand following above steps, or request them to be added by opening an appropriate issue at *GitHub*. *Galaxy* interactive tours can be contributed similarly, by opening a pull request and including tours in the tours folder of <https://github.com/bgruening/galaxy-rna-workbench> after approval.

## USE CASES

### de.NBI

The ‘German Network for Bioinformatics Infrastructure–(de.NBI)’ is an academic and non-profit infrastructure supported by the German Federal Ministry of Education and Research. As German partner of *ELIXIR* (<https://www.elixir-europe.org>) it provides bioinformatics services to users in life science research and biomedicine in Europe (6). The partners organize training events, courses and summer schools on tools, standards and compute services provided by de.NBI and *ELIXIR* to assist researchers to more effectively exploit their data. The *RNA Workbench* and also *RNA Workbench 2.0* have in part been developed by researchers funded by de.NBI with the aim to generate a free and easy to use platform for training and education. As such, the *RNA Workbench* is ready for and has been used in de.NBI training courses. With the publication of *RNA Workbench 2.0* this will become even easier, as trainers and trainees have access to a ready-to-use instance, including dedicated hardware, simply connecting via a web browser.

### B3Africa

The Bridging Biomolecular Researcher and Biobanking in Africa (B3Africa) created the *eB3Kit*, an informatics plat-

form for comprehensive management of samples and associated data (21) to support the establishment of research integrated biobanks (22). A key priority of the project is to strengthen the research capacity in resource constrained areas. As bioinformatics is a rapidly advancing field leading to constant changes in the demand for tools and procedures, the bioinformatics module has been designed to integrate a pre-existing platform satisfying the following key requirements. (i) An active community providing access to new tools, algorithms and training through a standardized interface, (ii) an accessible API enabling the B3Africa project to interact with the software without changing the supported codebase and (iii) the ability to download tools and databases for access without internet connection. Fulfilling these requirements, the *RNA Workbench* has been implemented in the *BIBBOX* appstore (7) and is used as the preferred solution to showcase both the *eB3Kit* and the *Galaksio* interface for simplified workflow management (23). Throughout the project successful showcases of the *eB3Kit* using the *RNA Workbench* have been conducted, e.g. in Lyon (France), Banjul (Gambia) and at Lake Naivasha in the Rift Valley region of Kenya.

## DISCUSSION

An active community developing and applying the *RNA workbench* in training (e.g. within de.NBI, *ELIXIR* and B3Africa) and research, the *RNA Workbench* has become an important resource for best practices in RNA and high-throughput sequencing bioinformatics in *Galaxy*.

In this work, we present an update to this resource with the creation of the ready-to-use webserver instance. Users benefit from this setup as they can now directly browse to <https://rna.usegalaxy.eu> and use a pre-configured instance of *RNA Workbench*, without needing to have any software installed on their own system except for a browser. This enables researchers not only to become familiar with a set of RNA-related bioinformatics tasks, running one of the provided tutorials and/or accompanying workflows, but also to compute and analyze data on dedicated hardware. For users concerned with data regulations, e.g. when working on patient data, or users with their own dedicated hardware, we also provide an updated *Docker* container, similar to the first version of *RNA Workbench*. A *RNA Workbench* instance started with this container provides the same tools, workflows, trainings and tours as the online instance and can easily be extended with additional tools via the *Galaxy* Tool Shed. As for the first version of *RNA Workbench*, each tool in the workbench is also available as a *BioConda* package as well as a *Docker/rkt* container (*BioContainers*). The *Docker* container offers a comprehensive virtualized *RNA workbench* that can be deployed on every standard Linux, Windows and OSX computer, but can at the same time employ high-performance- or cloud-computing infrastructure.

Similar to the first version, this release is developed and maintained by a constantly growing RNA and *Galaxy* community. This community approach helps to keep the workbench up-to-date and valuable for research. Moreover, all components such as tools, workflows, visualizations, interactive tours and training material can be easily integrated

into any available *Galaxy* instance for teaching, learning or exploratory purposes. Every user is encouraged to contribute and add to this collection, which is tightly integrated into the *Galaxy* Training Material, providing state-of-the-art learning material.

To our knowledge, the *RNA workbench* is a unique suite without direct competitors. Existing workbenches, such as *miARma-Seq* (24), the *UEA Small RNA Workbench* (25) or the *NCBI genome workbench*, are all tailored to specific analysis tasks. In addition, our focus on accessibility, flexibility in workflow assembly and application, training and the interaction with the community are all major benefits of *RNA Workbench 2.0*.

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