

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 12**

**Catalog No. NR-19736**

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**Contributor:**

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

**Manufacturer:**

BEI Resources

**Product Description:**

Clone plates are replicated using a BioMek® FX robot. 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 only confirms the clone plate orientation and viability of randomly picked clones. BEI Resources does not confirm or validate individual clone identities provided by the contributor.

The *Bacillus anthracis* (*B. anthracis*) Gateway® clone set consists of 58 plates which contain 5341 sequence validated clones from *B. anthracis*, strains Ames (5139 clones), Sterne (107 clones; contains plasmid pXO1 only) and A2012 (95 clones; contains plasmid pXO2 only) cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector pDONR™221 (Invitrogen™) with an ATG start codon and no 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 a Harvard-modified *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-19736.

**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-19736 was packaged aseptically in 96-well plates. 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 containing 50 µg/mL kanamycin

LB agar containing 50 µg/mL kanamycin

Incubation:

Temperature: 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 24 hours.

**Citation:**

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 12, NR-19736."

**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](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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**References:**

1. Read, T. D., et al. "The Genome Sequence of *Bacillus anthracis* Ames and Comparison to Closely Related Bacteria." *Nature* 423 (2003): 81-86. PubMed: 12721629.
2. Read, T. D., et al. "Comparative Genome Sequencing for Discovery of Novel Polymorphisms in *Bacillus anthracis*." *Science* 296 (2002): 2028-2033. PubMed: 12004073.

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**Table 1: *Bacillus anthracis*, Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 12 (QMG002853)<sup>1</sup>**

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
48872	A02	BA1872	lipoprotein, putative	Chromosome (NC_003997)	NP_844288.1	246211
51319	A03	BA0529	lipoprotein, putative	Chromosome (NC_003997)	NP_843064.1	246220
49490	A04	BA0527	hypothetical protein	Chromosome (NC_003997)	NP_843062.1	246231
51733	A05	BA0531	glutamate-1-semialdehyde-2,1-aminomutase	Chromosome (NC_003997)	NP_843066.1	246248
47872	A06	BA1407	hypothetical protein	Chromosome (NC_003997)	NP_843864.1	241889
47933	A07	BA1400	hypothetical protein	Chromosome (NC_003997)	NP_843857.1	241897
50499	A08	BA3748	phosphonate ABC transporter, permease protein	Chromosome (NC_003997)	NP_846009.1	241912
50836	A09	BA1397	aminoglycoside phosphotransferase family protein	Chromosome (NC_003997)	NP_843854.1	241922
51169	A10	BA1378	transcriptional regulator, AbrB family	Chromosome (NC_003997)	NP_843836.1	241934
48946	A11	BA1383	hypothetical protein	Chromosome (NC_003997)	NP_843841.1	241943
51634	A12	BA3775	hypothetical protein	Chromosome (NC_003997)	NP_846031.1	241954
48576	B01	BA0517	hypothetical protein	Chromosome (NC_003997)	NP_843053.1	246203
51072	B02	BA4759	electron transfer flavoprotein, alpha subunit	Chromosome (NC_003997)	NP_846965.1	246212
49162	B03	BA4751	4-hydroxybenzoyl-CoA thioesterase, putative	Chromosome (NC_003997)	NP_846958.1	246221
51507	B04	BA1893	nucleoside transporter NupC	Chromosome (NC_003997)	NP_844304.1	246232
50040	B05	BA1892	deoxyribose-phosphate aldolase	Chromosome (NC_003997)	NP_844303.1	246249
49912	B06	BA1425	ATP phosphoribosyltransferase	Chromosome (NC_003997)	NP_843882.1	241890
50216	B07	BA1429	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	Chromosome (NC_003997)	NP_843886.1	241898
48265	B08	BA1415	hypothetical protein	Chromosome (NC_003997)	NP_843872.1	241913
48451	B09	BA3782	hypothetical protein	Chromosome (NC_003997)	NP_846038.1	241923
48654	B10	BA3757	hypothetical protein	Chromosome (NC_003997)	NP_846017.1	241935
49152	B11	BA1394	flavodoxin	Chromosome (NC_003997)	NP_843851.2	241945
49175	B12	BA1380	transcriptional regulator, AsnC family	Chromosome (NC_003997)	NP_843838.1	241955
50964	C01	BA1884	2-dehydropantoate 2-reductase, putative	Chromosome (NC_003997)	NP_844298.1	246204
48934	C02	BA1895	cytidine deaminase	Chromosome (NC_003997)	NP_844306.1	246213

