

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 4**

**Catalog No. NR-19728**

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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-19728.

**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-19728 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 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 4, NR-19728."

**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 4 (QMG002845)<sup>1</sup>**

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX Clone ID
49956	A02	BA3090	pyrrolidone-carboxylate peptidase	Chromosome (NC_003997)	NP_845417.1	242658
50064	A03	BA3100	copper homeostasis protein CutC, putative	Chromosome (NC_003997)	NP_845427.1	242668
50246	A04	BA3089	transcriptional regulator, Sir2 family	Chromosome (NC_003997)	NP_845416.3	242678
48713	A05	BA3599	hypothetical protein	Chromosome (NC_003997)	NP_845869.1	242689
48930	A06	BA1200	hypothetical protein	Chromosome (NC_003997)	NP_843675.1	242699
49145	A07	BA3596	flavodoxin	Chromosome (NC_003997)	NP_845866.1	242709
51317	A08	BA3054	ABC transporter, permease protein	Chromosome (NC_003997)	NP_845384.1	242721
51624	A09	BA1205	hypothetical protein	Chromosome (NC_003997)	NP_843680.1	242731
49870	A10	BA3124	membrane protein, putative	Chromosome (NC_003997)	NP_845448.1	242742
47765	A11	BA4835	hypothetical protein	Chromosome (NC_003997)	NP_847038.1	245808
50542	A12	BA0573	hypothetical protein	Chromosome (NC_003997)	NP_843107.1	245816
49801	B01	BA0213	1-acyl-sn-glycerol-3-phosphate acyltransferase, putative	Chromosome (NC_003997)	NP_842773.1	247482
47631	B02	BA3050	hypothetical protein	Chromosome (NC_003997)	NP_845380.1	242659
47925	B03	BA3083	lipoprotein, putative	Chromosome (NC_003997)	NP_845412.1	242669
50305	B04	BA3071	phosphatase family protein	Chromosome (NC_003997)	NP_845401.1	242680
50405	B05	BA3078	hypothetical protein	Chromosome (NC_003997)	NP_845408.1	242690
48938	B06	BA3598	mutT/nudix family protein	Chromosome (NC_003997)	NP_845868.1	242701
49221	B07	BA3075	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845405.1	242711
49430	B08	BA3112	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845437.1	242722
49707	B09	BA3088	hypothetical protein	Chromosome (NC_003997)	NP_845415.1	242732
49902	B10	BA3106	hypothetical protein	Chromosome (NC_003997)	NP_845432.1	242744
50403	B11	BA2482	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_844857.1	245809
47960	B12	BA0613	hypothetical protein	Chromosome (NC_003997)	NP_843144.3	245817
49905	C01	BA4455	membrane protein, putative	Chromosome (NC_003997)	NP_846682.1	247484
50089	C02	BA1203	negative regulator of competence MecA	Chromosome (NC_003997)	NP_843678.2	242660
47932	C03	BA1207	hypothetical protein	Chromosome (NC_003997)	NP_843682.1	242671
50332	C04	BA3098	transcriptional regulator, IclR family	Chromosome (NC_003997)	NP_845425.1	242682

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX Clone ID
48780	C05	BA1211	hypothetical protein	Chromosome (NC_003997)	NP_843686.1	242691
50765	C06	BA3111	aminoglycoside phosphotransferase family protein	Chromosome (NC_003997)	NP_845436.1	242702
49240	C07	BA3044	mutT/nudix family protein	Chromosome (NC_003997)	NP_845375.1	242712
49467	C08	BA3086	Signal peptidase I	Chromosome (NC_003997)	NP_845414.1	242724
51658	C09	BA3053	ABC transporter, permease protein	Chromosome (NC_003997)	NP_845383.1	242733
49945	C10	BA3043	hypothetical protein	Chromosome (NC_003997)	NP_845374.1	242748
50458	C11	BA2485	hypothetical protein	Chromosome (NC_003997)	NP_844860.1	245810
50577	C12	BA0568	glycerol-3-phosphate ABC transporter, permease protein, putativ	Chromosome (NC_003997)	NP_843102.1	245818
49903	D01	BA4021	orotate phosphoribosyltransferase	Chromosome (NC_003997)	NP_846262.1	247486
50043	D02	BA3052	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_845382.1	242662
50157	D03	BA3094	membrane protein, putative	Chromosome (NC_003997)	NP_845421.1	242672
48503	D04	BA3046	hypothetical protein	Chromosome (NC_003997)	NP_845376.1	242683
50478	D05	BA3147	hypothetical protein	Chromosome (NC_003997)	NP_845468.1	242692
48944	D06	BA1209	protozoan/cyanobacterial globin family protein	Chromosome (NC_003997)	NP_843684.1	242703
51021	D07	BA3096	urea amidolyase-related protein	Chromosome (NC_003997)	NP_845423.1	242713
49580	D08	BA3099	Signal peptidase I U	Chromosome (NC_003997)	NP_845426.1	242726
49735	D09	BA3108	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845433.1	242734
47678	D10	BA2470	hypothetical protein	Chromosome (NC_003997)	NP_844846.1	245802
47778	D11	BA4801	hypothetical protein	Chromosome (NC_003997)	NP_847004.1	245811
48102	D12	BA4818	ribosomal protein L35	Chromosome (NC_003997)	NP_847021.1	245819
50126	E01	BA4467	hypothetical protein	Chromosome (NC_003997)	NP_846693.1	247490
47772	E02	BA3151	hypothetical protein	Chromosome (NC_003997)	NP_845470.1	242663
48058	E03	BA3082	hypothetical protein	Chromosome (NC_003997)	NP_845411.1	242673
50354	E04	BA3123	hypothetical protein	Chromosome (NC_003997)	NP_845447.1	242684
50512	E05	BA3073	hypothetical protein	Chromosome (NC_003997)	NP_845403.1	242694
50801	E06	BA3595	BNR repeat domain protein	Chromosome (NC_003997)	NP_845865.1	242704
49253	E07	BA3104	hypothetical protein	Chromosome (NC_003997)	NP_845430.1	242714
51447	E08	BA3105	acyl-CoA dehydrogenase, short-chain specific, putative	Chromosome (NC_003997)	NP_845431.1	242727
49842	E09	BA3115	transcriptional regulator, TetR family	Chromosome (NC_003997)	NP_845440.1	242736
50275	E10	BA2488	phosphoglycerate mutase	Chromosome (NC_003997)	NP_844863.1	245803
50487	E11	BA2473	hypothetical protein	Chromosome (NC_003997)	NP_844849.1	245812
50614	E12	BA4830	formamidopyrimidine-DNA glycosylase	Chromosome (NC_003997)	NP_847033.1	245820
50209	F01	BA4022	orotidine 5'-phosphate decarboxylase	Chromosome (NC_003997)	NP_846263.1	247492
50045	F02	BA3070	hypothetical protein	Chromosome (NC_003997)	NP_845400.1	242664
48090	F03	BA3594	cold shock protein CspB	Chromosome (NC_003997)	NP_845864.1	242675
50365	F04	BA3095	hypothetical protein	Chromosome (NC_003997)	NP_845422.1	242686
50623	F05	BA3119	membrane protein, putative	Chromosome (NC_003997)	NP_845443.1	242696
49070	F06	BA3041	transcriptional regulator, MarR family	Chromosome (NC_003997)	NP_845372.1	242705
49291	F07	BA3121	spore coat protein F	Chromosome (NC_003997)	NP_845445.1	242716

