# Investigation of RNA-RNA Interactions Using The RISE Database

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RNA-RNA interactions (RRIs) are essential to understanding the regulatory mechanisms of RNAs. Mapping RRIs *in vivo* in a transcriptome-wide manner remained challenging until the recent development of several sequencing-based technologies. However, RRIs generated from large-scale studies had not been systematically collected and analyzed before. This article introduces RISE, a database of the RNA Interactome from Sequencing Experiments. RISE provides a comprehensive collection of RRIs in human, mouse, and yeast, derived from transcriptome-wide sequencing experiments, as well as targeted sequencing studies and other public databases/datasets. To facilitate better understanding of the biological roles of these RRIs, RISE also offers rich functional annotations involving RNAs, and an interactive interface to explore the analysis results. Here, we provide a brief description of the RISE website and a step-by-step protocol for using RISE to study RRIs. © 2018 by John Wiley & Sons, Inc.

Keywords: interaction • RISE • RNA • RNA-RNA • RNA structure • sequencing experiments

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## INTRODUCTION

RNA molecules can fold into intrinsic complex structures by forming intramolecular and intermolecular base pairs. RNA-RNA interactions (RRIs) induced by intermolecular base pairing play essential roles in gene expression. A canonical example is the base pairing formed between the codons of mRNAs and the anticodons of tRNAs during protein synthesis (Ibba & Soll, 2000). Another example is the recognition of the intronic regions of pre-mRNAs by spliceosomal small nuclear RNAs (snRNAs; Guil & Esteller, 2015). In addition, RRIs are the basis of many post-transcriptional regulatory processes. For example, the 3' UTRs of mRNAs, and lncRNAs as well, can be targeted by miRNAs (Bartel, 2004), which often results in a complex regulatory competing endogenous RNA (ceRNA) network when different sites are targeted by the same set of miRNAs (Salmena, Poliseno, Tay, Kats, & Pandolfi, 2011).



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Although RRIs can be formed between all types of RNAs, previous studies have focused on limited RNA species, mainly mRNA-miRNA interactions. Only recently have technologies emerged to characterize comprehensive sets of RRIs involving other types of RNAs. These high-throughput methods can be mainly divided into two groups, i.e., (i) detecting RRIs for a target RNA/protein and (ii) detecting transcriptome-wide RRIs in a cell. The first group includes CLASH (Helwak, Kudla, Dudnakova, & Tollervey, 2013; Kudla, Granneman, Hahn, Beggs, & Tollervey, 2011), hiCLIP (Sugimoto et al., 2015), RIL-seq (Melamed et al., 2016), RIA-seq (Kretz et al., 2013), and RAP-RNA (Engreitz et al., 2014). Briefly, CLASH, hiCLIP, and RIL-seq first crosslink RNA duplexes with associated proteins by UV irradiation. The crosslinked ribonucleoprotein complexes are then affinity purified, and the ligated RNA sequences are subjected to high-throughput sequencing. A modified CLASH method additionally incorporates a small molecule crosslinker [i.e., 4'-aminomethyltrioxsalen (AMT)] to probe direct base pairing simultaneously (Liu et al., 2017). RIA-seq and RAP-RNA use antisense DNA probes of the target RNA to capture RNA duplexes, followed by high-throughput sequencing. The second group includes PARIS (Lu et al., 2016), SPLASH (Aw et al., 2016), LIGR-seq (Sharma, Sterne-Weiler, O'Hanlon, & Blencowe, 2016), and MARIO (Nguyen et al., 2016). The basic strategy of these transcriptome-wide methods is to first crosslink interacting RNA duplexes using nucleic acid crosslinker (e.g., AMT) treatment and UV irradiation, and then purify and capture the enriched RNA duplexes. The RRI regions can be identified by high-throughput sequencing of the ligated duplexes and downstream bioinformatics analysis.

We have recently developed RISE, a database of RNA Interactome from Sequencing Experiments, to aggregate and annotate RRIs derived from these high-throughput sequencing experiments together with those from other databases and publications (Gong et al., 2018). Currently, RISE contains 328,811 RRIs from human, mouse and yeast.

In this article, we will first introduce the basic structure of the RISE database. Then, we will show how to explore RISE by using an example target gene to search for its associated RRIs (Basic Protocol 1). Finally, we will demonstrate how the functional annotations of these RRIs can be useful in understanding their regulatory functions (Basic Protocol 2).

## STRATEGIC PLANNING

RISE can be accessed at *http://rise.zhanglab.net*. The homepage includes a welcome description, a search box, and a navigation bar. The navigation bar is on the left of the homepage, with hyperlinks to other pages (Fig. 1). Users can click the bar to go to any other section and return to the homepage by clicking "Search". The front page contains seven sections, including "News," "Search," "Statistics," "Downloads," "Help", "Links," and "Contact.". Here, we briefly describe five of these sections.

(1) The "News" module provides information about updates to the RISE database. Information on additional data or new functional features of RISE can be found here.
(2) The "Statistics" module provides information on the data source and simple statistics for all collected RRIs in both table view and graph view. RRIs are grouped into three categories: (i) transcriptome-wide sequencing studies, (ii) targeted sequencing studies, and (iii) other databases/datasets. The numbers of RRIs and involved genes in each group are shown in a table view (Fig. 2). More statistics are visualized using several graphs. The pie charts show the number of RRIs from different species, cell lines, and sources/studies (Fig. 3). The Circos plots (Krzywinski et al., 2009) show RRI distribution within and between different types of RNAs in human and mouse (Fig. 3).

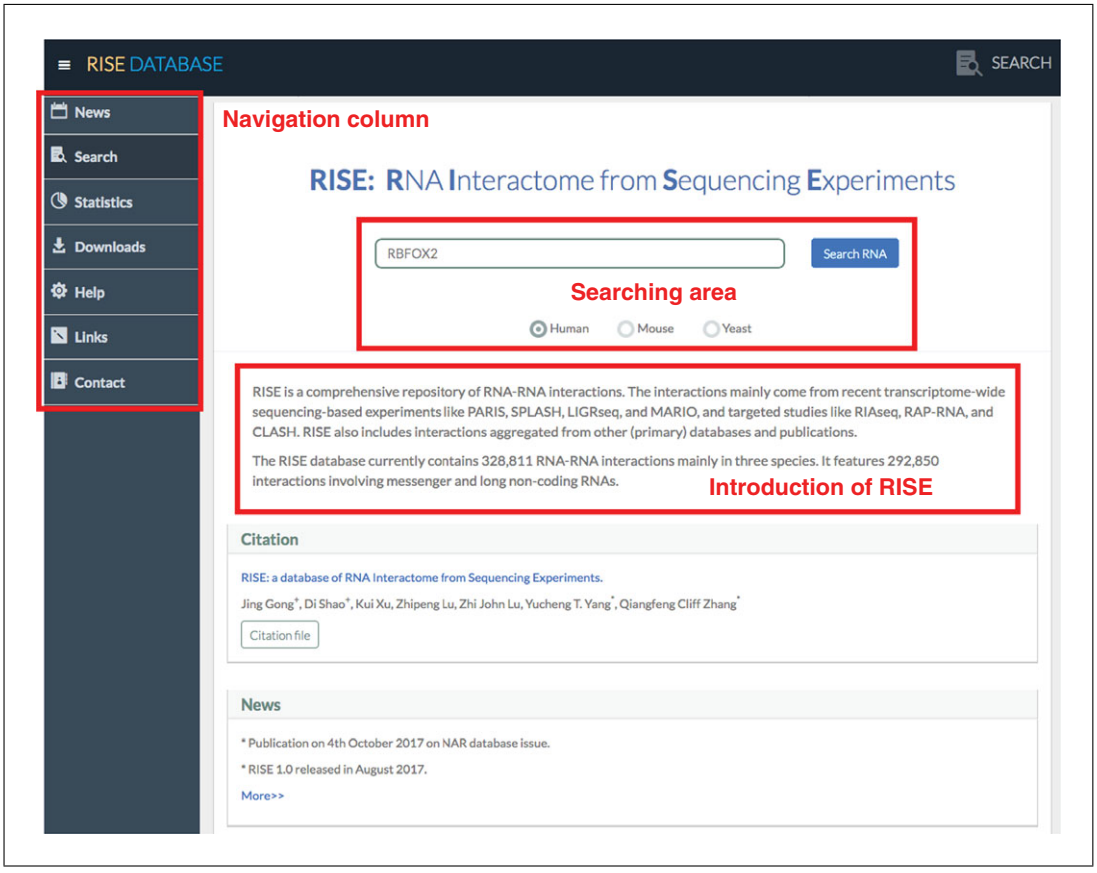



**Figure 1** The homepage of the RISE database. See Strategic Planning for detailed description of the navigation menu, and Basic Protocol 1 for how to search RISE with the search box.

