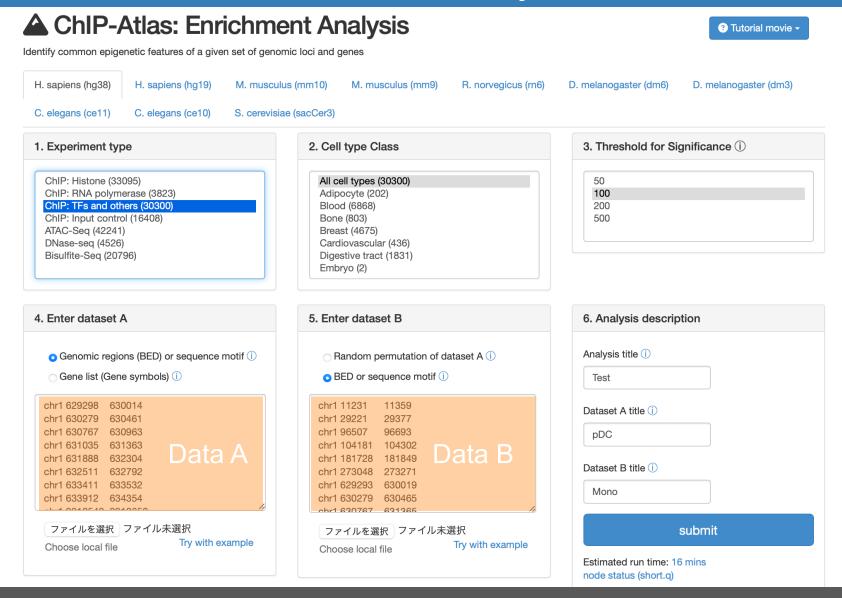
The manual for

△ ChIP-Atlas: Enrichment Analysis

- 1. Overview
- 2. Enrichment for the genomic loci (BED)
- 3. Enrichment around the genes (Gene symbol)
- 4. Tips

Overview of the Enrichment Analysis tool



The ChIP-Atlas "Enrichment Analysis" tool is useful to identify the transcription factors (TFs) whose bindings are enriched for given genomic coordinates or gene loci.

Overview of the Enrichment Analysis tool

ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.

Show 100 er	ntries		Search:								
			TFs		Test						
ID \$	Antigen class	Antigen	Cell class 🛊	Cell \$	Num of • peaks	Overlaps / pDC	Overlaps / Mono	Log P- ^ val	Log Q- ∳ val	Fold Enrichment	FE > 1?
SRX1023792	TFs and others	SPI1	Blood	Macrophages	112968	5339/11207	13014/18171	-324.0	-324.0	0.67	FALSE
SRX2770855	TFs and others	SPI1	Blood	Macrophages	88807	5420/11207	13855/18171	-324.0	-324.0	0.63	FALSE
SRX1287846	TFs and others	CDK8	Blood	MOLM-14	50649	5066/11207	13051/18171	-324.0	-324.0	0.63	FALSE
SRX1287844	TFs and others	CDK8	Blood	MOLM-14	50871	5084/11207	13250/18171	-324.0	-324.0	0.62	FALSE
SRX3031982	TFs and others	BRD4	Blood	MV-4-11	47241	5298/11207	14117/18171	-324.0	-324.0	0.61	FALSE
SRX9867025	TFs and others	BCL11B	Blood	TALL-1	31691	4185/11207	11317/18171	-324.0	-324.0	0.60	FALSE
SRX4484988	TFs and others	ERG	Blood	SEM	50919	4052/11207	10966/18171	-324.0	-324.0	0.60	FALSE
SRX9867024	TFs and others	BCL11B	Blood	TALL-1	29842	4111/11207	11167/18171	-324.0	-324.0	0.60	FALSE

The ChIP-Atlas "Enrichment Analysis" tool is useful for identifying the transcription factors (TFs) whose binding is enriched for given genomic coordinates or gene loci.

