

LNCipedia 5: towards a reference set of human long non-coding RNAs

Pieter-Jan Volders^{1,2,3,4,*}, Jasper Anckaert^{1,2,4}, Kenneth Verheggen^{1,3,4}, Justine Nuytens^{1,2,4}, Lennart Martens^{1,3,4}, Pieter Mestdagh^{1,2,4} and Jo Vandesompele^{1,2,4}

¹Cancer Research Institute Ghent (CRIG), 9000 Ghent, Belgium, ²Center for Medical Genetics (CMGG), Ghent University, 9000 Ghent, Belgium, ³VIB-UGent Center for Medical Biotechnology, 9000 Ghent, Belgium and ⁴Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium

Received September 03, 2018; Revised October 12, 2018; Editorial Decision October 15, 2018; Accepted October 17, 2018

ABSTRACT

While long non-coding RNA (lncRNA) research in the past has primarily focused on the discovery of novel genes, today it has shifted towards functional annotation of this large class of genes. With thousands of lncRNA studies published every year, the current challenge lies in keeping track of which lncRNAs are functionally described. This is further complicated by the fact that lncRNA nomenclature is not straightforward and lncRNA annotation is scattered across different resources with their own quality metrics and definition of a lncRNA. To overcome this issue, large scale curation and annotation is needed. Here, we present the fifth release of the human lncRNA database LNCipedia (<https://lncipedia.org>). The most notable improvements include manual literature curation of 2482 lncRNA articles and the use of official gene symbols when available. In addition, an improved filtering pipeline results in a higher quality reference lncRNA gene set.

INTRODUCTION

The human genome is pervasively transcribed, producing vast amounts of RNA transcripts, of which the majority does not encode protein (1). Long non-coding RNAs (lncRNAs) are typically defined as non-coding RNA transcripts longer than 200 nucleotides. First regarded as transcriptional noise, lncRNAs are now known to exhibit diverse functions through a wide array of mechanisms (2,3). In addition, deregulation of lncRNAs is associated with diseases including cancer (4).

The fundamental question of how many lncRNAs are embedded in the human genome has proven to be difficult to answer. While some studies report large transcriptomes

containing over 90 000 lncRNAs (5), more conservative resources such as GENCODE annotate only 16 000 lncRNAs (6). The challenges in the annotation of lncRNAs have led to the creation of several specialised lncRNAs databases. Notable examples are LncRNAWiki, a wiki-based resource that combines computational and manual curation (7,8) and NONCODE, a lncRNA annotation database covering 17 species of which human and mouse have the highest number of annotations (9). Of note, lncRNA annotation is not limited to human or laboratory animal species. The domestic-animal lncRNA database ALDB for instance, stores pig, chicken and cow lncRNAs (10). And even though lncRNAs are often regarded as evolutionary new, also plant lncRNAs have been discovered and are catalogued in the PLncDB database (11). A recent valuable effort by the European Bioinformatics Institute (EMBL-EBI) aims to unite all non-coding RNA annotation databases into a single compendium RNACentral (12).

While the advent of massively parallel RNA sequencing technologies drastically accelerated the identification of novel lncRNAs, functional annotation is lagging behind. In addition, the lack of official gene names for many lncRNAs makes it increasingly difficult to keep track of what is currently known of a particular. Several research groups have therefore turned to manual literature curation to annotate lncRNA with functional evidence or aberrant expression in disease contexts. Notable examples of such datasets are Lnc2Cancer (13), LncRNADisease (14), the recently published pan-cancer lncRNA co-expression atlas LncMAP (15) and the Mammal ncRNA Disease Repository (MNDR) that stores 3213 mammalian lncRNAs associated with diseases (16). Despite these clear advances in lncRNA annotation, current resources are unfortunately still incomplete and plagued with inaccurate transcript and gene models, with import consequences for the lncRNA research field (17). In addition, the coding potential of numerous genes is still debated, and as such the fundamental dif-

*To whom correspondence should be addressed. Tel: +32 9 332 6979; Fax: +32 9 332 6549; Email: pieterjan.volders@ugent.be

ferentiation between coding and non-coding RNA remains troublesome (18).

In 2012, we released LNCipedia, a database to collect human lncRNA sequences and annotation (19). Central to LNCipedia is the merging of redundant transcripts across the different data sources and grouping of the transcript into genes resulting in a highly consistent database. Through regular updates, LNCipedia offers a complete set of human lncRNAs without compromising the quality of the annotations. An example of this is the high-confidence gene set introduced in LNCipedia 3 (20) as a subset of the database with lncRNAs that lack coding potential by any metric. Here, we describe the development and novel features of LNCipedia 5, the latest update of the database. Following the release of valuable resources such as FANTOM CAT (21), we expanded our database with new lncRNAs. In addition to several small improvements, we introduced an improved filtering pipeline and support for official HGNC gene names. Importantly, an extensive manual literature curation effort resulted in the annotation of 2 482 lncRNA publications, providing insights into functions of 1555 human lncRNAs.

