

DSS-induced inflammation changes protein expression of intestinal *Escherichia coli* in a gnotobiotic mouse model

Supplementary Data:

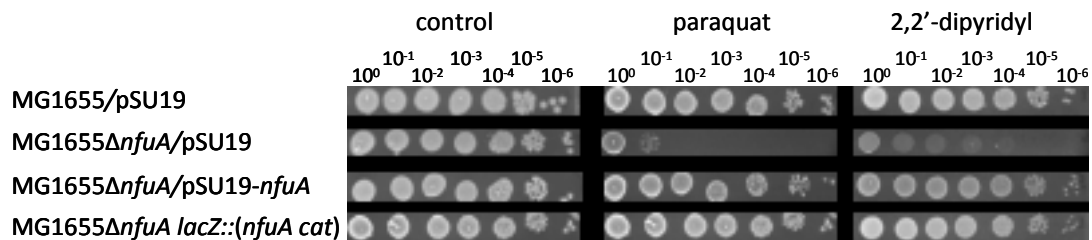


Fig. S1. Effect of the *nfuA* deletion on the growth of MG1655 in the presence of either paraquat or dipyridyl. The deletion was complemented either with the low copy number plasmid pSU19 carrying a copy of the *nfuA* gene under control of its own promoter region or by insertion of a similar fragment into the chromosomal *lacZ* gene by the method of Le Borgne et al. (1). Exponentially growing cells were sedimented by centrifugation, resuspended to equal optical densities and decimally diluted. Five μ l of each suspension were spotted onto LB-plates containing 20 μ g/ml chloramphenicol (control), or on plates which contained in addition either 300 μ M paraquat or 375 μ M 2,2'-dipyridyl. Photographs were taken after overnight incubation at 37°C. Each block depicts four dilution series from the same plate.

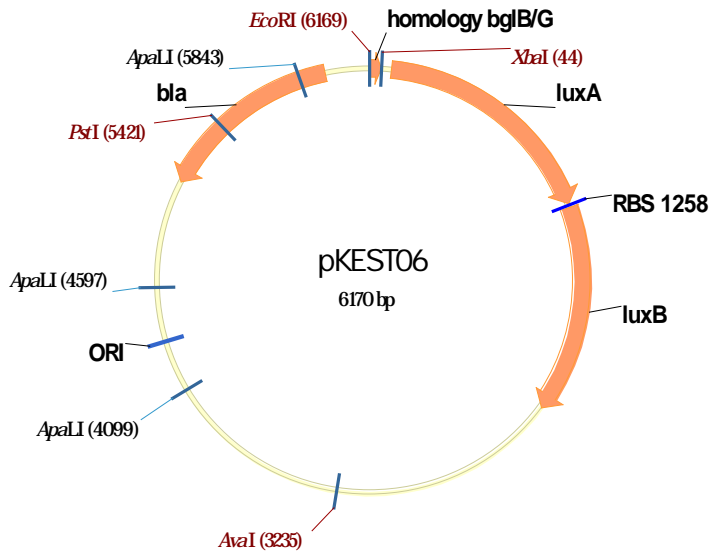


Fig. S2. pKEST06 vector used for expression of *luxAB* genes

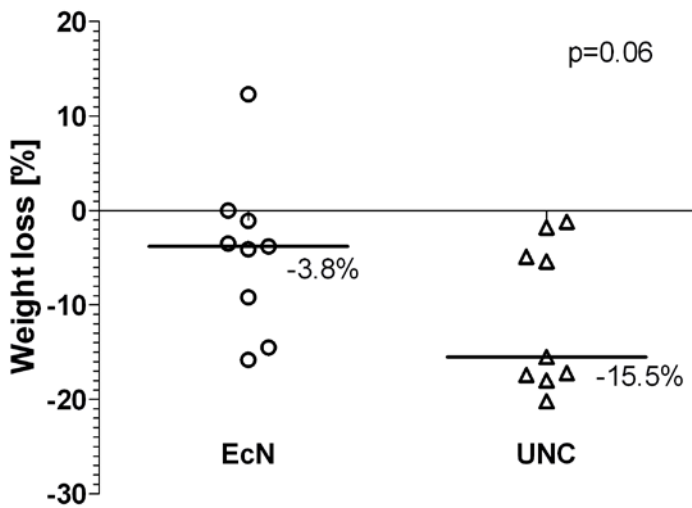


Fig. S3. EcN attenuates body weight loss caused by DSS-induced inflammation.

