

In Silico Signature Prediction Modeling in Cytolethal Distending Toxin-Producing *Escherichia coli* Strains

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In this study, cytolethal distending toxin (CDT) producer isolates genome were compared with genome of pathogenic and commensal *Escherichia coli* strains. Conserved genomic signatures among different types of CDT producer *E. coli* strains were assessed. It was shown that they could be used as biomarkers for research purposes and clinical diagnosis by polymerase chain reaction, or in vaccine development. *cdt* genes and several other genetic biomarkers were identified as signature sequences in CDT producer strains. The identified signatures include several individual phage proteins (holins, nucleases, and terminases, and transferases) and multiple members of different protein families (the lambda family, phage-integrase family, phage-tail tape protein family, putative membrane proteins, regulatory proteins, restriction-modification system proteins, tail fiber-assembly proteins, base plate-assembly proteins, and other prophage tail-related proteins). In this study, a sporadic phylogenetic pattern was demonstrated in the CDT-producing strains. In conclusion, conserved signature proteins in a wide range of pathogenic bacterial strains can potentially be used in modern vaccine-design strategies.

Keywords: biomarkers, cytolethal distending toxin, genomic signature, multiple alignments, pathogenic *Escherichia coli*

Introduction

The co-evolution of pathogenic bacteria and their hosts leads to the generation of functional pathogen-host interfaces. Well-adapted pathogens have evolved a variety of strategies for manipulating host cell functions to guarantee their successive colonization and survival. For instance, a group of gram-negative bacterial pathogens produces a toxin, known as cytolethal distending toxin (CDT) [1]. Among the vast majority of CDT producers are *Escherichia coli*, which is commonly found in the intestines of humans and other mammals. Most *E. coli* strains are harmless commensals; however, some isolates can cause severe diseases and are designated as pathogenic *E. coli*. Among the various pathogenic *E. coli* strains, some have acquired virulence determinants through the horizontal transfer of genes, such as the *cdt* genes encoding CDTs. CDTs were the first bacterial toxins identified that block the eukaryotic cell cycle and suppress cell proliferation, eventually resulting in cell death. The active subunits of CDT toxins exhibit features of type I deoxyribonuclease-like activity [2, 3].

In this study, comparative genome analysis of CDT-producer *E. coli* isolates with other pathogenic and commensal strains was performed. Alignments between multiple genomes led to the identification of a set of distinct (“signature”) sequence motifs. These signature sequences could be used to delineate single genomes or a specified group of associated genomes within a desired group, such as the CDT-producing *E. coli* (the target group in this study). While genomic signatures were conserved in the target group, which they were not conserved or were absent in other related or unrelated genomes (i.e., the background group). From a clinical point of view, conserved signature sequences could offer advantages in predicting and further designing novel CDT inhibitors to vaccine candidates [4].

On the other hand, phylogenetic trees can be constructed based on multiple sequence alignments. It is important that phylogeny based on an immense number of genes and whole-genome sequences are more reliable than those based on a single gene or a few selected loci [5]. Phylogenetic analysis can provide an overall classification of the target group among the background group. Alignment of whole-genome sequences yields detailed information on specific differences

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between genomes and, consequently, has shed new insights into phylogenetic relationships in recent years [6-9].

In this study, phylogenetic relationships of CDT⁺ strains with other pathogenic and commensal *E. coli* strains were assessed, and conserved signature genomic regions in the target group (CDT-producers) were annotated. This information could be used for developing molecular diagnostics assays, polymerase chain reaction primer and probe design in modern vaccines.

Methods

CDT⁺ strains

Several databases were used to identify bacterial strains harboring *cdt* genes. Data was extracted from the following resources: NCBI, National Center for Biotechnology Information GenBank; EMBL, European Molecular Biology Laboratory; DDBJ, DNA Data Bank of Japan; PDB, Protein Data Bank; RefSeq, NCBI Reference Sequence Database; and UniProtKB, Swiss-Prot Database.

Whole-genome sequences

All genomes analyzed in this study were downloaded from the NCBI file transfer protocol (FTP) site at: <ftp://ftp.ncbi.nih.gov/genomes>.

Reordering of draft genomes

Ordering and orienting contigs in draft genomes facilitates comparative genome analysis. Contig ordering can be predicted by comparison of a reference genome that is expected to have a conserved genome organization [10]. ProgressiveMauve (version 2.3.1) was used for ordering contigs in draft genomes. Mauve contig mover (MCM) offers advantages over methods that rely on matches in limited regions near the ends of contigs [11, 12]. The *E. coli* K-12 MG1655 strain (accession No. NC_000913.3) was used as a reference genome.

The MCM optional parameters were used in this study including default seed weight, use seed families: 15 determine Locally Collinear Blocks (LCBs); LCBs, full alignment, iterative refinement, sum-of-pairs LCB scoring, and min LCB weight: 200.

Multiple genome alignments

In this study, Gegenees software (version 2.2.1) was used for multiple-genome alignments. The software is written in JAVA, and making it compatible with several platforms. Limitations were not observed in the speed calculation, number and memory of the genomes that could be aligned. Gegenees software is also capable of performing fragmented alignments [4]. Multiple alignments of *E. coli* genomes were

created using a fragment size of 200 nucleotides, a step size of 100 parameters, and BLASTN, which was optimized for highly similar sequences.

Phylogenetic tree construction

A phylogram was produced in SplitsTree 4, using the neighbor-joining method and a distance matrix Nexus file exported from Gegenees software [13]. *E. albertii* TW07627 and *E. fergusonii* ATCC 35469 strains were set as the out-groups.

Identifying conserved signatures

CDT-producing isolates were set as the target group, and all other strains were used as the background group by using the in-group setting tab in Gegenees software. Because of the genomic diversity in CDT-producer *E. coli*, we repeated this procedure with five different strains, including *E. coli* 53638, *E. coli* IHE3034, *E. coli* RN587/1, *E. coli* STEC B2F1, and *E. coli* STEC C165-02, which were defined as separate reference strains.

The biomarker score (max/average) setting was also used. Biomarker scores were drawn graphically and loaded into the tabular view for further data analysis. In the tabular view, a score of 1.0 is the maximum biomarker score and is considered as a signature.

Assembling signature fragments

Several overlapping fragments were obtained, based on the sequences of each reference strain. To facilitate subsequent analysis steps, the overlapping fragments were assembled using DNA Dragon software, version 1.6.0 (<http://www.dna-dragon.com/>).

The settings were designed with minimum overlaps (100 bases) along the diagonal length, a minimum %-identity of complete overlapping fragments, and 100% full-search parameters.

BLAST

BLAST was done with sequences for each of the five reference strains by using NCBI BLASTX (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the putative protein domains. Furthermore, putative conserved domains were also detected. The results were confirmed using the UniProtKB Bank BLASTX program (<http://www.uniprot.org/blast/>).

Results

Strains

The sequences of 76 strains were downloaded from the NCBI site. Details regarding genome sizes, %GC content,

Table 1. Strains characteristics

Strain	DNA length (Mb)	<i>cdt</i> gene	GC%	Protein count	Gene count	Genome type, No. of subsequences/contigs	Pathotype, serotype, other characteristic	Accession No.
<i>Escherichia coli</i> 96.0497	5.01426	+	50.80	4,862	5,026	Draft, 13	Host: homo sapiens, O91:H21	NZ_AEQ00000000.2
<i>Escherichia coli</i> 3003	4.91733	+	50.7	4,825	4,982	Draft, 8	I.S: water, O157:H45	NZ_AFAF00000000.2
<i>Escherichia coli</i> 5412	5.38651	+	50.20	5,670	5,761	Draft, 373	Host: homo sapiens, SFO157	NZ_AMUJ000000000.1
<i>Escherichia coli</i> 53638	5.37179	+	50.99	4,803	5,218	Draft, 2	EIEC, O144	NZ_AAKB000000000.2
<i>Escherichia coli</i> ARS4.2123	4.98276	+	50.50	5,105	5,194	Draft, 209	I.S: water, O157:H16	NZ_AMUL000000000.1
<i>Escherichia coli</i> DEC3F	5.4079	+	50.30	5,541	5,692	Draft, 93	Host: homo sapiens, SF EHEC O157:H	NZ_AIFJ000000000.1
<i>Escherichia coli</i> KTE11	4.52715	+	50.50	4,109	4,214	Draft, 7	No published information	NZ_ANSR000000000.1
<i>Escherichia coli</i> KTE28	5.0544	+	50.40	4,673	4,760	Draft, 12	No published information	NZ_ANSY000000000.1
<i>Escherichia coli</i> KTE47	4.98747	+	50.60	4,694	4,798	Draft, 11	No published information	NZ_ANUB000000000.1
<i>Escherichia coli</i> KTE60	5.07079	+	50.50	4,664	4,756	Draft, 20	No published information	NZ_ANUJ000000000.1
<i>Escherichia coli</i> KTE137	5.00154	+	50.50	4,702	4,789	Draft, 99	No published information	NZ_ANYA000000000.1
<i>Escherichia coli</i> KTE178	5.30789	+	50.60	4,973	5,050	Draft, 11	No published information	NZ_ANTB000000000.1
<i>Escherichia coli</i> KTE180	5.12548	+	50.60	4,883	4,966	Draft, 112	No published information	NZ_ANYR000000000.1
<i>Escherichia coli</i> KTE209	5.11008	+	50.50	4,702	4,791	Draft, 3	No published information	NZ_ANXD000000000.1
<i>Escherichia coli</i> MS 21-1	5.30899	+	50.40	5,744	5,860	Draft, 206	No published information	NZ_ADTR000000000.1
<i>Escherichia coli</i> O157:H-493-89	5.05482	+	50.50	4,838	4,946	Draft, 204	Host: homo sapiens, O157:H-	NZ_AETY000000000.1
<i>Escherichia coli</i> O157:H43 T22	4.95898	+	50.80	4,859	4,935	Draft, 64	I.S: milk from healthy cattle, O157:H43	NZ_AHZD000000000.2
<i>Escherichia coli</i> RN587/1	5.06158	+	50.60	4,999	5,108	Draft, 73	EPEC, O157:H8	NZ_ADUS000000000.1
<i>Escherichia coli</i> STEC B2F1	4.98941	+	50.90	4,875	5,006	Draft, 37	STEC, O91:H21	NZ_AFDQ000000000.1
<i>Escherichia coli</i> STEC C165-02	5.00927	+	50.60	4,891	5,019	Draft, 30	STEC, O73:H16	NZ_AFDR000000000.1
<i>Escherichia coli</i> TA271	5.07582	+	50.70	5,081	5,197	Draft, 83	Host: some mammal	NZ_ADAZ000000000.1
<i>Escherichia coli</i> TW06591	5.47546	+	50.30	5,521	5,650	Draft, 45	Host: homo sapiens, O157:H-	NZ_AKLT000000000.1
<i>Escherichia coli</i> W26	5.11853	+	50.60	4,852	4,920	Draft, 165	Host: cow, I.S: feces	NZ_AGIA000000000.1
<i>Escherichia albertii</i> TW07627	4.74659	+	49.90	4,386	4,889	Draft, 43	Diarrhea genic	NZ_ABKX000000000.1
<i>Escherichia coli</i> APEC O1	5.49765	+	50.29	4,853	4,968	Complete, 3	ExPEC, O1:K1:H7, avian pathogenic	NC_008563.1
<i>Escherichia coli</i> IHE3034	5.10838	+	50.70	4,966	4,753	Complete, 1	ExPEC, O18:K1:H7, meningitis	NC_017628.1

Table 1. Continued

Strain	DNA length (Mb)	<i>cdt</i> gene	GC%	Protein count	Gene count	Genome type, No. of subsequences/contigs	Pathotype, serotype, other characteristic	Accession No.
<i>Escherichia coli</i> 042	5.35532	-	50.58	4,920	5,036	Complete, 2	EAEC, O44:H18	NC_017626.1
<i>Escherichia coli</i> 536	4.93892	-	50.50	4,619	4,779	Complete, 1	UPEC, O6:K15:H31	NC_008253.1
<i>Escherichia coli</i> 55989	5.15486	-	50.70	4,755	5,136	Complete, 1	EAEC	NC_011748.1
<i>Escherichia coli</i> ABU 83972	5.13296	-	50.60	4,795	4,905	Complete, 2	ExPEC UTI, OR:K5:H-	NC_017631.1
<i>Escherichia coli</i> APEC O78	4.79843	-	50.70	4,588	4,695	Complete, 1	ExPEC	NC_020163.1
<i>Escherichia coli</i> ATCC 8739	4.74622	-	50.90	4,199	4,408	Complete, 1	K12 derivative	NC_010468.1
<i>Escherichia coli</i> B REL606	4.62981	-	50.80	4,200	4,361	Complete, 1	Commensal, strain B	NC_012967.1
<i>Escherichia coli</i> BL21 DE3	4.55895	-	50.80	4,153	4,330	Complete, 1	Commensal, strain B	NC_012971.2
<i>Escherichia coli</i> BW2952	4.57816	-	50.80	4,079	4,262	Complete, 1	K12 derivative	NC_012759.1
<i>Escherichia coli</i> CFT073	5.23143	-	50.50	5,364	5,574	Complete, 1	ExPEC, UPEC, O6:K2:H1	NC_004431.1
<i>Escherichia coli</i> DH1	4.63071	-	50.80	4,160	4,375	Complete, 1	K12 derivative	NC_017625.1
<i>Escherichia coli</i> E24377A	5.24929	-	50.54	4,991	5,258	Complete, 7	ETEC, O139:H28	NC_009801.1
<i>Escherichia coli</i> ED1a	5.20955	-	50.70	4,911	5,321	Complete, 1	Commensal, O81	NC_011745.1
<i>Escherichia coli</i> ETEC H10407	5.32589	-	50.73	4,872	5,084	Complete, 5	ETEC, O78:H11	NC_017633.1
<i>Escherichia coli</i> HS	4.64354	-	50.80	4,374	4,626	Complete, 1	Commensal, O9	NC_009800.1
<i>Escherichia coli</i> IAI1	4.70056	-	50.80	4,345	4,629	Complete, 1	Commensal	NC_011741.1
<i>Escherichia coli</i> IAI39	5.13207	-	50.60	4,725	5,092	Complete, 1	ExPEC, UPEC, O7:K1	NC_011750.1
<i>Escherichia coli</i> JJ1886	5.30828	-	50.77	5,049	5,213	Complete, 6	ExPEC, UPEC	NC_022648.1
<i>Escherichia coli</i> K-12 DH10B	4.68614	-	50.80	4,124	4,352	Complete, 1	K12 derivative	NC_010473.1
<i>Escherichia coli</i> K-12 MG1655	4.64165	-	50.80	4,140	4,497	Complete, 1	Commensal, K12	NC_000913.3
<i>Escherichia coli</i> K-12 W3110	4.64633	-	50.80	4,213	4,436	Complete, 1	Commensal, K12	NC_007779.1
<i>Escherichia coli</i> KO11FL	5.02717	-	50.79	4,705	4,821	Complete, 2	Commensal	NC_017660.1
<i>Escherichia coli</i> LF82	4.77311	-	50.70	4,376	4,545	Complete, 1	AIEC	NC_011993.1
<i>Escherichia coli</i> LY180	4.8356	-	50.90	4,463	4,624	Complete, 1	Ethanologenic <i>E. coli</i>	NC_022364.1
<i>Escherichia coli</i> NA114	4.97146	-	51.20	4,873	4,975	Complete, 1	ExPEC, UPEC	NC_017644.1
<i>Escherichia coli</i> O7:K1 CE10	5.37873	-	50.58	5,080	5,269	Complete, 5	ExPec, Neonatal meningitis, O7:K1	NC_017646.1
<i>Escherichia coli</i> O26:H11 11368	5.85553	-	50.66	5,515	5,985	Complete, 5	EHEC, O26:H11	NC_013361.1
<i>Escherichia coli</i> O55:H7 CB9615	5.45235	-	50.48	5,117	5,367	Complete, 2	EPEC, O55:H7	NC_013941.1
<i>Escherichia coli</i> O83:H1 NRG 857C	4.89488	-	50.71	4,582	4,690	Complete, 2	AIEC, O83:H1	NC_017634.1
<i>Escherichia coli</i> O103:H2 12009	5.52486	-	50.68	5,117	5,541	Complete, 2	EHEC, O103:H2	NC_013353.1
<i>Escherichia coli</i> O104:H4 2011C-3493	5.43741	-	50.63	5,149	5,269	Complete, 4	EAEC/STEC, O104:H4	NC_018658.1
<i>Escherichia coli</i> O111:H-11128	5.76608	-	50.42	5,403	5,931	Complete, 6	EHEC, O111:H	NC_013364.1
<i>Escherichia coli</i> O127:H6 E2348 69	5.06968	-	50.55	4,647	5,011	Complete, 3	EPEC, O127:H6	NC_011601.1
<i>Escherichia coli</i> O157:H7 EC4115	5.70417	-	50.39	5,477	6,066	Complete, 3	EHEC, O157:H7	NC_011353.1
<i>Escherichia coli</i> O157:H7 EDL933	5.6394	-	50.45	5,772	5,920	Complete, 2	EHEC, O157:H7	NC_002655.2
<i>Escherichia coli</i> O157:H7 Sakai	5.59448	-	50.45	5,292	5,448	Complete, 3	EHEC, O157:H7	NC_002695.1
<i>Escherichia coli</i> O157:H7 TW14359	5.62274	-	50.46	5,363	5,586	Complete, 2	EHEC, O157:H7	NC_013008.1
<i>Escherichia coli</i> P12b	4.93529	-	50.90	4,379	4,567	Complete, 1	O15:H17	NC_017663.1
<i>Escherichia coli</i> PMV 1	5.21093	-	50.67	4,979	5,257	Complete, 2	ExPEC, O18:K1	NC_022370.1

Table 1. Continued

Strain	DNA length (Mb)	cat gene	GC%	Protein count	Gene count	Genome type, No. of subsequences/contigs	Pathotype, serotype, other characteristic	Accession No.
<i>Escherichia coli</i> S88	5.16612	-	50.66	4,823	5,187	Complete, 2	ExPEC, Neonatal Meningitis, O45:K1:H7	NC_011742.1
<i>Escherichia coli</i> SE11	5.15563	-	50.75	4,996	5,103	Complete, 7	Commensal, O152:H28	NC_011415.1
<i>Escherichia coli</i> SE15	4.83968	-	50.71	4,486	4,592	Complete, 2	Commensal, O150:H5	NC_013654.1
<i>Escherichia coli</i> SMS-3-5	5.21538	-	50.50	4,912	5,127	Complete, 5	Environmental isolate	NC_010498.1
<i>Escherichia coli</i> UM146	5.10756	-	50.61	4,783	4,891	Complete, 2	AIEC (adherent invasive)	NC_017632.1
<i>Escherichia coli</i> UMN026	5.3582	-	50.64	5,010	5,294	Complete, 3	ExPEC, UPEC, O7:K1	NC_011751.1
<i>Escherichia coli</i> UMNK88	5.66676	-	50.74	5,607	5,754	Complete, 6	Porcine ETEC, O149	NC_017641.1
<i>Escherichia coli</i> UT189	5.17997	-	50.61	5,162	5,272	Complete, 2	ExPEC, UPEC, O18:K1:H7	NC_007946.1
<i>Escherichia coli</i> W	5.00886	-	50.78	4,602	4,876	Complete, 3	Commensal, ATCC 9637	NC_017635.1
<i>Escherichia coli</i> Xuzhou21	5.51674	-	50.38	5,179	5,294	Complete, 3	EHEC, O157:H7	NC_017906.1
<i>Escherichia fergusonii</i> ATCC 35469	4.64386	-	49.88	4,314	4,543	Complete, 2	I.S: Feces, human	NC_011740.1

I.S, isolation source; EIEC, enteroinvasive *E. coli*; EHEC, enterohemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; STEC, Shiga toxin-producing *E. coli*; ExPEC, extraintestinal pathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; UPEC, uropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; AIEC, adherent invasive *E. coli*.

