

**Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 9 (Neuraminidase)**

**Catalog No. NR-45827**

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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-45827 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 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-45827 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 9 (Neuraminidase), NR-45827.”

**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).

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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 9 (NR-45827)<sup>1</sup>**

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
A01	A/chicken/Eastern China/JX037/2009 (H5N1)	AVIAN_H5N1_NA_M0000 02:1135630356638	HQ185380.1	305430869	3382	3'-5'
A02	A/chicken/Phichit/NIAH606988/2006 (H5N1)	AVIAN_H5N1_NA_M0000 46:1135630359281	AB450614.1	210144878	3381	3'-5'
A03	A/black-necked grebe/Germany/R1393/2007 (H5N1)	AVIAN_H5N1_NA_M0001 04:1135661843131	AM914000.1	219969199	3382	3'-5'
A04	A/chicken/Guangxi/1212/2006 (H5N1)	AVIAN_H5N1_NA_M0001 22:1135630354528	EF124330.1	118584245	3380	5'-3'
A05	A/muscovy duck/Son La/07-85/2007 (H5N1)	AVIAN_H5N1_NA_M0001 51:1135630359809	GU050493.1	267845215	3381	5'-3'
A06	A/duck/Ha Tinh/07-53/2007 (H5N1)	AVIAN_H5N1_NA_M0001 71:1135630357670	GU050430.1	267845199	3382	3'-5'
A07	A/ruddy turnstone/Delaware/103/2007 (H5N1)	AVIAN_H5N1_NA_M0001 89:1135630357869	CY077112.1	312602885	3441	3'-5'
A08	A/chicken/Shanxi/10/2006 (H5N1)	AVIAN_H5N1_NA_M0002 08:1135630356884	HM172172.1	295915804	3382	5'-3'
A09	A/wild duck/Hunan/211/2005 (H5N1)	AVIAN_H5N1_NA_M0002 24:1135630354394	EU329184.1	162296076	3381	3'-5'
A10	A/chicken/Vietnam/P22/2005 (H5N1)	AVIAN_H5N1_NA_M0002 45:1135630355473	AM183679.1	109941949	3382	5'-3'
A11	A/wild bird/Vietnam/434/2005 (H5N1)	AVIAN_H5N1_NA_M0002 70:1135630354767	DQ493054.1	93008534	3380	5'-3'
A12	A/duck/Vietnam/NCVD-16/2007 (H5N1)	AVIAN_H5N1_NA_M0002 89:1135630354422	CY030353.1	211998042	3381	3'-5'
B01	A/chicken/Eastern China/JX036/2009 (H5N1)	AVIAN_H5N1_NA_M0000 03:1135630356655	HQ185379.1	305430867	3381	5'-3'
B02	A/chicken/Yunnan/chuxiong01/2005 (H5N1)	AVIAN_H5N1_NA_M0000 50:1135630358282	EU635875.1	188485743	3382	5'-3'
B03	A/chicken/Laos/16/2008 (H5N1)	AVIAN_H5N1_NA_M0001 07:1135630355485	CY041000.1	238628235	3380	3'-5'
B04	A/chicken/Guangxi/1951/2006 (H5N1)	AVIAN_H5N1_NA_M0001 23:1135630354453	EF124338.1	118584261	3382	3'-5'

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Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
B05	A/chicken/Ha Nam/07-83/2007 (H5N1)	AVIAN_H5N1_NA_M0001 53:1135630359619	GU050477.1	267845211	3381	3'-5'
B06	A/duck/France/05066b/2005 (H5N1)	AVIAN_H5N1_NA_M0001 72:1135630357781	AJ972921.1	145207504	3442	5'-3'
B07	A/duck/Novosibirsk/02/2005 (H5N1)	AVIAN_H5N1_NA_M0001 93:1135630358427	DQ864710.1	112005131	3381	3'-5'
B08	A/chicken/Henan/A-7/2006 (H5N1)	AVIAN_H5N1_NA_M0002 09:1135630356848	HM172201.1	295915862	3381	5'-3'
B09	A/duck/Vietnam/NCVD05/2005 (H5N1)	AVIAN_H5N1_NA_M0002 25:1135630354408	CY034232.1	194589736	3381	3'-5'
B10	A/chicken/Vietnam/29/2007 (H5N1)	AVIAN_H5N1_NA_M0002 47:1135630355572	CY029633.1	172052999	3382	5'-3'
B11	A/duck/Vietnam/557/2005 (H5N1)	AVIAN_H5N1_NA_M0002 76:1135630354872	DQ493058.1	93008542	3380	5'-3'
B12	A/duck/Vietnam/NCVD-6/2007 (H5N1)	AVIAN_H5N1_NA_M0002 97:1135630357470	CY030281.1	211997871	3380	3'-5'
C01	A/duck/Phu Tho/07-48/2007 (H5N1)	AVIAN_H5N1_NA_M0000 06:1135630356763	GU050415.1	267845195	3380	5'-3'
C02	A/duck/Mong Cai/07-58/2007 (H5N1)	AVIAN_H5N1_NA_M0000 51:1135630358315	GU050445.1	267845203	3381	3'-5'
C03	A/peregrine falcon/Hong Kong/2142/2008 (H5N1)	AVIAN_H5N1_NA_M0001 08:1135630355540	CY036271.1	212509389	3380	5'-3'
C04	A/chicken/Vietnam/NCVD-20/2007 (H5N1)	AVIAN_H5N1_NA_M0001 24:1135630354457	CY030385.1	211998118	3380	5'-3'
C05	A/duck/Nghe An/07-49/2007 (H5N1)	AVIAN_H5N1_NA_M0001 55:1135630359658	GU050423.1	267845197	3382	3'-5'
C06	A/peregrine falcon/Hong Kong/810/2009 (H5N1)	AVIAN_H5N1_NA_M0001 79:1135630357492	AB521160.1	258612352	3380	5'-3'
C07	A/chicken/Sudan/2115-12/2006 (H5N1)	AVIAN_H5N1_NA_M0001 94:1135630358471	CY020679.1	133984064	3381	5'-3'
C08	A/chicken/Egypt/06495-NLQP/2006 (H5N1)	AVIAN_H5N1_NA_M0002 15:1135630355019	GQ184252.1	238800789	3382	3'-5'
C09	A/chicken/Korea/IS/2006 (H5N1)	AVIAN_H5N1_NA_M0002 35:1135630356005	EU233677.1	158538817	3382	5'-3'
C10	A/duck/Vietnam/NCVD-18/2007 (H5N1)	AVIAN_H5N1_NA_M0002 49:1135630355699	CY030369.1	211998080	3382	5'-3'
C11	A/duck/Vietnam/286/2005 (H5N1)	AVIAN_H5N1_NA_M0002 77:1135630354878	DQ493046.1	93008518	3382	3'-5'
C12	A/duck/Bac Lieu/07-06/2007 (H5N1)	AVIAN_H5N1_NA_M0002 98:1135630357426	GU186755.1	269315553	3380	3'-5'
D01	A/great crested grebe/Tyva/22/2010 (H5N1)	AVIAN_H5N1_NA_M0000 07:1135630356787	HQ131676.1	304419381	3382	5'-3'
D02	A/goose/Wien/1966/2006 (H5N1)	AVIAN_H5N1_NA_M0000 53:1135630358208	GQ355846.1	254546504	3381	5'-3'
D03	A/maillard/Maryland/802/2007 (H5N1)	AVIAN_H5N1_NA_M0001 10:1135630355066	CY053879.1	282269028	3441	3'-5'
D04	A/duck/Hai Duong/07-40/2007 (H5N1)	AVIAN_H5N1_NA_M0001 35:1135630358888	GU050391.1	267845189	3381	3'-5'
D05	A/muscovy duck/Vietnam/41/2007 (H5N1)	AVIAN_H5N1_NA_M0001 57:1135630359704	CY029681.1	172053113	3380	5'-3'
D06	A/turkey/Virginia/505477-17/2007 (H5N1)	AVIAN_H5N1_NA_M0001 80:1135630358174	GU051959.1	269820113	3378	3'-5'
D07	A/chicken/Domododovo/MK/2007 (H5N1)	AVIAN_H5N1_NA_M0002 01:1135630357074	FJ667187.1	224180928	3381	5'-3'
D08	A/chicken/Shanxi/2/2006 (H5N1)	AVIAN_H5N1_NA_M0002 16:1135630355055	DQ914816.1	117414801	3380	5'-3'
D09	A/chicken/Nam Dinh/07-32/2007 (H5N1)	AVIAN_H5N1_NA_M0002 38:1135630356083	GU050383.1	267845187	3381	5'-3'
D10	A/duck/Vietnam/NCVD-12/2007 (H5N1)	AVIAN_H5N1_NA_M0002 50:1135630355910	CY030329.1	211997985	3382	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
