

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 18**

**Catalog No. NR-19742**

This reagent is the tangible property of the U.S. Government.

**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:**

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-19742.

**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-19742 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 18, NR-19742."

**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).

**Disclaimers:**

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at [www.beiresources.org](http://www.beiresources.org).

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI

Resources are not liable for damages arising from the misidentification or misrepresentation of products.

**Use Restrictions:**

**This material is distributed for internal research, non-commercial purposes only.** This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

**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.

ATCC® is a trademark of the American Type Culture Collection



**Table 1: *Bacillus anthracis*, Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 18 (QMG002859)<sup>1</sup>**

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
48750	A02	BA5377	stage V sporulation protein AE	Chromosome (NC_003997)	NP_847550.1	248050
49011	A03	BA5319	crcB protein	Chromosome (NC_003997)	NP_847495.1	248060
49438	A04	BA5389	bacterial transferase hexapeptide domain protein	Chromosome (NC_003997)	NP_847562.1	248075
49993	A05	BA1764	hypothetical protein	Chromosome (NC_003997)	NP_844193.1	246875
50134	A06	BA1774	hypothetical protein	Chromosome (NC_003997)	NP_844200.1	246885
50556	A07	BA1777	hypothetical protein	Chromosome (NC_003997)	NP_844203.1	246895
51041	A08	BA1759	transporter, EamA family	Chromosome (NC_003997)	NP_844189.1	246910
51202	A09	BA4136	PDZ domain protein	Chromosome (NC_003997)	NP_846375.1	246918
49189	A10	BA1810	hypothetical protein	Chromosome (NC_003997)	NP_844232.1	246929
51833	A11	BA0081	DNA repair protein RadA	Chromosome (NC_003997)	NP_842650.1	246941
50190	A12	BA4808	CAAX amino terminal protease family protein	Chromosome (NC_003997)	NP_847011.1	245893
51247	B01	BA2989	transcriptional regulator, ArsR family	Chromosome (NC_003997)	NP_845322.1	248043
48775	B02	BA5320	crcB protein	Chromosome (NC_003997)	NP_847496.1	248052
51744	B03	BA5372	RNA polymerase sigma-54 factor	Chromosome (NC_003997)	NP_847545.1	248061
49484	B04	BA3031	hypothetical protein	Chromosome (NC_003997)	NP_845362.1	248079
50015	B05	BA0088	serine O-acetyltransferase	Chromosome (NC_003997)	NP_842656.1	246877
48003	B06	BA1812	hypothetical protein	Chromosome (NC_003997)	NP_844234.1	246886
48308	B07	BA4133	hypothetical protein	Chromosome (NC_003997)	NP_846372.1	246896
48841	B08	BA1746	hypothetical protein	Chromosome (NC_003997)	NP_844179.1	246911
49013	B09	BA0418	general stress protein 26	Chromosome (NC_003997)	NP_842961.1	246919
49216	B10	BA0077	transcriptional regulator CtsR	Chromosome (NC_003997)	NP_842646.1	246931
49562	B11	BA0078	hypothetical protein	Chromosome (NC_003997)	NP_842647.1	246942
47621	B12	BA0549	hypothetical protein	Chromosome (NC_003997)	NP_843084.1	245894
48618	C01	BA1083	hypothetical protein	Chromosome (NC_003997)	NP_843576.1	248044
48889	C02	BA3035	hypothetical protein	Chromosome (NC_003997)	NP_845366.1	248054
49065	C03	BA1084	hypothetical protein	Chromosome (NC_003997)	NP_843577.1	248062
49521	C04	BA3014	hypothetical protein	Chromosome (NC_003997)	NP_845345.1	248081
47886	C05	BA1741	hypothetical protein	Chromosome (NC_003997)	NP_844174.1	246878
48027	C06	BA4169	hypothetical protein	Chromosome (NC_003997)	NP_846407.1	246888
50613	C07	BA4173	protein-glutamine gamma-glutamyltransferase	Chromosome (NC_003997)	NP_846411.1	246897
51054	C08	BA0075	TIM-barrel protein, putative, NifR3 family	Chromosome (NC_003997)	NP_842644.2	246912

