Metatranscriptomics Analysis

Quality control and adapter trimming was performed with bcl-convert[1]. HUMAnN (HMP Unified Metabolic Analysis Network) was used to classify each read[2]. Reads were then placed into metabolic categories based on these taxonomic hits using the 'full_chocophlan.v201901_v31' and 'uniref50_annotated_v201901b' precompiled databases.

Summary data is provided for each sample within their project folders, including:

- *genefamilies.tsv*: gene family abundance data that has been normalized. This normalized abundance is scaled so that the sum of all gene family abundances for a single sample equals 1,000,000.
- *pathabundance.tsv*: this file is similar to the genefamilies.tsv file, but instead describes metabolic pathways rather than individual gene families. This normalized abundance is scaled so that the sum of all gene family abundances in a single sample equals 1,000,000.
- *taxonomy.tsv*: This file lists the microbial species (or higher taxa) detected in your sample during the initial MetaPhlAn step of HUMAnN. It provides the relative abundance of each taxon in the sample.

After initial HUMAnN analyses, gene family abundance tables (genefamilies.tsv) from individual samples were aggregated with humann_join_tables and normalized to relative abundance using humann_renorm_table (units: fractional abundance per sample). To enable pathway-level interpretation, gene families were regrouped into MetaCyc reactions via humann_regroup_table with UniRef50 mappings and renamed to human-readable identifiers using humann_rename_table.

The resulting table was used to identify the top ten most abundant metabolic reactions across all samples. These reactions were visualized as bar plots showing mean relative abundance. These images can be found within the 'Pathway Abundance - Top 10 Pathways' folder.

Tool	**Version**	**Parameters**
bcl-convert	4.2.4	default parameters
humann3	3.7	humann (default parameters) humann_join_tables (default parameters) humann_renorm_table (--units relab) humann_regroup_table (--groups uniref50_rxn) humann_rename_table (--names metacyc-rxn)

91 43rd Street
Suite 250
Pittsburgh, PA 15201
878-227-4915

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[1] bcl-convert: A proprietary Illumina software for the conversion of bcl files to basecalls.

[2] F Beghini, et al. (2021) Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife 2021;10:e65088