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SUPPORTING INFECTIOUS DISEASE RESEARCH

Vibrio cholerae Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 43

Catalog No. NR-19721

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Contributor:

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

Manufacturer:

BEI Resources

Product Description:

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 <u>pDONR™221</u> 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 <u>Invitrogen</u>[™]. Recombination was facilitated through an *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONR[™]221) to create an *att*L-containing entry clone. The entry clone contains recombinational cloning sites, *att*L1 and *att*L2 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[™] <u>Gateway[®] Technology Manual</u> for additional details.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 40 μ L of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) Broth containing 50 μ g/mL kanamycin supplemented with 15% glycerol.

<u>Note:</u> 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 cannot confirm or validate any clone not identified on the plate information table.

Packaging/Storage:

NR-19721 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 17 to 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources: *Vibrio cholerae* Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 43, NR-19721."

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. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 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:

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

 $\mathsf{ATCC}^{\circledast}$ is a trademark of the American Type Culture Collection.



Table 1:	Vibrio cholerae Gatewa	v [®] Clones, Plate 43
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Clone	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
		_0.19.11			s-adenosylmethionine:2-demethylmenaquinone	
234636	A02	171	VC2672	menG-2	methyltransferase	NP_232300.1
234655	A03	205	VC2666		conserved hypothetical protein	NP_232294.1
234674	A04	56	VC2652		conserved hypothetical protein	NP_232280.1
234698	A05	N/A	VCA0492		hypothetical protein, interruption	N/A
234250	A06	N/A	VCA0626		hypothetical protein	N/A
234270	A07	36	VC2313		hypothetical protein	NP_231944.1
234286	A08	N/A	VCA0162		hypothetical protein	N/A
234306	A09	N/A	VCA0669		sugar transporter family protein	N/A
234334	A10	470	VC1767		conserved hypothetical protein	NP_231402.1
236345	A11	204	VC1302		MutT-nudix family protein	NP_230947.1
236365	A12	224	VC1741		transcriptional regulator, TetR family	NP_231377.1
234618	B01	31	VC0387		hypothetical protein	NP_230041.1
234638	B02	41	VC0053		hypothetical protein	NP_229712.1
234657	B03	N/A	VCA0427		hypothetical protein	N/A
234678	B04	56	VC0382		hypothetical protein	NP_230036.1
234699	B05	253	VC0386	cysH	phosphoadenosine phosphosulfate reductase	NP_230040.1
234251	B06	319	VC0228		hypothetical protein	NP 229885.1
234271	B07	346	VC2226	purM	phosphoribosylformylglycinamidine cyclo-ligase	NP 231857.1
234290	B08	72	VC1737	infA	initiation factor IF-1	NP 231373.1
234307	B09	130	VC2659	frdD	fumarate reductase, 13 kDa hydrophobic protein	NP_232287.1
234336	B10	195	VC2720		conserved hypothetical protein	NP_232347.1
236349	B11	N/A	VCA1059		conserved hypothetical protein	N/A
236366	B12	553	VC1516		iron-sulfur cluster-binding protein	NP_231156.1
234619	C01	N/A	VCA0453		hypothetical protein	N/A
234640	C02	N/A	VC1470	tlcR-1	tlcR protein	N/A
234662	C03	N/A	VCA0467		hypothetical protein	N/A
234682	C04	N/A	VCA0973		hypothetical protein	N/A
234701	C05	87	VC2640		hypothetical protein	NP_232268.1
234253	C06	36	VC0915		hypothetical protein	NP_230562.1
234274	C07	N/A	VCA0660		hypothetical protein	N/A
234291	C08	379	VC2247	IpxB	lipid-A-disaccharide synthase	NP_231878.1
234312	C09	N/A	VCA0838	•	conserved hypothetical protein	N/A
236331	C10	N/A	VCA0666	sdaA-2	L-serine dehydratase 1	N/A
236350	C11	484	VC1046	hadHB	fatty oxidation complex, beta subunit	NP_230691.1
236369	C12	N/A	VCA0135		cyclic nucleotide binding protein, putative	N/A
234622	D01	N/A	VCA0500		hypothetical protein	N/A
234644	D02	184	VC0058		carbonic anhydrase, family 3	NP_229717.1
234663	D03	219	VC1431		hypothetical protein	NP_231074.1
234684	D04	242	VC0333		transcriptional reguator, TetR family	NP_229987.1
234704	D05	256	VC1426	potC	spermidine-putrescine ABC transporter, permease protein	NP_231069.1

