

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 6**

**Catalog No. NR-19730**

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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-19730.

**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-19730 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 6, NR-19730."

**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 6 (QMG002847)<sup>1</sup>**

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX Clone ID
51798	A02	BA0157	phosphoglucosamine mutase	Chromosome (NC_003997)	NP_842723.1	247813
50191	A03	BA0146	germination-specific N-acetylmuramoyl-L-alanine amidase	Chromosome (NC_003997)	NP_842713.1	247828
47703	A04	BA2100	hypothetical protein	Chromosome (NC_003997)	NP_844499.1	244103
50183	A05	BA2143	cbiX domain protein	Chromosome (NC_003997)	NP_844541.1	244112
48224	A06	BA5489	hypothetical protein	Chromosome (NC_003997)	NP_847651.1	244121
51111	A07	BA5505	UDP-glucose 4-epimerase	Chromosome (NC_003997)	NP_847665.1	244131
48881	A08	BA5452	hypothetical protein	Chromosome (NC_003997)	NP_847620.1	244142
49054	A09	BA2101	hypothetical protein	Chromosome (NC_003997)	NP_844500.1	244152
49264	A10	BA2142	siroheme synthase domain protein	Chromosome (NC_003997)	NP_844540.1	244161
49419	A11	BA5499	membrane protein, putative	Chromosome (NC_003997)	NP_847659.1	244173
47748	A12	BA3789	hypothetical protein	Chromosome (NC_003997)	NP_846045.1	241022
51636	B01	BA0181	drug resistance transporter, Bcr/CflA family	Chromosome (NC_003997)	NP_842745.1	247805
49833	B02	BA0148	spore germination protein GerD	Chromosome (NC_003997)	NP_842715.1	247816
50230	B03	BA4374	amino acid ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_846604.1	247830
47702	B04	BA2090	hypothetical protein	Chromosome (NC_003997)	NP_844489.1	244105
48022	B05	BA2096	hypothetical protein	Chromosome (NC_003997)	NP_844495.1	244113
48258	B06	BA2137	molybdopterin converting factor, subunit 1	Chromosome (NC_003997)	NP_844535.1	244123
48614	B07	BA5466	hypothetical protein	Chromosome (NC_003997)	NP_847633.1	244132
51482	B08	BA2138	nitrate transporter	Chromosome (NC_003997)	NP_844536.1	244143
51579	B09	BA2083	glycosyltransferase, MGT family	Chromosome (NC_003997)	NP_844483.1	244153
51776	B10	BA5441	6-phospho-beta-glucosidase	Chromosome (NC_003997)	NP_847609.1	244162
51920	B11	BA5503	sensor histidine kinase	Chromosome (NC_003997)	NP_847663.1	244174
49837	B12	BA1447	siroheme synthase, putative	Chromosome (NC_003997)	NP_843903.1	241023
49505	C01	BA0179	hypothetical protein	Chromosome (NC_003997)	NP_842743.1	247806
51819	C02	BA4404	exodeoxyribonuclease VII, large subunit	Chromosome (NC_003997)	NP_846632.1	247817
50249	C03	BA4391	glycerophosphoryl diester phosphodiesterase family protein	Chromosome (NC_003997)	NP_846621.1	247832

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
50111	C04	BA2128	respiratory nitrate reductase, gamma subunit	Chromosome (NC_003997)	NP_844526.1	244106
50195	C05	BA2080	hypothetical protein	Chromosome (NC_003997)	NP_844480.1	244114
48546	C06	BA5449	PTS system, cellobiose-specific IIB component	Chromosome (NC_003997)	NP_847617.1	244124
48633	C07	BA5442	PTS system, cellobiose-specific IIA component	Chromosome (NC_003997)	NP_847610.1	244133
48884	C08	BA2111	glyoxalase family protein	Chromosome (NC_003997)	NP_844509.1	244144
51592	C09	BA5450	proton/glutamate symporter family protein, putative	Chromosome (NC_003997)	NP_847618.1	244155
49323	C10	BA2091	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_844490.1	244163
49494	C11	BA2088	hypothetical protein	Chromosome (NC_003997)	NP_844487.1	244175
47812	C12	BA1439	hypothetical protein	Chromosome (NC_003997)	NP_843895.1	241024
49545	D01	BA4369	ribosomal-protein-alanine acetyltransferase, putative	Chromosome (NC_003997)	NP_846599.1	247808
49904	D02	BA4412	stage III sporulation protein AF	Chromosome (NC_003997)	NP_846640.1	247818
50252	D03	BA0161	gluconate operon transcriptional repressor	Chromosome (NC_003997)	NP_842727.1	247834
47908	D04	BA2098	hypothetical protein	Chromosome (NC_003997)	NP_844497.1	244107
48037	D05	BA2097	hypothetical protein	Chromosome (NC_003997)	NP_844496.1	244115
48545	D06	BA5444	PTS system, cellobiose-specific IIB component	Chromosome (NC_003997)	NP_847612.1	244125
48816	D07	BA5468	hypothetical protein	Chromosome (NC_003997)	NP_847635.1	244134
48901	D08	BA2129	transcriptional regulator, GntR family	Chromosome (NC_003997)	NP_844527.1	244146
49219	D09	BA2078	hypothetical protein	Chromosome (NC_003997)	NP_844478.1	244156
49339	D10	BA5485	hypothetical protein	Chromosome (NC_003997)	NP_847647.1	244167
49495	D11	BA2127	nitrate reductase delta chain	Chromosome (NC_003997)	NP_844525.1	244176
49860	D12	BA3838	membrane protein, putative	Chromosome (NC_003997)	NP_846089.1	241025
51765	E01	BA4382	dihydrolypoamide acetyltransferase	Chromosome (NC_003997)	NP_846612.1	247809
49925	E02	BA4410	stage III sporulation protein AH	Chromosome (NC_003997)	NP_846638.1	247820
47671	E03	BA5471	hypothetical protein	Chromosome (NC_003997)	NP_847637.1	244099
50151	E04	BA5494	hypothetical protein	Chromosome (NC_003997)	NP_847654.1	244108
48045	E05	BA5498	DNA-binding protein	Chromosome (NC_003997)	NP_847658.1	244116
50875	E06	BA5506	transcription antiterminator LytR	Chromosome (NC_003997)	NP_847666.1	244126
48825	E07	BA2099	hypothetical protein	Chromosome (NC_003997)	NP_844498.1	244136
51516	E08	BA5439	chromate ion transporter	Chromosome (NC_003997)	NP_847607.1	244147
49250	E09	BA2136	molybdopterin converting factor, subunit 2	Chromosome (NC_003997)	NP_844534.1	244157
51965	E10	BA2126	respiratory nitrate reductase, beta subunit	Chromosome (NC_003997)	NP_844524.2	244168
49601	E11	BA2089	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_844488.1	244177
47900	E12	BA3812	hypothetical protein	Chromosome (NC_003997)	NP_846064.1	241028
49623	F01	BA4372	hypothetical protein	Chromosome (NC_003997)	NP_846602.1	247810
50000	F02	BA4375	amino acid ABC transporter, permease protein	Chromosome (NC_003997)	NP_846605.1	247822
50049	F03	BA5504	DNA-binding response regulator	Chromosome (NC_003997)	NP_847664.1	244100
47935	F04	BA2095	hypothetical protein	Chromosome (NC_003997)	NP_844494.1	244109
48055	F05	BA2093	hypothetical protein	Chromosome (NC_003997)	NP_844492.1	244118
48536	F06	BA2092	hypothetical protein	Chromosome (NC_003997)	NP_844491.1	244127

