

PEACE: Parallel Environment for Assembly and Clustering of Gene Expression

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ABSTRACT

We present PEACE, a stand alone tool for the high-throughput *ab initio* clustering of transcript fragment sequences produced by Next Generation or Sanger Sequencing technologies, freely available from www.peace-tools.org. Installed and managed through a download-able user-friendly GUI, PEACE can process large data sets of transcript fragments of length 50 bases or greater, grouping the fragments by gene associations with a sensitivity compatible to leading clustering tools. Once clustered, the user can employ the GUI's analysis functions, allowing the easy collection of statistics and single out specific clusters for more comprehensive study or assembly. Using a novel minimum spanning tree based clustering method, PEACE is the equal of other leading tools in the literature. It produces results of quality virtually identical to those of the WCD tool when applied to Sanger sequences, significantly improved results over WCD when applied to the products of Next Generation Sequencing Technology, and significantly improved results over Cap3 in both cases. In short, PEACE provides an easily accessible user interface for interface with a clustering engine that is the match of the leading clustering tools in the literature.

INTRODUCTION

Understanding an organism's transcriptome, the set of (spliced) transcripts expressed by genes of the organism, is a vital step in understanding the full functional and organizational role of the genome in the life cycle of any eukaryote. Studying the transcriptome has led to gene discovery, provided information on splice variants, and helped shed light on the biological processes both controlling and controlled by the genome (1). However, to access those transcripts, we must deal with the fragmented data produced by both Next Generation and traditional Sanger sequencing technology.

In the past, access to a transcriptome sequence was primarily through the use of Expressed Sequence Tags

(ESTs), single-pass cDNA sequences derived from transcribed mRNAs and sequenced by Sanger Sequencing technology. More recently, Next Generation Sequencing (NGS) technology has begun to rapidly replace Sanger Sequencing, allowing for more comprehensive coverage of the transcriptome. For example, ESTs now being added to the GenBank dbEST are increasingly the product of NGS technologies such as 454 pyrosequencing, which enables the sequencing of novel and rare transcripts at a considerably higher rate of coverage than Sanger Sequencing (2, 3). From a computational perspective, this is a mixed blessing: while NGS provides immense quantities of new information, it also provides immensely larger data sets – and thus a need for fast, efficient analysis algorithms.

Given a set of transcript fragments sampled from across the genome, a necessary first step of the set's analysis is that of clustering: separating the fragments according to the transcript from which they were derived. Frequently performed implicitly by assembly tools, clustering the data as a “pre-assembly” step has a number of advantages. Most significantly: performing this step will allow the application of the assembly tool to individual clusters – saving significant amounts of time (4).

However, clustering is a computationally challenging problem. Even with the smaller number of ESTs produced using Sanger Sequencing, the runtime and memory requirements to cluster on the basis of pair-wise sequence alignments make such an approach infeasible in practice. The much larger data-set size produced by NGS technologies exacerbates this problem. To deal with this, PEACE combines our own version of the d^2 alignment-free sequence distance function (5) and the concept of a *minimum spanning tree* (6) to quickly and accurately find clusters of ESTs expressed from the same gene without reference to a sequenced genome. Compared against WCD, the leading clustering tool in the literature (4), as well as other tools designed for the same purpose (4, 7, 8, 9, 10, 11, 12, 13, 14), PEACE proves to be both more sensitive and more robust to sequencing error without sacrificing runtime. Nor are any of these tools designed for the ease of installation and use that PEACE provides.

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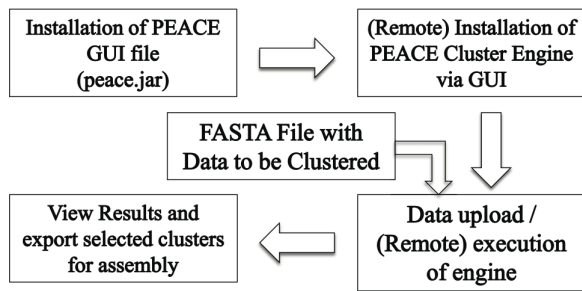


Figure 1. Overview of the procedure for clustering and analysis using PEACE.

In short, PEACE is a computational tool for the *ab initio* clustering of transcript fragments by gene association, applicable to both NGS and traditional Sanger Sequencing technologies. Available through the www.peace-tools.org website, the PEACE GUI allows the user to both easily install (locally or remotely) and run the clustering engine, as well as enabling transparent parallel processing and providing various tools for result analysis.

