Sequence analysis

**PSI-Search: iterative HOE-reduced profile SSEARCH searching**

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**Abstract**

Summary: Iterative similarity searches with PSI-BLAST position-specific score matrices (PSSMs) find many more homologs than single searches, but PSSMs can be contaminated when homologous alignments are extended into unrelated protein domains—homologous over-extension (HOE). PSI-Search combines an optimal Smith–Waterman local alignment sequence search, using SSEARCH, with the PSI-BLAST profile construction strategy. An optional sequence boundary-masking procedure, which prevents alignments from being extended after they are initially included, can reduce HOE errors in the PSSM profile. Preventing HOE improves selectivity for both PSI-BLAST and PSI-Search, but PSI-Search has ~4-fold better selectivity than PSI-BLAST and similar sensitivity at 50% and 60% family coverage. PSI-Search is also produces 2- to 4-fold fewer false-positives than JackHMMER, but is ~5% less sensitive.

Availability and implementation: PSI-Search is available from the authors as a standalone implementation written in Perl for Linux-compatible platforms. It is also available through a web interface (www.ebi.ac.uk/Tools/ssss/psisearch) and SOAP and REST Web Services (www.ebi.ac.uk/Tools/web/services).

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**1 INTRODUCTION**

PSI-BLAST (Altschul et al., 1997) uses an iterative strategy to construct a protein profile, in the form of a position-specific score matrix (PSSM), which dramatically improves homology detection in diverse protein families. Improved versions of PSI-BLAST have more accurate statistics and more sensitive consensus profiles (Agrawal et al., 2009; Altschul et al., 2005, 2009; Bharda et al., 2006; Li et al., 2011; Przybylski and Rost, 2008; Stojmirovic et al., 2008), but the most common cause of PSI-BLAST errors is contamination of the PSSM by extension of an homologous domain into a non-homologous region (homologous over-extension, HOE) (Gonzalez and Pearson, 2010a). Even searches with a single well-defined domain do not guarantee uncontaminated profiles (Kim et al., 2010). Some HOE errors can be reduced by ‘profile cleaning’; HangOut (Kim et al., 2010) focuses on long insertions, but requires insertion boundaries to be specified by the user, thus assuming a priori knowledge of the domain structure of the query protein.

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Here we present PSI-Search, an iterated profile search application for identifying distantly related protein sequences. PSI-Search is similar to PSI-BLAST, but substitutes a rigorous Smith–Waterman local alignment (Smith and Waterman, 1981) search strategy (SSEARCH, Pearson, 1991) to produce optimal local alignment scores from the profile PSSM. PSI-Search includes an optional alignment boundary-masking procedure that reduces HOE errors in the PSSM. SCANPS (Walth et al., 2008) implements a similar iterative search strategy using Smith–Waterman alignments; however, it does not currently scale to large protein databases and does not include boundary masking.

**2 METHODS**

In PSI-Search, library searches are performed with **search**, selected hit sequences from the result are processed with an automated sequence boundary-masking procedure, and PSSM profiles are built using **blastpgp**. The PSI-Search iteration workflow (Fig. 1a) iterates through search and alignment/PSSM construction steps:

1. The initial iteration is a normal **search** run with a sequence input.
2. During the second iteration, aligned sequences with statistically significant scores from the previous search are retrieved using **fastacidm**; details of the alignment boundaries are stored; sequence regions outside the boundaries are masked with ‘X’s to remove potential HOE regions; masked sequences are formatted into BLAST indexes using **formatdb** with an additional 10,000 random protein sequences created by **makeprotseq** (Rice et al., 2000); and a PSSM checkpoint constructed with a **blastpgp** search; finally **search** is run with the input sequence, using the generated PSSM, to complete the second iteration and output alignments.
3. Further iterations repeat Step (2). To avoid HOE, PSI-Search always uses the alignment boundary information from the first significant alignment in which a library sequence appears. Thus, if the first significant alignment with a library sequence aligns residues 25–125 at iteration i, later alignment boundaries at iteration i + 1 and beyond are ignored; only the initially aligned region (25–125) is used to form the PSSM.

**3 RESULTS**

Five iterative search strategies—PSI-BLAST (standard and HOE-reduced), PSI-Search (standard and HOE-reduced) and JackHMMER (Eddy, 2011)—were evaluated on the ReProtDom benchmark (Gonzalez and Pearson, 2010b) benchmark queries (500 sampled domain-embedded sequences) against the ReProtDom benchmark database using an E-value threshold of 0.001. JackHMMER is another iterative search tool that uses Hidden Markov Models.

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Weighted true-positives and false-positives are calculated as $1 - \frac{\text{fpf}}{\text{fpf} + \text{tpf}}$, where $\text{fpf}$ is the number of true positives (or false positives) at iteration 5 and $\text{tpf}$ is the total number of homologs for query $f$ in the RefProtDom benchmark database. Alignments containing HOEs with >50% of the alignment outside the homologous boundary are counted as both true and false positives.

(HMMs) (Johnson et al., 2010) rather than a PSSM. The output alignments from the fifth iteration were classified into true positives (TPs) and false positives (FPs, Fig. 1b). At 50% family coverage, PSI-Search reduces the weighted fraction of errors from 4.5% (PSI-BLAST) to 2.9% (PSI-Search). Reducing HOE improves sensitivity even more, to 1.7% for HOE-reduced PSI-BLAST and 0.5% for HOE-reduced PSI-Search. At 50% coverage, JackHMMER performs very well using its statistical alignment envelope, producing only 1% weighted FPs, but its selectivity is worse than PSI-Search or HOE-reduced PSI-Search at 60% and 75% coverage. Overall, HOE-reduced PSI-Search is 9-fold more sensitive than JackHMMER, HOE-reduced PSI-Search and PSI-Search respectively. Thus, (i) HOE-reduction greatly improves search selectivity with a small cost in sensitivity in both PSI-BLAST and PSI-Search; (ii) Both PSI-Search and JackHMMER are more sensitive and selective than PSI-BLAST; (iii) HOE-reduced PSI-Search is more selective, but slightly less sensitive, than JackHMMER. JackHMMER is the most sensitive tool, but HOE-reduced PSI-Search is the most selective iterative tool.

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Conflict of Interest: none declared.

REFERENCES


Schaffer, A. et al. (2009) HOE-reduced PSI-Search iteration workflow. (Fig. 1.)
