

***Vibrio cholerae* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 20**

Catalog No. NR-19698

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Contributor:

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Manufacturer:

BEI Resources

Product Description:

Production in the 96-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources does not confirm or validate individual mutants provided by the contributor.

The *Vibrio cholerae* (*V. cholerae*) Gateway® clone set consists of 46 plates which contain 3813 sequence validated clones from *V. cholerae*, strain El Tor N16961 cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector pDONR™221 with a native start codon and stop codon. The library was independently cloned and sequence verified by the Harvard Institute of Proteomics. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway® Clones can be obtained from Invitrogen™. Recombination was facilitated through an *attB* substrate (*attB*-PCR product or a linearized *attB* expression clone) with an *attP* substrate (pDONR™221) to create an *attL*-containing entry clone. The entry clone contains recombinational cloning sites, *attL1* and *attL2* to facilitate gene transfer into a destination vector, M13 forward and reverse priming sites for sequencing and a kanamycin resistance gene for selection. Please refer to the Invitrogen™ Gateway® Technology Manual for additional details.

Plate orientation and viability were confirmed for NR-19698.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

Packaging/Storage:

NR-19698 was packaged aseptically in a 96-well plate. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Media:

LB broth or agar containing 50 µg/mL kanamycin

Incubation:

Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C.

Atmosphere: Aerobic

Propagation:

1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at 37°C for 1 day.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Vibrio cholerae* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 20, NR-19698."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

1. Heidelberg, J. F., et al. "DNA Sequence of both Chromosomes of the Cholera Pathogen *Vibrio cholera*." *Nature* 406 (2000): 477-483. PubMed. 10952301.

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Table 1: *Vibrio cholerae* Gateway® Clones, Plate 20

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
199776	A02	154	VC2653	secB	protein-transport protein SecB	NP_232281.1
199800	A03	206	VC1441	ccoO	cytochrome c oxidase, subunit CcoO	NP_231084.1
199817	A04	602	VC2656	frdA	fumarate reductase, flavoprotein subunit	NP_232284.1
199108	A05	289	VC0091		O-methyltransferase-related protein	NP_229750.1
199127	A06	136	VC0097		flagellar protein FlIL, putative	NP_229756.1
199143	A07	169	VC1481		conserved hypothetical protein	NP_231122.1
199156	A08	N/A	VCA0516	fruA-2	PTS system, fructose-specific IIBC component	N/A
199827	A09	N/A	VCA0940		transcriptional regulator, DeoR family	N/A
199845	A10	312	VC0823		hypothetical protein	NP_230471.1
199886	A11	199	VC2632		fimbrial assembly protein PilO, putative	NP_232260.1
199905	A12	595	VC1405		methyl-accepting chemotaxis protein	NP_231048.1
199764	B01	146	VC0849		conserved hypothetical protein	NP_230496.1
199778	B02	157	VC0842		conserved hypothetical protein	NP_230490.1
199801	B03	477	VC1442	ccoN	cytochrome c oxidase, subunit CcoN	NP_231085.1
199078	B04	223	VC2706		conserved hypothetical protein	NP_232333.1
199112	B05	N/A	VCA0508	orfB	transposase OrfAB, subunit B	N/A
199129	B06	N/A	VCA0506		conserved hypothetical protein	N/A
199144	B07	506	VC2705		sodium-solute symporter, putative	NP_232332.1
199158	B08	581	VC2703		conserved hypothetical protein	NP_232330.1
199829	B09	262	VC2643	argB	acetylglutamate kinase	NP_232271.1
199890	B11	200	VC1408		transcriptional regulator, TetR family	NP_231051.1
199906	B12	224	VC0828	tcpA	toxin co-regulated pilin	NP_230476.1
199765	C01	315	VC1433		conserved hypothetical protein	NP_231076.1
199782	C02	161	VC0848	smpB	small protein B	NP_230495.1
199802	C03	N/A	VCA0980		hypothetical protein	N/A
199084	C04	229	VC2708	gmk	guanylate kinase	NP_232335.1
199115	C05	N/A	VCA0507	orfA	transposase OrfAB, subunit A	N/A
199132	C06	398	VC0080		conserved hypothetical protein	NP_229739.1
199146	C07	513	VC0082		conserved hypothetical protein	NP_229741.1
199159	C08	194	VC1465		hypothetical protein	NP_231108.1
199833	C09	274	VC2593	rplB	ribosomal protein L2	NP_232221.1
199849	C10	N/A	VCA0956		GGDEF family protein	N/A
199893	C11	511	VC1411	vceB	multidrug resistance protein VceB	NP_231054.1
199908	C12	N/A	VCA0952		transcriptional regulator, LuxR family	N/A
199767	D01	326	VC1439	ccoP	cytochrome c oxidase, subunit CcoP	NP_231082.1
199784	D02	N/A	VCA0976		hypothetical protein	N/A
199092	D04	250	VC0088		conserved hypothetical protein	NP_229747.1
199117	D05	117	VC1477	orfA	transposase OrfAB, subunit A	NP_231118.1
199133	D06	153	VC0071	asnC	transcriptional regulator AsnC	NP_229730.1
199149	D07	181	VC0078	ftn	ferritin	NP_229737.1
199162	D08	639	VC1486		ABC transporter, ATP-binding protein	NP_231127.1
199861	D10	397	VC1407	rhIE-1	ATP-dependent RNA helicase RhIE	NP_231050.1
199895	D11	520	VC1402		purine-binding chemotaxis protein Chew, putative	NP_231045.1
199770	E01	151	VC0832	tcpR	toxin co-regulated pilus biosynthesis protein R	NP_230480.1
199788	E02	183	VC2676		cell division protein FtsN, putative	NP_232304.1

