GUAVA Manual

Mayur Divate and Edwin Cheung

Version 1

Released on May 3, 2018

Table of Content

1. About GUAVA

GUAVA: a GUI tool for the Analysis and Visualization of ATAC-seq data

GUAVA is a standalone GUI tool for the processing, analysis, and visualization of ATAC-seq data from raw sequencing reads to ATAC-seq signals. GUAVA can compare ATAC-seq signals from two conditions to identify genomic loci with differentially enriched ATAC-seq signals. Furthermore, GUAVA provides results on gene ontology and pathways analysis. Since using GUAVA requires only several clicks and no learning curve, it will help novice bioinformatics researchers and biologist with minimal computer skills to analyze ATAC-seq data. Therefore, we believe that GUAVA is a powerful and time saving tool for ATAC-seq data analysis. The GUAVA setup contains a script to configure and install dependencies which facilitates the GUAVA installation. GUAVA works on Linux and Mac OS.

This document contains all the information that is required to install and use GUAVA.

GUAVA was developed in Edwin Cheung's laboratory at the University of Macau.

2. How to download GUAVA

GUAVA is hosted on GitHub and can be downloaded to your computer by performing the following steps.

Step 1: Go to the link: https://github.com/MayurDivate/GUAVA/releases.

Step 2: Click on 'Source code (zip)'.

Step 3: This will save the GUAVA zip package in your computer's downloads folder.

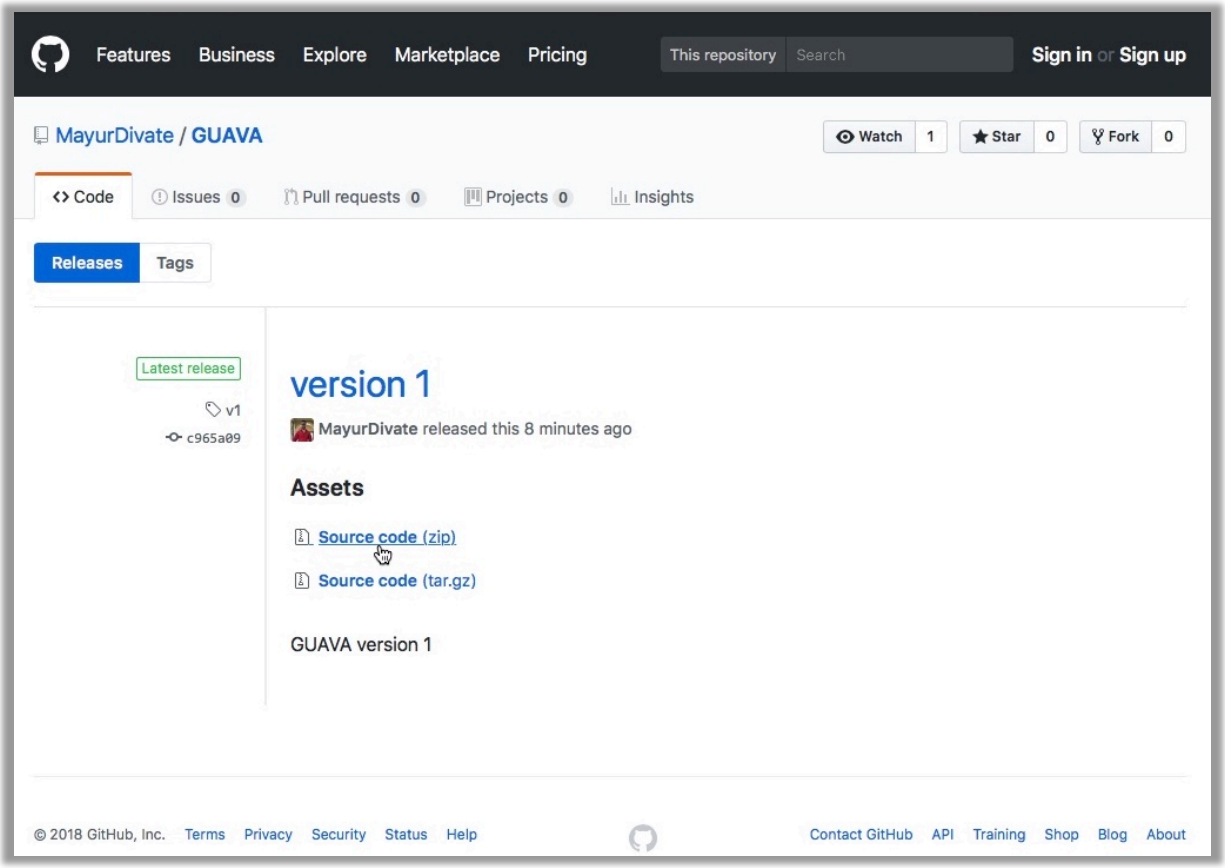


Figure1. GUAVA - GitHub project page. Screenshot shows the download page for GUAVA at GitHub. Users can download the GUAVA package by clicking on 'Source code (zip)'.

If you would like to download the source code for GUAVA, use the link below.
https://github.com/MayurDivate/GUAVASourceCode.

3. How to get help and report bugs

Sometimes users face difficulties in installing or using bioinformatic tools. Therefore, it is very important to have an active forum where users can report issues and share information with authors and other users. Thus, GUAVA has a forum at GitHub for this and users can report any issues and share information by going to this link: https://github.com/MayurDivate/GUAVA/issues.

4. How to open Terminal

After downloading GUAVA, users will need to install it using the Terminal. Here, we describe the procedure to open the Terminal for non-bioinformatics users.

MAC

1. Open Finder
2. Click on ‘Go’ in the menu bar and then select the ‘Utilities’ folder
3. Double-click on the Terminal icon

Linux

1. Press the ‘windows’ key on the keyboard
2. Type “Terminal” in the search box
3. Click on the Terminal icon

   OR press “**Ctrl** + **Alt** + **T**” at the same time which will open Terminal

5. Installation of GUAVA

GUAVA can be installed in the home folder. Before proceeding, first copy/move the downloaded GUAVA package to the home folder and unzip the package. If the downloaded package is in the folder 'Downloads', then type the following commands in the Terminal to unzip the package.

```
mv ~/Downloads/GUAVA-1.zip ~/
cd ~/
unzip GUAVA-1.zip
```

NOTE: If the downloaded GUAVA package is in a different folder than 'Downloads', you will have to use the complete path of that folder instead of ~/Downloads/GUAVA-1.zip. To copy the path, simply copy the downloaded package and paste it in the Terminal.

5.1 Installing dependencies for GUAVA

GUAVA depends on other tools in order to process ATAC-seq data (*e.g.* Bowtie for alignment). If any of the dependencies are not found on the system, GUAVA will not work properly. Therefore, to help users to install the dependencies, we have written a program called configure.sh, which automatically downloads and installs the dependencies. However, users will need to install R and MACS2 manually due to technical reasons.

5.1.1 Installing R

MAC

1. To download R, follow this link => https://cran.r-project.org/bin/macosx/.
2. Click on the R-X.X.X.pkg file link (*e.g.* R-3.4.3.pkg).
3. Double-click on the downloaded file and follow the instructions.

Linux

1. Open Terminal.
2. Type the command, 'sudo apt-get install r-base' and then press enter.

Note: To know more about R, follow the link https://cran.r-project.org/bin/linux/. Choose the appropriate Linux OS type.

5.1.2 Installing other dependencies

To run configure.sh, use the following commands in the Terminal.

```
cd ~/GUAVA-1
sh ./configure.sh
```

Note: This may take a while to finish. Also, you will need to press 'enter' several times to continue. Additionally, answer all questions with 'yes'

5.1.3 Installing MACS2

To install the last dependency, MACS2, use the following commands in the Terminal.

```
cd ~/GUAVA-1
python get-pip.py
pip install MACS2
```

Error: Sometime MACS2 fails to install **Numpy**. In such a situation run `pip install numpy` first, and then try to install MACS2.

NOTE: If you see the error message '**permission denied',** type 'sudo' at the beginning of the commands (*e.g. sudo python get-pip.py)*. Then, in order to proceed you will have to enter your password.

This is the end of the installation. GUAVA is now ready to process ATAC-seq data.

6. How to start GUAVA

After successfully installing GUAVA, users can start GUAVA by using the following commands in the Terminal. This will open the GUAVA home window where users can choose the program they want to use.

```
cd ~/GUAVA-1
java -jar GUAVA.jar
```

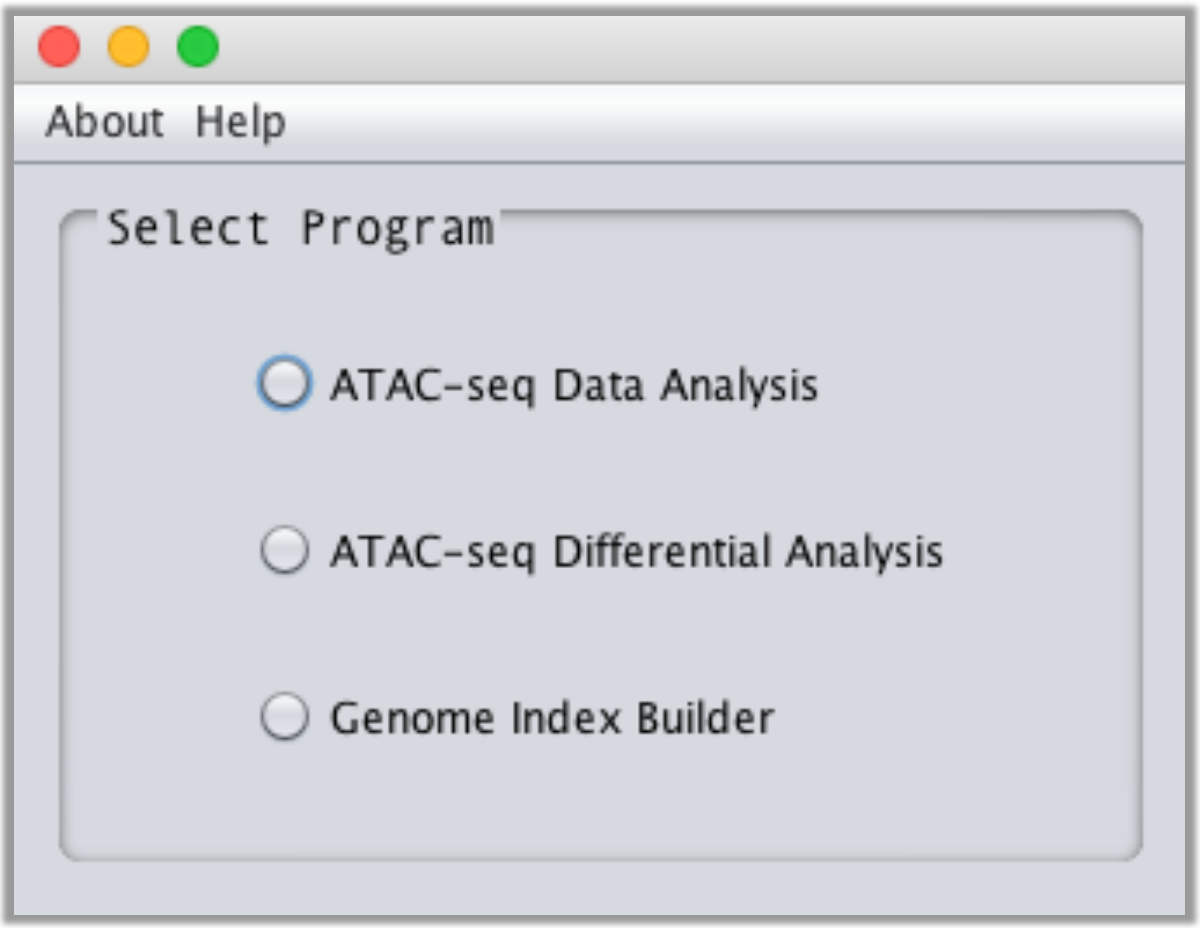


Figure 2. GUAVA–home window. Screenshot showing the GUAVA user interface which allows users to select the desired GUAVA program they wish to run.

7. The graphical user interface of GUAVA

As shown in Figure 2, the GUAVA tool consists of the following three programs:

1) ATAC-seq Data Analysis: to process raw ATAC-seq sequencing reads.

2) ATAC-seq Differential Analysis: to compare ATAC-seq signals.

3) Genome Index Builder: to create the Bowtie or Bowtie2 index of genome

When the GUAVA GUI is evoked, it will open the GUAVA home window (Figure 2). From here, users can choose one of the above programs to proceed further. Based on the selection of the program, the desired input window will open (Figures 3, 13 and 23).

7.1 ATAC-seq data analysis program GUI

The ATAC-seq data analysis program accepts raw ATAC-seq reads as an input. Before aligning reads to genome, the program trims adapter sequences from reads using cutadapt if the trimming option has been selected. In addition, it filters unsuitable reads such as duplicate reads. Users can also exclude certain chromosomes from the analysis using the 'Show Chromosomes' button (Figure 4). The program uses MACS2 to identify ATAC-seq peaks. Finally, it performs functional annotation of the ATAC-seq peaks.

