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SOFTWARE TOOL ARTICLE

REVISED RBP-Tar – a searchable database for experimental RBP

binding sites

[version 2; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: RNA-binding proteins (RBPs) play a critical role in regulating gene expression by binding to specific sites on RNA molecules. Identifying these binding sites is crucial for understanding the many functions of RBPs in cellular function, development and disease. Current experimental methods for identifying RBP binding sites, such as ultra-violet (UV) crosslinking and immunoprecipitation (CLIP), and especially the enhanced CLIP (eCLIP) protocol, were developed to identify authentic RBP binding sites experimentally.







Methods: To make this data more accessible to the scientific community, we have developed RBP-Tar (<https://ncbr.muni.cz/RBP-Tar>), a web server and database that utilises eCLIP data for 167 RBPs mapped on the human genome. The web server allows researchers to easily search and retrieve binding site information by genomic location and RBP name.

Use case: Researchers can produce lists of all known RBP binding sites on a gene of interest, or produce lists of binding sites for one RBP on different genomic loci.

Conclusions: Our future goal is to continue to populate the web server with additional experimental datasets from CLIP experiments as they become available and processed, making it an increasingly valuable resource for the scientific community.

Open Peer Review

Approval Status    

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1. **Xiaoyong Pan** , Shanghai Jiao Tong University, Shanghai, China
2. **Clifford A Meyer**, Harvard T.H. Chan School of Public Health, Dana-Farber Cancer Institute, Boston, USA
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Keywords

RNA Binding Proteins, CLIP, RBP, Web-server

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the **Bioinformatics** gateway.

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Author roles: **Gresova K:** Data Curation, Formal Analysis, Investigation, Methodology, Software, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Racek T:** Resources, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Martinek V:** Software, Writing – Original Draft Preparation, Writing – Review & Editing; **Cechak D:** Data Curation, Investigation, Methodology, Software, Writing – Original Draft Preparation, Writing – Review & Editing; **Svobodova R:** Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Alexiou P:** Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 1

In this revised version of the article, we have made improvements based on the reviewers' feedback to enhance the accuracy, completeness, and utility of our resource for RNA-binding protein (RBP) binding sites. Firstly, we re-examined and adjusted our metadata processing pipeline to ensure the inclusion of the merged peak files for each RBP. This adjustment led to the revision of the binding site numbers and file sizes, with new figures provided for an up-to-date webserver view.

Any further responses from the reviewers can be found at the end of the article

Introduction

RNA binding proteins (RBPs) are key players in a broad spectrum of RNA regulation, including all stages of the RNA lifecycle (Gerstberger *et al.* 2014; Gebauer *et al.* 2021). Eukaryotic genomes typically encode hundreds of RBPs. For example, over 1500 human RBPs involved in the maturation, transport, stability and translation of coding and non-coding RNA were recently characterised and manually curated (Gerstberger *et al.* 2014). Each RBP can typically target hundreds of RNAs in a complex coordinated fashion (Hogan *et al.* 2008). The general transcriptomic locations of thousands of RNA binding sites corresponding to hundreds of RBPs have been identified using a family of experimental techniques based on RBP CrossLinking, ImmunoPrecipitation and Sequencing (CLIP-Seq) (Chi *et al.* 2009; Licatalosi *et al.* 2008; Moore *et al.* 2014; Hafner *et al.* 2010). A thorough exploration of tens of RBPs binding characteristics in vitro has shown that RBPs can differentiate their binding sites with context preferences beyond narrowly defined binding sequence motif and secondary structure often involving complex binding configurations (Dominguez *et al.* 2018). Among the experimental techniques available to date, the enhanced CLIP (eCLIP) protocol (Van Nostrand *et al.* 2016) is particularly important, as it significantly reduces required amplification and increases specificity in identifying authentic binding sites. This improves the efficiency and accuracy of RBP binding site identification and allows for a deeper understanding of the role of RBPs in gene regulation.

