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## Is prokaryotic complexity limited by accelerated growth in regulatory overhead?

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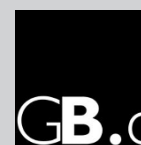
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# **Is prokaryotic complexity limited by accelerated growth in regulatory overhead?**

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## **Abstract**

### **Background**

Increased biological complexity is generally associated with the addition of new genetic information, which must be integrated into the existing regulatory network that operates within the cell. General arguments on network control, as well as several recent genomic observations, indicate that regulatory gene number grows disproportionately fast with increasing genome size.

### **Results**

We present two models for the growth of regulatory networks. Both predict that the number of transcriptional regulators will scale quadratically with total gene number. This appears to be in good quantitative agreement with genomic data from 89 fully sequenced prokaryotes. Moreover, the empirical curve predicts that any new non-regulatory gene will be accompanied by more than one additional regulator beyond a genome size of about 20,000 genes, within a factor of two of the observed ceiling.

### **Conclusions**

Our analysis places transcriptional regulatory networks in the class of accelerating networks. We suggest that prokaryotic complexity may have been limited throughout evolution by regulatory overhead, and conversely that complex eukaryotes must have bypassed this constraint by novel strategies.

## Background

An increase in organism complexity or functionality can be achieved by adding new functional genes, and/or by adding new regulatory regimes. Each case requires an expansion of the regulatory network to integrate new capabilities with existing ones. The ability to access more genes (or operons, i.e., co-regulated functional modules of genes) therefore not only involves a linear increase in regulator number, but also enforces an additional expansion of higher order regulation, if the system as a whole is to be coordinated and not descend into chaotic space. A fraction of the regulators (those that are not constitutively expressed) will themselves require regulation, and the impact of new gene products on the biology of the cell will need to be integrated by additional input into the existing regulatory framework. For example, if a new operon dealing with the metabolism of a particular sugar is introduced into the cell, not only is a new regulator that recognizes this sugar required (or at least advantageous), but the effect of the activity of this operon has to be coordinated with the metabolism of other substrates that feed into the cell's energy flux, as exemplified by the *lac* operon. A growing body of literature supports the notion that increases in complexity arise indeed from progressively more elaborate regulation of gene expression [1]. These considerations suggest that the numbers of regulators (or combinations thereof) must generally scale faster than linearly with the number of genes.

In agreement with this general prediction, it has been shown that regulatory gene number in prokaryotic genomes grows disproportionately fast [2-5]. In particular, a recent study analyzed the scaling of gene counts  $n_c$  for each of 44 functional protein categories in relation to the total number of genes  $n$ , across 64 bacterial genomes [5]. Surprisingly, almost all categories showed a power law dependence on total gene count,  $n_c \sim n^\alpha$ . Transcriptional regulators were the fastest growing class, with an exponent  $\alpha$  of approximately 2 ( $1.87 \pm 0.13$

for “transcription regulation” and  $2.07 \pm 0.21$  for “two component systems”). As linear increases in regulator numbers can theoretically provide a combinatorially explosive number of regulatory regimes, this observation suggests that the number of required regulatory states is increasing faster than the number of meaningful combinations of regulatory factors [5], although the upper limits on the size of genetic networks that may be imposed by this regulatory expansion were not considered.

Studies of such ‘accelerating growth’ networks have recently been prompted by observations that the Internet grows by adding links more quickly than sites [6]. However, the relative change over time is small and the Internet appears to remain scale free and well characterized by stationary statistics [7]. Similarly, the average number of links per substrate in metabolic networks of organisms appears to increase linearly with substrate number [8], while the average number of links of scientific collaboration networks increases linearly over time [9]. These observations have motivated models where accelerating growth in link number generates nonstationary statistics from random to scale-free to regular connectivity at particular network sizes, as the growing number of links gradually saturates the network and links all nodes together [10] (for an overview, see [11, 12]). If biological regulatory networks indeed feature accelerating growth, they will be characterized by sparse connectivity at low gene numbers. If these networks, optimized by evolution in the sparse regime, are unable to make the transition to the densely connected regime, the evolutionary record would show a strict size limit at some maximum network size. This is exactly what is observed: prokaryotic gene numbers appear restricted to below approximately 10,000 genes or a genome size of approximately 10 megabases [13].

