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Database issue

eRNAbase: a comprehensive database for decoding the regulatory eRNAs in human and mouse

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Abstract

Enhancer RNAs (eRNAs) transcribed from distal active enhancers serve as key regulators in gene transcriptional regulation. The accumulation of eRNAs from multiple sequencing assays has led to an urgent need to comprehensively collect and process these data to illustrate the regulatory landscape of eRNAs. To address this need, we developed the **eRNAbase** (http://bio.liclab.net/eRNAbase/index.php) to store the massive available resources of human and mouse eRNAs and provide comprehensive annotation and analyses for eRNAs. The current version of eRNAbase cataloged 10 399 928 eRNAs from 1012 samples, including 858 human samples and 154 mouse samples. These eRNAs were first identified and uniformly processed from 14 eRNA-related experiment types manually collected from GEO/SRA and ENCODE. Importantly, the eRNAbase provides detailed and abundant (epi)genetic annotations in eRNA regions, such as super enhancers, enhancers, common single nucleotide polymorphisms, expression quantitative trait loci, transcription factor binding sites, CRISPR/Cas9 target sites, DNase I hypersensitivity sites, chromatin accessibility regions, methylation sites, chromatin interactions regions, topologically associating domains and RNA spatial interactions. Furthermore, the eRNAbase provides users with three novel analyses including eRNA-mediated pathway regulatory analysis, eRNA-based variation interpretation analysis and eRNA-mediated TF–target gene analysis. Hence, eRNAbase is a powerful platform to query, browse and visualize regulatory cues associated with eRNAs.

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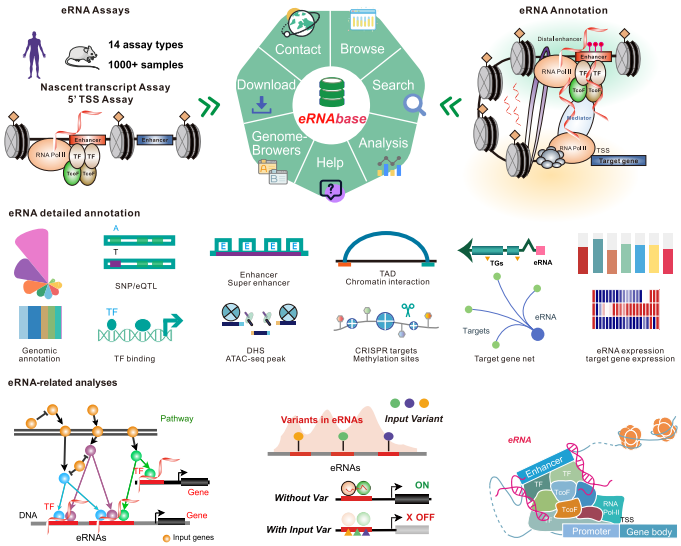
Graphical abstract



Introduction

Enhancers are distal *cis*-regulatory element that couples with promoters to form the enhancer-promoter regulation circuit to trigger cell-specific gene transcription patterns (1,2). Emerging evidence has revealed that active enhancers, especially super enhancers, possess transcription potentials, generating bi-directional non-coding transcripts defined as enhancer RNAs (eRNAs) (3–5). Previous studies reported that eRNAs with cell-specific expression manner played crucial regulatory roles in complex diseases (6). For instance, *CCAT1* eRNA within the oncogenic super-enhancer can coordinate the trafficking of the *MYC* gene to promote colon cancer cell proliferation (7). The overexpression of *NET1e* in cancer cells can promote tumor growth and induce resistance to anticancer compounds in breast cancer (8). Mechanistically, increasing evidence suggests that eRNAs mediate gene transcription in conjunction with transcription regulators and genetic variants. For instance, selective gene regulation programs of the transcriptional activator *PAX3-FOXO1* were determined by enhancer architecture and eRNA transcription from the super enhancer region (9). eRNA can act as a scaffold to activate *YAP/TEAD ER*-bound enhancers in breast cancer (10). eRNAs transcribed from enhancers are enriched for context-specific genetic variants, which can alter enhancer activities and contribute to pathogenesis (11–13). Moreover, studies demonstrated the regulatory roles of eRNAs in histone modification, chromatin accessibility and gene expression by constructing a chromatin loop, thereby communicating regulatory cues from enhancers to their target gene (14,15). These studies highlight the crucial role and significance of eRNA-mediated regulation, especially in cell-specific pathological processes, cancer cell proliferation and oncogenesis.

