

***Vibrio cholerae* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 15**

Catalog No. NR-19693

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For research use only. Not for human use.

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 EI 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.

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-19693 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 15, NR-19693.”

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/bmb15/index.htm.

Disclaimers:

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References:

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

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Table 1: *Vibrio cholerae* Gateway® Clones, Plate 15

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
198208	A02	397	VC2190	flgL	flagellar hook-associated protein FlgL	NP_229752.2
198217	A03	N/A	VCA0263		acetyltransferase, putative	NP_229958.1
198229	A04	209	VC1013		RnfG-related protein	NP_230048.1
200646	A05	N/A	VCA0016	glgB	1,4-alpha-glucan branching enzyme	NP_230083.1
200670	A07	697	VC2069	flhA	flagellar biosynthetic protein FlhA	NP_230186.1
200679	A08	708	VC1488		conserved hypothetical protein	NP_230194.1
200703	A10	753	VC0901		hypothetical protein	NP_230198.1
200721	A11	1147	VC0303		sensor histidine kinase	NP_230201.1
200733	A12	1297	VC0869	purL	phosphoribosylformylglycinamide synthase	NP_230208.1
198198	B01	379	VC2188	flaA	flagellin core protein A	NP_230209.1
198209	B02	163	VC1003		bacteriocin production protein	NP_230213.1
198218	B03	441	VC1005		conserved hypothetical protein	NP_230218.1
200647	B05	N/A	VCA0013	malP	maltodextrin phosphorylase	NP_230516.1
200659	B06	849	VC2750		GGDEF family protein	NP_230548.1
200671	B07	869	VC2621		extracellular nuclease-related protein	NP_230584.1
200680	B08	N/A	VCA0011	malT	malT regulatory protein	NP_230648.1
200705	B10	758	VC2077	feoB	ferrous iron transport protein B	NP_230649.1
200723	B11	1163	VC1416	vgrG-1	vgrG protein	NP_230650.1
198894	B12	253	VC1553	glpR	glycerol-3-phosphate regulon repressor	NP_230651.1
198199	C01	145	VC0567		conserved hypothetical protein	NP_230655.1
198210	C02	412	VC0543	recA	recA protein	NP_230656.1
198219	C03	184	VC0562	rimM	16S rRNA processing protein RimM	NP_230657.1
198231	C04	211	VC2181	lolB	outer membrane lipoprotein LolB	NP_230659.1
200649	C05	818	VC1710		conserved hypothetical protein	NP_230661.1
200660	C06	677	VC1521		sensor histidine kinase	NP_230662.1
200672	C07	N/A	VCA1084		toxin secretion ATP-binding protein	NP_230666.1
200681	C08	N/A	VCA0064	hutR	heme receptor HutR	NP_231059.1
200707	C10	761	VC1704	metE	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	NP_231080.1
200724	C11	786	VC1920	lon	ATP-dependent protease LA	NP_231091.1
198895	C12	33	VC1559		hypothetical protein	NP_231129.1
198200	D01	363	VC2185		GTP-binding protein	NP_231135.1
198212	D02	426	VC0558		hemolysin, putative	NP_231161.1
198220	D03	455	VC1021	luxO	LuxO repressor protein	NP_231193.1
198234	D04	597	VC0550	oadA-1	oxaloacetate decarboxylase, alpha subunit	NP_231199.1
200650	D05	666	VC1993	fadH	2,4-dienoyl-CoA reductase	NP_231328.1
200661	D06	854	VC0429		conserved hypothetical protein	NP_231340.1
200674	D07	705	VC2710	spoT	guanosine-3,5-bis(diphosphate) 3-pyrophosphohydrolase	NP_231345.1
200682	D08	922	VC1709		zinc protease, insulinase family	NP_231346.1
200696	D09	N/A	VCA0985		oxidoreductase-iron-sulfur cluster-binding protein	NP_231554.1
200725	D11	N/A	VCA0120		IcmF-related protein	NP_231627.1
198896	D12	257	VC2773		ParA family protein	NP_231667.1
198202	E01	N/A	VCA0270	dacA-2	D-alanyl-D-alanine carboxypeptidase	NP_231701.1

