0. Introduction for Identification of RNA Modifications

This module provides step-by-step functions required for epitranscriptome reads mapping and identification of RNA modifications.

Align Reads to Genome

Several commonly used aligners are wrapped to align epitranscriptome reads to genome. Currently, Tophat2, Bowtie2, STAR, HISAT2, bwa-mem.

Tools	Description	Input	Output	Time (test data)	Reference
Tophat2	Tophat2 is a spliced aligner, which aligns short reads by calling Bowtie2 but alows for variable-length indels with respect to the reference genome.			~50s	Kim *et al.*, 2013, Genome Biology
Bowtie2	Bowtie2 is a short read aligner which achieves a combination of high speed, sensitivity and accuracy by combining the strengths of the full-text minute index with the flexibility and speed of			~10 s	Langmead *et al.*, 2012, Nature Methods

	hardware-accelerated dynamic programming algorithms, therefore bowtie2 is suitable for large genomes	Epitranscriptome sequencing reads in FASTQ format and reference genome sequences in FASTA format	Read alignments in SAM/BAM format		
STAR	STAR is an ultrafast universal RNA-Seq aligner and can discover non-canonical splices and chimeric (fusion) transcripts			~16s	Dobin *et al.*, 2013, Bioinformatics
HISAT2	HISAT2 is an ultrafast spliced aligner with low memory requirements. It supports genomes of any size, including those larger than 4 billion bases			~8s	Kim *et al.*, 2015, Nature Methods
bwa-mem	bwa-mem is a relatively early aligner based on backward search with Burrows–Wheeler Transform			~10s	Li *et al.*, 2009, Bioinformatics

Identify RNA Modifications

Identify RNA Modifications implements three pipelines for MeRIP-Seq, CeU-Seq and RNA-BSSeq, respectively.

Tools	Description	Input	Output	Time (test data)	Reference
Peak Calling from the MeRIP-Seq data	Identify enriched genomic regions from MeRIP-Seq experiment	Read alignments of IP and input in SAM/BAM format and reference genome sequences in FASTA format	RNA modifications in BED format	~36s	Zhai *et al.*, 2018, Bioinformatics
Calling m^5C from the RNA-BSseq data	Perform bisulfite sequencing (BS-Seq) read mapping, comprehensive methylation calling using meRanTK	Sequencing reads in FASTQ format and reference genome sequences in FASTA format	m^5C sites in BED format	~10 mins using 2 threads	Rieder *et al.*, 2016, Bioinformatics
Calling Ψ from CeU-Seq data	Identify pseudouridylation from CeU-Seq	Read alignments in SAM/BAM format and cDNA sequences in FASTA format	Pseudoridylation sites in BED format	~1 mins	Li *et al.*, 2015, Nature Chemical Biology

1. Align reads to genome

Currently, deepEA wrapped five aligners to map epitranscriptome reads to genome, here, we take Tophat2 as an example to show how to use deepEA to run reads mapping, the other four aligners are similar.

