Sequence analysis

**HPC-CLUST:** distributed hierarchical clustering for large sets of nucleotide sequences

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ABSTRACT

Motivation: Nucleotide sequence data are being produced at an ever increasing rate. Clustering such sequences by similarity is often an essential first step in their analysis—intended to reduce redundancy, define gene families or suggest taxonomic units. Exact clustering algorithms, such as hierarchical clustering, scale relatively poorly in terms of run time and memory usage, yet they are desirable because heuristic shortcuts taken during clustering might have unintended consequences in later analysis steps.

Results: Here we present HPC-CLUST, a highly optimized software pipeline that can cluster large numbers of pre-aligned DNA sequences by running on distributed computing hardware. It allocates both memory and computing resources efficiently, and can process more than a million sequences in a few hours on a small cluster.

Availability and implementation: Source code and binaries are freely available at http://meringlab.org/software/hpc-clust/; the pipeline is implemented in C++ and uses the Message Passing Interface (MPI) standard for distributed computing.

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Supplementary Information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION

The time complexity of hierarchical clustering algorithms (HCA) is quadratic \( O(N^2) \) or even worse \( O(N^2 \log N) \), depending on the selected cluster linkage method (Day and Edelsbrunner, 1984). However, HCAs have a number of advantages that make them attractive for applications in biology: (i) they are well defined and should be reproducible across implementations, (ii) they require nothing but a pairwise distance matrix as input and (iii) they are agglomerative, meaning that sets of clusters at arbitrary similarity thresholds can be extracted quickly by post-processing, once a complete clustering run has been executed. Consequently, HCAs have been widely adopted in biology, in areas ranging from data mining to sequence analysis to evolutionary biology.

Apart from generic implementations, a number of hierarchical clustering implementations exist that focus on biological sequence data, taking advantage of the fact that distances between clustering implementations exist that focus on biological mining to sequence analysis to evolutionary biology.

Hierarchical clustering starts by considering every sequence separately and merging the two closest ones into a cluster. Then, iteratively, larger clusters are formed, by joining the closest clusters and/or clusters. The distance between two clusters with several sequences will depend on the clustering linkage chosen. In single linkage, it is the similarity between the two most similar sequences; in complete linkage, between the two most dissimilar sequences; and in average linkage, the average of all pairwise similarities. The latter method is also known as the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and is often used in the construction of phylogenetic guide trees.

In the type of approach used by CD-HIT and UCLUST, each input sequence is considered sequentially, and is either added to an existing cluster (if it is found to meet the clustering threshold) or is used as a seed to start a new cluster. Although this approach is extremely efficient, it can lead to some undesired characteristics (Sun et al., 2012): (i) it will create clusters with sequences that may be more dissimilar than the chosen clustering threshold; (ii) it can occur that a new cluster is created close to an existing cluster, but at a distance just slightly longer than the clustering threshold; at this point, any new sequences close to both clusters will be split among the two clusters, whereas previous sequences will have been added to only the first cluster; this effectively results in a reduction of the clustering threshold locally; and (iii) different sequence input orders will result in different sets of clusters due to different choices of the seed sequences. Point (i) also affects HCA using single linkage and to a lesser extent average linkage, but does not occur with complete linkage.

Here we present a distributed implementation of an HCA that can handle large numbers of sequences. It can compute single-, complete- and average-linkage clusters in a single run and produces a merge-log from which clusters can subsequently be parsed at any threshold. In contrast to CD-HIT, UCLUST and ESPRIT, which all take unaligned sequence data as their input, HPC-CLUST (like MOTHUR) takes as input a set of pre-aligned sequences. This allows for flexibility in the choice of alignment algorithm; a future version of HPC-CLUST may include the alignment step as well. For further details on implementation and algorithms, see the Supplementary Material.

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Material). Because CD-HIT and UCLUST use a different ap-
identical or nearly identical clustering results (see Supplementary

HPC-CLUST has been highly optimized for computation speed

runtime when using multiple threads. Identity threshold for clustering
was 98% identity

2 METHODS
For all benchmarks, we used one or more dedicated Dell Blade M605
compute nodes with 2 quad-core Opteron 2.33 GHz processors and 24
GB of random access memory. The most recent version of each software
pipeline was used: HPC-CLUST (v1.0.0), MOTHUR (v.1.29.2), ESPRIT
(Feb. 2011), CD-HIT (v4.6.1) and UCLUST (v6.0.307). Detailed infor-
mation on settings and parameters is available in the Supplementary
Material.

We compiled a dataset of publicly available full-length 16S bacterial
ribosomal RNA sequences from NCBI Genbank. Sequences were aligned
using INFERNAL v1.0.2 with a 16S model for bacteria from the
align package (Nawrocki et al., 2009). Importantly, INFERNAL uses a
profile alignment strategy that scales linearly O(N) with the number of
sequences, and can be trivially parallelized. Indels were removed and
sequences were trimmed between two well-conserved alignment columns,
such that all sequences had the same aligned length. The final dataset
consisted of 1 105 195 bacterial sequences (833 013 unique) of 1301 in
aligned length.

3 RESULTS
3.1 Clustering performance on a single computer
HPC-CLUST has been highly optimized for computation speed
and memory efficiency. It is by far the fastest of the exact clus-
tering implementations tested here, even when running on a
single computer (Fig. 1). Compared with MOTHUR, it produces
identical or nearly identical clustering results (see Supplementary
Material). Because CD-HIT and UCLUST use a different ap-
proach to clustering, they are not directly comparable and are
included for reference only.

In HPC-CLUST, the largest fraction of computation time is
spent calculating the pairwise sequence distances, the second lar-
gest in sorting the distances and the final clustering step is the
fastest. HPC-CLUST can make use of multithreaded execution
on multiple nodes and practically achieves optimal paralleliza-
tion in the distance calculation step. Additional benchmarks are
shown and discussed in the Supplementary Material.

3.2 Distributed clustering performance
Clustering the full dataset (833 013 unique sequences) to 97%
identity threshold required a total of 2 h and 42 min on a com-
pute cluster of 24 nodes with 8 cores each (192 total cores).
Owing to parallelization, the distance and sorting computation
took only 57 min (wall clock time), corresponding to >10000
min CPU time. The remaining 1 h and 45 min (wall clock time)
were spent collecting and clustering the distances. The combined
total memory used for the distance matrix was 59.8 or 2.6 GB per
node. The node on which the merging step was performed used a
maximum of 4.9 GB of memory when doing single-, complete-
and average-linkage clusterings in the same run

4 CONCLUSION
Clustering is often among the first steps when dealing with raw
sequence data, and therefore needs to be as fast and as memory
efficient as possible. The implementation of a distributed version
of hierarchical clustering in HPC-CLUST makes it now possible
to fully cluster a much larger number of sequences, essentially
limited only by the number of available computing nodes.

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