0. Introduction

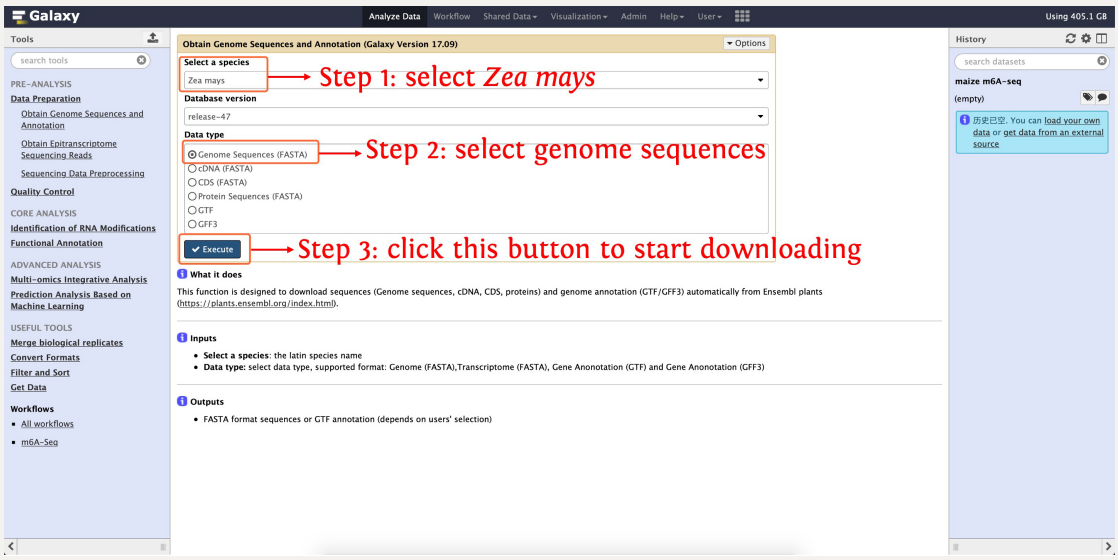
In this tutorial, we will show how to use deepEA (https://deepea.nwafu.edu.cn or http://39.101.176.205:4006) to perform comprehensive m^{6}A sequencing data analysis. The m^{6}A - immunoprecipitated (IP) and input (non-IP) samples with two biological replicates were extracted from the 14-day-old seedlings of maize (*Zea mays* L.) inbred lines B73, and then sequenced with high-throughput sequencing technology. The SRA accessions of four datasets are listed in the following table. The More information regarding these maize m^{6}A sequencing datasets is available in the reference (Luo *et al*., 2020).

SAMPLES	EXPERIMENTS	REPLICATES
SRR8383013	IP	Replicate 1
SRR8383014	IP	Replicate 2
SRR8383017	input	Replicate 1
SRR8383018	input	Replicate 2

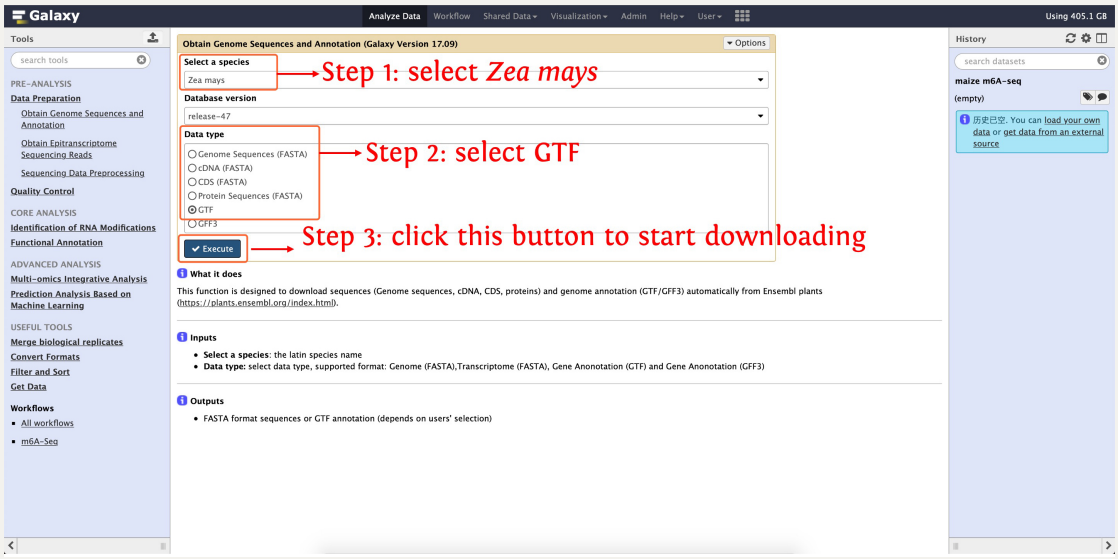
1. Download *Zea mays* reference genome sequences and annotation

Before analyzing m^{6}A sequencing data, we firstly use the function **Obtain Genome Sequences and Annotation** in **Data Preparation** module to download *Zea mays* B73 reference genome sequences and GTF annotation, the following two screenshots shows details about how to execute this step:

Step 1: download *Zea mays* reference genome sequences in FASTA format



Step 2: download *Zea mays* genome annotation file in GTF format

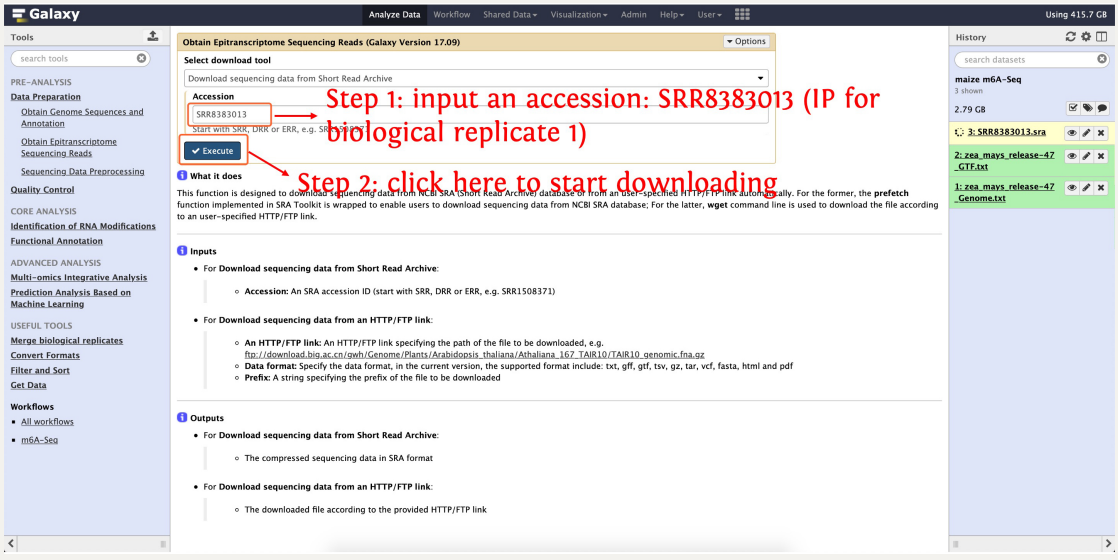


After that, reference genome sequences (named as **zea_mays_release-47_Genome.txt**) and annotation (named as **zea_mays_release-47_GTF.txt**) will be listed in your History Panel.

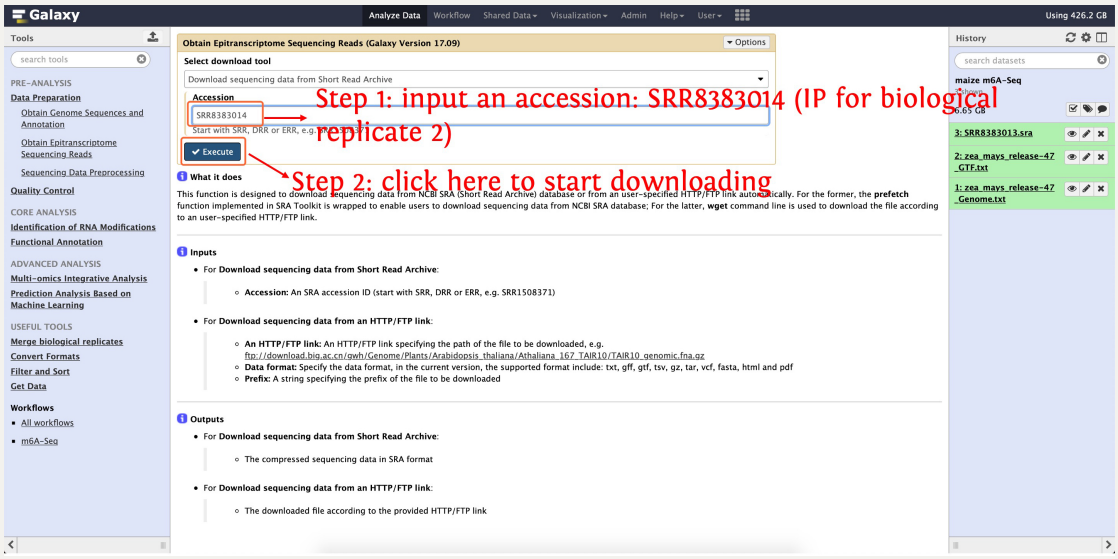
2. Download raw m^{6}A sequencing reads

After finishing downloading *Zea mays* B73 reference genome sequences and annotation, we start to download raw m^{6}A sequencing reads from NCBI SRA's database, this process can be finished by the function **Obtain Epitranscriptome Sequencing Reads** in **Data Preparation** module. Please see the following screenshots for details:

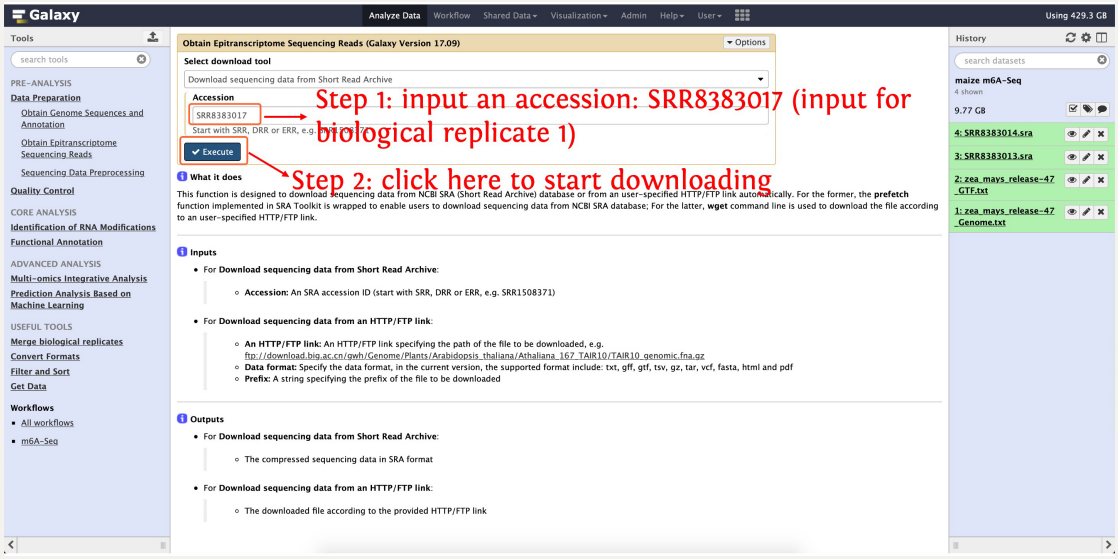
Step 1: download IP sample for biological replicate 1 (SRR8383013)



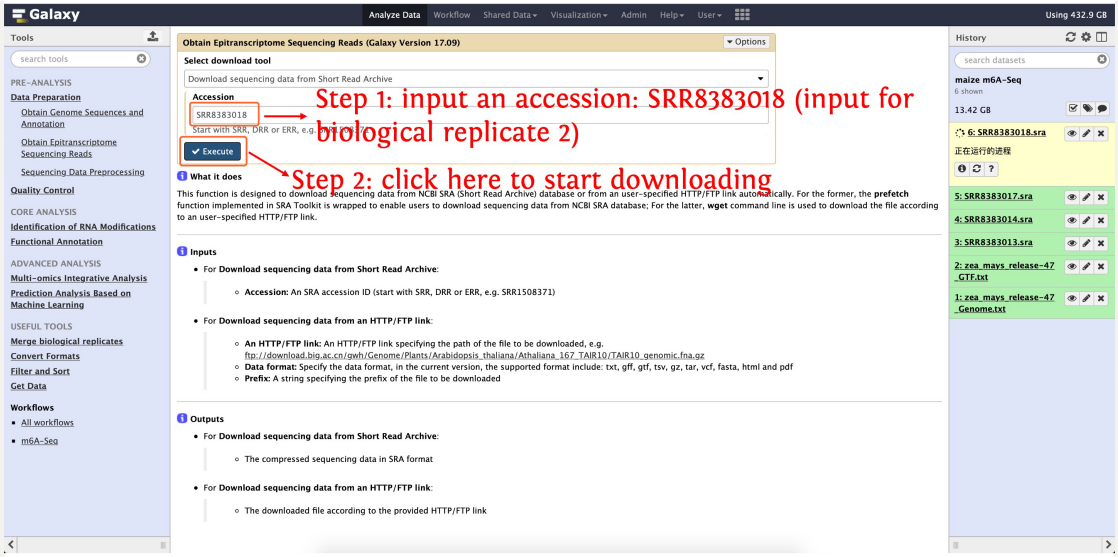
Step 2: download IP sample for biological replicate 2 (SRR8383014)



Step 3: download input sample for biological replicate 1 (SRR8383017)



Step 4: download input sample for biological replicate 2 (SRR8383018)



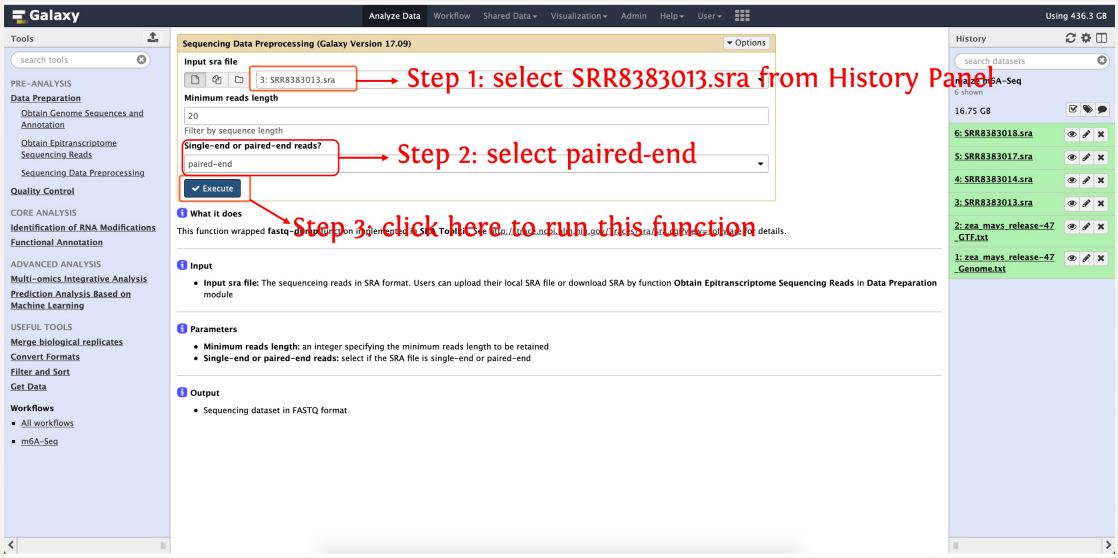
After finishing the above four steps, four raw m^{6}A sequencing reads named as **SRR8383013.sra, SRR8383013.sra, SRR8383013.sra, SRR8383013.sra**, respectively will be listed in your History Panel

3. Convert raw m^{6}A sequencing reads from SRA to FASTQ format

Here, let's convert raw m^{6}A sequencing reads from SRA to FASTQ format by the function **Sequencing Data Preprocessing** in **Data Preparation** module, please see the following four screenshots for details:

