Full Title:

Combining *Shigella* Tn-seq data with Gold-standard *E. coli* Gene Deletion Data Suggests Rare Transitions between Essential and Non-essential Gene Functionality

Short Title:

Transitions between Essential and Non-essential Functionality

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Abstract

Gene essentiality - whether or not a gene is necessary for cell growth - is a fundamental component of gene function. It is not well established how quickly gene essentiality can change, as few studies have compared empirical measures of essentiality between closely related organisms. Here we present the results of a Tn-seq experiment designed to detect essential protein coding genes in the bacterial pathogen *Shigella flexneri* 2a 2457T on a genome-wide scale. Superficial analysis of this data suggested that 451 protein-coding genes in this *Shigella* strain are critical for robust cellular growth on rich media. Comparison of this set of genes with a gold-standard data set of essential genes in the closely related *Escherichia coli* K12 BW25113 suggested that an excessive number of genes appeared essential in *Shigella* but non-essential in *E. coli.* Importantly, and in converse to this comparison, we found no genes that were essential in *E. coli* and non-essential in *Shigella*, suggesting that many genes were artefactually inferred as essential in *Shigella.* Controlling for such artefacts resulted in a much smaller set of discrepant genes. Among these, we identified three sets of functionally related genes; two of which have previously been implicated as critical for *Shigella* growth, but which are dispensable for *E. coli* growth. The data presented here highlight the small number of protein coding genes for which we have strong evidence that their essentiality status differs between the closely related bacterial taxa *E. coli* and *Shigella.* A set of genes involved in acetate utilization provides a canonical example. These results leave open the possibility of developing strain-specific antibiotic treatments targeting such differentially essential genes, but suggest that such opportunities may be rare in closely related bacteria.

Author Summary

Essential genes are those that encode proteins required for growth and survival in a particular environment. We performed experiments using transposons, genetic elements that disrupt gene function, to determine the set of essential genes in the pathogenic bacteria *Shigella flexneri*. We then compared our results to the well-characterized set of essential genes in the closely related, yet non-pathogenic, bacteria *Escherichia coli*. We found only a small number of genes that are important for growth in *Shigella flexneri*, yet not in *Escherichia* coli. We believe these findings are interesting for several reasons; they help us better understand how quickly the functions of proteins change over time; they suggest possible targets for developing strain-specific antibiotic treatments; and they expand our basic understanding of this pathogen's metabolic processes.

47 # Introduction

48 One general functional characteristic of a gene is essentiality - whether that gene is required
49 for cellular viability and growth. In haploid (e.g. bacterial) genomes, this characteristic can be
50 assessed by attempting to delete a specific gene from a genome. When such a deletion is not
51 possible, this gene is frequently termed "essential" [1], implying that the gene is necessary for
52 cell growth and viability. Gene disruption, although less precise, is more commonly used to
53 infer essentiality using a similar criterion. For example, genes that cannot be disrupted by
54 transposon insertion have been inferred as being essential (e.g. [2]).

55 One important question is how quickly essential functions change over evolutionary time. If
56 orthologous protein coding genes in two bacterial strains differ in their essentiality
57 classification, this suggests that either the biochemical nature of the protein has changed, or
58 that the cellular context in which the protein acts has changed [3]. It has been experimentally
59 established that such transitions can occur [4-6]. Here we examine how frequently proteins go
60 from being essential to non-essential and vice versa in nature.

61 A recent study quantified changes in the essentiality classifications of protein coding genes
62 between three alpha-proteobacteria: *Caulobacter crescentus*, *Brevundimonas subvibrioides*,
63 and *Agrobacterium tumefaciens* [3]. The analysis showed that although orthologous cell
64 components are well conserved, the essentiality of such components (e.g. those involved in
65 the cell cycle) had changed considerably, with only 106 orthologous genes being essential in
66 all three organisms, despite their relatively close evolutionary relationship (89%-93% identity
67 in 16S RNA genes).

68 In this study we combine dense transposon mutagenesis with high-throughput sequencing
69 (Tn-seq [7]) to quantify gene essentiality in *Shigella flexneri* 2a 2457T (hereafter referred to
70 as *Shigella*). We compare the essentiality classifications of protein coding genes in *Shigella*
71 with a gold-standard assessment of essentiality in the closely related strain *Escherichia coli*
72 K12 BW25113 (hereafter referred to as *E. coli*) [1]. These two stains are 99.5% identical in
73 their 16S RNA genes and share approximately 70% of their genomic content.

74 This proximity in evolutionary distance, and the use of a gold-standard data set, brings two
75 unique advantages that have not been available in other studies that have used Tn-Seq or
76 similar methods to quantify gene essentiality [3, 7-19]. First, by relying on the null hypothesis
77 that protein coding genes do maintain their essentiality characteristics, we can objectively
78 assess which quantitative features in the *Shigella* Tn-seq data best predict essentiality or non-
79 essentiality of their orthologous counterparts in *E. coli*; such a comparison to a gold-standard
80 has not yet been used to assess the quality and sensitivity of Tn-Seq data [20], although
81 several studies have validated a small number of Tn-seq-inferred growth defects using clean

deletion methods (e.g. [10]). Second, the use of very closely related taxa allows us to quantify on a much shorter time scale the fraction of the essential gene complement that has changed, providing a fine scale window into the rate with which orthologues change in their essential functions.

The data presented here suggest that the essential gene complement of *Shigella* and *E. coli* overlap considerably. Indeed, we find no strong evidence that there are any protein-coding genes that are essential in *E. coli* but not *Shigella*. Conversely, we do find a small number of genes that play critical roles for *Shigella* growth, but which have dispensable roles in *E. coli*, or which are absent entirely from *E. coli.* This implies that the functional correspondence, in terms of essentiality, has changed for only a small number of protein-coding genes.

However, our analysis also suggests that some protein-coding genes that we observe as undisrupted by transposon insertions are in fact not essential for cell growth. Instead, they are either essential for transposon insertion to occur successfully, or their disruption (but not clean deletion) is detrimental to cell growth. This result emphasizes that in high throughput transposon mutagenesis studies, false positive inferences of essentiality may be common, and that simply increasing the resolution or precision of a dataset cannot necessarily solve this problem.

Taken together, our data suggests that the essential gene complement is relatively static over short time scales. However, when protein-coding genes do change from being non-essential to being essential, this appears more likely to occur in pathogenic organisms, perhaps because host environments absolve the organism from manufacturing its own nutrients, or because such organisms have smaller population sizes and are prone to the accumulation of deleterious mutations. It would be interesting to see if this pattern is observed when comparing other pathogens to their free-living sister taxa. If antibiotics can be directed against the function or expression of such differentially essential genes, this may allow targeting such treatments toward specific bacterial strains.

Results and Discussion

A Transposon Mutagenesis Library Provides Fine-scale Resolution of Gene Essentiality

We generated a transposon insertion library by transforming a *Shigella icsA* mutant [21] with a plasmid containing a mini-Tn10 transposase with decreased hotspot activity ([22]; **Fig. 1A**, inset) inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) [23, 24]. After overnight growth on Tryptic Soy Broth (TSB) agar plates containing IPTG, we harvested approximately10^6 colonies carrying transposon insertions. We pooled and then split this

116 library of clones into six replicates. Three replicates were subject to additional growth step
117 inside the cytoplasm of HeLa cells for four hours. The resulting cells were then harvested, the
118 replicates were bar coded and libraries were prepared. We sequenced all six of these pools on
119 a single Illumina HiSeq lane. For all the analyses presented in this study, we have pooled the
120 data from all replicates and from both of these treatments, as we are focusing on *Shigella*
121 genes that are essential across any permissive growth conditions.

122 From this pool, we mapped insertions at 131,670 unique positions on the *Shigella*
123 chromosome (with many insertions occurring on both the forward and reverse strands but at
124 the same position), and 12,552 unique positions on the large *Shigella* virulence plasmid (see
125 **Methods**). The median distance between inserts on the chromosome was 17 base pairs (bp);
126 on the plasmid this distance was 9 bp. 95% of all inter-insert distances on the chromosome
127 were less than 107 bp; the corresponding figure for the plasmid was 59 bp (**Figs. 1A and B**).

128 Although the distribution of transposon inserts was relatively even across both the
129 chromosome (**S1 Fig.**), at smaller scales we found many regions in which few or no insertions
130 occurred. Quantitative analyses showed that regions containing no transposon insertions for
131 100bp or more were considerably enriched (see **Methods**; **Figs. 1C and D**). It is likely that
132 many of these regions are critical for cellular growth in *Shigella*. Indeed, we found that for
133 many of the protein-coding genes in these regions, the orthologous *E. coli* genes are known to
134 be essential (**Figs. 2** and **3**).

135 In contrast to the *Shigella* chromosome, we found that few open reading frames on the
136 virulence plasmid were devoid of insertions. Only six out of 263 plasmid ORFs had no inserts.
137 Two of these were replication proteins (CP0258 and CP0259), and two (CP0217 and CP0218)
138 were located within the plasmid stabilisation region. The remaining two, *mxiH* and *acp*, are
139 both less than 250 bp in length (the cut-off used here to classify ORFs as essential; see below),
140 and thus have a lower likelihood of being hit due simply to their smaller target size. The third
141 replication protein of the plasmid, CP0260, contained a single insert in its 858 bp length. The
142 absence of inserts in the plasmid replication or stabilisation regions is explained by the fact
143 that if such insertions did occur, the plasmid would be lost; such insertions would thus never
144 be sequenced. Thus, this data is consistent with the fact that the *Shigella* plasmid contains no
145 essential genes [25], and suggested that our transposon library provided a fine-scale
146 assessment of which *Shigella* chromosomal ORFs provide critical cellular functions.

147 Average Distance Between Inserts Clearly Delineates Essential and Non-essential 148 ORFs

149 We next quantified which transposon insertion patterns in the chromosome were good
150 predictors of the essentiality of open reading frames. To do so, we first identified 3,027

151 orthologous open reading frames present in both *E. coli* and *Shigella* for which we also had
152 data on essentiality from both the Keio [1, 26] and the Profiling of the Escherichia coli
153 Chromosome (PEC) [27] studies (**S1 Table**). We considered this combined gene set as a gold-
154 standard of essentiality, for two reasons: it is not subject to artefacts that might exist in Tn-seq
155 dataset, such as insertion biases or biases arising during sequencing library preparation (e.g.
156 [28]); and combining both the Keio and PEC datasets should result in few false positive or
157 false negative essentiality characterizations. This set consisted of 277 orthologues considered
158 essential by both studies, 2,717 genes considered non-essential by both studies, and 33 genes
159 for which the two studies disagree.

160 We next quantified several characteristics for each protein-coding gene in our Tn-seq data set,
161 including the total number of inserts per ORF, the mean distance between inserts, the length
162 of the 5' fraction of the ORF upstream of the first insertion, the largest uninterrupted region in
163 the ORF, and others (**S2 Fig.**). We took as a null hypothesis that generally, genes have
164 maintained their essentiality characteristics since the divergence of *E. coli* and *Shigella*. We
165 then tested which of these characteristics best predicted the essentiality status of their
166 orthologous counterparts in the gold-standard dataset of open reading frame essentiality in *E.*
167 *coli* (the Keio and PEC datasets).

168 We found that the best predictor of essentiality status in *E. coli* was the mean distance
169 between transposon insertions in their *Shigella* orthologues (Materials and Methods; **S2 Fig.**).
170 For the *Shigella* orthologues of the 277 *E. coli* essential genes, only four had a mean distance
171 between inserts of less than 150bp. 17 (6%) had a mean inter-insert distance less than 250bp.
172 In contrast, only 6% of the orthologues of non-essential *E. coli* genes had a mean distance
173 between inserts of greater than 250bp (**Fig. 3A**). We selected this mean inter-insert distance
174 of 250bp as a cut-off for classifying *Shigella* ORFs as essential, as it provided a balance
175 between protein coding genes classified as essential in *E. coli* but non-essential in *Shigella* (a
176 6% false negative rate) versus non-essential in *E. coli* and essential in *Shigella* (a 6% false
177 positive rate). By extension, genes that are less than 250bp in length and in which we do not
178 observe insertions were inferred as essential (26 ORFs in total, of which 12 were ribosomal
179 proteins and five were leader peptides). We note, importantly, that almost all of the predictors
180 we tested performed extremely well (**S2 Fig.**).

181 We next investigated in greater detail the disagreements in essentiality classification between
182 *E. coli* and *Shigella* (**Fig. 3B**). Of the17 *E. coli*-essential genes that this metric identified as
183 non-essential in *Shigella*, all are likely to be false negatives (i.e. in fact essential in *Shigella,*
184 but not classified as such by our criterion). All 17 have a mean distance between inserts of
185 greater than 100 bp (**Fig. 3A**), and nine are uninterrupted for more than 90% of their reading
186 frame. This suggests, surprisingly, that there are no genes that are essential in *E. coli* but

whose *Shigella* orthologues are non-essential. This similarity in essentiality is not due to the fact that we use a characteristic that most closely predicts essentiality in the gold standard dataset – this result is robustly corroborated by any meaningful metric that we used (e.g. using other mean distances between inserts as cut-offs for essentiality, using the total number of inserts, the longest uninterrupted gene fraction, or others (**S2 Fig.**)). Overall, this data gives us a very strong prior that genes have maintained their essentiality status (or near-lethal effects on growth) since the divergence of *E. coli* and *Shigella*.

Many Non-essential *E. coli* orthologues of Essential *Shigella* Genes Exhibit Impaired Growth

As a result of this strong prior, we thus expect that many of the discrepancies in essentiality between *E. coli* and *Shigella* are false positives due simply to the *Shigella* mutants being non-essential, but having significantly impaired growth. Indeed, of the 160 discrepant genes classified as non-essential in *E. coli* but essential in *Shigella*, 34% of the orthologous *E. coli* deletion genotypes exhibit low growth yields (less than 0.5 OD600 after 22 hours of growth in LB [1]). This contrasts strongly with the 2557 ORFs we classified as non-essential in *Shigella*: only 3.7% of the orthologous *E. coli* deletion genotypes had low growth yields (**Fig. 4**). Similar but less striking patterns were observed for growth in glucose minimal MOPS media (**S3 Fig**.).

It is important to note that Tn-seq assays have only limited power to differentiate essential genes from those whose deletion results in severe growth deficiencies. During the course of preparing the library for sequencing, we estimate that at least 20 generations of growth occurred. If a mutant has a growth rate even 60% that of the wild type, we would expect it to undergo only 12 doublings in contrast to the 20 of the wild type. This would result in a greater than 200-fold underrepresentation of such a mutant ($2^{12}/2^{20}$). In addition, this calculation does not take into account any effects that the mutations have on the length of the lag time, which might also have significant effects on the relative frequency of some mutants.

In light of this limited resolution power; given that our prior expectation is that essentiality status changes only rarely; and because we are specifically interested in genes that may have changed in essentiality status, from this point on we focus our analysis on essential *Shigella* genes whose orthologous *E. coli* deletion genotype exhibits robust growth yields (OD600 greater than 0.75 after 22 hours growth in LB (**Fig. 4**)). For these genes, we have relatively high confidence that while their deletion in *E. coli* has few effects on growth, their disruption in *Shigella* is lethal or results in a severe growth deficiency.

220 **Artefacts of the Transposon Screen Explain Some False Positive Discrepancies**

221 35 essential *Shigella* genes have orthologous *E. coli* deletion genotypes with growth yields
222 higher than 0.75 OD600 after 22 hours growth in LB [1] (**Fig. 4**). Careful inspection
223 suggested that some genes were present in this set due to differences in the growth conditions
224 between *E. coli* Keio and PEC collections and our own. For example, *fhuACD*, and *tonB* all
225 appeared in this set of genes (**Table 1**). All four are involved in iron acquisition, and it is
226 likely that iron was limiting in the solid agar media [29] used during the preparation of the
227 Tn-seq library, as compared to the liquid LB used to measure growth in the Keio study.

228

229 **Table 1. Genes artefactually inferred as essential in *Shigella.***

Gene(s)	Evidence for Artefactual Inference of *Shigella* Essentiality
acrAB, tolC, ybaB, ksgA, yebC, smpB (0.73)[1]	Affect kanamycin resistance [30-32]
dnaQ, holD, recD, *xseA*, *ruvA* (0.58), *ruvB* (0.60), *ruvC* (0.61), *recB* (0.58), *recC* (0.65)	Likely to affect the transposition process; *dnaQ, holD, ruvA,* and *ruvB* inferred as essential using Tn-seq in *Salmonella* [13]
priB	Deficient in plasmid maintenance [33, 34]
fhuACD, tonB	Involved in iron acquisition which is critical for growth in the iron limited media used in this study [29]
rpsT	S20 ribosomal subunit; new data indicates mutants have poor growth [35] (in conflict with Keio data)
miaA	tRNA dimethyl transferase; previous data indicates *E. coli* mutants have poor growth [36] (in conflict with Keio data)
ompA	Outer membrane porin; clean knockouts appear viable [37]; mutant forms are frequently lethal [38]
pitA	Metal phosphate transporter with ten transmembrane segments; transposon disruption of substrate transporters is three-fold more likely to be inferred as essential compared to clean deletion (see main text; **Fig**)
potB	Type I ABC transporter (Putrescine / spermidine transporter)
cysU	Type I ABC transporter (Sulfate / thiosulfate transporter)
sapB	Type I ABC transporter (unknown substrate)
ptsH	Short 306 bp reading frame
ydhR	Short 258 bp reading frame

230 [1] Gene deletions of orthologous *E. coli* genes with growth levels less than OD600 0.75 have these
231 levels in parentheses

232

233 For a second set of genes, the discrepancies are likely due to the differences in methodology
234 between the *E. coli* (precise gene deletions) and *Shigella* (transposon inactivation) studies
235 (**Table 1**). We inferred *acrAB* and *tolC* as essential. These genes act together as an efflux
236 pump, and mutations in these genes result in hypersensitivity to antibiotics [39]. Thus, clones
237 with transposon insertions in these genes are unlikely to survive during library growth under
238 kanamycin selection. A similar explanation likely underlies the fact that we inferred *ybaB,*
239 *ksgA, yebC,* and *smpB* as essential: these four play role in aminoglycoside resistance [30-32].

240 We also inferred *priB*, *dnaQ*, *holD*, *xseA*, and *recD* as being essential in *Shigella*, although
241 the *E. coli* deletion genotypes exhibit robust growth. All of these are involved in DNA
242 replication, recombination, and double strand break repair, all of which are essential processes
243 in the completion of the transposition process [40]. The related genes *recBC* and *ruvABC*
244 contained a single insert between the five of them, while the *E. coli* deletion genotypes all
245 exhibit only slightly impaired growth of 0.6 OD600 or more (**Table 1**). Certain *recBC*
246 mutants can have considerable effects on the rate of Tn10 excision [41, 42] and we speculate
247 that this may be one reason why we rarely observed insertions in these loci. It has also been
248 speculated that *ruv* mutants inhibit transposition [43]. We propose that after transposition
249 occurs, in order for the event to be successfully resolved, transcription of these genes is often
250 required, and the transposition itself precludes the formation of a proper transcript.

251 Thus, the dispensability of these ten genes in *E. coli*, and the similarity in their function,
252 suggests that they all affect successful transposon insertion rather than having critical effects
253 on growth. Notably, *priB*, *dnaQ*, *holD, ruvA,* and *ruvB* were also inferred as essential in the
254 closely related bacterium *Salmonella typhimurium* via a high-throughput transposon assay. In
255 the same study *ruvC, recB, recC,* and *ybaB,* were inferred as extremely important for growth
256 while *ksgA* and *yebC* were inferred as significantly impairing growth [13]. Again, the
257 majority of these knockouts in *E. coli* exhibit very robust growth (greater than 0.75 OD600
258 after 22h growth in LB). Given the roles that these genes are known to play in transposition
259 and antibiotic resistance, this suggests that the inference of essentiality may be due to
260 artefacts of the transposon screen.

261 For a third set of genes, the literature presents conflicting information on the growth
262 phenotypes, with studies that have individually assessed growth rates suggesting poor growth.
263 These include *rpsT* [35], *miaA* [36], and *ompA* [38] (**Table 1**).

264 There were also two open reading frames that we inferred as differentially essential as they
265 were completely uninterrupted in our data. However, these two open reading frames, *ydhR*
266 and *ptsH*, are very small and less likely to be disrupted, being 306 bp and 258 bp long,

267 respectively. It is probable, then, that this discrepancy is not driven by different physiological
268 roles that they play in *E. coli* as compared to *Shigella*.

269 Finally, we tested for other possible artefactual patterns in the data based on gene function.
270 We asked whether there were specific functional categories in which genes were more likely
271 to be inferred as essential using the transposon mutagenesis screen in *Shigella* as opposed to
272 clean deletions in *E. coli*. We found two functional categories of genes that showed clear
273 enrichment: genes involved in substrate transport and / or active transport, which were 3- and
274 2.1-fold enriched, respectively (**Fig**. **5**). We hypothesize that one reason for this enrichment is
275 that truncated versions of these proteins disturb the operation of the *sec* machinery, thereby
276 decreasing or stopping growth. Thus, we propose that the four active transporters we infer as
277 essential in *Shigella* but not *E. coli* (**Table 1**) are artefacts due to the transposition process
278 resulting in truncated proteins.

