

# SUBSET SEED EXTENSION TO PROTEIN BLAST

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**Abstract:** The seeding technique became central in the theory of sequence alignment and there are several efficient tools applying seeds to DNA homology search. Recently, a concept of subset seeds has been proposed for similarity search in protein sequences.

We experimentally evaluate the applicability of subset seeds to protein homology search. We advocate the use of multiple subset seeds derived from a hierarchical tree of amino acid residues. Our method computes, by an evolutionary algorithm, seeds that are specifically designed for a given protein family. The representation of seeds by deterministic finite automata (DFAs) is developed and built into the NCBI-BLAST software. This extended tool, named SeedBLAST, is compared to the original NCBI-BLAST and PSI-BLAST on several protein families. Our results demonstrate a superiority of SeedBLAST in terms of efficiency, especially in the case of twilight zone hits.

SeedBLAST is an open source software freely available <http://bioputer.mimuw.edu.pl/papers/sblast>. Supplementary material and user manual are also provided.

## 1 INTRODUCTION

**Motivation.** Since the time complexity of the optimal alignment problem is quadratic (e.g., the Smith-Waterman algorithm (Smith and Waterman, 1981)), thus too large for everyday tasks, most of sequence aligning is done using heuristics, typically with the ubiquitous BLAST software (Altschul et al., 1990; Altschul et al., 1997). It runs in three phases, and the first of which finds short initial alignments, so called *hot spots*. Theory of seeds may be applied here, trying to answer the question: which short aligned segments are relevant for the indication of a true global alignment?

In the case of nucleotide sequences, *spaced seeds* have been intensively investigated and have successful applications: an improvement of BLASTN (Brejová et al., 2004), sensitive alignment tools like PatternHunter (Ma et al., 2002; Li et al., 2004) and Yass (Noe and Kucherov, 2005), automaton based theory for modeling and analyzing seeds (Kucherov et al., 2006; Buhler et al., 2005). The idea of using

*multiple seeds* is also widely recognized (Li et al., 2004; Brejová et al., 2005; Sun and Buhler, 2004; Kucherov et al., 2005). In this paper we attempt to achieve similar results for protein homology search. However, techniques based on spaced seeds seem not to apply directly to protein sequences, as the alphabet size is bigger (20 amino acids) and strict letter identity is much less relevant.

**Related Research.** The first phase of BLAST is a search for *hot spots*, i.e., short initial alignments of a query and a subject. Quite different methods are applied to define a hot spot for DNA and protein sequences. In the case of DNA, a hot spot is a short sequence of identically matching nucleotides. Application of seeds enables the consideration of non-identical matchings as well, and thus finding out previously overlooked good initial alignments. The reported increase of the efficiency of homology search is therefore quite expectable (Kisman et al., 2005; Shiryev et al., 2007).

In the case of protein sequences, a hot spot is

defined through a *cumulative* contribution of amino acid matches, not necessarily identical. A short sequence of such matches is considered a hot spot if their additive contribution (score) exceeds a predefined threshold. It is thus not clear whether seed-based approaches may measure up with the cumulative scores in expressibility and effectiveness. A first attempt to compare the two approaches has been done in (Roytberg et al., 2009), with the conclusion that *subset seeds* (Kucherov et al., 2006) may offer an attractive alternative to the “cumulative” approach of BLAST (cf. also discussion and references therein concerning expressibility of different classes of seeds). It is also argued that the algorithmic cost may thus be reduced, as application of seeds allows the use of a direct indexing scheme based on hashing. In addition, a comparison of subset seed technique with the vector seed approach (Brown, 2004) has been reported.

A fundamental notion in seed theory is an alignment alphabet, whose letters correspond to matching two residues. In the case of nucleotide sequences, the alignment alphabet has 6 (or 12, if directional) letters. In the case of amino acid sequences, however, the alignment alphabet has at least 200 letters, which makes exploration of even medium length sequences costly and difficult. A way of approaching the problem is to reduce the alignment alphabet, exploiting similarities among various amino acids (Roytberg et al., 2009). By applying the *subset seed* the complexity of alignment description may be reduced, while maintaining the biological information content. The idea of subset seeds (Kucherov et al., 2006), can be viewed as an intermediate concept between ordinary spaced seeds and vector seeds. In this approach different types of matches (or mismatches) are distinguished, as a seed letter corresponds to a subset of matches. In the case of protein sequences, for instance, it might be beneficial to distinguish mutations inside some predefined amino acid groups (like aliphatic, aromatic, tiny, etc. (Livingstone and Barton, 1993)) from mutations between these groups.