(3) The "Downloads" module enables users to acquire all the data in RISE. The detailed explanation of the file format can also be found. All RRI data are grouped into different subsets according to species and source. The number of entries and the size of the file for each category are shown in a table. By clicking on a table item, users can download the chosen RRIs. The download files are provided in the BEDPE file format, defined in the BEDTOOLS suite (Quinlan & Hall, 2010) for connecting two relevant genomic regions. A hyperlink to the detailed description of the BEDPE format is provided.

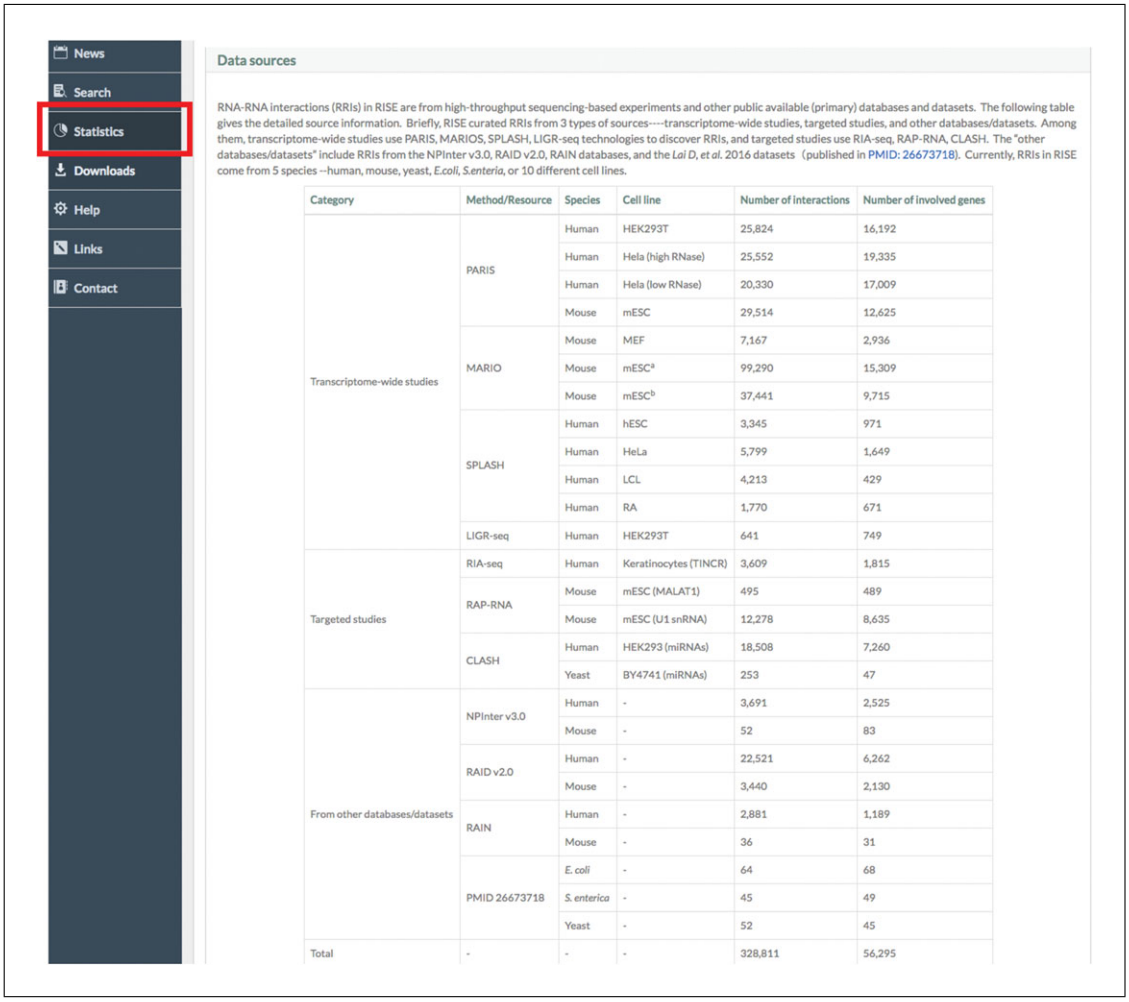
(4) The "Help" module provides a detailed guidance to RISE, including an introduction, a usage example demonstrated with RNA binding fox-1 homolog 2 (RBFOX2), frequently asked questions, and related references as well. Users are advised to briefly go through this section before exploring the RISE database.

(5) The "Links" module provides hyperlinks to all additional resources in RISE, including databases used for RRI curations, different resources for RRI annotation, and other related RNA databases/datasets.

## SEARCHING RNA-RNA INTERACTIONS

***BASIC PROTOCOL 1***

Users can query the RRIs of a specific gene in the search box on the front page. The result page contains several modules for further exploration, including a summary table of query gene, visualization of all RRIs using a Circos plot, and a table view for detailed information. Here, we use an example, RBFOX2, for a step-by-step demonstration of the RRI search.




| Category | Method/Resource | Species | Cell line | Number of interactions | Number of involved genes |
|---|---|---|---|---|---|
| Transcriptome-wide studies | PARIS | Human | HEK293T | 25,824 | 16,192 |
| | | Human | Hela (high RNase) | 25,552 | 19,335 |
| | | Human | Hela (low RNase) | 20,330 | 17,009 |
| | | Mouse | mESC | 29,514 | 12,625 |
| | MARIO | Mouse | MEF | 7,167 | 2,936 |
| | | Mouse | mESC[a] | 99,290 | 15,309 |
| | | Mouse | mESC[b] | 37,441 | 9,715 |
| | SPLASH | Human | hESC | 3,345 | 971 |
| | | Human | HeLa | 5,799 | 1,649 |
| | | Human | LCL | 4,213 | 429 |
| | | Human | RA | 1,770 | 671 |
| | LIGR-seq | Human | HEK293T | 641 | 749 |
| Targeted studies | RIA-seq | Human | Keratinocytes (TINCR) | 3,609 | 1,815 |
| | RAP-RNA | Mouse | mESC (MALAT1) | 495 | 489 |
| | | Mouse | mESC (U1 snRNA) | 12,278 | 8,635 |
| | CLASH | Human | HEK293 (miRNAs) | 18,508 | 7,260 |
| | | Yeast | BY4741 (miRNAs) | 253 | 47 |
| From other databases/datasets | NPInter v3.0 | Human | - | 3,691 | 2,525 |
| | | Mouse | - | 52 | 83 |
| | RAID v2.0 | Human | - | 22,521 | 6,262 |
| | | Mouse | - | 3,440 | 2,130 |
| | RAIN | Human | - | 2,881 | 1,189 |
| | | Mouse | - | 36 | 31 |
| | PMID 26673718 | *E. coli* | - | 64 | 68 |
| | | *S. enterica* | - | 45 | 49 |
| | | Yeast | - | 52 | 45 |
| Total | - | - | - | 328,811 | 56,295 |

**Figure 2** The statistics table view in the "Statistics" page of RISE. The table provides a summary of the curated RRI data, including the number of RRIs and involved genes from different categories, methods, species, and cell lines.

