SUPPLEMENTAL MATERIAL

Generation of hyper-immune bovine sera. Hyperimmune sera were obtained from cattle experimentally inoculated with *E. coli* O157 strains of both cattle and human origin (1), which included the strain EDL933, one of the sequenced O157 strains (2). The rationale was that exposure of these cattle to diverse O157 strains of both lineage I and II (3) might engender antibody responses against a wider variety of protein antigens expressed in the gastrointestinal tract (GIT) of cattle rendering the hyperimmune sera optimal for profiling the immunoproteome of this organism in the bovine reservoir. Cattle were first confirmed to be negative for O157 colonization using highly sensitive recto-anal swab culture techniques (1), and then inoculated once orally with 10^{10} CFU of each strain (1). Serum samples, collected from nine cattle that had remained culture positive for up to two months (1) following experimental inoculation, demonstrated high titers of anti-O157 lipopolysaccharide (LPS) antibodies (1). Preimmune sera were also collected from the same animals prior to inoculation.

Preliminary evaluation of reactivity of pooled hyperimmune sera. We assessed the quality of pooled hyperimmune sera, by reacting them with previously identified, secreted O157 proteins encoded in the locus of enterocyte effacement (LEE) (4). Cattle immunized with such proteins, including those expressed from the LEE, reportedly show decreased shedding of O157 (4). We first confirmed reactivity of hyper-immune cattle sera with O157 lipopolysaccharide (LPS) purified in our laboratory using a protocol described previously (5). We then amplified genes encoding full-length EspB from O157 strain EDL933 (test), and PilA from *Vibrio cholerae* El Tor N16961 (control), and

cloned them into the expression vector, pET-30b (Novagen, Inc., Madison, WI.) under control of the phage T7 promoter. Following confirmation of in-frame cloning via gene sequencing, we transformed plasmids into *E. coli* BL21(DE3). Recombinant clones were induced with isopropyl-β-D-thiogalactopyranoside (IPTG) and probed in a colony immunoblot assay with pooled, hyper-immune cattle sera adsorbed against purified O157 LPS, as described previously by our group (6). None of the above recombinant clones reacted with pooled, pre-immune cattle sera (data not shown).

Affinity-purification of PAbs from hyperimmune sera. Prior to affinity purification, we pooled hyperimmune sera from nine cattle to compensate for variations in individual immune responses and identify a wider complement of O157 proteins expressed within the GIT of bovine reservoirs. PAbs from 1 ml of pooled hyperimmune cattle sera were purified using HiTrap Protein G HP (5 ml) columns (Amersham Biosciences, Piscataway, NJ), as recommended by the manufacturer with a few modifications. Briefly, pooled sera were diluted 1:4 in binding buffer (0.02 M Sodium phosphate buffer, pH 7.0), and then loaded using a syringe onto a protein G column equilibrated with ten volumes of binding buffer. Protein G binds all IgG subclasses but not other immunoglobulin isotypes (7). Following a wash with ten volumes of binding buffer, bound IgG PAbs were eluted with 4 ml of elution buffer (0.1 M Glycine, pH 2.7), directly into five tubes each containing 200 µl of 1M Tris-HCl, pH 9.0. Affinity-purified IgG PAbs ("Bait" PAbs) were quantified using a nomograph (8) and prepared for coupling.

REFERENCES

1. Rice, D.H., Sheng, H.Q., Wynia, S.A., and Hovde, C.J. (2003) Rectoanal Mucosal Swab Culture Is More Sensitive Than Fecal Culture and Distinguishes *Escherichia coli* O157:H7-Colonized Cattle and Those Transiently Shedding the Same Organism. *J. Clin. Microbiol.* **41**, 4924-4929.

2. Perna, N.T., Plunkett, G., Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F., Evans, P.S., Gregor, J., Kirkpatrick, H.A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamousis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J., Yen, G., Schwartz, D.C., Welch, R.A., and Blattner,F.R. (2001) Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* **409**, 529-533.

3. Kim, J., Nietfeldt, J., and Benson, A.K. (1999) Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. *Proc. Natl. Acad. Sci.* **96**, 13288-13293.

4. Potter, A.A., Klashinsky, S., Li, Y., Frey, E., Townsend, H., Rogan, D., Erickson, G., Hinkley, S., Klopfenstein, T., Moxley, R.A., Smith, D.R., and Finlay, B.B. (2004) Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* **22**, 362-369.

5. Inzana,T.J. (1983) Electrophoretic heterogeneity and interstrain variation of the lipopolysaccharide of *Haemophilus influenzae*. *J Infect Dis* **148**, 492-499.

6. John, M., Kudva, I.T., Griffin, R.W., Dodson, A.W., McManus, B., Krastins, B., Sarracino, D., Progulske-Fox, A., Hillman, J.D., Handfield, M., Tarr, P.I., and Calderwood, S.B. (2005) Use of In Vivo-Induced Antigen Technology for Identification of *Escherichia coli* O157:H7 Proteins Expressed during Human Infection. *Infect. Immun.* **73**, 2665-2679.

7. Harlow, E.and Lane, D. (1998) Antibodies-A Laboratory Manual. (Cold Spring Harbor Laboratory, New York).

8. Bollag, D.M., Rozycki, M.D., and Edelstein, J.S. (1996) Protein Methods . (Wiley-Liss, Inc., New York).

9. Dziva, F., van Diemen, P.M., Stevens, M.P., Smith, A.J., and Wallis, T.S. (2004) Identification of *Escherichia coli* O157 : H7 genes influencing colonization of the bovine gastrointestinal tract using signature-tagged mutagenesis. *Microbiology* **150**, 3631-3645.

Supplementary Table 1. *In vivo*-expressed O157 proteins identified by PELS in cattle.

No.	Region in the EDL 933 genome / pO157 associated with the protein[1]	Protein/Function[2]	Bacterial cell localization[3]	No. peptide hits	Percent protein coverage	Identical or Related proteins expressed *in vivo* in:	
						Calves as identified by STM[4]	Humans as identified by IVIAT[4]
1.	Backbone	EcnB / entericidin B; bacteriolytic lipoprotein; putative toxin of osmotically regulated toxin-antitoxin system associated with programmed cell death	Extracellular	1	40%	-	-
2.	Backbone	OmpA / outer membrane protein 3a (II*;G;d); adhesin	Outer membrane	42	43%	-	-
3.	Backbone	Lpp (Murein-lipoprotein) / major outer membrane, murein sacculus, peptidoglycan lipoprotein precursor (OmpT-like)	Outer membrane	7	62%	-	MepA, MltB, Slt / murein sacculus synthesis
4.	Backbone	OmpC / outer membrane protein 1b (Ib;c); outer membrane constituents	Outer membrane	4	10%	-	-
5.	O-island # 43	Z1178 (Iha) / adhesin; exogenous ferric siderophore receptor R4	Outer membrane	4	8%	Z1182 / 1726 bp 5' of Z1178 (Iha) adhesin	-

6.	O-island # 140	ChuA / heme/hemoglobin receptor (Heme utilization/transport protein)	Outer membrane	1	2%	-	HemK / heme biosynthesis, CcmH / heme lyase
7.	O-island # 36	Z0975 / putative tail component of prophage CP-933K	Outer membrane	3	2%	Z0990 / type III secreted protein encoded within prophage CP-933K	Z0975
8.	O-island # 52	Z1931 / outer membrane protein 3b (a), protease VII / enzyme; outer membrane constituents encoded within prophage CP-933X	Outer membrane	7	15%	Z1930 / putative protease encoded within prophage CP-933X	-
9.	Backbone	TolC / Outer membrane channel; specific tolerance to colicin E1; segregation of daughter chromosomes; cell division	Outer membrane	3	5%	-	-
10.	Backbone	FhuE / outer membrane receptor for ferric iron uptake	Outer membrane	2	5%	-	FhuA / outer membrane protein receptor for ferrichrome, colicin M, and phages T1, T5, and phi80
11.	pO157	EspP / serine protease; putative exoprotein-precursor	Outer membrane	1	1%	-	-

12.	Backbone	OmpF / outer membrane protein 1a (Ia;b;F); outer membrane constituents	Outer membrane	2	6%	-	-
13.	Backbone	LamB / phage lambda receptor protein; maltose high-affinity receptor ; IS, phage, Tn; transport of small molecules: carbohydrates, organic acids, alcohols	Outer membrane	1	4%	-	-
14.	Backbone	HflK / protease specific for phage lambda cII repressor; enzyme; macromolecule degradation: degradation of proteins, peptides	Outer membrane	2	6%	-	HflX / GTP-binding subunit of protease specific for phage lambda cII repressor
15.	O-island # 148	EspB / LEE encoded type III secreted protein; formation of pore along with EspD on host cell membrane	Outer membrane	4	23%	EspD / LEE encoded type III secreted protein; formation of pore along with EspB on host cell membrane	-
16.	Backbone	HlpA / histone-like protein, located in outer membrane or nucleoid; factor; Nucleoid-related functions	Outer membrane	1	6%	-	-
17.	Backbone	OmpX / outer membrane protein X ; membrane; Cell envelop: Outer membrane constituents	Outer membrane	1	7%	-	-

18.	Backbone	YeaF / hypothetical protein ; unknown function	Outer membrane	1	5%	-	YeaA / hypothetical protein; unknown function
19.	Backbone	CirA / outer membrane receptor for iron-regulated colicin I receptor; porin; requires tonB gene product; Cell envelop: Outer membrane constituents	Outer membrane	1	2%	-	CirA
20.	O-island # 36	Z0955 / hypothetical protein encoded by prophage CP-933K; unknown function	Outer membrane	3	8%	-	-
21.	Backbone	FepA / outer membrane receptor for ferric enterobactin (enterochelin) and colicins B and D; Transport of small molecules: Cations	Outer membrane	1	2%	-	-
22.	Backbone	MalE / maltose-binding protein; substrate recognition for transport and chemotaxis; transport of small molecules: carbohydrates, organic acids, alcohols	Periplasm	2	8%	-	Mal F / maltose permease, MalS / alpha amylase, MaLY / enzyme that may degrade or block biosynthesis of endogenous mal inducer, MalZ / maltodextrin glucosidase