The availability of a large amount of data points produced from the same experimental technique can be very beneficial for applications such as machine learning, as it allows researchers to train and test models with more confidence. Here we present RBP-Tar (Tomáš Raček 2023), a centralised and searchable database of experimentally identified RBP binding sites that can significantly facilitate the study of the RBP mediated gene regulation. Using RBP-Tar, researchers can quickly and cleanly retrieve RBP binding sites constrained by both genomic location and associated RBP for hundreds of RBPs.

Methods

Implementation

Pipeline for reproducible data download and annotation

We have developed a reproducible and easy-to-use pipeline for downloading and annotating RBP eCLIP data from the ENCODE (Luo *et al.* 2020) database.

Metadata for eCLIP experiments, as well as additional files containing genomic coordinates are downloaded from the ENCODE database with the following parameters:

```
"status= released&
internal_tags=ENCORE&
assay_title=eCLIP&
biosample_ontology.term_name=K562&
biosample_ontology.term_name=HepG2&
files.file_type=bed+narrowPeak&
type=Experiment
files.analyses.status=released".
```

Following the download, information about the chromosome, start position, end position and strand of reads are extracted for each RBP binding site. Binding sites are filtered by length, excluding ones shorter than 20 and longer than 100 nucleotides. As a last step, genomic sequences of binding sites are retrieved.

The described pipeline is implemented as a set of python scripts and is freely available at [GitHub](#).

Using this pipeline, data for 168 RBPs on two cell lines (K562, HepG2) were downloaded. In total, 42 MB of data, representing more than 400 thousand binding sites, were thus processed.

Web server

Here we present RBP-Tar, a web server that can access the above curated dataset of RBP binding sites (<https://rbp-tar.biodata.ceitec.cz/>) and was built with Python (RRID:SCR_008394), the web development framework Flask, and a simple SQLite database (RRID:SCR_017672). The application’s source codes can be found on the project’s [GitHub](#) page, along with the requirements and instructions for the deployment if a user wants to run the application locally.

The web user interface allows searching and filtering based on the start and end position of the binding site, strand, chromosome, and protein name. It offers the download of the filtered data based on the search done by the user. Due to the size of the dataset, the view is limited to 10 000 results. However, the whole dataset can be conveniently downloaded as a gzipped CSV (14 MB) (https://rbp-tar.biodata.ceitec.cz/download_all).

Operation

The RBP-Tar web server can take as input any of the following user-provided parameters: [Start min, Start max] denote the limits of the low genomic coordinate of the locus of interest. Similarly, [End min, End max] denote the limits of the high coordinate. [Strand] and [Chromosome] can be used to narrow down the search to only one strand and a specific chromosome. Using these combinations of parameters, a user can easily search for binding sites on their favourite gene, exon, or even a whole chromosome. The last parameter is [Protein name], which brings out a drop-down menu of all the RBPs in the database. If this parameter is not set, all RBPs are queried.

Results are shown as a table with the [Chromosome, Start, End, Strand] genomic location of the binding site, followed by the [Protein name] of the associated RBP and the [Sequence] of the binding site contains the genomic sequence of the binding site. Results can be seen online in a table format or downloaded as a CSV file with one button click. We expect most users to download the results and use them for further downstream analyses.