Numerous eRNA-related databases have been developed recently to investigate the biological significance of eRNAs. For instance, HeRA and Animal-eRNAdb focus on detecting eRNAs expressed from well-annotated enhancer regions (16,17). Based on this strategy, Prof. Han's group also elaborated on the pan-cancer eRNA regulatory atlas in eRic and GPIeR (8,18). However, eRNAs show low-abundance characteristics in cells. Typical RNA-seq captures eRNAs with extremely low efficiency. Two categories of assays (TSS and nascent transcript assays) have been developed to fill this gap (19). HACER, PINTS and FANTOM5 provide a list of human eRNAs from these genome-wide RNA-seq assays, such as CAGE and GRO/PRO-seq (20,21). While these advanced databases have promoted eRNA research, they also face limitations, especially concerning the eRNA identification method and scale. More importantly, only a small subset of annotation information was provided in above databases, the comprehensive genetic and epigenetic annotation information of eRNAs is lacking. Because of the rapid accumulation of eRNA-associated high-throughput data from multiple experiments (e.g. Bru-seq, BruChase-seq, mNET-seq, NET-CAGE and PRO/GRO-cap), there is an urgent need to comprehensively collect and effectively process these data (22–26). Numerous studies showed that eRNAs as well as the related variants and TFs, have a strong influence on human diseases and biological processes (27–29). Integrating abundant (epi)genetic annotation and regulatory analyses will be extremely useful for elucidating the mechanism of action of eRNAs on transcriptional regulation. Therefore, a resource for eRNAs that provides comprehensive eRNA annotations and eRNA-associated regulatory analyses is highly desirable.

In this study, we developed the eRNAbase (http://bio.liclab.net/eRNAbase/index.php) to document a large number of available resources of human and mouse eRNAs and to provide comprehensive annotation and analyses for eRNAs. The current version of eRNAbase cataloged 10399928 eRNAs from 1012 samples including 858 human samples and 154 mouse samples. These eRNAs were first identified from 14 eRNA-related experiment types manually collected from GEO/SRA and ENCODE, including Bru-seq, CAGE, BruChase-seq, PRO/GRO-seq, PRO/GRO-cap, mNET-seq, NET-CAGE, csRNA-seq, BruUV-seq, Start-seq, CoPRO and RAMPAGE. Importantly, eRNAbase provides detailed and abundant (epi)genetic annotations in eRNA regions, such as super enhancers, enhancers, common single nucleotide polymorphisms (SNPs), expression quantitative trait loci (eQTL), risk SNPs, transcription factor binding sites (TFBSs), CRISPR/Cas9 target sites, DNase I hypersensitivity sites

(DHSs), chromatin accessibility regions, methylation sites, chromatin interactions regions, topologically associating domains (TADs) and RNA spatial interactions. Furthermore, eRNAbase provides users with three types of eRNA regulatory analyses, including eRNA-mediated pathway regulatory analysis, eRNA-based variation interpretation analysis and eRNA-mediated TF–target gene analysis. eRNAbase is a user-friendly database to query, browse and visualize information associated with eRNAs (Figure 1).

Materials and methods

Data processing and eRNA identification

In the current eRNAbase, we manually collected 1012 eRNA sequencing samples, including 858 human samples and 154 mouse samples. These samples were firstly collected from 14 eRNA-related experiments from GEO/SRA and ENCODE with the keywords, including 'Bru-seq, CAGE, BruChase-seq, PRO/GRO-seq, PRO/GRO-cap, mNET-seq, NET-CAGE, csRNA-seq, BruUV-seq, Start-seq, CoPRO and RAMPAGE' (30–32). To make these datasets more available, eRNAbase performed quality control for sequencing data and used a unified pipeline, named PINTS, to identify eRNAs (19). Briefly, we first used fastp (version: 0.12.4) to evaluate the sequencing quality and filter the low-quality reads with the parameters (-adapter_sequence -low_complexity_filter -l 14 -w 16) (33). Second, STAR (version: 2.7.10b) was used to align the human genome (Hg38) or mouse genome (Mm10) with the following parameters (STAR -runThreadN 16 -outSAMtype BAM SortedByCoordinate) (34). Third, pints_caller (version: 1.1.8) was used to identify bidirectional eRNA peaks from BAM files with the parameters (pints_caller –bam –exp-type -thread 16). Finally, distal eRNA peaks were filtered out using Bedtools (version: 2.30.0) with the parameters (bedtools intersect -a bidirectional_peaks.bed -b promoters.bed -v > bidirectional_peaks.distalTREs.bed). A total of 10399928 eRNAs were identified based on the aforementioned protocols.

