

**Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 2 (Neuraminidase)**

**Catalog No. NR-45091**

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**For research use only. Not for human use.**

**Contributor and Manufacturer:**

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

**Product Description:**

The Synfluenza clone set is part of a National Institute of Allergy and Infectious Diseases (NIAID) initiative to create 1000 influenza gene segment clones from 12 host subtypes that span the protein sequence diversity of influenza viruses between 2005 and 2010. Each clone is designed from GenBank sequences with consensus untranslated regions. The purpose of the project is to develop the ability to create and stockpile synthetic DNA encoding influenza gene segments. These segments can then be used to generate virus seed stocks and a library of clones for vaccine, diagnostic and basic research.<sup>1</sup>

The NIAID Genome Sequencing Center at the J. Craig Venter Institute constructed synthetic influenza neuraminidase (NA) and hemagglutinin (HA) genes using automated DNA synthesis and assembly. There are nine synthetic NA influenza clone plates (BEI numbers NR-45827 through NR-45833, NR-45090 and NR-45091) and six synthetic HA influenza clone plates (BEI numbers NR-45092 through NR-45097) in the set.

Each synthetic NA gene from NR-45091 was manufactured from five individually-designed, double-stranded DNA construct cassettes produced by assembly of eight chemically-synthesized oligonucleotides using the Gibson Assembly™ process.<sup>2-6</sup> The five cassettes were combined into the pUC19\_CmR (chloramphenicol) vector to establish gene segment clones in Transformax™ EPI300™ competent (Epicentre®) *Escherichia coli* (*E. coli*) cells. Detailed information for each clone on the plate is shown in Table 1.

**Material Provided:**

Each well of the 96-well plate contains approximately 200 µL of *E. coli* culture in Yeast Extract Tryptone media containing 12.5 µg/mL chloramphenicol supplemented with 10% glycerol.

**Note:** 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.

**Packaging/Storage:**

NR-45091 was packaged aseptically in a 96-well plate. The product is provided frozen and should be stored at -60°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:

Yeast Extract Tryptone broth or agar containing 12.5 µg/mL chloramphenicol

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 plate at 37°C for 18 to 24 hours.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 2 (Neuraminidase), NR-45091.”

**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).

**Disclaimers:**

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**References:**

1. D. Wentworth, Personal Communication.
2. Gibson, D. G. et al. "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome." *Science* 329 (2010): 52-56. PubMed: 20488990.

3. Gibson, D. G. et al. "Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases." *Nat. Methods* 6 (2009): 343-345. PubMed: 19363495.
4. Gibson, D. G. et al. "Chemical Synthesis of the Mouse Mitochondrial Genome." *Nat. Methods* 7 (2010): 901-903. PubMed: 20935651.
5. Gibson, D. G. et al. "Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome." *Science* 319 (2008): 1215-1220. PubMed: 18218864.
6. Dormitzer, P. R. et. al. "Synthetic Generation of Influenza Vaccine for Rapid Response to Pandemics." *Sci Transl Med.* 185 (2013): 1-12. PubMed: 23677594.

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**Table 1: Synfluenza Clone Set, Plate 2 (NR-45091)<sup>1</sup>**