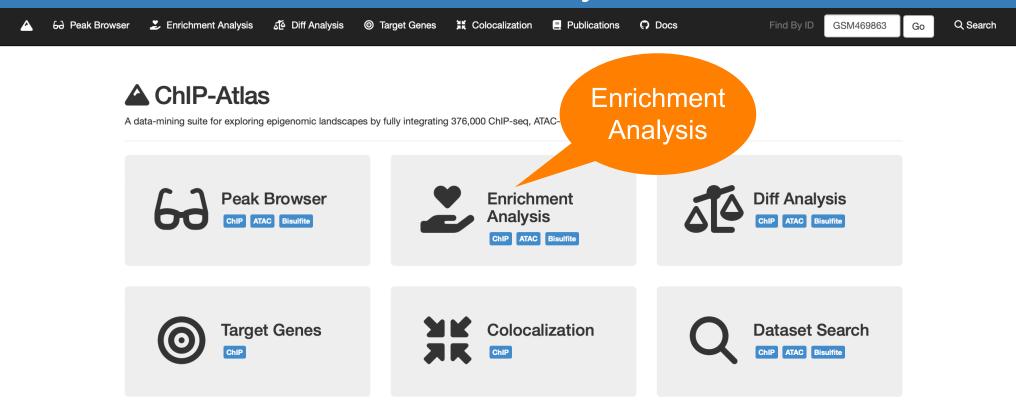
The manual for



- 1. Overview
- 2. Enrichment for the genomic loci (BED)
- 3. Enrichment around the genes (Gene symbol)
- 4. Tips

In this tutorial, you will identify transcription factors (TFs) whose bindings are enriched for the open chromatin regions obtained from public ATAC-seq data.

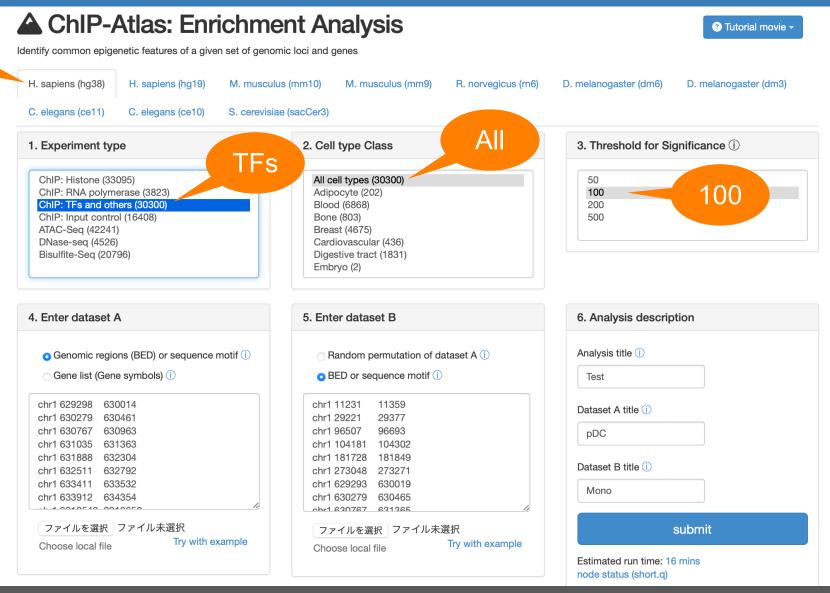
Access to the Enrichment Analysis tool



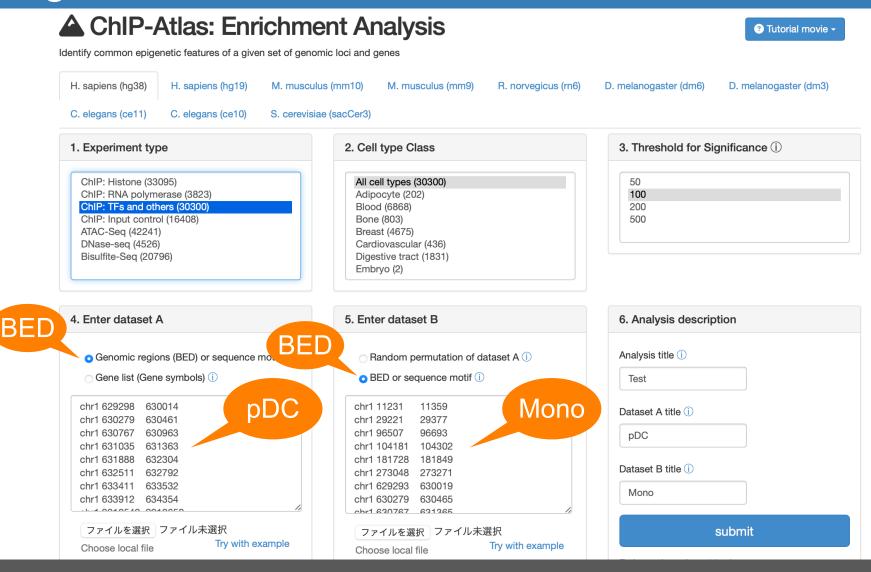
What's new

- Added Annotation tracks to Peak Browser, together with UI improvement (2023/10/25)
- Launched Diff Analysis tool enabling to detect differential peaks or differentially methylated regions (2023/10/25)
- New publication on the NAR web server issue! https://doi.org/10.1093/nar/gkac199 (2022/03/24)
- Added ATAC-Seq and Bisulfite-Seq, together with UI improvement including 'peak' icon! (2021/10/04)

