

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 3**

**Catalog No. NR-19727**

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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:**

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-19727.

**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-19727 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 3, NR-19727."

**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 3 (QMG002844)<sup>1</sup>**

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
49413	A02	BA3558	hypothetical protein	Chromosome (NC_003997)	NP_845831.1	242806
51614	A03	BA1185	3-oxoacyl-(acyl-carrier-protein) synthase II	Chromosome (NC_003997)	NP_843662.1	242817
51933	A04	BA3062	transcriptional regulator, GntR family/aminotransferase, class	Chromosome (NC_003997)	NP_845392.1	242827
47620	A05	BA0212	hypothetical protein	Chromosome (NC_003997)	NP_842772.1	247406
47697	A06	BA0221	hypothetical protein	Chromosome (NC_003997)	NP_842778.1	247416
48241	A07	BA4453	hypothetical protein	Chromosome (NC_003997)	NP_846681.1	247428
48301	A08	BA0229	hypothetical protein	Chromosome (NC_003997)	NP_842785.1	247436
51016	A09	BA0248	UV-endonuclease, putative	Chromosome (NC_003997)	NP_842801.1	247445
51223	A10	BA4465	comG operon protein 2	Chromosome (NC_003997)	NP_846691.1	247455
51388	A11	BA0240	4-hydroxyphenylpyruvate dioxygenase	Chromosome (NC_003997)	NP_842794.1	247464
49195	A12	BA4463	comG operon protein 4	Chromosome (NC_003997)	NP_846689.1	247472
49278	B01	BA3540	hypothetical protein	Chromosome (NC_003997)	NP_845817.1	242798
51175	B02	BA1193	oligopeptide ABC transporter, permease protein	Chromosome (NC_003997)	NP_843669.1	242807
49784	B03	BA3571	protease synthase and sporulation negative regulatory protein PA	Chromosome (NC_003997)	NP_845841.1	242818
50068	B04	BA3585	mgtC family protein	Chromosome (NC_003997)	NP_845855.1	242828
50291	B05	BA3989	3-oxoacyl-(acyl-carrier-protein) reductase	Chromosome (NC_003997)	NP_846231.1	247407
47723	B06	BA0230	hypothetical protein	Chromosome (NC_003997)	NP_842786.1	247418
50718	B07	BA1606	5'-3' exonuclease family protein	Chromosome (NC_003997)	NP_844054.1	247429
50901	B08	BA0233	oligopeptide ABC transporter, permease protein	Chromosome (NC_003997)	NP_842789.1	247437
48531	B09	BA4464	comG operon protein 3	Chromosome (NC_003997)	NP_846690.1	247446
48756	B10	BA0219	hypothetical protein	Chromosome (NC_003997)	NP_842777.1	247456
48993	B11	BA4471	hypothetical protein	Chromosome (NC_003997)	NP_846696.1	247465
51701	B12	BA4027	dihydroorotase	Chromosome (NC_003997)	NP_846268.1	247473
51100	C01	BA1188	tryptophanyl-tRNA synthetase	Chromosome (NC_003997)	NP_843665.1	242799
51243	C02	BA1194	oligopeptide ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_843670.1	242809
51680	C03	BA1132	isocitrate lyase	Chromosome (NC_003997)	NP_843617.1	242819
50067	C04	BA3559	hypothetical protein	Chromosome (NC_003997)	NP_845832.1	242830

