

**H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli***

**Catalog No. NR-19271**

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**For research use only. Not for use in humans.**

**Contributor:**

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

**Manufacturer:**

BEI Resources

**Product Description:**

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 does not confirm or validate individual mutants provided by the contributor.

The H1N1pdm09 Gateway® Clone Set contains Influenza A (H1N1)pdm09 open reading frames from two clinical isolates A/New York/1682/2009 (H1N1)pdm09 (CY039901 to CY039908) and A/New York/1669/2009 (H1N1)pdm09 (CY039893 to CY039900) cloned in *Escherichia coli* (*E. coli*) DH10B or Stbl4 cells. The clone set consists of 27 clones 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. The transmembrane regions of the hemagglutinin and neuraminidase coding sequences have been truncated. 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 inoculated well of the 96-well plate contains approximately 60 µL of culture in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

**Packaging/Storage:**

NR-19271 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 or agar containing 50 µg/mL kanamycin

Incubation:

Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C and *E. coli*, strain Stbl4 clones should be grown at 30°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 1 day.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli*, NR-19271.”

**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 \(BMBL\)](#). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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**Table 1: H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli* <sup>1</sup>**

Clone	Well Position	ORF Length	Description	Average Depth of Coverage	Class <sup>2</sup>	<i>E. coli</i> Strain	Growth Temperature
79143	A01	331	CDS(M2) MP-NY1682	5.5	A	DH10B	37°C
79114	A02	733	CDS(NS1) NS-NY1682	7.0	A	DH10B	37°C
79154	A03	933	NS-seg8-NY1682	8.1	CSPT	DH10B	37°C
79094	A04	1070	MP-seg7-NY1682	8.3	CSPT	DH10B	37°C
79177	A05	1366	CDS(-TM) NA-NY1682	8.6	BSC	DH10B	37°C
79147	A06	1450	wholeCDS NA-NY1682	8.2	BSC	DH10B	37°C
79186	A07	1501	NA-seg6-NY1682	8.1	CSPT	DH10B	37°C
79140	A08	2191	CDS PA-NY1682	5.5	A	DH10B	37°C
79179	A09	2320	CDS PB2-NY1682	8.4	BLM	DH10B	37°C
79067	A10	331	CDS(M2) MP-NY1669	5.7	A	DH10B	37°C
79032	A11	406	CDS(NS2) NS-NY1669	5.3	A	DH10B	37°C
79035	A12	1070	MP-seg7-NY1669	8.3	CSPT	DH10B	37°C
79039	B01	1608	NP-seg5-NY1669	8.6	CSPT	DH10B	37°C
79263	B02	406	CDS(NS2) NS-NY1682	5.0	A	DH10B	37°C
79294	B03	799	CDS(M1) MP-NY1682	6.9	A	DH10B	37°C
79302	B04	1821	HA-seg4-NY1682	8.5	CFC	DH10B	37°C
79287	B05	799	CDS(M1) MP-NY1669	6.9	A	DH10B	37°C
79215	B06	933	NS-seg8-NY1669	8.0	CSPT	DH10B	37°C
81821	B07	733	CDS(NS1) NS-NY1682	2.0	A	DH10B	37°C
82466	B08	1608	NP-seg5-NY1682	7.4	CSPT	Stbl4	30°C
82469	B09	1633	CDS(-TM) HA-NY1682	7.7	CFM	Stbl4	30°C
82475	B10	1741	wholeCDS HA-NY1682	6.0	BLM	Stbl4	30°C
82481	B11	2314	CDS PB1-NY1682	5.5	BLM	Stbl4	30°C
82485	B12	733	CDS(NS1) NS-NY1669	5.1	A	Stbl4	30°C
82489	C01	2384	PB2-seg1-NY1682	5.4	CSPT	Stbl4	30°C
82494	C02	1450	wholeCDS NA-NY1669	5.2	A	Stbl4	30°C
82500	C03	1501	NA-seg6-NY1669	6.7	CSPT	Stbl4	30°C

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

<sup>2</sup>**A:** 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).