

Software

Open Access

Triad pattern algorithm for predicting strong promoter candidates in bacterial genomes

Michael Dekhtyar*¹, Amelie Morin² and Vehary Sakanyan*^{2,3}

Address: ¹University of Tver, 33 Jelyabova, 170000 Tver, Russia, ²Laboratoire de Biotechnologie, UMR CNRS 6204, Université de Nantes, 2 rue de la Houssinière, 44322 Nantes, France and ³ProtNeteomix, 2 rue de la Houssinière, 44322 Nantes, France

Email: Michael Dekhtyar* - Michael.Dekhtyar@tversu.ru; Amelie Morin - Amelie.Morin@cancer.org.uk; Vehary Sakanyan* - Vehary.Sakanyan@univ-nantes.fr

* Corresponding authors

Published: 9 May 2008

Received: 19 November 2007

BMC Bioinformatics 2008, 9:233 doi:10.1186/1471-2105-9-233

Accepted: 9 May 2008

This article is available from: <http://www.biomedcentral.com/1471-2105/9/233>

© 2008 Dekhtyar et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Bacterial promoters, which increase the efficiency of gene expression, differ from other promoters by several characteristics. This difference, not yet widely exploited in bioinformatics, looks promising for the development of relevant computational tools to search for strong promoters in bacterial genomes.

Results: We describe a new triad pattern algorithm that predicts strong promoter candidates in annotated bacterial genomes by matching specific patterns for the group I σ^{70} factors of *Escherichia coli* RNA polymerase. It detects promoter-specific motifs by consecutively matching three patterns, consisting of an UP-element, required for interaction with the α subunit, and then optimally-separated patterns of -35 and -10 boxes, required for interaction with the σ^{70} subunit of RNA polymerase. Analysis of 43 bacterial genomes revealed that the frequency of candidate sequences depends on the A+T content of the DNA under examination. The accuracy of *in silico* prediction was experimentally validated for the genome of a hyperthermophilic bacterium, *Thermotoga maritima*, by applying a cell-free expression assay using the predicted strong promoters. In this organism, the strong promoters govern genes for translation, energy metabolism, transport, cell movement, and other as-yet unidentified functions.

Conclusion: The triad pattern algorithm developed for predicting strong bacterial promoters is well suited for analyzing bacterial genomes with an A+T content of less than 62%. This computational tool opens new prospects for investigating global gene expression, and individual strong promoters in bacteria of medical and/or economic significance.

Background

Efficient promoter recognition is crucial in the synthesis of the gene-encoded products required by bacteria to allow them to grow rapidly and to adapt to different environmental conditions. The general architecture and protein-DNA interaction interfaces appear to be conserved in RNA polymerases of different bacteria, to judge by a com-

parison of the resolved structures of the multi-subunit protein or its subunits [1]. This structural information suggests that the principles of DNA recognition by RNA polymerases are universal, and this constitutes a basis for *in silico* prediction of promoters that are recognized by families of sigma factors. Research in bioinformatics has developed approximate matching methods to detect con-

served sequences in nucleic acids [2-5], including promoter-specific sequences that are invaluable in helping to elucidate the overall organization of transcriptional signals and regulatory circuits in evolutionarily distant bacteria [6-14]. Most promoter prediction programs so far proposed use statistical or motif-based methods, and take into consideration what is known about experimentally defined promoter architectures, and extract conserved sequences from the genomes under analysis. Attempts have been made to improve promoter prediction by introducing statistical mechanical methods to measure the stress-induced destabilization or bendability of the duplex DNA region located upstream of the transcription initiation site required for the local dissociation of strands to start mRNA synthesis [15-17]. The steady increase in the number of sequenced bacterial genomes of medical and economic significance means that there is an increasing need for computational tools to predict promoters, especially those responsible for high-level gene expression in organisms, of which there has been little experimental investigation.

Many housekeeping genes in *Escherichia coli* are transcribed from promoters possessing the recognition elements referred to as -35 and -10 sites (boxes), for which the TTGACA and TATAAT consensi, respectively, have been identified by compiling characterized RNA polymerase-binding regions in the DNA [18,19]. The -35 and -10 sites, which are separated from each other by a 15–20-bp spacer [20], are specifically recognized by $E\sigma^{70}$ RNA polymerase, an RNA polymerase holoenzyme bearing the group I σ^{70} factor [21]. Experimental data have shown that high transcription rates of genes correlate with the level of conservation of three promoter parameters, with the consensus -35 and -10 hexanucleotide boxes, and with the 17 ± 1 -bp spacer separating them [22]. This fact has been widely used to construct vectors for protein overexpression in bacterial cells [23].

However, the strength of strong promoters also depends on a fourth parameter, an AT-rich UP element 17–20 bp length, which is located just upstream of the -35 site, and which is recognized by the α subunit of $E\sigma^{70}$ RNA polymerase, which was first discovered for ribosomal RNA promoters [24]. The C-terminal domain of this subunit binds both to the UP element and to transcription regulation proteins, whereas the N-terminal domain makes contact with other subunits during the assembly of RNA polymerase [25]. A 17-bp consensus 5'AAAWWT-WTTTTNNAAA (where W is A or T, and N can be any of the four bases) has been identified for the UP element by analyzing the patterns, selected by the SELEX method, which mediate increases of between 10- and 300-fold in gene expression in *E. coli* cells [26-28]. Two preferred sub-sites have been identified within the UP element. They are

centered approximately at the -42 and the -52 positions respectively, and appear to be specifically recognized by one or two monomers of a dimeric α subunit in the RNA polymerase.

It is noteworthy, that a virtual analysis of patterns located upstream from the consensus -35 had since long time suggested their functional significance [3]. The sequences reminiscent of UP element have been detected in the *E. coli* genome by the algorithms PWM [29] and PlatPram [30]. A software-based (GCG, version 9.0) dissection of regions located upstream of the *E. coli* promoters had made it possible to detect putative promoters with ≤ 4 mismatches in the full UP element consensus [28]. Several UP elements have also been visually identified, and characterized by their ability to direct high level gene expression *in vivo* or *in vitro* in *Bacillus subtilis* [31], *Geobacillus* (formerly *Bacillus*) *stearothermophilus* [32] and *Vibrio natrigens* [33]. Recently, a comparative analysis of $E\sigma^{70}$ -specific promoter and non-promoter regions indicated that upstream regions of *E. coli* ribosomal and T4 phage early promoters possess electrostatic elements that could be responsible for modulating promoter activities due to ADP-ribosylation of RNA polymerase α subunit [34]. However, no specific algorithms have yet been proposed to detect strong promoters in bacterial genomes, and so this remains an important task for genomic and proteomic research in microbiology.

In this study, we have developed a triad pattern algorithm that detects strong promoter candidates composed of a UP-element, and two consensi, -35 and -10 boxes, which are optimally distanced from each other. All four parameters are required for efficient DNA recognition, and the initiation of mRNA synthesis by an $E\sigma^{70}$ -like RNA polymerase. The data presented indicate that the frequency of strong promoters is a function of the A+T content of the corresponding genomes. The proposed prediction program is flexible, and can be modified by users to modulate the search stringency criteria depending on the promoter features of the genome under analysis. The accuracy of detection has been experimentally validated for putative strong promoters predicted in a hyperthermophilic bacterium *Thermotoga maritima*.

Implementation

Overview of the approach

The promoter activity in cells is determined by regulatory proteins (activators and repressors) that can recognize overlapping sequences specific for $E\sigma^{70}$ RNA polymerase sites, and thereby mask the true promoter strength. In addition, almost 20% of *E. coli* RNA polymerase $E\sigma^{70}$ -specific promoters possess an extended -10 sequence that might compensate for the absence of a clear -35 site [35]. Different prediction programs based on statistical and

motif-searching approaches have been developed to detect a variety of binding sites in DNA, and both position-specific weight matrices [36] and hidden Markov models [37] have been used to improve the accuracy of the prediction of promoter sequences in bacterial genomes [38-40]. These programs usually detect hexanucleotide dyad patterns of RNA polymerase-promoter binding sites, such as -35 and -10 boxes, and none of them is free of false-positives, which correspond to similar, non-promoter sequences in bacterial genomes [for a review, see [41]].

In this study, we exploited the strengths of the "triad pattern" approach to develop an algorithm able to detect strong promoters by matching three nucleotide sequences recognized by the σ^{70} and α subunits of bacterial RNA polymerase. Theoretically, the presence of a UP element may not be essential for relatively strong promoter activity if two -35 and -10 boxes are well conserved and optimally distanced. Similarly, the presence of a well conserved UP element may compensate for a poor -35 box in some promoters. However, it seems very likely that the strongest promoters probably possess all three essential sequences. The specific interaction between the UP element and the α subunit significantly amplifies the association of RNA polymerase with promoter DNA [27,28]. Therefore, to improve the filter to exclude possible false-positive due to short hexanucleotide similar sequences scattered throughout the genome, our algorithm starts by first matching the UP element, and only then identifying the -35 and -10 boxes located further downstream.

Design of the triad pattern algorithm

We designed an algorithm able to detect the triad nucleotide patterns in bacterial genomes. The core of the algorithm is the FIND_TRIAD procedure, which given an input nucleotide string, s , returns the substring s' of s , which is the best approximation of a given triad pattern of the form $(pat(1),L1)-(l1,l2)-(pat(2),L2)-(d1,d2)-(pat(3),L3)$, where each $pat(i)$, $i = 1,2,3$, is a nucleotide string, Li is its length, $l1$ and $l2$ are the minimum and maximum distances respectively between the first and the second patterns, and $d1$ and $d2$ are the minimum and maximum distances respectively between the second and the third patterns. To avoid making a "bad" approximation, three scores $Sc1$, $Sc2$ and $Sc3$ are used as input parameters for the procedure. The resulting substring, s' , can then be represented as $(spat(1),Ls1)-ls1-(spat(2),Ls2)-ls2-(spat(3),Ls3)$, where each $spat(i)$, $i = 1,2,3$, is a substring of s aligned to $pat(i)$, Lsi is its length, $ls1$ is the distance between $spat(1)$ and $spat(2)$, and $ls2$ is the distance between $spat(2)$ and $spat(3)$. This result for s' satisfies the following conditions:

(1) for each $i = 1,2,3$ the similarity score (weight) Wi of the match or alignment of $pat(i)$ and $spat(i)$ is not less than Sci (or the number of "mismatches" does not exceed $(Li - Sci)$);

(2) $(l1 \leq ls1 \leq l2)$ and $(d1 \leq ls2 \leq d2)$.