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Clone	Well	ORF	Locus ID	Symbol	Product	Accession
	Position	Length	V/00240	h in A	hin A hifunction of protoin	
234255	D06	320	VC0319	AIID	birA birunctional protein	NP_229973.1
234270	D07	N/A	VCA0925	pyrc oitD		
234294	D08	98	VC0797	CILD	citrate iyase, gamma subunit	NP_230446.1
234313	D09	440 N/A	VC2430		Dom X related protein	NP_232065.1
230332	D10	N/A	VCA0573		DamA-related protein	IN/A
230300	D11	N/A	VCA0596			IN/A
230375	D12	159	VC40644		hypethetical protein	IN/A
234024	E01	100	VC1436	ofe	nypolinetical protein	NP_231001.1
234040	E02	100	VC2660	eip		NP_232200.1
234666	E03	54	VC1440	CCOQ	cytochrome c oxidase, subunit CcoQ	NP_231083.1
234686	E04	N/A	VCA0494		nypothetical protein, interruption	
234705	E05	89	VC0383	h 4D	http://	NP_230037.1
234259	E06	316	VC0685	IYtB	IVE protein	NP_230334.2
234277	E07	63	VC0205	la una la A		NP_229862.1
234295	E08	N/A	VCA0183	nmpA	terrisiderophore reductase	N/A
234316	E09	162	VC0220		nypotnetical protein	NP_229877.1
236334	E10	N/A	VCA1067		aldenyde denydrogenase	N/A
236357	E11	218	VC1515		chaperone, formate dehydrogenase-specific, putative	NP_231155.1
236377	E12	N/A	VCA0600		repressor protein PhnR, putative	N/A
234628	F01	161	VC0052	purE	phosphoribosylaminoimidazole carboxylase, catalytic subunit	NP_229711.1
234650	F02	N/A	VCA0471		hypothetical protein	N/A
234668	F03	224	VC1435		conserved hypothetical protein	NP_231078.1
234690	F04	74	VC1452	rstC	RstC protein	NP_231095.1
234708	F05	256	VC0065	thiG	thiG protein	NP_229724.1
234261	F06	N/A	VCA0143		hypothetical protein	N/A
234280	F07	351	VC2250	lpxD	UDP-3-O-3-hydroxymyristoyl glucosamine N- acyltransferase	NP_231881.1
234300	F08	390	VC0437		GTP1-Obg family protein	NP_230091.2
234324	F09	177	VC0585	hpt	hypoxanthine phosphoribosyltransferase	NP_230235.1
236337	F10	196	VC2184	pth	peptidyl-tRNA hydrolase	NP_231815.1
236359	F11	512	VC2406	murE	UDP-N-acetylmuramoylalanyl-D-glutamate2,6- diaminopimelate ligase	NP_232036.1
236379	F12	582	VC1878	msbA	transport ATP-binding protein MsbA	NP_231512.1
234629	G01	38	VC1412		hypothetical protein	NP_231055.1
234652	G02	200	VC0854	grpE	heat shock protein GrpE	NP_230501.1
234670	G03	55	VC0038		hypothetical protein	NP_229697.1
234692	G04	244	VC1423		hypothetical protein	NP_231066.1
234246	G05	32	VC0096		hypothetical protein	NP_229755.1
234264	G06	332	VC1929	dctP-2	C4-dicarboxylate-binding periplasmic protein	NP_231563.1
234282	G07	66	VC1020		hypothetical protein	NP_230665.1
234302	G08	N/A	VCA0410		hypothetical protein	N/A
234330	G09	462	VC2295	nqrA	NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit	NP_231926.1
236339	G10	481	VC2037	yqkl	Na+-H+ antiporter	NP_231671.1
236361	G11	219	VC1038	udk	uridine kinase	NP_230683.1
236381	G12	244	VC0888		pseudouridine synthase Rlu family protein	NP_230535.1
234633	H01	41	VC2010		hypothetical protein	NP_231644.1
234654	H02	N/A	VCA0959		hypothetical protein	N/A
234672	H03	N/A	VCA0496		glutathione S-transferase, putative	N/A
234696	H04	252	VC2657	frdB	fumarate reductase, iron-sulfur protein	NP_232285.1
234248	H05	306	VC2042		histone deacetylase-AcuC-AphA family protein	NP_231676.1
234268	H06	339	VC0521	gcp	O-sialoglycoprotein endopeptidase, authentic frameshift	NP_230172.2

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Clone ID	Well Position	ORF Length	Locus ID	Symbol	Product	Accession Number
234284	H07	356	VC1773		conserved hypothetical protein	NP_231408.1
234304	H08	121	VC0327	rpIL	ribosomal protein L7-L12	NP_229981.1
234332	H09	191	VC1309	rimJ	ribosomal-protein-alanine acetyltransferase	NP_230953.1
236343	H10	484	VC2164		conserved hypothetical protein	NP_231795.1
236363	H11	523	VC0995	nagE	PTS system, N-acetylglucosamine-specific IIABC component	NP_230641.1