pfsearchV3: a code acceleration and heuristic to search PROSITE profiles

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ABSTRACT

Summary: The PROSITE resource provides a rich and well annotated source of signatures in the form of generalized profiles that allow protein domain detection and functional annotation. One of the major limiting factors in the application of PROSITE in genome and metagenome annotation pipelines is the time required to search protein sequence databases for putative matches. We describe an improved and optimized implementation of the PROSITE search tool pfsearch that, combined with a newly developed heuristic, addresses this limitation. On a modern x86_64 hyper-threaded quad-core desktop computer, the new pfsearchV3 is two orders of magnitude faster than the original algorithm.

Availability and implementation: Source code and binaries of pfsearchV3 are freely available for download at http://web.expasy.org/pftools/#pfsearchV3, implemented in C and supported on Linux. PROSITE generalized profiles including the heuristic cut-off scores are available at the same address.

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1 INTRODUCTION

Falling costs and continuing technological developments have led to a dramatic increase in the rate of sequencing of individual species genomes (Lindblad-Toh et al., 2011) and the diversity of the ecological niches sampled by metagenomic sequencing (Teeling and Glöckner, 2012). The identification, classification and functional annotation of the putative protein sequences encoded by these samples is essential to understand the diversity of the underlying biological systems, and will ultimately allow the construction of biological models that simulate and make testable predictions about their behaviour (Faust and Raes, 2012).

Most functional annotation is predicted using sequence homology-based methods that infer the function of uncharacterized protein sequences based on their similarity to characterized templates. These methods include generalized profiles and Hidden Markov Models (HMMs), which can detect more subtle homologies than pairwise sequence alignments (Park et al., 1998). The application of these computationally expensive methods on large datasets has been made feasible by the development of heuristics for sequence database search and faster more efficient code (e.g. Eddy, 2011).

Our PROSITE method combines manually constructed generalized profiles for efficient domain detection with rules for precise functional annotation (Sigrist et al., 2013). Here, we describe a new heuristic method and code optimization and parallelization for the PROSITE profile-sequence database search tool pfsearch. These developments increase the speed of pfsearch by two orders of magnitude using a modern x86_64 hyper-threaded quad-core computer (see Table 1 legend for specifications of the computer used in our tests), making the annotation of large sequence datasets using PROSITE feasible.

2 RESULTS AND DISCUSSION

2.1 Heuristics for generalized profiles

A major reduction in the execution time of sequence database searches can be achieved by an heuristic filter that selects sequences for the next CPU-expensive alignment step of the core algorithm. One such heuristic is the MSV algorithm of HMMER3, which computes the sum of multiple optimal ungapped local alignment segments (Eddy, 2011). Although extremely fast and convenient, the MSV heuristic filter cannot be directly transposed to generalized profiles that have a different model topology and are not bound to the probabilistic model restrictions of HMMs. We therefore developed a variant that is directly applicable to generalized profiles.

Our pfsearch heuristic, named prf/h, sums the maximal matching diagonals between the profile and the sequence, ignoring both gaps and the order of the matching diagonals. First, for each position i of the profile and j of the sequence, we define a score $S(i, j)$:

$$S(i, j) = \max \left[ S(i-1, j-1) + M(i, a_j), 0 \right]$$

where $M(i, a_j)$ is the match score read at position $i$ of the profile matrix table for residue $a_j$ observed at position $j$ of the sequence. Boundary scores $S(i, 0)$ and $S(0, j)$ are set to 0. Second, only the maximal scoring diagonal $S(i, j)$ is kept for every position $j$ of the sequence [the maximization part of Equation (2)]. All maxima are then summed to form the final heuristic score ($H_{score}$).

$$H_{score} = \sum_j (\max_i S(i, j))$$

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sequences. On a low quantile ensures a minimal loss of true-positive responding to the standard profile cut-offs (Fig. 1). The regression quantreg/), and use it to obtain the heuristic cut-offs corres-

one 5% quantile of the heuristic score distribution using the

An artificial set of mutants is generated from these, including

We randomly sample 200 sequences belonging to the original seed alignment for each profile (re-sampling if their number is <200), and generate a set of artificially mutated sequences from these, including indels, at various PAM distances. These artificial sequences (sharing from 40–85% sequence identity with their source) are then scored with both the standard profile scoring method and the heuristic (-heuristic) and the default mode of psearchV3, which uses all available cores with hyper-threading for a total of eight cores in our testing machine (no options -t and -k are used). NB: psearchV3 was run using an indexed sequence database (option -i); selecting this option reduces the execution time by 7 s in all experiments using the specified set of protein sequences.

The psearch and psearchV3 programs have been compiled on a Gentoo Linux (-mtune=x86_64 -march=x86_64 -fomit-frame-pointer -f2001) using gcc (4.6.3) and glibc (2.15) using the following compilation options: -O3 -enable-mmap -enable-thread-affinity. For compilation of the performance code, we use the -mtune=core2 -march=core2 -fno-omit-frame-pointer -f2001 -O2, and use sed to change the flag to -mtune=core2 -march=core2 -fno-omit-frame-pointer -O2 to support the core2 architecture and benefits from the SSE 4.1 instruction set when operating on a quad-core Intel® Core™ i7-3770 CPU @ 3.40 GHz with 8 Gb RAM running on Linux 3.2.0-4-amd64. The number of cores, the selection of the SSE and the selection or otherwise of the heuristic where specified at runtime with options -t, -s and -C, respectively, of psearchV3. Both psearch and psearchV3 have been run to produce the same output alignment, options -t and -k 2 respectively. (*) physical cores obtained with option -k and -t of psearchV3. (+) the default mode of psearchV3, which uses all available cores with hyper-threading for a total of eight cores in our testing machine (no options -t and -k are used). NB: psearchV3 was run using an indexed sequence database (option -i); selecting this option reduces the execution time by 7 s in all experiments using the specified set of protein sequences.