23.	Backbone	TolB / protein involved in the tonb-independent uptake of group A colicins; colicin-related functions	Periplasm	1	4%	-		-
24.	Backbone	FkpA / FKBP-type peptidyl-prolyl cis-trans isomerase (rotamase); enzyme; macromolecule synthesis, modification: proteins - translation and modification	Periplasm	1	6%	-		-
25.	Backbone	YbeJ / putative periplasmic binding transport protein; putative transport	Periplasm	1	6%	-		-
26.	Backbone	GlnH / periplasmic glutamine-binding protein; permease; transport; transport of small molecules: amino acids, amines	Periplasm	1	8%	-		GlnG / response regulator for *gln* operon; interacts with sensor GlnL GlnL / histidine protein kinase sensor for the GlnG regulator Both are involved in glutamine biosynthesis
27.	Backbone	Mdh / malate dehydrogenase; enzyme; energy metabolism, carbon: TCA cycle	Periplasm	1	4%	-		-

28.	Backbone	Z2267 / hypothetical protein yncE precursor; putative receptor	Periplasm	4	10%	-	-
29.	Backbone	YaeC / putative lipoprotein, D-methionine transport protein; D-methionine-binding lipoprotein metQ precursor	Periplasm	1	6%	-	-
30.	Backbone	SdhA / succinate dehydrogenase, flavoprotein subunit ; enzyme; energy metabolism, carbon: TCA cycle	Periplasm	1	3%	-	-
31.	Backbone	DppA / dipeptide transport protein ; transport; Transport of large molecules: Protein, peptide secretion	Periplasm	1	2%	-	-
32.	Backbone	YahO / hypothetical protein ; unknown function	Periplasm	1	9%	-	-
33.	O-island # 7	Z0269 / hypothetical protein; unknown function (Rhs Element Associated)	Periplasm	3	4%	-	-
34.	Backbone	RbsB / D-ribose periplasmic binding protein; Transport of small molecules: Carbohydrates, organic acids, alcohols	Periplasm	3	17%	-	-

35.	Backbone	OsmY / hyperosmotically inducible periplasmic protein; osmotic adaptation	Periplasm	7	26%	-	OsmY
36.	Backbone	Z3065 / hypothetical protein; unknown function	Periplasm	1	6%	-	-
37.	Backbone	Prc / carboxy-terminal protease for penicillin-binding protein 3; Macromolecule degradation: Degradation of proteins, peptides, glyco	Periplasm	1	2%	-	-
38.	Backbone	ArgT / lysine-, arginine-, ornithine-binding periplasmic protein; transport; Transport of small molecules: Amino acids, amines	Periplasm	1	6%	-	-
39.	Backbone	OppA / oligopeptide transport; periplasmic binding protein; transport; Transport of large molecules: Protein, peptide secretion	Periplasm	5	8%	-	OppB / oligopeptide transport permease protein; transport; Transport of large molecules: Protein, peptide secretion
40.	Backbone	SodC / superoxide dismutase precursor (Cu-Zn); enzyme; Protection responses: Detoxification	Periplasm	1	6%	-	-

41.	Backbone	XasA (GadC) / acid sensitivity protein, putative transporter; Probable glutamate/gamma-aminobutyrate antiporter	Inner membrane	6	3%	-		-
42.	Backbone	PspA / phage shock protein A; prophage and phage-related functions; adaptations, atypical conditions; negative regulatory gene for the psp operon	Inner membrane	1	7%	-		-
43.	Backbone	AtpF / ATP synthase, F0 sector, B chain; enzyme; ATP-proton motive force interconversion	Inner membrane	1	8%	-		-
44.	Backbone	AtpB / membrane-bound ATP synthase, F0 sector, subunit a ; enzyme; ATP-proton motive force interconversion	Inner membrane	3	21%	-		-
45.	Backbone	YleB / putative 2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase; unknown function	Inner membrane	1	5%	-	YleB	
46.	Backbone	CysK / cysteine synthase A, O-acetylserine sulfhydrolase A; enzyme; Amino acid biosynthesis: Cysteine	Inner membrane	2	8%	-		-

47.	Backbone	DnaC / chromosome replication; initiation and chain elongation; putative enzyme; Macromolecule synthesis, modification: DNA - replication, repair, restr./modific'n	Inner membrane	1	5%	-	-
48.	Backbone	DsbB / reoxidizes DsbA protein following formation of disulfide bond in P-ring of flagella; enzyme; Cell exterior constituents: Surface structures	Inner membrane	1	8%	-	DsbA /protein disulfide isomerase I, essential for cytochrome c synthesis and formate-dependent reduction; Proteins - translation and modification
49.	Backbone	ElaB / hypothetical protein; unknown function	Inner membrane	3	28%	-	-
50.	Backbone	GatD / galactitol-1-phosphate dehydrogenase; enzyme; Degradation of small molecules: Carbon compounds	Inner membrane	1	4%	-	-
51.	Backbone	KefC / K+ efflux antiporter, glutathione-regulated; transport; Transport of small molecules: Cations	Inner membrane	1	3%	-	KefC

52.	O-island # 57	Z2112 / putative ClpP-like protease encoded within prophage CP-933O; putative enzyme; Macromolecule degradation: Degradation of proteins, peptides, glyco (Phage or Prophage Related)	Inner membrane	1	7%	-	-
53.	Backbone	LacI / transcriptional repressor of the lac operon; regulator; Degradation of small molecules: Carbon compounds	Inner membrane	3	11%	-	-
54.	Backbone	GatZ / putative tagatose 6-phosphate kinase 1; unknown function	Inner membrane	4	10%	-	-
55.	Backbone	AcnB / aconitate hydrase B; Energy metabolism, carbon: TCA cycle	Inner membrane	1	2%	-	-
56.	Backbone	CydA / cytochrome d terminal oxidase, polypeptide subunit I; Energy metabolism, carbon: Electron transport	Inner membrane	1	3%	-	-
57.	Backbone	CreD / tolerance to colicin E2; putative membrane; Colicin-related functions	Inner membrane	1	6%	-	-

58.	Backbone	YgjD / putative O-sialoglycoprotein endopeptidase; unknown function	Inner membrane	1	5%	-	-
59.	Backbone	Z5187 / putative replicase; Macromolecule synthesis, modification: DNA - replication, repair, restr./modific'n	Inner membrane	1	8%	-	-
60.	Backbone	YtfQ / putative LACI-type transcriptional regulator; unknown function	Inner membrane	1	4%	-	-
61.	Backbone	FklB / FKBP-type 22KD peptidyl-prolyl cis-trans isomerase (rotamase); Macromolecule synthesis, modification:Proteins - translation and modification	Inner membrane	5	17%	-	-
62.	Backbone	RpsA / 30S ribosomal subunit protein S1; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Inner membrane	2	6%	-	-
63.	Backbone	Ssb / ssDNA-binding protein; factor; Macromolecule synthesis, modification: DNA - replication, repair, restriction./modification	Inner membrane	5	20%	-	-

64.	Backbone	SucC/ succinyl-CoA synthetase, beta subunit; enzyme; Energy metabolism, carbon: TCA cycle	Inner membrane	2	9%	-	-
65.	Backbone	RplK / 50S ribosomal subunit protein L11; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Inner membrane	5	26%	-	-
66.	Backbone	TpiA / triosephosphate isomerase; enzyme; Energy metabolism, carbon: Glycolysis	Inner membrane	1	6%	-	-
67.	Backbone	YgaM / hypothetical protein; unknown function	Inner membrane	2	12%	-	-
68.	Backbone	TrxA / thioredoxin 1; enzyme; biosynthesis of cofactors, carriers: thioredoxin, glutaredoxin, glutathione	Cytoplasm	18	43%	TrxC / putative thioredoxin-like protein	Ggt / biosynthesis of cofactors, carriers: thioredoxin, glutaredoxin, glutathione
69.	Backbone	DeaD / inducible ATP-independent RNA helicase; enzyme; macromolecule synthesis, modification: RNA synthesis, modification, DNA transcription	Cytoplasm	13	5%	-	-
70.	Backbone	DpS / DNA protection during starvation protein; global regulatory functions	Cytoplasm	2	22%	-	-

71.	Backbone	Tpx / thiol peroxidase; enzyme; protection responses: detoxification	Cytoplasm	1	9%	-	-
72.	Backbone	AceE / pyruvate dehydrogenase (decarboxylase component); enzyme; energy metabolism	Cytoplasm	2	4%	-	-
73.	Backbone	Gnd / gluconate-6-phosphate dehydrogenase, decarboxylating; enzyme; energy metabolism, carbon: oxidative branch, pentose pathway	Cytoplasm	2	6%	-	-
74.	Backbone	MalP / maltodextrin phosphorylase; enzyme; degradation of small molecules: carbon compounds	Cytoplasm	3	5%	-	Mal F / maltose permease, MalS / alpha amylase, MaLY / enzyme that may degrade or block biosynthesis of endogenous mal inducer, MalZ / maltodextrin glucosidase
75.	Backbone	HflC / protease specific for phage lambda cII repressor; enzyme; macromolecule degradation: degradation of proteins, peptides	Cytoplasm	1	5%	-	HflX / GTP-binding subunit of protease specific for phage lambda cII repressor

76.	Backbone	Eno / enolase; enzyme; energy metabolism, carbon: glycolysis, anaerobic respiration, gluconeogenesis	Cytoplasm	2	7%	-		-
77.	Backbone	MopB / GroES; 10 Kd chaperone binds to Hsp60 in presence of Mg-ATP, suppressing its ATPase activity; folding and ushering proteins: Chaperones	Cytoplasm	1	13%	-	MopB	
78.	Backbone	Z3260 / hypothetical protein; unknown function;putative fructose bis-phosphate aldolase	Cytoplasm	1	6%	-		-
79.	Backbone	Add / adenosine deaminase; enzyme; central intermediary metabolism: salvage of nucleosides and nucleotides	Cytoplasm	1	10%	-		-
80.	Backbone	DnaK / chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins; folding and ushering proteins: chaperones	Cytoplasm	4	9%	-	Z2654 / putative chaperone distantly related to Hsp70	
81.	Backbone	Pnp / polynucleotide phosphorylase; cytidylate kinase; macromolecule synthesis, modification: RNA synthesis, modification, DNA transcription	Cytoplasm	2	6%	-		-

82.	Backbone	RpsE / 30S ribosomal subunit protein S5; protein synthesis, modification	Cytoplasm	2	13%	-	-
83.	Backbone	RpsG / 30S ribosomal subunit protein S7, initiates assembly; protein synthesis, modification	Cytoplasm	1	8%	-	-
84.	Backbone	RpsH / 30S ribosomal subunit protein S8, initiates assembly; protein synthesis, modification	Cytoplasm	1	12%	-	-
85.	Backbone	TnaA / tryptophanase; degradation of small molecules: amino acids	Cytoplasm	3	9%	-	-
86.	Backbone	LpdA / lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; L-protein of glycine cleavage complex; enzyme; energy metabolism, carbon: pyruvate dehydrogenase	Cytoplasm	4	17%	-	-
87.	Backbone	TufA / protein chain elongation factor EF-Tu ; macromolecule synthesis, modification: proteins - translation and modification	Cytoplasm	2	10%	-	-

88.	Backbone	Ftn (FtnA) / ferritin (an iron storage protein) ; carrier; transport of small molecules: cations	Cytoplasm	1	9%	-		-
89.	Backbone	DapD / 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase; enzyme; amino acid biosynthesis: lysine	Cytoplasm	1	5%	-	DapD	
90.	Backbone	AceF / pyruvate dehydrogenase (dihydrolipoyltransacetylase component); enzyme; energy metabolism, carbon: pyruvate dehydrogenase	Cytoplasm	3	6%	-		-
91.	Backbone	AdhP / alcohol dehydrogenase; enzyme; energy metabolism, carbon: anaerobic respiration	Cytoplasm	1	7%	-	AdhP	
92.	Backbone	Z1099 / hypothetical protein; unknown function	Cytoplasm	1	13%	-		-
93.	Backbone	Tsf / protein chain elongation factor EF-Ts; macromolecule synthesis, modification: proteins - translation and modification	Cytoplasm	1	7%	-		-
94.	Backbone	RpoE / RNA polymerase, sigma-E factor; heat shock and oxidative stress; regulator; global regulatory functions	Cytoplasm	1	9%	-		-

95.	Backbone	YqjD / hypothetical protein; unknown function	Cytoplasm	1	18%	-	-
96.	Backbone	FabG / 3-oxoacyl-[acyl-carrier-protein] reductases; enzyme; fatty acid and phosphatidic acid biosynthesis	Cytoplasm	1	10%	-	-
97.	Backbone	CreA / hypothetical protein; unknown function	Cytoplasm	1	9%	-	-
98.	Backbone	LacZ / beta-D-galactosidase; enzyme; degradation of small molecules: carbon compounds	Cytoplasm	1	6%	-	-
99.	Backbone	OrdL / putative oxidoreductase; function unknown	Cytoplasm	1	4%	Z2702 / putative oxidoreductase, Fe-S subunit	YhjL, Z2723, Z3401 /putative oxidoreductase
100.	Backbone	FldA / flavodoxin 1; enzyme; energy metabolism, carbon: electron transport	Cytoplasm	1	11%	-	Z4018 / putative flavodoxin
101.	Backbone	FusA / GTP-binding protein chain elongation factor EF-G; macromolecule synthesis, modification: proteins - translation and modification	Cytoplasm	1	2%	-	-

102.	Backbone	RpoA / RNA polymerase, alpha subunit ; enzyme; macromolecule synthesis, modification: RNA synthesis, modification, DNA transcription	Cytoplasm	1	4%	-	-
103.	Backbone	YhdH / putative dehydrogenase ; unknown function	Cytoplasm	1	4%	-	YgjL, YidS, YliL, Z3775, Z4220 / putative dehydrogenase
104.	Backbone	SucA / 2-oxoglutarate dehydrogenase (decarboxylase component); enzyme; energy metabolism, carbon: TCA cycle	Cytoplasm	1	2%	-	-
105.	Backbone	AdhE / CoA-linked acetaldehyde dehydrogenase and iron-dependent alcohol dehydrogenase; pyruvate-formate-lyase deactivase; enzyme; energy metabolism, carbon: fermentation	Cytoplasm	1	2%	-	-
106.	Backbone	Pgk / phosphoglycerate kinase; enzyme; energy metabolism, carbon: glycolysis	Cytoplasm	1	6%	-	-
107.	Backbone	RplO / 50S ribosomal protein L15; protein synthesis, modification	Cytoplasm	1	11%	-	-

108.	Backbone	RplN / 50S ribosomal protein L14; protein synthesis, modification	Cytoplasm	2	33%	-	-
109.	Backbone	RplL / 50S ribosomal protein L7/L12 ; protein synthesis, modification	Cytoplasm	2	10%	-	-
110.	Backbone	RplJ / 50S ribosomal protein L10; protein synthesis, modification	Cytoplasm	1	9%	-	-
111.	Backbone	RplF / 50S ribosomal protein L6; protein synthesis, modification	Cytoplasm	1	9%	-	-
112.	Backbone	RplC / 50S ribosomal protein L3; protein synthesis, modification	Cytoplasm	1	10%	-	-
113.	Backbone	YnaF / putative universal stress protein; unknown function	Cytoplasm	1	11%	-	-
114.	Backbone	MalQ / 4-alpha-glucanotransferase (amylomaltase); enzyme; macromolecule degradation: degradation of polysaccharides	Cytoplasm	2	6%	-	Mal F / maltose permease, MalS / alpha amylase, MaLY / enzyme that may degrade or block biosynthesis of endogenous mal inducer, MalZ / maltodextrin glucosidase

