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genBlastA: Enabling BLAST to identify homologous gene sequences

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BLAST is an extensively used local similarity search tool for identifying homologous sequences. When a gene sequence (either protein sequence or nucleotide sequence) is used as a query to search for homologous sequences in a genome, the search results, represented as a list of high-scoring pairs (HSPs), are fragments of candidate genes rather than full-length candidate genes. Relevant HSPs ("signals"), which represent candidate genes in the target genome sequences, are buried within a report that contains also hundreds to thousands of random HSPs ("noises"). Consequently, BLAST results are often overwhelming and confusing even to experienced users. For effective use of BLAST, a program is needed for extracting relevant HSPs that represent candidate homologous genes from the entire HSP report. To achieve this goal, we have designed a graph-based algorithm, genBlastA, which automatically filters HSPs into well-defined groups, each representing a candidate gene in the target genome. The novelty of genBlastA is an edge length metric that reflects a set of biologically motivated requirements so that each shortest path corresponds to an HSP group representing a homologous gene. We have demonstrated that this novel algorithm is both efficient and accurate for identifying homologous sequences, and that it outperforms existing approaches with similar functionalities.

[Supplemental material is available online at www.genome.org.]
of filtering and assembling HSPs representing genes in tandem clusters is very important.

In this project, we developed a new graph-based algorithm, genBlastA, to directly address the above described challenge, among other issues, in filtering and assembling HSPs into genomic gene regions. A distinctive feature of genBlastA is that it does not rely on using ad hoc thresholds for filtering noise HSPs and on physical distance between target genes. Instead, genBlastA models the relationships and constraints among HSPs as a directed graph—designated the HSP graph—and models the HSP filtering and assembling problem as a search for the shortest paths in this graph. The novelty of this graph-based algorithm is an innovative edge length metric that reflects a set of biologically motivated requirements so that each shortest path corresponds to an HSP group representing a homologous gene. Unlike existing ad hoc grouping methods, this method filters and assembles HSPs on the basis of optimizing the path length to best capture the quality of a group of HSPs as a candidate gene. Consequently, our method is more robust, and it finds an optimal solution (with respect to a given length metric) without imposing a prior constraint (i.e., ad hoc thresholds) on gene structures.

We have tested the performance of genBlastA extensively in filtering and assembling HSPs found in the genomes of two closely related nematode species: *C. elegans* (Consortium 1998) and *Caenorhabditis briggsae* (Stein et al. 2003). These genomes were selected for testing because both have been extensively annotated. Our study shows that the performance of genBlastA is significantly better than that of WU-BLAST and the program by Cui et al. (2007).

**Results**

In this project, we developed the program genBlastA (described in Methods) that uses a novel graph-based algorithm that gives the program excellent capability for identifying HSP groups that represent orthologs (genes in different species but with same origin in evolution), paralogs (genes duplicated within a species), as well as novel genes (genes that have not yet been identified).

**Test gene set preparation and test strategy**

The data sets used for evaluation were obtained from WormBase (http://www.wormbase.org/), an integrated database for the biology and genomics of *C. elegans* and other nematode species including *C. briggsae* (Chen et al. 2005), release WS170. For testing the performance of genBlastA, we have selected a test gene set of 464 *C. elegans* genes that are representative of the *C. elegans* genome. To achieve this representation, the majority (300 genes) of these genes were taken from three representational contiguous regions of *C. elegans* chromosome I. These three regions are the left arm (containing 100 genes), the middle region (containing 100 genes), and the right arm (containing 100 genes) of chromosomal regions. To ensure that the test gene set contains representative genes of different complexities, we further included 164 additional genes, including genes with internal repetitive regions (Pfam domains) and genes that belong to large paralogous tan-
dem clusters. The executable file and the test gene set can be downloaded from http://genome.sfu.ca/projects/genBlastA/.

To evaluate the capability of genBlastA to identify and group HSPs into gene-like structures and the capability of identifying novel genes, we used C. elegans genome as the target database for C. elegans query genes (called EvsE test). To evaluate the performance of genBlastA in identifying homologous sequences in genomes of different but related species, we used C. briggsae genome as the target database for the same set of C. elegans query genes (called EvsB test). These two species split ~80–120 million yr ago (Coghlan and Wolfe 2002; Stein et al. 2003), around the same time as the human/mouse split (Waterston et al. 2002).

The query for genBlastA can be either protein sequences or cDNA sequences. Details for using genBlastA are described in the README file included in the software package. In our experiments, genBlastA was able to process all 464 test genes (with over 43,000 HSPs reported by BLAST in EvsE test) within only 1 min on a medium-speed PC (with a Pentium-IV 2.6-GHz CPU). Since these 464 genes are representative of the entire C. elegans genome and comprise 2% of the genome, we calculate that it would take less than 1 h to process the entire genome (which contains ~20,000 genes).

We compared the performance of genBlastA with two existing programs with similar functionalities—WU-BLAST (Lopez et al. 2003) and the program by Cui et al. (2007). WU-BLAST is available by an academic license. Since the HSP grouping function of the program by Cui et al. (2007) is not readily available, we implemented this program, called ML in the following text, based on their publication (Cui et al. 2007). ML requires a distance threshold to resolve different HSP groups. This threshold is not described in detail in their publication; therefore, we derived an optimal distance value based on simulation results. In our experiments, we found that ML performs best when the distance threshold is set to 1000 bp for our test cases described below (Supplemental Figs. 1–3). Therefore this distance was used for ML throughout our analysis.

For each query gene in the test gene set, we first ran TBLASTN against the C. elegans genome (for EvsE test) and the C. briggsae genome (for EvsB test) with two different BLAST settings: “ungapped” and “gapped,” while the gapped HSPs are generally longer with more gaps and mismatches and ungapped HSPs are generally shorter with much higher PIDs. We then carried out three sets of experiments, each with a different purpose.

1. Resolving paralogous genes in tandem clusters: This first experiment was designed to test the capability of these programs in addressing the major challenge that we have identified—resolving HSP groups that correspond to target gene families in the target genome. For this purpose, we selected 30 genes from the test gene set that belong to large gene families and these family members form tandem gene clusters.

2. Searching for orthologous groups: In this test, each gene in the test gene set was used as a query to identify the top-ranked HSP group, i.e., the candidate ortholog of the query gene. Since the top-ranked group is expected to be the most similar to the query gene, in the EvsE test, it is expected to map to the query gene itself; in the EvsB test, it should map to its C. briggsae ortholog.

3. Identifying novel genes: In the third experiment, we explored the utility of genBlastA for identifying novel (paralogous) genes, i.e., the genomic regions that show high similarity to known genes but have no gene annotations.

Resolving paralogous genes in tandem clusters

To test the three programs’ abilities to resolve tandem duplicate genes, we examined the HSP groups produced for 30 query genes in the test gene set that are members of large gene families. For our comparison, after we identified HSP groups using genBlastA, WU-BLAST, and ML, we retained all candidate regions with query coverage ≥50%. The HSP groups were then examined and divided into two categories: “specific” and “nonspecific” groups. An HSP group is called specific if the corresponding genomic region contains only one annotated gene and is called nonspecific if the region has multiple annotated genes. HSP groups with high similarity to the query and containing only single genes are likely to be true paralogs. The programs’ performance in resolving multiple paralogous genes is evaluated by comparing the ratio of specific groups over the total number of HSP groups examined. Figure 2 illustrates an example, in which there are five paralogous genes in a tandem gene cluster. As expected, WU-BLAST correctly identified only one target gene and failed to produce HSP groups corresponding to the rest of the four genes. ML produced three groups, two of which erroneously contain HSPs corresponding to other adjacent genes. ML missed groups for two target genes (T27B7.4 [nhr-115] and T27B7.6a [nhr-228]), and mistakenly grouped HSPs corresponding to T27B7.6a to the HSP group corresponding to T27B7.5 (nhr-227) (Fig. 2). In contrast, genBlastA successfully resolved all five genes, producing five groups of HSPs.

In summary, when BLAST was executed with the ungapped setting in the EvsE sets, the average ratio of specific HSP groups by genBlastA is ~80%, which is significantly higher than that produced by WU-BLAST (~20%) or ML (~40%) (Fig. 3). Similar results were observed when BLAST was performed with the gapped setting. Thus, in all cases, genBlastA was able to resolve more specific HSP groups in tandem duplicates compared to either WU-BLAST or ML. WU-BLAST usually generated numerous HSP groups, but they usually spanned regions with multiple genes (therefore nonspecific). Consequently, WU-BLAST groups together tandem paralogous genes, leading to poor performance in resolving tandem paralogous genes. ML had poor performance due to its use of a distance threshold. In particular, as the distance threshold increases, the ability of ML to resolve closely spaced paralogous groups decreases.

Searching for orthologous groups

In this test, the top-ranked HSP group corresponding to each query gene is evaluated by comparing to the expected gene as annotated in WormBase (WS170). First, we compared the accuracy rates of three programs when C. elegans genes were used as query genes to search for top-ranked genes in C. elegans genome. The accuracy rate is defined as the percentage of correctly assembled HSP groups. The accuracy rate for genBlastA is 97.2%, much higher than those of WU-BLAST and ML, which are 67.0% and 82.8%, respectively. For more accurate comparisons, the similarity or overlap between the HSP group and the expected gene were quantified. We used the following two criteria to evaluate the top-ranked HSP groups: (1) query coverage and (2) genomic span. Query coverage measures the similarity between the HSP group and the query gene. It is defined as the proportion of the query sequence covered by the HSPs in the HSP group identified by each of the three programs. A program should identify the HSP group that best covers the query gene. Genomic span measures the extent of overlap between the genomic region
given by the HSP group and the expected gene region in the target genome. We evaluated this using the Jaccard similarity: For the annotated target gene region RA and the reported gene region RR, their similarity is \(\frac{|RA \cap RR|}{|RA \cup RR|}\). This result is zero when two regions do not overlap.

**Query coverage test**

Figure 4, A and C, shows the average query coverage for 464 query genes in the test gene set. When BLAST was executed using the ungapped setting in the EvsE test (Fig. 4A) and the EvsB test (Fig. 4C), genBlastA identifies HSP groups with close to 100% query coverage and significantly outperformed both WU-BLAST and ML. Similarly, when BLAST was executed using the gapped setting, for both EvsE and EvsB tests, genBlastA outperformed both WU-BLAST and ML significantly, while WU-BLAST outperformed ML.

Taken together, genBlastA outperformed both WU-BLAST and ML in identifying orthologous HSP groups.

**Identifying novel genes**

Since genBlastA can be applied to effectively identify homologous genomic regions in a target genome, we reasoned that it can be used for identifying novel paralogous genes that have been missed by other approaches. To demonstrate this, we examined whether genBlastA can be used to identify HSP groups in the C. elegans genome that are homologous to the test genes and that do not overlap with any existing gene annotation, therefore, identifying putative novel genes or novel pseudogenes.

We evaluated all candidate homologous gene regions for the 464 query genes for ones that show both significant query gene

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**Figure 2.** Grouping HSPs into groups representing individual genes. genBlastA was able to resolve all five members, while ML resolved only two and WU only one. Gene models are shown in the Gene Models track. HSPs are shown as blue boxes in the All HSPs track. The color indicates different PIDs for the HSPs. Darker color indicates higher PID. The genBlastA Group, ML Group, and WU Group tracks show HSPs groupings that are returned by genBlastA, ML, and WU-BLAST, respectively.
coverage (>80%) and do not correspond to known genes. We found eight candidates. In particular, four of them contain putative novel genes that are relatively long (>300 amino acids) (Supplemental Table 1; Fig. 5). These putative novel genes will be tested in the laboratory to examine if they are real genes. Since the test gene set represents ~2% of *C. elegans* genome, we estimate that genBlastA will identify hundreds of novel homologous genes (including hundreds of long genes) in the entire genome. Our finding has thus demonstrated that genBlastA has the potential to identify novel paralogous genes.

**Discussion**

BLAST and related search programs have been widely used for identifying homologous sequences since they are sensitive and effective in finding homologous fragments for query genes. However, BLAST results often contain a large number of HSPs and can be challenging if not overwhelming for the end users. Our program genBlastA provides an effective way to interpret the large list of HSPs reported by BLAST in order to allow users to focus on targets they find interesting. genBlastA enables users to effectively identify homologous genomic regions that represent full-length candidate genes, rather than fragments of a gene (HSPs). Thus, genBlastA empowers users by allowing them to effectively identify candidate genes in target genomes. This will make BLAST and related programs even more useful.

Our analysis has clearly shown that genBlastA outperforms existing programs developed previously with similar objectives. In particular, genBlastA is very effective in grouping HSPs corresponding individual genes within tandem clusters of homologous genes. Both WU-BLAST and the program developed by Cui et al. (2007) failed in this task. Additionally, although ML performs better than WU-BLAST in resolving multiple paralogous genes in tandem clusters, the current ML program is not ready for
this job because the current ML program is not capable of removing random HSPs in the genomic regions.

The ability of effectively resolving HSP groups by genBlastA will enable users to take advantage of HSP groups, which are useful in several ways. First, genBlastA can be used by researchers to quickly locate candidate gene structures in the identified homologous genomic regions in the target genomes. Compared with the large collection of HSPs reported by BLAST and similar programs, ranked HSP groups provide much more useful information relevant to full-length target gene structures, instead of fragments of target genes. Since end users such as experimental biologists are usually more interested in genes, genBlastA makes search results more accessible and meaningful to them.

Second, genBlastA can be used to preprocess genomic DNA sequences for gene finding programs, including genewise (Birney et al. 2004) and exonerate (Slater and Birney 2005). Both genewise and exonerate are widely used for homology-based gene prediction. However, both programs, especially genewise, are computationally expensive when used to search for candidate genes in entire genomes. Their performance can be dramatically enhanced if their genomic search space is reduced. genBlastA, which is capable of identifying candidate genomic regions, can be used effectively to preprocess the genomic sequences in order to reduce search spaces. It can also be integrated into the program by Cui et al. (2007) to identify homologous genes.

Third, these HSPs can be used to resolve gene structures, either manually or computationally. Candidate gene models can be accurately defined by HSPs in each HSP group, intron–exon splicing information at the edges of HSPs, as well as the similarity between query and candidate genes. A gene prediction program based on this is being developed and will be reported separately.

Methods

Problem definition

In this work, we study the following problem: given a query (gene) sequence, which is a protein (gene product), and a database of target genomic sequences, we want to identify all homologous genomic regions containing target genes (genes in the target sequences that are homologous to the query gene). First, as a preprocessing step, we apply BLAST to find local alignments between the query sequence and the target sequences. This step produces a list of HSPs, with each HSP containing the following information: (1) the target segment T and its location in the target sequence, and the corresponding query segment Q and its location in the query sequence, (2) an E-value, and (3) a PID value. In the second step, we filter and group the HSPs such that each group of HSPs forms a candidate region containing the target gene, called candidate gene region. genBlastA focuses on the second step.

An example of a list of HSPs is shown in Figure 5A, where the correspondence between the target segment (T) and query segment (Q) in an HSP is illustrated by dotted lines. For example, \([Q_1,T_1]\) and \([Q_1,T_2]\) represent two different HSPs. HSPs may overlap in terms of their genomic positions and/or their query correspondences. Note the HSPs shown in this figure are only for illustration purposes, although our algorithm is able to properly handle HSPs with various kinds of relationships.