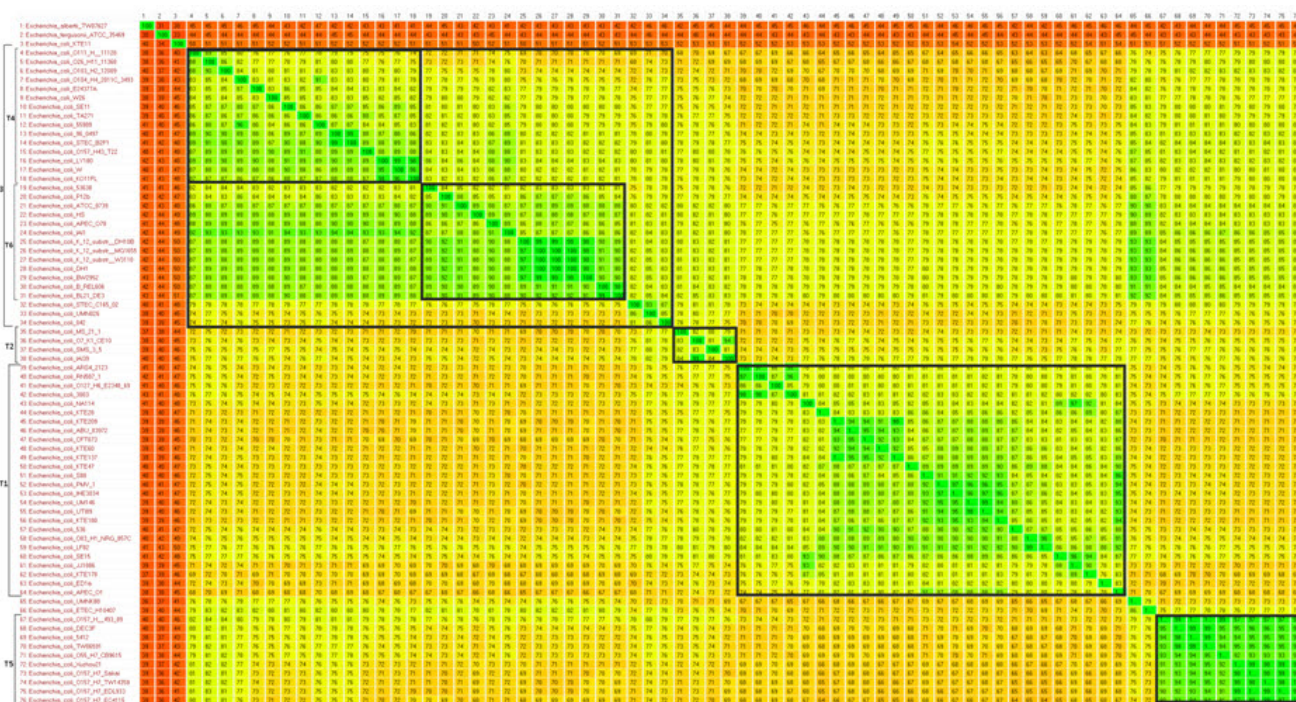


Fig. 1. Phylogenetic heat-plot overview of multiple-genome alignments. A heat plot based on a 200/100 BLASTN fragmented alignment was performed with Gegenees software. Six distinct genomic groups (T1–T6) recognized in cytolethal distending toxin (CDT)⁺ strains were observed sporadically among the strains that were studied, revealing the heterogeneous genomic nature of CDT-producing *Escherichia coli*.

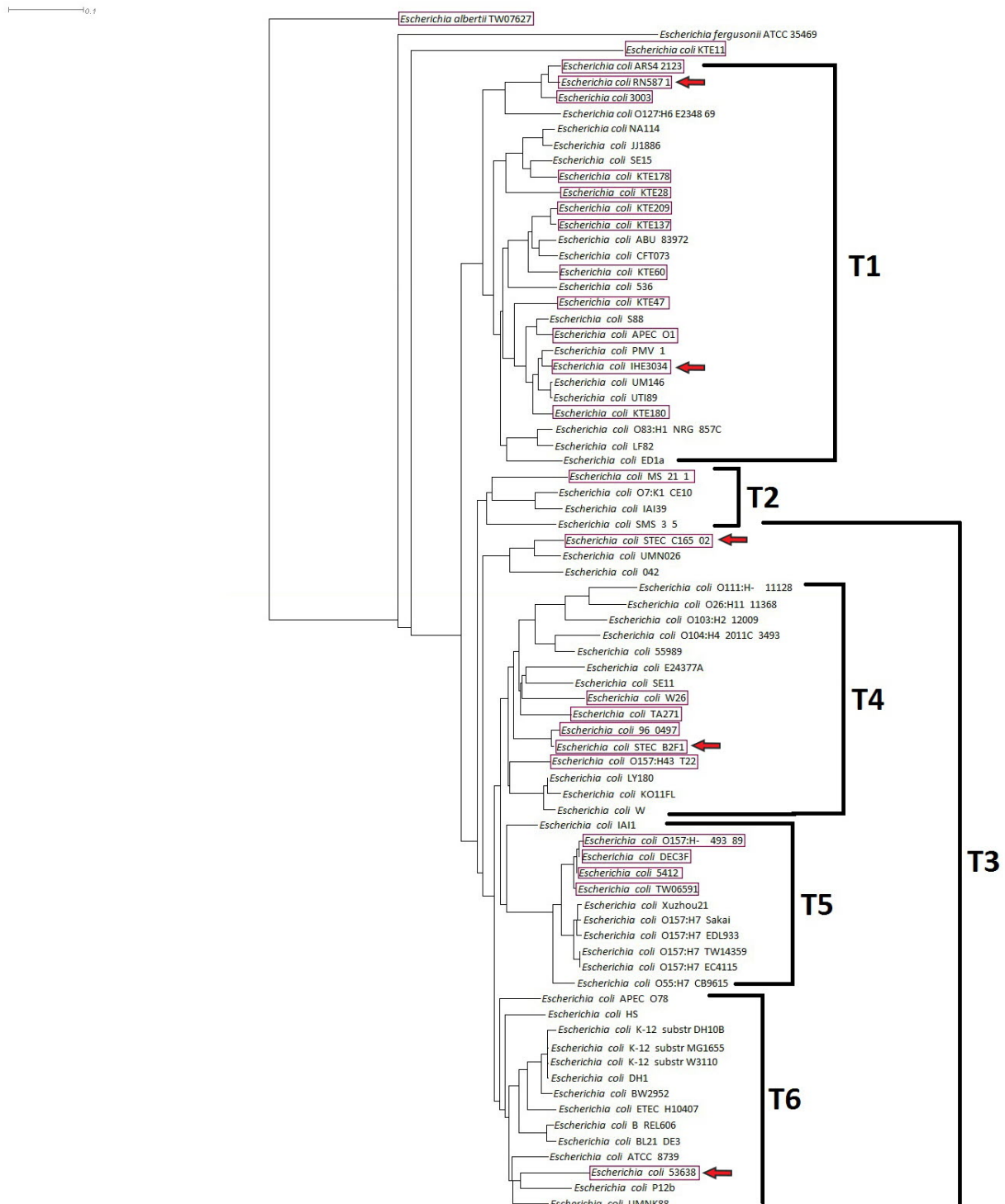


Fig. 2. Phylogram overview. A phylogram was generated using SplitsTree 4 software, using the neighbor-joining method and a distance-matrix Nexus file exported from Gegenees software. The *Escherichia albertii* TW07627 and *Escherichia fergusonii* ATCC 35469 strains were set as out-groups. In addition, six unique groups (T1–T6) were analyzed. In the phylogenetic overview, a sporadic pattern of cytolethal distending toxin (CDT)–producing strains was observed, as were specific clades. These strains were related and their similarities were shown. CDT⁺ strains are shown in boxes. The *Escherichia coli* strains that were set as reference strains for biomarker-detection studies are indicated with red arrows.

the number of encoded proteins, encoded genes, genome type, pathotype, serotype, other characteristics, and accession numbers are summarized in Table 1. Most data presented were extracted from NCBI GenBank and UniProt Bank and some information was extracted from original articles [14, 15]. The genomes of 24 strains were drafted, and a reordering process of the draft genomes was performed. Twenty-five CDT⁺ *E. coli* strains were analyzed, including *E. albertii* TW07627.

Phylogenetic analysis

A heat-plot based on a 200/100 BLASTN fragmented alignment drawn with Gegenees software is shown in Fig 1. A phylogenetic overview is also shown in the heat-plot. A more detailed phylogram was constructed with SplitsTree 4 software, as shown in Fig. 2.

CDT-producer *E. coli* strains were displayed a sporadic, phylogenomic pattern in the heat-plot, with a lack of a consensus pattern. Six distinct genomic groups of CDT⁺ strains (T1 to T6 in Fig. 2) were shown in the phylogram, all of which were sporadic among the strains in Fig 1. As a sporadic pattern of CDT-producing strains was observed in the bacterial population in the phylogram for specific clades, these strains were related and some degrees of similarity were also found.

Signature sequences in the target group

In total, 1,527 fragments representing 3.0% of the *E. coli* 53638-strain genome were identified as signature sequences. Biomarkers were restricted to 21 highly significant regions, designated A to U. When *E. coli* IHE3034 was set as the reference strain, 220 signature sequences (0.4%) were detected. Biomarkers were identified in six regions, designated A to F. However, 1,512 (2.9%) signature fragments were obtained, which were restricted to 18 regions (A to R) in the genome of *E. coli* RN587/1 when it was regarded as the reference strain. Moreover, 620 biomarker fragments (1.2%) were detected in the genome of *E. coli* STEC B2F1 when it was set as the reference strain, 16 biomarker regions (A to P) were recognized. In addition, when *E. coli* STEC C165-02 was used as the reference strain, 593 signature fragments (1.1%) were identified, which were restricted to eight regions (A to H). The signature regions for all reference strains are shown in Fig. 3, separately. In addition, the biomarker designation, domain description, BLASTX results and related putative conserved domains for each reference strain are provided in Supplementary Tables 1–6.

Conserved signature proteins

The most common biomarker proteins were distinguished by comparing BLASTX results for all reference strains

fragments (Table 2). The signature proteins identified included: CDT, holin, lambda-family proteins, nuclease, phage integrase family proteins, phage tail tape measure family proteins, putative membrane proteins, regulatory proteins, restriction-modification system proteins, tail fiber assembly proteins, baseplate assembly proteins, tail fiber protein and other prophage tail related proteins, terminuses and transferases. The nucleotide sequences of some proteins including anti-termination proteins, prophage DNA packaging and binding proteins, transposase and DNA transposition proteins, scaffold proteins, recombination-related domains, putative phage-replication proteins, hemolysin, helicase, glycol transferase, and glycohydrolase superfamilies, were detected as biomarkers in the target group, although these BLASTX results were not observed in all reference strains. Presumably, CDT-producer *E. coli* strains possess several hypothetical proteins whose functions are not yet defined and might be conserved proteins. The existence of these DNA biomarker sequences in reference strains is clear; however, the related proteins in some strains have not been determined.

Significant putative conserved domains and superfamilies

In the era of modern vaccines, finding conserved domains or epitopes has a great therapeutic value. Putative conserved domains were described as non-specific hits (NH), specific hits (SH), and multi-domains (MD), and it was shown in Supplementary Tables 1–6.

The putative conserved domains and superfamilies that were associated with some signature proteins are shown below.

- NH: PRK15251, DUF4102, CdtB, CDtoxinA, INT_P4, HP1_INT_C, Phage_integrase, INT_Lambda_C, Phage_integ_N, Methylase_S, Caudo_TAP, phage_tail_N, Tail_P2_I, gpI, phage_term_2, Terminase_3, Terminase_5, M, Phage_term_smal, COG5525, Terminase_GpA, Phage_Nu1, dexA, Phage_holin_2, DUF3751, Phage_attach, dcm, DNA_methylase, Cyt_C5_DNA_methylase, Dcm, Glycos_transf_2, and CESA_like
- SH: INT_REC_C, PhageMin_Tail, COG4220, Phage_fiber_2, HSDR_N, Glycos_transf_2, GT_2_like_d, PRK10018, and PLN02726
- MD: PRK09692, int, recomb_XerC, XerD, xerC, HsdS, N6_Mtase, HsdM, hsdM, rumA, P, Terminase_6, COG5484, PLN03114, COG5301, COG0610, hsdR, PRK10458, PRK10073, Glyco_tranf_2_3, WcaA, PRK10073, and PTZ00260
- Superfamilies: RICIN superfamily, EEP superfamily, DNA_BRE_C superfamily, DUF4102 superfamily, Phage_integ_N superfamily, MCP_signal superfamily,

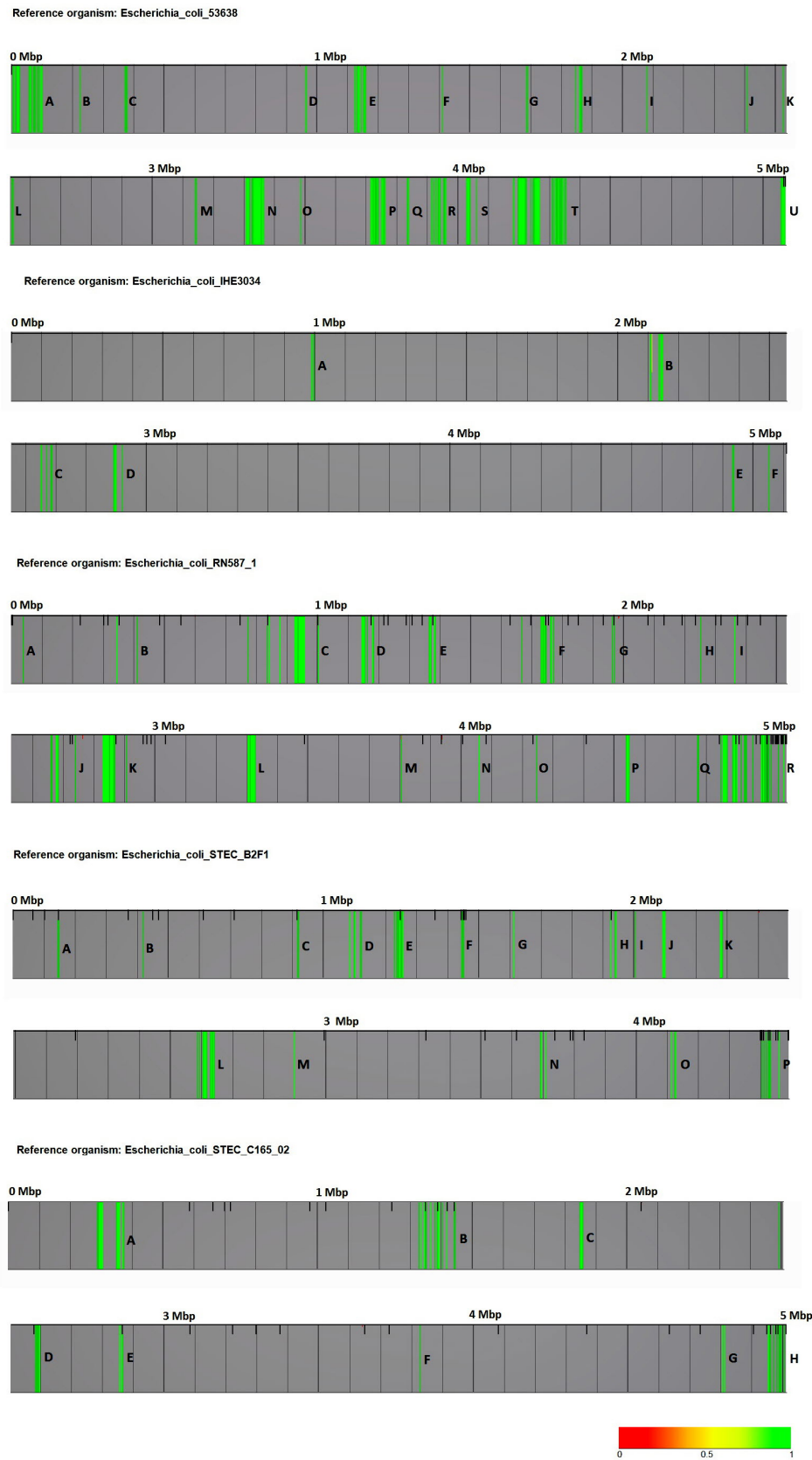


Fig. 3. Biomarker regions. Biomarker regions were illustrated in the whole-genome sequences of five different reference strains including *Escherichia coli* 53638, *E. coli* IHE3034, *E. coli* RN587/1, *E. coli* STEC B2F1, and *E. coli* STEC C165-02. The biomarker score (max/average) setting was used. A score of 1.0 is the maximum biomarker score, which was considered to represent a signature sequence, as indicated in green. STEC, Shiga toxin-producing *E. coli*.

