

1 **Whole genome sequence analysis of 91 *Salmonella***  
2 **Enteritidis isolates from mice caught on poultry farms in the**  
3 **mid 1990s**

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## 21 **Abstract**

22 *Salmonella enterica* serovar Enteritidis (SE), the most commonly reported serovar of  
23 human salmonellosis, has been frequently associated with poultry farms, eggs and egg  
24 products. Mice are known vectors of SE contamination in these facilities. The objective  
25 of this study was to use whole-genome sequencing (WGS) to analyze SE from mice  
26 obtained at poultry farms in Pennsylvania. Documenting pathogen diversity can identify  
27 reliable biomarkers for rapid detection and speed up outbreak investigations. We  
28 sequenced 91 SE isolates from 83 mice (62 spleen isolates, 29 intestinal isolates)  
29 caught at 15 poultry farms between 1995-1998 using an Illumina NextSeq 500. We  
30 identified 742 single nucleotide polymorphisms (SNPs) capable of distinguishing each  
31 isolate from one another. Isolates were divided into two major clades: there were more  
32 SNPs differences within Clade B than counterparts in Clade A. All isolates containing  
33 antimicrobial resistance genes belong to Subgroup B2. Clade-defining SNPs provided  
34 biomarkers distinguishing isolates from 12 individual subgroups, which were separated  
35 by farm location or year of collection. Nonsynonymous changes from the clade-defining  
36 SNPs proffered a better understanding of possible genetic variations among these  
37 isolates. For a broader view of SE diversity, we included data from NCBI Pathogen  
38 Detection Isolates Browser, in which subgroups in Clade B formed new SNP Clusters.

39

## 40 **Importance**

41 WGS and SNPs analyses are excellent and powerful tools for investigating SE  
42 phylogenies. Identifying the evolutionary relationships among SE isolates from mouse,  
43 poultry, environmental, and clinical isolates, along with patterns of genetic diversity,

44 advances understanding of SE and the role mice may play in SE contamination and  
45 spread among poultry population. Our data was able to identify SE isolates from  
46 different farms or years of collection. Moreover, the annotations of clade-defining SNPs  
47 provided information about possible protein functions among these SE isolates from  
48 each subgroup. Clade-defining or farm-unique biomarkers were useful for rapid  
49 detection and outbreak investigations.

50 **Keywords:** *Salmonella*, WGS, phylogenetics, mouse, poultry, egg

## 51 **Introduction**

52 *Salmonella enterica* serovar Enteritidis (SE) is a long-standing public health concern in  
53 the US (1); salmonellosis can result in hospitalization or death of infants, the elderly,  
54 and those with compromised immune systems (2, 3). This pathogen has been strongly  
55 associated with poultry farms, eggs, and egg products (4, 5). In 2010, SE linked to shell  
56 eggs resulted in an outbreak requiring the recall of a half billion eggs  
57 (<https://www.cdc.gov/salmonella/2010/shell-eggs-12-2-10.html>) (6).

58 One of the challenges in resolving foodborne outbreaks associated with SE is the  
59 extreme genomic homogeneity within a specific geographic location or ecology system  
60 and its broad host range (6, 7). Mice are important biological vehicles contributing to SE  
61 dissemination and amplification in chicken houses, especially among laying hens (8, 9).  
62 In fact, SE has been strongly correlated with rodent activity; chickens in caged housing  
63 where mice are present are more likely to carry SE (10). Understanding the evolutionary  
64 relationships among SE isolates from mice, poultry, environmental surfaces, and clinical  
65 cases is important both for outbreak investigations and for identifying strains with  
66 genetic markers for virulence or capacity for rapid host adaptation, such as mutations in  
67 the mismatch repair gene *mutS* that can contribute to rapid evolution in  
68 immunocompromised hosts (11).

69 Whole genome sequencing (WGS) methods have identified variations across otherwise  
70 indistinguishable isolates from eggs and egg products (6, 12), SE associated with reptile  
71 feeder mice (13), *S. Montevideo* from red and black pepper (14). Genome-wide single  
72 nucleotide polymorphisms (SNPs) detected by WGS are considered as the most  
73 valuable genetic markers for investigating the evolutionary relationships among SE

74 homogeneous isolates (1, 7, 15). Application of WGS have also been useful in other  
75 microorganisms, including *E. coli* (16), *Vibrio cholera* (17), and *Staphylococcus aureus*  
76 (18).

77 Importantly, WGS can be also applied to historic isolates, some of which have been  
78 stored for decades. Data from those historic isolates should allow us to understand the  
79 origin and persistence of important traits. In this current project, we sequenced 91 SE  
80 isolated from 82 mice at poultry farms during the 1990s, which lets us to compare both  
81 site and host-adaptions with those of isolates from more recent sampling. Documenting  
82 these genomes and fitting them into large-scale phylogeny projects such as  
83 GenomeTrakr  
84 (<https://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgram>  
85 [WGS/ucm363134.htm](https://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgram)) and NCBI Pathogen Detection Isolates Browser  
86 (<https://www.ncbi.nlm.nih.gov/pathogens/>) will refine our understanding of SE  
87 contamination and spread in poultry facilities (19). Further, identifying and  
88 characterizing biomarkers can facilitate the development of rapid and reliable tests that  
89 could guide appropriate interventions during future outbreaks.

## 90 **Materials and Methods**

### 91 **Bacterial isolates**

92 Ninety-one SE isolates from mouse spleens (n=62) and intestines (n=29), collected  
93 from 15 poultry farms in Pennsylvania during 1995-1998, are listed in Table 1. Among  
94 these isolates, eight pairs were isolated from the spleen and intestine of the same  
95 mouse; these were designated as m1 through m8. These isolates are archived under  
96 Bioproject Number PRJNA186035 (<https://www.ncbi.nlm.nih.gov/bioproject/186035>).

### 97 **Whole genome sequencing and assembly**

98 Genomic DNA was extracted after incubation of culture for 16 hours at 37 °C in  
99 Trypticase Soy Broth (TSB) using the DNeasy Blood and Tissue Kit (Qiagen Inc,  
100 Valencia, CA). Concentrations of DNA were measured using a Qubit 3.0 fluorometer  
101 (Life Technologies, MD). Libraries were prepared according to Nextera XT protocols  
102 and sequenced on the Illumina NextSeq 500 (Illumina, San Diego, CA) using NextSeq  
103 500/550 High Output Kit v2 (300 cycles). Raw reads were assembled *de novo* using  
104 SPAdes software v3.8.2 with default settings (20). We obtained chromosome draft  
105 genomes between 4.69M bps and 4.80M bps. These genomes were annotated using  
106 the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (21).  
107 We selected SE CFSAN051873 (spleen, 1996, Farm VIII) to serve as the reference  
108 genome, using the PacBio platform we obtained a fully closed genome for  
109 CFSAN051873 as follows (22). Genomic DNA was sheared into approximately 20-kb  
110 fragments using g-TUBE (Covaris, Inc., Woburn, MA). The library was prepared based  
111 on the 20-kb PacBio sample preparation protocol and sequenced using P6/C4  
112 chemistry on four single-molecule real-time (SMRT) cells with a 240-min collection time.

113 The continuous long-read data were *de novo* assembled using the PacBio hierarchical  
114 genome assembly process (HGAP version 3.0) with default parameters (23). The  
115 assembled sequence was annotated using PGAP (21).

## 116 **Genomic and phylogenetic analysis**

117 The Fastq data from NextSeq runs were put into the Center for Food Safety and Applied  
118 Nutrition (CFSAN) SNP pipeline v0.8 to create a SNP matrix (24) with SE  
119 CFSAN051873 (CP\_022003.1) as the reference genome. GARLI (Genetic Algorithm for  
120 Rapid Likelihood Inference: <https://code.google.com/archive/p/garli/>) v2.01 (25) was  
121 used to construct maximum-likelihood (ML) phylogenetic trees (ratematrix = 6rate;  
122 ratehetmodel = gamma). Multiple runs were performed (n=100) to ensure that results  
123 were consistent. To estimate support for each node, phylogenies were created for 1,000  
124 bootstrap replicates of the data set from GARLI. Python program SumTrees was used  
125 to generate one consensus tree with bootstrap values at a 70% threshold  
126 (<https://pythonhosted.org/DendroPy/programs/sumtrees.html>) and FigTree v 1.4.3 was  
127 used to export the figures (<http://tree.bio.ed.ac.uk/software/figtree/>). NCBI Pathogen  
128 Detection Isolates Browser (<https://www.ncbi.nlm.nih.gov/pathogens>) was used to show  
129 phylogenetic relationship among SE isolates from broader ranges of geographical  
130 locations and sources. Custom script was used to identify clade-defining SNPs and Tool  
131 for Rapid Annotation of Microbial SNPs (TRAMS) tool to perform annotations on clade-  
132 defining SNPs (26). The pairwise distance matrix, shown as number of SNP differences  
133 among isolates, was calculated using MEGA7 with 1,000 bootstrap iterations (27).

## 134 **Results**

### 135 **Phylogenetic analysis**

#### 136 **Overview**

137 We identified 742 SNPs and generated the maximum-likelihood phylogenetic tree  
138 arising from these SNPs, as depicted in Fig 1. Tree tips were marked using  
139 isolate name, source, year, farm, and NCBI Pathogen Detection Isolates Browser  
140 SNP Cluster. For example, CFSAN051866 was labeled as  
141 CFSAN051866\_spleen\_1996\_FarmVII\_SCA, which provides the following  
142 details: this bacterium was isolated from a mouse spleen in 1996, that mouse  
143 came from Farm VII, and the isolate fits within SNP Cluster A (28), which was  
144 designated according to the NCBI Pathogen Detection Isolates Browser (Table  
145 1). Subgroup names and the number of clade-defining SNPs were labeled on the  
146 internal branches. For example, Subgroup B1 had the most clade-defining SNPs  
147 (179 SNPs), while Subgroup A5 had only 6 clade-defining SNPs.

#### 148 **Phylogenetic Tree Construction**

149 We recognized two major clades: Clades A and B, which further subdivided into  
150 12 subgroups: A1 to A8 and B1 to B4. It was notable that all isolates carrying  
151 antimicrobial resistance genes belonged exclusively to Subgroup B2. Moreover,  
152 isolates in each subgroup had varied ranges of SNP differences. The maximum  
153 SNP differences within Subgroups A1 and A5 were 33 (CFSAN063779 and  
154 CFSAN063803) and 27 (CFSAN063788 and CFSAN063792) SNPs, respectively,  
155 while the maximum number in Subgroup A3 was only 6 SNPs (CFSAN051856  
156 and CFSAN051861). Subgroups A1 and A2 were the two largest subgroups,



157 containing 18 and 17 isolates, respectively. Subgroup B3 only contained two  
158 isolates, as the smallest group in the tree.

### 159 **Impact of Farm**

160 Not all subgroups in each clade showed the same pattern of geographic  
161 distribution, although some subgroups were exclusively comprised of isolates  
162 from a single farm. For example, all Subgroup A2 isolates were from Farm III and  
163 all Subgroup B1 isolates from Farm X. In contrast, Subgroups B3 only contained  
164 two from Farm I; A3 contained isolates from Farms V and VII.

165 Our phylogeny revealed that some isolates from different farms can be grouped  
166 together and were closely related: isolates in Subgroup A3 obtained from Farms  
167 V and VII with few to no SNP differences among them. For example,  
168 CFSAN051854 (CFSAN051854\_spleen(m7)\_1996\_FarmV\_SCA) and  
169 CFSAN051864 (CFSAN051864\_spleen\_1996\_FarmVII\_SCA) where zero SNP  
170 differences were observed (Table S1).

171 Isolates from some farms were only distantly related and, unsurprisingly, our  
172 phylogeny showed these belonging to different subgroups. For example,  
173 Subgroups B1 and B2 both contained isolates from Farm X, indicating that these  
174 were distantly related to the rest of the isolates from our sequencing.

175 There were several cases in which isolates from different farms were found to  
176 belong to the same subgroup: isolates from Subgroup B2, which contained 11  
177 clade-defining SNPs, came from Farms VI, X, and XI. Isolates in Subgroup A3  
178 were found at Farms V and VII, and there were only very small differences  
179 among their SNPs (Table S1).

180 Although isolates in Subgroups A1 and A5 were found at different farms, isolates  
181 from the same farm shared common ancestors. Specifically, all Subgroup A1  
182 isolates were from Farm XII and XV, isolates from Farm XII formed a cluster and  
183 shared a common ancestor, and another common ancestor was shared by all  
184 isolates collected from Farm XV.