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
199093	E04	68	VC2054	ccmD	heme exporter protein D	NP_231686.1
199119	E05	119	VC1479		hypothetical protein	NP_231120.1
199134	E06	N/A	VCA0518	fruB	PTS system, fructose-specific IIA-FPR component	N/A
199150	E07	522	VC1469	cri-1	phage replication protein Cri	NP_231112.1
199163	E08	207	VC0084		conserved hypothetical protein	NP_229743.1
199869	E10	N/A	VCA0945	malE	maltose ABC transporter, periplasmic maltose-binding protein	N/A
198058	E12	915	VC2212		hypothetical protein	NP_231843.1
199771	F01	340	VC2661		conserved hypothetical protein	NP_232289.1
199790	F02	185	VC2675	hslV	protease HslVU, subunit HslV	NP_232303.1
199094	F04	250	VC2055	ccmC	heme exporter protein C	NP_231687.1
199120	F05	N/A	VCA0517	fruK	1-phosphofructokinase	N/A
199136	F06	N/A	VCA0513		amino acid biosynthesis aminotransferase	N/A
199151	F07	N/A	VC1476	tlcR-2	tlcR protein	N/A
199164	F08	651	VC2052	ccmF	cytochrome c-type biogenesis protein CcmF	NP_231684.1
199839	F09	N/A	VCA0943	malG	maltose ABC transporter, permease protein	N/A
199874	F10	N/A	VCA0947	speG	spermidine n1-acetyltransferase	N/A
199899	F11	N/A	VCA0944	malF	maltose ABC transporter, permease protein	N/A
198060	F12	245	VC0575	petC	ubiquinol--cytochrome c reductase, cytochrome c1	NP_230226.1
199772	G01	152	VC0834	tcpS	toxin co-regulated pilus biosynthesis protein S	NP_230482.1
199794	G02	191	VC2662		conserved hypothetical protein	NP_232290.1
199810	G03	N/A	VC0846		integrase, degenerate	N/A
199095	G04	80	VC1487		conserved hypothetical protein	NP_231128.1
199122	G05	N/A	VCA0519	fruR	fructose repressor	N/A
199141	G06	165	VC2717		hypothetical protein	NP_232344.1
199152	G07	N/A	VC2049	ccmH	cytochrome c-type biogenesis protein CcmH, authentic frameshift	N/A
199821	G08	247	VC2598		RNA methyltransferase, TrmH family	NP_232226.1
199841	G09	297	VC2636		transcriptional regulator, LysR family	NP_232264.1
199876	G10	N/A	VCA0955		transcriptional regulator, MarR family	N/A
199900	G11	212	VC1421		conserved hypothetical protein	NP_231064.1
198068	G12	258	VC1034	udp-1	uridine phosphorylase	NP_230679.1
199775	H01	345	VC1424	potD-1	spermidine-putrescine ABC transporter, periplasmic spermidine-putrescine-binding protein	NP_231067.1
199799	H02	467	VC1447	rtxD	RTX toxin transporter	NP_231090.1
199106	H04	286	VC2719		ComF-related protein	NP_232346.1
199126	H05	336	VC1491	pyrD	dihydroorotate dehydrogenase	NP_231132.1
199142	H06	480	VC2712		xanthine-uracil permease family protein	NP_232339.1
199825	H08	247	VC2637		peroxiredoxin family protein-glutaredoxin	NP_232265.1
199881	H10	458	VC2641	argH	argininosuccinate lyase	NP_232269.1
199901	H11	578	VC1413		methyl-accepting chemotaxis protein	NP_231056.1