Databases and ontologies

**DASHR 2.0: integrated database of human small non-coding RNA genes and mature products**

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**Abstract**

**Motivation:** Small non-coding RNAs (sncRNAs, <100 nts) are highly abundant RNAs that regulate diverse and often tissue-specific cellular processes by associating with transcription factor complexes or binding to mRNAs. While thousands of sncRNA genes exist in the human genome, no single resource provides searchable, unified annotation, expression and processing information for full sncRNA transcripts and mature RNA products derived from these larger RNAs.

**Results:** Our goal is to establish a complete catalog of annotation, expression, processing, conservation, tissue-specificity and other biological features for all human sncRNA genes and mature products derived from all major RNA classes. DASHR (Database of small human non-coding RNAs) v2.0 database is the first that integrates human sncRNA gene and mature products profiles obtained from multiple RNA-seq protocols. Altogether, 185 tissues/cell types and sncRNA annotations and >800 curated experiments from ENCODE and GEO/SRA across multiple RNA-seq protocols for both GRCh38/hg38 and GRCh37/hg19 assemblies are integrated in DASHR. Moreover, DASHR is the first to contain both known and novel, previously un-annotated sncRNA loci identified by unsupervised segmentation (13 times more loci with 1 678 800 total). Additionally, DASHR v2.0 adds >3 200 000 annotations for non-small RNA genes and other genomic features (long-non-coding RNAs, mRNAs, promoters, repeats). Furthermore, DASHR v2.0 introduces an enhanced user interface, interactive experiment-by-locus table view, sncRNA locus sorting and filtering by biological features. All annotation and expression information directly downloadable and accessible as UCSC genome browser tracks.

**Availability and implementation:** DASHR v2.0 is freely available at https://lisanwanglab.org/DASHRv2.

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**Supplementary information:** Supplementary data are available at *Bioinformatics* online.
important cellular processes and that dysfunctional sncRNAs are associated with a variety of human diseases, including neurodegenerative diseases and cancers (Goodarzi et al., 2016; Li et al., 2016; Martens-Uzunova et al., 2013; Ng et al., 2016; Salta and De Strooer, 2017; Soares and Manuel, 2017; Steinbusch et al., 2017; Valen et al., 2011). These sncRNAs include not only the commonly studied microRNAs, but also small nucleolar and small nuclear RNAs (sno/snRNAs) (Steinbusch et al., 2017), Piwi-interacting (piRNAs) (Ng et al., 2016), transfer RNAs (tRNAs) (Goodarzi et al., 2016; Li et al., 2016), newly discovered classes such as tRNA fragments (Soares and Manuel, 2017), as well as sncRNAs derived from long non-coding RNAs (lncRNAs) (Martens-Uzunova et al., 2013; Salta and De Strooper, 2017; Soares and Manuel, 2017) and promoter regions (Valen et al., 2011). Thus, there is a strong need to systematically integrate and process expression data measuring diverse types of sncRNAs from different RNA-seq protocols and data sources including the sequencing read archive (SRA) (Kodama et al., 2012) and ENCODE consortium (Djebali et al., 2012).

The DASHR database aims to provide unified, searchable annotation and expression information for both primary sncRNA transcripts and mature RNA products and across eight major sncRNA classes including microRNAs (miRNAs), Piwi-interacting (piRNAs), small nuclear, nucleolar, cytoplasmic (sn-, sno-, scRNAs, respectively), transfer (tRNAs), tRNA fragments (tRFs) and ribosomal RNAs (rRNAs).

The current release of DASHR (v2.0) integrates >800 high-throughput sequencing datasets, both manually collected and curated from GEO/SRA (Kodama et al., 2012) and from ENCODE (Djebali et al., 2012; Sloan et al., 2016), with over 22 billion reads. DASHR v2.0 contains >133 000 annotation records for small RNA genes and mature sncRNA products and >1 680 000 detected sncRNA loci across 185 tissues and cell types for both GRCh37/hg19 and GRCh38/hg38 genomes. For all sncRNAs, annotations and expression data can be searched, browsed and downloaded. DASHR v2.0 will aid the broader scientific community in exploring both the genomic landscape of sncRNA abundance and processing and individual sncRNAs across tissues cell types.