279 Genes uniquely essential in *Shigella flexneri*

280 While many differences in essentiality classification between *Shigella* and *E. coli* are likely
281 due to (1) severe growth defects present in both *E. coli* and *Shigella* rather than strict
282 essentiality; and (2) differences in environmental conditions (e.g. iron) between the *E. coli*
283 and *Shigella* assays; and (3) artefacts of the *Shigella* transposon screen that do not occur in
284 the *E. coli* knockout screen, we do find a number of genes which we infer to be uniquely
285 essential to Shigella. We expect that the physiological differences between *E. coli* and
286 *Shigella* are driving these differences in gene essentiality (**Table 2**).

287 Among the set of genes essential in *Shigella* but dispensable in *E. coli* is *lysS*: this ORF has a
288 functional homologue in *E. coli* (*lysU* [45]), while in *Shigella flexneri* there is no homologue.
289 Also in this set of genes are *proA, proB, and proC*. These genes act in proline biosynthesis.
290 Given the rich media the cells were grown in, it is surprising that they would be essential. In
291 addition, as *proB* is involved in the first committed step of proline synthesis, its disruption
292 should not cause accumulation of toxic intermediates. However, the data provide strong
293 evidence that the disruption of any these three genes is either lethal or causes severe growth
294 defects (**Fig. 6**). Interestingly, the active proline transporter *putP* is absent from *Shigella* [46].
295 It is also known that in *Salmonella,* the cryptic proline transporter *proY* is silent [47], and we
296 hypothesize that this may also be true of this transporter in *Shigella.* Thus, inefficient proline
297 transport from the media might necessitate biosynthesis.

298 A suite of genes involved in acetate utilization (*aceE*, *aceF*, *ackA*, *pta*, and *pykF*) were all
299 inferred as essential in *Shigella* but dispensable in *E. coli.* The significantly detrimental effect
300 on growth that such mutants have has been noted previously using a completely different
301 approach [21]. The difference in essentiality between these two organisms is most likely due

302 to the absence of acetyl CoA synthetase from *Shigella,* and confirms the sensitivity and
303 relevance of our transposon mutagenesis assay for assaying differences between *E. coli* and
304 *Shigella* biology.

305 **Table 2. Genes inferred as uniquely essential in *Shigella*.** All gene deletions in
306 homologous *E. coli* genes show robust growth in rich media after 22 hours (greater than 0.75
307 OD600), suggesting that these genes are uniquely essential in *Shigella* as compared to *E. coli*.

Gene(s)	Function [44]	Evidence for Different Physiological Roles in E. coli and Shigella
lysS	Aminoacyl tRNA synthetase, tRNA modification	The *lysU* functional homologue is absent in Shigella [45]
proABC	Proline biosynthesis	The active proline transporter *putP* is absent from Shigella [46]. The cryptic transporter *proY* may be silent, as observed in Salmonella [47], possibly necessitating proline biosynthesis
ackA	acetate kinase	All affect acetate accumulation [48] and utilization [21], which is required for robust growth (Shigella lacks the acetyl CoA synthetase present in *E. coli* K12 [49])
pta	phosphotransacetylase	
aceEF	pyruvate dehydrogenase	
pykF	pyruvate kinase	
rfbF, rfbG, rfc, and *rfbI*	sugar nucleotide biosynthesis for LPS	No *E. coli* K12 orthologues, as this locus has been replaced by the laterally transferred *wbb* locus [50]
spr	Murein DD-endopeptidase	None known
tufB	Elongation factor EF-Tu	None known

308

309 For only two other orthologous gene pairs is there strong evidence of discrepant essentiality
310 status: *tufB* (two insert locations; **Fig. 7**) and *spr* (one insert at base pair 543 across 567 bp).
311 For neither of these genes do we have a hypothetical causal explanation. Interestingly, we
312 also found very few transposon insertions in the *tufB* paralogue *tufA* (three insert locations;
313 **Fig. 7**), suggesting that this gene, too, is important for *Shigella* growth despite its relative
314 dispensability in *E. coli* (0.72 OD600 after 22h in LB). We note that these two genes are
315 nearly identical in their sequence, which creates ambiguities in mapping some reads.
316 However, this does not explain the absence of reads mapping to either of them.
317 Understanding the molecular mechanisms driving these apparent disparities in growth
318 phenotypes between *Shigella* and *E. coli* is an important topic for future research.

319 Finally, the transposon insertion data indicated that a within single large operon, containing
320 the ORFs *rfbACEFGI / rfc,* four genes completely lacked insertions (*rfbF, rfbG, rfc,* and *rfbI*)
321 (**Fig. 8**). Only *rfbA* and *rfbC* in this operon have *E. coli* orthologues. The remaining genes lie
322 within a commonly laterally transferred region of the *E. coli* chromosome containing
323 *wbbHIJKL, wzxB* (*rfbX*), and *glf*. These were all laterally transferred into the K12 lineage [50],

replacing the *Shigella*-like *rfb* operon. The genes in this operon all play a role in sugar nucleotide biosynthesis necessary for O-antigen synthesis and production of the lipopolysaccharide component of the outer membrane [44]. This provides some evidence that specific aspects of this process have become essential in *Shigella*, despite these genes having been replaced by a laterally transferred set in *E. coli* K12.

Conclusions

By exploiting the extremely close evolutionary relationship of *Shigella flexneri* with *E. coli* K12, the bacterial strain that has been the most extensively and carefully characterized for its essential gene complement [1, 27], we were able to develop an objective metric to precisely quantify how the results of the Tn-seq data relate to essentiality.

A superficial analysis of our Tn-seq data suggested that a total of 451 ORFs in *Shigella* were essential for cellular growth in rich media. This is very much in line with what other Tn-Seq studies have found, with numbers ranging from 480 in *Caulobacter crescentus* [8] to 447 in *B. subvibrioides* to 372 in *Agrobacterium tumefaciens* [3]. However, it is considerably more than the number that had been found in *E. coli* using in-frame gene knockouts, which is on the order of 300 essential genes. In addition, we found that close to 100% of the reading frames that were classified as essential in *E. coli* K12 were also essential in *S. flexneri*, giving us a strong prior expectation that the essentiality classifications should match between these two taxa.

A more nuanced analysis suggested four explanations for artefactual discrepancies in essentiality between *E. coli* and *Shigella*: (1) many *Shigella* genes were not strictly essential but instead gene disruption caused severe growth impairment; (2) differences in experimental conditions (i.e. iron availability); (3) many of the genes we inferred as essential were important for antibiotic resistance or successful transposition, and are in fact dispensable for growth; and (4) transposon disruption of specific functional classes of genes may result in systematically different effects as compared to gene deletions, for example due to the production of truncated protein products. By carefully dissecting the functions of discrepant genes that do not appear to be artefactual, we were able to pinpoint several genes for which there is some evidence of differential physiological roles in *E. coli* and *Shigella*. Among others, these included *lysS,* three genes involved in proline biosynthesis, and a suite of genes involved in acetate utilization (**Table 2**). In addition to these, we found one large operon which appears to have an essential role in *Shigella* growth but which is missing completely in *E. coli.* Surprisingly, we found only two additional genes that are differentially essential (*tufB* and *spr*) (**Table 2**).

358 Even after attempting to decrease false positive inferences of gene essentiality in *Shigella*, it
359 appears to be considerably more common for genes to be dispensable for growth in *E. coli*,
360 but critical for growth in *Shigella*. We suggests that one reason *Shigella* more may have a
361 larger complement of essential genes than *E. coli* is that it frequently lives as an intracellular
362 pathogen, and may have lost some of the functional redundancy that is present in *E. coli*. This
363 may occur because host environments provide an abundance of nutrients, or because
364 pathogens requiring a small infectious dose, such as *Shigella* [51], have inherently smaller
365 population sizes and are more subject to genetic drift. A third possibility is that changes in
366 gene function or redundancy may have occurred through selection for increased virulence,
367 which has resulted in the inactivation of certain genes being selectively advantageous. Finally,
368 we note that the discrepancies in essentiality between these two bacteria may be exploited to
369 develop antibiotics that have strain-specific effects [21].

370 # Methods

371 ## Strains

372 For all experiments, *Shigella flexneri* 2457T Δ*icsA* was provided by M. B. Goldberg was used
373 as the parental strain. This strain is unable to exploit the host actin cytoskeleton for motility
374 and spreading [52]. Bacterial cells were grown in Tryptic Soy Broth (TSB) media. For
375 experiments using eukaryotic cells, HeLa cells were cultured in DMEM supplemented with
376 10 mM Hepes, 25 mM glucose, and 4 mM glutamine. *Shigella* were grown to exponential
377 phase in tryptic soy broth, coated with poly-L-lysine, and added at a multiplicity of infection
378 of 25, resulting in an infection rate of around 60%. *Shigella* was centrifuged onto HeLa cells
379 (600 × g for 5 min). At 30 min postinfection, we added gentamicin (100 µg/mL) to kill
380 extracellular bacteria. Bacterial cells were allowed to grow within HeLa cells for a total of 4
381 hours.

382 ## Transposon library

383 Using a Tn10 transposon with a T7 promoter [23, 24] we created a library consisting of
384 approximately 10^6 clones. This library was created by mating *E. coli* strain BW20767
385 containing the pJA1 transposon plasmid with a spontaneous nalidixic acid resistant clone of
386 *Shigella flexneri* 2457T Δ icsA for 5 hours. Transposase expression was induced by plating
387 onto TSB plates containing 0.2 mM isopropyl-β-D-thiogalactoside (IPTG). Colonies were
388 allowed to grow at 37°C for 18 hours on TSB agar plates. All colonies from these plates were
389 then pooled and 100µl aliquots of the transposon library were stored at -80°C.

390 Three replicate experiments were carried out on different days in which an aliquot of the
391 transposon library was grown for 18 hours in TSB to stationary phase, diluted 1:100 and

392 grown to exponential phase (0.7 OD600). This exponential phase culture was split into two:
393 part of the bacterial culture was pelleted and saved and other was used for infecting HeLa
394 cells (as described in [21]). After 3.5 hours, HeLa cells infected with the *Shigella* transposon
395 library were trypsinized and pelleted. Uninfected HeLa cells were also collected and used to
396 spike the original bacterial culture not used for HeLa infection in order to account for HeLa
397 DNA. All resulting DNA was extracted using the Bacterial Genomic Miniprep Kit (Sigma).

398 **Sequence library construction and sequencing**

399 To amplify the transposon region from these pools, we used one top strand primer annealing
400 to the transposon and a pool of three bottom strand primers each of which consisted of 10
401 random nucleotides followed by a pentamer of common nucleotides in *E. coli* [53]:
402 N_{10}GGTGC, N_{10}GATAT, and N_{10}AGTAC, using Phusion pfu (**S4 Fig**). A nested PCR was
403 then performed to add the P7 and P5 Illumina adapters, as well as a barcode. The products
404 from this second PCR were then size selected for inserts between 200bp and 300bp,
405 quantified using a Qubit, and sequenced on an Illumina HiSeq2000 at the D-BSSE
406 Quantitative Genomics Facility resulting in 49bp single end reads. We used a custom
407 sequencing primer on the P5 end of the molecule such that on both ends of the molecule,
408 reads started directly on the chromosome.

409 **Read mapping**

410 In total, we obtained 198,682,954 reads. We found that the number of reads at each location
411 in the genome varied by up to four orders of magnitude. For this reason, we considered only
412 whether an insertion had occurred at a specific location, and not on the number of reads we
413 obtained at a specific location, which is likely to be highly biased due to PCR artefacts. We
414 thus first deduplicated the reads using *tally* [54], and then used bowtie2 [55] to align the reads
415 to the *Shigella flexneri* 2a 2457T genome and the *Shigella flexneri* 2a str. 301 plasmid
416 pCP301. The sequence of the *S. flexneri* 2457T plasmid is not available. However, the *S.*
417 *flexneri* 2457T and 2a str. 301 plasmids are nearly identical in sequence (differing by 30
418 SNPs; see below). Sequence reads were not trimmed for quality as read quality is taken into
419 account in bowtie2. We used the --sensitive-local option to allow soft clipping on the 3' end
420 of the reads (so that reads that contained adapter sequences at the 3' end could map
421 successfully), and required at least 22bp of matching sequence at the 5' end of the read.

422 We checked for single nucleotide polymorphisms (SNPs) on both the chromosome and the
423 plasmid using the samtools mpileup and bcftools utilities [56, 57]. We retained as possible
424 SNPs only those sites that fulfilled the following three criteria: (1) the SNP was inferred as
425 homozygous (necessarily true, as *Shigella* is haploid); (2) the quality score was above 20; and
426 (3) at least three reads on both the reverse and forward strands confirmed the SNP. We found

427 99 SNPs on the chromosome (as compared to the reference *Shigella flexneri* 2457T in NCBI)
428 and 30 SNPs on the plasmid (as compared to the *Shigella flexneri* 2a str. 301 plasmid in
429 NCBI (in addition to 12 and 2 small indels, respectively). These are detailed in **S2 Table** and
430 **S3 Table,** respectively.

431 Within chromosomal protein coding regions, 44% of all SNPs were synonymous, while 32%
432 fell outside of genic regions (i.e. protein coding or RNA genes). These fractions are greater
433 than one would expect if such SNPs were randomly located on the genome. Only 24% of all
434 mutations in chromosomal coding regions are expected to be synonymous (not accounting for
435 mutational biases), and only 28% of the chromosome is annotated as nongenic (including
436 repeat regions, although for many of these regions, the absence of an annotation may be
437 erroneous). Additionally, only 2 of the 12 (17%) small chromosomal indels fell in coding
438 regions. This suggests that there was some selection against nonsynonymous substitutions
439 that occurred during the culturing and derivation of the *Shigella flexneri* 2a 2457T *virG*
440 mutant. More importantly, the small number of SNPs that we found suggests that few, if any,
441 reads remained unmapped due to sequence differences between the strain used in our
442 experiments and the sequenced GenBank strain.

443 In total, the reads mapped to 89,028 unique locations on the forward strand and 83,074 on the
444 reverse strand of the chromosome, for a total of 172,102 insertions. Some of these insertions
445 occurred at identical positions but on opposite strands, so in total, insertions occurred at
446 131,670 unique sites in the chromosome. Correspondingly, the reads mapped to 8,208 unique
447 locations on the forward strand and 8,585 unique locations on the reverse strand of the
448 plasmid, for a total of 12,552 unique sites. During the insertion of the Tn10 transposon, a 9 bp
449 target DNA sequence is duplicated [58]. We accounted for this duplication in calculating the
450 distances between insertions (by moving the inferred site of insertion for one direction (we
451 arbitrarily selected the antisense direction) backward by 9 bp). Similarly, this duplication was
452 accounted for in calculating various statistics of insertions within genes: sense insertions that
453 were inferred as occurring in the last 9 bp of a gene were ignored in calculating the mean
454 number of insertions per gene (as these bp are duplicated upstream of the insertion).
455 Antisense insertions occurring in the first 9 bp of a gene were ignored, as these bp are
456 duplicated downstream of the insertion.

457 Using the read frequencies at all unique insert locations, we found that the transposon
458 insertions occurred in a biased manner, integrating more often at sites similar to the known
459 9bp consensus NGCTNAGC [58], although this bias was relatively weak (**Figs. 1A** and **B**,
460 insets). This low level of bias is likely due to our using a transposon with reduced hotspot
461 activity [22]. In addition, we found that insertion frequency was slightly influenced by

462 nucleotides further downstream of this 9bp consensus (**Figs. 1A** and **B**, insets). Sequence
463 logos for this analysis were visualized using the R package seqLogo [59].

464 The median distance between inserts was 17 bp in the chromosome and 9 bp in the plasmid
465 (**Fig. 1B**), suggesting that the transposon libraries yielded a relatively fine-grained map of the
466 essential genomic complement for both the chromosome and the plasmid.

467 As expected given the variation in insertion densities across the chromosome, we found high
468 variance in the distribution of inter-insert distances. The total length of the *S. flexneri* genome
469 is 4,599,354 bp in total. Given that we observed 131,670 inserts, under a model of random
470 insertion, we would expect a median distance between inserts of 35bp, with 95% of all inter-
471 insert distances being less than 107 bp (under the assumption that these distances are
472 distributed in a geometric manner (i.e. a negative binomial with the number of successes set
473 to one). For the plasmid, we observed 12,552 inserts over 221,618 bp, such that we expect a
474 median distance of 18bp between inserts, and that 95% of all inter-insert distances are less
475 than 59 bp. However, as noted above, we found that on average transposons insertions were
476 separated by a median of 17bp on the chromosome and 9bp on the plasmid. Fitting a
477 geometric distribution to the observed data over 99% of the range of the inter-insert distances
478 (i.e. from 1 to 237 bp for the chromosome and from 1 to 78 bp for the plasmid) more exactly
479 quantified this over-dispersion, and showed that uninterrupted regions in the chromosome
480 greater than 100 bp were considerably enriched (**Fig. 1C**).

481 **Paired end read mapping and inference of IS element dynamics**

482 We used 100 bp paired end Illumina sequencing data from this same library to look for
483 structural rearrangements due to IS elements in the genome. However, this analysis was
484 complicated by the fact that many IS elements share close to 100% identity with others
485 around the genome. During these analyses we thus restricted our searches to regions of the
486 genome for which we had *a priori* expectations that they harboured a rearrangement (i.e. if
487 there were no inserts and the orthologous *E. coli* locus was non-essential or absent).
488 Specifically, we followed the following procedure: we extracted a 50 kilobase pair (Kbp)
489 region from the genome surrounding each hypothesized rearrangement (in all cases, this was
490 a deletion). We then used bowtie2 with the paired end option, allowing up to 10 Kbp inserts
491 to map all reads from our 100 bp PE dataset. From these mapped reads, we retained only read
492 pairs that had (1) mapping quality scores greater than 20; (2) at least one read that matched
493 perfectly (i.e. at all 101 bases of the read) to the genome; and (3) were unique in their length
494 at any specific location (thereby excluding artefacts such as PCR doublets). From these paired
495 reads we then inferred the insert size, which is plotted in **S5 Fig**. The vast majority of insert
496 sizes ranged between 100 and 400bp. However, some were much larger (e.g. up to 9,000 bp

497 in **S5B Fig.**). We inferred that these surrounded regions of the genome that must have been
498 deleted.

499 Such deletions would result in the set of genes contained within as being inferred as essential
500 because of their lack of transposon insertions. However, in the vast majority of cases, we
501 found that when large operons lacked insertions but had non-essential orthologous operons in
502 *E. coli*, or were missing entirely from *E. coli*, these operons were in fact missing from the
503 *Shigella* clone that we used, most likely due to the rapid dynamics of IS elements in this
504 bacterium [60]. For example, no sequence reads we obtained mapped to the *yeaKLMNOP*
505 operon, which spans a total of 9,240 bp. Upon further analysis using a paired end genomic
506 data set, we found that this region was clearly missing from our *Shigella* clone (**S6B Fig**.).
507 This was similarly true for several other operons, as well as for single genes. We did not
508 consider any region in which we identified a deletion in our downstream analyses.

509 Essential open reading frames

510 We identified 3,027 unambiguously ORFs that were present in both *E. coli* and *Shigella*
511 *flexneri* 2457T [61], and for which we had essentiality data. We used reciprocal shortest
512 distance [62] to find orthologues, with the requirement that the alignment of the two
513 hypothetical orthologues extend over at least 60% of the longer ORF. To establish a gold-
514 standard set of essential genes we combined the data from two studies of the effects of gene
515 deletion on growth in *E. coli* K12: the Keio collection [1] and the PEC study [27]. We
516 retained only those ORFs which we had data on essentiality from both studies. We then
517 quantified which transposon insertion patterns that most closely corresponded with the
518 essentiality delineations in theses studies. Specifically, we selected the feature that maximized
519 the number of true positive essential genes (maximizing the sensitivity) while minimizing the
520 number of FP (maximizing specificity) (this metric is a receiver operator characteristic for
521 which we quantified the area under the curve (AUC; **S2 Fig.**)). We selected from eleven non-
522 independent features: (1) the total number of insertions; (2) the mean number of bp between
523 insertions; (3) the median number of bp between insertions; (4) the number of bp in the 5' end
524 preceding the first insertion; (5) the number of bp in the 5' end preceding the first insertion
525 relative to the total bp in the gene; (6) the number of bp in the 5' end preceding the second
526 insertion; (7) the number of bp in the 5' end preceding the second insertion relative to the
527 total bp in the gene; (8) the number of bp in the longest uninterrupted stretch of the gene; (9)
528 the number of bp in the longest uninterrupted stretch of the gene relative to the total length of
529 the gene, and (10) the number of bp in the longest stretch of the gene interrupted by at most
530 one insertion; (11) the number of bp in the longest stretch of the gene interrupted by at most
531 one insertion, relative to the total length of the gene.

532 We found that for both the PEC dataset and the Keio dataset, the two best predictors of
533 essentiality were the mean distance between inserts (AUC = 0.972 for the PEC dataset, 0.952
534 for the Keio dataset, and 0.973 for the genes on which both datasets agreed on the essentiality
535 classifications); and the fraction of the gene that lay in the longest uninterrupted region (AUC
536 = 0.969 for the PEC dataset, 0.955 for the Keio dataset, and 0.971 for the genes on which both
537 datasets agreed on the essentiality classifications) (**S2 Fig.**). We selected mean distance as on
538 average, it marginally outperformed the other statistic on the gold standard data set.

539 We note that for eight of the 14 genes classified as essential solely in the Keio dataset, the
540 orthologous *Shigella* ORFs have mean distances less than 30 bp, suggesting that these genes
541 may be falsely annotated as essential in the Keio study. In contrast, nine of the ten genes
542 inferred as essential solely in the PEC dataset have mean distances greater than 200 bp; the
543 tenth has a mean distance of 189 bp.