Identification of TF binding sites of eRNA genomic loci

Identification of TFs that are recruited to eRNA genomic loci can provide valuable insights into the regulatory roles of eRNAs in transcriptional regulation (35). This study used two strategies to map potential TFBSs on eRNA genome loci via integrating TF ChIP-seq data and motif scanning methods (36). For the first strategy, 51 616 973 nonredundant TF binding peaks from 817 human TF ChIP-seq datasets and 32 985 444 nonredundant TF binding peaks from 648 mouse TF ChIP-seq datasets from multiple cells and tissues were collected from ReMap 2022 (37). Then, we used the genome intersection command of Bedtools to find the overlap regions between eRNAs and TF ChIP-seq peaks. Each overlapped region represented the potential TFBSs on eRNAs. For the second strategy of the motif scanning method, we first integrated the position weight matrices of ∼700 TF motifs from multiple sources, including JASPAR CORE 2020 vertebrates, Homeodomains, Jolma2013, UniPROBE and Wei2010. Then, we used Find Individual Motif Occurrences (FIMO) (version: 5.4.0), which was the constituent of the MEME Suite toolkit, to scan for the motif binding activities within each eRNA region (38). Finally, we identified potential TFs binding to eRNA genomic loci with a strict experiential *P*-value threshold of 1.0E-06 (39).

Moreover, we embedded 5 547 656 human TFBSs and 2 858 356 mouse TFBSs from UCSC genome browser and used the genome intersection command of Bedtools to identify the intersected TFBSs within eRNA regions (40).

Epigenetic and genetic annotation for eRNAs

Genome mutation events and epigenetic state changes of eRNAs were considered key issues in investigating the regulatory mechanisms of diseases from the transcription regulation facet. In this study, we provided abundant epigenetic and genetic annotations for unveiling eRNAs, such as super enhancers, enhancers, accessible chromatins, DHS, 3D chromatin interactions, TFBS, DNA methylation sites, common SNPs, risk SNPs and eQTLs. Furthermore, we also provided the eRNA-related gene editing sites of CRISPR for low-throughput knockout experiments. All the detailed information of (epi)genetic annotations was provided in Supplementary Table S1.

Super enhancer and enhancer

eRNAs are increasingly considered as a critical marker for active enhancers and super enhancers genome wide. Notably, cell-specific gene transcription patterns driver by distal enhancers, especially super enhancers, were demonstrated to play significant roles in maintaining cell identity (6,41). We manually collected and processed H3K27ac ChIP-seq data from GEO/SRA, ENCODE, Roadmap and GGR databases for the super enhancer category annotation. Briefly, we first used Bowtie software to align human (Hg38) or mouse (Mm10) genomes for H3K27ac ChIP-seq data and then used MACS2 software to call H3K27ac binding peaks. ROSE software was used to identify super enhancers by merging H3K27ac-binding peaks. We also collected human and mouse super enhancers from SEA and dbSuper (42,43). As a result, 2 678 273 human super enhancers and 11609 mouse super enhancers were integrated into the current eRNAbase. For enhancer category annotation, we collected the archived enhancer data from EnhancerAtlas, HACER, ENCODE, FANTOM5, DENDB and ENdb, including 14 797 266 human enhancers and 439 092 mouse enhancers (20,21,32,44,45).

Chromatin accessibility regions

Emerging evidence has proved that eRNA regions have high chromatin accessibility, which can recruit transcription regulators to mediate the activation of downstream genes. ATAC-seq and DNase-seq are considered as the golden methods to identify accessible chromatins or DHSs. We collected more than 130 000 000 chromatin accessibility regions from ATACdb, which was developed by our lab, to annotate accessible chromatins for eRNAs. In brief, we manually processed 2723 human and mouse ATAC-seq datasets from GEO/SRA and performed Bowtie2 and MACS2 to call chromatin accessibility peaks. We also collected 69 860 705 human DHSs from 293 samples and 9 802 229 mouse DHSs from 56 samples from ENCODE DNase-seq datasets.

Common SNP/risk SNP/eQTL

Genetic variants, especially SNPs within eRNAs determined eRNA activity and TF binding events, prominently affecting gene regulatory circuit states. eRNAbase provides multiple