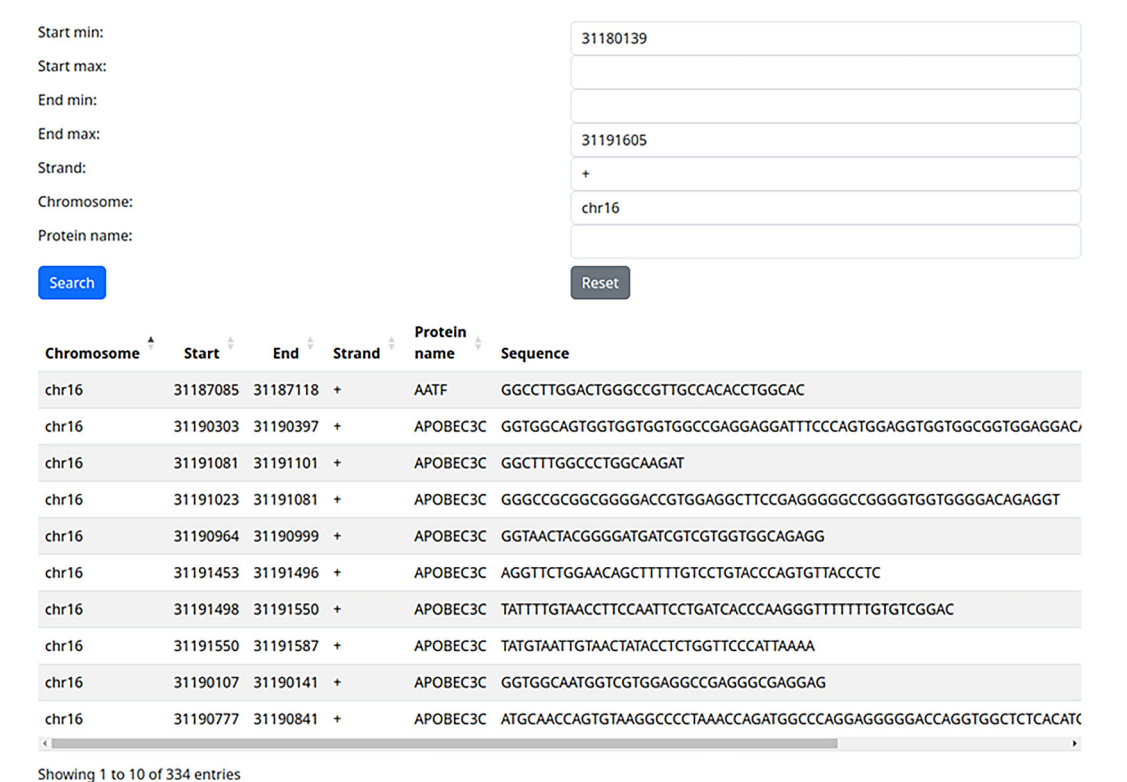


Figure 1. Extracting binding sites of all RNA-binding proteins on one locus (use case 1).

Chromosome	Start	End	Strand	Protein name	Sequence
chr16	31188069	31188116	+	FUS	CAGCATTTCGCTGAAGTGTGGAGTTGTCTCTGTGGAGACTCAAGTTAC
chr16	31187431	31187496	+	FUS	TGTGGCTCATGGGAAATAACCAAGTCTTAAACAGCACAAACTGAATTCGTGGACCAGGAAGGTC
chr16	31187615	31187647	+	FUS	AAGGTGTGGGTTTCGACTTAGGTGTCATTGC
chr16	31187136	31187183	+	FUS	AGAAACTGGAGAGTGCCTGCTGGAACACGTGGTGCCATCTTGGCTTT
chr16	31187068	31187135	+	FUS	ATAAGAGGGGTCTAGTAGGCCCTTGGACTGGGCCGTTGCCACACCTGGCACTTAGTGACCATCATG
chr16	31187041	31187068	+	FUS	TTTACCAATGGGTTTGCCCGAGGTTA
chr16	31187662	31187724	+	FUS	TTATATATGGCGCAATTTCAGATAAACATTGGTCAAAGATGGTCTCTGAAAAACAAATAGAG
chr16	31186989	31187024	+	FUS	GCCTGGTGTGTGCTAACCTGGAGCAGGTAGGGGTA
chr16	31186916	31186974	+	FUS	TAATTTAAATGTCAAAGGTTTGTAGGTGCCAGAACACCTCCAGAAAGGGGTAGGGT
chr16	31187996	31188057	+	FUS	TGTTCACTTTAATGGGTCTCCGTTTCCCTGCCACCTGTGCTGAGGACATTTCACAGCTG

Figure 2. Fused in Sarcoma (FUS) self-targeting identified by filtering by gene location and protein name.