Deterministic finite automata (DFAs) find many applications related to homology search and seed theory. Early BLAST implementations investigated two hashing methods (Altschul et al., 1990) to efficiently manipulate the dictionary of hot spots. One of these was a dictionary organized as a DFA; this method was preferred until it has been abandoned in 1997 in NCBI-BLAST (Neuwald, 1998). DFAs are also known to be useful in constructing a *perfect hashing* function, in computing the value of a hashing function, or retrieving a value from a dictionary (Hopcroft and Ullman, 1979; Aho and Corasick,

1975). In (Kucherov et al., 2006) a special type of DFA called *probability transducer* was used for computation of seed sensitivity.

Recently the concept of *hash seed* for protein homology search has been proposed in (Li et al., 2009). It also applies amino acid grouping (in this case based on BLOSUM matrix) to designing good seeds that allow for a *direct hashing* scheme. The subset seeds, as considered in this paper, can be regarded as another (potentially more powerful) type of hash seeds.

**Our Contribution.** The overall aim of this paper is to experimentally confirm the value of applying seed-based hot spot search, using the approach of (Roytberg et al., 2009). It appears to be especially interesting in cases when the search is restricted to a particular protein family, as this opens the possibility of designing specialized seeds in order to increase the efficiency of the homology search. In short, as our technical contribution we propose a method of computing a well-performing multiple space seed, and present an implementation of a new seed-based hot spot search routine. Furthermore, we advocate the use of deterministic finite automata (DFAs) as a seed representation. Finally, we experimentally confirm a supremacy of this new approach over the original NCBI-BLAST hot spot search.

We investigate, and search for, reduced alignment alphabets, called *seed alphabets*, that can be derived from hierarchical trees of amino acids. Such trees were designed, e.g., in (Li et al., 2003; Murphy et al., 2000); for our purposes we compute, (by amino acids clustering), a specific tree for a given protein family. An advantage of using hierarchical trees is that the alphabets are always *transitive* (i.e., each letter corresponds to a transitive set of matching pairs) and thus enable application of the direct hashing scheme.

We search for a well-performing alphabet and a multiple subset seed over it with the use of an evolutionary algorithm. The fitness evaluation is based on computing the seed sensitivity and selectivity in the way suggested in (Kucherov et al., 2006).

The multiple seed, represented as a DFA, is then used in the hot spot search of BLAST. We have implemented an extension to the NCBI-BLAST software, called SeedBLAST, that accepts a multiple subset seed as its input parameter. The extension is written in C++, relies on the template mechanism, and is prone to compiler optimizations (most functions can be *inlined*). An important advantage of our implementation is that being developed within the NCBI-BLAST framework, it inherits all stable and tested features of this implementation.

The first test results can be perceived as promis-

ing: although our multiple seed selection method is rather simplistic, our tool returns more interesting hits than the standard BLAST with comparable settings. Some returned hits tend to be long although having only medium E-value, the type of hits known to be dimmed and not reported by BLAST. This kind of hits is termed *twilight zone* after (Rost, 1999).

Furthermore, this methodology can be useful for searching for particular type of alignments. Given a set of alignments, one can construct a specific seed automaton and perform database search for this certain type of alignments. Following this idea we investigated the ability to align known structurally homologous domains of the Rhodopsin family of G-protein coupled receptors (GPCRs). The outcome of our experiment showed a significant difference between NCBI-BLAST and SeedBLAST, in favor to the latter: our method yielded much longer alignments covering up to 70% of the entire domain, even for proteins sharing low sequence identity (20-30%).

## 2 SUBSET SEED DESIGN

**General Approach.** Given a protein family, we assume that a small representative subset of this family has already been aligned well (for example manually by experts), and is available as a training set. The algorithm designing subset seed attempt to extract information about the structure of the family from this set, and use it to produce alignments for the entire family. In the first phase a hierarchical tree is constructed that represents similarities of amino acids. Then, a *seed alphabet* is designed, along with a set of *seeds*. This is a learning phase, and runs independently of our BLAST enhancement. Next, the seed alphabet along with the corresponding set of seeds is used by the SeedBLAST algorithm to find hot spots. Afterwards, the computation of SeedBLAST follows the standard BLAST scheme.

**Hierarchical Tree of Amino Acids.** Let  $\Sigma = \{A, C, D, \dots\}$  be the amino acid alphabet ( $|\Sigma| = 20$ ). A valid hierarchical tree of amino acids is a binary tree whose leaves are labeled bijectively by elements of  $\Sigma$ , and whose every internal (non-leaf) node has two children. An example of such a tree is shown in Figure 1. The specific tree used e. g. for Rhodopsin family is presented in supplementary material. Such a tree constitutes a parameter in our approach; we assume that it corresponds to some biologically significant hierarchical clustering of amino acid residues, c.f. (Murphy et al., 2000; Li et al., 2003).