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
49172	C03	BA4756	hypothetical protein	Chromosome (NC_003997)	NP_846962.1	246223
49558	C04	BA4738	membrane protein, putative	Chromosome (NC_003997)	NP_846947.1	246233
50207	C05	BA1856	membrane protein, putative	Chromosome (NC_003997)	NP_844273.1	246253
47890	C06	BA3776	hypothetical protein	Chromosome (NC_003997)	NP_846032.1	241891
47946	C07	BA1393	hypothetical protein	Chromosome (NC_003997)	NP_843850.1	241899
48289	C08	BA3752	hypothetical protein	Chromosome (NC_003997)	NP_846012.1	241915
48495	C09	BA3783	hypothetical protein	Chromosome (NC_003997)	NP_846039.1	241925
51176	C10	BA1432	histidinol phosphatase, putative	Chromosome (NC_003997)	NP_843888.1	241936
51470	C11	BA3784	phage major capsid protein, putative	Chromosome (NC_003997)	NP_846040.1	241946
51652	C12	BA1424	histidyl-tRNA synthetase, putative	Chromosome (NC_003997)	NP_843881.1	241956
48597	D01	BA4758	thioredoxin	Chromosome (NC_003997)	NP_846964.1	246205
48964	D02	BA0535	potassium channel protein, putative	Chromosome (NC_003997)	NP_843070.1	246215
51351	D03	BA1855	hypothetical protein	Chromosome (NC_003997)	NP_844272.1	246224
49665	D04	BA4740	DNA-binding protein	Chromosome (NC_003997)	NP_846949.1	246235
50238	D05	BA4239	hypothetical protein	Chromosome (NC_003997)	NP_846474.1	246255
49988	D06	BA3753	site-specific recombinase, resolvase family	Chromosome (NC_003997)	NP_846013.1	241892
50312	D07	BA1395	hypothetical protein	Chromosome (NC_003997)	NP_843852.1	241900
48290	D08	BA3764	hypothetical protein	Chromosome (NC_003997)	NP_846023.1	241917
48515	D09	BA3718	hypothetical protein	Chromosome (NC_003997)	NP_845981.1	241927
48769	D10	BA3780	hypothetical protein	Chromosome (NC_003997)	NP_846036.1	241937
51476	D11	BA1388	ditB protein	Chromosome (NC_003997)	NP_843846.1	241948
49409	D12	BA1418	acetolactate synthase, small subunit	Chromosome (NC_003997)	NP_843875.1	241961
48669	E01	BA4248	transcriptional regulator, PadR family	Chromosome (NC_003997)	NP_846483.1	246207
51168	E02	BA0532	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_843067.1	246216
49185	E03	BA0536	bacterioferritin comigratory protein	Chromosome (NC_003997)	NP_843071.1	246225
49844	E04	BA4243	hypothetical protein	Chromosome (NC_003997)	NP_846478.1	246237
47736	E05	BA3762	hypothetical protein	Chromosome (NC_003997)	NP_846021.1	241881
47885	E06	BA1396	hypothetical protein	Chromosome (NC_003997)	NP_843853.1	241893
47981	E07	BA1379	hypothetical protein	Chromosome (NC_003997)	NP_843837.1	241901
50835	E08	BA1376	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_843834.2	241918
50950	E09	BA3750	phosphonate ABC transporter, phosphonate-binding protein, putat	Chromosome (NC_003997)	NP_846011.1	241928
51285	E10	BA1421	3-isopropylmalate dehydrogenase	Chromosome (NC_003997)	NP_843878.1	241938
49067	E11	BA1381	hypothetical protein	Chromosome (NC_003997)	NP_843839.1	241949
49541	E12	BA3756	hypothetical protein	Chromosome (NC_003997)	NP_846016.1	241963
51010	F01	BA1881	rhodanese-like domain protein	Chromosome (NC_003997)	NP_844296.1	246208
49081	F02	BA4743	rrf2 family protein	Chromosome (NC_003997)	NP_846952.1	246217
49326	F03	BA4241	mutT/nudix family protein	Chromosome (NC_003997)	NP_846476.1	246227
49875	F04	BA4755	succinate dehydrogenase, cytochrome b558 subunit	Chromosome (NC_003997)	NP_846961.1	246241
49725	F05	BA3785	prophage LambdaBa01, prohead protease, putative	Chromosome (NC_003997)	NP_846041.1	241882