NEW FEATURES IN LNCIPEDIA 5

LNCipedia 5 content and filtering

New lncRNA annotation in LNCipedia 5 (Table 1) originates from Ensembl (22), RefSeq (23) and FANTOM CAT (21). In the last few years, the Ensembl lncRNA annotation increased substantially, mainly through the efforts of the GENCODE consortium (24,25). We therefore updated the Ensembl lncRNA annotation to version 92, the latest release at the time of development. Similarly, RefSeq annotation was updated to the NCBI Annotation Release 106. FANTOM CAT (21) lncRNAs constitute the third addition to LNCipedia. This interesting lncRNA resource is based on cap analysis of gene expression (CAGE) data, providing annotation with more accurate 5' end positions. As a result, almost no overlap between this and other resources can be seen on the transcript level, while 45% of the genes is shared with at least one other source in the database (Table 1, Supplementary Figure S1).

In LNCipedia 5, we also introduced a new filtering pipeline to remove transcripts originating from protein coding genes. Several resources annotate transcripts with truncated or partial ORFs that overlap coding ORFs, often referred to as processed transcripts, as long non-coding RNA. While it is possible that such transcripts are functional, many likely originate from problems in the RNA sequencing data analysis pipeline. We therefore opted to follow a strict definition where transcripts that have exons overlapping with coding ORFs in sense are not regarded as lncRNAs. In this way, 9203 genes were removed, while 455 new genes were added compared to LNCipedia 4 (Supplementary Figure S2). This brings the total number of lncRNA genes in the database to 56 946 (127 802 transcripts), constituting a 13% decrease in the number of genes compared to LNCipedia 4 (65 694 genes). The high-confidence set, a subset of transcripts that lack coding potential by any metric (20), presently contains 49 372 genes (107 039 transcripts), an increase of 5% compared to the 47 213 genes in

LNCipedia 4. This was not unexpected as transcripts overlapping annotated protein coding sequence often show increased coding potential and were as such never part of the high-confidence set.

Improved gene naming includes official gene symbols

In the first version of LNCipedia (19), only very few lncRNAs were annotated with an official gene symbol. Therefore, we introduced at that time a universal scheme that names a lncRNA after the nearest protein coding gene on the same strand. As the number of lncRNAs with a gene symbol provided by the HUGO Gene Nomenclature Committee (HGNC) has grown substantially (26), LNCipedia 5 now uses a hybrid solution for gene names. If an official gene symbol is available, it will be used as the primary identifier in LNCipedia; otherwise, the universal naming scheme will be used. HGNC gene symbols, full names and identifiers are automatically retrieved from the HGNC website and matched to transcripts in the database. Currently, 23% of the transcripts and 6% of the genes in LNCipedia are annotated with an official gene symbol.

Literature annotation provides insights into functions

Compared to protein coding genes, lncRNAs are poorly understood as the greater majority lacks functional annotation. Nevertheless, the number of PubMed articles with the keyword 'long non-coding RNA' has grown enormously of the past years (Figure 1A). Although the available literature is heavily skewed towards a few well-studied lncRNAs (Figure 1B), a few thousand lncRNAs have now been studied functionally. Unfortunately, the lack of official names or stable identifiers, such as Ensembl or RefSeq identifiers, for the majority of lncRNAs had led authors to use their own in-house identifiers. This poses an important challenge for annotators and complicates the functional annotation of lncRNAs. Several annotation groups have therefore used manual curation of lncRNA articles to collect useful resources of functional annotation (13–16). For LNCipedia, we combined manual and programmatical curation of thousands of lncRNA papers in PubMed and linked the papers to entries in our database (supplemental methods). In this way, we were able to associate 2482 PubMed articles with lncRNAs in LNCipedia raising the number of lncRNA genes with at least one published article to 1555. The articles associated with a specific lncRNA are displayed on the transcript and gene pages. In addition, the titles and abstracts of these articles can be searched by keyword.

Numerous small updates to improve usability

To accommodate users that visit the website on devices with small screens such as smartphones, a new and responsive design was implemented using Bootstrap (<https://getbootstrap.com/>).

