

***Bacillus anthracis* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 1**

Catalog No. NR-19725

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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-19725.

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-19725 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 1, NR-19725."

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 1 (QM002842)¹

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
47919	A02	BA0817	hypothetical protein	Chromosome (NC_003997)	NP_843335.1	240937
49995	A03	BA2712	hypothetical protein	Chromosome (NC_003997)	NP_845066.1	240946
50113	A04	BA5068	DNA-binding response regulator	Chromosome (NC_003997)	NP_847259.1	240954
48359	A05	BA2710	hypothetical protein	Chromosome (NC_003997)	NP_845064.1	240963
48674	A06	BA5071	hypothetical protein	Chromosome (NC_003997)	NP_847262.1	240973
51297	A07	BA5040	hypothetical protein	Chromosome (NC_003997)	NP_847232.1	240982
49027	A08	BA5078	hypothetical protein	Chromosome (NC_003997)	NP_847268.1	240990
49267	A09	BA2699	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845055.1	241000
49441	A10	BA0842	thiJ/pfpl family protein	Chromosome (NC_003997)	NP_843358.1	241012
47815	A11	BA2875	hypothetical protein	Chromosome (NC_003997)	NP_845216.1	239652
48082	A12	BA0987	hypothetical protein	Chromosome (NC_003997)	NP_843488.1	239664
47630	B01	BA2714	hypothetical protein	Chromosome (NC_003997)	NP_845068.1	240927
49668	B02	BA0834	transcriptional regulator, TetR family	Chromosome (NC_003997)	NP_843351.1	240938
48130	B03	BA2704	hypothetical protein	Chromosome (NC_003997)	NP_845059.1	240947
50167	B04	BA0856	amino acid ABC transporter, permease protein	Chromosome (NC_003997)	NP_843369.1	240956
50607	B05	BA0847	glutamate racemase	Chromosome (NC_003997)	NP_843363.1	240964
51074	B06	BA5077	hypothetical protein	Chromosome (NC_003997)	NP_847267.1	240974
48873	B07	BA2705	endo/excinuclease amino terminal domain protein	Chromosome (NC_003997)	NP_845060.1	240983
51565	B08	BA0835	multidrug resistance protein	Chromosome (NC_003997)	NP_843352.1	240991
51826	B09	BA0823	PTS system, sucrose-specific IIBC component	Chromosome (NC_003997)	NP_843341.1	241001
49465	B10	BA2700	hypothetical protein	Chromosome (NC_003997)	NP_845056.1	241014
49811	B11	BA0983	hypothetical protein	Chromosome (NC_003997)	NP_843484.1	239653
50173	B12	BA2878	hypothetical protein	Chromosome (NC_003997)	NP_845219.1	239665
49533	C01	BA5042	hypothetical protein	Chromosome (NC_003997)	NP_847233.1	240928
47971	C02	BA5080	hypothetical protein	Chromosome (NC_003997)	NP_847270.1	240939
50024	C03	BA5061	hypothetical protein	Chromosome (NC_003997)	NP_847252.1	240948
48262	C04	BA5052	spore germination protein GerPF-like protein	Chromosome (NC_003997)	NP_847243.1	240957
48589	C05	BA0820	hypothetical protein	Chromosome (NC_003997)	NP_843338.1	240965
51166	C06	BA5079	oxidoreductase, aldo/keto reductase family	Chromosome (NC_003997)	NP_847269.1	240976