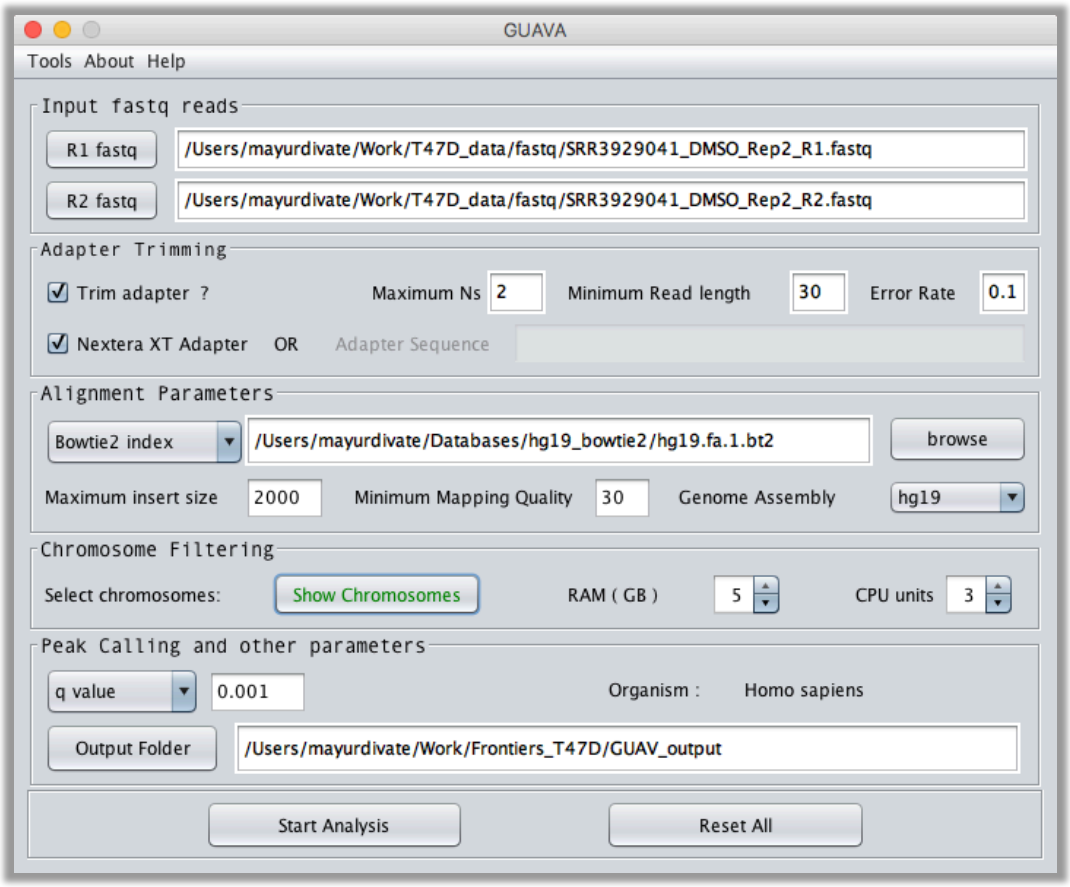


Figure 3. Input window of GUAVA ATAC-seq data analysis program. Screenshot shows the input window of the ATAC-seq data analysis program for uploading input

files such as fastq and genome index, and set parameters such as insert size, p/q value, *etc*.

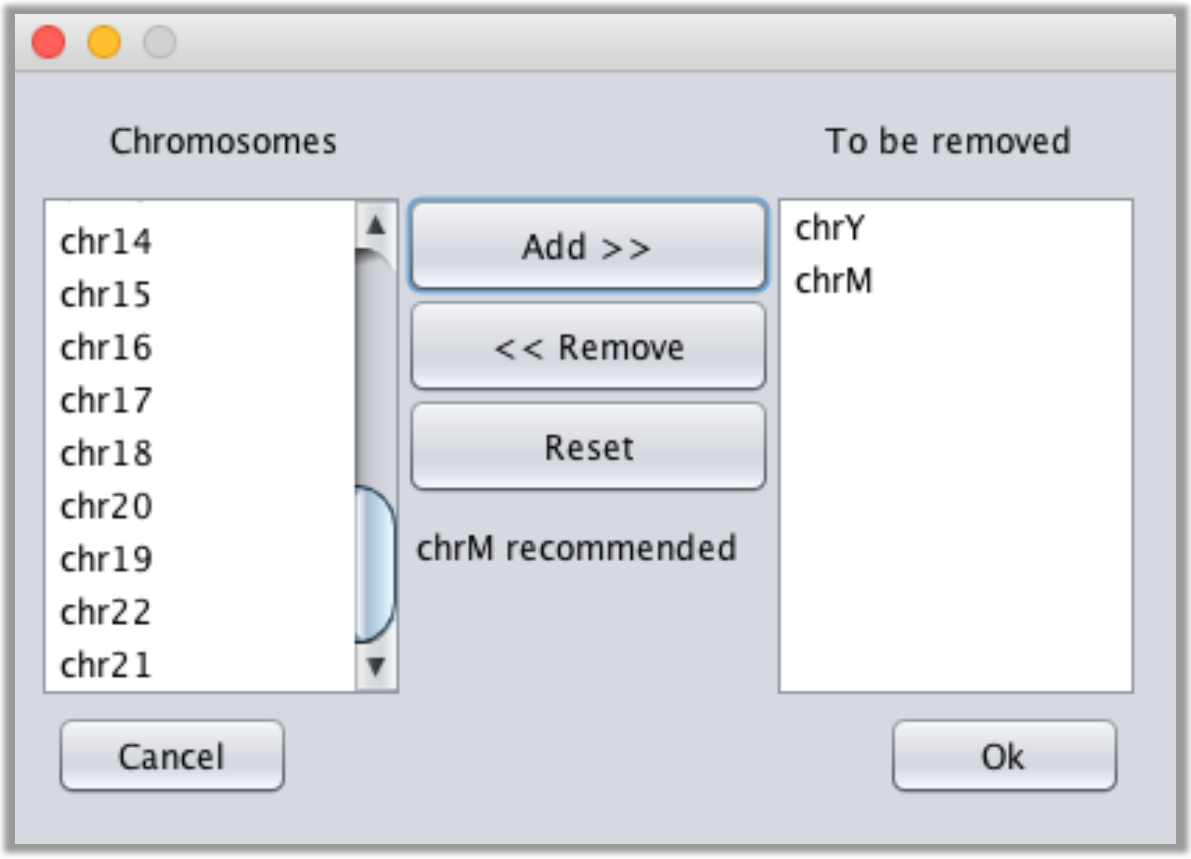


Figure 4. Interface to select chromosomes for alignment filtering. Here, users can add the desired chromosomes to the 'To be removed' list to discard reads aligning to those chromosomes they do not want in the analysis.

7.1.1 ATAC-seq data analysis program parameters

Below is a complete list of the buttons and parameters present in the input interface of the ATAC-seq data analysis program together with a description of their usage.

R1 fastq and R2 fastq: Buttons to upload read1 and read2 fastq files of ATAC-seq data.
Trim adapter: Check this option if reads contain adapter.
Maximum Ns: If any read contains more than the specified number of Ns after the adapter trimming, that read pair will be discarded (default is 2).
Minimum Read Length: If any read is shorter than the specified length after adapter trimming, that read pair will be discarded (default is 30 bp).
Error Rate: The allowed number of mismatches as a fraction of length. For example, if the error rate is 0.1 then 1 mismatch is allowed for a 10 bp match of adapter sequence (default is 0.1).

Nextera XT Adapter: Users can select this option if the adapter used for ATAC-seq is a Nextera XT adapter (default adapter).
Adapter sequence: An option to specify the custom adapter sequence when Nextera XT adapter was not used for library preparation.
Bowtie V1 or Bowtie V2 index: If users want to use Bowtie for read mapping they should select “Bowtie index” from the dropdown menu or select “Bowtie2 index” to use Bowtie2. Then, using the ‘browse’ button upload the appropriate genome index file (Bowtie or Bowtie2 index). Please see the section ‘9. How to create an index of genome fasta file’ to know more about the genome index and the genome index builder tool.
Maximum insert size: The maximum insert size in base pair that is allowed for a paired end alignment (default is 2,000 bp).
Maximum genomic hits or Minimum Mapping Quality: The maximum genomic hit (Bowtie) and Minimum Mapping Quality (Bowtie2) to discard reads pairs which have multiple alignments. Higher mapping quality gives more unique mapping for reads. The default maximum genomic hits = 1 and the mapping quality = 30.
Genome assembly: Select the correct genome build from the dropdown menu (***e.g.*** hg19) for genome assembly which will also be used for peak annotation and functional analysis.
Show chromosomes: A button to exclude reads mapping to specific chromosomes such as the mitochondrial chromosome. After clicking this button, it will open a new window (Figure 4) where users can select the desired chromosome(s) that will be excluded from analysis.
RAM: RAM in GB to be used by GUAVA (default is 1).
CPU units: Number of CPU units to be used by GUAVA (default is 1).
p or q value: Select the appropriate value from the dropdown menu and specify the cut off value in the box next to it. This will be used by MACS2 to filter peaks (default is q value).
Output folder: The folder where GUAVA ATAC-seq data analysis results are saved.
Reset All: The button to set all parameters to the default value.
Start Analysis: Clicking this button will start the ‘ATAC-seq data analysis’ program if all of the provided options are valid.

7.2 Output interface for ATAC-seq data analysis program

Once GUAVA has finished the analysis, it will show the results as a tabular output interface (Figure 5-12). GUAVA also facilitates the visualization of ATAC-seq signals on the IGV browser.

7.2.1. Alignment Statistics

This tab provides the reads mapping statistics (*e.g.* the total number of reads mapped and not mapped to the genome) along with the summary of the input files and parameters.

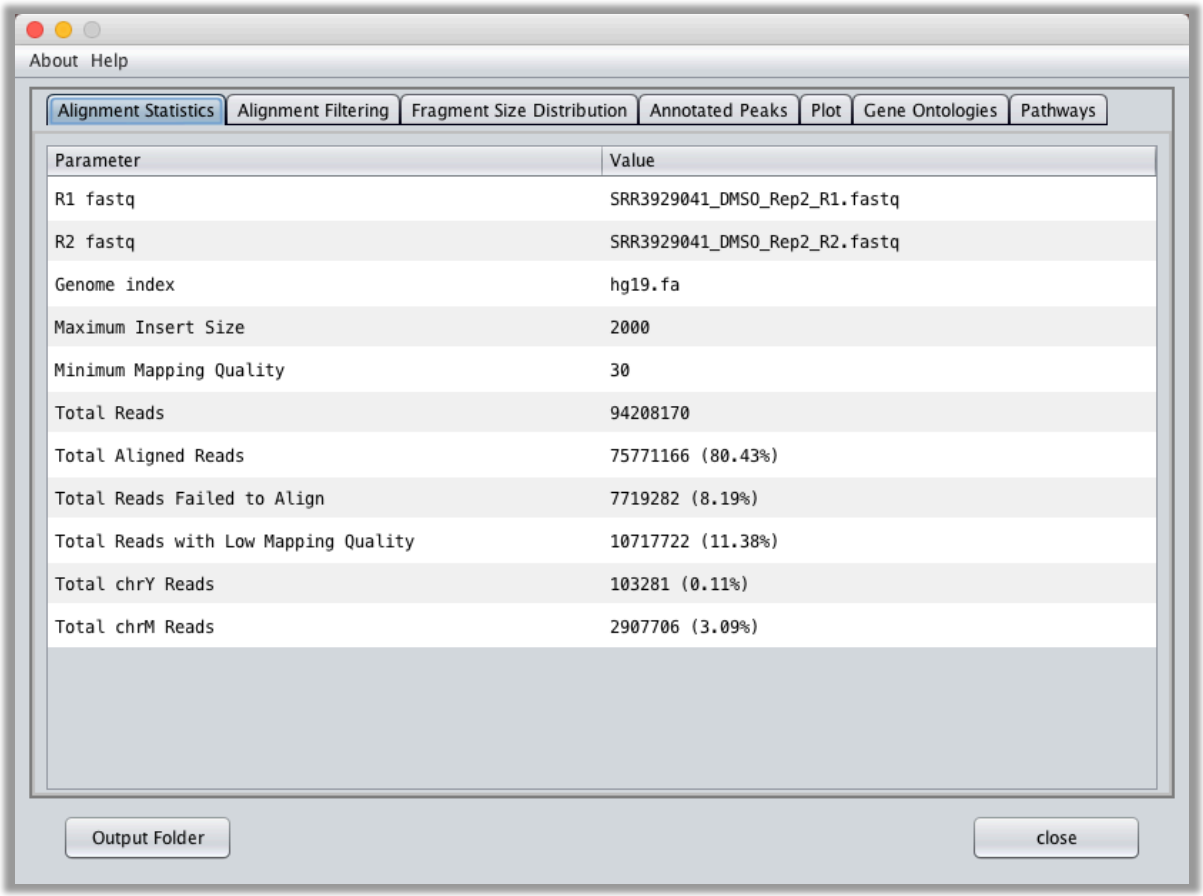


Figure 5. Input summary and alignment statistics.

7.2.2. Alignment Filtering

This tab contains two tables: 1) the alignment filtering statistics (duplicates, useful reads, *etc*), and 2) a summary of the MACS2 peak calling results.