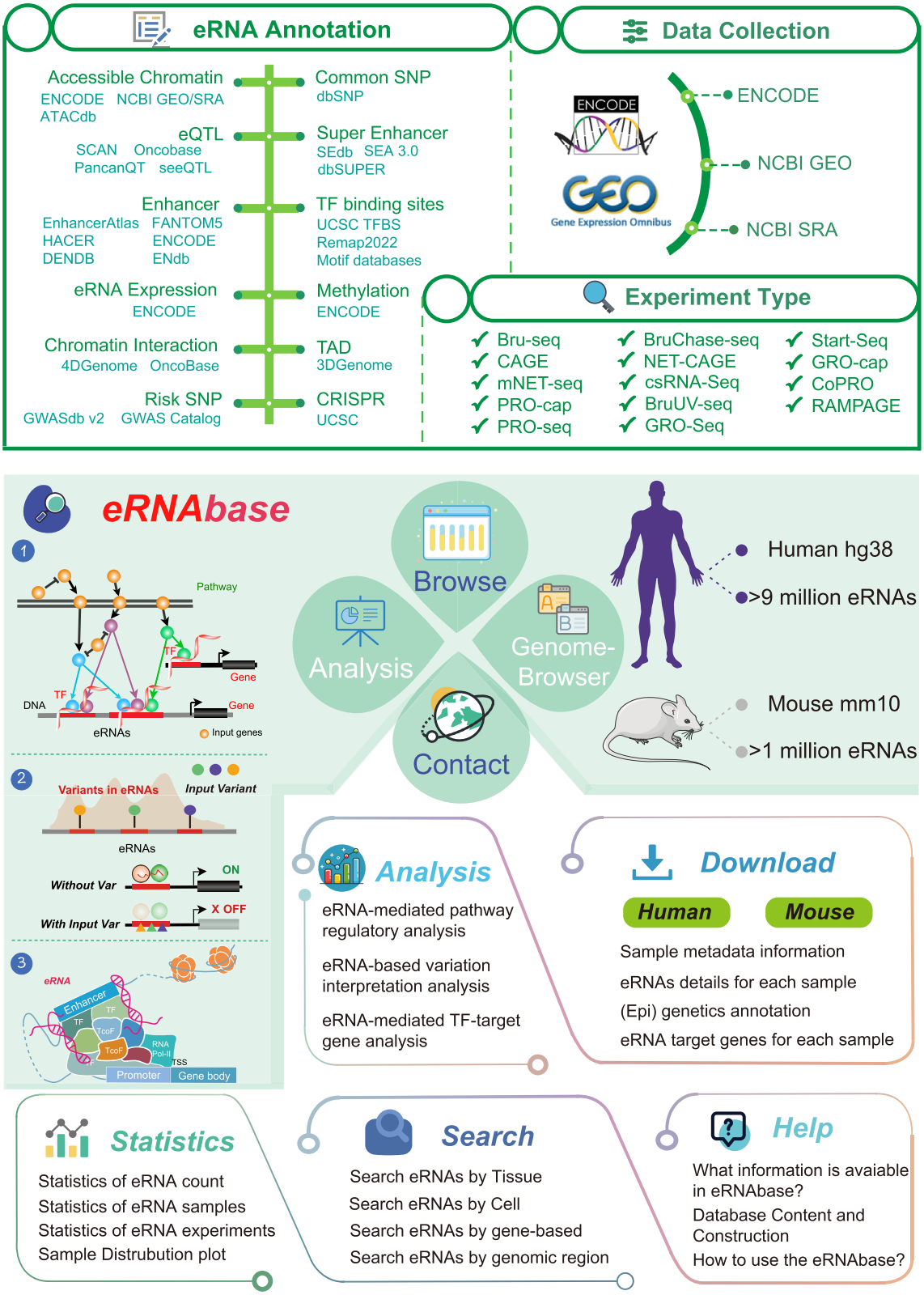


Figure 1. Data collection and construction of eRNAbase. eRNAs of eRNAbase were identified from 14 experiment types manually collected from GEO/SRA and ENCODE. Abundant (epi) genetic annotations, such as super enhancers, enhancers, open chromatins, common SNPs, eQTLs, risk SNPs, TFBS, CRISPR target sites, methylation sites, chromatin interactions regions and TADs. Moreover, eRNAbase contains analysis tools and functions to analyze, query, browse and visualize information associated with eRNAs.

SNP annotation information eRNAs from diverse sources. In detail, 37 302 978 human common SNPs from dbSNP were downloaded and filtered SNPs with a minimum allelic frequency (MAF) > 0.05 out via VCFTools (v0.1.13) (46). A total of 351 728 human risk SNPs from GWAS Catalog and GWASdb v2 were also curated (47). We also collected 11 995 221 human eQTLs from PancanQTL, seeQTL, SCAN, Oncobase and GTEx (48–52). Besides, 81432271 mouse common SNPs from dbSNP were also released in the current eRNAbase. In the analysis panel, we identified the linkage disequilibrium SNPs ($r^2 = 0.8$) for five super-populations (African, Ad Mixed American, East Asian, European and South Asian) via Plink (v1.9).

Chromatin interaction/methylation/RNA spatial interaction

Chromatin loops link the regulatory cues from distal and proximal elements, directing the cell-specific gene transcription (53). In eRNAbase, we collected 34 342 926 human chromatin interactions from 4DGenome and Oncobase, including data from ChIA-PET 3C, 4C, 5C and Hi-C. Furthermore, 72019 human TADs from 21 tissues or cell lines from 3D Genome Browser were embedded (54,55). Dynamic DNA methylation states, which are highly related to chromatin interaction conformation, also have the regulatory effects on eRNA activity (56). The eRNAbase recorded DNA methylation states from 166 855 665 methylation sites of whole-genome shotgun bisulfite sequencing and 30 392 523 methylation sites of the 450k array from ENCODE. Emerging RNA spatial interaction experiment, such as RIC-seq, enables the identification of the specific molecules that interact with eRNAs, shedding new light on the molecular mechanisms of enhancer function and gene regulation. We also collected the human RIC-seq derived eRNA interaction data from published datasets of GSE190214 and GSE221551. Totally, 1 026 628 pairs of RNA interactions from 9 tissues/cells were provided in eRNAbase.