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
A01	A/Pennsylvania/05/2007 (H3N2)	HUMAN_H3N2_NA_M000572	EU199416.1	158188233	3953	5'-3'
A02	A/New Hampshire/01/2009 (H3N2)	HUMAN_H3N2_NA_M000705	GQ385888.1	254555008	3953	5'-3'
A03	A/Canada/83/2006 (H3N2)	HUMAN_H3N2_NA_M000811	EU879070.1	194140568	3953	5'-3'
A04	A/Hong Kong/CUHK7546/2005 (H3N2)	HUMAN_H3N2_NA_M000960	EU857369.1	194269620	3953	5'-3'
A05	A/swine/Chonburi/05CB1/2005 (H1N1)	PORCINE_H1N1_NA_M000039	EU296604.1	163676449	3944	5'-3'
A06	A/swine/Tochigi/1/2008 (H1N2)	PORCINE_H1N2_NA_M000053	AB514932.1	291461513	3952	5'-3'
A07	A/swine/Italy/66945/2006 (H3N1)	PORCINE_H3N1_NA_M000002	EU037015.1	152207624	3944	5'-3'
A08	A/swine/Minnesota/578/2007 (H3N2)	PORCINE_H3N2_NA_M000023	FJ519963.1	217384821	3952	5'-3'
A09	A/swine/Guangdong/01/2005 (H3N2)	PORCINE_H3N2_NA_M000053	EF455564.1	149211808	3952	5'-3'
B01	A/Hanoi/ISBM69/2005 (H3N2)	HUMAN_H3N2_NA_M000574	AB281236.1	146197683	3953	5'-3'
B02	A/Thailand/CU-H16/2009 (H3N2)	HUMAN_H3N2_NA_M000709	GU271992.1	270358797	3953	5'-3'
B03	A/Thailand/340/2007 (H3N2)	HUMAN_H3N2_NA_M000812	AB501499.1	261399547	3953	5'-3'
B04	A/California/VRDL169/2009 (H3N2)	HUMAN_H3N2_NA_M000962	CY068195.1	302182536	3953	5'-3'
B05	A/swine/Thailand/CU-RA204/2010 (H1N1)	PORCINE_H1N1_NA_M000081	CY062294.1	295345603	3944	5'-3'
B06	A/swine/Guangxi/13/2006 (H1N2)	PORCINE_H1N2_NA_M000056	EF556200.1	146706474	3952	5'-3'
B07	A/swine/Korea/CY07/2007 (H3N2)	PORCINE_H3N2_NA_M000003	EU798835.1	190403833	3952	5'-3'
B08	A/swine/Minnesota/66743/2006 (H3N2)	PORCINE_H3N2_NA_M000027	FJ519968.1	217384831	3952	5'-3'
B09	A/swine/Korea/CAN04/2005 (H3N2)	PORCINE_H3N2_NA_M000057	EU798830.1	190403823	3952	5'-3'
C01	A/Sendai-H/441/2007 (H3N2)	HUMAN_H3N2_NA_M000576	AB441989.1	213385039	3953	5'-3'
C02	A/Qingdao/1231/2009 (H3N2)	HUMAN_H3N2_NA_M000724	CY050116.1	301638007	3953	5'-3'
C03	A/California/VRDL305/2009 (H3N2)	HUMAN_H3N2_NA_M000824	CY068459.1	302183177	3953	5'-3'
C04	A/California/VRDL335/2009 (H3N2)	HUMAN_H3N2_NA_M000981	CY068611.1	302183913	3953	5'-3'
C05	A/swine/Hong Kong/1562/2005 (H1N2)	PORCINE_H1N2_NA_M000001	GQ229338.1	239618793	3952	5'-3'
C06	A/swine/Hong Kong/2314/2009 (H1N2)	PORCINE_H1N2_NA_M000058	CY061743.1	295424519	3952	5'-3'
C07	A/swine/Nakhon pathom/NIAH586-1/2005 (H3N2)	PORCINE_H3N2_NA_M000005	EU296614.1	163676459	3949	5'-3'
C08	A/swine/British Columbia/28103/2005 (H3N2)	PORCINE_H3N2_NA_M000030	DQ469976.1	94404617	3952	5'-3'
C09	A/swine/Minnesota/5947/2007 (H3N2)	PORCINE_H3N2_NA_M000060	FJ519966.1	217384827	3952	5'-3'
D01	A/Kuwait/AF03/2008 (H3N2)	HUMAN_H3N2_NA_M000593	CY037313.1	224016416	3953	5'-3'
D02	A/Hong Kong/CUHK5567/2005 (H3N2)	HUMAN_H3N2_NA_M000739	EU857339.1	194269560	3953	5'-3'
D03	A/Nagasaki/N07/2005 (H3N2)	HUMAN_H3N2_NA_M000910	AB271004.1	124484547	3953	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
D04	A/Nevada/05/2007 (H3N2)	HUMAN_H3N2_NA_M001018	EU516184.1	168827320	3953	5'-3'
D05	A/swine/Minnesota/44837-3/2009 (H1N2)	PORCINE_H1N2_NA_M000014	HQ424892.1	310693823	3952	5'-3'
D06	A/swine/Guangxi/17/2005 (H1N2)	PORCINE_H1N2_NA_M000062	EF556202.1	146706501	3952	5'-3'
D07	A/swine/Chonburi/05CB2/2005 (H3N2)	PORCINE_H3N2_NA_M000007	EU296622.1	163676467	3949	5'-3'