hg38



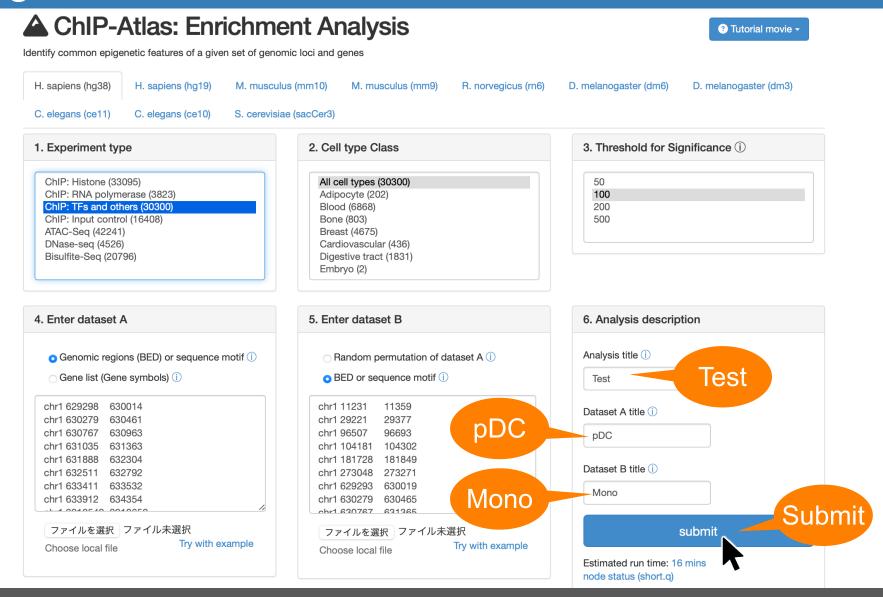
Set to examine the enrichment of TF peaks in all cell types with >100 MACS2 scores.



Enter the tab-delimited genomic coordinates in BED format. For example:

Dataset A: ATAC-seq peaks of plasmacytoid dendritic cells (pDC; SRX790284)

Dataset B: ATAC-seq peaks of monocytes (Mono; SRX7902871)



Enter the titles of this project (Test) and datasets A (pDC) and B (Mono), which will be used to label the result data. Click on the "submit" button.

Running

△ 6-3 Peak Browser

Enrichment Analysis

Diff Analysis

Target Genes

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▲ ChIP-Atlas: Enrichment Analysis

identify common epigenetic features of a given set of genomic loci and genes

Result page URL will be available for a week from the time when 'status' is 'finished'.



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▲ ChIP-Atlas: Enrichment Analysis

identify common epigenetic features of a given set of genomic loci and genes

Result page URL will be available for a week from the time when 'status' is 'finished'.

Project title	Test
Request ID	wabi_chipatlas_2024-0121-1545-55-143-974837
Submitted at:	15:45:55 (Jan-21-2024)
Estimated finishing time:	16:01:55 (Jan-21-2024)
Current time:	16:10:59 (Jan-21-2024) finished
Status	finished
Result URL:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_chipatlas_2024-0121-1545-55-143-974837?info=result&format=html
Download TSV:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_c_vatlas_2024-0121-1545-55-143-974837?info=result&format=tsv

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When the "Status" changes to "finished", click on the result URL. Take a note of the URL so that you can review the results for up to one month.

ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.

Show 100 entries

Search:

Test

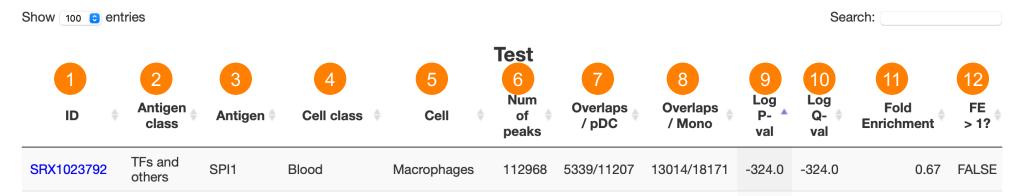
ID \$	Antigen class	Antigen 	Cell class 🍦	Cell 🛊	Num of \$ peaks	Overlaps / pDC	Overlaps / Mono	Log P- ^ val	Log Q- ♦ val	Fold Enrichment	FE > 1?
SRX1023792	TFs and others	SPI1	Blood	Macrophages	112968	5339/11207	13014/18171	-324.0	-324.0	0.67	FALSE
SRX2770855	TFs and others	SPI1	Blood	Macrophages	88807	5420/11207	13855/18171	-324.0	-324.0	0.63	FALSE
SRX1287846	TFs and others	CDK8	Blood	MOLM-14	50649	5066/11207	13051/18171	-324.0	-324.0	0.63	FALSE
SRX1287844	TFs and others	CDK8	Blood	MOLM-14	50871	5084/11207	13250/18171	-324.0	-324.0	0.62	FALSE
SRX3031982	TFs and others	BRD4	Blood	MV-4-11	47241	5298/11207	14117/18171	-324.0	-324.0	0.61	FALSE
SRX9867025	TFs and others	BCL11B	Blood	TALL-1	31691	4185/11207	11317/18171	-324.0	-324.0	0.60	FALSE
SRX4484988	TFs and others	ERG	Blood	SEM	50919	4052/11207	10966/18171	-324.0	-324.0	0.60	FALSE
SRX9867024	TFs and others	BCL11B	Blood	TALL-1	29842	4111/11207	11167/18171	-324.0	-324.0	0.60	FALSE

The result is displayed. Each line indicates TF ChIP-seq data whose bindings are enriched for open chromatin regions of pDC or Mono.

Link to the download

ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.



For example, the first line (SRX1023792 1) indicates that this is the SPI1 ChIP-seq data in macrophages (2345). This data has 112,968 peaks (6), of which 5,339 peaks overlap with the ATAC-seq peaks of pDC (n = 11,207), whereas 13,014 peaks overlap with the ATAC-seq peaks of Mono (n = 18,171)creating the 2×2 cross table, the following calculations were performed:

- **9** Log₁₀[*P* values] calculated **using** two-tailed Fisher's exact test (Min = -324).
- $\mathbf{10}$ Log₁₀[Q values] calculated **using** Benjamini-Hochberg method (Min = -324).
- 11 Fold-enrichment = pDC / Mono = (5,339 / 11,207) / (13,014 / 18,171)
- 12 TRUE: The fold-enrichment (FE) > 1.

FALSE: The fold-enrichment (FE) ≤ 1 .

By default, this table is sorted by $Log_{10}[P \text{ values}]$ in the 9th column.

ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.

Show 100 entries Search:

	Test											
ID \$	Antigen _{\$\phi\$} class	Antigen 🌲	Cell class 🍦	Cell 💠	Num of \$ peaks	Overlaps / pDC	Overlaps / Mono	FE	< 1	Fold Enrichment	FE > 1?	
SRX1023792	TFs and others	SPI1	Blood	Macrophages	112968	5339/11207	13014/18171	-324.0	-324.0	0.67	FALSE	
SRX2770855	TFs and others	SPI1	Blood	Macrophages	88807	5420/11207	13855/18171	-324.0	-324.0	0.63	FALSE	
SRX1287846	TFs and others	CDK8	Blood	MOLM-14	50649	5066/11207	13051/18171	-324.0	-324.0	0.63	FALSE	
SRX1287844	TFs and others	CDK8	Blood	MOLM-14	50871	5084/11207	13250/18171	-324.0	-324.0	0.62	FALSE	
SRX3031982	TFs and others	BRD4	Blood	MV-4-11	47241	5298/11207	14117/18171	-324.0	-324.0	0.61	FALSE	
SRX9867025	TFs and others	BCL11B	Blood	TALL-1	31691	4185/11207	11317/18171	-324.0	-324.0	0.60	FALSE	
SRX4484988	TFs and others	ERG	Blood	SEM	50919	4052/11207	10966/18171	-324.0	-324.0	0.60	FALSE	
SRX9867024	TFs and others	BCL11B	Blood	TALL-1	29842	4111/11207	11167/18171	-324.0	-324.0	0.60	FALSE	

This result indicates that SPI1 is one of the most enriched TFs for the open chromatin regions of Mono because the fold-enrichment (FE) is less than 1.

ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.