### 185 **Impact of Isolation Year**

186 Isolates in each subgroup were collected during the same year, with only two  
187 exceptions: A1 contained isolates from 1995 and 1996, and A5 contained  
188 isolates from 1997 and 1998. In Subgroup A1, isolates from 1995 were grouped  
189 together sharing common ancestor, which also applied to those from 1996 in  
190 Subgroup A1. In another case, all Subgroup A5 isolates were collected from  
191 1998 except CFSAN063788, which was from 1997.

### 192 **Impact of Isolation Organ**

193 As expected, isolates from the same mouse appeared very closely related: SNP  
194 differences ranged from zero (m8) to two SNPs (m2). Most subgroups contained  
195 isolates from both organs. Although Subgroups A4, A8, B3, and B4 only  
196 contained isolates originating from spleens, our phylogenetic analyses did not  
197 reveal any organ-defining SNPs that could be reliably used to distinguish  
198 between SE isolates taken from spleens and those obtained from intestines.

### 199 **Pathogen Detection SNP Cluster analysis**

200 At the time of this research (Dec 7<sup>th</sup>, 2017), the NCBI Pathogen Detection  
201 Isolates Browser (<https://www.ncbi.nlm.nih.gov/pathogens>) contained more than  
202 94,000 *Salmonella enterica* genomes. At the time of our analysis, 86 of our

203 isolates fit into five existing Pathogen Detection SNP Clusters, as follows. All 68  
204 isolates, but CFSAN063803, within our eight Clade A subgroups belonged to one  
205 single Pathogen Detection SNP Cluster, which was designated as SNP Cluster A  
206 (SCA, at the time designated as SCA PDS000002757.323) (28). CFSAN063803  
207 did not fit within any of the established SNP Cluster at that time. The four  
208 subgroups we recognized as Clade B belonged to four different Pathogen  
209 Detection SNP Clusters, which were designated as SCB, SCC, SCD, and SCE,  
210 respectively.

211 The data from Pathogen Detection Isolates Browser matched our phylogenetic  
212 analysis. Among our sequenced isolates, some farms contained isolates that  
213 were distantly related according to Pathogen Detection Isolates Browser data.  
214 For example, isolates collected from mice at Farm I, which we identified as  
215 Subgroups A8, B3, and B4, were members of three existing Pathogen Detection  
216 SNP Clusters: SCA, SCB, and SCC, respectively.

217 Our isolates in SCA had been collected from mice at 12 different farms between  
218 1995 and 1998. However, SCA also encompassed 5,468 genomes already in the  
219 Pathogen Detection Isolates Browser. This provides the opportunity to explore  
220 additional levels of relatedness across SE isolates, as well as identify patterns  
221 across multiple years. For example, in the Pathogen Detection phylogenetic tree,  
222 Subgroup A1 isolates from 1995 shared a common ancestor with SE  
223 NYVetLIRN-37 (Sequence Read Archive (SRA) number: SRR6107632), which  
224 was isolated from dust taken from a poultry coop at Massachusetts in April 2017  
225 (<https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1>

226 124/PDS000002757.351). Another example, SE WAPHL\_SAL-A00192, which  
227 was isolated from an avian source from Washington in 2003, shared a common  
228 ancestor with Subgroup A5 isolates  
229 (<https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1>  
230 [124/PDS000002757.351](https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1)).

231 It was notable that isolates from egg yolk and chicken drag swab appeared  
232 closely related to isolates in Subgroups A4 and A6. For example, SE  
233 CRJJGF\_00137 (egg yolk, 2002, US, SRR1686612) and SE OH-10-18938-5  
234 (chicken drag swab, 2010, Ohio, SRR5278942) were closely related to  
235 CFSAN051834 and CFSAN051835 in Subgroup A4  
236 (<https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1>  
237 [124/PDS000002757.351](https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1)).

238 The SCB (designated at that time as PDS000004690.16) encompassed a total of  
239 24 isolates including those five isolates of our Subgroup B4. These 24 isolates in  
240 SCB were obtained from human, animal, food, and environmental sources in US  
241 and Canada (Figure 2). Within SCB, our Subgroup B4 isolates were clustered  
242 together and shared a most recent common ancestor with five NCBI isolates  
243 collected from human stool (SE PNUSAS011122, US, 2016), turkey (SE  
244 SA19943269, Canada), and chicken drag swab (SE OH-15-14655, OH, US,  
245 2015, SE OH-12-29345, OH, US, 2012 & SE OH-13-28244, OH, US, 2013). The  
246 remaining 14 isolates in SCB formed a separate cluster, these were 13 clinical  
247 isolates and one environmental isolate that all shared a different common

248 ancestor from the rest of SCB. The minimum distance between isolates in SCB  
249 was one SNP while the maximum number was 104.  
250 SCC (designated at that time as PDS000011158.1) consisted of two isolates  
251 from Subgroup B3. No other genomes from Pathogen Detection Isolates Browser  
252 fit within SCC. Similarly, no other NCBI genome fit within SCD (designated at that  
253 time as PDS000011157.1), which contained only Subgroup B1 isolates.  
254 SCE (designated at that time as PDS000004693.11) comprised 20 isolates from  
255 chicken, mouse, and human. These isolates had been collected from the states  
256 of Tennessee, Georgia, and Pennsylvania, in the US. Eight of the Subgroup B2  
257 isolates that fit within SCE shared a common ancestor (Figure 3). Intriguingly, all  
258 SCE isolates, with the exception isolate PNUSAS014592, carried at least one of  
259 following antimicrobial resistance genes: *tetA*, *aadA*, *bla<sub>TEM-1</sub>*  
260 ([https://www.ncbi.nlm.nih.gov/pathogens/isolates#/tree/Salmonella/PDG000000000](https://www.ncbi.nlm.nih.gov/pathogens/isolates#/tree/Salmonella/PDG0000000002.1056/PDS000004693.11/)  
261 [02.1056/PDS000004693.11/](https://www.ncbi.nlm.nih.gov/pathogens/isolates#/tree/Salmonella/PDG0000000002.1056/PDS000004693.11/)).

## 262 **Clade-defining SNPs**

263 We identified clade-defining SNPs and annotations identifying  
264 synonymous/nonsynonymous changes in amino acids, positions in reference  
265 genes, strands, and gene functions are presented in Table 3.

## 266 **Clade A polymorphisms**

267 We identified 11 SNPs that defined Subgroup A1, including seven  
268 nonsynonymous changes, three synonymous changes, and one nonsense  
269 mutation. Type VI secretion protein IcmF (reference locus tag BCA92\_14555)  
270 contained one C to A mutation, which resulted in amino acid changing A to D.

271 Another unique genetic signature change within Subgroup A1 occurred in the  
272 colanic acid synthesis gene *wcaF* (BCA92\_08715), which changed C to T  
273 change. The nonsense mutation resulted in a stop codon which interrupted *hpaE*  
274 (BCA92\_14790), encoding for enzymes involved in catabolism in the aromatic  
275 pathway.

276 In Subgroup A2, which contained isolates exclusively from Farm III, we  
277 discovered 19 clade-defining SNPs, including 16 in coding region. The LysR  
278 family transcriptional regulator (dBCA92\_19265) contained one G to A mutation  
279 resulting in a stop codon.

280 Other notable findings in other subgroups included nonsynonymous mutations in  
281 *zwf* (Subgroup A3, BCA92\_10040, oxidoreductase in glucose metabolism), *asnB*  
282 (Subgroup A3, BCA92\_16545, asparagine synthase B), *ushA* (Subgroups A4&A5  
283 and A6, BCA92\_17470, 5'-nucleotidase), and *frsA* (Subgroup A6, BCA92\_18395,  
284 esterase).

### 285 **Clade B polymorphisms**

286 Among the 179 clade-defining SNPs in Subgroup B1, 146 SNPs were in coding  
287 regions, including 85 nonsynonymous mutations and four nonsense mutations.  
288 Subgroup B2, which contained isolates carrying resistance genes, contained 11  
289 SNPs with nine in coding regions. Among the isolates in Subgroups B3 and B4,  
290 we identified multiple nonsynonymous mutations, including *deoD*  
291 (BCA92\_20135, purine-nucleoside phosphorylase), *cysQ* (BCA92\_20970,  
292 3'(2'),5'-bisphosphate nucleotidase activity and magnesium ion binding), *hisD*  
293 (BCA92\_08915, histidinol dehydrogenase and zinc ion binding), *tolA*

- 294 (BCA92\_16260, cell envelope integrity protein in transporter activity), and *fimH*
- 295 (BCA92\_19875, fimbrial adhesion).

## 296 **Discussion**

297 The dissemination of SE via mice, particularly on poultry farms, is considered to be one  
298 of the most serious threats to poultry industry today (2). Here, we characterized a set of  
299 91 SE that (i) represented two organs in mice that have been associated with  
300 dissemination of SE among poultry and hence to humans, (ii) were isolated at 15 farms  
301 in Pennsylvania during the mid-1990s, which was a time during which few SE isolates  
302 from mice have previously been sequenced, and (iii) analyzed in combination with the  
303 open access NCBI Pathogen Detection Isolates Browser. These steps allow us to  
304 construct a more nuanced picture of SE dissemination during the 1990s, and also  
305 identify connections between historic isolates and current SE phenotypes.

306 Our study demonstrated that WGS not only reliably distinguishes among closely related  
307 SE isolates from mice and trace a genome back to its farm of origin and year of  
308 isolation, but also allows sufficient resolution to distinguish between SE isolates, even  
309 those collected from different organs (spleens and intestines) of individual mouse. In  
310 addition, our analyses showed that (i) isolates carrying antimicrobial resistance genes  
311 formed a separate subgroup, which could indicate a shared mechanism which enables  
312 that feature, (ii) open access WGS database contributes comprehensive perspectives to  
313 our understanding of selected isolates, and (iii) new clade-defining markers and NCBI  
314 Pathogen Detection Isolates Browser SNP Clusters were identified, offering tool with  
315 high resolution in outbreak investigations and rapid detections to identify specific clade  
316 related to certain years or locations.

317 Our results strongly suggested it was possible for unique ecologies of SE to develop on  
318 individual farms, although local adaptation is not inevitable. Farms I and X exemplify this



319 range of possibilities: Farm I exhibited heterogeneous isolates while isolates from Farm  
320 X were shown to be highly similar. Isolates can spread from one location to another in  
321 multiple ways: insects (29), wild birds (30), wild animals (31, 32), and even wind (33)  
322 can move contamination from one place to another. However, among these possible  
323 transmission routes, mice are ubiquitous pests (8-10), and their behaviors may help  
324 shape those unique local ecologies: mice migrate periodically and also defend their  
325 territories. Understanding the genetic relatedness among the SE carried by mice and  
326 the SE found in veterinary, food, and human sampling will help improve safety and  
327 security in poultry industry.

328 **WGS data identified a subgroup consisting exclusively of isolates carrying**  
329 **antimicrobial resistance genes.**

330 Previously, WGS has been used to differentiate drug-resistant *S. enterica* isolates from  
331 different locations, which can exhibit notable differences in resistant-relevant genotypic  
332 and phenotypic characteristics (34). Other research has shown WGS can be valuable in  
333 predicting phenotypic resistance among both *S. enterica* (34, 35) and *E. coli* (36). In the  
334 current study, WGS analyses revealed that all our Subgroup B2 isolates carried *bla*<sub>TEM-1</sub>  
335 and *tetA*. It is possible that Subgroup B2 isolates share specific genetic features that  
336 permit them to obtain and carry antimicrobial resistance genes via horizontal gene  
337 transfer, or make it more likely for those genes to be maintained. For example, bacteria  
338 that carry non-functional Clustered Regularly Interspaced Short Palindromic Repeats  
339 (CRISPR) /cas system could acquire plasmids carrying antimicrobial resistance genes.  
340 Possession of a fully-functioning CRISPR/cas system is reversely correlated with  
341 antimicrobial resistance in bacteria (37-39).

342 **Open access genome databases allow greatly expanded genomic and**  
343 **phylogenetic investigations**

344 In the NCBI Pathogen Detection Isolates Browser, comprehensive data was available  
345 for each genome, including up to 40 columns of detail such as WGS run qualities,  
346 outbreak relatedness, and antimicrobial resistance genotypes. The Browser also  
347 assigns specific cluster ID numbers computed based on SNP distances. Although these  
348 cluster numbers can change as new information is added to the Browser, this feature  
349 allows researchers to quickly identify isolates most closely related to target isolates,  
350 which can assist in recognizing possible connections among clinical illness cases. The  
351 phylogenetic analyses from the Browser were consistent with our phylogenetic tree.  
352 Multiple subgroups in the current study formed distinct SNP Clusters containing isolates  
353 exclusively from our collection, like B3 isolates in SCC. We identified clinical isolates  
354 and poultry related isolates closely related to our isolates, such as SE PNUSAS011122  
355 (human stool, US, 2016) and SE OH-15-14655 (chicken drag swab, OH, US, 2015) with  
356 B4 isolates in SCB. Our data has the potential to bridge surveillance data with long-  
357 term and large-scale genomics and phylogenetics studies (19).