Start min:	<input type="text"/>
Start max:	<input type="text"/>
End min:	<input type="text"/>
End max:	<input type="text"/>
Strand:	<input type="text"/>
Chromosome:	<input type="text" value="chr1"/>
Protein name:	<input type="text" value="AATF"/>
<input type="button" value="Search"/>	<input type="button" value="Reset"/>

Chromosome	Start	End	Strand	Protein name	Sequence
chr1	179025917	179026005	+	AATF	GGCGAGAGGTTGGCTGGGAATGCCGGGCCGGGGTGGGCCGGAGCCGCTGTGGCGGGC
chr1	1223085	1223161	-	AATF	TGTGTGTCGTGTATAGTGTGCCGTGCGTGTGCTCGTAGTACGTGTGCGTGCCTGTGTG
chr1	173866893	173866918	-	AATF	TAGTGGACAGAAGGGATTCTGAAA
chr1	173864832	173864871	-	AATF	GCTGATGAGCAATATTAAGTCTTCGCTCCTATCTGATG
chr1	1222709	1222787	-	AATF	AAGCCGTGCTGACCTAGGCCGCCGCCAGGAGTCCGGTGGGGAGCGTGTGAGCTGTGCGCTG
chr1	1052401	1052449	+	AATF	TGTGTGTATATGTGGGGGGGACATGTAGATATGCGTGTGTGACTATGC
chr1	228144241	228144296	+	AATF	GGATTAGGAGGGCATCTCAGGTCCTTCCCACGTGTGCTCGTCTGCCACAGGC
chr1	42177684	42177754	-	AATF	TAATGGAGTGTTCCTTGAAATGAATCACTGGGAAGCCAGCCATGGTAAGGGCTGGTGAGGTT
chr1	75787114	75787138	+	AATF	ATTACTACTTTAGCTCTAGAATTA
chr1	1320775	1320874	-	AATF	CCCTGTGGGGAGCAGGGATGGGTGGGCCTGGCCGAGGTGAGCCCCTGCATGGTGGGGTCC

Figure 3. Extracting all binding sites of a single protein on a single chromosome (use case 2).

Use cases

Use case 1: all known RBP binding sites on the gene of interest

The first and potentially most common use case would be the query of all known RBP binding sites on a gene of interest. For example, we can query our web server with the coordinates of the Fused in Sarcoma (FUS) gene (chr16: 31180139-31191605, +) and leave the protein field empty. After this search, all 334 known RBP binding sites on this gene are returned and can be easily downloaded in a CSV file for further analysis (Figure 1).

In fact, the gene we used encodes the RBP FUS, which plays important roles, among others, in neurodegeneration and cancer progression. We can use the filter [Protein Name] to identify the potential self-targeting of FUS on itself. Indeed, we can thus identify 19 potential FUS self-targeting binding sites identified *via* eCLIP (Figure 2). Of course, the biological relevance of this type of finding is left to the users, as is the further validation of high-throughput derived RBP binding sites.

Use case 2: training/testing dataset for one RBP

A potential user may want to develop an RBP binding site machine learning tool that would be able to predict binding sites based on a sequence. It is important to make sure that training and testing sets for their machine learning method are not overlapping. Using our web server, they can download all binding sites for a specific RBP, for example, on chromosome 1, and use them as a training set (Figure 3). Then do the same for chromosome 2 and use it as an independent testing set, thus ensuring that training and testing set do not overlap.

Conclusion

Recent advancements in experimental techniques, such as eCLIP, have greatly expanded our understanding of RBP binding preferences and their role in gene regulation. The development of centralised and searchable databases of experimentally identified RBP binding sites allows researchers to access and analyse the binding preferences of RBPs easily. This information can be used to identify known binding sites on genes of interest and aid in training machine-learning models for RBP binding site prediction. This paper presents RBP-Tar, a centralised web server that can retrieve RBP Target sites with location and RBP constraints. RBP-Tar has been designed to be easily accessible by non-experts. It is still confined to a single source of data, which is helpful for avoiding experimental design effects, but makes its scope limited. We plan to expand the web server with other sources of data, as well as ways for the user to be able to take into account provenance and experimental variation.

