Goal for this tutorial

- Identify top small noncoding RNAs that are expressed in a particular tissue
- Analyze publicly available brain dataset from ENCODE in hg38, and identify highly expressed sncRNA in this data.
- Compare and validate the analyze results in vivo using a brain dataset from DASHR in hg38
- Note: refer to "SPAR_output_and_report_page.pdf" if you want to under the outputs more



analysis, annotation, visualization of small RNA sequencing experiments

discover and chararacterize small RNAs from sequencing

analyze public datasets Analyze public datasets **Click here to** start Genome Human (Dec. 2013 hg38, GRCh38) ▼ Select DASHR dataset (small RNA-seq) (Human hg38): Select DASHR dataset Analyze DASHR Select ENCODE data portal dataset (short total RNA-seq) (Human hg38): frontal-cortex-rep1_ENCSR000AFS Analyze ENCODE



analysis, annotation, visualization of small RNA sequencing experiments

discover and chararacterize small RNAs from sequencing

analyze public datasets analyze your own data

Step 3: Click "Analyze ENCODE"

to proceed

Analyze public datasets

Genome Human (Dec. 2013 hg38, GRCh38) ▼	Step 1: Select hg38 as reference genome					
Select DASHR dataset (small RNA-seq) (Human hg38): Select DASHR dataset Analyze DASHR						
Select ENCODE data portal dataset (short total RNA-seq) (Human hg38): Step 2: Select from dropdown, a frontal cortex data from ENCODE						

Progress of SPAR



analysis, annotation, visualization of small RNA sequencing experiments

discover and chararacterize small RNAs from sequencing

Running job ca8ac37

Job id is shown

Input files:

http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.pos.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigWighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigwighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.neg.bigwighttp://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/

SPAR

Input files are listed

```
Genome: hg38

Fri, 26 Jan 2018 15:37:49 -0500 .... Loading PLUS strand: http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_EFri, 26 Jan 2018 15:37:49 -0500 .... Starting SPAR run

Fri, 26 Jan 2018 15:37:49 -0500 .... Starting SPAR run

Fri, 26 Jan 2018 15:37:49 -0500 .... Analysis parameters: min. fold change=2; min. coverage=10; min. size=14; max. size=44
bash run_SPAR.sh SPAR_out/ca8ac37/tracks/raw.pos.bigWig SPAR_out/ca8ac37 pos SPAR_out/ca8ac37/config.hg38.sh 2>&1

Fri, 26 Jan 2018 15:37:55 -0500 .... DONE.

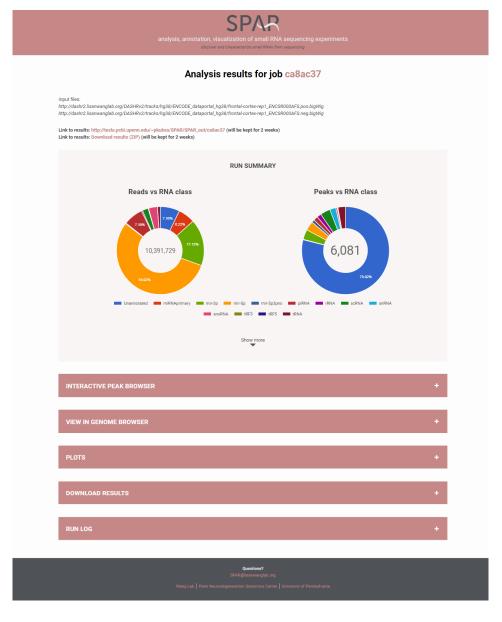
Fri, 26 Jan 2018 15:37:55 -0500 .... DONE.

Fri, 26 Jan 2018 15:37:55 -0500 .... Sending results to the web server.....

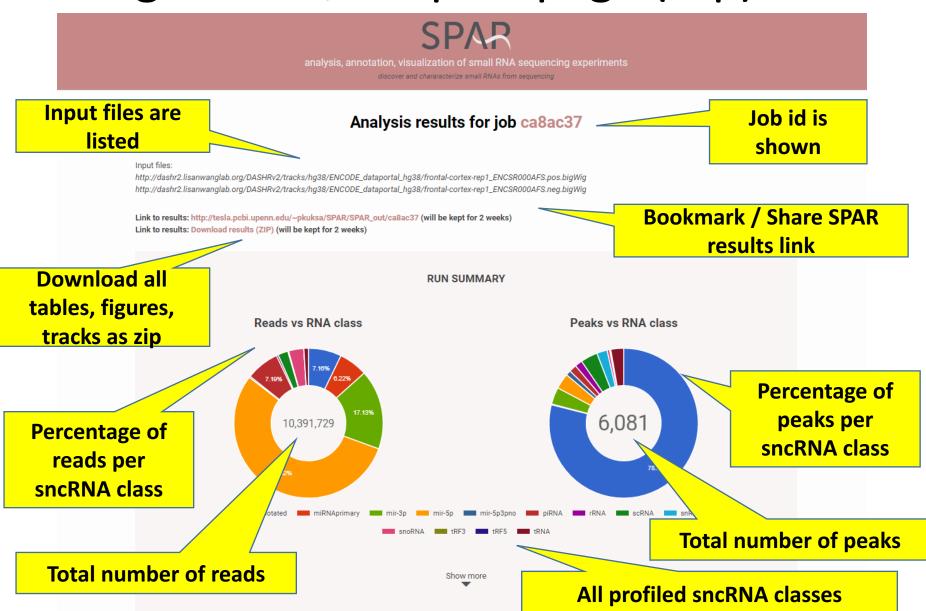
Parameters used in

SPAR analyses
```