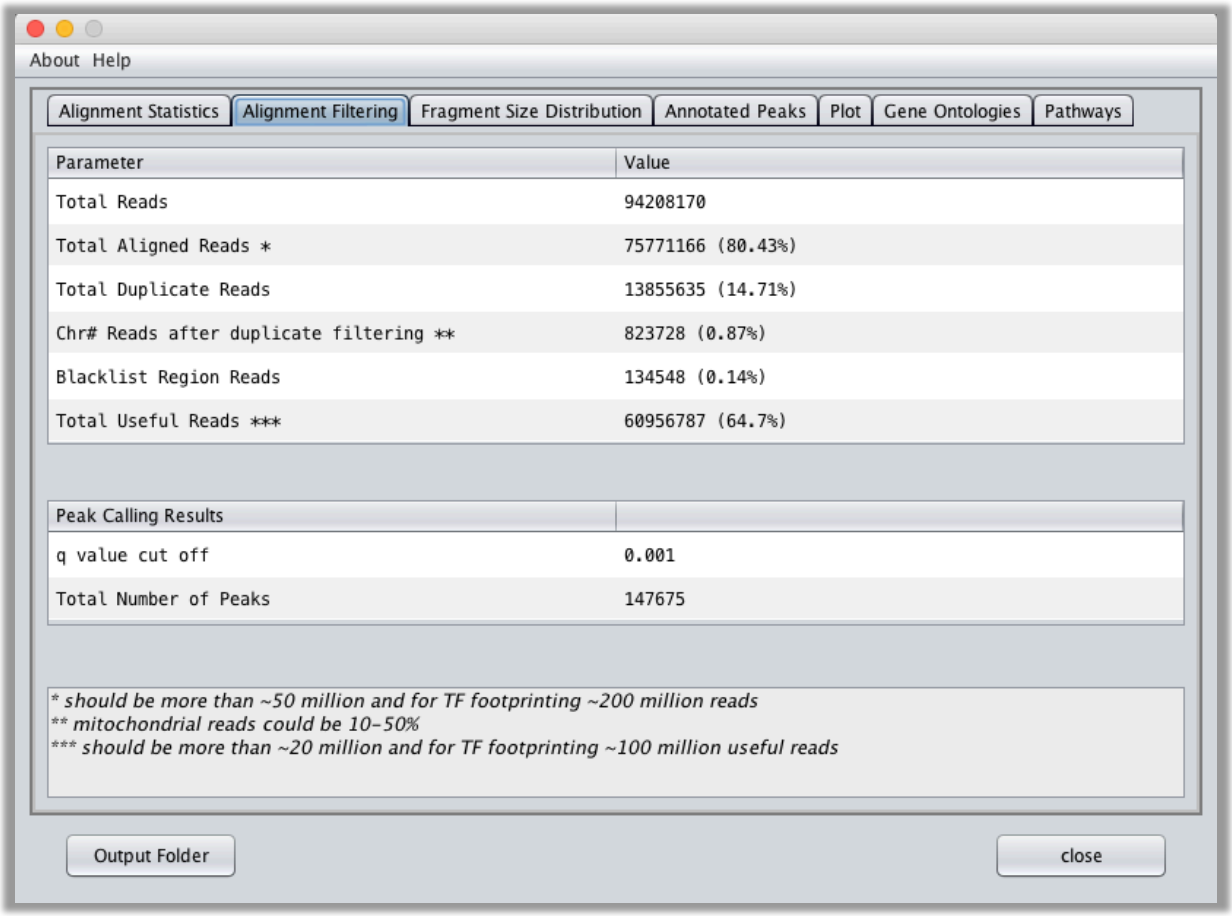
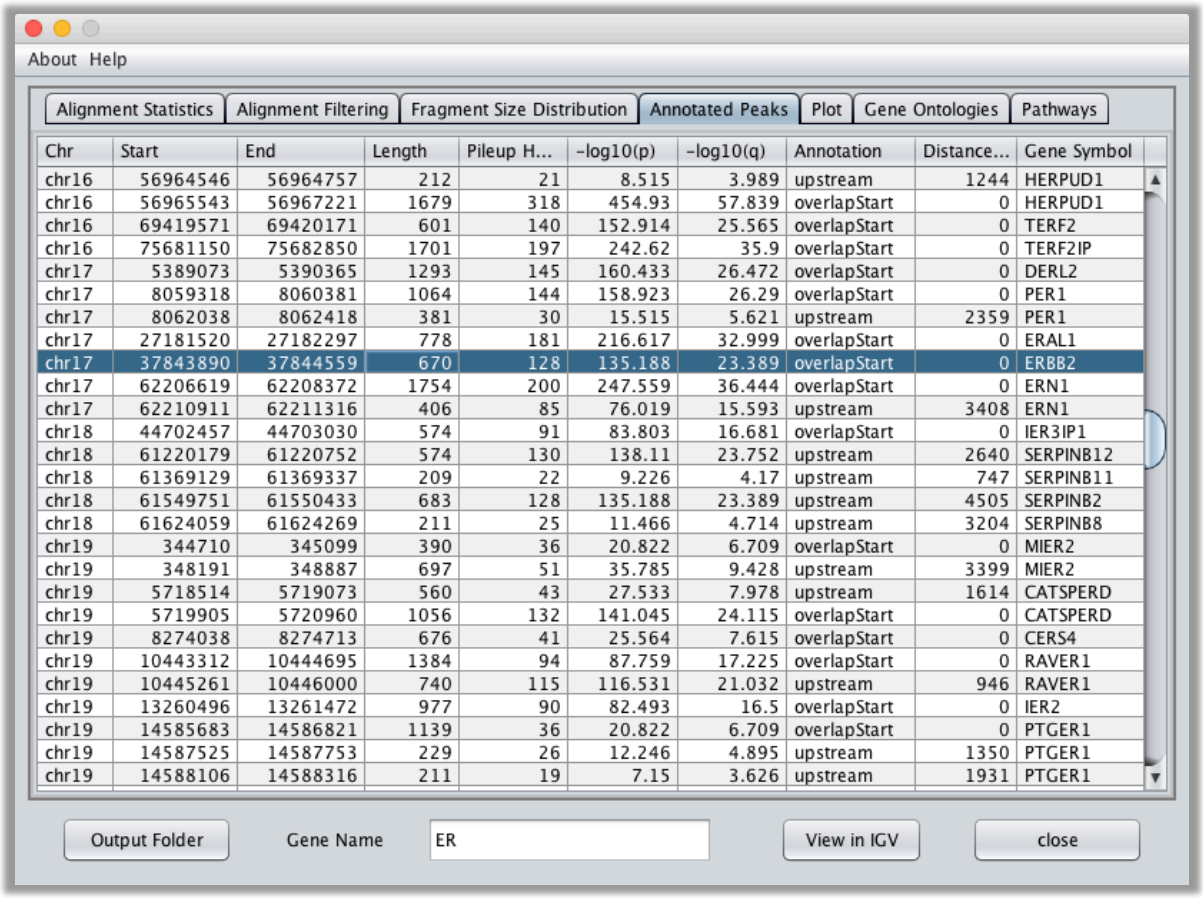


Figure 6. Read filtering and peak calling summary.

7.2.3. Annotated Peaks

This window shows the ATAC-seq annotated peaks. It contains information on peak location, nearest gene, and distance to TSS. This window also provides easy access to the IGV for visualizing peaks and automatically generated normalized ATAC-seq signals by GUAVA. Users can search their peak of interest by typing the symbol of the nearest gene in the search box at the bottom.



Chr	Start	End	Length	Pileup H...	-log10(p)	-log10(q)	Annotation	Distance...	Gene Symbol
chr16	56964546	56964757	212	21	8.515	3.989	upstream	1244	HERPUD1
chr16	56965543	56967221	1679	318	454.93	57.839	overlapStart	0	HERPUD1
chr16	69419571	69420171	601	140	152.914	25.565	overlapStart	0	TERF2
chr16	75681150	75682850	1701	197	242.62	35.9	overlapStart	0	TERF2IP
chr17	5389073	5390365	1293	145	160.433	26.472	overlapStart	0	DERL2
chr17	8059318	8060381	1064	144	158.923	26.29	overlapStart	0	PER1
chr17	8062038	8062418	381	30	15.515	5.621	upstream	2359	PER1
chr17	27181520	27182297	778	181	216.617	32.999	overlapStart	0	ERAL1
chr17	37843890	37844559	670	128	135.188	23.389	overlapStart	0	ERBB2
chr17	62206619	62208372	1754	200	247.559	36.444	overlapStart	0	ERN1
chr17	62210911	62211316	406	85	76.019	15.593	upstream	3408	ERN1
chr18	44702457	44703030	574	91	83.803	16.681	overlapStart	0	IER3IP1
chr18	61220179	61220752	574	130	138.11	23.752	upstream	2640	SERPINB12
chr18	61369129	61369337	209	22	9.226	4.17	upstream	747	SERPINB11
chr18	61549751	61550433	683	128	135.188	23.389	upstream	4505	SERPINB2
chr18	61624059	61624269	211	25	11.466	4.714	upstream	3204	SERPINB8
chr19	344710	345099	390	36	20.822	6.709	overlapStart	0	MIER2
chr19	348191	348887	697	51	35.785	9.428	upstream	3399	MIER2
chr19	5718514	5719073	560	43	27.533	7.978	upstream	1614	CATSPERD
chr19	5719905	5720960	1056	132	141.045	24.115	overlapStart	0	CATSPERD
chr19	8274038	8274713	676	41	25.564	7.615	overlapStart	0	CERS4
chr19	10443312	10444695	1384	94	87.759	17.225	overlapStart	0	RAVER1
chr19	10445261	10446000	740	115	116.531	21.032	upstream	946	RAVER1
chr19	13260496	13261472	977	90	82.493	16.5	overlapStart	0	IER2
chr19	14585683	14586821	1139	36	20.822	6.709	overlapStart	0	PTGER1
chr19	14587525	14587753	229	26	12.246	4.895	upstream	1350	PTGER1
chr19	14588106	14588316	211	19	7.15	3.626	upstream	1931	PTGER1

Figure 7. A table containing peak annotation information.

7.2.4. Visualization of ATAC-seq peaks using the Integrated Genome Viewer (IGV)

Users can visualize their peak of interest from the table (Figure 7) by selecting it and then clicking the 'View in IGV' button. This will automatically load the normalized ATAC-seq signals and peaks to the IGV browser as shown below.

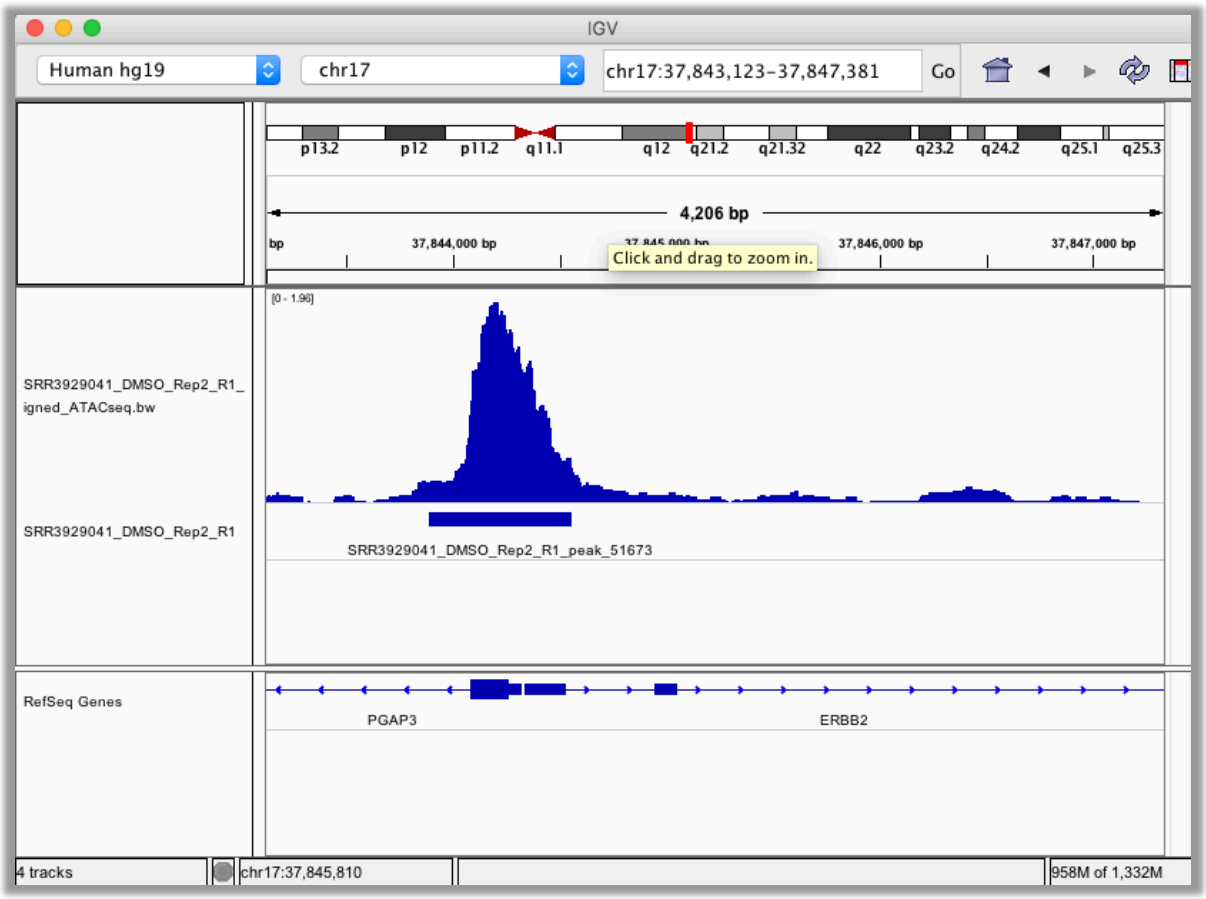


Figure 8. Visualization of ATAC-seq peaks with IGV.

7.2.5. Fragment Size Distribution

This plot shows the observed fragment size distribution for an ATAC-seq sample.

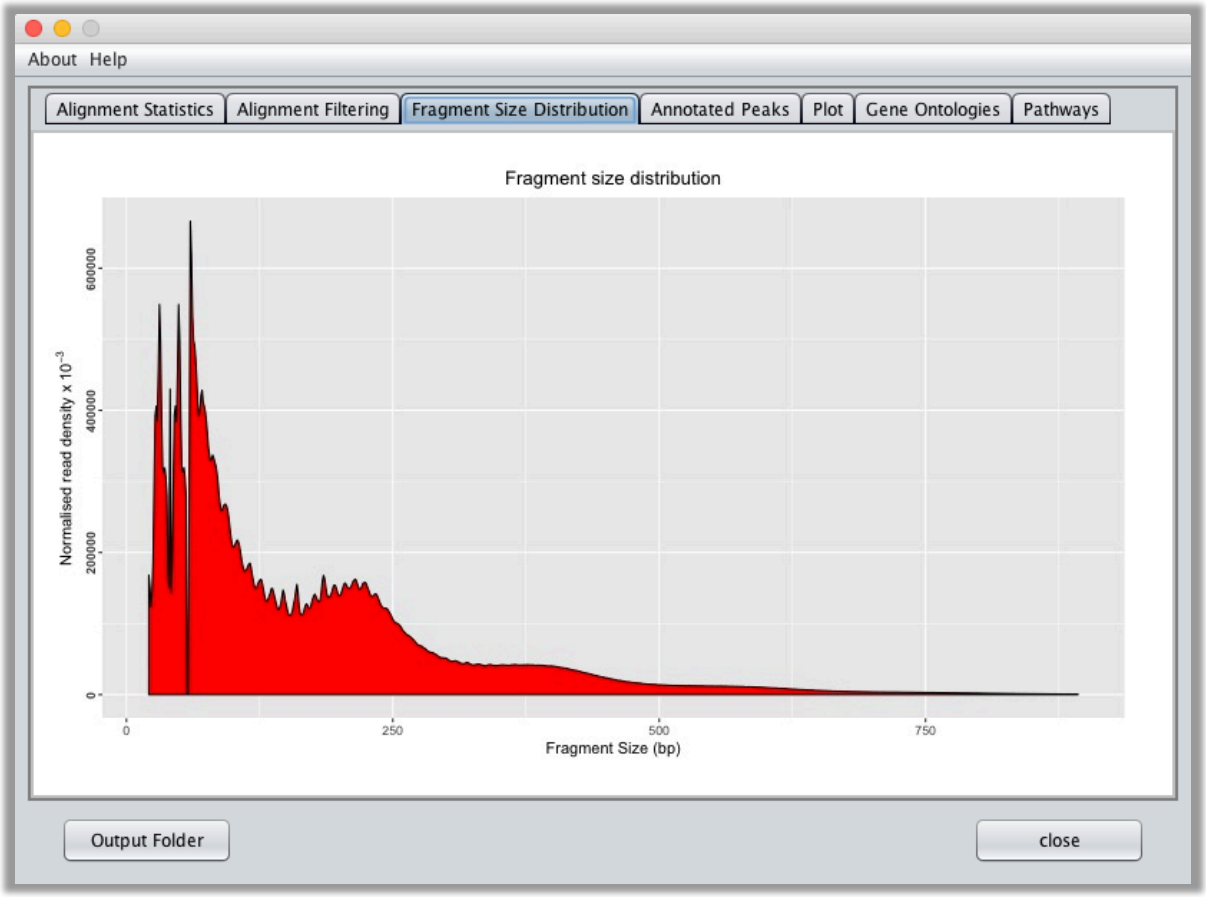


Figure 9. Graph showing the fragment size distribution.