Data availability

Source data

All data used in RBP-Tar has been downloaded from ENCODE and Ensembl projects in June 2024. RBP eCLIP metadata were downloaded from https://www.encodeproject.org/metadata/?status=released&internal_tags=ENCORE&assay_title=eCLIP&biosample_ontology.term_name=K562&biosample_ontology.term_name=HepG2&files.file_type=bed+narrowPeak&type=Experiment&files.analysis.status=released. A list of all downloaded files containing genomic coordinates can be found here: https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip/blob/main/csv/coord_links.txt. The reference genome was downloaded from http://ftp.ensembl.org/pub/release-97/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.toplevel.fa.gz

Columns 'chrom', 'chromStart', 'chromEnd' and 'strand' from downloaded files containing genomic coordinates can be found here: https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip/tree/main/csv. The whole dataset (curated and including sequence) can be downloaded from <https://rbp-tar.biodata.ceitec.cz/> as a gzipped CSV (14 MB) (https://rbp-tar.biodata.ceitec.cz/download_all).

Software availability

RBP-Tar

Software: <https://rbp-tar.biodata.ceitec.cz/>

Source code: <https://github.com/sb-ncbr/RBP-Tar/releases/tag/v1.0.1>

Archived source code at time of publication: <https://doi.org/10.5281/zenodo.12720685> (Raček 2024)

License: MIT

eCLIP RBP

Source code: https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip

Archived source code at the time of publication: <https://doi.org/10.5281/zenodo.12704072> (Grešová & Raček 2024)

License: [Apache 2.0](#)

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[Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 05 September 2024

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Clifford A Meyer

Harvard T.H. Chan School of Public Health, Dana-Farber Cancer Institute, Boston, USA

The revision is not satisfactory as most of the reviewers' suggestions for improving the website were not implemented. We hope that these will be implemented in future versions.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epigenetics, Chromatin analysis, Gene regulation, Single cell

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Nov 2024

Panagiotis Alexiou

Thank you for the continued feedback on our manuscript and webserver resource. We appreciate the reviewer's commitment to ensuring that the resource becomes more robust and user-friendly. In response to the earlier suggestions, we have implemented the following improvements in our webserver, as outlined below:

1. Inclusion of p-values: We have added a p-value column to our data, providing an additional layer of statistical context to aid users in evaluating the significance of RNA binding protein binding sites.
2. Filtering and sorting functionality by p-value: To enhance usability, we have introduced the functionality to filter and sort binding site data based on p-values. This improvement allows users to more easily interpret and prioritize results according to their significance.

We recognize that many of the enhancements suggested in previous rounds, such as expanding the dataset scope and incorporating broader quality control metrics, require a

significant overhaul and are part of our longer-term development roadmap. Our current updates reflect initial, impactful steps toward addressing reviewer concerns, and we remain committed to further expanding the features and datasets in future versions.

Competing Interests: No competing interests were disclosed.

Reviewer Report 23 August 2024

<https://doi.org/10.5256/f1000research.169056.r313334>

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Xiaoyong Pan 

Shanghai Jiao Tong University, Shanghai, Shanghai, China

The authors addressed my comments.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 18 December 2023

<https://doi.org/10.5256/f1000research.143817.r224195>

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Clifford A Meyer

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This paper describes a database of RNA binding protein binding sites based on 2 ENCODE cell lines. The website allows users to search for data by querying a gene or genomic interval.

The scope of the database is very limited. It is not clear why the data is restricted to eCLIP data

and not CLIP data in general. Although the authors make the argument that eCLIP data is of good quality, quality will vary from sample to sample and there are likely to be high quality CLIP data sets as well as low quality eCLIP ones.

It appears that the processed peaks are downloaded from ENCODE, but the methods used by ENCODE to identify these peaks are not described, and statistics such as p-values, false discovery rates and enrichment relative to background are not provided. Quality controls are also not provided, so it is not possible to know if the experiment worked.