In addition to the GRCh37/hg19 reference genome, LNCipedia now supports GRCh38/hg38 as well. All genomic positions are automatically remapped to both reference genomes (supplemental methods), ensuring the annotations are available with both. The user can choose the desired reference genome and all coordinates on the websites

Table 2. Sequence ontology terms used to annotate lncRNA subclasses

| Classification | Gene level SO term | Transcript level SO term |
|-------------------|---|-----------------------------------|
| intergenic | lincRNA_gene (SO:0001641) | lincRNA (SO:0001463) |
| antisense | antisense_lincRNA_gene (SO:0002182) | antisense_lincRNA (SO:0001904) |
| intronic | sense_intronic_ncRNA_gene (SO:0002184) | sense_intronic_ncRNA (SO:0002131) |
| sense-overlapping | sense_overlap_ncRNA_gene (SO:0002183) | sense_overlap_ncRNA (SO:0002132) |
| bidirectional | bidirectional_promoter_lincRNA (SO:0002185) | NA |

with the Integrative Genomics Viewer (IGV) (29) is provided, allowing researchers to directly overlay (RNA) sequencing data with LNCipedia annotation. Finally, LNCipedia is also available as a UCSC genome browser track hub (30).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Thomas Vanneville for his contributions to the design of the web-interface. In addition, we would like to thank Manuel Luybaert (Biogazelle), Chris Conley (Huntsman Cancer Institute), Dries Rombaut (Ghent University), Francisco Avila Cobos (Ghent University) and various LNCipedia users for useful suggestions or notification of problems with the website.

FUNDING

Ghent University [to P.V., J.V.]; UGent-GOA LNCCA; Fund for Scientific Research Flanders [FWO to P.M.]. Funding for open access charge: Ghent University. Conflict of interest statement. None declared.

REFERENCES

1. Djebali,S., Davis,C.A., Merkel,A., Dobin,A., Lassmann,T., Mortazavi,A., Tanzer,A., Lagarde,J., Lin,W., Schlesinger,F. *et al.* (2012) Landscape of transcription in human cells. *Nature*, **489**, 101–108.

2. Marchese,F.P., Raimondi,I. and Huarte,M. (2017) The multidimensional mechanisms of long noncoding RNA function. *Genome Biol.*, **18**, 206.

3. Mattick,J.S. (2018) The state of long non-coding RNA biology. *Noncoding RNA*, **4**, 17.

4. Huarte,M. (2015) The emerging role of lncRNAs in cancer. *Nat. Med.*, **21**, 1253–1261.

5. Iyer,M.K., Niknafs,Y.S., Malik,R., Singhal,U., Sahu,A., Hosono,Y., Barrette,T.R., Prensner,J.R., Evans,J.R., Zhao,S. *et al.* (2015) The landscape of long noncoding RNAs in the human transcriptome. *Nat. Genet.*, **47**, 199–208.

6. Harrow,J., Frankish,A., Gonzalez,J.M., Tapanari,E., Diekhans,M., Kokocinski,F., Aken,B.L., Barrell,D., Ziadina,A., Searle,S. *et al.* (2012) GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res.*, **22**, 1760–1774.

7. Ma,L., Li,A., Zou,D., Xu,X., Xia,L., Yu,J., Bajic,V.B. and Zhang,Z. (2015) LncRNAWiki: harnessing community knowledge in collaborative curation of human long non-coding RNAs. *Nucleic Acids Res.*, **43**, D187–D192.

8. Members,B.I.G.D.C. (2018) Database resources of the BIG data center in 2018. *Nucleic Acids Res.*, **46**, D14–D20.

9. Fang,S., Zhang,L., Guo,J., Niu,Y., Wu,Y., Li,H., Zhao,L., Li,X., Teng,X., Sun,X. *et al.* (2018) NONCODEV5: a comprehensive annotation database for long non-coding RNAs. *Nucleic Acids Res.*, **46**, D308–D314.

10. Li,A., Zhang,J., Zhou,Z., Wang,L., Liu,Y. and Liu,Y. (2015) ALDB: a domestic-animal long noncoding RNA database. *PLoS One*, **10**, e0124003.

11. Jin,J., Liu,J., Wang,H., Wong,L. and Chua,N.H. (2013) PLncDB: plant long non-coding RNA database. *Bioinformatics*, **29**, 1068–1071.

12. Consortium,T.R.N.A. (2017) RNACentral: a comprehensive database of non-coding RNA sequences. *Nucleic Acids Res.*, **45**, D128–D134.

13. Ning,S., Zhang,J., Wang,P., Zhi,H., Wang,J., Liu,Y., Gao,Y., Guo,M., Yue,M., Wang,L. *et al.* (2015) Lnc2Cancer: a manually curated database of experimentally supported lncRNAs associated with various human cancers. *Nucleic Acids Res.*, **44**, D980–D985.

14. Chen,G., Wang,Z., Wang,D., Qiu,C., Liu,M., Chen,X., Zhang,Q., Yan,G. and Cui,Q. (2012) LncRNADisease: a database for long-non-coding RNA-associated diseases. *Nucleic Acids Res.*, **41**, D983–D986.