eRNA expression/CRISPR

Cell-specific eRNA expression patterns could help find key regulators in pathological processes. To provide global normalized eRNA expression in tissues, we downloaded the same-batch BAM files of human and mouse total RNA-seq from ENCODE project. Then, we used SAMtools (version: 1.13) and Bedtools to call the read count number and calculate the log-normalized CPM (counts per million) in eRNA regions. The eRNAbase provided eRNA expression in 30 human tissues/cells and 11 mouse tissues. Knockout experiments were the efficient method to investigate the biological functions of RNAs or DNA regulatory elements. We also provided CRISPR/Cas9 target sites. These sites were annotated for predicted specificity (off-target effects) and predicted efficiency (on-target cleavage) using various algorithms through the tool CRISPOR from UCSC.

Assigning target genes of eRNAs

Identifying eRNA downstream target genes is important to reveal the biological function and regulatory mechanisms of eRNAs. We used two strategies (geneMapper and BETA (version: 1.0.7)) based on genome distances to comprehensively annotate the potential target genes (57,58). For the geneMapper method, we input eRNA regions in the bed format file and used a Python script (ROSE geneMapper.py) to assign downstream target genes. The target genes were classified into three strategies including overlap, proximal and closest. For the BETA method, we also input eRNA regions into BETA script and obtained the target genes with distance and score thresholds. The target genes from the aforementioned two methods were integrated and used for further analysis. Furthermore, we provided the target gene expression in humans and mice from GTEx, ENCODE, TCGA, CCLE and FANTOM5.

User interface

Search interface for eRNAs

eRNAbase is a comprehensive platform for users to search, browse, visualize and analyze eRNAs of interest. In the 'Search' interface, eRNAbase provides four query methods for searching eRNAs based on eRNA characteristics and sample information: 'Search by Genomic Region' (input genomic position and select the optional sample metadata), 'Search by Target Genes' (input gene symbols of interest and select species and methods), 'Search by Tissue Type' (input tissue of interest and select species) and 'Search by Cell Type' (input cell type of interest and select species) (Figure 2A and C). Brief information of the search results is displayed in a table on the result page. For instance, for the methods based on eRNA regions or target genes, the result table returned the eRNA information including eRNA ID, eRNA region, sample ID, tissue/cell type and experiment type. For methods based on tissue or cell type, the result table described the basic information of samples such as 'Browse' page, including sample ID, species, tissue type, biosample type, cell type and experiment type (Figure 2B). Users can click the sample ID to obtain the summarized regulatory information for searching the eRNA sample. In the sample page, eRNAbase provides information on sample overview, eRNA genomic annotation, eRNA detail, annotation data source and software parameters (Figure 2D). In the genomic annotation section, eRNAbase provides genomic proportions and peak distribution statistics calculated using ChIPseeker. The eRNA 'Details' section provides comprehensive annotation for eRNAs, including eRNA ID, genome location, size, bidirectional genome location and detailed (epi)genetic information in eRNA regions (Figure 2D). Users can click the eRNA ID to obtain details regarding the eRNA (Figure 2E).

On the 'Details' page, eRNAbase provides six sections to list the comprehensive annotation for eRNAs: eRNA overview, eRNA annotation, eRNA-associated genes, TFs binding to eRNAs, overlap with other eRNAs and eRNA expression (Figure 2E). In the section of eRNA annotation, eRNAbase provides a large amount of (epi)genetic annotation information, including super enhancers, enhancers, common SNPs, risk SNPs, CRISPRs, eQTLs, TFBS, DHS, DNA methylation sites, TAD, 3D chromatin interactions and RNA spatial interactions. Alternatively, for the results of searches by eRNA regions or target genes, users can obtain the eRNAs of interest directly.

User-friendly interface for browsing eRNA

The 'Data-Browse' page is organized as an interactive and alphanumerically sortable table that allows users to quickly browse samples and customize filters through 'Species,' 'Tissue type,' 'Biosample Type,' 'Cell Type' and 'Experiment type.'