Germfree mice (129/SvEv) were monoassociated with EcN or UNC and received sterile drinking water with 3.5% DSS. The mice were weighed at the beginning and at the end of the experiment. Data are expressed as medians ($p=0.06$).

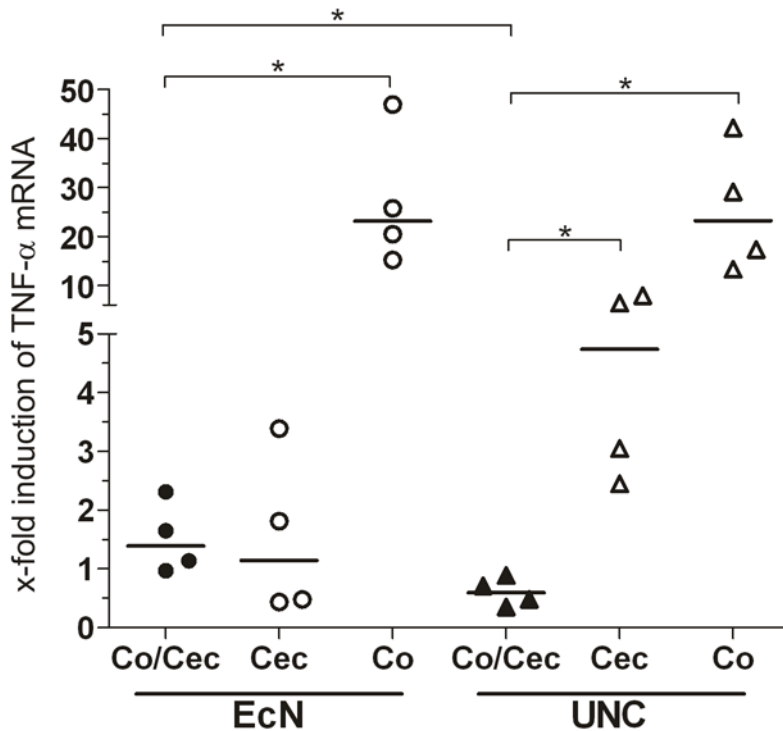


Fig. S4. DSS treatment causes elevated transcription of the inflammation marker TNF- α . Germfree mice (129/SvEv) monoassociated with EcN or UNC received sterile drinking water with 3.5% DSS (open symbols) or without (filled symbols). Induction of the cytokine was measured by quantitative PCR using as template total RNA isolated from intestinal epithelial cells of the respective tissue of those animals whose samples were not used for histological scoring. Amplification was normalized against the geometrical means of amplification reactions with *Hprt1* (hypoxanthine guanine phosphoribosyl transferase) and *Rpl13a* (Ribosomal protein L13A) (2). Primers were designed to be specific for cDNA by placing at least one primer onto an exon/intron boundary. Data are expressed as medians, * $p \leq 0.05$.

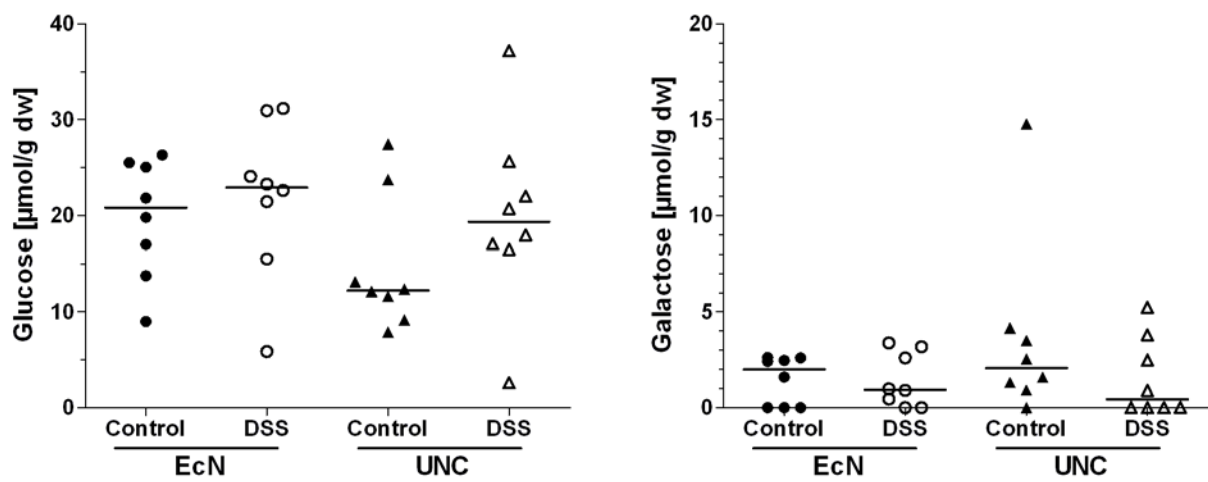


Fig. S5. Concentrations of carbohydrates in the cecal contents. Germfree mice (129/SvEv) monoassociated with EcN or UNC received sterile drinking water with 3.5% DSS or without. Concentrations of carbohydrates were measured in the cecal water. Data are expressed as medians (no significant differences).

Table S1. Influence of inflammation (DSS treatment) on protein expression in cecal bacteria of monoassociated mice

Swiss-Prot accession no.	Gene	Protein description ^a	Fold change (DSS vs. control)
Proteins differentially expressed in <i>E. coli</i> Nissle			
P0A9B2	<i>gapA</i>	Glyceraldehyde-3-phosphate dehydrogenase A	-7.06
P40120	<i>mdoD</i>	Glucans biosynthesis protein D	-6.46
P75733	<i>ybfM</i>	Uncharacterized protein YbfM	-5.31
P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	-5.1, -8.23
P77376	<i>ydgJ</i>	Uncharacterized oxidoreductase YdgJ	-5.08
P0ABB0	<i>atpA</i>	ATP synthase subunit alpha	-4.73, -6.49, -5.42, -4.51
P0A8F0	<i>upp</i>	Uracil phosphoribosyltransferase	-4.69
P0ACE0	<i>hybC</i>	Hydrogenase-2 large chain	-4.67
P0AGD3	<i>sodB</i>	Superoxide dismutase [Fe]	-4.52
P0A8N5	<i>lysU</i>	Lysyl-tRNA synthetase, heat inducible	-4.06
P02925	<i>rbsB</i>	D-ribose-binding periplasmic protein	-4.04, -5.67, -7.11
P0A858	<i>tpiA</i>	Triosephosphate isomerase	-4.03
P27302	<i>tktA</i>	Transketolase 1	-3.97
P0A7R1	<i>rplI</i>	50S ribosomal protein L9	-3.88
P06720	<i>melA</i>	Alpha-galactosidase	-3.82
P00509	<i>aspC</i>	Aspartate aminotransferase	-3.71, -8.82
P0AG55	<i>rplF</i>	50S ribosomal protein L6	-3.69, -3.68
P22259	<i>pckA</i>	Phosphoenolpyruvate carboxykinase [ATP]	-3.46
P0AE08	<i>ahpC</i>	Alkyl hydroperoxide reductase subunit C	-3.41
P0CE47	<i>tufA</i>	Elongation factor Tu 1	-3.24, -3.52, -4.55