As processed RBP data is downloaded from ENCODE this resource does not add much to what is already available on the ENCODE website. A resource that processed the raw read data from other data resources, such as GEO, would be more valuable. There are over 2,000 eCLIP samples and over 6,000 CLIP samples in GEO.

The search features are useful, although it is hard to interpret the results. Some ranking of the results from most significant to least is needed. It would also be helpful to enable filtering based on some binding sites statistics.

Overall, the resource is limited in terms of the data included and the website features. It would be helpful to include quality control metrics to let users know which samples are more informative.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Partly

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

No

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epigenetics, Chromatin analysis, Gene regulation, Single cell

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Jul 2024

Panagiotis Alexiou

This paper describes a database of RNA binding protein binding sites based on 2 ENCODE cell lines. The website allows users to search for data by querying a gene or genomic interval. The scope of the database is very limited. It is not clear why the data is restricted to eCLIP data and not CLIP data in general. Although the authors make the argument that eCLIP data is of good quality, quality will vary from sample to sample and there are likely to be high quality CLIP data sets as well as low quality eCLIP ones.

We agree that the scope of the database is limited at this point and we aim to widen it in the future updates of the app. ENCODE/ENCORE has a comprehensive eCLIP experimental list, and only 3 iCLIP libraries (https://www.encodeproject.org/encore-matrix/?type=Experiment&status=released&internal_tags=ENCORE). We chose eCLIP to keep the experimental procedure more consistent in case of different methods introducing different biases. In a future version, we will explore using different datasets from multiple sources.

It appears that the processed peaks are downloaded from ENCODE, but the methods used by ENCODE to identify these peaks are not described, and statistics such as p-values, false discovery rates and enrichment relative to background are not provided. Quality controls are also not provided, so it is not possible to know if the experiment worked.

Methods used by ENCODE to identify peaks from eCLIP data are described here: <https://www.encodeproject.org/eclip/> while the detailed description of data processing pipeline can be downloaded from here: https://www.encodeproject.org/documents/739ca190-8d43-4a68-90ce-1a0ddfffc6fd/@@download/attachment/eCLIP_analysisSOP_v2.2.pdf. This pipeline ensures that only high-quality peaks are identified.

As processed RBP data is downloaded from ENCODE this resource does not add much to what is already available on the ENCODE website. A resource that processed the raw read data from other data resources, such as GEO, would be more valuable. There are over 2,000 eCLIP samples and over 6,000 CLIP samples in GEO.

We agree that a resource that processed the raw read data from other data resources, such as GEO, would be more valuable, we will consider adding other data resources in the future updates of the webserver. The addition of our app to what is already available on the ENCODE website is advanced filtering of binding sites based on the genome position.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 December 2023

<https://doi.org/10.5256/f1000research.143817.r224199>

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1. In the paper, the author claim they download narrowPeak metadata from https://www.encodeproject.org/metadata/?status=released&internal_tags=ENCORE&assay_title=eCLIP&, and the link summary is provided in the file https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip/blob/main/csv/coord_links.txt. In the ENCODE dataset, the majority of RNA-binding proteins (RBPs) have biological replicate(s) or technical replicate(s), with each replicate having an associated narrowPeak file. Additionally, there is another merged peak file containing reproducible peaks as determined by entropy-ordered peaks between two replicates. However, it is observed that only one file per RBP from K562 or HepG2, which is both the merged peak file. For eCLIP experiment on K562 against QKI, I just find ENCFF190XSX that is from replicate 1. A careful examination of the data is recommended to ensure accuracy and completeness.
2. After narrowPeak download, the author filter the peak by length, excluding ones shorter than 20 and longer than 100 nucleotides. It's important to note that must short (rarely more than 4–6-nucleotide-long) sequence elements within RNA targets that are recognized and bound by RNA-binding proteins (Gerstberger, S et al. (2014 ¹). In my perspective, employing a filter based on fold change and p-value, in accordance with the eCLIP-seq Processing Pipeline of ENCODE (https://www.encodeproject.org/documents/739ca190-8d43-4a68-90ce-1a0ddfffc6fd/@@download/attachment/eCLIP_analysisSOP_v2.2.pdf) is powerful.
3. If this job is for the benefit of biologists, why not use transcripts as a reference? Because RBP binds to transcripts. And it's too simplistic for systems biologists, the author can refer to the POSTAR3 data.