Figure 2. Main interfaces and usage of eRNAbase. **(A)** The navigation bar of eRNAbase. **(B)** Data browse page of eRNAbase. **(C)** Users can search eRNAs of interest via four queries. **(D)** Sample interface of eRNA sets from human K562 cell line, including sample overview, eRNA detail, genome proportion plot, peak distribution plot and chromosome distribution plot. **(E)** Detailed annotation information of eRNAs of interest, including eRNA overview information, annotation statistic, eRNA-mediated network, (epi)genetic annotation interactive table, eRNA associated genes, target gene expression, eRNA expression and TF binding information. **(F)** The eRNAbase provides three online analysis tools to help dissect eRNA-related regulation. **(G)** Genome browser interface. **(H)** Download interface.

Users can use the 'Show entries' drop-down menu to change the number of the displayed records per page (Figure 2A and B). Then, users can click on 'Sample ID' to view the eRNAs for a given sample.

eRNA-related online analysis tool

The eRNAbase provides three analysis tools to help biologists comprehensively explore the biological function and mechanisms of eRNAs (Figure 2F). Pathway analysis is an effective strategy to identify abnormal biological processes of diseases. While TFs are frequently located at the end of pathways, their downstream regulatory events remain unknown. In eRNA-mediated pathway regulatory analysis, users can submit a list of genes of interest to obtain enriched pathways through a hypergeometric test. Within each pathway, the eRNAbase locates the terminal TFs and terminal TF-bound eRNAs, forming genes/pathways/TFs/eRNA regulatory axes. The genetic variants located at eRNAs determine eRNA activity and TF binding affinity, inducing aberrant gene expression programs. In eRNA-based variation interpretation analysis, users can submit a variant ID (rsID) or genomic region (bed format) to eRNAbase to locate the eRNAs that overlapped with the variant or variants within the submitted genomic region. The results from this analysis also displayed the annotation information of submitted variant(s), including genomic coordinates, variant type, linkage disequilibrium SNP and disease information. Moreover, TF binding activity is highly related to enhancer activity and eRNA biogenesis. The eRNAs can also serve as the scaffolds or intermediates for TF–target gene regulation. In eRNA-mediated TF–target gene analysis, users can input paired lists of TFs and genes of interest and then select species. The eRNAbase can identify eRNA-mediated TF-gene pair(s) in different tissue types. Meanwhile, users can set different TF identification methods through the 'Type' option, including motif and ChIP-seq methods.

Genome browser and data download

The eRNAbase uses the JBrowse genome browser to visualize the regulatory information of human and mouse eRNAs by customizing tracks of interest, such as eRNA regions, genes, super-enhancer and variants (Figure 2G). The eRNA regions, eRNA downstream target genes and all annotation data are available for individual or batch download on the 'Download' page. Additionally, we also provided the merged eRNA sets of human and mouse on the 'Download' page. Users can quickly download data of interest (Figure 2H).

Case study

Search for CCAT1 eRNA

Colon cancer-associated transcript 1 (*CCAT1*) is a widely studied eRNA transcribed from the super enhancer region of the 8q24 locus (59,60). Increasing evidence has suggested that *CCAT1* participates in various biological processes, such as cell proliferation, invasion, metastasis and apoptosis via functioning as a regulator in transcription regulation in multiple cancers. We performed a search case to illustrate the regulatory information for *CCAT1* eRNA in eRNAbase. Briefly, we first input *CCAT1* and selected the 'ROSE genemapper' method in the target gene search (Figure 3A and B). The results showed that >4000 eRNA records were searched out, indicating that *CCAT1* was a hot region to produce eRNA transcript (Figure 3C). *CCAT1* was first identified from colon cancers, and then we selected the 'Sample-01–013520459' eRNA in HCT-116 cell lines of colon cancer, which was overlapped with the target gene *CCAT1* (Figure 3C).

The eRNAbase provides comprehensive epigenetic and genetic annotations for eRNAs. Users can click the eRNA ID to explore the potential regulatory information. For example, in the 'Details' page of eRNA 'Sample-01–013520459', the results showed that the 571-bp-long eRNA covered multiple annotations and was high-expressed in HCT-116 cell line (Figure 3D), suggesting that this eRNA might play crucial roles in transcription regulation. Some colon cancer-related TF binding events were also detected within this eRNA, such as *TP53*, *FOXO3s1* and *OCT1* (Figure 3E, upper) (61,62). *CCAT1* was demonstrated as a key super enhancer-driven transcript. The results showed that 327 super enhancers overlapped with this eRNA (Figure 3E, middle). Notably, previous studies reported that *CCAT1* maintained the chromosome loop of *MYC*, promoting the expression of *MYC* (60). In the annotation result of chromatin interaction, some colon-specific validated interactions within *MYC* promoters were identified (Figure 3E, bottom). These results indicated that the eRNAbase has a strong ability to explore the potential regulatory mechanisms for eRNAs.