Landing results page (full view)



Landing results / outputs page (top)



Landing results / outputs page (top)



analysis, annotation, visualization of small RNA sequencing experiments

discover and chararacterize small RNAs from sequencing

Analysis results for job ca8ac37

Input files:

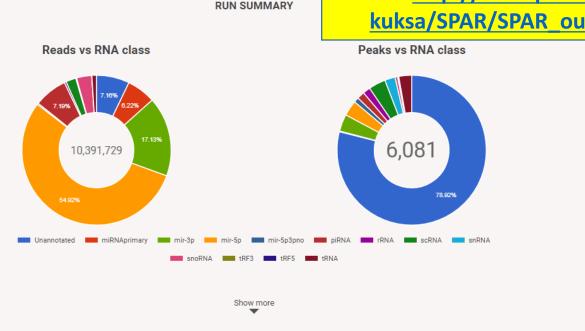
http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR000AFS.pos.bigWig

http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/frontal-cortex-rep1_ENCSR0004

Link to results: http://tesla.pcbi.upenn.edu/~pkuksa/SPAR/SPAR_out/ca8ac37 (will be kept for 2 weeks)
Link to results: Download results (ZIP) (will be kept for 2 weeks)

To view a report (in html / pdf): Click "Link to

results: http://tesla.pcbi.upenn.edu/~p kuksa/SPAR/SPAR out/ca8ac37



Landing results → SPAR report page

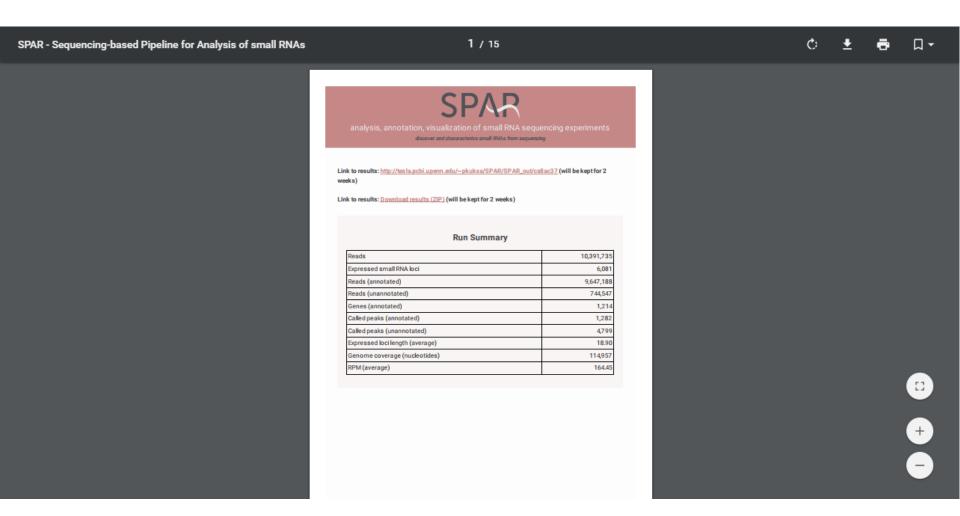
Index of /~pkuksa/SPAR/SPAR_out/ca8ac37

<u>Name</u>	<u>Last modified</u>	<u>Size</u>	<u>Description</u>
Parent Directory		-	
DASHR_comparison/	26-Jan-2018 15:38	-	
ENCODE_comparison/	26-Jan-2018 15:39	-	
figures/	26-Jan-2018 15:39	-	
inputs/	26-Jan-2018 15:37	-	
logs/	26-Jan-2018 15:39	-	
results/	26-Jan-2018 15:39		Report is available in html / pdf
tracks/	26-Jan-2018 15-2		
report.html	26 Jan-2018 15:39	40K	
report.pdf	26-Jan-2018 15:39	1.8M	
results.html	26-Jan-2018 15:39	2.4K	
run_summary.html	26-Jan-2018 15:39	584	
SPAR.hg38.config	26-Jan-2018 15:37	2.0K	

