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FeatureScan: revealing property-dependent similarity of nucleotide sequences

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ABSTRACT

FeatureScan is a software package aiming to reveal novel types of DNA sequence similarity by comparing physico-chemical properties. Thirty-eight different parameters of DNA double strands such as charge, melting enthalpy, conformational parameters and the like are provided. As input FeatureScan requires two sequences, a pattern sequence and a target sequence, search conditions are set by selecting a specific DNA parameter and a threshold value. Search results are displayed in FASTA format and directly linked to external genome databases/browsers (ENSEMBL, NCBI, UCSC). An Internet version of FeatureScan is accessible at http://genome.gbf.de/featurescan/. As part of the HOBIT initiative (http://hobit.sourceforge.net/) FeatureScan is also accessible as a web service at its above home page. Currently, several preloaded genomes are provided at this Internet website (*Homo sapiens*, *Mus musculus*, *Rattus norvegicus* and four strains of *Escherichia coli*) as target sequences. Standalone executables of FeatureScan are available on request.

INTRODUCTION

The principle of similarity measurement lies behind any recognition method or sequence analysis. Motifs, weight matrices, Markov models significantly differ in mathematical background (1–4), but they all rely on more or less sophisticated statistics of four mnemonic letters (A, T, G, C). Even the most complex of such methods are still not able to specifically and accurately solve the problem of detecting regulatory DNA sequences.

FeatureScan is based *ab initio* on a different principle. It works with numerical sequences which describe specific properties of DNA and utilizes methods from signal theory (5) to compare them. One convincing argument of many in favour of such an approach is that it has been observed that the binding sites for the HFN1 transcription factor require its own specific melting characteristic of the surrounding region (6). For an appropriate modelling of this fact, it appears adequate to consider melting enthalpy of DNA rather than the bare alphabetic sequence.

METHODS

Algorithmically, FeatureScan originates from proven methodologies in image analysis (7) and speech recognition (8). The current implementation is based on a convolution method (9) and can be described briefly in three main steps (for the detailed theoretical background see our earlier publication (10)).

First is a transformation of nucleotide sequences (pattern and target sequence) into numerical form, which we refer to as signals. At this step users have to decide which property may play an important role in their specific cases [see (11) and below].

Second is a computation of the correlation integral (1) of two signals f and g, which can be rewritten using Fourier transformants F and G yielding (3). Assuming having direct and inverse Fourier transformations implemented (in our case it is optionally hardware implemented), this step is reduced to just a multiplication. The final step is looking for shift values y which will define possible matches of the sequences. The difference between correlation (3) and

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autocorrelation (2) integrals must be less than the predefined threshold.

$$\mathrm{Corr}(y) = \int f(x)\cdot g(x-y)\,\mathrm{d}x, \quad \mathbf{1}$$

$$\mathrm{AutoCorr} = \int g(x)\cdot g(x)\,\mathrm{d}x, \quad \mathbf{2}$$

$$\mathrm{Corr}(y) = \mathrm{InverseFourier}T\{F(y)\cdot\overline{G(y)}\}. \quad \mathbf{3}$$

PROGRAMME/WEBSITE DESCRIPTION

FeatureScan is available as a local standalone application and via Internet, both having equivalent core functionality. The local, command-line version lends itself to extensive batch processing. The web service (following HOBIT standards, http://hobit.sourceforge.net/) offers an additional advantage: using the optional hardware acceleration (fast Fourier transformation PowerFFT card from Eonic, www.eonic.com) boosts the entire procedure up to 470 times compared with a 1.7 GHz AMD processor PC alone. This allows scanning, for example, through the entire human genome in ~3 min. The core programme is written in compliance with ANSI C standards and can be compiled on many platforms [tested under Windows, Linux (RedHat, SuSe), FreeBSD]. Currently, there are different implementation-dependent limitations with respect to the maximum length of the sequences (see FeatuteScan help page).

To run FeatureScan via Internet, users have to provide a pattern sequence (field 1 in Figure 1a) and a target sequence (field 2 in Figure 1a). All sequences must be in one of the following formats: EMBL, FASTA or plain text. A target sequence can either be uploaded or selected from the preloaded human, mouse, rat or *Escherichia coli* genomes. A pattern sequence is assumed not to be longer than a target sequence. In field 3 (Figure 1a) it is specified which of the 38 DNA parameters will be used in the analysis. A threshold value needs to be entered in field 4 (Figure 1a) which defines the stringency of the analysis run. It must be noted here that due to the entirely different theoretical background the similarity values are not always suited for a 1:1 comparison to BLAST-type similarity values [for further details see (11)]. For a first brief inspection of the programme the user may wish to choose our example.