358 **Genetic variations in clade-defining SNPs showed possible unique genotypic and**  
359 **phenotypic features.**

360 Distinctive genetic features are extremely useful for epidemiologic investigations.  
361 Finding such genetic identifiers can help rapidly determine outbreak lineages and  
362 accurately distinguish highly clonal clades (6). The nonsynonymous changes we  
363 identified in this study suggested that a combination of several genetic factors has  
364 facilitated the survival and growth of SE, resulting in different contamination risks for

365 each subgroup. For example, the *icmF* we identified in Subgroup A1 was part of Type  
366 VI Secretion System, which is known to be required for full virulence in mice (40, 41).  
367 Similarly, *fimH* alleles have been associated with the abilities of *Salmonella* to bind onto  
368 avian or mammalian cells (42). Despite the clonal structure of SE, isolates vary greatly  
369 in the ability to contaminate eggs, which is biologically independent of phage types  
370 those isolates belong to (15, 43, 44). The heterogeneity of metabolic profiles in SE  
371 isolates might provide an explanation for the variation in contamination capability (15).  
372 The accumulation of mutations that affect gene function is a significant part of the  
373 process by which *S. enterica* becomes host adapted (45). Such host adaptations may  
374 well be occurring at some of the farms where we collected SE from local mice, with  
375 important consequences for the safety and security of the poultry supply chain. Notably,  
376 serovars Enteritidis, Gallinarum, and Pullorum can circulate within the same farm, and  
377 sometimes within the same bird, as evidenced by field analyses conducted in South  
378 America (46). Therefore, WGS also has potential for detecting evolutionary trends within  
379 SE that could threaten the poultry industry supply chain. Our data also pave the way for  
380 research on poultry pathogenic serovars *S. Gallinarum* and *S. Pullorum*, which diverged  
381 independently from an Enteritidis-like ancestor (3, 47, 48).

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385 and Drug Administration support on this project.

## 386 **Figure Legends**

387

388 Figure 1. Maximum likelihood phylogenetic tree of 91 *S. Enteritidis* isolates from mice  
389 spleens and intestines. We constructed the phylogenetic tree using 742 single  
390 nucleotide polymorphisms (SNPs). All sequenced isolates were divided into two major  
391 clades, Clade A and B, which were further grouped into 12 subgroups.

392

393 Figure 2. Phylogenetic tree of SNP Cluster B (SCB, designated at that time as  
394 PDS000004690.16) from NCBI Pathogen Detection Isolates Browser. The phylogenetic  
395 tree encompassed 24 isolates including our five sequenced isolates belonging to  
396 Subgroup B4.

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398 Figure 3. Phylogenetic tree of SNP Cluster E (SCE, designated at that time as  
399 PDS000004693.11) from NCBI Pathogen Detection Isolates Browser. The phylogenetic  
400 tree encompassed 20 isolates including our eight sequenced isolates belonging to  
401 Subgroup B2.

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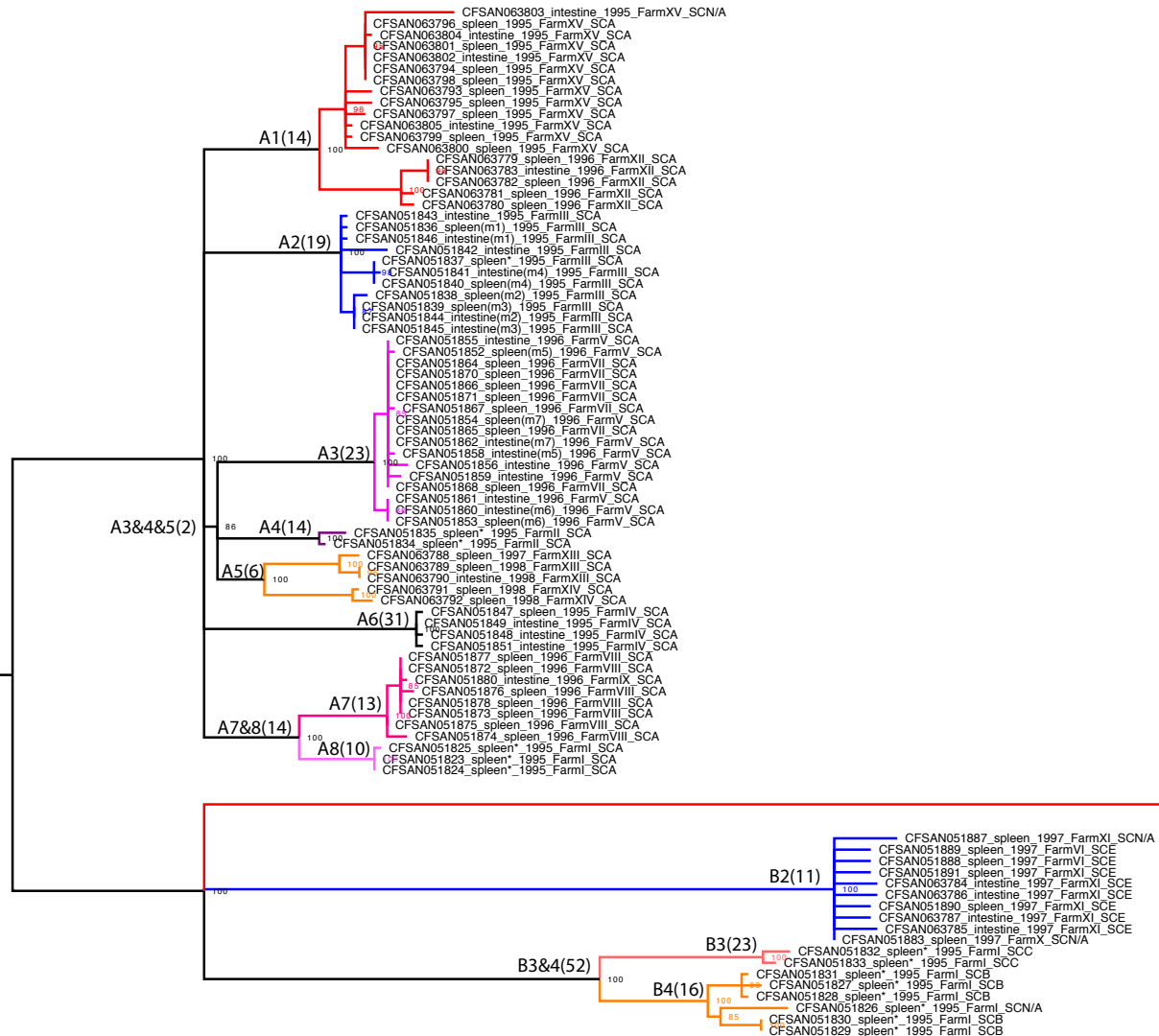


Figure 1. Maximum likelihood phylogenetic tree of 91 *S. Enteritidis* isolates from mice spleens and intestines. We constructed the phylogenetic tree using 742 single nucleotide polymorphisms (SNPs). All sequenced isolates were divided into two major clades, Clade A and B, which were further grouped into 12 subgroups.

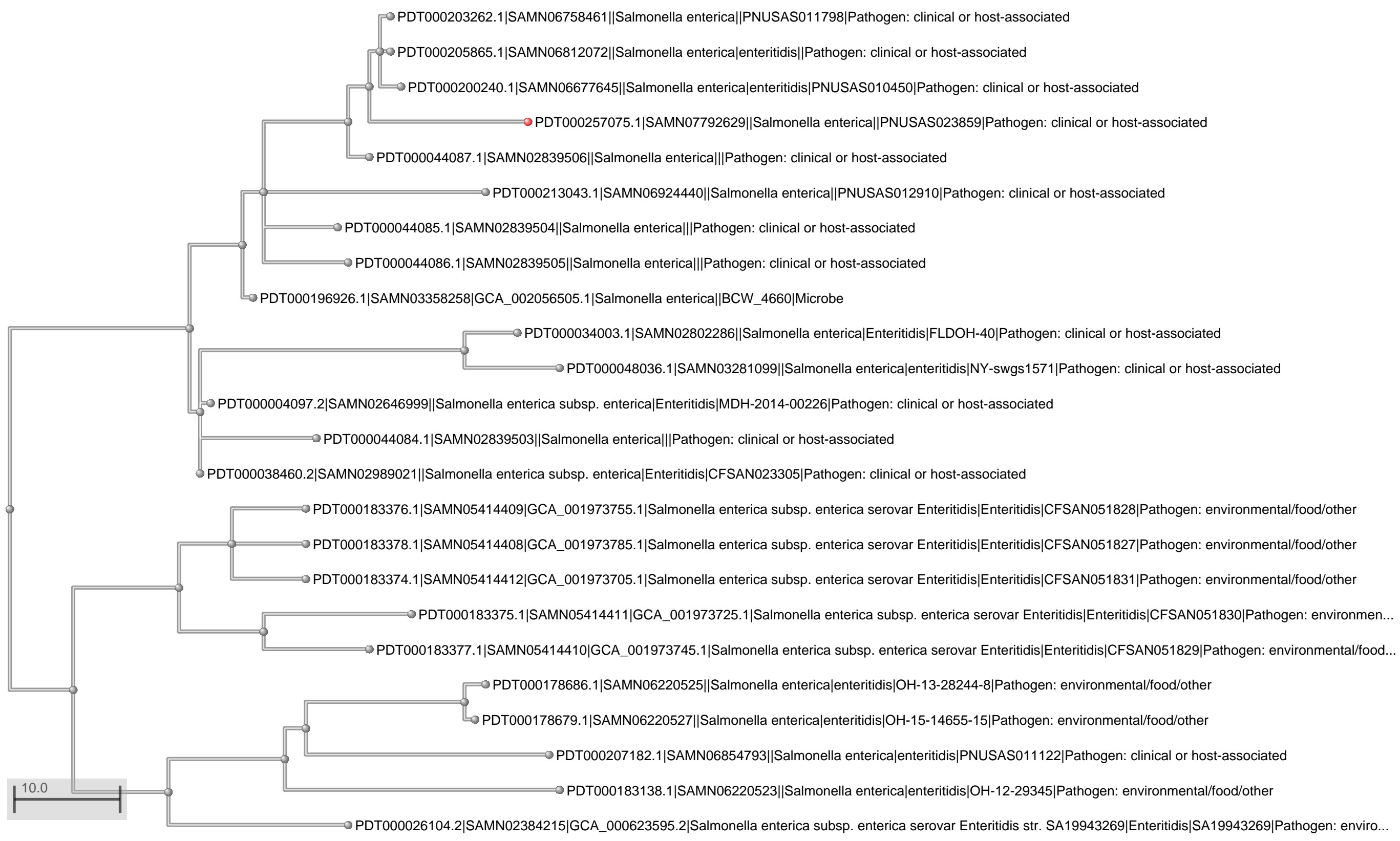




Table 1. The metadata and general genomic information of 91 sequenced *S. Enteritidis* in current study.

Strain	Year	Source	Farm ID	Clade	SNP Cluster	Contig# (>500bp)	AMR Gene	SRA Accession
CFSAN051823	1995	Spleen*	I (ST11)	A8	A	37		SRR5063209
CFSAN051824	1995	Spleen*	I (ST11)	A8	A	36		SRR5063211
CFSAN051825	1995	Spleen*	I (ST11)	A8	A	40		SRR5063208
CFSAN051826	1995	Spleen*	I (ST3632)	B4	N/A	39		SRR5063210
CFSAN051827	1995	Spleen*	I (ST3632)	B4	B	37		SRR5063216
CFSAN051828	1995	Spleen*	I (ST3632)	B4	B	38		SRR5064765
CFSAN051829	1995	Spleen*	I (ST3632)	B4	B	40		SRR5064766
CFSAN051830	1995	Spleen*	I (ST3632)	B4	B	37		SRR5064767
CFSAN051831	1995	Spleen*	I (ST3632)	B4	B	35		SRR5064768
CFSAN051832	1995	Spleen*	I (ST11)	B3	C	38		SRR5064769
CFSAN051833	1995	Spleen*	I (ST11)	B3	C	39		SRR5064773
CFSAN051834	1995	Spleen*	II (ST11)	A4	A	39		SRR5064774
CFSAN051835	1995	Spleen*	II (ST11)	A4	A	36		SRR5064771
CFSAN051836(m1)	1995	Spleen	III (ST11)	A2	A	39		SRR5064772
CFSAN051837	1995	Spleen*	III (ST11)	A2	A	39		SRR5064777
CFSAN051838(m2)	1995	Spleen	III (ST11)	A2	A	38		SRR5064775
CFSAN051839(m3)	1995	Spleen	III (ST11)	A2	A	37		SRR5064776
CFSAN051840(m4)	1995	Spleen	III (ST11)	A2	A	40		SRR5064779
CFSAN051841(m4)	1995	Intestine	III (ST11)	A2	A	39		SRR5064781
CFSAN051842	1995	Intestine	III (ST11)	A2	A	36		SRR5064783
CFSAN051843	1995	Intestine	III (ST11)	A2	A	41		SRR5064782
CFSAN051844(m2)	1995	Intestine	III (ST11)	A2	A	42		SRR5064786
CFSAN051845(m3)	1995	Intestine	III (ST11)	A2	A	40		SRR5064785
CFSAN051846(m1)	1995	Intestine	III (ST11)	A2	A	44		SRR5064787
CFSAN051847	1995	Spleen	IV (ST11)	A6	A	37		SRR5064784
CFSAN051848	1995	Intestine	IV (ST11)	A6	A	58		SRR5064788
CFSAN051849	1995	Intestine	IV (ST11)	A6	A	41		SRR5064792
CFSAN051851	1995	Intestine	IV (ST11)	A6	A	38		SRR5064795

<b>CFSAN051852(m5)</b>	1996	Spleen	V (ST11)	A3	A	40		SRR5064794
<b>CFSAN051853(m6)</b>	1996	Spleen	V (ST11)	A3	A	43		SRR5064790
<b>CFSAN051854(m7)</b>	1996	Spleen	V (ST11)	A3	A	70		SRR5064797
<b>CFSAN051855</b>	1996	Intestine	V (ST11)	A3	A	39		SRR5064802
<b>CFSAN051856</b>	1996	Intestine	V (ST11)	A3	A	38		SRR5064799
<b>CFSAN051858(m5)</b>	1996	Intestine	V (ST11)	A3	A	44		SRR5064800
<b>CFSAN051859</b>	1996	Intestine	V (ST11)	A3	A	45		SRR5064803
<b>CFSAN051860(m6)</b>	1996	Intestine	V (ST11)	A3	A	45		SRR5064804
<b>CFSAN051861</b>	1996	Intestine	V (ST11)	A3	A	39		SRR5064801
<b>CFSAN051862(m7)</b>	1996	Intestine	V (ST11)	A3	A	39		SRR5064806
<b>CFSAN051864</b>	1996	Spleen	VII (ST11)	A3	A	39		SRR5064810
<b>CFSAN051865</b>	1996	Spleen	VII (ST11)	A3	A	40		SRR5064809
<b>CFSAN051866</b>	1996	Spleen	VII (ST11)	A3	A	41		SRR5064812
<b>CFSAN051867</b>	1996	Spleen	VII (ST11)	A3	A	40		SRR5064811
<b>CFSAN051868</b>	1996	Spleen	VII (ST11)	A3	A	37		SRR5064813
<b>CFSAN051870</b>	1996	Spleen	VII (ST11)	A3	A	39		SRR5064814
<b>CFSAN051871</b>	1996	Spleen	VII (ST11)	A3	A	49		SRR5064817
<b>CFSAN051872</b>	1996	Spleen	VIII (ST11)	A7	A	99		SRR5064815
<b>CFSAN051873</b>	1996	Spleen	VIII (ST11)	A7	A	35		SRR5064816
<b>CFSAN051874</b>	1996	Spleen	VIII (ST11)	A7	A	39		SRR5064818
<b>CFSAN051875</b>	1996	Spleen	VIII (ST11)	A7	A	39		SRR5064821
<b>CFSAN051876</b>	1996	Spleen	VIII (ST11)	A7	A	39		SRR5064820
<b>CFSAN051877</b>	1996	Spleen	VIII (ST11)	A7	A	40		SRR5064854
<b>CFSAN051878</b>	1996	Spleen	VIII (ST11)	A7	A	115		SRR5064855
<b>CFSAN051880</b>	1996	Intestine	IX (ST11)	A7	A	40		SRR5064857
<b>CFSAN051881(m8)</b>	1997	Intestine	X (ST11)	B1	D	43		SRR5065189
<b>CFSAN051882</b>	1997	Intestine	X (ST11)	B1	D	39		SRR5065192
<b>CFSAN051883</b>	1997	Spleen	X (ST11)	B2	N/A	46	<i>bla<sub>TEM-1</sub>, tetA</i>	SRR5065190
<b>CFSAN051884</b>	1997	Spleen	X (ST11)	B1	D	40		SRR5065191
<b>CFSAN051885</b>	1997	Spleen	X (ST11)	B1	D	40		SRR5065194
<b>CFSAN051886(m8)</b>	1997	Spleen	X (ST11)	B1	N/A	38		SRR5065196
<b>CFSAN051887</b>	1997	Spleen	XI (ST11)	B2	N/A	40	<i>bla<sub>TEM-1</sub></i>	SRR5065195
<b>CFSAN051888</b>	1997	Spleen	VI (ST11)	B2	E	42	<i>bla<sub>TEM-1</sub>, tetA</i>	SRR5065193

<b>CFSAN051889</b>	1997	Spleen	VI (ST11)	B2	E	53	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5065198
<b>CFSAN051890</b>	1997	Spleen	XI (ST11)	B2	E	42	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5065197
<b>CFSAN051891</b>	1997	Spleen	XI (ST11)	B2	E	41	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5065199
<b>CFSAN063779</b>	1996	Spleen	XII (ST11)	A1	A	15		SRR5884037
<b>CFSAN063780</b>	1996	Spleen	XII (ST11)	A1	A	15		SRR5884036
<b>CFSAN063781</b>	1996	Spleen	XII (ST11)	A1	A	15		SRR5970532
<b>CFSAN063782</b>	1996	Spleen	XII (ST11)	A1	A	14		SRR5884033
<b>CFSAN063783</b>	1996	Intestine	XII (ST11)	A1	A	14		SRR5884041
<b>CFSAN063784</b>	1997	Intestine	XI (ST11)	B2	E	32	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5884042
<b>CFSAN063785</b>	1997	Intestine	XI (ST11)	B2	E	28	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5884050
<b>CFSAN063786</b>	1997	Intestine	XI (ST11)	B2	E	28	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5884043
<b>CFSAN063787</b>	1997	Intestine	XI (ST11)	B2	E	29	<i>bla</i> <sub>TEM-1</sub> , <i>tetA</i>	SRR5884057
<b>CFSAN063788</b>	1997	Spleen	XIII (ST11)	A5	A	22		SRR5819771
<b>CFSAN063789</b>	1998	Spleen	XIII (ST11)	A5	A	18		SRR5819768
<b>CFSAN063790</b>	1998	Intestine	XIII (ST11)	A5	A	78		SRR5819773
<b>CFSAN063791</b>	1998	Spleen	XIV (ST11)	A5	A	18		SRR5819769
<b>CFSAN063792</b>	1998	Spleen	XIV (ST11)	A5	A	20		SRR5819774
<b>CFSAN063793</b>	1995	Spleen	XV (ST11)	A1	A	17		SRR5819770
<b>CFSAN063794</b>	1995	Spleen	XV (ST11)	A1	A	14		SRR5819775
<b>CFSAN063795</b>	1995	Spleen	XV (ST11)	A1	A	14		SRR5819777
<b>CFSAN063796</b>	1995	Spleen	XV (ST11)	A1	A	15		SRR5819776
<b>CFSAN063797</b>	1995	Spleen	XV (ST11)	A1	A	15		SRR5819784
<b>CFSAN063798</b>	1995	Spleen	XV (ST11)	A1	A	18		SRR5819788
<b>CFSAN063799</b>	1995	Spleen	XV (ST11)	A1	A	14		SRR5819790
<b>CFSAN063800</b>	1995	Spleen	XV (ST11)	A1	A	15		SRR5819779
<b>CFSAN063801</b>	1995	Spleen	XV (ST11)	A1	A	19		SRR5819785
<b>CFSAN063802</b>	1995	Intestine	XV (ST11)	A1	A	25		SRR5819789
<b>CFSAN063803</b>	1995	Intestine	XV (ST11)	A1	N/A	30		SRR5819781
<b>CFSAN063804</b>	1995	Intestine	XV (ST11)	A1	A	47		SRR5819782
<b>CFSAN063805</b>	1995	Intestine	XV (ST11)	A1	A	25		SRR5819778

Spleen\* Spleen cultured but intestine not cultured, unknown about intestine.



SNP Cluster Number  
A PDS000002757.323  
B PDS000004690.16  
C PDS000011158.1  
D PDS000011157.1  
E PDS000004693.11

Table 2. The number of SNP differences (standard deviation) between 12 subgroups.

	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>A4</b>	<b>A5</b>	<b>A6</b>	<b>A7</b>	<b>A8</b>	<b>B1</b>	<b>B2</b>	<b>B3</b>
<b>A2</b>	44(5)										
<b>A3</b>	50(6)	45(6)									
<b>A4</b>	40(5)	36(5)	39(6)								
<b>A5</b>	44(5)	40(5)	43(6)	32(5)							
<b>A6</b>	54(6)	50(6)	55(7)	46(6)	50(6)						
<b>A7</b>	51(6)	47(6)	52(6)	43(6)	47(6)	57(7)					
<b>A8</b>	50(6)	45(6)	51(6)	41(6)	45(6)	55(7)	24(5)				
<b>B1</b>	92(8)	90(8)	95(8)	86(8)	90(8)	99(8)	97(8)	95(8)			
<b>B2</b>	84(8)	82(8)	87(8)	78(8)	82(8)	91(8)	89(8)	87(8)	33(5)		
<b>B3</b>	137(9)	135(9)	140(10)	131(9)	135(9)	144(10)	142(10)	140(10)	86(8)	78(8)	
<b>B4</b>	133(9)	131(9)	137(9)	127(9)	132(9)	141(10)	138(10)	137(9)	83(8)	75(7)	46(6)

Table 3 Variable genes observed that define subgroups in phylogenetic tree of *S. Enteritidis*