7.2.6. Plot

This tab contains a bar chart which illustrates the distribution of the annotated peaks in various genomic locations such as the promoter, intron, exon, UTR, *etc*.

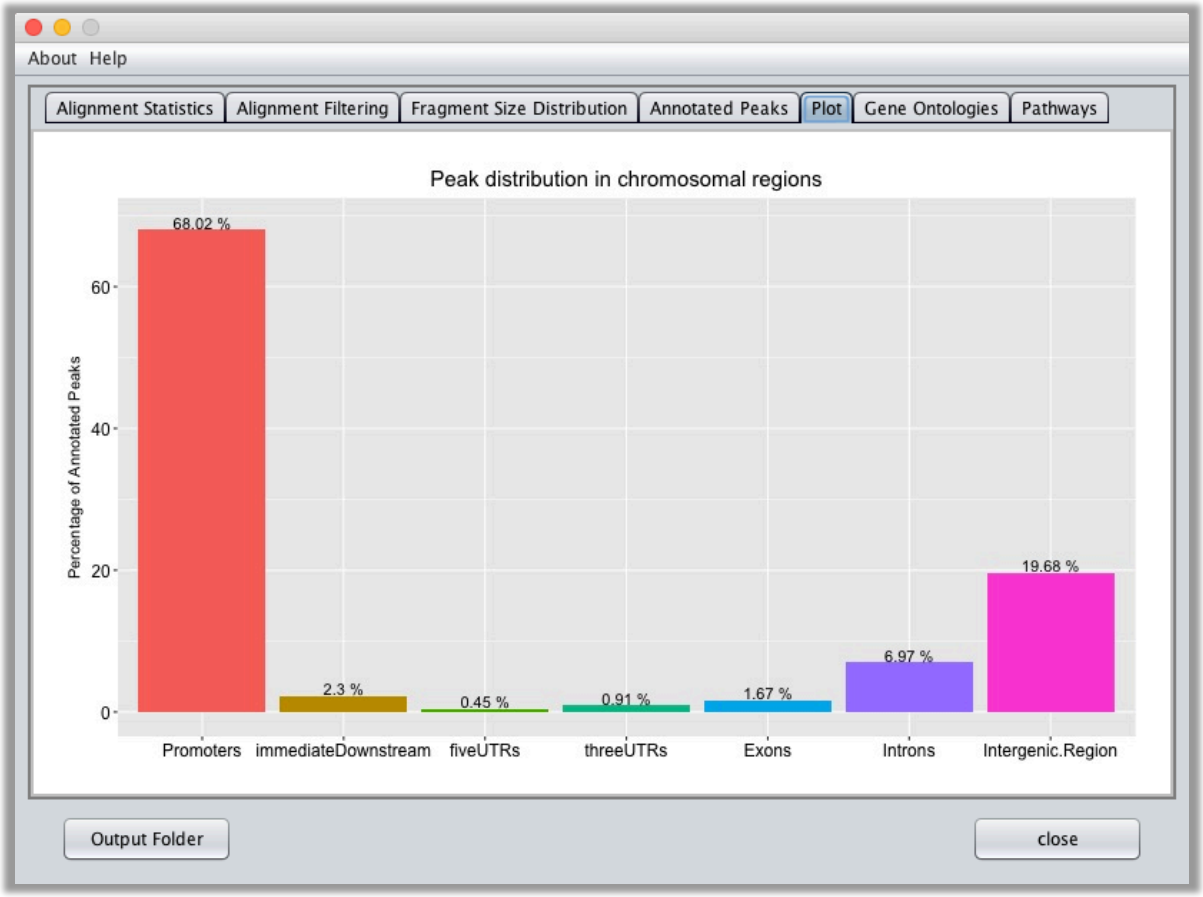
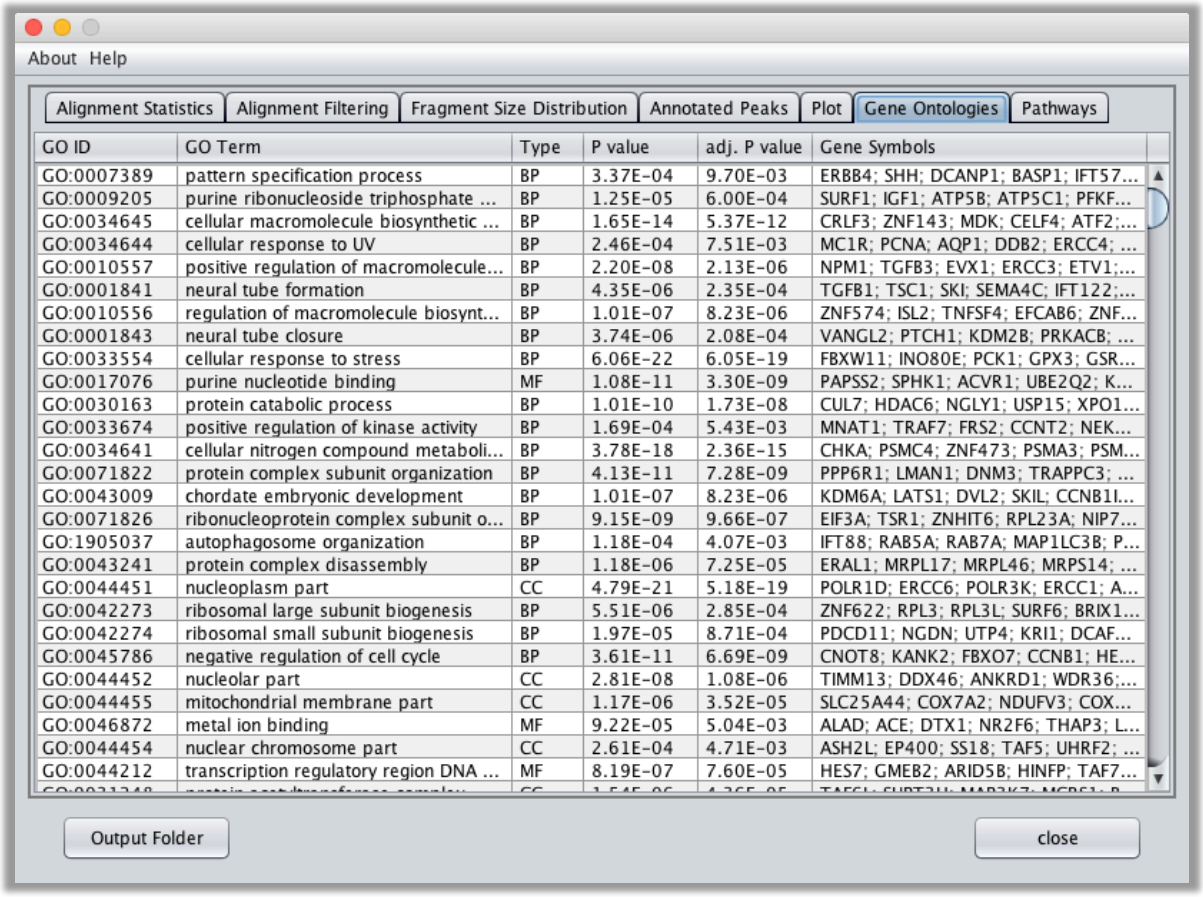


Figure 10: **Bar chart showing the distribution of annotated ATAC-seq peaks in various genomic regions.**

7.2.7. Gene Ontologies

This tab shows the list of the over-represented gene ontologies associated with ATAC-seq peaks.

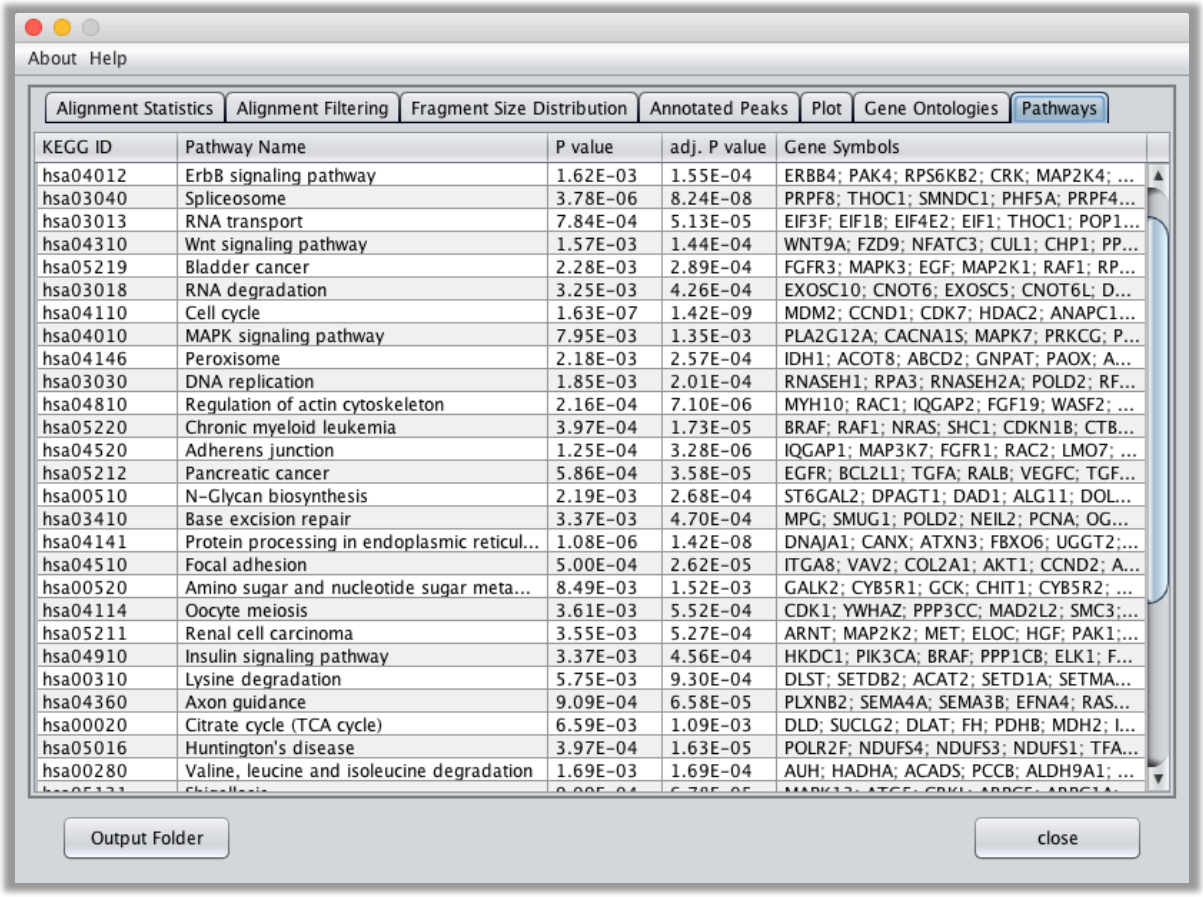


GO ID	GO Term	Type	P value	adj. P value	Gene Symbols
GO:0007389	pattern specification process	BP	3.37E-04	9.70E-03	ERBB4; SHH; DCANP1; BASP1; IFT57...
GO:0009205	purine ribonucleoside triphosphate ...	BP	1.25E-05	6.00E-04	SURF1; IGF1; ATP5B; ATP5C1; PFKF...
GO:0034645	cellular macromolecule biosynthetic ...	BP	1.65E-14	5.37E-12	CRLF3; ZNF143; MDK; CELF4; ATF2;...
GO:0034644	cellular response to UV	BP	2.46E-04	7.51E-03	MC1R; PCNA; AQP1; DDB2; ERCC4; ...
GO:0010557	positive regulation of macromolecule...	BP	2.20E-08	2.13E-06	NPM1; TGFB3; EVX1; ERCC3; ETV1;...
GO:0001841	neural tube formation	BP	4.35E-06	2.35E-04	TGFB1; TSC1; SKI; SEMA4C; IFT122;...
GO:0010556	regulation of macromolecule biosynt...	BP	1.01E-07	8.23E-06	ZNF574; ISL2; TNFSF4; EFCAB6; ZNF...
GO:0001843	neural tube closure	BP	3.74E-06	2.08E-04	VANGL2; PTCH1; KDM2B; PRKACB; ...
GO:0033554	cellular response to stress	BP	6.06E-22	6.05E-19	FBXW11; INO80E; PCK1; GPX3; GSR...
GO:0017076	purine nucleotide binding	MF	1.08E-11	3.30E-09	PAPSS2; SPHK1; ACVR1; UBE2Q2; K...
GO:0030163	protein catabolic process	BP	1.01E-10	1.73E-08	CUL7; HDAC6; NGLY1; USP15; XPO1...
GO:0033674	positive regulation of kinase activity	BP	1.69E-04	5.43E-03	MNAT1; TRAF7; FRS2; CCNT2; NEK...
GO:0034641	cellular nitrogen compound metaboli...	BP	3.78E-18	2.36E-15	CHKA; PSMC4; ZNF473; PSMA3; PSM...
GO:0071822	protein complex subunit organization	BP	4.13E-11	7.28E-09	PPP6R1; LMAN1; DNM3; TRAPPC3; ...
GO:0043009	chordate embryonic development	BP	1.01E-07	8.23E-06	KDM6A; LATS1; DVL2; SKIL; CCNB1I...
GO:0071826	ribonucleoprotein complex subunit o...	BP	9.15E-09	9.66E-07	EIF3A; TSR1; ZNHIT6; RPL23A; NIP7...
GO:1905037	autophagosome organization	BP	1.18E-04	4.07E-03	IFT88; RAB5A; RAB7A; MAP1LC3B; P...
GO:0043241	protein complex disassembly	BP	1.18E-06	7.25E-05	ERAL1; MRPL17; MRPL46; MRPS14; ...
GO:0044451	nucleoplasm part	CC	4.79E-21	5.18E-19	POLR1D; ERCC6; POLR3K; ERCC1; A...
GO:0042273	ribosomal large subunit biogenesis	BP	5.51E-06	2.85E-04	ZNF622; RPL3; RPL3L; SURF6; BRIX1...
GO:0042274	ribosomal small subunit biogenesis	BP	1.97E-05	8.71E-04	PDCD11; NGDN; UTP4; KRI1; DCAF...
GO:0045786	negative regulation of cell cycle	BP	3.61E-11	6.69E-09	CNOT8; KANK2; FBXO7; CCNB1; HE...
GO:0044452	nucleolar part	CC	2.81E-08	1.08E-06	TIMM13; DDX46; ANKRD1; WDR36;...
GO:0044455	mitochondrial membrane part	CC	1.17E-06	3.52E-05	SLC25A44; COX7A2; NDUFV3; COX...
GO:0046872	metal ion binding	MF	9.22E-05	5.04E-03	ALAD; ACE; DTX1; NR2F6; THAP3; L...
GO:0044454	nuclear chromosome part	CC	2.61E-04	4.71E-03	ASH2L; EP400; SS18; TAF5; UHRF2; ...
GO:0044212	transcription regulatory region DNA ...	MF	8.19E-07	7.60E-05	HES7; GMEB2; ARID5B; HINFP; TAF7...