Below, we present mathematical arguments that substantiate our intuitive expectation of accelerated growth of regulatory networks; and we confirm that our predictions are in good

agreement with experimental results. We further calculate a rough estimate of the size limit that this accelerated growth of regulatory networks may impose on prokaryotic genomes.

We wish to point out that both models outlined below rely on a number of plausible, yet unproven assumptions. These models will undoubtedly need adjustments once more detailed information on the evolutionary mechanism of genome expansion is available. While they will not encapsulate all biological details correctly, the models may serve as illustrations of the kind of mechanisms that might cause the observed accelerating growth of regulatory networks.

## Results and Discussion

### Model I: Regulatory integration of new genes

We can derive a specific prediction of the relationship between regulator numbers  $R$  and the numbers of genes  $N$  from simple assumptions about the evolution of regulatory networks. Consider a new gene that is added to the genome, e.g., by gene duplication or horizontal gene transfer. Initially, the new gene will be free to drift in its pattern of interactions with other genes. The available space of regulatory interactions that it can explore is the full set of genes already present in the genome. This evolutionary search is undirected. Thus, *a priori*, each regulatory interaction of the new gene with any previous gene has the same probability (termed  $p$ ) to be selectively favorable. For each gene added to a genome containing  $N$  genes, we thus expect  $p N$  interactions to become fixed. Some genes may be integrated into the regulatory network only via existing regulatory factors. However, we expect that some of the new genes have to be regulated specifically; thus, a fraction  $v$  of the new interactions will correspond to new regulatory factors. In sum, adding one new gene results in the fixation of  $\Delta R = v p N$  new regulators, with  $v p = c$  constant; or

equivalently, adding  $\Delta N$  new genes results in  $\Delta R = c N \Delta N$  new regulators. Some (but perhaps only a minority) of the  $\Delta R$  new regulators will themselves require regulation [14], and reciprocally the activities of the newly regulated functional units will have to be integrated back into the regulatory network of the cell, both leading to additional higher order terms dependent on the degree of required connectivity of the system as a whole. In a first approximation, we will ignore these; their inclusion will further accelerate regulatory network growth.

Starting from a hypothetical empty genome and adding one gene at a time, we can estimate the total number of regulators as a sum over all  $\Delta R$  terms:

$$R = \sum_{n=0}^N cn = \frac{cN(N+1)}{2} \approx \frac{c}{2} N^2 \quad (1)$$

Thus, the number of regulators  $R$  scales approximately quadratically with increasing gene number. As prokaryotic operon size decreases only slowly with increasing genome size [15],  $N$  in Eq.1 can also be interpreted as operon number, which simply changes the scaling factor  $c$ . In eukaryotes,  $N$  includes the numbers of different splice variants.

## **Model II: Homology based interactions of new regulators**

An alternative theoretical approach focuses not on the regulation required by newly added genes, but on the transcriptional regulators themselves. Any given transcription factor, which is newly added to a genome, will be retained under one condition: that it establishes fitness enhancing interaction with potential binding sites present in the genome.

We assume that the nucleotide sequences of potential binding sites are approximately random. The probability of finding or developing a match to the given transcription factor specificity among potential transcription factor binding sequences is then

proportional to the total amount of such sequences. This scaling is analogous to that known from sequence similarity searches, such as BLAST or FASTA: the probability of finding a random sequence match scales linearly with the length of the target sequence.

The total amount of potential transcription factor binding sequences will scale linearly with the number of genes. Thus, the average number of matches between our new transcription factor and potential binding sequences scales linearly with total gene number. If the evolutionary search is undirected, i.e., each interaction is *a priori* equally likely to provide a fitness benefit, then the probability of retaining a newly added transcription factor also scales linearly with gene number:  $\Delta R = c'N$ , again leading to Equation (1).