Table 2. Significant signature proteins in five reference *Escherichia coli* strains

Signature protein	Reference strain				
	<i>Escherichia coli</i> 53638	<i>Escherichia coli</i> IHE3034	<i>Escherichia coli</i> RN587/1	<i>Escherichia coli</i> STEC_B2F1	<i>Escherichia coli</i> STEC_C165_02
Cytolethal distending toxin	Cytolethal distending toxin A Cytolethal distending toxin B Cytolethal distending toxin subunit C	Cytolethal distending toxin, subunit C Cytolethal distending toxin, subunit B Cytolethal distending toxin, subunit A	Cytolethal distending toxin A/C family protein	Cytolethal distending toxin C Cytolethal distending toxin A/C family protein	Cytolethal distending toxin A/C family protein
Holin	Phage holin, lambda family	Holin, lambda family	Holing	^a	Phage holin, lambda family
Nuclease	Exodeoxyribonuclease 8	Exonuclease family protein	Exonuclease family protein Hypothetical protein ECRN5871_4153, [HNH endonuclease family protein]	Endonuclease/Exonuclease/phosphatase family protein Type I site-specific deoxyribonuclease, HsdR family	Restriction endonuclease family protein Type I site-specific deoxyribonuclease, HsdR family protein Hypothetical protein ECSTECC16502_0280, [HNH endonuclease] Endonuclease/Exonuclease/phosphatase family protein
Phage integrase	Phage integrase Prophage integrase Integrase for prophage CP-933T	Integrase/recombinase, phage integrase family Site-specific recombinase, phage integrase family	Integrase Phage integrase family protein Prophage lambda integrase Integrase domain protein	Phage integrase family protein Prophage lambda integrase	Integrase Prophage CP4-57 integrase
Putative membrane protein	Putative membrane protein Hypothetical protein Ec53638_1156, [membrane protein]	Hypothetical protein ECOK1_2122, [membrane protein] Hypothetical protein ECOK1_2557, [membrane protein]	Outer membrane autotransporter barrel domain protein	Putative membrane protein Hypothetical protein ECSTECB2F1_3192, [membrane protein] OmpA-like transmembrane domain protein Outer membrane porin protein LC Outer membrane protein lom	Putative membrane protein
Regulatory proteins	Phage regulatory protein Cro Transcriptional regulator, AlpA family Putative phage regulatory protein, Rha family	Putative transcriptional regulator DicA157 Putative regulatory protein Cox	Regulatory protein CII Prophage CP4-57 regulatory protein family protein Transcriptional regulator, LacI family	Transcriptional regulator, AraC family	4-Hydroxyphenylacetate catabolism regulatory protein HpaA Prophage CP4-57 regulatory protein family protein
Restriction-modification system	Putative type I restriction-modification system, S subunit Type I restriction-modification system specificity subunit Type I restriction-modification enzyme, R subunit Type I restriction-modification system, M subunit	^a	Type II restriction enzyme EcoRII Modification methylase EcoRII Type I restriction enzyme specificity protein Type I restriction-modification system, M subunit	Type I restriction modification DNA specificity domain protein	Type I restriction-modification system specificity determinant Type III restriction enzyme, res subunit

Table 2. Continued

Signature protein	Reference strain				
	<i>Escherichia coli</i> 53638	<i>Escherichia coli</i> IHE3034	<i>Escherichia coli</i> RN587/1	<i>Escherichia coli</i> STEC_B2F1	<i>Escherichia coli</i> STEC_C165_02
Tail fiber assembly family, baseplate assembly proteins, Tail fiber protein and Tail tape measure protein	Tail fiber assembly protein Phage P2 baseplate assembly protein gpV Putative tail fiber protein Tail fiber Phage tail tape measure protein family	Tail fiber protein Phage tail tape measure protein	Caudovirales tail fiber Assembly family protein Hypothetical protein ECRN5871_3504,[tail fiber assembly protein] Baseplate assembly protein V, W Long tail fiber protein p37 domain protein Tail fiber domain protein Phage tail tape measure protein, TP901 family, core region	Tail fiber assembly Hypothetical protein ECSTECB2F1_0901, [tail fiber assembly protein, caudovirales tail fiber assembly protein] Caudovirales tail fiber assembly family protein Prophage tail fiber family protein Phage tail fiber repeat family protein Phage tail tape measure protein, lambda family	Caudovirales tail fiber assembly family protein Tail fiber Tail fiber domain protein Phage tail fiber repeat family protein
Terminase	Phage terminase large subunit Terminase	^a	Phage small terminase subunit Terminase, ATPase subunit Terminase, endonuclease subunit Terminase large subunit Terminase small subunit	Phage terminase large subunit family protein	Terminase small subunit Terminase B protein domain protein Terminase B protein
Transferase	Pyruvyl transferase Glycosyl transferase domain protein, group 2 family Glycosyltransferase, sugar-binding region containing DXD motif	^a	Hypothetical protein ECRN5871_3051, [nucleotidyl transferase, PF08843 family] D12 class N6 adenine-specific DNA methyltransferase family protein Hypothetical protein ECRN5871_0025, [N-acetyltransferase CN5]	Putative teichuronic acid biosynthesis glycosyltransferase tuaG Glucose-1-phosphate thymidyltransferase RTX toxin acyltransferase family protein Acetyl-CoA acetyltransferase	Acetyltransferase family protein Hypothetical protein ECSTECC16502_1295, [acetyltransferase]

^aThere are lots of hypothetical proteins with unknown function in desired genome which they have mentioned but their roles have not been defined yet.

Methylase_Ssuperfamily, Caudo_TAPsuperfamily, phage_tail_Nsuperfamily, Tail_P2_Isuperfamily, Terminase_3superfamily, Terminase_5superfamily, Phage_term_smalsuperfamily, Terminase_GpAsuperfamily, Phage_Nu1superfamily, DnaQ-like-exosuperfamily, Phage_holin_2superfamily, DUF3751 superfamily, Phage_fiber_2superfamily, Gifsy-2 superfamily, HSDR_Nsuperfamily, Cyt_C5_DNA_methylase superfamily, MethyltransfD12superfamily, Glyco_transf_GTA type superfamily, and Glyco_transf_GTA typesuperfamily

Discussion

The synchronic evolution of bacterial pathogens and virulence-associated determinants encoded by horizontally transferred genetic elements has been observed in several

species. However, *E. coli* is a normal member of the intestinal microflora of humans and animals. *E. coli* strains have acquired virulence factors by the attainment of particular genetic loci through horizontal gene transfer, transposons, or phages. These elements frequently encode multiple factors that enable bacteria to colonize the host and initiate disease development [16]. CDTs belong to one such class of virulence-associated factors. CDT was first identified in *E. coli* by Johnson and Lior in 1988 [17]; since then several studies have been reported that CDTs can be produced by intestinal and extra-intestinal pathogenic bacteria [18].

In this study, the genomes of 25 CDT⁺ *E. coli* strains were acquired from several gene banks. Multiple genome comparisons with 49 CDT⁻ *E. coli* strains, including EPEC (enteropathogenic *E. coli*), ETEC (enterotoxigenic *E. coli*), STEC (Shiga toxin-producing *E. coli*), EAEC (enteroagg-

regative *E. coli*), EIEC (enteroinvasive *E. coli*), AIEC (adherent invasive *E. coli*), UPEC (uropathogenic *E. coli*), ExPEC (extraintestinal pathogenic *E. coli*), EHEC (enterohemorrhagic *E. coli*), environmental strains and commensal strains were performed.

In fact, phylogenetic analysis based on whole-genome information is more accurate than those based on one gene or a set of limited genes. In this study, CDT-producing strains were not shown a phylogenomic relationship or pattern. Indeed, while they might carry the same or similar virulence gene sets, they also possess their own divergent genomic structures. This is probably because of their complex and distinct evolutionary pathways, indicating an independent acquisition of mobile genetic elements during their evolution.

The sporadic pattern in the phylogenomic dendrogram confirmed previous findings that CDT⁺ strains are heterogeneous. The heterogeneous nature of CDT-producing strains might arise from horizontal gene transfer through mobile genetic elements. These genetic exchanges that occur in bacteria provide genetic diversity and versatility [19].

A significant challenge in comparative genomics is the utilization of large datasets to identify specific sequence signatures that are biologically important or are useful in diagnosis [4, 20]. In this study, we define CDT-producing *E. coli* as the target group and found regions that were conserved that could serve as genomic signatures for the target group. Because of the heterogeneous genomic nature of CDT⁺ *E. coli*, five reference strains were selected instead of one, including EIEC, ExPEC, EPEC, STEC B2F1, and STEC C165-02. Moreover, in the phylogenomic overview, these five reference strains were selected from different clades of the phylogenetic tree, representing the T1-T6 groups.

The findings presented in this study indicate that the major conserved biomarkers beyond CDT were exonuclease, phage integrase, putative membrane, and tail-fiber proteins. Furthermore, with signature proteins of a targeted group, it was shown that phage-related proteins and virulence-associated factors could be commonly transferred by phages. Moreover, in the putative conserved domains of biomarker proteins, phage-related superfamilies were frequently observed. As a result, *cdt* genes were used as a signature sequences in CDT-producing *E. coli* strains, and it was shown that they can be used as a powerful biomarker.

In this study, the most significant signature proteins in the five *E. coli* strains were identified using *in-silico* whole-genome sequences. It was demonstrated that conserved signature proteins were expressed in a wide range of pathogenic bacterial strains, which could be used in future studies in a broad range of research applications and in modern vaccine-design strategies.

Supplementary materials

Supplementary data including six tables can be found with this article online at <http://www.genominfo.org/src/sm/gni-15-69-s001.pdf>.

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SUPPLEMENTARY INFORMATION

***In Silico* Signature Prediction Modeling in Cytolethal Distending Toxin-Producing *Escherichia coli* Strains**

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Supplementary Table 1. Signature details based on *Escherichia coli* 53638 reference

Region	Biomarker range in <i>Escherichia coli</i> 53638 genome between	Protein obtained by NCBI Blasts [Identical protein in other <i>Escherichia coli</i> strains]	Blast score	Blast Identity (%)	Blast E-value	Putative conserved domains non-specific hits (NH), specific hits (SH), multi domains (MD)
A	0 Mbp to 1 Mbp	Invasion plasmid antigen	829	73	0.0	SH: NEL, NEL superfamily, MD: PRK15387, COG4886, PLN00113 SH: DnaB, DnaB superfamily, NH: DnaB_C, RecA-like_NTPases superfamily, MD: phage_DnaB, PRK06904, DnaB, DnaB_C SH: HTH_XRE, NH: HTH_19, HTH_XRE superfamily, MD: PRK09706, HipB NH: Phage_attach, Gifsy-2 superfamily NH: Packaging_FI, Packaging_FI superfamily NH: Phage_Ci_repr, Phage_Ci_repr superfamily NH: phage_tail_N, phage_tail_N superfamily SH: ParE, NH: Plasmid_stabil, Plasmid_stabil superfamily MD: PRK09709 NH: Gam, Gam superfamily NH: tape_meas_lam_C, tape_meas_lam_C superfamily, NH: TMP_2, TMP_2 superfamily, MD: COG5281 NH: COG4220, Phage_Nu1, Phage_Nu1 superfamily NH: Minor_tail_Z, Minor_tail_Z superfamily NH: Phage_antitermQ, Phage_antitermQ superfamily NH: DUF1133, DUF1133 superfamily
		Replicative DNA helicase homolog	885	100	0.0	
		Replication protein	199	100	6e-60	
		Hypothetical protein Ec53638_0018	562	100	0.0	
		Hypothetical protein Ec53638_0019	94.4	98	1e-24	
		Conserved hypothetical protein	249	100	1e-82	
		Putative bacteriophage protein	745	100	0.0	
		Addiction module antitoxin, Axe family	154	100	1e-47	
		Plasmid stabilization system protein, RelE/ParE family	216	100	3e-71	
		Phage Head-Tail Attachment	252	99	2e-85	
		Putative DNA-packaging protein	223	100	5e-74	
		Bacteriophage CI repressor protein	267	100	1e-91	
		Putative tail fiber protein	481	100	6e-171	
		Conserved domain protein	77.4	100	1e-19	
		Hypothetical bacteriophage protein	205	99	7e-68	
		Putative tail component of prophage	203	98	6e-67	
		Putative tail fiber protein	99.4	100	3e-26	
		Exodeoxyribonuclease 8	153	100	2e-44	
		Host-nuclease inhibitor protein Gam	153	100	2e-48	
		Gifsy-1 prophage Vmth	226	100	3e-70	
Hypothetical protein Ec53638_0122, [gp41 domain protein]	278	100	3e-97			
Putative phage protein	202	100	2e-68			
Conserved hypothetical protein	197	100	5e-67			
Putative Prophage Qin DNA packaging protein NU1 homolog	204	100	8e-71			
Prophage minor tail protein Z (GPZ)	195	100	8e-65			
Putative bacteriophage protein	142	100	5e-44			
Phage antitermination Q type 1 family	135	100	5e-43			
Conserved hypothetical protein	107	100	2e-31			
B	0 Mbp to 1 Mbp	No significant results				
C	0 Mbp to 1 Mbp	Hypothetical protein Ec53638_3910	130	100	1e-41	
D	0 Mbp to 1 Mbp	No significant results				
E	1 Mbp to 2 Mbp	gp27	343	100	9e-119	NH: DUF3486, DUF3486 superfamily NH: COG4373, Terminase_1 superfamily, MD: 17 NH: Tail_P2_I, gpl, Tail_P2_I superfamily NH: COG4373, Terminase_1 superfamily, MD: P, Terminase_6 NH: Mor, Mor superfamily NH: FI, COG3497, Phage_sheath_1 superfamily, NH: GATase1_DJ-1, GAT_1 superfamily NH: phage_P2_V_gpV, Phage_base_V superfamily
		Putative DNA packaging protein Gp17	405	100	3e-139	
		Tail fiber	271	100	3e-91	
		Phage protein	282	100	5e-92	
		Putative DNA packaging protein Gp17	481	100	2e-171	
		Hypothetical protein Ec53638_1156, [membrane protein]	275	100	1e-93	
		DNA-binding protein RdgB	115	100	1e-32	
		Hypothetical protein Ec53638_1176	177	100	1e-57	
		Phage tail sheath protein	340	100	1e-116	
		Baseplate	217	100	1e-72	
		Bcv gene product	274	100	2e-93	
		Conserved hypothetical protein	179	100	2e-57	
		Putative type I restriction-modification system, S subunit	352	100	9e-120	
F	1 Mbp to 2 Mbp					

Supplementary Table 1. Signature details based on *Escherichia coli* 53638 reference

G	1 Mbp to 2 Mbp	ISCps8, transposase Transposase	809 200	100 100	0.0 9e-68	SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
H	1 Mbp to 2 Mbp	Cytolial distending toxin A Cytolial distending toxin B Cytolial distending toxin subunit C Hypothetical protein Ec53638_1905 Putative phage protein	486 545 370 158 253	100 100 100 100 100	9e-166 0.0 3e-122 2e-50 2e-81	NH: CDtoxinA, RICIN superfamily NH: PRK15251, CdtB, EEP superfamily NH: CDtoxinA, RICIN superfamily
I	2 Mbp to 3 Mbp	ISCps8, transposase	809	100	0.0	SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
J	2 Mbp to 3 Mbp	Transposase, IS1111 family ISAfe1, transposase	599 594	100 99	0.0 0.0	SH: Transposase_20, Transposase_20 superfamily, NH: DEDD_Tnp_IS110, DEDD_Tnp_IS110 superfamily, MD: COG3547
K	2 Mbp to 3 Mbp	No significant results				
L	2 Mbp to 3 Mbp	Transposase, IS1111 family ISAfe1, transposase	580 576	100 99	0.0 0.0	SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
M	3 Mbp to 4 Mbp	Transposase, IS1111 family ISAfe1, transposase	604 600	100 99	0.0 0.0	SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
N	3 Mbp to 4 Mbp	Gp33 TerL Putative phage-associated protein, HI1409 family Phage Mu protein F domain protein Phage protein gp13 Phage protein gp12 Conserved hypothetical protein Transglycosylase SLT domain protein Conserved hypothetical protein Phage P2 baseplate assembly protein gpV Putative bacteriophage protein Putative tail fiber protein Hypothetical bacteriophage protein Phage integrase Conserved hypothetical protein Putative tail component of prophage Invasion plasmid antigen, probably secreted by the Mxi-Spa machinery Dead box helicase Hypothetical protein Ec53638_3354 Hypothetical protein Ec53638_3355 Arc DNA binding domain protein Protein of unknown function Phage anti-repressor protein AntiB Type I restriction-modification system specificity subunit Conserved hypothetical protein Phage regulatory protein Cro gpH Bcv gene product Tail fiber assembly protein Type I restriction-modification enzyme, R subunit Type I restriction-modification system, M subunit	736 1,016 530 288 711 221 888 451 456 761 586 410 1,079 1,628 247 690 65.5 557 162 198 471 534 348 102 95.5 163 101 77.4 210 148	100 100 100 100 100 100 100 99 99 100 64 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	0.0 0.0 1e-176 5e-89 0.0 7e-66 0.0 9e-146 8e-148 0.0 0.0 1e-131 0.0 0.0 2e-80 0.0 4e-12 0.0 2e-48 4e-61 5e-168 0.0 6e-121 2e-29 1e-26 1e-49 5e-27 7e-19 7e-65 1e-44	NH: DUF1073, COG3567, Phage_portal superfamily, NH: phge_rel_HI1409, phge_rel_HI1409 superfamily, NH: COG3566, DUF2213, DUF2213 superfamily MD: COG2369 NH: DUF3383, DUF3383 superfamily SH: LT_GEWL, NH: SLT, Lysozyme_like superfamily, MD: MfE, mltD, PHA00368, NH: DUF2612, DUF2612 superfamily SH: INT_REC_C, DNA_BRE_C superfamily NH: COG4688, COG4688 superfamily NH: NEL, NEL superfamily, MD: PRK15387, COG4886, Golgin_A5 NH: Phage_pRha, Phage_pRha superfamily SH: DUF45, NH: COG1451, superfamily: DUF45 NH: COG3561, AntA, AntA superfamily NH: Methylase_S, Methylase_S superfamily, MD: HsdS NH: Vapl, THE_XRE superfamily, MD: PRK09706 MD: N6_Mfase, HsdM, hsdM, rumA SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
O	3 Mbp to 4 Mbp	ISCps8, transposase	809	100	0.0	SH: Transposase_20, Transposase_20 superfamily, MD: COG3547

Supplementary Table 1. Signature details based on *Escherichia coli* 53638 reference