Any non-leaf node  $v$  of  $T$  is represented by a set

of (labels of) leaves in the subtree rooted in  $v$ . This set is denoted by  $\Sigma_v$ . In particular, the root is labeled by the whole set  $\Sigma$ . There are precisely  $|\Sigma| - 1 = 19$  non-leaf nodes.

Our basic intuition is as follows. Think of a leaf labeled by  $A \in \Sigma$  as a representation of the exact match  $A-A$ . Then a node  $v$  represents all matches  $A-B$  for  $A, B \in \Sigma_v$ .

The tree is obtained from the training set of alignments in the following way: first, for each pair of amino acids the number of times they have been aligned one with another is counted, and then, using those counts, the amino acids are hierarchically clustered through neighbor-joining method.

**Seed Alphabets and Seeds.** From now on we assume a fixed hierarchical tree  $T$ . The tree nodes are partially ordered by a natural ordering induced by the tree structure (we call it *tree ordering*). This coincides with the inclusion ordering of the labeling sets:  $v_1 \leq v_2 \iff \Sigma_{v_1} \subseteq \Sigma_{v_2}$ . We assume here for technical convenience that the leaves are labeled by singletons  $\{A\}$  instead of single amino-acids  $A \in \Sigma$ . Below we consider sets of nodes of  $T$ , ordered by inclusion as well. Certain sets of nodes will be *seed letters* (potential elements of a *seed alphabet*).

A *seed letter* is defined as any subset  $\alpha$  of nodes such that:

- (i) (*maximality*)  $\alpha$  contains all leaves and
- (ii) (*downward closedness*) whenever  $v \in \alpha$  and  $v' < v$  then  $v' \in \alpha$ .

Hence, a single seed letter  $\alpha$  is defined as a lower set of a maximal antichain wrt. the tree ordering. This antichain contains the maximal elements of  $\alpha$  wrt. the tree ordering and may be visualized by a *horizontal cut through the tree*  $T$ . Seed letters are naturally ordered by inclusion. The smallest one is the "exact match" seed letter  $\#$ , containing only the leaves. The largest one is the "don't care" seed letter  $\_$ , containing all the nodes of  $T$ . One particular seed letter, denoted by  $@$ , is obtained by removing from  $\_$  the root node. We place an additional restriction on alphabets that we use, that they must contain both  $\#$  and  $\_$ .

The maximal elements of a seed letter  $\alpha$  wrt. the tree ordering form a partition of  $\Sigma$ . Thus  $\alpha$  represents naturally an equivalence relation on  $\Sigma$ :  $A$  and  $B$  are related iff they belong jointly to some node of  $\alpha$ ; i.e., iff there exists some  $v \in \alpha$  such that  $A \in \Sigma_v$  and  $B \in \Sigma_v$ . We feel free to write  $(A, B) \in \alpha$  in this case. The induced equivalence is identity relation in case of  $\#$  and full relation in case of  $\_$ . The inclusion ordering of seed letters coincides with the inclusion of the induced equivalences.

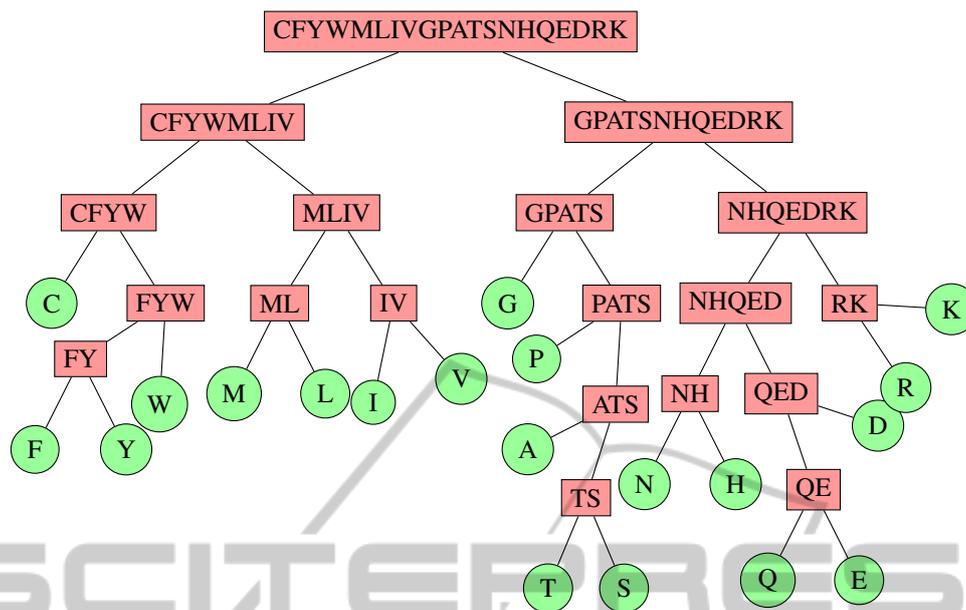


Figure 1: The hierarchical tree of amino acids proposed by (Li et al., 2003).

