

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

2020-12-10

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¹ **Abstract**

² **Importance**

3 Introduction

4 Assigning amplicon sequences to Operational Taxonomic Units (OTUs) is an important
5 step in characterizing the composition of microbial communities across large datasets.
6 OptiClust, a *de novo* OTU clustering method in the mothur program, has been shown to
7 produce higher quality OTU assignments than other methods and at comparable or faster
8 speeds (1, 2). A notable difference between *de novo* clustering and database-dependent
9 methods is that OTU assignments clustered with *de novo* methods are not stable when
10 new sequences are added to a dataset (3). However, in some cases one may wish to
11 incorporate new samples into a previously clustered dataset without performing clustering
12 again on all sequences, such as when deploying a machine learning model where OTUs
13 are features (4). To provide an efficient and robust method to fit amplicon sequence data
14 to existing OTUs, we developed the OptiFit algorithm as a new component of the mothur
15 program.

- 16 • TODO: Describe OptiClust and vsearch.

17 Results

- 18 • TODO: brief description of OptiFit algorithm. the method we came up with is the
19 result. OptiClust paper had toy example to walk through the algorithm.
- 20 • TODO: Paragraph at beginning to describe datasets: why picked, how processed.

21 We used four published datasets isolated from soil (5), marine (6), mouse (7), and human
22 (8) samples.

- 23 • 16S rRNA gene sequence datasets from human gut, mouse gut, marine, and soil
24 environments as well as the greengenes, silva, and RDP public databases were
25 processed with mothur and clustered *de novo* with mothur's OptiClust algorithm.
- 26 • 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. OTU quality was evaluated using the Matthews Correlation Coefficient (MCC) with a sequence dissimilarity threshold of 0.03% as described previously (3, 9).

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.

Reference clustering with split datasets

- TODO: compare optifit performance for split dataset vs public database
- TODO: double-check with Sarah that MCCs are from full OTU dataset or just query sequence OTUs
- TODO: double-check reference fractions aren't flipped (fraction mapped plot looks weird)
- 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

- TODO: move this into first section with public database.
- vsearch is more permissive than mothur (radius vs diameter with 0.03 dissimilarity threshold).
- 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

74 vsearch.

75 – When closed-reference clustering against the greengenes database, vsearch

76 was able to map more query sequences to the reference than mothur’s OptiFit

77 algorithm.

78 – In terms of runtime, OptiFit generally performed faster than vsearch when

79 reference clustering, while vsearch *de novo* clustering outperformed OptiClust.

80 Discussion

81 Materials and Methods

82 Sequence Data Processing Steps

83 Benchmarking

84 Data and Code Availability

85 TODO: cite all these dependencies

86 We implemented the analysis workflow in Snakemake and relied on Python, R, and GNU

87 bash. Dependencies include mothur v1.45.0, vsearch v2.13.3, numpy, R Markdown, the

88 Tidyverse metapackage, and the conda environment manager. A reproducible version of

89 the workflow, manuscript, and conda environment is available at **TODO: UPDATED REPO**

90 **LINK**.

91 Acknowledgements

92 KLS received support from the NIH Training Program in Bioinformatics (T32 GM070449).

93 The funders had no role in study design, data collection and interpretation, or the decision

94 to submit the work for publication.

Author Contributions

KLS wrote the analysis code, evaluated the algorithm, and wrote the original draft of the manuscript. SLW designed and implemented the OptiFit algorithm and assisted in debugging the analysis code. MBM and GAD contributed analysis code. PDS conceived the study, supervised the project, and assisted in debugging the analysis code. All authors reviewed and edited the manuscript.

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