Assembling MAGs using KBase

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ABSTRACT

This protocol describes the steps to run a KBase narrative to assemble MAGs from raw reads.

DEC 03, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x54v9pop4g3e/v1

Protocol Citation: John Beckley 2023. Assembling MAGs using KBase. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9pop4g3e/v1

Protocol status: Working
We use this protocol and it's working

Created: Dec 03, 2023

Last Modified: Dec 03, 2023

PROTOCOL integer ID:
91746

Keywords: metagenomics,
Nanopore sequencing, WGS

1 Create an acount with KBase, login, and start a new narrative.

2 Import metagenomic data as SRA reads by selecting the “Import SRA File as Reads from Web” app. Provide the SRA file URL, the sequencing technology, and an output name for the file to be generated. Run the app and locate output in My Data.

3 To assess quality of the reads, run the “Assess Read Quality with FastQC” app. Load the uploaded data from the SRA upload and run the app.

4 Review the quality scores and determine if any of the reads need to be removed for low quality scores.

5 To trim reads of adapters and to remove low quality reads, load the data into the “Trim Reads with Trimmomatic” app. There are several parameters to adjust, but these can be left at the default unless specific criteria are needed for the analysis. Define an output file name and run the app.

6 Review the quality scores and compare to the pre-trimmed quality report.

7 Prior to assembly, the reads can be used to assign taxonomy. Load the trimmed data file into the “Classify Taxonomy of Metagenomic Reads with Kaiju” app. Select the taxonomic level of classification and the database to be used and run the app.

8 Analyze the data to look at the taxonomic classifications present in the sample.

9 To run the assembly, loaded the trimmed reads into the “Assemble Reads with metaSPAdes” app. Select the minimum contig length, identify an output object, and run the app.

10 Bin the contigs by loading the assembled read object into the “Bin Contigs using MaxBin2” app. Choose an output object and run the app.

11 10. Load the result of the binning into the “Assess Genome Quality with CheckM” and run the app.

12 11. Analyze and determine which bins are acceptable for extraction. This would be any bins with more than 75% completion and less than 5% contamination.

13 12. Extract the selected genomes using the “Extract Bins as Assemblies from BinnedContigs”.

14 13. Metagenome assembled genomes are now ready to be annotated and further analyzed in KBase, or exported for analysis by other methods.