Certain families of seed letters will be allowed as seed alphabets. Essentially, we forbid two letters  $\alpha_1, \alpha_2$  that are incomparable by inclusion. A *seed alphabet* is a family  $\mathbb{A}$  of seed letters totally ordered by inclusion: for each  $\alpha_1, \alpha_2 \in \mathbb{A}$ , either  $\alpha_1 \subseteq \alpha_2$  or  $\alpha_2 \subseteq \alpha_1$ . Alphabets with this property are called *hierarchical* in (Roytberg et al., 2009). We used this assumption as it leads to a nice mathematical formalization, namely the family of seed alphabets forms a constrained independence system (Korte and Hausmann, 1978; Cheng and Xu, 1995). We show that even with this restriction very efficient seeds can be obtained. Thus, in this paper we will not consider non-hierarchical alphabets. Note that, again, the seed alphabets may be naturally ordered by inclusion as well.

We define a *seed* over a seed alphabet  $\mathbb{A}$  as a finite word over  $\mathbb{A}$ . A *multiple seed* is a pair consisting of a seed alphabet and a set of seeds over that alphabet.

We say that a seed  $s = s_1 s_2 \dots s_n$  aligns two amino acid sequences  $a = a_1 a_2 \dots a_n, b = b_1 b_2 \dots b_n$ , if and only if for all  $i \in \{1, 2, \dots, n\}, (a_i, b_i) \in s_i$ .

Foreground sensitivity (or just sensitivity) of a multiple seed  $M$ , denoted by  $\text{sens}^F(M)$ , is the number of positions in the training set of alignments matched by at least one of the seeds from  $M$ , divided by the total number of positions. Foreground sensitivity is computed directly from the training set.

Background sensitivity of a seed corresponds to the probability of matching two aligned random sequences. We assume that the background model for amino acid sequences is given as a Markov chain.

For our experiments, the Markov chain models of orders 1, 2 and 3 were learned from the TrEMBL database (Boeckmann et al., 2003) using GenRGenS Java tool (Ponty et al., 2006). The background sensitivity of a seed was computed with the use of Markovian probability transducer as described in (Kucherov et al., 2006). Background sensitivity of a multiple seed  $M$ , denoted by  $\text{sens}^B(M)$ , is estimated from above by the sum of background sensitivities of each of the individual seeds in  $M$  (the estimation is sharp only if seed occurrences are independent).

**Evolutionary Approach.** Optimizing multiple seeds is recognized as a highly non-trivial task (Yang et al., 2004; Buhler et al., 2005; Ma and Yao, 2008). In the case of hierarchical subset seeds the combinatorial structure of seed alphabets suggests hardness of the optimization problem (see (Roytberg et al., 2009) for details). Therefore we decided to use an efficient heuristic algorithm.

In the proposed approach seed alphabets and seeds are simultaneously chosen through an application of a genetic algorithm. The genetic algorithms are used to solve various optimization problems (Mitchell, 1996). They work by first generating a random multiset (*initial population*) of potential solutions, evaluating the function being optimized (*fitness function*) for each one of them, culling a percentage of them with low values of such function, cloning and slightly altering (mutating) the rest at random – and repeating this process until a satisfactory solution is obtained.

In our case, the potential solutions are pairs: a seed alphabet, and a set of seeds. A mutation applies thus either to the alphabet, or to one of the seeds. Mutating the alphabet is one of the following: deleting a randomly chosen letter (except for the top # and bottom - one), altering a letter (by adding a tree node to it, or removing a tree node - but only if that would not violate the constraint that the alphabet must be hierarchical), or adding a random (non-conflicting) letter. While modifying the letter one has to respect its definition, i.e. the (i) *maximality* and (ii) *downward closedness* conditions. Mutating the set of seeds means either deleting one of the seeds, adding a random seed, or replacing a random letter in a random seed by one of its neighbors in the alphabet. Algorithm 1 explains the details.

A multiple seed may contain individual seeds of different lengths in general. However to simplify and speed-up the computations we have decided to fix the length; all individual seeds computed by the evolutionary algorithms have the same length  $W = 5$ .

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**Algorithm 1:** Genetic Algorithm.