### *Necessary Resources*

*Hardware*

A computer with internet access

*Software*

Any up-to-date web browser (e.g., Firefox, Chrome, Opera, Safari, Internet Explorer)

*Files*

None

### *Input gene name and select a species*

1. First, start the web browser and connect to the website *http://rise.zhanglab.net*. The homepage of the RISE database consists of a brief introduction to RISE, a search box, and a navigation bar with links to other functional modules (Fig. 1). Users can search RRIs of a specific gene on this page with the search box. They can also look for additional information in the RISE database by using the navigation bar.

2. Enter the name of the query gene (i.e., `RBFOX2`) in the search box. The server can automatically complete the full gene name according to the first few letters entered, and provide some candidate gene names in the drop-down box, in which the users

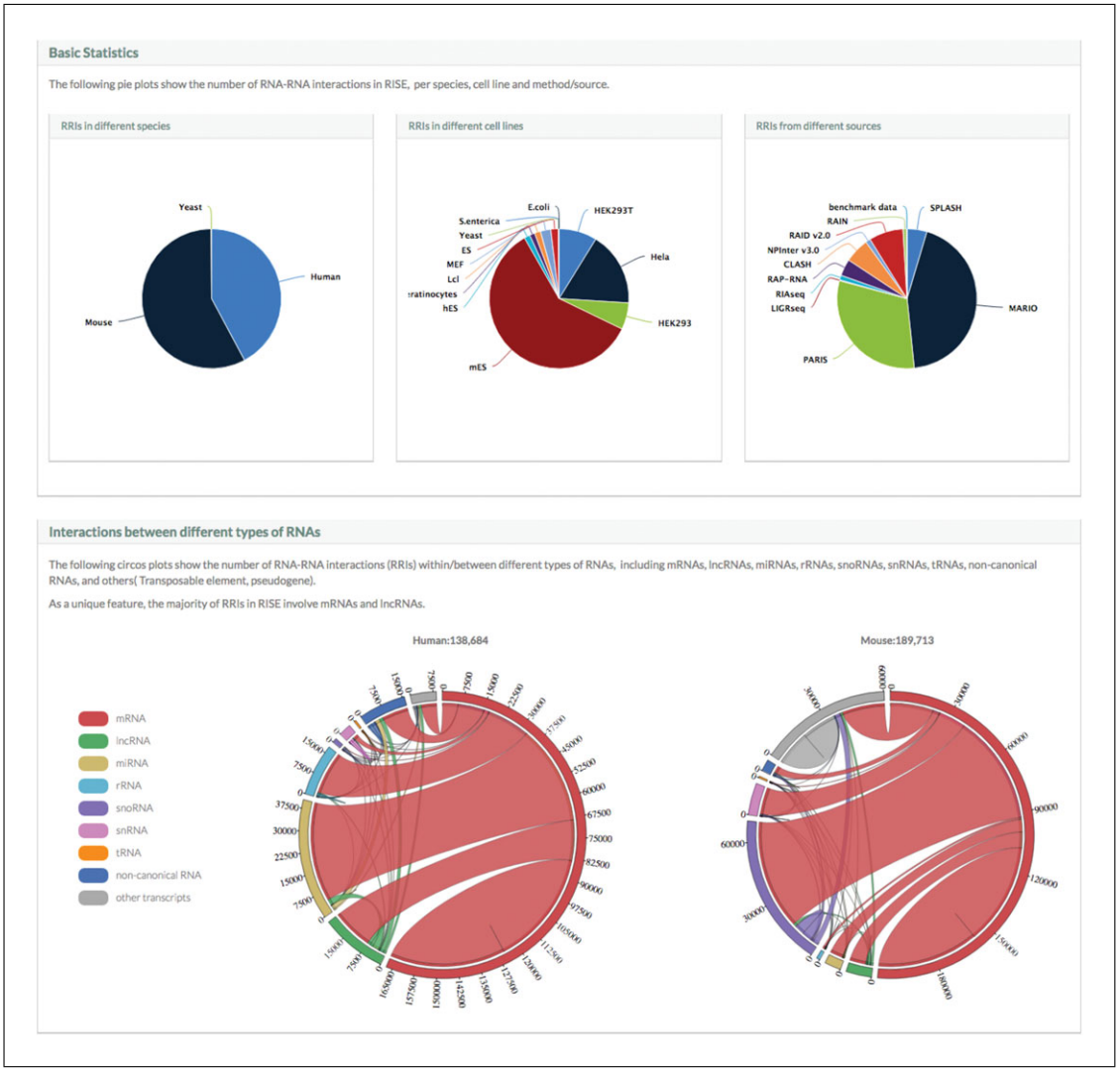



**Figure 3** The statistics plots in the "Statistics" page of RISE. The pie plots (upper) show the distribution of RISE's RRI data in different species, cell lines, and sources. The Circos plots (bottom) show the number of RRIs within/between different types of RNAs.

can select the exact gene name they want to search (Fig. 4). Note that gene-name matching is case-insensitive.

3. After entering the gene name, users need to select a species of interest from human, mouse, and yeast. Note that "Human" is the default selection (Fig. 4). Then, click the "Search RNA" button on the right of the search box. The page will be refreshed to the result layout.

### *Gene summary*

The first part of the search results page is an information table about the query gene (Fig. 5). The table lists some basic information, including gene name, gene aliases, species, Ensembl gene ID, genomic location of the RNA, exon count, gene type, RRI number (the total number of RRIs involving this gene), and RefSeq summary. The information on the gene annotation is obtained from Ensembl v84. In the case where multiple isoforms exist, the longest transcript of the gene will be used (Zerbino et al., 2018). The RefSeq summary provides an overall description about the gene and its biological functions (O'Leary et al., 2016).

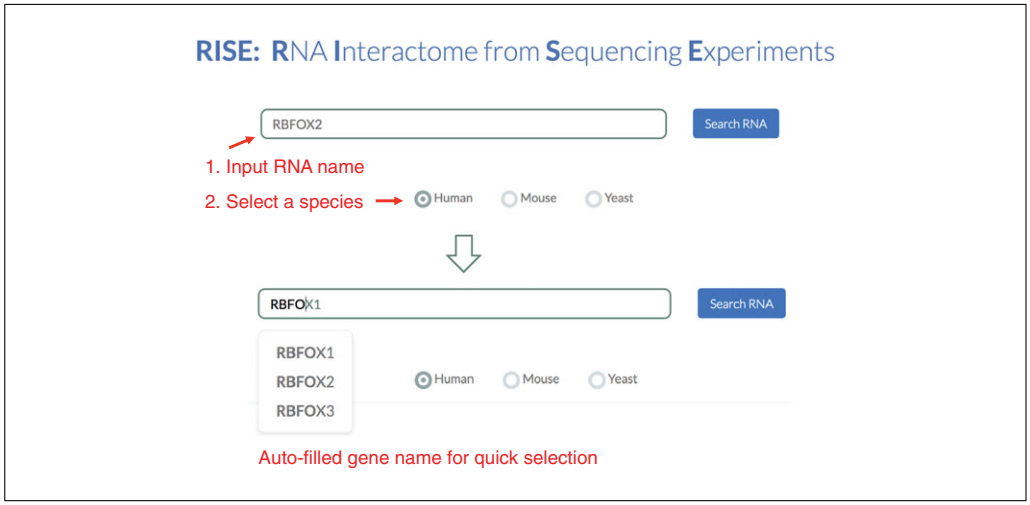



**Figure 4** The search of RRIs for a specific RNA in RISE. Users need to enter a query RNA name and select the species ("Human" is the default species).

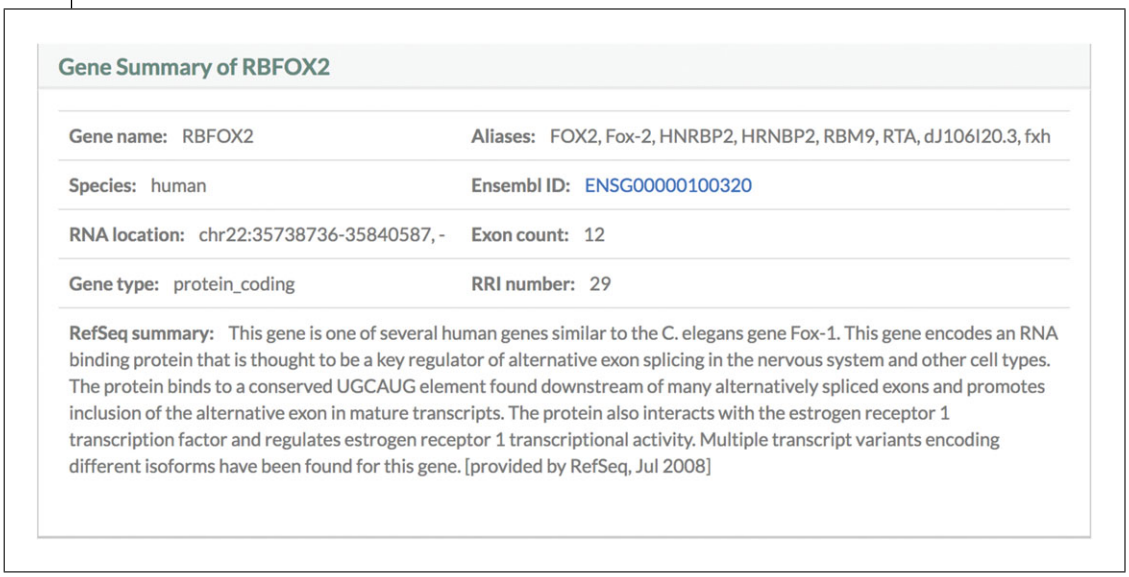



**Figure 5** The gene summary section in a result page. The table provides the basic information of the query gene RBFOX2.

### *Overview of RRIs by Circos plot*

This section provides an integrative visualization of the query gene using a Circos plot, which has been widely used for genomics visualizations (Krzywinski et al., 2009). Here, we show the RRIs between RBFOX2 and its partners (Fig. 6). When exploring the results, users can always adjust their view using the buttons on the upper left. Users can zoom in/out and move the plot by clicking the ± buttons and the four arrows for four different directions, respectively. The plot can be reset to its original state by clicking the refresh button. The Circos plot contains six functional annotation tracks to help users explore the genomic context of the interacting regions, and study the potential regulatory roles of the RRIs. Annotations are encoded using different colors in each track. Users can hold the mouse on the label in the track, and a floating hint box with detailed explanation of the label will appear (Fig. 7). Here, we give a brief introduction on each track using this example:

4. *Gene name*: In this track, the query gene is in bold black, and labeled with asterisks (i.e., `***RBFOX2***`). Different types of genes (e.g., protein-coding, lncRNA,

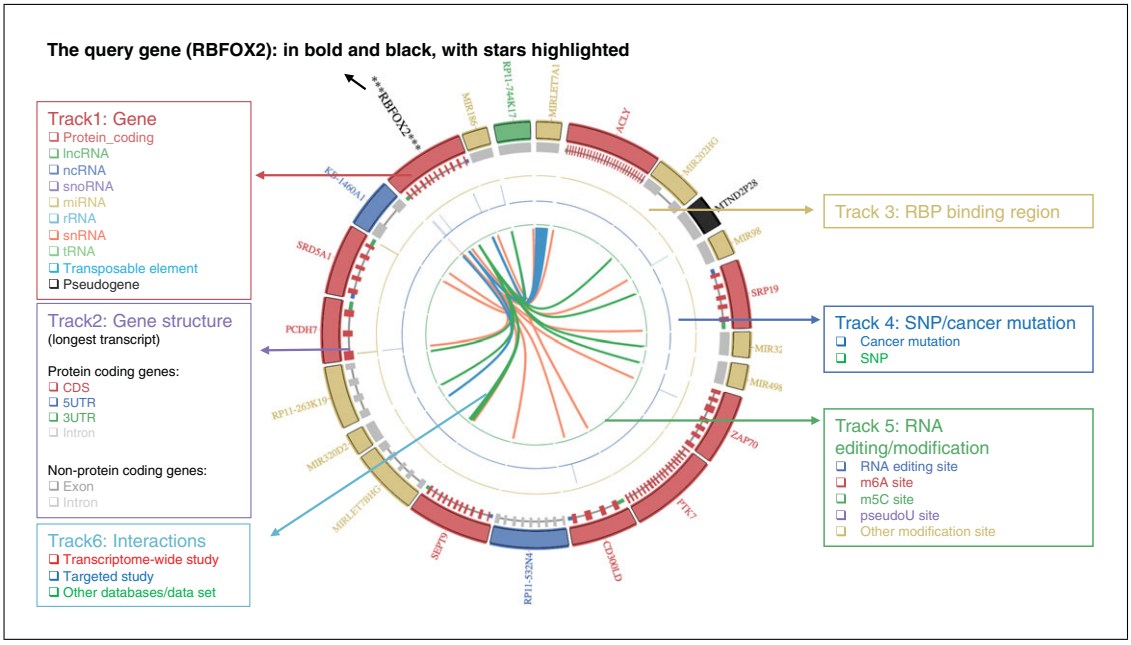



**Figure 6** Interactive visualization of RRIs and annotations using a Circos plot. From the outside to the inside, the six tracks are: (i) gene name, (ii) gene structure, (iii) RBP binding sites, (iv) SNPs/pan-cancer mutations, (v) RNA modification/editing sites, and (vi) RNA-RNA interactions. The track legends list different types of available elements.