P27248	<i>gcvT</i>	Aminomethyltransferase	-3.22
P0ABP8	<i>deoD</i>	Purine nucleoside phosphorylase deoD-type	-3.16
P09373	<i>pflB</i>	Formate acetyltransferase 1	-3.15
P0A7D7	<i>purC</i>	Phosphoribosylaminoimidazole-succinocarboxamide synthase	-3.01
P28248	<i>dcd</i>	Deoxycytidine triphosphate deaminase	3.00
P19926	<i>agp</i>	Glucose-1-phosphatase	3.14
P0A8E7	<i>yajQ</i>	UPF0234 protein YajQ	3.15
P76177	<i>ydgH</i>	Protein YdgH	3.24
P63020	<i>nfuA</i>	Fe/S biogenesis protein NfuA	3.75
P02413	<i>rplO</i>	50S ribosomal protein L15	4.00
P0A6F5	<i>groL</i>	60 kDa chaperonin	4.13
P0A7G6	<i>recA</i>	Protein RecA	4.46
P0A6Q3	<i>fabA</i>	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	5.58
P0A9C5	<i>glnA</i>	Glutamine synthetase	5.58, 6.16
P02358	<i>rpsF</i>	30S ribosomal protein S6	9.51
Proteins differentially expressed in <i>E. coli</i> UNC			
P0A9B2	<i>gapA</i>	Glyceraldehyde-3-phosphate dehydrogenase A	-8.41
P62623	<i>ispH</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-7.47
P0AB71	<i>fbaA</i>	Fructose-bisphosphate aldolase class 2	-6.92
P0A858	<i>tpiA</i>	Triosephosphate isomerase	-6.10
P0AEZ3	<i>minD</i>	Septum site-determining protein mind	-5.56, -6.97
P07862	<i>ddlB</i>	D-alanine--D-alanine ligase B	-5.48
P0A7J7	<i>rplK</i>	50S ribosomal protein L11	-5.16
P0A8F0	<i>upp</i>	Uracil phosphoribosyltransferase	-5.09
P09148	<i>galT</i>	Galactose-1-phosphate uridylyltransferase	-5.05

P0A9L3	<i>fkfB</i>	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase	-4.96
P0A6F5	<i>groL</i>	60 kDa chaperonin	-4.49
P0AGD3	<i>sodB</i>	Superoxide dismutase [Fe]	-4.41, -5.43
P0AAT9	<i>ybeL</i>	Uncharacterized protein YbeL	-4.20
P0ABA6	<i>atpG</i>	ATP synthase gamma chain	-4.17, -4.63
P0ABB0	<i>atpA</i>	ATP synthase subunit alpha	-4.16
P76541	<i>eutL</i>	Ethanolamine utilization protein EutL	-4.11
P08331	<i>cpdB</i>	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	-4.09, -8.58
P40120	<i>mdoD</i>	Glucan biosynthesis protein D	-4.02
P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	-3.86, -6.68
P0A6T3	<i>galK</i>	Galactokinase	-3.81
P67910	<i>hldD</i>	ADP-L-glycero-D-manno-heptose-6-epimerase	-3.68
P0ACE0	<i>hybC</i>	Hydrogenase-2 large chain	-3.58
P04982	<i>rbsD</i>	D-ribose pyranase	-3.55
P0AG18	<i>purE</i>	Phosphoribosylaminoimidazole carboxylase catalytic subunit	-3.51
P0A799	<i>pgk</i>	Phosphoglycerate kinase	-3.46, -3.61
P0A9J6	<i>rbsK</i>	Ribokinase	-3.46
P0A8N5	<i>lysU</i>	Lysyl-tRNA synthetase, heat inducible	-3.44
P0A6P9	<i>eno</i>	Enolase	-3.38, -4.5
P0CE47	<i>tufA</i>	Elongation factor Tu 1	-3.30, -3.75, -4.06, -4.83, -26.15
P11875	<i>argS</i>	Arginyl-tRNA synthetase	-3.26
P27248	<i>gcvT</i>	Aminomethyltransferase	-3.23
P02358	<i>rpsF</i>	30S ribosomal protein S6	-3.15
P0AE08	<i>ahpC</i>	Alkyl hydroperoxide reductase subunit C	-3.10