Show 100 entries



Test

ID \$	Antigen class	Antigen 🍦	Cell class	Cell	\$ Num of peaks	Overlaps / pDC	Overlaps / Mono	Log P- ^ val	Log Q-	Fold Enrichment	FE > \$
SRX1491252	TFs and others	TCF4	Blood	GEN2.2	48362	6418/11207	6792/18171	FE	> 1	1.53	TRUE
SRX1491250	TFs and others	TCF4	Blood	CAL-1	29027	4342/11207	4025/18171	-201.6	-200.2	1.75	TRUE
SRX220960	TFs and others	TCF4	Blood	CAL-1	4012	825/11207	315/18171	-125.5	-124.3	4.25	TRUE
SRX158106	TFs and others	TCF3	Blood	RPMI 8402	6547	620/11207	302/18171	-72.9	-71.9	3.33	TRUE
SRX725589	TFs and others	TAL1	Blood	T-ALL	22746	1572/11207	1378/18171	-68.4	-67.5	1.85	TRUE
SRX158111	TFs and others	TAL1	Blood	RPMI 8402	11091	774/11207	503/18171	-61.0	-60.1	2.49	TRUE
SRX475794	TFs and others	SPI1	Blood	GM12878	23939	2675/11207	2989/18171	-53.5	-52.6	1.45	TRUE
SRX1041802	TFs and others	GATA3	Blood	Jurkat	13079	958/11207	757/18171	-52.1	-51.2	2.05	TRUE

TFs enriched for pDC are filtered by searching with the keyword "TRUE" in the text box. The most enriched factor is TCF4, which is an essential factor for the differentiation of pDCs.

Download the data



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▲ ChIP-Atlas: Enrichment Analysis

identify common epigenetic features of a given set of genomic loci and genes

Result page URL will be available for a week from the time when 'status' is 'finished'.

Project title	Test
Request ID	wabi_chipatlas_2024-0121-1545-55-143-974837
Submitted at:	15:45:55 (Jan-21-2024)
Estimated finishing time:	16:01:55 (Jan-21-2024)
Current time:	16:10:59 (Jan-21-2024)
Status	finished
Result URL:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_chipatlas_2024-0121-1545-55-143-974837?info=result&format=html
Download TSV:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_chipatlas_2024-0121-1545-55-143-974837?info=result&format=tsv

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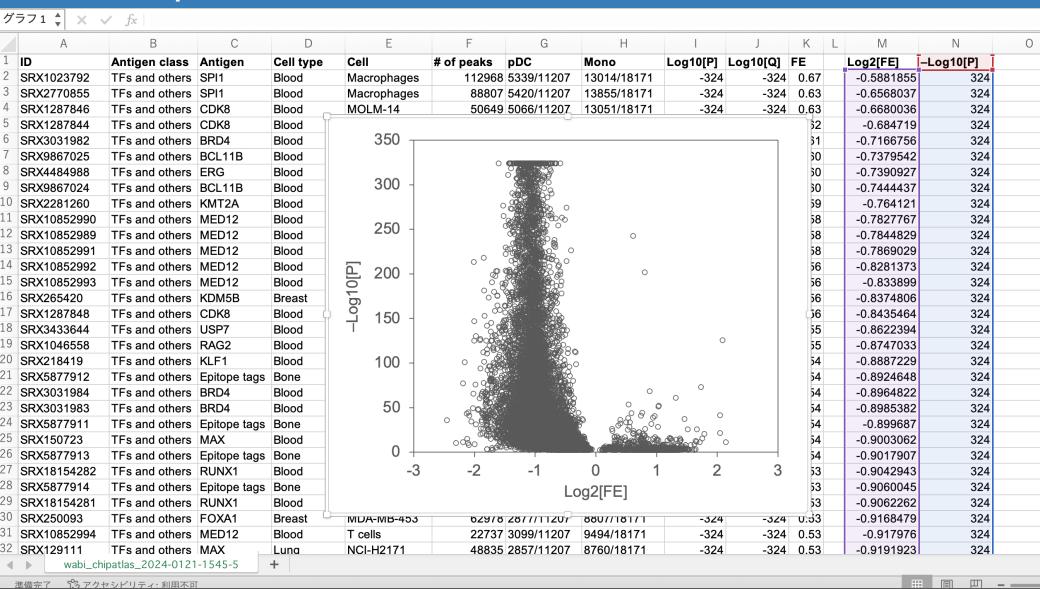
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The table can be downloaded in the Tab-Separated Values (TSV) format and used for further analysis, such as drawing the volcano plot shown on the next slide.

