

***Vibrio cholerae* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 2**

Catalog No. NR-19680

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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-19680.

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-19680 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 2, NR-19680.”

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 2

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
175200	A02	317	VC2380		cobalamin biosynthesis protein CbiB, putative	NP_232010.1
175313	A03	196	VC1894		conserved hypothetical protein	NP_231528.1
175325	A04	402	VC1205	hutI	imidazolonepropionase	NP_230850.1
175432	A05	261	VC0780	vibD	vibriobactin synthase component D	NP_230429.1
175260	A06	160	VC2388		hypothetical protein	NP_232018.1
175455	A07	269	VC2391	dapB	dihydrodipicolinate reductase	NP_232021.1
175645	A08	403	VC1200		trypsin, putative	NP_230845.1
175739	A09	197	VC0770		conserved hypothetical protein	NP_230419.1
175664	A10	144	VC2378		conserved hypothetical protein	NP_232008.1
175676	A11	437	VC0736	aceA	isocitrate lyase	NP_230385.1
175777	A12	223	VC1195		lipoprotein, putative	NP_230840.1
175281	B01	173	VC1902	dsbB	disulfide bond formation protein B	NP_231536.1
175204	B02	116	VC1897		Hit family protein	NP_231531.1
175408	B03	244	VC0746		RNA methyltransferase, TrmH family	NP_230395.1
175329	B04	204	VC0761		conserved hypothetical protein	NP_230410.1
175152	B05	288	VC0745		inositol monophosphate family protein	NP_230394.1
175349	B06	N/A	VCA0749	gipC	anaerobic glycerol-3-phosphate dehydrogenase, subunit C	NA
175176	B07	315	VC1207		hypothetical protein	NP_230852.1
175731	B08	N/A	VCA0769		conserved hypothetical protein	NA
175466	B09	N/A	VCA0767		transcriptional regulator, TetR family	NA
175754	B10	214	VC1213		transcriptional regulator, LuxR family	NP_230858.1
175680	B11	155	VC1199		conserved hypothetical protein	NP_230844.1
175508	B12	39	VC2372		hypothetical protein	NP_232002.1
175372	C01	429	VC0755	pepB	peptidase B	NP_230404.2
175297	C02	N/A	VCA0755		hypothetical protein	NA
175228	C03	132	VC2392	mutT	mutator MutT protein	NP_232022.1
175424	C04	261	VC2384		conserved hypothetical protein	NP_232014.1
175248	C05	358	VC0778	viuG	vibriobactin and enterobactin ABC transporter, permease protein	NP_230427.1
175353	C06	N/A	VCA0757	artM	arginine ABC transporter, permease protein	NA
175180	C07	106	VC1208		conserved hypothetical protein	NP_230853.1
175548	C08	N/A	VCA0759	artI	arginine ABC transporter, periplasmic arginine-binding protein	NA
175569	C09	N/A	VCA0765	ybjU	L-allo-threonine aldolase	NA
175482	C10	254	VC1186	sanA	sanA protein	NP_230831.1
175769	C11	219	VC1183		hypothetical protein	NP_230828.1
175606	C12	372	VC1216		GGDEF family protein	NP_230861.1
175376	D01	231	VC2379	pfs	MTA-SAH nucleosidase	NP_232009.1
175392	D02	239	VC2382		conserved hypothetical protein	NP_232012.1
175317	D03	N/A	VCA0772	tyrP	tyrosine-specific transport protein	NA
175148	D04	N/A	VCA0746		hypothetical protein	NA
175252	D05	157	VC2395		hypothetical protein	NP_232025.1
175168	D06	308	VC0781		transcriptional regulator, LysR family	NP_230430.1
175272	D07	376	VC0759	gcpE	gcpE protein	NP_230408.1
175561	D08	327	VC1219	pheS	phenylalanyl-tRNA synthetase, alpha chain	NP_230864.1