Each genomic sequence has two strands—positive and negative. Each strand is considered a separate target sequence by genBlastA. Their only difference is the direction of alignment between the target gene and the query gene. Because each target sequence is independent and has its own list of HSPs, we process each target sequence separately to obtain the candidate gene regions for that sequence. Finally, all candidates for all target sequences are ranked into a single ranked list by their score as computed by our algorithm (discussed later). From now on, for brevity, all discussions will be based on a query sequence and a single positive-strand target sequence.

In this report, due to space limitation, we briefly present a novel graph-based method genBlastA to model the best grouping of HSPs as the problem of searching for shortest paths in a graph. Details of genBlastA algorithm are described in the Supplemental Data.

HSP groups

With each HSP target segment that matches a query segment, a sequential group of HSP target segments can collectively match a larger piece of the query sequence. We are interested in those groups of HSPs, which correspond to genes that are homologous to the query gene. Such groups are termed HSP groups. In general, there are different numbers of HSP groups in the target sequence for each query gene. If the query gene is not conserved in the target genome, then no HSP group can be found. If the query gene belongs to a multigene family (or the query gene has many paralogous genes), there will be multiple HSP groups in the target sequence, each representing a candidate region encoding a paralogous gene.

Consider the example in Figure 5A. \(T_3\) and \(T_4\) are in the same
order as their query segments. So \( [Q_2, T_4] \) can be in the same group as \( [Q_2, T_3] \). In fact, by merging \( T_3 \) and \( T_4 \) into one continuous target region, and merging their query segments into one continuous query region, we have a larger, thus better alignment. Figure 5B shows a possible grouping of HSPs that satisfies the sequential ordering and co-linearity requirements. Note that Group 1 and Group 3 have incomplete query gene coverage because a large portion of the query sequence is not covered by their query segments. In contrast, Group 2 covers the entire query sequence. A good HSP group should have large query coverage.

For a group of HSPs, the combined region of their query segments should cover the query sequence as much as possible. In Figure 5B, Group 2 is better than either Group 1 or Group 3 because it covers a larger region of the query sequence.

**Graph modeling**

An HSP graph is a graph representation that captures the above requirements on HSP groups. Each HSP is represented by a node, with edges that model the sequential ordering of the HSP target segments and edges that skip HSPs. An HSP grouping is modeled by grouping the nodes on a path, such that each group covers as many query segments as possible while preserving colinearity. By using a length metric (Supplemental Data), we will show that an optimal HSP group is a shortest path in the HSP graph.

Figure 5C shows the HSP graph for the HSPs in Figure 5A. The dotted edges are skip edges. Each path in the graph represents a way of selecting HSPs along the path. With skip edges, the HSP graph provides a complete search space for all possible groupings of HSPs. The number of skip edges can be very large. However, after introducing a length metric on edges (Supplemental Data), we will show that many skip edges can be removed without affecting the result. Our program genBlastA will not construct such skip edges, thus dramatically increasing the efficiency of genBlastA.

In Figure 5D, to distinguish these two types of edges, we add a vertical bar to each separating edge. For example, \( H_1 \rightarrow H_2 \) is a separating edge, which means that its source node and destination node should belong to different HSP groups. The skip edge \( H_1 \rightarrow H_2 \) is an extension edge, and the skip edge \( H_1 \rightarrow H_2 \) is a separating edge.

With extension edges and separating edges, each path in the HSP graph represents a way of filtering and grouping HSPs: As we traverse a path, following an extension edge extends the current HSP group to include the destination node, and following a separating edge ends the current HSP group at its source node and starts a new HSP group at its destination node. If an extension edge is a skip edge, following the edge will skip over the nodes on the paths that are shortcut by the edge. In this sense, the HSP graph provides a complete search space for filtering and grouping HSPs.

The single-source shortest path algorithm for a directed acyclic graph can be done efficiently in \( O(E) \) time, where \( E \) is the number of edges (Manber 1989). Executing this algorithm once for each possible starting node \( H_1 \), the total running time is \( O(E + V) \), where \( V \) is the number of end nodes of separating edges and is bounded by the number of HSPs.

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**Homologous gene search using genBlastA**