15. Li,Y., Li,L., Wang,Z., Pan,T., Sahni,N., Jin,X., Wang,G., Li,J., Zheng,X., Zhang,Y. *et al.* (2018) LncMAP: Pan-cancer atlas of long noncoding RNA-mediated transcriptional network perturbations. *Nucleic Acids Res.*, **46**, 1113–1123.

16. Cui,T., Zhang,L., Huang,Y., Yi,Y., Tan,P., Zhao,Y., Hu,Y., Xu,L., Li,E. and Wang,D. (2018) MNDR v2.0: an updated resource of ncRNA-disease associations in mammals. *Nucleic Acids Res.*, **46**, D371–D374.

17. Uszczynska-Ratajczak,B., Lagarde,J., Frankish,A., Guigó,R. and Johnson,R. (2018) Towards a complete map of the human long non-coding RNA transcriptome. *Nat. Rev. Genet.*, **19**, 535–548.

18. Abascal,F., Juan,D., Jungreis,I., Martinez,L., Rigau,M., Rodriguez,J.M., Vazquez,J. and Tress,M.L. (2018) Loose ends: almost one in five human genes still have unresolved coding status. *Nucleic Acids Res.*, **46**, 7070–7084.

19. Volders,P.J., Helsens,K., Wang,X., Menten,B., Martens,L., Gevaert,K., Vandesompele,J. and Mestdagh,P. (2013) LNCipedia: a database for annotated human lncRNA transcript sequences and structures. *Nucleic Acids Res.*, **41**, D246–D251.

20. Volders,P.J., Verheggen,K., Menschaert,G., Vandepoele,K., Martens,L., Vandesompele,J. and Mestdagh,P. (2015) An update on LNCipedia: a database for annotated human lncRNA sequences. *Nucleic Acids Res.*, **43**, D174–D180.

21. Hon,C.C., Ramiłowski,J.A., Harshbarger,J., Bertin,N., Rackham,O.J., Gough,J., Denisenko,E., Schmeier,S., Poulsen,T.M., Severin,J. *et al.* (2017) An atlas of human long non-coding RNAs with accurate 5' ends. *Nature*, **543**, 199–204.

22. Zerbino,D.R., Achuthan,P., Akanni,W., Amodé,M.R., Barrell,D., Bhai,J., Billis,K., Cummins,C., Gall,A., Girón,C.G. *et al.* (2018) Ensembl 2018. *Nucleic Acids Res.*, **46**, D754–D761.

23. O'Leary,N.A., Wright,M.W., Brister,J.R., Ciufio,S., Haddad,D., McVeigh,R., Rajput,B., Robertse,B., Smith-White,B., Ako-Adjei,D. *et al.* (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.*, **44**, D733–D745.

24. Derrien,T., Johnson,R., Bussotti,G., Tanzer,A., Djebali,S., Tilgner,H., Guernec,G., Martin,D., Merkel,A., Knowles,D.G. *et al.* (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res.*, **22**, 1775–1789.

25. Lagarde,J., Uszczynska-Ratajczak,B., Carbonell,S., Pérez-Lluch,S., Abad,A., Davis,C., Gingeras,T.R., Frankish,A., Harrow,J., Guigó,R. *et al.* (2017) High-throughput annotation of full-length long noncoding RNAs with capture long-read sequencing. *Nat. Genet.*, **49**, 1731–1740.

26. Yates,B., Braschi,B., Gray,K.A., Seal,R.L., Tweedie,S. and Bruford,E.A. (2017) Genenames.org: the HGNC and VGNC resources in 2017. *Nucleic Acids Res.*, **45**, D619–D625.
27. Uszczynska-Ratajczak,B., Lagarde,J., Frankish,A., Guigó,R. and Johnson,R. (2018) Towards a complete map of the human long non-coding RNA transcriptome. *Nat. Rev. Genet.*, **19**, 535–548.
28. Eilbeck,K., Lewis,S.E., Mungall,C.J., Yandell,M., Stein,L., Durbin,R. and Ashburner,M. (2005) The Sequence Ontology: a tool for the unification of genome annotations. *Genome Biol.*, **6**, R44.
29. Thorvaldsdóttir,H., Robinson,J.T. and Mesirov,J.P. (2013) Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform.*, **14**, 178–192.
30. Raney,B.J., Dreszer,T.R., Barber,G.P., Clawson,H., Fujita,P.A., Wang,T., Nguyen,N., Paten,B., Zweig,A.S., Karolchik,D. *et al.* (2013) Track data hubs enable visualization of user-defined genome-wide annotations on the UCSC Genome Browser. *Bioinformatics*, **30**, 1003–1005.