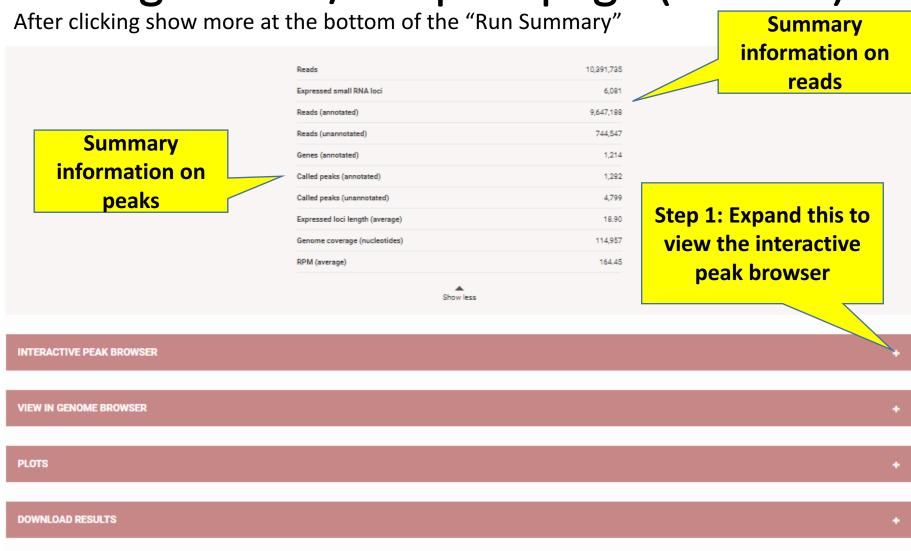
Apache/2.2.3 (CentOS) Server at tesla.pcbi.upenn.edu Port 80

SPAR report.pdf

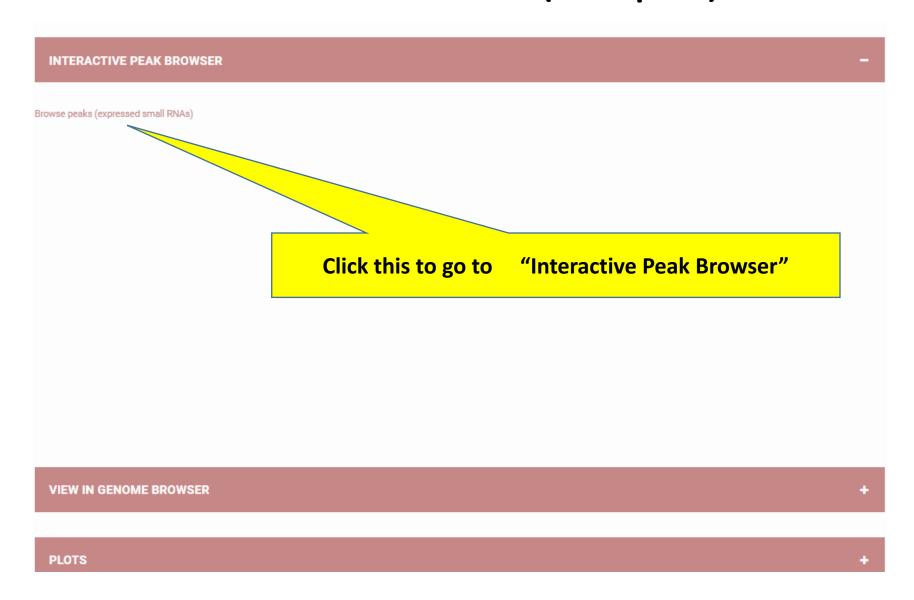
A 15 page report containing tables and figures for download and sharing



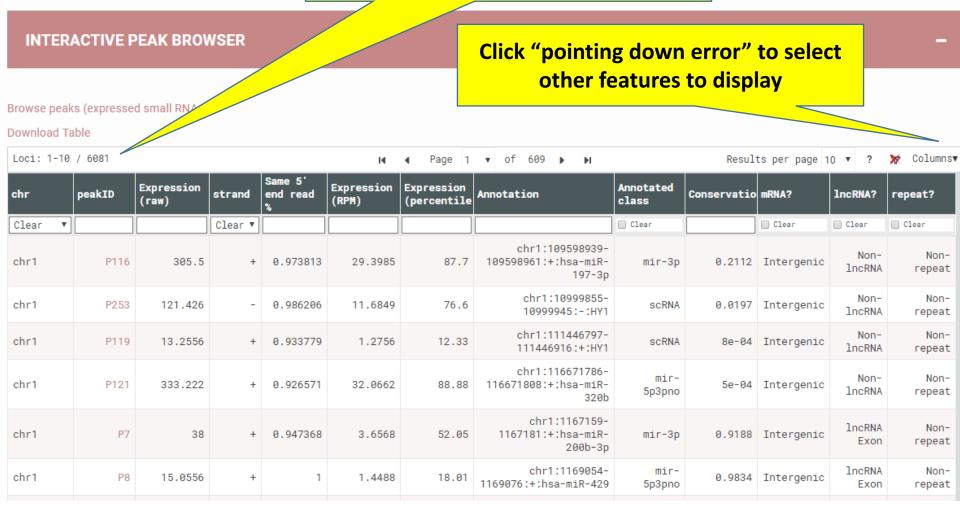
Landing results / outputs page (bottom)



