Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 8 (Hemagglutinin)

Catalog No. NR-45097

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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.[1]

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 HA gene from NR-45097 was manufactured from seven individually-designed, double-stranded DNA construct cassettes produced by assembly of eight chemically-synthesized oligonucleotides using the Gibson Assembly™ process.[2-6] The seven cassettes were combined into the pSMART®-LCKan vector (Lucigen®) to establish gene segment clones in One Shot® TOP10 competent (Invitrogen™) *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 25 µg/mL kanamycin 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-45097 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 25 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 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 8 (Hemagglutinin), NR-45097."

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/bmbl5/index.htm.

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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.

Support Provided by NIAID

Table 1: HA Synfluenza Clone Set, Plate 8, (NR-45097)[1]

Well	Strain	Clone Name	Locus (CDS)	Gene ID[3]	Vector Total Size	Insert Orientation
A01	A/swine/Illinois/53612-1/2009 (H3N2)	PORCINE_H3N2_HA_M000041:1135630363645	HQ734186.1	317015208	3744	5'-3'
A02	A/swine/Alberta/14722/2005 (H3N2)	PORCINE_H3N2_HA_M000054:1135630363295	DQ469970.1	94404585	3745	3'-5'
A03	A/swine/Italy/50127/2007 (H3N2)	PORCINE_H3N2_HA_M000067:1135630362913	EU982296.1	195957946	3745	3'-5'
B01	A/swine/Minnesota/578/2007 (H3N2)	PORCINE_H3N2_HA_M000044:1135630363613	FJ519974.1	217384843	3745	3'-5'
B02	A/swine/British Columbia/28103/2005 (H3N2)	PORCINE_H3N2_HA_M000056:1135630363247	DQ469978.1	94404587	3744	5'-3'
B03	A/swine/Oklahoma/011506/2007 (H3N2)	PORCINE_H3N2_HA_M000068:1135630362950	CY045575.1	257127241	3745	3'-5'
C01	A/swine/Illinois/53612-4/2009 (H3N2)	PORCINE_H3N2_HA_M000045:1135630363595	HQ734195.1	317015214	3746	3'-5'
C02	A/swine/Quebec/4001/2005 (H3N2)	PORCINE_H3N2_HA_M000058:1135630363212	EU826543.2	239840472	3746	5'-3'
D01	A/swine/Minnesota/761/2007 (H3N2)	PORCINE_H3N2_HA_M000049:1135630363526	FJ519975.1	217384845	3745	3'-5'
D02	A/swine/Minnesota/1300/2007 (H3N2)	PORCINE_H3N2_HA_M000059:1135630363195	HQ315643.1	307940670	3745	3'-5'
E01	A/swine/Oklahoma/001142/2009 (H3N2)	PORCINE_H3N2_HA_M000050:1135630363432	CY045551.1	257127184	3745	3'-5'
E02	A/swine/Minnesota/65767/2006 (H3N2)	PORCINE_H3N2_HA_M000060:1135630362765	FJ519971.1	217384837	3746	5'-3'
F01	A/swine/Kansas/015252/2009 (H3N2)	PORCINE_H3N2_HA_M000051:1135630363418	CY045559.1	257127203	3745	5'-3'
F02	A/swine/Chonburi/05CB2/2005 (H3N2)	PORCINE_H3N2_HA_M000063:1135630362809	EU296621.1	163676491	3745	5'-3'
G01	A/swine/Minnesota/5947/2007 (H3N2)	PORCINE_H3N2_HA_M000052:1135630363360	FJ519976.1	217384847	3744	5'-3'
G02	A/swine/Hungary/13509/2007 (H3N2)	PORCINE_H3N2_HA_M000065:1135630362875	FJ798772.1	224979402	3746	3'-5'
H01	A/swine/Minnesota/1145/2007 (H3N2)	PORCINE_H3N2_HA_M000053:1135630363345	FJ410140.1	209973703	3743	5'-3'
H02	A/swine/Italy/10659-1/2007 (H3N2)	PORCINE_H3N2_HA_M000066:1135630362905	EU982295.1	195957944	3745	5'-3'

[1]All information in this table was provided by J. Craig Venter Institute at the time of deposition.
[2]All clones contain full length inserts, HA inserts are 1716 to 1803 base pairs, NA inserts are 1453 to 1557 base pairs.
[3]Genbank gene ID

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