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
198213	E02	N/A	VCA0258		hypothetical protein	NP_231709.1
198221	E03	184	VC1010	gloA	lactoylglutathione lyase	NP_231759.1
198235	E04	213	VC1011	nth	endonuclease III	NP_231812.1
200651	E05	820	VC1692	torA	trimethylamine-N-oxide reductase	NP_231816.2
200662	E06	N/A	VCA1092		methyl-accepting chemotaxis protein	NP_231819.1
200675	E07	876	VC2646	ppc	phosphoenolpyruvate carboxylase	NP_231821.1
200683	E08	N/A	VCA1095	cheA-3	chemotaxis protein CheA	NP_231822.1
200697	E09	N/A	VCA0014	malQ	4-alpha-glucanotransferase	NP_231828.1
200726	E11	N/A	VCA0975		ATP-dependent protease LA-related protein	NP_232227.1
198898	E12	N/A	VCA1100		ABC transporter, permease protein	NP_232249.1
198203	F01	146	VC1009		conserved hypothetical protein	NP_232263.1
198214	F02	434	VC2197	flgE	flagellar hook protein FlgE	NP_232274.2
198223	F03	195	VC1016		RnfB-related protein	NP_232312.1
198236	F04	624	VC2191	flgM	flagellar hook-associated protein FlgM	NP_232337.1
200653	F05	821	VC2599	vacB	ribonuclease R	NP_232376.1
200663	F06	862	VC0535	mutS	DNA mismatch repair protein MutS	NP_232380.1
200676	F07	N/A	VCA0511	nrdD	anaerobic ribonucleoside-triphosphate reductase	NP_232399.1
200685	F08	N/A	VCA1063	speF	ornithine decarboxylase, inducible	N/A
200698	F09	N/A	VCA0510		hypothetical protein	N/A
200716	F10	N/A	VCA1056		methyl-accepting chemotaxis protein	N/A
200729	F11	790	VC1437		cation transport ATPase, E1-E2 family	N/A
198900	F12	N/A	VCA1091	cheR-3	chemotaxis protein methyltransferase CheR	N/A
198204	G01	N/A	VCA0264		transcriptional regulator, putative	N/A
198215	G02	172	VC0557	luxS	autoinducer-2 production protein	N/A
198225	G03	198	VC1002	dedD	dedD protein	N/A
198238	G04	N/A	VCA0268		methyl-accepting chemotaxis protein	N/A
200655	G05	835	VC2635	mrcA	penicillin-binding protein 1A	N/A
200667	G06	868	VC1494	pepN	aminopeptidase N	N/A
200677	G07	894	VC2033	adhE	alcohol dehydrogenase-acetaldehyde dehydrogenase	N/A
200686	G08	940	VC0394	uvrA	excinuclease ABC, subunit A	N/A
200699	G09	737	VC0937		exopolysaccharide biosynthesis protein, putative	N/A
200731	G11	803	VC2684	metL	aspartokinase II-homoserine dehydrogenase, methionine-sensitive	N/A
198903	G12	48	VC2754		hypothetical protein	N/A
198206	H01	395	VC0547		aspartokinase, alpha and beta subunits	N/A
198216	H02	N/A	VCA0278	glyA-2	serine hydroxymethyltransferase	N/A
198226	H03	504	VC1004	purF	amidophosphoribosyltransferase	N/A
200645	H04	808	VC0093	plsB	glycerol-3-phosphate acyltransferase	N/A
200656	H05	674	VC2128		flagellar hook-length control protein FliK, putative	N/A
200668	H06	N/A	VCA0018	vgrG-2	vgrG protein	N/A
200678	H07	N/A	VCA1112		conserved hypothetical protein	N/A
200687	H08	720	VC1448	rtxB	RTX toxin transporter	N/A
200720	H10	801	VC1015		RnfC-related protein	N/A
200732	H11	N/A	VCA0987	ppsA	phosphoenolpyruvate synthase	N/A