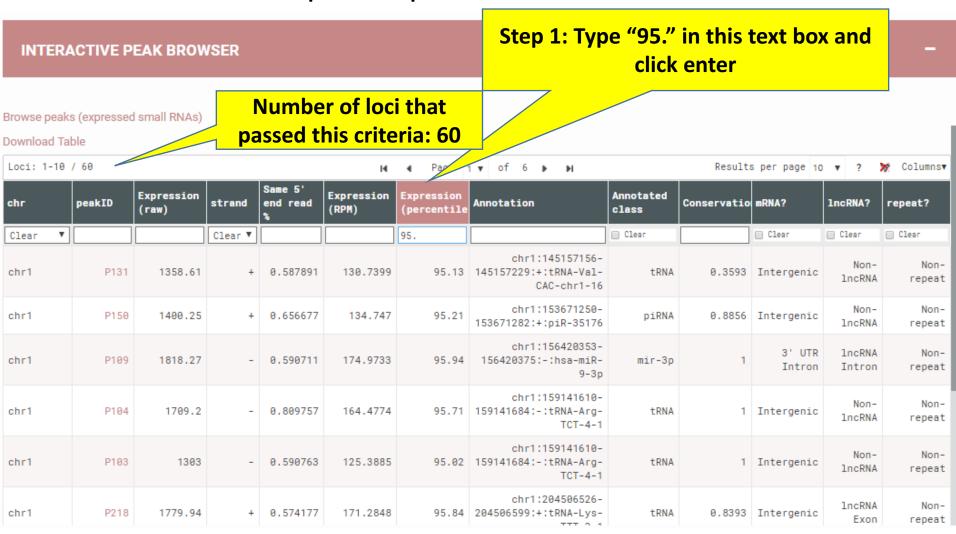
RUN LOG



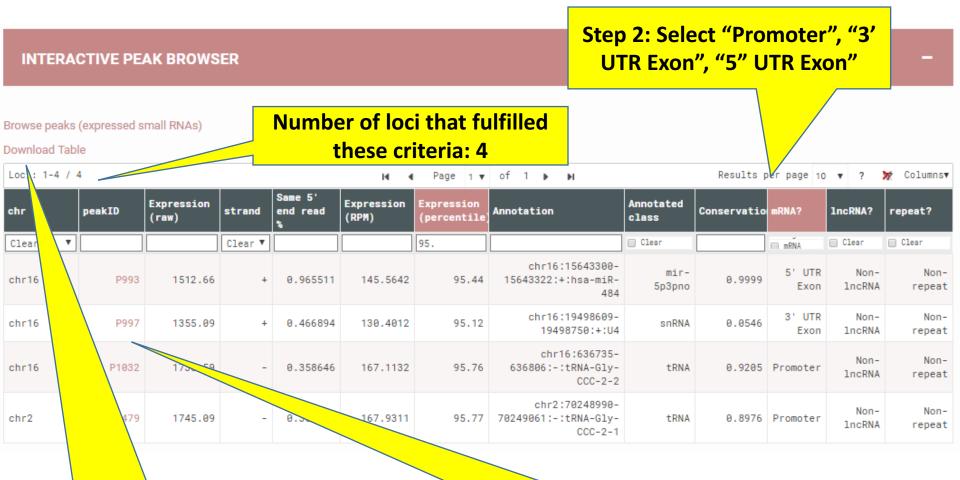
Total number of called peaks (annotated or unannotated)



Filter sncRNA loci with the top 5% of expression



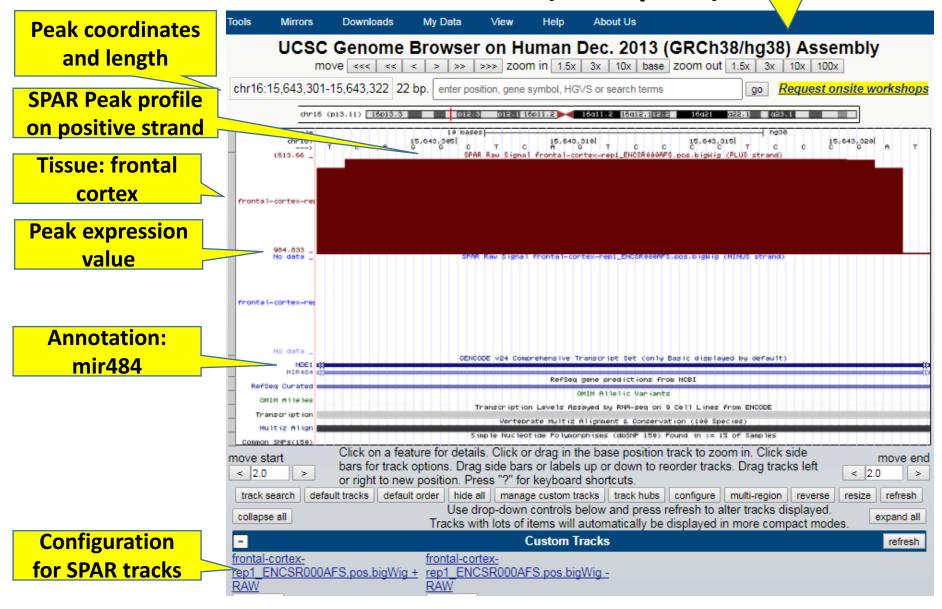
Further filter sncRNA loci that only present in Promoter, 3' or 5' UTR exonic regions



