PanGraphRNA User Manual

(version 1.0)

- PanGraphRNA is an efficient, flexible and web-based Galaxy platform that can be easily used to construct graph pangenomes from genetic variations at individual, subpopulation, and population levels. It can assist researchers to select appropriate graph pangenomes using various performance metrics for both real and simulation experiments.
- Currently, PanGraphRNA is composed of four functional modules: **Graph Pangenome Preparation Module, Construction Module, Evaluation Module, and Application Moudule**.
- PanGraphRNA was powered with an advanced packaging technology, which enables compatibility and portability.
- PanGraphRNA project is hosted on https://github.com/cma2015/PanGraphRNA
- PanGraphRNA docker image is available at https://hub.docker.com/r/malab/pangraphrna

Graph Pangenome Evaluation Module

This module assesses the performance of graph pangenomes against the SLR strategy with six read mapping-relevant measurements: *Unique alignment rate*, *Multiple alignment rate*, *Error mapping rate*, *F1 score*, *Recall* and *Precision*.

Tools	Description	Input	Output	Time (test data)	Reference
Basic Alignment Statistics	Obtain the bascic alignment information among multiple read-genome alignment results	HISAT2 alignment report in TXT format and graph pangenome files	Basic alignment statistics matrix in TXT format	~1 mins	/

Tools	Description	Input	Output	Time (test data)	Reference
Mapping Error Measurement	Perform RNA-seq reads simulation and measure mapping errors	Ground truth files and graph pangenome files	Measurement of mapping errors in CSV format	~10 mins	Flux and HISAT2
F1 Score Calculation	Perform RNA-seq reads simulation and calculate F1 score	Graph pangenome files	Calculation of F1 score in CSV format	~10 mins	Flux and HISAT2

Basic Alignment Statistics

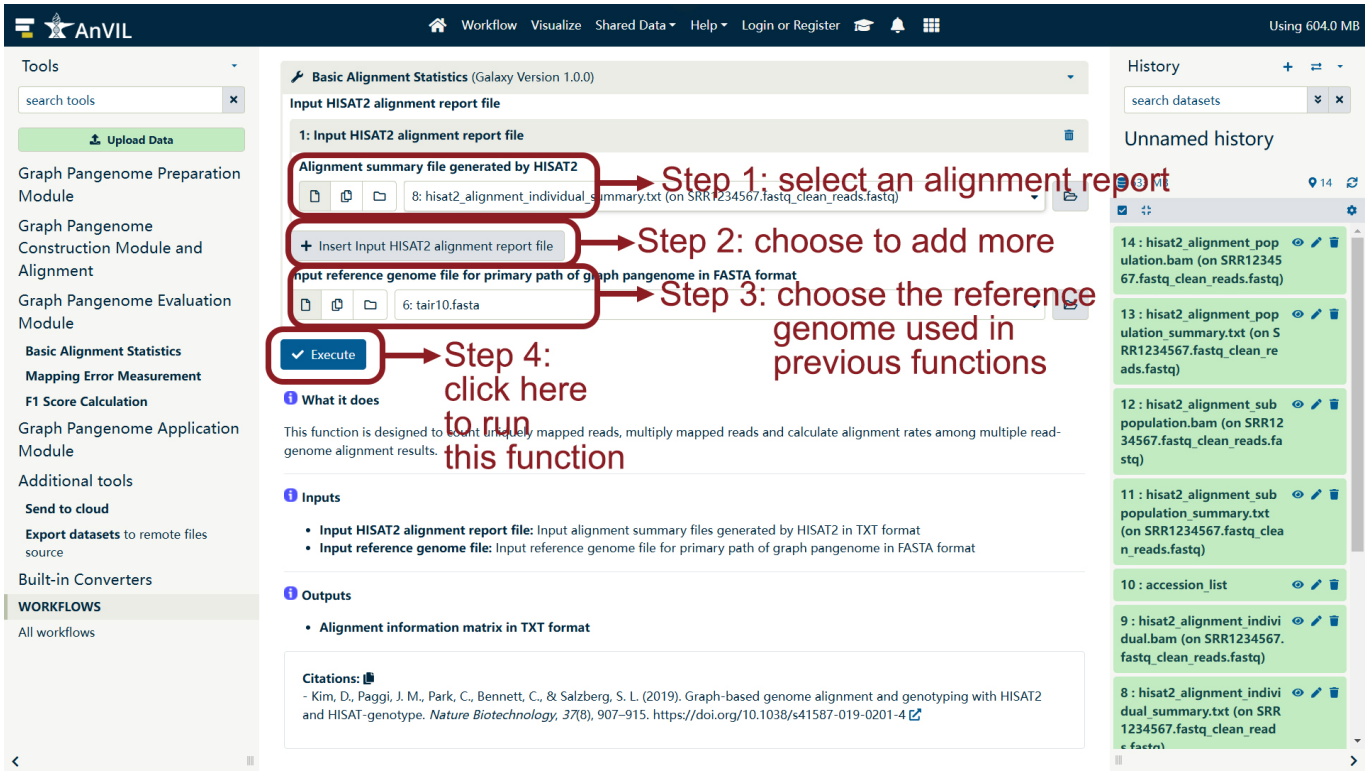
This function is designed to count uniquely mapped reads, multiply mapped reads and calculate alignment rates among multiple read-genome alignment results.