Volcano plot



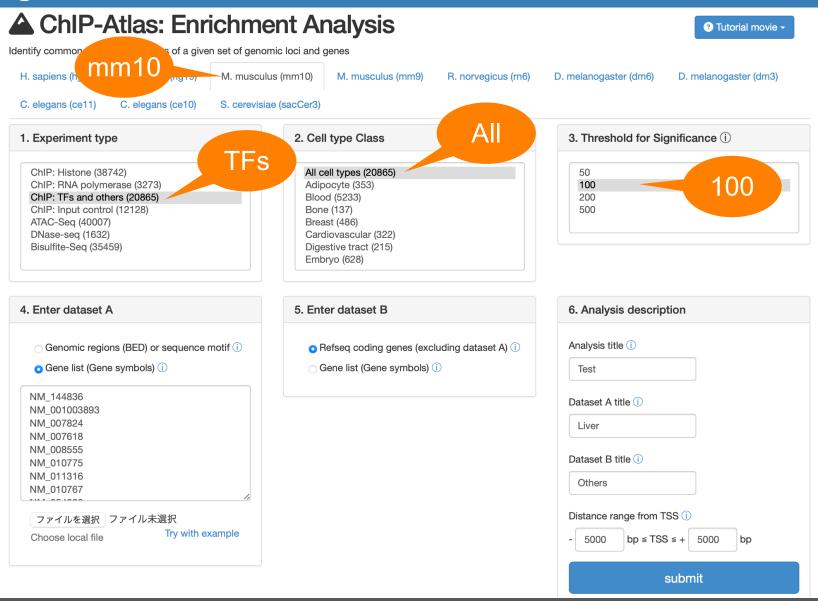
Volcano plots are useful to visualize the distribution of *P* and FE values simultaneously. This can be done on Microsoft Excel[®] using a downloaded TSV file.

The manual for

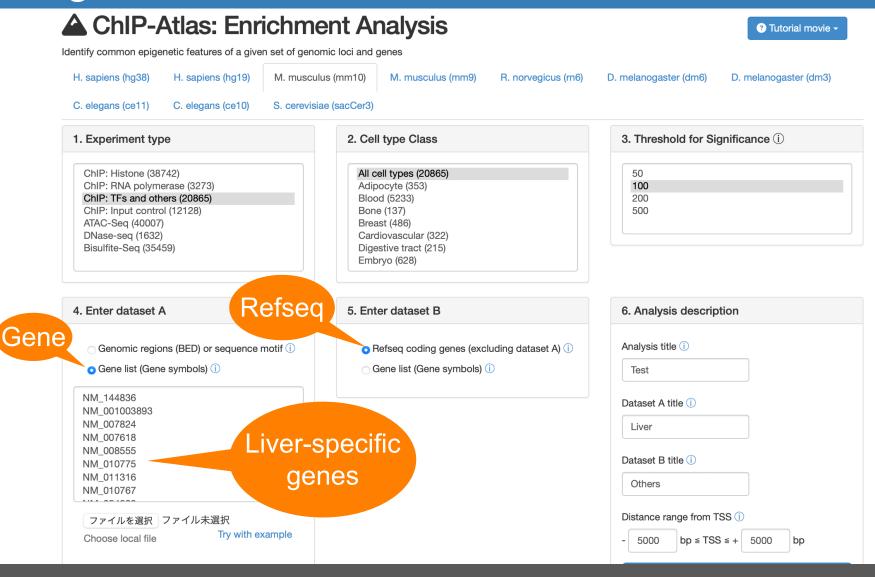


- 1. Overview
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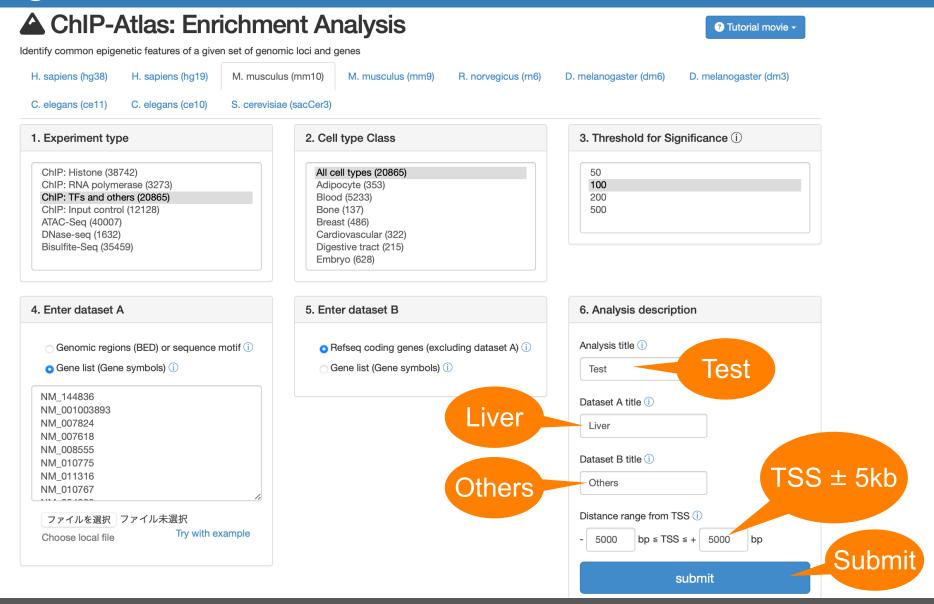
Next, let us try to identify the TFs whose bindings are enriched around the genes that are specifically expressed in liver cells.



