Neo-formation of chromosomes in bacteria

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Summary

The genome of bacteria is classically separated into the essential, stable and slow evolving chromosomes and the accessory, mobile and rapidly evolving plasmids. This distinction was blurred with the characterization of multipartite genomes constituted of a standard chromosome and additional essential replicons, generally thought to be adapted plasmids. Whereas chromosomes are exclusively transmitted from mother to daughter cell during the bacterial cell cycle, in general, the duplication of plasmids is unlinked to the cell cycle and their transfer to daughter cell is random. Hence, the integration of a replicon within the cell cycle is key to determining its essential nature. Here we show that the content in genes involved in the replication, partition and segregation of the replicons and in the cell cycle discriminates the bacterial replicons into chromosomes, plasmids, and another class of essential genomic elements that function as supernumerary chromosomes. These are unlikely to derive directly from plasmids. Rather, together with the chromosome, they are neochromosomes in a divided genome resulting from the fission of the fused and rearranged ancestral chromosome and plasmid. Having a divided genome appears to extend and accelerate the exploration of the genome evolutionary landscape, producing complex regulation and leading to novel eco-phenotypes and species diversification (*e.g.*, symbionts and/or pathogens among Burkholderiales , Rhizobiales and Vibrionales).

Chromosomes are the only components of the genome that encode the necessary information for replication and life of the cell/organism under normal growth conditions. Their number varies across taxa, a single chromosome being the standard in bacteria[1]. Evidence accumulated over the past thirty years is proving otherwise: bacterial genomes can be distributed on more than one chromosome-like autonomously replicating genomic element (replicon)[2,3,4]. The largest, primary, essential replicon (ER) in a multipartite genome corresponds to a *bona fide* chromosome and the additional, secondary, ERs (SERs) are expected to derive from accessory replicons (plasmids[5,6]). The most popular model of SER formation posits that a plasmid acquired by a mono-chromosome progenitor bacterium is stabilized in the genome through the transfer from the chromosome of genes essential to the cell viability[4,7,8]. The existence in SERs of plasmid-like replication and partition systems[7,9-14] as well as experimental results[15] support this view. Yet, the duplication and maintenance processes of SERs contrast with the typical behaviour of plasmids for which both the timing of replication initiation and the centromere movement are random[16,17]. Indeed, the SERs share many characteristic features with chromosomes: enrichment in Dam methylation sites of the replication origin[9,18], presence of initiator titration sites[9,19], synchronization of the replication with the cell cycle[9,20-28], KOPS-guided FtsK translocation[29], FtsK-dependent dimer resolution system[29], MatP/matS macrodomain organisation system[30], and similar fine-scale segregation dynamics[22,28]. Within a multipartite genome, the replication of the chromosome and that of the SER(s) are initiated at different time points[22-28] and use replicon-specific systems[7,9-11,31,32]. Yet, they are coordinated, hence maintaining the genome stoichiometry[21,22,25,26,28]. In the few species where this was studied, the replication of the SER is initiated after that of the chromosome[22-28] under various modalities. In *V. cholerae* N16961, the replication of a short region of the chromosome licenses the SER duplication[33,34], and the advancement of the SER replication and segregation triggers the divisome assembly[35]. In turn, the altering of the chromosome replication does not affect the replication initiation control of the SER in α-proteobacterium *Ensifer/Sinorhizobium meliloti*[28].

Beside the exploration of the replication/segregation mechanistic, studies of multipartite genomes, targeting a single bacterial species or genus[3,7,8,10,15] or using a more extensive set of taxa[4,36], relied on flawed[4,36,37] (replicon size, nucleotide composition, coding of core essential genes for growth and survival) (Fig. 1) and/or biased[36] (presence of plasmid-type systems for genome maintenance and replication initiation) criteria to characterize the SERs. While clarifying the functional and evolutionary contributions of each type of replicon to a multipartite genome in given bacterial lineages[7,10,32,36], these studies produce no absolute definition of SERs[4,36] or universal model for their emergence[4,7,15,32,36]. We thus set about investigating the nature(s) and origin(s) of these replicons using as few assumptions as possible.

Replicon inheritance systems as diagnostic features

We reasoned that the key property discriminating chromosomes from plasmids is their transmission only from mother to daughter cells during the bacterial cell cycle. The functions involved in the replication, partition and maintenance of a replicon, *i.e.*, its inheritance systems (ISs), thence are expected to reflect the replicon degree of integration into the host cycle. We did not limit our study to a particular multipartite genome or a unique gene family. Rather, we performed a global analysis encompassing all bacterial replicons whose complete sequence was available in public sequence databases (Fig. 2). We first faced the challenge of identifying all IS functional homologues. The inheritance of genetic information requires functionally diverse and heterogeneous actuators depending on the replicon type and the characteristics of the organism. Also, selecting sequence orthologues whilst avoiding false positives (*e.g.*, sequence paralogues) can be tricky since remote sequence homology most likely prevails among chromosome/plasmid protein-homologue pairs. Starting from an initial dataset of 5125 replicons, we identified 358,624 putative IS functional homologues, overall corresponding to 1711 Pfam functional domains (Fig. 3a), using a query set of 47,604 chromosomal and plasmidic IS-related proteins selected from the KEGG and ACLAME databases. We then inferred 7013 homology groups using a clustering procedure and named the clusters after the most frequent annotation found among their proteins (Fig. 3b,c). Most clusters were characterized by a single annotation whilst the remaining few (4.7%) each harboured from 2 to 710 annotations, the most frequent annotation in a cluster generally representing more than half of all annotations (Fig. 3c). The removal of false positives left 267,497 IS protein homologues distributed in 6096 clusters (Fig. 3d) and coded by 4928 replicons (Supplementary data Table S1) out of the initial replicon dataset. Following the Genbank/RefSeq annotations, our final dataset comprised 2016 complete genome sets corresponding to 3592 replicons (2016 chromosomes, 129 SERs, and 1447 plasmids) and 1336 plasmid genomes, irregularly distributed across the bacterial phylogeny (Fig. 4a). Multi-ER genomes are observed in 5.0% of all represented bacterial genera and constitute 5.7% of the complete genomes (averaged over genera) available at the time of study (Fig. 4b). They are yet to be observed in most bacterial phyla, possibly because of the poor representation of some lineages. They are merely incidental (0.2% in Firmicutes) or reach up to almost one third of the genomes (30.1% in β-Proteobacteria) depending on the lineage. Although found in ten phyla, they occur more than once per genus in only three of them: Bacteroidetes, Proteobacteria and Spirochaetae.

Exploration of the replicon diversity

We explored the differences and similarities of the bacterial replicons with regard to their IS usage using a data mining and machine learning approach (Methods). The 6096 retained IS clusters were used as distinct variables to ascribe each of the 4928 replicons with a vector according to its IS usage profile. We transformed these data

into bipartite graphs depending on the number of proteins from the IS clusters coded by each replicon. Bipartite graphs display both the vectors (replicons) and the variables (protein clusters) together with their respective connections, and allow the interactive exploration of the data. The majority of the replicons are interconnected (Fig. 5) as testimony of the shared evolutionary history of their IS sequences. Chromosomes and plasmids form overall distinct groups and communities with varying degree of connectivity depending on their functional specificities (Fig. 5a) as well as on the bacterial taxonomy of their hosts (Fig. 5c). They nonetheless share many ISs, bearing witness to the continuity of the genomic material and the extensive exchange of genetic material within bacterial genomes. However, the occurrence of poorly IS cluster-connected plasmids within a group of chromosomes does not consistently reflect a true relationship, and rather results from shared connections to a very small number (as low as one) of common ISs. While being interconnected to both chromosomes and plasmids *via* numerous IS clusters, the SERs generally stand apart from either these types of replicons and gather at the chromosome-plasmid interface (Fig. 5a,b). Their IS usage is neither chromosome-like nor plasmid-like, suggesting that they may constitute a separate category of replicons. This is most tangible in the case of the proteobacterial lineages where SERs occur most frequently (Fig. 5b). All SERs in the β- and γ-Proteobacteria, and most in the α-Proteobacteria are linked to some remarkable chromosome-type IS clusters, such as AcrA, IciA, FtsE, HN-S and Lrp, as well as to plasmid-like ParA/ParB-, Rep- and PSK-like IS clusters. A similar pattern is observed for the SERs in actinobacterium *Nocardiopsis dassonvillei,* firmicute *Butyrivibrio proteoclasticus*, and chloroflexi *Sphaerobacter thermophilus* and *Thermobaculum terrenum* (Fig. 3b). Interestingly, DnaG-annotated clusters connect the SERs present in all but one *Burkholderia* species (β-Proteobacteria) as well as the chromosomes of all other bacteria. Since the reduced genome of SER-less *B. rhizoxinica* has adapted to intracellular life, the *Burkholderia* SERs likely originated from a single event prior to the diversification of the genus, possibly in relation to the speciation event that gave rise to his lineage. The second SERs harboured by only some *Burkholderia* species exhibit a higher level of interconnection to plasmids, as do the SERs in α-proteobacterium *Sphingobium*, cyanobacterium *Cyanothece*, *Deinococcus radiodurans* (Deinococcus-Thermus) and *Ilyobacter polytropus* (Fusobacteria). This points to an incomplete stabilisation of the SERs into the genome that may reflect a recent, ongoing, event of integration and/or differing selective pressures at play depending on the bacterial lineages. At odds with these observations, some SERs group unambiguously with chromosomes. The SERs in α-Proteobacteria *Asticcacaulis excentricus* and *Paracoccus denitrificans*, Bacteroidetes *Prevotella intermedia* and *P. melaninogenica*, acidobacterium *Chloracidobacterium thermophilum*, and cyanobacterium *Anabaena* bear higher levels of interconnection to chromosomes than to plasmids or other SERs. Indeed, the SERs in *Prevotella* spp. are hardly linked to plasmids, and the few plasmid-like IS proteins that *C. thermophilum* SER codes (mostly Rep, Helicase and PSK), albeit found in plasmids occurring in other phyla, are observed in none of the Acidobacteria plasmids. An extreme situation

is met in *Leptospira* spp. (Spirochaetae) whose SERs are each linked to only three or four (out of six) chromosome-like IS clusters, always including ParA and ParB. Interestingly, the ParA cluster appears to be specific to Spirochaetae chromosomes with the notable exception of one plasmid found in Leptospiraceae *Turneriella parva*.

IS-based relationships of the replicons

We submitted the bipartite graph of the whole dataset to a community structure detection algorithm (INFOMAP) that performs a random walk along the edges connecting the graph vertices. We expected the replicon communities to be trapped in high-density regions of the graph. We also performed a dimension reduction by Principal Component Analysis followed by a hierarchical clustering procedure (WARD). The clustering solutions were meaningful (high values reached by the stability criterion scores), and biologically relevant (efficient separation of the chromosomes from the plasmids; high *homogeneity* values) using either method (Table 1). In another experiment, we considered each genus as a unique sample and averaged the variables over the replicons of the different species for each replicon type. The aim was to control for the disparity in taxon representation of the replicons. This dataset produced overall similar albeit slightly less stable clusters (lower *homogeneity* values). Taxonomically homogeneous clusters of chromosomes were best retrieved using the coupling of dimension reduction and hierarchical clustering with a large enough number of clusters (*homogeneity* scores up to 0.93). In turn, the community detection algorithm was more efficient in recovering the underlying taxonomy of replicons (higher value of *completeness*), and was sole able to identify small and scattered plasmid clusters (Supplementary data Tables S6 and S7). The plasmid clusters obtained using PCA+WARD lacked taxonomical patterning and, although highly stable, only reflected the small Euclidian distances existing among the plasmid replicons (*e.g.*, one cluster of 2656 plasmids had a stability score of 0.975). The clusters obtained with INFOMAP mirrored the taxonomical structure of the data, suggesting that the taxonomic signal, expected to be associated to the chromosomes, is preserved among the IS protein families functionally specifying the plasmids. The presence of a majority of the SERs amongst the chromosome clusters generated by INFOMAP confirmed the affinities between these two genomic elements, and the clear individuation of the SERs from the plasmids. However, the larger number of IS of chromosomes often caused the PCA+WARD approach to place SERs into plasmid clusters. The SERs in *Butyrivibrio*, *Deinococcus*, *Leptospira* and *Rhodobacter* spp. grouped consistently with plasmids while the SERs in *Vibrionaceae* and *Brucellaceae* formed specific clusters (Table 2). Burkholderiales and *Agrobacterium* SERs, whose homogenous clusters tended to be unstable, exhibited a higher affinity to plasmids overall. The SERs of *Asticaccaulis*, *Paracoccus* and *Prevotella* spp. associated stably with chromosomes using the two clustering methods (Table 2a,b) and appear to possess IS profiles that set them apart from both the plasmids and the other SERs. We reached similar conclusions when performing a PCA+WARD clustering using the 117 functional annotations of the IS protein clusters rather than the IS clusters themselves

(Tables 1 and 3). *Prevotella* multipartite genomes (*P. intermedia* and *P. melaninogenica*) are notably remarkable: their chromosomes are more similar to plasmids than to other groups of chromosomes, in particular to single chromosomes of other *Prevotella* species (*P. denticola* and *P. ruminicola*).

SER-specifying IS functions

Next, we searched which of the IS functions are specific to the SERs. We performed several logistic regression analyses to identify over- or under-represented ISs and to assess their respective relevance to each class of replicons. Because of their comparatively small number, all SERs were assembled into a single group despite their disparity. A hundred and one IS functionalities (96% of KEGG-annotated chromosome-like functions and 72% of ACLAME-annotated plasmid-like functions) were significantly enriched in one replicon category over the other (Table 4). The large majority of the IS functions differentiates the chromosomes from the plasmids. These are only determined by ISs corresponding to ACLAME annotations Rep, Rop and TrfA, involved in initiation of plasmid replication, and ParA and ParB, dedicated to plasmid partition. Some KEGG-annotated functions, *e.g.,* DnaA, DnaB or FtsZ, appear to be more highly specific to chromosomes than others such as DnaC, FtsE or H-NS (lower *OR* values). Strikingly, very few functions distinguished significantly the chromosomes from the SERs, by contrast with plasmids (Table 4). Chromosome-signature ISs are also present on the SERs, and a few of them are enriched to the same order of magnitude in both classes but not in plasmids (signalled by stars in Table 4). The main divergence between SERs and chromosomes lies in the distribution patterns of the ACLAME-annotated ISs, more present on the SERs. This bias suggests a stronger link of SERs to plasmids. Also, this pattern may arise from the unbalanced taxon representation in our SER dataset due to a single bacterial lineage. For example, the presence of RepC is likely to be specific to Rhizobiales SERs[38].

Since the IS profiles constitute replicon-type signatures, we searched for new putative SERs or chromosomes among the extra-chromosomal replicons. We used the IS functions as features to perform different supervised classification analyses with various training sets (Table 5). We first observed that the SER class was consistent (overall high values of the probability for a SER to be assigned to its own class in Extended Data Tables 5 and 6), confirming that the ISs are robust genomic markers for replicon characterisation. The low SER probability score presented by a few SERs (Table 6) likely resulted from a low number of carried ISs (*e.g., Leptospira*) or the absence of lineage-specific ISs (*e.g.*, Vibrionaceae SER idiosyncratic replication initiator RtcB). We detected a number of candidate SERs among the plasmids (Table 7a), most of which are known to be essential to the cell functioning and/or to the fitness of the organism (*cf.* Supplementary data). Whereas most belong to bacterial lineages known to harbour multipartite genomes, novel taxa emerge as putative hosts to complex genomes (Rhodospirillales and, to a lesser extent, Actinomycetales). In contrast, our analyses confirmed only one putative SER (*Ruegeria* sp. TM1040) within the *Roseobacter* clade[39]. Remarkably, we identified eight candidate chromosomes: two

plasmids (also identified as candidate SERs) that encode ISs hardly found in extra-chromosomal elements (*e.g.*, DnaG, DnaB, ParC and ParE), and six SERs that part of, or all, our analyses associate to standard chromosomes (Table 7b). Notably, *P. intermedia* SER shows a very high probability (> 0.98) to be a chromosome while its annotated chromosome, unique of its kind, falls within the plasmid class. This approach can thus be extended to test the type of replicon for (re)annotation purposes.

Neochromosomes and divided genomes formation

SERs clearly stand apart from plasmids, including those that occur consistently in a bacterial species, *e.g.*, *Lactobacillus salivarius* pMP118-like plasmids[40]. The replicon size proposed as a primary classification criterion to separate the SERs from the plasmids[4,36] proves to be inoperative. The IS profiles accurately identify *Leptospira* and *Butyrivibrio* SERs despite their plasmid-like size and assign unambiguously the chromosomes in the reduced genomes of endosymbionts (sizes down to 139 kb) to the chromosome class. Conversely, they assign *Rhodococcus jostii* RHA1 1.12 Mb-long pRHL1 replicon to the plasmid class, and do not discriminate the megaplasmids (>350 kb[4]) from smaller plasmids. Plasmids may be stabilized in a bacterial population by rapid compensatory adaptation that alleviates the fitness cost incurred by their presence in the cell[41-43]. This phenomenon involves mutations either on the chromosome only, on the plasmid only, or both, and does not preclude the segregational loss of the plasmid. On the contrary, SERs code for chromosome-type IS proteins that integrate them constitutively in the species genome and the cell cycle. The SERs thence qualify as essential replicons regardless of their size and of the phenotypical/ecological, possibly essential, functions that they encode and which vary across host taxa.

Yet, SERs also carry plasmid-like ISs, suggesting a role for plasmids in their formation. The prevalent opinion assumes that SERs derive from the amelioration of megaplasmids[4,7,8,10,36]. A plasmid bringing novel functions for the adaptation of its host to a new environment is stabilized into the bacterial species genome through the transfer from the chromosome of essential genes[4,7]. However, the generalized presence of chromosome-like ISs in SERs of all taxa with multipartite genomes is unlikely to derive from the action of environment- and lineage-specific selective forces. Also, bacteria with similar lifestyle and exhibiting some phylogenetic relatedness may not all harbour multiple ERs (*e.g.*, *α*-proteobacterial nitrogen-fixing legume symbionts). Furthermore, the gene shuttling from chromosome to plasmid fails to account for the situation met in the multipartite genomes of *Asticaccaulis excentricus, Paracoccus denitrificans* and *Prevotella* species. Their chromosome-type ISs are evenly distributed between the chromosome and the SER whereas their homologues in the mono- or multi-partite genomes of most closely related species are primarily chromosome-coded (see Table 8 for an example). This pattern, mirrored in their whole gene content[45,46], hints at the stemming of the two essential replicons from a single chromosome by either a splitting event or a duplication followed by massive gene

loss. Neither mechanism informs on the presence of plasmid-type maintenance machinery on one of the replicons. The severing of a chromosome generates a single true replicon carrying the chromosome replication origin and an origin-less remnant, whilst the duplication of the chromosome produces two chromosomal replicons with identical maintenance systems. Whereas multiple copies of the chromosome are known to cohabit constitutively in polyploid bacteria[44], the co-occurrence of dissimilar chromosomes bearing identical replication initiation and partition systems is yet to be described in bacteria.

We propose that the requirement for maintenance system compatibility between co-occurring replicons is the driving force behind the presence of plasmid-type replication initiation and maintenance systems in SERs. Indeed, genes encoding chromosome-like replication initiators (DnaA) are hardly found on SERs. When they are, in *Paracoccus denitrificans*, *Prevotella intermedia* and *P. melaninogenica*, the annotated chromosome in the corresponding genome does not carry one. Similarly, chromosomal centromeres (*parS*) are found on a single replicon within a multipartite genome, which is the chromosome in all genomes but one. In *P. intermedia* (GCA_000261025.1), both replication initiation and partition systems define the SER (CP003503) as the *bona fide* chromosome and the chromosome (CP003502) as an extra-chromosomal replicon. The harmonious coexistence of different replicons in a cell requires that they use divergent enough maintenance systems. In the advent of a chromosome fission or duplication, the involvement of an autonomously self-replicating element different from the chromosome is mandatory to provide one of the generated DNA molecules with the non-chromosomal maintenance machinery.

Plasmid-first and chromosome-first hypotheses can be reconciled into a unified, general Fusion-Shuffling-Scission model of SER emergence where a chromosome and a plasmid combine into a co-integrate. Plasmids are known to merge or to integrate chromosomes in both experimental settings[47,48] and the natural environment[43,49,50]. They can thus replicate with the chromosome[47,48] and persist in the bacterium lineage through several generations[47,48]. The co-integrate may resolve into its original components[47,48] or give rise to novel genomic architectures[47,48,49,50]. The co-integration state likely facilitates inter-replicon gene exchanges and rearrangements that may lead to the translocation of large chromosome fragments to the resolved plasmid[49]. Multiple cell divisions, and possibly several merging-resolution rounds, could provide time and opportunity for the plasmid-chromosome reassortment to take place, and multiple essential replicons and a viable divided genome to form ultimately. In the novel genome, one ER retains the chromosome-like origin of replication and centrosome, and the other the plasmidic counterparts. Both differ from the chromosome and plasmid that gathered in the progenitor host at the onset. The novel ERs thus constitute neo-chromosomes that carry divergent maintenance machineries and can cohabit and function in the same cell. Depending on the number of cell cycles spent as co-integrate, the level of reorganisation, the acquisition of genetic material, and the environmental selective pressure, the final essential replicons may exhibit

diverse modalities of genome integration.
The Fusion-Shuffling-Scission model of genome evolution accounts for the extreme plasticity met in divided genomes and the eco-phenotypic flexibility of their hosts. Indeed, having a divided genome appears to extend and accelerate the exploration of the genome evolutionary landscape, producing complex expression regulation and leading to novel eco-phenotypes and species diversification, *e.g.*, Burkholderiaceae and Vibrionaceae. Furthermore, it may explain the observed separation of the replicons according to taxonomy. Chromosomes and plasmids thus appear as extremes on a continuum of a lineage-specific genetic material.

Methods

To understand the relationships between the chromosomal and plasmidic replicons, we focused on the distribution of Inheritance System (IS) genes for each replicon and built networks linking the replicons given their IS functional orthologues (Fig. 2).

Retrieval of IS functional homologues

A sample of proteins involved in the replication and segregation of bacterial replicons and of the bacterial cell cycle was constructed using datasets available from the ACLAME[51] and KEGG[52] databases. Gene ontologies for "replication", "partition", "dimer resolution", and "genome maintenance" (Supplementary data Table S2) were used to select related ACLAME plasmid protein families (Supplementary data Table S3) using a semi-automated procedure. KEGG orthology groups were selected following the KEGG BRITE hierarchical classification (Supplementary data Table S4). Then, the proteins belonging to the relevant 92 ACLAME protein families and 71 KEGG orthology groups (3,847 and 43,757 proteins, respectively) were retrieved and pooled. Using this query set amounting to a total of 47,604 proteins, we performed a *blastp* search of the 6,903,452 protein sequences available from the 5,125 complete sequences of bacterial replicons downloaded from NCBI Reference Sequence database (RefSeq)[53] on 30/11/2012. We identified 358,624 putative homologues using BLAST default parameters[54] and a 10^{-5} significance cut-off value. We chose this *E*-value threshold to enable the capture of similarities between chromosome and plasmid proteins whilst minimizing the production of false positives, *i.e.*, proteins in a given cluster exhibiting small *E*-values despite not being functionally homologous. Using RefSeq ensured the annotation consistency of the genomes included in our dataset. used.

Clustering of IS functional homologues

Using this dataset, we inferred clusters of IS functional homologues by coupling of an *all-versus-all blastp* search using a 10^{-2} *E*-value threshold, and a TRIBE-MCL[55] clustering procedure. As input to TRIBE-MCL, we used the matrix of log transformed *E*-value $d(p_i, p_j) = -\log_{10}\left(e_{value}(p_i, p_j)\right)$ obtained from the comparisons of all

possible protein pairs. Using a granularity value of 4.0 (see below), we organized the 358,624 IS homologues into 7013 clusters, each comprising from a single to 1990 proteins (Fig. 3A). We annotated IS homologues according to their best match (BLAST hit with the lowest *E*-value) among the proteins of the query set, *i.e.*, according to one of the 117 functions of the query set (71 from KEGG and 46 from ACLAME). Then, we named the clusters of functional homologues using the most frequent annotation among the proteins in the cluster. We used the number of protein annotations in a cluster to determine the cluster quality, a single annotation being optimal. To select the best granularity and to estimate the consistency of the clusters in terms of functional homologues, we computed the weighted Biological Homogeneity Index (*wBHI*, modified from the *BHI*[56], each cluster being weighted by its size) and the Conservation Consistency Measure (*CCM*, similar to the *BHI* but using the functional domains of the proteins to define the reference classes), which both take into account the size distribution of the clusters (*cf.* Supplementary Data for details on index calculation). The former gives an estimation of the overall consistency of clusters annotations according to the protein annotations whereas the latter gives an estimation of cluster homogeneity according to the protein domains identified beforehand. To build the sets of functional domains, we performed an *hmmscan*[57] procedure against the Pfam database[58] to each of the 358,624 putative IS homologues. We annotated each protein according to the domain match(es) with *E*- value < 10^{-5} (individual *E*-value of the domain) and *c-E*-value < 10^{-5} (conditional *E*- value that measures the statistical significance of each domain). If two domains overlapped, we only considered the domain exhibiting the smallest *E*-value. WE estimated *wBHI* and *CCM* indices for the clustering of the IS homologues and compared with values obtained for random clusters simulated according to the cluster size distribution of the IS proteins, irrespective of their length or function. For each of the clustering obtained for different granularities, we constructed a random clustering following the original cluster size distribution (assessed with a χ^2 test) and composed with simulated proteins according to the distributions of the type and number of functional domains of the data collected from the 358,624 IS homologues. Overall, the clusters obtained using a granularity of 4.0 with the TRIBE-MCL algorithm appeared to be homogenous in terms of proteins similarities toward their best BLAST hits and their functional domain distributions (Fig. 7).

Assessment of the homogeneity of IS functional homologues

The homogeneity towards the functions of the proteins in the query set relied on the assumption that the first BLAST cut-off (10^{-5} *E*-value) was stringent enough to capture only functional homologues to the query proteins. Potential bias might nevertheless arise from query proteins possessing a supplementary functional domain unrelated to the IS role, or from the selection of proteins belonging to the same superfamily but differing in function. To address these issues, we calculated the functional vectors associated to each KEGG group or ACLAME family of the query set, as well as those

for all obtained clusters. For a protein P_i, we defined the associated functional vector with respect to its set of identified domains D_{P_i} and to the set of all identified domains $D=\{d_1,...,d_X\}$ as:

$$v_{P_i} = \left(n_{d_1}^{P_i}, \dots, n_{d_x}^{P_i}\right)$$

where $n_{d_i}^{P_i}$ is the number of time d_i is found in D_{P_i}. The functional vector associated to a given cluster of proteins C_i could then be defined as:

$$v_{C_i} = \left(n_{d_1}^{C_i}, \dots, n_{d_x}^{C_i}\right)$$

where $n_{d_i}^{C_i}$ is defined as:

$$n_{d_i}^{C_i} = \frac{1}{|C_i|} \sum_{P_i \in C_i} n_{d_x}^{P_j}$$

For each cluster C_0, the cosine distance between its associated vector v_{C_0} and the associated vector v_{C_a} of the corresponding KEGG group or ACLAME family annotations C_a was then computed as:

$$d_{cosine}\left(v_{C_a}, v_{C_0}\right) = 1 - \frac{\sum_{i=1}^{X} n_{d_i}^{C_0} . n_{d_i}^{C_a}}{\sqrt{\sum_{i=1}^{X} n_{d_i}^{{C_0}^2}} \cdot \sqrt{\sum_{i=1}^{X} . n_{d_i}^{{C_a}^2}}}$$

For each cluster C_0, the cosine distance between its associated vector !!! and the associated vector !!! of the corresponding KEGG group or ACLAME family annotations C_a was then computed as:

$$d_{cosine}\left(v_{C_a}, v_{C_0}\right) = 1 - \frac{\sum_{i=1}^{X} n_{d_i}^{C_0} . n_{d_i}^{C_a}}{\sqrt{\sum_{i=1}^{X} n_{d_i}^{{C_0}^2}} \cdot \sqrt{\sum_{i=1}^{X} . n_{d_i}^{{C_a}^2}}}$$

The $d_{cosine}\left(v_{C_a}, v_{C_0}\right)$ values were compared with those obtained using random clusters C_r of the same size than C_0. For each C_0 and its corresponding C_a, 200 random clusters and their associated distances $d_{cosine}\left(v_{C_a}, v_{C_r}\right)$, from whichthe corresponding empirical distribution D_e was constructed, were computed. C_0 is then considered as noise if $d_{cosine}\left(v_{C_a}, v_{C_0}\right) \notin Q_{10\%}^{D_e}$ where $Q_{10\%}^{D_e}$ is the 0.1-quantile of D_e.

Unsupervised analyses of the replicon space

We represented Bacterial replicons (Supplementary data Table S1) as vectors according to their content in IS genes. The number of IS protein clusters retained for the analysis determined the vector dimension and the number of proteins in a replicon assigned to each cluster gave the value of each vector component. We built matrices $P = \begin{bmatrix} p_{1,1} & \cdots & p_{1,m} \\ \vdots & \ddots & \vdots \\ p_{n,1} & \cdots & p_{n,m} \end{bmatrix}$, where n is the number of replicons, m the number of protein clusters, and $p_{i,j}$ the number of proteins of the jth cluster encoded by a gene present on the ith replicon. We constructed several datasets to explore both the replicon type and the host taxonomy effects on the separation of the replicons in the analyses (Supplementary Data Table S5). The taxonomic representation bias was taken into account by normalizing the data with regard to the host genus: a consensus vector was

built for each bacterial genus present in the datasets. The value of each vector attribute was calculated as the mean of the attribute values in the replicon vectors that belong to the same genus.

As a first approach, we transformed data into bipartite graphs whose vertices are the replicons and the proteins clusters. The graphs were spatialized using the force-directed layout algorithm ForceAtlas2[59] implemented in Gephi[60]. Bipartite graphs are a powerful way of representing the data by naturally drawing the links between the replicons while enabling the detailed analysis of the IS cluster- based connections of each replicon by applying forces to each node with regard to its connecting edges. To investigate further the IS-based relationships of the replicons, we applied the community structure detection algorithm INFOMAP[61] using the *igraph* python library[62]. We also performed A WARD hierarchical clustering[63] after a dimension reduction of the data using a Principal Component Analysis[64]. To select of an optimal number of principal components, we relied on the cluster stabilities measurement using a *stability criterion*[65] and retained the first 30 principal components (57% of total variance). For consistency purpose, the number of clusters in the WARD analysis was chosen to match that obtained with the INFOMAP procedure. The number of clusters used was assessed by the stability index by Fang & Wang[66] (Table 1). The quality of the projection and clustering results were confirmed using the V-measure indices[67] (*homogeneity*, *completeness*, *V-measure*) as external cluster evaluation measures (Table 1). The *homogeneity* indicates how uniform clusters are towards a class of reference. The *completeness* indicates whether reference classes are embedded within clusters. The *V-measure* is the harmonic mean between these two indices and indicates the quality of a clustering solution relative to the classes of reference. These three indices vary between 0 and 1, with values closest to 1 reflecting the good quality of the clustering solution. The type of replicons (*i.e.,* plasmid and chromosome) and the taxonomic affiliation (phylum or class) for chromosomes or plasmids were used as references classes (Supplementary Data Table S5). Additionally, the *stability criterion*[65] of individual clusters, weighted by their size, for a given clustering result was evaluated using the bootstrapping of the original dataset as re-sampling scheme. Individual Jaccard coefficient for each replicon were computed as the number of times that a given replicon of a cluster in a clustering solution is also present in the closest cluster in the resampled datasets.

Functional characterization of the replicons and genomes

In order to characterize the functional bias of the replicons, 117 IS functionalities (46 from ACLAME and 71 from KEGG) were considered. When equivalent in plasmids and chromosomes, functions from ACLAME and KEGG databases were considered to be distinct. A $n*m$ matrix $F = \begin{bmatrix} f_{1,1} & \cdots & f_{1,m} \\ \vdots & \ddots & \vdots \\ f_{n,1} & \cdots & f_{n,m} \end{bmatrix}$ with n the number of replicons and m the number of IS functionalities, was used as input to the projection algorithms. $f_{i,j}$

represents the number of times that genes coding for proteins annotated with the jth function are present on the ith replicon. Several datasets were analysed using PCA dimension reduction of the data followed by WARD hierarchical clustering (Table 1).

Logistic regression analyses

Several reference classes of replicons and complete genomes were considered for comparison (Supplementary data Table S8). Ambiguous, *i.e.*, potentially adapted, plasmids belonging to INFOMAP clusters of plasmid replicons partially composed of SERs and/or chromosomes were removed from the plasmid class. When appropriate, the taxonomic representation bias was taken into account by normalizing the data with regard to the host genus as before. Logistic regressions[68] were performed for the 117 IS functions using the R glm package coupled to the python binder rpy2. The computed P_{value} measured the probability of a functionality to be predictive of a given group of replicons/genomes and the *Odd-Ratio* estimated how the functionality occurrence influenced the belonging of a replicon/genome to a given group.

Supervised classification of replicons and genomes

In order to identify putative ill-defined SERs and chromosomes amongst plasmids, we performed supervised classification analyses using random forest procedures[69]. We used the IS functionalities as the set of features and the whole sets of chromosomes, plasmids and SER as sets of samples to build four classification studies (Table 5) and detect SER candidates (plasmids *vs.* SERs) and chromosome candidates (chromosomes *vs.* SERs or chromosomes *vs.* plasmids). Because of the unbalanced size of the training classes (SERs *vs.* chromosomes and plasmids), iterative sampling procedures were performed using 1000 random subsets of the largest class, with a size similar to that of the smallest class. The ensuing results were averaged to build the class probabilities and relative importance of the variables. We also used the whole set of plasmids when compared to SERs, to identify more robust SER candidates. The discarding of plasmids in the iterative procedure increases the classifier sensitivity while reducing the rate of false negatives by including more plasmid-annotated putative true SERs, whereas it decreases the classifier precision while increasing the rate of false positives. The ExtraTreeClassifier (a classifier similar to Random Forest) class from the Scikit-learn python library[70] was used to perform the classifications, with *K=1000*, *max_feat=sqrt(number of variables)* and *min_split=1*. For each run, the *feature_importances* and *estimate_proba* functions were used to compute, respectively, the relative contribution of the input variables and the class probabilities of replicons/genomes. The statistical probability of a replicon/genome belonging to a class was calculated as the average predicted class of the trees in the forest. The relative contribution of the input variables was estimated according to Breiman[71]. The choices of the number of trees in the forest *K*, the number of variables selected for each split *max_feat*, and the minimum number of samples required to split an internal node *min_split* were cross-validated using a *Leave-One-Out* scheme. The performance of the

Extremely-randomized-trees classification procedures was assessed using a stratified 10-fold cross-validation procedure following Han *et al.* [72], and the out-of-bag estimate (OOB score)[70,73] computed using the *oob_score* function of Scikit-learn python library.

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Table 1. Evaluation of the replicon IS-based clusterings

		INDEX[a]	USING IS PROTEIN SEQUENCES				USING IS FUNCTIONS	
			INFOMAP		PCA+ WARD[b]		PCA+ WARD[b]	
PROCEDURE	Dataset[c]		V^R	$\bar{V}^R_{genus}$	V^R	$\bar{V}^R_{genus}$	V^R_f	$\bar{V}^R_{f,genus}$
	Parameters		500 iterations		$\begin{cases} k = 200 \\ pc = 30 \end{cases}$	$\begin{cases} k = 200 \\ pc = 30 \end{cases}$	$\begin{cases} k = 50 \\ pc = 4 \end{cases}$	$\begin{cases} k = 20 \\ pc = 4 \end{cases}$
	Number of clusters		223	77	175	75	49	19
	PCA explained variance				57%	58%	87%	85%
	Stability criterion (Δ^{Kl}) [d]		0.82	0.76	0.85	0.74	0.80	0.71
EVALUATED SEPARATION	Chromosomes *vs.* Plasmids	*homogeneity*	0.82	0.63	0.93	0.83	0.85	0.68
		completeness	0.15	0.15	0.25	0.20	0.30	0.23
		V-measure	0.25	0.24	0.43	0.32	0.44	0.35
	Chromosomes *per* host phylum	*homogeneity*	0.93	0.69	0.93	0.80	0.50	0.44
		completeness	0.60	0.61	0.35	0.40	0.27	0.33
		V-measure	0.73	0.65	0.51	0.53	0.35	0.38
	Chromosomes *per* host class	*homogeneity*	0.85	0.64	0.93	0.80	0.47	0.37
		completeness	0.80	0.82	0.16	0.58	0.36	0.41
		V-measure	0.82	0.72	0.66	0.67	0.41	0.39
	Plasmids *per* host phylum	*homogeneity*	0.88	0.78	0.06	0.01	0.02	0.02
		completeness	0.33	0.35	0.16	0.14	0.10	0.30
		V-measure	0.48	0.48	0.08	0.02	0.03	0.03
	Plasmids *per* host class	*homogeneity*	0.84	0.74	0.07	0.02	0.03	0.02
		completeness	0.43	0.51	0.28	0.36	0.25	0.28
		V-measure	0.57	0.60	0.12	0.03	0.05	0.03

[a] *V-measure* according to Rosenberg & Hirschberg[67]
[b] k: number of input clusters; pc: principal components used in WARD
[c] V_f^R: Ensemble of all IS function-based replicon vectors (v_f^r); $\bar{V}_{f,genus}^R$: Ensemble of IS function-based genus-normalized replicon vectors ($v_{f,genus}^r$)
[d] Stability criterion according to Hennig[66]

Table 2. IS protein cluster-based unsupervised classification of SERs

a. INFOMAP clustering solution

Bacterial genus	C [a]	CHR%	SER%	PLD%	*wBHI* [b]	$\overline{\Delta^C}$ [c]	$\overline{\Delta^r}$ [d]
Agrobacterium	3	38	35	27	0.90	0.47	0.61
Aliivibrio	1	0	100	0	1.00	0.95	1.00
Anabaena	1	98	1	1	1.00	0.90	1.00
Asticcacaulis	1	96	1	3	1.00	0.97	1.00
Brucella	1	0	95	5	1.00	0.87	1.00
Burkholderia	2	64	17	19	0.99	0.77	0.99
Butyrivibrio	1	0	50	50	1.00	0.83	1.00
Chloracidobacterium	1	91	<1	9	0.82	0.86	0.00
Cupriavidus	1	73	18	9	0.99	0.72	1.00
Cyanothece	1	0	6	94	0.89	0.61	0.33
Deinococcus	1	0	4	96	0.71	0.61	1.00
Ilyobacter	1	91	<1	9	0.82	0.86	0.25
Leptospira	1	0	88	12	1.00	1.00	1.00
Nocardiopsis	1	91	<1	9	0.97	0.97	1.00
Ochrobactrum	1	0	95	5	1.00	0.87	*n.a.n.* [e]
Paracoccus	1	96	1	3	1.00	0.97	1.00
Photobacterium	1	96	1	3	0.99	0.79	1.00
Prevotella	1	96	2	2	0.95	0.92	1.00
Pseudoalteromonas	1	96	1	3	0.99	0.79	0.56
Ralstonia	1	73	18	9	0.99	0.72	1.00
Rhodobacter	1	0	40	60	1.00	0.71	1.00
Ensifer (*Sinorhizobium*)	2	0	2	98	0.96	0.65	0.67
Sphaerobacter	1	0	50	50	1.00	1.00	1.00
Sphingobium	2	77	1	22	0.95	0.90	0.50
Thermobaculum	1	91	<1	9	0.82	0.86	1.00
Variovorax	1	73	18	9	0.99	0.72	0.90
Vibrio	1	0	100	0	1.00	0.95	0.89

b. PCA+WARD clustering solution

Bacterial genus	C [a]	CHR%	SER%	PLD%	*wBHI* [b]	$\overline{\Delta^C}$ [c]	$\overline{\Delta^r}$ [d]
Agrobacterium	2	0	29	71	0.94	0.76	1.00
Aliivibrio	2	0	56	44	1.00	0.60	0.33
Anabaena	1	98	2	0	0.97	0.84	0.00
Asticcacaulis	1	88	8	4	1.00	0.88	1.00
Brucella	2	0	33	67	0.96	0.53	0.97
Burkholderia	7	0	79	21	0.97	0.69	0.84
Butyrivibrio	1	<1	1	99	0.27	0.98	1.00
Chloracidobacterium	1	<1	1	99	0.27	0.98	1.00
Cupriavidus	2	0	92	8	1.00	0.69	0.92
Cyanothece	1	<1	1	99	0.27	0.98	1.00
Deinococcus	1	<1	1	99	0.27	0.98	1.00
Ilyobacter	1	<1	1	99	0.27	0.98	1.00
Leptospira	1	<1	1	99	0.27	0.98	1.00
Nocardiopsis	1	0	2	98	0.58	0.40	1.00
Ochrobactrum	1	0	100	0	1.00	1.00	1.00
Paracoccus	1	88	8	4	1.00	0.88	1.00
Photobacterium	1	0	100	0	1.00	0.55	1.00
Prevotella	2	95	5	0	1.00	0.73	0.50
Pseudoalteromonas	2	<1	1	99	0.28	0.82	0.83
Ralstonia	1	0	68	32	1.00	0.81	0.83
Rhodobacter	2	0	6	94	0.65	0.43	0.58
Ensifer (*Sinorhizobium*)	2	0	21	79	0.94	0.46	0.25
Sphaerobacter	1	0	20	80	0.93	0.52	0.50
Sphingobium	1	0	39	61	1.00	0.66	0.83
Thermobaculum	1	<1	1	99	0.27	0.98	1.00
Variovorax	1	0	67	33	1.00	0.48	0.00
Vibrio	2	0	56	44	1.00	0.60	0.79

[a] number of clusters containing SERs of a given bacterial genus
[b] weighted biological homogeneity index value for the phylum of the replicons in the clusters
[c] mean value of the cluster stability estimator, weighted by the cluster sizes
[d] mean value of the SER stability estimator for a given bacterial genus
[e] "*n.a.n.*", standing for "not a number", indicates that the replicon appeared in none of the bootstrap replications performed in the clustering procedure

Table 3. Function-based unsupervised classification of SERs using PCA+WARD

Bacterial genus	C[a]	CHR%	SER%	PLD%	*wBHI*[b]	$\overline{\Delta^C}$[c]	$\overline{\Delta^r}$[d]
Agrobacterium	3	64	21	15	0.86	0.60	0.81
Aliivibrio	1	0	70	30	1.00	0.70	0.67
Anabaena	1	77	4	19	0.21	0.64	1.00
Asticcacaulis	1	99	1	0	0.51	0.60	1.00
Brucella	1	43	32	25	0.75	0.80	1.00
Burkholderia	6	31	42	27	0.92	0.68	0.81
Butyrivibrio	1	77	4	19	0.21	0.64	1.00
Chloracidobacterium	1	1	<1	99	0.29	0.98	0.00
Cupriavidus	1	5	95	0	1.00	0.66	0.66
Cyanothece	1	1	<1	99	0.29	0.98	1.00
Deinococcus	1	1	<1	99	0.29	0.98	1.00
Ilyobacter	1	77	4	19	0.21	0.64	1.00
Leptospira	1	1	<1	99	0.29	0.98	1.00
Nocardiopsis	1	77	4	19	0.21	0.64	1.00
Ochrobactrum	1	90	8	2	1.00	0.40	0.73
Paracoccus	1	92	5	3	0.89	0.32	0.53
Photobacterium	1	0	70	30	1.00	0.70	0.36
Prevotella	2	86	3	11	0.34	0.62	0.50
Pseudoalteromonas	1	77	4	19	0.21	0.64	0.29
Ralstonia	1	0	70	30	1.00	0.70	0.22
Rhodobacter	2	27	32	41	0.84	0.73	0.83
Ensifer (*Sinorhizobium*)	2	25	48	27	0.86	0.76	0.63
Sphaerobacter	1	100	<1	0	0.35	0.60	1.00
Sphingobium	2	62	17	21	0.34	0.64	0.86
Thermobaculum	1	77	4	19	0.21	0.64	1.00
Variovorax	1	0	70	30	1.00	0.70	1.00
Vibrio	4	31	37	32	0.97	0.57	0.92

[a] number of clusters containing SERs of a given bacterial genus
[b] weighted biological homogeneity index value for the phylum of the replicons in the clusters
[c] mean value of the cluster stability estimator, weighted by the sizes of the clusters
[d] mean value of the SER stability estimator for a given bacterial genus

Table 4. IS usage comparison between classes of replicons

		IS function[a]		Chromosomes *vs.* Plasmids		Chromosomes *vs.* SERs		SERs *vs.* Plasmids	
				P_value[b]	*OR*[c]	*P_value*[b]	*OR*[c]	*P_value*[b]	*OR*[c]
KEGG entries (chromosome-like)	REPLICATION	CbpA		8.20E-27	2,608.4	9.90E-13	22.8	5.60E-07	36.1
		Dam		6.90E-16	16.7	3.60E-02	2.0	2.40E-02	4.3
		DiaA		1.50E-15	81.9	1.20E-03	38.4		
		DnaA		3.00E-44	2,118.9	1.10E-19	239.6	3.50E-03	8.3
		DnaB		1.10E-43	1,992.9	5.10E-19	429.4	8.20E-03	3.7
		DnaB2		6.70E-03	12.6				
		DnaC	*	6.00E-12	2.6			4.60E-02	1.5
		DnaG		2.10E-50	1,861.5	1.90E-21	205.3	2.50E-03	4.5
		DnaI		5.20E-03	18.0				
		Dps		9.10E-21	65.3	3.50E-05	8.4	8.70E-03	6.7
		Fis		5.80E-07	180.9	3.30E-03	7.9	1.40E-02	25.1
		Hda		7.30E-07	149.1	5.30E-03	7.9	1.90E-02	18.0
		Hfq		1.40E-12	121.7	3.00E-04	6.9	8.10E-04	19.3
		H-NS	*	1.10E-05	2.8			3.80E-04	2.8
		HupA		2.70E-04	15.1				
		HupB		1.20E-53	97.6	2.30E-08	6.7	2.40E-07	11.6
		IciA	*	7.10E-20	3.2			4.50E-07	1.8
		IhfA, HimA		1.70E-12	63.8	1.40E-03	10.5	4.90E-02	6.9
		IhfB, HimD		1.20E-14	68.4	4.90E-04	8.4	8.40E-03	9.9
		Lrp	*	1.60E-19	8.4			5.40E-11	8.1
		Rob	*	6.30E-19	5.3			3.40E-08	4.2
		SeqA		1.60E-03	25.9				
		ssb		5.90E-41	298.3	5.00E-18	160.6		
	PARTITION	Fic	*	3.10E-09	10.3			8.60E-03	7.2
		GidA, MnmG, MTO1		5.20E-13	1,477.2	2.90E-08	110.6	4.30E-02	18.2
		GidB, RsmG		6.70E-17	6,059.9	2.20E-15	252.5	9.00E-03	32.2
		MreB		1.30E-21	1,598.2	3.90E-12	40.1	1.40E-05	24.1
		MreC		2.90E-11	1,311.2	1.30E-08	46.3	8.90E-03	32.8
		MreD		1.80E-08	459.2	6.80E-05	19.8	1.90E-02	18.2
		Mrp		6.60E-17	2,599.3	1.30E-14	35.0	2.50E-05	86.2
		MukB	*	2.30E-03	27.4			1.90E-02	18.2
		MukE	*	3.10E-03	21.0			1.90E-02	18.2
		MukF	*	3.70E-03	19.6			1.90E-02	18.2
		ParA, Soj		2.70E-38	9.9	9.00E-06	2.6	8.40E-06	3.8
		ParB, Spo0J		2.50E-44	13.7	3.00E-03	2.1	2.30E-06	4.1
		ParC		3.00E-27	4,149.3	3.00E-16	134.0	4.60E-04	12.3
		ParE		7.30E-26	5,842.4	5.70E-15	350.1	2.40E-04	15.8
		RodA, MrdB		2.80E-12	1,233.1	9.70E-10	33.0	2.60E-03	55.3
		TrmFO, Gid		1.50E-06	182.5	4.40E-03	8.3	1.90E-02	18.0
		XerC		1.70E-43	55.0	3.10E-08	8.8	1.80E-04	6.7
		XerD		1.30E-38	26.6	4.10E-08	3.4	2.50E-06	6.2
	SEGREGATION	ScpA		1.40E-11	789.4	5.70E-07	42.9	2.10E-02	16.6
		ScpB		7.50E-32	102.5	1.80E-07	25.8		
		SepF		1.80E-07	68.8	1.40E-02	12.3		
		SlmA, Ttk		3.80E-09	52.3	1.20E-02	4.6	1.50E-02	7.5
		Smc		1.60E-08	3,090.5	1.40E-05	131.9		
		SulA	*	3.30E-06	17.5			1.50E-02	10.7
	CELL DIVISION	AcrA		6.60E-19	2.8	1.70E-02	1.1	5.30E-10	2.7
		AmiA, AmiB, AmiC		6.40E-36	46.4	2.90E-10	8.9	4.60E-03	3.0
		DivIC, DivA		4.90E-05	90.5	4.70E-02	8.1		
		DivIVA		4.10E-06	128.0	1.10E-02	13.4		
		EzrA		1.00E-02	13.7				
		FtsA		9.50E-12	742.7	2.20E-08	24.6	2.50E-03	41.7
		FtsB		1.10E-06	167.2	5.40E-03	16.1		
		FtsE		4.20E-24	2.3	1.30E-06	1.1	4.00E-11	1.9
		FtsI		9.80E-09	47.0	7.00E-16	3.9	2.20E-07	76.7
		FtsK, SpoIIIE		2.80E-37	76.9	2.70E-08	15.8	1.40E-02	4.2
		FtsL		1.20E-05	91.5	2.70E-02	9.8		
		FtsN		1.60E-04	53.0				
		FtsQ		1.70E-15	2,135.0	1.30E-11	99.3	9.00E-03	28.8
		FtsW, SpoVE		5.70E-16	4,266.4	4.40E-16	87.7	8.20E-04	55.0
		FtsX		9.30E-12	972.9	1.30E-08	13.8	4.80E-04	146.2
		FtsZ		3.10E-31	2,747.0	1.20E-19	101.6	9.70E-04	16.5
		MinC		4.40E-09	172.3	1.20E-02	3.0	5.80E-05	76.8
		MinD		3.10E-19	42.8	1.60E-04	2.3	5.40E-11	81.5
		MinE		9.00E-09	152.9	3.10E-02	2.6	5.90E-05	75.2
		ZapA		8.20E-09	602.8	7.40E-06	17.3	7.30E-03	56.1
		ZipA		7.90E-05	66.0				
ACLAME Families (plasmid-like)		CdsD				4.40E-02	0.1		
	REPLICATION	DNA helicase		5.80E-21	33.6	2.70E-04	4.1	1.30E-04	9.8
		Helicase-1		1.60E-27	71.1	1.90E-13	20.0	1.10E-04	4.6
		DNA repair		2.20E-04	34.0			5.70E-04	43.6
		primase, LtrC		3.10E-02	1.8				
		RepA		5.90E-03	0.7				
		RepAEB		1.70E-16	0.0	1.90E-04	0.1		
		RepC						9.60E-03	2.7
		RepCJE				4.40E-02	0.1		
		RepRSE		1.30E-02	0.0	4.90E-02	0.1		
		RNA polymerase		3.20E-02	6.3				
		Rop		3.20E-02	0.0	4.40E-02	0.1		
		RuvB		1.20E-08	433.0	5.70E-08	17.7	1.40E-05	37.8
		TrfA		1.40E-02	0.3				
	PARTITION	ATPase, TyrK, ExoP		2.20E-20	19.4			8.50E-07	9.3
		CopG				2.70E-02	0.2	4.60E-03	23.1
		DNA-binding protein				4.40E-02	0.1		
		FtsK, SpoIIIE		1.90E-07	6.0			9.90E-05	9.8
		ParA, ParM		1.50E-10	0.4	4.00E-06	0.3		
		ParB		5.70E-12	0.1	1.40E-05	0.2		
		serine recombinase		2.50E-06	1.4	1.50E-03	2.9	1.80E-02	0.4
		Tyrosine recombinase		3.40E-04	3.3			7.40E-04	8.7
		Xer-like Tyrosine		7.60E-11	2.0			6.30E-03	1.6
	MAINTENANCE	Ccd (PSK)		4.60E-02	3.9				
		HicAB (PSK)		4.30E-05	25.2			4.80E-03	15.1
		HigBA (PSK)		3.30E-15	3.4	2.40E-02	1.5	1.20E-03	2.5
		MazEF (PSK)		1.20E-11	5.2	2.90E-02	2.6		
		ParC (PSK)				4.40E-02	0.1		
		ParDE (PSK)		5.50E-08	2.3			7.80E-05	3.4
		PhD, Doc (PSK)		3.20E-07	11.9			2.90E-03	8.8
		plasmid maintenance				4.40E-02	0.1		
		RelBE (PSK)		2.70E-08	3.5			6.10E-04	4.2
		VapBC/Vag (PSK)		1.20E-09	3.9			1.40E-05	5.8

OR colour code

- $10^4 \leq OR$
- $10^3 \leq OR < 10^4$
- $10^2 \leq OR < 10^3$
- $10^1 \leq OR < 10^2$
- $10^0 \leq OR < 10^1$
- $10^{-1} \leq OR < 10^0$
- $10^{-2} \leq OR < 10^{-1}$
- $10^{-3} \leq OR < 10^{-2}$
- $10^{-4} \leq OR < 10^{-3}$
- $OR < 10^{-4}$

[a] Stars indicate chromosome-like KEGG-annotated functionalities which level of bias is of the same order of magnitude in chromosomes and SERs when compared to plasmids.

[b] Model significance. $0 < P_value < 0.01$: significant (darker shade of grey); $0.01 < P_value < 0.05$: poorly significant (lighter shade of grey); $0.05 < P_value$: non significant (not shown)

[c] *OR*: Odd-ratio favouring the first class (blue) or the second class (green)

Table 5. Performance of the ERT classification procedures

TRAINING SET [a]	CV_{score} [b]	$\sigma_{CV_{score}}$ [c]	OOB_{score} [d]	$\sigma_{OOB_{score}}$ [e]
$\{E_{SER}, E'_{plasmid}\}$	0.96	-	0.96	-
$\{E_{SER}, E'_{plasmid}\}^{it}$	0.92	0.02	0.93	0.02
$\{E_{chromosome}, E'_{plasmid}\}$	1.00	-	1.00	-
$\{E_{SER}, E_{chromosome}\}^{it}$	0.98	0.00	0.98	0.01

[a] $E_{chromosome}$ and E_{SER} are host genus-normalized sets of chromosomes or SERs, respectively, as in Table S8. $E'_{plasmid}$ is derived from the INFOMAP clustering solution, by discarding plasmids belonging to clusters also harbouring SERs or chromosomes, and normalized according to host genus. *it* designates the iterative procedure.
[b] Cross-validation score or mean of iteration cross-validation scores.
[c] Standard deviation of iteration cross-validation scores.
[d] Out-of bag estimate or mean of iteration Out-of bag estimates.
[e] Standard deviation of iteration Out-of bag estimates.

Table 6. SER probability to belong to the *SER* class

Genus	$\bar{P}_{SER}(SER)$[a]
Agrobacterium	0.90
Aliivibrio	0.87
Anabaena	0.94
Asticcacaulis	0.95
Brucella	0.92
Burkholderia	0.89
Butyrivibrio	0.83
Chloracidobacterium	0.88
Cupriavidus	0.94
Cyanothece	0.86
Deinococcus	0.78
Ensifer /Sinorhizobium	0.90
Ilyobacter	0.88
Leptospira	0.54
Nocardiopsis	0.90
Ochrobactrum	0.96
Paracoccus	0.96
Photobacterium	0.95
Prevotella	0.92
Pseudoalteromonas	0.91
Ralstonia	0.95
Rhodobacter	0.69
Sphaerobacter	0.88
Sphingobium	0.73
Thermobaculum	0.78
Variovorax	0.83
Vibrio	0.76

[a] SER probability, averaged per host genus, to belong to the *SER* class in the supervised classification using $\{E_{SER}, E_{plasmid}\}^{it}$ as training set.

Table 7. Identification of ERs among the extra-chromosomal replicons

a. Candidate-SERs identified among plasmids

REPLICON	PROBABILITY[a]
Acaryochloris marina MBIC11017 plasmid pREB1 [CYANOBACTERIA : Chroococcales] (CP000838)	0.578
Acaryochloris marina MBIC11017 plasmid pREB2 [CYANOBACTERIA : Chroococcales] (CP000839)	0.582
Agrobacterium sp. H13-3 plasmid pAspH13-3a [α-PROTEOBACTERIA : Rhizobiales] (CP0022)	0.565
Arthrobacter chlorophenolicus A6 plasmid pACHL01 [ACTINOBACTERIA : Actinomycetales] (CP001342)	0.648
Azospirillum brasilense Sp245 plasmid AZOBR_p1 [α-PROTEOBACTERIA : Rhodospirillales] (HE577328)	0.878
Azospirillum brasilense Sp245 plasmid AZOBR_p2 [α-PROTEOBACTERIA : Rhodospirillales] (HE577329)	0.591
Azospirillum brasilense Sp245 plasmid AZOBR_p3 [α-PROTEOBACTERIA : Rhodospirillales] (HE577330)	0.603
Azospirillum lipoferum 4B plasmid AZO_p1e [α-PROTEOBACTERIA : Rhodospirillales] (FQ311869)	0.722
Azospirillum lipoferum 4B plasmid AZO_p2 [α-PROTEOBACTERIA : Rhodospirillales] (FQ311870)	0.609
Azospirillum lipoferum 4B plasmid AZO_p4 [α-PROTEOBACTERIA : Rhodospirillales] (FQ311872)	0.645
Azospirillum sp. B510 plasmid pAB510a [α-PROTEOBACTERIA : Rhodospirillales] (AP010947)	0.732
Azospirillum sp. B510 plasmid pAB510c [α-PROTEOBACTERIA : Rhodospirillales] (AP010949)	0.545
Azospirillum sp. B510 plasmid pAB510d [α-PROTEOBACTERIA : Rhodospirillales] (AP010950)	0.530
Burkholderia phenoliruptrix BR3459a plasmid pSYMBR3459 [β-PROTEOBACTERIA : Burkholderiales] (CP003865)	0.663
Burkholderia phymatum STM815 plasmid pBPHY01 [β-PROTEOBACTERIA : Burkholderiales] (CP001045)	0.733
Burkholderia sp. YI23 plasmid byi_1p [β-PROTEOBACTERIA : Burkholderiales] (CP003090)	0.846
Clostridium botulinum A3 str. Loch Maree plasmid pCLK [FIRMICUTES : Clostridiales] (CP000963)	0.531
Clostridium botulinum Ba4 str. 657 plasmid pCLJ [FIRMICUTES : Clostridiales] (CP001081)	0.531
Cupriavidus metallidurans CH34 megaplasmid [β-PROTEOBACTERIA : Burkholderiales] (CP000353)	0.883
Cupriavidus necator N-1 plasmid BB1p [β-PROTEOBACTERIA : Burkholderiales] (CP002879)	0.500
Cupriavidus pinatubonensis JMP134 megaplasmid [β-PROTEOBACTERIA : Burkholderiales] (CP000092)	0.513
Deinococcus geothermalis DSM 11300 plasmid1 [DEINOCOCCUS-THERMUS : Deinococcales] (CP000358)	0.622
Deinococcus gobiensis I-0 plasmid P1 [DEINOCOCCUS-THERMUS : Deinococcales] (CP002192)	0.812
Ensifer/Sinorhizobium fredii HH103 plasmid pSfHH103e [α-PROTEOBACTERIA : Rhizobiales] (HE616899)	0.915
Ensifer/Sinorhizobium fredii NGR234 plasmid pNGR234b [α-PROTEOBACTERIA : Rhizobiales] (CP000874)	0.894
Ensifer/Sinorhizobium medicae WSM419 plasmid pSMED01 [α-PROTEOBACTERIA : Rhizobiales] (CP000739)	0.942
Ensifer/Sinorhizobium medicae WSM419 plasmid pSMED02 [α-PROTEOBACTERIA : Rhizobiales] (CP000740)	0.836
Ensifer/Sinorhizobium meliloti 1021 plasmid pSymA [α-PROTEOBACTERIA : Rhizobiales] (AE006469)	0.818
Ensifer/Sinorhizobium meliloti 1021 plasmid pSymB [α-PROTEOBACTERIA : Rhizobiales] (AL591985)	0.949
Ensifer/Sinorhizobium meliloti BL2C plasmid pSINMEB01 [α-PROTEOBACTERIA : Rhizobiales] (CP002741)	0.800
Ensifer/Sinorhizobium meliloti BL2C plasmid pSINMEB02 [α-PROTEOBACTERIA : Rhizobiales] (CP002742)	0.961
Ensifer/Sinorhizobium meliloti Rm41 plasmid pSYMA [α-PROTEOBACTERIA : Rhizobiales] (HE995407)	0.922
Ensifer/Sinorhizobium meliloti Rm41 plasmid pSYMB [α-PROTEOBACTERIA : Rhizobiales] (HE995408)	0.960
Ensifer/Sinorhizobium meliloti SM11 plasmid pSmeSM11c [α-PROTEOBACTERIA : Rhizobiales] (CP001831)	0.877
Ensifer/Sinorhizobium meliloti SM11 plasmid pSmeSM11d [α-PROTEOBACTERIA : Rhizobiales] (CP001832)	0.947
Methylobacterium extorquens AM1 megaplasmid [α-PROTEOBACTERIA : Rhizobiales] (CP001511)	0.538
Novosphingobium sp. PP1Y plasmid Mpl [α-PROTEOBACTERIA : Sphingomonadales] (FR856861)	0.523
Pantoea sp. At-9b plasmid pPAT9B01 [γ-PROTEOBACTERIA : Enterobacteriales] (CP002434)	0.527
Paracoccus denitrificans PD1222 plasmid1 [α-PROTEOBACTERIA : Rhodobacterales] (CP000491)	0.769
Ralstonia solanacearum GMI0 plasmid pGMI0MP [β-PROTEOBACTERIA : Burkholderiales] (AL646053)	0.861
Ralstonia solanacearum Po82 megaplasmid [β-PROTEOBACTERIA : Burkholderiales] (CP002820)	0.865
Ralstonia solanacearum PSI07 megaplasmid [β-PROTEOBACTERIA : Burkholderiales] (FP885891)	0.827
Rhizobium etli CFN 42 plasmid p42e [α-PROTEOBACTERIA : Rhizobiales] (CP000137)	0.700
Rhizobium etli CFN 42 plasmid p42f [α-PROTEOBACTERIA : Rhizobiales] (CP000138)	0.555
Rhizobium etli CIAT 652 plasmid pA [α-PROTEOBACTERIA : Rhizobiales] (CP0010)	0.701
Rhizobium etli CIAT 652 plasmid pC [α-PROTEOBACTERIA : Rhizobiales] (CP001077)	0.792
Rhizobium leguminosarum bv. trifolii WSM1325 plasmid pR132501 [α-PROTEOBACTERIA : Rhizobiales] (CP001623)	0.711
Rhizobium leguminosarum bv. trifolii WSM1325 plasmid pR132502 [α-PROTEOBACTERIA : Rhizobiales] (CP001624)	0.741
Rhizobium leguminosarum bv. *trifolii* WSM2304 plasmid pRLG201 [α-PROTEOBACTERIA : Rhizobiales] (CP001192)	0.777
Rhizobium leguminosarum bv. *trifolii* WSM2304 plasmid pRLG202 [α-PROTEOBACTERIA : Rhizobiales] (CP001193)	0.630
Rhizobium leguminosarum bv. *viciae* 3841 plasmid pRL11 [α-PROTEOBACTERIA : Rhizobiales] (AM236085)	0.731
Rhizobium leguminosarum bv. *viciae* 3841 plasmid pRL12 [α-PROTEOBACTERIA : Rhizobiales] (AM236086)	0.718
Ruegeria sp. TM1040 megaplasmid [α-PROTEOBACTERIA : Rhodobacterales] (CP000376)	0.667
Streptomyces cattleya NRRL 8057 plasmid pSCA [ACTINOBACTERIA : Actinomycetales] (FQ859184)	0.727
Streptomyces cattleya NRRL 8057 plasmid pSCATT [ACTINOBACTERIA : Actinomycetales] (CP003229)	0.702
Streptomyces clavuligerus ATCC 27064 plasmid pSCL4 [ACTINOBACTERIA : Actinomycetales] (CM000914)	0.642
Streptomyces clavuligerus ATCC 27064 plasmid pSCL4 [ACTINOBACTERIA : Actinomycetales] (CM001019)	0.642
Thermus thermophilus HB8 plasmid pTT27 [DEINOCOCCUS-THERMUS : Thermales] (AP008227)	0.500
Thermus thermophilus JL-18 plasmid pTTJL1801 [DEINOCOCCUS-THERMUS : Thermales] (CP0033)	0.557
Tistrella mobilis KA081020-065 plasmid pTM2 [α-PROTEOBACTERIA : Rhodospirillales] (CP003238)	0.578
Tistrella mobilis KA081020-065 plasmid pTM3 [α-PROTEOBACTERIA : Rhodospirillales] (CP003239)	0.797

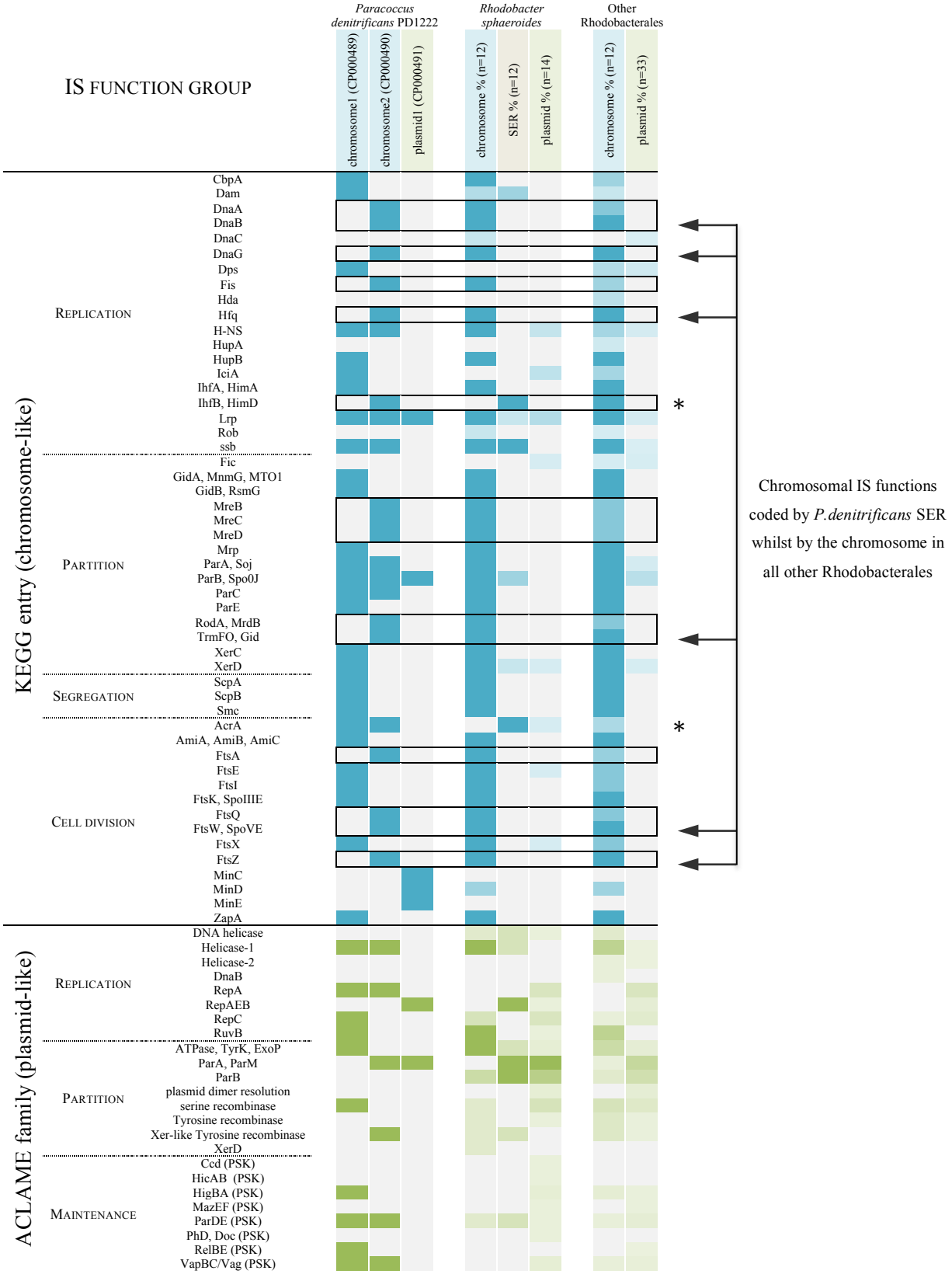
b. Candidate chromosomes identified among extra-chromosomal replicons

REPLICON	PROBABILITY[a]
Anaeba sp. 90 chromosome chANA02 [CYANOBACTERIA : Chroococcales] (CP003285)	0.638
Asticcacaulis excentricus CB 48 chromosome 2 [α-PROTEOBACTERIA : Caulobacterales] (CP002396)	0.637
Azospirillum brasilense Sp245 plasmid AZOBR_p1 [α-PROTEOBACTERIA : Rhodospirillales] (HE577328)	0.774
Methylobacterium extorquens AM1 megaplasmid [α-PROTEOBACTERIA : Rhizobiales] (CP001511)	0.669
Nocardioides dassonvillei DSM 43111 chromosome 2 [ACTINOBACTERIA : Actinomycetales] (CP002041)	0.539
Paracoccus denitrificans PD1222 chromosome2 [α-PROTEOBACTERIA : Rhodobacterales] (CP000490)	0.778
Prevotella intermedia 17 chromosome II [BACTEROIDETES : Bacteroidales] (CP0033)	0.984
Prevotella melaninogenica ATCC 845 chromosome II [BACTEROIDETES : Bacteroidales] (CP002123)	0.698

[a] Probability for an extra-chromosomal replicon, *i.e.*, plasmid or SER, to belong to the SER (A) or Chromosome (B) class according to the supervised classification procedures. Colour code varies from probability value = 0.500 (yellow) to probability value = 1.000 (blue).

Table 8. IS profiles of *Paracoccus denitrificans vs. Rhodobacter sphaeroides* (Rhodobacterales).

Frames show functions that are coded only by the SER in *P. denitrificans* whilst by the chromosome in all other Rhodobacterales. The asterisks indicate the two chromosome-like functions coded only by the SER in *R. sphaeroides*.



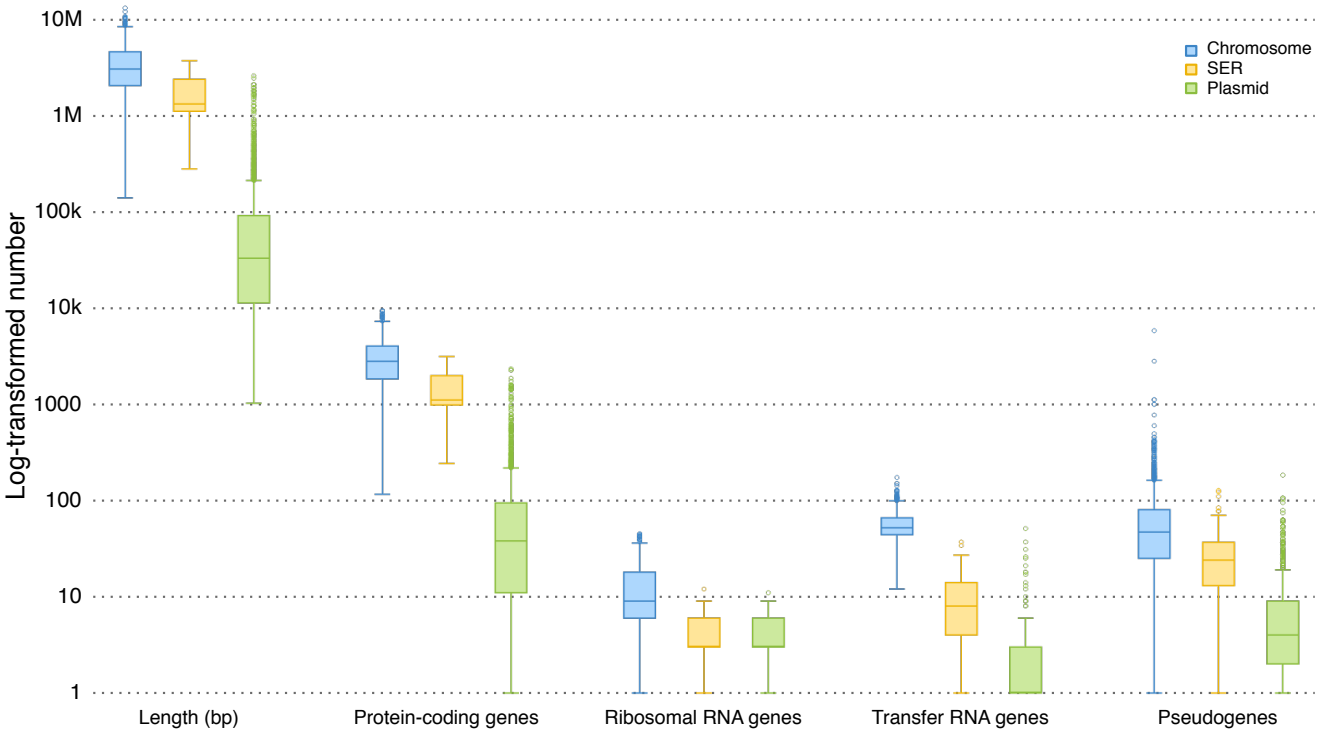


Figure 1. Structural features of the replicons

Boxplots of the length, number of protein-coding genes, number of rRNA genes, number of tRNA genes and number of pseudogenes for the 2016 chromosomes (green), 129 SERs (blue), and 2783 plasmids (yellow) included in the final dataset (4928 replicons).

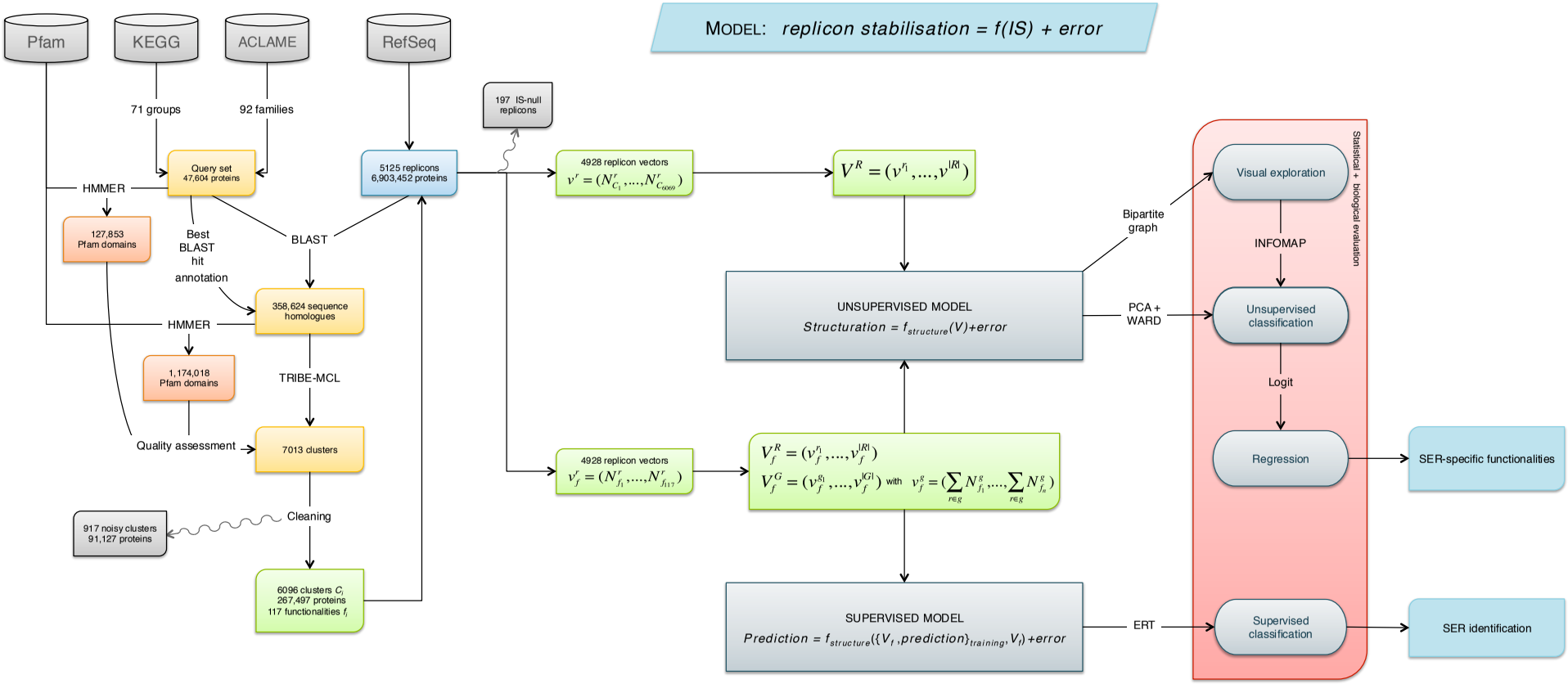


Figure 2. Analytical procedure

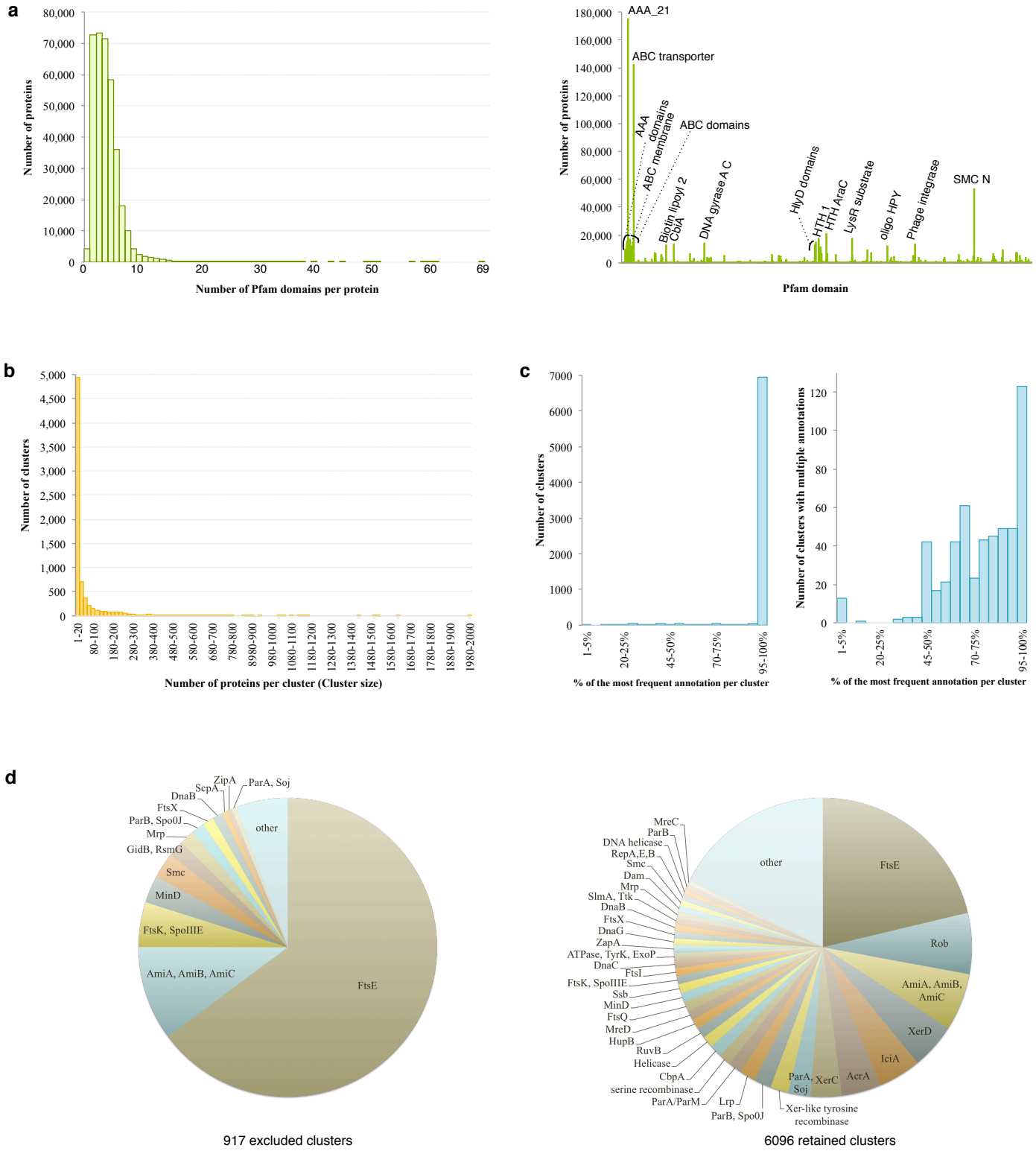


Figure 3. Properties of the IS clustering

(a) Frequency distribution of the 358,624 putative IS protein homologues according to their number of functional domains (0 to 69) per protein (left), and occurrences of the 1711 functional Pfam domains across the 358,624 putative IS homologues (right). The 20 top most frequently encountered functional domains are indicated. (b) Size distribution of the 7013 clusters, each comprising from a single to 1990 proteins. (c) Pourcentage distribution of the most frequent annotation per cluster among all clusters (left) and among clusters with multiple annotations (right). (d) Distribution of the most frequent annotation per cluster among the 917 excluded clusters (left) and the 6096 clusters retained for the analysis (right).

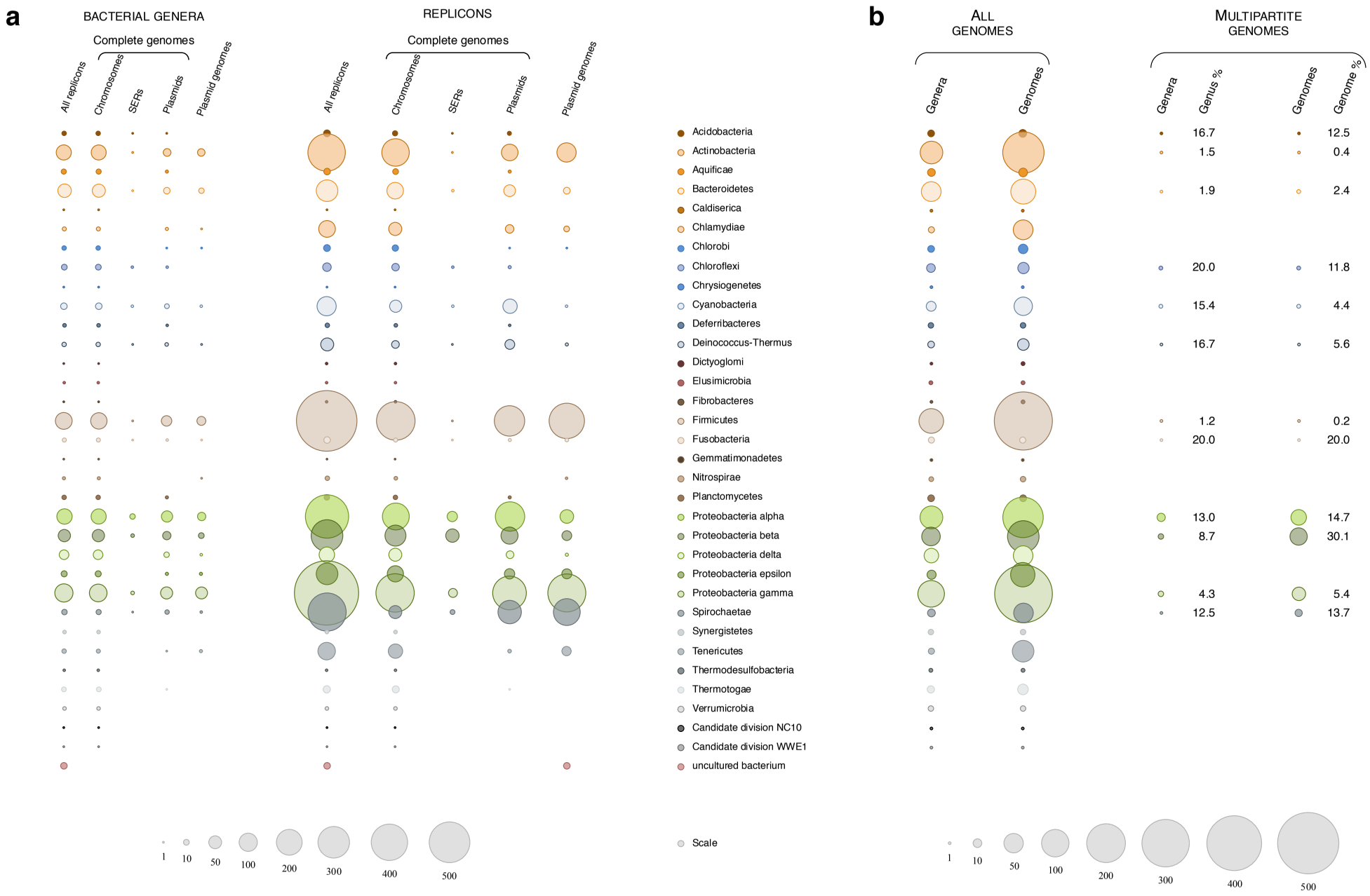


Figure 4. Taxonomic structure of the replicon dataset

Numbers of replicons (a) or complete genomes (b), and represented bacterial genera are shown according to datasets and host phylum or class (Proteobacteria). Surfaces represent the numbers of bacterial genera, replicons or genomes within each category. Percentages of multipartite genomes and corresponding bacterial genera are calculated for each host phylum or class (Proteobacteria).

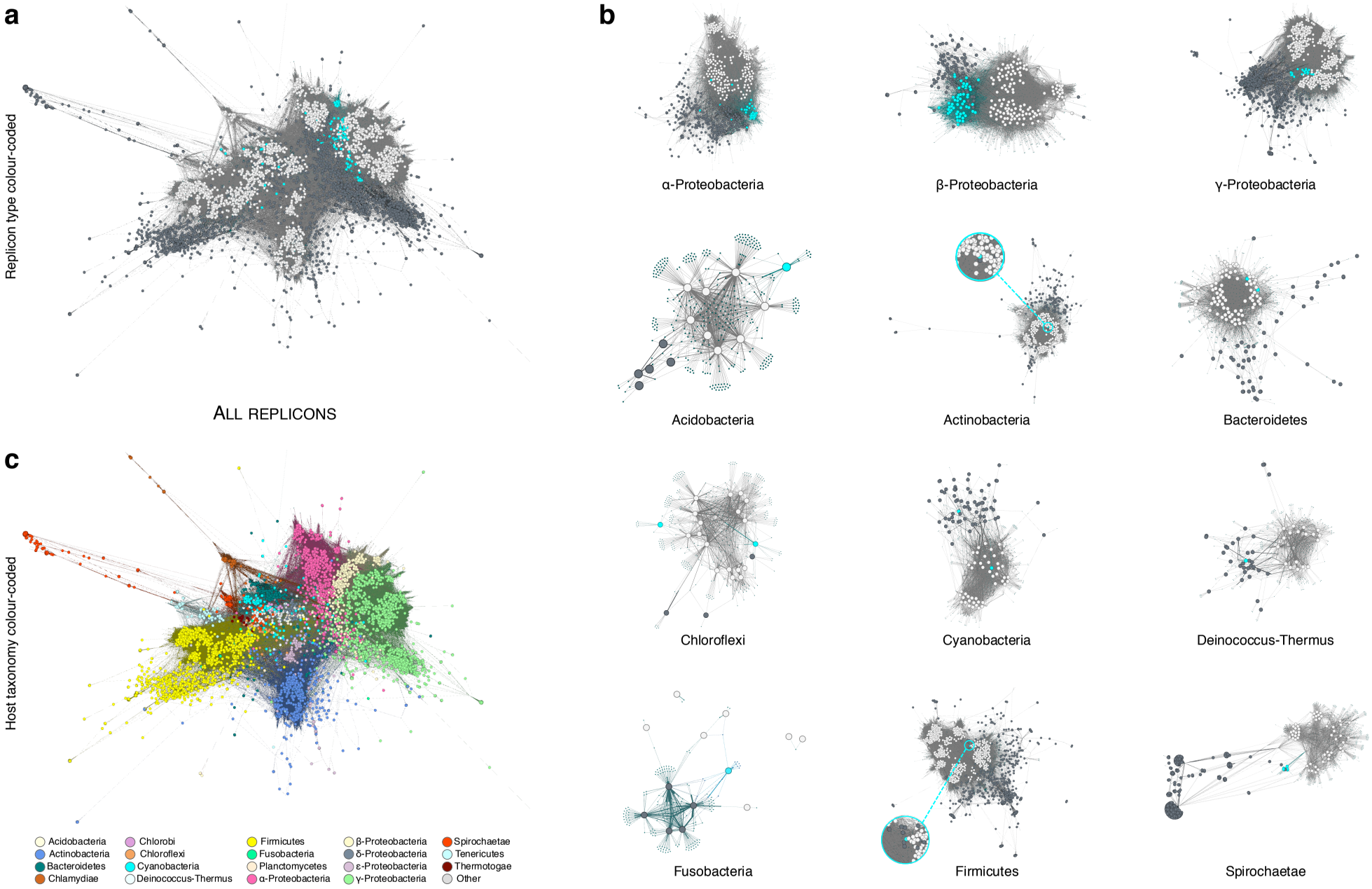


Figure 5. Visualisation of the replicon IS-based relationshipss

Gephi-generated bipartite-graphs for the whole dataset (a and b) or groups of replicons following the host taxonomy (c). Nodes correspond to the replicons (large dots) or the clusters of IS proteins (small dots). Edges linking replicons and protein clusters reflect the presence on a replicon of at least one protein of a protein cluster. Colouring according to replicon type (a and c): chromosomes (white), plasmids (grey), and SERs (blue), or according to host taxonomy (b).

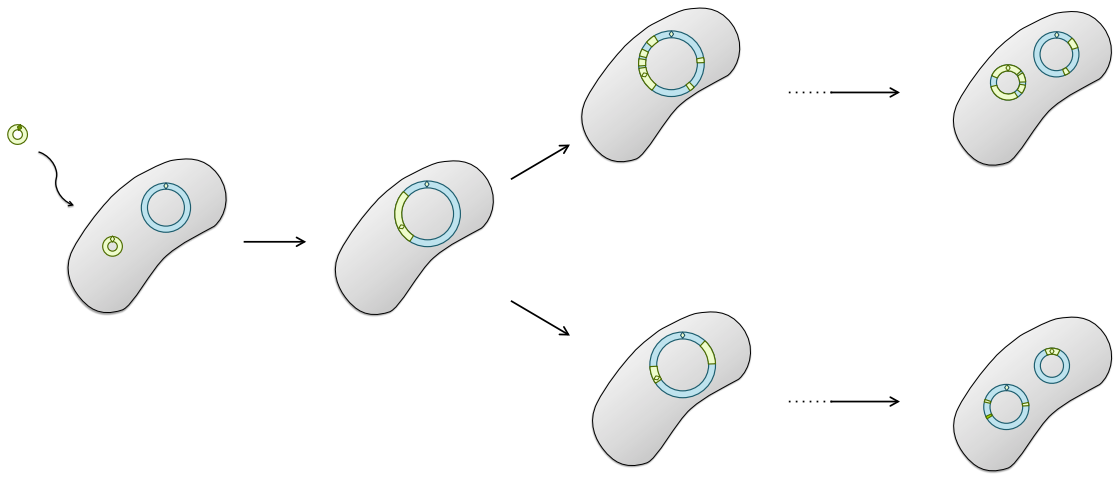


Figure 6. Fusion-Shuffling-Scission model of genome evolution

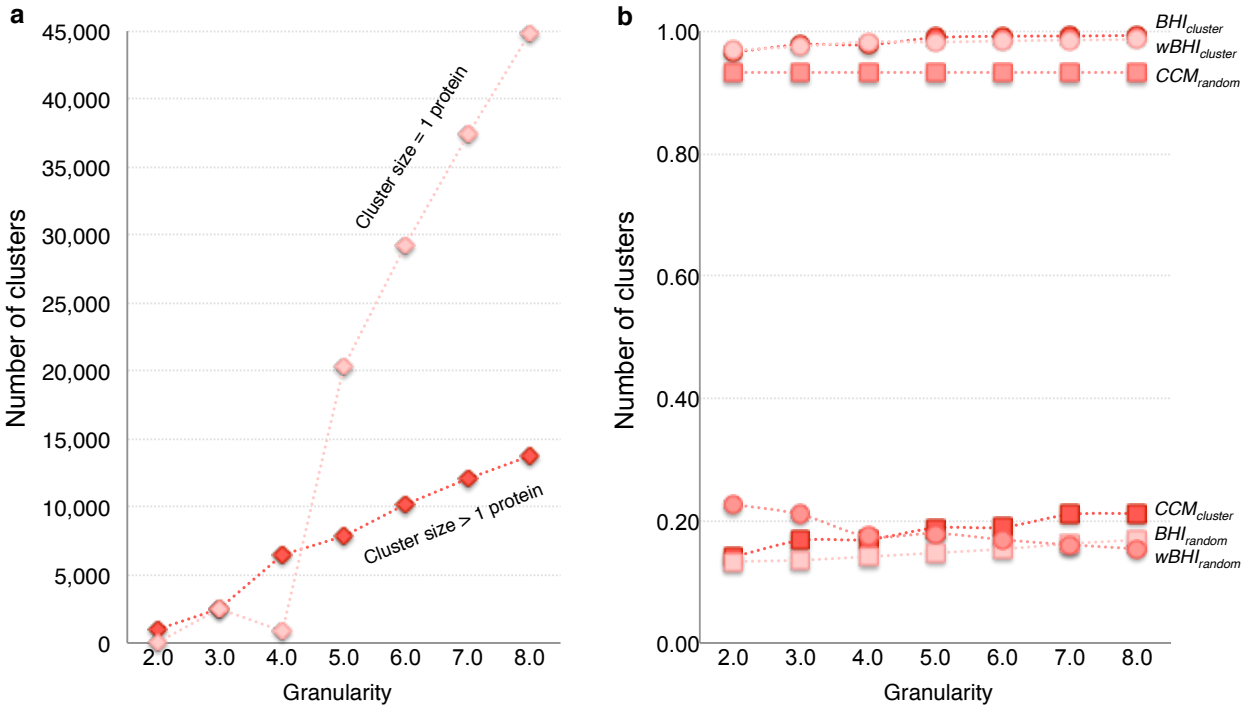


Figure 7. Influence of granularity on the clustering

(a) Number of clusters with more than one protein (dark diamonds) or clusters holding a single protein (pale diamonds). (b) *BHI* (dark), *wBHI* (pale) and *CCM* (medium) scores obtained with random clusters (squares) and normal clusters (circles), respectively.