P	3 Mbp to 4 Mbp	<p>Invasion plasmid antigen Putative bacteriophage protein Phage terminase large subunit Putative bacteriophage protein Putative tail fiber protein Hypothetical protein Ec53638_3420 Putative tail component of prophage Putative bacteriophage protein Hypothetical protein Ec53638_3782 Conserved hypothetical protein Bacteriophage lysis protein Exodeoxyribonuclease 8 Host-nuclease inhibitor protein Gam gpH Bcv gene product Tail fiber assembly protein Hypothetical protein Ec53638_3785 Phage Mu protein F like protein Putative tail fiber protein Tail fiber assembly protein Conserved hypothetical protein Putative phage protein</p>	836 528 937 317 574 77.0 239 688 478 337 52.8 162 143 180 98.6 102 277 266 146 137 130 141	83 100 100 100 100 100 99 99 100 100 100 100 100 100 57 100 100 100 100 100 100 100	0.0 0.0 0.0 3e-100 0.0 6e-18 3e-77 0.0 1e-174 2e-118 2e-09 7e-48 9e-45 6e-56 5e-26 3e-28 1e-98 5e-93 5e-46 5e-43 2e-40 2e-45	<p>SH: NEL, NEL superfamily, MD: PRK15370 NH: phage_term_2, Terminase_3, Terminase_3 superfamily NH: V, V superfamily NH: DedA, PRK10847, SNARE_assoc superfamily MD: PRK09709 NH: Gam, Gam superfamily NH: DUF1627, DUF1627 superfamily NH: Phage_Mu_F, Phage_Mu_F superfamily, MD: COG2369 NH: phage_tail_N, phage_tail_N superfamily NH: Caudo_TAP, Caudo_TAP superfamily NH: DUF1133, DUF1133 superfamily</p>
Q	3 Mbp to 4 Mbp	<p>Transcriptional regulator, AlpA family Hypothetical protein Ec53638_3914 Prophage integrase Conserved hypothetical protein Hypothetical protein Ec53638_3910</p>	114 108 256 379 119	98 100 100 100 100	1e-32 1e-30 4e-86 2e-131 4e-37	<p>NH: Phage_Alpa, Phage_Alpa superfamily, MD: PRK09692 SH: DUF4102, DUF4102 superfamily, NH: INT_P4, DNA_BRE_C superfamily, MD: PRK09692</p>
R	3 Mbp to 4 Mbp	<p>Putative tail component of prophage Invasion plasmid antigen Hypothetical bacteriophage protein Putative tail fiber protein Putative tail component of prophage Phage antitermination Q type 1 family Hypothetical protein Ec53638_4012 ISCs8, transposase Hypothetical bacteriophage protein Vonserved hypothetical protein BRO family, N- domain protein Conserved domain protein Hypothetical protein Ec53638_4059 Putative tail fiber protein Putative Prophage Qin DNA packaging protein NU1 homolog Hypothetical bacteriophage protein</p>	80.1 821 412 666 195 141 81.6 809 393 496 516 129 83.6 113 134 96.3	100 74 100 100 99 100 100 100 100 100 100 100 100 100 100 100	1e-17 0.0 2e-148 0.0 6e-60 5e-44 8e-22 0.0 6e-137 3e-176 0.0 1e-40 2e-23 4e-32 6e-42 2e-28	<p>SH: NEL, NEL superfamily, MD: PRK15370, COG4886 NH: Phage_antitermQ, Phage_antitermQ superfamily SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 MD: PRK05643, DnaN SH: ORF6N, ORF6N superfamily SH: Bro-N, NH: Bro-N, Bro-N superfamily, MD: COG3617 NH: Phage_NinH, Phage_NinH superfamily NH: phage_tail_N, phage_tail_N superfamily NH: COG4220, Phage_Nu1, Phage_Nu1 superfamily</p>
S	4 Mbp to 5 Mbp	<p>Putative membrane protein Conserved hypothetical protein Pyruvyl transferase</p>	944 909 774	100 100 100	0.0 0.0 0.0	<p>NH: MATE_Wzx_like, MATE_Wzx_like superfamily, NH: Polysacc_synt, Polysacc_synt superfamily, MD: RfbX, spore_V_B NH: FrhB_FdhB_C, FrhB_FdhB_C superfamily, MD: PRK09326, FrhB SH: PS_pyruv_trans, PS_pyruv_trans superfamily</p>

Supplementary Table 1. Signature details based on *Escherichia coli* 53638 reference

			Glycosyl transferase domain protein, group 2 family	202	100	1e-57	SH: Glyco_tranf_GTA_type, NH: Glycos_transf_2, PLN02726, PRK10018, Glyco_tranf_GTA_type superfamily, MD: Wcaa, PRK10073, Glyco_tranf_2_3, PgaC_lcaA, PTZ00260 MD: Caps_synth, OCH1 SH: EpsG, 7TMR_DISM_7TM superfamily SH: Glyco_tranf_GTA_type, NH: Glycos_transf_2, PRK10018, PRN02726, Glyco_tranf_GTA_type superfamily, MD: Wcaa, PLN02726, glyco2_xrt_Gpos1, PTZ00260, Glyco_tranf_2_3 SH: GLF superfamily, SH: NAD-GALP_mutase, PRK07208 superfamily, MD: Gif, UDP-GALP_mutase, PRK07208 NH: GT_2_like_b, Glycos_transf_2, Glyco_tranf_GTA_type superfamily, MD: COG1216, Wcaa SH: Transposase_20, Transposase_20 superfamily, MD: COG3547
			Glycosyltransferase, sugar-binding region containing DXD motif	539	99	1e-177	
			Putative membrane protein	680	100	0.0	
			Cps23Fh	695	100	0.0	
			UDP-galactopyranose mutase	758	99	0.0	
			WfbU	620	99	0.0	
			IScps8, transposase	810	100	0.0	
T	4 Mbp to 5 Mbp		gpH	705	100	0.0	SH: DUF3751, DUF3751 superfamily
			Putative phage gene	811	100	0.0	
			Conserved hypothetical protein	228	100	4e-69	NH: DUF2590, DUF2590 superfamily
			Phage tail tape measure protein, family	1,292	100	0.0	SH: PhageMin_Tail, MCP_signal superfamily
			Conserved hypothetical protein	185	100	2e-54	SH: DUF2765, DUF2765 superfamily
			Bacteriophage lysis protein	238	100	5e-72	SH: Phage_lysis, Phage_lysis superfamily
			Phage lysozyme	306	100	7e-96	NH: endolysin_autolysin, COG3772, Phage_lysozyme_Lysozyme_like superfamily
			Phage holin, lambda family	171	100	5e-49	
			Tail tube	311	100	2e-97	NH: DUF2597, DUF2597 superfamily
			Tail sheath	774	100	0.0	NH: DUF2586, DUF2586 superfamily
			Putative phage gene	498	100	2e-162	NH: P2_Phage_GpR, P2_Phage_GpR superfamily
			Conserved hypothetical protein	324	100	9e-102	
			Phage head completion protein (GPL)	321	100	9e-101	NH: Phage_GPL, Phage_GPL superfamily
			Putative repressor protein CI	373	100	1e-118	
			Putative DNA-binding protein Ner	170	100	3e-49	NH: Nlp, HTH_35, PRK10344, HTH_35 superfamily
			Phage transposase	579	100	0.0	NH: HTH_Tnp_Mu_1, HTH_Tnp_Mu_1 superfamily,
			Packaging protein	450	100	7e-145	NH: M, Phage_term_smal, Phage_term_smal superfamily
			Phage major capsid protein, P2 family	730	100	0.0	NH: N, major_capsid_P2, Phage_cap_P2, Phage_cap_P2 superfamily
			Scaffold	666	100	0.0	NH: O, Phage_GPO, Phage_GPO superfamily
			Terminase	1,232	100	0.0	NH: Terminase_5, Terminase_5 superfamily, MD: P, Terminase_6, COG5484
			Phage portal protein, pbsx family	674	100	0.0	NH: O, portal_PBSX, COG5518, Phage_portal, Phage_portal superfamily
			Phage transcriptional activator, Ogr/delta	186	100	1e-54	
			Hypothetical protein Ec53638_4365	176	100	6e-51	
			Hypothetical protein Ec53638_4366	162	100	3e-46	
			Putative phage replication protein	254	100	4e-70	
			Hypothetical protein Ec53638_4288	102	98	3e-26	
			Putative phage gene	1,133	100	0.0	
			Conserved hypothetical protein	217	100	9e-66	NH: DUF4406, DUF4406 superfamily
			DNA adenine methylase	641	100	0.0	NH: dam, PRK10904, Dam, MethyltransfD12, MethyltransfD12 superfamily
T	4 Mbp to 5 Mbp		Hypothetical protein Ec53638_4374	161	100	2e-46	
			Hypothetical protein Ec53638_4375	372	100	6e-121	
			Hypothetical protein Ec53638_4376	293	100	2e-92	
			Hypothetical protein Ec53638_4378	171	100	7e-50	

Supplementary Table 1. Signature details based on *Escherichia coli* 53638 reference

	<p>Hypothetical protein Ec53638_4377 Hypothetical protein Ec53638_4380 Integrase for prophage CP-933T</p> <p>Hypothetical bacteriophage protein Conserved hypothetical protein Putative tail fiber protein Putative tail component of prophage CP-933K Flagellin FlIC Putative DNA-packaging protein Phage Head-Tail Attachment Hypothetical protein Ec53638_4317 Phage transposase PaaX family protein Tail fiber protein Bcv gene product Putative phage replication protein Gifsy-1 prophage VmTH</p> <p>Putative phage regulatory protein, Rha family DNA transposition protein Gifsy-1 prophage VmTH Prophage minor tail protein Z (GPZ) Putative Prophage Qin DNA packaging protein NU1 homolog Hypothetical protein Ec53638_4368, [inositol Monophosphatase 1 (IMPase 1) (IMP 1)](or 4)] Protein gp42 Bacteriophage Mu Gam like protein</p>	<p>71.2 133 711</p> <p>393 496 586 202 469 223 252 387 488 266 273 262 149 229</p> <p>204 203 163 195 134 139</p> <p>140 122</p>	<p>100 100 100</p> <p>100 100 100 100 100 100 99 100 100 100</p> <p>100 100 100 100 100 100 100 100 100 100 100</p>	<p>3e-15 8e-37 0.0</p> <p>6e-137 2e-176 0.0 3e-63 8e-164 5e-74 2e-85 1e-137 3e-170 3e-90 4e-91 7e-89 2e-43 1e-71</p> <p>4e-66 2e-66 3e-48 7e-65 6e-42 2e-44</p> <p>5e-41 4e-37</p>	<p>NH: HP1_INT_C, Phage_integrase, DNA_BRE_C superfamily, MD: int, recomb_XerC, XerD, xerC DM: PRK05643, DnaN NH: ORF6N, ORF6N superfamily NH: phage_tail_N, phage_tail_N superfamily SH: FlIC, FlIC superfamily, MD: PRK08026 NH: Packaging_Fl, Packaging_Fl superfamily NH: Phage_attach, Gifsy-2 superfamily SH: rve, rve superfamily SH: HTH_36, HTH_36 superfamily, MD: PaaX_trns_reg NH: tape_meas_lam_C, tape_meas_lam_C superfamily, MD: COG5281 NH: Phage-MuB_C, Phage-MuB_C superfamily SH: TMP_2, TMP_2 superfamily, MD: COG5281 NH: Minor_tail_Z, Minor_tail_Z superfamily NH: COG4220, Phage_Nu1, Phage_Nu1 superfamily NH: COG4396, Phage_Mu_Gam, Phage_Mu_Gam superfamily</p>
U	<p>5 Mbp to end of the genome</p>	<p>761 575 410 456 1,126</p> <p>246 137 142 146 236</p>	<p>100 100 100 100 100</p> <p>99 100 100 100 100</p>	<p>0.0 0.0 6e-135 5e-152 0.0</p> <p>6e-80 7e-43 3e-42 4e-44 3e-70</p>	<p>NH: DUF2612, DUF2612 superfamily SH: TTSSLRR, TTSSLRR superfamily, SH: NEL, NEL superfamily, MD: PRK15370, COG4886 NH: DUF4376, DUF4376 superfamily NH: Caudo_TAP, Caudo_TAP superfamily NH: phage_tail_N, phage_tail_N superfamily</p>

Supplementary Table 2. Signature details based on *Escherichia coli* IHE3034 reference

Region	Biomarker range in <i>Escherichia coli</i> IHE3034 genome between	Protein obtained by NCBI Blastx [identical protein in other <i>Escherichia coli</i> strains]	Blast score	Blast Identity (%)	Blast E-value	Putative conserved domains non-specific hits (NH), specific hits (SH), multi domains (MD)
A	0 Mbp to 1 Mbp	Tail fiber protein Phage tail tape measure protein	559 135	100 100	0.0 4e-39	
B	2 Mbp to 3 Mbp	Cytolethal distending toxin, subunit C Cytolthal distending toxin, subunit B Cytolthal distending toxin, subunit A Exonuclease family protein Hypothetical protein ECOK1_2135, [conserved domain protein] Hypothetical protein ECOK1_2134 Hypothetical protein ECOK1_2122, [membrane protein] Putative transcriptional regulator Dica157 Antitermination protein	382 520 483 350 120 147 164 370 98.6	100 100 99 100 100 100 100 100 100	1e-127 1e-179 5e-166 1e-116 4e-36 3e-47 2e-52 3e-106 2e-27	NH: CDtoxinA, RICIN superfamily NH: PRK15251, CdtB, EEP superfamily NH: CDtoxinA, RICIN superfamily NH: dexA, DnaQ-like-exo superfamily
C	2 Mbp to 3 Mbp	Integrase/recombinase, phage integrase family Putative regulatory protein Cox Hypothetical protein ECOK1_2557, [membrane protein] Hypothetical protein ECOK1_2558 Hypothetical protein ECOK1_2602, [type VI secretion protein] Hypothetical protein ECOK1_2601, [double zinc ribbon family protein] Hypothetical protein ECOK1_2600 Holin, lambda family Hypothetical protein ECOK1_2581	693 196 131 83.2 585 194 675 58.9 375	100 100 100 98 100 100 100 100 100	0.0 1e-59 1e-36 9e-20 0.0 4e-60 0.0 3e-12 1e-133	NH: HP1_INT_C, Phage_integrase, DNA_BRE_C superfamily MD: int, XerD, recomb_XerD, xerD NH: Phage_Cox, Phage_Cox superfamily NH: VI_minor_1, DUF_3121 superfamily NH: Glyco_hydro_108, Glyco_hydro_108 superfamily NH: PG_binding_3, PG_binding_3 superfamily MD: zliS
D	2 Mbp to 3 Mbp	Hypothetical protein ECOK1_2812 Hypothetical protein ECOK1_2814 Hypothetical protein ECOK1_2815 Hypothetical protein ECOK1_2816 Hypothetical protein ECOK1_2809 Enterohemolysin 1	519 2,219 1,837 103 222 317	100 100 99 100 100 100	8e-169 0.0 0.0 6e-25 2e-72 2e-110	NH: V, V superfamily MD: PRK03918, COG1340, SMC_N, SMC_prok_B
E	4 Mbp to 5 Mbp	Site-specific recombinase, phage integrase family Protein cII Hypothetical protein ECOK1_4790, [beta family protein, Enterobacteria phage P4]	223 531 700	100 100 100	6e-65 0.0 0.0	NH: INT_P4, DNA_BRE_C superfamily, MD: PRK09692 NH: Beta_protein, Beta_protein superfamily
F	5 Mbp to end of the genome	Hypothetical protein ECOK1_4914	133	100	9e-42	

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

Region	Biomarker range in <i>Escherichia coli</i> RN587/1 genome between	Protein obtained by NCBI BlastX [identical protein in other <i>Escherichia coli</i> strains]	Blast score	Blast Identity (%)	Blast E-value	Putative conserved domains non-specific hits (NH), specific hits (SH), multi domains (MD)
A	0 Mbp to 1 Mbp	Cytolysin distending toxin A/C family protein	205	100	2e-66	NH: CDtoxinA, RICIN superfamily
B	0 Mbp to 1 Mbp	Type II restriction enzyme EcoRII	833	100	0.0	SH: EcoRII-C, EcoRII-C superfamily, NH: EcoRII_N, Bfil_C_EcoRII_N_B3 superfamily
		Modification methylase EcoRII	949	100	0.0	NH: dcm, DNA_methylase, Cyt_C5_DNA_methylase, Dcm, Cyt_C5_DNA_methylase superfamily, MD: PRK10458
C	Around 1 Mbp	Outer membrane autotransporter barrel domain protein	214	100	3e-66	NH: PL1_Passenger_AT, PL1_Passenger_AT superfamily, NH: DUF4353, DUF4353 superfamily, MD: PHA03255
		Hypothetical protein ECRN5871_0833	710	100	0.0	
		Hypothetical protein ECRN5871_0834	600	100	0.0	
		Hypothetical protein ECRN5871_0832	79.3	97	6e-18	
		Phage Tail Collar domain protein	528	100	0.0	NH: DUF3751, DUF3751 superfamily
		Caudovirales tail fiber assembly family protein	400	100	3e-136	SH: Caudo_TAP, Caudo_TAP superfamily
		Hypothetical protein ECRN5871_0827	442	100	6e-152	
		Phage protein	206	100	4e-67	NH: DUF2586, DUF2586 superfamily
		Pentapeptide repeats family protein	1,107	100	0.0	NH: SopA, SopA superfamily, NH: SopA_C, SopA_C superfamily, MD: Pentapeptide_4, PRK15377, COG1357
		hdmiD	475	100	3e-167	
		Hypothetical protein ECRN5871_0812, [phage tail protein, P2	316	100	1e-110	NH: P2_Phage_GpR, P2_Phage_GpR superfamily
		Phage tail completion protein R (GpR)]				
		Phage protein	273	100	1e-95	NH: DUF2597, DUF2597 superfamily
		Hypothetical protein ECRN5871_0702	69.7	100	1e-16	
		Phage protein	375	100	2e-131	NH: DUF2586, DUF2586 superfamily, MD: PAT1, PHA03247
		Phage small terminase subunit	163	100	1e-50	NH: M, Phage_smal_term superfamily
		Long tail fiber protein p37 domain protein	119	100	2e-35	
		Retron EC67 protein domain protein	850	100	0.0	SH: RT_Bac_retron_II, RT_like superfamily, MD: RVT_1
		Hypothetical protein ECRN5871_0823, [baseplate J-like protein]	814	100	0.0	NH: XkdT, Baseplate_J superfamily
		Hypothetical protein ECRN5871_0822, [PF10761 family protein]	77.8	100	6e-18	NH: DUF2590, DUF2590 superfamily
Phage tail tape measure protein, TP901 family, core region	775	100	0.0			
Hypothetical protein ECRN5871_0798	296	100	7e-101			
Hypothetical protein ECRN5871_0799	276	100	4e-93			
Regulatory protein CII	38.1	100	3e-04			
Hypothetical protein ECRN5871_0797	119	100	6e-34			
Hypothetical protein ECRN5871_0829	702	100	0.0	NH: Q, portal_PBSX, Phage_portal, Phage_portal superfamily		
Phage portal protein, PBSX family	342	100	3e-119	SH: DUF1311, DUF1311 superfamily, MD: PHA02067, LprI		
Hypothetical protein ECRN5871_0691	501	100	0.0	MD: P, Terminase_6		
Terminase, ATPase subunit	605	100	0.0	NH: Rhs_assc_core, Rhs_assc_core superfamily		
Protein rhsB	355	100	2e-113	NH: Phage_GPA, Phage_GPA superfamily		
Replication protein A	350	100	2e-118			
Hypothetical protein ECRN5871_0824	273	100	5e-95			
Integrase	495	100	1e-177	NH: HP1_INT_C, DNA_BRE_C superfamily, MD: int, recomb_XerD, XerD,		
Scaffold domain protein	473	100	6e-171	NH: O, Phage_GPO, Phage_GPO superfamily, MD: InCA		
Repressor protein CI	338	100	4e-120	NH: Phage_Cl_repr, Phage_Cl_repr superfamily		
Hypothetical protein ECRN5871_0819	129	98	3e-40			