P02925	<i>rbsB</i>	D-ribose-binding periplasmic protein	-3.03, -4.43, -5.17, -7.13, -7.39, -8.84
P0A7D7	<i>purC</i>	Phosphoribosylaminoimidazole- succinocarboxamide synthase	-3.03
P0A864	<i>tpx</i>	Thiol peroxidase	3.00
P35340	<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F	3.03
P09373	<i>pflB</i>	Formate acetyltransferase 1	3.03, 3.11, 3.18, 4.2
P0A7E5	<i>pyrG</i>	CTP synthase	3.05
P63020	<i>nfuA</i>	Fe/S biogenesis protein NfuA	3.13
P27302	<i>tktA</i>	Transketolase 1	3.13, 3.58, 3.82
P0AC38	<i>aspA</i>	Aspartate ammonia-lyase	3.17, 4.85
P63284	<i>clpB</i>	Chaperone protein ClpB	3.31
P0A853	<i>tnaA</i>	Tryptophanase	3.36, 5.93
P00722	<i>lacZ</i>	Beta-galactosidase	3.75, 4.77
P0AC41	<i>sdhA</i>	Succinate dehydrogenase flavoprotein subunit	4.0
P77804	<i>ydgA</i>	Protein YdgA	4.09
P42632	<i>tdcE</i>	Keto-acid formate acetyltransferase	4.49
P0A6Q3	<i>fabA</i>	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	6.18
P0AFF6	<i>nusA</i>	Transcription elongation protein nusA	6.38
P0A9C5	<i>glnA</i>	Glutamine synthetase	6.87

^aProteins with differential expression factors ≥ 3 ($p \leq 0.05$) in cecal samples (inflamed DSS vs. control group) are listed in this table.

Table S2. Comparison of strain-specific bacterial protein expression between mice monoassociated with UNC vs. mice monoassociated with EcN.

Swiss-Prot accession no.	Gene	Protein description ^a	Fold change UNC vs. EcN
Proteins differentially expressed in control group			
P06720	<i>melA</i>	Alpha-galactosidase	-11.19
P19926	<i>agp</i>	Glucose-1-phosphatase	-11.17
P00805	<i>ansB</i>	L-asparaginase 2	-10.64; -13.96; -29.66
P0AES2	<i>gudD</i>	Glucarate dehydratase	-9.62
P21420	<i>nmpC</i>	Putative outer membrane porin protein NmpC	-8.29
P0A855	<i>tolB</i>	Protein TolB	-7.67
P0A853	<i>tnaA</i>	Tryptophanase	-7.03
P0AEK4	<i>fabI</i>	Enoyl-[acyl-carrier-protein] reductase [NADH]	-6.76
P0A915	<i>ompW</i>	Outer membrane protein W	-6.49
P10121	<i>ftsY</i>	Cell division protein FtsY	-6.11
P23256	<i>malY</i>	Protein MalY	-5.75
P17169	<i>glmS</i>	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	-5.69
P26646	<i>yhdH</i>	Putative quinone oxidoreductase YhdH	-5.53
P0A825	<i>glyA</i>	Serine hydroxymethyltransferase	-5.31
P16456	<i>selD</i>	Selenide, water dikinase	-5.29
P61889	<i>mdh</i>	Malate dehydrogenase	-5.11; -6.73
P23893	<i>hemL</i>	Glutamate-1-semialdehyde 2,1-aminomutase	-5.09
P0AAT9	<i>ybeL</i>	Uncharacterized protein YbeL	-5.06
P75733	<i>ybfM</i>	Uncharacterized protein YbfM	-5.04
P0ABH0	<i>ftsA</i>	Cell division protein FtsA	-4.98