115.	Backbone	YhbP / hypothetical protein; unknown function	Cytoplasm	1	8%	-	-
116.	Backbone	OsmC / osmotically inducible protein; phenotype; osmotic adaptation	Cytoplasm	1	15%	-	OsmY / hyperosmotically inducible protein; osmotic adaptation
117.	Backbone	Hns / DNA-binding protein HLP-II (HU, BH2, HD, NS); pleiotropic regulator; regulator; basic proteins - synthesis, modification	Cytoplasm	3	17%	-	-
118.	Backbone	SbmC / hypothetical protein; unknown function	Cytoplasm	1	12%	-	-
119.	Backbone	AtpA / membrane-bound ATP synthase, F1 sector, alpha-subunit; enzyme; ATP-proton motive force interconversion	Cytoplasm	11	23%	-	-
120.	Backbone	AtpG / membrane-bound ATP synthase, F1 sector, gamma-subunit; enzyme; ATP-proton motive force interconversion	Cytoplasm	1	4%	-	-
121.	Backbone	MopA / GroEL, chaperone Hsp60, peptide-dependent ATPase, heat shock protein; Folding and ushering proteins: Chaperones	Cytoplasm	6	17%	-	-

122.	Backbone	Crp / cyclic AMP receptor protein; regulator; Global regulatory functions	Cytoplasm	2	12%	-		-
123.	Backbone	CspA / cold shock protein 7.4, transcriptional activator of hns; regulator; Adaptations, atypical conditions	Cytoplasm	1	20%	-		-
124.	Backbone	CspC / cold shock protein; unknown function	Cytoplasm	3	20%	5'of *cspC*		-
125.	Backbone	CysN / ATP-sulfurylase (ATP:sulfate adenylyltransferase), subunit 1, probably a GTPase; enzyme; Central intermediary metabolism: Sulfur metabolism	Cytoplasm	1	3%	-	CysD / ATP:sulfurylase (ATP:sulfate adenylyltransferase), subunit 2; enzyme; Central intermediary metabolism: Sulfur metabolism	
126.	Backbone	HupA / DNA-binding protein HU-alpha (HU-2); Basic proteins - synthesis, modification	Cytoplasm	4	32%	-		-
127.	Backbone	FrdB / fumarate reductase, anaerobic, iron-sulfur protein subunit; enzyme; Energy metabolism, carbon: Anaerobic respiration	Cytoplasm	1	4%	-		-

128.	Backbone	GapA / glyceraldehyde-3-phosphate dehydrogenase A; enzyme; Energy metabolism, carbon: Glycolysis	Cytoplasm	5	22%	-		-
129.	Backbone	FolE / GTP cyclohydrolase I; enzyme; Biosynthesis of cofactors, carriers: Folic acid	Cytoplasm	2	13%	-	FolE	
130.	Backbone	GlnA / glutamine synthetase; enzyme; Amino acid biosynthesis: Glutamine	Cytoplasm	1	3%	-	GlnG / response regulator for *gln* operon; interacts with sensor GlnL GlnL / histidine protein kinase sensor for the GlnG regulator Both are involved in glutamine biosynthesis	
131.	Backbone	GpmA / phosphoglyceromutase 1; enzyme; Energy metabolism, carbon: Glycolysis	Cytoplasm	2	11%	-		-
132.	Backbone	GrpE / phage lambda replication; host DNA synthesis; heat shock protein; protein repair; IS, phage, Tn; Other or unknown	Cytoplasm	1	8%	-		-

133.	Backbone	HisB / imidazoleglycerolphosphate dehydratase and histidinol-phosphate phosphatase; enzyme; Amino acid biosynthesis: Histidine	Cytoplasm	1	5%	-	HisS/ histidine tRNA synthetase; enzyme; Amino acyl tRNA syn; tRNA modific'n
134.	Backbone	HslV / heat shock protein hslVU, proteasome-related peptidase subunit; enzyme; macromolecule degradation: Degradation of proteins, peptides, glyco	Cytoplasm	1	7%	-	-
135.	Backbone	IbpA / heat shock protein; factor; Adaptations, atypical conditions	Cytoplasm	1	9%	-	-
136.	Backbone	IbpB / heat shock protein; factor; Adaptations, atypical conditions	Cytoplasm	2	8%	-	-
137.	Backbone	InfC / Initiation factor IF-3; Macromolecule synthesis, modification: Proteins - translation and modification	Cytoplasm	2	34%	-	-
138.	Backbone	HimD / integration host factor (IHF), beta subunit; site-specific recombination; factor; Macromolecule synthesis, modification: DNA - replication, repair, restr./modific'n	Cytoplasm	2	13%	-	-