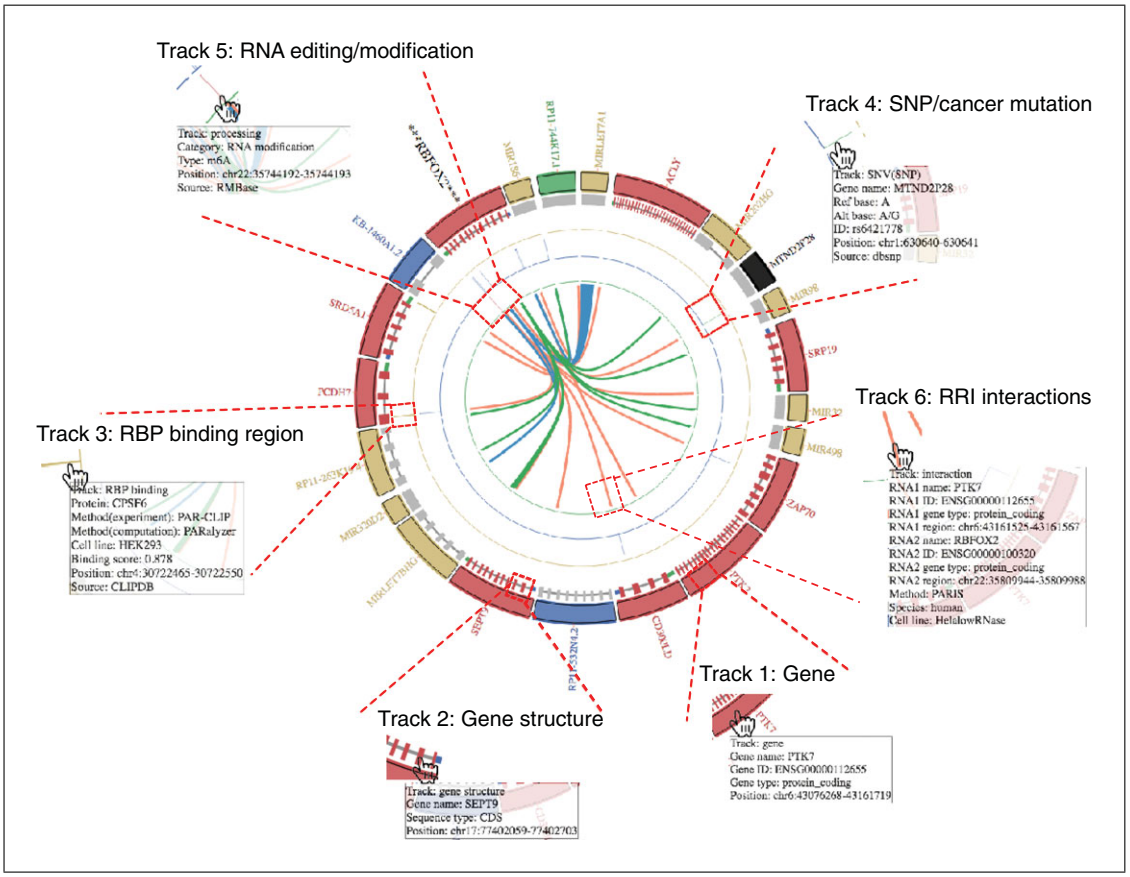



**Figure 7** Exemplified floating box to describe the feature label under each track.

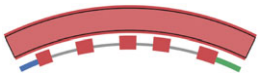
| Gene structure |  | | |  | | |
|---|---|---|---|---|---|---|
| Genomic element | Start coordinate | End coordinate | Length (bp) | Start coordinate | Length (bp, $log_2$ scaled) | End coordinate |
| 5' UTR | 112,861,222 | 112,861,376 | 153 | 112,861,222 | 8 | 112,861,229 |
| CDS 1 | 112,861,377 | 112,861,417 | 41 | 112,861,230 | 6 | 112,861,235 |
| Intron 1 | 112,861,418 | 112,862,507 | 1,090 | 112,861,236 | 11 | 112,861,246 |
| CDS 2 | 112,862,508 | 112,862,583 | 76 | 112,861,247 | 7 | 112,861,253 |
| Intron 2 | 112,862,584 | 112,864,456 | 1,873 | 112,861,254 | 11 | 112,861,264 |
| CDS 3 | 112,864,457 | 112,864,528 | 72 | 112,861,265 | 7 | 112,861,271 |
| Intron 3 | 112,864,529 | 112,864,620 | 92 | 112,861,272 | 7 | 112,861,278 |
| CDS 4 | 112,864,621 | 112,864,732 | 112 | 112,861,279 | 7 | 112,861,285 |
| Intron 4 | 112,864,733 | 112,867,403 | 2,671 | 112,861,286 | 12 | 112,861,297 |
| CDS 5 | 112,867,404 | 112,867,534 | 131 | 112,861,298 | 8 | 112,861,305 |
| 3' UTR | 112,867,535 | 112,869,788 | 2,254 | 112,861,306 | 12 | 112,861,317 |

**Figure 8** The conversion of the width of different genomic elements from raw coordinates to $log_2$ scaled for a transcript of SRP19 (ENSG00000153037).

ncRNA, snoRNA, miRNA, rRNA, snRNA, tRNA, pseudogene, transposable element, etc.) are assigned with different colors.

5. *Gene structure*: This track depicts the details of the gene structure of the involved RNA using its annotation from Ensembl. For genes with multiple isoforms, the longest transcript is displayed here. Each gene structure element is shown as a block with different colors and varied width. The length of transcripts depicted here is on a $log_2$ scale. To explain how we did length scaling, we provide an example showing a transcript of SRP19, which contains five exons and four introns (Fig. 8).

6. *RBP (RNA binding protein) binding*: This track provides RBP binding information of the interaction regions. The RBP binding sites located in any interacting regions will be shown with a yellow segment. In this example, there are two RBP binding sites located in SRD5A1 and PCDH7, respectively.

7. *SNP/mutation site*: This track displays SNPs and Pan-cancer mutations in green and blue segments. Each segment represents a SNP/mutation site overlapping with interacting regions.

8. *RNA editing/modification site*: This track contains RNA editing/modification sites within interacting regions, classified into five different types: RNA editing, $m^6A$, $m^5C$, pseudoU, and others. Each type is represented by segment with different colors. In this example, there is a $m^6A$ modification site in an interacting region of RBFOX2.

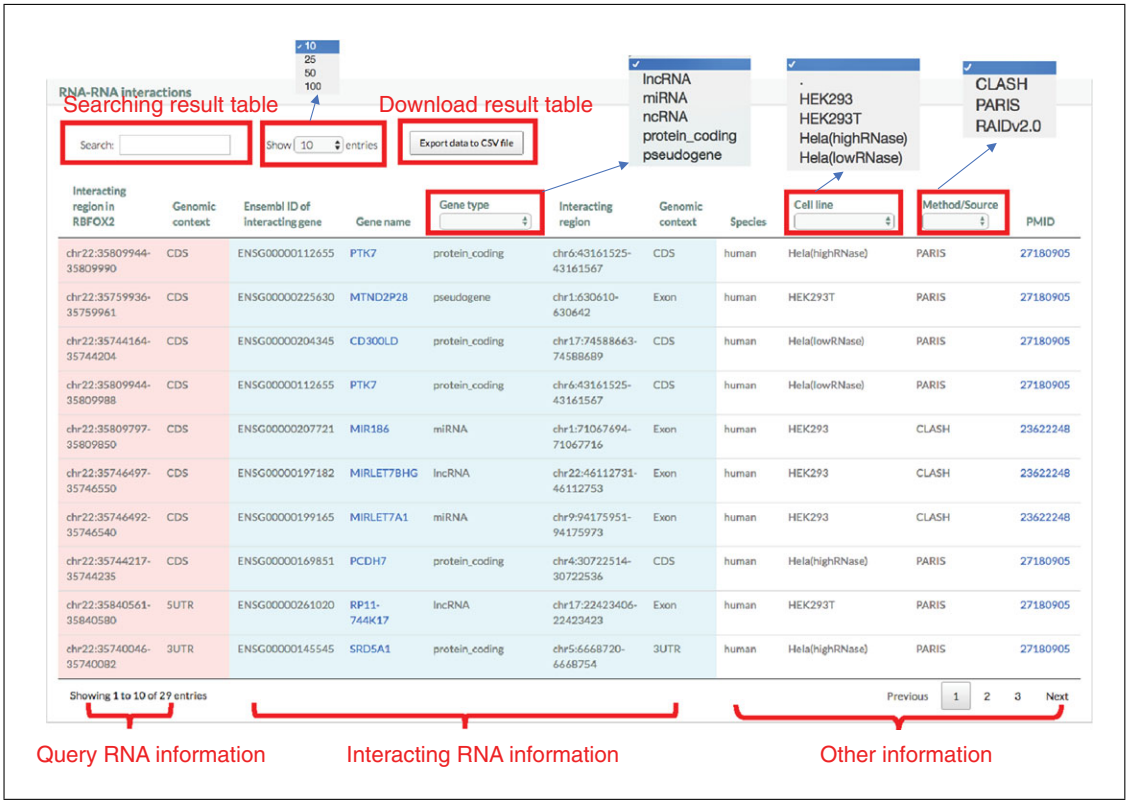



**Figure 9** The integrated table view of the RRIs for a query RNA, RBFOX2. The table provides regional details of the query RNA and the interacting RNA partner. See text and next few figures for details.