Set to examine the enrichment of TF peaks in all cell types with >100 MACS2 scores.



You can enter the gene symbols or IDs in the text box of "dataset A", e.g., the genes specifically expressed in the liver (🔀 Download) obtained from the tissue-specific gene database, RefEx. The other Refseq coding genes are set as the background.



Enter the titles of this project (Test) and datasets A (Liver) and B (Others), which will be used to label the result data. Regions of $\pm 5,000$ bp are specified to examine the TF binding. Click on the "submit" button.

Run status



Enrichment Analysis

Diff Analysis فأه

Target Genes

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▲ ChIP-Atlas: Enrichment Analysis

identify common epigenetic features of a given set of genomic loci and genes

Result page URL will be available for a week from the time when 'status' is 'finished'.

Project title	Test
Request ID	wabi_chipatlas_2024-0121-1545-55-143-974837
Submitted at:	15:45:55 (Jan-21-2024)
Estimated finishing time:	16:01:55 (Jan-21-2024)
Current time:	16:10:59 (Jan-21-2024) finished
Status	finished
Result URL:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_chipatlas_2024-0121-1545-55-143-974837?info=result&format=html
Download TSV:	http://ddbj.nig.ac.jp/wabi/chipatlas/wabi_c/_atlas_2024-0121-1545-55-143-974837?info=result&format=tsv

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ChIP-Atlas / Enrichment Analysis

Search for proteins significantly bound to your data.

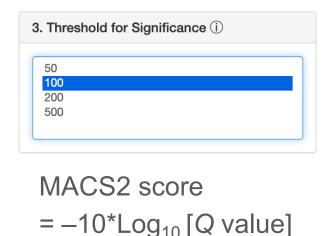
Show 100 entries

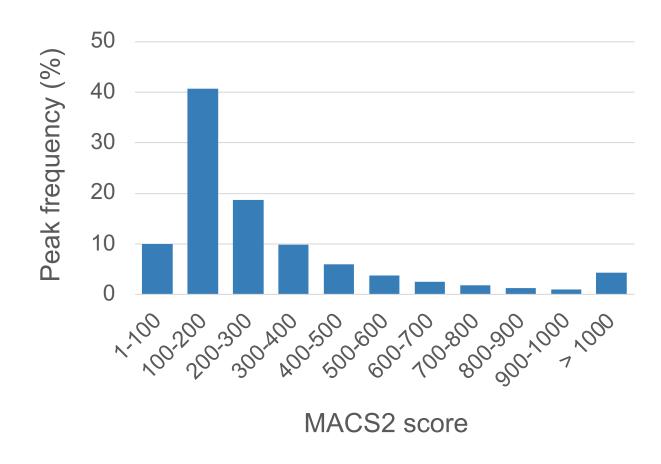
Test

ID \$	Antigen class	Antigen	Cell class	Cell	\$ Num of peaks	Overlaps / Liver	Overlaps / Others	Log P- ^ val	Log Q- ∲ val	Fold Enrichment	FE > \$ 1?
SRX2375602	TFs and others	Prox1	Liver	Liver	22246	120/176	2947/19280	-54.5	-50.2	4.46	TRUE
SRX5028147	TFs and others	Ncor1	Liver	Liver	20087	114/176	2813/19280	-50.4	-46.4	4.44	TRUE
SRX6817423	TFs and others	Hnf4a	Liver	Liver	24867	136/176	4494/19280	-50.2	-46.4	3.32	TRUE
SRX5028173	TFs and others	Ncor1	Liver	Liver	22448	125/176	3619/19280	-49.9	-46.2	3.78	TRUE
SRX377391	TFs and others	Esr1	Liver	Liver	20828	137/176	4639/19280	-49.5	-45.9	3.24	TRUE
SRX5028171	TFs and others	Ncor1	Liver	Liver	26423	132/176	4226/19280	-49.3	-45.8	3.42	TRUE
SRX20201041	TFs and others	Hnf4a	Liver	Liver	22824	131/176	4151/19280	-49.2	-45.7	3.46	TRUE
ODV0047400	TFs and	Llof4a	1	Lhan	04400	400/470	4040/40000	40.0	440	0.05	TDUE

The most enriched TFs, include Prox1, Ncor1, and Hnf4a, which are known to be essential for liver development and function. This tool is therefore useful for identifying the TFs that organize cell type-specific genes.