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**Input:** Protein family F  
**Output:** A multiple seed for family F  
**begin**  
 Population  $\leftarrow$  a multiset of 100 randomly chosen multiple seeds (the initial population);  
**while** *Not Run Out Of Time* **do**  
   **foreach** *multiple seed*  $M \in$  Population **do**  
      $f \leftarrow \text{fitness}_F(M)$ ;  
     Randomly, based on  $f$ , choose one of the following:  

- Population  $\leftarrow$  Population  $\setminus \{M\}$ ;  
   - with increasing probability for low values of  $f$
- Population  $\leftarrow$  Population  $\setminus \{M\} \cup \{\text{Mutate}(M)\}$ ;
- Population  $\leftarrow$  Population  $\cup \{\text{Mutate}(M)\}$ ; - with increasing probability for high values of  $f$

**end**  
   **end**  
**end**  
**return** *the member of Population that maximizes fitness<sub>F</sub>*  
**end**

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The most important aspect of every optimization algorithm, a genetic algorithm being no exception, is the fitness function chosen. Usually, what we want to

obtain is a seed that has as low background sensitivity as possible, while at the same time having as high foreground sensitivity as possible. So, the first idea might be to choose the following function:

$$\text{fitness}_1(M) = \frac{\text{sens}^F(M)}{\text{sens}^B(M)}.$$

This, however, yields unsatisfactory results - the evolution just results in a smallest multiple seed possible, with minuscule foreground and background sensitivity.

The fitness function has to reflect the trade-off between foreground sensitivity and background sensitivity. It should be noted that both of these play similar role to NCBI-BLAST '-f' parameter (i.e. the threshold for the cumulative score of three hit positions). The '-f' parameter allows one to adjust the length of computation, and the quality of results. With SeedBLAST it has been split in two - the  $\text{sens}^F(M)$  part is responsible for the quality of results, while  $\text{sens}^B(M)$  is responsible for the length of computation. Keeping that in mind, we can select a fitness function that can match our needs - using it, we can in effect specify 'give me the best results you can achieve within a given time-frame' - or, the opposite - 'give me results at least this good, and I don't care how long it takes to compute them'. Or everything in-between.

An example of fitness function that adheres to the first approach might be as follows:

$$\text{fitness}_2(M) = \begin{cases} 0 & \text{if } \text{sens}^B(M) > c \\ \text{sens}^F(M) & \text{otherwise} \end{cases}$$

The second approach is fulfilled by the following fitness function:

$$\text{fitness}_3(M) = \begin{cases} \text{sens}^F(M) & \text{if } \text{sens}^F(M) < c \\ \frac{\text{sens}^F(M)}{\text{sens}^B(M)} & \text{otherwise} \end{cases}$$

Another appropriate fitness functions and their evolution paths are presented in supplementary material.

For further tests, described in the rest of the paper, we have chosen the function  $\text{fitness}_3$ , with  $c = 0.15$ ; except for the performance evaluation, where we prefer to use  $\text{fitness}_2$  (in order to make the fair comparison with NCBI-BLAST).

This decision was taken through trial and error - there is no guarantee that this is the optimal choice. The multiple seed that was computed and used for further experiments exhibits foreground sensitivity equal to 0.179906, and background sensitivity equal to 0.01047971. The underlying alphabet is presented in supplementary material; the whole multiple seed, consisting of 3686 individual seeds, is not subject to a concise presentation.

### 3 SeedBLAST: EXTENSION OF NCBI-BLAST

Given a query, the goal of the first phase of the BLAST algorithm is to index all subwords of length  $W$  (chosen as a parameter). Not only exact subwords are indexed but also their predefined neighborhoods, with respect to a metric determined by the cumulative score according to the BLOSUM matrix. With each query, the occurrences of the neighborhoods are stored in a dictionary-type data structure; current version of NCBI-BLAST uses a hash table.

The size of neighborhood is crucial as it must be stored in a dictionary. BLAST uses a threshold on the BLOSUM score of an alignment of a segment pair. The threshold represents the trade-off between sensitivity and time and memory efficiency since it has a direct impact on the number of analyzed hits. The default threshold was adjusted experimentally by the BLAST developers and currently equals 11 in protein NCBI-BLAST.

We seek to describe the neighborhood using our selected multiple seed. In principle, the method may be applied to any multiple seed, possibly containing words of different lengths. However, in the case study described in the following section, all the seeds have the same length  $W = 5$ . Moreover, all individual seeds are constructed over the same alphabet. This assumption greatly simplifies the seed design and allows to construct a single automaton for looking for all hot spots simultaneously.