Search for *NPPA* super enhancer

Super enhancers-mediated chromatin circuits play crucial roles in cell identity and diseases, whereas eRNA signals within super enhancer region can be used to infer its activities. Heart-specific expressed *NPPA* is controlled by a shared super-enhancer (63). We obtained the super-enhancer region of NPPA from SEdb 2.0 and searched eRNAs based on the method of genomic region search (Figure 3F). As a result, four eRNAs in diverse samples overlapped with the input super enhancer (Figure 3G). We selected the eRNAs with eRNA ID 'Sample-01-08170014' as the example. In the eRNA detail page, the results showed that a lot of heart tissue-specific annotation information was enriched in this eRNA region, such as DHS and chromatin interactions, indicating that the eRNAbase could support tissue-specific research (Figure 3H). In addition, *NPPA* were identified by the eRNA-associated gene network. The expression heatmap showed that *NPPA* was highly expressed in heart tissue, which coincided with the biomarker feature of *NPPA*. TF binding to the eRNA module listed the potential binding TFs in eRNA regions, including the validated transcription regulators of *EGR1*, *EP300*, *HDAC1* and *SP1* (Figure 3I) (64–66). The aforementioned results also illustrate the availability and biological value of the eRNAbase.

Discussion

eRNA is a type of noncoding RNA transcribed from distal enhancers, which mediates cell-specific gene expression (67). Numerous studies have revealed that aberrant eRNA expression patterns can disrupt the chromatin circuits of disease genes to promote disease development (68). The eRNA data needs to be collected and processed to investigate the regulatory landscape of eRNA. Some advanced databases have been developed to illustrate eRNA regulation. For in-