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
47637	C05	BA0281	hypothetical protein	Chromosome (NC_003997)	NP_842832.1	247408
50503	C06	BA4451	hypothetical protein	Chromosome (NC_003997)	NP_846679.1	247419
48237	C07	BA3981	KH domain protein	Chromosome (NC_003997)	NP_846223.1	247430
48352	C08	BA4019	hypothetical protein	Chromosome (NC_003997)	NP_846261.1	247438
51062	C09	BA0216	sugar-binding transcriptional regulator, LacI family	Chromosome (NC_003997)	NP_842774.1	247447
51249	C10	BA4466	comG operon protein 1	Chromosome (NC_003997)	NP_846692.1	247457
51450	C11	BA1602	hypothetical protein	Chromosome (NC_003997)	NP_844051.1	247466
49363	C12	BA4457	shikimate kinase	Chromosome (NC_003997)	NP_846684.1	247474
49341	D01	BA1166	hypothetical protein	Chromosome (NC_003997)	NP_843645.1	242800
49455	D02	BA3586	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845856.1	242810
49891	D03	BA3550	hypothetical protein	Chromosome (NC_003997)	NP_845824.1	242820
50158	D04	BA3545	phosphoglycerate mutase, putative	Chromosome (NC_003997)	NP_845821.1	242832
47672	D05	BA0238	hypothetical protein	Chromosome (NC_003997)	NP_842792.1	247410
47797	D06	BA1607	hypothetical protein	Chromosome (NC_003997)	NP_844055.1	247422
50749	D07	BA4444	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_846672.1	247431
50922	D08	BA4023	dihydroorotate dehydrogenase	Chromosome (NC_003997)	NP_846264.1	247439
48579	D09	BA1601	lipoprotein, putative	Chromosome (NC_003997)	NP_844050.1	247448
48813	D10	BA4501	hypothetical protein	Chromosome (NC_003997)	NP_846726.1	247458
49009	D11	BA4503	transcriptional regulator, Fur family	Chromosome (NC_003997)	NP_846728.1	247467
51797	D12	BA4448	glycine cleavage system P-protein, subunit 1	Chromosome (NC_003997)	NP_846676.1	247475
51108	E01	BA3068	hypothetical protein	Chromosome (NC_003997)	NP_845398.1	242801
51415	E02	BA3581	degT/dnrJ/eryC1/strS family protein	Chromosome (NC_003997)	NP_845851.1	242811
51725	E03	BA1162	alpha-amylase family protein	Chromosome (NC_003997)	NP_843643.1	242821
50265	E04	BA1180	hypothetical protein	Chromosome (NC_003997)	NP_843657.1	242834
50376	E05	BA0222	deoxyribonuclease, TatD family, putative	Chromosome (NC_003997)	NP_842779.1	247411
50625	E06	BA4504	cation ABC transporter, permease protein, putative	Chromosome (NC_003997)	NP_846729.1	247423
48259	E07	BA3988	acyl carrier protein	Chromosome (NC_003997)	NP_846230.1	247432
48426	E08	BA3982	ribosomal protein S16	Chromosome (NC_003997)	NP_846224.1	247440
51077	E09	BA0234	oligopeptide ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_842790.1	247449
51339	E10	BA4026	carbamoyl-phosphate synthase, small subunit	Chromosome (NC_003997)	NP_846267.1	247459
49024	E11	BA4461	comG operon protein 6, putative	Chromosome (NC_003997)	NP_846687.1	247468
49446	E12	BA3980	16S rRNA processing protein RimM	Chromosome (NC_003997)	NP_846222.1	247476
49348	F01	BA3539	hypothetical protein	Chromosome (NC_003997)	NP_845816.1	242802
49476	F02	BA3546	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845822.1	242812
49978	F03	BA3563	membrane protein, putative	Chromosome (NC_003997)	NP_845835.1	242822
50472	F04	BA1133	trifolitoxin immunity domain protein	Chromosome (NC_003997)	NP_843618.1	242840
47684	F05	BA0243	hypothetical protein	Chromosome (NC_003997)	NP_842797.1	247412
50619	F06	BA1599	rhodanese-like domain protein	Chromosome (NC_003997)	NP_844048.1	247425
50769	F07	BA4506	membrane protein, putative	Chromosome (NC_003997)	NP_846731.1	247433
48417	F08	BA1598	hypothetical protein	Chromosome (NC_003997)	NP_844047.1	247442

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
51106	F09	BA3985	signal recognition particle-docking protein FtsY	Chromosome (NC_003997)	NP_846227.1	247451
51345	F10	BA4449	glycine cleavage system T protein	Chromosome (NC_003997)	NP_846677.1	247460
51667	F11	BA0244	major facilitator family transporter	Chromosome (NC_003997)	NP_842798.1	247469
49506	F12	BA0224	invasion protein lagB domain protein	Chromosome (NC_003997)	NP_842781.1	247478
51129	G01	BA3566	alcohol dehydrogenase, zinc-containing	Chromosome (NC_003997)	NP_845838.1	242803
51423	G02	BA3554	penicillin-binding protein, putative	Chromosome (NC_003997)	NP_845827.1	242813
51795	G03	BA3578	methyltransferase, putative	Chromosome (NC_003997)	NP_845848.1	242823
50489	G04	BA3583	enoyl-CoA hydratase/isomerase family protein	Chromosome (NC_003997)	NP_845853.1	242842
47683	G05	BA0239	hypothetical protein	Chromosome (NC_003997)	NP_842793.1	247414
48120	G06	BA4446	hypothetical protein	Chromosome (NC_003997)	NP_846674.1	247426
48299	G07	BA4468	hypothetical protein	Chromosome (NC_003997)	NP_846694.1	247434
51004	G08	BA0241	fumarylacetoacetate hydrolase family protein	Chromosome (NC_003997)	NP_842795.1	247443
48647	G09	BA0217	hypothetical protein	Chromosome (NC_003997)	NP_842775.1	247452
48856	G10	BA4445	transcriptional regulator, GntR family	Chromosome (NC_003997)	NP_846673.1	247461
49167	G11	BA1600	hypothetical protein	Chromosome (NC_003997)	NP_844049.1	247470
51834	G12	BA0246	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-ala	Chromosome (NC_003997)	NP_842799.1	247479
49354	H01	BA3562	hypothetical protein	Chromosome (NC_003997)	NP_845834.1	242804
49642	H02	BA3580	O-acetyltransferase, putative	Chromosome (NC_003997)	NP_845850.1	242816
50044	H03	BA3067	DNA-binding response regulator	Chromosome (NC_003997)	NP_845397.1	242826
50279	H04	BA3987	ribonuclease III	Chromosome (NC_003997)	NP_846229.1	247405
50447	H05	BA4024	dihydroorotate dehydrogenase, electron transfer subunit	Chromosome (NC_003997)	NP_846265.1	247415
50632	H06	BA0218	yitT family protein	Chromosome (NC_003997)	NP_842776.1	247427
50882	H07	BA4028	aspartate carbamoyltransferase	Chromosome (NC_003997)	NP_846269.1	247435
48506	H08	BA4462	hypothetical protein	Chromosome (NC_003997)	NP_846688.1	247444
51161	H09	BA0235	oligopeptide ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_842791.1	247453
48899	H10	BA1603	hypothetical protein	Chromosome (NC_003997)	NP_844052.1	247463
51694	H11	BA4029	uracil permease	Chromosome (NC_003997)	NP_846270.1	247471

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