9. *RNA-RNA interaction*: This track illustrates the links connecting the interacting regions of the query gene and its interaction partners. The colors of the links are assigned according to their sources, i.e., transcriptome-wide sequencing studies (red), targeted sequencing studies (blue), and other databases or datasets (green).

10. For all these six tracks, find a detailed description of the annotations by holding the mouse on the blocks, segments, or interaction links (Fig. 7). Taking the interacting region as an example, a floating window will appear when putting the mouse cursor over the link. The information is given in the following order: RNA name, Ensembl gene ID, gene type, and duplex region of two interacting RNAs, together with experimental method, species, and cell line.

### *RRI table*

This section provides details for the query gene and all RRIs involving this gene (Fig. 9). Above the table is a search box with a selection box to set the number of entries shown on each page, and a data export button.

11. Use the search box to filter the results. For example, to check whether another RNA (e.g., TP53 and MIRLET7A1) has specific interactions with the query RNA (i.e., RBFOX2), the user can input the target in the search box (Fig. 10). Searching with more than one term is also supported, in which case users need to type multiple terms separated by spaces, thus keeping entries that meet all query terms. The table will be refreshed in real time, automatically changing the displayed entries. If there are a large number of queries, users can adjust the number of entries in the selection box to view more in one page.

12. Download the result table by clicking "Export data to CSV file" for more downstream analysis (Fig. 10).

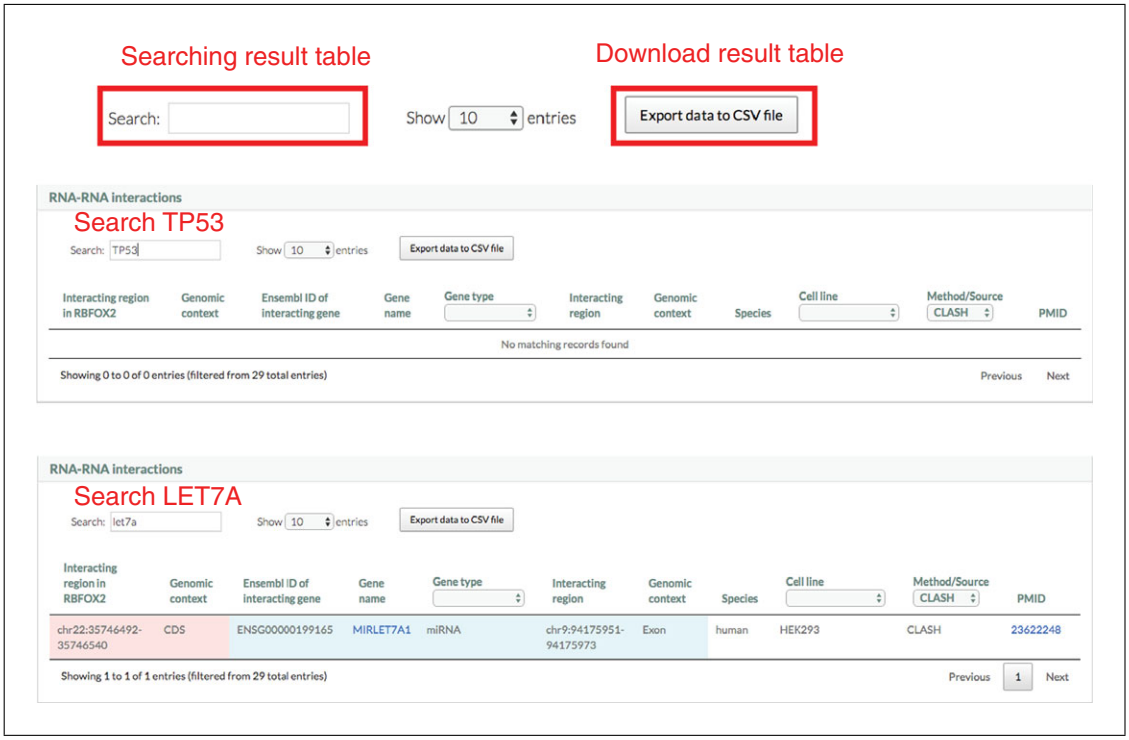



**Figure 10** Two filtering examples in the RRI table of RBFOX2 search result. In this case, TP53 (upper) does not interact with RBFOX2 while MIRLET7A1 (bottom) does.

This RRI table (Fig. 9) contains eleven columns shown in three different background colors: (i) the first two columns (with red background) denote the query RNA information; (ii) the third to seventh columns (with blue background) denote the RNA information of interacting partners; and (iii) eighth to the last columns (with white background) denote other information. The first part includes the interacting regions of the query gene (i.e., RBFOX2) and its genomic context. The second part indicates the Ensembl gene ID of the interacting partner gene, the gene name, the gene type, the interacting region, and genomic context. The third part includes species, cell line, experimental method/source, and PMID. Among these eleven columns, "Gene type," "Cell line" (note that the RRIs from other databases do not have cell line information), and "Method/source" have their own selection buttons.

13. Filter the search results by clicking on these buttons, and choose one from the floating selection box.

14. Finally, click a gene in the "Gene name" column to be directed to the result page of this gene. In addition, users can access the reference paper in PubMed by clicking the PMID in the "PMID" column.

## *BASIC PROTOCOL 2*

## EXPLORING FUNCTIONAL ANNOTATIONS

To facilitate functional exploration of interacting regions, RISE provides multiple types of molecular annotations: (i) RBP binding, (ii) RNA editing, (iii) RNA modification, (iv) SNP, (v) pan-cancer mutation, and (vi) gene expression level.