2 Materials and methods

2.1 Database overview

Table 1 summarizes contents and features provided by DASHR v2.0. Some major new features and contents include:

1. 365 more experiments across 34 smRNA-seq studies from GEO/SRA and integration of 240 short total RNA-seq experiments from ENCODE (Fig. 1A), increasing the coverage of various tissues/cell types (185 total) with a total of 802 integrated small RNA sequencing experiments;
2. sncRNA gene and mature product annotations for GRCh37/hg19 and GRCh38/hg38 (Fig. 2A–C) genome assemblies;
3. integration of biological features of sncRNAs including evolutionary conservation, co-localization with other genomic features, and tissue specificity;
4. integration of biological features of sncRNAs including evolutionary conservation, co-localization with other genomic features, and tissue specificity;
5. novel, previously unannotated sncRNA loci consistently detected across tissues and cell types (Fig. 2E);

Table 1. Advances and improvements provided by DASHR v2.0

| Features                        | DASHR v1.0                          | DASHR v2.0                          |
|---------------------------------|-------------------------------------|-------------------------------------|
| **Release date**                | August 2015                         | September 2017                      |
| **Genome Assembly**             | GRCh37/hg19                         | GRCh37/hg19                         |
| **Data collection:**            |                                     |                                     |
| Curated GEO/SRA experiments     | 42                                  | 197 DASHR1-GEO                      |
| ENCODE experiments              | 0                                   | 365 DASHR2-GEO                      |
| **sncRNA genes and mature products** | 48 075                              | 72 ENCODE-GEO                       |
| Non-small RNA genes and mature products | 0                                   | 168 ENCODE-portal                   |
| **Annotated sncRNA loci**       | 84 514                              | 68 135                              |
| **Unannotated sncRNA loci**     | 0                                   | 1 469 297                           |
| **Biological features of sncRNAs** | Expression and specificity           | Expression, 5p specificity, conservation, tissue specificity, co-localization within regions of interest |
| Enhanced web interface feature  | –                                   | Experiment-by-loci table per data collection – filter sncRNA products by features |
| Compare hg19 and hg38 results   | –                                   | Allow users to compare the genomic contexts of sncRNAs in both hg19 and hg38 |
6. the ability to compare data and annotations across GRCh37/hg19 and GRCh38/hg38 (Fig. 3A);
7. interactively browse and filter sncRNA loci by one or more features, including expression, processing specificity, conservation scores and tissue specificity (Fig. 3B); and an enhanced web interface (Fig. 3A–B, Supplementary Figs S1–S3).

DASHR v2.0 is substantially more comprehensive than existing non-coding RNA databases (Supplementary Table S1) (Chung et al.,...
2.2 Data collections

All smRNA-seq experiments integrated into DASHR v2.0 have been organized into four data collections (Table 1, Supplementary Tables S2–S5, Supplementary Methods):

1. DASHR1-GEO data collection - consists of all 197 smRNA-seq experiments originally included in DASHR v1.0 (Leung et al., 2016); to incorporate these experiments into DASHR 2.0, the raw sequencing reads were re-processed for both hg19 and hg38 genome builds and to include additional features introduced in DASHR 2.0 (Table 1; Supplementary Methods);

2. DASHR2-GEO data collection - consists of 365 new Illumina smRNA-seq datasets curated from GEO/SRA (last curation date: August 2017) (Kodama et al., 2012);

3. ENCODE-GEO data collection – consists of all 72 short total RNA sequencing datasets (whole-cell) available in the 2012 ENCODE transcriptome data (GSE24565) (Djebali et al., 2012);

4. ENCODE-portal data collection - consists of all 168 small RNA-seq datasets from ENCODE portal (Sloan et al., 2016)
v2.0 data collection includes significantly more (3× more, 605 new datasets) sncRNA experiments with greatly increased tissue/cell type diversity compared to DASHR v1.0 (Fig. 1A). Overall, 70% of the datasets in DASHR v2.0 were derived from experiments performed on tissues, with the remaining spanning 51 cell types and 48 cell lines.

All small RNA sequencing experiments were processed and integrated into DASHR v2.0 following our previously described approach (Leung et al., 2013, 2016; Kuksa et al., 2018). Thus, these sncRNA expression and processing information are comparable to each other (see Supplementary Methods). All data collections are available in both GRCh37/hg19 and GRCh38/hg38 reference genomes. Note that the sequencing experiments in DASHR1-GEO and DASHR2-GEO data collections were generated using the TruSeq Small RNA Library Preparation Kit (Illumina), while the ENCODE-GEO and ENCODE-portal experiments were generated using a different, short total RNA-seq protocol (Djebali et al., 2012) (Fig. 1A, see Supplementary Methods).

2.3 Database contents
DASHR v2.0 contains 802 sequencing experiments comprising a total of 22 billion reads. Over 79 and 80% of the trimmed reads (i.e. reads that included a 3' adapter) were mapped to the GRCh37/hg19 and GRCh38/hg38 reference genomes. Note that the sequencing experiments in DASHR1-GEO and DASHR2-GEO data collections were generated using the TruSeq Small RNA Library Preparation Kit (Illumina), while the ENCODE-GEO and ENCODE-portal experiments were generated using a different, short total RNA-seq protocol (Djebali et al., 2012) (Fig. 1A, see Supplementary Methods).