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

C	Around 1 Mbp	Hypothetical protein ECRN5871_0801 Retron EC67 DNA adenine methylase Phage tail tape measure protein, TP901 family, core region Replication protein A Phage major capsid protein, P2 family Holin Phage head completion protein family protein Phage protein Hypothetical protein ECRN5871_0700	202 204 200 201 48.9 93.6 98.2 152 107	100 100 100 100 100 100 100 100 100 100 100	2e-68 3e-67 2e-62 2e-62 1e-08 8e-27 4e-28 1e-48 2e-32	NH: DUF3850, DUF3850 superfamily NH: Dam, PRK10904, Methyltransferase D12, dam, Methyltransferase D12 superfamily MD: PRK14960 NH: Phage_holin_2, Phage_holin_2 superfamily NH: DUF2765, DUF2765 superfamily
D	1 Mbp to 2 Mbp	Hypothetical protein ECRN5871_1072, [type III secretion system protein] Hypothetical protein ECRN5871_4139, [type III secretion system protein] Bacteriophage CI repressor helix-turn-helix domain protein Exonuclease family protein Avirulence protein A domain protein Hypothetical protein ECRN5871_1040 Hypothetical protein ECRN5871_1039 Hypothetical protein ECRN5871_1038 Eae-like protein Hypothetical protein ECRN5871_1027 Phage integrase family protein ea59 protein	594 321 431 676 551 185 237 155 223 224 227	100 65 100 100 100 100 100 100 100 100 100	1e-174 2e-89 2e-134 0.0 2e-173 8e-54 1e-71 2e-45 2e-66 2e-72 2e-67	MD: PRK15386 NH: Phage_Cl_repr, Phage_Cl_repr superfamily NH: DUF1482, DUF1482 superfamily NH: INT_Lambda_C, DNA_BRE_C superfamily SH: AAA_21, NH: COG3910, ABC_ATPase superfamily, MD: AAA_15
E	1 Mbp to 2 Mbp	Hypothetical protein ECRN5871_4153, [HNH endonuclease family protein] Prophage lambda integrase Hypothetical protein ECRN5871_4172 Hypothetical protein ECRN5871_4173 Hypothetical protein ECRN5871_4177, [Rz1 lytic protein] Prophage lambda integrase Reverse transcriptase recF/RecN/SMC N terminal domain protein	1,043 576 234 829 301 91.3 104 331 1,100	100 100 100 100 100 98 100 100 100	0.0 0.0 3e-68 0.0 5e-97 3e-24 1e-28 6e-107 0.0	NH: INT_Lambda_C, DNA_BRE_C superfamily NH: NA37, PRK00378, NA37 superfamily NH: Phage_integ_N, Phage_integ_N superfamily NH: RT_Bac_retron_I, RT_like superfamily SH: AAA_23, NH: ABC_RecF, COG3910, AAA_21, ABC_ATPase superfamily, MD: COG3950, recF, recf, AAA_15 NH: TIGR02646, TIGR02646 superfamily NH: COG4688, COG4688 superfamily NH: PRK15131, PMI_type1, PLN02288, ABD superfamily, MD: Mana SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 MD: pcrA, UvrD, UvrD, UvrD-helicase
F	1 Mbp to 2 Mbp	Hypothetical protein ECRN5871_4523, [TIGR02646 family protein] Hypothetical protein ECRN5871_4556 Hypothetical protein ECRN5871_4549 Transposase for insertion sequence element IS1111A Transposase IS116/IS110/IS902 family protein Hypothetical protein ECRN5871_4552, [UvrD/REP helicase domain protein] tnpA	424 775 91.7 336 338 273 71.2 206	100 100 98 100 100 100 100 100	9e-142 0.0 1e-24 5e-118 1e-117 3e-91 6e-18 6e-65	NH: PRK15131, PMI_type1, PLN02288, ABD superfamily, MD: Mana SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 SH: Transposase_20, Transposase_20 superfamily, MD: COG3547 MD: pcrA, UvrD, UvrD, UvrD-helicase NH: ABC_SMC_barmotin, AAA_23, ABC_ATPase superfamily,

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

G	1 Mbp to 2 Mbp	protein SMC mdaB domain protein Hypothetical protein ECRN5871_4526 Hypothetical protein ECRN5871_4525 Hypothetical protein ECRN5871_4553 Peptidoglycan domain protein Tail fiber domain protein	172 137 103 58.9 374 131	100 100 100 61 100 100	4e-57 1e-40 3e-30 1e-13 2e-133 9e-41	MD: SMC_N, Smc, PRK14272, recf NH: Flavodoxin_2, PRK00871, FMIN_red superfamily NH: Glyco_hydro_108, Glyco_hydro_108 superfamily, NH: PG_binding_3, PG_binding_3 superfamily, MD: zlis
H	2 Mbp to 3 Mbp	Tail fiber domain protein	349	100	3e-116	NH: dexA, DnaQ_like_exo superfamily, MD: PRK09709
I	2 Mbp to 3 Mbp	Exonuclease family protein	111	100	6e-32	
J	2 Mbp to 3 Mbp	Non-LEE-encoded effector EspJ Phage protein Zinc-binding domain of primase-helicase family protein	268 1,586	100 100	3e-78 0.0	NH: Phage_ASH, Phage_ASH superfamily SH: Prim_Zn_Ribbon, NH: Prim_Zn_Ribbon, Prim_Zn_Ribbon superfamily, NH: Toprim_3, Toprim superfamily, MD: COG4643
K	2 Mbp to 3 Mbp	Hypothetical protein ECRN5871_3674 Hypothetical protein ECRN5871_3673 Hypothetical protein ECRN5871_3672 Prophage CP4-57 regulatory protein family protein Integrase	432 271 216 151 833	100 100 99 100 100	2e-140 2e-84 5e-65 2e-43 0.0	NH: Phage_Alpa, AlpA, Phage_Alpa superfamily SH: INT_P4, DNA_BRE_C superfamily, NH: DUF4102, DUF4102 superfamily, MD: PRK09692, XerC
		Hypothetical protein ECRN5871_3699 Phage portal protein, PBSX family Terminase, ATPase subunit	343 284 1,238	100 100 100	1e-120 1e-86 0.0	NH: DUF2544, DUF2544 superfamily NH: Q, COG5518, portal_PBSX, Phage_portal superfamily SH: Terminase_5, Terminase_5 superfamily, MD: P, Terminase_6, COG5484
		Presumed capsid-scaffolding protein Phage major capsid protein, P2 family	439 632	100 100	7e-144 0.0	NH: Phage_GPO, O, Phage_GPO superfamily NH: N, Phage_cap_P2, major_capsid_P2, Phage_cap_P2 superfamily
		Terminase, endonuclease subunit Head completion/stabilization protein Hypothetical protein ECRN5871_3522 Hypothetical protein ECRN5871_3519	420 290 417 215	100 100 99 100	4e-138 2e-91 3e-137 3e-65	NH: M, Phage_term_smal, Phage_term_smal superfamily NH: Phage_GPL, Phage_GPL superfamily
		Hypothetical protein ECRN5871_3516, [PF05449 family protein] Hypothetical protein ECRN5871_3515, [PF11860 family protein]	209 408	100 100	1e-62 2e-132	NH: DUF754, DUF754 superfamily SH: DUF3380, DUF3380 superfamily
		P2 phage tail completion protein R family protein Phage virion morphogenesis protein hicB family protein Baseplate assembly protein V	318 300 230 377	100 100 100 100	9e-101 3e-94 5e-70 5e-121	NH: P2_Phage_GpR, P2_Phage_GpR superfamily SH: Phage_tail_S, NH: tail_comp_S, Phage_tail_S superfamily NH: HicB, HicB superfamily, MD: HicB
		Baseplate assembly protein W Baseplate J-like family protein Phage tail protein I Phage tail fiber repeat family protein	236 330 397 1,291	100 100 100 100	3e-72 5e-102 2e-127 0.0	NH: W, COG3628, GPW_gp25, GPW_gp25 superfamily NH: J, COG3948, Baseplate_J, Baseplate_J superfamily NH: gpl_tail_P2_I, tail_P2_I superfamily NH: DUF3751, DUF3751 superfamily, SH: Phage_fiber_2, Phage_fiber_2 superfamily, MD: COG5301
		Tail fiber domain protein Phage Tail Collar domain protein	219 244	68 58	7e-62 1e-69	NH: DUF3751, DUF3751 superfamily NH: DUF3751, DUF3751 superfamily
		Hypothetical protein ECRN5871_3504, [tail fiber assembly protein]	470	100	6e-153	NH: DUF4376, DUF4376 superfamily, NH: Caudo_TAP, Caudo_TAP superfamily

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

L	3 Mbp to 4 Mbp	<p>Enoyl-CoA hydratase/carnithine racemase-like protein</p> <p>D12 class N6 adenine-specific DNA methyltransferase family protein</p> <p>Major tail sheath protein</p> <p>Phage major tail tube protein</p> <p>Phage tail tape measure protein, TP901 family, core region</p> <p>Phage P2 GpU family protein</p> <p>Phage late control gene D family protein</p> <p>Caspase domain protein</p> <p>Hypothetical protein ECRN5871_3490</p> <p>Hypothetical protein ECRN5871_3489</p> <p>Integrase</p> <p>Phage portal protein, PBSX family</p> <p>Hypothetical protein ECRN5871_3481, [RatA]</p> <p>Aldose 1-epimerase family protein</p> <p>Hypothetical protein ECRN5871_3053</p> <p>Hypothetical protein ECRN5871_3052</p> <p>Hypothetical protein ECRN5871_3051, [nucleotidyl transferase, PF08843 family]</p> <p>Hypothetical protein ECRN5871_3050, [ATPase, tellurite resistance protein TerB]</p> <p>Biotin carboxylase</p> <p>DEAD/DEAH box helicase family protein</p> <p>Hypothetical protein ECRN5871_3043, [ATP-dependent helicase]</p> <p>Hypothetical protein ECRN5871_3042, [PF09983 domain protein]</p> <p>Hypothetical protein ECRN5871_3041</p> <p>Hypothetical protein ECRN5871_3040</p> <p>Hypothetical protein ECRN5871_3039, [PF12128 family protein]</p> <p>DNA-binding prophage protein</p> <p>DNA-binding protein Ner</p> <p>Integrase domain protein</p>	<p>321</p> <p>528</p> <p>546</p> <p>328</p> <p>1,687</p> <p>287</p> <p>727</p> <p>671</p> <p>1,759</p> <p>259</p> <p>511</p> <p>320</p> <p>202</p> <p>139</p> <p>1,435</p> <p>380</p> <p>679</p> <p>1,535</p> <p>619</p> <p>988</p> <p>407</p> <p>607</p> <p>777</p> <p>352</p> <p>2,302</p> <p>597</p> <p>145</p> <p>450</p> <p>255</p> <p>303</p> <p>147</p>	<p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p> <p>100</p>	<p>4e-101</p> <p>7e-174</p> <p>2e-178</p> <p>5e-103</p> <p>0.0</p> <p>7e-89</p> <p>0.0</p> <p>0.0</p> <p>0.0</p> <p>0.0</p> <p>0.0</p> <p>2e-169</p> <p>2e-106</p> <p>5e-63</p> <p>1e-42</p> <p>0.0</p> <p>1e-123</p> <p>0.0</p> <p>0.0</p> <p>0.0</p> <p>0.0</p> <p>2e-130</p> <p>0.0</p> <p>0.0</p> <p>6e-111</p> <p>0.0</p> <p>0.0</p> <p>5e-42</p> <p>6e-161</p> <p>4e-87</p> <p>3e-104</p> <p>1e-41</p>	<p>SH: Dam, NH: dam, PRK10904, MethyltransferaseD12, MethyltransferaseD12 superfamily</p> <p>NH: Fl, COG3497, Phage_sheath_1 superfamily</p> <p>NH: Fl, Phage_tube, COG3498, tail_tube, Phage_tube superfamily</p> <p>NH: tape_meas_TP901, PhageMin_Tail, MCP_signal superfamily, MD: COG5283, NH: PHA01399, PHA01399 superfamily</p> <p>NH: COG3499, Phage_P2_GpU, Phage_P2_GpU superfamily</p> <p>NH: D, COG3500, Phage_GPD, Phage_GPD superfamily</p> <p>SH: INT_P4, DNA_BRE_C superfamily, NH: DUF4102, DUF4102 superfamily, MD: PRK09692</p> <p>NH: Q, portal_PBSX, COG5518, Phage_portal superfamily</p> <p>MD: PRK15316</p> <p>NH: Aldose_epim_Ec_YphB, GalM, Aldose_epim superfamily</p> <p>MD: AAA_13, COG4694, SMC_prok_B</p> <p>NH: COG2253, DUF1814 superfamily</p> <p>SH: TerB-N, TerB-N superfamily, SH: TerB-C, TerB-C superfamily, NH: terB, terB_like superfamily</p> <p>NH: DUF2791, AAA superfamily</p> <p>SH: DEAD, NH: DEADc, DEADc superfamily, NH: HELIcC, HELIcC superfamily, NH: HELIcC, Helicase_C, Helicase_C superfamily, MD: Lhr, PRK13767, DEXH_lig_assoc, DEXDc, PTZ00110, PLN00206</p> <p>SH: Tropomyosin_1, Cep57_CLD, ApoLP_III_like superfamily, NH: SPEC, SPEC superfamily, NH: iSH2_PI3K_IA_R, iSH2_PI3K_IA_R superfamily, MD: DUF3584, Smc, PRK03918, SMC_prok_B, AIP3</p> <p>SH: Integrase_1, Integrase_1 superfamily, NH: Phage_int_SAM_2, Phage_int_SAM_2 superfamily</p> <p>NH: Nlp, HTH_35, PRK10344, HTH_35 superfamily</p> <p>NH: INT_P4, Phage_integrase, DNA_BRE_C superfamily, MD: PRK09692, XerC, SH: DUF4102, DUF4102 superfamily</p> <p>NH: Doc, DOC_P1, Fic superfamily</p> <p>NH: EspA, EspA superfamily</p> <p>NH: COG5613, COG5613 superfamily</p>
M	3 Mbp to 4 Mbp	Death on curing protein	255	100	4e-87	NH: Doc, DOC_P1, Fic superfamily
N	4 Mbp to 5 Mbp	espA-like secreted family protein	303	100	3e-104	NH: EspA, EspA superfamily
		Secretion system effector C (SseC) like family protein	147	100	1e-41	NH: COG5613, COG5613 superfamily

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

O	4 Mbp to 5 Mbp	No significant results					
P	4 Mbp to 5 Mbp	Anaerobic C4-dicarboxylate transporter dcuA Fumarylacetoacetate (FAA) hydrolase family protein	721 610	100 100	0.0 0.0	NH: Dcu, PRK09412, DcuB, DcuA, DcuB, DcuA, DcuB superfamily SH: FAA_hydrolase, NH: PRK10691, HpaG-N-term, FAA_hydrolase superfamily, MD: MhpD, HpaG-C-term, PRK15203	
		Dihydrodipicolinate synthetase family protein	600	100	0.0	SH: DHSDPS-like, NH: DapA, PRK03170, DHSDPS, dapA, PLN02417, Aldolase_Class_I superfamily	
		Transcriptional regulator, LacI family	466	100	2e-152	SH: HTH_Laci, NH: HTH_Laci, HTH_Laci superfamily, NH: PBP1_Laci_like_7, Periplasmic_Binding_Protein_Type_I superfamily, NH: Peripla_BP_3, MD: PurR, PRK10703, ccpA, Peripla_BP_1	
		Sugar (and other) transporter family protein	941	100	0.0	SH: MFS, MFS superfamily, NH: 2_A_01_02, PRK11195 superfamily, MD: 2A0115, PRK11551, Sugar_tr, ProP, synapt_SV2	
		Porin B	837	100	0.0	NH: OprB, OprB superfamily	
Q	4 Mbp to 5 Mbp	Type I restriction enzyme specificity protein Type I restriction-modification system, M subunit Type I site-specific deoxyribonuclease, HsdR family	213 256 139	100 100 100	2e-69 7e-82 2e-40	NH: Methylase_5, Methylase_S superfamily, MD: HsdS, sufB MD: HsdM, hsdM, N6_Mtase SH: HSDR_N, HSDR_N superfamily, MD: COG0610, hsdR	
R	4.5 Mbp to end of the genome	Hypothetical protein ECRN5871_0098 Hypothetical protein ECRN5871_0099 Hypothetical protein ECRN5871_0100 sel1 repeat family protein Hypothetical protein ECRN5871_0138, [secretion protein EspT] Hypothetical protein ECRN5871_0139 Hypothetical protein ECRN5871_0129 Hypothetical protein ECRN5871_0128, [secretion protein EspM] Transposase IS116/IS110/IS902 family protein	1,119 1,435 814 1,603 406 546 435 103 233	100 100 99 100 100 99 100 100 100	0.0 0.0 0.0 0.0 4e-136 0.0 0.0 1e-149 7e-27 2e-72	NH: DLP_2, Dynamis_N, Ras_like_GTPase superfamily NH: DLP_2, PRK09866, Ras_like_GTPase superfamily NH: DLP_2, Ras_like_GTPase superfamily NH: IpaB_Evca, IpaB_Evca superfamily NH: DUF1076, DUF1076 superfamily SH: IpaB_Evca, IpaB_Evca superfamily, NH: sifB, sif superfamily SH: Transposase_20, Transposase_20 superfamily, MD: COG3547	
		Hypothetical protein ECRN5871_0087 Cysteine protease domain, Yopt-type domain protein Hypothetical protein ECRN5871_0025, [N-acetyltransferase GCN5] Hypothetical protein ECRN5871_0026 Hypothetical protein ECRN5871_0137 Superoxide dismutase [Cu-Zn] 1	390 1,030 332 205 399 344	100 100 100 100 100 100	4e-134 0.0 2e-114 1e-65 4e-142 2e-121	NH: DUF3491, DUF3491 superfamily SH: COG4453, DUF_1778 NH: DUF1076, DUF1076 superfamily NH: PRK15388, SodC, Cu-Zn_Superoxide_Dismutase, Sod_Cu, PLN02386, Cu-Zn_Superoxide_Dismutase superfamily	
		Hypothetical protein ECRN5871_0110 Transposase for insertion sequence element IS1111A	161 292	100 86	8e-51 3e-101	NH: DEDD_Tnp_IS110, DEDD_Tnp_IS110 superfamily, SH: Transposase_20, Transposase_20 superfamily, MD: COG3547	
		Retron EC67 protein domain protein Phage portal protein, PBSX family yadA Hypothetical protein ECRN5871_0131	257 95.9 316 114	100 100 100 100	4e-88 3e-24 3e-111 4e-33	NH: O, Phage_portal superfamily MD: PTZ00102	
R	4.5 Mbp to end of the genome	Terminase large subunit Terminase small subunit Serine protease eatA	226 206 345	100 100 100	5e-71 7e-68 8e-112	NH: COG5525, Terminase_GpA, Terminase_GpA superfamily SH: COG4220, NH: Phage_Nu1, Phage_Nu1 superfamily, MD: PLN03114 MD: Peptidase_S6	

