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Nephele DADA2 Workflow

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Protocol status: Working

We use this protocol and it's working

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Abstract

A protocol outlining the workflow of utilizing DADA2 on Nephele with a provided practice dataset. DADA2 is a pipeline that provides species assignments using Amplicon Sequence Variants or ASVs which compare variants to a predetermined sequence through assumption that the most amplified sequence is the correct one.

Downloading the Dataset

- 1 To download the practice dataset provided by Nephele, visit:
https://nephele.niaid.nih.gov/user_guide_data/
- 2 Download the first set of sequences listed as well as the mapping file.
- 2.1 The reference provided for this set of sequences is listed as: Experimental Microbial Dysbiosis Does Not Promote Disease Progression in SIV-Infected Macaques. NCBI BioProject: [PRJNA417022](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA417022)
- 3 Once downloaded, unzip the downloaded file and ensure all .fastq.gz files are present.

Running DADA2

- 4 Click the **Home** tab.
- 5 Choose the **Analyze** option.
- 6 Under **Amplicon Metagenomics**, select "Start job" option under **DADA2**.
- 7 Select **Paired End FASTQ**, as this is a paired-end dataset.
- 8 Click "Upload from my computer" and add all .fastq.gz files from the downloaded dataset. Start upload
- 9 Select "Next" and upload the corresponding Mapping File.
- 10 After reaching the Pipeline Selection page, click **Select** and enter a description of the job.
- 11 Keep Filter and Trim, Merge Pairs, and Analysis settings defaulted. Do not check Ion Torrent Data. Click **Validate and Submit** **Note: This is for following the exact tutorial*



instructions. If you would like to make changes to these pre-sets, proceed with these changes.

- 12 Nephele will send an email upon job completion.

Analysis of the Output

- 13 From the **My Workspace** tab, hover to the view results icon under **Actions** for the completed DADA2 job.
- 14 Under **Reported Statistics, Reported Files, Reported Images, Reported Tables, and Reported Charts** and explore outputs.
- 14.1 **Reported files** of note include: **Rooted tree, Rarefaction curve, Alpha diversity, PCoA binomial, BCoA Bray-Curtis, and ASV heatmap.**
- 14.2 **Reported images** of note include: **Quality profiles** of forward and reverse reads
- 14.3 **Reported tables** of note include: **Read counts by sample** and **Top species table**
- 14.4 **Reported charts** of note include: **Reads in vs. reads out** and **Track reads**

Protocol references

<https://nephele.niaid.nih.gov/index>