D11	A/chicken/Vietnam/398/2005 (H5N1)	AVIAN_H5N1_NA_M0002 78:1135630354902	DQ493052.1	93008530	3377	5'-3'
D12	A/duck/Bac Lieu/07-09/2007 (H5N1)	AVIAN_H5N1_NA_M0003 00:1135630358524	GU052528.1	269820233	3381	3'-5'
E01	A/teal/Germany/Wv632/2005 (H5N1)	AVIAN_H5N1_NA_M0000 08:1135630356817	CY061887.1	295054822	3442	3'-5'
E02	A/swan/Wien/2323/2006 (H5N1)	AVIAN_H5N1_NA_M0000 54:1135630358232	GQ355861.1	254546534	3381	5'-3'
E03	A/duck/Eastern China/L22/2008 (H5N1)	AVIAN_H5N1_NA_M0001 11:1135630355086	HM370120.1	298353175	3381	5'-3'
E04	A/chicken/Vietnam/NCVD-41/2007 (H5N1)	AVIAN_H5N1_NA_M0001 37:1135630358956	CY030511.1	211998410	3380	3'-5'
E05	A/chicken/Bac Giang/07-74/2007 (H5N1)	AVIAN_H5N1_NA_M0001 67:1135630360089	GU050469.1	267845209	3380	5'-3'
E06	A/mallard/Manitoba/458/2005 (H5N1)	AVIAN_H5N1_NA_M0001 81:1135630358154	EF426681.1	125634634	3441	5'-3'
E07	A/chicken/East Java/UT6016/2006 (H5N1)	AVIAN_H5N1_NA_M0002 02:1135630357092	GQ122499.1	238627452	3381	3'-5'
E08	A/chicken/Ningxia/24/2006 (H5N1)	AVIAN_H5N1_NA_M0002 17:1135630354979	HM172205.1	295915870	3382	3'-5'
E09	A/duck/Vietnam/DT-9/2007 (H5N1)	AVIAN_H5N1_NA_M0002 39:1135630356052	FJ811999.1	225219877	3380	5'-3'
E10	A/chicken/Vietnam/TY9/2005 (H5N1)	AVIAN_H5N1_NA_M0002 54:1135630356039	EU118143.1	156523801	3382	5'-3'
E11	A/duck/Cambodia/D14AL/2006 (H5N1)	AVIAN_H5N1_NA_M0002 79:1135630354914	HQ200451.1	306493953	3382	5'-3'
E12	A/chicken/Bac Lieu/07-10/2007 (H5N1)	AVIAN_H5N1_NA_M0003 02:1135630358556	GU186763.1	269315551	3381	3'-5'
F01	A/chicken/Thailand/CU-353/2008 (H5N1)	AVIAN_H5N1_NA_M0000 18:1135630359679	CY047453.1	261254160	3382	5'-3'
F02	A/swan/Bavaria/14/2006 (H5N1)	AVIAN_H5N1_NA_M0000 65:1135630358689	EF165580.1	119352665	3382	3'-5'
F03	A/duck/Yunnan/5820/2005 (H5N1)	AVIAN_H5N1_NA_M0001 12:1135630355033	EF124214.1	118584013	3382	5'-3'
F04	A/muscovy duck/Bac Ninh/07-69/2007 (H5N1)	AVIAN_H5N1_NA_M0001 38:1135630358864	GU050351.1	267845179	3380	3'-5'
F05	A/muscovy duck/Bac Gieng/07-66/2007 (H5N1)	AVIAN_H5N1_NA_M0001 68:1135630360057	GU050453.1	267845205	3380	5'-3'
F06	A/mallard/Ontario/499/2005 (H5N1)	AVIAN_H5N1_NA_M0001 83:1135630358121	EF564746.1	146746669	3441	5'-3'
F07	A/duck/Eastern China/89/2005 (H5N1)	AVIAN_H5N1_NA_M0002 05:1135630356943	EU429765.1	167859495	3381	3'-5'
F08	A/goose/Shantou/3624/2006 (H5N1)	AVIAN_H5N1_NA_M0002 20:1135630354321	EF124325.1	118584235	3441	3'-5'
F09	A/muscovy duck/Vietnam/NCVD-29/2007 (H5N1)	AVIAN_H5N1_NA_M0002 41:1135630355638	CY030440.1	211998251	3381	5'-3'
F10	A/chicken/Vietnam/11/2005 (H5N1)	AVIAN_H5N1_NA_M0002 65:1135630355623	CY016877.1	115952224	3381	3'-5'
F11	A/chicken/Vietnam/NCVD11/2005 (H5N1)	AVIAN_H5N1_NA_M0002 80:1135630354266	CY036721.1	218663929	3381	3'-5'
F12	A/duck/Vietnam/6/2007 (H5N1)	AVIAN_H5N1_NA_M0003 04:1135630358592	CY029593.1	172052904	3380	5'-3'
G01	A/open-billed stork/Nakhonsawan/BBA2611M/2005 (H5N1)	AVIAN_H5N1_NA_M0000 20:1135630359997	EF112332.1	132653854	3382	3'-5'
G02	A/buzzard/Bavaria/5/2006 (H5N1)	AVIAN_H5N1_NA_M0000 66:1135630358677	EF165572.1	119352649	3380	5'-3'
G03	A/duck/Yunnan/6607/2005 (H5N1)	AVIAN_H5N1_NA_M0001 14:1135630354997	EF124216.1	118584017	3382	3'-5'
G04	A/muscovy duck/Vietnam/NCVD-22/2007 (H5N1)	AVIAN_H5N1_NA_M0001 41:1135630359323	CY030401.1	211998156	3382	5'-3'

