

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 16**

Catalog No. NR-19740

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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-19740.

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-19740 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 16, NR-19740."

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.

Disclaimers:

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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 16 (QMG002857)¹

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
48484	A02	BA4723	hypothetical protein	Chromosome (NC_003997)	NP_846934.1	246393
51647	A03	BA4704	ATP-dependent Clp protease, ATP-binding subunit ClpX	Chromosome (NC_003997)	NP_846917.1	246404
49426	A04	BA2379	DNA polymerase, DinB family	Chromosome (NC_003997)	NP_844761.1	246415
49819	A05	BA0495	hypothetical protein	Chromosome (NC_003997)	NP_843032.1	246427
47771	A06	BA2619	hypothetical protein	Chromosome (NC_003997)	NP_844981.1	244190
48023	A07	BA2621	hypothetical protein	Chromosome (NC_003997)	NP_844983.1	244202
48206	A08	BA2118	hypothetical protein	Chromosome (NC_003997)	NP_844516.1	244213
48349	A09	BA2166	hypothetical protein	Chromosome (NC_003997)	NP_844561.1	244223
48692	A10	BA2168	single-stranded DNA-binding protein	Chromosome (NC_003997)	NP_844562.1	244233
51387	A11	BA5509	UDP-N-acetylglucosamine 2-epimerase	Chromosome (NC_003997)	NP_847668.1	244242
51611	A12	BA2627	cytochrome P450	Chromosome (NC_003997)	NP_844989.1	244252
51219	B01	BA2392	hydrolase, alpha/beta fold family	Chromosome (NC_003997)	NP_844770.1	246386
51657	B02	BA2393	ABC transporter, permease protein, putative	Chromosome (NC_003997)	NP_844771.2	246394
48703	B03	BA0497	hypothetical protein	Chromosome (NC_003997)	NP_843034.1	246405
51827	B04	BA2381	sodium/alanine symporter family protein	Chromosome (NC_003997)	NP_844763.1	246416
49829	B05	BA4710	hypothetical protein	Chromosome (NC_003997)	NP_846922.1	246428
49899	B06	BA2629	acetyltransferase, CYSE/LACA/LPXA/NODL family	Chromosome (NC_003997)	NP_844991.1	244191
50258	B07	BA2165	membrane protein, putative	Chromosome (NC_003997)	NP_844560.1	244203
50698	B08	BA4969	glucose uptake protein	Chromosome (NC_003997)	NP_847164.1	244214
50816	B09	BA5406	transcriptional regulator, LysR family	Chromosome (NC_003997)	NP_847579.1	244224
51153	B10	BA2625	hypothetical protein	Chromosome (NC_003997)	NP_844987.1	244234
48927	B11	BA5402	hypothetical protein	Chromosome (NC_003997)	NP_847575.1	244243
49093	B12	BA2173	hypothetical protein	Chromosome (NC_003997)	NP_844566.1	244253
48327	C01	BA4707	hypothetical protein	Chromosome (NC_003997)	NP_846920.1	246387
48490	C02	BA2347	hypothetical protein	Chromosome (NC_003997)	NP_844730.1	246395
51684	C03	BA4705	trigger factor	Chromosome (NC_003997)	NP_846918.1	246406
49538	C04	BA2376	hypothetical protein	Chromosome (NC_003997)	NP_844758.1	246417
49852	C05	BA2385	hypothetical protein	Chromosome (NC_003997)	NP_844766.1	246430