P0AFF6	<i>nusA</i>	Transcription elongation protein NusA	-4.72
P12758	<i>udp</i>	Uridine phosphorylase	-4.63
P0ADS6	<i>yggE</i>	Uncharacterized protein YggE	-4.40
P0A850	<i>tig</i>	Trigger factor	-4.20; -10.58
Q8FAD9	<i>arcA</i>	Arginine deiminase	-4.19; -4.92; -10.75
P15639	<i>purH</i>	Bifunctional purine biosynthesis protein PurH	-4.18
P27248	<i>gcvT</i>	Aminomethyltransferase	-4.13; -7.19; -12.15
P77376	<i>ydgJ</i>	Uncharacterized oxidoreductase YdgJ	-3.87
P0A6Y8	<i>dnaK</i>	Chaperone protein DnaK	-3.83
P0AG55	<i>rplF</i>	50S ribosomal protein L6	-3.70
P00722	<i>lacZ</i>	Beta-galactosidase	-3.67; -9.27
P0A786	<i>pyrB</i>	Aspartate carbamoyltransferase catalytic chain	-3.66
P0ABT2	<i>dps</i>	DNA protection during starvation protein	-3.64
P0AC81	<i>gloA</i>	Lactoylglutathione lyase	-3.46
P35340	<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F	-3.30; -3.48
P0AET8	<i>hdhA</i>	7-alpha-hydroxysteroid dehydrogenase	-3.25
P0A862	<i>tpx</i>	Thiol peroxidase	-3.19
P37773	<i>mpl</i>	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	-3.16
P0AGF6	<i>tdcB</i>	Threonine dehydratase catabolic	-3.08
P0CE47	<i>tufA</i>	Elongation factor Tu 1	3.07; 4.21
P0A8G6	<i>wrbA</i>	Flavoprotein WrbA	3.20, 4.84
P08200	<i>icd</i>	Isocitrate dehydrogenase [NADP]	3.22
P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	3.25; 4.25
P63224	<i>gmhA</i>	Phosphoheptose isomerase	3.27
P02358	<i>rpsF</i>	30S ribosomal protein S6	3.32

P00509	<i>aspC</i>	Aspartate aminotransferase	3.34; 3.76
P0A794	<i>pdxJ</i>	Pyridoxine 5'-phosphate synthase	3.37
P02925	<i>rbsB</i>	D-ribose-binding periplasmic protein	3.40; 3.55; 3.93; 4.24; 4.89
P0A7G6	<i>recA</i>	Protein RecA	3.60
P0A9L3	<i>fkfB</i>	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase	3.67
P27302	<i>tktA</i>	Transketolase 1	4.13
P07862	<i>ddlB</i>	D-alanine--D-alanine ligase B	4.14
P62623	<i>ispH</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	4.31
P0ABA6	<i>atpG</i>	ATP synthase gamma chain	4.32
P0A817	<i>metK</i>	S-adenosylmethionine synthase	4.34
P0A6F5	<i>groL</i>	60 kDa chaperonin	4.42
P0A858	<i>tpiA</i>	Triosephosphate isomerase	4.54
P0A7L0	<i>rplA</i>	50S ribosomal protein L1	4.57
P04982	<i>rbsD</i>	D-ribose pyranase	4.69
P09127	<i>hemX</i>	Putative uroporphyrinogen-III C methyltransferase	5.45
P09148	<i>galT</i>	Galactose-1-phosphate uridylyltransferase	5.30
P67910	<i>hldD</i>	ADP-L-glycero-D-manno-heptose-6-epimerase	5.84
P0AEZ3	<i>minD</i>	Septum site-determining protein MinD	6.32
P0A9C3	<i>galM</i>	Aldose 1-epimerase	7.04
P0ACF8	<i>hns</i>	DNA-binding protein H-NS	14.52

Proteins differentially expressed in DSS group

P02358	<i>rpsF</i>	30S ribosomal protein S6	-16.72
P23256	<i>malY</i>	Protein MalY	-9.52
P00805	<i>ansB</i>	L-asparaginase 2	-9.19; -10.76; -22.90
P0A9A9	<i>fur</i>	Ferric uptake regulation protein	-8.93; -20.62