Figure 11: Over-represented gene ontology terms associated with ATAC-seq peaks.

7.2.8. Pathways

This tab shows the list of the over-represented KEGG pathways associated with ATAC-seq peaks.



KEGG ID	Pathway Name	P value	adj. P value	Gene Symbols
hsa04012	ErbB signaling pathway	1.62E-03	1.55E-04	ERBB4; PAK4; RPS6KB2; CRK; MAP2K4; ...
hsa03040	Spliceosome	3.78E-06	8.24E-08	PRPF8; THOC1; SMNDC1; PHF5A; PRPF4...
hsa03013	RNA transport	7.84E-04	5.13E-05	EIF3F; EIF1B; EIF4E2; EIF1; THOC1; POP1...
hsa04310	Wnt signaling pathway	1.57E-03	1.44E-04	WNT9A; FZD9; NFATC3; CUL1; CHP1; PP...
hsa05219	Bladder cancer	2.28E-03	2.89E-04	FGFR3; MAPK3; EGF; MAP2K1; RAF1; RP...
hsa03018	RNA degradation	3.25E-03	4.26E-04	EXOSC10; CNOT6; EXOSC5; CNOT6L; D...
hsa04110	Cell cycle	1.63E-07	1.42E-09	MDM2; CCND1; CDK7; HDAC2; ANAPC1...
hsa04010	MAPK signaling pathway	7.95E-03	1.35E-03	PLA2G12A; CACNA1S; MAPK7; PRKCG; P...
hsa04146	Peroxisome	2.18E-03	2.57E-04	IDH1; ACOT8; ABCD2; GNPAT; PAOX; A...
hsa03030	DNA replication	1.85E-03	2.01E-04	RNASEH1; RPA3; RNASEH2A; POLD2; RF...
hsa04810	Regulation of actin cytoskeleton	2.16E-04	7.10E-06	MYH10; RAC1; IQGAP2; FGF19; WASF2; ...
hsa05220	Chronic myeloid leukemia	3.97E-04	1.73E-05	BRAF; RAF1; NRAS; SHC1; CDKN1B; CTB...
hsa04520	Adherens junction	1.25E-04	3.28E-06	IQGAP1; MAP3K7; FGFR1; RAC2; LMO7; ...
hsa05212	Pancreatic cancer	5.86E-04	3.58E-05	EGFR; BCL2L1; TGFA; RALB; VEGFC; TGF...
hsa00510	N-Glycan biosynthesis	2.19E-03	2.68E-04	ST6GAL2; DPAGT1; DAD1; ALG11; DOL...
hsa03410	Base excision repair	3.37E-03	4.70E-04	MPG; SMUG1; POLD2; NEIL2; PCNA; OG...
hsa04141	Protein processing in endoplasmic reticul...	1.08E-06	1.42E-08	DNAJA1; CANX; ATXN3; FBXO6; UGGT2;...
hsa04510	Focal adhesion	5.00E-04	2.62E-05	ITGA8; VAV2; COL2A1; AKT1; CCND2; A...
hsa00520	Amino sugar and nucleotide sugar meta...	8.49E-03	1.52E-03	GALK2; CYB5R1; GCK; CHIT1; CYB5R2; ...
hsa04114	Oocyte meiosis	3.61E-03	5.52E-04	CDK1; YWHAZ; PPP3CC; MAD2L2; SMC3;...
hsa05211	Renal cell carcinoma	3.55E-03	5.27E-04	ARNT; MAP2K2; MET; ELOC; HGF; PAK1;...
hsa04910	Insulin signaling pathway	3.37E-03	4.56E-04	HKDC1; PIK3CA; BRAF; PPP1CB; ELK1; F...
hsa00310	Lysine degradation	5.75E-03	9.30E-04	DLST; SETDB2; ACAT2; SETD1A; SETMA...
hsa04360	Axon guidance	9.09E-04	6.58E-05	PLXNB2; SEMA4A; SEMA3B; EFNA4; RAS...
hsa00020	Citrate cycle (TCA cycle)	6.59E-03	1.09E-03	DLD; SUCLG2; DLAT; FH; PDHB; MDH2; I...
hsa05016	Huntington's disease	3.97E-04	1.63E-05	POLR2F; NDUFS4; NDUFS3; NDUFS1; TFA...
hsa00280	Valine, leucine and isoleucine degradation	1.69E-03	1.69E-04	AUH; HADHA; ACADS; PCCB; ALDH9A1; ...

Figure 12: Over represented KEGG pathways associated with ATAC-seq peaks.

NOTE: The above results are stored in the output folder. To access the output folder, click on the '**Output Folde**r' button located at the bottom-left corner.

7.3 ATAC-seq differential analysis program

The ATAC-seq differential analysis program compares ATAC-seq signals from two different conditions. It provides users results for the differentially enriched signals, as well as the peak annotation and functional analysis for the differentially enriched peaks. There are two input windows for this program. The first window is to upload the ATAC-seq signals for the two different conditions and replicates (Figures 13 and 14). The second window allows users to specify the differential analysis related parameters such as fold change and p value. (Figure 14).

When user select the ATAC-seq differential analysis (*i.e.* the second option) in the home window (Figure 2), the following input window is displayed (Figure 13). Below describes how to use the ATAC-seq differential analysis program.

Step 1: Load input files for the differential analysis.

Use the 'add file' and 'remove' buttons to add and delete input files, respectively. After adding input files, select the appropriate condition and replicate number from the drop-down menu. Then, click 'Next' to specify differential analysis parameters.

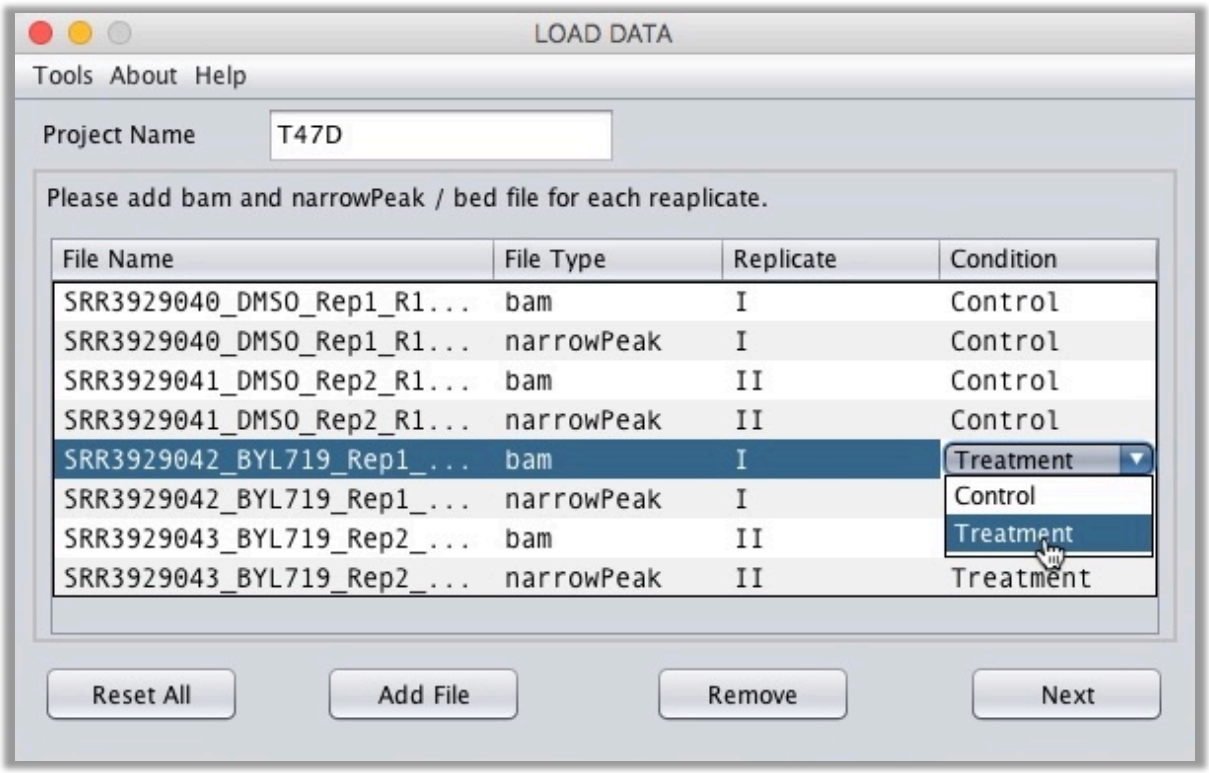


Figure 13. GUAVA ATAC-seq differential analysis input interface 1.

Step 2: Set differential analysis parameters.

Choose the appropriate genome build (*e.g.* hg19), log2 (fold change) cut off, p value, number of CPUs, TSS to peak upstream and downstream distance cut off, and the output folder as shown in Figure 14. Once all these parameters have been entered, users can start the differential analysis.

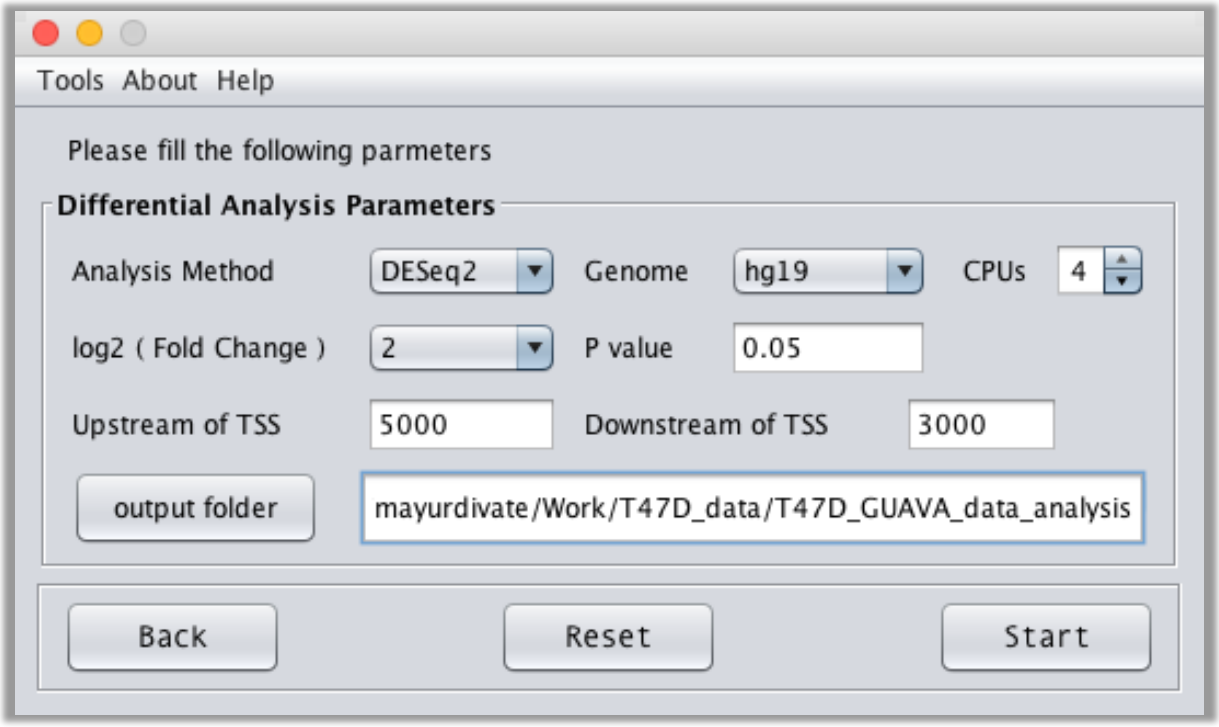


Figure 14. GUAVA ATAC-seq differential analysis input interface 2.

7.3.1 ATAC-seq differential analysis program parameters

Below is the complete list of buttons and parameters present in the input interface (Figures 13 and 14) of the ATAC-seq differential analysis program together with a description of their usage.

Analysis method: The select method for differential analysis is DESeq2.
log2 (Fold Change): The log2 fold change cut off to define differentially enriched peaks. (Default is 2)
P value: The p value cut off to select the most significant differentially enriched peaks. (Default is 0.05)
Upstream of TSS: If a peak is present within a specified distance (in base pair) upstream from the TSS of a gene, then that gene will be associated with the peak for functional analysis. (Default is 5000 bp)
Downstream of TSS: If a peak is present within a specified distance (in base pair) downstream from the TSS of a gene, then that gene will be associated with the peak for functional analysis. (Default is 3000 bp)
Output folder: The folder where GUAVA differential analysis results are saved.

7.4 Output interface of ATAC-seq differential analysis program

Once differential analysis has finished, GUAVA will show the results as a tabular output interface (Figures 15-22). GUAVA also facilitates the visualization of ATAC-seq signals on the IGV browser.

7.4.1. Summary

This tab provides a summary of the input parameters and files that were used to run differential analysis.