PEACE: INSTALLATION AND USE

The PEACE GUI can be launched by downloading and executing the Jar file available on the PEACE website (www.peace-tools.org) to any machine running the standard Java Virtual Machine (JVM). Once running, the user can employ the GUI to install the clustering engine and perform a clustering of a data file in FASTA format, view an initial analysis of the clusters, and produce files containing subsets of the clusters as input to assembly tools such as Cap3 (9). A typical (first) use of PEACE must be performed in the following manner (see Figure 1):

Tool Installation: (First use only.) To install the PEACE clustering engine onto a local or remote machine, the user selects from within the GUI the appropriate menu tab (Figure 2(a)), which then starts an install wizard that will prompt for the appropriate information. Figure 2(b) illustrates the request for server information; the user has chosen to install the PEACE computational tool on a remote machine and is providing the necessary connection information. Server information is persistent between GUI sessions, giving the user access to PEACE on the target machine as needed.

Job Processing: After importing the target sequence file into the GUI, the user starts a new job by following the wizard menus. Figure 2(c) illustrates the process of specifying the number of processors available (if running on a machine supporting the OpenMPI protocol – which will be determined during job installation). Once executed, the GUI will manage the job thread, alert the user when the job is completed (or when the user next runs the GUI after completion), and copy the final results back to the local machine if necessary.

Result Analysis: Once the resulting clusters have been computed, the user has several options for analysis:

- **Export:** The user can export the contents of one or more clusters into a FASTA format file, obtaining a subset of

the original target file containing the sequences corresponding to the selected clusters ready for processing by an assembly tool (e.g. Cap3 (9)).

- **View Clustering:** The user may view a list of clusters, expanding selected clusters to a list of all individual sequences (illustrated in Figure 2(d)).
- **Classified Summary Graph:** The user may view a distribution of cluster sizes. Further, the user can set up a *classifier*, associating certain patterns with specific colors. These patterns were matched against the fragment header information from the original FASTA file, allowing the overlay of a colored cluster size distributions. For example, if the sequence names contain unique string patterns denoting different cDNA libraries, the classifier can help the user to determine and visualize the differential expression profiles of different libraries for a given cluster. The method of setting up these classifiers, and the resulting histogram, is illustrated in Figure 2(e).

Extensive documentation for the tool has been posted on the PEACE website, as well as links to several tutorial videos demonstrating PEACE use and capabilities.

METHODS

The clustering performed by PEACE is based on the use of minimum spanning trees (MSTs), known to be an effective approach for narrow band single linkage clustering (15, 16). Using a graph structure to model the fragment relationships and the d^2 distance measure to assign edge weights (5), we can employ Prim's algorithm (6) to efficiently calculate an MST from which we can infer a high-quality clustering solution.

The d^2 distance measure used to assign edge weights is an alignment-free measurement of sequence distance that can be calculated significantly faster than a Smith-Waterman alignment (5). d^2 works by comparing the frequency of words (strings of a fixed length) appearing in a limited region of each string. Fragments overlapping by a sufficient length will share neighborhoods of enough similarity to ensure a small distance even in the presence of a moderate number of base errors. In practice we employ our own variation of d^2 , the *two-pass d^2 algorithm*, which heuristically searches for a neighborhood of maximum similarity and then finds the d^2 score based on that neighborhood (see Supplementary Materials for details).

Fragment input is modeled as a weighted, undirected graph: the fragments are represented as nodes, with d^2 sequence distances assigned to the connecting edges as weights. Conceptually, we want to remove each edge exceeding a threshold score from the complete graph and define our partitions by the remaining connected components. An edge with a large weight connects fragments which are likely unrelated; once such edges are removed the components define a series of overlaps. Those fragments that can still be connected by some path correspond to the same gene. However, such an approach requires the calculation of all edge weights. That task is infeasible both in terms of runtime and memory usage for the data set sizes we expect to process.

PEACE approaches the problem by generating a minimum spanning tree of the described graph, then removing edges