## Genomic analysis

We then proceeded to examine the actual relationship between the numbers of transcriptional regulators (defined here as those utilizing sequence specific binding to DNA or RNA) and genome size in prokaryotes. We analysed 89 completely sequenced bacterial and archaeal genomes, ranging from *Mycoplasma genitalium* (containing just 480 genes) to *Bradyrhizobium japonicum* (8317 genes), thereby spanning almost the entire range of observed genome sizes. For each genome, we estimated the number of regulatory proteins by searching all genes for matches to Pfam profiles of protein domains [16] with known regulatory or signalling functions and/or known to be involved in DNA or RNA binding. Although all of these genomes contain many genes with unknown function, some of which may have potentially unidentified regulatory domains, this should not unduly bias the analysis unless the proportion of unidentified regulators varies with genome size, which appears unlikely.

In agreement with the predictions from the above models, and in agreement with previous studies [2, 5], we find the increase of regulatory genes with total gene number to be



most consistent with a quadratic function: the double logarithmic plot in Fig. 1 is well described by a straight line with slope 1.96 (95%-confidence interval 1.81 - 2.11,  $r^2=0.88$ ), giving the empirical relationship  $R = 0.0000163 N^{1.96}$ . The fitted curve is not significantly different from  $R = 0.0000120 N^2$ . Importantly, the relationship remains consistent with a quadratic even when more or less stringent definitions of regulatory protein domains are employed, or when all proteins annotated as regulators are included (data not shown; see also [5]). While the size range of fully sequenced prokaryotic genomes spans hardly more than one order of magnitude, the high  $r^2$  value together with the tight confidence interval for the exponent are good evidence that the true scaling behaviour is a higher order function (i.e. higher than linear), and most likely to be close to a quadratic.

From Fig. 1, it is evident that regulatory networks are sparsely connected. In *E. coli*, each transcriptional regulator targets on average 5 operons [17], corresponding to approximately 8.5 genes [15]. If this relation can be extrapolated to other genomes, Fig. 1 suggests that each gene is connected to on average  $C = 0.06\%$  of all other genes (see Methods).

Previous studies of the transcriptional network in *Escherichia coli* have found a modular structure [17, 18]: densely co-regulated sets of genes form partially overlapping functional modules, which are controlled by global regulators. If new genes explored regulatory interactions predominantly within modules that are sparsely connected into the rest of the network, then this would give a largely linear relationship between  $R$  and  $N$ , with a small quadratic term for module interconnectivity. The fact that the relationship between  $R$  and  $N$  appears to be close to a pure quadratic (Fig. 1) then suggests that new genes explore regulatory interactions with a significant proportion of the genome, not just within modules. This is consistent with the finding that modules do not represent closed systems: many

genes are regulated not only by within-module transcription factors, but also by factors that control genes across several modules [17].

## Regulatory networks

The observed increase of average link number with network size means that transcription networks feature accelerating growth in connectivity, and hence nonstationary (or size dependent) statistics. This constitutes a significant difference from the more usual classes of exponential or scale-free networks with stationary (size independent) statistics. The non-linear relationship predicted in Eq. 1 and confirmed in Fig. 1 differs from the growth behavior described for previously studied metabolic and man-made networks, which largely feature non-accelerating growth and stationary statistics [6, 8, 9, 19, 20]. This may be a consequence of fundamental differences: the Internet, the World Wide Web, and scientific collaboration networks, among other generally studied networks, have not been subject to selection for specific dynamical functions, as opposed to gene regulatory networks [21]. While metabolic networks are of course related to the regulatory networks governing them (and are indeed optimized for a closely related function), they are dominated by the most highly connected substrates (such as water, ATP, and ADP) [8]; links involving such ubiquitous reactants contain little information on network control.

The scaling law in Eq. 1 is based on two simple suppositions: that each new gene explores a space of possible interactions which is proportional in size to the total number of genes; and that *a priori* each new interaction is equally likely to lead to the fixation of a new regulator. We can develop this into a simple explicit network model by presuming that most regulatory interactions are between non-regulatory genes (which for prokaryotes may be a reasonable first-order approximation [18]). These genes form the nodes of the network, while the links between nodes are regulatory interactions. In this case, total gene number  $N$

in Eq. 1 is replaced by the number of non-regulatory genes  $S$ . If we presume that new regulatory genes explore outbound regulatory interactions with all existing nodes with equal probability, then this means that the inbound regulatory links to regulated genes are exponentially distributed and described by a random Erdős-Rényi style compact distribution network [22]. Interestingly, it has been shown that the number of transcriptional regulators controlling one gene follows an exponential distribution in *E. coli* [17], as is expected in Erdős-Rényi networks [22]. Existing regulatory networks are of course far from random, but are highly optimized by natural selection. However, if selective forces affect all nodes with equal probability, then the resulting network topology resembles that of a random network.

### Limited complexity of prokaryotes

Regardless of the exact nature of the distributions, the non-linear scaling confirmed in Fig. 1 places prokaryote transcriptional networks firmly in the class of accelerating networks. Regulators are the fastest growing class of proteins [5], and their scaling behaviour has profound implications for the ability of prokaryotes to evolve more complex genetic programs. In particular, the accelerating growth of the regulatory overhead must eventually impose an inherent upper size limit on prokaryote genomes, which we can roughly estimate as being at the point where functional gain is outweighed by regulatory cost, as follows. The total gene number  $N$  is composed of both regulatory genes  $R$  and non-regulatory genes  $S$ , and thus  $\Delta N = \Delta R + \Delta S$  for any increase in genome size  $\Delta N$ . In small genomes, growth occurs with the addition of many more non-regulatory than regulatory genes, and  $\Delta R \ll \Delta S$ . However, as genomes enlarge, there comes a point where each new non-regulatory gene will be accompanied by the addition of more than one regulatory gene,  $\Delta R > \Delta S$ . Satisfaction of this constraint roughly indicates when the required regulatory overhead outweighs the gains afforded by additional non-regulatory genes: genome expansion becomes inefficient. From

$\Delta R = c N \Delta N$  (either inferred directly from Fig. 1, or from the derivation of Eq. 1, with  $c = 2.40 \times 10^{-5}$  from a fit with slope 2.00), we find that this regime is reached when  $c N \Delta N \approx 1/2$ , or  $N >$  approximately 20,000 genes. The latter figure is within a factor of two of the observed ceiling of around 10,000 genes in prokaryotes [13]. It may also be noteworthy that the observed limit coincides with the point where the number of operons equals the total number of regulatory interactions affecting them, where the latter is estimated as 5 x the number of regulators [17]. Regardless of the precise limit, the inescapable conclusion stemming from accelerated regulatory network growth is that there is a limit to genome size imposed by regulatory overhead. It may be more than coincidental that the predicted and observed limits are similar.

Our results then suggest that gene numbers and the complexity of prokaryotes may have been constrained by the architecture of their regulatory networks. It is evident that prokaryotes have never reached the size and complexity of multicellular eukaryotes, whose genomes contain 14000-50000 individually regulated protein-coding genes [23-26], that are subject to alternative splicing to produce many more isoforms, in addition to large numbers of noncoding RNA genes [27]. The low complexity of prokaryotes has previously been attributed to biochemical or environmental factors, e.g., the type of energy metabolism or the absence of particular proteins such as cell-cell signaling proteins or homeobox proteins which are unique to higher eukaryotes. However, such stratagems should have been available to prokaryotes; like eukaryotes, they have had over 4 billion years of evolutionary history in which to explore protein structural and chemical space, aided by lateral gene transfer. It is also often assumed that the increased complexity of eukaryotes is a result of control systems which exploit the increased possibilities afforded by combinatorics of regulatory factors, and of the introduction of new levels of control. However, it would not be difficult to imagine the evolution of larger cis-regulatory regions and new regulatory protein

recognition sites in prokaryotic genes. Moreover, the introduction of new levels of control requires the introduction of new regulatory systems and pathways, so the regulatory load problem cannot be avoided in that way.

So how might the developmentally complex eukaryotes have bypassed this constraint? The only general way to relieve the problem is either to reduce the level of connectivity of the regulatory network (which is the opposite of what might be expected in a complex system), or to fundamentally change the nature of  $R$ , so that the numbers of regulatory factors may be expanded faster than the numbers of regulated proteins. Given that noncoding RNA accounts for about 97% of all transcriptional output in humans [28], and that many complex genetic phenomena in higher organisms are RNA-directed [29], it seems likely that RNA (which is utilized only for a few specific functions in prokaryotes) has been co-opted by the eukaryotes to solve this problem, enabling the programming of large numbers of different cell states and developmental trajectories in complex organisms like humans [30]. The regulatory advantage of RNA is its ability to convey sequence-specific signals (like a zip code or bit string) to receptive targets, while requiring 1.5 orders of magnitude less genomic sequence and correspondingly lower metabolic costs than proteins [29, 31].

## Conclusions

General arguments on the scaling of regulatory networks, as well as two alternative models, predict a faster than linear increase of transcriptional regulatory overhead with gene number. This is confirmed by genomic data. Both models and the empirical data are most consistent with a quadratic growth of transcription regulator number with total gene number. This links transcriptional networks to the emerging field of accelerating networks. The observed non-linear scaling implies a limit on network growth, and therefore on genome size

and complexity, within any given regulatory architecture. Our rough prediction of this limit lies within a factor of two of the biologically observed size limit of prokaryotes. This implicates regulatory network structure as a defining feature distinguishing prokaryotes from complex eukaryotes.

## Methods

### Data mining

Profiles in Pfam [16] (<http://pfam.wustl.edu>) were identified that were DNA or RNA binding and either had known regulatory function or demonstrated sequence specific binding. Pfam was searched by keywords such as “DNA bind”, “RNA bind”, “regulator” and “transcription factor”. In this way almost half of all Pfam profiles were examined (see Supplemental Table 1 for a list of included Pfam profiles). Viral and Eukaryal profiles were included if the profiles fitted the above criteria. TIG immunoglobulin-like domains were excluded as they bind substrates apart from DNA.

Complete, annotated genes were downloaded from NCBI [32]. hmmpfam from Hmmer 2.1.2 was used to identify all proteins that fit any of the selected Pfam profiles. The expectation cutoff for a valid profile match was set at  $10^{-4}$ . The results were parsed and counted using a Perl script. A list of species, gene numbers, and regulator numbers is provided as Supplemental Table 2. Graphs (not shown) using different definitions of regulatory proteins were also made using COG functional categories, subsets of the Pfam profiles in Supplemental Table 1 and functional classification from genome annotation. All such graphs showed similar behaviours to Figure 1.

## Connectivity

The average connectivity of genes was estimated as follows. If on average each of  $R$  regulators connects to five operons, as is the case in *E. coli* [15], then one operon is accessed by  $5R/(N_{op}) = 8R/N$  regulators (substituting the asymptotic relationship  $N_{op} \approx 0.6 \times N$  [17]). Each of these regulators is also linked to four other operons. Assuming independence of the regulator connections, each gene in the original operon is therefore directly linked to  $4 \times 8R/N$  other operons, or  $4 \times 8R/(N \times 0.6) = 53R/N$  genes. As a proportion of the total  $N$ , any one gene is therefore connected to  $C = 53R/N^2$  other genes. Substituting  $R/N^2$  as estimated from Fig. 1, this gives  $C = 0.06\%$  (gene connectivity).

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## Figure Legends

### Figure 1

Double-logarithmic plot of transcriptional regulator number against total gene number for bacteria (green circles) and archaea (blue triangles). The overall distribution is well described by a straight line with slope 1.96 ( $r^2 = 0.88$ , 95% confidence interval: 1.81 – 2.11), corresponding to a quadratic relationship between regulator number and genome size. The inset shows the same data before log-transformation.

## Description of additional data files

### Croft\_Suppl\_Tab1.xls

Format: MS Excel

Title: Pfam profiles of regulatory proteins

Description: Name and description of each Pfam profile used to identify transcriptional regulators in each genome.

### Croft\_Suppl\_Tab2.xls

Format: MS Excel

Title: Genomic data for bacteria and archaea

Description: Completely sequenced and annotated prokaryotes used for this study, number of protein coding genes and observed regulators. Bacterial species are shown in green, while archaeal species are shown in blue.

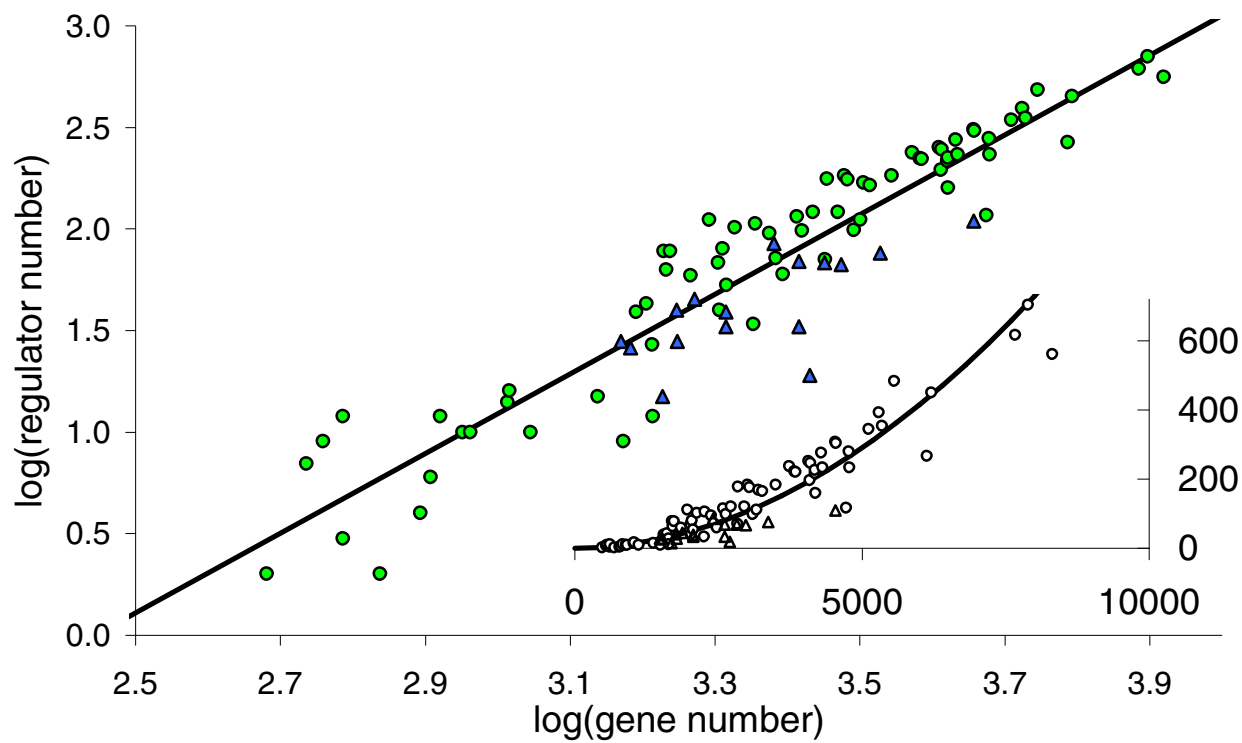


Figure 1

**Additional files provided with this submission:**

Additional file 1: Croft\_Suppl\_Tab1.xls : 19KB

<http://genomebiology.com/imedia/6242531952294365/sup1.xls>

Additional file 2: Croft\_Suppl\_Tab2.xls : 19KB

<http://genomebiology.com/imedia/9810393722943652/sup2.xls>

## Croft\_Suppl\_Tab1

Name	Description
ANTAR	ANTAR domain
AraC_binding	Arabinose operon regulatory protein
Arc	Arc-like DNA binding domain
Arg_repressor	Arginine repressor, DNA binding domain
ARID	ARID/BRIGHT DNA binding domain
ASNC_trans_reg	AsnC family
AT_hook	AT hook motif
Baculo_IE-1	Baculovirus immediate-early protein (IE-0)
CarD_TRCF	CarD-like/TRCF domain
Carla_C4	Carlavirus putative nucleic acid binding protein
CAT_RBD	CAT RNA binding domain
crp	Bacterial regulatory proteins, crp family
CSD	'Cold-shock' DNA-binding domain
CsrA	Global regulator protein family
deoR	Bacterial regulatory proteins, deoR family
DM-domain	DM DNA binding domain
dsDNA_bind	Double-stranded DNA-binding domain
dsrm	Double-stranded RNA binding motif
Fe_dep_repress	Iron dependent repressor, N-terminal DNA binding domain
filament_head	Intermediate filament head (DNA binding) region
FINO	Fertility inhibition protein (FINO)
FUR	Ferric uptake regulator family
GATA	GATA zinc finger
GerE	Bacterial regulatory proteins, luxR family
gntR	Bacterial regulatory proteins, gntR family
Herpes_ICP4_N	Herpesvirus ICP4-like protein N-terminal region
Histone_HNS	H-NS histone family
HLH	Helix-loop-helix DNA-binding domain
HrcA	HrcA protein C terminal domain
HSF_DNA-bind	HSF-type DNA-binding
HTH_1	Bacterial regulatory helix-turn-helix protein, lysR family
HTH_10	HTH DNA binding domain
HTH_3	Helix-turn-helix
HTH_4	Ribbon-helix-helix protein, copG family
HTH_5	Bacterial regulatory protein, arsR family
HTH_6	Helix-turn-helix domain, rpiR family
HTH_8	Bacterial regulatory protein, Fis family
HTH_AraC	Bacterial regulatory helix-turn-helix proteins, araC family
HTH_psq	helix-turn-helix, Psq domain
IclR	Bacterial transcriptional regulator
KilA-N	KilA-N domain
lacI	Bacterial regulatory proteins, lacI family
LexA_DNA_bind	LexA DNA binding domain
LytTR	LytTr DNA-binding domain

MarR	MarR family
merR	MerR family regulatory protein
Mga	M protein trans-acting positive regulator (MGA)
Mu_DNA_bind	Mu DNA-binding domain
myb_DNA-binding	Myb-like DNA-binding domain
NusB	NusB family
PadR	Transcriptional regulator PadR-like family
PAS	PAS domain
PC4	Transcriptional Coactivator p15 (PC4)
PRD	PRD domain
PurA	PurA ssDNA and RNA-binding protein
response_reg	Response regulator receiver domain
rrm	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)
RseC_MucC	Positive regulator of sigma(E), RseC/MucC
S1FA	DNA binding protein S1FA
SAND	SAND domain
SBP	SBP domain
SeqA	SeqA protein
SfsA	Sugar fermentation stimulation protein
Sigma54_activat	Sigma-54 interaction domain
sigma54_DBD	Sigma-54, DNA binding domain
sigma70_r1_1	Sigma-70 factor, region 1.1
sigma70_r2	Sigma-70 region 2
SIS	SIS domain
SpoVT_AbrB	SpoVT / AbrB like domain
SRF-TF	SRF-type transcription factor (DNA-binding and dimerisation domain)
Sua5_yciO_yrdC	yrdC domain
Tat	Transactivating regulatory protein (Tat)
T-box	T-box
TCP	TCP family transcription factor
TEA	TEA/ATTS domain family
tetR	Bacterial regulatory proteins, tetR family
trans_reg_C	Transcriptional regulatory protein, C terminal
TrpBP	Tryptophan RNA-binding attenuator protein
Vir_DNA_binding	Viral DNA-binding protein, all alpha domain
zf-C2H2	Zinc finger, C2H2 type
zf-C2HC	Zinc finger, C2HC type
zf-C4	Zinc finger, C4 type (two domains)
zf-Dof	Dof domain, zinc finger
zf-NF-X1	NF-X1 type zinc finger
Zfx_Zfy_act	Zfx / Zfy transcription activation region

## Croft\_Suppl\_Tab2

organism	genes	regulatory genes
<i>Mycoplasma genitalium</i>	480	2
<i>Buchnera aphidicola</i> Sg	545	7
<i>Buchnera</i> sp.	574	9
<i>Ureaplasma urealyticum</i>	611	3
<i>Wigglesworthia brevipalpis</i>	611	12
<i>Mycoplasma pneumoniae</i>	688	2
<i>Mycoplasma pulmonis</i>	782	4
<i>Tropheryma whipplei</i> twist	808	6
<i>Rickettsia prowazekii</i>	834	12
<i>Chlamydia trachomatis</i>	894	10
<i>Chlamydia muridarum</i>	916	10
<i>Treponema pallidum</i>	1031	14
<i>Mycoplasma penetrans</i>	1037	16
<i>Chlamydophila pneumoniae</i> AR39	1110	10
<i>Rickettsia conorii</i>	1374	15
<i>Thermoplasma acidophilum</i>	1478	28
<i>Helicobacter pylori</i> J99	1491	9
<i>Thermoplasma volcanium</i>	1526	26
<i>Aquifex aeolicus</i>	1553	39
<i>Mycobacterium leprae</i>	1605	43
<i>Campylobacter jejuni</i>	1634	27
<i>Borrelia burgdorferi</i>	1637	12
<i>Methanopyrus kandleri</i>	1687	15
<i>Streptococcus pyogenes</i>	1696	78
<i>Haemophilus influenzae</i>	1709	63
<i>Bifidobacterium longum</i>	1729	78
<i>Pyrococcus abyssi</i>	1765	40
<i>Methanococcus jannaschii</i>	1770	28
<i>Thermotoga maritima</i>	1846	59
<i>Methanobacterium thermoautotrophicum</i>	1869	45
<i>Streptococcus mutans</i>	1960	111
<i>Pasteurella multocida</i>	2014	68
<i>Neisseria meningitidis</i> MC58	2025	40
<i>Streptococcus pneumoniae</i> R6	2043	80
<i>Pyrococcus horikoshii</i>	2064	33
<i>Pyrococcus furiosus</i>	2065	39
<i>Fusobacterium nucleatum</i>	2068	53
<i>Streptococcus agalactiae</i> 2603	2124	102
<i>Chlorobium tepidum</i> TLS	2252	34
<i>Lactococcus lactis</i>	2266	106



<i>Clostridium tetani</i> E88	2373	95
<i>Archaeoglobus fulgidus</i>	2407	85
<i>Staphylococcus epidermidis</i> ATCC 12228	2419	72
<i>Thermosynechococcus elongatus</i>	2475	60
<i>Thermoanaerobacter tengcongensis</i>	2588	115
<i>Halobacterium</i> sp.	2605	69
<i>Pyrobaculum aerophilum</i>	2605	33
<i>Staphylococcus aureus</i> MW2	2632	98
<i>Aeropyrum pernix</i>	2694	19
<i>Clostridium perfringens</i>	2723	121
<i>Sulfolobus tokodaii</i>	2826	68
<i>Xylella fastidiosa</i>	2831	71
<i>Listeria monocytogenes</i>	2846	177
<i>Corynebacterium efficiens</i> YS-314	2950	121
<i>Sulfolobus solfataricus</i>	2977	67
<i>Lactobacillus plantarum</i>	3009	183
<i>Listeria innocua</i>	3043	175
<i>Deinococcus radiodurans</i>	3102	99
<i>Synechocystis</i> PCC6803	3169	111
<i>Brucella melitensis</i>	3198	169
<i>Brucella suis</i> 1330	3264	164
<i>Methanosarcina mazei</i>	3371	76
<i>Oceanobacillus iheyensis</i>	3496	183
<i>Caulobacter crescentus</i>	3737	237
<i>Vibrio cholerae</i>	3828	223
<i>Clostridium acetobutylicum</i>	3848	221
<i>Bacillus halodurans</i>	4066	252
<i>Yersinia pestis</i> KIM	4090	196
<i>Bacillus subtilis</i>	4100	247
<i>Shigella flexneri</i> 2a	4180	216
<i>Xanthomonas campestris</i>	4181	225
<i>Mycobacterium tuberculosis</i> CDC1551	4187	160
<i>Escherichia coli</i> K12	4289	275
<i>Xanthomonas citri</i>	4312	233
<i>Vibrio vulnificus</i> CMCP6	4537	308
<i>Methanosarcina acetivorans</i>	4540	109
<i>Salmonella typhimurium</i> LT2	4553	304
<i>Leptospira interrogans</i>	4727	117
<i>Salmonella typhi</i>	4767	279
<i>Shewanella oneidensis</i>	4778	233
<i>Ralstonia solanacearum</i>	5116	345
<i>Agrobacterium tumefaciens</i> C58	5301	392
<i>Pseudomonas putida</i> KT2440	5350	353

<i>Pseudomonas aeruginosa PA01</i>	5565	484
<i>Nostoc sp.</i>	6129	267
<i>Sinorhizobium meliloti</i>	6205	449
<i>Streptomyces avermitilis</i>	7671	617
<i>Streptomyces coelicolor</i>	7897	704
<i>Bradyrhizobium japonicum</i>	8317	560