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
49955	C06	BA2163	HD domain protein	Chromosome (NC_003997)	NP_844558.1	244193
50314	C07	BA2162	hypothetical protein	Chromosome (NC_003997)	NP_844557.1	244205
50717	C08	BA0748	oxidoreductase, short chain dehydrogenase/reductase family	Chromosome (NC_003997)	NP_843273.1	244216
48432	C09	BA2154	stage V sporulation protein S	Chromosome (NC_003997)	NP_844552.1	244225
51172	C10	BA4976	molybdopterin biosynthesis protein MoeB, putative	Chromosome (NC_003997)	NP_847171.1	244236
51406	C11	BA2171	PBS lyase HEAT-like repeat domain protein	Chromosome (NC_003997)	NP_844564.1	244244
51695	C12	BA5512	UDP-N-acetyl-D-mannosamine dehydrogenase	Chromosome (NC_003997)	NP_847671.1	244254
51442	D01	BA2346	major facilitator family transporter	Chromosome (NC_003997)	NP_844729.1	246388
51541	D02	BA2380	alkaline serine protease, subtilase family	Chromosome (NC_003997)	NP_844762.1	246396
51685	D03	BA0493	acetylornithine deacetylase, putative	Chromosome (NC_003997)	NP_843030.1	246408
49598	D04	BA0513	hypothetical protein	Chromosome (NC_003997)	NP_843049.1	246419
47607	D05	BA2616	hypothetical protein	Chromosome (NC_003997)	YP_003329040.1	244182
47861	D06	BA2150	hypothetical protein	Chromosome (NC_003997)	NP_844548.1	244194
50372	D07	BA5400	transcriptional regulator, MerR family	Chromosome (NC_003997)	NP_847573.1	244207
48261	D08	BA4971	molybdopterin converting factor, subunit 1	Chromosome (NC_003997)	NP_847166.1	244217
50880	D09	BA2610	D-alanine--D-alanine ligase	Chromosome (NC_003997)	NP_844972.1	244226
48763	D10	BA2145	nitrite reductase [NAD(P)H], small subunit	Chromosome (NC_003997)	NP_844543.1	244237
48970	D11	BA2626	hypothetical protein	Chromosome (NC_003997)	NP_844988.1	244245
51693	D12	BA2628	permease, putative	Chromosome (NC_003997)	NP_844990.1	244256
48421	E01	BA2377	DNA-binding protein HU	Chromosome (NC_003997)	NP_844759.1	246389
51556	E02	BA2369	isochorismate synthase Dhbc	Chromosome (NC_003997)	NP_844751.1	246398
51728	E03	BA4689	folylpolyglutamate synthase	Chromosome (NC_003997)	NP_846902.1	246410
49673	E04	BA2383	hypothetical protein	Chromosome (NC_003997)	NP_844764.1	246421
47644	E05	BA2157	hypothetical protein	Chromosome (NC_003997)	NP_844554.1	244184
50081	E06	BA2115	hypothetical protein	Chromosome (NC_003997)	NP_844513.1	244195
48155	E07	BA2614	hypothetical protein	Chromosome (NC_003997)	NP_844976.1	244208
50752	E08	BA5472	D-amino acid aminotransferase	Chromosome (NC_003997)	NP_847638.1	244218
48604	E09	BA2176	hypothetical protein	Chromosome (NC_003997)	NP_844569.1	244229
51204	E10	BA5405	membrane protein, putative	Chromosome (NC_003997)	NP_847578.1	244238
51523	E11	BA5412	membrane protein, putative	Chromosome (NC_003997)	NP_847584.1	244246
51707	E12	BA4974	molybdopterin biosynthesis protein MoeA	Chromosome (NC_003997)	NP_847169.1	244258
51456	F01	BA2359	exonuclease SbcD, putative	Chromosome (NC_003997)	NP_844741.1	246390
48521	F02	BA0471	hypothetical protein	Chromosome (NC_003997)	NP_843010.1	246399
49026	F03	BA4700	organic hydroperoxide resistance protein	Chromosome (NC_003997)	NP_846913.1	246411
49773	F04	BA4701	GTP-binding protein	Chromosome (NC_003997)	NP_846914.1	246423
49651	F05	BA2148	hypothetical protein	Chromosome (NC_003997)	NP_844546.1	244185
47898	F06	BA2613	hypothetical protein	Chromosome (NC_003997)	NP_844975.1	244198
50433	F07	BA2174	hypothetical protein	Chromosome (NC_003997)	NP_844567.1	244209
48266	F08	BA2151	hypothetical protein	Chromosome (NC_003997)	NP_844549.1	244219

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
51050	F09	BA2117	metallo-beta-lactamase family protein	Chromosome (NC_003997)	NP_844515.1	244230
48815	F10	BA5407	transcriptional regulator, MerR family	Chromosome (NC_003997)	NP_847580.1	244239
49019	F11	BA2622	hypothetical protein	Chromosome (NC_003997)	NP_844984.1	244247
49237	F12	BA4972	molybdopterin converting factor, subunit 2	Chromosome (NC_003997)	NP_847167.1	244261
48414	G01	BA0470	prophage LambdaBa04, DNA packaging protein, putative	Chromosome (NC_003997)	NP_843009.1	246391
48535	G02	BA0486	hypothetical protein	Chromosome (NC_003997)	NP_843025.1	246401
49387	G03	BA4713	phosphoesterase, putative	Chromosome (NC_003997)	NP_846925.1	246413
51981	G04	BA0501	PTS system, N-acetylglucosamine-specific IIBC component, putative	Chromosome (NC_003997)	NP_843038.1	246424
47745	G05	BA2615	hypothetical protein	Chromosome (NC_003997)	NP_844977.1	244186
47909	G06	BA2160	hypothetical protein	Chromosome (NC_003997)	NP_844556.1	244200
48192	G07	BA2177	hypothetical protein	Chromosome (NC_003997)	NP_844570.1	244211
48332	G08	BA0751	membrane protein, putative	Chromosome (NC_003997)	NP_843275.1	244221
48635	G09	BA2159	hypothetical protein	Chromosome (NC_003997)	NP_844555.1	244231
51322	G10	BA2121	hypothetical protein	Chromosome (NC_003997)	NP_844519.2	244240
51586	G11	BA5475	nucleoside transporter, NupC family	Chromosome (NC_003997)	NP_847639.1	244248
49308	G12	BA2119	glutathione peroxidase	Chromosome (NC_003997)	NP_844517.1	244263
51474	H01	BA0511	hypothetical protein	Chromosome (NC_003997)	NP_843047.1	246392
51613	H02	BA0502	penicillin-binding protein, putative	Chromosome (NC_003997)	NP_843039.1	246402
51774	H03	BA4718	major facilitator family transporter	Chromosome (NC_003997)	NP_846930.1	246414
49817	H04	BA4714	HAM1 protein	Chromosome (NC_003997)	NP_846926.1	246425
49742	H05	BA2169	transcriptional regulator, TetR family	Chromosome (NC_003997)	NP_844563.1	244187
50251	H06	BA5476	hypothetical protein	Chromosome (NC_003997)	NP_847640.1	244201
50640	H07	BA2152	N-acetyltransferase family protein	Chromosome (NC_003997)	NP_844550.1	244212
50796	H08	BA2122	hypothetical protein	Chromosome (NC_003997)	NP_844520.1	244222
51140	H09	BA2149	hypothetical protein	Chromosome (NC_003997)	NP_844547.1	244232
48885	H10	BA2172	hypothetical protein	Chromosome (NC_003997)	NP_844565.1	244241
49037	H11	BA4970	hypothetical protein	Chromosome (NC_003997)	NP_847165.1	244249

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