### *Necessary Resources*

*Hardware*

A computer with internet access

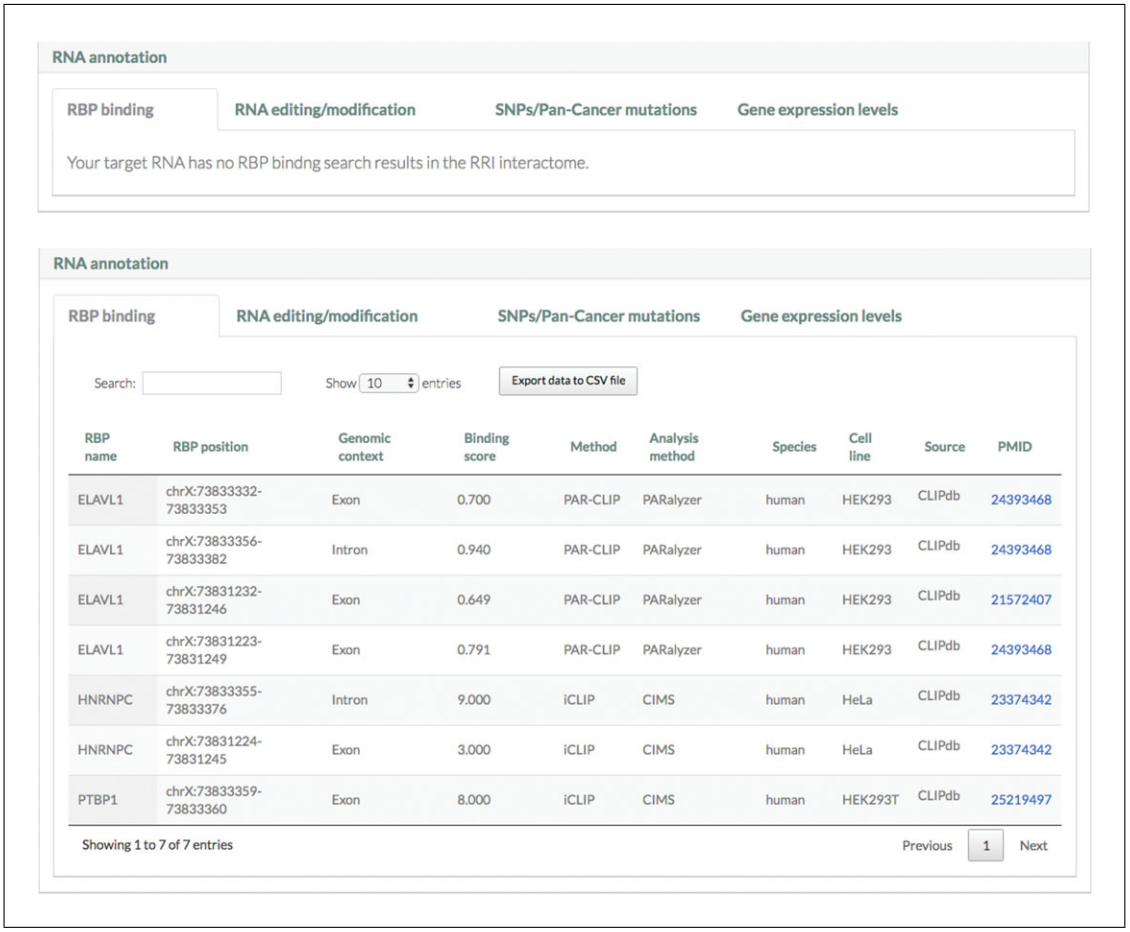



**Figure 11** The "RBP binding" annotation sub-module for two query RNAs, RBFOX2 (top) and XIST (bottom).

*Software*

Any up-to-date web browser (e.g., Firefox, Chrome, Opera, Safari, Internet Explorer)

*Files*

None

### *Functional annotations of RRIs*

1. Use the "RBP binding" module to identify RBP binding sites located in the interacting regions (Fig. 11). In the case of RBFOX2, there are no RBP binding sites in the interacting regions. Here, we demonstrate the usage of this sub-module with another transcript, XIST, with interacting regions covering several RBP binding sites. From left to right, the table shows the name of the RBP, the position of the RBP binding region on the gene, the genomic context of the binding site, the binding score calculated in CLIPdb (Yang et al., 2015), the CLIP-seq experimental method to detect the binding site, the computational method for binding site identification, the species, the cell line, the source database (i.e., CLIPdb), and the PMID of the reference that describes the experiment.

2. Use the "RNA editing" sub-module, which shows RNA editing sites located in the RRI regions (Fig. 12). Again there is no RNA editing site in its RRI regions for RBFOX2. So we use another example, TP53, whose interacting regions cover three RNA editing sites. There are five columns in the RNA editing table, including the type of RNA editing (i.e., the change of base before and after editing), the editing

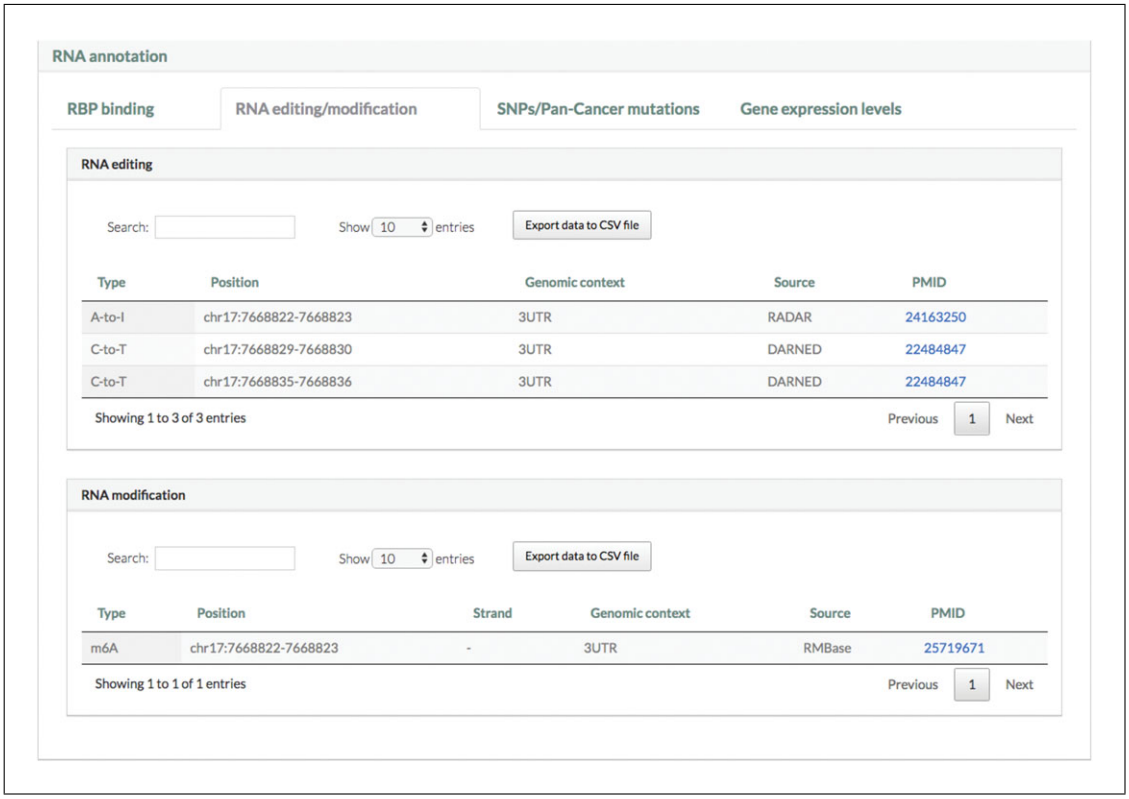



**Figure 12** The "RNA editing/modification" annotation sub-module for a query RNA, TP53.

site position, the genomic context of the editing site, the source database, and the PMID of the reference that describes the experiment.

3. Use the "RNA modification" sub-module to show RNA modification sites located in the interacting regions (Fig. 12). There are six columns in this table, including modification type ($m^6A$, $m^5C$, pseudoU and others), the modification site position, the strand (+ or −), the genomic context, the source database, and the PMID of the reference that describes the experiment.

4. Use the "SNP" sub-module to identify SNPs located in the interacting regions (Fig. 13). Here again, we show the example from TP53. There are six columns in this table, including the SNP position, the genomic context, the reference base, the alternative base, the source database (i.e., dbSNP), and the PMID of the reference.

5. Explore the "pan-cancer mutation" sub-module, which provides cancer somatic mutations located in the interacting regions (Fig. 13). This sub-module includes somatic mutations from more than 40 cancer types. There are seven columns in this table, including the coordinate of the mutation site, the genomic context of the mutation site, the reference base, the mutated base, the cancer type (i.e., full name and its abbreviation), the source (i.e., where the mutation comes from), and the PMID of the reference.

6. The "gene expression level" sub-module provides the expression levels of the query gene across various cell lines and tissue types. We provide expression levels from 34 cell lines and tissue types in human, and 18 cell lines and tissue types in mouse. Expression levels are shown in a bar plot. The *x* axis represents cell and tissue types in the corresponding species, and the height of a bar represents the expression level (FPKM value). Check the FPKM value of a certain cell and or a tissue type by holding the mouse on the bar. The data source is showed below the bar plot with a hyperlink pointing to the paper in PubMed. (Fig. 14).