Location	Accession	Annotation	Locus_tag	Gene	Positions in coding	Nucleotide change	Amino acid change	Synonymous / Nonsynonymous	Strand	Product name
<i>A1 (18 samples / 14 SNPs)</i>										
66674	NZ_CP022003.1	coding	BCA92_00310		2562	TCG -> TCA	S -> S	S	+	transcriptional regulator
292426	NZ_CP022003.1	coding	BCA92_01355		84	CGT -> TGT	R -> C	N	-	methyl-accepting chemotaxis protein II
484015	NZ_CP022003.1	coding	BCA92_02350		689	GAC -> GGC	D -> G	N	-	50S ribosomal protein L11 methyltransferase
1081137	NZ_CP022003.1	coding	BCA92_05455		705	GCC -> GTC	A -> V	N	-	ribonucleoside-diphosphate reductase subunit alpha
1327257	NZ_CP022003.1	coding	BCA92_06615		181	CTG -> TTG	L -> L	S	+	IMP dehydrogenase
1408128	NZ_CP022003.1	coding	BCA92_07015		323	CCT -> CTT	P -> L	N	-	glucose-specific phosphotransferase enzyme IIA component
1648417	NZ_CP022003.1	coding	BCA92_08220		490	CTG -> CTA	L -> L	S	-	hypothetical protein
1755377	NZ_CP022003.1	coding	BCA92_08715	<i>wcaF</i>	251	GCT -> GTT	A -> V	N	+	colanic acid biosynthesis acetyltransferase WcaF
2549901	NZ_CP022003.1	intergenic								
2732526	NZ_CP022003.1	intergenic								
2803507	NZ_CP022003.1	coding	BCA92_14555		512	GCC -> GAC	A -> D	N	+	type VI secretion protein IcmF
2845163	NZ_CP022003.1	coding	BCA92_14790	<i>hpaE</i>	1001	TGG -> TAG	W -> *	nonsense	-	5-carboxymethyl-2-hydroxyruconate semialdehyde dehydrogenase
3031053	NZ_CP022003.1	coding	BCA92_15710		399	GCG -> ACG	A -> T	N	-	glutathione ABC transporter permease
3837497	NZ_CP022003.1	intergenic								
<i>A2 (11 samples / 19 SNPs)</i>										
92535	NZ_CP022003.1	coding	BCA92_00440		33	CAG -> CAC	Q -> H	N	+	alpha-xylosidase
444373	NZ_CP022003.1	coding	BCA92_02080		96	CGC -> CGT	R -> R	S	+	30S ribosomal protein S19
584188	NZ_CP022003.1	intergenic								
586670	NZ_CP022003.1	coding	BCA92_02880	<i>pnp</i>	410	GCG -> GTG	A -> V	N	+	polyribonucleotide nucleotidyltransferase
635474	NZ_CP022003.1	intergenic								
847071	NZ_CP022003.1	coding	BCA92_04270		138	CGC -> CGT	R -> R	S	+	transcriptional regulator
901519	NZ_CP022003.1	coding	BCA92_04520	<i>fucl</i>	525	GAA -> AAA	E -> K	N	-	L-fucose isomerase
1033097	NZ_CP022003.1	coding	BCA92_05185		376	CAG -> CAA	Q -> Q	S	-	hydrogenase formation protein HypD
1156296	NZ_CP022003.1	coding	BCA92_05925		193	CGA -> AGA	R -> R	S	+	late control protein D
1384377	NZ_CP022003.1	coding	BCA92_06875		1098	GCG -> GCT	A -> A	S	+	alcohol dehydrogenase EutG
1724432	NZ_CP022003.1	coding	BCA92_08600		263	TGG -> TAG	W -> *	nonsense	-	DNA-binding response regulator
2294640	NZ_CP022003.1	coding	BCA92_11725		124	GCC -> GCA	A -> A	S	-	hydrogenase formation protein
2852680	NZ_CP022003.1	coding	BCA92_14830		1189	GAT -> AAT	D -> N	N	+	two-component sensor histidine kinase
3172034	NZ_CP022003.1	coding	BCA92_16425		826	CGG -> TGG	R -> W	N	+	two-component sensor histidine kinase
3749568	NZ_CP022003.1	coding	BCA92_19265		307	TGG -> TGA	W -> *	nonsense	-	LysR family transcriptional regulator
3782361	NZ_CP022003.1	coding	BCA92_19405	<i>leuC</i>	181	ATG -> GTG	M -> V	N	+	3-isopropylmalate dehydratase large subunit
3976348	NZ_CP022003.1	coding	BCA92_20320		1523	CGT -> CCT	R -> p	N	-	methyl-accepting chemotaxis protein II
4629508	NZ_CP022003.1	intergenic								
4645676	NZ_CP022003.1	coding	BCA92_23640		998	AAC -> AGC	N -> S	N	+	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis enzyme MnmG
<i>A3 (17 samples / 23 SNPs)</i>										
499284	NZ_CP022003.1	coding	BCA92_02430		1084	CTG -> TTG	L -> L	S	+	DUF3971 domain-containing protein
537836	NZ_CP022003.1	coding	BCA92_02620		323	CGC -> CAC	R -> H	N	-	glutamate synthase small subunit
943216	NZ_CP022003.1	intergenic								
983144	NZ_CP022003.1	coding	BCA92_04915	<i>mutS</i>	892	AAC -> AAT	N -> N	S	-	DNA mismatch repair protein MutS
1175265	NZ_CP022003.1	coding	BCA92_05960		312	CTC -> TTC	L -> F	N	-	SsrA-binding protein
1270601	NZ_CP022003.1	coding	BCA92_06430		87	CTG -> CTA	L -> L	S	+	IscS subfamily cysteine desulfurase
1407550	NZ_CP022003.1	coding	BCA92_07010		148	GAT -> TAT	D -> Y	N	+	cytoplasmic protein
1532601	NZ_CP022003.1	intergenic								
1991481	NZ_CP022003.1	coding	BCA92_10040		729	TTT -> CTT	F -> L	N	-	50S ribosomal protein L16 arginine hydroxylase

2029562	NZ_CP022003.1	intergenic									
2407662	NZ_CP022003.1	intergenic									
2699062	NZ_CP022003.1	coding	BCA92_13935	<i>zwf</i>	794	CAG -> CTG	Q -> L	N	-	glucose-6-phosphate dehydrogenase	
2725497	NZ_CP022003.1	coding	BCA92_14075		1134	GTT -> TTT	V -> F	N	-	glycoside hydrolase 105 family protein	
3155979	NZ_CP022003.1	intergenic									
3174194	NZ_CP022003.1	coding	BCA92_16430		329	GCG -> GAG	A -> E	N	+	DNA-binding response regulator	
3201347	NZ_CP022003.1	coding	BCA92_16545	<i>asnB</i>	854	CAG -> CTG	Q -> L	N	+	asparagine synthase B	
3211689	NZ_CP022003.1	coding	BCA92_16625		429	CTG -> CTA	L -> L	S	+	glutamate/aspartate ABC transporter substrate-binding protein	
3660045	NZ_CP022003.1	intergenic									
4138301	NZ_CP022003.1	coding	BCA92_21210		1020	CAG -> TAG	Q -> *	nonsense	-	GTPase HflX	
4159701	NZ_CP022003.1	coding	BCA92_21310		175	CGT -> TGT	R -> C	N	+	succinate dehydrogenase/fumarate reductase iron-sulfur subunit	
4290798	NZ_CP022003.1	intergenic									
4330100	NZ_CP022003.1	intergenic									
4403772	NZ_CP022003.1	coding	BCA92_22425	<i>gldA</i>	1066	GAC -> AAC	D -> N	N	+	glycerol dehydrogenase	
<i>A3&amp;4&amp;5 (24 samples / 2 SNPs)</i>											
3378656	NZ_CP022003.1	pseudogene	BCA92_17470	<i>ushA</i>	491	GTG -> GGG	V -> G	N	-	bifunctional UDP-sugar hydrolase/5'-nucleotidase	
4325151	NZ_CP022003.1	intergenic									
<i>A4 (2 samples / 14 SNPs)</i>											
272586	NZ_CP022003.1	coding	BCA92_01265		1197	GGC -> GGA	G -> G	S	+	phosphoesterase PA-phosphatase	
366931	NZ_CP022003.1	coding	BCA92_01675		1925	GCC -> GTC	A -> V	N	+	4-alpha-glucanotransferase	
889829	NZ_CP022003.1	intergenic									
1371258	NZ_CP022003.1	coding	BCA92_06810	<i>tkt</i>	210	TCT -> CCT	S -> P	N	-	transketolase	
1447810	NZ_CP022003.1	intergenic									
1544666	NZ_CP022003.1	coding	BCA92_07710		302	CCA -> CTA	P -> L	N	+	2-succinyl-6-hydroxy-2, 4-cyclohexadiene-1-carboxylate synthase	
1995406	NZ_CP022003.1	coding	BCA92_10055		1191	GCC -> TCC	A -> S	N	-	adenylosuccinate lyase	
2438031	NZ_CP022003.1	intergenic									
2745105	NZ_CP022003.1	intergenic									
2797811	NZ_CP022003.1	coding	BCA92_14515		234	CGG -> TGG	R -> W	N	-	hypothetical protein	
2980813	NZ_CP022003.1	coding	BCA92_15430		1526	TTA -> TGA	L -> *	nonsense	-	ATP-dependent endonuclease	
3172203	NZ_CP022003.1	coding	BCA92_16425		995	GCC -> GTC	A -> V	N	+	two-component sensor histidine kinase	
3760541	NZ_CP022003.1	coding	BCA92_19310	<i>ddl</i>	819	ATT -> GTT	I -> V	N	-	D-alanine--D-alanine ligase	
3791617	NZ_CP022003.1	coding	BCA92_19450		530	GAG -> GTG	E -> V	N	-	arabinose operon regulatory protein	
<i>A5 (5 samples / 6 SNPs)</i>											
1143752	NZ_CP022003.1	coding	BCA92_05850		94	AGG -> AGT	R -> S	N	-	hypothetical protein	
1344925	NZ_CP022003.1	coding	BCA92_06700		1216	CTG -> CTA	L -> L	S	-	beta-barrel assembly-enhancing protease	
1629966	NZ_CP022003.1	coding	BCA92_08125		1203	GCA -> ACA	A -> T	N	-	microcin C ABC transporter ATP-binding protein YejF	
3172160	NZ_CP022003.1	coding	BCA92_16425		952	ACC -> CCC	T -> P	N	+	two-component sensor histidine kinase	
3471679	NZ_CP022003.1	coding	BCA92_17940		67	TTA -> TTG	L -> L	S	-	tRNA guanosine(34) transglycosylase Tgt	
3775456	NZ_CP022003.1	coding	BCA92_19375		256	GGA -> GGT	G -> G	S	-	acetolactate synthase small subunit	
<i>A6 (4 samples / 31 SNPs)</i>											
84482	NZ_CP022003.1	coding	BCA92_00390		2709	GCT -> ACT	A -> T	N	-	intestinal colonization autotransporter adhesin Misl	
534862	NZ_CP022003.1	coding	BCA92_02610		28	TCA -> TCG	S -> S	S	-	cytosine permease	
624925	NZ_CP022003.1	coding	BCA92_03070	<i>pf1B</i>	1937	CGC -> CAC	R -> H	N	+	formate acetyltransferase	
642429	NZ_CP022003.1	coding	BCA92_03180		198	TCG -> TCT	S -> S	S	+	ribosomal RNA large subunit methyltransferase G	
681388	NZ_CP022003.1	coding	BCA92_03360		595	CTG -> CTA	L -> L	S	-	hypothetical protein	
764440	NZ_CP022003.1	coding	BCA92_03790		159	GAC -> GAT	D -> D	S	+	hypothetical protein	
774876	NZ_CP022003.1	coding	BCA92_03855		79	GGA -> GGG	G -> G	S	-	16S rRNA (uracil(1498)-N(3))-methyltransferase	
812123	NZ_CP022003.1	coding	BCA92_04070		76	ACG -> TCG	T -> S	N	+	2-octaprenyl-6-methoxyphenyl hydroxylase	
1172189	NZ_CP022003.1	coding	BCA92_05955		9325	GGT -> GGA	G -> G	S	-	Ig-like domain repeat protein	

1210085	NZ_CP022003.1	coding	BCA92_06140	870	TTG -> CTG	L -> L	S	-	protein acetyltransferase
1721423	NZ_CP022003.1	coding	BCA92_08580	1563	CTC -> ATC	L -> I	N	-	hypothetical protein
1740362	NZ_CP022003.1	coding	BCA92_08645	2453	CAC -> AAC	H -> N	N	-	diguanylate cyclase/phosphodiesterase
2210643	NZ_CP022003.1	intergenic							
2356200	NZ_CP022003.1	coding	BCA92_12000	492	GTC -> ATC	V -> I	N	-	colanic acid/biofilm transcriptional regulator
2464926	NZ_CP022003.1	coding	BCA92_12610	165	GCC -> GCA	A -> A	S	+	oxidoreductase
2521018	NZ_CP022003.1	coding	BCA92_12900	<i>trpB</i> 1178	GCG -> GAG	A -> E	N	+	tryptophan synthase subunit beta
2676025	NZ_CP022003.1	intergenic							
3109364	NZ_CP022003.1	coding	BCA92_16080	766	GTG -> TTG	V -> L	N	+	molybdenum-dependent transcriptional regulator
3171711	NZ_CP022003.1	coding	BCA92_16425	503	GAT -> GCT	D -> A	N	+	two-component sensor histidine kinase
3211370	NZ_CP022003.1	coding	BCA92_16625	110	AGC -> AAC	S -> N	N	+	glutamate/aspartate ABC transporter substrate-binding protein
3289174	NZ_CP022003.1	coding	BCA92_17010	656	GGC -> GAC	G -> D	N	+	iron-enterobactin transporter permease
3353022	NZ_CP022003.1	coding	BCA92_17345	587	ATC -> ACC	I -> T	N	-	2-hydroxy-3-oxopropionate reductase
3371497	NZ_CP022003.1	coding	BCA92_17435	385	CTC -> TTC	L -> F	N	+	paraslipin
3378738	NZ_CP022003.1	pseudogene	BCA92_17470	<i>ushA</i> 573	GAG -> TAG	E -> *	nonsense	-	bifunctional UDP-sugar hydrolase/5'-nucleotidase
3556348	NZ_CP022003.1	coding	BCA92_18320	340	CAG -> CAT	Q -> H	N	-	LysR family transcriptional regulator
3569685	NZ_CP022003.1	coding	BCA92_18395	<i>frsA</i> 701	ACC -> ATC	T -> I	N	-	esterase
3692990	NZ_CP022003.1	coding	BCA92_18995	109	GTC -> ATC	V -> I	N	+	RNA 2',3'-cyclic phosphodiesterase
3819853	NZ_CP022003.1	coding	BCA92_19590	5	CAT -> CGT	H -> R	N	-	MFS transporter
4252918	NZ_CP022003.1	coding	BCA92_21725	10483	GGT -> GGC	G -> G	S	-	Ig-like domain repeat protein
4299506	NZ_CP022003.1	intergenic							
4471528	NZ_CP022003.1	coding	BCA92_22755	278	GGT -> GAT	G -> D	N	+	rhamnulokinase