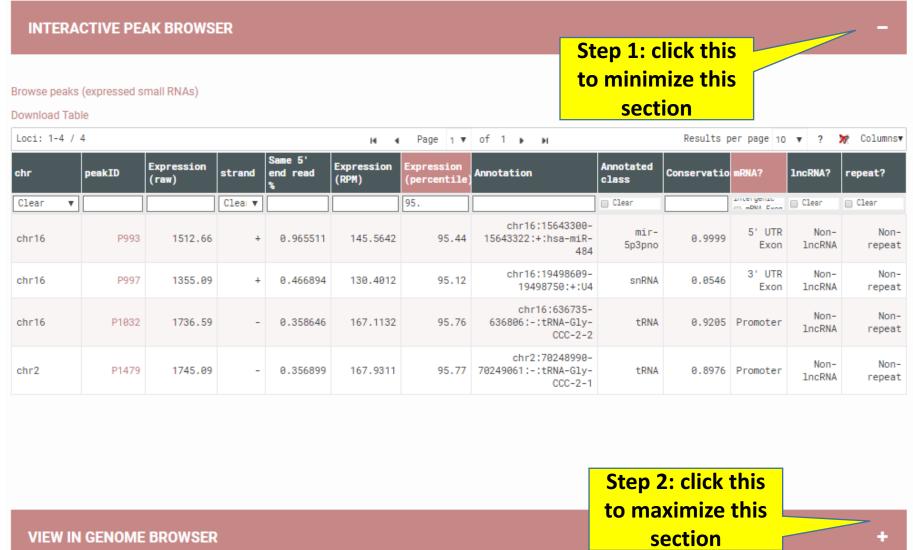
Step 3: Export these 4 filtered sncRNAs

Optional: click this link to visualize this peak P993 in UCSC genome browser

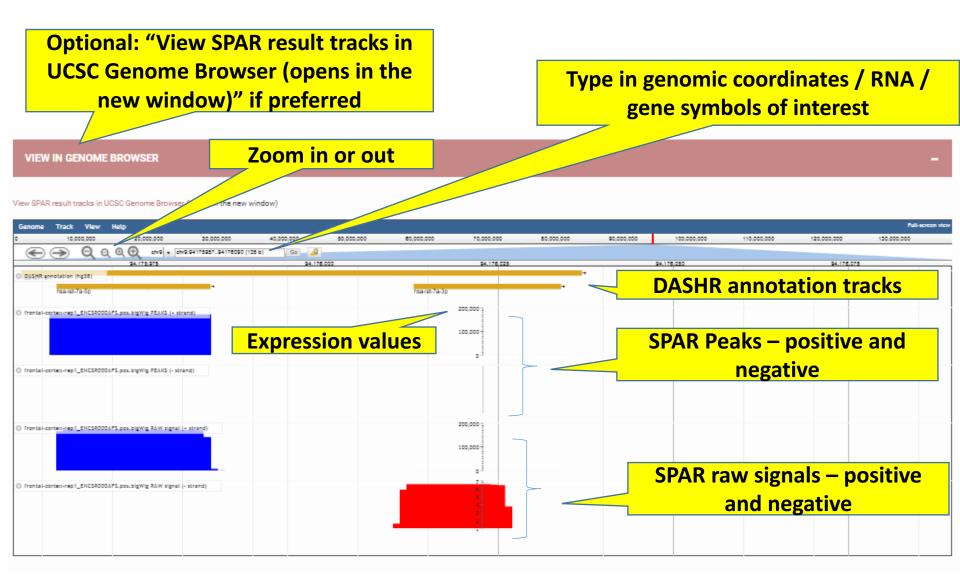
Hg38 reference genome



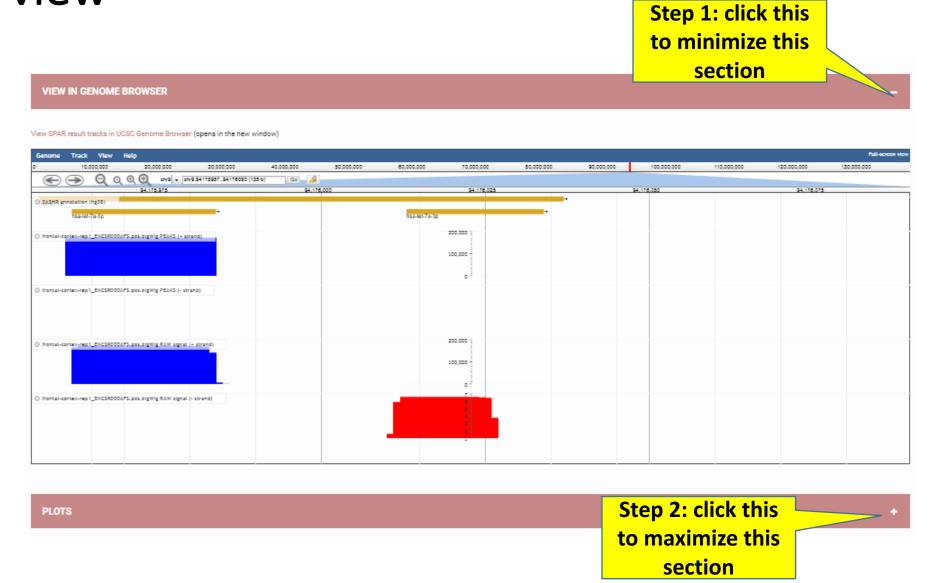
Finish exploring Interactive Peak Browser, next view peaks in genome browser