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
51298	F07	BA5435	glycosyl transferase, group 4 family protein	Chromosome (NC_003997)	NP_847603.1	244137
49012	F08	BA5438	hypothetical protein	Chromosome (NC_003997)	NP_847606.1	244148
51730	F09	BA5448	PTS system, cellobiose-specific IIC component	Chromosome (NC_003997)	NP_847616.1	244158
51848	F10	BA5483	hypothetical protein	Chromosome (NC_003997)	NP_847645.1	244170
49687	F11	BA2114	RNA polymerase sigma-70 factor, ECF subfamily	Chromosome (NC_003997)	NP_844512.1	244179
49896	F12	BA1457	DNA-binding response regulator	Chromosome (NC_003997)	NP_843913.1	241029
51799	G01	BA0163	gluconate permease	Chromosome (NC_003997)	NP_842728.1	247811
50013	G02	BA4411	stage III sporulation protein AG	Chromosome (NC_003997)	NP_846639.1	247824
47667	G03	BA2141	hypothetical protein	Chromosome (NC_003997)	NP_844539.1	244101
50162	G04	BA5440	hypothetical protein	Chromosome (NC_003997)	NP_847608.1	244110
50659	G05	BA2084	hypothetical protein	Chromosome (NC_003997)	NP_844484.1	244119
48547	G06	BA5482	hypothetical protein	Chromosome (NC_003997)	NP_847644.1	244128
48849	G07	BA5446	S-adenosylmethionine decarboxylase proenzyme	Chromosome (NC_003997)	NP_847614.1	244138
51547	G08	BA5497	efflux transporter, RND family, MFP subunit	Chromosome (NC_003997)	NP_847657.1	244149
49249	G09	BA2131	hypothetical protein	Chromosome (NC_003997)	NP_844529.1	244159
49371	G10	BA2124	hypothetical protein	Chromosome (NC_003997)	NP_844522.1	244171
47700	G11	BA1412	hypothetical protein	Chromosome (NC_003997)	NP_843869.1	241020
47927	G12	BA3817	hypothetical protein	Chromosome (NC_003997)	NP_846069.1	241032
49636	H01	BA0166	hypothetical protein	Chromosome (NC_003997)	NP_842731.1	247812
50028	H02	BA0173	ABC transporter, permease protein	Chromosome (NC_003997)	NP_842737.1	247826
50087	H03	BA5496	ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_847656.1	244102
48004	H04	BA2094	hypothetical protein	Chromosome (NC_003997)	NP_844493.1	244111
48186	H05	BA5490	hypothetical protein	Chromosome (NC_003997)	NP_847652.1	244120
48613	H06	BA5447	PTS system, cellobiose-specific IIA component	Chromosome (NC_003997)	NP_847615.1	244130
48865	H07	BA2140	hypothetical protein	Chromosome (NC_003997)	NP_844538.1	244140
49047	H08	BA2086	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_844486.1	244150
51745	H09	BA5443	PTS system, cellobiose-specific IIC component	Chromosome (NC_003997)	NP_847611.1	244160
51871	H10	BA2085	transcriptional regulator, GntR family	Chromosome (NC_003997)	NP_844485.1	244172
49752	H11	BA1442	adenylylsulfate kinase	Chromosome (NC_003997)	NP_843898.1	241021

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