#### A7 (8 samples / 13 SNPs)

212601	NZ_CP022003.1	coding	BCA92_01020	575	AAC -> AAC	N -> N	Same as referenc	+	fimbrial assembly protein
738389	NZ_CP022003.1	coding	BCA92_03650	695	GGT -> GGT	G -> G	Same as referenc	+	amidohydrolase
900194	NZ_CP022003.1	coding	BCA92_04515	719	GCG -> GCG	A -> A	Same as referenc	-	L-fuculokinase
1392314	NZ_CP022003.1	coding	BCA92_06920	89	CAT -> CAT	H -> H	Same as referenc	-	hypothetical protein
1662042	NZ_CP022003.1	coding	BCA92_08285	671	GAC -> GAC	D -> D	Same as referenc	+	DNA-binding transcriptional regulator GalS
1710502	NZ_CP022003.1	coding	BCA92_08530	405	CCT -> CCT	P -> P	Same as referenc	+	GntR family transcriptional regulator
2972241	NZ_CP022003.1	coding	BCA92_15395	410	GGC -> GGC	G -> G	Same as referenc	-	ATP-dependent Clp protease ATP-binding subunit ClpA
3059259	NZ_CP022003.1	coding	BCA92_15835	1	GTG -> GTG	V -> V	Same as referenc	+	mechanosensitive channel protein
3292066	NZ_CP022003.1	coding	BCA92_17025	<i>entF</i> 329	GGC -> GGC	G -> G	Same as referenc	-	non-ribosomal peptide synthetase
3302049	NZ_CP022003.1	coding	BCA92_17060	323	GCT -> GCT	A -> A	Same as referenc	-	DNA-binding transcriptional regulator RamA
3400882	NZ_CP022003.1	coding	BCA92_17570	926	CTA -> CTA	L -> L	Same as referenc	+	efflux transporter periplasmic adaptor subunit
3622599	NZ_CP022003.1	coding	BCA92_18690	75	GAC -> GAC	D -> D	Same as referenc	+	Rcs stress response system protein RcsF
3917407	NZ_CP022003.1	coding	BCA92_20020	196	CCC -> CCC	P -> P	Same as referenc	+	fimbrial protein SthA

#### A7&8 (11 samples / 15SNPs)

47216	NZ_CP022003.1	coding	BCA92_00220	182	TCC -> TCC	S -> S	Same as referenc	-	hypothetical protein
47216	NZ_CP022003.1	coding	BCA92_00225	4	GAC -> GAC	D -> D	Same as referenc	+	ilvB operon leader peptide IvbL
742751	NZ_CP022003.1	intergenic							
1061287	NZ_CP022003.1	coding	BCA92_05350	85	TTG -> TTG	L -> L	Same as referenc	+	alanine--tRNA ligase
1593242	NZ_CP022003.1	intergenic							
2589729	NZ_CP022003.1	coding	BCA92_13275	<i>cydB</i> 580	AGC -> AGC	S -> S	Same as referenc	+	cytochrome d ubiquinol oxidase subunit II
2597837	NZ_CP022003.1	coding	BCA92_13315	828	TGT -> TGT	C -> C	Same as referenc	+	K+/H+ antiporter
2832926	NZ_CP022003.1	coding	BCA92_14720	244	TAT -> TAT	Y -> Y	Same as referenc	-	protein-disulfide reductase
3064961	NZ_CP022003.1	coding	BCA92_15860	<i>dinG</i> 388	AGA -> AGA	R -> R	Same as referenc	-	ATP-dependent DNA helicase DinG
3124704	NZ_CP022003.1	coding	BCA92_16160	699	GTA -> GTA	V -> V	Same as referenc	-	cation transporter
3325008	NZ_CP022003.1	coding	BCA92_17205	198	GCC -> GCC	A -> A	Same as referenc	-	outer membrane usher protein
3378960	NZ_CP022003.1	pseudogene	BCA92_17470	<i>ushA</i> 795	TAG -> TAG	*	Same as referenc	-	bifunctional UDP-sugar hydrolase/5'-nucleotidase
3496206	NZ_CP022003.1	coding	BCA92_18055	14	CCC -> CCC	P -> P	Same as referenc	-	anti-RssB factor
3917922	NZ_CP022003.1	coding	BCA92_20025	95	GGC -> GGC	G -> G	Same as referenc	+	fimbrial assembly protein



1132290	NZ_CP022003.1	coding	BCA92_05765	193	TGC -> CGC	C -> R	N	+	DinI family protein	
1153814	NZ_CP022003.1	coding	BCA92_05915	997	TGC -> GGC	C -> G	N	+	phage tail tape measure protein	
1165234	NZ_CP022003.1	coding	BCA92_05955	2370	ATC -> CTC	I -> L	N	-	Ig-like domain repeat protein	
1167383	NZ_CP022003.1	coding	BCA92_05955	4519	TGG -> TGT	W -> C	N	-	Ig-like domain repeat protein	
1186125	NZ_CP022003.1	coding	BCA92_06035	<i>trmD</i>	294	GGC -> GGA	G -> G	S	+	tRNA (guanosine(37)-N1)-methyltransferase TrmD
1189044	NZ_CP022003.1	intergenic								
1189098	NZ_CP022003.1	intergenic								
1221880	NZ_CP022003.1	coding	BCA92_06205	<i>rpoE</i>	128	TCG -> TTG	S -> L	N	+	ECF RNA polymerase sigma-E factor
1255004	NZ_CP022003.1	intergenic								
1263328	NZ_CP022003.1	coding	BCA92_06390	384	TTT -> TTG	F -> L	N	+	nickel transporter	
1289858	NZ_CP022003.1	coding	BCA92_06505	1912	TCG -> GCG	S -> A	N	+	dimethyl sulfoxide reductase subunit A	
1365643	NZ_CP022003.1	coding	BCA92_06785	671	GCG -> GTG	A -> V	N	+	oxidoreductase FeS-binding subunit	
1380936	NZ_CP022003.1	coding	BCA92_06860	211	CAT -> TAT	H -> Y	N	+	ethanolamine utilization protein EutN	
1387797	NZ_CP022003.1	coding	BCA92_06890	573	GGC -> GGA	G -> G	S	+	ethanolamine ammonia-lyase heavy chain	
1397007	NZ_CP022003.1	coding	BCA92_06950	855	GGA -> GGC	G -> G	S	+	iron-dependent peroxidase	
1477635	NZ_CP022003.1	intergenic								
1485223	NZ_CP022003.1	coding	BCA92_07425	1107	TCC -> TCT	S -> S	S	+	amidophosphoribosyltransferase	
1524616	NZ_CP022003.1	coding	BCA92_07630	621	TCC -> TCT	S -> S	S	+	NADH-quinone oxidoreductase subunit F	
1564854	NZ_CP022003.1	coding	BCA92_07815	632	ACA -> ATA	T -> I	N	-	type III secretion system effector deubiquitinase SseL	
1612441	NZ_CP022003.1	coding	BCA92_08025	936	CGT -> CGC	R -> R	S	+	cytochrome c biogenesis protein CcmH	
1646199	NZ_CP022003.1	coding	BCA92_08210	961	ACC -> GCC	T -> A	N	+	PTS fructose transporter subunit EHBC	
1660947	NZ_CP022003.1	coding	BCA92_08280	868	CCG -> TCG	P -> S	N	+	DUF418 family protein	
1697466	NZ_CP022003.1	coding	BCA92_08465	130	TTT -> TTC	F -> F	S	-	lipoprotein	
1722080	NZ_CP022003.1	coding	BCA92_08585	98	GGA -> GAA	G -> E	N	-	hypothetical protein	
1753620	NZ_CP022003.1	coding	BCA92_08705	<i>wcaD</i>	483	AAC -> AAT	N -> N	S	+	putative colanic acid polymerase WcaD
1771599	NZ_CP022003.1	coding	BCA92_08780	<i>rfbB</i>	781	AAC -> CAC	N -> H	N	+	dTDP-glucose 4,6-dehydratase
1771638	NZ_CP022003.1	coding	BCA92_08780	<i>rfbB</i>	820	TGT -> AGT	C -> S	N	+	dTDP-glucose 4,6-dehydratase
1781330	NZ_CP022003.1	coding	BCA92_08830	898	AGA -> GGA	R -> G	N	+	transporter	
1795919	NZ_CP022003.1	coding	BCA92_08895	<i>hisA</i>	703	ACC -> ACT	T -> T	S	-	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
1809044	NZ_CP022003.1	coding	BCA92_08950	2335	TCA -> TCG	S -> S	S	-	E3 ubiquitin--protein ligase	
1809044	NZ_CP022003.1	coding	BCA92_08955	57	CCT -> CCC	P -> P	S	+	hypothetical protein	
1811339	NZ_CP022003.1	coding	BCA92_08965	1662	GAA -> GAG	E -> E	S	+	thiosulfate reductase	
1813310	NZ_CP022003.1	intergenic								
1822810	NZ_CP022003.1	coding	BCA92_09030	923	GAC -> GCC	D -> A	N	-	propanediol utilization protein	
1854608	NZ_CP022003.1	tRNA	BCA92_09220	53	N/A	N/A	N/A	N/A	tRNA-Asn	
1891422	NZ_CP022003.1	coding	BCA92_09450	25	CCG -> CCA	P -> P	S	-	recombinase	
1905319	NZ_CP022003.1	intergenic								
1923249	NZ_CP022003.1	intergenic								
1925726	NZ_CP022003.1	coding	BCA92_09700	<i>grxB</i>	636	GTT -> ATT	V -> I	S	-	glutaredoxin 2
1979211	NZ_CP022003.1	coding	BCA92_09980	612	CAT -> CAC	H -> H	N	+	lipoprotein-releasing system ATP-binding protein LolD	
2005269	NZ_CP022003.1	intergenic								
2010154	NZ_CP022003.1	intergenic								
2011688	NZ_CP022003.1	coding	BCA92_10165	636	GGC -> GGT	G -> G	S	+	hypothetical protein	
2084889	NZ_CP022003.1	coding	BCA92_10605	718	TGG -> TGA	W -> *	nonsense	-	L-cystine transporter	
2111961	NZ_CP022003.1	coding	BCA92_10755	108	ACT -> ACC	T -> T	S	+	phosphoenolpyruvate synthase	
2127652	NZ_CP022003.1	coding	BCA92_10815	469	ATC -> ATA	I -> I	S	-	MFS transporter	
2174741	NZ_CP022003.1	coding	BCA92_11065	267	CCA -> CCG	P -> P	S	+	EscJ/YscJ/HrcJ family type III secretion inner membrane ring protein	
2190356	NZ_CP022003.1	coding	BCA92_11160	486	AGC -> GGC	S -> G	N	-	Bcr/CfiA family drug resistance efflux transporter	
2198111	NZ_CP022003.1	coding	BCA92_11210	987	CCT -> TCT	P -> S	N	-	alkene reductase	
2216104	NZ_CP022003.1	coding	BCA92_11315	337	GCC -> GCT	A -> A	S	-	electron transport complex subunit R <sub>sxC</sub>	
2216131	NZ_CP022003.1	coding	BCA92_11315	364	GAT -> GAC	D -> D	S	-	electron transport complex subunit R <sub>sxC</sub>	
2216191	NZ_CP022003.1	coding	BCA92_11315	424	GCT -> GCC	A -> A	S	-	electron transport complex subunit R <sub>sxC</sub>	