Input

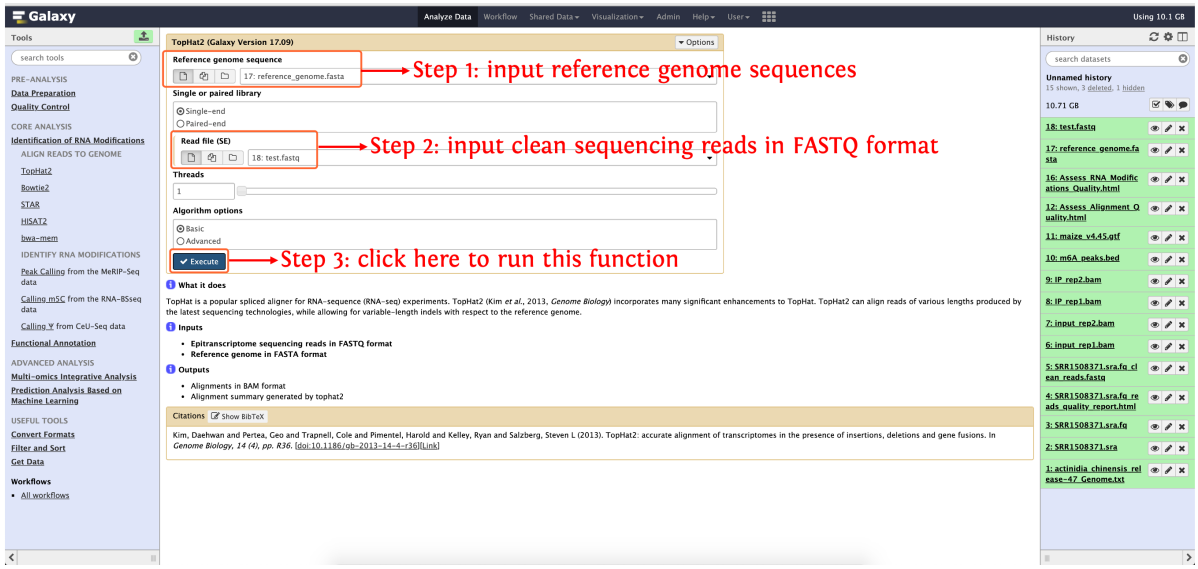
- **Epitranscriptome sequencing reads in FASTQ format**
- **Reference genome in FASTA format**

Output

- Alignments in BAM format
- Alignment summary generated by tophat2

How to use this function

- **Step 1**: upload the data in directory `test_data/Identification_of_RNA_Modifications/Align_Reads_to_Genome/` to history panel, if you are not clear about how to upload local data to deepEA server, please see here for details
- **Step 2**: see the following screenshot to run this function



2. Peak calling from the MeRIP-Seq data

Peak calling is used to identify enriched genomic regions in MeRIP-seq or ChIP-seq experiments. The function is implemented using the **peakCalling** function in PEA package (zhai *et al*., 2018)

Input

- **IP sample:** The IP experiment in BAM format
- **Input sample:** The input control experiment in BAM format
- **Reference genome:** The Reference genome sequences with FASTA format
- **Reference annotation file:** The Reference genome annotation file with GTF/GFF3 format (required for methods: **exomePeak**, **MeTPeak** and **BayesPeak**)

Output

- **The enriched peak region matrix in BED format**
  - For **SlidingWindow** method:

Chromosome	Start(1-based)	End	Bin number	Mean FDR	Max FDR	Minimum FDR	Mean Ratio	Max Ratio	Minimum Ratio
1	67476	67575	4	0.0136	0.0328	0.0001	-1.0012	-0.6334	-1.581
1	330776	330875	4	0.0215	0.0381	0.0007	-1.576	-1.4077	-1.788
1	389201	389300	4	0.0024	0.0070	0.0002	-1.115	-1.0598	-1.190

  - For **exomePeak** metod:

Chromosome	Start (0-based)	End	Gene ID	P.value	Strand
1	30663	30723	AT1G01040	0.0026	+
1	73831	74096	AT1G01160	2.5e-30	+
1	117530	117710	AT1G01300	2.4e-07	+

