Short Communication

Sequence search algorithms for single pass sequence identification: does one size fit all?

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Abstract

Bioinformatic tools have become essential to biologists in their quest to understand the vast quantities of sequence data, and now whole genomes, which are being produced at an ever increasing rate. Much of these sequence data are single-pass sequences, such as sample sequences from organisms closely related to other organisms of interest which have already been sequenced, or cDNAs or expressed sequence tags (ESTs). These single-pass sequences often contain errors, including frameshifts, which complicate the identification of homologues, especially at the protein level. Therefore, sequence searches with this type of data are often performed at the nucleotide level. The most commonly used sequence search algorithms for the identification of homologues are Washington University’s and the National Center for Biotechnology Information’s (NCBI) versions of the BLAST suites of tools, which are to be found on websites all over the world. The work reported here examines the use of these tools for comparing sample sequence datasets to a known genome. It shows that care must be taken when choosing the parameters to use with the BLAST algorithms. NCBI’s version of gapped BLASTn gives much shorter, and sometimes different, top alignments to those found using Washington University’s version of BLASTn (which also allows for gaps), when both are used with their default parameters. Most of the differences in performance were found to be due to the choices of default parameters rather than underlying differences between the two algorithms. Washington University’s version, used with defaults, compares very favourably with the results obtained using the accurate but computationally intensive Smith–Waterman algorithm. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: BLASTn; sequence search algorithm; EST; cDNA; yeast; single pass sequence

Introduction

Sequence search algorithms are the keystone of bioinformatic tools, the most popular of which is probably the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) algorithm. When a biologist obtains a new sequence, one of the first tasks he/she undertakes is to ‘BLAST’ it against a database of choice in order to check the sequence or to try to discover more about it. If this sequence is ‘single pass’ (sequenced only once), such as an EST or a sample shotgun sequence, then it may contain undetected sequencing errors, such as single nucleotide insertions or deletions. These frameshift errors naturally cause problems when the sequence is translated into protein. Alternatively, the sequence of interest may not code for protein; for example, when upstream regions are compared to elucidate promoter regions or the sequence codes for rRNA or tRNA molecules. In these instances, nucleotide sequences are often compared to sequences from closely related species, most commonly by using BLASTn. These types of sequence data are now being produced at a phenomenal rate, and so this sequence ‘identification’ is often automated. If automated, the default BLAST parameters tend to be used, as they are often optimized to give the best results with a range of sequences, as well as allowing for consistency of results between runs.
Single sequences are also usually analysed with BLAST using the default parameters, as when web-based forms are used they often do not allow for BLASTn parameters to be changed. This is due to the problems in calculating the sum statistics for BLAST when gaps are allowed in the alignments. When run with anything other than the default parameters, the Washington University version of BLASTn displays the following:

‘WARNING:

Precomputed values for Lambda, K, and H are unavailable for the +1, −3 scoring matrix, when used with gap penalties of −5 and −2. Unless overridden on the command line, the values computed for ungapped alignments will be used instead, but may yield P-values that are unduly low.’

There are two versions of BLAST which allow gaps, available on the web (or for download): from Washington University (wuBLAST) (Altschul et al., 1990) and from the National Centre for Biotechnology Information (ncbiBLAST) (Altschul et al., 1997). These vary in the default parameters available for BLASTn (see Table 1) as well as how the algorithms introduce gaps into the alignment.

The *Saccharomyces cerevisiae* genome sequence has been available for some time (Goffeau et al., 1996) but there are still many gaps in our knowledge of its genes and of its relationship with other members of the genus *Saccharomyces*. In order to try and fill in some of these gaps, we are performing sample shotgun sequencing on the genomes of other members of the genus. The sequences used for the comparisons described here are from the *Saccharomyces sensu stricto* yeast *S. bayanus*, which is closely related to *S. cerevisiae* (Ryu et al., 1996; Naumov 1987; Fischer et al., 2000). This work was the first step in identifying gene homologues between *S. bayanus* and *S. cerevisiae*. To this end, coding regions in *S. bayanus* were identified by comparing the sample sequences against a database of *S. cerevisiae* coding regions (obtained from the KEGG ftp site ftp://kegg-genome.ad.jp/pub/ genomes/sequences/S.cerevisiae).

### Materials and methods

#### Sequence search algorithms

Washington University’s Blast version 2.0a19MP, available from [http://blast.wustl.edu/](http://blast.wustl.edu/), was used with default parameters as well as with the default parameters of ncbiBLAST, as detailed in Table 1.

National Center for Biotechnology Information’s gapped Blast version 2.0.9 (Altschul et al., 1997) (available from [http://www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)) was used with default parameters as well as with the default parameters of wuBLAST, as shown in Table 1.

Fasta 3 (Pearson and Lipman, 1988) and the SSearch implementation of Smith–Waterman (Smith and Waterman, 1981) (available from [http://www.ebi.ac.uk/FTP/](http://www.ebi.ac.uk/FTP/)) came from the same

| Algorithm          | Washington Uni’s BLASTn | ncbiBLASTn |
|--------------------|-------------------------|------------|
|                    | wuBLASTn                | ncbiBLASTn |
|                    | (wuBLASTn)              | (ncbiBLASTn) |
| Mismatch           | −4                      | −3         |
| Gap opening penalty| −10                     | −5         |
| Filter             | False                   | True       |

1. Match—positive score.
2. Mismatch—negative score/penalty.
3. Gap opening—this penalty applies per gap.
4. Gap extension—a gap extension penalty is added for each missing nucleotide in the gap.

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**Table 1. Parameters used in the different BLAST comparisons**
suite of tools. These were also used with their
default parameters (see Table 3).

All sequence search algorithms were run on two
processors of a four-processor SGI Origin and an
SGI O2.

Data

The S. cerevisiae DNA protein-coding database was
taken from Kegg (Kyoto Encyclopaedia of Genes
and Genomes) ftp site ([ftp://kegg-genome.ad.jp/pub/genomes/sequences/S.cerevisiae]) The S. bayanus
sequences were from shotgun-cloned sample
sequences, sequenced by the Washington University
Sequencing Centre, and kindly made available by
Mark Johnston. There are 909 sequences with an
average length of 403 nucleotides.

Results

In a trial of sample sequence identification, using
the two versions of BLASTn (default parameters), it
was discovered that they found not only different
lengths and numbers of database matches (hits) but
also different hits and even different top hits.

The graph in Figure 1 shows a comparison of
the number of hits found for wuBLASTn and
ncbiBLASTn for a sample of 909 sample shotgun
sequences from S. bayanus, ‘BLASTed’ against the
DNA database of S. cerevisiae coding regions.
These species are closely related, so that homologue
identification should be possible at the DNA-sequence level. As may be seen from Figure 1, ncbiBLASTn (mean = 16.1) normally finds more hits than wuBLASTn (mean = 5.5). However, there are a number of sequences for which wuBLASTn finds a greater number of hits as, by default, filtering for simple sequences is not switched on in wuBLASTn.

Table 1. Summary of the effects of exchanging BLASTn parameters between ncbiBLASTn and WuBLASTn

| Algorithm          | Washington Uni’s BLASTn | ncbiBLASTn | ncbiBLASTn | WuBLASTn |
|-------------------|-------------------------|------------|------------|----------|
| Origin of parameters | wuBLASTn                | ncbiBLASTn | ncbiBLASTn | WuBLASTn |
| No. of hits (range) | 0–259                   | 0–125      | 0–128      | 0–234    |
| Mean no. of hits  | 5.5                     | 11.9       | 16.1       | 11.6     |
| Total no. of hits | 5365                    | 10794      | 14644      | 10592    |
| Total no. of hits in common (as % of total) | 1517 (28.27%) | 2475 (22.93%) | 1517 (10.35%) | 2475 (23.37%) |

Table 2. Summary of the effects of exchanging BLASTn parameters between ncbiBLASTn and WuBLASTn

| Parameters | wuBLASTn | ncbiBLASTn | Smith-Waterman | Fasta |
|-----------|----------|------------|----------------|-------|
| Match     | 5        | 1          | 5              | 5     |
| Mismatch  | –4       | –3         | –4             | –4    |
| Gap opening penalty | –10 | –5         | –16            | –16   |
| Gap extension penalty | –10 | –2         | –4             | –4    |

Figure 2 shows a comparison of alignment
lengths for those alignments that were found in
common between the two versions of BLASTn for
the same query sequence. In all cases, wuBLASTn
(mean = 251.71 bp) finds alignments of the
same length or longer than ncbiBLASTn
(mean = 136.12 bp). The average length of the
query sequences is only 403 nt and many sequences
are only partially coding, if at all. These results were
unexpected and invited a more detailed analysis.

Using the same set of sequences from S. bayanus,
the two BLAST2 versions were compared again
but, this time, their parameters were changed to the
default values of the other algorithm. Therefore,
wuBLASTn was used with NCBI’s parameters
(wu_ncbiPar) and ncbiBLASTn with those of
wuBLASTn’s parameters (ncbi_wuPar), as shown
in Table 1. The results of this comparison are
shown in Table 2.

