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**INTRODUCTION**

Recent technological advancements in single-cell genomics have encouraged the classification and functional assessment of microorganisms from a wide span of the biosphere’s phylogeny. Environmental processes of interest to the DOE, such as bioremediation and carbon cycling, can be elucidated through the genomic lens of these unculturable microbes. However, contamination can occur at various stages of the single-cell sequencing process. Contaminated data can lead to wasted time and effort on meaningless analyses, inaccurate or erroneous conclusions, and pollution of public databases.

A fully automated decontamination tool is necessary to prevent these instances and increase the throughput of the single-cell sequencing process.

**BACKGROUND**

Screening single-cell datasets for contaminants is currently a very manually-intensive procedure. The processing time for one highly-trained scientist to decontaminate one single cell dataset is several hours. The manual single cell decontamination procedure contains both homology and feature-based screening procedures, the consensus of which can be used to classify a sequence as contaminated or clean. The process includes blasting ribosomal RNA sequences and protein coding genes, as well as visual analysis of k-mer frequency plots and GC content. These tools are available through the IMG website.

An existing software tool, DeconSeq3, automatically removes sequence contaminants. However, not only do all sources of contamination need to be known before runtime, the databases for the contaminants need to be selected as input to DeconSeq.

The Single Cell Data Decontamination Pipeline is a fully-automated software tool which classifies unscreened contigs from single cell datasets through a combination of homology and feature-based methodologies using the organism’s nucleotide sequences and known NCBI taxonomy. The software is freely available to download and install, and can be run on any system.

**RESULTS**

Results of automated screening of single cell datasets with the Single Cell Data Decontamination Pipeline vary depending on whether the known NCBI taxonomy can be used to classify contigs with blast homology bining. The pipeline was calibrated for a large specificity rate in order to produce a very clean dataset.

Most of the Endophyte datasets have known taxonomy deeper than domain, and thus are screened using both the blast homology and 5-mer binning tools. The MDM datasets have no known taxonomy deeper than domain, thus only 5-mer binning is used to classify the sequences.

The average complete running time for the pipeline is 12 minutes per 1.5 megabase of sequence data, using 16 cores.

**REFERENCES**


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