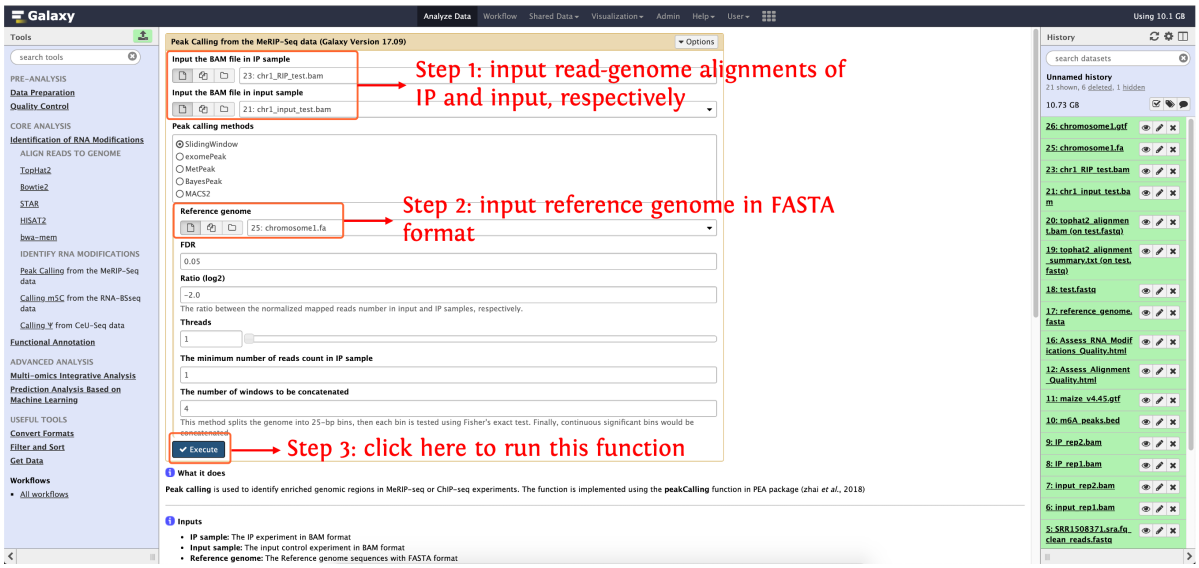
  - For **MetPeak** method: it's the same as **exomePeak**
  - For **BayesPeak** method:

chr	start	end	PP	job
1	3748	3848	0.0231	2
1	6848	6948	0.0178	2
1	6898	6998	0.9960	1

- For **macs2** method: please see macs2

How to use this function

- **Step 1**: upload the data in directory `test_data/Identification_of_RNA_Modifications/Peak Calling from the MeRIP-Seq data/` to history panel, if you are not clear about how to upload local data to deepEA server, please see here for details
- **Step 2**: see the following screenshot to run this function



3. Calling m^{5}C from the RNA-BSseq data

This function integrated meRanTK (Rieder *et al*., 2016, *Bioinformatics*) to perform RNA bisulfite sequencing (BS-Seq) read mapping, comprehensive methylation calling.

Input

- **FASTQ file:** The FASTQ format sequencing file

Output

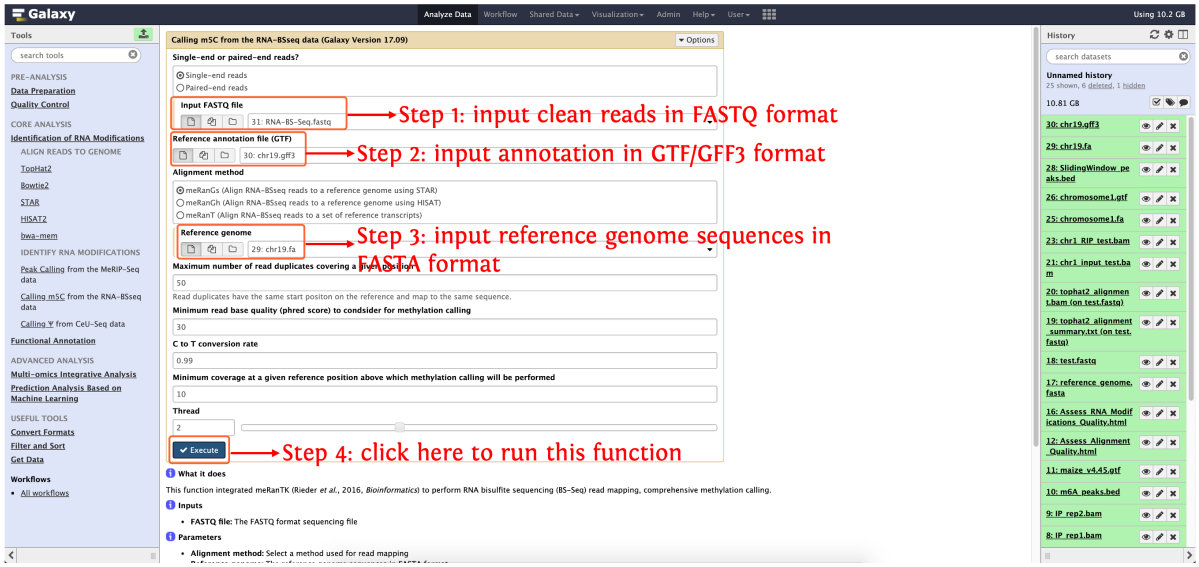
- **m5C_out_peaks:** The detected m^{5}C sites