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Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
G05	A/duck/Thai Ninh/07-86/2007 (H5N1)	AVIAN_H5N1_NA_M0001 69:1135630360028	GU050501.1	267845217	3381	5'-3'
G06	A/duck/Pennsylvania/454069/2005 (H5N1)	AVIAN_H5N1_NA_M0001 86:1135630357918	EF607896.1	156777920	3441	5'-3'
G07	A/goose/Shantou/1621/2005 (H5N1)	AVIAN_H5N1_NA_M0002 06:1135630357060	DQ095668.1	70905384	3382	3'-5'
G08	A/duck/Hunan/689/2006 (H5N1)	AVIAN_H5N1_NA_M0002 21:1135630354364	FJ784761.1	224181330	3381	5'-3'
G09	A/duck/Vietnam/NCVD-43/2007 (H5N1)	AVIAN_H5N1_NA_M0002 43:113563035688	CY030525.1	211998446	3381	3'-5'
G10	A/muscovy duck/Vietnam/33/2007 (H5N1)	AVIAN_H5N1_NA_M0002 66:1135630355516	CY029641.1	172053018	3381	3'-5'
G11	A/duck/Vinh Long/07-26/2007 (H5N1)	AVIAN_H5N1_NA_M0002 81:1135630354247	GU050367.1	267845183	3382	5'-3'
G12	A/duck/Can Tho/07-28/2007 (H5N1)	AVIAN_H5N1_NA_M0003 05:1135630358627	GU050375.1	267845185	3381	3'-5'
H01	A/chicken/Thailand/ICRC-V143/2007 (H5N1)	AVIAN_H5N1_NA_M0000 43:1135630359241	EU233418.1	159139253	3380	3'-5'
H02	A/tufted duck/Bavaria/26/2006 (H5N1)	AVIAN_H5N1_NA_M0000 68:1135630358607	GU046713.1	260677721	3381	3'-5'
H03	A/duck/Yunnan/4873/2006 (H5N1)	AVIAN_H5N1_NA_M0001 17:1135630354936	CY030883.1	198285691	3381	5'-3'
H04	A/chicken/Guangxi/463/2006 (H5N1)	AVIAN_H5N1_NA_M0001 49:1135630359288	EF124272.1	118584129	3381	3'-5'
H05	A/duck/Vietnam/43/2007 (H5N1)	AVIAN_H5N1_NA_M0001 70:1135630357691	CY029689.1	172053132	3380	3'-5'
H06	A/ruddy turnstone/Delaware/509531/2007 (H5N1)	AVIAN_H5N1_NA_M0001 87:1135630357890	GU186519.1	269315609	3441	3'-5'
H07	A/chicken/Liaoning/A-11/2006 (H5N1)	AVIAN_H5N1_NA_M0002 07:1135630356999	HM172196.1	295915852	3381	3'-5'
H08	A/chicken/West Bengal/80995/2008 (H5N1)	AVIAN_H5N1_NA_M0002 23:1135630354379	EU871812.2	311355706	3381	3'-5'
H09	A/duck/Cambodia/D1KC/2006 (H5N1)	AVIAN_H5N1_NA_M0002 44:1135630355456	HQ200594.1	306493919	3380	5'-3'
H10	A/duck/Vietnam/376/2005 (H5N1)	AVIAN_H5N1_NA_M0002 67:1135630355566	DQ493053.1	93008532	3382	5'-3'
H11	A/muscovy duck/Ca Mau/07-04/2007 (H5N1)	AVIAN_H5N1_NA_M0002 84:1135630354337	GU186747.1	269315555	3380	5'-3'
H12	A/duck/Vietnam/37/2007 (H5N1)	AVIAN_H5N1_NA_M0003 06:1135630358644	CY029657.1	172053056	3381	3'-5'

<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