Sequence analysis

orfipy: a fast and flexible tool for extracting ORFs

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Abstract

Summary: Searching for open reading frames is a routine task and a critical step prior to annotating protein coding regions in newly sequenced genomes or \textit{de novo} transcriptome assemblies. With the tremendous increase in genomic and transcriptomic data, faster tools are needed to handle large input datasets. These tools should be versatile enough to fine-tune search criteria and allow efficient downstream analysis. Here we present a new python based tool, orfipy, which allows the user to flexibly search for open reading frames in genomic and transcriptomic sequences. The search is rapid and is fully customizable, with a choice of FASTA and BED output formats.

Availability and implementation: orfipy is implemented in python and is compatible with python v3.6 and higher. Source code: https://github.com/urmi-21/orfipy. Installation: from the source, or via PyPi (https://pypi.org/project/orfipy) or bioconda (https://anaconda.org/bioconda/orfipy).

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Open reading frames (ORFs) are sequences that have potential to be translated into proteins. They are delineated by start sites, at which translation is initiated by assembly of a ribosome complex, and stop sites, at which translation is terminated and the ribosome complex disassembles (Sieber et al., 2018).

Accurate annotation of the protein coding regions in sequenced genomes remains a challenging task in bioinformatics. For simpler prokaryotic genomes, ORFs correspond to the potential coding sequences (CDS) (Sieber et al., 2018). In eukaryotes, where gene splicing is prevalent, eukaryotic CDS prediction is a much more challenging task (Seetharam et al., 2019; Sieber et al., 2018).

Transcriptomic data is critical in addressing this challenge, where presence of an ORF in a mature transcript may indicate a potential protein coding gene (Mahmood et al., 2020; Martinez et al., 2020; Seetharam et al., 2019). These data are key to identifying potential orphan genes (Seetharam et al., 2019), young genes unique to a species (Singh and Wurtele, 2020; Tautz and Domazet-Lošo, 2011; Vakirlis et al., 2020); standard \textit{ab initio} gene-prediction models are trained on canonical gene features and do not work well for identifying orphan genes, which are often sparse in canonical gene features (Heames et al., 2020; Ruiz-Orera et al., 2013; Seetharam et al., 2019).

Depending on data (genomic, transcriptomic or metagenomic) and researcher interest, the computational problem of ORF prediction may be stated in multiple ways (Sieber et al., 2018), yet existing tools lack the flexibility to allow users to fine-tune or customize the search for ORF sequences. Here we present orfipy, an efficient tool for extracting ORFs from nucleotide sequences. orfipy provides rapid, flexible searches in multiple output formats to allow easy downstream analysis of ORFs.

2 Implementation

orfipy is written in python, with the core ORF search algorithm implemented in cython to achieve faster execution times. orfipy uses the pyfastx library (Du et al., 2020) for efficient parsing of input FASTA/FASTQ file. orfipy can leverage multiple cpu-cores to process FASTA sequences in parallel, based on available memory and cpu cores (Supplementary Data).

2.1 Input, flexible search and output

orfipy takes nucleotide sequences in a multi-FASTA/FASTQ, plain or gz-compressed, file as input. Users can provide input parameters that include minimum and maximum size of ORFs, list of start and stop codons and/or a user-defined codon table (Supplementary Data). For efficient and flexible downstream analysis (Fig. 1A, B), orfipy provides multiple output types including BED format. BED files reduce disk space use by storing only the coordinates of the ORFs, and are useful in developing more scalable, flexible downstream analysis pipelines. orfipy also adds relevant information about codon use and ORF types, and can group the output by
longest ORF contained in each transcript, or can list each reading frame in each transcript.

orfipy enables researchers to fully fine-tune ORF searches using a variety of options (Fig. 1A). For example, users can limit ORF searching to a specific start codon or choose to output ORFs without an inframe start codon. orfipy labels each ORF for users to easily comprehend results (Supplementary Data).

2.2 Comparison with existing tools
We compared orfipy with two popular ORF searching tools, getorf (Rice et al., 2000) and OrfM (Woodcroft et al., 2016). What sets orfipy apart is its flexibility and the options to fine-tune ORF searches and output (Fig. 1A, B). Runtimes (Fig. 1C, D) depend on software, environment, input (FASTA input is shown) and output-type. In all scenarios except using a PC to analyze the A. thaliana genome, orfipy is much faster than getorf, and comparable to OrfM, with OrfM being faster for FASTQ input (Supplementary Data).

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