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
51325	C07	BA0848	hypothetical protein	Chromosome (NC_003997)	NP_843364.1	240984
49109	C08	BA2713	mutT/nudix family protein	Chromosome (NC_003997)	NP_845067.1	240992
49297	C09	BA5043	mutT/nudix family protein	Chromosome (NC_003997)	NP_847234.1	241002
49482	C10	BA2701	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845057.1	241016
47997	C11	BA1007	hypothetical protein	Chromosome (NC_003997)	NP_843508.1	239656
48201	C12	BA1002	hypothetical protein	Chromosome (NC_003997)	NP_843503.1	239666
49565	D01	BA0838	NAD(P)H dehydrogenase, quinone family	Chromosome (NC_003997)	NP_843354.1	240930
49696	D02	BA5062	lipoprotein, putative	Chromosome (NC_003997)	NP_847253.1	240940
48167	D03	BA5072	hypothetical protein	Chromosome (NC_003997)	NP_847263.1	240949
50263	D04	BA0857	amino acid ABC transporter, ATP-binding protein	Chromosome (NC_003997)	NP_843370.1	240958
50709	D05	BA0821	transcriptional regulator, RpiR family, putative	Chromosome (NC_003997)	NP_843339.1	240966
48721	D06	BA0832	sugE protein, putative	Chromosome (NC_003997)	NP_843349.1	240977
48958	D07	BA0827	hypothetical protein	Chromosome (NC_003997)	NP_843344.1	240985
51577	D08	BA1467	flavoheomprotein	Chromosome (NC_003997)	NP_843922.1	240993
51829	D09	BA0845	amino acid permease family protein	Chromosome (NC_003997)	NP_843362.1	241003
49527	D10	BA0837	lipoprotein, putative	Chromosome (NC_003997)	NP_843353.1	241018
49909	D11	BA5193	transcriptional regulator, DeoR family	Chromosome (NC_003997)	NP_847376.1	239657
50186	D12	BA2879	hypothetical protein	Chromosome (NC_003997)	NP_845220.1	239667
47846	E01	BA3851	hypothetical protein	Chromosome (NC_003997)	NP_846101.1	240931
49774	E02	BA5069	LPXTG-site transpeptidase family protein	Chromosome (NC_003997)	NP_847260.1	240942
50032	E03	BA5066	hypothetical protein	Chromosome (NC_003997)	NP_847257.1	240950
48274	E04	BA5048	conserved hypothetical protein TIGR00278	Chromosome (NC_003997)	NP_847239.1	240959
48590	E05	BA0833	sugE protein, putative	Chromosome (NC_003997)	NP_843350.1	240967
51216	E06	BA5051	cytochrome d ubiquinol oxidase, subunit II	Chromosome (NC_003997)	NP_847242.1	240978
51380	E07	BA2715	oxidoreductase, DadA family	Chromosome (NC_003997)	NP_845069.1	240986
49164	E08	BA5070	cell wall surface anchor family protein	Chromosome (NC_003997)	NP_847261.1	240994
49311	E09	BA2707	acetyltransferase, GNAT family	Chromosome (NC_003997)	NP_845061.1	241004
47786	E10	BA0998	hypothetical protein	Chromosome (NC_003997)	NP_843499.1	239648
49944	E11	BA2883	lipoprotein, putative	Chromosome (NC_003997)	NP_845224.1	239659
48254	E12	BA5236	hypothetical protein	Chromosome (NC_003997)	NP_847415.1	239668
47858	F01	BA0816	hypothetical protein	Chromosome (NC_003997)	NP_843334.1	240933
48110	F02	BA0858	small acid-soluble spore protein	Chromosome (NC_003997)	NP_843371.1	240943
48197	F03	BA5057	hypothetical protein	Chromosome (NC_003997)	NP_847248.1	240951
50508	F04	BA0855	amino acid ABC transporter, amino acid-binding protein	Chromosome (NC_003997)	NP_843368.1	240960
48611	F05	BA5044	hypothetical protein	Chromosome (NC_003997)	NP_847235.1	240969
48741	F06	BA5081	hypothetical protein	Chromosome (NC_003997)	NP_847271.1	240979
48979	F07	BA5060	hypothetical protein	Chromosome (NC_003997)	NP_847251.1	240987
51639	F08	BA0840	major facilitator family transporter	Chromosome (NC_003997)	NP_843356.1	240995
49319	F09	BA0828	hypothetical protein	Chromosome (NC_003997)	NP_843345.1	241006
49778	F10	BA1009	hypothetical protein	Chromosome (NC_003997)	NP_843510.1	239649
48061	F11	BA5235	hypothetical protein	Chromosome (NC_003997)	NP_847414.1	239660

Clone	Well Position	Locus ID	Description	Source	Accession Number	FLEX CloneID
50261	F12	BA5207	hypothetical protein	Chromosome (NC_003997)	NP_847390.1	239669
49634	G01	BA5049	carbonic anhydrase, prokaryotic type, putative	Chromosome (NC_003997)	NP_847240.1	240934
49976	G02	BA2716	hypothetical protein	Chromosome (NC_003997)	NP_845070.1	240944
50085	G03	BA5076	hypothetical protein	Chromosome (NC_003997)	NP_847266.1	240952
48285	G04	BA2711	hypothetical protein	Chromosome (NC_003997)	NP_845065.1	240961
48653	G05	BA2696	hypothetical protein	Chromosome (NC_003997)	NP_845053.1	240971
51284	G06	BA0824	hypothetical protein	Chromosome (NC_003997)	NP_843342.1	240980
51405	G07	BA0819	germination protein gerN	Chromosome (NC_003997)	NP_843337.1	240988
49273	G08	BA5047	autoinducer-2 production protein LuxS	Chromosome (NC_003997)	NP_847238.1	240998
49352	G09	BA0830	transcriptional regulator, AsnC family	Chromosome (NC_003997)	NP_843347.1	241008
47795	G10	BA1005	hypothetical protein	Chromosome (NC_003997)	NP_843506.1	239650
49961	G11	BA5238	PAP2 family protein	Chromosome (NC_003997)	NP_847417.1	239661
48280	G12	BA0984	hypothetical protein	Chromosome (NC_003997)	NP_843485.1	239670
49660	H01	BA2708	membrane protein, putative	Chromosome (NC_003997)	NP_845062.1	240936
48109	H02	BA0841	hypothetical protein	Chromosome (NC_003997)	NP_843357.1	240945
48244	H03	BA5065	FeoA family protein	Chromosome (NC_003997)	NP_847256.1	240953
50568	H04	BA5058	lipoprotein, putative	Chromosome (NC_003997)	NP_847249.1	240962
50863	H05	BA0829	transporter, EamA family	Chromosome (NC_003997)	NP_843346.1	240972
48866	H06	BA2698	hypothetical protein	Chromosome (NC_003997)	NP_845054.1	240981
48965	H07	BA0825	hypothetical protein	Chromosome (NC_003997)	NP_843343.1	240989
51804	H08	BA5050	cytochrome d ubiquinol oxidase, subunit I	Chromosome (NC_003997)	NP_847241.1	240999
49403	H09	BA5059	hypothetical protein	Chromosome (NC_003997)	NP_847250.1	241010
49799	H10	BA5202	hypothetical protein	Chromosome (NC_003997)	NP_847385.1	239651
50025	H11	BA5221	ABC transporter, permease protein	Chromosome (NC_003997)	NP_847402.1	239663

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