2.4 Biological features of sncRNAs in DASHR v2.0
In addition to the sncRNA features included in DASHR v1.0 (expression and specificity of 5' RNA cleavage) (Leung et al., 2016), DASHR v2.0 incorporates new features to further characterize all sncRNA loci in a genome-wide manner (Table 1, Fig. 3B). These include evolutionary conservation scores, co-localization of sncRNA loci within specific genomic elements and genes, and tissue specificity scores.

2.4.1 Evolutionary conservation
58% of piRNA loci, 18% of tRF loci and 79% of tRNA-derived sncRNA loci are conserved (phastCons > .5, see Supplementary Methods). Overall, 33% of sncRNA loci are evolutionary conserved.

2.4.2 Co-localization with genomic features
We computed co-localization of the non-small RNA genes and other genomic elements (repeats, promoters, exons and introns) with each sncRNA locus (Supplementary Fig. S4). Co-localization information for each sncRNA locus includes the IDs and coordinates of each co-localized element. Overall, 32 and 27% of sncRNA loci are localized within lncRNA and mRNA genes, respectively, across all data collections.

2.4.3 Tissue specificity
For each sncRNA locus in DASHR v2.0, the tissue specificity Q-score was computed at the study (i.e. tissue/cell type) level (Schug et al., 2005). Q-score < 7 generally indicates a tissue-specific sncRNA locus. On average, 13% of expressed tRFs, 9% of expressed tRNA-derived sncRNAs and 9% of expressed piRNAs in each tissue are tissue-specific (Supplementary Fig. S5). Overall, an average of 34% of expressed RNAs in each tissue/cell type are tissue-specific.
2.4.4 New sncRNA mature product annotations

In addition to eight major sncRNA classes (mRNAs, piRNAs, sn-, sno-, scRNAs, tRNAs, tRFs and rRNAs) included in DASHR v1.0 (Leung et al., 2016), DASHR v2.0 includes new sncRNA annotations (Supplementary Methods) for tRNA-derived RNA fragments (tRFs) from the tRFdb database (Kumar et al., 2015) and 719 annotations for newly described snoRNA mature products from snorNAsome database (Jorjani et al., 2016). The piRNA annotations have been expanded to include piRNAbank database records (Sai Lakshmi and Agrawal, 2008) with a total of 26 649 (GRCh37/hg19) and 23 116 (GRCh38/hg38) piRNAs.

On average, around 200 tRF loci from tRFdb are present in each tissue/cell type in DASHR v2.0, with 89% (897) of annotated tRFdb tRF fragments detected at least one DASHR v2.0 tissue/cell type. 50% (356) of the newly described snoRNA mature products are present in at least one of the datasets, with 72 snoRNA loci in each dataset on average. For piRNAs, 40% (9517) of annotated piRNA loci have been detected in one or more of DASHR v2.0 datasets.

2.4.5 Inclusion of both annotated and previously unannotated snoRNA loci

As new snoRNA classes and loci continue to be discovered (Martens-Uzunova et al., 2013; Rother and Meister, 2011; Salta and De Strooper, 2017), integration of both annotated and novel, previously unannotated snoRNA loci in DASHR v2.0 will provide a unique resource for RNA researchers for further experimental studies and validation. Therefore, in DASHR v2.0, we characterized snoRNA loci by identifying snoRNA peaks using unsupervised segmentation followed by the annotation of the detected peaks (Supplementary Methods) and incorporated both annotated and unannotated snoRNA loci in each data collection. Figure 2D shows the number of snoRNA loci per snoRNA class, and Figure 2E, the number of unannotated snoRNA loci in each DASHR v2.0 data collection for GRCh38/hg38. Note that the composition of snoRNA classes (Fig. 2D) and the length of the unannotated snoRNA loci (Supplementary Fig. S6) vary across data collections, as different RNA-seq protocols were used in DASHR1-DASHR2-GEO compared to ENCODE experiments.

20% of annotated snoRNA loci have highly specific S' start positions (S’ specificity > .9), and 51% of previously unannotated snoRNA loci also have highly specific S' start positions. 31% of the annotated loci in DASHR v2.0 are highly conserved (100-way phastCons > .8), and 24% of the annotated loci are tissue-specific (Q-score < 7). The unannotated snoRNA loci display similar characteristics: 21% are conserved (phastCons > .5) and 44% of these are tissue-specific.