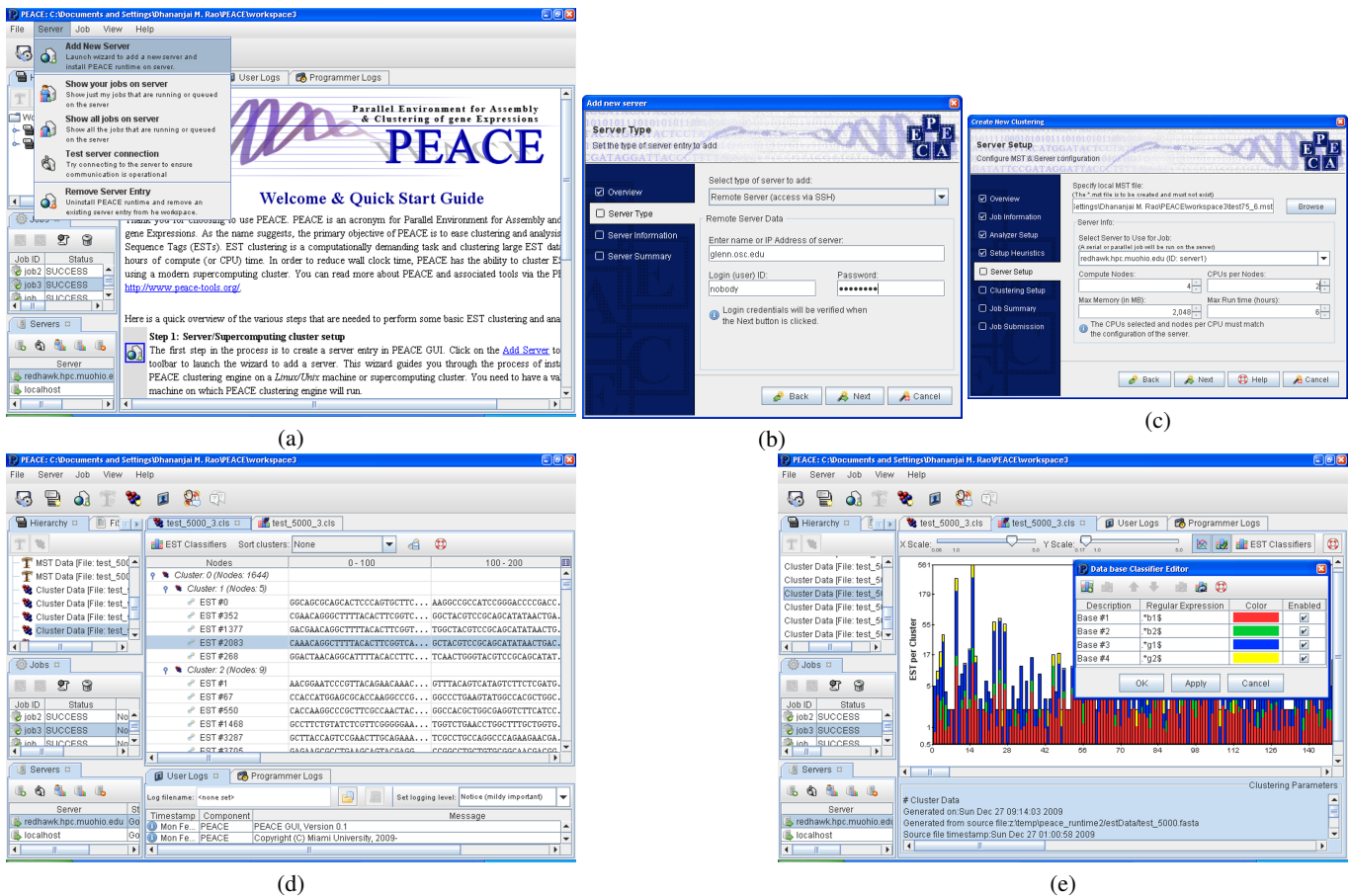


Figure 2. Screenshots of the PEACE GUI during execution, including (a) GUI Welcome and server installation menu; (b) setup wizard for installing the computational tool on a remote server; (c) execution wizard for starting a selected job to be executed in parallel mode; (d) basic cluster output; and (e) histogram view of cluster results and classifier editor for setting up differential expression profiles.

exceeding our threshold. By using Prim's algorithm we are able to calculate edge weights on-the-fly (reducing memory requirements) and can skip the calculation of a majority of edge distances using the u/v and t/v filtering heuristics employed in WCD (4). These heuristics allow us to quickly dismiss many of the edges as too large without the need to apply the full d^2 algorithm (see Sections A and B of the Supplementary Materials for more details).

