Pipelines

1 Pipeline for genome decontamination (DeCon)

1.1 Introduction

Pipeline DeCon is designed to retrieve genomic sequences of target **phylum** from metagenomic assembly of paired next generation sequencing (NGS) reads. First, NGS reads are mapped to assembly by minimap2 (Li 2018), generating BAM file. Second, SprayNPray (Garber et al. 2022) is used to compute coverage, GC content and coding density of each contigs. Third, all contigs are searched against non-redundant (nr) database by DIAMOND (Buchfink et al. 2015) and assigned to phyla by MEGAN (Huson et al. 2007). Forth, contigs below 400 base pair (bp) are removed. Then a decision tree classifier is trained, taking coverage, GC content and coding density as training features and phylum assignment as target value. This classifier is used to compute phylum assignment of contigs that DIAMOND and MEGAN failed to compute assignments. Fifth, contigs assigned to the target phylum are retrieved. QUAST (Gurevich et al. 2013) and BUSCO (Simão et al. 2015) are used to evaluate retrieved genome. Distributions of contig coverage and GC content of retrieved genome are plotted.

1.2 Dependencies

Softwares

R

Python

minimap2

SAMtools

SprayNPray

DIAMOND

MEGAN (blast2rma rma2info scripts)

seqkit

QUAST

BUSCO

Databases

DIAMOND database (nr)

MEGAN database

BUSCO database

Python modules

numpy

pandas

scikit-learn

R packages

reticulate

stringr

ggplot2

ggExtra

1.3 Usage

Modify configuration file (templated as DeCon_conf.R), and run

Rscript path/DeCon_pipeline.R path/DeCon_main.R path/DeCon_main.py path/DeCon_conf.R

2 Pipeline for calling protein-coding genes from genome (ProtGeneCall)

2.1 Introduction

Pipeline ProtGeneCall is designed to call protein-coding genes from genome, combining protein-genome alignments, transcriptome-genome alignments and *ab initio* gene predictions. First, repeat elements are identified by RepeatModeler (Smit et al. 2015b) and masked by RepeatMasker (Smit et al. 2015a). Masked genome is used for downstream analysis. Second, proteins of closely related species are mapped to the masked genome by miniprot (Li 2023). Third, paired RNA-sequencing (RNA-seq) reads are mapped to masked genome by Hisat2 (Kim et al. 2019). Forth, transcriptome-genome alignments are computed by

StringTie (Pertea et al. 2015). Fifth, gene structures are predicted from transcriptome-genome alignments by TransDecoder (Haas et al. 2016), combining searching against UniRef and PfamA databases. Sixth, AUGUSTUS (Stanke et al. 2003) is trained with gene structures from TransDecoder to compute gene predictions. Seventh, BRAKER (Hoff et al. 2019) is trained with RNA-seq mapping to call genes. Eighth, GALBA (Hoff et al. 2019) is trained with proteins of closely related species. Ninth, protein-genome alignments from miniprot, transcript-genome alignments from Hisat2-StringTie, and *ab initio* gene predictions from TransDecoder, AUGUSTUS, BRAKER and GALBA are integrated into consensus gene structures by EvidenceModeler (Haas et al. 2008). Tenth, genes supported by only one *ab initio* predictor and lack protein/RNA-seq evidence are removed. Eleventh, PASA (Haas et al. 2008) is run twice to update filtered gene structures from EvidenceModeler. Twelfth, genes with in-frame stop codons are removed and the predicted peptide set is evaluated by BUSCO (Simão et al. 2015).

2.2 Dependencies

Softwares

R

Python

RepeatModeler

RepeatMasker

miniprot

Hisat2

SAMtools

StringTie

TransDecoder

HMMER

DIAMOND

AGAT

AUGUSTUS

BLAST+

GALBA

BRAKER

EvidenceModeler

BUSCO

gffread

seqkit

MAKER

Databases

DIAMOND database (UniRef)

Pfam-A

External scrpts

cufflinks_gtf_to_alignment_gff3.pl from EvidenceModeler

augustus_GFF3_to_EVM_GFF3.pl from EvidenceModeler

gth2gtf.pl from AUGUSTUS

computeFlankingRegion.pl from AUGUSTUS

gff2gbSmallDNA.pl from AUGUSTUS

gtf2aa.pl from AUGUSTUS

simplifyFastaHeaders.pl from AUGUSTUS

aa2nonred.pl from AUGUSTUS

filterGenesIn.pl from AUGUSTUS

autoAug.pl from AUGUSTUS

evm_evidence.py in this GitHub

R packages

stringr

parallel

2.3 Usage

Modify configuration file (templated as ProtGeneCall_conf.R), and run

Rscript path/ProtGeneCall_pipeline.R path/ProtGeneCall_main.R path/ProtGeneCall_conf.R

3 Pipeline for calling repeat elements from genome (RepCall)

3.1 Introduction

Pipeline RepCall is designed to call repeat elements genes from genome. First, miniature inverted-repeat transposable elements (MITE) are called by MITE-Hunter (Han et al. 2010). Second, long terminal repeats (LTRs) are identified by incorporating LTR_FINDER_parallel (Ou et al. 2019), LTRharvest (Ellinghaus et al. 2008) and LTR_retriever (Ou et al. 2018). Third, identified MITEs and LTRs are masked by RepeatMasker (Smit et al. 2015a). Forth, RepeatModeler (Smit et al. 2015b) is used to further identify repeats in the masked genome. Fifth, the locations of MITEs, LTRs and repeats from RepeatModeler are identified by RepeatMasker and all repeats are incorporated into a consensus library.

3.2 Dependencies

Softwares

R

seqkit

MITE-Hunter

LTR_FINDER_parallel

LTRharvest

LTR_retriever

RepeatMasker

RepeatModeler

3.3 Usage

Modify configuration file (templated as RepCall_conf.R), and run

Rscript path/RepCall_pipeline.R path/RepCall_main.R path/RepCall_conf.R

4 Pipeline for calling non-coding RNA (ncRNAcall)

4.1 Introduction

Pipeline ncRNAcall is designed to call non-coding RNA (ncRNA) from genome. First, transfer RNA (tRNA) is identified by tRNAscan-SE (Lowe et al. 1997). Second, microRNA is called by miRNAture (Velandia-Huerto et al. 2021). Third, target genes of microRNA are identified by searching microRNA

against annotated three prime untranslated regions (3'UTR) by miRanda (Enright et al. 2003). Forth, Infernal (Nawrocki et al. 2013) searches against Rfam (Kalvari et al. 2021) database to call other non-coding RNA, *e.g.* ribosomal RNA (rRNA) and small nuclear RNA (snRNA). Fifth, all results are incorporated together.

4.2 Dependencies

Softwares

R

tRNAscan-SE

biocode

miRNAture

miRanda

bedtools

seqkit

Infernal

Databases

miRNAture database

Rfam database

R packages parallel

stringr

4.3 Usage

Modify configuration file (templated as ncRNAcall_conf.R), and run

Rscript path/ncRNAcall_pipeline.R path/ncRNAcall_main.R path/ncRNAcall_conf.R

References

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