

# **OptiFit: a fast method for fitting amplicon sequences to existing OTUs**

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<sup>1</sup> **Abstract**

<sup>2</sup> **Importance**

## 3 Introduction

## 4 Results

- 16S rRNA gene sequence datasets from human gut, mouse gut, marine, and soil environments as well as the greengenes, silva, and RDP public databases were processed with mothur and clustered *de novo* with mothur's OptiClust algorithm.
- All clustering was performed at a distance threshold of 0.03 and repeated with 100 different random seeds.
- The Matthews Correlation Coefficient was calculated to serve as a measure of OTU quality.

### 12 Reference clustering with public databases

- Public reference databases were clustered *de novo* using OptiClust, then query datasets were fit to the *de novo* OTUs using OptiFit.
  - In open-reference mode, OTU quality was similar between fitting the datasets to reference OTUs with OptiFit and clustering the datasets *de novo* with OptiClust. This held true for all datasets and reference databases.
  - However, in closed-reference mode, OTU quality was slightly worse when fitting to greengenes and silva, and much worse when fitting to RDP as compared to OptiClust. OptiFit was able to map more query sequences to reference OTUs created with the greengenes and silva databases than with RDP.
  - In terms of runtime, closed-reference OptiFit outperformed OptiClust, while OptiClust out-performed open-reference OptiFit.

### 24 Reference clustering with split datasets

- Datasets were randomly split into a reference fraction and a query fraction. Reference sizes from 10% to 80% of the sequences were created, with the remaining sequences

used for the query. Reference sequences were clustered *de novo* with OptiClust, then query sequences were fit to the *de novo* OTUs with OptiFit.

- OTU quality from fitting split datasets was highly similar to that from *de novo* clustering the whole dataset.
- Closed-reference OptiFit with split datasets was faster than OptiClust on whole datasets.
  - \* OptiClust performed faster than open-reference OptiFit only when the OptiFit reference fraction was 30% or less.
- Different methods for selecting the sequences to be used as the reference were tested; a simple random sample, weighting sequences by relative abundance, and weighting by similarity to other sequences in the dataset.
  - \* OTU quality was similar with the simple and abundance-weighted sampling, but slightly worse with similarity-weighted sampling.
  - \* The fraction of query sequences that are able to be fit to the reference OTUs in closed-reference mode decreases as the reference fraction increases.

## Comparison to vsearch

- To compare to existing software, vsearch was used to cluster OTUs *de novo* or with reference-based clustering to the greengenes database.
  - For all datasets and clustering methods (*de novo*, open reference, and closed reference), mothur's clustering algorithms produced higher quality OTUs than vsearch.
  - When closed-reference clustering against the greengenes database, vsearch was able to map more query sequences to the reference than mothur's OptiFit algorithm.
  - In terms of runtime, OptiFit generally performed faster than vsearch when reference clustering, while vsearch *de novo* clustering outperformed OptiClust.

## 53 **Discussion**

## 54 **Materials and Methods**

### 55 **The OptiFit Algorithm**

### 56 **Analysis Workflow**

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## 61 **Author Contributions**

## 62 **References**