RESULTS

PEACE has been tested on both simulated and real data from NGS and Sanger Sequencing technologies, comparing results against those produced by the WCD clustering tool (4) and the Cap3 assembly tool (9) (the latter of which implicitly calculates a clustering in the process of assembly). For our simulation tests we used the **ESTSim** tool (17) to generate simulated transcript fragments of varying length under different models of error (Supplementary Materials, Section D.1), generating the fragments from the list of 100 zebra fish genes used in the WCD testing (4). Tool parameters were taken to match, as closely as possible, those used in the WCD study (see Supplementary Materials). The most important method of quality assessment is *sensitivity* (the fraction of fragment pairs from the same gene that were

correctly clustered together). But we also look at the *Jaccard Index* (which balances sensitivity with the number of false positives by looking at the ratio of true positives to the sum of true positives, false positives, and true negatives), *Type 1 error* (the fraction of genes that were divided between clusters), and *Type 2 error* (the fraction of clusters containing two or more genes). Specificity was not an issue in simulated tests, as both PEACE and WCD were completely successful in separating unrelated ESTs (save when dealing with recently duplicated genes). In Figure 3 we plot these four tests as a function of error rate, and observe the almost identical results between PEACE and WCD. In Figure 4 we plot the runtime for PEACE and WCD, again observing almost identical results when run sequentially – but significantly faster runtime for PEACE on multiple processor when holding the EST/processor ratio constant (ranging from a 65% improvement for two processors too a 17% improvement for 12 processors).

We further note, as a point of interest that, we can significantly improve the quality of PEACE simulation results through the increase of the threshold value (see Supplementary Materials, Section C) – achieving a significant improved in PEACE sensitivity without an adverse effect on the Jaccard index (i.e. a significant increase in the incorrect merging of clusters). However, the improvement does not carry over to the application of real data, where we observe a significant drop in

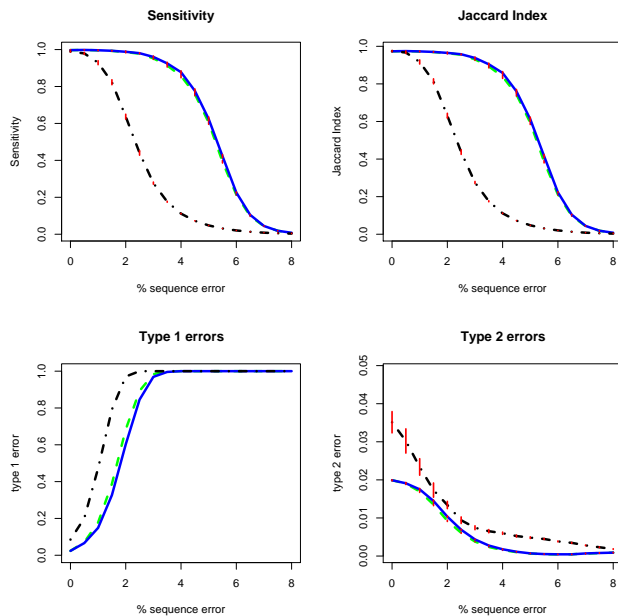


Figure 3. Comparisons of Sensitivity, Jaccard Index, Type 1 error and Type 2 error, based on the average over 30 simulated Sanger Sequence ESTs sets derived from 100 zebra fish genes (see Supplementary Materials, Section C, for more details). Blue/Solid = PEACE, Green/Dash = WCD(version 0.5.1), Black/Dot-Dash = Cap3; vertical ticks = 95% confidence intervals on estimates. Intervals are not presented for Type 1 error due to the effective lack of variance.

the Jaccard Index. Due to this drop we have opted to leave the lower threshold value as the default, but users might consider the higher value, thus decreasing the risk of incorrectly splitting clusters, if planning on using on the subsequent use of a assembly tool that capable of breaking up the incorrectly merged clusters during transcript reconstruction.

In applying the tools to real data Sanger data, we used the Human Benchmark Data Set used to test EasyCluster (14), and the A076941.fa *Arabidopsis thaliana* data set used to test WCD (4, 18), (see Table 1). We notice essentially identical results for PEACE and WCD in quality, both significantly better than Cap3 in Sensitivity and Type 1 error rate, while slightly worse in the Jaccard Index and Type 2 error. In runtime we see some inconsistency, with PEACE showing a 60% runtime improvement in the first data set, but requiring 20% more time in the Arabidopsis data set. In Section D.1 of the Supplementary Materials we present runtimes several more sets, observing that while PEACE appears to be significantly more faster on the smaller sets, WCD does overtake it for larger sets.