P0AAT9	<i>ybeL</i>	Uncharacterized protein YbeL	-8.54
P0ABT2	<i>dps</i>	DNA protection during starvation protein	-8.16
P0ADS6	<i>yggE</i>	Uncharacterized protein YggE	-8.13
P06720	<i>melA</i>	Alpha-galactosidase	-7.24
P19926	<i>agp</i>	Glucose-1-phosphatase	-6.69
P0AES2	<i>gudD</i>	Glucarate dehydratase	-6.47; -12.5
P21420	<i>nmpC</i>	Putative outer membrane porin protein NmpC	-6.20
P12758	<i>udp</i>	Uridine phosphorylase	-6.05
P0A915	<i>ompW</i>	Outer membrane protein W	-5.99
P26646	<i>yhdH</i>	Putative quinone oxidoreductase YhdH	-5.93
P16456	<i>selD</i>	Selenide, water dikinase	-5.83
P0AEK4	<i>fabI</i>	Enoyl-[acyl-carrier-protein] reductase [NADH]	-5.54
P18843	<i>nadE</i>	NH(3)-dependent NAD(+) synthetase	-4.75
Q8FAD9	<i>arcA</i>	Arginine deiminase	-4.61; -6.77; -8.72; -15.2
P0A786	<i>pyrB</i>	Aspartate carbamoyltransferase catalytic chain	-4.60
P0AET8	<i>hdhA</i>	7-alpha-hydroxysteroid dehydrogenase	-4.58
P69441	<i>adk</i>	Adenylate kinase	-4.41
P0A9C5	<i>glnA</i>	Glutamine synthetase	-4.40
P11875	<i>argS</i>	Arginyl-tRNA synthetase	-4.34
P0A853	<i>tnaA</i>	Tryptophanase	-4.16
P61889	<i>mdh</i>	Malate dehydrogenase	-4.14; -8.13
P76576	<i>yfgM</i>	UPF0070 protein YfgM	-4.10
P0A9J6	<i>rbsK</i>	Ribokinase	-4.09
P08331	<i>cpdB</i>	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	-3.48
P37903	<i>uspF</i>	Universal stress protein F	-3.47

P0AC81	<i>gloA</i>	Lactoylglutathione lyase	-3.41
P0A855	<i>tolB</i>	Protein TolB	-3.28; -3.32
P0A9Q7	<i>adhE</i>	Aldehyde-alcohol dehydrogenase	-3.12
P0A6K6	<i>deoB</i>	Phosphopentomutase	3.04
P14407	<i>fumB</i>	Fumarate hydratase class I, anaerobic	3.14; 3.70
P77318	<i>ydeN</i>	Uncharacterized sulfatase YdeN	3.38
P0A8N5	<i>lysU</i>	Lysyl-tRNA synthetase, heat inducible	3.40
P0CE47	<i>tufA</i>	Elongation factor Tu 1	3.41
P0A6A3	<i>ackA</i>	Acetate kinase	3.52
P00509	<i>aspC</i>	Aspartate aminotransferase	3.82; 4.26; 6.01; 6.16
P27302	<i>tktA</i>	Transketolase 1	3.86; 8.04
P0A858	<i>tpiA</i>	Triosephosphate isomerase	3.88
P02925	<i>rbsB</i>	D-ribose-binding periplasmic protein	3.98; 4.81; 37.35
P0A794	<i>pdxJ</i>	Pyridoxine 5'-phosphate synthase	4.39
P0A8G6	<i>wrbA</i>	Flavoprotein WrbA	5.27
P0A817	<i>metK</i>	S-adenosylmethionine synthase	5.53; 7.47
P10121	<i>ftsY</i>	Cell division protein FtsY	5.87
P00722	<i>lacZ</i>	Beta-galactosidase	6.06; 6.29; 7.88; 32.21; 49.06
P63224	<i>gmhA</i>	Phosphoheptose isomerase	6.40
P02931	<i>ompF</i>	Outer membrane protein F	6.50; 18.71
P09148	<i>galT</i>	Galactose-1-phosphate uridylyltransferase	6.52
P0A6Y8	<i>dnaK</i>	Chaperone protein DnaK	7.56
P0A7L0	<i>rplA</i>	50S ribosomal protein L1	9.00
P0ACF8	<i>hns</i>	DNA-binding protein H-NS	34.21

^a **Proteins with differential expression factors ≥ 3 ($p \leq 0.05$) in cecal samples (UNC vs. EcN)**

References

1. **Le Borgne, S., B. Palmeros, F. Bolivar, and G. Gosset.** 2001. Improvement of the pBRINT-Ts plasmid family to obtain marker-free chromosomal insertion of cloned DNA in *E. coli*. *Biotechniques* **30**:252-254, 256.
2. **Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman.** 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* **3**:RESEARCH0034.