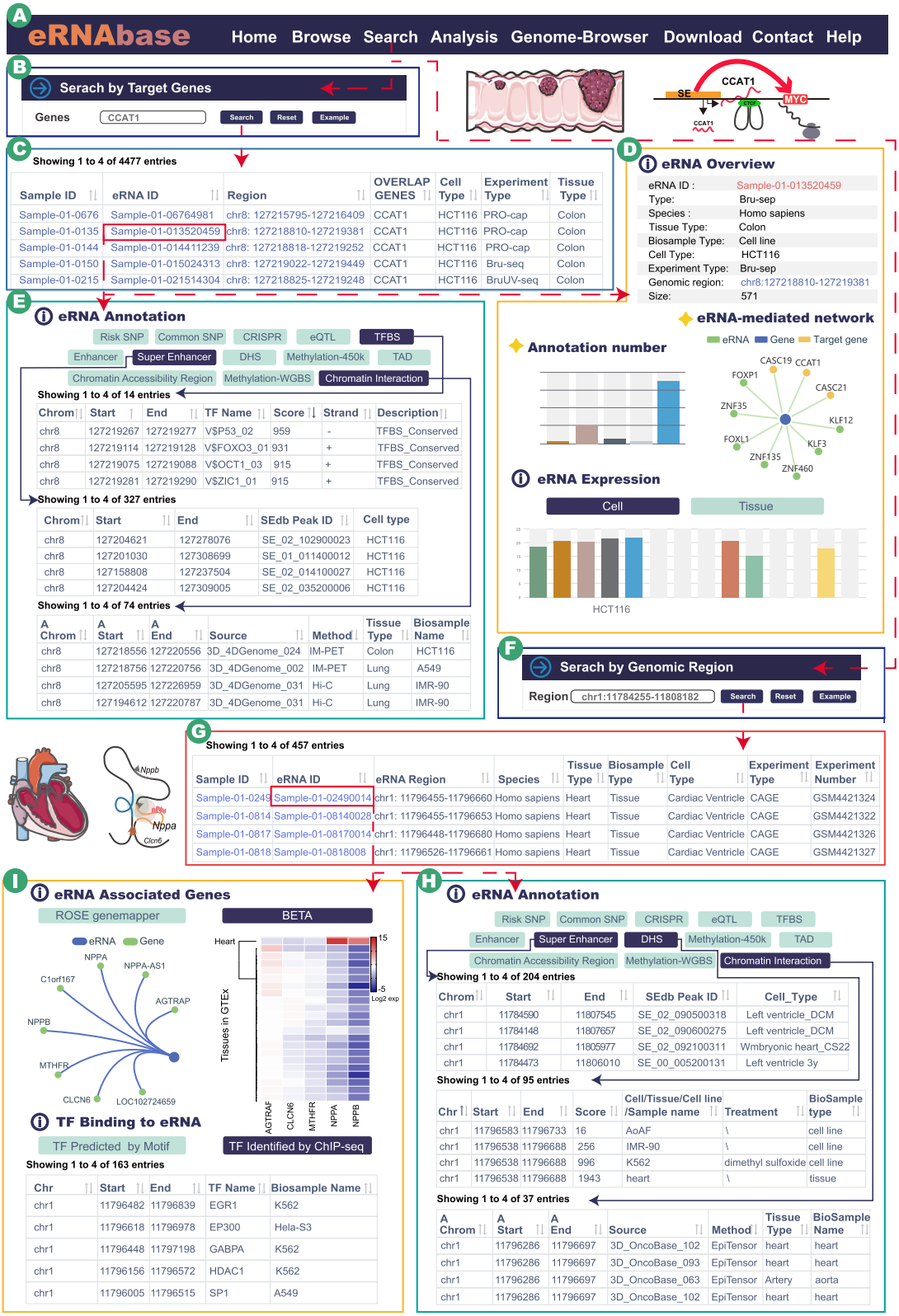


Figure 3. Case study panels of eRNAbase. **(A)** The navigation bar of eRNAbase. **(B)** Case study of *CCAT1* query in target gene search. *CCAT1* is a well-investigated eRNAs that plays key roles in cancers, especially in colon cancer. **(C)** Interactive result table of *CCAT1* search, including the sample ID, eRNA ID, regions, genes and sample metadata information. (D-E) eRNA detailed information of the selected eRNA 'Sample-01–013520459", including eRNA overview, eRNA-mediated network, (epi)genetic annotation interactive table (TFBS, Super enhancer and Chromatin interaction), eRNA associated genes and eRNA expression. **(F)** Case study of *NPPA* super enhancer query in genomic region search. **(G)** Interactive result table of genomic region search. (**H–I**) eRNA detailed annotation information of the selected eRNA 'Sample-01–02490014'.

stance, HACER, FANTOM5 and PINTS cataloged eRNAs derived from nascent transcript sequencing data, including CAGE and GRO/PRO-seq (19–21). Conversely, HeRA and Animal-eRNAdb detected the high-expression transcripts located within enhancer regions from RNA-seq data (16,17). Prof. Han's group also developed eRIC and GPIeR to reveal cancer-related eRNA transcripts (8,18). These databases have promoted eRNA research, but they have limitations, especially regarding eRNA identification methods, scale and annotation. The eRNAbase was highlighted in eRNA scale and annotation (Supplementary Table S2). Briefly, the eRNAbase extends the eRNA scale to >5 times the existing eRNA databases, recording 10 399 928 eRNAs from 1012 samples including 858 human samples and 154 mouse samples. Moreover, abundant epigenetic and genetic annotations within eRNAs are integrated to help users explore potential functions and the regulation of eRNAs in diseases and biological processes. Overall, eRNAbase serves as a powerful platform for eRNA-related regulation enriched with abundant annotation information.

The eRNAbase is a user-friendly database to query, browse and visualize information associated with eRNAs, which has multiple highlights and advantages compared with the existing databases as follows: (i) eRNAs were first identified from 14 eRNA-related experiment types manually collected from GEO/SRA and ENCODE, including Bru-seq, CAGE, BruChase-seq, PRO/GRO-seq, PRO/GRO-cap, mNET-seq, NET-CAGE, csRNA-seq, BruUV-seq, Start-seq, CoPRO and RAMPAGE; (ii) comprehensive genetic and epigenetic annotations within eRNA regions include TF bindings, super-enhancers, enhancers, common SNPs, eQTLs, CRISPR editing sites, risk SNPs, DHS, DNA methylation sites, 3D chromatin interactions, RNA spatial interactions and TADs; (iii) quality control and unified software parameters are used to process sequencing data; (iv) the visualization function is used to annotate eRNA regions; (v) novel and useful online analysis tools are available to investigate eRNA-mediated pathways, variations and TF regulation; (vi) a customized genome browser provides users with an intuitive view of the regulatory information of eRNA adjacent regions and embeds several annotation tracks and (vii) the user-friendly database displays eRNA regions and associated annotation information with interactive tables.

In summary, the eRNAbase is a comprehensive resource for eRNAs, with the largest scale and the most comprehensive annotation details for both human and mouse eRNAs. The current version of the eRNAbase also provides online analysis tools, allowing researchers to explore regulatory patterns of eRNAs across multi-omics facets. In forthcoming versions of the eRNAbase, we aim to update this useful resource by extending eRNA scales from more experiments (e.g. RNA-seq and single-cell data) and by adding more epigenetic and genetic annotations (e.g. functional variants and somatic mutations). Moreover, the current version of eRNAbase used PINTS to identify eRNAs in all datasets, which was designed for detecting eRNA TSSs and candidate enhancers. This pipeline may lose the information of the entire eRNA transcription unit. We will use more powerful pipelines to identify eRNAs in the next updated versions. We are confident that eRNAbase will emerge as a useful and effective platform for exploring potential functions and the regulation of eRNAs in diseases and various biological processes.

Data availability

The research community can access information freely in the eRNAbase without registration or logging in. The URL of eRNAbase is http://bio.liclab.net/eRNAbase/index.php.

Supplementary data

Supplementary Data are available at NAR Online.

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Conflict of interest statement

None declared.

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