D08	A/swine/Minnesota/7931/2007 (H3N2)	PORCINE_H3N2_NA_M000032	FJ519967.1	217384829	3952	5'-3'
D09	A/swine/Jilin/37/2008 (H3N2)	PORCINE_H3N2_NA_M000061	GU215036.1	269930174	3952	5'-3'
E01	A/New York/3051/2009 (H3N2)	HUMAN_H3N2_NA_M000627	CY050486.1	268515936	3953	5'-3'
E02	A/Kyrgyzstan/WRAIR1121P/2008 (H3N2)	HUMAN_H3N2_NA_M000746	CY069407.1	302424516	3953	5'-3'
E03	A/Malindi/7579/2008 (H3N2)	HUMAN_H3N2_NA_M000915	HQ214387.1	306494511	3953	5'-3'
E04	A/Texas/12/2007 (H3N2)	HUMAN_H3N2_NA_M001026	EU516020.1	168825197	3953	5'-3'
E05	A/swine/Oklahoma/053259/2008 (H1N2)	PORCINE_H1N2_NA_M000032	CY045545.1	257127169	3952	5'-3'
E06	A/swine/Hainan/1/2005 (H1N2)	PORCINE_H1N2_NA_M000063	EF556204.1	146706526	3952	5'-3'
E07	A/swine/Minnesota/1145/2007 (H3N2)	PORCINE_H3N2_NA_M000008	FJ410142.1	209973707	3952	5'-3'
E08	A/swine/Illinois/53612-3/2009 (H3N2)	PORCINE_H3N2_NA_M000036	HQ734193.1	317015198	3952	5'-3'
E09	A/swine/Ratchaburi/NIAH9426/2005 (H3N2)	PORCINE_H3N2_NA_M000062	EU296620.1	163676465	3952	5'-3'
F01	A/New York/3006/2009 (H3N2)	HUMAN_H3N2_NA_M000628	CY050590.1	268516197	3953	5'-3'
F02	A/Peru/WRAIR1508P/2007 (H3N2)	HUMAN_H3N2_NA_M000755	CY070146.1	302634490	3953	5'-3'
F03	A/Yamagata/K34/2006 (H3N2)	HUMAN_H3N2_NA_M000923	AB271789.1	126364478	3953	5'-3'
F04	A/Cheongju/H397/2008 (H3N2)	HUMAN_H3N2_NA_M001040	FJ009483.1	196481119	3950	5'-3'
F05	A/swine/Italy/116114/2010 (H1N2)	PORCINE_H1N2_NA_M000047	CY067664.1	312839916	3952	5'-3'
F06	A/swine/Hong Kong/294/2009 (H1N2)	PORCINE_H1N2_NA_M000064	GQ229346.1	239618795	3952	5'-3'
F07	A/swine/Oklahoma/011506/2007 (H3N2)	PORCINE_H3N2_NA_M000010	CY045577.1	257127245	3952	5'-3'
F08	A/swine/Oklahoma/008722/2007 (H3N2)	PORCINE_H3N2_NA_M000039	CY045569.1	257127226	3952	5'-3'
G01	A/Miyagi/N1292/2005 (H3N2)	HUMAN_H3N2_NA_M000682	AB271523.1	124484452	3953	5'-3'
G02	A/Texas/12/2008 (H3N2)	HUMAN_H3N2_NA_M000762	FJ532090.1	216960239	3953	5'-3'
G03	A/British Columbia/EFA0401/2009 (H3N2)	HUMAN_H3N2_NA_M000949	CY065754.1	299758552	3953	5'-3'
G05	A/swine/Korea/S5/2005 (H1N2)	PORCINE_H1N2_NA_M000048	DQ666927.1	109501325	3967	5'-3'
G06	A/swine/South Dakota/2/2010 (H1N2)	PORCINE_H1N2_NA_M000065	CY078422.1	315320455	3952	5'-3'
G07	A/swine/Chachoengsao/NIAH586/2005 (H3N2)	PORCINE_H3N2_NA_M000017	EU296612.1	163676457	3952	5'-3'
G08	A/swine/Heilongjiang/10/2007 (H3N2)	PORCINE_H3N2_NA_M000045	HM765434.1	301752279	3952	5'-3'
H01	A/Argentina/135/2005 (H3N2)	HUMAN_H3N2_NA_M000695	EU879077.1	194140582	3953	5'-3'
H02	A/Sendai-H/271/2007 (H3N2)	HUMAN_H3N2_NA_M000802	AB441988.1	213385037	3953	5'-3'
H03	A/Colorado/UR06-0558/2007 (H3N2)	HUMAN_H3N2_NA_M000950	CY026253.1	157368207	3953	5'-3'
H05	A/swine/Korea/S11/2005 (H1N2)	PORCINE_H1N2_NA_M000049	DQ666935.1	109501339	3952	5'-3'
H06	A/swine/Gent/100/2007 (H1N2)	PORCINE_H1N2_NA_M000072	FJ791297.1	224979318	3952	5'-3'
H07	A/swine/Alberta/14722/2005 (H3N2)	PORCINE_H3N2_NA_M000021	DQ469968.1	94404615	3952	5'-3'
H08	A/swine/Jilin/19/2007 (H3N2)	PORCINE_H3N2_NA_M000047	GU215028.1	269930155	3952	5'-3'

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

<sup>2</sup>All clones contain full length inserts, HA inserts are 1716 to 1803 base pairs, NA inserts are 1453 to 1557 base pairs.

<sup>3</sup>Genbank gene ID