

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 15 (Neuraminidase)

Catalog No. NR-45833

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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 NA gene from NR-45833 was manufactured from five individually-designed, double-stranded DNA construct cassettes produced by assembly of eight chemically-synthesized oligonucleotides using the Gibson Assembly™ process.^{2,6} The five 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-45833 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 15 (Neuraminidase), NR-45833.”

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.

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 15 (NR-45833)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/Qingdao/1346/2009 (H3N2)	HUMAN_H3N2_NA_M001028:1135661843962	CY050129.1	301638033	3451	5'-3'
A02	A/swine/Ohio/24366/2007 (H1N1)	PORCINE_H1N1_NA_M00022:1135630358999	EU409949.1	188572591	3441	5'-3'
A03	A/swine/Shandong/275/2009 (H1N1)	PORCINE_H1N1_NA_M00055:1135661845154	GU086067.1	261889591	3442	5'-3'
A04	A/swine/Minnesota/SG-00239/2007 (H1N2)	PORCINE_H1N2_NA_M00008:1135630355653	CY041905.1	242397889	3449	3'-5'
A05	A/swine/Doetlingen/IDT4735/2005 (H1N2)	PORCINE_H1N2_NA_M00034:1135661843677	EU053135.1	153957955	3450	3'-5'
A06	A/swine/Shanghai/1/2007 (H1N2)	PORCINE_H1N2_NA_M00057:1135630357344	FJ374514.1	209486611	3452	5'-3'
A07	A/swine/Illinois/53612-2/2009 (H3N2)	PORCINE_H3N2_NA_M00019:1135630358743	HQ734190.1	317015196	3449	3'-5'
A08	A/swine/Guangdong/166/2006 (H3N2)	PORCINE_H3N2_NA_M00048:1135661842682	EU273785.1	162532818	3449	5'-3'
B01	A/Thailand/CU231/2006 (H3N2)	HUMAN_H3N2_NA_M001035:1135661844998	EU021283.1	154224722	3450	3'-5'
B02	A/swine/Ohio/02026/2008 (H1N1)	PORCINE_H1N1_NA_M00023:1135630358978	HM461780.1	304272285	3440	5'-3'
B03	A/swine/Beijing/21/2008 (H1N1)	PORCINE_H1N1_NA_M00059:1135661845230	FJ536763.1	218138407	3441	3'-5'
B04	A/swine/Minnesota/3236/2007 (H1N2)	PORCINE_H1N2_NA_M00009:1135630355675	FJ519969.1	217384833	3449	5'-3'
B05	A/swine/Korea/PZ4/2006 (H1N2)	PORCINE_H1N2_NA_M00035:1135661843740	EU798825.1	190403813	3450	5'-3'
B06	A/swine/Hong Kong/NS1889/2009 (H1N2)	PORCINE_H1N2_NA_M00059:1135661844652	CY061839.1	295424738	3450	5'-3'
B07	A/swine/Minnesota/66960/2006 (H3N2)	PORCINE_H3N2_NA_M00024:1135630357596	FJ519962.1	217384819	3449	3'-5'

Product Information Sheet for NR-45833

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B08	A/swine/Korea/CAS07/2005 (H3N2)	PORCINE_H3N2_NA_M00056:1135661842482	EU798831.1	190403825	3450	5'-3'
C01	A/Thailand/CU46/2006 (H3N2)	HUMAN_H3N2_NA_M001036:1135661845016	EU021269.1	154224708	3450	5'-3'
C02	A/swine/Guangdong/02/2008 (H1N1)	PORCINE_H1N1_NA_M00033:1135661847304	HM223596.1	296882234	3441	3'-5'
C03	A/swine/Shandong/101/2008 (H1N1)	PORCINE_H1N1_NA_M00065:1135661844834	GU086059.1	261889589	3441	5'-3'
C04	A/swine/Saraburi/NIAH13021/2005 (H1N2)	PORCINE_H1N2_NA_M00010:1135661843261	AB434338.1	216409325	3447	3'-5'
C05	A/swine/Korea/PZ7/2006 (H1N2)	PORCINE_H1N2_NA_M00036:1135630356565	EU798826.1	190403815	3449	5'-3'
C06	A/swine/Cloppenburg/IDT4777/2005 (H1N2)	PORCINE_H1N2_NA_M00067:1135661844866	EU053143.1	153958061	3450	3'-5'
C07	A/swine/Minnesota/761/2007 (H3N2)	PORCINE_H3N2_NA_M00028:1135630357827	FJ519964.1	217384823	3449	3'-5'
C08	A/swine/Ratchaburi/NIAH874/2005 (H3N2)	PORCINE_H3N2_NA_M00063:1135661842358	EU296618.1	163676463	3450	3'-5'
D01	A/Thailand/CU228/2006 (H3N2)	HUMAN_H3N2_NA_M001039:1135661845035	EU021275.1	154224714	3449	5'-3'
D02	A/swine/Ratchaburi/NIAH101942/2008 (H1N1)	PORCINE_H1N1_NA_M00035:1135630360011	AB514941.1	291461531	3441	5'-3'
D03	A/swine/Germany/SIV04/2008 (H1N1)	PORCINE_H1N1_NA_M00075:1135661845935	FN429079.1	283481680	3441	3'-5'
D04	A/swine/South Dakota/31813/2009 (H1N2)	PORCINE_H1N2_NA_M00012:1135630356058	GU480921.1	284223817	3449	5'-3'
D05	A/swine/Korea/S14/2006 (H1N2)	PORCINE_H1N2_NA_M00039:1135661843793	DQ666943.1	109501353	3448	3'-5'
D06	A/swine/Kitzen/IDT6142/2007 (H1N2)	PORCINE_H1N2_NA_M00070:1135630357554	GQ161146.1	238057623	3449	3'-5'
D07	A/swine/North Carolina/R08-001877-D08-013371/2008 (H3N2)	PORCINE_H3N2_NA_M00037:1135630358056	CY041849.1	242397782	3449	5'-3'
E01	A/Cheongju/H380/2007 (H3N2)	HUMAN_H3N2_NA_M001044:1135661844788	FJ009478.1	196481109	3450	5'-3'
E02	A/swine/Chonburi/06CB2/2006 (H1N1)	PORCINE_H1N1_NA_M00038:1135630359978	EU296606.1	163676451	3441	5'-3'
E03	A/swine/Hungary/19774/2006 (H1N1)	PORCINE_H1N1_NA_M00076:1135661845775	FJ798780.1	224979383	3441	5'-3'
E04	A/swine/Texas/050593/2008 (H1N2)	PORCINE_H1N2_NA_M00016:1135630356020	CY045529.1	257127131	3449	5'-3'
E05	A/swine/Korea/Asan04/2006 (H1N2)	PORCINE_H1N2_NA_M00042:1135661844107	EU798824.1	190403811	3450	5'-3'
E06	A/swine/Sichuan/01/2006 (H3N2)	PORCINE_H3N2_NA_M00002:1135630358362	EU655690.1	194338963	3448	5'-3'
E07	A/wild boar/Germany/WS169/2006 (H3N2)	PORCINE_H3N2_NA_M00040:1135661845271	AM746617.1	164511470	3450	5'-3'
F01	A/Cheongju/H396/2007 (H3N2)	HUMAN_H3N2_NA_M001047:1135661845477	FJ009482.1	196481102	3450	3'-5'
F02	A/swine/Hong Kong/2433/2009 (H1N1)	PORCINE_H1N1_NA_M00043:1135661847049	CY061823.1	295424700	3441	5'-3'
F03	A/swine/Hubei/S1/2009 (H1N1)	PORCINE_H1N1_NA_M00078:1135661845816	GU121820.1	261889840	3441	3'-5'
F04	A/swine/Oklahoma/010226-16/2008 (H1N2)	PORCINE_H1N2_NA_M00023:1135630356355	CY045593.1	257127283	3449	5'-3'
F05	A/swine/North Carolina/44837-4/2009 (H1N2)	PORCINE_H1N2_NA_M00044:1135661844173	HQ424895.1	310693830	3450	3'-5'
F06	A/swine/Thailand/S1/2005 (H3N2)	PORCINE_H3N2_NA_M00004:1135661845617	FJ688269.1	223056342	3447	5'-3'
F07	A/swine/Damme/IDT5673/2006 (H3N2)	PORCINE_H3N2_NA_M00041:1135630357946	GQ161148.1	238057625	3449	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G01	A/Thailand/CU124/2006 (H3N2)	HUMAN_H3N2_NA_M001048:1135630358122	EU021285.1	154224724	3450	3'-5'
G02	A/swine/Hong Kong/2990/2009 (H1N1)	PORCINE_H1N1_NA_M000047:1135661847034	CY061871.1	295424814	3441	3'-5'
G03	A/swine/Hong Kong/1435/2009 (H1N2)	PORCINE_H1N2_NA_M00002:1135661843075	CY061655.1	295424312	3449	5'-3'
G04	A/swine/Oklahoma/44837-5/2010 (H1N2)	PORCINE_H1N2_NA_M000029:1135630356398	HQ424898.1	310693837	3448	3'-5'
G05	A/swine/Texas/008648/2008 (H1N2)	PORCINE_H1N2_NA_M000045:1135630356989	CY045585.1	257127264	3450	3'-5'
G06	A/swine/Minnesota/66853/2006 (H3N2)	PORCINE_H3N2_NA_M000009:1135630358394	FJ519961.1	217384817	3449	5'-3'
G07	A/swine/Korea/CY04/2007 (H3N2)	PORCINE_H3N2_NA_M000044:1135630354953	EU798833.1	190403829	3449	3'-5'
H01	A/swine/Guangdong/628/2006 (H1N1)	PORCINE_H1N1_NA_M000005:1135661843118	GU086051.1	261889587	3442	3'-5'
H02	A/swine/Hong Kong/1105/2009 (H1N1)	PORCINE_H1N1_NA_M000051:1135630357853	CY061639.1	295424274	3441	3'-5'
H03	A/swine/Guangdong/1222/2006 (H1N2)	PORCINE_H1N2_NA_M000005:1135661843027	GU086083.1	261889595	3450	3'-5'
H04	A/swine/North Carolina/44837-2/2009 (H1N2)	PORCINE_H1N2_NA_M000033:1135630356516	HQ424889.1	310693816	3449	3'-5'
H05	A/swine/Miyazaki/1/2006 (H1N2)	PORCINE_H1N2_NA_M000052:1135630357273	AB441172.1	192806781	3448	5'-3'
H06	A/swine/Guangdong/211/2006 (H3N2)	PORCINE_H3N2_NA_M000012:1135661846250	GU086123.1	261889605	3448	3'-5'
H07	A/swine/Jilin/5/2007 (H3N2)	PORCINE_H3N2_NA_M000046:1135630354849	GU215020.1	269930136	3450	5'-3'

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

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

³Genbank gene ID