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
51263	C09	BA1755	BNR repeat domain protein	Chromosome (NC_003997)	NP_844185.1	246920
49274	C10	BA0085	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	Chromosome (NC_003997)	NP_842654.1	246932
49644	C11	BA4140	methyltransferase, putative	Chromosome (NC_003997)	NP_846379.1	246943
50201	C12	BA4789	LPXTG-motif cell wall anchor domain protein, putative	Chromosome (NC_003997)	NP_846994.1	245895
51277	D01	BA5318	endonuclease/exonuclease/phosphatase family	Chromosome (NC_003997)	NP_847494.1	248045
51591	D02	BA3029	succinylornithine transaminase, putative	Chromosome (NC_003997)	NP_845360.1	248055
49214	D03	BA5385	mutT/nudix family protein	Chromosome (NC_003997)	NP_847558.1	248064
49641	D04	BA3040	nitroreductase family protein	Chromosome (NC_003997)	NP_845371.1	248083
50039	D05	BA1758	transcriptional regulator, GntR family	Chromosome (NC_003997)	NP_844188.1	246879
50379	D06	BA1770	hypothetical protein	Chromosome (NC_003997)	NP_844198.1	246889
50730	D07	BA0043	4-diphosphocytidyl-2C-methyl-D-erythritol kinase	Chromosome (NC_003997)	NP_842612.1	246903
48947	D08	BA1775	hypothetical protein	Chromosome (NC_003997)	NP_844201.1	246913
49046	D09	BA1768	hypothetical protein	Chromosome (NC_003997)	NP_844196.1	246921
49368	D10	BA0099	ribosomal protein L10	Chromosome (NC_003997)	NP_842667.1	246935
51867	D11	BA0089	cysteinyl-tRNA synthetase	Chromosome (NC_003997)	NP_842657.1	246944
47730	D12	BA0586	hypothetical protein	Chromosome (NC_003997)	NP_843120.1	245896
48639	E01	BA3036	hypothetical protein	Chromosome (NC_003997)	NP_845367.1	248046
48888	E02	BA3011	glyoxalase family protein	Chromosome (NC_003997)	NP_845343.1	248056
49283	E03	BA5375	stage V sporulation protein AC	Chromosome (NC_003997)	NP_847548.1	248066
47634	E04	BA4635	hypothetical protein	Chromosome (NC_003997)	NP_846852.1	246870
47924	E05	BA1757	hypothetical protein	Chromosome (NC_003997)	NP_844187.1	246880
48105	E06	BA0074	DNA-binding protein	Chromosome (NC_003997)	NP_842643.1	246890
48736	E07	BA1748	hypothetical protein	Chromosome (NC_003997)	NP_844181.1	246906
51068	E08	BA1762	membrane protein, putative	Chromosome (NC_003997)	NP_844191.1	246914
51281	E09	BA0079	phosphotransferase domain protein	Chromosome (NC_003997)	NP_842648.1	246922
51643	E10	BA1737	metallo-beta-lactamase family protein	Chromosome (NC_003997)	NP_844172.1	246937
49671	E11	BA1745	phosphoglycerate mutase family protein	Chromosome (NC_003997)	NP_844178.1	246947
50250	E12	BA4809	CAAX amino terminal protease family protein	Chromosome (NC_003997)	NP_847012.1	245897
51371	F01	BA3037	hypothetical protein	Chromosome (NC_003997)	NP_845368.1	248047
51663	F02	BA1124	peptidase, M48 family	Chromosome (NC_003997)	NP_843610.1	248057
49328	F03	BA5374	lipoprotein, putative	Chromosome (NC_003997)	NP_847547.1	248067
49933	F04	BA1744	hypothetical protein	Chromosome (NC_003997)	NP_844177.1	246871
50074	F05	BA0084	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	Chromosome (NC_003997)	NP_842653.1	246881
50492	F06	BA4168	inositol monophosphatase family protein	Chromosome (NC_003997)	NP_846406.1	246891
48777	F07	BA0100	ribosomal protein L7/L12	Chromosome (NC_003997)	NP_842668.1	246907
48981	F08	BA0090	hypothetical protein	Chromosome (NC_003997)	NP_842658.1	246915
49053	F09	BA0097	ribosomal protein L11	Chromosome (NC_003997)	NP_842665.1	246923
49440	F10	BA0073	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokin	Chromosome (NC_003997)	NP_842642.1	246938

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
49871	F11	BA4170	hypothetical protein	Chromosome (NC_003997)	NP_846408.1	246953
47732	F12	BA2456	hypothetical protein	Chromosome (NC_003997)	NP_844832.1	245898
48659	G01	BA5357	hypothetical protein	Chromosome (NC_003997)	NP_847531.1	248048
48980	G02	BA5324	membrane protein, putative	Chromosome (NC_003997)	NP_847500.1	248058
49365	G03	BA5359	prophage LambdaBa03, prohead protease, putative	Chromosome (NC_003997)	NP_847533.1	248069
47641	G04	BA1773	hypothetical protein	Chromosome (NC_003997)	NP_844199.1	246872
47949	G05	BA1769	hypothetical protein	Chromosome (NC_003997)	NP_844197.1	246882
48216	G06	BA1807	hypothetical protein	Chromosome (NC_003997)	NP_844230.1	246892
50927	G07	BA1803	response regulator	Chromosome (NC_003997)	NP_844227.1	246908
51082	G08	BA1808	aspartate--ammonia ligase	Chromosome (NC_003997)	NP_844231.1	246916
49134	G09	BA4636	D-tyrosyl-tRNA(Tyr) deacylase	Chromosome (NC_003997)	NP_846853.1	246925
51719	G10	BA1802	sensor histidine kinase	Chromosome (NC_003997)	NP_844226.1	246939
49913	G11	BA1742	hypothetical protein	Chromosome (NC_003997)	NP_844175.1	246955
50388	G12	BA4805	RNA methyltransferase, TrmH family	Chromosome (NC_003997)	NP_847008.1	245899
51469	H01	BA3017	hypothetical protein	Chromosome (NC_003997)	NP_845348.1	248049
51718	H02	BA5364	enolase	Chromosome (NC_003997)	NP_847538.1	248059
49396	H03	BA3034	hypothetical protein	Chromosome (NC_003997)	NP_845365.1	248073
47770	H04	BA1805	hypothetical protein	Chromosome (NC_003997)	NP_844229.1	246874
50114	H05	BA0098	ribosomal protein L1	Chromosome (NC_003997)	NP_842666.1	246883
50491	H06	BA4137	phospholipase, putative	Chromosome (NC_003997)	NP_846376.1	246893
48790	H07	BA0072	dihydroneopterin aldolase	Chromosome (NC_003997)	NP_842641.1	246909
49003	H08	BA1776	hypothetical protein	Chromosome (NC_003997)	NP_844202.1	246917
51327	H09	BA1743	hypothetical protein	Chromosome (NC_003997)	NP_844176.1	246926
49504	H10	BA0096	transcription antitermination protein NusG	Chromosome (NC_003997)	NP_842664.1	246940
47616	H11	BA4793	hypothetical protein	Chromosome (NC_003997)	NP_846996.1	245892

<sup>1</sup>All information in this table was provided by J. Craig Venter Institute at the time of deposition.