544 tRNA disruptions

545 We found insertions in 27 out of 99 tRNAs, with tRNAs for certain amino acids being
546 considerably overrepresented (**S2 Table**).

547 Additional analyses of differentially classified essential genes

548 We also tested for the enrichment of certain functional categories in the set of genes that were
549 classified as being essential in *Shigella* but not *E. coli*. This differs from the analysis present
550 in **Fig. 5** in that we are asking whether across a broad set of functions, are specific categories
551 enriched for *Shigella*-essential genes. In **Fig. 5** we ask whether *within* a single functional
552 category, is there a much higher fraction of *Shigella* essential genes than we would expect,
553 given the fraction of genes in that functional category that are essential in *E. coli.*

554 Thus, for this analysis, we separated genes by primary functional category and secondary
555 subcategory using the MultiFun designations (e.g. the primary functional category cell
556 processes divided into the secondary subcategories of cell division, SOS, stress, protection,
557 and motility). We then calculated the fraction of *Shigella*-essential genes within each
558 secondary subcategory and compared this to the total fraction of *Shigella*-essential genes
559 within the primary category (e.g. we calculated the fraction of essential genes in Ribosomal
560 Function (the secondary category) and the fraction of *Shigella*-essential genes in all other
561 categories in Cell Structure (the primary category) (**S6 Fig**.). We tested for enrichment
562 (depletion) using a Fisher exact test.

563 We also examined gene conservation. Highly conserved genes were considered to be those
564 present in more than 50% of all gamma-proteobacteria [61]. We found that genes classified as
565 uniquely essential in *Shigella* were much more conserved across gamma-proteobacteria (79%

566 highly conserved) compared to genes that were found non-essential in both *E. coli* and
567 *Shigella* (36% highly conserved; p=1.0e-33, Wilcox rank sum test).

Availability of supporting data

All read data are in the SRA with accession numbers XXX.

List of abbreviations used

bp - base pairs; *Shigella – Shigella flexneri* 2a 2457T; *E. coli – Escherichia coli* BW25113; ORF – open reading frame; PEC - Profiling the E coli Chromosome database

Competing interests

The authors declare no competing interests.

Authors' contributions

NEF, DB, and OKS conceived and designed the transposon mutagenesis. NEF performed the mutagenesis and sequencing. OKS analysed the data with input from DB and NEF. NEF and OKS wrote the paper.

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Endnotes

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765

766 # Table and Figure legends

767 **Table 1. Genes artefactually inferred as essential in *Shigella***. Listed here are those genes
768 that were likely inferred as essential largely due to the gene disruptions having direct effects
769 on (1) antibiotic resistance, (2) successful transposition events, (3) differences between the
770 growth conditions of the *E. coli* and *Shigella* essentiality studies, or (4) systematically
771 different effects of gene disruption versus gene deletion.

772 **Table 2. Genes inferred as uniquely essential in *Shigella*.** Listed here are genes inferred as
773 essential in *Shigella*, but which have orthologous *E. coli* deletion genotypes that exhibit
774 robust growth (greater than 0.75 OD600 after 22h growth in LB). The genes in the *rfb* operon
775 have no orthologues in *E. coli* K12 (see main text).

776 **Fig 1**. **Histograms of distances between inserts on the chromosome for (A) the *Shigella***
777 **chromosome and (B) the *Shigella* virulence plasmid**. The median distance between inserts
778 is indicated by the dotted line. The insets in (A) and (B) indicate the slight but detectable
779 biases in transposon insert location using a weight matrix motif. The reverse cumulative plots
780 show the observed fractions of distances between inserts for the chromosome (C) and the
781 plasmid (D). In blue, the observed frequencies are plotted. In black, the expected frequencies
782 are plotted, given a geometric distribution (negative binomial with the number of successes
783 set to one) of inter-insert distances (see main text). For both the chromosome and the plasmid,
784 there are considerably more large regions uninterrupted by transposons than one would expect
785 given the geometric null model, observed as a shift of the curve to the right.

786 **Fig 2**. **Orthologous genes known to be essential in *E. coli* are also essential in *Shigella.*** A
787 region of the *Shigella* chromosome is shown, with genes whose orthologues are known to be
788 essential for growth in *E. coli* (coloured in white) [1, 27], or non-essential (coloured in grey).
789 The unique locations of transposon insertions are plotted as vertical black segments. In the
790 genome region shown here, none of the genes essential in *E. coli* have orthologues that are
791 interrupted in *Shigella*.

792 **Fig 3. Differences in essentiality classification between *E. coli* K12 and *Shigella.*** (A)
793 Cumulative distributions showing the mean distances between inserts for ORFs depending on
794 whether their orthologues are known to be non-essential (black curve) or essential (blue
795 curve) in *E. coli*. All ORFs that are completely uninterrupted by transposons have been
796 plotted at the very right end of the x-axis. The dotted vertical line indicates the cut-off that we
797 used to delineate essentiality in *Shigella* (a mean distance between transposons of 250 bp or
798 more). The 17 blue points to the left of the dotted vertical line indicate ORFs that are essential
799 in *E. coli* but not *Shigella* by our metric. These are likely to be false positives (i.e. non-
800 essential in both *Shigella* and *E. coli*), as all have inter-insert distances greater than 100 bp

801 (see main text). Black points to the right of the dotted vertical line indicate ORFS that we
802 classify as essential in *Shigella* but not in *E. coli*. Many of these ORFs have *E. coli*
803 orthologues whose deletion genotypes exhibit robust growth, suggesting that their essentiality
804 status has changed. (B) A Venn diagram showing the overlap between essential orthologous
805 ORFs in *E. coli* and *Shigella*.

806 **Fig 4**. **Orthologous gene pairs that are non-essential in *E. coli* but inferred as essential in**
807 ***Shigella* (blue) tend to exhibit low growth yields in *E. coli*.** ORFs that we infer to be
808 uniquely essential in *Shigella* consistently have *E. coli* orthologues with low growth
809 phenotypes in LB media after 22 hours (apparent as a strong leftward shift in the cumulative
810 curve). For genes inferred as uniquely essential in *Shigella,* 34% of the orthologous *E. coli*
811 deletion genotypes exhibit low growth yields (less than 0.5 OD600 after 22 hours of growth
812 in LB). For genes we classified as non-essential in *Shigella* and *E. coli* only 3.7% exhibit low
813 growth yields. Thus, some genes we infer as essential in *Shigella* may not be strictly essential,
814 but instead be required for robust growth. Despite this enrichment for low-growth phenotypes,
815 there are many genes which we infer as essential in *Shigella*, but which have *E. coli*
816 orthologues whose deletion genotypes exhibit robust growth (OD600 greater than 0.75 after
817 22 hours growth in LB).

818 **Fig 5**. **Transposon disruption of *Shigella* genes with transport-related functions are**
819 **more likely to be inferred as essential compared to clean deletions of similarly**
820 **functioning genes in *E. coli*.** We classified genes according to function using the MultiFun
821 functional classification system [63]. For any category containing more than ten essential *E.*
822 *coli* genes, we also calculated the number of *Shigella*-essential genes. As expected, most
823 categories show a relative excess of *Shigella*-essential genes, as we inferred approximately
824 50% more genes as being essential in *Shigella* versus *E. coli* (**Fig. 3B**). However, two
825 functional categories show a clear excess above this level: substrate transport and active
826 transport, showing a 3- and 2.1-fold increased probability of inferring a gene as being
827 essential in *Shigella* as opposed to *E. coli*. This provides evidence that genes in these
828 functional categories may be more likely to be inferred as artefactually essential. For each
829 functional category (y-axis), we show the number of genes in that category (to the right of
830 each bar); the number of genes found to be essential in *E. coli* (within each bar); and the level
831 of enrichment of essential genes in *Shigella* (x-axis).

832 **Fig 6. Three genes involved in proline biosynthesis (*proABC*) appear uniquely essential**
833 **in *Shigella***. The orthologous *E. coli* deletion strains exhibit robust growth (OD600 greater
834 than 0.75 after 22 hours growth in LB), but are essential by our criteria**.** *proA* and *proC*
835 completely lack transposon insertions*,* while *proB* contains only two insertions near the 3'end,

836 which leaves approximately 70% of the gene intact, including the entire kinase and substrate-
837 binding domain.

838 **Fig 7. Both elongation factor paralogues *tufA* and *tufB* appear differentially essential in**
839 ***Shigella* as compared to *E. coli***. The orthologous *E. coli* deletion strains of *tufA* and *tufB*
840 exhibit robust growth (OD600 of 0.72 and 0.78 after 22 hours in LB), but are essential by our
841 criteria**.** Both genes contain insertions only at the 5' or 3' ends of the genes. Genes that are
842 essential in both *E. coli* and *Shigella* are coloured in white. Those inferred as being essential
843 in *Shigella* but for which the orthologous deletion genotypes exhibit robust growth in *E. coli*
844 are indicated in blue. Genes inferred as essential in *Shigella* and which do not exhibit robust
845 growth in *E. coli* are coloured in light blue. tRNA genes are indicated in dark grey.

846 **Fig 8. The region of the genome containing the *rfb* operon is largely uninterrupted by**
847 **transposon insertions.** *rfbI*, *rfc*, *rfbG*, and *rfbF* are completely uninterrupted by transposon
848 insertions; *rfbE* is uninterrupted over 90% of its length. None of these genes have orthologous
849 counterparts in *E. coli* K12 due to a lateral transfer event that occurred at this locus (see main
850 text). This operon encodes genes active in O-antigen biosynthesis.

851 **S1 Table**. Full table of gene characteristics and orthologue relationships used in the analyses.

852 **S2 Table.** List of chromosomal SNPs and indels observed in the *Shigella* strain used here that
853 differ from the GenBank sequence NC004741.

854 **S3 Table.** List of plasmid SNPs and indels observed in the *Shigella* strain used here that are
855 different from the GenBank sequence of the *Shigella flexneri* 2a strain 301 virulence plasmid
856 pCP301 (NC_004851).

857 **S4 Table**. Table listing tRNA genes and the number of insertions in each.

858 **S1 Fig. Distribution of transposon insertions across the genome.** We observed little bias
859 on the chromosomal level of insert locations.

860 **S2 Fig. ROC curves showing the predictive power of various features.** To select a feature
861 that was the best predictor of essentiality in *E. coli* orthologues*,* used only ORFs that we had
862 data on essentiality from both the Keio and PEC studies. We then selected transposon
863 insertion patterns that most closely match the essentiality delineations in theses studies.
864 Specifically, we selected the feature that maximized the number of true positive "essential"
865 genes (maximizing the sensitivity) while minimizing the number of FP (maximizing
866 specificity). We selected from eleven (non-independent) features shown here: (1) the total
867 number of insertions; (2) the mean number of bp between insertions; (3) the median number
868 of bp between insertions; (4) the number of bp in the 5' end preceding the first insertion; (5)
869 the number of bp in the 5' end preceding the first insertion relative to the total bp in the gene;

870 (6) the number of bp in the 5' end preceding the second insertion; (7) the number of bp in the
871 5' end preceding the second insertion relative to the total bp in the gene; (8) the number of bp
872 in the longest uninterrupted stretch of the gene; (9) the number of bp in the longest
873 uninterrupted stretch of the gene relative to the total length of the gene, and (10) the number
874 of bp in the longest stretch of the gene interrupted by at most one insertion; (11) the number
875 of bp in the longest stretch of the gene interrupted by at most one insertion, relative to the
876 total length of the gene. See the **Methods** section for more details of this analysis.

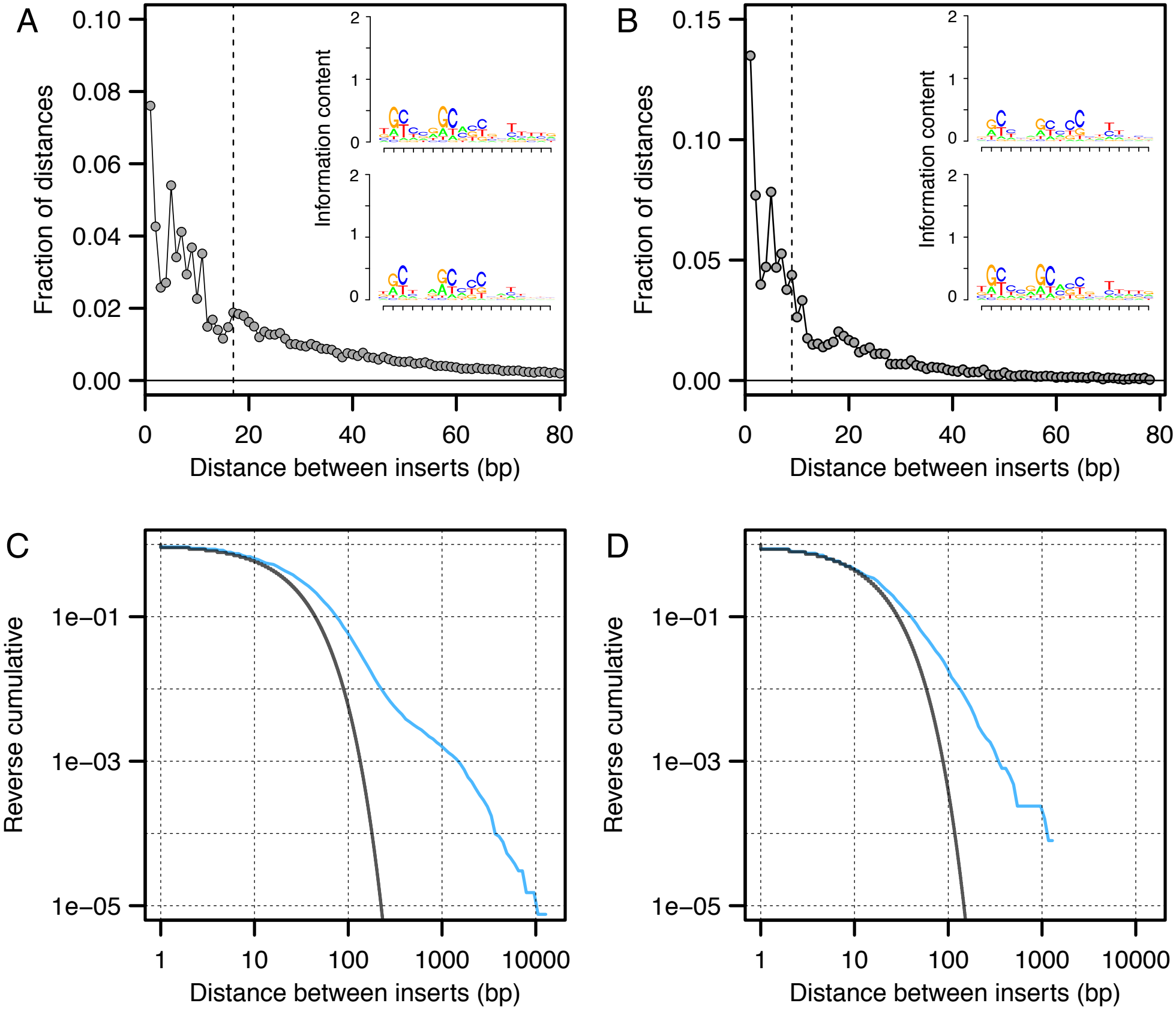
877 **S3 Fig**. **Analogous plots to that shown in Fig. 3, for growth in minimal glucose MOPS**
878 **media after (A) 24 and (B) 48 hours.** In both cases, we find that the shift is less pronounced
879 than that observed for LB.

880 **S4 Fig**. **Schematic of the primer positions used for Illumina sequencing of transposon**
881 **insertions**.

882 **S5 Fig. Inferred fragment lengths of perfectly mapped reads across several genomic**
883 **regions.** For each plot, the inferred fragment lengths are arranged by increasing length
884 (ranked on the y-axis). Thus, very long fragments are present at the top of the y-axis. Most
885 fragments have lengths between 100bp and 400bp; a small number have lengths over 1000bp
886 or more. It is very likely that these are not the true insert sizes, but appear that way because of
887 large scale deletions in our *Shigella* clone compared to the clone present in the NCBI genome
888 database; see **Methods** for more details. (A) A region of the chromosome in which a
889 complicated series of rearrangements has occurred, leading to paired end reads perfectly
890 matching to different locations in this region. 45 mapped read pairs span more than 1.5 Kbp, a
891 size that is not concordant with the majority of insert sizes. (B) A genomic region where an
892 approximately 10Kbp deletion occurred, removing a region containing the *yeaKLMNOP*
893 operon. 92 mapped read pairs span more than 8.5Kbp. This region is flanked by two IS
894 elements. (C) A region where an approximately 4Kbp deletion occurred, removing two genes
895 with no *E. coli* K12 orthologues. 68 mapped read pairs span more than 4 Kbp, and again this
896 region is flanked by two IS elements. (D) A genomic region where an approximately 2Kbp
897 deletion occurred, removing *yhdW*. 244 mapped read pairs span more than 2Kbp, and the
898 region is flanked by two IS elements. (E) A deletion in the region of the chromosome
899 containing *S4145 (yiaN)*. 232 mapped read pairs spanned more than 1.8 Kbp, and this region
900 is also flanked by two IS elements. (F) A region of the chromosome containing the *rfb* operon.
901 Most of the genes within this operon are uninterrupted by transposons. However, we find no
902 evidence that this is due to a deletion of this region in our *Shigella* clone, as we find no reads
903 mapping across the region; a small number of reads map within the region; and the closest IS
904 elements are 15 Kb upstream of *rfbJ* and 20 Kb downstream of *rfbA*. The genes in this operon
905 have no orthologues in *E. coli* K12.

906 **S6 Fig**. **Functional categories of non-essential *E. coli* genes that are enriched (or**
907 **depleted) for essential *Shigella* genes.** We classified genes according to function using the
908 MultiFun classification system [63]. For this analysis we considered only genes that are non-
909 essential in *E. coli*. We find that genes uniquely essential in *Shigella* are enriched in some
910 functional categories. For example, of the 27 ribosomal proteins identified as non-essential in
911 *E. coli*, we identify approximately 45% as being essential in *Shigella.* In contrast, of the 565
912 membrane proteins identified as non-essential in *E. coli*, we find that less than 10% are
913 essential in *Shigella.* Thus, ORFs uniquely essential in *Shigella* are far more likely to function
914 in the ribosome than one would expect. The number of non-essential *E. coli* genes is indicated
915 above each bar; the probability of finding the level of enrichment (or depletion) that we
916 observe in each secondary category is indicated for cases in which this probability is less than
917 0.025, using a Fisher exact test. (A) All non-essential genes in *E. coli*. (B) An identical
918 analysis excluding all non-essential genes in *E. coli* that exhibit very low growth yields
919 (OD600 less than 0.5 after 22 hours of growth LB). In both cases, the only subcategory
920 notably enriched for essential genes is that containing ribosomal proteins. The only categories
921 appreciably depleted for genes with essential function are genes with function in RNA
922 processing and to some extent, energy production.

923 **S7 Fig. Transposon insertion locations across the entire *Shigella* chromosome. Each**
924 **insertion site is indicated by a vertical red line.** ORFs are indicated in light green; rRNAs
925 in dark green; and tRNAs in dark blue. The annotation is taken from the GenBank sequence
926 of *Shigella flexneri* 2a 2457T.


A
B
C
D
Fraction of distances
Information content
Distance between inserts (bp)
Reverse cumulative
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0.08
0.06
0.04
0.02
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0
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40
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80
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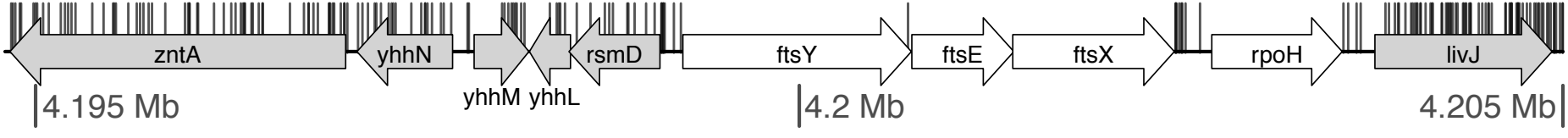
1e−05
1
10
100
1000
10000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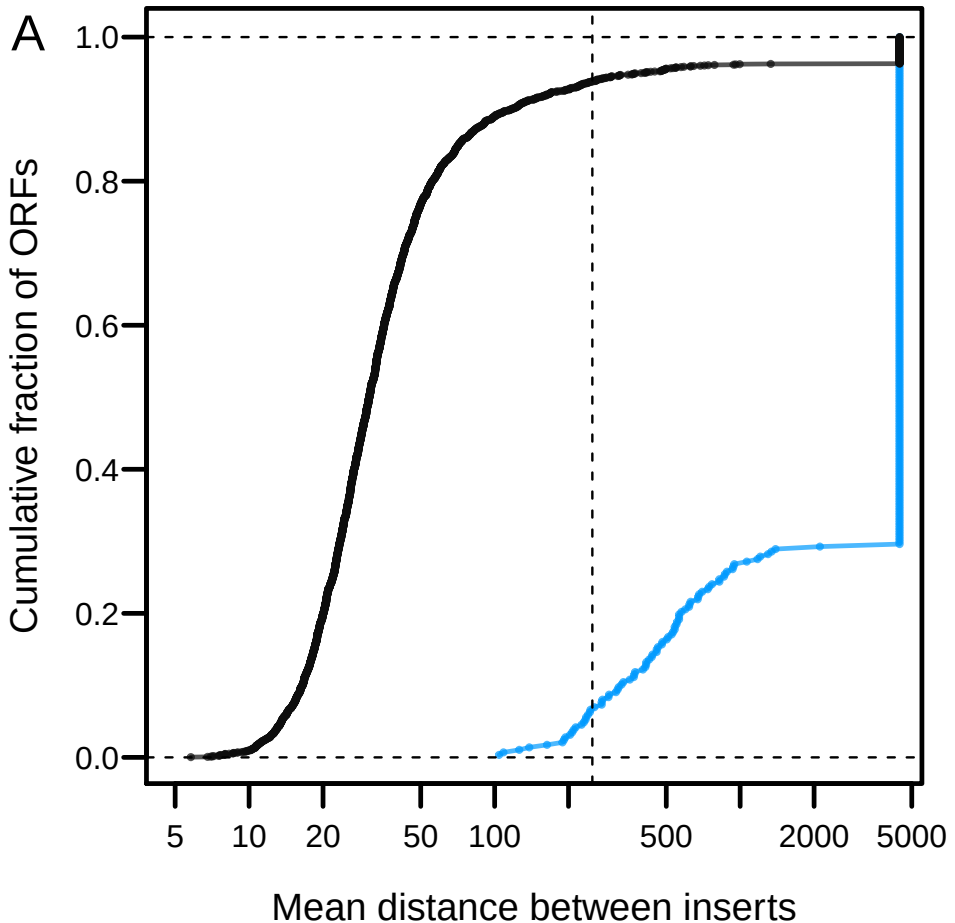
A
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0.8
0.6
0.4
0.2
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Cumulative fraction of ORFs
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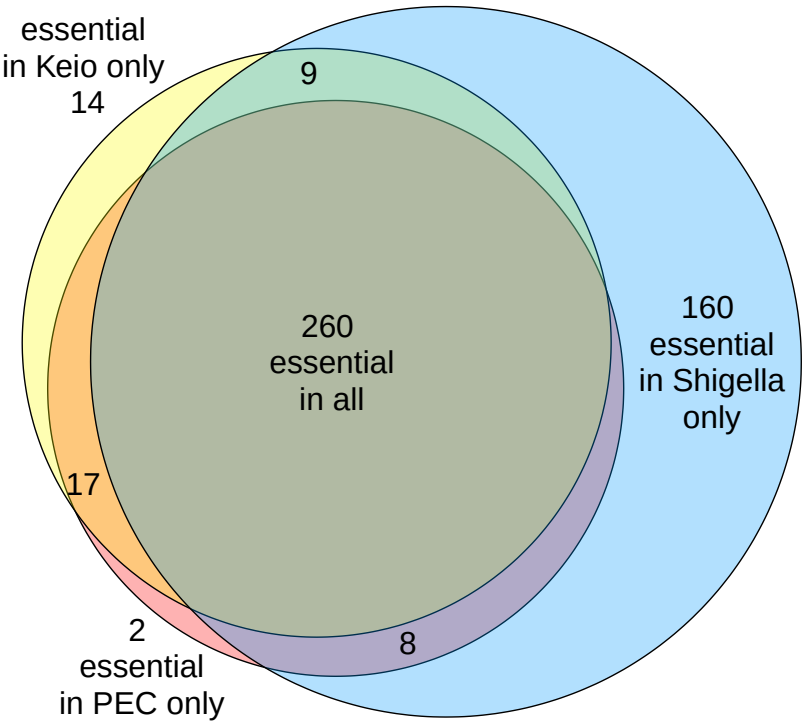
10
20
50
100
500
2000
5000
Mean distance between inserts


B
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in Keio only
14
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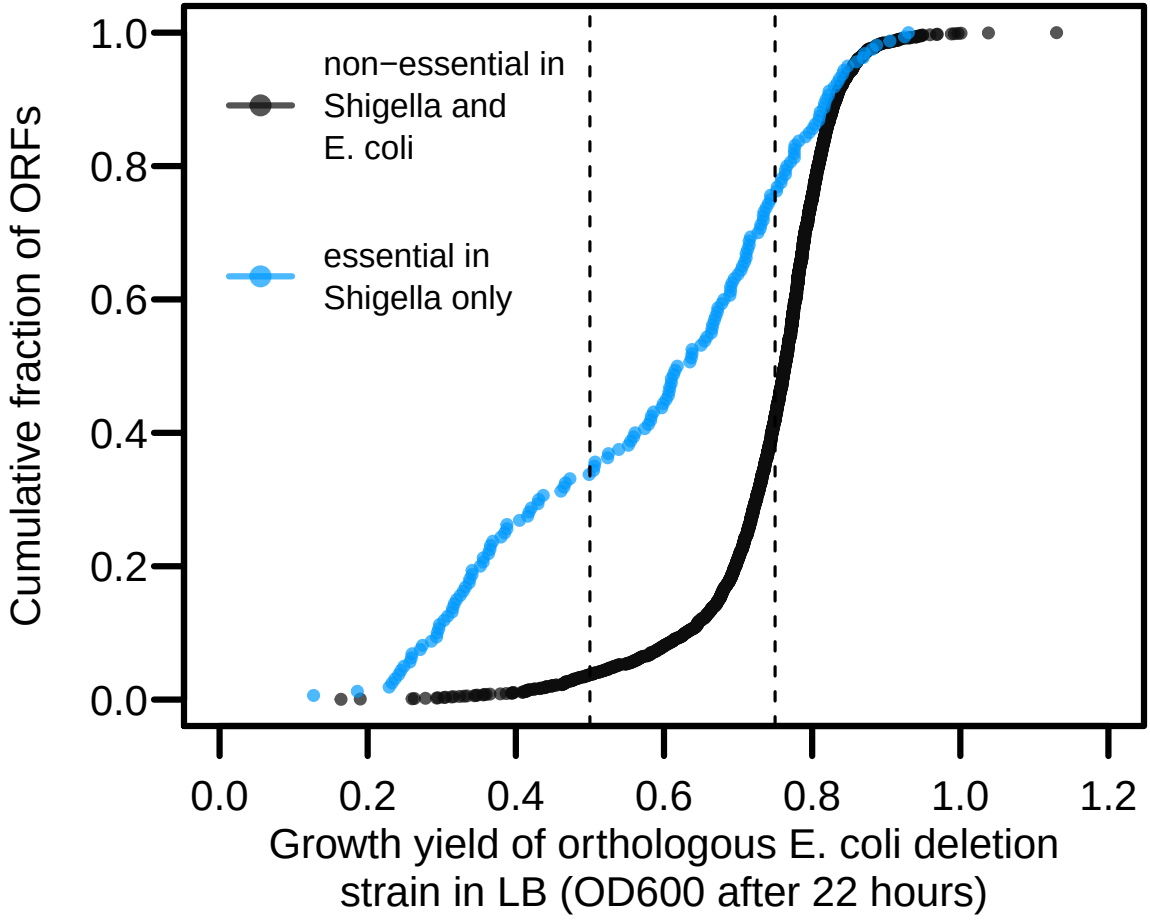
260
essential
in all
160
essential
in Shigella
only
17
2
essential
in PEC only
8


non−essential in Shigella and E. coli
essential in Shigella only
Cumulative fraction of ORFs
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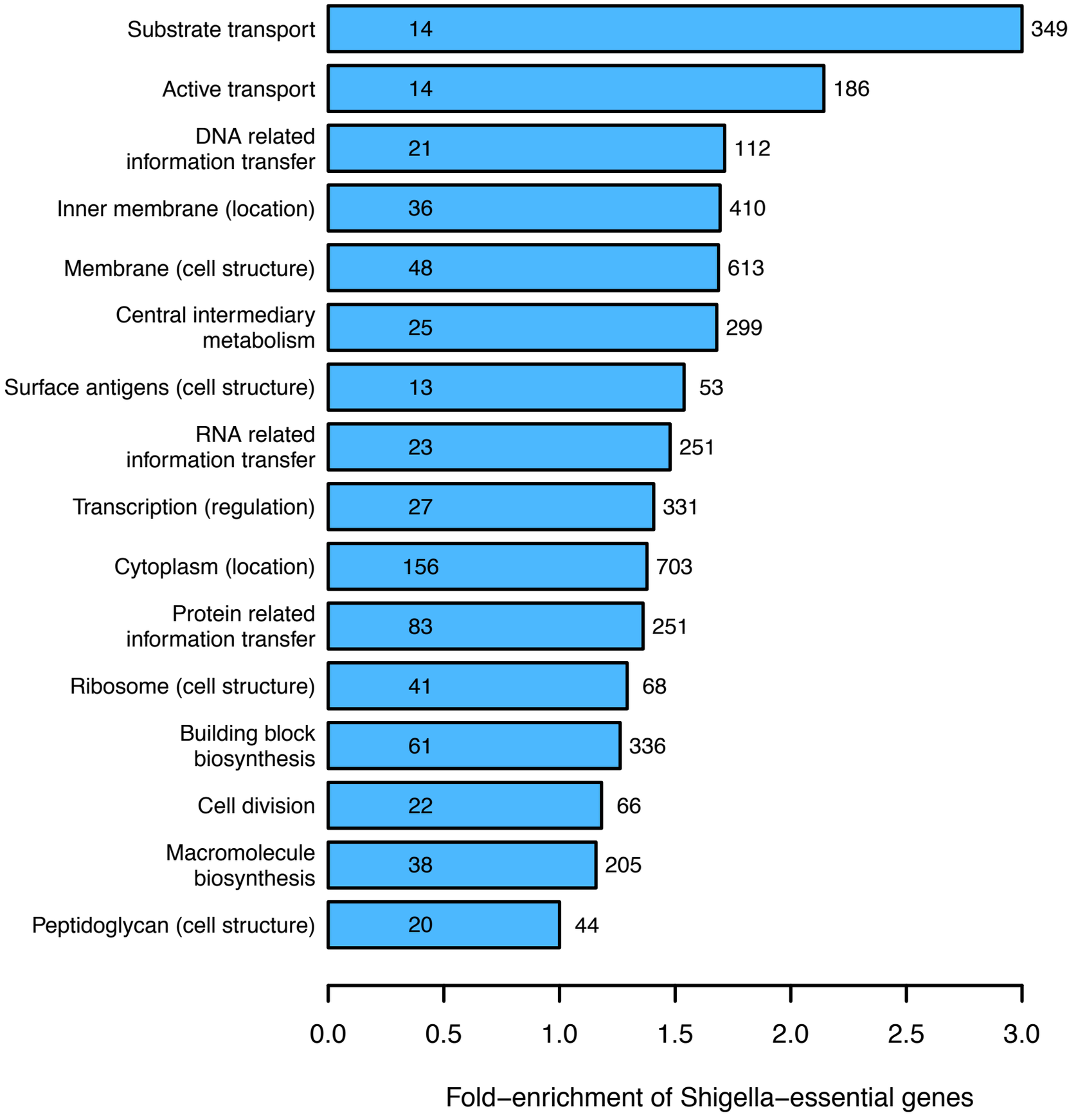
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Growth yield of orthologous E. coli deletion strain in LB (OD600 after 22 hours)


Substrate transport
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Active transport
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DNA related information transfer
21
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Inner membrane (location)
36
410
Membrane (cell structure)
48
613
Central intermediary metabolism
25
299
Surface antigens (cell structure)
13
53
RNA related information transfer
23
251
Transcription (regulation)
27
331
Cytoplasm (location)
156
703
Protein related information transfer
83
251
Ribosome (cell structure)
41
68
Building block biosynthesis
61
336
Cell division
22
66
Macromolecule biosynthesis
38
205
Peptidoglycan (cell structure)
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1.0
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2.0
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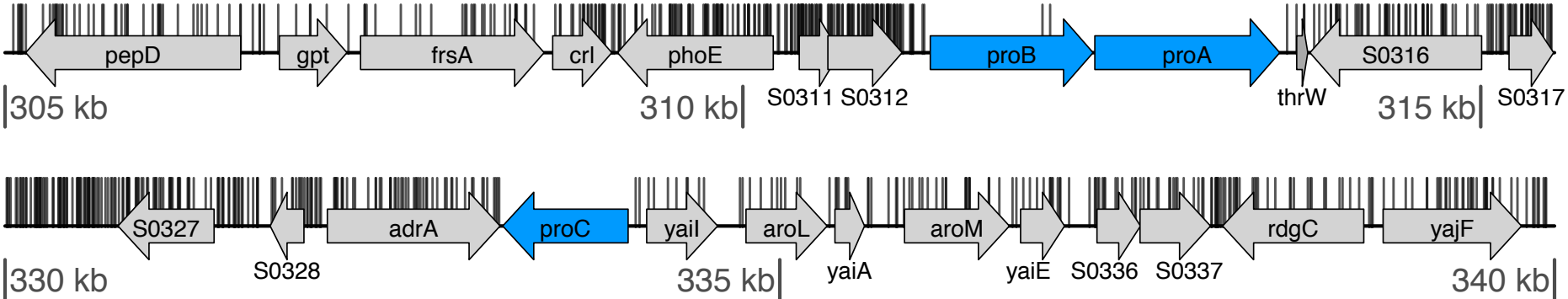
3.0
Fold−enrichment of Shigella−essential genes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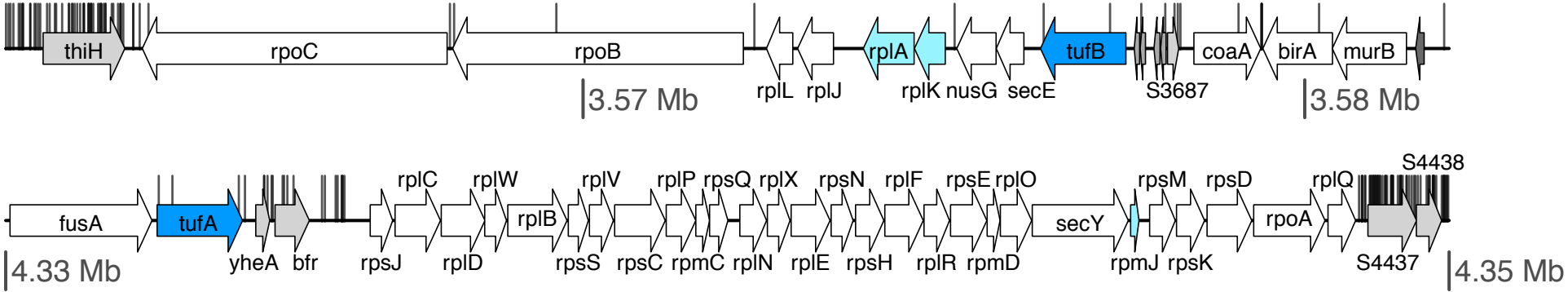
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rplB
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rpoA
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bfr
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rpsS
rpsC
rpmC
rplN
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hisF
hisI
wzzB
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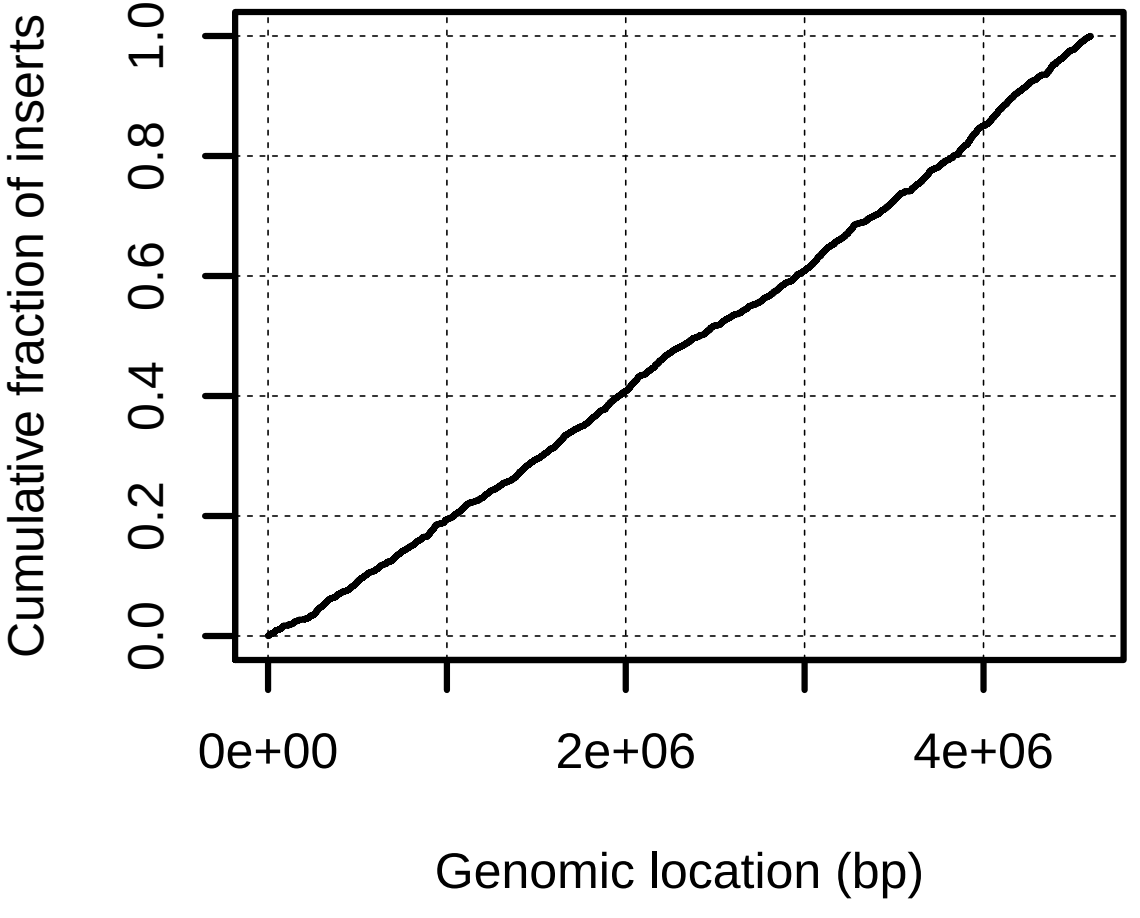
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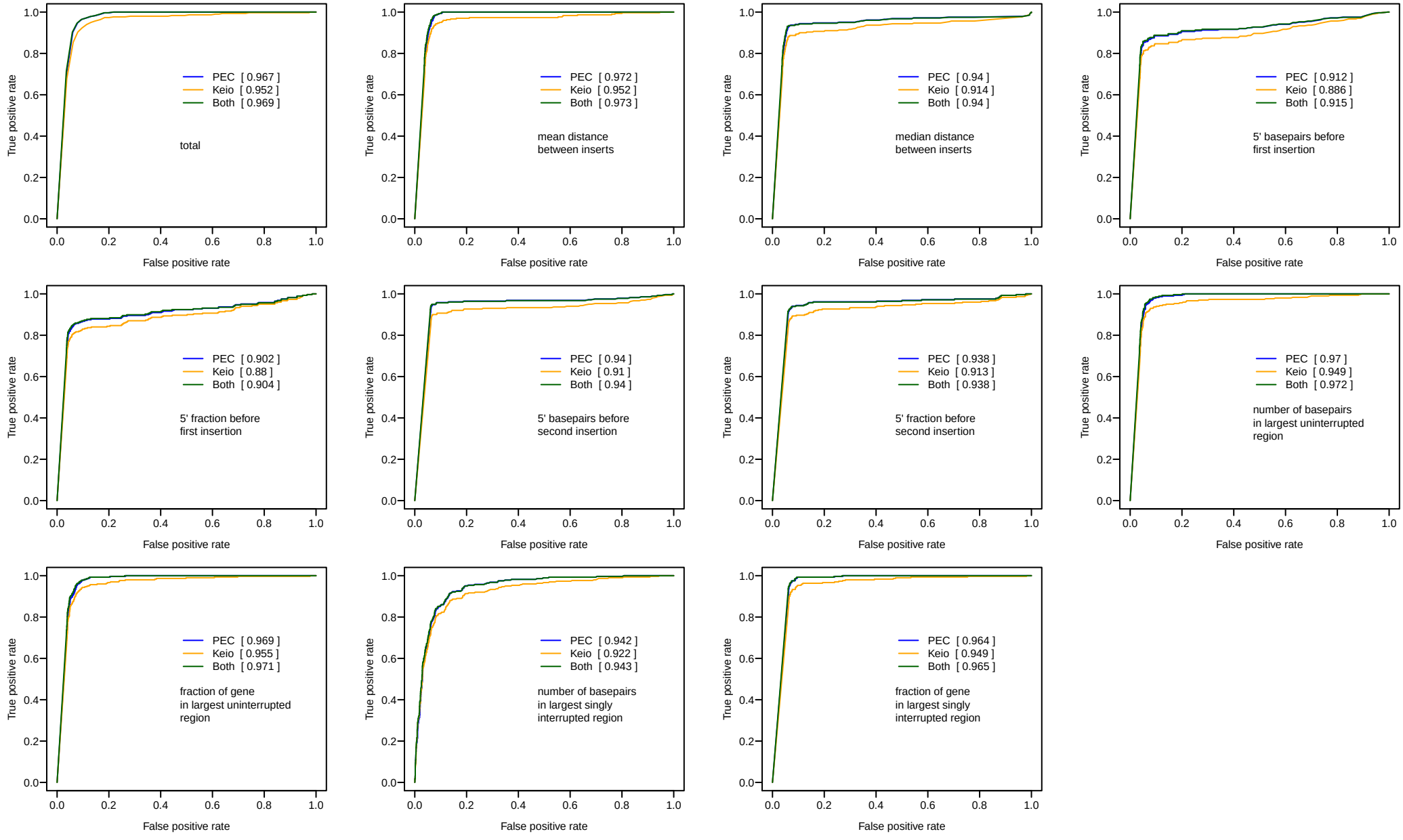
Genomic location (bp)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False positive rate
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Keio [0.952]
Both [0.969]
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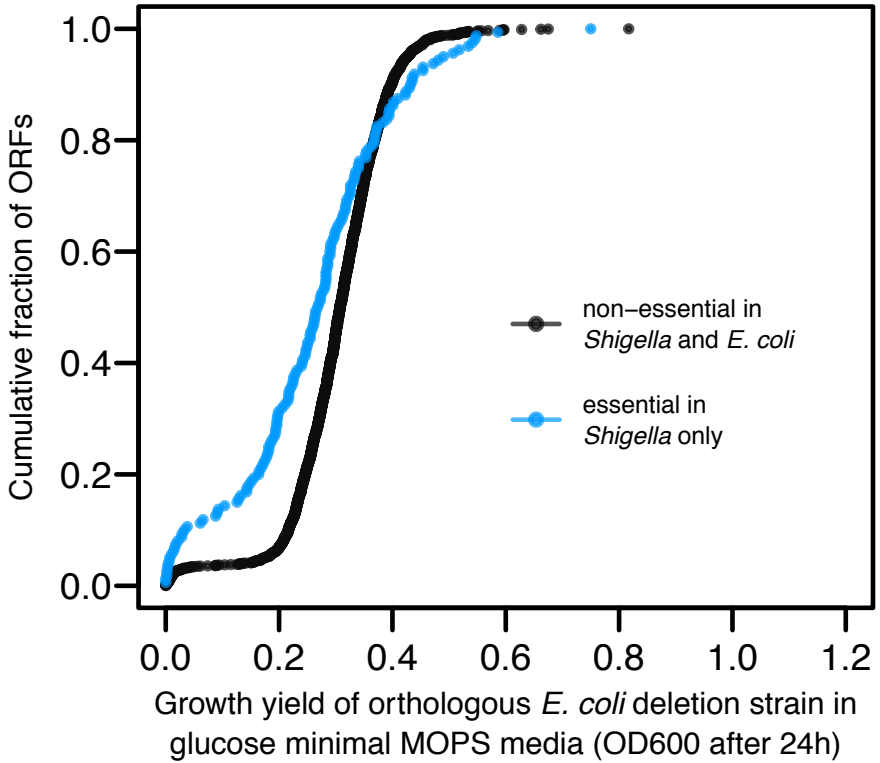
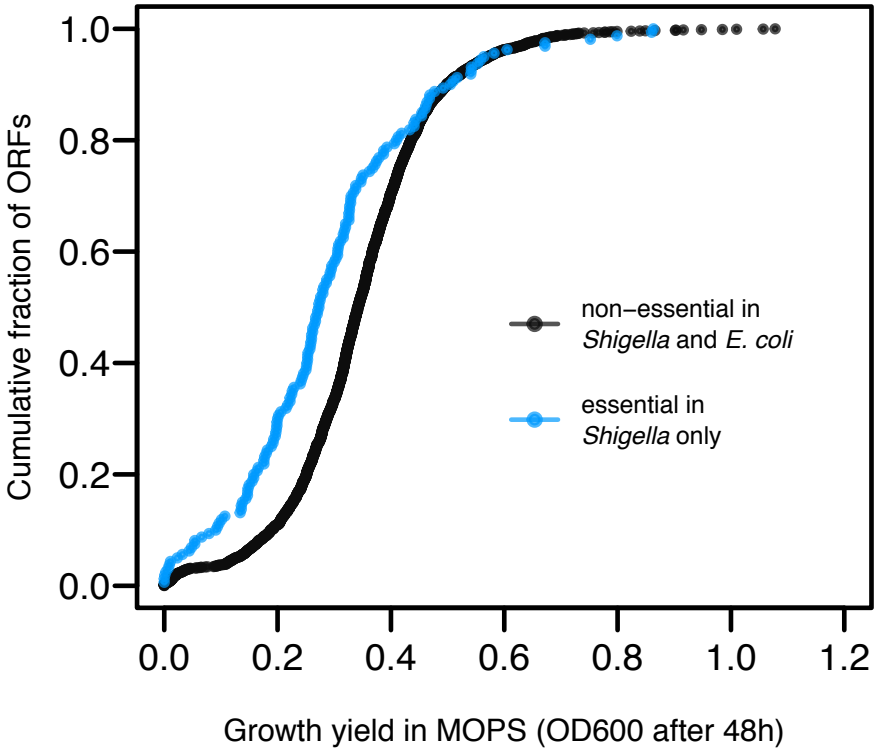
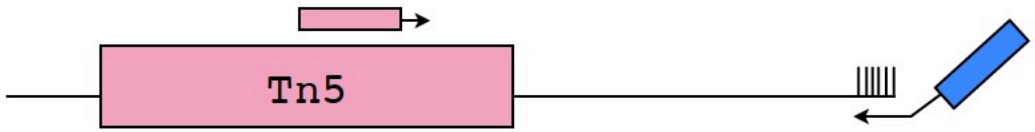
PEC [0.972]
Keio [0.952]
Both [0.973]
mean distance between inserts
PEC [0.94]
Keio [0.914]
Both [0.94]
median distance between inserts
PEC [0.912]
Keio [0.886]
Both [0.915]
5' basepairs before first insertion
PEC [0.902]
Keio [0.88]
Both [0.904]
5' fraction before first insertion
PEC [0.94]
Keio [0.91]
Both [0.94]
5' basepairs before second insertion
PEC [0.938]
Keio [0.913]
Both [0.938]
5' fraction before second insertion
PEC [0.97]
Keio [0.949]
Both [0.972]
number of basepairs in largest uninterrupted region
PEC [0.969]
Keio [0.955]
Both [0.971]
fraction of gene in largest uninterrupted region
PEC [0.942]
Keio [0.922]
Both [0.943]
number of basepairs in largest singly interrupted region
PEC [0.964]
Keio [0.949]
Both [0.965]
fraction of gene in largest singly interrupted region