An important step of the procedure is selecting a suitable property. As mentioned before, 38 parameters of DNA double strand, comprising physico-chemical (melting temperature, entropy and others) and conformational (roll, tilt, slide, twist and others) DNA characteristics, have been collected in a public database (6). A brief overview of a few of the parameters is given in Table 1 and of all of them on the FeatureScan help page. It should be pointed out that the datasets cannot be ordered by their recognition power (by selectivity, false positive rate and others) to be able to recommend one universally accepted best parameter. In numerous examples it has been demonstrated that various functions of different DNA *loci* are implicated by various DNA physical properties (6). Thus, we do not recommend any 'best-recognizing' parameter to the user. Instead, we suggest the user to speculate about the possible background of his specific task and then choose the most relevant parameter.

As a last rescue, we would recommend to start with '*MeltEnthalpyBreslauer*', because melting often plays a role in DNA processing.

Results of the search are either displayed in a table or in FASTA format, which makes further analysis more comfortable (Figure 1b). The name line (following '>') consists of consecutive number, similarity value and starting position of a matched subsequence in the submitted target sequence. For pre-loaded genome sequences, links to the corresponding sequences in the external databases are also added to the name.

FeatureScan WEB SERVICE

To take full advantage of the accelerating power of the FFT card and to avoid the tedious routine of typing sequences and parameters on an Internet page, the FeatureScan web service was developed. Such Internet services allow user programmes to directly call FeatureScan on a remote machine as if it was a local function. The key point of such web services is an interface (between user and server programmes), the standardized description of the number and format of parameters which are passed on from the user programme to FeatureScan and a returned result. Our implementation of the interface complies with the rules of the HOBIT initiative (http://hobit.sourceforge.net/).

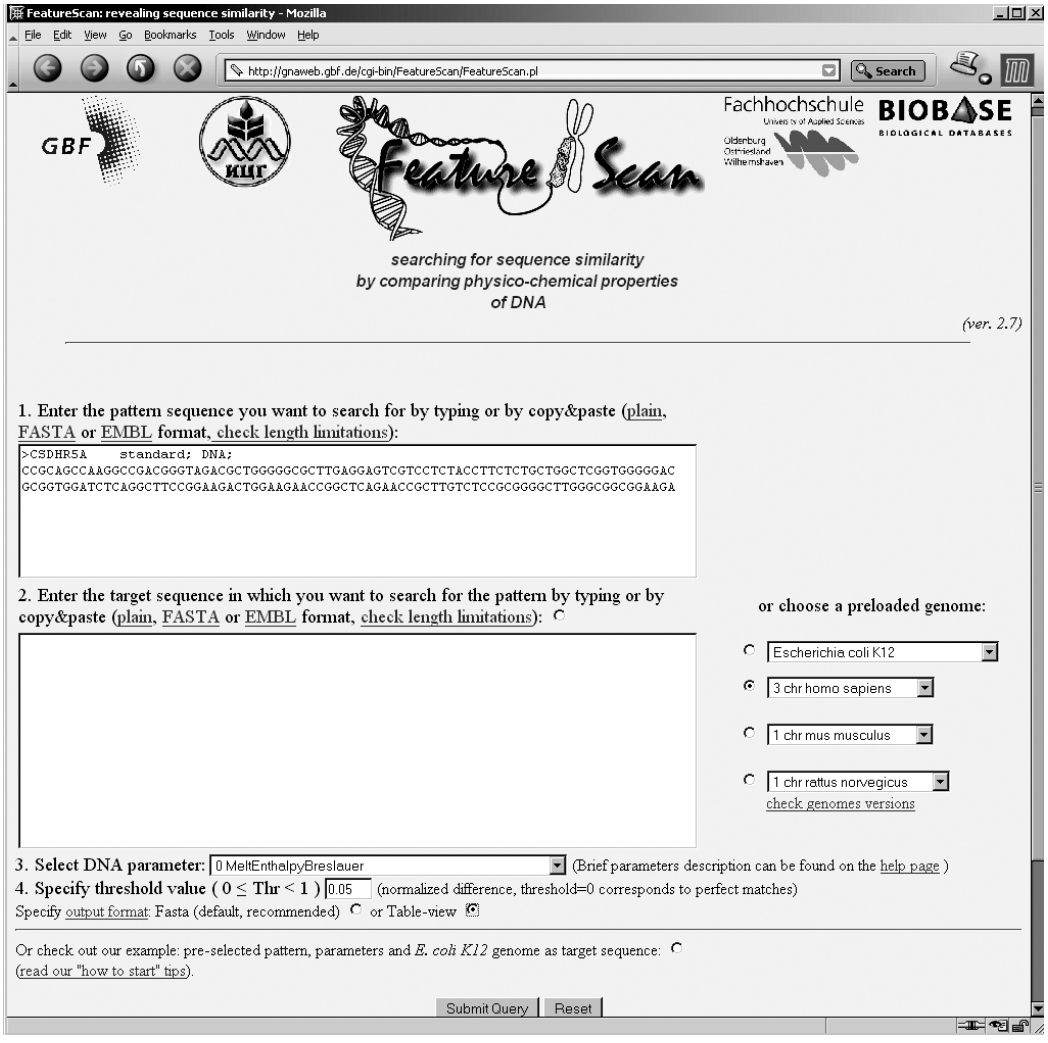
The FeatureScan web service is implemented in a Client–Server architecture. Any user client programme set up according to the rules can send a request to the database, and the job will be tagged with a unique identifier. The server programme connects to the database, picks up a task from the queue and returns a result. By querying the database, the user programme will track the status of the task and may pick up results. A client application, written in C++ (Windows), can be downloaded from the FeatureScan web page (multiplatform Java client to follow soon).