Supplementary Table 3. Signature details based on *Escherichia coli* RN587/1 reference

	ST44 protein	133	100	4e-42	NH: Transposase_mut, Transposase_mut superfamily, MD: COG3328
	ygeA	162	100	5e-53	
	Baseplate assembly protein V	140	100	8e-44	NH: gpV, phage_P2_V, Phage_base_V, Phage_base_V superfamily
	Transposase, IS605 OrfB family	62.4	100	8e-15	
	Putative transposase	136	100	7e-44	
	Cysteine protease domain, YopT-type domain protein	92.0	100	1e-23	

Supplementary Table 4. Signature details based on *Escherichia coli* STEC_B2F1 reference

Region	Biomarker range in <i>Escherichia coli</i> STEC_B2F1 genome between	Protein obtained by NCBI Blastx [identical protein in other <i>Escherichia coli</i> strains]	Blast score	Blast Identity (%)	Blast E-value	Putative conserved domains non-specific hits (NH), specific hits (SH), multi domains (MD)
A	0 Mbp to 1 Mbp	Hypothetical protein ECSTECB2F1_0150 Hypothetical protein ECSTECB2F1_0149, [transposase]	80.1 82.4	100 100	1e-21 5e-22	
B	0 Mbp to 1 Mbp	Protein 40A	582	100	0.0	
C	0 Mbp to 1 Mbp	Collagen triple helix repeat family protein Tail fiber assembly Hypothetical protein ECSTECB2F1_0901, [tail fiber assembly protein, caudovirales tail fiber assembly protein] Outer membrane protein lom Phage integrase family protein Prophage tail fiber family protein	487 144 251	100 100 100	3e-166 6e-42 1e-79	SH: collagen, collagen superfamily NH: Ail_Lom, PRK15240, COG3637, OMP_b-brl superfamily NH: phage_tail_N, phage_tail_N superfamily
D	1 Mbp to 2 Mbp	Putative endopeptidase Hypothetical protein ECSTECB2F1_1098 Hypothetical protein ECSTECB2F1_1099 Hypothetical protein ECSTECB2F1_1078, [PF03235 family protein] Caudovirales tail fiber assembly family protein Prophage lambda integrase Prophage lambda integrase Prophage lambda integrase	63.9 78.2 86.7 726 125 275 139 110	100 100 98 100 100 100 100 100	2e-12 3e-18 3e-21 0.0 5e-37 2e-93 6e-42 2e-31	 NH: DUF 262, DUF 262 superfamily NH: INT_Lambda_C, DNA_BRE_C superfamily NH: Phage_integ_N, Phage_integ_N superfamily MD: PRK09940, COG4753
E	1 Mbp to 2 Mbp	Transcriptional regulator, AraC family Collagen triple helix repeat family protein Hypothetical protein ECSTECB2F1_1516, [tail fiber assembly protein, caudovirales tail fiber assembly protein] Hypothetical protein ECSTECB2F1_1255 Host specificity protein J Antitermination protein Q Hypothetical protein ECSTECB2F1_1251 Hypothetical protein ECSTECB2F1_1263 Hypothetical protein ECSTECB2F1_1515 Helix-turn-helix family protein Outer membrane protein lom Prophage tail fiber family protein Collagen triple helix repeat family protein	497 228 221 89.4 188 308 28.5 207 197 60.1 130 127 82.8 85.9	100 100 100 100 100 100 100 100 100 100 100 96 100	3e-175 2e-70 4e-70 1e-23 5e-62 1e-99 0.13 6e-69 2e-61 9e-14 6e-41 6e-40 2e-22 4e-22	 SH: DUF3672, DUF3672 superfamily NH: ORF6N, ORF6N superfamily MD: PRK09706 NH: Ail_Lom, PRK15240, COG3637, OMP_b-brl superfamily NH: phage_tail_N, phage_tail_N superfamily
F	1 Mbp to 2 Mbp	Phage integrase family protein Hypothetical protein ECSTECB2F1_1296 Hypothetical protein ECSTECB2F1_1297 Phage integrase family protein	212 91.3 56.2 141	100 100 100 100	1e-69 7e-26 6e-12 5e-43	 NH: INT_Lambda_C, DNA_BRE_C superfamily
G	1 Mbp to 2 Mbp	Hypothetical protein ECSTECB2F1_1685	902	100	0.0	
H	1 Mbp to 2 Mbp	BRO family, N-terminal domain protein paaX-like family protein Phage tail fiber repeat family protein DNA-binding protein Roi DNA-damage-inducible protein I Hypothetical protein ECSTECB2F1_2003	537 271 355 245 59.7 69.7	100 100 100 100 100 100	0.0 0.0 3e-92 2e-120 2e-83 6e-14 4e-18	SH: Bro-N, NH: Bro-N, Bro-N superfamily, MD: COG3617 SH: HTH_36, HTH_36 superfamily, MD: PaaX_tms_reg SH: Phage_fiber_2, Phage_fiber_2 superfamily NH: PRK10597, Dintl superfamily

Supplementary Table 4. Signature details based on *Escherichia coli* STEC_B2F1 reference

I		outer membrane protein lom	136	100	2e-42	NH: Ail_Lom, PRK15240, COG6337, OMP_b-brl superfamily
J	2 Mbp to 3 Mbp 2 Mbp to 3 Mbp	Flagellin Putative membrane protein Glycosyl transferase family 2 family protein Hypothetical protein ECSTECB2F1_2214 Polysaccharide biosynthesis family protein Erythromycin biosynthesis sensory transduction protein eryC1 wbtB Putative teichuronic acid biosynthesis glycosyltransferase tuag Glucose-1-phosphate thymidyltransferase	229 632 572 328 764 753 164 525 556	100 100 100 99 100 99 100 100	4e-74 0.0 0.0 8e-103 0.0 0.0 4e-47 2e-170 0.0	Glyco_transf_GTA type superfamily NH: Glycos_transf_2, CESA_like, Glyco_transf_GTA type superfamily, MD: PRK10073 SH: MATE_like_10, NH: PRK15099, MATE_like superfamily, MD: RfbX NH: AHBA_syn, DegT_Dnrd_EryC1, WeeC, PRK11658, NHT_00031, AAT_I superfamily, MD: PRK15407, c SH: Glycos_transf_2, GT_2_like_d, PRK10018, PLN02726, Glyco_transf_GTA type superfamily, MD: Glyco_tranf_2_3, WcaA, PRK10073, PTZ00260 NH: G1P_TT_short, NTP_transferase, GalU, galU, PRK10122, Glyco_transf_GTA type superfamily, MD: rmlA, PRK15480, RfbA
K	2 Mbp to 3 Mbp	Hypothetical protein ECSTECB2F1_2378 Hypothetical protein ECSTECB2F1_2379 clp protease family protein Bacteriophage P4 DNA primase Hypothetical protein ECSTECB2F1_2371 Hemagglutination activity domain protein	99.8 94.7 1,288 203 132	98 100 100 100 100	1e-25 6e-24 0.0 8e-65 3e-40	SH: S14_ClpP_1, NH: ClpP, CLP_protease, clpP, Clp_protease_like superfamily, MD: SDH_sah, SppA
L	2 Mbp to 3 Mbp	Hemagglutination activity domain protein Hypothetical protein ECSTECB2F1_3213 Sulfatase family protein Hypothetical protein ECSTECB2F1_3190, [arylsulfatase] Outer membrane porin protein LC RTX toxin acyltransferase family protein Hemolysin secretion/activation protein ShlB/FhaC/HecB family pr Hypothetical protein ECSTECB2F1_3193 Transcriptional regulator, AraC family Serine protease eatA Hypothetical protein ECSTECB2F1_3192, [membrane protein] Hypothetical protein ECSTECB2F1_3199 Hypothetical protein ECSTECB2F1_3200 Neurotensin receptor R8 tonB-dependent vitamin B12 receptor Acetyl-CoA acetyltransferase	1,036 105 769 261 611 361 746 130 377 1,121 60.5 89.4 133 133 268 44.7	99 99 99 100 100 100 100 100 100 100 100 100 100 100 100 100 100	0.0 3e-26 0.0 3e-81 0.0 1e-118 0.0 0.0 9e-37 9e-128 0.0 9e-13 0.0 5e-24 1e-40 2e-40 9e-88 6e-07	SH: Fil_haemagg_2_fil_hemagg_20aa, Fil_haemagg_2 superfamily, MD: FhaB, PRK15319, Hia, PRK12688 NH: Sulfatase, Sulfatase superfamily, MD: AsIA, PRK13759, chol_sulfatase NH: Sulfatase, Sulfatase superfamily, MD: AsIA NH: PRK10554, Porin_1, OmpC, gram_neg_porins, OM_channels superfamily NH: Haemagg_act, SH: Haemagg_act, Haemagg_act superfamily NH: HlyC, SH: HlyC, HlyC superfamily, MD: FhaC, ShlB NH: PRK09750, DUF1187, DUF1187 superfamily MD: HTH_ARAC, PRK09940, AraC MD: Peptidase_S6, AidA NH: ligand_gated_channel, Plug, Plug superfamily, OM_channels superfamily, MD: Btub, TonB-B12 NH: Thiolase_C, thiolase, Thiolase_C superfamily, cond-enzymes superfamily, MD: PRK05790, PaaI, AcCoA-C

Supplementary Table 4. Signature details based on *Escherichia coli* STEC_B2F1 reference

			76.6	97	9e-20	Actrans
M	2 Mbp to 3 Mbp	Hypothetical protein ECSTECB2F1_3178 ompA-like transmembrane domain protein	135	100	2e-41	NH: COG3637, OMP_b-brl, OMP_b-brl superfamily
N	3 Mbp to 4 Mbp	Hypothetical protein ECSTECB2F1_3480 Replication gene A protein Cytolethal distending toxin C Endonuclease/Exonuclease/phosphatase family protein Cytolethal distending toxin A/C family protein Caudovirales tail fiber assembly family protein Hypothetical protein ECSTECB2F1_4300 Phage tail fiber repeat family protein	453	100	6e-161	NH: CDtoxinA, RICIN superfamily NH: PRK15251, CdtB, EEP superfamily NH: CDtoxinA, RICIN superfamily
O	4 Mbp to end of the genome	Hypothetical protein ECSTECB2F1_4680 Putative membrane protein Hypothetical protein ECSTECB2F1_4682 Type I restriction modification DNA specificity domain protein Type I site-specific deoxyribonuclease, HsdR family	564	100	0.0	NH: ResIII, DEXDc superfamily, MD: hsdR, COG0610
P	4 Mbp to end of the genome	Host specificity protein J Hypothetical protein ECSTECB2F1_1326, [hok/gef family protein] Prophage tail fiber family protein Major tail protein V Hypothetical protein ECSTECB2F1_1281 Hemin receptor domain protein Minor tail protein M Phage tail tape measure protein, lambda family Phage terminase large subunit family protein	374	100	7e-119	SH: DUF3672, DUF3672 superfamily
			63.2	100	2e-13	NH: phage_tail_N, phage_tail_N superfamily
			89.7	100	3e-22	SH: BID_2, Big_2 superfamily, MD: COG5492
			337	100	6e-118	
			130	100	9e-41	
			40.0	100	9e-06	NH: COG4718, Phage_min_tail, Phage_min_tail superfamily
			144	99	4e-46	
			67.0	100	1e-14	NH: COG5525, Terminase_GpA superfamily
			140	100	6e-42	

Supplementary Table 5. Signature details based on *Escherichia coli* STEC_C165_02 reference

Region	Biomarker range in <i>Escherichia coli</i> STEC_C165_02 genome between	Protein obtained by NCBI Blastx [identical protein in other <i>Escherichia coli</i> strains]	Blast score	Blast Identity (%)	Blast E-value	Putative conserved domains non-specific hits (NH), specific hits (SH), multi domains (MD)
A	0 Mbp to 1 Mbp	Restriction endonuclease family protein	723	100	0.0	SH: COG4127, HsdM_N, NH: Mrr_cat, UPF0020, Mrr_cat superfamily, HsdM_N superfamily, MD: Mrr, HsdM, N6_Mtase SH: Methyltransf_26, AdoMet_Mtase superfamily, HsdM_N, HsdM_N superfamily, MD: N6_Mtase, hsdM SH: HsdM_N, NH: UPF0020, HsdM_N superfamily, MD: HsdM, N6_Mtase, COG0610, hsdR, DEXDc
		TIR protein	411	100	2e-131	
		N-6 DNA Methylase family protein	974	100	0.0	
		Type I site-specific deoxyribonuclease, HsdR family protein	1,409	100	0.0	
		Hypothetical protein ECSTECC16502_0289, [ABC transporter ATP-binding protein]	1,107	100	0.0	
		Hypothetical protein ECSTECC16502_0290	106	98	4e-27	
		Hypothetical protein ECSTECC16502_0291	74.3	100	4e-16	
		Type I restriction-modification system specificity determinant	1,176	100	0.0	
		Putative membrane protein	364	100	6e-115	
		Type III restriction enzyme, res subunit	2,261	100	0.0	
		Hypothetical protein ECSTECC16502_0339	1,450	100	0.0	
		Metallo-beta-lactamase superfamily protein	569	100	0.0	
		4-Hydroxyphenylacetate catabolism regulatory protein HpaA	618	100	0.0	
		4-Hydroxyphenylacetate permease	850	100	0.0	
Hypothetical protein ECSTECC16502_0280, [HNH endonuclease]	516	99	0.0			
Filamentation induced by cAMP protein Fic	138	100	2e-42			
B	1 Mbp to 2 Mbp	Phage virion morphogenesis protein	70.5	100	1e-14	
		Hypothetical protein ECSTECC16502_1311	133	98	1e-37	
		AAA ATPase	795	100	0.0	NH: GP4d_helicase, RecA_like superfamily, MD: AAA_15, COG3950
		Putative membrane protein	852	100	0.0	
		Hypothetical protein ECSTECC16502_4950, [Rz1 lytic protein]	62.0	100	1e-12	
		Hypothetical protein ECSTECC16502_4968	442	100	4e-159	NH: PRK14512, S14_ClpP_1, ClpP, clpP, ClpP_protease_like superfamily
		Acetyltransferase family protein	394	100	4e-141	
		Hypothetical protein ECSTECC16502_4986	51.2	100	2e-10	
		Hypothetical protein ECSTECC16502_4996	466	100	2e-167	NH: DUF2829, DUF2829 superfamily
		isaA	43.9	95	6e-06	
		Hypothetical protein ECSTECC16502_1295, [acetyltransferase]	120	100	2e-35	
		Integrase	208	100	3e-68	MD: int
		Tail fiber	176	100	4e-55	
		DNA-invertase	92.8	100	2e-23	NH: SR_Resniv, Resolvase, Ser_Recombinase superfamily, MD: PinR
Caudovirales tail fiber assembly family protein	338	100	6e-118	SH: Caudo_TAP, Caudo_TAP superfamily		
Tail fiber domain protein	201	100	4e-65			

Supplementary Table 5. Signature details based on *Escherichia coli* STEC_C165_O2 reference

		<p>Recombination enhancement Phage Tail Collar domain protein DNA-invertase Hypothetical protein ECSTEC16502_4984, [lysogeny establishment protein] Phage tail fiber repeat family protein</p>	<p>298 134 28.9 120 138</p>	<p>99 95 100 100 100</p>	<p>3e-104 2e-41 0.061 6e-37 1e-41</p>	<p>NH: Collar, Collar superfamily SH: Phage_fiber_2, Phage_fiber_2 superfamily, MD: COG5301</p>
C	1 Mbp to 2 Mbp	<p>Hypothetical protein ECSTEC16502_1393 Hypothetical protein ECSTEC16502_1803 Hypothetical protein ECSTEC16502_1804 Hypothetical protein ECSTEC16502_1805 Hypothetical protein ECSTEC16502_1809 Resolvase, N terminal domain protein Hypothetical protein ECSTEC16502_1807 Hypothetical protein ECSTEC16502_2561 Phage holin, lambda family Peptidoglycan domain protein</p>	<p>264 122 213 156 138 126 194 68.9 374</p>	<p>100 100 100 100 100 100 100 100 100</p>	<p>4e-87 3e-34 1e-72 6e-49 7e-41 5e-40 3e-62 8e-16 3e-133</p>	<p>NH: Glyco_hydro_108, Glyco_hydro_108 superfamily, NH: PG_binding_3, PG_binding_3 superfamily, MD: zlls</p>
D	2 Mbp to 3 Mbp	<p>Hypothetical protein ECSTEC16502_2576 Hypothetical protein ECSTEC16502_2577 gp41 domain protein Hypothetical protein ECSTEC16502_2586 Hypothetical protein ECSTEC16502_2559 Hypothetical protein ECSTEC16502_2587, [DNA-binding protein] Hypothetical protein ECSTEC16502_2588</p>	<p>188 74.7 312 32.0 75.9 168 133</p>	<p>100 100 99 100 97 100 100</p>	<p>5e-62 3e-18 2e-110 0.006 1e-19 1e-55 8e-41</p>	<p>NH: PHA00675, PHA00675 superfamily NH: DUF1627, DUF1627 superfamily NH: INT_P4,DNA_BRE_C superfamily, MD: PRK09692</p>
E	2 Mbp to 3 Mbp	<p>Prophage CPA-57 integrase Hypothetical protein ECSTEC16502_2827 Hypothetical protein ECSTEC16502_2828 Prophage CPA-57 regulatory protein family protein Terminase small subunit Hypothetical protein ECSTEC16502_2842 Major head protein Prophage CPA-57 integrase</p>	<p>108 168 99.4 136 374 101 288 237</p>	<p>100 100 100 99 100 100 100 100</p>	<p>1e-47 6e-53 5e-27 1e-40 2e-132 5e-28 8e-97 6e-77</p>	<p>NH: Phage_Alpa, Alpa, Phage_Alpa superfamily SH: COG4220, NH: Phage_Nu1, Phage_Nu1 superfamily NH: Phage_cap_E, Phage_cap_E superfamily NH: INT_P4,DNA_BRE_C superfamily, SH: DUF4102, DUF4102 superfamily, MD: PRK09692</p>
F	3 Mbp to 4 Mbp	<p>Hypothetical protein ECSTEC16502_2843 DNA topoisomerase IV, A subunit</p>	<p>128 177</p>	<p>100 100</p>	<p>2e-40 6e-54</p>	<p>NH: TOP4c, TOP4c superfamily, MD: parC_Gneg, PRK05561, GyrA, TOP4c, DNA_topoisolv_52, PLN03128</p>
G	4 Mbp to 5 Mbp	<p>Cytolethal distending toxin A/C family protein Endonuclease/Exonuclease/phosphatase family protein Hypothetical protein ECSTEC16502_4757</p>	<p>456 546 189</p>	<p>100 100 99</p>	<p>3e-154 0.0 3e-62</p>	<p>NH: CDtoxinA, RICIN superfamily NH: PRK15251, CdtB, EEP superfamily</p>
H	4.5 Mbp to end of the genome	<p>upf89.5 Putative lipoprotein humD Hot protein Hypothetical protein ECSTEC16502_1327 Terminase B protein domain protein</p>	<p>194 185 122 87.0 96.7 565</p>	<p>100 100 100 98 98 100</p>	<p>4e-59 2e-57 3e-34 1e-21 3e-25 0.0</p>	<p>NH: PRK10276, peptidase_S24_S26 superfamily, MD: LexA SH: DNA_pol3_theta, NH: PRK10969, DNA_pol3_theta superfamily</p>