Cumulative fraction of ORFs
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Growth yield of orthologous *E. coli* deletion strain in glucose minimal MOPS media (OD600 after 24h)
non−essential in *Shigella* and *E. coli*
essential in *Shigella* only


Cumulative fraction of ORFs
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Growth yield in MOPS (OD600 after 48h)
non−essential in *Shigella* and *E. coli*
essential in *Shigella* only



1: Arbitrary PCR

using phusion PCR enzyme, so that no A's added at 3' end

```
1_transp_deep_seq_F1: TCTATCGCCTTCTTGACGAG
```

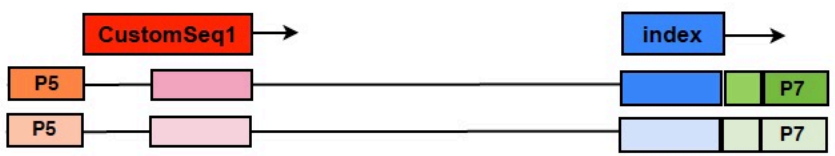
```
1_transp_deep_seq_R2: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNNggtgc
1_transp_deep_seq_R3: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNNgatat
1_transp_deep_seq_R4: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNNagtac
```

2: Nested PCR (addition of P7 and P5)

using phusion PCR enzyme, so that no A's added at 3' end

```
2_transp_deep_seq_ FP5: AATGATACGGCGACCACCGAGATCT actggtcggcg CATTAGGGGATTCATCAG
```

```
Rev_P7 6_02
CAAGCAGAAGACGGCATACGAGAT agtctt GTGACTGGAGTTCAGACGTGT
CAAGCAGAAGACGGCATACGAGAT gtatct GTGACTGGAGTTCAGACGTGT
CAAGCAGAAGACGGCATACGAGAT tcatgg GTGACTGGAGTTCAGACGTGT
CAAGCAGAAGACGGCATACGAGAT cgcgac GTGACTGGAGTTCAGACGTGT
CAAGCAGAAGACGGCATACGAGAT acgata GTGACTGGAGTTCAGACGTGT
CAAGCAGAAGACGGCATACGAGAT tataga GTGACTGGAGTTCAGACGTGT
```



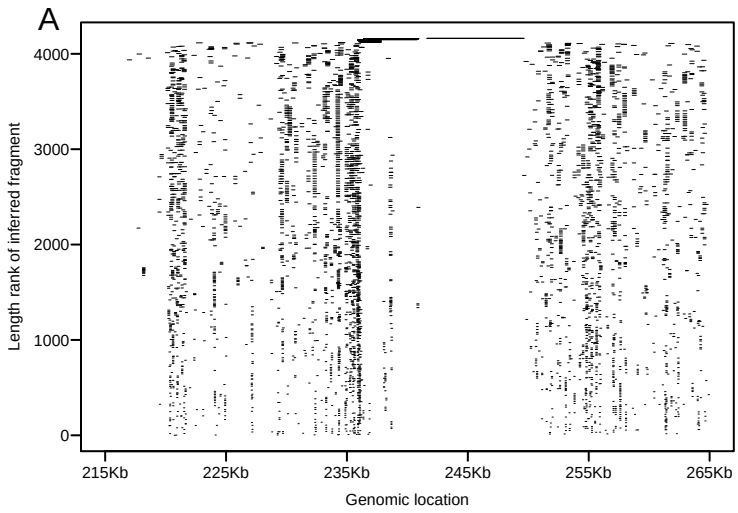
double stranded PCR product,
gel purify band/smear at (300-400bp)
(insert at 200-300bp)

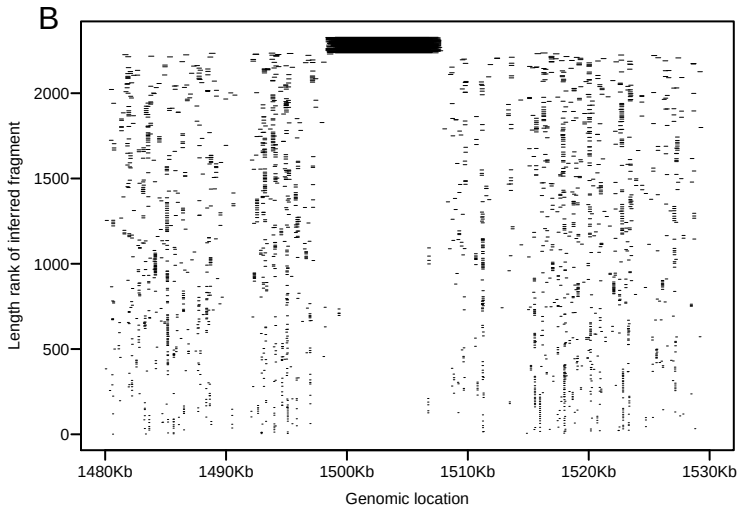
3: Sequencing on Illlumina chip

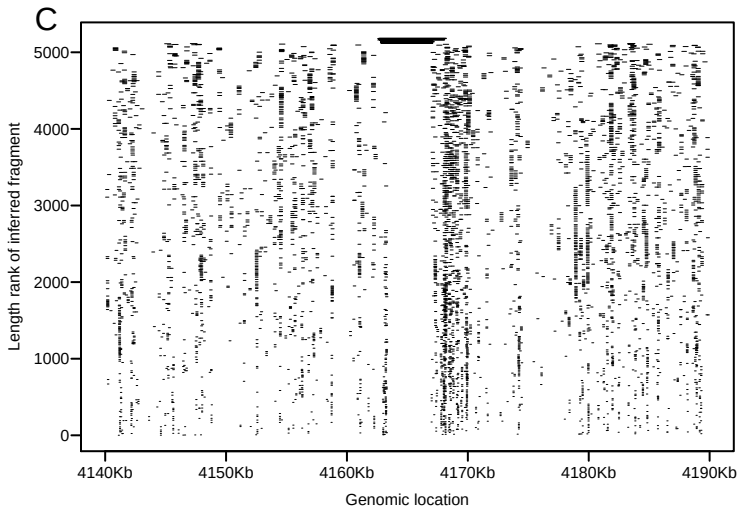
```
transp_deep_seq_Custom seq 1: actggtcggcgCATTAGGGGATTCATCAG
```

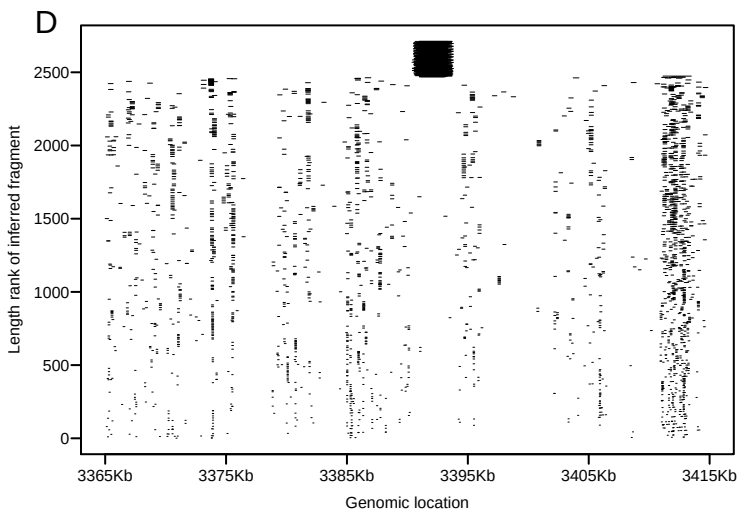
4: Barcode is read on Illlumina chip

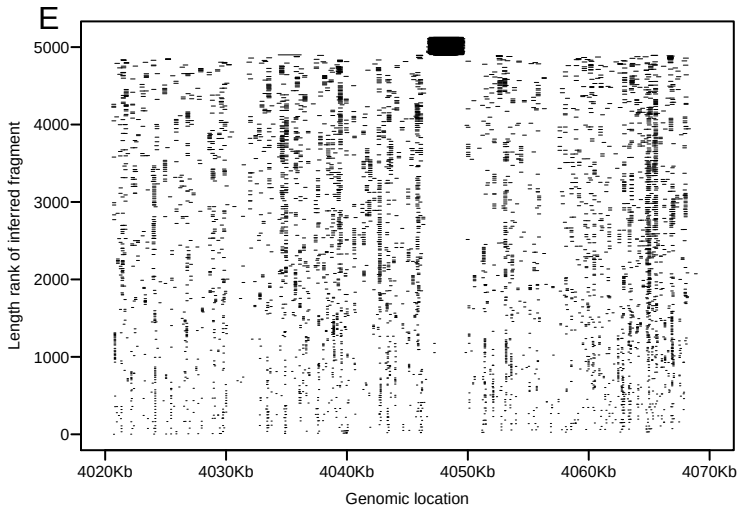
```
transp_deep_seq_Index seq: GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
```

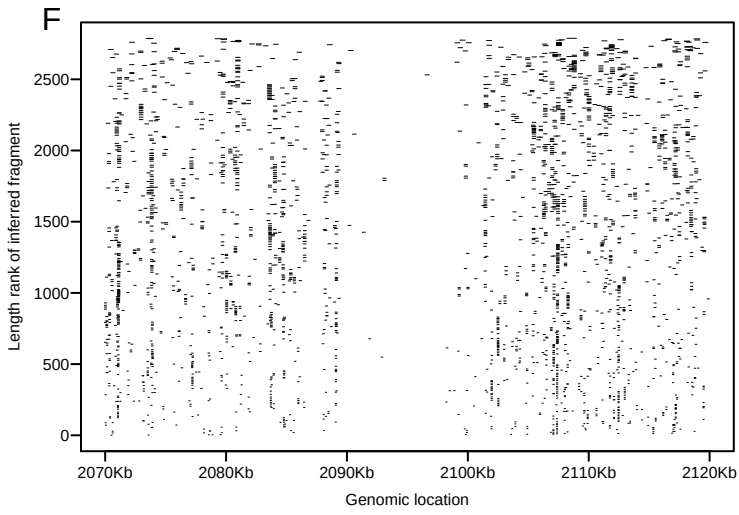

A
Length rank of inferred fragment
0
1000
2000
3000
4000
215Kb
225Kb
235Kb
245Kb
255Kb
265Kb
Genomic location

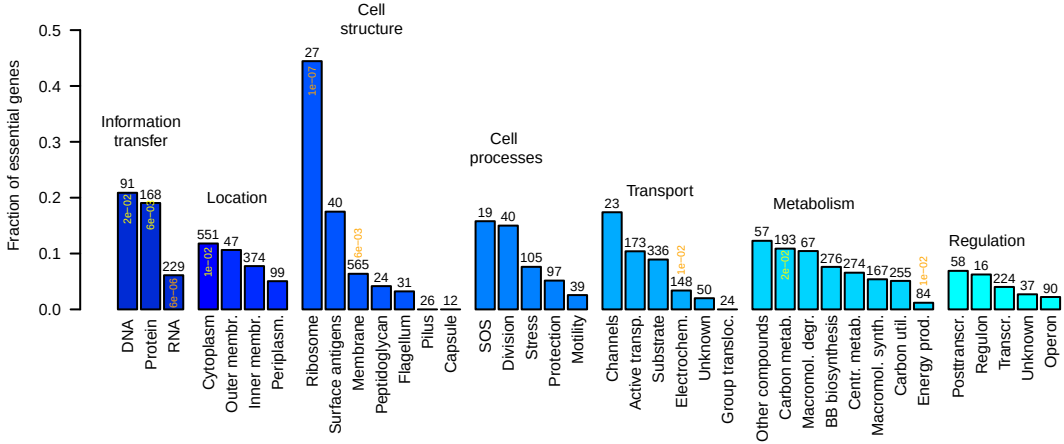

B
Length rank of inferred fragment
0
500
1000
1500
2000
1480Kb
1490Kb
1500Kb
1510Kb
1520Kb
1530Kb
Genomic location

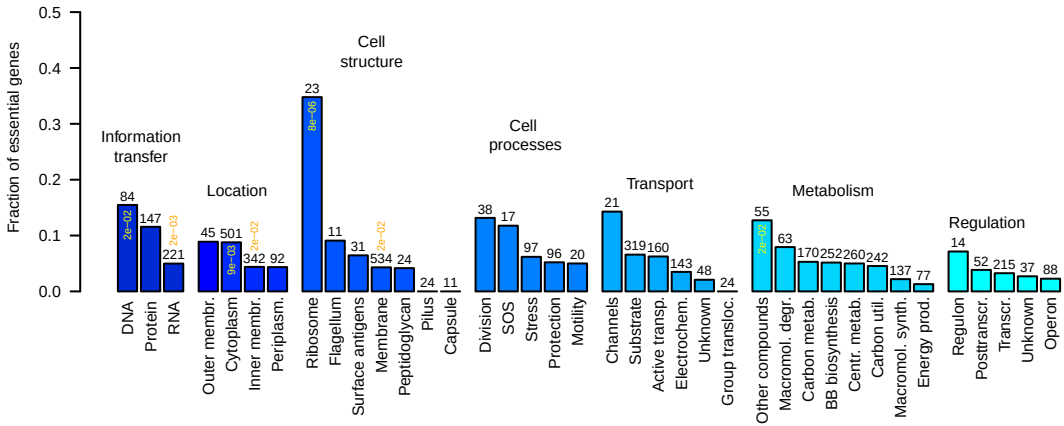

C
Length rank of inferred fragment
0
1000
2000
3000
4000
5000
4140Kb
4150Kb
4160Kb
4170Kb
4180Kb
4190Kb
Genomic location


D
Length rank of inferred fragment
0
500
1000
1500
2000
2500
3365Kb
3375Kb
3385Kb
3395Kb
3405Kb
3415Kb
Genomic location

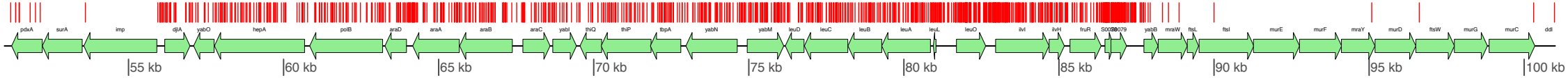

E
Length rank of inferred fragment
0
1000
2000
3000
4000
5000
4020Kb
4030Kb
4040Kb
4050Kb
4060Kb
4070Kb
Genomic location


F
Length rank of inferred fragment
0
500
1000
1500
2000
2500
2070Kb
2080Kb
2090Kb
2100Kb
2110Kb
2120Kb
Genomic location


Fraction of essential genes
0.0
0.1
0.2
0.3
0.4
0.5
Information transfer
Location
Cell structure
Cell processes
Transport
Metabolism
Regulation
91
2e−02
168
6e−04
229
6e−05
551
1e−02
47
374
99
27
1e−07
40
565
2e−03
24
31
26
12
19
40
105
97
39
23
173
336
148
1e−02
50
24
57
193
2e−02
67
276
274
167
255
84
1e−02
58
16
224
37
90
DNA
Protein
RNA
Cytoplasm
Outer membr.
Inner membr.
Periplasm.
Ribosome
Surface antigens
Membrane
Peptidoglycan
Flagellum
Pilus
Capsule
SOS
Division
Stress
Protection
Motility
Channels
Active transp.
Substrate
Electrochem.
Unknown
Group transloc.
Other compounds
Carbon metab.
Macromol. degr.
BB biosynthesis
Centr. metab.
Macromol. synth.
Carbon util.
Energy prod.
Posttranscr.
Regulon
Transcr.
Unknown
Operon