2232095	NZ_CP022003.1	coding	BCA92_11390	182	TGG -> TAG	W -> *	nonsense	-	amidohydrolase
2254370	NZ_CP022003.1	coding	BCA92_11505	584	CGC -> CTC	R -> L	N	-	choline ABC transporter permease
2256726	NZ_CP022003.1	coding	BCA92_11520	337	GAT -> GAG	D -> E	N	-	DMSO reductase maturation protein DsmD
2274959	NZ_CP022003.1	coding	BCA92_11605	1157	GGC -> GAC	G -> D	N	+	dipeptidyl carboxypeptidase II
2333624	NZ_CP022003.1	coding	BCA92_11905	233	TAC -> TGC	Y -> C	N	+	EamA family transporter
2337952	NZ_CP022003.1	intergenic							
2371331	NZ_CP022003.1	intergenic							
2380433	NZ_CP022003.1	coding	BCA92_12115	749	GTG -> GCG	V -> A	N	+	hypothetical protein
2438493	NZ_CP022003.1	coding	BCA92_12425	327	ACA -> ACG	T -> T	S	+	hypothetical protein
2467879	NZ_CP022003.1	coding	BCA92_12625	145	GAT -> AAT	D -> N	N	+	aromatic alcohol reductase
2496756	NZ_CP022003.1	coding	BCA92_12775	72	TGC -> TGT	C -> C	S	+	osmotically-inducible lipoprotein B
2529496	NZ_CP022003.1	intergenic							
2549474	NZ_CP022003.1	ncRNA	BCA92_13060	134	N/A	N/A	N/A	N/A	N/A
2581346	NZ_CP022003.1	intergenic							
2593804	NZ_CP022003.1	coding	BCA92_13290	<i>treA</i> 1534	ACC -> GCC	T -> A	N	+	trehalase
2595691	NZ_CP022003.1	coding	BCA92_13305	<i>emtA</i> 475	GCC -> GCA	A -> A	S	-	murein transglycosylase
2625180	NZ_CP022003.1	coding	BCA92_13455	1476	GTC -> ATC	V -> I	N	-	TerC family protein
2631795	NZ_CP022003.1	coding	BCA92_13500	1009	ATC -> ATT	I -> I	S	-	cell division protein FtsI
2633083	NZ_CP022003.1	coding	BCA92_13510	110	AAA -> AGA	K -> R	N	-	DUF2627 domain-containing protein
2636289	NZ_CP022003.1	coding	BCA92_13535	447	GTC -> GTA	V -> V	S	+	MFS transporter
2663553	NZ_CP022003.1	coding	BCA92_13695	290	GGC -> GAC	G -> D	N	-	DNA breaking-rejoining protein
2664866	NZ_CP022003.1	coding	BCA92_13710	380	GTC -> GAC	V -> D	N	-	DUF2514 domain-containing protein
2696527	NZ_CP022003.1	coding	BCA92_13930	<i>edd</i> 305	AAA -> AGA	K -> R	N	-	phosphogluconate dehydratase
2737304	NZ_CP022003.1	coding	BCA92_14130	<i>motB</i> 187	CTG -> CTA	L -> L	S	-	flagellar motor protein MotB
2748251	NZ_CP022003.1	intergenic							
2749728	NZ_CP022003.1	coding	BCA92_14210	123	TGG -> GGG	W -> G	N	-	YecA family protein
2753476	NZ_CP022003.1	intergenic							
2786139	NZ_CP022003.1	intergenic							
2817536	NZ_CP022003.1	coding	BCA92_14635	360	TGC -> TGA	C -> *	nonsense	+	acetylneuraminate ABC transporter
2877805	NZ_CP022003.1	coding	BCA92_14980	59	GCA -> GTA	A -> V	N	-	hypothetical protein
2893251	NZ_CP022003.1	coding	BCA92_15040	326	GAA -> GCA	E -> A	N	+	DUF159 family protein
2975968	NZ_CP022003.1	coding	BCA92_15415	835	GGC -> GGT	G -> G	S	-	macrolide ABC transporter permease/ATP-binding protein MacB
2992160	NZ_CP022003.1	intergenic							
3003806	NZ_CP022003.1	coding	BCA92_15555	433	CCG -> CCT	P -> P	S	-	polyamine ABC transporter ATP-binding protein
3081209	NZ_CP022003.1	coding	BCA92_15945	<i>potG</i> 226	TTC -> TTA	F -> L	N	-	molybdopterin synthase sulfur carrier subunit
3112990	NZ_CP022003.1	coding	BCA92_16100	<i>moaD</i> 759	AAA -> AAT	K -> N	N	+	UDP-glucose 4-epimerase
3126229	NZ_CP022003.1	coding	BCA92_16165	<i>galE</i> 733	CAT -> TAT	H -> Y	N	+	LysR family transcriptional regulator
3191555	NZ_CP022003.1	intergenic							
3196084	NZ_CP022003.1	intergenic							
3228078	NZ_CP022003.1	coding	BCA92_16700	457	GGA -> GGC	G -> G	S	-	galactonate dehydratase
3248933	NZ_CP022003.1	intergenic							
3251648	NZ_CP022003.1	coding	BCA92_16825	<i>dpiB</i> 253	GTG -> GTT	V -> V	S	-	histidine kinase
3256983	NZ_CP022003.1	coding	BCA92_16845	<i>citF</i> 1258	ATG -> CTG	M -> L	N	+	citrate lyase subunit alpha
3347362	NZ_CP022003.1	coding	BCA92_17325	587	CCG -> CTG	P -> L	N	-	uracil/xanthine transporter
3379696	NZ_CP022003.1	pseudogene	BCA92_17470	<i>ushA</i> 1531	GAT -> GAG	D -> E	N	-	bifunctional UDP-sugar hydrolase/5'-nucleotidase
3399407	NZ_CP022003.1	coding	BCA92_17565	246	AGT -> GGT	S -> G	N	-	DNA-binding transcriptional repressor AcrR
3407165	NZ_CP022003.1	coding	BCA92_17605	208	AGA -> AGC	R -> S	N	-	50S ribosomal protein L31
3431995	NZ_CP022003.1	intergenic							
3439044	NZ_CP022003.1	coding	BCA92_17760	57	ATA -> ATG	I -> M	N	+	cytochrome o ubiquinol oxidase subunit IV
3511771	NZ_CP022003.1	coding	BCA92_18120	<i>prpE</i> 1710	CGC -> TGC	R -> C	N	-	propionate--CoA ligase
3513417	NZ_CP022003.1	coding	BCA92_18125	1429	ATC -> ATT	I -> I	S	-	2-methylcitrate dehydratase
3561008	NZ_CP022003.1	coding	BCA92_18345	13	CAG -> CAA	Q -> Q	S	-	MFS transporter
3608574	NZ_CP022003.1	coding	BCA92_18615	471	ACC -> GCC	T -> A	N	-	class I SAM-dependent methyltransferase



3657928	NZ_CP022003.1	coding	BCA92_18860	<i>dapD</i>	117	GAT -> GAC	D -> D	S	+	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
3664856	NZ_CP022003.1	coding	BCA92_18880		975	TAT -> GAT	Y -> D	N	-	carbohydrate diacid regulon transcriptional regulator CdaR
3671905	NZ_CP022003.1	coding	BCA92_18915		877	GCG -> GCA	A -> A	S	-	ClC family H(+)/Cl(-) exchange transporter
3675086	NZ_CP022003.1	coding	BCA92_18930		240	ACC -> CCC	T -> P	N	-	fimbrial protein
3716332	NZ_CP022003.1	coding	BCA92_19115		163	ACC -> GCC	T -> A	N	+	S-adenosylmethionine decarboxylase proenzyme
3725950	NZ_CP022003.1	intergenic								
3783348	NZ_CP022003.1	coding	BCA92_19405	<i>leuC</i>	1168	AGT -> GGT	S -> G	N	+	3-isopropylmalate dehydratase large subunit
3792361	NZ_CP022003.1	coding	BCA92_19455	<i>araB</i>	89	AGC -> AAC	S -> N	N	+	ribulokinase
3817746	NZ_CP022003.1	intergenic								
3824894	NZ_CP022003.1	coding	BCA92_19615		144	GTT -> TTT	V -> F	N	-	hypothetical protein
3830021	NZ_CP022003.1	coding	BCA92_19635		772	ATA -> GTA	I -> V	N	+	ATP-dependent acyl-CoA ligase
3838386	NZ_CP022003.1	coding	BCA92_19670		36	GAC -> AAC	D -> N	N	-	4-hydroxy-tetrahydrodipicolinate reductase
3867089	NZ_CP022003.1	intergenic								
3932075	NZ_CP022003.1	coding	BCA92_20095		296	AAC -> AGC	N -> S	N	+	energy-dependent translational throttle protein EttA
3943047	NZ_CP022003.1	coding	BCA92_20145	<i>deoA</i>	139	GGT -> GGG	G -> G	S	-	thymidine phosphorylase
3976262	NZ_CP022003.1	coding	BCA92_20320		1437	ATG -> GTG	M -> V	N	-	methyl-accepting chemotaxis protein II
4002577	NZ_CP022003.1	intergenic								
4006268	NZ_CP022003.1	coding	BCA92_20455		92	ACA -> ATA	T -> I	N	-	aspartate racemase
4019804	NZ_CP022003.1	coding	BCA92_20550		183	CTA -> CTG	L -> L	S	+	hypothetical protein
4033372	NZ_CP022003.1	coding	BCA92_20645		460	GGC -> AGC	G -> S	N	+	alcohol dehydrogenase
4040680	NZ_CP022003.1	coding	BCA92_20680		423	TTC -> CTC	F -> L	N	-	LPS export ABC transporter permease LptF
4081583	NZ_CP022003.1	coding	BCA92_20895		1046	ACG -> ATG	T -> M	N	-	metalloprotease PmbA
4177866	NZ_CP022003.1	coding	BCA92_21425		219	GCT -> GCC	A -> A	S	+	phosphatase PAP2 family protein
4179845	NZ_CP022003.1	coding	BCA92_21440		100	GTT -> TTT	V -> F	N	+	hypothetical protein
4180343	NZ_CP022003.1	coding	BCA92_21445		111	CTG -> CTA	L -> L	S	+	AraC family transcriptional regulator
4196744	NZ_CP022003.1	coding	BCA92_21520		440	GGT -> GAT	G -> D	N	-	melibiose/sodium symporter
4247272	NZ_CP022003.1	coding	BCA92_21725		4837	GAT -> GAC	D -> D	S	-	Ig-like domain repeat protein
4327025	NZ_CP022003.1	coding	BCA92_22050	<i>rrf</i>	106	C -> G	N/A	N/A	N/A	5S ribosomal RNA
4345465	NZ_CP022003.1	coding	BCA92_22130		1247	CTG -> CAG	L -> Q	N	+	phosphomethylpyrimidine synthase
4446379	NZ_CP022003.1	coding	BCA92_22620		790	CAA -> CAG	Q -> Q	S	-	autoinducer 2 import ATP-binding protein LsrA
4465675	NZ_CP022003.1	coding	BCA92_22730		303	GGA -> GGC	G -> G	S	+	hypothetical protein
4571318	NZ_CP022003.1	coding	BCA92_23255	<i>corA</i>	472	CGC -> CGT	R -> R	S	-	magnesium transporter CorA
4579946	NZ_CP022003.1	coding	BCA92_23315		443	AAC -> AGC	N -> S	N	-	adenylate cyclase
4580032	NZ_CP022003.1	coding	BCA92_23315		529	ACT -> ACC	T -> T	S	-	adenylate cyclase
4594203	NZ_CP022003.1	coding	BCA92_23385		580	ACC -> ACT	T -> T	S	-	dTDP-4-amino-4,6-dideoxy-D-glucose transaminase
4603094	NZ_CP022003.1	intergenic								
4656020	NZ_CP022003.1	intergenic								

*B2 (10 samples / 11 SNPs)*

1090131	NZ_CP022003.1	coding	BCA92_05535		193	GCG -> GCA	A -> A	S	-	transcriptional regulator
1750882	NZ_CP022003.1	coding	BCA92_08690		267	TAC -> TAT	Y -> Y	S	+	colanic acid biosynthesis glycosyltransferase WcaA
2070127	NZ_CP022003.1	coding	BCA92_10520	<i>astB</i>	115	CGT -> TGT	R -> C	N	+	succinylarginine dihydrolase
2371470	NZ_CP022003.1	intergenic								
2584589	NZ_CP022003.1	coding	BCA92_13245		1446	CTG -> CTA	L -> L	S	+	hydrogenase 2 large subunit
2748916	NZ_CP022003.1	coding	BCA92_14205		597	AGC -> AGT	S -> S	S	+	tyrosine transporter TyrP
2818725	NZ_CP022003.1	intergenic								
4066198	NZ_CP022003.1	coding	BCA92_20815		704	CTA -> CCA	L -> P	N	+	PTS trehalose transporter subunit IIBC
4233664	NZ_CP022003.1	coding	BCA92_21680		1368	CTG -> TTG	L -> L	S	-	Na <sup>+</sup> /H <sup>+</sup> antiporter
4267308	NZ_CP022003.1	coding	BCA92_21765		1758	TCG -> TCA	S -> S	S	+	excinuclease ABC subunit A
4302445	NZ_CP022003.1	coding	BCA92_21935		630	GAA -> GAG	E -> E	S	+	lysine-sensitive aspartokinase 3