#### 3.1 Hot Spot Search using DFA

A *trie*, or a *prefix tree*, is a dictionary with a tree-structured transition graph, in which the start node is the root and all the leaves are final nodes (Liang, 1983). Tries are especially convenient when the keys are short strings: the tree edges are labeled by letters, and retrieving a value assigned to a given key  $w$  is done by following the  $w$ -labeled path in the tree, thus very efficient.

It is assumed that labels of edges outgoing from a node are all different. A trie may be thus seen as an acyclic DFA recognizing a finite language (the language contains labels of all the paths going from the root to a leaf). Upon acceptance, the automaton in addition returns the value assigned to a word read (being a key). In our case, the value will be typically a set of positions in a query.

In our algorithm, to be described below, we construct a number of different tries (automata). To optimize for memory, on the implementation level we always conform to the *Mealy paradigm* of keeping

values attached to transitions, not vertices.

In a preprocessing phase a trie  $S$  is constructed to represent the multiple seed. Its input alphabet is the seed alphabet  $\mathbb{A}$ .

Next, we proceed with constructing a trie  $Q$ , over the input alphabet  $\Sigma$ , that keeps all subwords of length  $W$  from a given query. For each such word we store in  $Q$  pointers to all positions in query where it appears. This will reduce operations in the following phases. It is worth noting that  $Q$  may be used to process jointly multiple queries. Analogously, NCBI-BLAST also permits many queries to be stored jointly in its hash table.

As a consecutive step, a trie  $N$  is built to store neighborhoods. Its alphabet is  $\Sigma$ , and language is given by

$$N = Q \times S :=$$

$$\{w \mid \text{for some } q \in Q \text{ and } s \in S, s \text{ aligns } q \text{ and } w\}.$$

The trie  $N$  is constructed by systematically traversing a *product* of  $Q$  and  $S$ . The value assigned to a word  $w$  in  $N$  denotes, similarly as in  $Q$ , a set of positions in the query. It is given by the union of values assigned to  $q$  in  $Q$ , for all  $q$  ranging over

$$\{q \in Q \mid \text{for some } s \in S, s \text{ aligns } q \text{ and } w\}.$$

On the implementation level, the union is represented by a suitable pointer data structure.

Finally we construct an automaton  $H$  over the alphabet  $\Sigma$ , whose aim is to find hot spots in the subject sequences. Operation of  $H$  is similar to a pattern-matching automaton. It is built on the basis of the automaton  $N$ , by adding additional edges outgoing from the final (leaf) nodes. To easily explain the construction, we recall that each node of  $N$  is uniquely determined by the labeling of the path from the root to that node. Fix a leaf determined by  $w$  and a letter  $\alpha \in \Sigma$ ; the outgoing  $\alpha$ -labeled edge will point to a node determined by the longest suffix of  $w\alpha$  that belongs to  $N$ . Clearly, in contrast to all other automata,  $H$  may have cycles.

Having constructed  $H$ , next BLAST phases remain unchanged. Each subject sequence is traversed along, starting from the root of  $H$ . At each step, the value assigned to the current node (state) of  $H$  informs whether any hot spots are found at the current position in a subject. If so, the hot spots are stored for further processing in the following phases of BLAST.

## 4 EXPERIMENTS

**Datasets.** We used a dataset extracted from the Pfam database, that contains expert-made protein structural families and their multi-alignments,

later extended to larger families using *profile-HMMs* (Bateman et al., 2002; Finn et al., 2008).

A protein family, exhibiting low identity percentage, has been selected from Pfam (namely PF00001). This family contains, amongst other G-protein-coupled receptors (GPCRs), members of the opsin family, which have been considered to be typical members of the rhodopsin superfamily. They share several motifs, mainly the seven transmembrane helices (7tm\_1 domain). This domain will be the main focus of our experiment.

The rhodopsin-like GPCRs themselves represent a widespread protein family that includes hormone, neurotransmitter and light receptors, all of which transduce extracellular signals through interaction with guanine nucleotide-binding (G) proteins. Although their activating ligands vary widely in structure and character, the receptors are believed to adopt a common structural framework comprising 7 transmembrane helices.

The expert-made multi alignment of 7tm\_1 domains from 64 of the family members was downloaded (the whole family contains 16975 proteins), and used as a training set to obtain a multiple seed. The latter was subsequently used by the SeedBLAST algorithm to compute pair-wise alignments of the 7tm\_1 domain of all the family members. The results were compared with those obtained by the standard BLAST algorithm.

For a fair comparison, it had to be ensured that both algorithms actually run with the same background sensitivity. Thus, the '-f' parameter of NCBI-BLAST was adjusted in the course of the experiment to obtain similar background sensitivity to that of the multiple seed used by SeedBLAST. The table shows typical values of the '-f' parameter together with the corresponding values of background sensitivity:

f parameter of NCBI-BLAST	SeedBLAST background sensitivity
11 (default)	0.002195
10	0.005342
9	0.00816
8	0.012276
7	0.018163

The background sensitivity of the seed used by SeedBLAST was 0.01047971; this corresponds to 8 or 9 as the value of the '-f' parameter in the NCBI-BLAST invocations.

