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16S Data Analysis Protocol

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

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Abstract

Learn how to upload sequence data into BV-BRC and analyze the results.

16S Barcoding, Library Prep, Flow Cell Priming

1 To begin 16S barcoding, library preparation, and Nanopore flow cell priming for sequencing results, please follow the steps **described here.**

1.1 The protocol was created by Emily Gailey as apart of the NCSU Biotechnology Program and all credits go to her.

BV-BRC File Preparation & Upload

2 Upon reaching this step, 16S samples should be barcoded, pooled together to achieve a concentration of 50-100 ng, and injected into a PromethION flow cell for sequencing.

3 When the sequence is complete, raw sequence reads will be available to download within a results folder.

4 Select the "fastq_pass" folder to access successfully run barcodes.

5 Based on your specific analysis goal, select and download your files of interest (Our group selected 6 total barcodes for investigation: #1, #3, #5, #7, #9, & #11).

6 Download the fastq.gz files. Then, for each barcode, you will want to concatenate the sequences. Read this tutorial to learn how to concatenate files. **Concatenate Sequence Files**

7 Once the desired files are concatenated, visit **bv-brc.org** and ensure you have an account created.

8 Once logged into BV-BRC, select "upload" on the bottom right corner of the screen and select your newly concatenated files. It is important to change the "Upload type" to "Reads" prior to starting the upload.

9 After the files are successfully uploaded, under “Tools & Services” at the top toolbar on the BV-BRC homepage select “Taxonomic Classification”.

10 Under “Single Read Library” select the barcode file you wish to analyze.

11 Click the arrow to move this file into the “Selected Libraries” box.

12 Add all barcode files to the same job.

13 Adjust the sequencing type to “16S Ribosomal RNA”.

14 Select your output folder and output name. Then, click "Submit"!

15 Monitor the job progress under “Workspaces > My Jobs”. Five barcodes will likely take ~15 minutes to complete.

Analyze BV-BRC Results

16 Open the ”My jobs” page. After the job has been completed select the job and then click “View” on the green sidebar.

17 Explore the outputs of each individual barcode as well as the alpha and beta diversity comparison files.

18 Analysis through the "Sankey" output files will show a phylogenetic tree-style output of the microbial families present in a give barcode/sample. This output allows for analysis of bacteria present, and how closely related certain strains are to one another.

19 Analysis using the "Krona" output files gives a pie chart-style report of total diversity present in the barcode/sample. This tool allows for specific percentages of abundance for a given strain of microbe.

20 We encourage others to explore the results and outputs of BV-BRC taxonomic runs. Analyze what is present and draw scientific conclusions.

21 You have successfully generated 16S barcoded libraries from samples, inserted it into a Nanopore flow cell for sequencing, concatenated files given from our sequencing report, uploaded them into BV-BRC, and observed trends to draw conclusions!

22 Congratulations! You are now a metagenomic scientist!

Protocol references

Emily Gailey: ONT 16S Barcoding & Flow Cell Priming Protocol: https://www.protocols.io/view/ont-16s-barcoding-amp-flow-cell-priming-q26g7pd11gwz/v1