Input

- **Input HISAT2 alignment report file:** Input alignment summary files generated by HISAT2 in TXT format
- **Input reference genome file:** Input reference genome file for primary path of graph pangenome in FASTA format

Output

- **Alignment information matrix in TXT format**



Mapping Error Measurement

Mapping error measurement is designed to identify incorrectly mapped reads and record their error types in RNA-seq read-genome alignments. This function also enables **Flux** features (Griebel, T., *et al.*, 2012) for generating simulated reads and performs read-genome alignment using **HISAT2** (Kim, D., *et al.*, 2019). For more details, see https://bio.tools/the_flux_simulator and https://daehwankimlab.github.io/hisat2/manual for details.

Measuring mapping errors

The alignment results for different reference genomes were compared with the ground truth, and the five error types were defined according to the read alignment type and position.

- Five **mapping error** types:

Mapping error type	Groundtruth alignment result (read type)	Alignment result (read type)
Mapped to UnMapped	Mapped read	Unmapped read
UnMapped to Mapped	Unmapped read	Mapped read

Mapping error type	Groundtruth alignment result (read type)	Alignment result (read type)
Unique to MultiMapper	Uniquely mapped read	Multiply mapped read
MultiMapper to Unique	Multiply mapped read	Uniquely mapped read
Different Mapping Loci	Mapped to A region	Mapped to B region

Input

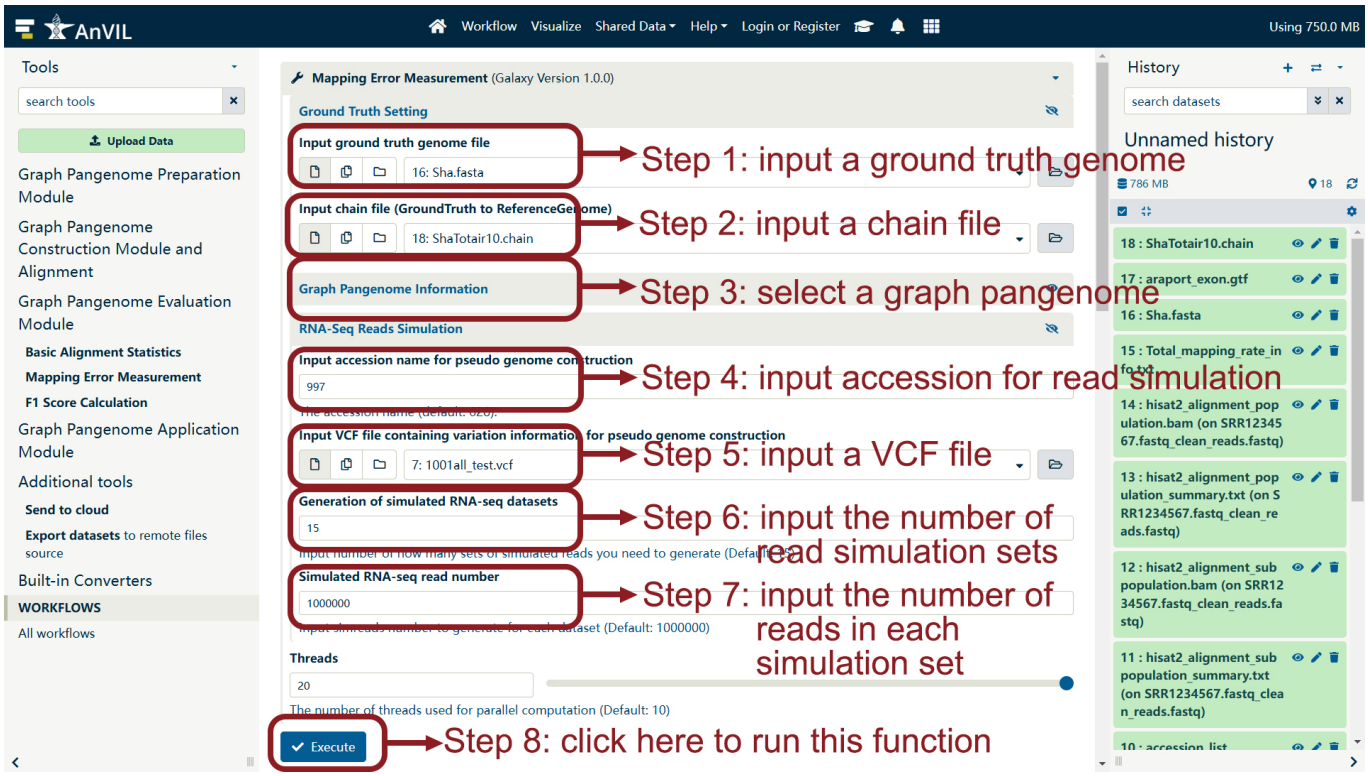
- **Ground Truth Setting**
- **Input ground truth genome file:** Input ground truth reference genome in FASTA format
- **Input chain file:** Input chain file of ground truth genome (GroundTruth to ReferenceGenome) in CHAIN format
- **Graph Pangenome Information**
- **Input reference genome file:** Input reference genome file that has been used for graph pangenome construction in FASTA format
- **Input VCF file:** Input vcf file that has been used for graph pangenome construction in VCF format
- **Input exon annotation file:** Input exon annotation file (GTF file) of reference genome file used for constructing the primary path of graph pangenome in GTF format
- **RNA-Seq Reads Simulation**
- **Input VCF file:** Input VCF file containing variation information for pseudo genome construction in VCF format