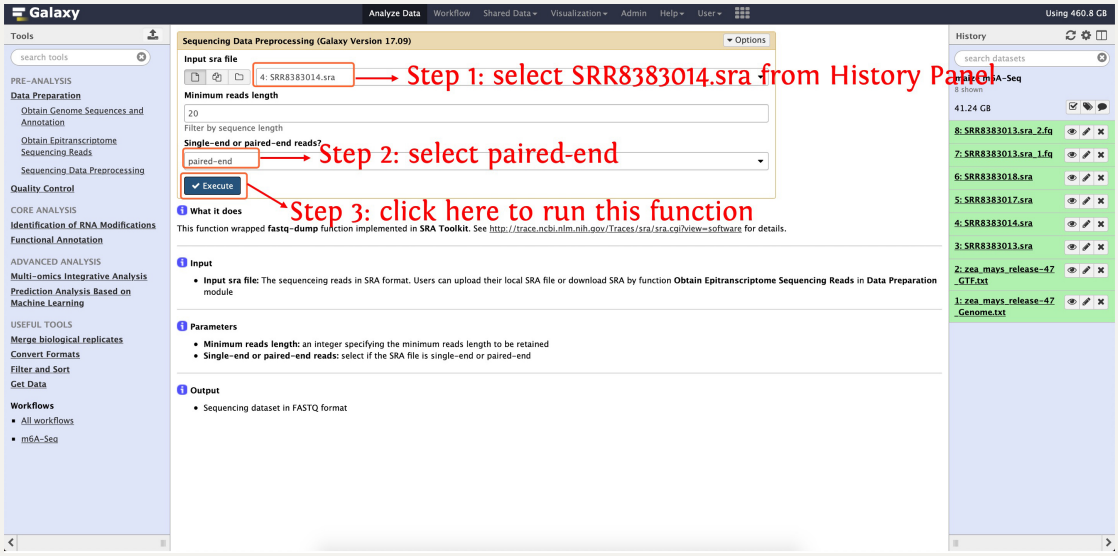
Step 1: process SRR8383013.sra

This step will cost ~12 minutes



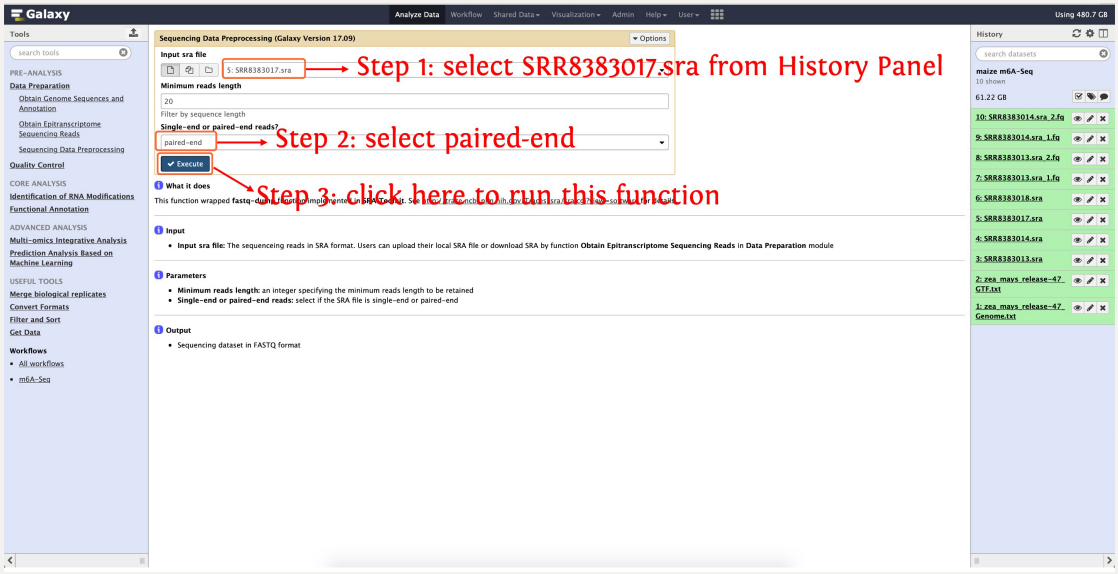
Step 2: process SRR8383014.sra

This step will cost ~26 minutes



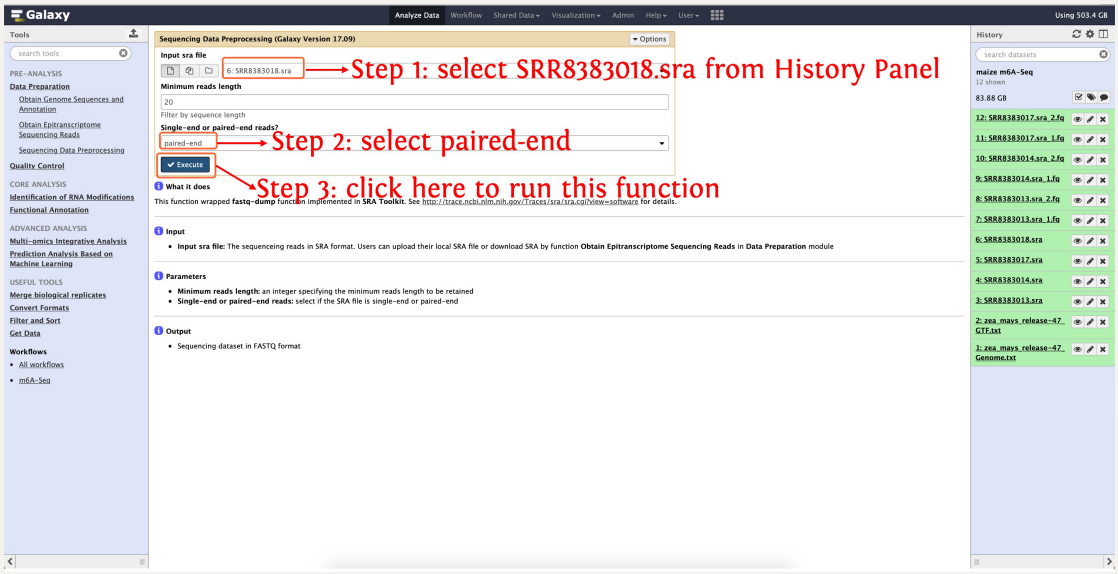
Step 3: process SRR8383017.sra

This step will cost ~24 minutes



Step 4: process SRR8383018.sra

This step will cost ~23 minutes



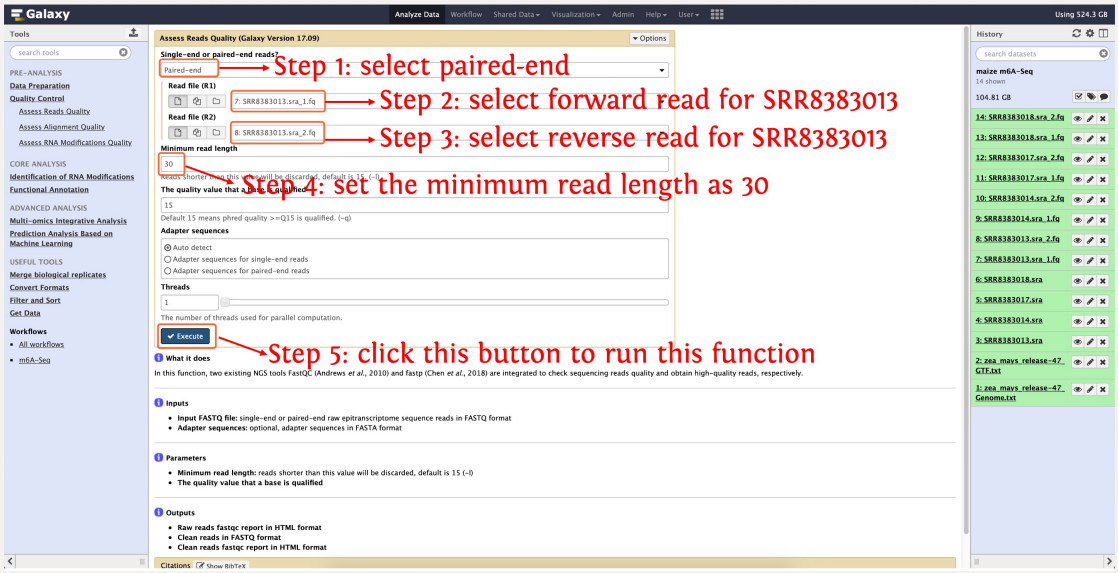
For each SRA accession, two FASTQ format files (forward reads and reverse reads) will be generated as this experiment is paired-end sequencing.

4. Trim raw m^{6}A sequencing reads

Before align m^{6}A sequencing reads to genome, trim low-quality reads is necessary in all NGS (Next Generation Sequencing) analyses as which may cause incorrect mapping of reads to a reference genome, and even result in incorrect identification of RNA modifications. deepEA provided the function **Assess Reads Quality** in **Quality Control** module to filter raw reads to clean reads, please see the following screenshots for details:

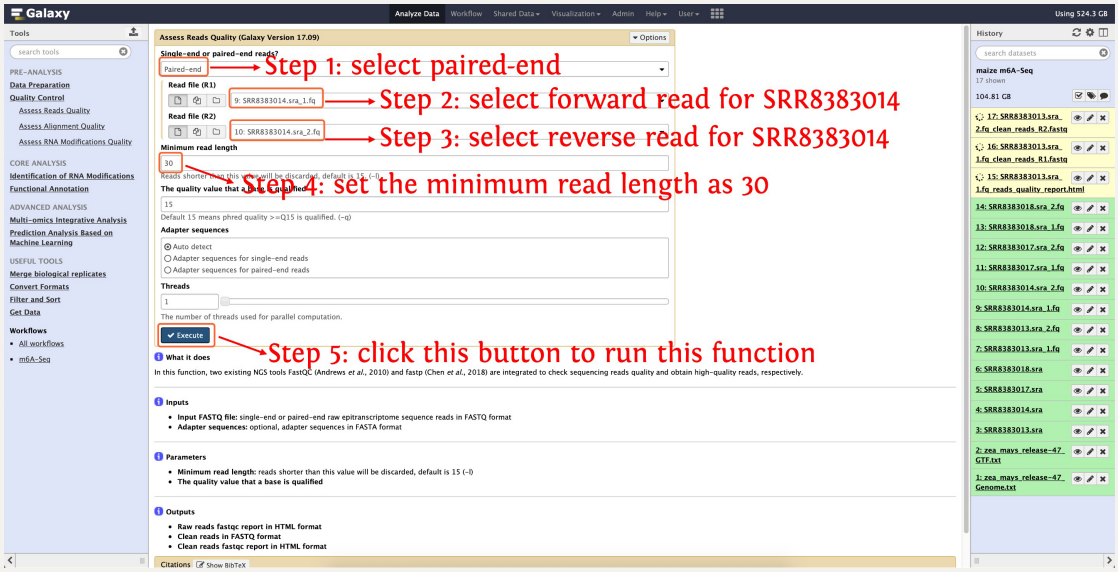
Step 1: trim SRR8383013

This step will cost ~16 minutes



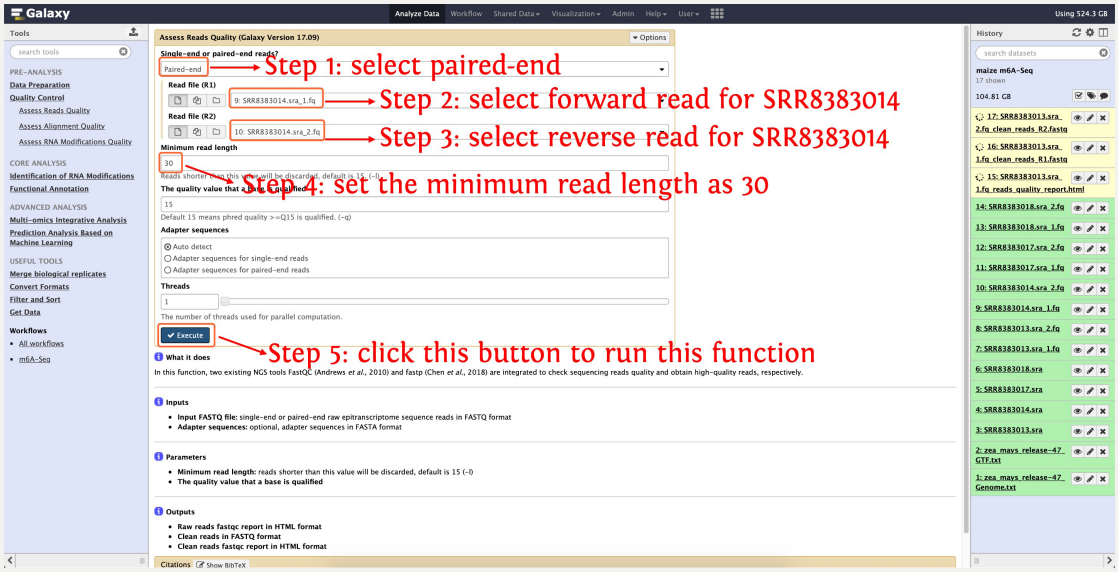
Step 2: trim SRR8383014

This step will cost ~12 minutes



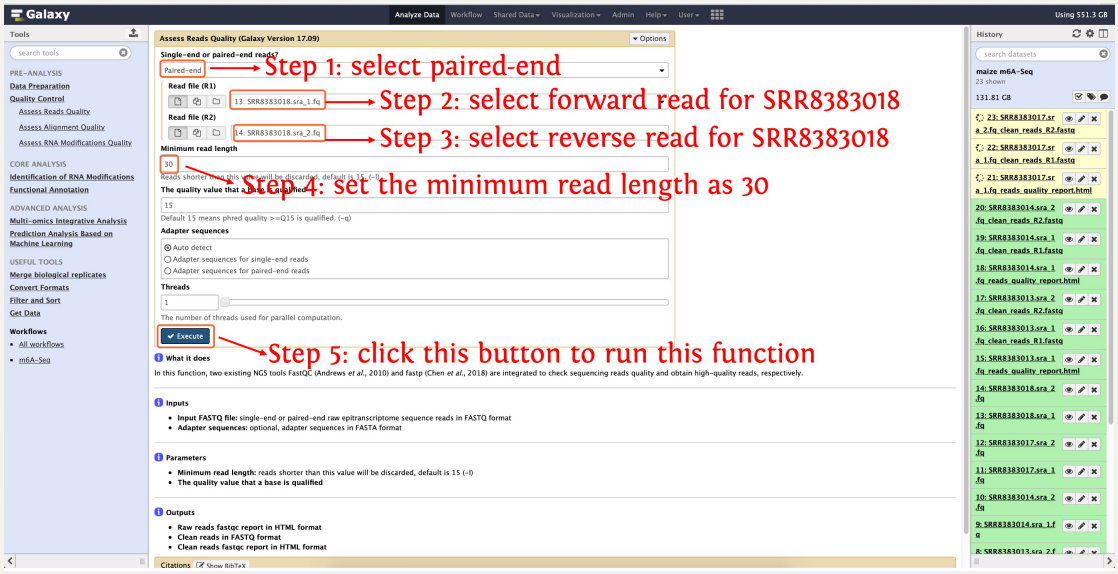
Step 3: trim SRR8383017

This step will cost ~13 minutes

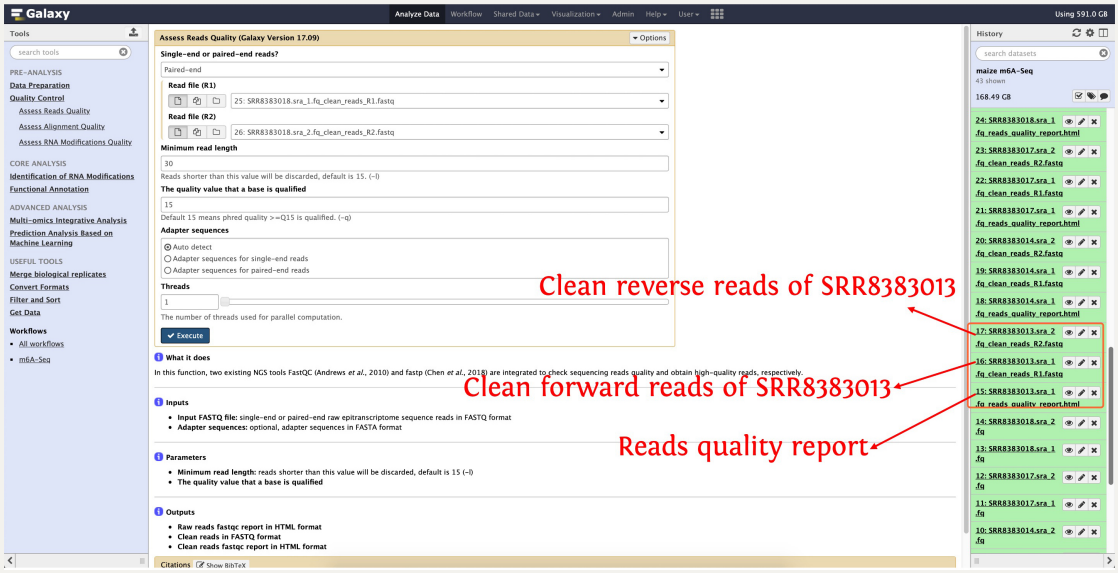


Step 4: trim SRR8383018

This step will cost ~12 minutes



For each SRA accession, three files will be output, please see the following screenshot for detail:

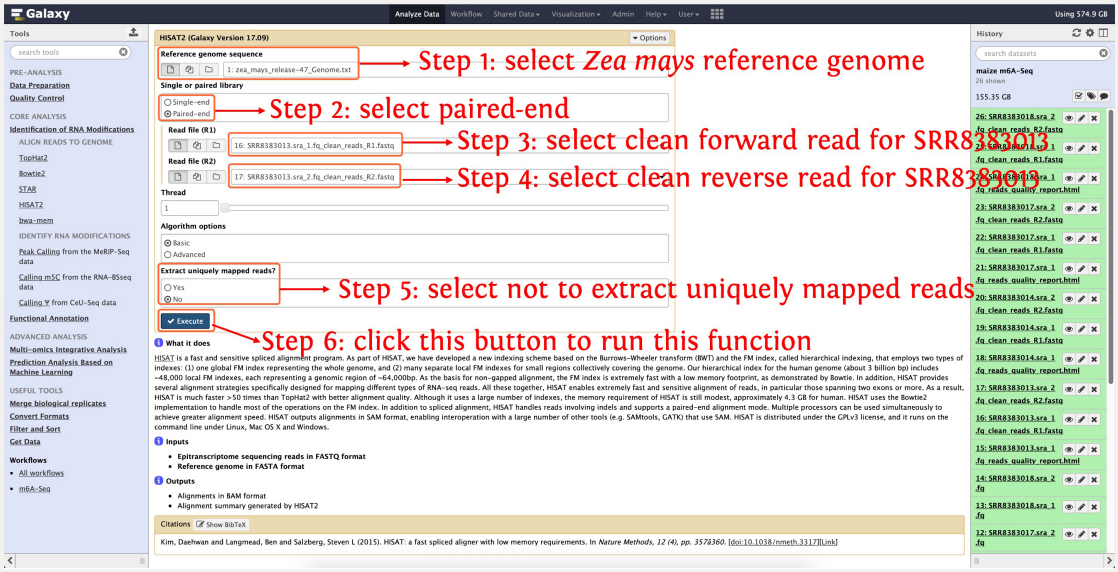


5. Align clean m^{6}A sequencing reads to *Zea mays* B73 reference genome with HISAT2

Here, we start to align clean reads to reference genome with HISAT2 provided in module **Identification of RNA Modifications**, see the following screenshots for details:

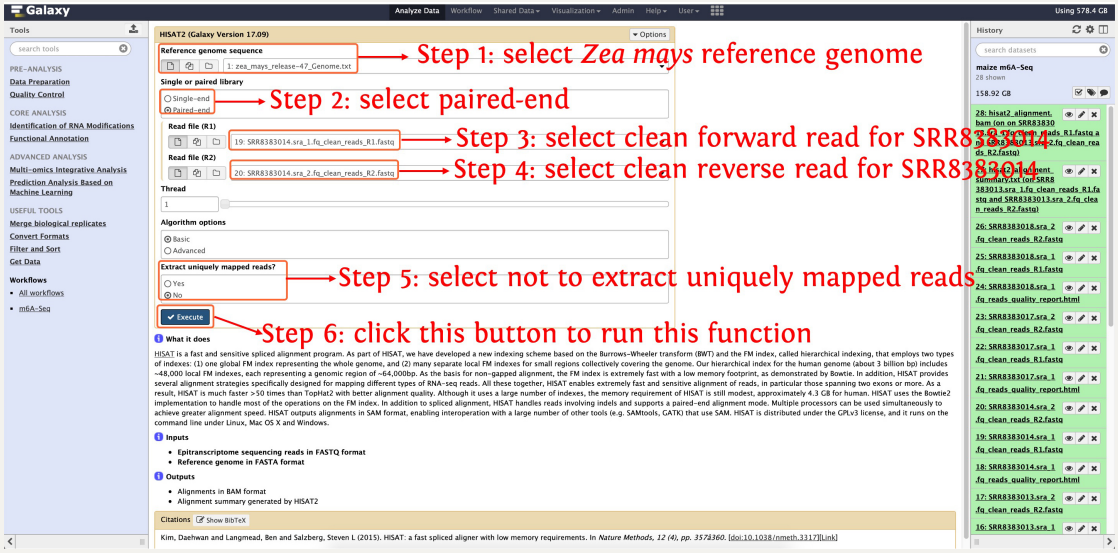
Step 1: align SRR8383013

This step will cost ~48 minutes



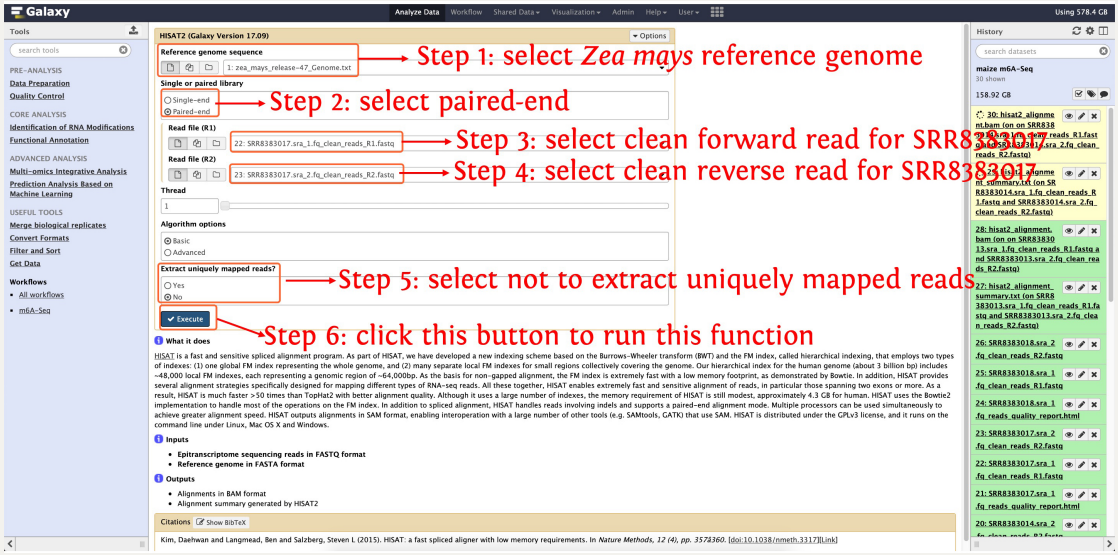
Step 2: align SRR8383014

This step will cost ~40 minutes



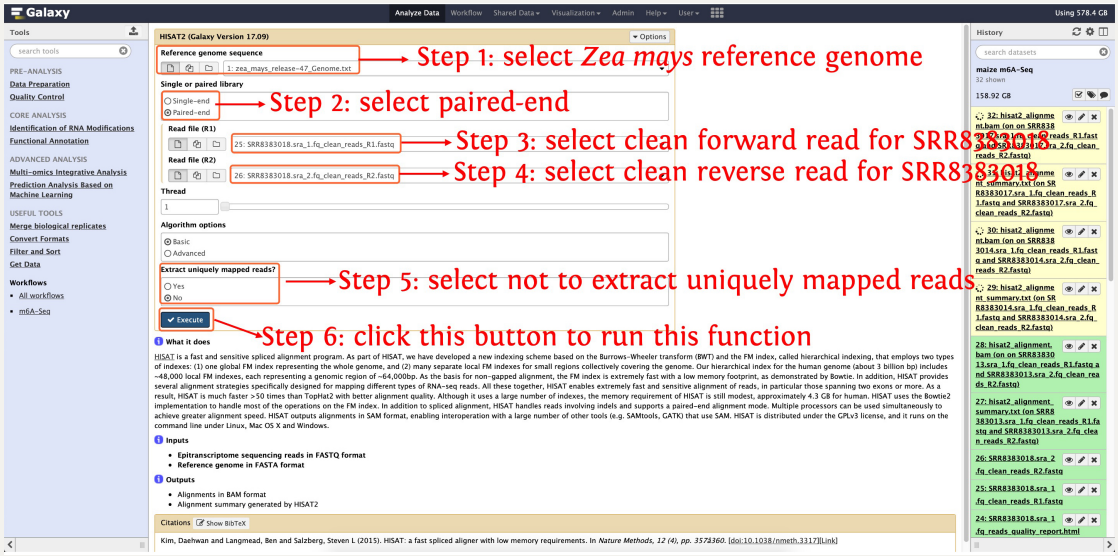
Step 3: align SRR8383017

This step will cost ~44 minutes

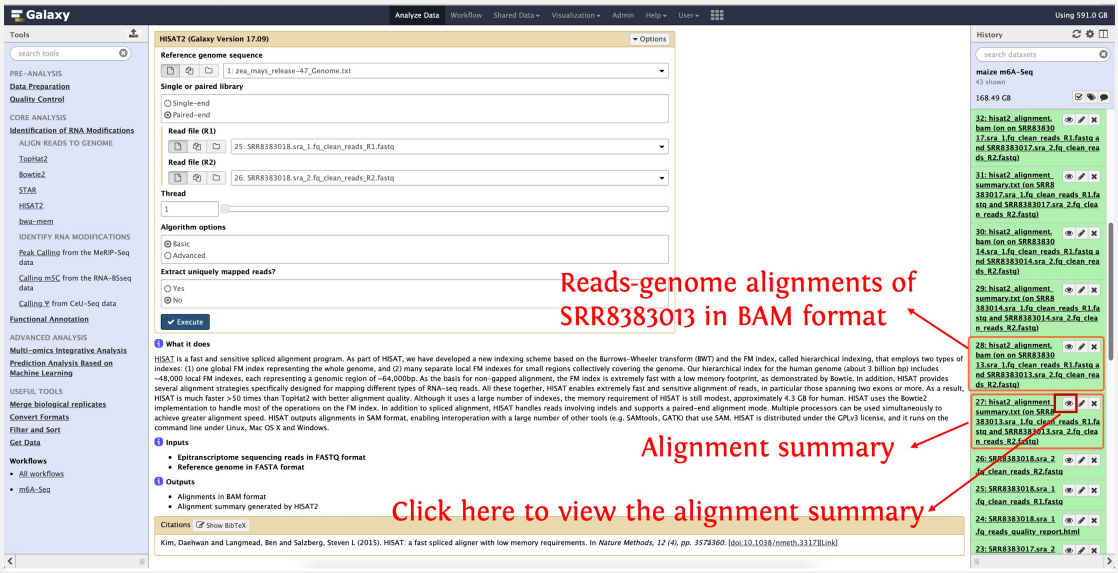


Step 4: align SRR8383018

This step will cost ~42 minutes



Then for each SRA accession, reads-genome alignments in BAM format and alignment summary will be generated, see the following screenshot:

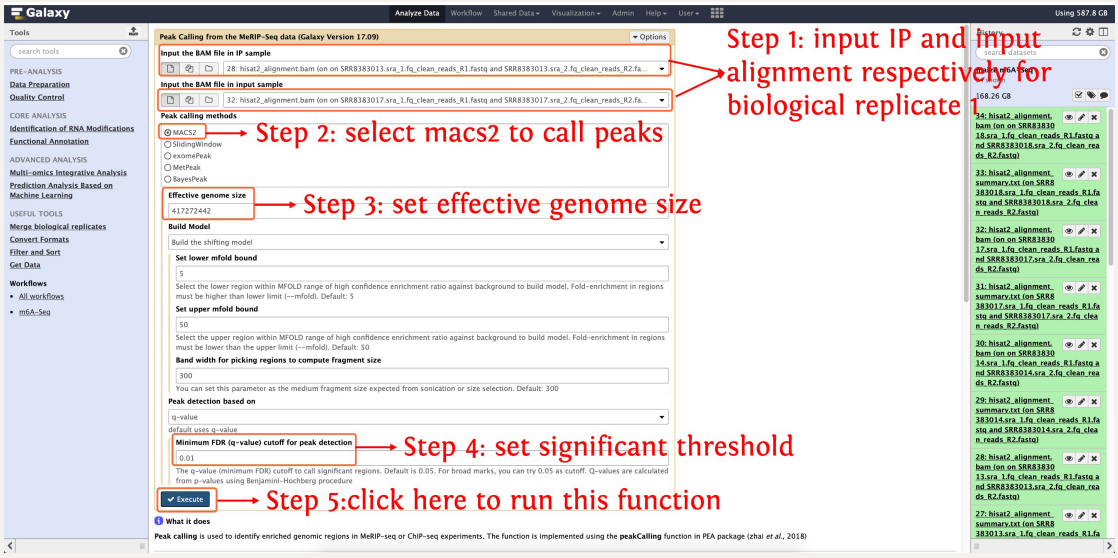


6. Call m^{6}A enriched peaks with macs2

After finishing aligning reads to genome, let's start to call m^{6}A enriched peaks with macs2, the following screenshots show details about parameter settings:

Step 1: call m^{6}A peaks for biological replicate 1

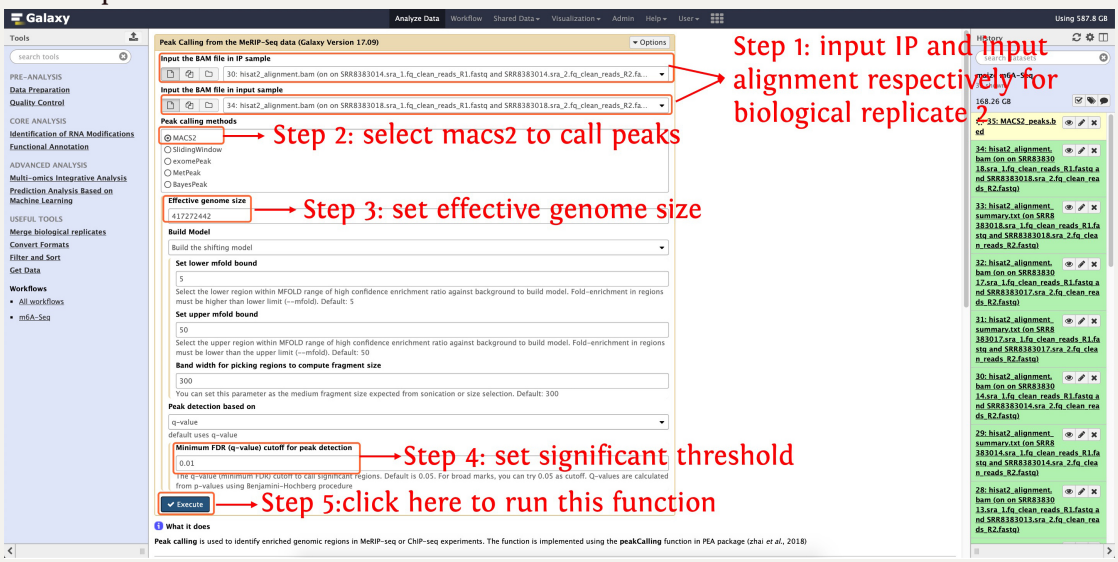
This step will cost ~8 minutes



This step will generate ~16,400 peaks for biological replicate 1

Step 2: call m^{6}A peaks for biological replicate 2

This step will cost ~7 minutes

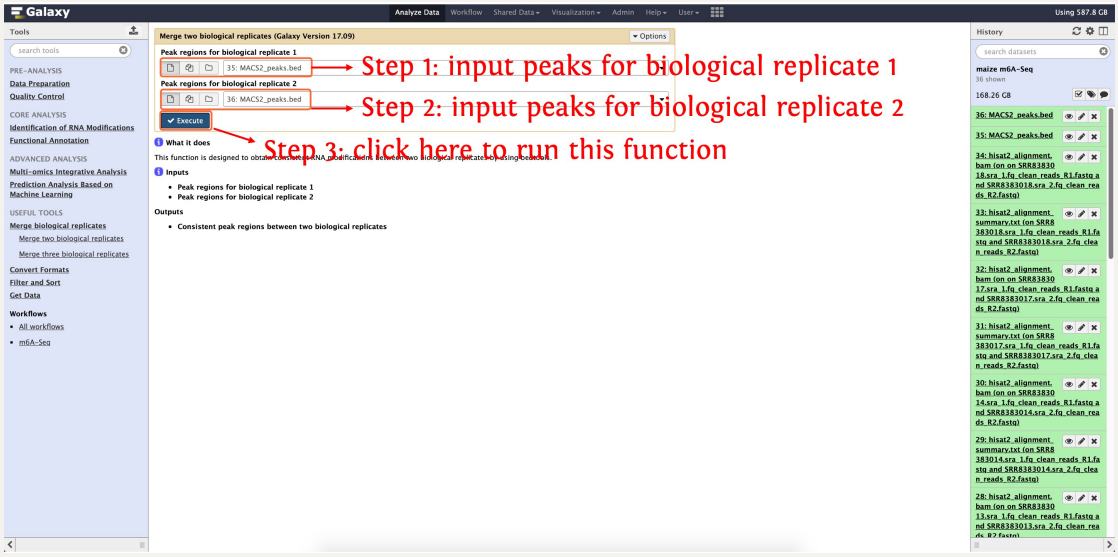


This step will generate ~16,100 peaks for biological replicate 2

Step 3: obtain consistent peaks between two biological replicates

After finishing peak calling for two replicates, deepEA also provided a function **Merge two biological replicates** to obtain consistent peaks between two biological replicates, see the following screenshots for details:

This step will cost ~2 seconds



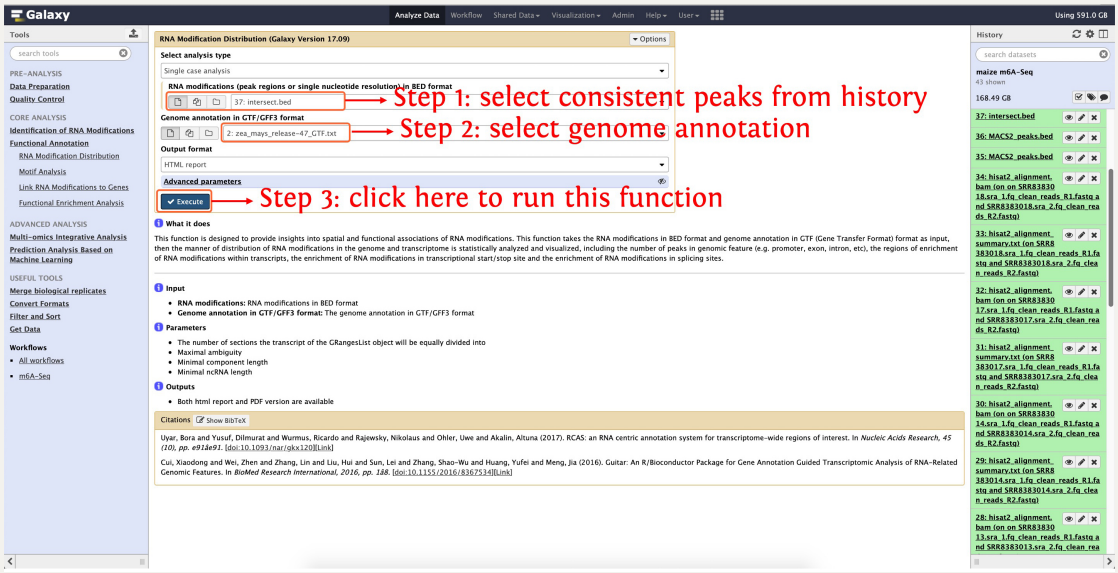
Then the consistent peaks named as **intersect.bed** (about ~14,000 consistent peaks) will be shown in your History Panel.

7. Perform functional annotation for m^{6}A

m^{6}A distribution

The distribution of m^{6}A in the genome and transcriptome can be visualized by the function **RNA Modification Distribution** in **Functional Annotation** module, see the following screenshot for detail:

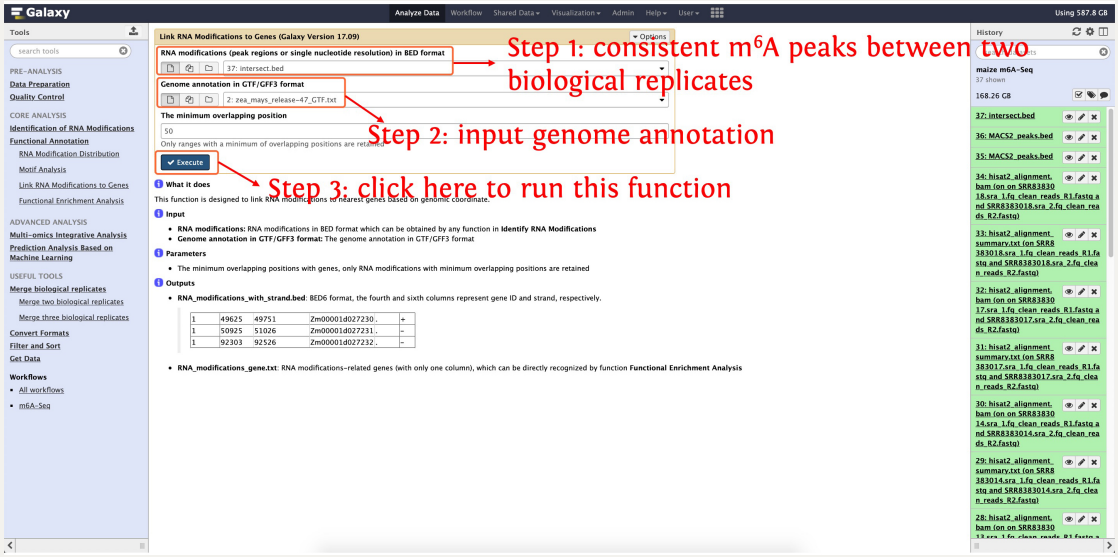
This step will cost ~5 minutes



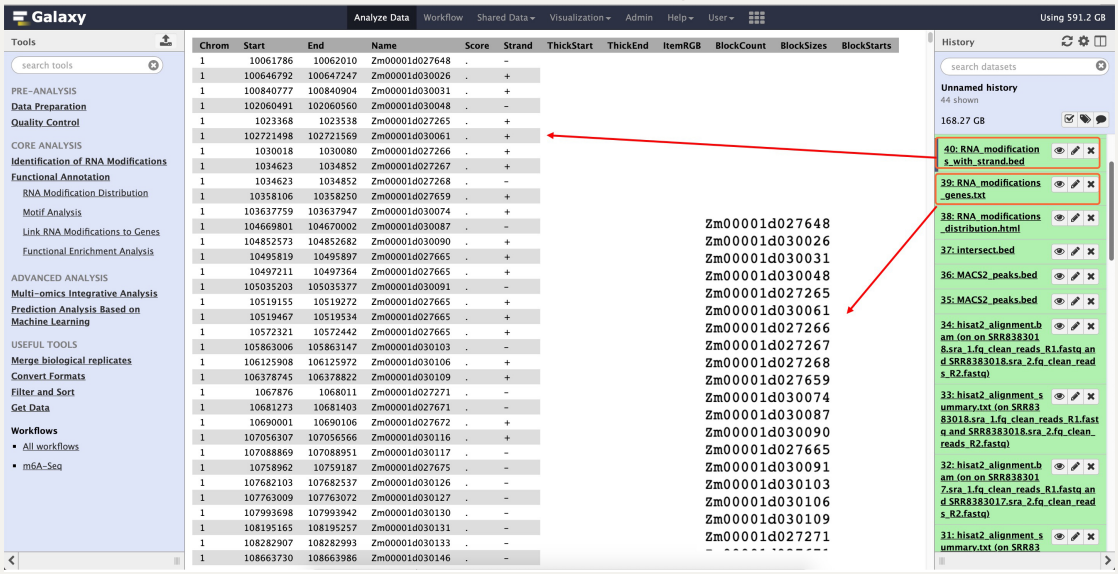
Then an interactively HTML will be generated, please see here to preview this results

Link m^{6}A modifications with genes

This step will cost ~2 minutes



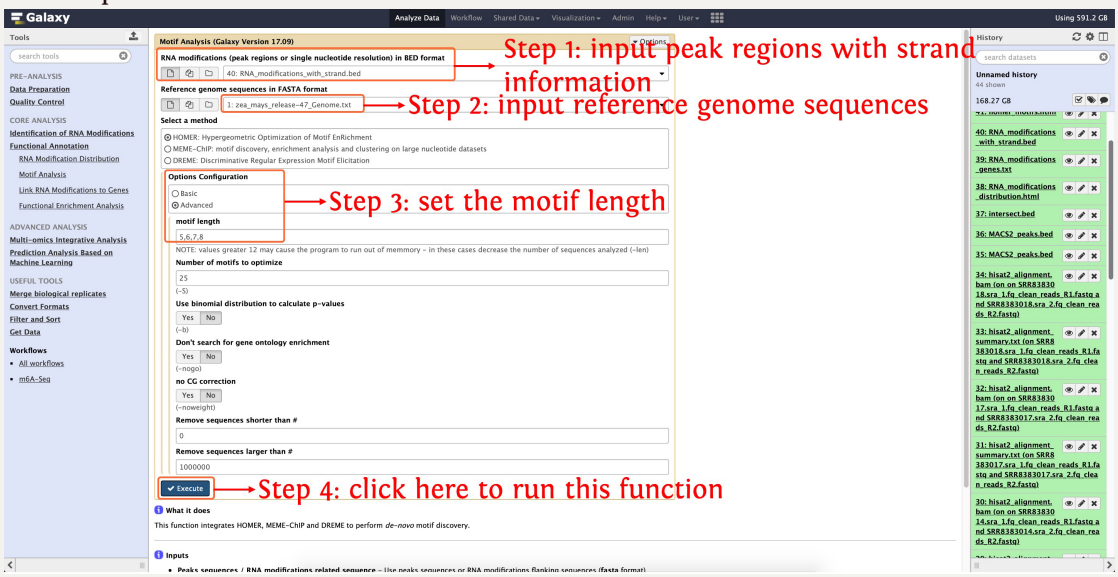
This output for this function are shown in the following the screenshot



De-novo motif discovery

This following screenshot shows how to use homer to perform *de-novo* motif discovery