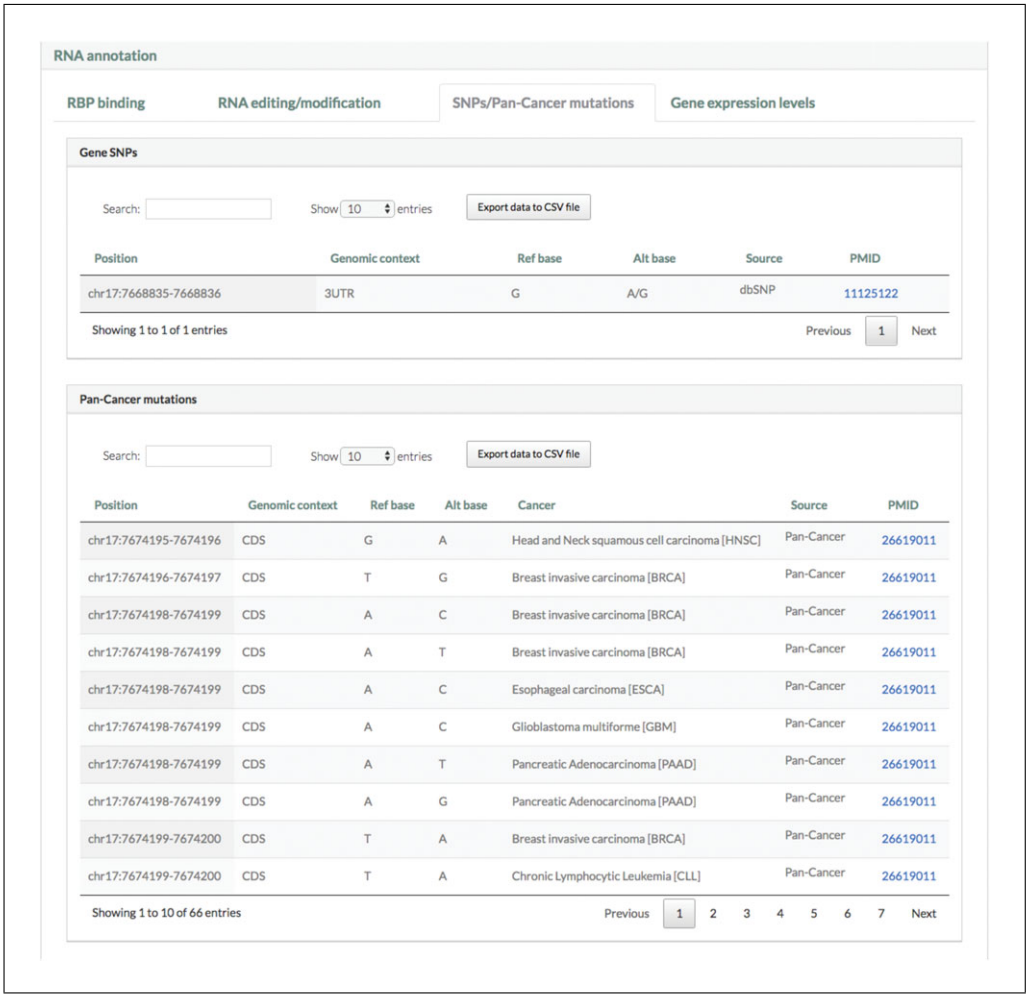



**Figure 13** The “SNP/pan-cancer mutation” annotation sub-module for a query RNA, TP53.

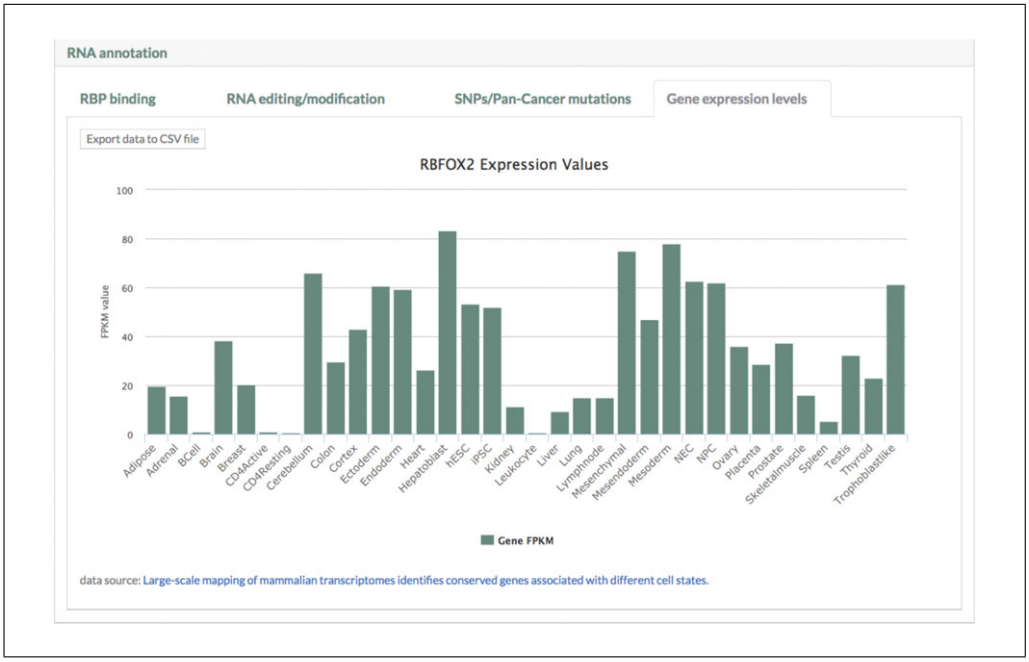



**Figure 14** The “gene expression level” annotation sub-module for a query RNA, RBFOX2.

## COMMENTARY

### Background Information

Databases that aim at collecting RRIs have been developed in recent years, including NPInter v3.0 (Hao et al., 2016), RAID v2.0 (Yi et al., 2017), and RAIN (Junge et al., 2017). The main limitation of these databases is that the RRIs in them are largely miRNA related. In addition, they usually contain little information on the collected RRIs, such as the cell and tissue types, the experimental methods, etc. NPInter is more informative, but still only provides limited molecular annotations for the RRIs. RISE differs from these existing similar databases mainly in two aspects: (i) RISE provides a comprehensive view on RRIs of diverse RNA types; (ii) RISE annotates RRIs with extensive functional annotations, including RNA editing/modification sites, pan-cancer mutations, and gene expression level, etc.

As mentioned above, there have been several sequencing-based experiments for transcriptome-wide RRI detection using different protocols. It is thus interesting to perform systematic comparison on the RRIs identified from different experiments. Indeed, we found substantial heterogeneity in the RRI datasets derived from different experiments (Gong et al., 2018). We observed limited overlaps of RRIs among different experimental technologies even for the same cell line. These results may suggest the dynamic and cell-specific nature of RNA interactions—and they also suggest that different sequencing-based experiments may have certain biases towards the RRIs they can identify. They may also indicate that the RRIs from these sequencing-based experiments are far from saturation. In the future, we need more powerful technologies and more comprehensive datasets to derive a complete understanding of the RNA interactions.

### Troubleshooting

RISE was implemented with Hyper Text Markup Language (HTML), Cascading Style Sheets (CSS), and Hypertext Preprocessor (PHP), and has been tested on all major web browsers. Currently, RISE does not support searching with gene IDs or genomic coordinates. We also suggest that users try more gene aliases for failed searches. The gene aliases can be accessed at *https://www.ncbi.nlm.nih.gov/gene*.

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