Supplementary Table 5. Signature details based on *Escherichia coli* STEC_C165_02 reference

	Putative membrane protein	80.9	100	6e-21	NH: Phage_ASH, Phage_ASH superfamily
	Hypothetical protein ECSTEC16502_1333	155	100	3e-49	
	Terminase B protein	283	100	1e-94	
	VRR-NUC domain protein	165	100	2e-53	
	Hypothetical protein ECSTEC16502_4927	57.4	100	2e-13	

Supplementary Table 6. AAlphabetic abbreviation and description of putative conserved domains

Alphabetic Abbreviation	Description
17	Large terminase protein
2_A_01_02	Multidrug resistance protein
2A0115	Benzoate transporter; [Transport and binding proteins, Carbohydrates, organic alcohols]
52	DNA topoisomerase II medium subunit; Provisional
AAA_13	AAA domain; This family of domains contain a P-loop motif
AAA_15	AAA ATPase domain; This family of domains contain a P-loop motif
AAA_21	AAA domain
AAA_23	AAA domain
ABC_RecF	ATP-binding cassette domain of RecF; RecF is a recombinational DNA repair ATPase
ABC_SMC_barmotin	ATP-binding cassette domain of barmotin, a member of the SMC protein family
ACCoA-C-Actrans	Acetyl-CoA acetyltransferases
AHBA_syn	3-Amino-5-hydroxybenzoic acid synthase family (AHBA_syn)
AidA	Type V secretory pathway, adhesin AidA [Cell envelope biogenesis]
Ail_Lom	Enterobacterial Ail/Lom protein; This family consists of several bacterial and phage Ail_Lom proteins
AIP3	Actin interacting protein 3; Aip3p/Bud6p is a regulator of cell and cytoskeletal polarity
Aldose_epim_Ec_YphB	Aldose 1-epimerase, similar to Escherichia coli YphB
AlpA	Predicted transcriptional regulator [Transcription]
AntA	AntA/AntB antirepressor
AraC	AraC-type DNA-binding domain-containing proteins [Transcription]
AsIA	Arylsulfatase A and related enzymes [Inorganic ion transport and metabolism]
Baseplate_J	Baseplate J-like protein; The P2 bacteriophage J protein lies at the edge of the baseplate
Beta_protein	Beta protein; This family includes the beta protein from Bacteriophage T4
BID_2	Bacterial Ig-like domain 2
Bro-N	BRO family, N-terminal domain; This family includes the N-terminus of baculovirus BRO
btuB	Vitamin B12/cobalamin outer membrane transporter; Provisional
BtuB	Outer membrane cobalamin receptor protein [Coenzyme metabolism]
Caps_synth	Capsular polysaccharide synthesis protein
Caudo_TAP	Caudovirales tail fibre assembly protein
ccpA	catabolite control protein A
CdtB	CdtB, the catalytic DNase I-like subunit of cytolethal distending toxin (CDT) protein
CDtoxInA	Cytolethal distending toxin A/C family
Cep57_CLD	Centrosome localisation domain of Cep57
CESA_like	CESA_like is the cellulose synthase superfamily; The cellulose synthase (CESA) superfamily
choI_sulfatase	Choline-sulfatase;
clpP	ATP-dependent Clp endopeptidase, proteolytic subunit ClpP
ClpP	Protease subunit of ATP-dependent Clp proteases
Clp_protease	Clp protease; The Clp protease has an active site catalytic triad
COG0436	Aspartate/tyrosine/aromatic aminotransferase [Amino acid transport and metabolism]
COG0610	Type I site-specific restriction-modification system, R (restriction) subunit and related proteins
COG1216	Predicted glycosyltransferases [General function prediction only]
COG1340	Uncharacterized archaeal coiled-coil protein [Function unknown]
COG1357	Pentapeptide repeats containing protein [Function unknown]
COG1451	Predicted metal-dependent hydrolase [General function prediction only]
COG1479	Uncharacterized conserved protein [Function unknown]

Supplementary Table 6. Aalphabetic abbreviation and description of putative conserved domains

COG2253	Uncharacterized conserved protein [Function unknown]
COG2369	Uncharacterized protein, homolog of phage Mu protein gp30 [Function unknown]
COG3328	Transposase and inactivated derivatives [DNA replication, recombination, and repair]
COG3440	Predicted restriction endonuclease [Defense mechanisms]
COG3497	Phage tail sheath protein F1 [General function prediction only]
COG3498	Phage tail tube protein FII [General function prediction only]
COG3499	Phage protein U [General function prediction only]
COG3500	Phage protein D [General function prediction only]
COG3547	Transposase and inactivated derivatives [DNA replication, recombination, and repair]
COG3561	Phage anti-repressor protein [Transcription]
COG3566	Uncharacterized protein conserved in bacteria [Function unknown]
COG3567	Uncharacterized protein conserved in bacteria [Function unknown]
COG3586	Uncharacterized conserved protein [Function unknown]
COG3617	Prophage antirepressor [Transcription]
COG3628	Phage baseplate assembly protein W [General function prediction only]
COG3637	Opacity protein and related surface antigens [Cell envelope biogenesis, outer membrane]
COG3772	Phage-related lysozyme (muraminidase) [General function prediction only]
COG3910	Predicted ATPase [General function prediction only]
COG3948	Phage-related baseplate assembly protein [General function prediction only]
COG3950	Predicted ATP-binding protein involved in virulence [General function prediction only]
COG4127	Uncharacterized conserved protein [Function unknown]
COG4220	Phage DNA packaging protein, Nu1 subunit of terminase
COG4373	Mu-like prophage FluMu protein gp28 [General function prediction only]
COG4396	Mu-like prophage host-nuclease inhibitor protein Gam [General function prediction only]
COG4453	Uncharacterized protein conserved in bacteria [Function unknown]
COG4643	Uncharacterized protein conserved in bacteria [Function unknown]
COG4688	Uncharacterized protein conserved in bacteria [Function unknown]
COG4694	Uncharacterized protein conserved in bacteria [Function unknown]
COG4718	Phage-related protein [Function unknown]
COG4753	Response regulator containing CheY-like receiver domain and AraC-type DNA-binding domain
COG4886	Leucine-rich repeat (LRR) protein [Function unknown]
COG5281	Phage-related minor tail protein [Function unknown]
COG5283	Phage-related tail protein [Function unknown]
COG5301	Phage-related tail fibre protein [General function prediction only]
COG5484	Uncharacterized conserved protein [Function unknown]
COG5492	Bacterial surface proteins containing Ig-like domains [Cell motility and secretion]
COG5518	Bacteriophage capsid portal protein [General function prediction only]
COG5525	Phage terminase, large subunit GpA [Replication, recombination and repair]
COG5613	Uncharacterized conserved protein [Function unknown]
Collagen	Collagen triple helix repeat (20 copies)
Collar	Phage Tail Collar Domain
Cu-Zn_Superoxide_Dismutase	Copper/zinc superoxide dismutase (SOD)
Cyt_C5_DNA_methylase	Cytosine-C5 specific DNA methylases
D	tail protein; Provisional
dam	DNA adenine methylase (dam)

Supplementary Table 6. Alphabetical abbreviation and description of putative conserved domains

Dam	Site-specific DNA methylase [DNA replication, recombination, and repair]
dapA	Dihydrodipicolinate synthase; Dihydrodipicolinate synthase is a homotetrameric enzyme
DapA	Dihydrodipicolinate synthase/N-acetylneuraminate lyase
dcm	DNA-methyltransferase (dcm)
Dcm	Site-specific DNA methylase [DNA replication, recombination, and repair]
Dcu	Anaerobic c4-dicarboxylate membrane transporter family protein
DcuA_DcuB	Anaerobic c4-dicarboxylate membrane transporter
DcuB	Anaerobic C4-dicarboxylate transporter [General function prediction only]
DEAD	DEAD/DEAH box helicase; Members of this family include the DEAD and DEAH box helicases
DEADc	DEAD-box helicases. A diverse family of proteins involved in ATP-dependent RNA unwinding
Deda	Uncharacterized membrane-associated protein [Function unknown]
DEDD_Tnp_IS110	Transposase; Transposase proteins are necessary for efficient DNA transposition
DegT_DnrJ_EryC1	DegT/DnrJ/EryC1/StrS aminotransferase family
dexA	Exonuclease
DEXdc	DEAD-like helicases superfamily
DEXH_lig_assoc	DEXH box helicase, DNA ligase-associated
DHDPS	Dihydrodipicolinate synthetase family; This family has a TIM barrel structure
DHDPS-like	Dihydrodipicolinate synthase family; Dihydrodipicolinate synthase family
DLP_2	Dynammin-like protein including dynamins, mitofusins, and guanylate-binding proteins
DnaB	Replicative DNA helicase [DNA replication, recombination, and repair]
DnaB_C	DnaB helicase C terminal domain
DNA_methylase	C-5 Cytosine-specific DNA methylase
DnaN	DNA polymerase sliding clamp subunit (PCNA homolog) [DNA replication, recombination]
DNA_pol3_theta	DNA polymerase III, theta subunit
DNA_topoisoIV	DNA gyrase/topoisomerase IV, subunit A
Doc	Prophage maintenance system killer protein [General function prediction only]
DOC_P1	Death-on-curing family protein
DUF1073	Protein of unknown function (DUF1073)
DUF1076	Protein of unknown function (DUF1076); This family consists of several hypothetical bacterial proteins
DUF1133	Protein of unknown function (DUF1133)
DUF1187	Protein of unknown function (DUF1187)
DUF1311	Protein of unknown function (DUF1311)
DUF1482	Protein of unknown function (DUF1482)
DUF1524	Protein of unknown function (DUF1524)
DUF1627	Protein of unknown function (DUF1627)
DUF2213	Uncharacterized protein conserved in bacteria (DUF2213)
DUF2544	Protein of unknown function (DUF2544)
DUF2586	Protein of unknown function (DUF2586)
DUF2590	Protein of unknown function (DUF2590); This family of proteins has no known function
DUF2597	Protein of unknown function (DUF2597)
DUF2612	Protein of unknown function (DUF2612); This is a phage protein family
DUF262	Protein of unknown function DUF262
DUF2765	Protein of unknown function (DUF2765); This family of proteins has no known function
DUF2791	P-loop Domain of unknown function (DUF2791); This is a family of proteins found in archaea
DUF2829	Protein of unknown function (DUF2829)

Supplementary Table 6. Aalphabetic abbreviation and description of putative conserved domains

DUF3380	Protein of unknown function (DUF3380)
DUF3383	Protein of unknown function (DUF3383)
DUF3486	Protein of unknown function (DUF3486)
DUF3491	Protein of unknown function (DUF3491); This family of proteins is functionally uncharacterized
DUF3584	Protein of unknown function (DUF3584); This protein is found in bacteria and eukaryotes
DUF3672	Fibronectin type III protein; This domain family is found in bacteria and viruses
DUF3751	Phage tail-collar fibre protein; This domain family is found in bacteria and viruses
DUF3850	Domain of Unknown Function with PDB structure (DUF3850)
DUF4102	Domain of unknown function (DUF4102)
DUF4353	Domain of unknown function (DUF4353)
DUF4376	Domain of unknown function (DUF4376)
DUF4406	Protein of unknown function (DUF4406)
DUF45	Protein of unknown function DUF45
DUF754	Protein of unknown function (DUF754); This domain appears to be found in a group of prophage
Dynamain_N	Dynamain family
EcoRII-C	EcoRII C terminal; The C-terminal catalytic domain of the Restriction Endonuclease EcoRII
EcoRII-N	Restriction endonuclease EcoRII, N-terminal
ElaC	Metal-dependent hydrolases of the beta-lactamase superfamily III [General function prediction]
endolysin_autolysin	Endolysins and autolysins are found in viruses and bacteria, respectively
EpsG	EpsG family
EspA	EspA-like secreted protein; EspA is the prototypical member of this family
FAA_hydrolase	Fumarylacetoacetate (FAA) hydrolase family
FhaB	Large exoproteins involved in heme utilization or adhesion
FhaC	Hemolysin activation/secretion protein [Intracellular trafficking and secretion]
Fl	Major tail sheath protein; Provisional
Fli	Major tail tube protein; Provisional
Fil_haemagg_2	Haemagglutinin repeat
fil_hemag_20aa	Adhesin HecA family 20-residue repeat (two copies)
Flavodoxin_2	Flavodoxin-like fold; This family consists of a domain with a flavodoxin-like fold
FliC	Flagellin protein; This domain family is found in bacteria
FrhB	Coenzyme F420-reducing hydrogenase, beta subunit [Energy production and conversion]
FrhB_FdhB_C	Coenzyme F420 hydrogenase/dehydrogenase, beta subunit C terminus
G1P_TT_short	G1P_TT_short is the short form of glucose-1-phosphate thymidyltransferase
GalM	Galactose mutarotase and related enzymes [Carbohydrate transport and metabolism]
galU	UTP-glucose-1-phosphate uridylyltransferase
GalU	UDP-glucose pyrophosphorylase [Cell envelope biogenesis, outer membrane]
Gam	Host-nuclease inhibitor protein Gam; The Gam protein inhibits RecBCD nuclease
GATase1_DJ-1	Type 1 glutamine amidotransferase (GATase1)-like domain found in Human DJ-1
Glf	UDP-galactopyranose mutase [Cell envelope biogenesis, outer membrane]
GLF	UDP-galactopyranose mutase
glyc2_xrtf_Gpos1	putative glycosyltransferase, exosortase G-associated
Glyco_hydro_108	Glycosyl hydrolase 108; This family acts as a lysozyme (N-acetylmuramidase)
Glycos_transf_2	Glycosyl transferase family 2; Diverse family, transferring sugar from UDP-glucose
Glyco_tranf_2_3	Glycosyltransferase like family 2
Glyco_tranf_GTA_type	Glycosyltransferase family A (GT-A) includes diverse families of glycosyl transferases

Supplementary Table 6. Alphabetic abbreviation and description of putative conserved domains

Golgin_A5	Golgin subfamily A member 5
GP4d_helicase	GP4d_helicase is a homohexameric 5'-3' helicases
gpl	Bacteriophage P2-related tail formation protein [General function prediction only]
gpV	Phage P2 baseplate assembly protein gpV [General function prediction only]
GPW_gp25	Gene 25-like lysozyme; This family includes the phage protein Gene 25 from T4
gram_neg_porins	Porins form aqueous channels for the diffusion of small hydrophilic molecules
GT_2_like_b	Subfamily of Glycosyltransferase Family GT2 of unknown function
GT_2_like_d	Subfamily of Glycosyltransferase Family GT2 of unknown function
GyFA	Type IIA topoisomerase (DNA gyrase/topo II, topoisomerase IV)
Haemagg_act	Haemagglutination activity domain
Helicase_C	Helicase conserved C-terminal domain; The Prosite family is restricted to DEAD/H helicases
HELJcC	Helicase superfamily c-terminal domain; associated with DEXDc-, DEAD-, and DEAH-box proteins
Hia	Autotransporter adhesin [Intracellular trafficking and secretion / Extracellular structures]
HicB	Predicted nuclease of the RNase H fold, HicB family [General function prediction only]
HipB	Predicted transcriptional regulators [Transcription]
HlyC	RTX toxin acyltransferase family; (hemolysin-activating protein)
HNH_2	HNH endonuclease
HP1_INT_C	Phage HP1 integrase, C-terminal catalytic domain. Bacteriophage HP1 and related integrases
HpaA	4-Hydroxyphenylacetate catabolism regulatory protein HpaA; putative transcriptional protein
HpaG-C-term	4-Hydroxyphenylacetate degradation bifunctional isomerase/decarboxylase, C-terminal subunit
HpaG-N-term	4-Hydroxyphenylacetate degradation bifunctional isomerase/decarboxylase, N-terminal subunit
HpaX	4-Hydroxyphenylacetate permease
HsdM	Type I restriction-modification system methyltransferase subunit [Defense mechanisms]
HsdM_N	HsdM N-terminal domain; This domain is found at the N-terminus of the methylase subunit
hsdR	Type I site-specific deoxyribonuclease, HsdR family
HSDR_N	Type I restriction enzyme R protein N terminus (HSDR_N)
HsdS	Restriction endonuclease S subunits [Defense mechanisms]
HTH_18	Helix-turn-helix domain
HTH_19	Helix-turn-helix domain; Members of this family contains a DNA-binding helix-turn-helix domain
HTH_35	Winged helix-turn-helix DNA-binding
HTH_36	Helix-turn-helix domain
HTH_ARAC	helix_turn_helix, arabinose operon control protein
HTH_LacI	Helix-turn-helix (HTH) DNA binding domain of the LacI family of transcriptional regulators
HTH_LACI	Helix_turn_helix lactose operon repressor
HTH_Tnp_Mu_1	Mu DNA-binding domain; This family consists of MuA-transposase and repressor protein CI
HTH_XRE	Helix-turn-helix XRE-family like proteins
IncA	IncA protein
int	Integrase; Provisional
Int	Integrase
Integrase_1	Integrase; This is a family of DNA-binding prophage integrases found in Proteobacteria.
INT_Lambda_C	Lambda integrase, C-terminal catalytic domain
INT_P4	Bacteriophage P4 integrase. P4-like integrases are found in temperate bacteriophages
INT_REC_C	DNA breaking-rejoining enzymes, integrase/recombinases, C-terminal catalytic domain
IpaB_Evca	IpaB/Evca family; This family includes IpaB, which is an invasion plasmid antigen
ISH2_P13K_IA_R	Inter-Src homology 2 (ISH2) helical domain of Class IA Phosphoinositide 3-kinase Regulatory protein

