mlPEA User Manual

(version 1.0)

- mlPEA is a user-friendly and multi-functionality platform specifically tailored to the needs of streamlined processing of m^{6}A-Seq data in a reference genome-free manner. By taking advantage of machine learning (ML) algorithms, mlPEA enhanced the m^{6}A-Seq data analysis by constructing robust computational models for identifying high-quality transcripts and high-confidence m^{6}A-modified regions.
- mlPEA comprises four functional modules: **Data Preprocessing, Transcriptome Construction, m^{6}A Calling, and Functional Exploration**.
- mlPEA was powered with an advanced packaging technology, which enables compatibility and portability.
- mlPEA project is hosted on https://github.com/cma2015/mlPEA
- mlPEA Docker image is available at https://hub.docker.com/r/malab/mlpea

Functional Exploration

This module provided five functions to perform functional exploration of m^{6}A-Seq data

Functions	Description	Input	Output	Time (test data)	Reference
ML-based Transcript Annotation	Predict the coding region of assembled transcripts	Assembled transcripts in FASTA format	Prediction scores of translation initiation and termination sites of assembled transcripts in txt format	~3 min	In-house scripts
Function Annotation	Annotate the functions of coding transcripts	RNA modifications in BED format and assembled transcripts in FASTA format	Functions corresponding to transcripts in txt format	~3 min	
Differential Methylation	Identify differential methylation modifications under multiple conditions	Assembled transcripts in FASTA format and alignment file in bam format	RNA differential modifications in BED format	~5 min	In-house scripts
Enrichment Analysis	Perform GO or KEGG enrichment analysis for any species	Transcript list and function annotation results	The enriched GO/KEGG terms	~3 min	
Motif Discovery	Integrate MEME-ChIP and HOMER to performed *de novo* motif discovery	RNA modifications in BED format and assembled transcriptome sequences in FASTA format	Discovered motifs in HTML format	~1 min	

ML-based Transcript Annotation

In this function, we utilized **TranslationAI**, a deep neural network to directly predict and analyze translation initiation (TIS) and termination sites (TSS) from transcripts.

Input

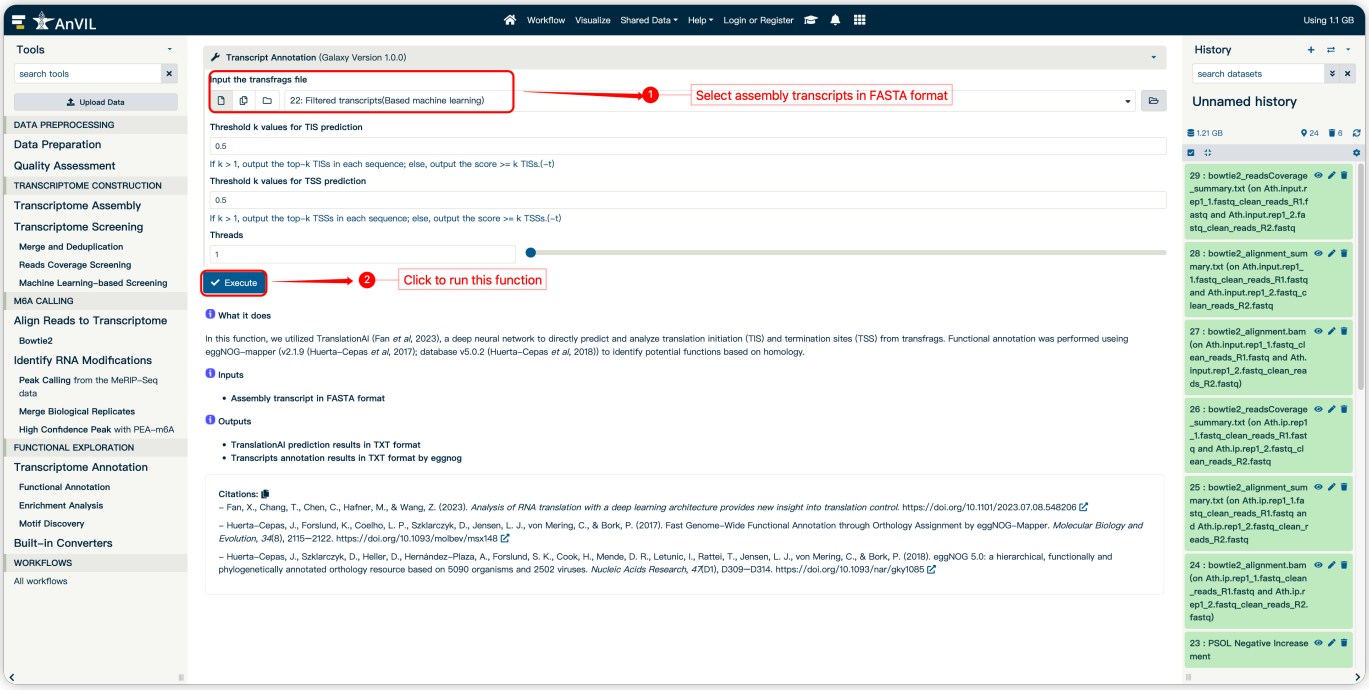
- **Assembly transcript in FASTA format**

Output

- **TranslationAI prediction results in TXT format**
- **Transcripts annotation results in TXT format**

How to use this function

- The following screenshot shows us how to use this function.



Function Annotation

In this function, functional annotation was performed useing eggNOG-mapper (database v5.0.2) to identify potential functions based on homology.

Input

- **Assembled transcript in FASTA format**

Output

- **Assembled transcripts function annotation results in TXT format by eggNOG**

How to use this function

- The following screenshot shows us how to use this function.

Differential Methylation

In this function, for pair-wised m^6A-Seq data, mIPEA can identify differentially methylated regions (DMRs) using **QNB**, with the negative-binomial distribution model to capture the within-group variability of m^6A methylation level across all samples.

Input

- **Assembled transcript in FASTA format**
- **Alignment reaults in BAM format**

Output

- **The differential peak region matrix in TXT format**

How to use this function

- The following screenshot shows us how to use this function.

Enrichment Analysis

In this function, for a set of m^6A-modified transcripts of interest, Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) enrichment analysis is performed utilizing the R package **clusterProfiler**.

Input

- **Assembled transcripts function annotation results in TXT format by eggNOG**
- **RNA modifications transcript list**

Output

- The enriched GO/KEGG terms
- A PDF focument of top enriched GO/KEGG terms

How to use this function

- The following screenshot shows us how to use this function.

Motif Discovery

This function integrates MEME-ChIP and HOMER to perform *de novo* motif discovery.

Input

- **RNA modifications regions in BED format**

- **Assembled transcript in FASTA format**

Output

- An HTML report generated by MEME-ChIP or HOMER

How to use this function

- The following screenshot shows us how to use this function.