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
175658	D09	N/A	VCA0738		hypothetical protein	NA
175583	D10	352	VC0739	queA	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	NP_230388.1
175496	D11	260	VC2399	ftsQ	cell division protein FtsQ	NP_232029.1
175610	D12	N/A	VCA0731		hypothetical protein	NA
175096	E01	N/A	VCA0754		lipase-related protein	NA
175208	E02	N/A	VCA0756		transcriptional regulator, LysR family	NA
175321	E03	188	VC1209		elongation factor P family protein	NP_230854.2
175240	E04	350	VC0777	viuD	vibriobactin and enterobactin ABC transporter, permease protein	NP_230426.1
175341	E05	404	VC0748		aminotransferase NifS, class V	NP_230397.1
175172	E06	N/A	VCA0743		conserved hypothetical protein	NA
175365	E07	438	VC0775	vibH	vibriobactin synthetase, amide synthase subunit VibH	NP_230424.1
175565	E08	77	VC0765		conserved hypothetical protein	NP_230414.1
175747	E09	207	VC0731		antioxidant, AhpC-Tsa family	NP_230380.1
175668	E10	N/A	VCA0768		ATP-dependent RNA helicase, DEAD box family	NA
175599	E11	N/A	VCA0737	luxP	luxP protein	NA
175692	E12	443	VC1185		GGDEF family protein	NP_230830.1
175192	F01	316	VC2409		conserved hypothetical protein	NP_232039.1
175212	F02	127	VC0749		NifU-related protein	NP_230398.1
175416	F03	256	VC2415	pdhR	pyruvate dehydrogenase complex repressor	NP_232045.1
175244	F04	N/A	VCA0752	trxC	thioredoxin 2	NA
175345	F05	N/A	VCA0740		conserved hypothetical protein	NA
175264	F06	374	VC1905	ald	alanine dehydrogenase	NP_231539.1
175368	F07	N/A	VCA0758	artQ	arginine ABC transporter, permease protein	NA
175651	F08	405	VC1190		phosphoribosylaminoimidazole-succinocarboxamide synthase, putative	NP_230835.1
175474	F09	N/A	VCA0760	artP	arginine ABC transporter, ATP-binding protein	NA
175672	F10	N/A	VCA0766		cytochrome c554	NA
175602	F11	N/A	VCA0739		conserved hypothetical protein	NA
175694	F12	162	VC2366	menG-1	s-adenosylmethionine:2-demethylmenaquinone methyltransferase	NP_231996.1
175285	G01	383	VC1899		hypothetical protein	NP_231533.1
175305	G02	188	VC0747		conserved hypothetical protein	NP_230396.1
175136	G03	286	VC1893		conserved hypothetical protein	NP_231527.1
175333	G04	N/A	VCA0751	fabH-2	3-oxoacyl-(acyl-carrier-protein) synthase III	NA
175160	G05	305	VC2396	envA	UDP-3-O-3-hydroxymyristoyl N-acetylglucosamine deacetylase	NP_232026.1
175268	G06	164	VC1904	lrp	leucine-responsive regulatory protein	NP_231538.1
175544	G07	N/A	VCA0761		hypothetical protein	NA
175654	G08	132	VC1191		hypothetical protein	NP_230836.1
175575	G09	350	VC1194		conserved hypothetical protein	NP_230839.1
175761	G10	218	VC1226		thiopurine methyltransferase	NP_230871.1
175684	G11	444	VC2403	murD	UDP-N-acetylmuramoylalanine--D-glutamate ligase	NP_232033.2
175524	G12	44	VC1221		hypothetical protein	NP_230866.1
175289	H01	179	VC1892		conserved hypothetical protein	NP_231526.1
175309	H02	398	VC1906		hypothetical protein	NP_231540.1
175236	H03	139	VC0753		ferredoxin	NP_230402.1
175337	H04	205	VC2387		conserved hypothetical protein	NP_232017.1
175256	H05	373	VC0757		conserved hypothetical protein	NP_230406.1
175357	H06	N/A	VCA0748	glpB	anaerobic glycerol-3-phosphate dehydrogenase, subunit B	NA
175553	H07	316	VC2371		conserved hypothetical protein	NP_232001.1
175735	H08	489	VC2374	gltD-1	glutamate synthase, small subunit	NP_232004.1
175663	H09	416	VC1184		NifS-related protein	NP_230829.1
175595	H10	98	VC1222	himA	integration host factor, alpha subunit	NP_230867.1
175688	H11	161	VC1217		conserved hypothetical protein	NP_230862.1