View in Genome Browser (Output)



understand the data in a genomewide view



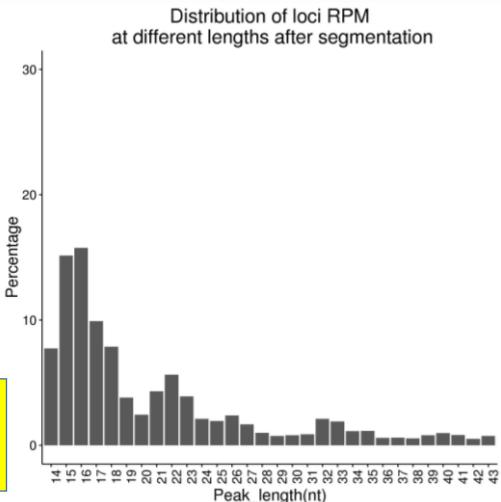
Plots (Output)

PLOTS

4 different plot sessions

SEGMENTATION ength distribution of rpm of segmented loc Genomic length distribution of identified small malloci LENGTHEXPRESSION Rpm distribution of identified small ma loci Percentile distribution of identified small malo cross different lengths PROCESSING 5p end positional offset between identified loci Processing specificity at 5p end of identified sma Read pipeup at 5p end of identified small rna loci GENOMEWIDE Proportion of expressed annotated small ma gene-Genomewide distribution patterns of small rna loci Circular genome data visualization Genomewide distribution of expressed small mailor Proportion of mapped reads across all loci Click to display,

Click to display, corresponding plot will be shown in the right



Plots (Output)

PLOTS

4 different plot sessions

SEGMENTATION

Length distribution of rpm of segmented loci

Genomic length distribution of identified small ma loci

LENGTHEXPRESSION

Rpm distribution of identified small ma loci

Percentile distribution of identified small malloci across different lengths

PROCESSING

5p end positional offset between identified loci and small ma gene

Processing specificity at 5p end of identified small ma loci

Read pipeup at 5p end of identified small rna loci

GENOMEWIDE

Proportion of expressed annotated small rna genes

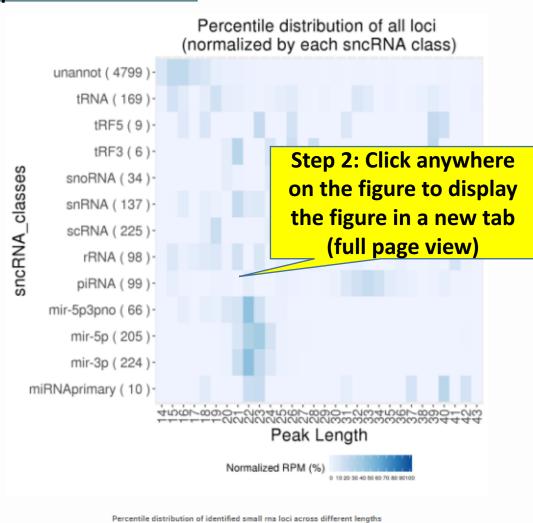
Genomewide distribution patterns of small ma loci

Circular genome data visualization

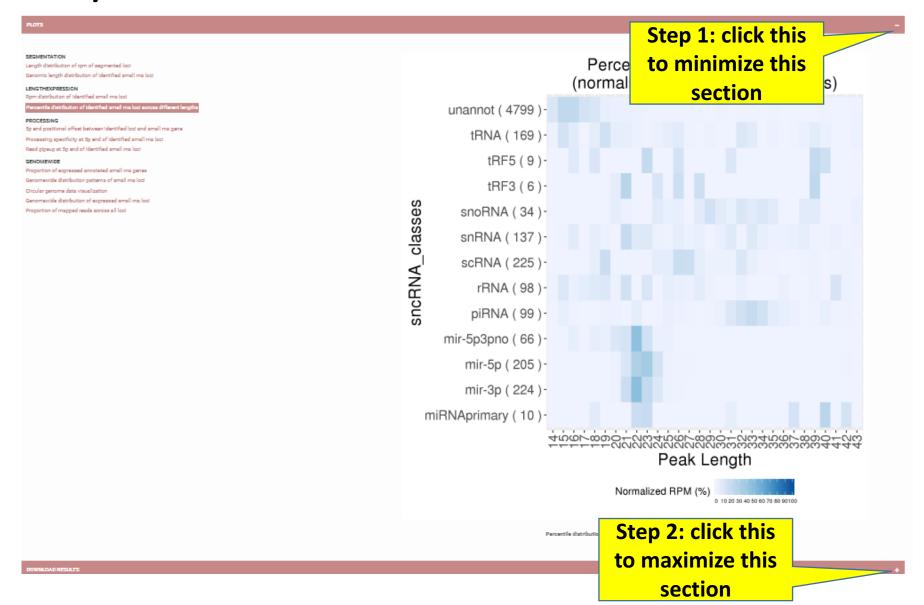
Genomewide distribution of expressed small ma loci

