

Influenza A Gateway® Clone Set, Recombinant in *Escherichia coli*

Catalog No. NR-19436

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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 Influenza A Gateway® Clone Set contains genes from 28 human and avian influenza A strains. Eighty-seven viable clones were constructed in vector pDONR™221. The full annotated coding sequence (CDS) for each genomic segment has been cloned (stop codons removed) and the sequence verified. 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 an *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.

Material Provided:

Each well of the 96-well plate contains approximately 40 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-19436 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 or agar containing 50 µg/mL kanamycin

Incubation:

Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 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 the temperatures indicated above. All clones should be grown for 18 to 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Influenza A Gateway® Clone Set, Recombinant in *Escherichia coli*, NR-19436."

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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Table 1: Influenza A Gateway® Clones

Clone	Well Position	ORF Lengths	Locus ID	Average Depth of Coverage	Class ¹
65691	A01	793	CY021806.1	1.544766709	BLM
63891	A02	1689	CY020517.1	2.251036116	BLM
65845	A03	793	CY020518.1	2	A
65735	A04	697	CY020521.1	2	A
65127	A05	1729	CY020765.1	4.546558704	BLM
65163	A06	1531	CY020768.1	2.948399739	A1
65423	A07	1447	CY020767.1	1.362128542	BSC
65777	A08	727	CY020769.1	2	A
64031	A09	2280	CY020868.1	18.86798246	BLM
66640	A10	2308	CY020867.1	3.055025997	BSC
66366	A11	1735	CY020861.1	3.931988473	BSC
66164	A12	1447	CY020863.1	1.205943331	A1
66126	B01	793	CY020862.1	2	BLM
65017	B02	727	CY020865.1	2	BLM
66119	B03	1447	CY022623.1	1.350380097	BLM
65055	B04	793	CY022622.1	2	A
66323	B06	1723	CY022693.1	4.71677307	BLM
64910	B07	1531	CY022696.1	1.16525147	BLM
65472	B08	793	CY022694.1	2	A
65669	B09	1717	CY024818.1	4.709959231	BLM
65787	B11	793	CY024819.1	1.586380832	BSC
65921	B12	1735	CY017749.1	4.647838617	BLM
66069	C01	1531	CY017752.1	4.828216852	A
65847	C02	1447	CY017751.1	1.309606082	BLM
64965	C03	727	CY017753.1	2	BLM
64121	C04	1701	CY021477.1	11.95002939	BLM
66691	C05	1531	CY021480.1	3.755062051	BLM
65086	C06	793	CY021478.1	2	BSC
66205	C07	727	CY021481.1	2	A
64144	C09	1497	CY015487.1	10.01002004	BSC
66304	C10	1531	CY015487.1	4.70346179	BLM
65033	C11	1456	CY015486.1	4.04532967	A
65840	D01	727	CY015488.1	2	A
63548	D02	1567	CY022720.1	11.07211232	A
65335	D03	1447	CY022719.1	1.362128542	BLM
65441	D05	1444	CY021431.1	1.399584488	BLM
65069	D06	793	CY021430.1	2	BLM
65440	D07	727	CY021433.1	-	A1
63898	D08	2274	CY017281.1	5.461741425	BLM
66028	D09	1735	CY017275.1	4.591354467	BLM
64922	D10	1531	CY017278.1	4.882429784	BLM
65088	D11	793	CY017276.1	1.963430013	BSC
66616	D12	727	CY017279.1	4.206327373	A

Clone	Well Position	ORF Lengths	Locus ID	Average Depth of Coverage	Class ¹
65881	E01	1723	CY021125.1	4.891468369	A
63669	E02	1457	CY021127.1	8.164722032	A
65025	E03	793	CY021126.1	2	A
65863	E04	727	CY021129.1	2	A
64054	E05	2274	CY021627.1	5.045734389	BLM
64999	E06	793	CY021622.1	2	A
65369	E07	727	CY021625.1	2	A
64238	E08	2280	CY020748.1	7.487719298	BLM
63956	E09	1766	CY020741.1	9.879388448	A
65795	E10	1447	CY020743.1	1.362819627	BLM
64963	E11	793	CY020742.1	2	BSC
65344	E12	727	CY020745.1	-	A1
65827	F02	1447	CY020855.1	3.713890809	BSC
65769	F03	793	CY020854.1	2	A
65466	F04	727	CY020857.1	2	A
65019	F06	793	CY020782.1	2	A
65831	F07	727	CY020785.1	2	A
64148	F08	2274	CY020795.1	6.440633245	CHM
65991	F09	1729	CY020789.1	4.652400231	BLM
63808	F10	1555	CY020792.1	9.88681672	A
63843	F11	1413	CY020791.1	20.80820948	BLM
66678	F12	793	CY020790.1	1.868852459	BLM
63948	G02	2280	CY020812.1	9.195614035	BLM
66598	G03	2308	CY020811.1	3.613518198	BLM
65006	G04	793	CY020806.1	2	BLM
65345	G05	727	CY020809.1	2	BLM
64076	G06	2326	CY016649.1	5.793277311	D
65304	G07	1447	CY016645.1	1.317208017	BSC
65779	G08	793	CY016644.1	2	A
65092	G09	727	CY016647.1	2	A
66085	G11	1444	CY016629.1	1.334487535	BLM
65067	G12	793	CY016628.1	2	A
65293	H01	727	CY016631.1	2	BSC
63813	H02	924	CY001425.1	11.49190939	CFC
63908	H03	2280	CY001687.1	7.627631579	BLM
64142	H04	2151	CY001685.1	9.915853092	BLM
63780	H05	1401	CY001682.2	10.5267666	BLM
65793	H06	1531	CY017792.1	1.201175702	BLM
64286	H07	1410	CY017791.1	7.75248227	BLM
65783	H08	793	CY017790.1	2	BSC
65022	H09	727	CY017793.1	2	A
65797	H10	793	CY022646.1	2	BSC
64962	H11	727	CY022649.1	2	A
64275	H12	759	CY012113.1	7.971014493	BSC

¹A: Full-length sequence validation, 2X or greater coverage, 100% sequence identity with the reference ORF.

A1: Full-Length sequence validation - 1X or greater sequence coverage, 100% sequence identity with the reference ORF.

BLM: B class clone with substitutions in CDS only at ≤ 0.2% mutation rate.

BSC: B class clone with substitutions in CDS only leading to silent mutations.

C: Full-length sequence validation - sequence variation (less than 100% sequence identity with the reference ORF); becomes invalid.

CFC: C class clone with frameshift mutations in CDS only.

CHM: C class clone with substitutions in CDS only at >0.2% mutation rate.

D: Partial sequence validation - single contig with missing end-sequence (less than 90% sequence identity with the reference ORF).