139.	Backbone	AdK / adenylate kinase activity; pleiotropic effects on glycerol-3-phosphate acyltransferase activity; enzyme; Nucleotide biosynthesis: Purine ribonucleotide biosynthesis	Cytoplasm	4	12%	-	-
140.	Backbone	MalK / ATP-binding component of transport system for maltose; transport; Transport of small molecules: Carbohydrates, organic acids, alcohols	Cytoplasm	1	3%	-	Mal F / maltose permease, MalS / alpha amylase, MaLY / enzyme that may degrade or block biosynthesis of endogenous mal inducer, MalZ / maltodextrin glucosidase
141.	Backbone	MukE / hypothetical protein; Unknown function	Cytoplasm	1	9%	-	-
142.	Backbone	NuoB / NADH dehydrogenase I chain B; enzyme; Energy metabolism, carbon: Aerobic respiration	Cytoplasm	1	4%	-	NuoL / NADH dehydrogenase I chain L ; enzyme; Energy metabolism, carbon: Aerobic respiration NuoM / NADH dehydrogenase I chain M; enzyme; Energy metabolism, carbon: Aerobic respiration

143.	Backbone	NuoG / NADH dehydrogenase I chain G; enzyme; Energy metabolism, carbon: Aerobic respiration	Cytoplasm	1	1%	-	NuoL / NADH dehydrogenase I chain L ; enzyme; Energy metabolism, carbon: Aerobic respiration NuoM / NADH dehydrogenase I chain M; enzyme; Energy metabolism, carbon: Aerobic respiration
144.	Backbone	SucB / 2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component); enzyme; Energy metabolism, carbon: TCA cycle	Cytoplasm	7	12%	-	-
145.	Backbone	PckA / phosphoenolpyruvate carboxykinase; enzyme; Central intermediary metabolism: Gluconeogenesis	Cytoplasm	1	1%	-	-
146.	Backbone	PPk / polyphosphate kinase; enzyme; Central intermediary metabolism: Phosphorus compounds	Cytoplasm	1	3%	-	-

147.	Backbone	ProQ / ProQ protein that influences osmotic activation of ProP; putative factor; Transport of small molecules: Amino acids, amines	Cytoplasm	1	30%	-	-
148.	Backbone	PyrG / CTP synthetase; enzyme; Central intermediary metabolism: Nucleotide interconversions	Cytoplasm	1	2%	-	-
149.	O-island # 57	Z2118 / putative endopeptidase Rz of prophage CP-933O; putative enzyme; Lysis (Phage or Prophage Related)	Cytoplasm	1	14%	-	-
150.	O-island # 50	Z1824 / hypothetical protein encoded by prophage CP-933N; unknown function	Cytoplasm	1	1%	-	-
151.	Backbone	FumB / fumarase B= fumarate hydratase Class I; anaerobic isozyme; enzyme; Energy metabolism, carbon: TCA cycle	Cytoplasm	1	2%	-	-
152.	Backbone	AceA / isocitrate lyase; enzyme; Central intermediary metabolism: Glyoxylate bypass	Cytoplasm	1	4%	-	-

153.	Backbone	TopA / DNA topoisomerase type I, omega protein; Macromolecule synthesis, modification: DNA - replication, repair, restr./modific'n	Cytoplasm	1	1%	-	TopA	
154.	Backbone	PepD / aminoacyl-histidine dipeptidase (peptidase D); Macromolecule degradation: Degradation of proteins, peptides, glyco	Cytoplasm	2	5%	-		-
155.	Backbone	Udp / uridine phosphorylase; Central intermediary metabolism: Salvage of nucleosides and nucleotides	Cytoplasm	1	5%	-		-
156.	Backbone	YgaU / hypothetical protein; unknown function	Cytoplasm	6	39%	-		-
157.	Backbone	DnaJ / chaperone with DnaK; heat shock protein; Folding and ushering proteins: Chaperones	Cytoplasm	2	4%	-		-
158.	Backbone	Crr / PTS system, glucose-specific IIA component; Transport of small molecules: Carbohydrates, organic acids, alcohols	Cytoplasm	4	62%	-		-

159.	O-island # 122	Z4317 / unknown protein encoded by ISEc8; Unknown function (Insertion Sequence Associated)	Cytoplasm	1	1%	-		-
160.	Backbone	Z1419 / hypothetical protein; unknown function	Cytoplasm	1	6%	-		-
161.	Backbone	yjgF / hypothetical protein; unknown function	Cytoplasm	1	8%	-		-
162.	Backbone	PpiB / peptidyl-prolyl cis-trans isomerase B (rotamase B); Macromolecule synthesis, modification: Proteins - translation and modification	Cytoplasm	1	8%	-		-
163.	Backbone	YlbA / hypothetical proteins; unknown function	Cytoplasm	1	4%	-		-
164.	Backbone	ChaC / cation transport regulator; Transport of small molecules: Cations	Cytoplasm	1	8%	-		-
165.	Backbone	SgaU / putative hexulose-6-phosphate isomerase; putative enzyme; Central intermediary metabolism: Pool, multipurpose conversions of intermediary metabolites	Cytoplasm	1	6%	-		SgaB / hypothetical protein; unknoiwn function