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX Clone ID
49606	F08	BA3600	acetyltransferase, CYSE/LACA/LPXA/NODL family	Chromosome (NC_003997)	NP_845870.1	242728
49841	F09	BA3047	hypothetical protein	Chromosome (NC_003997)	NP_845377.1	242738
47687	F10	BA2471	hypothetical protein	Chromosome (NC_003997)	NP_844847.1	245804
47879	F11	BA2455	hypothetical protein	Chromosome (NC_003997)	NP_844831.1	245813
48341	F12	BA4800	hypothetical protein	Chromosome (NC_003997)	NP_847003.1	245823
50233	G01	BA1605	cation transporter, putative	Chromosome (NC_003997)	NP_844053.1	247493
47811	G02	BA1198	hypothetical protein	Chromosome (NC_003997)	NP_843674.1	242665
50585	G03	BA3593	exonuclease family protein	Chromosome (NC_003997)	NP_845863.2	242676
48695	G04	BA3092	transposase, IS605 family, OrfA	Chromosome (NC_003997)	NP_845419.1	242687
48912	G05	BA3049	transcriptional regulator, GntR family	Chromosome (NC_003997)	NP_845379.1	242697
49112	G06	BA3113	hypothetical protein	Chromosome (NC_003997)	NP_845438.1	242707
49347	G07	BA3079	hypothetical protein	Chromosome (NC_003997)	NP_845409.1	242718
51509	G08	BA3114	membrane protein, putative	Chromosome (NC_003997)	NP_845439.1	242729
51861	G09	BA1196	MATE efflux family protein	Chromosome (NC_003997)	NP_843672.1	242739
47761	G10	BA0598	hypothetical protein	Chromosome (NC_003997)	NP_843131.1	245806
50533	G11	BA0588	formate dehydrogenase accessory protein FdhD	Chromosome (NC_003997)	NP_843122.1	245814
48355	G12	BA4799	hypothetical protein	Chromosome (NC_003997)	NP_847002.1	245825
47614	H01	BA3122	hypothetical protein	Chromosome (NC_003997)	NP_845446.1	242657
47864	H02	BA3126	hypothetical protein	Chromosome (NC_003997)	NP_845449.1	242667
48158	H03	BA3127	small, acid-soluble spore protein, alpha/beta family	Chromosome (NC_003997)	NP_845450.1	242677
50364	H04	BA3081	hypothetical protein	Chromosome (NC_003997)	NP_845410.1	242688
50663	H05	BA3118	metallo-beta-lactamase family protein	Chromosome (NC_003997)	NP_845442.1	242698
50840	H06	BA3074	membrane protein, putative	Chromosome (NC_003997)	NP_845404.1	242708
49397	H07	BA3120	hypothetical protein	Chromosome (NC_003997)	NP_845444.1	242720
49684	H08	BA1210	hypothetical protein	Chromosome (NC_003997)	NP_843685.1	242730
49853	H09	BA3597	membrane protein, putative	Chromosome (NC_003997)	NP_845867.1	242740
50310	H10	BA0574	hypothetical protein	Chromosome (NC_003997)	NP_843108.1	245807
47942	H11	BA4834	hypothetical protein	Chromosome (NC_003997)	NP_847037.1	245815

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