2.4.6 Non-small RNA genes and other genomic features

To allow researchers to identify snRNAs from specific genomic elements or regions of interest such as lincRNAs or promoters of mRNA genes, DASHR v2.0 incorporates annotations for non-small RNA genes [lncRNAs based on LNCipedia 4.1 (Volders et al., 2015) and mRNAs] and other genomic elements including promoters and repeat elements. Information on co-localization of snoRNA loci with these genomic elements has been incorporated into DASHR v2.0 for each snoRNA locus (Fig. 3B).

The annotated snoRNA loci in DASHR v2.0 derive from a variety of genomic elements, including mRNA introns (19%), intergenic lncRNAs (24%), promoters (1.3%), UTRs (5%) and intergenic regions (48%).

Novel, previously unannotated snoRNA loci are similarly distributed across various types of genomic regions: intronic regions (16%), exonic regions (5%), intergenic lncRNAs (12%), promoters (1.6%), UTRs (10%) and intergenic regions (55%).

3 Results

DASHR aims to provide a simple and unified resource to the scientific community allowing users to:

1. query and compare the expression and processing information for snoRNA genes and mature snoRNA products of interest (‘Search by snoRNA name/ID’, Supplementary Figs S1 and S2, Fig. 3A);
2. retrieve annotations across snoRNA classes simultaneously (‘Search by genomic coordinates’, Supplementary Figs S1 and S2);
3. view expression data for any genomic interval (‘View genomic region’, Supplementary Figs S1 and S2);
4. screen for specifically/alternatively processed, conserved, or tissue-specific snoRNA loci (Fig. 3B);
5. browse and download all or a selected/filtered set of snoRNAs and mature snoRNA products for specific human tissues or cell types (‘Browse’, Fig. 3B);
6. view snoRNA loci, expression, annotation data as UCSC genome browser tracks (Supplementary Fig. S7).

Several of the above features are new in DASHR v2.0. First, DASHR v2.0 introduces a ‘Browse’ function, enabling users to first select a data collection, then select the experiments and/or snoRNA classes they want to view (Fig. 3B). All snoRNA loci records are then viewed in an interactive table that allows users to quickly filter and locate their snoRNAs of interest using one or more features including chromosomal position, snoRNA length, expression value, S' processing specificity, tissue specificity and evolutionary conservation scores. Each snoRNA record in the table can be viewed in DASHR or in the UCSC genome browser (Fig. 3B). DASHR v2.0 also allows users to identify snoRNA loci co-localized within specific genomic elements (e.g. lincRNA, mRNA, promoter, UTR, etc.).

In addition to genome-wide raw coverage tracks, users can now download all detected snoRNA loci in each data collection along with their various features including per-tissue expression, conservation, specificity of snoRNA processing and tissue specificity scores (Supplementary Fig. S3, see Section 5).

4 Conclusions

The current release (v2.0) of DASHR is from September 2017 and is freely available for use at http://lisanwanglab.org/DASHRv2. This release improves the number of curated and processed experiments by 4-fold (total: 802 small RNA experiments) and increases the number of tissues/cell types represented in the database for a total of 185 tissues/cell types (Fig. 1). DASHR v2.0 has been expanded to include annotations for both GRCh37/hg19 and GRCh38/hg38 genomes. Importantly, DASHR v2.0 includes 372 194 previously unannotated snoRNA loci consistently expressed across tissues and cell types. Each snoRNA locus is annotated with additional biological information including conservation scores, co-localized genomic elements and tissue specificity scores. DASHR v2.0 will continue to aid the broader scientific community in exploring individual snoRNA loci and the genome-wide landscape of small RNA abundance and processing across tissues and cell types. We plan to
continuously increase and update the features and data available through this database by curating GEO/SRA, integrating data from large-scale genomic studies, e.g. FANTOM5 (Kawaij et al., 2017), and data generated from different small RNA-seq protocols focusing on small RNAs such as miRNA-seq (Djebali et al., 2012) and single cell small RNA-seq (Faridani et al., 2016).

5 Availability

The database is freely available at https://lisanwanglab.org/DASHRv2. Currently, users can download all resources in DASHR v2.0 from the ‘Download’ page (Supplementary Fig. S3, Fig. 4B). These include:

1. sncRNA loci data table summarizing expression and other features of each loci in each tissue;
2. Annotation table for all sncRNA entries and mature products;
3. Sequence table containing raw RNA sequences for all sncRNA entries in DASHR;
4. 4D conversion table, which contains cross-referenced IDs for each sncRNA entry;
5. Expression tables with raw read counts (RAW) and reads per million (RPM) across all tissues in DASHR;
6. Sequencing coverage in bedGraph format for each tissue;
7. Files with the detailed information on all sRNA-seq experiments included in each data collection in DASHR.

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