The advantages of such an architecture lies in its flexibility and scalability. Both, user client programme and server programme may be developed independently to meet specific demands, advanced functionality or improved performance. To add on to the overall existing performance one has only to run another copy of the server application. Currently, two server programmes are running in Braunschweig and in Emden (Germany).

VALIDATION

To demonstrate aspects of the nature of this novel similarity measure we carried out a series of experiments with artificial and genomic sequences as published in Refs (11,13). Tests on randomly generated data showed that the method performs as in theory. It is robust to interspersed 'noise' nucleotides, able to detect complex multi-sequence elements, has single nucleotide resolution and is easily applicable to genome-wide analysis. Thirty-eight different DNA parameters show a wide range of sensitivities *versus* letter mismatches, obviously

(a)



(b)

Results page

```
Your progress bar
|-------------------------------------------------------------------------------------------|
****Ready

Threshold=0.050000
DNA coding scheme used - MeltEnthalpyBreslauer
Your pattern:
>  CSDHR5A    standard; DNA; ROD;
CCGCAGCCAAGGCCGACGGGTAGACGCTGGGGGCGCTTGAGGAGTCGTCCTCTACCTTCTCTGCTGGCTCGGTGGGGGAC
GCGGTGGATCTCAGGCTTCCGGAAGACTGGAAGAACCGGCTCAGAACCGCTTGTCTCCGCGGGGCTTGGGCGGCGGAAGA

Query sequence is  chr3, length=199505740

Hits found:
```

No	Position (bp)	Similarity distance	References	Sequence
0	56230	0.04875	Ensembl UCSC	AAAAACACAAAAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCA GGGAACCCGGGGGGCGGAGCTTGCAGTGAGCCAAGATGGCGCCACCGCCCTCCAGCCTGGGCGACAGGGC
1	56231	0.04680	Ensembl UCSC	AAAACACAAAAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAG GGAACCCGGGGGGCGGAGCTTGCAGTGAGCCAAGATGGCGCCACCGCCCTCCAGCCTGGGCGACAGGGCC
2	56247	0.04846	Ensembl UCSC	GCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGGGAACC AGCTTGCAGTGAGCCAAGATGGCGCCACCGCCCTCCAGCCTGGGCGACAGGGCCAGACTCCGTCTCAAAA
3	56248	0.04991	Ensembl UCSC	CCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGGGAACCC GCTTGCAGTGAGCCAAGATGGCGCCACCGCCCTCCAGCCTGGGCGACAGGGCCAGACTCCGTCTCAAAAA
4	56249	0.04941	Ensembl UCSC	CGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGGGAACCCG CTTGCAGTGAGCCAAGATGGCGCCACCGCCCTCCAGCCTGGGCGACAGGGCCAGACTCCGTCTCAAAAAA

Figure 1. FeatureScan web interface (**a**) and sample output (**b**).

