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Authors
Ciobanu, Doina
Clum, Alicia
Singh, Vasanth
et al.

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Doina Ciobanu\(^{1\ast}\), Alicia Clum\(^1\), Vasanth Singan\(^1\), Asaf Salamov\(^1\), James Han\(^1\), Alex Copeland\(^1\), Igor Grigoriev\(^1\), Timothy James\(^2\), Steven Singer\(^3\), Tanja Woyke\(^1\), Rex Malmstrom\(^1\), and Jan-Fang Cheng\(^1\)

\(^1\)DOE Joint Genome Institute, Walnut Creek, California
\(^2\)University of Michigan, Ann Arbor, Michigan
\(^3\)DOE JointBioEnergy Institute, Emeryville, California

\(*\)Email Address: dgiobanu@lbl.gov

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Single Cell Genomics and Transcriptomics for Unicellular Eukaryotes

Doina Ciobanu1*(dcioiubanu@lbl.gov), Alicia Clum1, Vasanth Singan1, Asaf Salamov1, James Han1, Alex Copeland1, Igor Grigoriev1, Timothy James2, Steven Singer3, Tanja Woyke1, Rex Malmstrom4, and Jan-Fang Cheng1
1DOE Joint Genome Institute, Walnut Creek, California; 2University of Michigan, Ann Arbor, Michigan; 3DOE Joint BioEnergy Institute, Emeryville, California

Unicellular eukaryotes have complex genomes with a high degree of plasticity that allow them to adapt quickly to environmental changes. Their genome organization and eukaryotic transcriptional mechanisms are frequently studied by biologists and biotechnologists. The vast majority of unicellular eukaryotic microorganisms are unculturable or uncultivated, and thus not sequenced so far. To this day their contribution to the dynamics of the environmental communities remains to be understood. Here, we present four components of our approach to isolate, sequence and analyze eukaryotic microorganisms: target isolation and genome transcription recovery for sequencing; sequence analysis for single cell and genome transcriptomics, and genome annotation. We have tested some of our tools and some are being still tested, using six species: an unbanded protist from a cold enriched core identified as Platophrya, a close relative of Plulgus; the fungus Metschnikowia bicuspidata, a parasite of water flea Daphnia; the mycoparasitic fungi Piptocephalis cylindrospora, a parasite of Cereosmyces and Mucor; Caulochytrium protistosphaeria, a parasite of Sordaria; Rozella allomycis, a parasite of the water mild Allozyme; and the microalgae Chlamydomonas reinhardtii.

Single Cell Isolation and Critical Steps

Several lysis methods has been tested for single cell transcriptions. Selection criteria were: compatibility with high-throughput format; compatibility with the downstream process and chemistry; transcriptome recovery; time; cost; purity of the extracts. Commercial kits, virus direct lysis and UIC-based lysis were tested on single cells.

Single Cell Transcriptomics Method Development Critical Steps

1. Several lysis methods has been tested for single cell transcriptomics. Selection criteria were: compatibility with high-throughput format; compatibility with the downstream process and chemistry; transcriptome recovery; time; cost; purity of the extracts. Commercial kits, virus direct lysis and UIC-based lysis were tested on single cells.
2. Eight Reverse Transcription Methods were tested on purified total RNA in amounts equivalent to 1000, 100 and 1 ng of RNA. These methods tested were: Superscript III, Superscript II, Superscript I, and Single Cell RT. Primerscript with gDNA eraser (D), for all following manufacturer protocol: Superscript II and Superscript III with essential chemistry modifications (E); B1 and B1i respectively; SmartScript II (Nature Methods,VoI 10:1016-1098 (4)); SmartScript II modified protocol and components (H).
3. Reverse Transcript Quality Check was done using six C. reinhardtii 44++ genes, shown to have a high correlation with rRNAseq transcriptome analysis (Cell,VoI 216-1855 May 2012).
4. Second Strand Synthesis was performed differently for different RT methods. Efficiency was estimated in preliminary tests, not shown here.
5. Amplification of the cDNA was tested by T1-ITV, PCR or MDA. First method was dropped from further experiments due to much higher costs, however, it did show a higher efficiency than PCR or MDA.
6. For the library construction these methods are being tested: Illumina Fragment 50pb, Mondrian (Illumina SP) or ultra low input and Nexera XP for low input.

Single Cell Eukaryote Sequencing at JGI

Genome Assembly

Co-Assembly Strategy Comparison for Compost Prostot on Normalized Data

Several assembly strategies were tested using raw and normalized data for single cells and co-assembly. The current assembly strategy for these projects is to use SPAdes without normalization. This is the same approach that is used now on microbial single cell projects at JGI.

Prokaryote Analysis

Annotation pipeline was tested for each species with length > 500bp. For gene annotation we used an ab initio method genewise, with parameters specifically trained for eukaryotic organisms, as well as protein-homology based methods, like genewise and性nes, and using alternative genetic code 6.

Fungal Analysis: For Piptocephalis was used RNA-RNA annotation pipeline on a combined assembly of 3 single cells.

RESULTS: GENOME ANALYSIS

Prokaryote: Prosthutammin pluss 94,876 transcripts with at least 1 read mapped:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size MB</th>
<th>Genes</th>
<th>Annotation Genome Size MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas putida</td>
<td>974.5</td>
<td>9609</td>
<td>1,383</td>
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References: