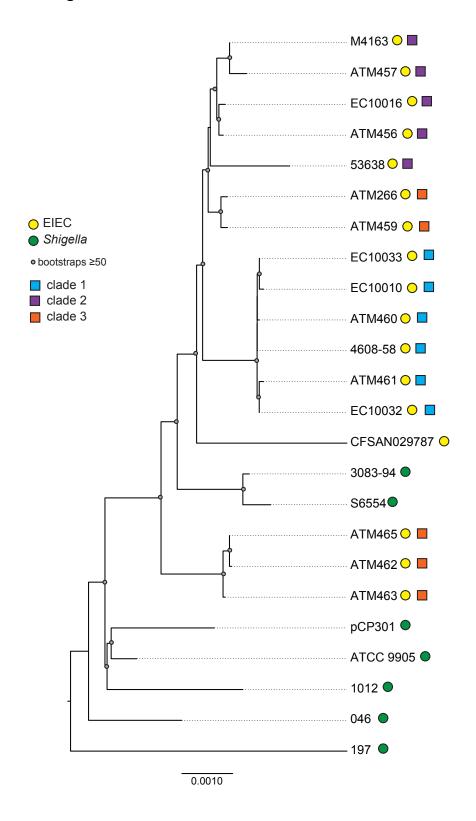
1 Supplemental Figure



- 4 Figure S1. Virulence plasmid phylogeny. The Shigella virulence plasmid, pCP301 5 (NC 004851.1), was aligned to the EIEC and Shigella genomes that contained genes 6 that exhibited similarity to those on the plasmid. The alignment was performed using 7 Mugsy (1) and the regions from each genome that aligned to pCP301 were 8 concatenated to generate a 24 kb sequence for each genome. These regions were 9 compared by constructing a maximum-likelihood phylogeny using RAxML v7.2.8 (2) that 10 was visualized using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The 11 EIEC genomes are indicated by a yellow circle and Shigella genomes are indicated by a 12 green circle. The EIEC lineages are indicated by blue (lineage 1), purple (lineage 2), 13 and orange (lineage 3) squares. The scale bar indicates the distance of 0.001 14 nucleotide substitutions per site. Bootstrap values ≥50 are designated by a grey circle. 15 16 17
 - 1. **Angiuoli SV, Salzberg SL.** 2011. Mugsy: fast multiple alignment of closely related whole genomes. Bioinformatics **27**:334-342.

18 19

20

2. **Stamatakis A.** 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**:2688-2690.

Table S1. Genomes included in the phylogenomic analysis

Isolate ID		Molecular Serotype	Pathovar	Phylogroup	Accession No.
HS	09	O9:H4	commensal	A	NC_009800.1
H10407	O78:H11:K80	O78:H11	ETEC	Α	FN649414.1
BL21	NA	O7:H-	lab adapted	Α	NC 012947.1
K-12, BW2952	NA	O16:H48	lab adapted	Α	NC 012759.1
ATCC 8739	NA	O146:H20	lab adapted	Α	NC_010468.1
S. dysenteriae 1012	NA	SD2:H14	Shigella	B1	AAMJ00000000.1
S. dysenteriae S6554	NA	SB1?:H45	Shigella	B1	AMJS00000000.1
S. flexneri 2a 2457T	NA	SF2a:H14	Shigella	B1	NC_004741.1
S. flexneri 2a 301	NA	SF2a:H14	Shigella	B1	NC_004337.2
S. flexneri CCH060	NA	SF6:H45	Shigella	B1	AKMW00000000.1
S. boydii 3083-94	NA	SB18:H45	Shigella	B1	NC_010658.1
S. boydii ATCC 9905	NA	SB7:H14	Shigella	B1	AERN00000000.1
S. sonnei 046	NA	SS:H16	Shigella	B1	NC_007384.1
SE11	NA	O173:H28	commensal	B1	NC_011415.1
55989	O104:H4	O104:H4	EAEC	B1	NC_011748.1
11368	O26:H11	O26:H11	EHEC/STEC	B1	NC_013361.1
11128	O111:H-	O111:H8	EHEC/STEC	B1	NC_013364.1
E110019	O111:H9	O111:H9	AEEC	B1	AAJW00000000.2
B171	O111:NM	O111:H2	EPEC2	B1	AAJX00000000.2
E24377A	O139:H28	O139v:H28	ETEC	B1	NC_009801.1
B7A	O148:H28	O148:H28	ETEC	B1	AAJT00000000.2
IAI1	08	O8/SB17:H19	fecal isolate	B1	NC_011741.1
TY-2482	O104:H4	O104:H4	O104 outbreak	B1	AFOG00000000.1
ED1a	O81	O81:H27	commensal	B2	CU928162.2
E2348/69	O127:H6	O127:H6	EPEC1	B2	NC_011601.1
536	O6:K15:H31	O6:H31	UPEC/ExPEC	B2	NC_008253.1
S88	O45:K1	O45v:H7	UPEC/ExPEC	B2	NC_011742.1
UTI89	NA	O18:H7	UPEC/ExPEC	B2	NC_007946.1
CFT073	O6:K2:H1	O6:H1	UPEC/ExPEC	B2	AE014075.1
042	O44:H18	O44:H18	EAEC	D	FN554766.1
UMN026	O17:K52:H18	O17:H18	UPEC/ExPEC	D	NC_011751.1
S. dysenteriae 197	NA	SD1:H18	Shigella	Е	NC_007606.1
EDL933	O157:H7	O157:H7	EHEC/STEC	Е	NC_002655.2
Sakai	O157:H7	O157:H7	EHEC/STEC	E	NC_002695.1
CB9615	O55:H7	O55:H7	AEEC	E	NC_013941.1
IA139	O7:K1	O7:H45	UPEC/ExPEC	F	NC_011750.1
SMS_3_5	O19:H34	O153:H34	other	F	CP000970.1

NA indicates that data was not applicable or could not be determined.

Table S2. Predicted protein functions of EIEC genes

		Table S2. Predicted protein functions of EIEC genes
LS-BSR Gene Cluster	Gene	Predicted Protein Function
EIEC vs. other E. c	oli and	Shigella Shi
Cluster_178041	pyk	pyruvate kinase
Cluster_178088		putative membrane protein
Cluster_178120		carboxymuconolactone decarboxylase family protein
		hypothetical protein
		hypothetical protein
		dihydrouridine synthase family protein
	torT	TMAO reductase system periplasmic protein TorT
EIEC vs. other E. c	oli (not i	including Shigella)
Cluster_16689	· ·	4Fe-4S dicluster domain protein
Cluster_171880		hypothetical protein
Cluster 171882		conjugal transfer/type IV secretion DotA/TraY family protein
Cluster_171887		hypothetical protein
Cluster_167621		phage-related minor tail family protein
Cluster_168446		enterobacterial exodeoxyribonuclease VIII family protein
Cluster_168480		phage major capsid protein, HK97 family
		hypothetical protein
		hypothetical protein
Cluster_169741		bacteriophage CI repressor helix-turn-helix domain protein
Cluster_169827		metallo-beta-lactamase superfamily protein
Cluster_170182		hypothetical protein
Cluster_173349		phage gp6-like head-tail connector family protein
Cluster_175432		hypothetical protein
Cluster_175571	rpoZ	DNA-directed RNA polymerase, omega subunit
Cluster_168990		hypothetical protein
Cluster_168992		hypothetical protein
Cluster_168993		hypothetical protein
Cluster_168995		hypothetical protein
Cluster_168997		hypothetical protein
Cluster_169044		glycosyl transferase 2 family protein
Cluster_169048		glycosyl transferase 2 family protein
Cluster_169049		CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase family protein
Cluster_169935		oxidoreductase family, C-terminal alpha/beta domain protein
Cluster_169939		D5 N terminal like family protein
Cluster_169940		conserved hypothetical protein
Cluster_170184		integrating conjugative element, PFL_4695 family domain protein
Cluster_170187		hypothetical protein
Cluster_170188		response regulator
Cluster_170194		conserved hypothetical family protein
Cluster_170195	ssb	single-stranded DNA-binding family protein
Cluster_171910		type IV leader peptidase family protein
Cluster_171911		transglycosylase SLT domain protein
Cluster_178041	pyk	pyruvate kinase
Cluster_178069		kinase/pyrophosphorylase family protein
Cluster_178088		putative membrane protein

Cluster_178120 Cluster_169052 Cluster_170093 Cluster_170303 Cluster_170304 Cluster_170515 Cluster_170570 Cluster_17062 Cluster_170665 Cluster_1707 Cluster_170798 Cluster_17074 Cluster_171074 Cluster_1711 Cluster_171226 Cluster_171408 Cluster_171408 Cluster_171408 Cluster_171446 Cluster_171446 Cluster_171915 Cluster_171915 Cluster_171916 Cluster_171917 Cluster_171918 Cluster_171918 Cluster_171920 Cluster_171920 Cluster_171920 Cluster_172064 Cluster_17208 Cluster_17209 Cluster_17212 Cluster_17213 Cluster_17213 Cluster_17216 Cluster_175616 Cluster_175616 Cluster_175616 Cluster_175612 Cluster_175616 Cluster_175612 Cluster_175616 Cluster_175612 Cluster_175616 Cluster_175612 Cluster_175616 Cluster_175616 Cluster_175612 Cluster_170112 Cluster_170113 Cluster_1723 Cluster_172355	galE btuB	carboxymuconolactone decarboxylase family protein UDP-glucose 4-epimerase GalE hypothetical protein arylsulfotransferase family protein DSBA-like thioredoxin domain protein conserved hypothetical protein hypothetical protein hypothetical protein hypothetical protein hypothetical protein flxA-like family protein flmbrial family protein haemagglutinin family protein methyltransferase domain protein methyltransferase domain protein tonB-dependent vitamin B12 receptor methyltransferase domain protein transposase IS66 family protein hypothetical protein oligogalacturonate-specific porin family protein FCD domain protein antitoxin Phd_YefM, type II toxin-antitoxin system family protein type IV pilus biogenesis family protein type IVB pilus formation outer membrane protein, R64 PilN family pilM family protein guar fermentation stimulation protein fimbrial family protein sugar fermentation stimulation protein fimbrial family protein hypothetical protein hypothetical protein hypothetical protein hypothetical protein terminase small subunit hypothetical protein conserved hypothetical protein
Cluster_170112		description family protein
_		e, , ,
-		
Cluster_172355		plasmid stabilisation system family protein
Cluster_178247		YecM family protein
Cluster_176594		phage baseplate assembly V family protein
Cluster_179014		hypothetical protein
Cluster_173627		hypothetical protein

01 / /=000=	
Cluster_178285	RNA polymerase sigma factor, sigma-70 family protein
Cluster_181210	bacterial mobilization family protein
Cluster_181725	hypothetical protein
Cluster_172738	PTS system sugar-specific permease component family protein
Cluster_181221	hypothetical protein
Cluster_183530	dihydrouridine synthase family protein
Cluster_183668	hypothetical protein
Cluster_183746	efflux pump membrane family protein
Cluster_183764	phosphotransferase system, EIIC family protein
Cluster_18658	phage integrase family protein
Cluster_186711	torT TMAO reductase system periplasmic protein TorT
E1E0 1.0/: #	
EIEC and Shigella	
Cluster_16689	4Fe-4S dicluster domain protein
Cluster_167621	phage-related minor tail family protein
Cluster_168446	enterobacterial exodeoxyribonuclease VIII family protein
Cluster_168480	phage major capsid protein, HK97 family
Cluster_168972	hypothetical protein
Cluster_168973	hypothetical protein
Cluster_168990	hypothetical protein
Cluster_168992	hypothetical protein
Cluster_168993	hypothetical protein
Cluster_168995	hypothetical protein
Cluster_168997	hypothetical protein
Cluster_169044	glycosyl transferase 2 family protein
Cluster_169048	glycosyl transferase 2 family protein
Cluster_169049	CDP-Glycerol:Poly(glycerophosphate) glycerophosphotransferase family protein
Cluster_169052	galE UDP-glucose 4-epimerase GalE
Cluster_169741	bacteriophage CI repressor helix-turn-helix domain protein
Cluster_169827	metallo-beta-lactamase superfamily protein
Cluster_169935	oxidoreductase family, C-terminal alpha/beta domain protein
	D5 N terminal like family protein
_ Cluster_169940	conserved hypothetical protein
_ Cluster_170093	hypothetical protein
	description family protein
_ Cluster_170113	conserved hypothetical protein
	hypothetical protein
Cluster 170184	integrating conjugative element , PFL_4695 family domain protein
Cluster_170187	hypothetical protein
Cluster_170188	response regulator
Cluster_170194	conserved hypothetical family protein
Cluster_170195	ssb single-stranded DNA-binding family protein
Cluster 170303	arylsulfotransferase family protein
Cluster_170304	DSBA-like thioredoxin domain protein
Cluster_170515	conserved hypothetical protein
Cluster_170570	hypothetical protein
Cluster_17062	hypothetical protein
Cluster 170665	hypothetical protein
Cluster_170005	flxA-like family protein
	iiAA-iike laitiiiy proteiti

Olivetes 470700		final sial family and the
Cluster_170798		fimbrial family protein
Cluster_170859		haemagglutinin family protein
Cluster_171074		SIS domain protein
Cluster_1711		methyltransferase domain protein
Cluster_171226	btuB	tonB-dependent vitamin B12 receptor
Cluster_171408		methyltransferase domain protein
Cluster_171424		transposase IS66 family protein
Cluster_171433		hypothetical protein
Cluster_171446		oligogalacturonate-specific porin family protein
Cluster_171449		FCD domain protein
Cluster_171880		hypothetical protein
Cluster_171882		conjugal transfer/type IV secretion DotA/TraY family protein
Cluster_171887		hypothetical protein
Cluster_171910		type IV leader peptidase family protein
Cluster_171911		transglycosylase SLT domain protein
Cluster_171915		antitoxin Phd_YefM, type II toxin-antitoxin system family protein
Cluster_171916		addiction module toxin, RelE/StbE family protein
Cluster_171917		type IV pilus biogenesis family protein
Cluster_171918		pilin accessory family protein
Cluster_171919		type IVB pilus formation outer membrane protein, R64 PilN family
		pilM family protein
		putative membrane protein
	sfsA	sugar fermentation stimulation protein
		fimbrial family protein
Cluster_17209		fimbrial family protein
Cluster_17212		fimbrial family protein
Cluster_17213	folK	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase
Cluster_172174		phosphoribosyl transferase domain protein
Cluster_172190		hypothetical protein
Cluster_172200		hypothetical protein
Cluster_172206		ash family protein
Cluster 1723		hypothetical protein
Cluster_172355		plasmid stabilisation system family protein
Cluster_172738		PTS system sugar-specific permease component family protein
Cluster_173349		phage gp6-like head-tail connector family protein
Cluster_173627		hypothetical protein
Cluster 175432		hypothetical protein
Cluster 175571	rpoZ	DNA-directed RNA polymerase, omega subunit
Cluster_175612	IPOZ	type III secretion apparatus protein, Yscl/HrpB, C-terminal domain
Cluster_175616		type III secretion low calcium response chaperone LcrH/SycD
Cluster 176582		phage gp6-like head-tail connector family protein
Cluster_176594		phage baseplate assembly V family protein
-	nyk	
Cluster_178041	pyk	pyruvate kinase
Cluster_178069		kinase/pyrophosphorylase family protein
Cluster_178088		putative membrane protein
Cluster_178120		carboxymuconolactone decarboxylase family protein
Cluster_178247		YecM family protein
Cluster_178285		RNA polymerase sigma factor, sigma-70 family protein
Cluster_179011		terminase small subunit

Cluster_179012 Cluster_179014 hypothetical protein hypothetical protein

 Table S3.
 Predicted protein functions of EIEC clade-specific genes

	Table	e CO. I reducted protein functions of ETEO clade-specific genes
LS-BSR Gene	Gene	Predicted Protein Function
Cluster		
Clade 1		ADO transportant familia prostain
Cluster_171822		ABC transporter family protein
Cluster_171824		transposase family protein
Cluster_171826		colicin pore forming domain protein
Cluster_171827		colicin E1 (microcin) immunity family protein
Cluster_173138		UreE urease accessory , C-terminal domain protein
Cluster_174773		hypothetical protein
Cluster_174373		fimbrial family protein
Cluster_174374		PapC C-terminal domain protein
Cluster_174375		fimbrial family protein
Cluster_174408		ribosome inactivating family protein
Cluster_174512		bacterial transcriptional regulator family protein
Cluster_174580		hypothetical protein
Cluster_174582		gene 9 domain protein
Cluster_17470	ribH	6,7-dimethyl-8-ribityllumazine synthase
Cluster_174762		hypothetical protein
Cluster_176485		methyltransferase domain protein
Cluster_177135		hypothetical protein
Cluster_177152		UbiE/COQ5 methyltransferase family protein
Cluster_17865		glutathione S-transferase, C-terminal domain protein
Cluster_178767		tetratricopeptide repeat family protein
Cluster_178830		fimbrial family protein
Cluster 178850		coA-transferase III family protein
	oxc	oxalyl-CoA decarboxylase
		hypothetical protein
		FGGY-pentulose kinase family protein
		glycosyl transferases group 1 family protein
		HAD hydrolase, IA, variant 1 family protein
Cluster 179214		hypothetical protein
		hypothetical protein
		hypothetical protein
		competence CoiA-like family protein
Cluster 180732		hypothetical protein
Cluster_180737		helix-turn-helix domain protein
Cluster_180738		hypothetical protein
Cluster 180727		hypothetical protein
Cluster 180726		conserved hypothetical protein
Cluster_180680		bacterial DNA-binding family protein
Cluster_181364		RelB antitoxin family protein
Cluster 181369		conserved hypothetical protein
Cluster_181372		hypothetical protein
Cluster_181375		hypothetical protein
Cluster_181376		resolvase, N terminal domain protein
Cluster_181377		conserved hypothetical protein
Cluster_181379		hypothetical protein
Cluster_181380		outer membrane efflux family protein
Cluster_181359		DNA methylase family protein
Cluster_181363		putative membrane protein
Olusici_101303		patative membrane protein

01 1 101055		10440/10440/10000
Cluster_181355		transposase IS116/IS110/IS902 family protein
Cluster_181347		PAS fold family protein
Cluster_183234		SIS domain protein
Cluster_183235	pyk	pyruvate kinase
Cluster_183237		opacity-associated A LysM-like domain protein
Cluster_18297		biofilm development YmgB/AriR family protein
Cluster_182952		helix-turn-helix domain protein
Cluster_184798		putative membrane protein
Cluster_184808		periplasmic binding protein-like domain protein
Cluster_184859		InsA N-terminal domain protein
Cluster_184884		RND transporter, hydrophobe/amphiphile efflux-1 family protein
Cluster_184888		cytochrome c family protein
Cluster_184908		binding-protein-dependent transport system inner membrane component family protein
Cluster_185221		hypothetical protein
Cluster_185250		formate dehydrogenase family accessory protein FdhD
Cluster 185308		glycyl-radical enzyme activating family protein
Cluster_18561		binding-protein-dependent transport system inner membrane component family protein
		HNH endonuclease family protein
		conserved hypothetical family protein
Cluster_18465	pgmB	
Cluster_184656	fmt	methionyl-tRNA formyltransferase
Cluster_184619		ribonuclease, Rne/Rng family domain protein
Cluster_184624		diguanylate cyclase domain protein
Cluster_187913	ubiG	3-demethylubiquinone-9 3-O-methyltransferase
Cluster_187956	abio	NADH dehydrogenase family protein
Cluster_18798		DeoC/LacD aldolase family protein
Cluster_188215		caudovirales tail fiber assembly family protein
Cluster_188216		hypothetical protein
Cluster_187732		polysaccharide pyruvyl transferase family protein
Cluster_191020		polysaccharide biosynthesis family protein
Cluster 191023		
_		hypothetical protein
Cluster_191024		hypothetical protein
Cluster_191027		glycosyl transferase 2 family protein
Cluster_191028		mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase
Cluster_191061		hypothetical protein
Cluster_191062		hypothetical protein
Cluster_191085		hypothetical protein
Cluster_191382		KR domain protein
Cluster_191530		conserved hypothetical protein
Cluster_190944		WGR domain protein
Cluster_190926		outer membrane beta-barrel domain protein
Cluster_193389		DctM-like transporters family protein
Cluster_193410		PapG carbohydrate binding domain protein
Cluster_193450	neuC	UDP-N-acetyl-D-glucosamine 2-epimerase, UDP-hydrolysing
Cluster_193451		GDSL-like Lipase/Acylhydrolase family protein
Cluster_193754		branched-chain amino acid transport system/ permease component family protein
Cluster_193756		periplasmic binding and sugar binding domain of Lacl family protein
Cluster_193912		putative membrane protein
Cluster_194039		hypothetical protein
Cluster_194075		conserved hypothetical protein
Cluster_194123		haemagglutinin family protein
_		

Cluster 193182		KDPG and KHG aldolase family protein
Cluster_197538		hypothetical protein
Cluster_197540		mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase
Cluster_197305		BRO family, N-terminal domain protein
Cluster_197316		hypothetical protein
Cluster 203828		conserved hypothetical protein
Cluster 203846		type II secretion system (T2SS), K family protein
Cluster 203867		hypothetical protein
Cluster_203873		hypothetical protein
		hypothetical protein
Cluster_203905		YcfA-like family protein
Cluster_203927		HMGL-like family protein
		conserved hypothetical protein
Cluster_204129		prokaryotic DksA/TraR C4-type zinc finger family protein
Cluster_204133		putative peptidoglycan domain protein
 Cluster_203574		Major Facilitator Superfamily protein
Cluster_203598		hypothetical protein
Cluster_20672	hisF	imidazoleglycerol phosphate synthase, cyclase subunit
Cluster_206720		acyl-CoA reductase family protein
Cluster_20675	hisB	histidinol-phosphatase
Cluster_206757		hypothetical protein
Cluster_20677	hisD	histidinol dehydrogenase
Cluster_20678	hisG	ATP phosphoribosyltransferase
Cluster_206784		hypothetical protein
Cluster_206797		hypothetical protein
Cluster_207165		Gram-negative porin family protein
Cluster_207229		cobinamide kinase / cobinamide phosphate guanyltransferase family protein
Cluster_207285	gnd	6-phosphogluconate dehydrogenase
Cluster_207289		N-terminal domain of galactosyltransferase family protein
Cluster_207292		3-beta hydroxysteroid dehydrogenase/isomerase family protein
Cluster_207293	rfbC	dTDP-4-dehydrorhamnose 3,5-epimerase
Cluster_207294	rfbB	dTDP-glucose 4,6-dehydratase
Cluster_207343		phage tail tape measure protein, TP901 family, core region
Cluster_207358		P2 phage tail completion R family protein
Cluster_207396		nucleoside transporter family protein
Cluster_20746		prokaryotic cytochrome b561 family protein
Cluster_207650		hypothetical protein
Cluster_207652		conserved hypothetical protein
Cluster_207676		NinF family protein
Cluster_207677		conserved hypothetical protein
Cluster_207681		hypothetical protein
Cluster_207694		hypothetical protein
Cluster_207699		hypothetical protein
Cluster_207748	ptsP	phosphoenolpyruvate-protein phosphotransferase
Cluster_207820	xseA	exodeoxyribonuclease VII, large subunit
Cluster_207827	yfgL in a C	outer membrane assembly lipoprotein YfgL
Cluster_207845	iscS	cysteine desulfurase IscS
Cluster_207922	ffh	signal recognition particle protein
Cluster_207935		phage tail tape measure protein, TP901 family, core region
Cluster_207939		caudovirales tail fibre assembly family protein
Cluster_207945		AIPR family protein

Cluster_207948		phage lysis regulatory, LysB family protein
Cluster 207959		hypothetical protein
Cluster_207961		hypothetical protein
Cluster_207966		conserved hypothetical protein
Cluster 207970		hypothetical protein
_		
Clade 2		
Cluster_171845		hypothetical protein
Cluster_171850		hypothetical protein
Cluster_174308		mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase
Cluster_176267		IstB-like ATP binding family protein
Cluster_176265		TolA C-terminal family protein
Cluster_193388		tripartite ATP-independent periplasmic transporters, DctQ component family protein
Cluster_193436	iutA	ferric aerobactin receptor
Cluster_193448		alpha-2,8-polysialyltransferase family protein
Cluster_197534		putative membrane protein
Cluster_197545		glycosyl transferases group 1 family protein
Cluster_203862		hypothetical protein
Cluster_20700		hypothetical protein
Clade 3		
Cluster_181381		hypothetical protein
Cluster_183353		hypothetical protein
Cluster_188221		hypothetical protein
Cluster_197682		LysR substrate binding domain protein
Cluster_197733		conserved hypothetical protein
Cluster_197619		hypothetical protein
Cluster_197635		response regulator
Cluster_204204		bacterial flagellin C-terminal helical region family protein
Cluster_208196		PGAP1-like family protein
Cluster_208198		hypothetical protein
Cluster_208008		hypothetical protein
Cluster_212088		hypothetical protein
Cluster_212334		conserved hypothetical protein

phage virion morphogenesis protein

Cluster_207946