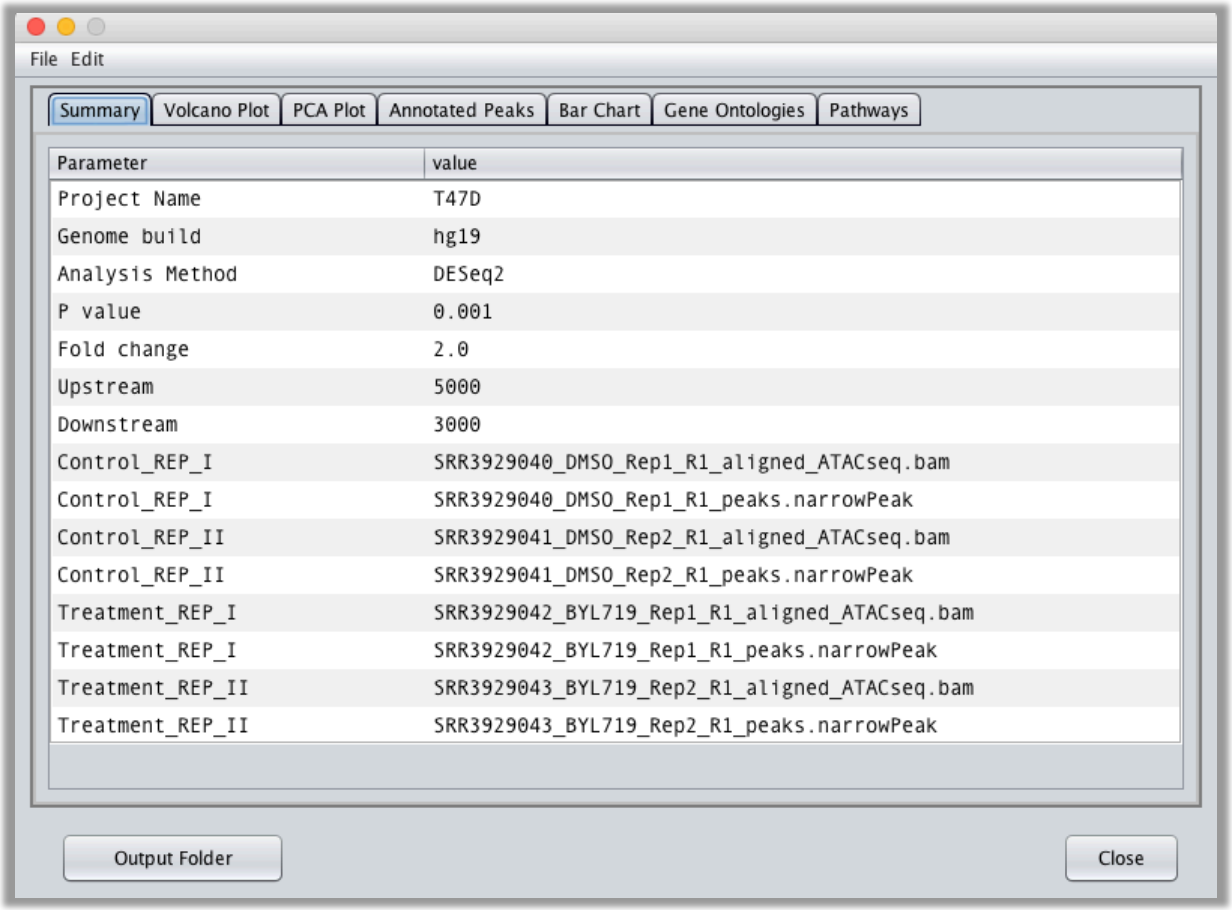


Figure 15. Input summary. Screenshot showing a summary of the parameters and input files used in the differential analysis.

7.4.2. Volcano Plot

This graph shows the summary of differential analysis. The red and green colors indicate peaks with reduced and increased chromatin accessibility, respectively.

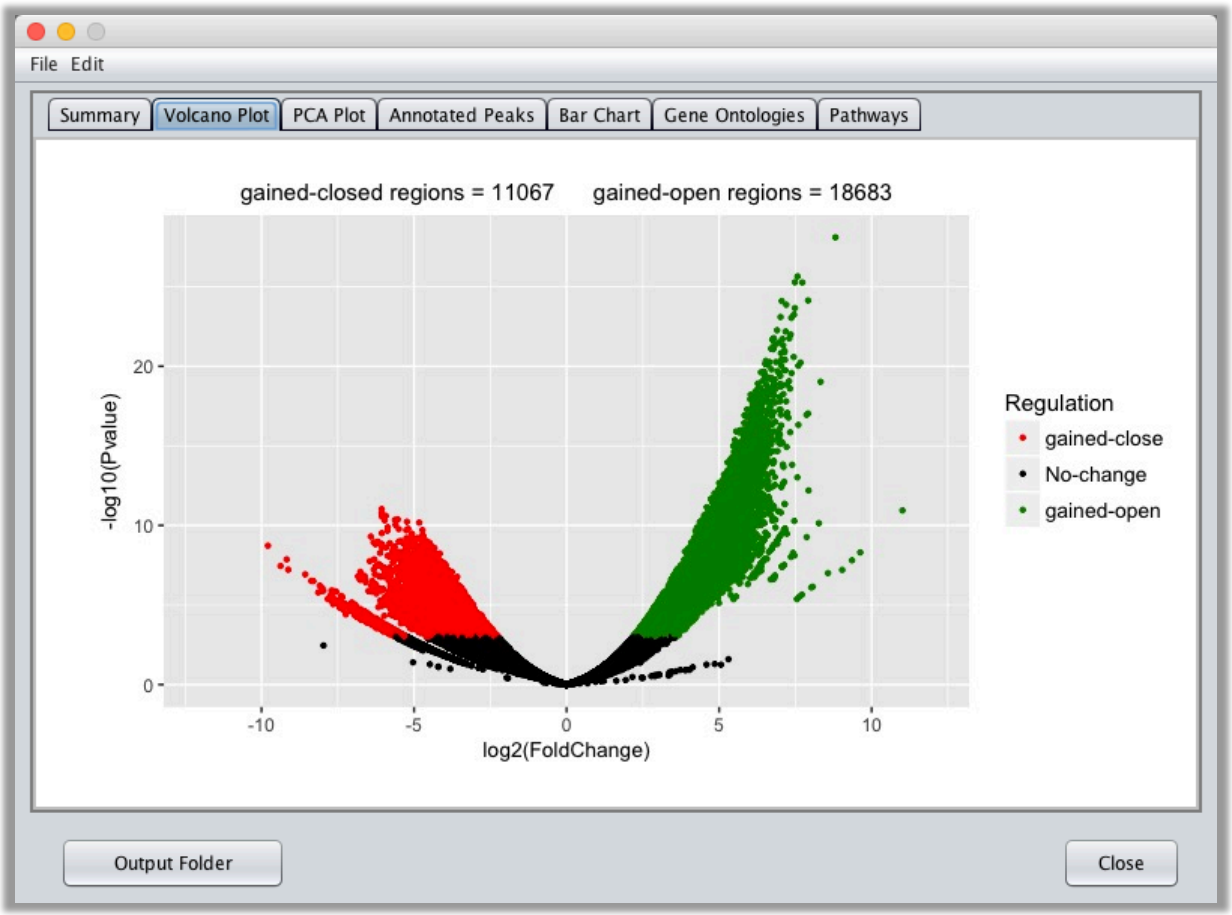


Figure 16. A volcano plot showing the differential ATAC-seq signals.

7.4.3. PCA Plot

This graph shows the principal component analysis (PCA) of the samples used in the differential analysis.

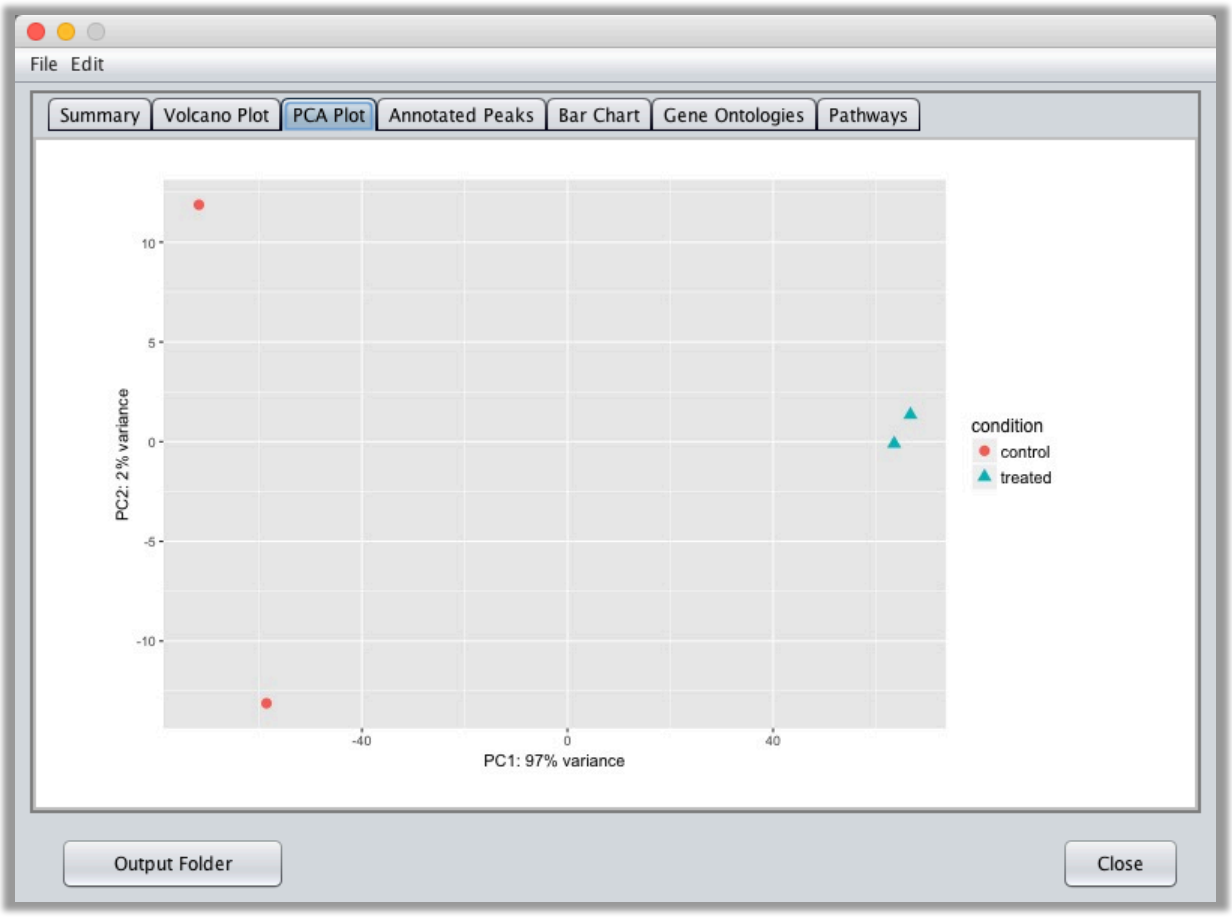
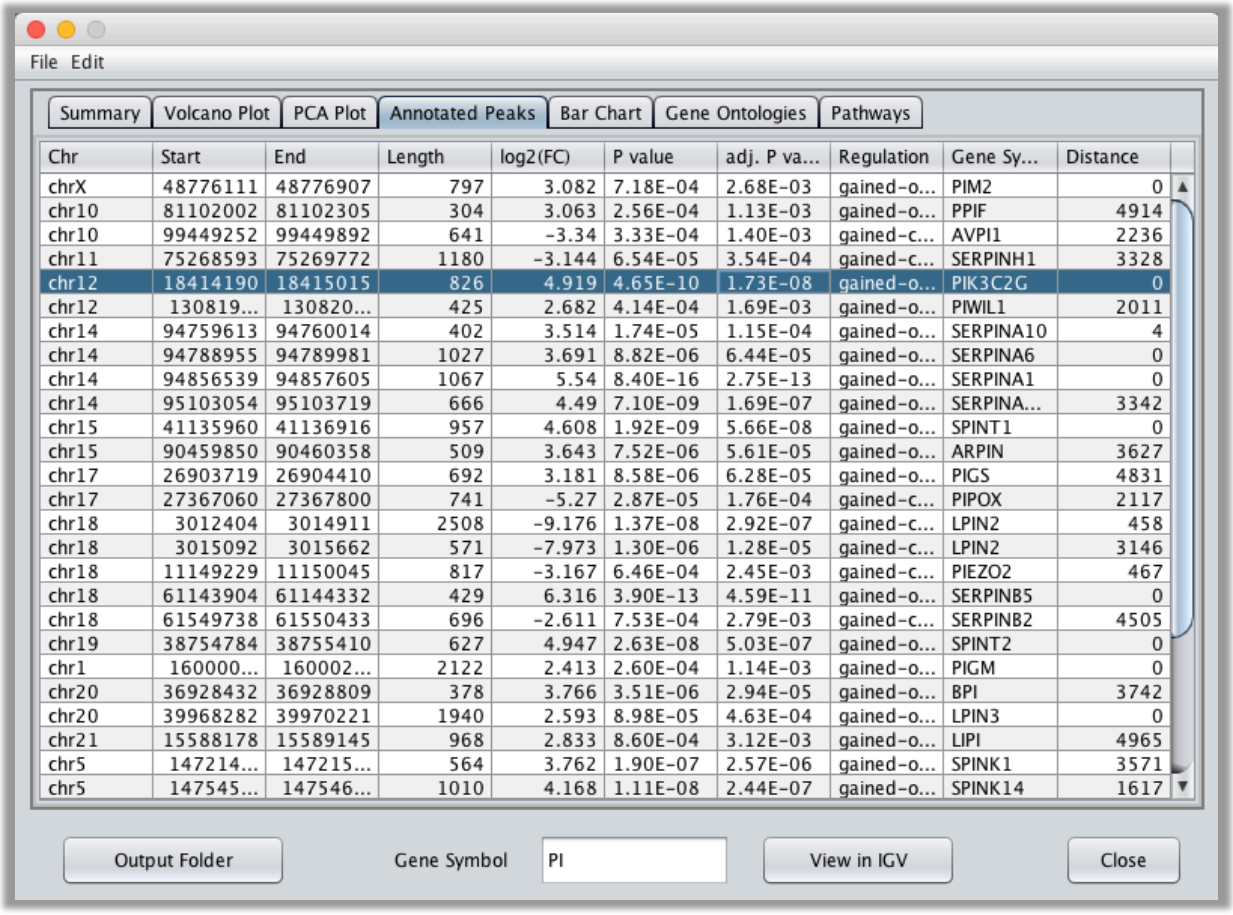


Figure 17. A PCA plot illustrating the variance between the control and treated samples.

7.4.4. Annotated Peaks

This tab provides a table with differentially enriched peaks and easy access to visualize ATAC-seq signals from control and treatment samples.