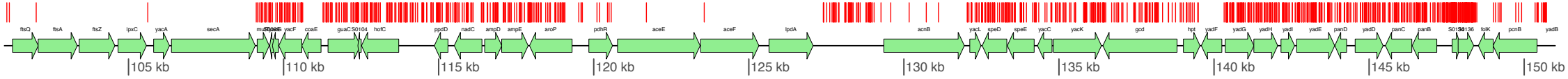

Fraction of essential genes
0.0
0.1
0.2
0.3
0.4
0.5
Information transfer
Location
Cell structure
Cell processes
Transport
Metabolism
Regulation
84
2e−02
147
1e−03
221
45
501
9e−03
2e−02
342
92
23
8e−06
11
31
534
2e−02
24
24
11
38
17
97
96
20
21
319
160
143
48
24
55
2e−02
63
170
252
260
242
137
77
14
52
215
37
88
DNA
Protein
RNA
Outer membr.
Cytoplasm
Inner membr.
Periplasm.
Ribosome
Flagellum
Surface antigens
Membrane
Peptidoglycan
Pilus
Capsule
Division
SOS
Stress
Protection
Motility
Channels
Substrate
Active transp.
Electrochem.
Unknown
Group transloc.
Other compounds
Macromol. degr.
Carbon metab.
BB biosynthesis
Centr. metab.
Carbon util.
Macromol. synth.
Energy prod.
Regulon
Posttranscr.
Transcr.
Unknown
Operon

51 – 101 Kb

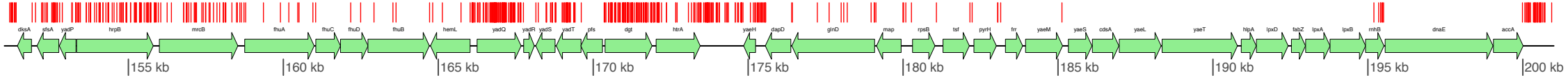

pdxA
surA
imp
djlA
yabO
hepA
polB
araD
araA
araB
araC
yabI
thiQ
thiP
tbpA
yabN
yabM
leuD
leuC
leuB
leuA
leuL
leuO
ilvI
ilvH
fruR
S0038
S0079
yabB
mraW
ftsL
ftsI
murE
murF
mraY
murD
ftsW
murG
murC
ddl
55 kb
60 kb
65 kb
70 kb
75 kb
80 kb
85 kb
90 kb
95 kb
100 kb

5 kb

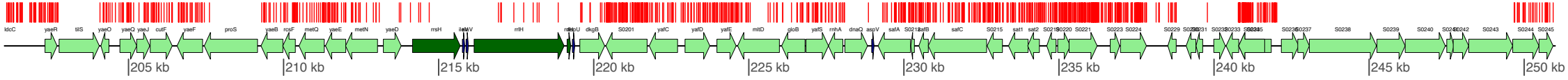
101 – 151 Kb


ftsQ
ftsA
ftsZ
lpxC
yacA
secA
yacF
coaE
guaC
S0104
hofC
ppdD
nadC
ampD
ampE
aroP
pdhR
aceE
aceF
lpdA
acnB
yacL
speD
speE
yacC
yacK
gcd
hpt
yadF
yadG
yadH
yadI
yadE
panD
yadD
panC
panB
folK
pcnB
yadB
105 kb
110 kb
115 kb
120 kb
125 kb
130 kb
135 kb
140 kb
145 kb
150 kb
5 kb

151 – 201 Kb


dksA
sfsA
yadP
hrpB
mrcB
fhuA
fhuC
fhuD
fhuB
hemL
yadQ
yadR
yadS
yadT
pfs
dgt
htrA
yaeH
dapD
glnD
map
rpsB
tsf
pyrH
frr
yaeM
yaeS
cdsA
yaeL
yaeT
hlpA
lpxD
fabZ
lpxA
lpxB
rnhB
dnaE
accA
155 kb
160 kb
165 kb
170 kb
175 kb
180 kb
185 kb
190 kb
195 kb
200 kb
5 kb

201 – 251 Kb


ldcC
yaeR
tilS
yaeQ
yaeQ
yaeJ
cutF
yaeF
proS
yaeB
rcsF
metQ
yaeE
metN
yaeD
rrsH
rrlH
dkgB
S0201
yafC
yafD
yafE
mltD
gloB
yafS
rnhA
dnaQ
aspV
safA
safB
safC
S0215
sat1
sat2
S0220
S0221
S0223
S0224
S0229
S0231
S0233
S0237
S0238
S0239
S0240
S0242
S0243
S0244
S0245
205 kb
210 kb
215 kb
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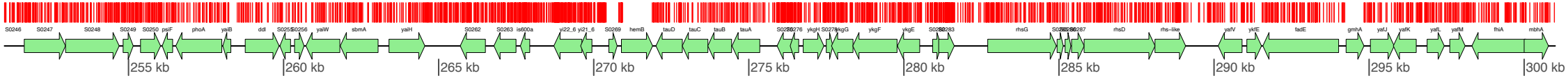
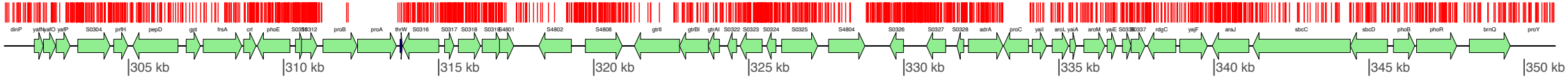
225 kb
230 kb
235 kb
240 kb
245 kb
250 kb
5 kb

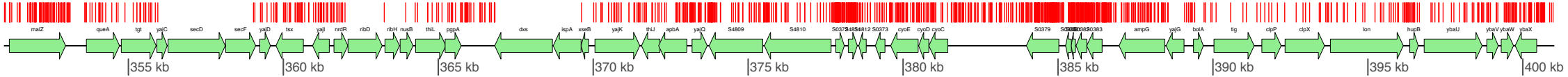
251 – 301 Kb


S0246
S0247
S0248
S0249
S0250
psiF
phoA
yaiB
ddl
S0255
S0256
yaiW
sbmA
yaiH
S0262
S0263
is600a
yi22_6
yi21_6
S0269
hemB
tauD
tauC
tauB
tauA
S0276
ykgH
ykgG
ykgF
ykgE
S0283
rhsG
S0287
rhsD
rhs-like
yafV
ykfE
fadE
gmhA
yafJ
yafK
yafL
yafM
fhiA
mbhA
255 kb
260 kb
265 kb
270 kb
275 kb
280 kb
285 kb
290 kb
295 kb
300 kb
5 kb

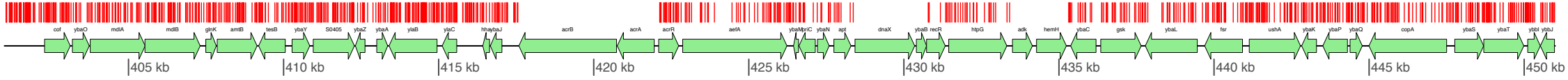
301 – 351 Kb


dinP
yafN
yafO
yafP
S0304
prfH
pepD
gpt
frsA
crl
phoE
S0312
proB
proA
thrW
S0316
S0317
S0318
S4801
S4802
S4808
gtrII
gtrBI
gtrAI
S0322
S0323
S0324
S0325
S4804
S0326
S0327
S0328
adrA
proC
yaiI
aroL
yaiA
aroM
yaiE
S0337
rdgC
yajF
araJ
sbcC
sbcD
phoB
phoR
brnQ
proY
305 kb
310 kb
315 kb
320 kb
325 kb
330 kb
335 kb
340 kb
345 kb
350 kb
5 kb

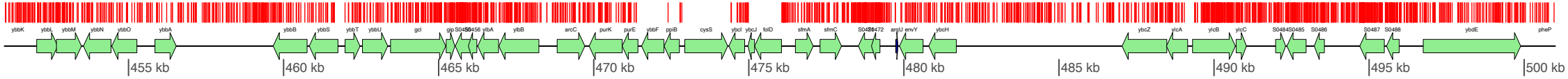
351 – 401 Kb


malZ
queA
tgt
yajC
secD
secF
yajD
tsx
yajI
nrdR
ribD
ribH
nusB
thiL
pggA
dxs
ispA
xseB
yajK
thiJ
apbA
yajQ
S4809
S4810
S0373
cyoE
cyoD
cyoC
S0379
S0383
ampG
yajG
holA
fig
clpP
clpX
lon
hupB
ybaU
ybaV
ybaW
ybaX
355 kb
360 kb
365 kb
370 kb
375 kb
380 kb
385 kb
390 kb
395 kb
400 kb
5 kb

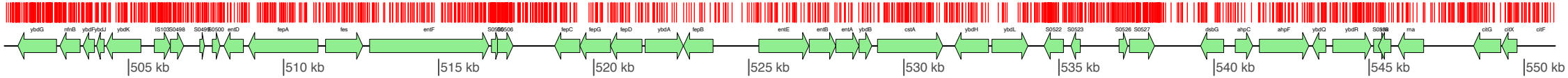
401 – 451 Kb


cof
ybaO
mdlA
mdlB
glnK
amtB
tesB
ybaY
S0405
ybaZ
ybaA
ylaB
ylaC
hha
ybaJ
acrB
acrA
acrR
aefA
ybaM
yriC
ybaN
apt
dnaX
ybaB
recR
htpG
adk
hemH
ybaC
gsk
ybaL
fsr
ushA
ybaK
ybaP
ybaQ
copA
ybaS
ybaT
ybbl
ybbJ
405 kb
410 kb
415 kb
420 kb
425 kb
430 kb
435 kb
440 kb
445 kb
450 kb
5 kb

451 – 501 Kb

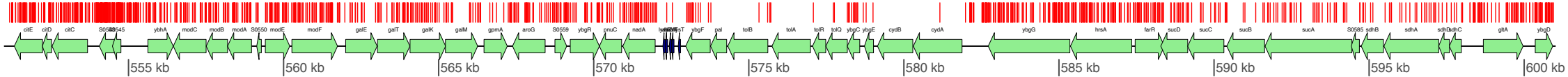

ybbK
ybbL
ybbM
ybbN
ybbO
ybbA
ybbB
ybbS
ybbT
ybbU
gcl
gip
S0456
ylbA
ylbB
arcC
purK
purE
ybbF
ppiB
cysS
ybcI
ybcJ
folD
sfmA
sfmC
argU
envY
ybcH
ybcZ
ylcA
ylcB
ylcC
S0484
S0485
S0486
S0487
S0488
ybdE
pheP
455 kb
460 kb
465 kb
470 kb
475 kb
480 kb
485 kb
490 kb
495 kb
500 kb
5 kb

501 – 551 Kb



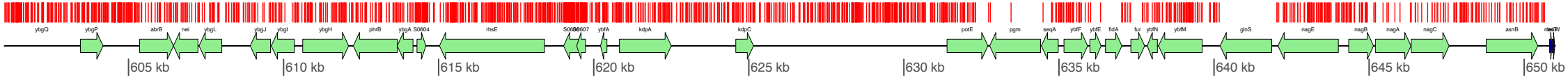
5 kb

551 – 601 Kb

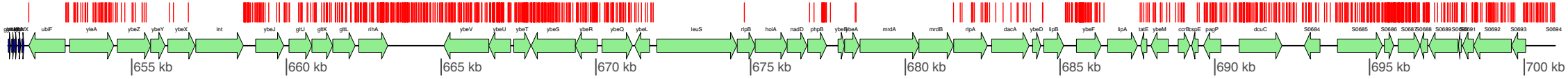


5 kb

601 – 651 Kb

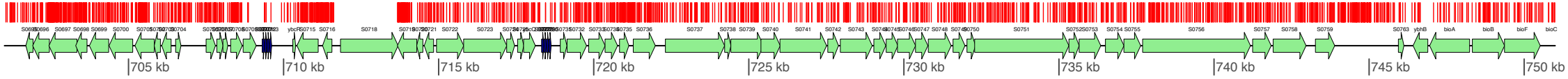

ybgQ
ybgP
abrB
nei
ybgL
ybgJ
ybgI
ybgH
phrB
ybgA
S0604
rhsE
S0606
S0607
ybfA
kdpA
kdpC
potE
pgm
segA
ybfF
ybfE
fldA
fur
ybfN
ybfM
glnS
nagE
nagB
nagA
nagC
asnB
605 kb
610 kb
615 kb
620 kb
625 kb
630 kb
635 kb
640 kb
645 kb
650 kb
5 kb

651 – 701 Kb



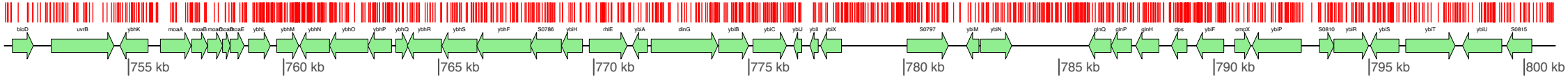
5 kb

701 – 751 Kb



5 kb

751 – 801 Kb



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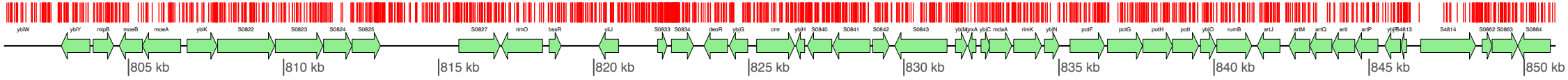
801 – 851 Kb


ybiW
ybiY
mipB
moeB
moeA
ybiK
S0822
S0823
S0824
S0825
S0827
rimO
bssR
yfiJ
S0833
S0834
deoR
ybjG
cmr
ybjH
S0840
S0841
S0842
S0843
ybjM
MzrA
ybjC
mdaA
rimK
ybjN
potF
potG
potH
potI
ybjO
rumB
artJ
artM
artQ
artI
artP
ybjR
S4813
S4814
S0862
S0863
S0864
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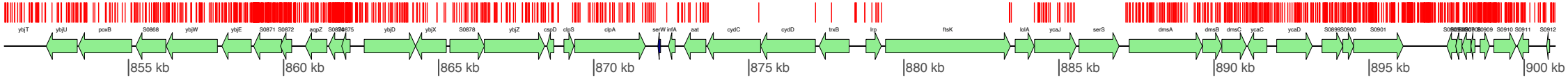
810 kb
815 kb
820 kb
825 kb
830 kb
835 kb
840 kb
845 kb
850 kb
5 kb

851 – 901 Kb


ybjT
ybjU
poxB
S0868
ybjW
ybjE
S0871
S0872
aqpZ
S0875
ybjD
ybjX
S0878
ybjZ
cspD
clpS
clpA
serW
infA
aat
cydC
cydD
trxB
lrp
ftsK
lolA
ycaJ
serS
dmsA
dmsB
dmsC
ycaC
ycaD
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S0900
S0901
S0909
S0910
S0911
S0912
855 kb
860 kb
865 kb
870 kb
875 kb
880 kb
885 kb
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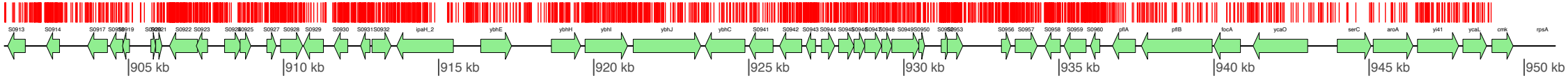
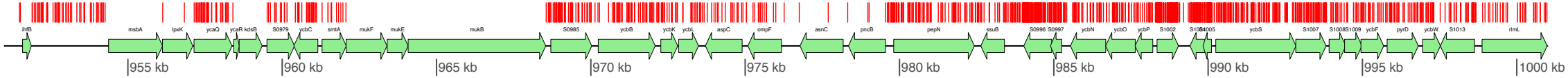
895 kb
900 kb
5 kb

901 – 951 Kb



5 kb

951 – 1001 Kb


ihfB
msbA
lpxK
ycaQ
ycaR
kdsB
S0979
ycbC
smtA
mukF
mukE
mukB
S0985
ycbB
ycbK
ycbL
aspC
ompF
asnC
pncB
pepN
ssuB
S0996
S0997
ycbN
ycbO
ycbP
S1002
S1004
S1005
ycbS
S1007
S1008
S1009
ycbF
pyrD
ycbW
S1013
rlmL
955 kb
960 kb
965 kb
970 kb
975 kb
980 kb
985 kb
990 kb
995 kb
1000 kb
5 kb

1001 – 1051 Kb

uup
pqiA
pqiB
ymbA
rmf
fabA
S1021
ycbG
ompA
sulA
S1027
S1028
yccF
helD
mqsA
S1032
yccV
S1035
yccK
yccA
serT
hyaA
hyaB
hyaC
hyaD
hyaE
hyaF
appC
appB
appA
erp
yccZ
ymcA
ymcB
ymcC
ymcD
cspH
ymcE
yccM
torT
torR
torC
1.005 Mb
1.01 Mb
1.015 Mb
1.02 Mb
1.025 Mb
1.03 Mb
1.035 Mb
1.04 Mb
1.045 Mb
1.05 Mb
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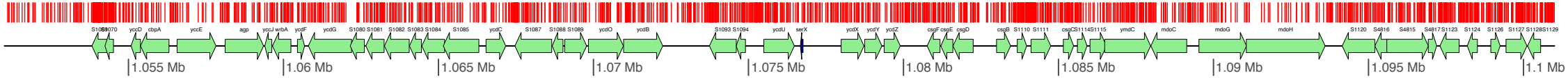
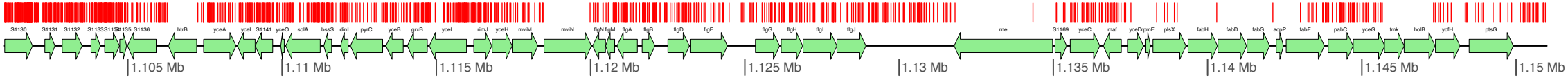
1051 – 1101 Kb



5 kb

1101 – 1151 Kb


S1130
S1131
S1132
S1133
S1134
S1135
S1136
htrB
yceA
yceI
S1141
yceO
solA
bssS
dinI
pyrC
yceB
cnxB
yceL
rimJ
yceH
mviM
mviN
flgN
flgM
flgA
flgB
flgD
flgE
flgG
flgH
flgI
flgJ
rne
S1169
yceC
maf
yceD
rpmF
plsX
fabH
fabD
fabG
acpP
fabF
pabC
yceG
tmk
holB
ycfH
ptsG
1.105 Mb
1.11 Mb
1.115 Mb
1.12 Mb
1.125 Mb
1.13 Mb
1.135 Mb
1.14 Mb
1.145 Mb
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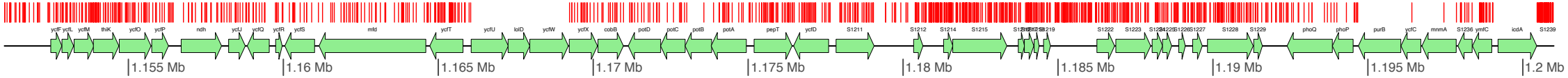
5 kb

1151 – 1201 Kb


ycfF
ycfL
ycfM
thiK
ycfO
ycfP
ndh
ycfJ
ycfQ
ycfR
ycfS
mfd
ycfT
ycfU
loiD
ycfW
ycfX
cobB
potD
potC
potB
potA
pepT
ycfD
S1211
S1212
S1214
S1215
S1219
S1222
S1223
S1227
S1228
S1229
phoQ
phoP
purB
ycfC
mnmA
S1236
ymfC
icdA
S1239
1.155 Mb
1.16 Mb
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1.175 Mb
1.18 Mb
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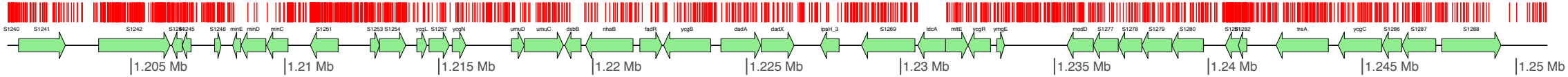
1.19 Mb
1.195 Mb
1.2 Mb
5 kb

1201 – 1251 Kb


S1240
S1241
S1242
S1244
S1245
S1246
minE
minD
minC
S1251
S1253
S1254
ycgL
S1257
ycgN
umuD
umuC
dsbB
nhaB
fadR
ycgB
dadA
dadX
ipaH_3
S1269
ldcA
mltE
ycgR
ymgE
modD
S1277
S1278
S1279
S1280
S1281
S1282
treA
ycgC
S1286
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S1288
1.205 Mb
1.21 Mb
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1.225 Mb
1.23 Mb
1.235 Mb
1.24 Mb
1.245 Mb
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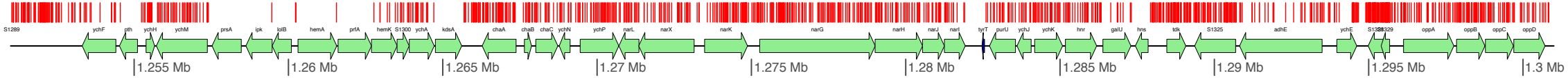
5 kb

1251 – 1301 Kb


S1289
ychF
pth
ychH
ychM
prsA
ipk
lplB
hemA
prfA
hemK
S1300
ychA
kdsA
chaA
chaB
chaC
ychN
ychP
narL
narX
narK
narG
narH
narJ
narI
tyrT
purU
ychJ
ychK
hnr
galU
hns
tdk
S1325
adhE
ychE
S1328
S1329
oppA
oppB
oppC
oppD
1.255 Mb
1.26 Mb
1.265 Mb
1.27 Mb
1.275 Mb
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1.285 Mb
1.29 Mb
1.295 Mb
1.3 Mb
5 kb