How to use this function

- **Step 1**: upload the data in directory `test_data/Identification_of_RNA_Modifications/Calling m5C from the RNA-BSseq data/` to history panel, if you are not clear about how to upload local data to deepEA server,

please see here for details

- **Step 2**: see the following screenshot to run this function



4. Calling Ψ from CeU-Seq data

This function is used to identify pseudouridylation from CeU-Seq (Li *et al*., 2015). To be specific, for any given position on a reference transcript, the stop rate of position i was calculated using the equation N_i_stop/(N_i_stop + N_i_readthrough), where N_i_stop (stop reads) is the number of reads with the mapping position starting at base i+1 (one nucleotide 3' to position i), and N_i_readthrough (readthrough reads) is the number of reads reading through position i; Then a position i is identified to be Ψ only when all of the following criteria were met:

- the stop reads of position i (N_i_stop) must be no less than 5 in the N3-CMC(+) sample;
- the stop rate in N3-CMC(−) samples must be less than 0.10;
- the difference of stop rate for position i between the N3-CMC(+) samples and the matched N3-CMC(−) samples must be at least 0.30.

Input

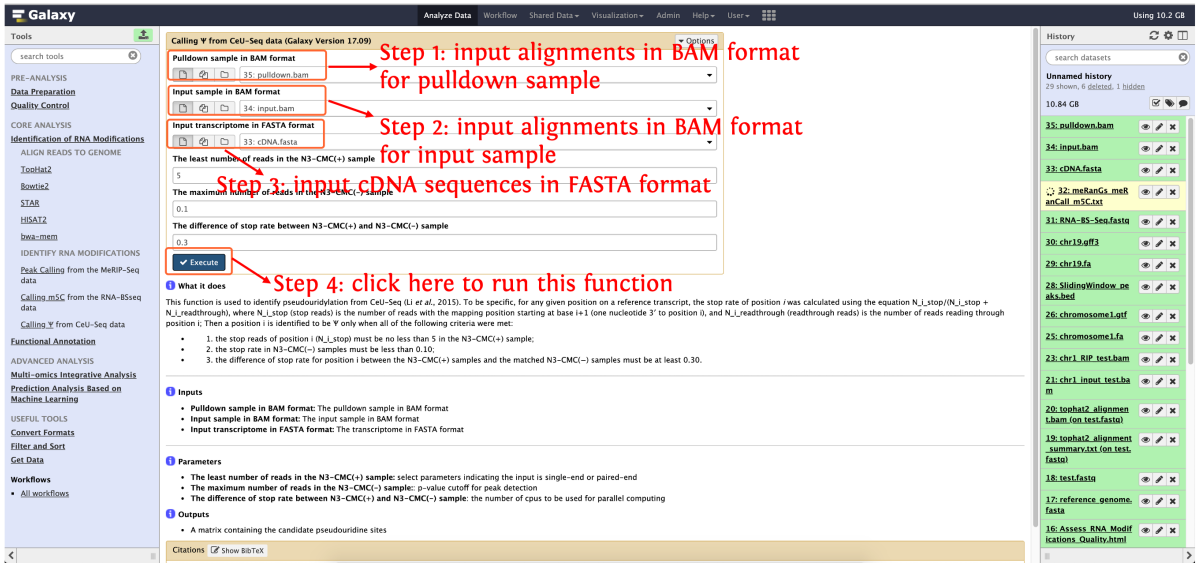
- **Pulldown sample in BAM format:** The pulldown sample in BAM format
- **Input sample in BAM format:** The input sample in BAM format
- **Input transcriptome in FASTA format:** The transcriptome in FASTA format