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
50133	F06	BA1374	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_843832.1	241894
50486	F07	BA1403	bacitracin resistance protein	Chromosome (NC_003997)	NP_843860.2	241904
48281	F08	BA1387	D-alanyl carrier protein	Chromosome (NC_003997)	NP_843845.1	241919
48530	F09	BA3781	hypothetical protein	Chromosome (NC_003997)	NP_846037.1	241929
48844	F10	BA3777	hypothetical protein	Chromosome (NC_003997)	NP_846033.1	241939
51480	F11	BA1392	N-acyl-L-amino acid amidohydrolase	Chromosome (NC_003997)	NP_843849.1	241950
51862	F12	BA1422	3-isopropylmalate dehydratase, large subunit	Chromosome (NC_003997)	NP_843879.1	241964
48785	G01	BA1864	hypothetical protein	Chromosome (NC_003997)	NP_844281.1	246209
51321	G02	BA1866	hypothetical protein	Chromosome (NC_003997)	NP_844283.1	246218
51463	G03	BA1887	enterotoxin	Chromosome (NC_003997)	NP_844299.1	246228
50021	G04	BA4245	membrane protein, putative	Chromosome (NC_003997)	NP_846480.1	246245
49758	G05	BA3778	prophage LambdaBa01, major tail protein, putative	Chromosome (NC_003997)	NP_846034.1	241884
47907	G06	BA1433	hypothetical protein	Chromosome (NC_003997)	NP_843889.1	241895
48182	G07	BA3746	hypothetical protein	Chromosome (NC_003997)	NP_846007.1	241907
50817	G08	BA1416	branched-chain amino acid aminotransferase	Chromosome (NC_003997)	NP_843873.1	241920
48529	G09	BA3763	hypothetical protein	Chromosome (NC_003997)	NP_846022.1	241931
51306	G10	BA3760	prophage LambdaBa01, TPR domain protein, putative	Chromosome (NC_003997)	NP_846019.1	241940
49075	G11	BA1405	hypothetical protein	Chromosome (NC_003997)	NP_843862.1	241951
49587	G12	BA1382	isochorismatase family protein	Chromosome (NC_003997)	NP_843840.1	241965
51033	H01	BA4741	membrane protein, putative	Chromosome (NC_003997)	NP_846950.1	246210
49141	H02	BA1863	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_844280.1	246219
51500	H03	BA4240	acetyl-CoA acetyltransferase	Chromosome (NC_003997)	NP_846475.1	246230
50023	H04	BA4742	aquaporin Z	Chromosome (NC_003997)	NP_846951.1	246247
47840	H05	BA1399	hypothetical protein	Chromosome (NC_003997)	NP_843856.1	241885
50145	H06	BA1435	CDP-diacylglycerol--serine O-phosphatidyltransferase, putative	Chromosome (NC_003997)	NP_843891.1	241896
48234	H07	BA3761	hypothetical protein	Chromosome (NC_003997)	NP_846020.1	241911
48437	H08	BA3769	prophage LambdaBa01, transcriptional regulator, AbrB family	Chromosome (NC_003997)	NP_846027.1	241921
51162	H09	BA1419	ketol-acid reductoisomerase	Chromosome (NC_003997)	NP_843876.1	241932
48932	H10	BA1406	transcriptional regulator, MarR family	Chromosome (NC_003997)	NP_843863.1	241941
51496	H11	BA1386	dltD protein	Chromosome (NC_003997)	NP_843844.1	241952

All information in this table was provided by J. Craig Venter Institute at the time of deposition.