References

1. Gerstberger S, Hafner M, Tuschl T: A census of human RNA-binding proteins. *Nat Rev Genet.* 2014; **15** (12): 829-45 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Partly

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Partly

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: The authors need solve some issues before published

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Jul 2024

Panagiotis Alexiou

Reviewer Comment:

In the paper, the author claim they download narrowPeak metadata from

https://www.encodeproject.org/metadata/?status=released&internal_tags=ENCORE&assay_title=eCLIP&biosample_

, and the link summary is provided in the file [https://github.com/ML-Bioinfo-](https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip/blob/main/csv/coord_links.txt)

[CEITEC/rbp_encode_eclip/blob/main/csv/coord_links.txt](https://github.com/ML-Bioinfo-CEITEC/rbp_encode_eclip/blob/main/csv/coord_links.txt). In the ENCODE dataset, the majority of RNA-binding proteins (RBPs) have biological replicate(s) or technical replicate(s), with each

replicate having an associated narrowPeak file. Additionally, there is another merged peak file containing reproducible peaks as determined by entropy-ordered peaks between two replicates.

However, it is observed that only one file per RBP from K562 or HepG2, which is both the merged peak file. For eCLIP experiment on K562 against QKI, I just find ENCF190XSX that is from replicate 1. A careful examination of the data is recommended to ensure accuracy and completeness.

Author Response: We would like to thank the reviewer for the insightful finding. We have carefully examined the downloaded metadata and adjusted the processing pipeline to ensure the merged peak file is used for every RBP. In the methods, section "Pipeline for reproducible data download and annotation", we have added the parameter "files.analyses.status=released" that is ensuring all available files are downloaded. Updated data have been uploaded to the RBP-Tar webserver and the manuscript has been updated with the current number of binding sites and size of files. We have provided new figures with up-to-date views of the web server.

Reviewer Comment:

After narrowPeak download, the author filter the peak by length, excluding ones shorter than 20 and longer than 100 nucleotides. It's important to note that must short (rarely more than 4–6-nucleotide-long) sequence elements within RNA targets that are recognized and bound by RNA-binding proteins (Gerstberger, S et al. (2014) [1](#)). In my perspective, employing a filter based on fold change and p-value, in accordance with the eCLIP-seq Processing Pipeline of ENCODE (

https://www.encodeproject.org/documents/739ca190-8d43-4a68-90ce-1a0ddfffc6fd/@download/attachment/eCLIP_analysisSOP_v2.2.pdf) is powerful.

Author Response: We appreciate the reviewer's perspective on filtering criteria. Our decision to filter based on length aimed to exclude potential artifacts and prioritize biologically relevant binding sites. While fold change and p-value are valuable parameters, our focus was on ensuring the inclusion of robust binding sites within the specified length range. We will consider incorporating additional filtering parameters in future iterations leading to a new version of the web server.

Reviewer Comment:

If this job is for the benefit of biologists, why not use transcripts as a reference? Because RBP binds to transcripts. And it's too simplistic for systems biologists, the author can refer to the POSTAR3 data.

Author Response: Our choice to focus on genomic coordinates was driven by the goal of providing a comprehensive resource for accessing RBP binding sites across the genome. While transcripts offer valuable context, our approach allows for flexibility in analyzing binding sites within different genomic regions and across multiple transcripts. However, we recognize the importance of incorporating transcript-level information and will explore integrating such data in future updates of the webserver to enhance the utility of our tool for systems biologists.

Competing Interests: No competing interests were disclosed.

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