Output

- A matrix containing the candidate pseudouridine sites

How to use this function

- **Step 1**: upload the data in directory `test_data/Identification_of_RNA_Modifications/Calling pseudouridylation from CeU-Seq/` to history panel, if you are not clear about how to upload local data to deepEA server, please see here for details
- **Step 2**: see the following screenshot to run this function


Galaxy
Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Using 10.2 GB
Tools
search tools
PRE-ANALYSIS
Data Preparation
Quality Control
CORE ANALYSIS
Identification of RNA Modifications
ALIGN READS TO GENOME
TopHat2
Bowtie2
STAR
HISAT2
bwa-mem
IDENTIFY RNA MODIFICATIONS
Peak Calling from the MeRIP-Seq data
Calling m5C from the RNA-BSseq data
Calling Ψ from CeU-Seq data
Functional Annotation
ADVANCED ANALYSIS
Multi-omics Integrative Analysis
Prediction Analysis Based on Machine Learning
USEFUL TOOLS
Convert Formats
Filter and Sort
Get Data
Workflows
All workflows
Calling Ψ from CeU-Seq data (Galaxy Version 17.09)
Options
Pulldown sample in BAM format
35: pulldown.bam
Input sample in BAM format
34: input.bam
Input transcriptome in FASTA format
33: cDNA.fasta
The least number of reads in the N3-CMC(+) sample
5
The maximum number of reads in the N3-CMC(-) sample
0.1
The difference of stop rate between N3-CMC(+) and N3-CMC(-) sample
0.3
Execute
Step 1: input alignments in BAM format for pulldown sample
Step 2: input alignments in BAM format for input sample
Step 3: input cDNA sequences in FASTA format
Step 4: click here to run this function
What it does
This function is used to identify pseudouridylation from CeU-Seq (Li et al., 2015). To be specific, for any given position on a reference transcript, the stop rate of position i was calculated using the equation N_i_stop/(N_i_stop + N_i_readthrough), where N_i_stop (stop reads) is the number of reads with the mapping position starting at base i+1 (one nucleotide 3′ to position i), and N_i_readthrough (readthrough reads) is the number of reads reading through position i; Then a position i is identified to be Ψ only when all of the following criteria were met:
1. the stop reads of position i (N_i_stop) must be no less than 5 in the N3-CMC(+) sample;
2. the stop rate in N3-CMC(−) samples must be less than 0.10;
3. the difference of stop rate for position i between the N3-CMC(+) samples and the matched N3-CMC(−) samples must be at least 0.30.
Inputs
Pulldown sample in BAM format: The pulldown sample in BAM format
Input sample in BAM format: The input sample in BAM format
Input transcriptome in FASTA format: The transcriptome in FASTA format
Parameters
The least number of reads in the N3-CMC(+) sample: select parameters indicating the input is single-end or paired-end
The maximum number of reads in the N3-CMC(−) sample:: p-value cutoff for peak detection
The difference of stop rate between N3-CMC(+) and N3-CMC(-) sample: the number of cpus to be used for parallel computing
Outputs
A matrix containing the candidate pseudouridine sites
Citations
Show BibTeX
History
search datasets
Unnamed history
29 shown, 6 deleted, 1 hidden
10.84 GB
35: pulldown.bam
34: input.bam
33: cDNA.fasta
32: meRanGs_meRanCall_m5C.txt
31: RNA-BS-Seq.fastq
30: chr19.gff3
29: chr19.fa
28: SlidingWindow_peaks.bed
26: chromosome1.gtf
25: chromosome1.fa
23: chr1_RIP_test.bam
21: chr1_input_test.bam
20: tophat2_alignment.bam (on test.fastq)
19: tophat2_alignment_summary.txt (on test.fastq)
18: test.fastq
17: reference_genome.fasta
16: Assess_RNA_Modifications_Quality.html