For each of the three patterns, one can either forbid insertions/deletions or allow them. In the former case, $Lsi = Li$ and the weight = Wi are computed as the sum of matching pairwise symbols, whereas in the latter case, the difference $|Lsi - Li|$ between $spat(i)$ and $pat(i)$ is bounded by a value Ri for the permissible deletions/insertions (gaps), an optimum alignment, and its weight, Wi , are computed by the standard dynamic programming method for global string alignment [42]. In both cases, a symbol scoring matrix $Mi(x,j)$ is used to define the weight of the symbol x in the position j , $1 \leq j \leq Lsi$, of $spat(i)$. If symbol x occurs in position j of $pat(i)$, then $Mi(x,j) = 1$, otherwise $Mi(x,j) \leq 1$. To choose the best approximation of the triad pattern from substrings satisfying conditions (i) and (ii), FIND_TRIAD uses a total score function with the form:

$$tot_sc = C1 * nsc1(L1, W1) + D12 * nsc_dist12(l1, l2, ls1) + C2 * nsc2(L2, W2) + D23 * nsc_dist23(d1, d2, ls2) + C3 * nsc3(L3, W3), \quad (1)$$

where $nsci(Li, Wi)$, $i = 1,2,3$, are normalized scores of matching (alignments) of $pat(i)$ and $spat(i)$, $0 \leq nsci(Li, Wi) \leq 1$, and $nsc_dist12(l1, l2, ls1)$ and $nsc_dist23(d1, d2, ls2)$ are the normalized scores of the distances between the first and the second, and the second and the third patterns, respectively, and $0 \leq nsc_dist12(l1, l2, ls1)$, $nsc_dist23(d1, d2, ls2) \leq 1$. The linear coefficients $C1$, $C2$, $C3$, $D12$, and $D13$ are chosen so that their sum is equal to 1. They indicate the relative importance of the corresponding sub-patterns of the triads; and the distances between them. So, the best value of tot_sc is 1.

Application of the algorithm to searching for strong promoter candidates

Here we describe the main parameters of the FIND_TRIAD procedure used to detect strong promoter candidates in bacterial genomes. In this study, a bacterial promoter is assumed to be a nucleotide sequence, located upstream from genes encoding proteins, tRNAs or rRNAs that could be recognized by an RNA polymerase holoenzyme containing a major σ factor (using *E. coli* $E\sigma^{70}$ RNAP as the reference). The triad patterns defined for strong promoter candidates include three specific nucleotide subregions: (i), a UP element, which is a 17-nt prefix of the strong promoter, and has the following consensus pattern: $pat(1) = P_{UP} = aaaWWtWtttNNNaaa$; (ii) the -35 site, which is located downstream of the UP element, and has

the pattern $pat(2) = P_{-35} = \underline{tcttgacat}$ (underlining indicates a commonly used consensus for group I σ^{70} factors; however, the σ_4 domain of these factors appears to be in contact with 9 nucleotides in the region extending from -30 to -38 [43,44]); (iii) the -10 site, which is located downstream of the -35 site, and has the pattern $pat(3) = P_{-10} = \underline{tataat}$ (this site is highly conserved). We used the following boundaries for the distances between the sub-regions: $l1 = 0$, $l2 = 5$ (these boundaries were extracted from the examples of UP-elements in [25-27]), $d1 = 14$, $d2 = 20$ (these boundaries are standard for the distance between the -35 site and the -10 site). To search for the first pattern $pat(1)$ of the UP-element, the simple matching algorithm was chosen with an *a* and *t* mismatch score of 0.5. The reason is that in the full UP-element consensus and the consensuses of two of its subsides – distal and proximal – in some places do not distinguish between *a* and *t*. We assumed that the consensus for the -35 site of length 9 is less conserved than that of the -10 site, and so in order to detect the second pattern $pat(2)$ of the -35 site we used a dynamic programming algorithm to search for optimal alignment, with boundaries for the number of permissible deletions/insertions of $R2 = 2$. For the most of -35 sites, which were detected by algorithm, no insertions/deletions were applied. However, this scoring system allowed us to identify some stronger promoter candidates. Thus, the insertion of C between two AA in the sequence TCTTGAAT of TM1016, increases the score of a putative promoter (see below). The -10 site is better conserved, and so we used the straightforward matching algorithm to detect this site.

To define the total score function, tot_sc (formula 1), we chose the following normalized scores for the three patterns and for the distance between the -35 site and -10 site (no information was available about the best values for the distance between the UP element and the -35 site):

$$nsc1(17,W1) = nsc_up = 1 - (17 - W1)/20, \quad (2)$$

$$nsc2(9,W2) = nsc_35 = 1 - (9 - W2)/10, \quad (3)$$

$$nsc3(6,W3) = nsc_10 = 1 - (6 - W3)^2/10, \quad (4)$$

and the values of the normalized distance score, $nsc_dist23(14,20,l2) = nsc_dist$, are defined as follows:

$ls2$: distance between the -35 and -10 sites, nt	17	16,18	15,19	14,20
nsc_dist	1	0.95	0.85	0.7

We also chose linear coefficients $C1 = 0.3$, $C2 = C3 = 0.25$, $D12 = 0$, and $D23 = 0.2$. These coefficients indicate the relative importance of corresponding sub-regions for evaluating the total score of a candidate sequence. They were chosen empirically, after preliminary tests with several

annotated genomes, assuming a higher significance of the UP element, equal significance of the -10 and -35 boxes, and lower significance of the distance between them. In this application, the value $D12 = 0$ means that we ignore the variations of the distance between a putative UP element and -35 box because *a priori* it is not known what value is the best in the interval 0–5 nt.

Formulas 2, 3 and 4 reflect the lack of exact matching for the different sub-regions. If the -10 box is highly conserved and is essential for initiation of transcription [22], then the penalty for its mismatches is higher than for those of the other parameters. For example, for 2 mismatches, the penalty is $(6 - 4)^2/10 = 0.4$ for the -10 site, whereas it is $(9 - 7)/10 = 0.2$ for the -35 site, and $(17 - 15)/20 = 0.1$ for the UP element. The choice of the normalized score functions in equations 2, 3 and 4 is based on empirical observations, and on common sense, and may seem to be arbitrary. We want to stress that, in fact, the total score function tot_sc also has a further role: it does not significantly change the set of the best candidates identified by the algorithm. This set is defined by the three score bounds $Sc1 = scup$ for UP element, $Sc2 = sc35$ for -35 site, and $Sc3 = sc10$ for -10 site. The total score affects only the ordering of these candidates amongst themselves.

The general scheme of the algorithm is as follows. It has the following input: (i) the name of a genome file in GenBank format; (ii) three parameters of scores: *scup*, *sc35* and *sc10*, determining the minimum acceptable similarity between candidate sequences of the UP element, the -35 box, and the -10 box, respectively, and the *E. coli* consensus patterns. For each gene in the genome input file that is not inside an operon, the algorithm runs in two steps:

- (i) it extracts a 300-bp DNA region, *s*, upstream of the annotated coding sequences for tRNA, rRNA or proteins (we limited the search to 300 bp, since most *E. coli* promoters fall within this length inter-gene space [41,45]);
- (ii) then it uses the FIND_TRIAD procedure to identify the best strong promoter candidate within *s* that satisfies conditions (1) and (2) above. If such a candidate is found, it is added to the output list of strong promoters.

We recommend to read attentively the "ReadMe" information [see Additional file 1] before to start proceeding the "strong_promoters.doc" software [see Additional file 2]. The algorithm is implemented by a program that produces the results in two forms: (i) a Text-format table which lists all strong promoter candidates in a genome, and provides additional information about the operon organization of genes located downstream (for example, see Fig. 1); (ii) a Word-format table which lists strong promoter candidate sequences. A 20-nt sequence preceding a

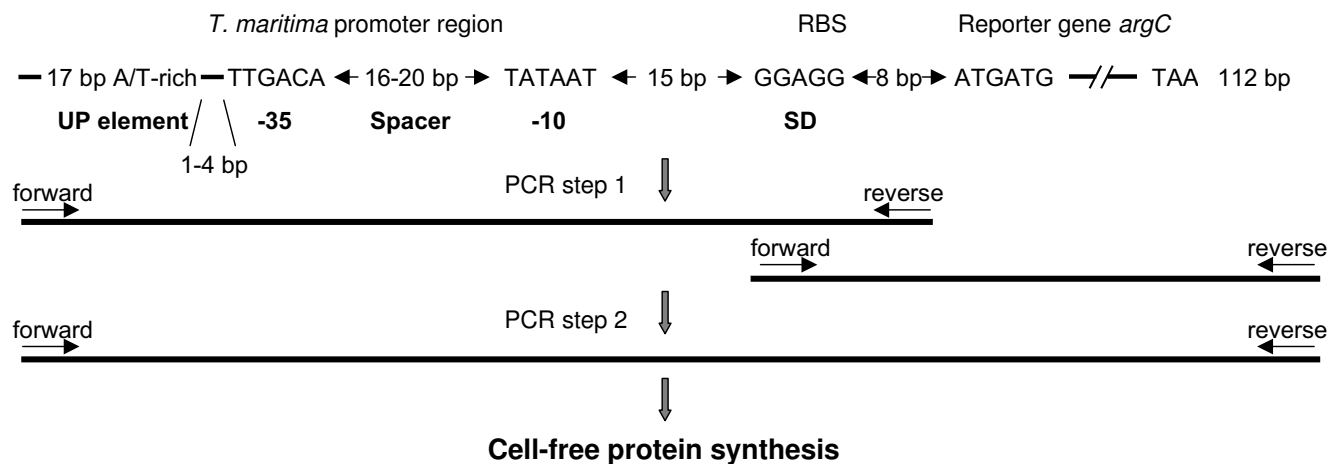


Figure 2
Diagram of the fusion DNA constructs used to express the *G. stearothermophilus argC*-reporter gene from putative strong promoters of *T. maritima* in a cell-free system. The *argC* gene was amplified with forward 5'-GGAG-GGGAACATATGATGAA and reverse 5'-GGACCACCGCGCTACTGCCG primers from pHAV2 [32] by conserving a 112-bp downstream region carrying transcriptional terminators of the vector DNA.

both *in vivo* and *in vitro*, and was amplified from the plasmid pHAV2 [32].

Cell-free protein synthesis

PCR-generated linear DNA fragments carrying a promoter region fused to the *argC* reporter gene were used to evaluate the promoter strength in a coupled transcription-translation system, as described previously [49]. The cell-free extracts were prepared from the *E. coli* strain BL21 (DE3) Star *recBCD* (our laboratory construction) as described by Pratt [50]. Protein synthesis was carried in the presence of pyruvate oxidase to generate ATP [51]. Typically, 50 ng of PCR-amplified DNA was added to a pre-mix containing all necessary compounds and 10 μCi of [α^{35} S]-L-methionine (specific activity 1000 Ci/mmol, 37 TBq/mmol, Amersham-Pharmacia Biotech), and *E. coli* S30 cell-free extracts. The reaction mixture was incubated at 37°C for 90 min, and heated to 65°C for 10 min. After centrifuging, the supernatant was precipitated with acetone, and then protein samples were separated by SDS-PAGE and bound to 3 MM paper. The ArgC protein synthesized *in vitro* was quantified by counting the radioactivity of the corresponding band with a PhosphorImager 445 SI (Molecular Dynamics).

The bacterial genome sequences were extracted from available data banks. The logo of *T. maritima* promoter consensus sequences was generated at the WebLogo site as described in [52,53].

Results

The number of strong promoters reflects the A+T content of bacterial genomes

In our algorithm, 26 of the 32 symbols used to evaluate matches in the three promoter-specific patterns, namely in the UP element and the -35 and -10 boxes, are *a* and *t*. One could expect the number of genes transcribed from potential strong promoters to depend on the A+T content of a given genome. To find out whether this is indeed the case, we compared the frequency of candidates in 300-bp regions located upstream of genes of annotated bacterial genomes and in random sequences of the same regions generated by computing. First, we calculated the (A+T)% in all 300-bp regions preceding each gene or operon in the annotated genomes (Table 1). The A+T content in these DNA regions was found to be slightly higher than that of the entire genomes of almost all bacteria that have been analyzed. Next, we generated 10,000 random sequences with the same A+T content for all the 300-bp regions of each genome. The algorithm was applied to detect strong promoter candidates in the 300-bp real genomic and random-generated regions of 43 bacterial genomes.

We tested different matching stringencies and empirically found that the score parameters $s_{UP} = 13$, $s_{35} = 5.5$ and $s_{10} = 4.5$ satisfied the criteria required for scaled comparative analysis without grossly exaggerating the number of candidate sequences identified in the various genomes. This analysis revealed that the real genomes with an A+T content of less than 50% contained many more potential strong promoters than their simulated counterparts (see

Table 1: A+T content of bacterial genomes and 300-bp regions located upstream of genes and the percentage of strong promoter candidates predicted in 300-bp real genomic and random-generated regions of the same content.

N°	Genome	(A+T)% of		% of candidates in	
		Bacterial genomes*	300-bp genomic regions	300-bp genomic regions	300-bp random sequences
1	<i>Deinococcus radiodurans</i> RI (AE000513)	32.99	34.19	0.19	0
2	<i>Caulobacter crescentus</i> (AE005673)	32.77	34.40	0.05	0
3	<i>Ralstonia solanacearum</i> GM11000 (AL646052)	34.51	34.50	0.20	0
4	<i>Pseudomonas aeruginosa</i> PA01 (AE004091)	33.44	35.38	0.27	0
5	<i>Xanthomonas campestris</i> pv. <i>campestris</i> (AE008922)	34.93	35.64	0.05	0
6	<i>Mycobacterium tuberculosis</i> (AL123456)	34.39	35.69	0.18	0
7	<i>Xanthomonas axonopodis</i> pv. <i>citri</i> 306 (AE008923)	35.23	36.02	0.05	0
8	<i>Mesorhizobium loti</i> (NC002678)	37.25	39.09	0.13	0
9	<i>Sinorhizobium meliloti</i> 1021 (AL591688)	37.27	39.66	0.24	0
10	<i>Mycobacterium leprae</i> TN (AL450380)	42.20	43.14	0.29	0
11	<i>Agrobacterium tumefaciens</i> C58 (AE007869)	45.64	43.20	0.74	0
12	<i>Brucella melitensis</i> 16 M chromosome I (AE008917)	42.84	45.73	1.02	0
13	<i>Treponema pallidum</i> (AE000520)	47.22	47.01	0.37	0.3
14	<i>Chlorobium tepidum</i> TLS (AE006470)	43.47	47.50	1.50	0.31
15	<i>Salmonella typhimurium</i> LT2 (AE006468)	47.78	51.08	3.54	0.7
16	<i>Neisseria meningitidis</i> serogroup B MC58 (AE002098)	48.47	52.20	5.03	0.93
17	<i>Escherichia coli</i> O157:H7 (AE005174)**	49.50	52.54	4.80	1.1
18	<i>Methanobacterium thermoautotrophicum</i> ΔH (AE000666)	50.46	53.11	4.26	1.23
19	<i>Synechocystis</i> PCC6803 (AB001339)	52.28	53.71	2.89	1.7
20	<i>Thermotoga maritima</i> (AE000512)	53.75	54.66	3.27	2.15
21	<i>Vibrio cholerae</i> chromosome I (AE003852)	52.30	54.94	3.22	2.4
22	<i>Yersinia pestis</i> CO92 (AL590842)	52.36	55.77	6.78	3.0
23	<i>Aquifex aeolicus</i> (AE000657)	57.73	57.70	4.72	4.15
24	<i>Bacillus halodurans</i> C-125 (BA000004)	56.31	58.65	8.70	5.1
25	<i>Bacillus subtilis</i> (AL009126)	56.48	59.30	10.28	5.8
26	<i>Mycoplasma pneumoniae</i> M129 (U00089)	59.99	61.71	5.25	9.7
27	<i>Chlamydia muridarum</i> (AE002160)	59.69	61.73	9.01	9.7
28	<i>Pasteurella multocida</i> PM70 (AE004439)	59.60	62.31	11.42	10.9
29	<i>Chlamydomonas reinhardtii</i> J138 (BA000008)	59.42	62.80	14.77	12.5
30	<i>Streptococcus pneumoniae</i> (AE005672)	60.30	62.88	15.83	13.0
31	<i>Streptococcus pyogenes</i> SF370 serotype M1 (AE004092)	61.49	63.99	16.87	14.3
32	<i>Thermoanaerobacter tengcongensis</i> MB4T (AE008691)	62.43	64.11	17.74	14.8
33	<i>Listeria innocua</i> Clip11262 (AL592022)	62.56	64.30	12.07	15.5
34	<i>Haemophilus influenzae</i> Rd (L42023)	61.85	64.45	15.61	16.0
35	<i>Mycoplasma genitalium</i> G37 (L43967)	68.31	69.50	15.99	35.0
36	<i>Staphylococcus aureus</i> N315 (BA000018)	67.16	69.71	35.25	36.1
37	<i>Campylobacter jejuni</i> (AL111168)	69.45	71.36	32.07	41.8
38	<i>Clostridium acetobutylicum</i> (AE001437)	69.07	71.83	45.08	44.2
39	<i>Borrelia burgdorferi</i> (AE000783)	71.40	73.18	40.00	54.1
40	<i>Rickettsia prowazekii</i> Madrid E (AJ235269)	71.00	73.26	50.06	55.2
41	<i>Clostridium perfringens</i> 13 (BA000016)	71.43	74.74	53.94	58.1
42	<i>Ureaplasma urealyticum</i> (AF222894)	74.50	76.05	50.85	65.35
43	<i>Buchnera aphidicola</i> Sg (AE013218)	74.67	78.36	58.05	74.5

* A+T content of bacterial genomes was calculated from corresponding genomic DNA sequences available in gene banks.

** Similar values were found for the *E. coli* K12 genome.

Table 1). The percentage of candidate sequences was very low in the bacterial genomes with an A+T content of between 33% and 47%, and these sequences were completely absent in the corresponding 300-bp, random-generated sequences. When the A+T content increased from 47% to 78%, the percentage of strong promoter candidates increased dramatically, whereas the difference between the real and random sequences decreased, and virtually disappeared when the A+T content exceeded 62%. There were two exceptions where the genomes analyzed did not display this pattern at an A+T content of less than 62%. One was *M. pneumoniae*, the genome of which had an A+T content of about 60%, and in which the promoters had no -35 consensus [54]. The other example is the hyperthermophilic species *A. aeolicus* (~58% AT-rich genome). This species is very close to the Archaea, and occupies a unique position in the bacterial kingdom [55].

Our data show that the number $N(A+T)$ of strong promoter candidates in 300-bp random-generated sequences corresponding to upstream regions of bacterial genes satisfies the "exponential low" of the form $N(A+T) = \exp [c_1(A+T) + c_2]$. The distribution of strong promoter candidates in real genomes indicates that the critical point of the A+T content is close to 62% (Fig. 3). Above this level, the number of random sequences reminiscent of strong promoter patterns increases markedly.

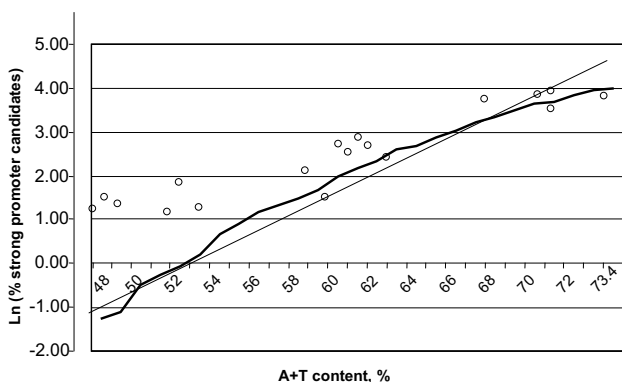


Figure 3
The number of strong promoter candidate sequences is a function of the A+T content of bacterial genomes. For the score parameters $sUp = 13$, $s35 = 5.5$, $s10 = 4.5$ and constants $c_1 = 0.22$ and $c_2 = -11.7$, the picture displays a linear graph of the "exponential low" (thin line), which approximates fairly closely to the curve $\ln [N(A+T)]$, shown as a thick line. The logarithm of the percentage of strong promoter candidates in real genomes is shown by ().

Strong promoter candidate sequences are located upstream of gene-coding regions

Another important aspect of the quality of detection is the location of candidate sequences with regard to coding regions in the genome analyzed. We compared the frequencies of strong promoter-like patterns identified upstream and downstream of the initiation codon in all the genomes. The frequency of candidate sequences was clearly greater in the upstream region of ORFs in most of the genomes with an A+T content of less than 62% (Table 2). No difference was detected in *T. pallidum* (~47% AT-rich genome), which belongs to a distinct phylum of Spirochetes that appear to use different DNA patterns for the promotion and regulation of transcription [56].

The fact that more candidate sequences were identified upstream of ORFs highlights the fact that they are not randomly distributed in bacterial genomes, which suggests that the detection of strong promoter candidates in genomes with an A+T content of less than 62% is fairly reliable.

Experimental validation of virtual prediction: analysis of putative strong promoters of T. maritima

Taking our cue from the results of the virtual prediction, we sought to find out whether, and if so, to what extent the putative promoters are functional in a biological context. To do this we used reporter-gene technology, which relies on the fusion of an assayable sequence with a promoter being investigated, and the subsequent evaluation of promoter strength in a cell-free system (see Fig. 2). The genome of the hyperthermophilic bacterium *T. maritima* [57] was used to evaluate the feasibility of the algorithm experimentally.

63 candidate sequences were detected in the *T. maritima* genome using the matching scores described above. We increased the penalty for mismatching of -35 and -10 boxes by raising the scores of $s35$ and $s10$ to 6 and 5, respectively. This reduced the number of candidate sequences to 34 (Table 3). In this shorter list, 28 *T. maritima* strong promoter candidates possessed a total score higher than the 0.8475 calculated for the reference strong promoter, *Ptac*, that does not have a typical UP element [48]. 15 of these candidates had a total score higher than 0.8775, as estimated for *PargC*, another reference strong promoter that has a well defined UP element [32,49]. It is worth mentioning that 6 candidate DNA regions in *T. maritima* had a total score higher than 0.91, a value estimated for *E. coli* promoters that govern the transcription of 16S ribosomal RNA, and which were used as models for studying the stimulating effect of the UP element on gene expression [58].

Table 2: Number of sequences reminiscent of strong promoters in regions located upstream and downstream of the initiation codon of genes in bacterial genomes.

N°	Genome	Length, bp	Number of genes	Upstream region	Downstream region
1	<i>Deinococcus radiodurans</i> RI (AE000513)	2648638	2681	5	1
2	<i>Caulobacter crescentus</i> (AE005673)	4016947	3787	2	0
3	<i>Ralstonia solanacearum</i> GM11000 (AL646052)	3716413	3477	7	0
4	<i>Pseudomonas aeruginosa</i> PA01 (AE004091)	6264403	5570	15	2
5	<i>Xanthomonas campestris</i> pv. <i>campestris</i> (AE008922)	5076188	4197	2	0
6	<i>Mycobacterium tuberculosis</i> (AL123456)	4411529	3922	7	0
7	<i>Xanthomonas axonopodis</i> pv. <i>citri</i> 306 (AE008923)	5175554	4344	2	0
8	<i>Mesorhizobium loti</i> (NC 002678)	7036074	6693	9	0
9	<i>Sinorhizobium meliloti</i> 1021 (AL591688)	3654135	3375	8	0
10	<i>Mycobacterium leprae</i> TN (AL450380)	3268203	2770	8	1
11	<i>Agrobacterium tumefaciens</i> C58 (AE007869)	2841581	2701	20	1
12	<i>Brucella melitensis</i> 16 M chromosome I (AE008917)	2117144	2059	21	4
13	<i>Treponema pallidum</i> (AE000520)	1138011	1083	4	3
14	<i>Chlorobium tepidum</i> TLS (AE006470)	2154946	2329	35	13
15	<i>Salmonella typhimurium</i> LT2 (AE006468)	4857432	4608	163	61
16	<i>Neisseria meningitidis</i> serogroup B MC58 (AE002098)	2272351	2226	112	45
17	<i>Escherichia coli</i> O157:H7 (AE005174)	5528445	5478	263	79
18	<i>Methanobacterium thermoautotrophicum</i> delta H (AE000666)	1751377	1900	81	24
19	<i>Synechocystis</i> PCC6803 (AB001339)	3573470	1074	31	6
20	<i>Thermotoga maritima</i> (AE000512)	1860725	1926	63	10
21	<i>Vibrio cholerae</i> chromosome I (AE003852)	2961149	2887	93	37
22	<i>Yersinia pestis</i> CO92 (AL590842)	4653728	4042	274	61
23	<i>Aquifex aeolicus</i> (AE000657)	1551335	1503	71	37
24	<i>Bacillus halodurans</i> C-125 (BA000004)	4202353	4125	359	87
25	<i>Bacillus subtilis</i> (AL009126)	4214814	4182	430	111
26	<i>Mycoplasma pneumoniae</i> M129 (U00089)	816394	705	37	14
27	<i>Chlamydia muridarum</i> (AE002160)	1069411	954	86	31
28	<i>Pasteurella multocida</i> PM70 (AE004439)	2257487	1996	228	64
29	<i>Chlamydia pneumoniae</i> J138 (BA000008)	1226565	1097	162	51
30	<i>Streptococcus pneumoniae</i> (AE005672)	2160837	2306	365	156
31	<i>Streptococcus pyogenes</i> SF370 serotype M1 (AE004092)	1852441	1731	292	115
32	<i>Thermoanaerobacter tengcongensis</i> MB4T (AE008691)	2689445	2632	467	248
33	<i>Listeria innocua</i> Clip11262 (AL592022)	3011208	3529	426	229
34	<i>Haemophilus influenzae</i> Rd (L42023)	1830138	1775	277	94
35	<i>Mycoplasma genitalium</i> G37 (L43967)	580074	519	83	63
36	<i>Staphylococcus aureus</i> N315 (BA000018)	2814816	2638	930	418
37	<i>Campylobacter jejuni</i> (AL111168)	1641481	1684	540	353
38	<i>Clostridium acetobutylicum</i> (AE001473)	3940880	3738	1685	916
39	<i>Borrelia burgdorferi</i> (AE000783)	910724	875	350	292
40	<i>Rickettsia prowazekii</i> Madrid E (AJ235269)	1111523	885	443	252
41	<i>Clostridium perfringens</i> 13 (BA000016)	3031430	2779	1499	772
42	<i>Ureaplasma urealyticum</i> (AF222894)	751719	645	328	236
43	<i>Buchnera aphidicola</i> Sg (AE013218)	641454	584	339	225

We selected 13 candidate promoter sequences for further analysis by evaluation of the ArgC thermostable protein production in a coupled transcription-translation system. These sequences all exhibited a total score ≥ 0.8475 , apart from TM1490 (see Table 3). The amplified DNA regions were connected to the reporter gene *argC*, and used directly to assess promoter activity *in vitro* (see Fig. 2). All putative promoters of *T. maritima* were found to be active; the protein yield ranged from 0.3 to 2.7-times that of the reference *Ptac* promoter (Fig. 4). The gene expression from the promoter PTM1272 was similar to that of *Ptac*,

whereas PTM0032 was reduced almost threefold. However, higher expression was detected from the other 11 promoters; the greatest expression level was observed for PTM0477, PTM1016, PTM1429 and PTMt45. Reporter gene expression was also higher for the strong promoter *PargC*, which carries the UP element.

We next aligned experimentally analyzed promoters of *T. maritima* (Fig. 5). The most conserved sequence was the -10 box, which was identical to the *E. coli* consensus. The -35 box was also highly conserved, except that cytosine

Table 3: Strong promoter candidates identified in *T. maritima* MSB8*.

Downstream located gene(s)**	Strong promoter candidate sequence***	Total score****
TM_0013 conserved hypothetical protein Operon: 2 genes	ACAATTTTTATCTGATATTTTTTTTCACAttcaccatagtcgatTATAAC <--- 97 bp --->aatctggagggtgacaatATG	0,8475
TM_0110 transcriptional regulator, XylR-related Operon: 6 genes	ACCTTGATTTTAAATTATTTCTGCATataattaatgtgaaCATAAT <--- 10 bp --->aaaaggaggaatcgaagTTG	0,805
TM_0280 hypothetical protein Operon: 6 genes	GCAATATTTGTCCAGAAATATACTTGATTtaacaaaaatggacaatgTAGAAT <--- 37 bp --->aaggaggaatcgtttATG	0,88
TM_0339 hypothetical protein Operon: 3 genes	AGAAAAATTTTTTTGGAGACTTGACAaaatatttgtaatttcTAAAAAT <--- 5 bp --->gcaggagggtgacaaaatATG	0,8975
TM_0373 dnaK protein Operon: 2 genes	TTTTACAAATCTCATACGACCCCTTGACAtccattctgtgcctcacTATAAT <--- 21 bp --->tctaaggagggtgacacaATG	0,94
TM_0657 rubrerythrin Operon: 3 genes	TAATGTAACTATTCAAATCATTACAgtttataattatgtggTAAAAAT <--- 22 bp --->ataggagggtgcagggtATG	0,8125
TM_0682 hypothetical protein Operon: 3 genes	GAATACTCTGTCAGAAAAGATTCGTGATCAtctttcacctcgtgtagTATAAT <--- 7 bp --->gagtattctctacacaATG	0,915
TM_1016 hypothetical protein	TAAAAATTTTCATGAAAAATTTCTTGAATtctgtgacaaaagggtTTAAT <--- 5 bp --->gccggagggtgatgtgagATG	0,9175
TM_1167 hypothetical protein	GAAAAGTTACAGAAAAAGTACCCTTGTTAtctgaaagggtgaaaaatggTAAAAAT <--- 61 bp --->tacaggaggggcgggagATG	0,865
TM_t27 tRNA-Asn	TCATTCATTTTACCATCGAGTCCACTTGAAAttcaggaagggtatgtagTACAAT <--- 0 bp --->tatccgtggaggttcc	0,8675
TM_1205 conserved hypothetical protein Operon: 13 genes	GTTTTTATCTCTACTAATTAGGTTGACAttattgattcagaagagTAAAAAT <--- 40 bp --->ccgaggagggtgatgaGTG	0,88
TM_1318 Operon: 2 genes	AGAAACAATTTTGGAAATTTGATCCATGGACAttattaccttaatgGATAAT <--- 0 bp --->ttaacaggagATG	0,8325
TM_t34 tRNA-Leu	AGAAAAATTTCCGATGAGGGTACTTGAAAagggtgaaaacctgtgcTATTAT <--- 0 bp --->atatgtcggaggttcc	0,855
TM_1429 glycerol uptake facilitator protein Operon: 3 genes	GCATTGTGATTTTTGTAACTATATTGACAtaaaaaaaagggtttgTATAAT <--- 107 bp --->caaggaggattgggaaaATG	0,9175
TM_t39 tRNA-Ala Operon: 3 genes	AAAAATAAAAAGTCCTTCTGGGGATTGACCattttctgactcatgcTATAAT <--- 50 bp --->taataaaaagcagggtggg	0,8725
TM_1667 xylose isomerase Operon: 2 genes	AAGTATATCCTAAAAAATATTTGAAAtgatacccaagattttaTATAAT <--- 16 bp --->ttagggagggtttacATG	0,905
TM_1780 argininosuccinate synthase Operon: 6 genes	GAAAATAACAGTGAAAAACACTTCATataatcatttcaataatccTATAAT <--- 15 bp --->aaaggagggttcatATG	0,875
TM_0150 (complem.) ribosomal protein L32 Operon: 5 genes	AAAAATGTAAAGAAGAGAAACTTGAATctttgaaaaacatcaTATACT <--- 210 bp --->acgaggaggataaaagATG	0,855
TM_0477 (complem.) outer membrane protein alpha	ACAAAAAACTTTAGAAAACTCTTGAATttcctttggacgggagggTATAAT <--- 28 bp --->gaaggagggtttgtcccATG	0,9425
TM_0625 (complem.) hypothetical protein	ATATTCGTTCTGAATGAAGTTTTACATttcatcaaatattttggtTATAGT <--- 152 bp --->attggaggcaaatagaaATG	0,805
TM_0656 (complem.) conserved hypothetical protein Operon: 2 genes	AACTTAAGTAACACAAAATTAACCTTGACAacgaaaggggggtgggTATAAT <--- 42 bp --->aagggttgggaactttGTG	0,8925
TM_0755 (complem.) conserved hypothetical protein Operon: 2 genes	AGAAATCTTTGAAAATCTAGAAAtcaaacgtcgtttccagTATACT <--- 101 bp --->aatggagggtctctgtATG	0,85
TM_0971 (complem.) hypothetical protein	AAATATAAATCTGAATTTACTAAATTCACAttgcaaatcatcattTATAAT <--- 10 bp --->aggaatctcaagggggaATG	0,895
TM_1015 (complem.) glutamate dehydrogenase	ATAATTTTGC AATTTTATCTCTATACAtctcacatcacctccggctaTATATT <--- 104 bp --->ttcgaggggggaaatgtATG	0,855

Table 3: Strong promoter candidates identified in *T. maritima* MSB8*. (Continued)

TM_1067 (complem.) oligopeptide ABC transporter, periplasmic	GGATTATTTTATACTGAAAGCCCTTGACCttgtgtatgtttgtgaTATTAT <--- 45 bp --->ataacgcaggggggtgATG	0,92
TM_1271 (complem.) type IV pilin-related protein	GGGTGATATTTCAACATTTAAATCTTGACAttctaccatgtcaaggtgTATAAT <--- 35 bp --->cccgggaggtggattttATG	0,9525
TM_1286 (complem.) 5- methyltetrahydropteroyltriglutamate...	GTTTATGCAAATTTTCCTTCTGTTAACCAgtttacacacacatgtggTATCAT <--- 19 bp --->aatggaggtgaaaagggTTG	0,8625
TM_t31 (complem.) tRNA-Leu	AAGTTTTGATTTTTGTAAGGTTGAAAtaactcttctgacgatgtggTATAAT <--- 0 bp --->aaaaaaaggagcc	0,86
TM_1412 (complem.) hypothetical protein	ATATGGAAGTTCAAAAAACATCTTGCTTcagagtggtttgtggTATAAA <--- 24 bp --->aataattccttagaggtATG	0,865
TM_1419 (complem.) myo-inositol-1-phosphate synthase-... Operon: 3 genes	AGAAAAC TATTGGTAAAGCACTTGAAAtatatgactgtaaaacctgaTATAAT <--- 61 bp --->ctaaggaggtgaaacatATG	0,87
TM_1439 (complem.) hypothetical protein Operon: 3 genes	TAGTATTCTACCCTAAACTCTTTCAttctggattcgataatTGTAAT <--- 222 bp --->tgagagtgaaaaaggccATG	0,835
TM_t45 (complem.) tRNA-Ser	AAAAGAAGGAAGAAAAATGAAAACCTTGAAACaaggaaacgattgagtgTATAAT <--- 1 bp --->ttttctgggtggagagga	0,865
TM_1786 (complem.) hypothetical protein	GTATTATTCATTCTAAAAACTTGAAAactgaccaataaagatTAGAAT <--- 44 bp --->cacaaggggggttttcATG	0,855
TM_1850 (complem.) hypothetical protein	AAACGATTCTTCTAAAATGTGTTCTTGATTgtatcactgttatgtTATAAA <--- 43 bp --->aaaaaggaggtgaaactATG	0,855
TM_t11 tRNA-Thr Operon: 2 genes	GAAAAGGTTATCAGGAAATATCTTGAATagaaaaggttcgtgtgtTAAAAT <--- 0 bp --->aaccacagaggcgagca	0,8825
TM_1272 glutamyl tRNA-Gln amidotransferase... Operon: 3 genes	TTTCACATTTTGCATTATACACCTTGACAtggtagaatgtcaagatTTTAAT <--- 99 bp --->ataatccacagagaggGTG	0,8975
TM_0032 (complem.) transcriptional regulator, XylR-related Operon: 1 genes	AATATTAGAATTTGAACTATAATTCGAAAtaattcctgtttattcactCATAAT <--- 79 bp --->agcaggaggaatatggaGTG	0,86
TM_1490 (complem.) ribosomal protein L14 Operon: 22 genes	GGTGAAAATATGCCCAGGAAACGTTTGACTggaatagttgtgagcgaTAAAAT <--- 259 bp --->aaggaggggtgaaatcATG	0,845

* The genome annotation of *T. maritima* AE000512 used for analysis was dated 28th December 2005.

** The gene order for the first 34 candidate sequences is shown on both strands as described in the annotated genome [49]. The complementary strand is noted as (complem).

*** The spacer between -35 and -10 sites and the region located downstream of the -10 site are shown in lowercase; the initiation codons of the ORFs are shown in capital letters at the end of the corresponding sequences.

**** The first 34 candidate sequences were detected with the score parameters $sUP = 13$, $s35 = 6$, $s10 = 5$; TMt11, TM1272, TM0032 and TM1490 were detected with $sUP = 12$, $s35 = 6$, $s10 = 5$ and used for analysis in a cell-free system (see Fig. 3).

preceded the -35 site in 9 promoters, and no significant preference was detected for the nucleotides at the 5th and 6th positions. An 18-bp spacer appeared to be more representative than a 17-bp distance between the -35 and -10 boxes. Although all candidates possessed an AT-rich region upstream of the -35 site, some of them had only one A-rich tract, suggesting that they harbor only a single sub-site of a putative UP element. In any case, the high score attributed to 11 identified promoters was corroborated by elevated activity *in vitro*. Taken together, the alignment data and the expression data from the cell-free system, suggest that *E. coli* RNA polymerase efficiently recognizes putative strong promoters of *T. maritima*, and that the presence of an UP-like element might contribute to the strength of the promoter.

Two regions, (2.4 and 4.2) of the four domains of σ^{70} are involved in the recognition of the -10 and -35 boxes of *E. coli* promoters, respectively [59]. Several amino acids involved in contact with DNA have been also identified in the α subunit [60]. These DNA-binding regions in both σ^{70} and α subunits of *E. coli* and *T. maritima* RNA polymerases share high similarity (data not shown), which highlights the fact that -35 and -10 boxes and UP-like element all contribute to the high promoter activity in the thermophilic host.

Discussion

Bacterial promoters can be arbitrarily classified as weak, moderate and strong promoters, depending on the level of expression of mRNAs or of the corresponding proteins. We have developed an algorithm that can predict strong promoters in bacterial genomes by matching the triad pat-

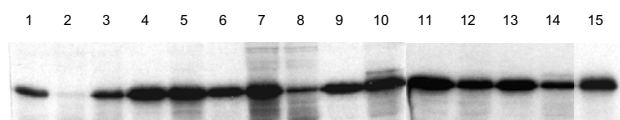


Figure 4
Assessment of the strength of *T. maritima* strong promoter candidates in a cell-free system. Lanes 1 – *PtaC* (reference); 2 – *PTM0032*; 3 – *PTM0373*; 4 – *PTM0477*; 5 – *PTM1016*; 6 – *PTM1067*; 7 – *PTM1271*; 8 – *PTM1272*; 9 – *PTM1429*; 10 – *PTM1490*; 11 – *PTM1667*; 12 – *PTM1780*; 13 – *PTMt45*; 14 – *PTMt11*; 15 – *PargC*. Similar results were obtained in 3 experiments.

tern specific for the group I σ^{70} factor of *E. coli* RNA polymerase. The first step in the proposed triad pattern approach involves matching the UP element located 300 bp upstream of a gene-coding sequence, and then matching two optimally separated -35 and -10 boxes.

The accuracy of the computational prediction of bacterial promoters depends on the A+T content of the genomes, which means that the matrix has to be adjusted to account for this factor in the DNA under analysis [29]. The data presented highlight the fact that the detection accuracy is lower in genomes with a high A+T content. The number of potential strong promoters identified in 43 bacterial genomes, is a direct function of their A+T content; this implies that the accuracy of the prediction is lower for genomes with A+T content higher than 62%.

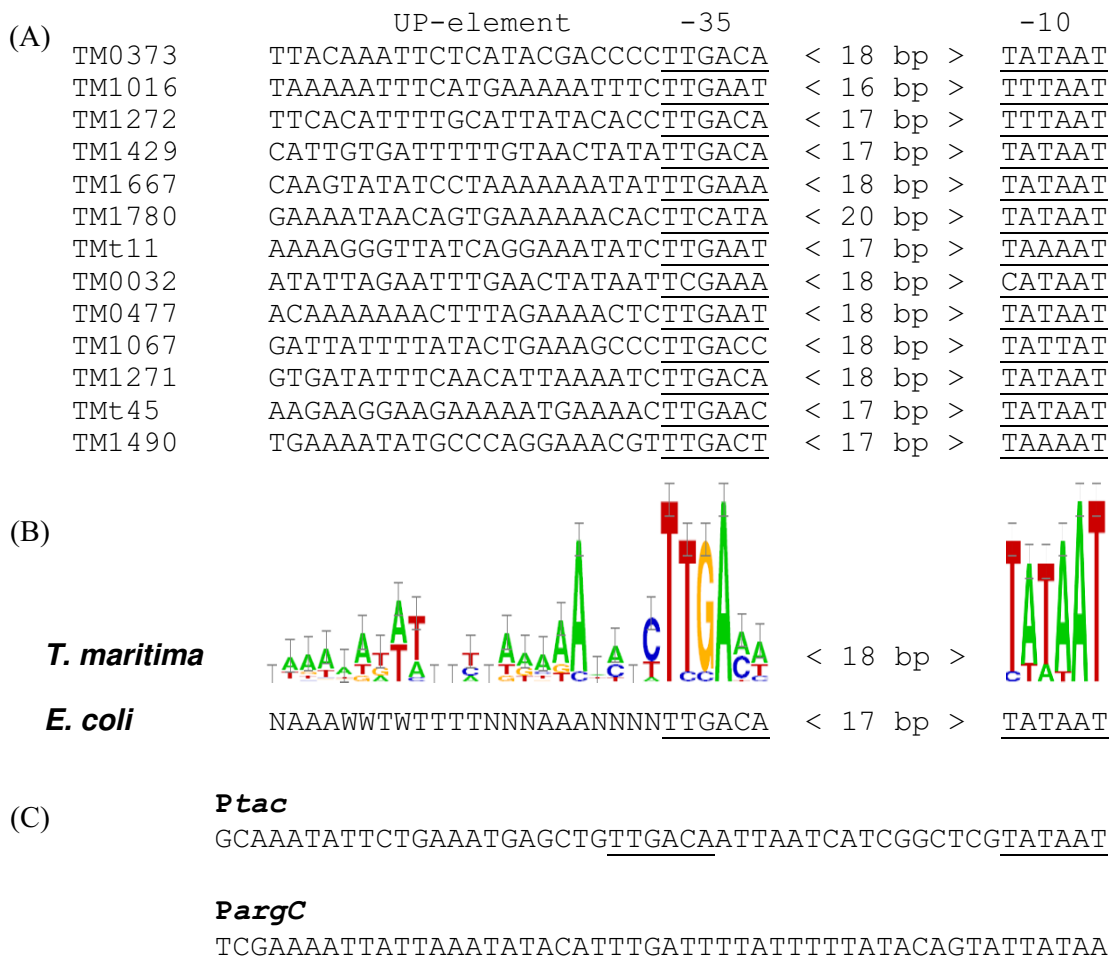


Figure 5
Organization of strong bacterial promoters. (A), Alignment of 13 promoter candidates of *T. maritima*; (B) consensus sequences of *T. maritima* and *E. coli* strong promoters; consensus of the *E. coli* UP element is described in [26, 27]; (C) the strong promoters *PtaC* and *PargC* were used as references in this study.

The choice of the matching score is yet another difficulty in identifying DNA-binding sites including promoters, as the highest score may not be the one most biologically relevant for genome-scale predictions [61,62]. It is therefore helpful to use additional criteria to eliminate false-positives. It looks as if the total score of 0.8475, calculated for the reference promoter *P_{tac}*, can be used as a reasonable criterion for identifying real strong promoters recognized by an $E\sigma^{70}$ -like RNA polymerase. In particular, using the scores applied to genomes analysis (see Tables 1 and 2), the algorithm detects 7 potential strong promoters in *M. tuberculosis* (~34% AT-rich genome) that encodes a variety of σ factors, including σ^A that recognizes the promoters possessing typical -10 and -35 boxes [63]. However, none of the predicted strong promoters had a total score in excess of 0.8475, and visual inspection indicated that none of these promoters possesses an UP-like sequence, suggesting that this gene expression-stimulating element is absent in *M. tuberculosis*.

The possibility of applying linear PCR-generated molecules for cell-free protein synthesis, without needing to perform DNA cloning in bacteria, is a prerequisite for assessing gene expression on a genome-wide scale. As a first step in this direction, we tested reporter-gene fusions to evaluate the strength of the promoters identified in the genome of *T. maritima*. Though this approach does not exclude possible masking effects of *E. coli* repressors or activators in the extracts, it is relatively simple, timesaving and informative, all of which are major advantages for evaluating computational predictions. Using the two well-characterized strong promoters (*P_{tac}* and *P_{argC}*) as references, high activity has been demonstrated for 11 out of 13 candidate sequences of *T. maritima*. This is quite a low proportion; however, it suggests that the detection accuracy by the triad pattern algorithm might be close to 85%. The limitations of the algorithm in terms of specificity and sensitivity of the virtual prediction of putative strong promoters might be further experimentally evaluated by analysis of bacterial genomes with high-throughput methods.

This study offers the first insight into the organization and distribution of strong promoters in hyperthermophilic organisms, which probably constitute the longest lineage in the microbial world [64]. Overall, strong promoters of hyperthermophiles are similar to those of mesophilic origin. We have recently shown that the *T. maritima* RNA polymerase α subunit binds to the *P_{argG}* promoter described here under *PTM1780* [65]. It has been found that the substitution of arginine in the hyperthermophilic α subunit, corresponding to the position Arg265 in the *E. coli* subunit and crucial for DNA recognition [60,66], or the deletion of an AT-rich sequence located upstream of the -35 site, decreases the binding affinity for DNA [65].

The *P_{argG}* promoter harbors a UP-like element, and is able to direct high gene expression *in vitro*. Moreover, this element appears to compensate for a poor -35 box or non-optimal 20-bp spacer of this promoter (see Table 3 and Fig. 5). Hence, these observations, along with the data obtained using other *T. maritima* promoters, allow us to assume that the presence of a UP-like element with less than 5 mismatches out of 17 nucleotides is essential for the strength of most strong promoters. This is consistent with the conservation of DNA interaction amino acids in the α subunit of the hyperthermophilic RNA polymerase. However, sequence-independent upstream DNA interactions within the C-terminal domain of the α subunit could often be required to initiate transcription in *E. coli* cells [67]. Therefore, the functional significance of the UP-like element in gene expression remains to be proven experimentally in hyperthermophilic organisms.

The strong promoters of *T. maritima* direct the transcription of genes involved in tRNA, ribosome synthesis, energy metabolism, transport, and cell movement (see Table 3). However, to our surprise, we found that 15 of the 38 best candidates promote the transcription of hypothetical proteins. The previously uncharacterized hypothetical protein TM1016 (total score 0.9175) turns out to share 28% identity with a biopolymer transport protein of *Vibrio vulnificus* [68]. In this context, recent studies of the *T. maritima* transcriptome have indicated that ABC transporters could play a major role in its ecology [69]. Further characterization of highly expressed hypothetical genes identified in our study might help to elucidate their role in the biology of this hyperthermophilic organism.

The strong promoter candidates prediction could contribute to the wide-scale genome expression analysis of evolutionarily distant bacteria, especially of those that possess an A+T DNA content lower than 62%. As a complement to DNA microarrays, it could help to elucidate the overall response of bacterial genomes to various environmental stresses. Moreover, the triad pattern algorithm can be used to extract the DNA region that carries translational signals; this is useful for investigating ORFs located downstream from the corresponding strong promoters (see Table 3). Thus, almost half of the *T. maritima* ORFs transcribed from putative strong promoters are preceded by a highly conserved Shine-Dalgarno site located 7–9 nucleotides from the ATG initiation codon, which is a characteristic feature of elevated protein synthesis in gram-negative and gram-positive bacteria [70]. This information will be useful for comparing highly synthesized mRNAs with the production of the corresponding proteins using high-throughput transcriptomic and proteomic methods, which is an important challenge in the fields of basic and applied microbiology [71]. Furthermore, the characterization of proteins whose expression is governed by strong

promoters looks like a promising approach to selecting candidate vaccines against microbial diseases and/or to identifying potential new antibacterial targets in the fight against nosocomial infections.

Further quantitative assessment of a dynamic and complicated mechanism of protein-DNA and protein-protein interactions involved in transcription might help to develop a more advantageous multi-pattern tool using both DNA and protein parameters to provide a comprehensive prediction of the strength of promoter activity in bacterial cells.

Conclusion

The triad pattern algorithm developed predicts strong promoter candidates by matching UP-like elements and identifying the presence of -35 and -10 boxes optimally distanced from each other in the annotated bacterial genomes. The presence of strong promoters is a function of the A+T content of the bacterial genome, and the number of false-positives is greater for genomes that have an A+T content higher than 62%. The prediction algorithm has been validated by cell-free experimental dissection of putative *T. maritima* promoters. The data indicate that strong promoters govern the transcription of genes coding vital functions, and of genes coding as-yet unknown functions in this hyperthermophilic bacterium. This algorithm is simple to use and flexible, and it could be further adapted to meet the requirements of a genome of interest if its promoter-specific motifs differ from consensus recognized by σ^{70} -like RNA polymerase.

Availability and requirements

The algorithm is freely accessible for non-commercial use at the web-site <http://www.protneteomix.com>. It takes several seconds to analyze the annotated genome sequence available from databases.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MD developed the algorithm and performed the computational analysis; AM conducted cell-free experiments; VS designed the project, contributed to the development of the algorithm and data analysis, and wrote the manuscript.

Additional material

Additional file 1

ReadMe. Contains information to use the algorithm.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2105-9-233-S1.pdf>]

Additional file 2

Software "strong_promoters.doc". The Text-format provides the list of putative strong promoter sequences with total and individual scores obtained for each consensus. The Word-format provides the tabulated list of putative strong promoters and their total score.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2105-9-233-S2.doc>]

Acknowledgements

At the time of the study AM was a research fellow supported by Pays de la Loire. MD acknowledges support from the Conseil Régional des Pays de la Loire and ProtNeteomix for his visit to Nantes University. We should like to thank anonymous reviewers whose suggestions allowed us to improve the manuscript. This study was supported by the "Post-Génome programme des Pays de la Loire", by the EU project EUR-INTAFAR (n°LSHM-CT-2004-512138), and by the R&D program of ProtNeteomix.

References

1. Darst SA: **Bacterial RNA polymerase.** *Curr Opin Struct Biol* 2001, **11**:155-162.
2. Queen C, Wegman MN, Korn LJ: **Improvements to a program for DNA analysis: a procedure to find homologies among many sequences.** *Nucleic Acids Res* 1982, **10**:449-456.
3. Galas DJ, Eggert M, Waterman MS: **Rigorous pattern-recognition methods for DNA sequences.** *J Mol Biol* 1985, **186**:117-128.
4. Staden R: **Methods for discovering novel motifs in nucleic acid sequences.** *Comput Appl Biosci* 1989, **5**:293-298.
5. Bailey TL, Elkan C: **Fitting a mixture model by expectation maximization to discover motifs in biopolymers.** *Proc Int Conf Intell Syst Mol Biol* 1994, **2**:28-36.
6. Alexandrov N, Mironov A: **Application of a new method of pattern recognition in DNA sequence analysis: a study of E. coli promoters.** *Nucleic Acids Res* 1990, **18**:1847-1852.
7. Demeler B, Zhou G: **Neural network optimization for E. coli promoter prediction.** *Nucleic Acids Res* 1991, **19**:1593-1599.
8. Cardon LR, Stormo GD: **Expectation maximization algorithm for identifying protein-binding sites with variable lengths from unaligned DNA fragments.** *J Mol Biol* 1992, **223**:159-170.
9. Horton PB, Kanehisa M: **An assessment of neural network and statistical approaches for prediction of E. coli promoter sites.** *Nucleic Acids Res* 1992, **20**:4331-4338.
10. Thieffry D, Salgado H, Huerta AM, Collado-Vides J: **Prediction of transcriptional regulatory sites in the complete genome sequence of Escherichia coli K-12.** *Bioinformatics* 1998, **14**:391-400.
11. Vanet A, Marsan L, Labigne A, Sagot M-F: **Inferring regulatory elements from a whole genome. An analysis of Helicobacter pylori σ^{80} family of promoter signals.** *J Mol Biol* 2000, **297**:335-353.
12. Leung SW, Melish C, Robertson D: **Basic gene grammars and DNA-chartparser for language processing of Escherichia coli promoter DNA sequence.** *Bioinformatics* 2001, **17**:226-236.
13. Gordon L, Chervonenkis AY, Gammerman AJ, Shahmuradov IA, Solov'yev VV: **Sequence alignment kernel for recognition of promoter regions.** *Bioinformatics* 2003, **19**:1964-1971.
14. Jacques P-E, Rodrigue S, Gaudreau L, Goulet J, Brzezinski R: **Detection of prokaryotic promoters from the genomic distribution of hexanucleotide pairs.** *BMC Bioinformatics* 2006, **7**:423. (doi:10.1186/1471-2105-7-423)
15. Benham CJ: **Sites of predicted stress-induced DNA duplex destabilization occur preferentially at regulatory loci.** *Proc Natl Acad Sci USA* 1993, **90**:2999-3003.
16. Kanhere A, Bansal M: **A novel method for prokaryotic promoter prediction based on DNA stability.** *BMC Bioinformatics* 2005, **6**:1-10.
17. Wang H, Benham CJ: **Promoter prediction and annotation of microbial genomes based on DNA sequence and structural**

- responses to superhelical stress. *BMC Bioinformatics* 2006, **7**:248. (doi:10.1186/1471-2105-7-248)
18. Hawley D, McClure WR: **Compilation and analysis of *Escherichia coli* promoter DNA sequences.** *Nucleic Acids Res* 1983, **11**:2237-2255.
 19. Harley C, Reynolds R: **Analysis of *E. coli* promoter sequences.** *Nucleic Acids Res* 1987, **15**:2343-2361.
 20. O'Neil M, Chiafari F: ***Escherichia coli* promoters. II. A spacing-class dependent promoter search protocol.** *J Biol Chem* 1989, **264**:5531-5534.
 21. Helmann JD: **The extracytoplasmic function (ECF) sigma factors.** *Adv Microb Physiol* 2002, **46**:47-110.
 22. deHaseth PL, Zupancic ML, Record MT Jr: **RNA-polymerase-promoter interactions: the comings and goings of RNA polymerase.** *J Bacteriol* 1998, **180**:3019-3025.
 23. Makrides SC: **Strategies for achieving high-level expression of genes in *Escherichia coli*.** *Microbiol Rev* 1996, **60**:512-538.
 24. Ross W, Gosink KK, Salomon J, Igarashi K, Zou C, Ishihama A, Severinov K, Gourse RL: **A third recognition element in bacterial promoters: DNA binding by the α subunit of RNA polymerase.** *Science* 1993, **262**:1407-1413.
 25. Ross W, Ernst A, Gourse RL: **Fine structure of *E. coli* RNA polymerase-promoter interactions: α subunit binding to the UP element minor groove.** *Genes & Dev* 2001, **15**:491-506.
 26. Estrem ST, Gaal T, Ross W, Gourse RL: **Identification of an UP element consensus sequence for bacterial promoters.** *Proc Natl Acad Sci USA* 1998, **95**:9761-9766.
 27. Aiyar SE, Gourse RL, Ross W: **Upstream A-tracts increase bacterial promoter activity through interactions with the RNA polymerase alpha subunit.** *Proc Natl Acad Sci USA* 1998, **95**:14652-14657.
 28. Estrem ST, Ross W, Gaal T, Chen ZWS, Niu W, Ebright RH, Gourse RL: **Bacterial promoter architecture: sub-site structure of UP elements and interactions with the C-terminal domain of the RNA polymerase α subunit.** *Genes & Dev* 1999, **13**:2134-2147.
 29. Hertz GZ, Stormo GD: ***Escherichia coli* promoter sequences: analysis and prediction.** *Methods Enzymol* 1996, **273**:30-42.
 30. Tutukina MN, Shakunov KS, Masulis IS, Ozoline ON: **Intragenic promoter-like sites in the genome of *Escherichia coli* discovery and functional implication.** *J Bioinform Comput Biol* 2007, **5**:549-560.
 31. Fredrick K, Caramori T, Chen Y, Galizzi A, Helmann JD: **Promoter architecture in the flagellar regulon of *Bacillus subtilis*: high-level expression of flagellin by the Σ D RNA polymerase requires an upstream promoter element.** *Proc Natl Acad Sci USA* 1995, **92**:2582-2586.
 32. Savchenko A, Weigel P, Dimova D, Lecocq M, Sakanyan V: **The *Bacillus stearothermophilus* argCJBD operon harbours a strong promoter as evaluated in *Escherichia coli* cells.** *Gene* 1998, **212**:167-177.
 33. Aiyar SE, Gaal T, Gourse RL: **rRNA promoter activity in the fast-growing bacterium *Vibrio natrigens*.** *J Bacteriol* 2002, **184**:1349-1358.
 34. Sorokin AA, Osypov AA, Dzhylyadin TR, Beskaravainy PM, Kamzolova SG: **Electrostatic properties of promoter recognized by *E. coli* RNA polymerase Esigma70.** *J Bioinform Comput Biol* 2006, **4**:455-467.
 35. Mitchell JE, Zheng D, Busby SJ, Minchin SD: **Identification and analysis of "extended" promoters in *Escherichia coli*.** *Nucleic Acids Res* 2003, **31**:4689-4695.
 36. Stormo GD, Schneider TD, Gold L, Ehrenfeucht A: **Use of the "Perception" algorithm to distinguish translational initiation sites in *E. coli*.** *Nucleic Acids Res* 1982, **10**:2997-3011.
 37. Baldi P, Chauvin Y, Hunkapiller T, McClure MA: **Hidden Markov models of biological primary sequence information.** *Proc Natl Acad Sci USA* 1994, **91**:1059-1063.
 38. Jarmer H, Larsen TS, Krpgh A, Saxild HH, Brunak S, Knudsen S: **Sigma A recognition sites in the *Bacillus subtilis* genome.** *Microbiology* 2001, **147**:2417-2424.
 39. Petersen L, Larsen TS, Ussery DW, On SL, Krogh A: **RpoD promoters in *Campylobacter jejuni* exhibit a strong periodic signal instead of a -35 box.** *J Mol Biol* 2003, **326**:1361-1372.
 40. Munch R, Hiller K, Grote A, Scheer M, Klein J, Schobert M, Jahn D: **Virtual Footprint and PRODORIC: an integrative framework for regulon prediction in prokaryotes.** *Bioinformatics* 2005, **21**:4187-4189.
 41. Vanet A, Marsan L, Sagot M-F: **Promoter sequences and algorithmic methods for identifying them.** *Res Microbiol* 1999, **150**:779-799.
 42. Waterman MS: **Sequence alignments in the neighborhood of the optimum with general application to dynamic programming.** *Proc Natl Acad Sci USA* 1983, **80**:3123-3124.
 43. Campbell EA, Muzzin O, Chlenov M, Sun JL, Olson CA, Weinman O, Trester-Zedlitz ML, Darst SA: **Structure of the bacterial RNA polymerase promoter specificity sigma subunit.** *Mol Cell* 2002, **9**:527-539.
 44. Vassylyev DG, Sekine S, Laptenko O, Lee J, Vassylyeva MN, Borukhov S, Yokoyama S: **Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 Å resolution.** *Nature* 2002, **417**:712-719.
 45. Salgado H, Santos-Zavalets A, Gama-Castro S, Millán-Zárate D, Díaz-Peredo E, Sánchez-Solano F, Pérez-Rueda E, Bonavides-Martínez C, Collado-Vides J: **RegulonDB (version 3.2): transcriptional regulation and operon organization in *Escherichia coli* K-12.** *Nucleic Acids Res* 2001, **29**:72-74.
 46. Sakanyan V, Charlier D, Legrain C, Kochikyan A, Mett I, Piérard A, Glansdorff N: **Primary structure, partial purification and regulation of key enzymes of the acetyl cycle of arginine biosynthesis in *Bacillus stearothermophilus*: dual function of ornithine acetyltransferase.** *J Gen Microbiol* 1993, **139**:393-402.
 47. Karaivanova IM, Weigel P, Takahashi M, Fort C, Versavaud A, Van Duyn G, Charlier D, Hallet JN, Glansdorff N, Sakanyan V: **Mutational analysis of the thermostable arginine repressor from *Bacillus stearothermophilus*: dissecting residues involved in DNA binding properties.** *J Mol Biol* 1999, **291**:843-855.
 48. De Boer HA, Comstock LJ, Vasser M: **The tac promoter: a functional hybrid derived from the trp and lac promoters.** *Proc Natl Acad Sci USA* 1983, **80**:21-25.
 49. Snappyan M, Lecocq M, Guevel L, Arnaud MC, Ghochikyan A, Sakanyan V: **Dissecting DNA-protein and protein-protein interactions involved in bacterial transcriptional regulation by a sensitive protein array method combining a near-infrared fluorescence detection.** *Proteomics* 2003, **3**:647-657.
 50. Pratt JM: **Coupled transcription-translation in prokaryotic cell-free systems.** In *Transcription and translation: a practical approach* Edited by: Hames BD, Higgins SJ. New York: IRL Press; 1984:179-209.
 51. Kim DV, Swartz JR: **Prolonging cell-free protein synthesis with a novel ATP regeneration system.** *Biotechnol Prog* 2000, **16**:385-390.
 52. Schneider TD, Stephens RM: **Sequence Logos: a new way to display consensus sequences.** *Nucleic Acids Res* 1990, **18**:6097-6100.
 53. Crooks GE, Hon G, Chandonia JM, Brenner SE: **WebLogo: A sequence logo generator.** *Genome Res* 2004, **14**:1188-1190 [<http://www.bio.cam.ac.uk/seqlogo/logo.cgi>].
 54. Weiner J III, Herrmann R, Browning GF: **Transcription in *Mycoplasma pneumoniae*.** *Nucleic Acids Res* 2000, **28**:4488-4496.
 55. Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Aujay M, Huber R, Feldman RA, Short JM, Olsen GJ, Swanson RV: **The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*.** *Nature* 1998, **392**:353-358.
 56. Giacani L, Hevner K, Centurion-Lara A: **Gene organization and transcriptional analysis of the trpJ, trpI, trpG, and trpF loci in *Treponema pallidum* strains Nichols and Sea 81-4.** *J Bacteriol* 2005, **187**:6084-6093.
 57. Nelson KE, Clayton RA, Gill SR, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Nelson WC, Ketchum KA, McDonald L, Utterback TR, Malek JA, Linher KD, Garrett MM, Stewart AM, Cotton MD, Pratt MS, Phillips CA, Richardson D, Heidelberg J, Sutton GG, Fleischmann RD, Eisen JA, White O, Salzberg SL, Smith HO, Venter JC, Fraser CM: **Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*.** *Nature* 1999, **399**(6734):323-329.
 58. Paul BJ, Ross W, Gaal T, Gourse RL: **rRNA transcription in *Escherichia coli*.** *Annu Rev Genet* 2004, **38**:749-770.
 59. Dove SL, Darst SA, Hochschild A: **Region 4 of sigma as a target for transcription regulation.** *Mol Microbiol* 2003, **48**:863-874.
 60. Murakami K, Fujita N, Ishihama A: **Transcription factor recognition surface on the RNA polymerase alpha subunit is**

- involved in contact with the DNA enhancer element. *EMBO J* 1996, **15**(16):4358-4367.
61. Eskin E, Keich U, Gelfand MS, Pevzner PA: **Genome-wide analysis of bacterial promoter regions.** *Pac Symp Biocomput* 2003:29-40.
 62. Huerta AM, Collado-Vides J: **Sigma70 promoters in Escherichia coli: specific transcription in dense regions of overlapping promoter-like signals.** *J Mol Biol* 2003, **333**:261-278.
 63. Manganelli R, Proveddi R, Rodrigue S, Beaucher J, Gaudreau L, Smith I: **σ factors and global regulation in Mycobacterium tuberculosis.** *J Bacteriol* 2004, **186**:895-902.
 64. Woese CR, Kandler O, Wheelies ML: **Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya.** *Proc Natl Acad Sci USA* 1990, **87**:4576-4579.
 65. Braun F, Marhuenda FB, Morin A, Guevel L, Fleury F, Takahashi M, Sakanyan V: **Similarity and divergence between the RNA polymerase alpha subunits from hyperthermophilic Thermotoga maritima and mesophilic Escherichia coli bacteria.** *Gene* 2006, **380**:120-126.
 66. Gaal T, Ross W, Blatter EE, Tang H, Jia X, Krishnan VV, Assa-Munt N, Ebright RH, Gourse RL: **DNA-binding determinants of the alpha subunit of RNA polymerase: novel DNA-binding domain architecture.** *Genes & Dev* 1996, **10**:16-26.
 67. Ross W, Gourse RL: **Sequence-independent upstream DNA- α CTD interactions strongly stimulate Escherichia coli RNA polymerase-lacUV5 promoter association.** *Proc Natl Acad Sci USA* 2005, **102**:291-296.
 68. Kim YR, Lee SE, Kim CM, Kim SY, Shin EK, Shin DH, Chung SS, Choy HE, Progulsk-Fox A, Hillman JD, Handfield M, Rhee JH: **Characterization and pathogenic significance of Vibrio vulnificus antigens preferentially expressed in septicemic patients.** *Infect Immun* 2003, **71**:5461-5471.
 69. Johnson MR, Connors SB, Montero CI, Chou CJ, Shockley KR, Kelly RM: **The Thermotoga maritima phenotype is impacted by syntrophic interaction with Methanococcus jannaschii in hyperthermophilic coculture.** *Appl Environ Microbiol* 2006, **72**:811-818.
 70. Vellanoweth RL, Rabinowitz JC: **The influence of ribosome-binding-site elements on translational efficiency in Bacillus subtilis and Escherichia coli in vivo.** *Mol Microbiol* 1992, **6**:1105-1114.
 71. Boyce JD, Cullen PA, Adler B: **Genomic-scale analysis of bacterial genes and protein expression in the host.** *Emerg Infect Dis* 2004, **10**:1357-1362.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