Tips: Threshold for significance



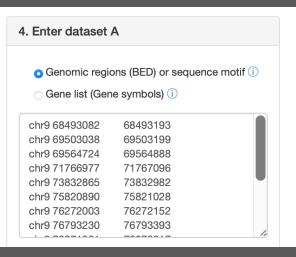


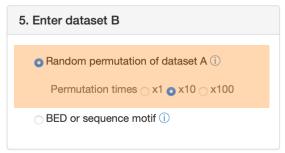
The 'Threshold for Significance' panel of the Enrichment Analysis tool specifies the cut-off value of the MACS2 scores that indicates the statistical significance of each peak call. This histogram shows the relative frequencies of the ChIP/ATAC/DNase-seq peaks according to the MACS2 score. We recommend a threshold value of >100 to display reliable data.

Tips: Acceptable BED data

Input BED file must be described as follows:

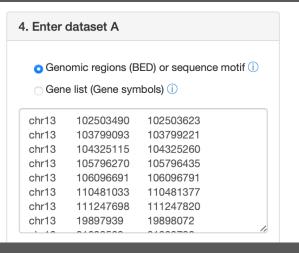
Chromosome<tab>Begin<tab>End

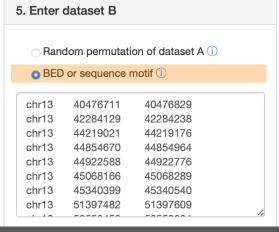




BED vs Random background:

Suitable if you have an input BED file, but do not have a file for comparison, such as the SNPs loci and evolutionary conserved regions. Increasing the number of 'permutation times' will generate less biased random backgrounds; however, the computation time will be longer.





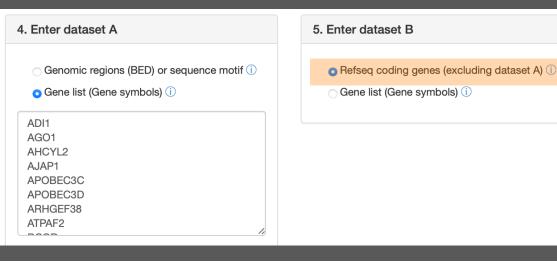
BED vs BED:

Suitable for comparing two sets of BED files, such as the two cell types of ATAC-seq peaks or hypomethylated regions and two types of histone mark peaks from an identical cell type.

Tips: Acceptable gene list

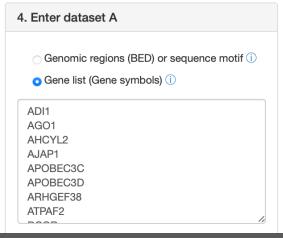
Official gene symbols and IDs shown here are acceptable; however, the synonymous symbols are not.

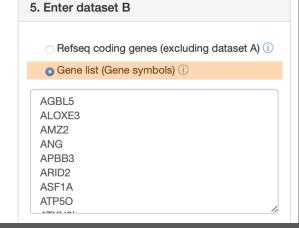
Official symbol	Synonym	Refseq ID	Ensembl gene ID	Ensembl transcript ID	UniProt ID
Pou5f1	Oct4	NM_013633	ENSMUSG00000024406	ENSMUST00000025271	P20263
Trp53	p5 3	NM_011640	ENSMUSG00000059552	ENSMUST00000005371	P02340



Genes vs Other Refseq coding genes:

Suitable if you have a list of input genes, but do not have a list for comparison, such as the genes specifically expressed in a tissue or cell type.





Genes vs Genes:

Suitable for comparing two sets of gene lists, such as the up- vs down-regulated genes in response to certain mutations or drug administration.

Tips: API

```
$ 1s
dataA.bed
$ curl -X POST \
  -d "format=text" \
  -d "result=www" \
  -d "genome=hg19" \
  -d "antigenClass=TFs and others" \
  -d "cellClass=Pluripotent stem cell" \
  -d "threshold=50" \
  -d "typeA=bed" \
  --data-urlencode "bedAFile@dataA.bed" \
  -d "typeB=rnd" \
  -d "bedBFile=empty" \
  -d "permTime=10" \
  -d "title=10 times random permutation of my data" \
  -d "descriptionA=my data" \
  -d "descriptionB=random permutation 10 times" \
  -d "distanceUp=0" \
  -d "distanceDown=0" \
  https://ddbj.nig.ac.jp/wabi/chipatlas/
```

The API of the Enrichment Analysis tool is available for batch analysis of multiple queries. See <u>here</u> for details.