Chr	Start	End	Length	log2(FC)	P value	adj. P va...	Regulation	Gene Sy...	Distance
chrX	48776111	48776907	797	3.082	7.18E-04	2.68E-03	gained-o...	PIM2	0
chr10	81102002	81102305	304	3.063	2.56E-04	1.13E-03	gained-o...	PPIF	4914
chr10	99449252	99449892	641	-3.34	3.33E-04	1.40E-03	gained-c...	AVPI1	2236
chr11	75268593	75269772	1180	-3.144	6.54E-05	3.54E-04	gained-c...	SERPINH1	3328
chr12	18414190	18415015	826	4.919	4.65E-10	1.73E-08	gained-o...	PIK3C2G	0
chr12	130819...	130820...	425	2.682	4.14E-04	1.69E-03	gained-o...	PIWIL1	2011
chr14	94759613	94760014	402	3.514	1.74E-05	1.15E-04	gained-o...	SERPINA10	4
chr14	94788955	94789981	1027	3.691	8.82E-06	6.44E-05	gained-o...	SERPINA6	0
chr14	94856539	94857605	1067	5.54	8.40E-16	2.75E-13	gained-o...	SERPINA1	0
chr14	95103054	95103719	666	4.49	7.10E-09	1.69E-07	gained-o...	SERPINA...	3342
chr15	41135960	41136916	957	4.608	1.92E-09	5.66E-08	gained-o...	SPINT1	0
chr15	90459850	90460358	509	3.643	7.52E-06	5.61E-05	gained-o...	ARPIN	3627
chr17	26903719	26904410	692	3.181	8.58E-06	6.28E-05	gained-o...	PIGS	4831
chr17	27367060	27367800	741	-5.27	2.87E-05	1.76E-04	gained-c...	PIPOX	2117
chr18	3012404	3014911	2508	-9.176	1.37E-08	2.92E-07	gained-c...	LPIN2	458
chr18	3015092	3015662	571	-7.973	1.30E-06	1.28E-05	gained-c...	LPIN2	3146
chr18	11149229	11150045	817	-3.167	6.46E-04	2.45E-03	gained-c...	PIEZO2	467
chr18	61143904	61144332	429	6.316	3.90E-13	4.59E-11	gained-o...	SERPINB5	0
chr18	61549738	61550433	696	-2.611	7.53E-04	2.79E-03	gained-c...	SERPINB2	4505
chr19	38754784	38755410	627	4.947	2.63E-08	5.03E-07	gained-o...	SPINT2	0
chr1	160000...	160002...	2122	2.413	2.60E-04	1.14E-03	gained-o...	PIGM	0
chr20	36928432	36928809	378	3.766	3.51E-06	2.94E-05	gained-o...	BPI	3742
chr20	39968282	39970221	1940	2.593	8.98E-05	4.63E-04	gained-o...	LPIN3	0
chr21	15588178	15589145	968	2.833	8.60E-04	3.12E-03	gained-o...	LIPI	4965
chr5	147214...	147215...	564	3.762	1.90E-07	2.57E-06	gained-o...	SPINK1	3571
chr5	147545...	147546...	1010	4.168	1.11E-08	2.44E-07	gained-o...	SPINK14	1617

Figure 18**. Annotation of the differentially enriched ATAC-seq signals.** Differentially enriched peaks associated with a particular gene can be searched by typing the gene symbol in the search box provided at the bottom.

7.4.5. Visualization of ATAC-seq signals from the control and treatment samples using IGV

To visualize ATAC-seq signals from the control and treatment samples, select a differentially enriched peak from the 'Annotated Peaks' tab and then click on the 'View in IGV' button. This will automatically load normalized signals from all the input samples on the IGV browser.

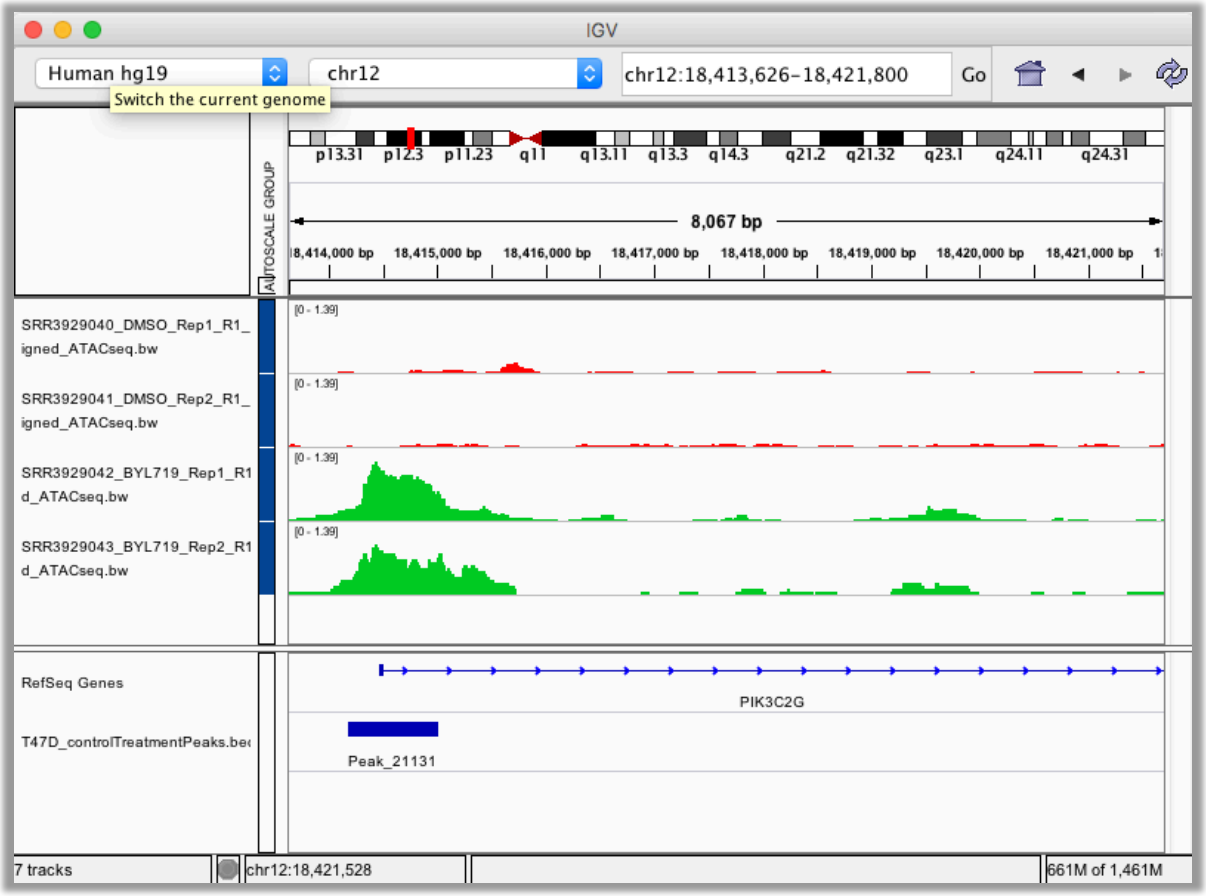


Figure 19. ATAC-seq signal visualization using IGV.

7.4.6. Bar Chart

This tab contains a bar chart that illustrates the distribution of the annotated peaks in various genomic locations such as the promoter, intron, exon, UTR, *etc*.

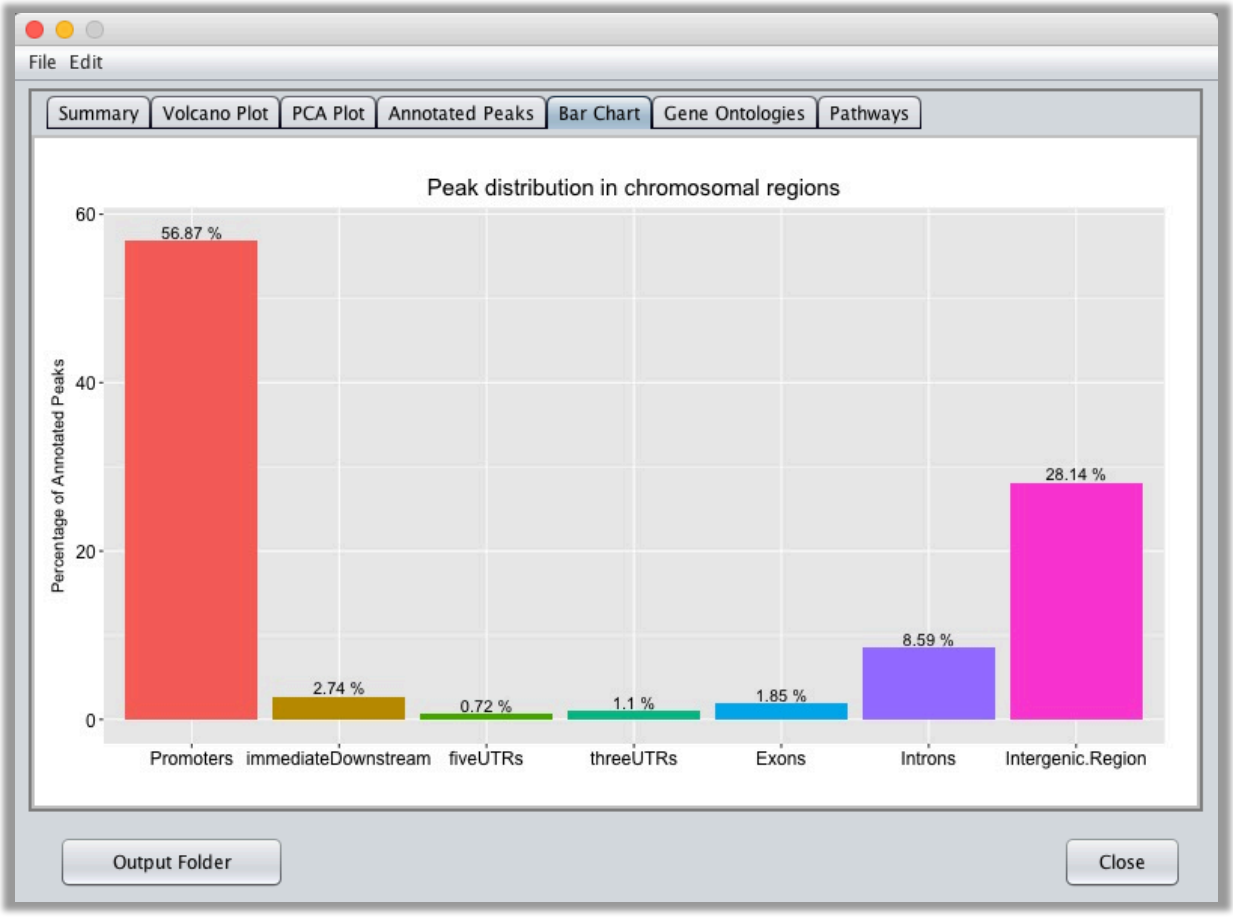
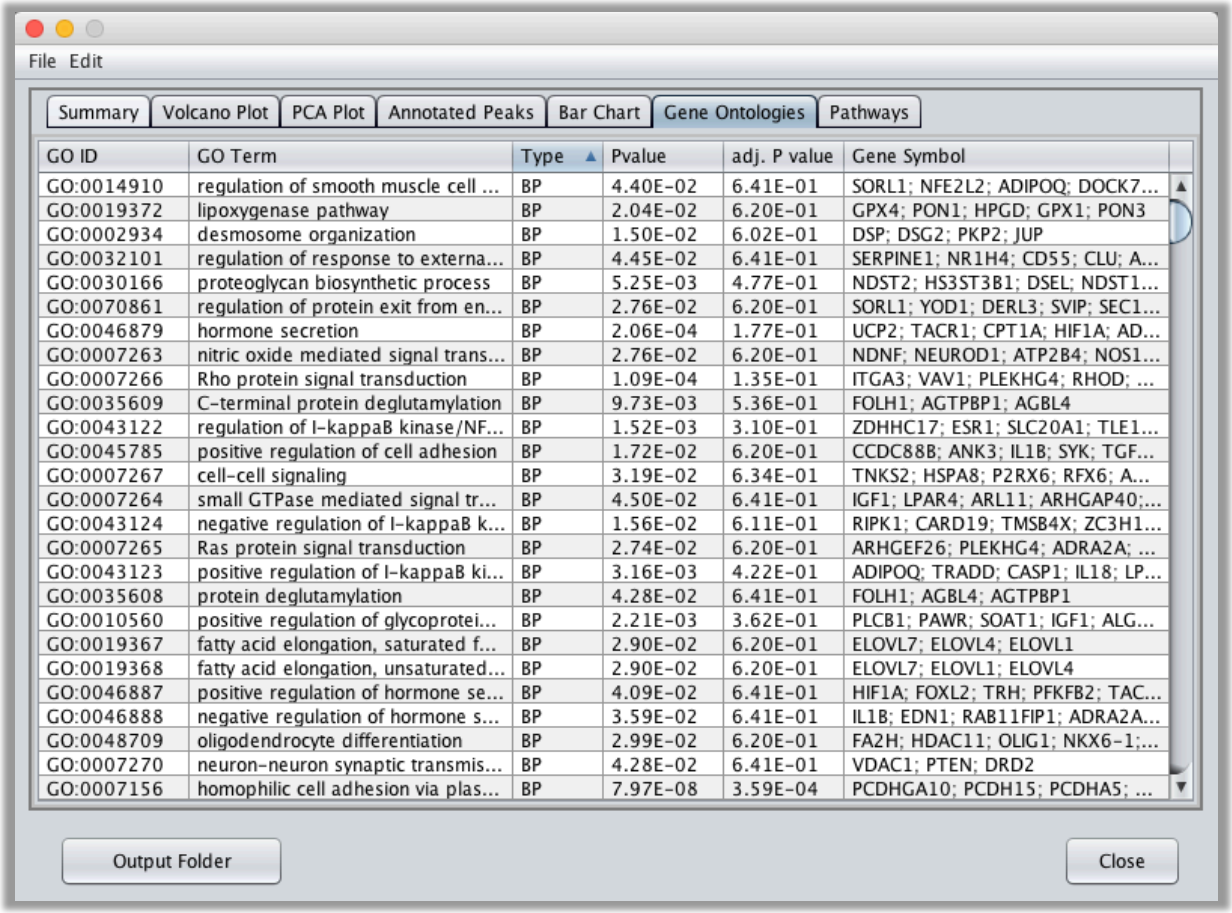


Figure 20. Bar chart showing the distribution of the differentially enriched ATAC-seq peaks in various genomic regions such as promoters, introns, exons, *etc*.

7.4.7. Gene Ontologies

This tab shows the list of the over-represented gene ontologies associated with differentially enriched peaks.

