LABORATORY FOR HEALTH, OMICS AND PATHWAY EXPLORATION

16S rRNA Amplicon Analysis with QIIME 2 and DADA2

A reproducible computational workflow for ASV inference, taxonomy assignment, microbiome diversity and quality review.

DOCUMENT	VERSION	ISSUED
HOPE-PR-05	1.0	09 November 2026

HOPE Lab member access copy: This analytical workflow is used in relevant laboratory research and training and is provided to help HOPE Lab students and members consult the main analysis stages. Record the exact software release and project-specific settings used.

Use and responsibility: This document is a student reference, not an institutional authorization or a universally validated SOP. It does not replace supervisor instruction, current standards, local risk assessment, institutional approval, equipment-specific validation or applicable safety procedures. Anyone outside HOPE Lab who consults or adapts this material must independently assess its suitability, obtain any required approvals, validate the method for their own setting, and assume responsibility for its application and interpretation. HOPE Lab makes no guarantee of fitness for third-party use and accepts no responsibility for external implementation or outcomes to the extent permitted by applicable law.

Digital-workflow scope: This document covers computational analysis of existing digital sequence files only. It contains no wet-lab specimen-processing, DNA-extraction, PCR or sequencing instructions.

Purpose and scope

This computational workflow processes demultiplexed 16S rRNA amplicon FASTQ files to infer amplicon sequence variants (ASVs), assign taxonomy and assess microbial community diversity. It starts from digital sequence files delivered by a sequencing provider and excludes specimen collection, DNA extraction, PCR, sequencing and organism cultivation.

Required digital inputs

- Demultiplexed single-end or paired-end FASTQ files from an approved sequencing provider.
- A sample metadata table with unique sample identifiers, study groups, sample types, batches and control status.
- Marker region, primer information, read layout, sequencing platform and provider quality report.
- A QIIME 2 installation with compatible demux, DADA2, feature-classifier, feature-table, diversity and export functionality.
- A reference database and classifier appropriate to the sequenced 16S region; record release, classifier training details and licence.

Computational workflow

1. Preserve the original FASTQ files as read-only data. Create a working copy and record file names, sizes, checksums where available, provider date, sample IDs and read layout.

2. Prepare and validate the QIIME 2 manifest. Check that sample identifiers match the metadata exactly and that paired-end files are correctly paired.

3. Import the demultiplexed reads using the manifest format that matches the sequencing quality encoding and read layout. Record the exact QIIME 2 release and import command.

4. Generate a demultiplexing summary and inspect per-base quality, read length, sample balance and read-count distribution before selecting trimming or truncation settings.

5. Select primer removal and truncation settings from the observed quality profiles and amplicon design. For paired-end analysis, verify sufficient overlap before denoising.

6. Apply the appropriate DADA2 denoising action (single-end or paired-end). Preserve the resulting feature table, representative sequences and denoising statistics.

7. Review read retention, feature counts, sample depth and controls. Investigate unusual losses or unexpected control profiles before proceeding.

8. Assign taxonomy with a classifier trained for the sequenced marker region. Record database release, classifier identity and confidence settings.

9. Perform the diversity analyses specified in the study plan. Select appropriate sampling depth and metrics; report exclusions and assumptions.

10. Export relevant tables and visualizations, retain provenance artifacts and preserve a record of software versions and parameters.

```
qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]' --input-path manifest.tsv --input-format
PairedEndFastqManifestPhred33V2 --output-path demux.qza
qiime demux summarize --i-data demux.qza --o-visualization demux.qzv
```

```
qiime dada2 denoise-paired --i-demultiplexed-seqs demux.qza --p-trim-left-f 0 --p-trim-left-r 0 --p-trunc-len-f <F_TRUNC>
--p-trunc-len-r <R_TRUNC> --o-table table.qza --o-representative-sequences rep-seqs.qza --o-denoising-stats denoising-stats.qza
```

Replace the placeholder truncation values with settings justified by the quality plots and amplicon design. Do not copy another project's values without re-evaluation.

Quality controls

Control / check	Purpose
Negative extraction or PCR controls	Review potential reagent-associated or process contamination where such controls were included in the original study.
Mock community, where available	Compare observed and expected composition to evaluate pipeline behaviour.
Sequencing-provider QC	Retain run-level quality and demultiplexing records.
Metadata integrity	Check one-to-one sample IDs, group labels, batch variables and control designation.
DADA2 denoising statistics	Review input, filtered, denoised, merged and non-chimeric read counts where applicable.

Reproducibility and reporting

Report sequencing region, primer information, read layout, quality-control decisions, QIIME 2 release, DADA2 plugin version, classifier/database release, trimming/truncation rationale, sample exclusions, diversity metrics and all transformations applied. Preserve QIIME 2 artifacts and provenance with the project records.

References and further reading

1. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019;37:852-857. doi:10.1038/s41587-019-0209-9

2. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13:581-583. doi:10.1038/nmeth.3869

3. QIIME 2 documentation: q2-dada2 plugin reference and tutorials. https://amplicon-docs.qiime2.org/en/stable/references/plugins/dada2/

4. QIIME 2 Library: q2-dada2 plugin releases and documentation. https://library.qiime2.org/plugins/qiime2/q2-dada2/overview

Document status: Version 1.0. This reference should be checked against current literature, institutional requirements and the approved local method before use.