Proportion of mapped reads across all loci

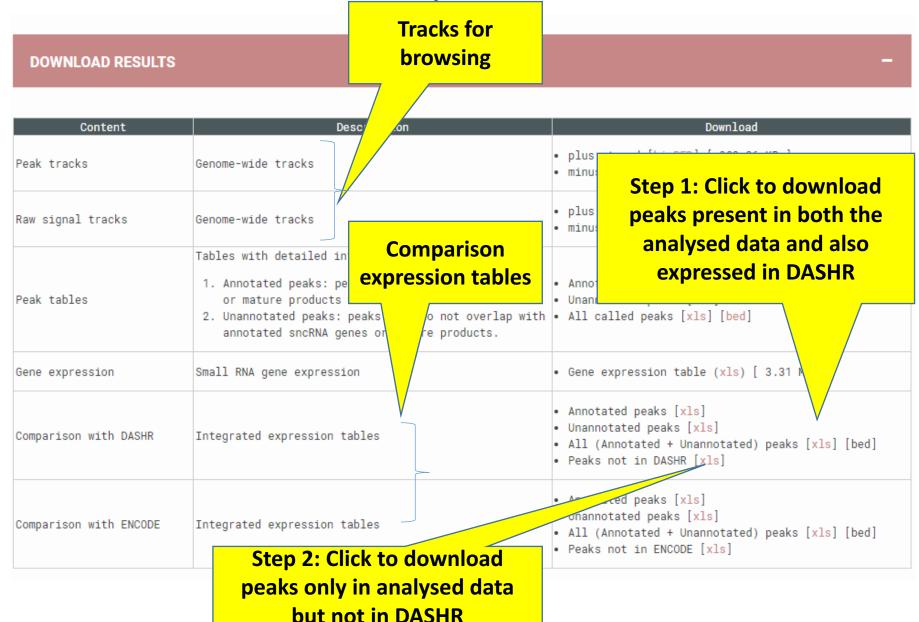
Step 1: Click to display, corresponding plot will be shown in the right



Finish viewing the plots, want to perform own analyses with SPAR results – visit download



Download results (Top) – bulk download



Download results (Bottom) – download by sncRNA class

Step 2: Click to download, e.g. all unannotated loci from SPAR

Total number of reads falling onto this RNA class

DOWNLOAD PEAK TABLES BY RNA

RN/ ss	Peaks	Genes	Reads	Percentage of reads
Annotated	1,282	1,214	9,647,188	92.84
Unannotated	4,799	4,799	744,547	7.16
miRNAprimary	10	10	646,698	6.22
mir-3p	224	224	1,779,736	17.13
mir-5p	205	205	5,706,729	54.92
mir-5p3pno	66	66	30,553	0.29
piRNA	99	99	747,600	7.19
rRNA	98	81	42,675	0.41
scRNA	225	215	227,677	2.19
snRNA	137	132	17,896	0.17
snoRNA	34	29	341,572	3.29
tRF3	6	6	1,323	0.01
tRF5	9	9	998	0.01
trna —	169	138	103,725	1.00

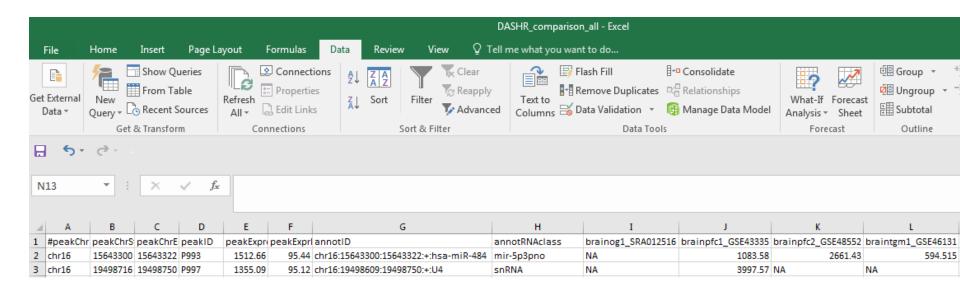
Step 1: Click to download, e.g. tRNA only loci results from SPAR

Download results (Output)

We found earlier that P1032, P993, P997, P1479 are of interest, not sure if these peaks are found in any of the DASHR brain tissues.

First excel file downloaded:

1) DASHR_comparison_all.xls – if any of the above peaks are found in this file, meaning they can be present in any DASHR tissue; not necessarily expressed in brain.