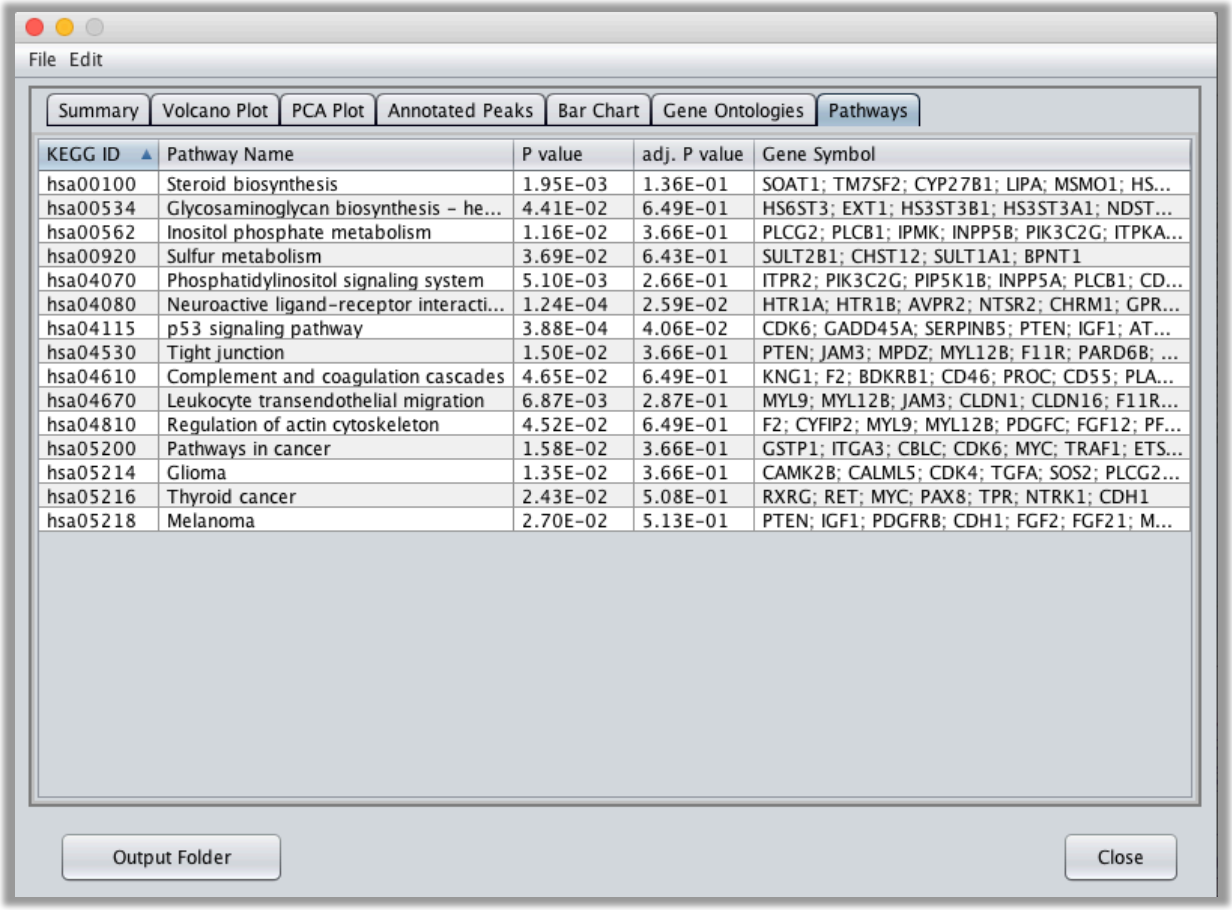


GO ID	GO Term	Type	Pvalue	adj. P value	Gene Symbol
GO:0014910	regulation of smooth muscle cell ...	BP	4.40E-02	6.41E-01	SORL1; NFE2L2; ADIPOQ; DOCK7...
GO:0019372	lipoxygenase pathway	BP	2.04E-02	6.20E-01	GPX4; PON1; HPGD; GPX1; PON3
GO:0002934	desmosome organization	BP	1.50E-02	6.02E-01	DSP; DSG2; PKP2; JUP
GO:0032101	regulation of response to externa...	BP	4.45E-02	6.41E-01	SERPINE1; NR1H4; CD55; CLU; A...
GO:0030166	proteoglycan biosynthetic process	BP	5.25E-03	4.77E-01	NDST2; HS3ST3B1; DSEL; NDST1...
GO:0070861	regulation of protein exit from en...	BP	2.76E-02	6.20E-01	SORL1; YOD1; DERL3; SVIP; SEC1...
GO:0046879	hormone secretion	BP	2.06E-04	1.77E-01	UCP2; TACR1; CPT1A; HIF1A; AD...
GO:0007263	nitric oxide mediated signal trans...	BP	2.76E-02	6.20E-01	NDNF; NEUROD1; ATP2B4; NOS1...
GO:0007266	Rho protein signal transduction	BP	1.09E-04	1.35E-01	ITGA3; VAV1; PLEKHG4; RHOD; ...
GO:0035609	C-terminal protein deglutamylation	BP	9.73E-03	5.36E-01	FOLH1; AGTPBP1; AGBL4
GO:0043122	regulation of I-kappaB kinase/NF...	BP	1.52E-03	3.10E-01	ZDHHC17; ESR1; SLC20A1; TLE1...
GO:0045785	positive regulation of cell adhesion	BP	1.72E-02	6.20E-01	CCDC88B; ANK3; IL1B; SYK; TGF...
GO:0007267	cell-cell signaling	BP	3.19E-02	6.34E-01	TNKS2; HSPA8; P2RX6; RFX6; A...
GO:0007264	small GTPase mediated signal tr...	BP	4.50E-02	6.41E-01	IGF1; LPAR4; ARL11; ARHGAP40;...
GO:0043124	negative regulation of I-kappaB k...	BP	1.56E-02	6.11E-01	RIPK1; CARD19; TMSB4X; ZC3H1...
GO:0007265	Ras protein signal transduction	BP	2.74E-02	6.20E-01	ARHGEF26; PLEKHG4; ADRA2A; ...
GO:0043123	positive regulation of I-kappaB ki...	BP	3.16E-03	4.22E-01	ADIPOQ; TRADD; CASP1; IL18; LP...
GO:0035608	protein deglutamylation	BP	4.28E-02	6.41E-01	FOLH1; AGBL4; AGTPBP1
GO:0010560	positive regulation of glycoprotei...	BP	2.21E-03	3.62E-01	PLCB1; PAWR; SOAT1; IGF1; ALG...
GO:0019367	fatty acid elongation, saturated f...	BP	2.90E-02	6.20E-01	ELOVL7; ELOVL4; ELOVL1
GO:0019368	fatty acid elongation, unsaturated...	BP	2.90E-02	6.20E-01	ELOVL7; ELOVL1; ELOVL4
GO:0046887	positive regulation of hormone se...	BP	4.09E-02	6.41E-01	HIF1A; FOXL2; TRH; PFKFB2; TAC...
GO:0046888	negative regulation of hormone s...	BP	3.59E-02	6.41E-01	IL1B; EDN1; RAB11FIP1; ADRA2A...
GO:0048709	oligodendrocyte differentiation	BP	2.99E-02	6.20E-01	FA2H; HDAC11; OLIG1; NKX6-1;...
GO:0007270	neuron-neuron synaptic transmis...	BP	4.28E-02	6.41E-01	VDAC1; PTEN; DRD2
GO:0007156	homophilic cell adhesion via plas...	BP	7.97E-08	3.59E-04	PCDHGA10; PCDH15; PCDHA5; ...

Figure 21. Over represented gene ontology terms associated with differentially enriched peaks.

7.4.8. Pathway

This tab shows the list of the over-represented KEGG pathways associated with differentially enriched peaks.



KEGG ID	Pathway Name	P value	adj. P value	Gene Symbol
hsa00100	Steroid biosynthesis	1.95E-03	1.36E-01	SOAT1; TM7SF2; CYP27B1; LIPA; MSMO1; HS...
hsa00534	Glycosaminoglycan biosynthesis - he...	4.41E-02	6.49E-01	HS6ST3; EXT1; HS3ST3B1; HS3ST3A1; NDST...
hsa00562	Inositol phosphate metabolism	1.16E-02	3.66E-01	PLCG2; PLCB1; IPMK; INPP5B; PIK3C2G; ITPKA...
hsa00920	Sulfur metabolism	3.69E-02	6.43E-01	SULT2B1; CHST12; SULT1A1; BPNT1
hsa04070	Phosphatidylinositol signaling system	5.10E-03	2.66E-01	ITPR2; PIK3C2G; PIP5K1B; INPP5A; PLCB1; CD...
hsa04080	Neuroactive ligand-receptor interacti...	1.24E-04	2.59E-02	HTR1A; HTR1B; AVPR2; NTSR2; CHRM1; GPR...
hsa04115	p53 signaling pathway	3.88E-04	4.06E-02	CDK6; GADD45A; SERPINB5; PTEN; IGF1; AT...
hsa04530	Tight junction	1.50E-02	3.66E-01	PTEN; JAM3; MPDZ; MYL12B; F11R; PARD6B; ...
hsa04610	Complement and coagulation cascades	4.65E-02	6.49E-01	KNG1; F2; BDKRB1; CD46; PROC; CD55; PLA...
hsa04670	Leukocyte transendothelial migration	6.87E-03	2.87E-01	MYL9; MYL12B; JAM3; CLDN1; CLDN16; F11R...
hsa04810	Regulation of actin cytoskeleton	4.52E-02	6.49E-01	F2; CYFIP2; MYL9; MYL12B; PDGFC; FGF12; PF...
hsa05200	Pathways in cancer	1.58E-02	3.66E-01	GSTP1; ITGA3; CBLC; CDK6; MYC; TRAF1; ETS...
hsa05214	Glioma	1.35E-02	3.66E-01	CAMK2B; CALML5; CDK4; TGFA; SOS2; PLCG2...
hsa05216	Thyroid cancer	2.43E-02	5.08E-01	RXRG; RET; MYC; PAX8; TPR; NTRK1; CDH1
hsa05218	Melanoma	2.70E-02	5.13E-01	PTEN; IGF1; PDGFRB; CDH1; FGF2; FGF21; M...

Figure 22. Over represented KEGG pathways associated with differentially enriched peaks.

NOTE: The above results are stored in the output folder. To access output folder, click on the 'Output Folder' button located at the bottom-left corner.

8. How to download a genome fasta file

Fasta is a flat file format for representing nucleotide or protein sequences. The genome fasta file is a fasta file that contains the nucleotide sequences from all of the chromosomes of a particular organism. The genome fasta file is required for mapping reads with any sequence aligner tool. For users who do not know where to find the genome fasta file, they can use the UCSC link below and choose the desired organism to download the fasta file.

Step 1.	Go to the link: http://hgdownload.soe.ucsc.edu/downloads.html.
Step 2.	Click on the desired organism (*e.g.* human).
Step 3.	Click on the 'Full data set' under the appropriate genome build (*e.g.* hg19).
Step 4.	Scroll down and then click on the chromFa.tar.gz to download genome sequence.
Step 5.	Open the Terminal.
Step 6.	Type the following commands to extract the chromosome files and merge them into a single file.

```
tar -zxvf -d /path/to/chromFa.tar.gz

cat chromFa/*fa > GenomeBuild.fasta
```

That's it, your genome fasta file is ready.

9. How to create an index of genome fasta file

Read aligners use a special set of files called the genome index that is generated from the genome fasta file. Index files are used to speed up the read mapping process so that the aligner can map millions of reads within a few hours. Therefore, users need to create a genome index before read mapping. Please note that the genome index format is different for each aligner. Please also refer to the 'Download genome fasta file' section to find more information about downloading the genome fasta file. If there is already a genome fasta file, then use the 'Genome Index Builder' program to create genome index. To run this program, users need to select an aligner, genome fasta file, and the output folder. Below is the instruction to use 'Genome Index Builder' program.

Step 1. Choose the 'Genome Index Builder' program from the home window (Figure 2).

Step 2. Select the appropriate aligner (Bowtie or Bowtie2).

Step 3. Click on the 'Genome Fasta' button to load the genome fasta file.

Step 4. Click on the 'output folder' button to select the folder to store the index files.

Step 5. Click on the 'Start' button to start program.

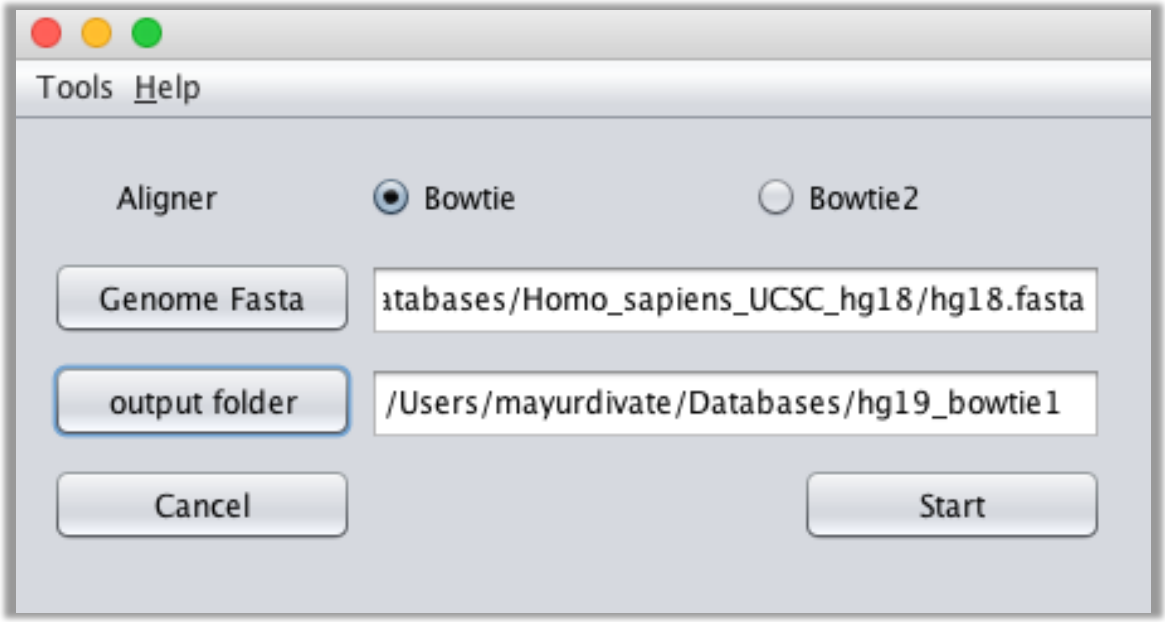


Figure 23. GUAVA Genome Index Builder program. Screenshot showing the interface for the 'Genome Index Builder' program. Here, users select the aligner, genome fasta file, and the output folder, and then click on the 'Start' button to run the program.