The ‘hits in common’, as shown in Table 2, were
calculated between the two versions of BLASTn
with their original parameters and between the two
versions with exchanged parameters. Figure 3
shows that wu_ncbiParBLASTn (i.e. wuBLASTn
with NCBI’s default parameters) usually finds more
hits than ncbi_wuParBLASTn. Figure 4 shows that

NCBI (with wuBLASTn’s default parameters, i.e. ncbi_wuPar) finds the longer alignments. However, it is not a complete role reversal, as may be seen from Table 2. The mean number of hits for wu_ncbiParBLASTn is lower than for ncbiBLASTn, even though they are using mostly the same parameters. Also, the mean number of hits for ncbi_wuParBLASTn is still much higher than for wuBLASTn. The same story is repeated for average alignment lengths. Therefore, not all the differences are due to the matrices and gap penalties used. Other underlying differences between the two versions of BLASTn must be responsible, but the matrix and gap penalties play a very important role.

These comparisons show that the database sequence matches found by the two versions of BLASTn were different, but not which algorithm is better at actually detecting homologues at the nucleotide level. To find out which of the two BLAST versions found the ‘right’ hits, they were both compared to FASTA and the ‘gold standard’ of sequence search algorithms—Smith–Waterman, using default parameters again (see Table 3). The results of this second comparison are summarized in Tables 4 and 5.

The same top ‘hit’ is found by all four methods 536 times for the 909 sample sequences. It should be noted that not all of these sample sequences are coding, so that not all sequences will find matches to the *S. cerevisiae* coding regions. Not surprisingly, FASTA and Smith–Waterman, since they are based on the same algorithm and use almost the same default parameters, find the most hits in common. wuBLASTn and Smith–Waterman have the next most in common, with ncbiBLASTn finding far fewer hits in common with Smith–Waterman. This pattern is repeated with the alignment lengths, although wuBLASTn actually produces, on average, slightly longer alignments than FASTA.
NcbiBLASTn’s alignments are very much shorter (see Table 5).

**Discussion**

The fact that ncbiBLASTn finds different top ‘hits’ and shorter alignments to the other algorithms is probably due to the matrix and gap penalties used, which do not allow for the gaps needed to extend alignments for this type of data. As may be seen from Table 3, wuBLASTn, FASTA and Smith–Waterman all use the same match/mismatch scores, although their gap penalties differ. Therefore, all three need fewer matches than ncbiBLASTn to allow for the insertion of a gap, as may be seen in Figure 5. Of course, the ability to extend alignments by inclusion of gaps and mismatches depends on the value of X, the extension threshold (Altschul et al., 1990). However, the opportunity to change this parameter is rarely given on web-based BLAST servers. WuBLASTn’s non-affine gap penalties mean that the programme tends to open gaps but not extend them, so that it will include short gaps, but not long ones. This allows for the frameshift errors found in single-pass sequence data, but not for long insertions or deletions.

It, has been suggested by Wolfe and co-workers (Wolfe and Shields, 1997; Keogh et al., 1998; Seoighe and Wolfe, 1999) that the *Saccharomyces sensu stricto* species have undergone a complete genome duplication during the course of their evolution. If this is so then it is not possible to know whether the homologue or the paralogue of a particular sample sequence has been found (this is because there may be differences in the pattern of gene loss during the evolution of the two species). Therefore, comparing WuBLASTn and ncbiBLASTn’s ‘top hits’ with Smith–Waterman does not give us the definitive answer; it is merely a guide. The correct homologue of a sample sequence cannot be definitely identified, until the whole genome has been sequenced.

**Conclusion**

WuBLASTn appears to be reasonably good at identifying the coding sequences in close homologues at the DNA sequence level (assuming the results of Smith–Waterman to be correct) and more effective than ncbiBLASTn, when default parameters are used. The main difference being that WuBLASTn finds longer alignments. The ability of WuBLASTn and ncbiBLASTn to detect distant homologues was not the subject of this trial.

**Table 4. Comparison of top alignments found for the sequence search algorithms as compared to Smith–Waterman**

| Top hits in common (out of 909) | WuBLASTn = Smith–Waterman | FASTA = Smith–Waterman | ncbiBLASTn = Smith–Waterman | WuBLASTn = ncbiBLASTn |
|---------------------------------|---------------------------|------------------------|----------------------------|-----------------------|
| All found no hits               | 0                         | 0                      | 0                          | 0                     |
| All found same top hits         | 536                        | 677                    | 641                        | 556                   |
| WuBLASTn = ncbiBLASTn           | 557                        |                        |                            |                       |

**Figure 5. Number of nucleotide matches needed to allow for a gap in an alignment**

**Table 5. Distribution and average alignment length for the 536 top hits found in common for all four sequence-search algorithms**

| Length of alignment (range) | WuBLASTn | ncbiBLASTn | Smith–Waterman | Fasta |
|----------------------------|-----------|------------|----------------|-------|
| Mean length                | 331.5     | 215.0      | 335.5          | 329.8 |
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