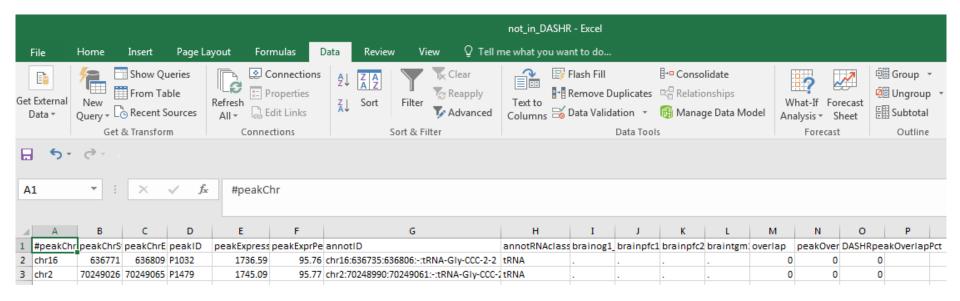


P993 and P997 were found in this table. We extracting the four brain related DASHR tissues (now in column I to L). Only column J (a prefronal cortex brain data) have both peaks expressed.

Download results (Output)

Second excel files downloaded:

not_in_DASHR.xls (for sanity check only)



P1032 and P1479 were found in this table, i.e. not expressed in any of the DASHR tissues, but present in the ENCODE prefrontal cortex data.

Conclusion – out of the 4 sncRNAs we identified, two were in silico confirmed to be highly expressed across different brain datasets in both DASHR and ENCODE.

Run log (Output)

Step 1: click this to minimize "Download results"

DOWNLOAD RESULTS

Content	Description	Download		
Peak tracks	Genome-wide tracks	plus strand [bigBED] [382.36 KB] minus strand [bigBED] [384.94 KB]		
Raw signal tracks	Genome-wide tracks	plus strand [bigWig] [5.86 MB] minus strand [bigWig] [3.68 MB]		
Peak tables	Tables with detailed information: 1. Annotated peaks: peaks overlapping smcRNA genes or mature products with annotations. 2. Unannotated peaks: peaks that do not overlap with annotated smcRNA genes or mature products.	Annotated peaks [xls] Unannotated peaks [xls] All called peaks [xls] [bed]		
Gene expression	Small RNA gene expression	Gene expression table (xls) [3.31 MB]		
Comperison with DASHR	Integrated expression tables	Annotated peaks [xis] Unannotated peaks [xis] All (Annotated + Unannotated) peaks [xis] [bed] Peaks not in DASHR [xis]		
Comparison with ENCODE	Integrated expression tables	Annotated peaks [xls] Unannotated peaks [xls] All (Annotated + Unannotated) peaks [xls] [bed] Peaks not in ENCODE [xls]		

DOWNLOAD PEAK TABLES BY RNA CLASS

RNA class	Peaks	Genes	Reads	Percentage of reads
Annotated	1,282	1,214	9,647,188	92.84
Unannotated	4,799	4,799	744,547	7.16
miRNAprimary	18	18	040,098	6.22
mir-3p	224	224	1,779,736	
mir-5p	285	285	5,786,729	
mir-5p3pno	00	00	39,553	
p1RNA	99	99	747,688	
rRNA	98	81	42,675	
scRNA	225	215	227,677	
snRNA	137	132	17,896	8.17
snoRNA	34	29	341,572	
tRF3	6	6	1,323	
tRF5	9	9	998	
tRNA	109	138	183,725	1.99

RUN LOG

Questions? SPAR@itsenwengleb.org Step 2: click this to maximize "Run log"

Run log (Output)

Run log displayed agin

RUN LOG

```
Genome: hg38
Fri, 26 Jan 2018 15:37:49 -0500 .... Loading PLUS strand: http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/fron
Fri, 26 Jan 2018 15:37:49 -0500 .... Loading MINUS strand: http://dashr2.lisanwanglab.org/DASHRv2/tracks/hg38/ENCODE_dataportal_hg38/fron
Fri, 26 Jan 2018 15:37:49 -0500 .... Starting SPAR run
Fri, 26 Jan 2018 15:37:49 -0500 .... Analysis parameters: min. fold change=2; min. coverage=10; min. size=14; max. size=44
bash run_SPAR.sh SPAR_out/ca8ac37/tracks/raw.pos.bigWig SPAR_out/ca8ac37 pos SPAR_out/ca8ac37/config.hg38.sh 2>&1
Fri, 26 Jan 2018 15:37:49 -0500 .... Processing job ca8ac37............
Fri, 26 Jan 2018 15:37:55 -0500 ... DONE.

Fri, 26 Jan 2018 15:37:55 -0500 ... Sending results to the web server...
Fri, 26 Jan 2018 15:39:38 -0500 ... Processed 10,391,735 reads in 109 seconds (95,337 reads / second)

Fri, 26 Jan 2018 15:39:38 -0500 ... Creating plots
Fri, 26 Jan 2018 15:39:46 -0500 ... DONE creating plots
```

SPAR run log

Questions?

SPAR@lisanwanglab.org

Wang Lab Penn Neurodegeneration

nomics Center | University of Pennsylvania

Send questions to this email: SPAR@lisanwanglab.org

END