

***Vibrio cholerae* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 3**

**Catalog No. NR-19681**

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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.

Plate orientation and viability were confirmed for NR-19681.

**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-19681 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 3, NR-19681.”

**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](http://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 cholerae*." *Nature* 406 (2000): 477-483. PubMed: 10952301.

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**Table 1: *Vibrio cholerae* Gateway® Clones, Plate 3**

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
175633	A02	122	VC1187		hypothetical protein	NP_230832.1
175896	A03	266	VC1887		hypothetical protein	NP_231521.1
175816	A04	239	VC0129		conserved hypothetical protein	NP_229787.1
176008	A05	426	VC2362	thrC	threonine synthase	NP_231992.1
176099	A06	516	VC1874		conserved hypothetical protein	NP_231508.1
175848	A07	255	VC0103		conserved hypothetical protein	NP_229762.1
175956	A08	N/A	VCA0819	groES-2	chaperonin, 10 Kd subunit	N/A
176137	A09	228	VC1883		ABC transporter, ATP-binding protein	NP_231517.1
175983	A10	406	VC1884		conserved hypothetical protein	NP_231518.1
197509	A11	259	VC2350	deoC	deoxyribose-phosphate aldolase	NP_231980.1
197521	A12	334	VC1845	ruvB	Holliday junction DNA helicase RuvB	NP_231479.1
175621	B01	386	VC0762		conserved hypothetical protein	NP_230411.1
175716	B02	N/A	VCA0764		MutT-nudix family protein	N/A
175992	B03	122	VC0142		hypothetical protein	NP_229794.1
175912	B04	275	VC0134		conserved hypothetical protein	NP_229792.1
176011	B05	N/A	VCA0823	ectC	ectoine synthase	N/A
176101	B06	205	VC0136		conserved hypothetical protein	NP_229794.1
175944	B07	318	VC2363	thrB	homoserine kinase	NP_231993.1
176036	B08	440	VC2452		TrmA family RNA methyltransferase, putative	NP_232081.1
175880	B09	260	VC1863		amino acid ABC transporter, periplasmic amino acid-binding protein	NP_231497.1
175987	B10	N/A	VCA0827	phhB	pterin-4-alpha-carbinolamine dehydratase	N/A
197511	B11	268	VC1860	xth	exodeoxyribonuclease III	NP_231494.1
197522	B12	N/A	VCA0721		hypothetical protein	N/A
175625	C01	110	VC0742		conserved hypothetical protein	NP_230391.1
175800	C02	238	VC2368	fexA	aerobic respiration control protein FexA	NP_231998.1
176071	C03	182	VC0114		conserved hypothetical protein	NP_229773.1
176001	C04	423	VC1873		conserved hypothetical protein	NP_231507.1
176091	C05	514	VC0132		hypothetical protein	NP_229790.1
175840	C06	254	VC0730	cutC	copper homeostasis protein	NP_230379.1
175948	C07	71	VC0101		hypothetical protein	NP_229760.1
176040	C08	163	VC0115		conserved hypothetical protein	NP_229774.1
175884	C09	45	VC1881		hypothetical protein	NP_231515.1
176062	C10	N/A	VCA0812		leucine aminopeptidase-related protein	N/A
197513	C11	279	VC1825		transcriptional regulator	NP_231459.1
197524	C12	105	VC1841		conserved hypothetical protein	NP_231475.1
175706	D01	453	VC2405	murF	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-alanyl-D-alanyl ligase	NP_232035.1
175540	D02	56	VC1227		hypothetical protein	NP_230872.1
175808	D03	236	VC0727	phoU	phosphate transport system regulatory protein PhoU	NP_230376.1
176004	D04	136	VC2455		hypothetical protein	NP_232084.1
176095	D05	198	VC0131		conserved hypothetical protein	NP_229789.1
175936	D06	311	VC0128	xerC	integrase-recombinase XerC	NP_229786.1
176028	D07	434	VC1890	ndh	NADH dehydrogenase	NP_231524.1
176125	D08	222	VC0113		methyltransferase-related protein	NP_229772.1

Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
175979	D09	109	VC0140		conserved hypothetical protein	NP_229793.1
176065	D10	178	VC1889		ribosomal-protein-serine acetyltransferase, putative	NP_231523.1
197515	D11	283	VC2353		conserved hypothetical protein	NP_231983.1
197525	D12	N/A	VCA0710	torT	periplasmic protein TorT	N/A
175709	E01	N/A	VCA0734		hypothetical protein	N/A
175637	E02	395	VC0773	vibC	vibriobactin-specific isochorismate synthase	NP_230422.1
175904	E03	273	VC0726	pstB-1	phosphate ABC transporter, ATP-binding protein	NP_230375.1
176083	E04	N/A	VCA0813		aminopeptidase	N/A
175832	E05	252	VC1875	kdsB	3-deoxy-manno-octulosonate cytidyltransferase	NP_231509.1
175940	E06	N/A	VC0124	lppL	lipopeptide	N/A
176032	E07	160	VC2365		hypothetical protein	NP_231995.1
176131	E08	223	VC0112	cycA	cytochrome c4	NP_229771.1
176054	E09	N/A	VCA0822		aspartokinase, putative	N/A
197497	E10	251	VC1245		vitamin B12 ABC transporter, ATP-binding protein BtuD, putative	NP_230890.1
197517	E11	N/A	VCA0719		sensor histidine kinase	N/A
197528	E12	N/A	VCA0714	dgkA	diacylglycerol kinase	N/A
175528	F01	301	VC0732		transcriptional regulator, LysR family	NP_230381.1
175641	F02	127	VC2367		hypothetical protein	NP_231997.1
175997	F03	125	VC2361		formate acetyl transferase-related protein	NP_231991.1
176087	F04	N/A	VCA0817		hypothetical protein	N/A
175928	F05	N/A	VCA0814	speB	agmatinase	N/A
176024	F06	156	VC0139		DPS family protein	NP_229792.1
176117	F07	220	VC1870		hypothetical protein	NP_231504.1
175968	F08	389	VC0117	hemY	hemY protein	NP_229776.1
176058	F09	176	VC1880		conserved hypothetical protein	NP_231514.1
197503	F10	257	VC2355		conserved hypothetical protein	NP_231985.1
197518	F11	N/A	VCA0722		hypothetical protein	N/A
197529	F12	356	VC1837	tolA	tolA protein	NP_231471.1
175532	G01	N/A	VCA0733		hypothetical protein	N/A
175720	G02	N/A	VCA0762		conserved hypothetical protein	N/A
176075	G03	N/A	VCA0811		chitinase, putative	N/A
175824	G04	246	VC1869	pflA	pyruvate formate-lyase 1 activating enzyme	NP_231503.1
176015	G05	430	VC2454		GGDEF family protein	NP_232083.1
176105	G06	517	VC1865		hypothetical protein	NP_231499.1
175856	G07	256	VC1864		amino acid ABC transporter, ATP-binding protein	NP_231498.1
175972	G08	106	VC0100	glpE	glpE protein	NP_229759.1
176143	G09	232	VC0127		conserved hypothetical protein	NP_229785.1
197505	G10	259	VC1856		conserved hypothetical protein	NP_231490.1
197519	G11	328	VC2345	serB	phosphoserine phosphatase	NP_231975.1
197530	G12	139	VC1812		conserved hypothetical protein	NP_231446.1
175629	H01	393	VC1228	purT	phosphoribosylglycinamide formyltransferase 2	NP_230873.1
175724	H02	185	VC1215	pgsA	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	NP_230860.1
176079	H03	191	VC1885		hypothetical protein	NP_231519.1
175920	H04	N/A	VCA0828	phhA	phenylalanine-4-hydroxylase	N/A
176016	H05	143	VC0106		conserved hypothetical protein	NP_229765.1
176109	H06	220	VC0111		conserved hypothetical protein	NP_229770.1
175952	H07	338	VC0135	lypA	lysophospholipase L2	NP_229793.1
176050	H08	N/A	VCA0825	ectA	L-2,4-diaminobutyric acid acetyltransferase	N/A
175888	H09	260	VC1862		amino acid ABC transporter, permease protein	NP_231496.1
197507	H10	259	VC1824		PTS system, nitrogen regulatory IIA component, putative	NP_231458.1
197520	H11	99	VC1823	frwB	PTS system, fructose-specific IIB component	NP_231457.1