Table 1. Description of a few DNA parameters, including those which were used in Refs (10–12) and which describe the three major groups of DNA features

Parameter name	Type	Unit	Comment
MeltEnthalpyBreslauer	Physico-chemical	kcal/mol	Describes the melting of DNA double strands, values taken from (12)
normMeltEnthalpyBreslauer	Physico-chemical	kcal/mol	Normalized values of melting enthalpy
Roll	Conformational angle	Degree	Describes respective bend angle of DNA (graphical view is given on the website)
Twist	Conformational angle	Degree	Similar to roll
Major groove depth	Conformational linear dimension	Å (Ångstrom)	Describes the depth of the major groove of DNA
Minor groove depth	Conformational linear dimension	Å (Ångstrom)	Describes the depth of the minor groove of DNA

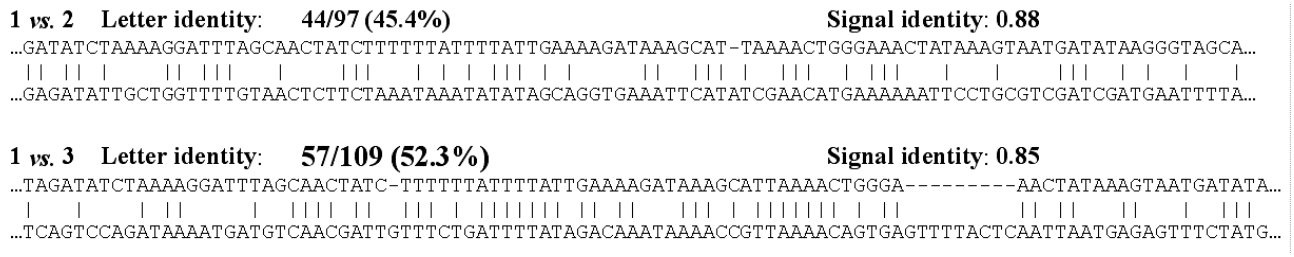


Figure 2. Similarity revealed by FeatureScan and BLAST of promoters of *E.coli* genes belonging to class 6.3 'Surface antigens' (according to GenProtEC database (7): (i) promoter region of UDP-D-glucose:(galactosyl)lipopolysaccharide glucosyltransferase gene; (ii) promoter region of UDP-D-galactose:(glucosyl)lipopolysaccharide-alpha-1,3-D-galactosyltransferase gene; and (iii) promoter region of UDP-D-galactose:(glucosyl)lipopolysaccharide-1, 6-D-galactosyltransferase gene. (ii) versus (iii): Letter identity, 0.52; signal identity, 0.89; alignment not shown.

reflecting respective changes in physical properties behind them.

A fully annotated *E.coli* genome was chosen to investigate the ability of our technique to reveal functional similarities between promoter regions (11). We built up a similarity matrix of *E.coli* promoters. Subsequent statistical analysis of the detected promoter similarities and of the cellular functions using the GenProtEC *E.coli* database (14) identified an enrichment of several functional categories of genes, the promoters of which showed high signal similarity. It was interesting to observe that among the functional classes most populated by similar promoters were classes of genes which encode membrane-specific proteins such as surface antigens and transporters. Cross checked with BLAST, these promoters showed similarity only at a background level (see typical example in Figure 2). One may speculate that the conformational parameter 'roll', which was used in this analysis, best reflects the requirements for specific 3D structure of the promoters for effective binding and assembling of the transcription machinery.

We performed a comparison of two evolutionary close genomes—human and chimpanzee. Similarity between promoters of homologous genes was calculated by ClustalW and FeatureScan (the latter by using DNA property melting enthalpy). We found that homologous promoters are much closer by their melting characteristics than by the number of conserved nucleotides (I. V. Deyneko, A. E. Kel, J. M. Kalybaeva, H. Blöcker and G. Kauer, unpublished data). This observation may indicate that mismatches in promoters were introduced in a correlated manner during the course of evolution, so that the DNA property 'melting enthalpy' of the promoters is retained.

Such promising advantages of property-dependant similarity measures encourage us to look for further application. For example, SNP mutations which are seen by Blast-type methods as single nucleotide mismatches, may introduce a number of different changes to DNA characteristics (melting temperature, conformation and others). FeatureScan is designed and can specifically spot such alterations and thus provide new insights into biological functionalities.

CONCLUSION

Here we present web resources for sequence analysis based on a novel type of sequence similarity. We show that it has certain specific advantages over letter-based methods. We think that none of the alternate approaches can entirely replace the other and that both will evolve in parallel. FeatureScan can be easily applied to the analysis of other information-bearing biomolecules by just using an appropriate (physical nature reflective) letter-to-signal encoding scheme. In the future, methods utilizing signal-theoretical similarity may be further automated and trained specifically for recognition of promoters, binding sites and others. It should be noted that utilizing specific hardware enables one to carry out search procedures with huge amounts of eukaryotic data in acceptable time.

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Conflict of interest statement. None declared.

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