1301 – 1351 Kb

oppF
S1335
cls
kch
S1338
S1339
ycil
tonB
yciA
yciB
yciC
yciD
yciE
yciF
yciG
trpA
trpB
trpC
trpD
trpE
trpL
yciV
yciO
yciL
btuR
yciK
sohB
yciN
topA
cysB
acnA
ribA
pgpB
yciS
yciM
pyrF
yciH
osmB
S1371
rnb
yciW
fabI
yciD
sapF
sapD
sapC
sapB
sapA
1.305 Mb
1.31 Mb
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1.325 Mb
1.33 Mb
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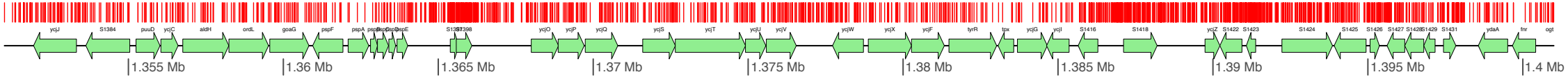
1.345 Mb
1.35 Mb
5 kb

1351 – 1401 Kb


ycjJ
S1384
puuD
ycjC
aldH
ordL
goaG
pspF
pspA
pspE
S1398
ycjO
ycjP
ycjQ
ycjS
ycjT
ycjU
ycjV
ycjW
ycjX
ycjF
tyrR
tpx
ycjG
ycjI
S1416
S1418
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ogt
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1.365 Mb
1.37 Mb
1.375 Mb
1.38 Mb
1.385 Mb
1.39 Mb
1.395 Mb
1.4 Mb
5 kb

1401 – 1451 Kb

S1430
S1439
S1440
S1441
S1443
zntB
dbpA
ydaO
ymaF
ydbK
S1453
S1454
ydbH
ldhA
S1462
ydbD
S1464
S1465
S1466
S1468
S1469
acpD
hrpA
S1473
aldA
cytB
ydcA
S1482
S1483
trg
S1486
S1487
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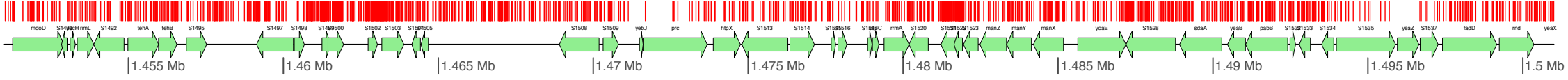
1451 – 1501 Kb



5 kb

1501 – 1551 Kb

S1542
S1543
S1544
yeaP
yoaJ
yeaO
yeaN
yeaL
S1551
yeaK
S1554
yeaG
yeaF
S1557
yeaD
gapA
yeaS
S1561
S1562
S1563
S1565
S1566
S1567
S1568
ydjE
ydjB
ansA
sppA
ydjA
selD
topB
S1578
S1579
S1581
S1582
S1584
S1588
ynjA
ydjZ
ydjY
ydjX
1.505 Mb
1.51 Mb
1.515 Mb
1.52 Mb
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1.55 Mb
5 kb

1551 – 1601 Kb

xthA
S1596
astD
ydjS
spy
S1601
S1602
nadE
osmE
celA
celC
celF
ydjC
katE
S1614
S1619
ynjC
S1621
S1622
S1623
pfkB
S1625
S1628
thrS
infC
rpmI
rpsT
pheM
pheS
pheT
ihfA
btuC
btuE
btuD
nlpC
S1641
S1642
ydiE
aroH
ydiA
ppsA
1.555 Mb
1.56 Mb
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1.585 Mb
1.59 Mb
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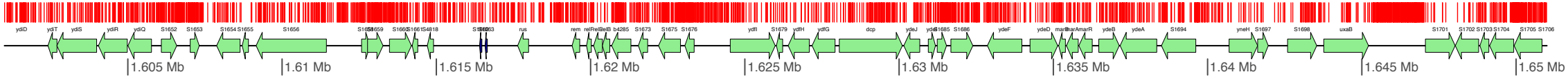
5 kb

1601 – 1651 Kb


ydiD
ydiT
ydiS
ydiR
ydiQ
S1652
S1653
S1654
S1655
S1656
S1659
S1660
S1661
S4818
rus
rem
b4285
S1673
S1675
S1676
ydfI
S1679
ydfH
ydfG
dcp
ydeJ
S1685
S1686
ydeF
ydeD
ydeB
ydeA
S1694
yneH
S1697
S1698
uxaB
S1701
S1702
S1703
S1704
S1705
S1706
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1.61 Mb
1.615 Mb
1.62 Mb
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1.63 Mb
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1.64 Mb
1.645 Mb
1.65 Mb
5 kb

1651 – 1701 Kb

ydeW
S1709
ydeY
ydeZ
S1714
S1715
S1716
S1717
rspA
S1719
S1720
yntC
S1723
S1727
S1728
S1730
S1731
S1732
S1736
S1738
S1739
S1742
S1743
S1744
S1745
bioD
mlc
yntL
asr
S1751
S1752
S1753
tqsA
pntB
pntA
S1757
S1758
ydgB
ydgC
rstA
S1762
rstB
tus
1.655 Mb
1.66 Mb
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1.675 Mb
1.68 Mb
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1.69 Mb
1.695 Mb
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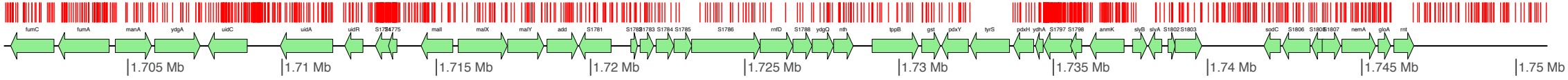
5 kb

1701 – 1751 Kb


fumC
fumA
manA
ydgA
uidC
uidA
uidR
S1774
S1775
malI
malX
malY
add
S1781
S1782
S1783
S1784
S1785
S1786
rnfD
S1788
ydgQ
nth
tppB
gst
pdxY
tyrS
pdxH
ydhA
S1797
S1798
anmK
slyB
slyA
S1802
S1803
sodC
S1806
S1807
nemA
gloA
rnt
1.705 Mb
1.71 Mb
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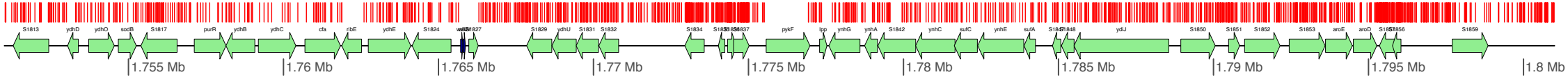
1751 – 1801 Kb



5 kb

1801 – 1851 Kb

5 kb

1851 – 1901 Kb

S1908
S1909
S1910
S1911
S1912
S1915
S1917
holE
S1919
S1920
ptrB
yebE
yebF
purT
eda
edd
zwf
yebK
pykA
msbB
yebA
znuA
znuC
znuB
ruvB
ruvA
ruvC
yebC
ntpA
aspS
S1944
S1946
ipaH_4
S1948
S1949
S1950
S1952
S1953
S1954
S1955
S1957
S1958
S1959
S1960
S1961
S1962
1.855 Mb
1.86 Mb
1.865 Mb
1.87 Mb
1.875 Mb
1.88 Mb
1.885 Mb
1.89 Mb
1.895 Mb
1.9 Mb
5 kb

1901 – 1951 Kb

S1963
sitA
sitB
sitC
sitD
S1968
S1969
S1970
S1971
S1972
S1973
S1975
S1978
S1980
S1981
S1986
S1987
S1989
S1990
S1991
S1992
S1998
yecN
yecO
yecP
bisZ
yecK
cutC
yecM
argS
S2009
S2011
tlhE
tlhB
S2015
S2018
S2019
S2020
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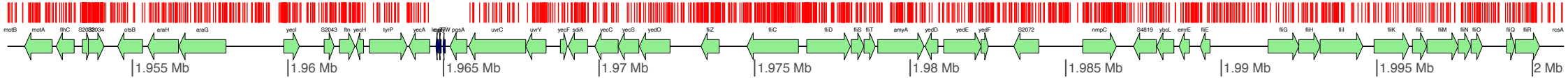
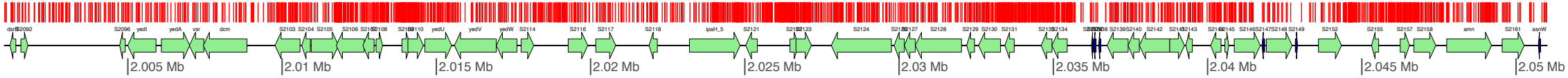
cheY
cheB
cheR
tap
tar
cheW
cheA
1.905 Mb
1.91 Mb
1.915 Mb
1.92 Mb
1.925 Mb
1.93 Mb
1.935 Mb
1.94 Mb
1.945 Mb
1.95 Mb
5 kb

1951 – 2001 Kb



5 kb

2001 – 2051 Kb



5 kb

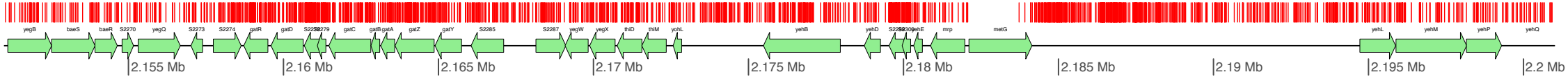
2051 – 2101 Kb

asnU
cbl
nac
asnV
erfK
cobT
cobS
S2172
S2173
is629a
is629b
yeeX
S2182
S2184
S2185
S2187
S2191
sbcB
yeeD
yeeE
yeeF
yeeY
S2198
hisG
hisD
hisC
hisB
hisH
hisA
hisF
hisI
wzzB
gnd
S2214
S2215
S2216
rfbJ
rfbI
rfc
rfbG
rfbF
rfbE
rfbC
rfbA
rfbD
2.055 Mb
2.06 Mb
2.065 Mb
2.07 Mb
2.075 Mb
2.08 Mb
2.085 Mb
2.09 Mb
2.095 Mb
2.1 Mb
5 kb

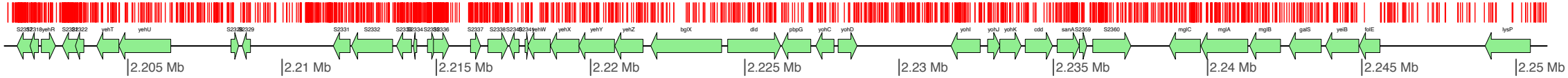
2101 – 2151 Kb

rfbB
galF
wcaM
wcaL
wcaK
wzxC
wcaJ
cpsG
cpsB
wcaI
wcaH
wcaG
gmd
wcaF
S2243
wcaB
S2248
wzb
wza
yegH
asmA
dcd
udk
S2256
alkA
S2261
S2262
issfl4
yegO
2.105 Mb
2.11 Mb
2.115 Mb
2.12 Mb
2.125 Mb
2.13 Mb
2.135 Mb
2.14 Mb
2.145 Mb
2.15 Mb
5 kb

2151 – 2201 Kb


yegB
baeS
baeR
S2270
yegQ
S2273
S2274
gatR
gatD
S2278
S2279
gatC
gatB
gatA
gatZ
gatY
S2285
S2287
yegW
yegX
thiD
thiM
ychL
yehB
yehD
S2298
S2299
yehE
mrp
metG
yehL
yehM
yehP
yehQ
2.155 Mb
2.16 Mb
2.165 Mb
2.17 Mb
2.175 Mb
2.18 Mb
2.185 Mb
2.19 Mb
2.195 Mb
2.2 Mb
5 kb

2201 – 2251 Kb


yehR
yehT
yehU
S2331
S2332
yehW
yehX
yehY
yehZ
bglX
dld
pbpG
yohC
yohD
yohI
yohJ
yohK
cdd
sanA
S2360
mglC
mglA
mglB
galS
yeiB
folE
lysP
2.205 Mb
2.21 Mb
2.215 Mb
2.22 Mb
2.225 Mb
2.23 Mb
2.235 Mb
2.24 Mb
2.245 Mb
2.25 Mb
5 kb

2251 – 2301 Kb

yeiE
yeiH
nfo
yeiI
yeiJ
rihB
yeiL
yeiM
yeiN
yeiC
fruA
fruK
fruB
yeiO
S2385
S2386
yeiP
yeiQ
yeiR
S2390
spr
rtn
yejA
yejB
yejE
yejF
yejG
bcr
rsuA
yejH
rplY
yejK
yejL
yejM
proL
yejO
narP
ccmH
dsbE
ccmF
ccmE
ccmD
ccmC
ccmB
ccmA
nagC
napB
napH
2.255 Mb
2.26 Mb
2.265 Mb
2.27 Mb
2.275 Mb
2.28 Mb
2.285 Mb
2.29 Mb
2.295 Mb
2.3 Mb
5 kb

2301 – 2351 Kb

napG
napA
napD
napF
eco
yojI
alkB
ada
yojL
ompC
yojN
rcsB
rcsC
S2434
S2435
S2436
S2438
S2440
S2442
gyrA
ubiG
S2447
S2448
nrdA
nrdB
yfaE
insA
yfaH
glpQ
glpT
glpA
2.305 Mb
2.31 Mb
2.315 Mb
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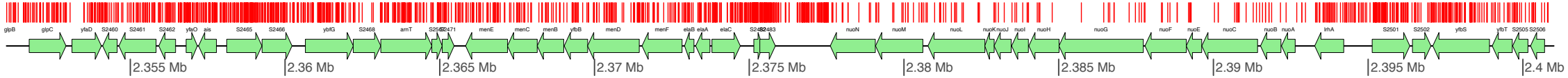
2.325 Mb
2.33 Mb
2.335 Mb
2.34 Mb
2.345 Mb
2.35 Mb
5 kb

2351 – 2401 Kb



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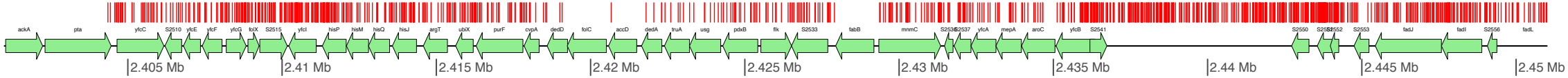
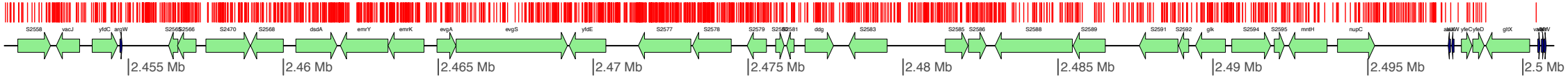
2401 – 2451 Kb



5 kb

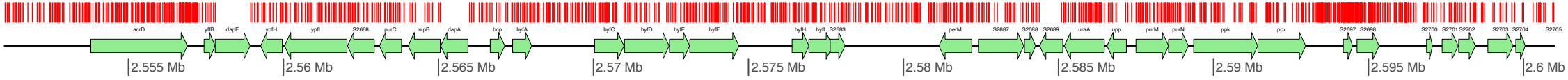
2451 – 2501 Kb


S2558
vacJ
yfdC
argW
S2565
S2566
S2470
S2568
dsdA
emrY
emrK
evgA
evgS
yfdE
S2577
S2578
S2579
S2581
ddg
S2583
S2585
S2586
S2588
S2589
S2591
S2592
glk
S2594
S2595
mntH
nupC
yfeC
yfeD
gltX
2.455 Mb
2.46 Mb
2.465 Mb
2.47 Mb
2.475 Mb
2.48 Mb
2.485 Mb
2.49 Mb
2.495 Mb
2.5 Mb
5 kb

2501 – 2551 Kb

5 kb

2551 – 2601 Kb


acrD
yfiB
dapE
ypfH
ypfI
S2668
purC
nlpB
dapA
bcp
hyfA
hyfC
hyfD
hyfE
hyfF
hyfH
hyfI
S2683
perM
S2687
S2688
S2689
uraA
upp
purM
purN
ppk
ppx
S2697
S2698
S2700
S2701
S2702
S2703
S2704
S2705
2.555 Mb
2.56 Mb
2.565 Mb
2.57 Mb
2.575 Mb
2.58 Mb
2.585 Mb
2.59 Mb
2.595 Mb
2.6 Mb
5 kb

2601 – 2651 Kb

S2706
parA
S2708
S2709
S2710
S2711
S2715
trbL
trbJ
S2719
repC
S2721
S2722
S2723
guaA
guaB
xseA
S2728
engA
S2730
S2731
hisS
ispG
yfgA
yfgB
ndk
S2738
eseB
pepB
yfhJ
fdx
hscA
hscB
iscA
S2748
yfhO
S2750
S2751
suhB
S2753
csiE
hcaT
hcaR
hcaA1
hcaA2
hcaC
hcaB
2.605 Mb
2.61 Mb
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2.625 Mb
2.63 Mb
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2.64 Mb
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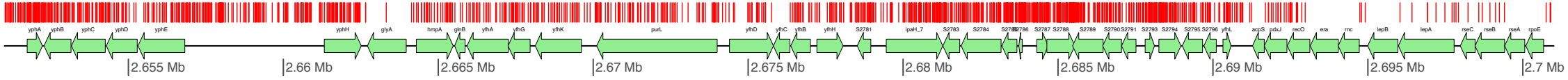
2651 – 2701 Kb



5 kb

2701 – 2751 Kb

yfiC
srmB
yfiE
yfiK
yfiD
ung
yfiF
trxC
yfiP
yfiQ
pssA
yfiM
kgtP
rrfG
rrfG
gltW
rrsG
S2827
S2828
riuD
yfiH
clpB
S2832
pheL
pheA
tyrA
aroF
yfiL
S2840
yfiB
rplS
trmD
rimM
rpsP
ffh
S2848
yfiD
grpE
ppnK
recN
smpB
S2859
S2860
2.705 Mb
2.71 Mb
2.715 Mb
2.72 Mb
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2.73 Mb
2.735 Mb
2.74 Mb
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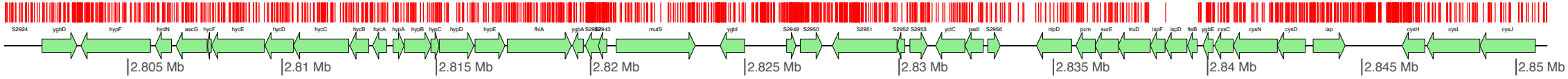
2.75 Mb
5 kb

2751 – 2801 Kb

5 kb

2801 – 2851 Kb


S2924
ygbD
hypF
hydN
ascG
hycF
hycE
hycD
hycC
hycB
hycA
hypA
hypB
hypC
hypD
hypE
fhlA
ygbA
S2943
mutS
ygbI
S2949
S2950
S2951
S2952
S2953
ycIC
padI
S2956
nlpD
pcm
surE
truD
ispF
ispD
ftsB
ygbE
cysC
cysN
cysD
iap
cysH
cysI
cysJ
2.805 Mb
2.81 Mb
2.815 Mb
2.82 Mb
2.825 Mb
2.83 Mb
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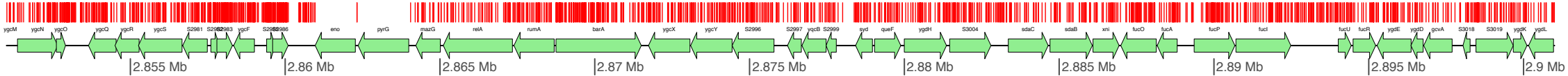
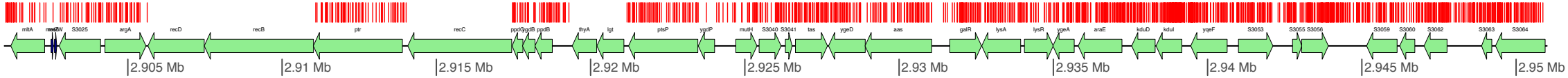
5 kb

2851 – 2901 Kb



5 kb

2901 – 2951 Kb


mltA
S3025
argA
recD
recB
ptr
recC
ppdG
ppdB
thyA
lgt
ptsP
ygdP
mutH
S3040
S3041
tas
ygeD
aas
galR
lysA
lysR
ygeA
araE
kduD
kdul
yqeF
S3053
S3055
S3056
S3059
S3060
S3062
S3063
S3064
2.905 Mb
2.91 Mb
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2.94 Mb
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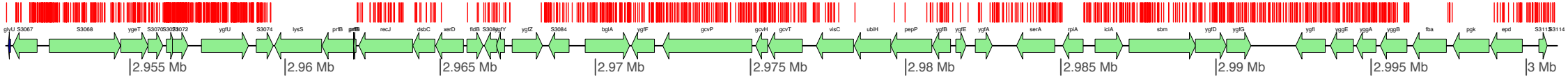
2951 – 3001 Kb


glyU
S3067
S3068
ygeT
S3070
S3071
S3072
ygfU
S3074
lysS
prfB
recJ
dsbC
xerD
flhB
S3083
ygfY
ygfZ
S3084
bglA
ygfF
gcvP
gcvH
gcvT
visC
ubiH
pepP
ygfB
ygfE
ygfA
serA
rpiA
iciA
sbm
ygfD
ygfG
ygfI
yggE
yggA
yggB
fba
pgk
epd
S3113
S3114
2.955 Mb
2.96 Mb
2.965 Mb
2.97 Mb
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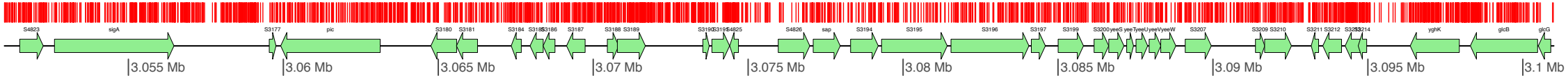
2.985 Mb
2.99 Mb
2.995 Mb
3 Mb
5 kb