Parameters

- **Generation of simulated RNA-seq datasets:** Input number of how many sets of simulated reads you need to generate (Default: 15)
- **Simulated RNA-seq read number:** Input simreads number to generate for each dataset (Default: 1000000)
- **Threads:** The number of threads used for parallel computation (Default: 10)

Output

- **Measurement of mapping errors in CSV format**



F1 Score Calculation

Mapping error measurement is designed to calculate **Recall**, **Precision** and **F1 score** based on RNA-seq read-genome alignments. This function also enables **Flux** features (Griebel, T., *et al.*, 2012) for generating simulated reads and performs read-genome alignment using **HISAT2** (Kim, D., *et al.*, 2019). For more details,
see https://bio.tools/the_flux_simulator and https://daehwankimlab.github.io/hisat2/manual for details.

Measuring F1 score

- **Recall** = 100 X *Num.correct* / *Num.reads*
- **Precision** = 100 X *Num.correct* / *Num.unique*
- **F1 score** = (2 X *Recall* X *Precision*) / (*Recall* + *Precision*)
- *Num.reads* represent the total number of reads in the simulated RNA-seq data; *Num.correct* represent the number of correctly aligned reads, matching the coordinates of the simulated read generation position; and *Num.unique* represent the number of uniquely mapped reads, with the uniquely mapped tag in the HISAT2 alignment result being "NH:i:1".

Input

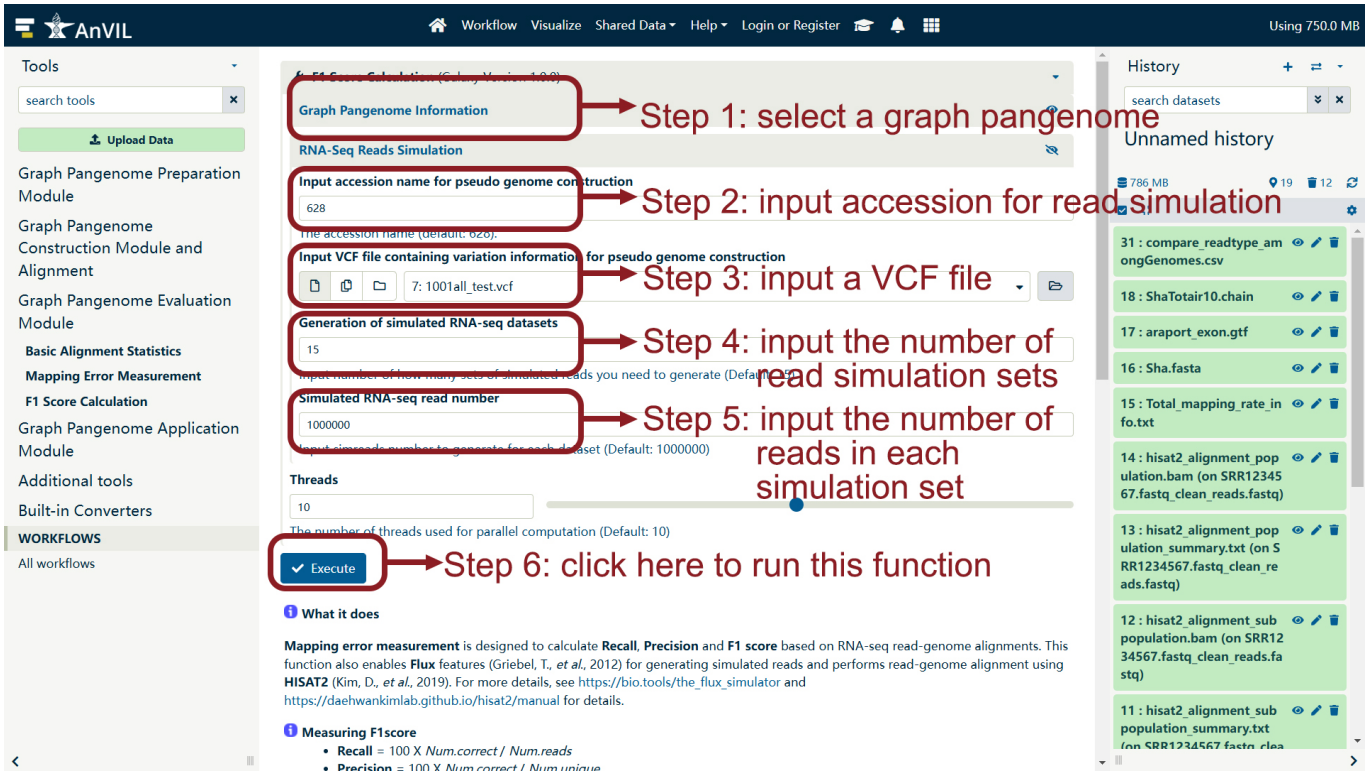
- **Graph Pangenome Information**
- **Input FASTA files:** Input the reference genome file that have been used for graph pangenome construction in FASTA format
- **Input VCF files:** Input the vcf file that have been used for graph pangenome construction in VCF format
- **Input GTF files:** Input the exon annotion file of reference genome that have been used for graph pangenome construction in GTF format
- **RNA-Seq Reads Simulation**
- **Input VCF files:** Input the vcf file containing the accession name for pseudo genome construction in VCF format

Parameters

- **Generation of simulated RNA-seq datasets:** Input number of how many sets of simulated reads you need to generate (Default: 15)
- **Simulated RNA-seq read number:** Input simreads number to generate for each dataset (Default: 1000000)
- **Threads:** The number of threads used for parallel computation (Default: 10)

Output

- **Calculation of F1 score in CSV format**


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 750.0 MB
Tools
search tools
Upload Data
Graph Pangenome Preparation Module
Graph Pangenome Construction Module and Alignment
Graph Pangenome Evaluation Module
Basic Alignment Statistics
Mapping Error Measurement
F1 Score Calculation
Graph Pangenome Application Module
Additional tools
Built-in Converters
WORKFLOWS
All workflows
Graph Pangenome Information
RNA-Seq Reads Simulation
Input accession name for pseudo genome construction
628
Input VCF file containing variation information for pseudo genome construction
7: 1001all_test.vcf
Generation of simulated RNA-seq datasets
15
Simulated RNA-seq read number
1000000
Threads
10
The number of threads used for parallel computation (Default: 10)
Execute
Step 1: select a graph pangenome
Step 2: input accession for read simulation
Step 3: input a VCF file
Step 4: input the number of read simulation sets
Step 5: input the number of reads in each simulation set
Step 6: click here to run this function
What it does
Mapping error measurement is designed to calculate Recall, Precision and F1 score based on RNA-seq read-genome alignments. This function also enables Flux features (Griebel, T., et al., 2012) for generating simulated reads and performs read-genome alignment using HISAT2 (Kim, D., et al., 2019). For more details, see https://bio.tools/the_flux_simulator and https://daehwankimlab.github.io/hisat2/manual for details.
Measuring F1score
Recall = 100 X Num.correct / Num.reads
History
search datasets
Unnamed history
786 MB
19
12
31 : compare_readtype_amongGenomes.csv
18 : ShaTotair10.chain
17 : araport_exon.gtf
16 : Sha.fasta
15 : Total_mapping_rate_info.txt
14 : hisat2_alignment_population.bam (on SRR1234567.fastq_clean_reads.fastq)
13 : hisat2_alignment_population_summary.txt (on SRR1234567.fastq_clean_reads.fastq)
12 : hisat2_alignment_subpopulation.bam (on SRR1234567.fastq_clean_reads.fastq)
11 : hisat2_alignment_subpopulation_summary.txt