The Hscore distribution measured using PROSITE profiles on UniProtKB linearly correlates with the raw score distribution obtained using the standard psearch (R² ≈ 0.9 on average). To determine the appropriate Hscore cut-offs with respect to the normalized score cut-offs of each calibrated profile (Sigrist et al., 2002), we apply the following procedure. We randomly sample 200 sequences belonging to the original seed alignment for each profile (re-sampling if their number is <200), and generate a set of artificially mutated sequences from these, including indels, at various PAM distances. These artificial sequences (sharing from 40–85% sequence identity with their source) are then scored with both the standard profile scoring method and the heuristic (Fig. 1). We calculate the regression line on the lower 5% quantile of the heuristic score distribution using the quanteg R package (http://cran.r-project.org/web/packages/quanteg/), and use it to obtain the heuristic standard profile cut-offs corresponding to the standard profile cut-offs (Fig. 1). The regression on a low quantile ensures a minimal loss of true-positive sequences.

This method to fix the Hscore cut-offs was automatically applied on the PROSITE profiles. Manual inspection showed that this method was appropriate for the majority of the profiles, although in some cases, the Hscore cut-off could be manually increased to further accelerate the search. A minority of very short or ‘exotic’ profiles cannot be used with the heuristic. For these, no Hscore cut-off is defined in the profile, and the psearch software skips the heuristic search step.

The heuristic reduces the mean search database size by 96.7% (median 99.1%). The recovery of true positives is ≥98% for >99% of the PROSITE profiles with an associated Hscore cut-off (the lowest measured recovery is 92.6%). The majority of the missing true positives correspond to fragmentary sequences in UniProtKB.

### Table 1. Execution times to search the PROSITE profile PS0255 (CYTOCHROME_B5_2.) against 16 544 936 UniProtKB sequences (5 358 014 649 residues)

|                     | SSE2     | SSE4.1   | SSE2     | SSE4.1 |
|---------------------|----------|----------|----------|--------|
| psearch (v2.4)      | 51 s     | n.a.     | n.a.     | n.a.   |
| psearchV3 (1 core*) | 33 s     | 20 m     | 1 m 55 s | 1 m 44 s |
| psearchV3 (2 cores*)| 16 m 54 s| 10 m 23 s| 0 m 58 s | 0 m 53 s |
| psearchV3 (4 cores*)| 9 m 14 s | 5 m 40 s | 0 m 31 s | 0 m 28 s |
| psearchV3 (8 cores*)| 9 m 04 s | 5 m 28 s | 0 m 28 s | 0 m 27 s |

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All run times have been measured on a quad-core Intel® Core™ i7-3770 CPU @ 3.40 GHz with 8 Gb RAM running on Linux 3.2.0-4-amd64. The number of cores, the selection of the SSE and the selection or otherwise of the heuristic where specified at runtime with options -t, -s and -C, respectively, of pfsearchV3. Both pfsearch and pfsearchV3 have been run to produce the same output alignment, options -t and -k respectively. (*) physical cores obtained with option -k and -t of pfsearchV3. (+) the default mode of pfsearchV3, which uses all available cores with hyper-threading for a total of eight cores in our testing machine (no options -t and -k are used). NB: pfsearchV3 was run using an indexed sequence database (option -i); selecting this option reduces the execution time by 7 s in all experiments using the specified set of protein sequences.

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### 2.2 Software optimization and performance of the new pfsearch

Psearch has been rewritten and optimized in C from the original Fortran. The code will run on any x86_64 POSIX compliant architecture and benefits from the SSE 4.1 instruction set when available. However with the current source code, only Linux operating systems may benefit from CPU core affinity and file to memory mapping optimization, detected at compile time. The optimization process entirely reformatted the memory structure to allow vectorization. High level assembly code (intrinsic functions) was used to enforce the SSE2 and SSE4.1 instruction sets, leading to a 2-fold acceleration of the original Fortran (Table 1). SSE4.1 is particularly effective in reducing the execution time of the core psearch algorithm, while both SSE4.1 and SSE2 show similar performance on the heuristic filter (Table 1). This acceleration scales up with multithreading: on a dual hyper-threaded quad-core machine, we measured an average 10-fold improvement. The scaling is clearly related to the number of physical cores, with hyper-threading having only a minimal effect on performance (Table 1).
Multithreading implementation is straightforward because profile alignment versus a database is in itself an embarrassingly parallel task. For pfsearchV3, we implemented a master–slave mechanism to analyse and adapt the load before each phase of the algorithm (heuristic, filter, alignment), thus providing more equitable shares between threads. This has some constraints: sequences are read several times, but above all, they are no longer accessed sequentially, so an index of the sequences has to be either computed or loaded at start.

By combining the heuristic with our code optimization, we achieved a 100× increase in the speed of pfsearch on average. To search 16,544,936 UniProtKB sequences (5,358,014,649 residues) required a mean of 98 s/profile (median of 73 s/profile). A typical example of the runtime acceleration achieved is shown in Table 1.

The heuristic version of pfsearch can be used to annotate large sets of complete sequences in a reasonable amount of time on a modern workstation. For example, the human proteome can be searched with the totality of the PROSITE profile models in <4 hours, and this time can be drastically reduced on machines with a large number of CPU cores and/or computer clusters. For fragmented sequences, users may inactivate the heuristic to minimize loss of true-positive matches, in which case the speed of execution will be determined by the number of available CPU cores. We also plan to implement our heuristic search method in the HAMAP pipeline that provides high quality functional annotation for protein families (Pedruzzi et al., 2013).

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