3001 – 3051 Kb

5 kb

3051 – 3101 Kb



5 kb

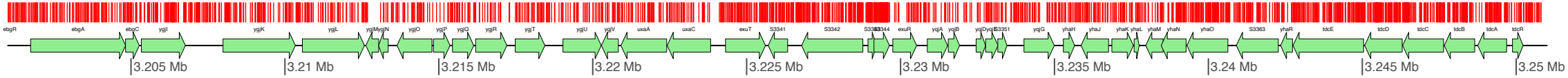
3101 – 3151 Kb

glcC
S3222
S3223
S3225
S3226
S3227
S3228
S3229
S3230
yghQ
yghR
yghS
yghT
pilB
gsp
S3237
hybG
hypA
hybE
hybD
hybC
hybB
hybA
S3245
S3247
S3249
yghA
yghA
S3255
metC
yghB
yqhC
yqhD
dkgA
yqhG
yqhH
S3263
sufI
plsC
parC
S3268
3.105 Mb
3.11 Mb
3.115 Mb
3.12 Mb
3.125 Mb
3.13 Mb
3.135 Mb
3.14 Mb
3.145 Mb
3.15 Mb
5 kb

3151 – 3201 Kb

5 kb

3201 – 3251 Kb


ebgR
ebgA
ebgC
ygjI
ygjK
ygjL
ygjM
ygjN
ygjO
ygjP
ygjQ
ygjR
ygjT
ygjU
ygjV
uxaA
uxaC
exuT
S3341
S3342
S3343
S3344
exuR
ygjA
ygjB
ygjD
ygjE
S3351
ygjG
yhaH
yhaJ
yhaK
yhaL
yhaM
yhaN
yhaO
S3363
yhaR
tdcE
tdcD
tdcC
tdcB
tdcA
tdcR
3.205 Mb
3.21 Mb
3.215 Mb
3.22 Mb
3.225 Mb
3.23 Mb
3.235 Mb
3.24 Mb
3.245 Mb
3.25 Mb
5 kb

3251 – 3301 Kb

S3374
yhaD
garR
yhaU
yhaG
agaR
agaZ
agaV
agaW
agaE
agaF
agaC
agaD
agaI
yraI
S3399
S3401
yraK
yraL
yraM
yraN
yraO
yraP
yraQ
yraR
yhbO
yhbP
yhbQ
yhbS
yhbT
yhbU
yhbV
yhbW
mtr
deaD
yhbM
pnp
rpsO
truB
rbfA
infB
nusA
3.255 Mb
3.26 Mb
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3.28 Mb
3.285 Mb
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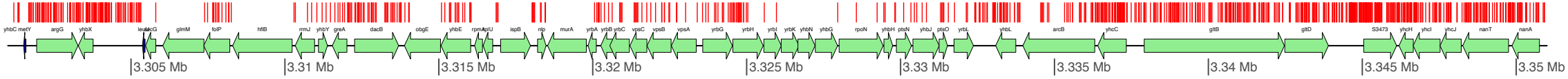
5 kb

3301 – 3351 Kb



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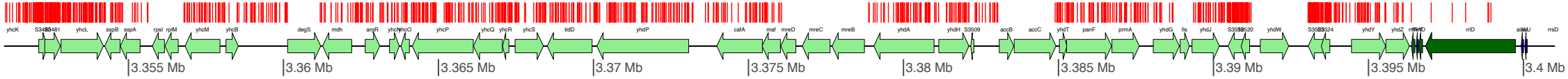
3351 – 3401 Kb



5 kb

3401 – 3451 Kb

yrdA
yrdB
aroE
yrdC
yrdD
smg
smf
def
fmt
sun
trkA
S3547
S3548
S3549
lysC
pgi
yjbE
yjbF
yjbG
yjbH
yjbA
xylE
malG
malF
malE
malK
lamB
malM
ubiC
ubiA
plsB
dgkA
lexA
dinF
yjbJ
yjbK
yjbM
yjbN
pspG
qor
dnaB
air
tyrB
3.405 Mb
3.41 Mb
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3.425 Mb
3.43 Mb
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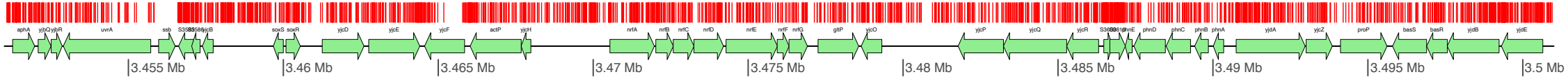
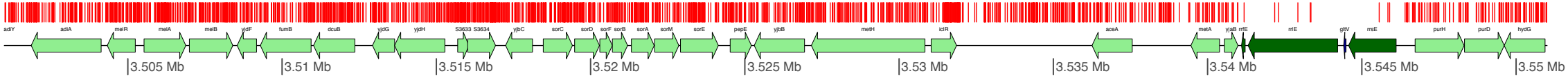
3451 – 3501 Kb

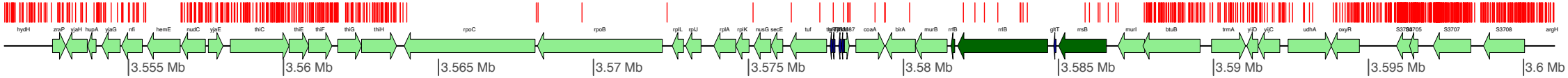


5 kb

3501 – 3551 Kb


adiY
adiA
melR
metA
melB
ydiF
fumB
dcuB
ydiG
ydiH
S3633
S3634
ybiC
sorC
sorD
sorF
sorB
sorA
sorM
sorE
pepE
ybiB
metH
icIR
aceA
metA
yiaB
rrlE
rrlE
gltV
rrsE
purH
purD
hydG
3.505 Mb
3.51 Mb
3.515 Mb
3.52 Mb
3.525 Mb
3.53 Mb
3.535 Mb
3.54 Mb
3.545 Mb
3.55 Mb
5 kb

3551 – 3601 Kb


hydH
zraP
yjaH
hupA
yjaG
nfi
hemE
nudC
yjaE
thiC
thiE
thiF
thiG
thiH
rpoC
rpoB
rplL
rplJ
rplA
rplK
nusG
secE
tuf
coaA
birA
murB
rrfB
rrlB
gltT
rrsB
murI
btuB
trmA
yijD
yijC
udhA
oxyR
S3707
S3708
argH
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3.56 Mb
3.565 Mb
3.57 Mb
3.575 Mb
3.58 Mb
3.585 Mb
3.59 Mb
3.595 Mb
3.6 Mb
5 kb

3601 – 3651 Kb

argB
argC
argE
ppc
yijP
yijO
frwD
pflC
pflD
frwB
frwC
katG
metF
metL
metB
metJ
yiiX
rpmE
priA
cytR
ftsN
hslV
hslU
menA
menG
yiiU
glpF
glpK
glpX
fpr
yiiT
yiiS
yiiQ
tpiA
cdh
sbp
pfkA
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3.63 Mb
3.635 Mb
3.64 Mb
3.645 Mb
3.65 Mb
5 kb

3651 – 3701 Kb

fieF
cpxP
cpxR
cpxA
yiiM
S3760
S3761
kdgT
sodA
rhaR
rhaS
rhaB
rhaA
rhaD
yiiL
frvA
frvB
frvX
frvR
S3775
S3776
fdhD
fdoG
fdoH
fdoI
fdhE
yiiF
yiiE
S4827
S4828
S3784
S3785
yiiD
yihZ
rbn
yihX
yihW
yihV
yihU
yihT
yihS
yihR
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yihL
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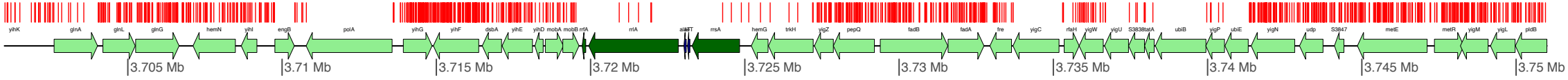
3.695 Mb
3.7 Mb
5 kb

3701 – 3751 Kb


yihK
glnA
glnL
glnG
hemN
yihI
engB
polA
yihG
yihF
dsbA
yihE
yihD
mobA
mobB
rrfA
rrlA
alaT
rrsA
hemG
trkH
yigZ
pepQ
fadB
fadA
fre
yigC
rfaH
yigW
yigU
S3838
tatA
ubiB
yigP
ubiE
yigN
udp
S3847
metE
metR
yigM
yigL
pldB
3.705 Mb
3.71 Mb
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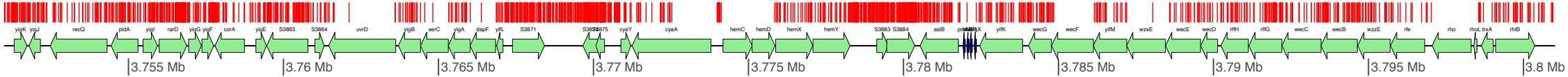
3.725 Mb
3.73 Mb
3.735 Mb
3.74 Mb
3.745 Mb
3.75 Mb
5 kb

3751 – 3801 Kb



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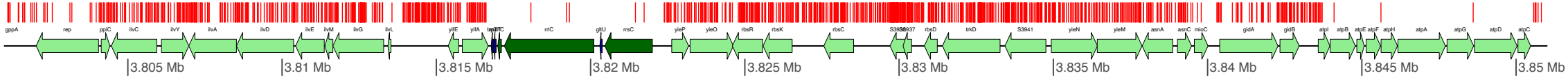
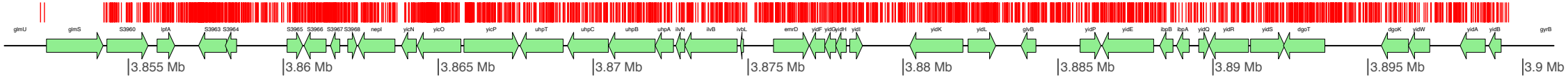
3801 – 3851 Kb


gppA
rep
ppiC
ilvC
ilvY
ilvA
ilvD
ilvE
ilvM
ilvG
ilvL
yifE
yifA
rrlC
gltU
rrsC
yieP
yieO
rbsR
rbsK
rbsC
S3938
S3937
rbsD
trkD
S3941
yieN
yieM
asnA
asnC
mioC
gidA
gidB
atpI
atpB
atpE
atpF
atpH
atpA
atpG
atpD
atpC
3.805 Mb
3.81 Mb
3.815 Mb
3.82 Mb
3.825 Mb
3.83 Mb
3.835 Mb
3.84 Mb
3.845 Mb
3.85 Mb
5 kb

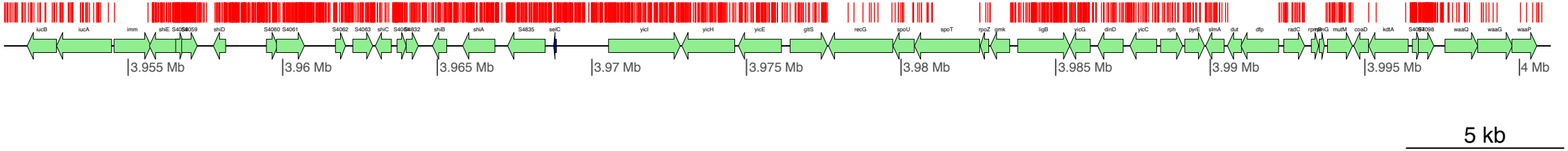
3851 – 3901 Kb


glmU
glmS
S3960
lpfA
S3963
S3964
S3965
S3966
S3967
S3968
nepI
yicN
yicO
yicP
uhpT
uhpC
uhpB
uhpA
ilvN
ilvB
ivbL
emrD
yidF
yidG
yidH
yidI
yidK
yidL
glvB
yidP
yidE
ibpB
ibpA
yidQ
yidR
yidS
dgoT
dgoK
yidW
yidA
yidB
gyrB
3.855 Mb
3.86 Mb
3.865 Mb
3.87 Mb
3.875 Mb
3.88 Mb
3.885 Mb
3.89 Mb
3.895 Mb
3.9 Mb
5 kb

3901 – 3951 Kb

5 kb

3951 – 4001 Kb


iucB
iucA
imm
shiE
S4058
S4059
shiD
S4060
S4061
S4062
S4063
shiC
S4064
S4832
shiB
shiA
S4835
selC
yicI
yicH
yicE
gltS
recG
spoU
spoT
rpoZ
gmk
ligB
yicG
dinD
yicC
rph
pyrE
slmA
dut
dfp
radC
rpmH
rnpA
rnpB
mutM
coaD
kdtA
S4097
S4098
waaQ
waaG
waaP
3.955 Mb
3.96 Mb
3.965 Mb
3.97 Mb
3.975 Mb
3.98 Mb
3.985 Mb
3.99 Mb
3.995 Mb
4 Mb
5 kb

4001 – 4051 Kb

waaL
waaY
waaJ
waaD
waaI
rfaC
rfaF
rfaD
kbl
tdh
S4112
S4113
yibD
yibQ
yibP
yibO
yibN
grxC
secB
gpsA
cysE
yibK
lldD
lldR
lldP
S4130
S4131
yibL
mtlR
mtlD
mtlA
yibI
yibH
yibF
selA
selB
yiaY
aldB
S4143
S4144
S4145
S4147
S4148
yiaK
4.005 Mb
4.01 Mb
4.015 Mb
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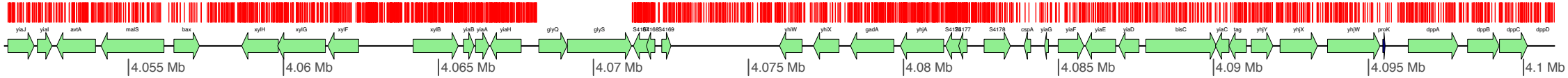
4.03 Mb
4.035 Mb
4.04 Mb
4.045 Mb
4.05 Mb
5 kb

4051 – 4101 Kb


yiaJ
yiaI
avtA
malS
bax
xylH
xylG
xylF
xylB
yiaB
yiaA
yiaH
glyQ
glyS
S4168
S4169
yhiW
yhiX
gadA
yhjA
S4178
cspA
yiaG
yiaF
yiaE
yiaD
bisC
yiaC
tag
yhjY
yhjX
yhjW
proK
dppA
dppB
dppC
dppD
4.055 Mb
4.06 Mb
4.065 Mb
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4.075 Mb
4.08 Mb
4.085 Mb
4.09 Mb
4.095 Mb
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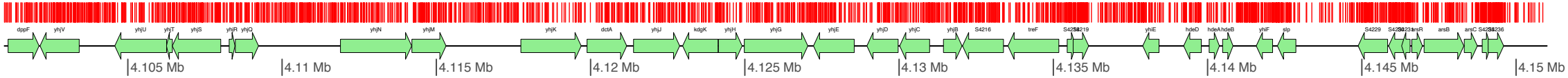
5 kb

4101 – 4151 Kb

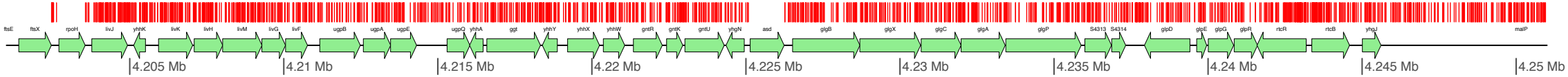


5 kb

4151 – 4201 Kb

gor
yhiR
prlC
yhiD
uspA
yhiO
pilA
yhiN
S4249
S4250
S4254
S4252
S4253
S4255
S4836
S4837
S4838
yhiJ
yhiI
yhiH
yhhJ
S4263
yhhG
nikE
nikD
nikC
nikB
nikA
yhhU
yhhT
yhhS
S4273
yhhQ
yhhP
zntA
yhhN
yhhM
yhhL
rsmD
ftsY
4.155 Mb
4.16 Mb
4.165 Mb
4.17 Mb
4.175 Mb
4.18 Mb
4.185 Mb
4.19 Mb
4.195 Mb
4.2 Mb
5 kb

4201 – 4251 Kb



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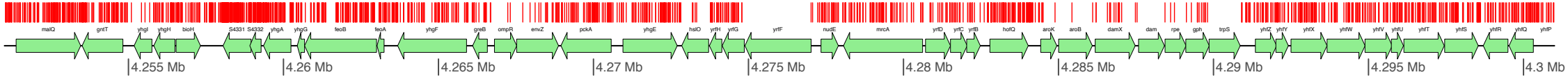
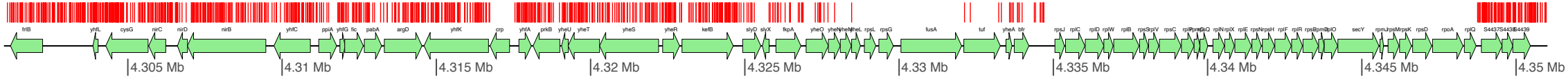
4251 – 4301 Kb



5 kb

4301 – 4351 Kb


frlB
yhfL
cysG
nirC
nirD
nirB
yhfC
ppiA
yhfG
fic
pabA
argD
yhfK
crp
yhfA
prkB
yheU
yheT
yheS
yheR
ketB
slyD
slyX
fkpA
yheO
yheN
yheM
yheL
rpsL
rpsG
fusA
tuf
yheA
bfr
rpsJ
rplC
rplD
rplW
rplB
rpsS
rplV
rpsC
rplP
rpmC
rplQ
rplN
rplX
rplE
rpsN
rpsH
rplF
rplR
rpsE
rpmD
rplO
secY
rpmJ
rpsM
rpsK
rpsD
rpoA
rplQ
S4437
S4438
S4439
4.305 Mb
4.31 Mb
4.315 Mb
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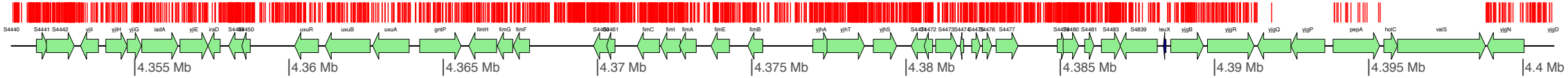
4.325 Mb
4.33 Mb
4.335 Mb
4.34 Mb
4.345 Mb
4.35 Mb
5 kb

4351 – 4401 Kb



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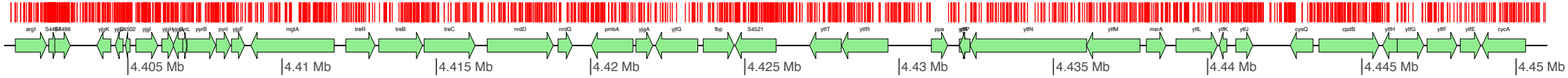
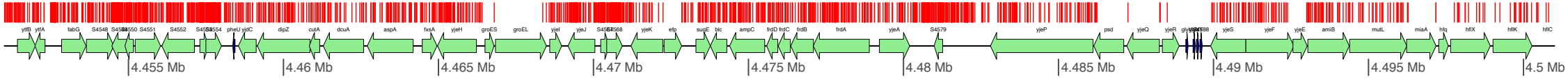
4401 – 4451 Kb


argI
S4498
yjgK
S4502
yjgI
pyrB
pyrI
yjgF
mgtA
treR
treB
treC
nrdD
nrdG
pmbA
yjgA
yjfG
fbp
S4521
ytfT
ytfR
ppa
ytfN
ytfM
msrA
ytfL
ytfK
ytfJ
cysQ
cpdB
ytfH
ytfG
ytfF
ytfE
cycA
4.405 Mb
4.41 Mb
4.415 Mb
4.42 Mb
4.425 Mb
4.43 Mb
4.435 Mb
4.44 Mb
4.445 Mb
4.45 Mb
5 kb

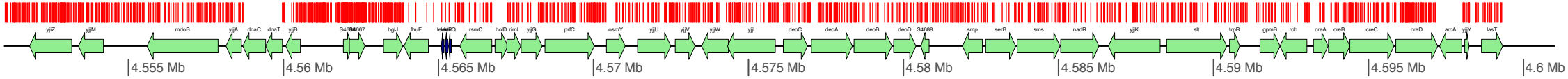
4451 – 4501 Kb


ytfB ytfA fabG S4548 S4549 S4550 S4551 S4552 S4553 S4554 pheU ydtC dipZ cutA dcuA aspA fxsA yjeH groES groEL yjeI yjeJ S4567 S4568 yjeK efp sugE hlc ampC frdD frdC frdB frdA yjeA S4579 yjeP psd yjeQ yjeR yjeS yjeF yjeE amiB mutL miaA hfq hflX hflK hflC
4.455 Mb
4.46 Mb
4.465 Mb
4.47 Mb
4.475 Mb
4.48 Mb
4.485 Mb
4.49 Mb
4.495 Mb
4.5 Mb
5 kb

4501 – 4551 Kb

5 kb

4551 – 4601 Kb


yjiZ
yjiM
mdoB
yjiA
dnaC
dnaT
yjiB
bglJ
fhuF
rsmC
holD
rimI
yjiG
prfC
osmY
yjiU
yjiV
yjiW
yjiI
deoC
deoA
deoB
deoD
smp
serB
sms
nadR
yjiK
slt
trpR
gpmB
rob
creA
creB
creC
creD
arcA
yjiY
lasT
4.555 Mb
4.56 Mb
4.565 Mb
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4.6 Mb
5 kb