Supplementary Table 6. Alphabetic abbreviation and description of putative conserved domains

J	Baseplate assembly protein; Provisional
Lactamase_B_2	Beta-lactamase superfamily domain; This family is part of the beta-lactamase superfamily
LexA	SOS-response transcriptional repressors (RecA-mediated autopeptidases)
Lhr	Lhr-like helicases [General function prediction only]
ligand_gated_channel	TonB dependent/Ligand-Gated channels
LprI	Uncharacterized protein conserved in bacteria, putative lipoprotein [Function unknown]
LT_GEWL	Lytic Transglycosylase (LT) and Goose Egg White Lysozyme (GEWL) domain
M	Terminase endonuclease subunit; Provisional
major_capsid_P2	Phage major capsid protein, P2 family
ManA	Phosphomannose isomerase [Carbohydrate transport and metabolism]
MATE_lik_e_10	Uncharacterized subfamily of the multidrug and toxic compound extrusion (MATE) proteins
MATE_Wzx_lik_e	Wzx, a subfamily of the multidrug and toxic compound extrusion (MATE)-like proteins
Methylase_5	Type I restriction modification DNA specificity domain
Methyltransf_26	Methyltransferase domain; This family contains methyltransferase domains
MethyltransfD12	D12 class N6 adenine-specific DNA methyltransferase
MFS	The Major Facilitator Superfamily (MFS) is a large and diverse group of secondary transporters
MFS_1	Major Facilitator Superfamily
MhpD	2-Keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)
Minor_tail_Z	Prophage minor tail protein Z (GPZ); This family consists of several prophage minor tail
mitD	Membrane-bound lytic murein transglycosylase D; Provisional
MitE	Soluble lytic murein transglycosylase and related regulatory proteins
Mor	Mor transcription activator family; Mor (Middle operon regulator)
Mrr	Restriction endonuclease [Defense mechanisms]
Mrr_cat	Restriction endonuclease; Prokaryotic family found in type II restriction enzymes
N	Capsid protein; Provisional
N_6_Mtase	N-6 DNA Methylase; Restriction-modification (R-M) systems
NA37	37-kD nucleoid-associated bacterial protein
NAD_binding_8	NAD(P)-binding Rossmann-like domain
NarK	Nitrate/nitrite transporter [Inorganic ion transport and metabolism]
NEL	C-terminal novel E3 ligase, LRR-interacting
NHT_00031	Aminotransferase, LLPSF_NHT_00031 family
Nlp	Predicted transcriptional regulator [Transcription]
NTP_transferase	Nucleotidyl transferase
O	Capsid-scaffolding protein; Provisional
OCH1	Mannosyltransferase OCH1 and related enzymes [Cell envelope biogenesis, outer membrane]
Ogr_Delta	Ogr/Delta-like zinc finger; This is a viral family of phage zinc-binding transcriptional proteins
OMP_b-brl	Outer membrane protein beta-barrel domain
OmpC	Outer membrane protein (porin) [Cell envelope biogenesis, outer membrane]
OprB	Carbohydrate-selective porin [Cell envelope biogenesis, outer membrane]; OprB family
ORF6N	ORF6N domain; This domain was identified by Iyer and colleagues
P	Terminase ATPase subunit; Provisional
P2_Phage_GpR	P2 phage tail completion protein R (GpR)
Paal	Acetyl-CoA acetyltransferase [Lipid metabolism]
PaaX_trns_reg	Phenylacetic acid degradation operon negative regulatory protein PaaX
Packaging_FI	DNA packaging protein FI; This family includes the lambda phage DNA-packaging protein FI

Supplementary Table 6. Alphabetic abbreviation and description of putative conserved domains

parC_Gneg	DNA topoisomerase IV, A subunit, proteobacterial; Operationally
ParE	Plasmid stabilization system protein [General function prediction only]
PAT1	Topoisomerase II-associated protein PAT1
PBP1_LacI_like_7	Ligand-binding domain of uncharacterized DNA-binding regulatory proteins
pcrA	ATP-dependent DNA helicase PcrA
Pentapeptide_4	Pentapeptide repeats (9 copies)
Peptidase_S6	Immunoglobulin A1 protease; This family consists of immunoglobulin A1 protease proteins
Peripla_BP_1	Periplasmic binding proteins and sugar binding domain of LacI family
Peripla_BP_3	Periplasmic binding protein-like domain; Thi domain is found in a variety of transcriptional proteins
Pgac_IcaA	Poly-beta-1,6 N-acetyl-D-glucosamine synthase
PG_binding_3	Predicted Peptidoglycan domain; This family contains a potential peptidoglycan binding domain
PHA00368	Internal virion protein D
PHA00675	Hypothetical protein
PHA01399	Membrane protein P6
PHA02067	Hypothetical protein
PHA03247	Large tegument protein UL36; Provisional
PHA03255	BDF3; Provisional
Phage_Alpa	Prophage CP4-57 regulatory protein (Alpa)
Phage_antitermQ	Phage antitermination protein Q; This family consists of several phage antitermination proteins
Phage_ASH	Ash protein family; This family was identified by Iyer and colleagues
Phage_attach	Phage Head-Tail Attachment
Phage_base_V	Phage-related baseplate assembly protein
Phage_cap_E	Phage major capsid protein E
Phage_cap_P2	Phage major capsid protein, P2 family
Phage_CI_repr	Bacteriophage CI repressor helix-turn-helix domain
Phage_Cox	Regulatory phage protein cox
phage_DnaB	Phage replicative helicase, DnaB family, HK022 subfamily
Phage_fiber_2	Phage tail fibre repeat; This repeat is found in the tail fibres of phage
Phage_GPA	Bacteriophage replication gene A protein (GPA)
Phage_GPD	Phage late control gene D protein (GPD)
Phage_GPL	Phage head completion protein (GPL)
Phage_GPO	Phage capsid scaffolding protein (GPO) serine peptidase
Phage_holin_2	Phage holin family 2; Holins are a diverse family of proteins
Phage_integ_N	Bacteriophage lambda integrase, N-terminal domain
Phage_integrase	Phage integrase family
Phage_int_SAM_2	Phage integrase, N-terminal; This is a family of DNA-binding prophage integrases
Phage_lysis	Bacteriophage Rz lysis protein; This protein is involved in host lysis
Phage_lysozyme	Phage lysozyme; This family includes lambda phage lysozyme and Escherichia coli endolysin
Phage_min_tail	Phage minor tail protein; This family consists of a series of phage minor tail proteins
PhageMin_Tail	Phage-related minor tail protein
Phage-MuB_C	Mu B transposition protein, C terminal; The C terminal domain of the B transposition protein
Phage_Mu_F	Phage Mu protein F like protein; Members of this family are found in double-stranded DNA
Phage_Mu_Gam	Bacteriophage Mu Gam like protein; This family consists of bacterial and phage Gam proteins
Phage_NinH	Phage NinH protein; This family consists of several phage NinH proteins
Phage_Nu1	Phage DNA packaging protein Nu1; Terminase, the DNA packaging enzyme of bacteriophage lambda

Supplementary Table 6. Alphabetic abbreviation and description of putative conserved domains

Phage_P2_GpE	Phage P2 GpE; This family consists of several phage and bacterial proteins
Phage_P2_GpU	Phage P2 GpU; This family consists of several bacterial and phage proteins
phage_P2_V	phage baseplate assembly protein V
Phage_portal	Phage portal protein; Bacteriophage portal proteins form a dodecamer
Phage_pRha	Phage regulatory protein Rha (Phage_pRha)
phage_rel_H1409	phage-related protein, H1409 family
phage_tail_N	Prophage tail fibre N-terminal; This domain is found at the N-terminus of prophage tail fibre
Phage_tail_S	Phage virion morphogenesis family; Protein S of phage P2
phage_term_2	phage terminase, large subunit, PBSX family
Phage_term_small	Phage small terminase subunit; This family consists of several phage small terminase subunit
Phage_tube	Phage tail tube protein FI; The major structural components of the contractile tail
PinR	Site-specific recombinases, DNA invertase Pin homologs [DNA replication, recombination]
PL1_Passenger_AT	Pertactin-like passenger domains (virulence factors)
Plasmid_stabil	Plasmid stabilisation system protein
PLN00113	leucine-rich repeat receptor-like protein kinase
PLN00206	DEAD-box ATP-dependent RNA helicase; Provisional
PLN02288	Mannose-6-phosphate isomerase
PLN02386	Superoxide dismutase [Cu-Zn]
PLN02417	Dihydrodipicolinate synthase
PLN02726	Dolichyl-phosphate beta-D-mannosyltransferase
PLN03114	ADP-ribosylation factor GTPase-activating protein AGD10; Provisional
PLN03128	DNA topoisomerase 2; Provisional
Plug	TonB-dependent Receptor Plug Domain
PMI_typeI	Phosphomannose isomerase type I
Polysacc_synt	Polysaccharide biosynthesis protein; Members of this family are integral membrane proteins
Porin_1	Gram-negative porin
portal_PBSX	Phage portal protein, PBSX family
Prim_Zn_Ribbon	Zinc-binding domain of primase-helicase
PRK00055	Ribonuclease Z; Reviewed
PRK00378	Nucleoid-associated protein NdpA; Validated
PRK00871	Glutathione-regulated potassium-efflux system ancillary protein Keff; Provisional
PRK03170	Dihydrodipicolinate synthase; Provisional
PRK03918	Chromosome segregation protein; Provisional
PRK05561	DNA topoisomerase IV subunit A; Validated
PRK05643	DNA polymerase III subunit beta; Validated
PRK06904	Replicative DNA helicase
PRK07208	Hypothetical protein; Provisional
PRK08026	Flagellin; Validated
PRK09326	F420H2 dehydrogenase subunit F; Provisional
PRK09412	Anaerobic C4-dicarboxylate transporter; Reviewed
PRK09678	DNA-binding transcriptional regulator; Provisional
PRK09685	DNA-binding transcriptional activator FcaR; Provisional
PRK09692	Integrase; Provisional
PRK09706	Transcriptional repressor DcaA; Reviewed
PRK09709	Exonuclease VIII; Reviewed

Supplementary Table 6. Aalphabetic abbreviation and description of putative conserved domains

PRK09737	EcoKI restriction-modification system protein HsdS; Provisional
PRK09750	Hypothetical protein; Provisional
PRK09866	Hypothetical protein; Provisional
PRK09940	Transcriptional regulator YdeO; Provisional
PRK10018	Putative glycosyl transferase; Provisional
PRK10073	putative glycosyl transferase; Provisional
PRK10122	GalU regulator GalF; Provisional
PRK10159	Outer membrane phosphoprotein protein E; Provisional
PRK10276	DNA polymerase V subunit UmuD; Provisional
PRK10344	DNA-binding transcriptional regulator Nlp; Provisional
PRK10458	DNA cytosine methylase; Provisional
PRK10554	Outer membrane porin protein C; P provisional
PRK05790	Putative acyltransferase; Provisional
PRK10597	DNA damage-inducible protein I; Provisional
PRK10691	Hypothetical protein; Provisional
PRK10703	DNA-binding transcriptional repressor PurR; Provisional
PRK10847	Hypothetical protein; Provisional
PRK10904	DNA adenine methylase; Provisional
PRK10969	DNA polymerase III subunit theta; Reviewed
PRK11551	Putative 3-hydroxyphenylpropionic transporter MhpT; Provisional
PRK11658	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase; Provisional
PRK12688	Flagellin; Reviewed
PRK13759	Arylsulfatase; Provisional
PRK13767	ATP-dependent helicase; Provisional
PRK14272	Phosphate ABC transporter ATP-binding protein; Provisional
PRK14512	ATP-dependent Clp protease proteolytic subunit; Provisional
PRK14960	DNA polymerase III subunits gamma and tau; Provisional
PRK15099	O-Antigen translocase; Provisional
PRK15131	Mannose-6-phosphate isomerase; Provisional
PRK15203	4-Hydroxyphenylacetate degradation bifunctional isomerase/decarboxylase; Provisional
PRK15240	Resistance to complement killing; Provisional
PRK15251	Cytolethal distending toxin subunit CdtB
PRK15316	RataA-like protein; Provisional
PRK15319	AIDA autotransporter-like protein ShdA; Provisional
PRK15370	E3 ubiquitin-protein ligase SirP; Provisional
PRK15377	E3 ubiquitin-protein ligase SopA; Provisional
PRK15386	Type III secretion protein GogB; Provisional
PRK15387	E3 ubiquitin-protein ligase SspH2
PRK15388	Cu/Zn superoxide dismutase; Provisional
PRK15407	Lipopolysaccharide biosynthesis protein RfBh; Provisional
PRK15480	Glucose-1-phosphate thymidyltransferase RfBA; Provisional
ProP	Permeases of the major facilitator superfamily
PS_pyruv_trans	Polysaccharide pyruvyl transferase
PTZ00102	Disulphide isomerase; Provisional
PTZ00110	Helicase; Provisional

Supplementary Table 6. Aalphabetic abbreviation and description of putative conserved domains

PTZ00260	Dolichyl-phosphate beta-glucosyltransferase; Provisional
PurR	Transcriptional regulators [Transcription]
Q	Portal vertex protein; Provisional
recF	recF protein
recF	Recombination protein F; Reviewed
recomb_XerC	Tyrosine recombinase XerC; The phage integrase family describes a number of recombinases
recomb_XerD	Tyrosine recombinase XerD (The phage integrase family)
Resolvase	Resolvase, N terminal domain; The N-terminal domain of the resolvase family
ResIII	Type III restriction enzyme, res subunit
RfbA	dTDP-glucose pyrophosphorylase [Cell envelope biogenesis, outer membrane]
RfbX	Membrane protein involved in the export of O-antigen and teichoic acid
Rhs_assoc_core	RHS repeat-associated core domain; This model represents a conserved unique core sequence
rmlA	Glucose-1-phosphate thymidyltransferase
RNase_Z	Ribonuclease Z
RT_Bac_retron_II	RT_Bac_retron_II: Reverse transcriptases (RTs) in bacterial retrotransposons or retrons
rumA	23S rRNA m(5)U1939 methyltransferase; Reviewed
rve	Integrase core domain
RVT_1	Reverse transcriptase (RNA-dependent DNA polymerase)
S14_ClpP_1	Caseinolytic protease (ClpP) is an ATP-dependent, highly conserved serine protease
SDH_sah	Serine dehydrogenase proteinase; This family of archaeobacterial proteins
ShlB	Haemolysin secretion/activation protein ShlB/FhaC/HecB
sifB	Secreted effector protein SifB; Provisional
SLT	Transglycosylase SLT domain; This family is distantly related to pfam00062
Smc	Chromosome segregation ATPases [Cell division and chromosome partitioning]
SMC_N	RecF/RecN/SMC N terminal domain; This domain is found at the N terminus of SMC proteins
SMC_prok_B	Chromosome segregation protein SMC, common bacterial type
SopA_C	SopA-like catalytic domain; This domain is found in the Escherichia coli Type III secretion system
SodC	Cu/Zn superoxide dismutase [Inorganic ion transport and metabolism]
Sod_Cu	Copper/zinc superoxide dismutase (SODC)
SPEC	Spectrin repeats, found in several proteins involved in cytoskeletal structure
spore_V_B	Stage V sporulation protein B; SpoVB is the stage V sporulation protein B
SppA	Periplasmic serine proteases (ClpP class) [Posttranslational modification, protein turnover]
SR_ResInv	Serine Recombinase (SR) family, Resolvase and Invertase subfamily, catalytic domain
sufB	FeS assembly protein SufB; This protein, SufB, forms a cytosolic complex SufBCD
Sugar_tr	Sugar (and other) transporter
Sulfatase	Sulfatase
synapt_SV2	Synaptic vesicle protein SV2
tail_comp_S	Phage virion morphogenesis (putative tail completion) protein
Tail_P2_I	Phage tail protein (Tail_P2_I); These sequences represent the family of phage P2 protein I
tail_tube	Phage contractile tail tube protein, P2 family; The tails of some phage are contractile
tape_meas_lam_C	Phage tail tape measure protein, lambda family
Tape_meas_lam_C	Lambda phage tail tape-measure protein (Tape_meas_lam_C)
tape_meas_TP901	Phage tail tape measure protein, TP901 family, core region
terB	Tellurite resistance protein terB; This family contains uncharacterized bacterial proteins
TerB-N	TerB-N; The TerB-N domain is found N terminus to TerB, and TerB-C containing proteins

Supplementary Table 6. Aalphabetic abbreviation and description of putative conserved domains

TerB-C	TerB-C domain; TerB-C occurs C terminal of TerB in TerB-N containing proteins
Terminase_3	Phage terminase large subunit; Initiation of packaging of double-stranded viral DNA
Terminase_5	Putative ATPase subunit of terminase (gpp-like)
Terminase_6	Terminase-like family; This family represents a group of terminase proteins
Terminase_GpA	Phage terminase large subunit (GpA)
thiolase	Thiolase are ubiquitous enzymes
Thiolase_C	Thiolase, C-terminal domain
TIGR02646	TIGR02646 family protein (uncharacterized protein family)
TMP_2	Prophage tail length tape measure protein; This family represents a conserved region
TonB-B12	TonB-dependent vitamin B12 receptor
TOP4c	DNA Topoisomerase, subtype IIA; domain A'; bacterial DNA topoisomerase IV, GyrA, ParC
Toprim_3	Toprim domain; The toprim domain is found in a wide variety of enzymes; toprim primase
Transposase_20	Transposase IS116/IS110/IS902 family
Transposase_mut	Transposase, Mutator family
TTSSLRR	Type III secretion system leucine rich repeat protein
UDP-GALP_mutase	UDP-galactopyranose mutase
UPF0020	Putative RNA methylase family UPF0020; This domain is probably a methylase
uvrD	DNA-dependent helicase II; Provisional
UvrD	Superfamily I DNA and RNA helicases [DNA replication, recombination, and repair]
UvrD-helicase	UvrD/REP helicase N-terminal domain
V	Virion protein; Provisional
Vapl	Plasmid maintenance system antidote protein [General function prediction only]
VI_minor_1	Type VI secretion-associated protein, VC_A0118 family
W	Baseplate wedge subunit; Provisional
WcaA	Glycosyltransferases involved in cell wall biogenesis
WeeC	Predicted pyridoxal phosphate-dependent enzyme
xerC	Site-specific tyrosine recombinase XerC; Reviewed
XerC	Integrase [DNA replication, recombination, and repair]
XerD	Site-specific recombinase XerD [DNA replication, recombination, and repair]
XkdIT	Uncharacterized homolog of phage Mu protein gp47 [Function unknown]
zIIS	Lysozyme family protein [General function prediction only]