166.	Backbone	MelA / alpha-galactosidase; enzyme; Degradation of small molecules: Carbon compounds	Cytoplasm	1	2%	-	-
167.	Backbone	Z2778 / putative aldehyde dehydrogenase; unknown function	Cytoplasm	1	3%	-	-
168.	Backbone	KatE / catalase; hydroperoxidase HPII(III); enzyme; Protection responses: detoxification	Cytoplasm	1	2%	-	-
169.	Backbone	RplY / 50S ribosomal subunit protein L25; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	16%	-	-
170.	Backbone	RhlB / putative ATP-dependent RNA helicase; unknown function	Cytoplasm	1	2%	-	-
171.	Backbone	Z0516 / riboflavin synthase subunit beta; 6,7-dimethyl-8-ribityllumazine synthase /note=DMRL_synthase; enzyme; Biosynthesis of cofactors, carriers: Riboflavin	Cytoplasm	1	10%	-	-

172.	Backbone	RplA / 50S ribosomal subunit protein L1, regulates synthesis of L1 and L11; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	10	52%	-	-
173.	Backbone	RplP / 50S ribosomal subunit protein L16; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	12%	-	-
174.	Backbone	RplQ / 50S ribosomal subunit protein L17; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	24%	-	-
175.	Backbone	RplR / 50S ribosomal subunit protein L18; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	9%	-	-
176.	Backbone	RplS / 50S ribosomal subunit protein L19; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	23%	-	-

177.	Backbone	RplB / 50S ribosomal subunit protein L2; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	5%	-	-
178.	Backbone	RplU / 50S ribosomal subunit protein L21; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	14%	-	-
179.	Backbone	RplX / 50S ribosomal subunit protein L24; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	6	32%	-	-
180.	Backbone	RpmA / 50S ribosomal subunit protein L27; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	11%	-	-
181.	Backbone	RpmB / 50S ribosomal subunit protein L28; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	15%	-	-

182.	Backbone	RplD / 50S ribosomal subunit protein L4, regulates expression of S10 operon; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	4	21%	-	-
183.	Backbone	RplE / 50S ribosomal subunit protein L5; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	9%	-	-
184.	Backbone	RplL / 50S ribosomal subunit protein L7/L12; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	8	38%	-	-
185.	Backbone	RpiA / ribosephosphate isomerase, constitutive; enzyme; Central intermediary metabolism: Non-oxidative branch, pentose pathway	Cytoplasm	2	17%	-	-
186.	Backbone	Frr / ribosome releasing factor; factor; Macromolecule synthesis, modification: Proteins - translation and modification	Cytoplasm	1	11%	-	-

187.	Backbone	RpsJ / 30S ribosomal subunit protein S10; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	3	40%	-	-
188.	Backbone	RpsK / 30S ribosomal subunit protein S11; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	18%	-	-
189.	Backbone	RpsN / 30S ribosomal subunit protein S14; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	12%	-	-
190.	Backbone	RpsP / 30S ribosomal subunit protein S16; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	6	41%	-	-
191.	Backbone	RpsQ / 30S ribosomal subunit protein S17; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	8%	-	-

192.	Backbone	RpsS / 30S ribosomal subunit protein S19; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	2	10%	-	-
193.	Backbone	RpsB / 30S ribosomal subunit protein S2; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	5%	-	-
194.	Backbone	RpsU / 30S ribosomal subunit protein S21; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	1	14%	-	-
195.	Backbone	RpsC / 30S ribosomal subunit protein S3; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	7	45%	-	-
196.	Backbone	RpsD / 30S ribosomal subunit protein S4; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	6	24%	-	-

197.	Backbone	RpsL / 30S ribosomal subunit protein S12; structural component; Macromolecule synthesis, modification: Ribosomal proteins	Cytoplasm	3	17%	-	-
198.	Backbone	SodB / superoxide dismutase, iron; enzyme; Protection responses: Detoxification	Cytoplasm	1	13%	-	-
199.	Backbone	SucD / succinyl-CoA synthetase, alpha subunit; enzyme; Energy metabolism, carbon: TCA cycle	Cytoplasm	2	8%	-	-
200.	Backbone	TalB / transaldolase B; enzyme; Central intermediary metabolism: Non-oxidative branch, pentose pathway	Cytoplasm	6	19%	-	-
201.	Backbone	Tig / trigger factor; a molecular chaperone involved in cell division; factor; Cell division	Cytoplasm	5	13%	-	-
202.	Backbone	Z0751 / hypothetical protein/ unknown function	Cytoplasm	4	32%	-	-
203.	Backbone	WrbA / trp repressor binding protein; affects association of trp repressor and operator; regulator; Amino acid biosynthesis: Tryptophan	Cytoplasm	1	10%	-	-

204.	Backbone	YaiE / hypothetical protein/ unknown function	Cytoplasm	2	14%	-	-
205.	Backbone	YbaA / hypothetical protein/ unknown function	Cytoplasm	1	15%	-	-
206.	Backbone	YdfH / hypothetical protein/ unknown function	Cytoplasm	1	7%	-	-
207.	Backbone	YjbJ / hypothetical protein/ unknown function	Cytoplasm	4	46%	-	-

[1] Based on homology to the sequenced, *E. coli* O157 strain EDL933 genome, and plasmid, pO157.

[2] Putative functions assigned to hypothetical proteins using the Conserved Domain Database (CDD).

[3]Bacterial cell localization of proteins determined by PSORTb v.2.0/PSLpred/ PSORTdb, and SignalP 3.0 prediction program.

[4]STM,signature tagged mutagenesis [9]; IVIAT, *in vivo* induced antigen technology [6].