We tested the three tools on short-read data using the MetaSim tool of Richter *et al.* (19). Encoded into MetaSim are sequence generation and error models corresponding to several technologies, including the 454 short-read technology. Leaving Cap3 and WCD at their default values, but employing the *adaptive d²* strategy for short read sequences (see Section C in the supplementary materials), we find a that PEACE performs significantly better than the other two tools on 454 data using the default MetaSim model (with sequence sizes ranging in size from 200bp to 370bp, at an average size of

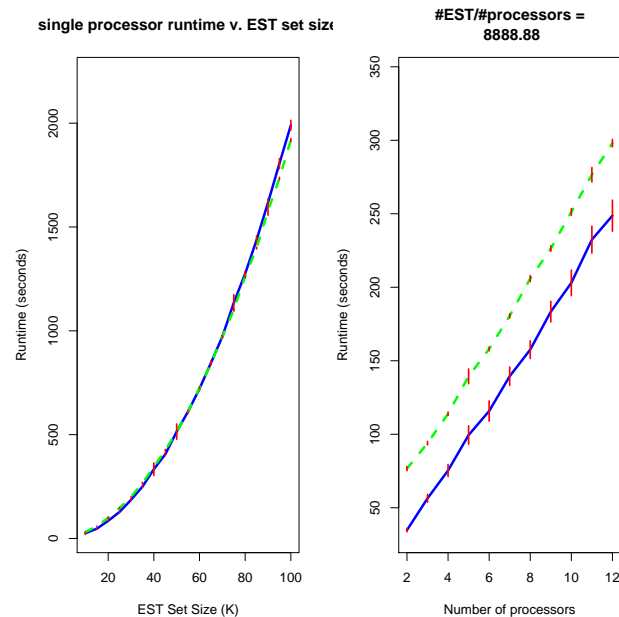


Figure 4. Comparisons of Runtime: On left, we compare the sequential runtime of PEACE (blue) and WCD (green) on simulate sets ranging in size from 10K sequences to 100K sequences. (Cap3 runtime is not reported, as the time spent on clustering cannot be differentiated from the time spent on assembly.) On right, a comparison when run in parallel, ranging the number of processors from 2 to 12 while holding the EST/Processor ratio steady at the constant 8888. All values represent the average of 30 runs; vertical ticks = 95% confidence intervals on estimates. All runs were done on a 3.0 GHz Intel Xeon EM64T CPU with a 2 MB cache and 800 MHz front side bus, model number Xeon LV 3.0 (2005).

250bp and a standard deviation of 17bp). Over thirty runs, we see an average sensitivity 0.884 for PEACE, 0.331 for WCD, and 0.004 for Cap3. For the Jaccard Index we get essentially the same numbers. For 100 transcript PEACE creates 128 clusters (as opposed to 6,370 and 19245 clusters for WCD and Cap3), and creates only 3 singletons (as opposed to 5,850 and 15432). We do note that it is likely that the WCD results could be considerably improved by the adjustment of a few parameters, but were unable to determine how to do that. (Runtime of PEACE was somewhat slower than that of WCD, but given the significant improvement in quality of results this is unimportant.)

CONCLUSIONS

Here we have presented PEACE, a stand alone tool for the high-throughput clustering of transcript fragments capable of dealing with sequences as short as 50 bases. PEACE, available at www.peace-tools.org, is open-source and managed through a user friendly GUI that enables both local and remote installation and execution in sequential or parallel mode. Based on a novel algorithm for the clustering of the fragments by gene association, PEACE shows significant improvement in sensitivity, over the competing WCD tool (4) when applied to NGS reads, matches WCD when applied to Traditional Sanger Sequencing output, and shows an order of magnitude in improvement over the clustering performed in the course of assembly by the Cap3 tool (9).

		Sensitivity	Jaccard	Type 1 error	Type 2 error	Number of Clusters	Number of Singletons	Single processor runtime (s)
Bicyclist Human Benchmark (111 Genes)	PEACE	0.998	0.672	0.153	0.042	118	21	293
	WCD	0.998	0.672	0.144	0.044	113	16	804
	Cap3	0.657	0.643	1.000	0.001	2269	1827	NA
WCD A076941 Benchmark (13240 genes)	PEACE	0.932	0.475	0.351	0.027	18825	8951	1166
	WCD	0.933	0.476	0.350	0.027	18787	8553	966
	Cap3	0.826	0.802	0.486	0.014	25042	14916	NA

Table 1. Comparisons of runs on the EasyCluster human Benchmark Data Set and the WCD A076941 Arabidopsis thaliana data set using the standard quality measurements.

As a clustering tool based on sequence distance, PEACE faces certain inherent limitations. For example, PEACE cannot handle duplicate genes; like WCD, it is unable to separate clusters corresponding to genes with a greater than 88% similarity. Similarly, other natural biological effects (e.g. the trans-splicing of transcripts), effects from poorly cleaned transcript data (e.g. the failure to remove sequencing adapters or post-transcriptional poly(A)/(T) tails), and the presence of low-complexity repeats can cause similar effects in these clustering tools. The problems can be handled through the application of the assembler, and the ability to apply any assembler to small cluster (as opposed to the data set as a whole) results in a significant reduction in overall assembly time.

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