This step will cost ~6 minutes

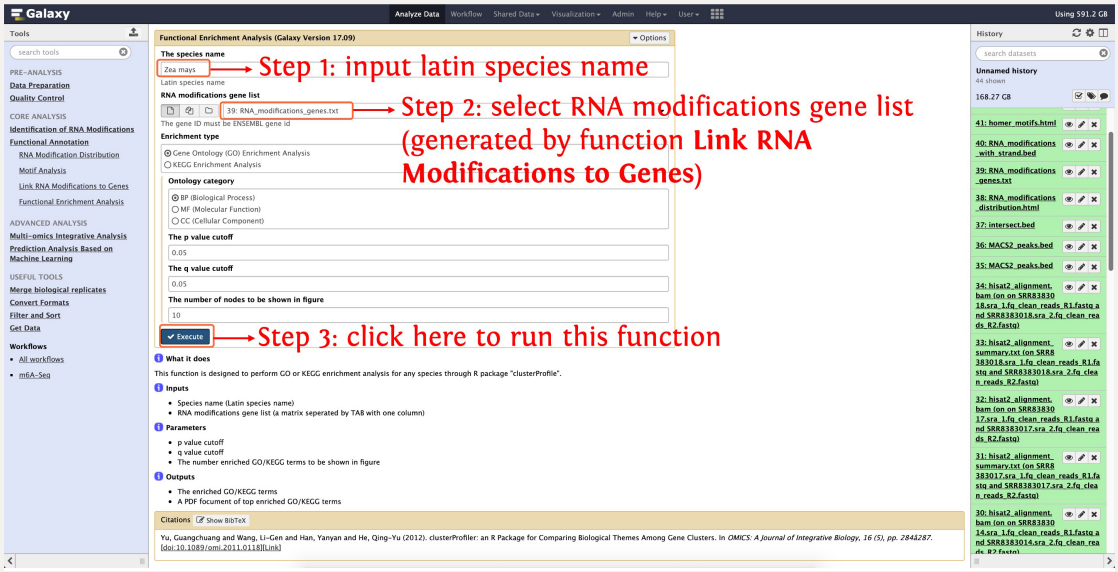


Then an HTML document will be generated, please click here to preview this results

GO functional enrichment analysis

deepEA provided the function **Functional Enrichment Analysis** to perform GO enrichment analysis, see the following screenshot for detail:

This step will cost ~1 minute

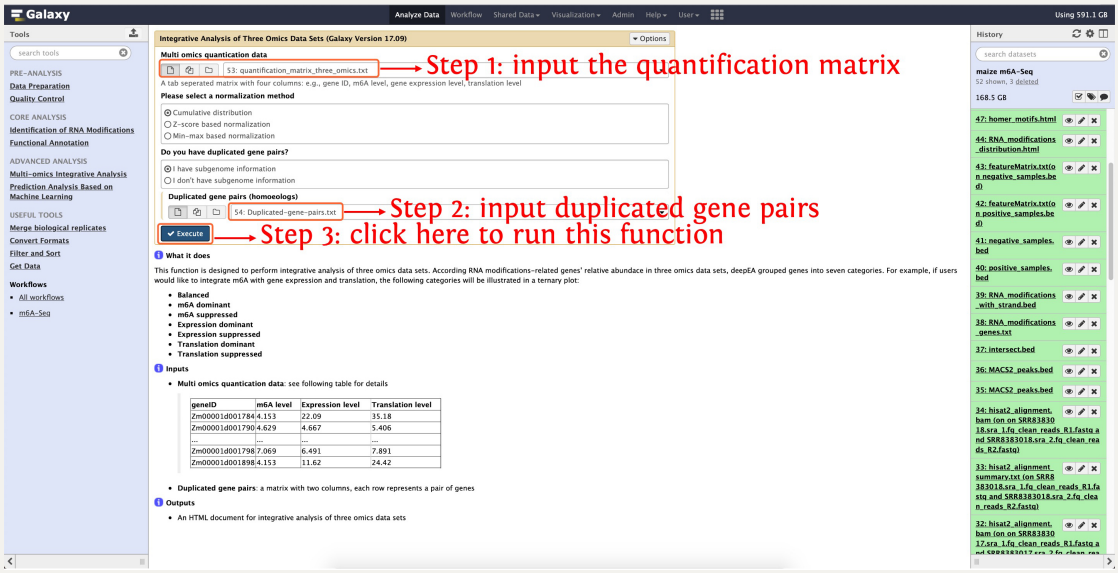


Then a figure in PDF format and a TAB separated matrix will be generated, please click here for details.

7. Multi-omics integrative analysis

To run this module, you have to download test data provided by deepEA, and then upload the data in directory `test_data/Multi-omics Integrative Analysis/` to deepEA server. If you are not sure how to upload local data into deepEA server, please see here for details. Then you can run the function **Integrative Analysis of Three Omics Data Sets** in **Multi-omics Integrative Analysis** module as the following screenshot shows:

This step will cost ~6 seconds



Then an interactively HTML document will be output, click here to preview this results.

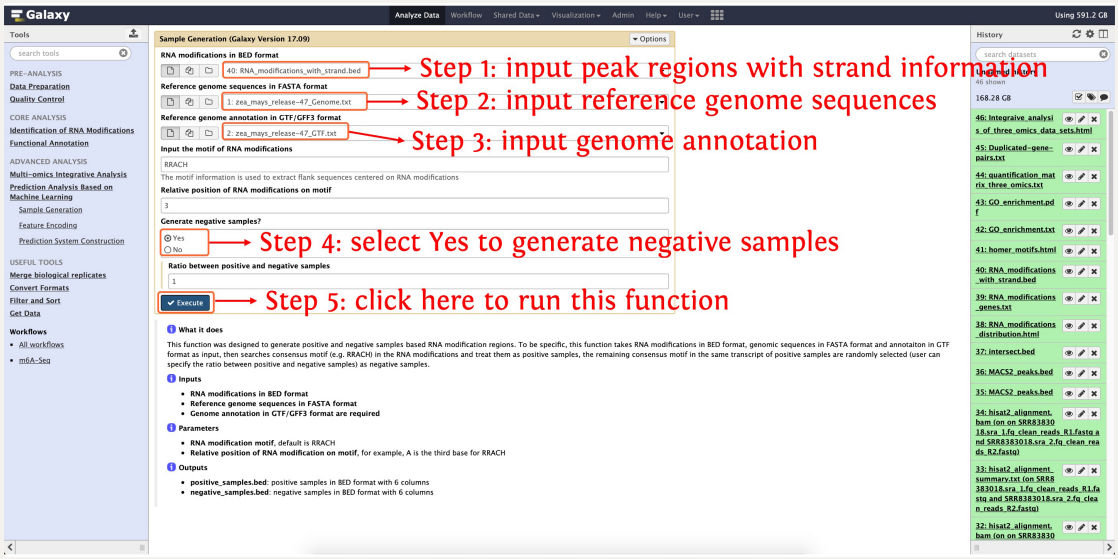
8. Build an m^{6}A predictor based on machine learning

The following four steps shows us how to use **Prediction Analysis Based on Machine Learning** module to build an m^{6}A predictor and perform cross-validation experiments.

Step 1: generate positive and negative samples for m^{6}A predictor construction

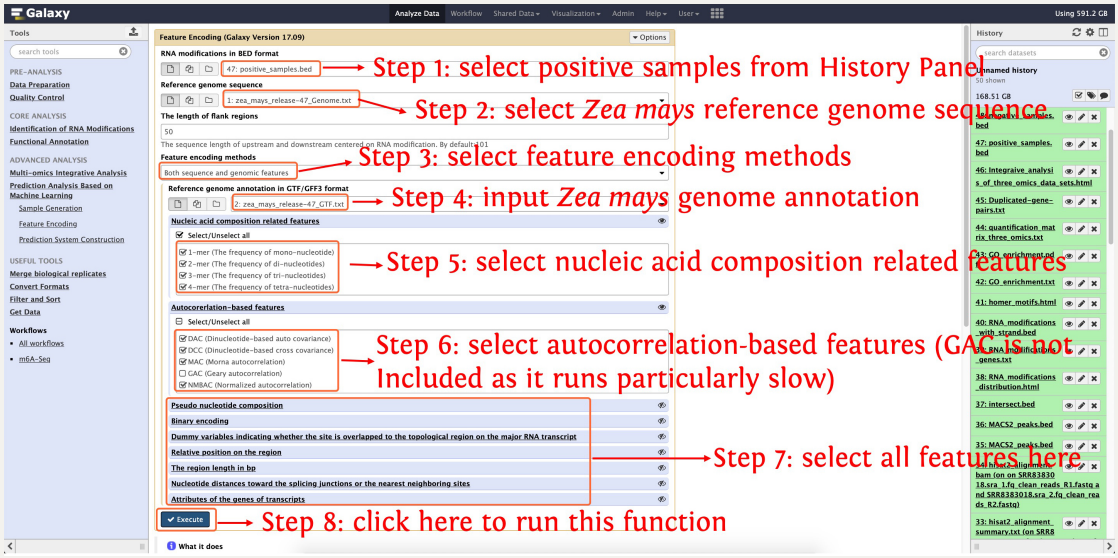
The function **Sample Generation** can be used to generate positive and negative samples.