*B3 (2 samples / 23 SNPs)*

7774	NZ_CP022003.1	coding	BCA92_00030		575	CGC -> CAC	R -> H	N	-	MR-MLE family protein
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74071	NZ_CP022003.1	intergenic											
138368	NZ_CP022003.1	coding	BCA92_00665	391	GGT -> GGC	G -> G	S	-	murein hydrolase activator EnvC				
157210	NZ_CP022003.1	coding	BCA92_00745	15	AAG -> GAG	K -> E	N	-	hypothetical protein				
790283	NZ_CP022003.1	coding	BCA92_03940	201	GGC -> GGA	G -> G	S	+	alpha/beta hydrolase				
989552	NZ_CP022003.1	intergenic											
997019	NZ_CP022003.1	coding	BCA92_04990	<i>spaK</i> 199	CAA -> AAA	Q -> K	N	+	surface presentation of antigens protein SpaK				
1727639	NZ_CP022003.1	coding	BCA92_08610	1348	CAG -> CAA	Q -> Q	S	-	MFS transporter				
1905554	NZ_CP022003.1	intergenic											
2195899	NZ_CP022003.1	intergenic											
2484053	NZ_CP022003.1	coding	BCA92_12710	124	TCG -> GCG	S -> A	N	+	ABC transporter ATP-binding protein				
2584509	NZ_CP022003.1	coding	BCA92_13245	1366	GCG -> ACG	A -> T	N	+	hydrogenase 2 large subunit				
2614203	NZ_CP022003.1	coding	BCA92_13405	76	ATT -> GTT	I -> V	N	+	hypothetical protein				
2897817	NZ_CP022003.1	coding	BCA92_15070	1591	AGC -> AGA	S -> R	N	-	phage tail protein				
3314285	NZ_CP022003.1	intergenic											
3576390	NZ_CP022003.1	coding	BCA92_18425	480	GCC -> GCT	A -> A	S	+	transpeptidase				
3812870	NZ_CP022003.1	coding	BCA92_19540	141	ACC -> GCC	T -> A	N	-	glutathione-regulated potassium-efflux system protein KefC				
3940879	NZ_CP022003.1	coding	BCA92_20135	<i>deoD</i> 175	GAA -> GAC	E -> D	N	-	purine-nucleoside phosphorylase				
3970776	NZ_CP022003.1	coding	BCA92_20305	39	CGG -> TGG	R -> W	N	-	PTS system mannose/fructose/N-acetylgalactosamine-transporter subunit IIB				
4077799	NZ_CP022003.1	coding	BCA92_20865	493	GCG -> GCA	A -> A	S	-	hypothetical protein				
4098712	NZ_CP022003.1	coding	BCA92_20970	536	GCC -> GAC	A -> D	N	-	3'(2'),5'-bisphosphate nucleotidase CysQ				
4366160	NZ_CP022003.1	tRNA	BCA92_22240	47	C -> T	N/A	N/A	N/A	tRNA-Thr				
4545348	NZ_CP022003.1	coding	BCA92_23125	457	GTG -> GTT	V -> V	S	-	3-octaprenyl-4-hydroxybenzoate decarboxylase				
<i>B3&amp;4 (8 samples / 52 SNPs)</i>													
105233	NZ_CP022003.1	coding	BCA92_00480	379	TAC -> TAT	Y -> Y	S	-	guanylate kinase				
286760	NZ_CP022003.1	coding	BCA92_01315	890	CCG -> CTG	P -> L	N	+	hypothetical protein				
417133	NZ_CP022003.1	coding	BCA92_01895	25	CGC -> AGC	R -> S	N	+	cell filamentation protein Fic				
524011	NZ_CP022003.1	coding	BCA92_02545	753	GCT -> GCC	A -> A	S	+	cell division protein ZapE				
809540	NZ_CP022003.1	ncRNA	BCA92_04045	<i>ssrS</i> 131	C -> A	N/A	N/A	N/A	N/A				
912776	NZ_CP022003.1	coding	BCA92_04565	517	GTG -> GTA	V -> V	S	-	NADPH-dependent 7-cyano-7-deazaguanine reductase QueF				
1078356	NZ_CP022003.1	coding	BCA92_05445	441	AAT -> GAT	N -> D	N	-	glycine betaine/L-proline ABC transporter ATP-binding protein				
1174644	NZ_CP022003.1	intergenic											
1256808	NZ_CP022003.1	intergenic											
1370967	NZ_CP022003.1	coding	BCA92_06805	212	GCG -> GTG	A -> V	N	+	hypothetical protein				
1389082	NZ_CP022003.1	coding	BCA92_06895	478	ATG -> TTG	M -> L	N	+	ethanolamine ammonia-lyase light chain				
1459551	NZ_CP022003.1	coding	BCA92_07290	<i>fadI</i> 1141	TTC -> CTC	F -> L	N	+	acetyl-CoA C-acyltransferase FadI				
1499099	NZ_CP022003.1	coding	BCA92_07495	190	TGG -> TGT	W -> C	N	-	thiol:disulfide oxidoreductase				
1683776	NZ_CP022003.1	coding	BCA92_08395	493	TTC -> CTC	F -> L	N	+	transporter				
1690707	NZ_CP022003.1	coding	BCA92_08425	269	CTG -> CGG	L -> R	N	+	osmoprotectant uptake system permease				
1713836	NZ_CP022003.1	coding	BCA92_08545	1056	GTA -> ATA	V -> I	N	-	nucleoside permease				
1798704	NZ_CP022003.1	coding	BCA92_08915	<i>hisD</i> 21	GCC -> TCC	A -> S	N	-	histidinol dehydrogenase				
1883583	NZ_CP022003.1	intergenic											
1893633	NZ_CP022003.1	intergenic											
1941777	NZ_CP022003.1	coding	BCA92_09795	1569	GGC -> GGT	G -> G	S	+	flagellar hook-associated protein FlgK				
2084714	NZ_CP022003.1	coding	BCA92_10605	543	GTG -> ATG	V -> M	N	-	L-cystine transporter				
2240677	NZ_CP022003.1	coding	BCA92_11425	<i>pntA</i> 867	GGC -> GGT	G -> G	S	+	NAD(P)(+) transhydrogenase (Re/Si-specific) subunit alpha				
2274445	NZ_CP022003.1	coding	BCA92_11605	643	CGC -> TGC	R -> C	N	+	dipeptidyl carboxypeptidase II				
2281091	NZ_CP022003.1	coding	BCA92_11645	112	GTG -> GTA	V -> V	S	-	multiple antibiotic resistance regulatory periplasmic protein MarB				
2551856	NZ_CP022003.1	coding	BCA92_13105	<i>narI</i> 28	AAA -> AAG	K -> K	S	-	respiratory nitrate reductase subunit gamma				
2571089	NZ_CP022003.1	coding	BCA92_13190	301	GTG -> GTA	V -> V	S	-	glutamyl-tRNA reductase				
2650520	NZ_CP022003.1	intergenic											
2652657	NZ_CP022003.1	intergenic											
2677521	NZ_CP022003.1	intergenic											

3039272	NZ_CP022003.1	intergenic									
3043866	NZ_CP022003.1	intergenic									
3138555	NZ_CP022003.1	coding	BCA92_16260	<i>tolA</i>	631	GTA -> GCG	V -> A	N	-	cell envelope integrity protein TolA	
3138556	NZ_CP022003.1	coding	BCA92_16260	<i>tolA</i>	632	GTA -> GCG	V -> A	N	-	cell envelope integrity protein TolA	
3171264	NZ_CP022003.1	coding	BCA92_16425		56	CCG -> CTG	P -> L	N	+	two-component sensor histidine kinase	
3230616	NZ_CP022003.1	coding	BCA92_16710	<i>leuS</i>	1228	GAA -> AAA	E -> K	N	+	leucine--tRNA ligase	
3315626	NZ_CP022003.1	intergenic									
3369806	NZ_CP022003.1	coding	BCA92_17425		284	CCG -> CTG	P -> L	N	-	iron export ABC transporter permease subunit FetB	
3379070	NZ_CP022003.1	pseudogene	BCA92_17470	<i>ushA</i>	905	GGC -> GTC	G -> V	N	-	bifunctional UDP-sugar hydrolase/5'-nucleotidase	
3412411	NZ_CP022003.1	coding	BCA92_17635		1022	GCT -> GTT	A -> V	N	-	ammonium transporter	
3528864	NZ_CP022003.1	coding	BCA92_18190		519	GCC -> TCC	A -> S	N	-	site-specific DNA-methyltransferase	
3564570	NZ_CP022003.1	intergenic									
3646276	NZ_CP022003.1	coding	BCA92_18795	<i>rseP</i>	700	CGC -> CGT	R -> R	S	-	RIP metalloprotease RseP	
3818274	NZ_CP022003.1	intergenic									
3822909	NZ_CP022003.1	intergenic									
3884341	NZ_CP022003.1	coding	BCA92_19875		602	GGT -> GCT	G -> A	N	-	fimbrial adhesin FimH	
3975167	NZ_CP022003.1	coding	BCA92_20320		342	ACC -> CCC	T -> P	N	-	methyl-accepting chemotaxis protein II	
3991859	NZ_CP022003.1	coding	BCA92_20385		1685	CGC -> CTC	R -> L	N	+	type I restriction endonuclease	
4001324	NZ_CP022003.1	coding	BCA92_20425		552	TTA -> TTG	L -> L	S	+	MFS transporter	
4070632	NZ_CP022003.1	coding	BCA92_20825		1609	TAC -> CAC	Y -> H	N	+	anaerobic ribonucleoside triphosphate reductase	
4103620	NZ_CP022003.1	intergenic									
4114107	NZ_CP022003.1	coding	BCA92_21080	<i>araD</i>	231	GAG -> TAG	E -> *	nonsense	-	L-ribulose-5-phosphate 4-epimerase	
4320783	NZ_CP022003.1	coding	BCA92_22020		612	GAT -> TAT	D -> Y	N	-	bifunctional isocitrate dehydrogenase kinase/phosphatase	
<i>B4 (6 samples / 16 SNPs)</i>											
579355	NZ_CP022003.1	coding	BCA92_02850		20	ACA -> ATA	T -> I	N	+	ribosome maturation factor	
1157947	NZ_CP022003.1	tmRNA	BCA92_05935	<i>ssrA</i>	339	A -> G	N/A	N/A	N/A		
1266389	NZ_CP022003.1	pseudogene	BCA92_06405	<i>asrA</i>	612	GTC -> GCC	V -> A	N	-	anaerobic sulfite reductase subunit A	
1280375	NZ_CP022003.1	intergenic									
1464794	NZ_CP022003.1	coding	BCA92_07315		448	GGC -> AGC	G -> S	N	+	chorismate synthase	
1751108	NZ_CP022003.1	coding	BCA92_08690		493	GCC -> TCC	A -> S	N	+	colanic acid biosynthesis glycosyltransferase WcaA	
1754958	NZ_CP022003.1	coding	BCA92_08710	<i>wcaE</i>	594	GGC -> GGA	G -> G	S	+	colanic acid biosynthesis glycosyltransferase WcaE	
2761556	NZ_CP022003.1	coding	BCA92_14290		447	GAT -> AAT	D -> N	N	-	cystine ABC transporter substrate-binding protein	
3155980	NZ_CP022003.1	intergenic									
3234488	NZ_CP022003.1	coding	BCA92_16730		264	GCC -> ACC	A -> T	N	-	threonine-phosphate decarboxylase	
3246138	NZ_CP022003.1	coding	BCA92_16785	<i>lipA</i>	882	ATG -> ATA	M -> I	N	+	lipoyl synthase	
3840659	NZ_CP022003.1	coding	BCA92_19680	<i>citX</i>	422	ACG -> ATG	T -> M	N	-	phosphoribosyl-dephospho-CoA transferase	
3843024	NZ_CP022003.1	coding	BCA92_19690	<i>citE</i>	705	CCG -> TCG	P -> S	N	-	citrate lyase subunit beta	
3947396	NZ_CP022003.1	coding	BCA92_20160		481	TGG -> CGG	W -> R	N	+	glycine radical enzyme activase	
4352414	NZ_CP022003.1	coding	BCA92_22190	<i>rpoC</i>	46	CTG -> CTA	L -> L	S	-	DNA-directed RNA polymerase subunit beta	
4610825	NZ_CP022003.1	coding	BCA92_23470		1243	TCC -> TCT	S -> S	S	-	ketol-acid reductoisomerase	