As the alignment concerned only the domain fragment of each protein, and the domain is already known to be the same in each protein, every alignment found should be considered biologically significant.

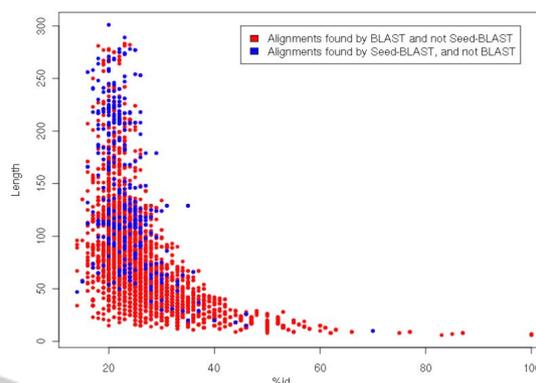


Figure 2: Symmetric difference between outputs of BLAST and SeedBLAST.

**Comparing Efficiency.** Figure 2 shows the symmetric difference between hits found by NCBI-BLAST, and those found by SeedBLAST (that is, alignments found by one of the algorithms but not the other).

We observe that alignments found by SeedBLAST are in general longer than those found by BLAST, and thus provide better coverage of the domain. Especially many alignments that have not been found by BLAST lie in the so-called twilight zone (Rost, 1999) – namely long alignments with low identity percentage, and thus low E-value, that nevertheless are biologically significant. The reason why SeedBLAST constructs longer alignments is that it detects much more biologically significant hot-spots. A supremacy of SeedBLAST becomes more apparent in view of Figure 3. To obtain this diagram, pairs of domains for which BLAST and SeedBLAST found different alignments were chosen from the set of all alignments, and the coverage of domains by these alignments was computed. We conclude that SeedBLAST is much more efficient in providing biologically significant alignments than the standard BLAST algorithm.

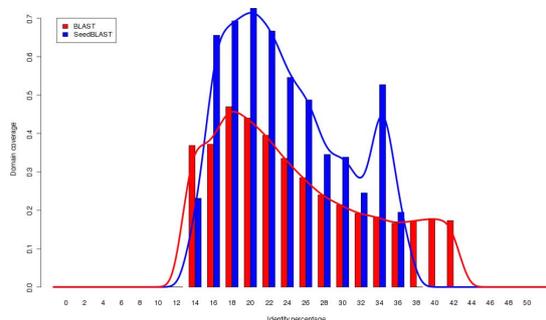


Figure 3: Domain coverage by alignments found by BLAST and SeedBLAST.

The reason for SeedBLAST's improved efficiency

is the inclusion of subset seeds specifically tuned for the domain under consideration. When one aims at aligning different family of proteins appropriate multiple seed should be designed and used in SeedBLAST. We conclude that SeedBLAST appears much more efficient than BLAST in recognizing protein domains, as it is both more effective in covering the entire domain as well as much less likely to cover anything beyond the sought-for domain.

Table 1:  $f$ : -f Parameter of NCBI-BLAST  $c$ : corresponding background sensitivity (parameter  $c$ ) used in seed design,  $sens^B(M)$ : actual background sensitivity of the multiple seed SeedBLAST, NCBI-BLAST: running time of NCBI-BLAST, SeedBLAST: running time of SeedBLAST.

$f$	$c$	$sens^B(M)$	NCBI-BLAST (sec.)	Seed-BLAST (sec.)
15	0.000306	0.00030564	1.20	0.45
11	0.002195	0.00211789	3.32	1.40
8	0.012276	0.01156471	12.17	4.74
5	0.026406	0.02622019	23.84	12.40

**Comparing Running Time.** In addition, SeedBLAST and NCBI-BLAST were compared with respect to their running time (preprocessing, i. e. seed design phase is not included). For a fair comparison, again, we had to ensure that both algorithms actually run with the same background sensitivity.

In case of SeedBLAST, we had to be able to control the background sensitivity of the multiple seed used. This led us to choose the fitness function:

$$fitness_2(M) = \begin{cases} 0 & \text{if } sens^B(M) > c \\ sens^F(M) & \text{otherwise} \end{cases}$$

(cf. Section 2) that seems to suit best to this purpose: the parameter  $c$  corresponds directly to the desired background sensitivity of the multiple seed.

In case of NCBI-BLAST, its background sensitivity can be adjusted by the '-f' parameter. For the test, we picked several different values of the '-f' parameter, and then calculated the background sensitivities induced by these values. These background sensitivities were taken as the value of the  $c$  parameter in the above fitness function, exploited in the computation of multiple seeds used by SeedBLAST.

The results are summarized in the Table 1. In fact, because of the unpredictable nature of multiple seed evolution, we can't control the background sensitivity of a multiple seed exactly. This gives SeedBLAST a slight advantage over the other, represented by the difference between the  $c$  parameter (equal to the actual

background sensitivity NCBI-BLAST runs with), and the background sensitivity of an obtained multiple seed. Still, even accounting for this slight difference, the results show that SeedBLAST algorithm is over two times faster than NCBI-BLAST on average. As the SeedBLAST is an extension of NCBI-BLAST we conclude, that the speed-up is achieved due to faster hot-spot identification stage. We argue that the multiple seed approach enables to detect biologically significant hot-spots, e.g. those corresponding to functional residues (Oliveira et al., 1993).

It is worth mentioning here that the performance of SeedBLAST (being the extension of standard NCBI-BLAST implementation) is comparable with the performance of subset seed based tools that use parallel implementation or specialized hardware (Petterlongo et al., 2008; Nguyen and Lavenier, 2008).

**Comparison with PSI-BLAST.** One can see the close similarity between the seed approach and position specific scoring matrices used to improve homology search. Therefore we decided to compare the selectivity and sensitivity of SeedBLAST to the efficiency of popular PSI-BLAST algorithm. The experiment was performed on two protein families (Surface antigen - PF01617 and Globin - PF00042). The performance of both algorithms on Globin family was almost identical (data not shown). On the other hand on Antigen family SeedBLAST achieved much better selectivity while keeping the same level of sensitivity (c. f. Fig.4).

## 5 FURTHER RESEARCH

Further experiments using in-sample, out-of-sample tests and larger families are needed. At this stage the performance of SeedBLAST is comparable with BLAST running with low threshold for hot spots. We hope that this result can be improved if more advanced methods for construction of multiple seeds are used, and longer seeds (i.e., of length  $> 5$ ) are included.

The remaining goal is to develop a method of seed construction that would keep sensitivity high and improve selectivity. That would eventually reduce the running time and memory requirements for greater seed lengths.

Protein homology search requires unavoidably storing a large dictionary in memory. Hence it seems to be worth pursuing ideas from (Kahveci and Singh, 2001), where a novel method of compression, based on *wavelets*, was proposed for dictionaries of words. Another possible improvement could be integration

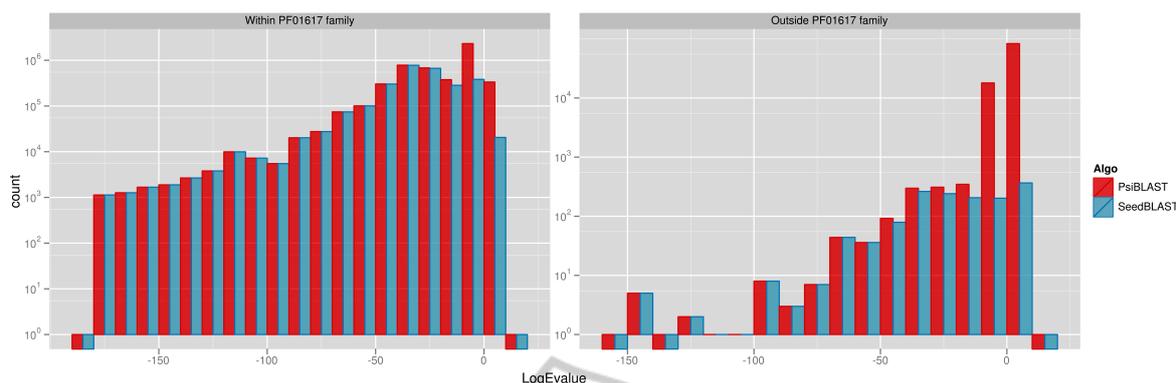


Figure 4: Histogram of found alignments grouped by logarithm of their E-value. We can observe that within the Antigen family SeedBLAST finds all of the alignments that PSI-BLAST does (except for a small fraction of some non-significant ones with E-values of 1 and more). Histogram on the right side shows results of alignment of proteins which we know to be unrelated with Antigens. We can see that SeedBLAST finds less non-homology-related alignments than PSI-BLAST does.

of the *cache-conscious* hashing DFA to improve efficiency of page-swapping, as described in (Cameron et al., 2006). However, we would like to recall here that our overall goal of investigating the seed-based hot spot search was to reduce the need for large information storage by choosing only those hits that seem important.

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