This step will cost ~**5 hours**, please wait patiently



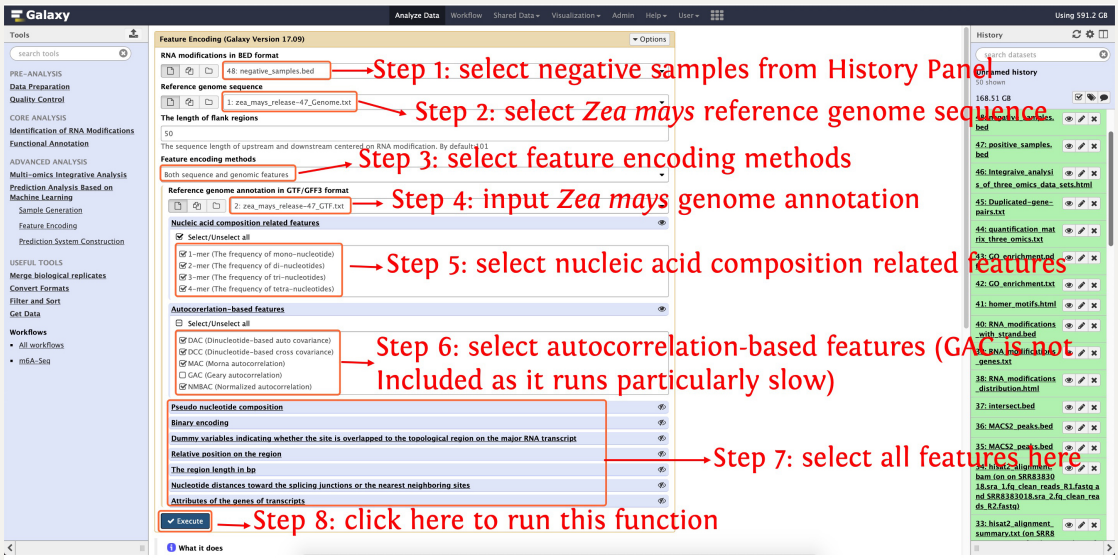
Step 2: encoding positive samples by function Feature Encoding

This step will cost ~13 minutes

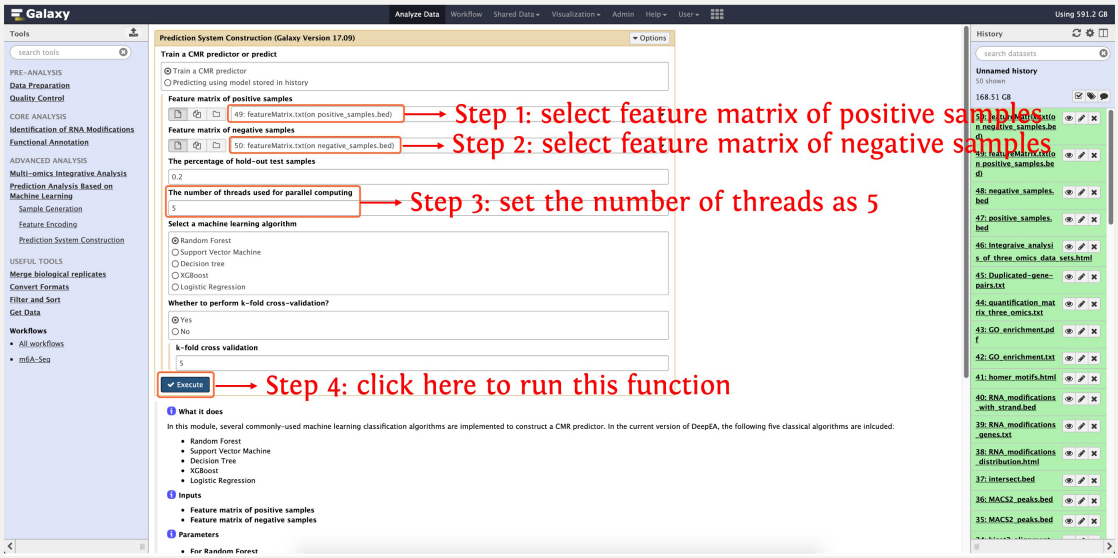


Step 3: encoding negative samples by function Feature Encoding

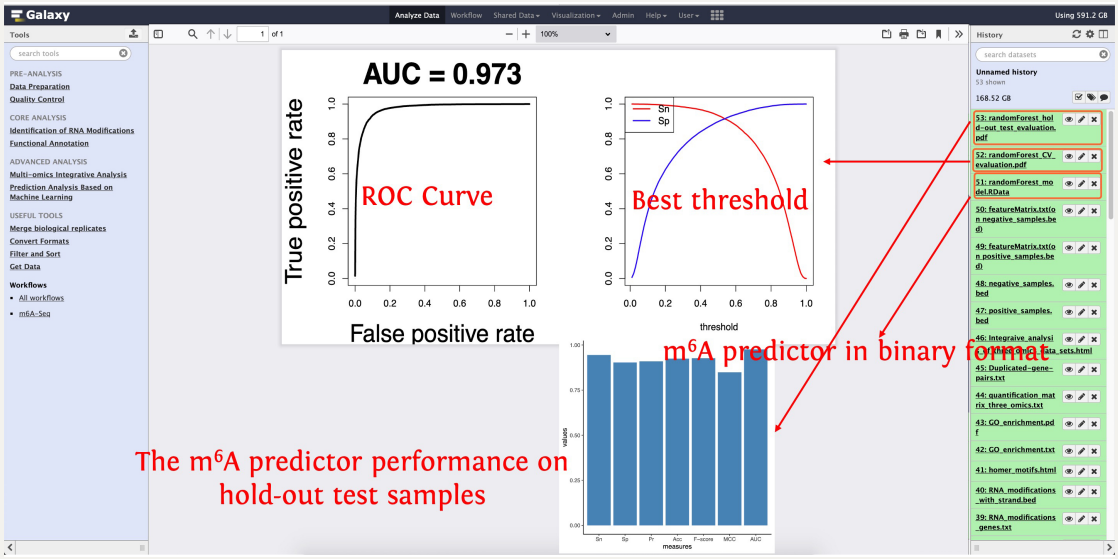
This step will cost ~13 minutes



Step 4: m^{6}A predictor construction and evaluation



After finishing predictor construction and evaluation, three documents will be output, please see the following screenshot:

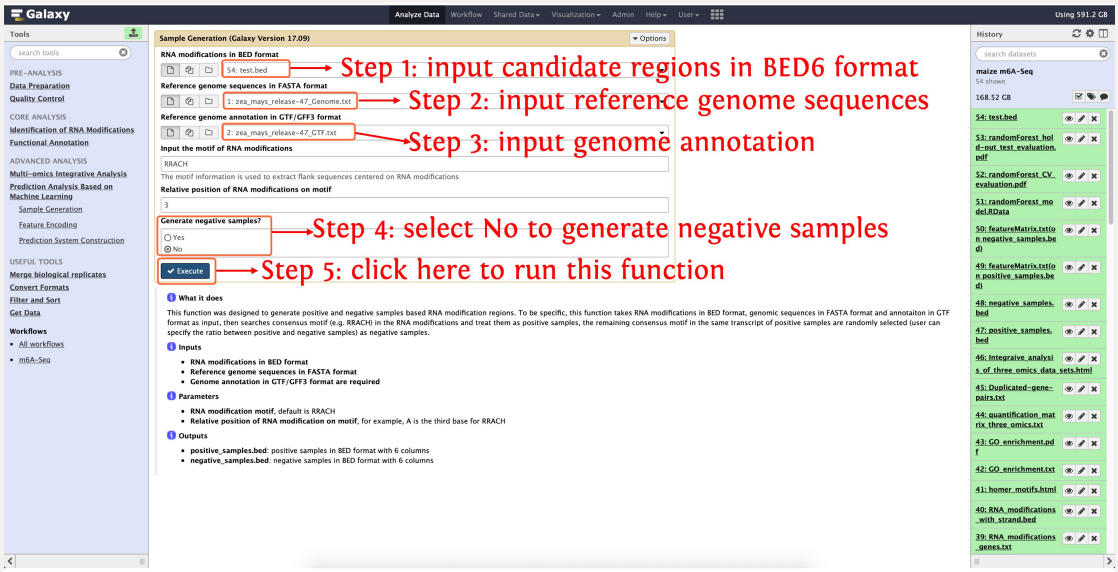


9. Predict candidate m^{6}A using the m^{6}A predictor

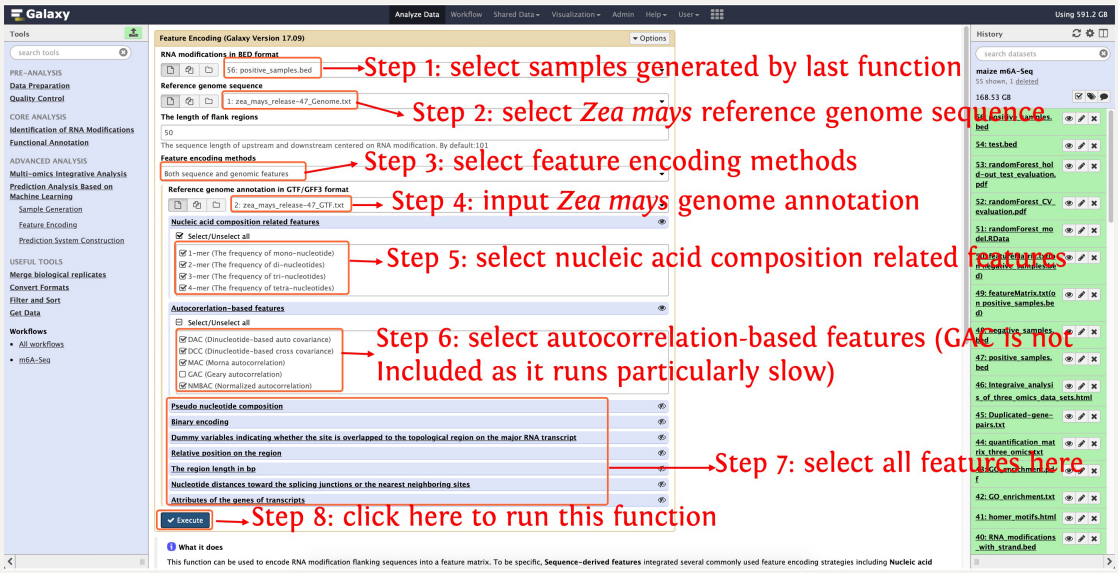
After building an m^{6}A predictor, we can use the predictor to prioritize candidate m^6 modifications, to do this, you have to prepare a file in BED format, see the following table:

CHR	START	END	GENEID	NA	STRAND
1	49625	49751	Zm00001d027230	.	+
1	50925	51026	Zm00001d027231	.	-
1	92303	92526	Zm00001d027232	.	-

Then upload this file into deepEA server and run the function **Sample Generation** in **Prediction Analysis Based on Machine Learning** module as the following screenshot shows:



Then encoding the candidate samples using the same feature encoding methods as m^6A predictor construction:



After encoding the candidate samples, we can predict the candidate m^6A: