

**Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 11 (Neuraminidase)**

**Catalog No. NR-45829**

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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-45829 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-45829 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 11 (Neuraminidase), NR-45829.”

**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](http://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 11 (NR-45829)<sup>1</sup>**

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
A01	A/chicken/Gunung Kidal/BBVW/2005 (H5N1)	AVIAN_H5N1_NA_M0006 38:1135630355304	DQ493000.1	93008426	3381	3'-5'
A02	A/great egret/Hong Kong/807/2008 (H5N1)	AVIAN_H5N1_NA_M0006 68:1135630359378	CY036239.1	212508782	3381	3'-5'
A03	A/chicken/Banten/UT6025/2006 (H5N1)	AVIAN_H5N1_NA_M0006 80:1135630360199	GQ122517.1	238627458	3380	3'-5'
A04	A/chicken/Laos/31/2006 (H5N1)	AVIAN_H5N1_NA_M0007 00:1135630356129	CY041046.1	238628344	3380	5'-3'
A05	A/grebe/Tyva/2/2010 (H5N1)	AVIAN_H5N1_NA_M0007 16:1135630357170	HQ630837.1	312435232	3381	5'-3'
A06	A/duck/Hunan/1964/2007 (H5N1)	AVIAN_H5N1_NA_M0007 50:1135630355829	FJ784769.1	224181346	3381	3'-5'
A07	A/mallard/Hokkaido/24/2009 (H5N1)	AVIAN_H5N1_NA_M0007 63:1135630355272	AB530994.1	264160596	3440	3'-5'
A08	A/duck/Guiyang/3834/2005 (H5N1)	AVIAN_H5N1_NA_M0007 81:1135630359125	EF124302.1	118584189	3380	3'-5'
A09	A/chicken/Hubei/2856/2007 (H5N1)	AVIAN_H5N1_NA_M0007 94:1135630360179	FJ784771.1	224181350	3380	3'-5'
A10	A/duck/Eastern China/51/2005 (H5N1)	AVIAN_H5N1_NA_M0008 09:1135630357276	EU429763.1	167859491	3382	5'-3'
A11	A/chicken/Laos/1/2008 (H5N1)	AVIAN_H5N1_NA_M0008 20:1135630356753	AB435523.1	189046950	3381	3'-5'
A12	A/crested myna/Hong Kong/1178/2009 (H5N1)	AVIAN_H5N1_NA_M0008 39:1135630356317	AB521164.1	258612360	3381	5'-3'
B01	A/chicken/Egypt/0841-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0006 39:1135630355338	GQ184269.1	238800823	3381	5'-3'
B02	A/little egret/Hong Kong/8863/2007 (H5N1)	AVIAN_H5N1_NA_M0006 69:1135630359396	CY036207.1	212508059	3381	3'-5'
B03	A/chicken/South Kalimantan/UT6028/2006 (H5N1)	AVIAN_H5N1_NA_M0006 83:1135630360204	GQ122573.1	238627474	3381	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
B04	A/duck/Laos/P0127/2007 (H5N1)	AVIAN_H5N1_NA_M0007 01:1135630356167	CY040912.1	238628026	3380	5'-3'
B05	A/chicken/Primorje/1/2008 (H5N1)	AVIAN_H5N1_NA_M0007 20:1135630356847	EU672460.1	188526736	3381	5'-3'
B06	A/lesser kestrel/Heilongjiang/194/2007 (H5N1)	AVIAN_H5N1_NA_M0007 51:1135630355808	GU220788.1	269930243	3380	5'-3'
B07	A/goose/Yunnan/5540/2006 (H5N1)	AVIAN_H5N1_NA_M0007 67:1135630355364	CY030947.1	198285843	3381	5'-3'
B08	A/chicken/Anhui/1089/2007 (H5N1)	AVIAN_H5N1_NA_M0007 82:1135630359153	FJ784762.1	224181332	3381	3'-5'
B09	A/pheasant/Shantou/2239/2006 (H5N1)	AVIAN_H5N1_NA_M0008 00:1135630358103	EF124212.1	118584009	3381	5'-3'
B10	A/mallard/Huadong/S/2005 (H5N1)	AVIAN_H5N1_NA_M0008 10:1135630357259	EU195394.1	158144815	3382	3'-5'
B11	A/chicken/Guiyang/237/2006 (H5N1)	AVIAN_H5N1_NA_M0008 25:1135630356678	EF124326.1	118584237	3381	5'-3'
B12	A/feral pigeon/Hong Kong/3409/2009 (H5N1)	AVIAN_H5N1_NA_M0008 40:1135630356176	AB557630.1	294831532	3381	5'-3'
C01	A/chicken/Guangxi/683/2006 (H5N1)	AVIAN_H5N1_NA_M0006 41:1135630355710	EF124274.1	118584133	3381	3'-5'
C02	A/little egret/Hong Kong/8550/2007 (H5N1)	AVIAN_H5N1_NA_M0006 70:1135630359929	CY036199.1	212507655	3381	5'-3'
C03	A/pigeon/Laos/NCVD-36/2007 (H5N1)	AVIAN_H5N1_NA_M0006 89:1135630360108	CY030479.1	211998340	3382	3'-5'
C04	A/chicken/Laos/P0072/2007 (H5N1)	AVIAN_H5N1_NA_M0007 02:1135630356205	CY040905.1	238628010	3379	5'-3'
C05	A/duck/Hunan/8/2008 (H5N1)	AVIAN_H5N1_NA_M0007 42:1135630354069	GU182168.1	268527122	3380	3'-5'
C06	A/chicken/Hubei/3002/2007 (H5N1)	AVIAN_H5N1_NA_M0007 53:1135630355752	FJ784773.1	224181354	3381	5'-3'
C07	A/chicken/Yunnan/493/2005 (H5N1)	AVIAN_H5N1_NA_M0007 72:1135630359505	DQ095665.1	70905378	3381	5'-3'
C08	A/duck/Eastern China/37/2006 (H5N1)	AVIAN_H5N1_NA_M0007 83:1135630359120	EU429778.1	167859521	3381	5'-3'
C09	A/goose/Guiyang/4180/2005 (H5N1)	AVIAN_H5N1_NA_M0008 01:1135630358079	EF124328.1	118584241	3381	5'-3'
C10	A/duck/Eastern China/40/2005 (H5N1)	AVIAN_H5N1_NA_M0008 11:1135630357223	EU429757.1	167859479	3381	5'-3'
C11	A/chicken/West Bengal/239020/2010 (H5N1)	AVIAN_H5N1_NA_M0008 26:1135630356700	CY061296.1	309354818	3381	5'-3'
C12	A/oriental magpie robin/Hong Kong/9298/2009 (H5N1)	AVIAN_H5N1_NA_M0008 41:1135630356186	AB557634.1	294831540	3381	5'-3'
D01	A/muscovy duck/Vietnam/54/2007 (H5N1)	AVIAN_H5N1_NA_M0006 44:1135630355968	CY029745.1	172053265	3380	3'-5'
D02	A/duck/Yunnan/6490/2006 (H5N1)	AVIAN_H5N1_NA_M0006 72:1135630359907	CY030899.1	198285729	3380	3'-5'
D03	A/duck/Laos/P0117/2007 (H5N1)	AVIAN_H5N1_NA_M0006 90:1135630357521	CY040920.1	238628045	3380	3'-5'
D04	A/duck/Laos/A0617/2007 (H5N1)	AVIAN_H5N1_NA_M0007 04:1135630356098	CY040960.1	238628140	3382	3'-5'
D05	A/chicken/Hunan/8/2008 (H5N1)	AVIAN_H5N1_NA_M0007 43:1135630354204	GU182160.1	268527124	3380	5'-3'
D06	A/duck/Hubei/2911/2007 (H5N1)	AVIAN_H5N1_NA_M0007 54:1135630355930	FJ784772.1	224181352	3382	3'-5'
D07	A/duck/Hunan/344/2006 (H5N1)	AVIAN_H5N1_NA_M0007 73:1135630359541	EF124254.1	118584093	3380	5'-3'
D08	A/duck/Eastern China/48/2006 (H5N1)	AVIAN_H5N1_NA_M0007 85:1135630359077	EU429779.1	167859523	3381	3'-5'

Product Information Sheet for NR-45829

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
D09	A/chicken/Guiyang/3055/2005 (H5N1)	AVIAN_H5N1_NA_M0008 02:1135630358067	EF124305.1	118584195	3380	3'-5'
D10	A/mallard/Huadong/lk/2005 (H5N1)	AVIAN_H5N1_NA_M0008 12:1135630357239	EU195402.1	158144833	3380	3'-5'
D11	A/chicken/India/WB-NIV96526/2009 (H5N1)	AVIAN_H5N1_NA_M0008 27:1135630356713	GQ917230.1	259023903	3381	5'-3'
D12	A/chicken/Nigeria/OG2/2007 (H5N1)	AVIAN_H5N1_NA_M0008 42:1135630356241	FM164816.1	194719719	3381	3'-5'
E01	A/duck/Hai Phong /208/2006 (H5N1)	AVIAN_H5N1_NA_M0006 47:1135630355949	GU052504.1	269820227	3382	3'-5'
E02	A/goose/Yunnan/6193/2006 (H5N1)	AVIAN_H5N1_NA_M0006 73:1135630359887	CY030979.1	198285919	3380	5'-3'
E03	A/chicken/Laos/P0169/2007 (H5N1)	AVIAN_H5N1_NA_M0006 91:1135630357601	CY034714.1	199580493	3380	3'-5'
E04	A/chicken/Laos/A0464/2007 (H5N1)	AVIAN_H5N1_NA_M0007 06:1135630356115	CY040944.1	238628102	3380	3'-5'
E05	A/chicken/Kulon Progo/BBVW/2005 (H5N1)	AVIAN_H5N1_NA_M0007 44:1135630354176	DQ493001.1	93008428	3381	3'-5'
E06	A/duck/Henan/1647/2006 (H5N1)	AVIAN_H5N1_NA_M0007 55:1135630355896	FJ784764.1	224181336	3381	3'-5'
E07	A/duck/Eastern China/JS017/2009 (H5N1)	AVIAN_H5N1_NA_M0007 76:1135630359398	HQ185383.1	305430875	3380	5'-3'
E08	A/duck/Fujian/1734/2005 (H5N1)	AVIAN_H5N1_NA_M0007 86:1135630359105	DQ095669.2	171473784	3380	3'-5'
E09	A/duck/Hunan/29/2006 (H5N1)	AVIAN_H5N1_NA_M0008 05:1135630358015	HM172177.1	295915814	3381	5'-3'
E10	A/chicken/Guiyang/1655/2006 (H5N1)	AVIAN_H5N1_NA_M0008 16:1135630357205	EF124327.1	118584239	3381	3'-5'
E11	A/quail/Shantou/911/2005 (H5N1)	AVIAN_H5N1_NA_M0008 29:1135630356538	DQ095667.1	70905382	3381	3'-5'
E12	A/duck/Eastern China/22/2005 (H5N1)	AVIAN_H5N1_NA_M0008 43:1135630356259	EU429783.1	167859531	3380	3'-5'
F01	A/chicken/Lang Son/200/2005 (H5N1)	AVIAN_H5N1_NA_M0006 51:1135630359194	GU186718.1	269315561	3380	3'-5'
F02	A/muscovy duck/Vietnam/NCVD-46/2007 (H5N1)	AVIAN_H5N1_NA_M0006 75:1135630359845	CY030546.1	211998503	3381	3'-5'
F03	A/chicken/Laos/P0171/2007 (H5N1)	AVIAN_H5N1_NA_M0006 92:1135630357582	CY034722.1	199580512	3381	5'-3'
F04	A/chicken/Laos/P0130/2007 (H5N1)	AVIAN_H5N1_NA_M0007 09:1135630357396	CY040976.1	238628178	3381	5'-3'
F05	A/goose/Shantou/18442/2005 (H5N1)	AVIAN_H5N1_NA_M0007 46:1135630354226	EF124244.1	118584073	3381	3'-5'
F06	A/chicken/Tarutung/BPPVI/2005 (H5N1)	AVIAN_H5N1_NA_M0007 58:1135630355993	DQ493017.1	93008460	3381	5'-3'
F07	A/chicken/Eastern China/XH222/2008 (H5N1)	AVIAN_H5N1_NA_M0007 77:1135630359440	HQ185381.1	305430871	3380	5'-3'
F08	A/duck/Guiyang/3996/2005 (H5N1)	AVIAN_H5N1_NA_M0007 88:1135630359045	EF124303.1	118584191	3381	3'-5'
F09	A/goose/Hubei/65/2005 (H5N1)	AVIAN_H5N1_NA_M0008 06:1135630357377	HM172168.1	295915796	3381	5'-3'
F10	A/quail/Vietnam/15/2005 (H5N1)	AVIAN_H5N1_NA_M0008 17:1135630356957	CY017053.1	116070388	3380	5'-3'
F11	A/chicken/Shantou/810/2005 (H5N1)	AVIAN_H5N1_NA_M0008 30:1135630356410	DQ095666.1	70905380	3381	5'-3'
F12	A/chicken/Surat/Gujrat/India/9257/2006 (H5N1)	AVIAN_H5N1_NA_M0008 44:1135630356283	EU189941.1	158347989	3381	5'-3'
G01	A/muscovy duck/Vietnam/211/2005 (H5N1)	AVIAN_H5N1_NA_M0006 54:1135630359179	EU930966.1	195934308	3381	5'-3'
G02	A/muscovy duck/Vietnam/39/2007 (H5N1)	AVIAN_H5N1_NA_M0006 76:1135630359862	CY029673.1	172053094	3382	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
G03	A/chicken/Laos/NCVD-38/2007 (H5N1)	AVIAN_H5N1_NA_M0006 98:1135630357646	CY030487.1	211998355	3381	3'-5'
G04	A/duck/Vietnam/8/2005 (H5N1)	AVIAN_H5N1_NA_M0007 10:1135630357135	DQ366324.1	86753779	3385	3'-5'
G05	A/scaly-breasted munia/Hong Kong/45/2007 (H5N1)	AVIAN_H5N1_NA_M0007 47:1135630354129	CY036055.1	212504947	3382	3'-5'
G06	A/duck/Hunan/11/2007 (H5N1)	AVIAN_H5N1_NA_M0007 61:1135630355242	HM172203.1	295915866	3380	5'-3'
G07	A/goose/Guangxi/532/2006 (H5N1)	AVIAN_H5N1_NA_M0007 78:1135630359463	EF124273.1	118584131	3381	5'-3'
G08	A/duck/Hubei/Hangmei01/2006 (H5N1)	AVIAN_H5N1_NA_M0007 89:1135630359035	EU594351.1	193227291	3380	3'-5'
G09	A/long-tailed shrike/Hong Kong/2762/2007 (H5N1)	AVIAN_H5N1_NA_M0008 07:1135630357324	CY036159.1	212506710	3381	5'-3'
G10	A/chicken/Vietnam/TY25/2005 (H5N1)	AVIAN_H5N1_NA_M0008 18:1135630356920	EU118144.1	156523803	3381	3'-5'
G11	A/duck/Cambodia/D1KP/2006 (H5N1)	AVIAN_H5N1_NA_M0008 32:1135630356500	HQ200592.1	306493921	3382	5'-3'
G12	A/chicken/Hunan/21/2005 (H5N1)	AVIAN_H5N1_NA_M0008 45:1135630356291	HM172170.1	295915800	3381	3'-5'
H01	A/goose/Guiyang/1636/2006 (H5N1)	AVIAN_H5N1_NA_M0006 66:1135630359354	EF124319.1	118584223	3381	5'-3'
H02	A/duck/Vietnam/205/2005 (H5N1)	AVIAN_H5N1_NA_M0006 77:1135630359771	EU930918.1	195934422	3380	5'-3'
H03	A/duck/Laos/22/2006 (H5N1)	AVIAN_H5N1_NA_M0006 99:1135630357774	CY041044.1	238628340	3382	5'-3'
H04	A/chicken/Laos/P0001/2007 (H5N1)	AVIAN_H5N1_NA_M0007 13:1135630357117	CY040894.1	238627984	3380	5'-3'
H05	A/duck/Hunan/856/2006 (H5N1)	AVIAN_H5N1_NA_M0007 48:1135630354095	EF124255.1	118584095	3380	5'-3'
H06	A/mallard/Italy/3401/2005 (H5N1)	AVIAN_H5N1_NA_M0007 62:1135630355225	CY021399.1	134048377	3441	5'-3'
H07	A/chicken/Malaysia/5223/2007 (H5N1)	AVIAN_H5N1_NA_M0007 79:1135630359491	CY029777.1	166782745	3382	3'-5'
H08	A/chicken/Henan/1362/2006 (H5N1)	AVIAN_H5N1_NA_M0007 93:1135630360144	FJ784763.1	224181334	3381	5'-3'
H09	A/duck/Anhui/56/2005 (H5N1)	AVIAN_H5N1_NA_M0008 08:1135630357312	HM172175.1	295915810	3380	5'-3'
H10	A/duck/Yunnan/47/2006 (H5N1)	AVIAN_H5N1_NA_M0008 19:1135630357052	HM172186.1	295915832	3381	5'-3'
H11	A/grey heron/Hong Kong/779/2009 (H5N1)	AVIAN_H5N1_NA_M0008 34:1135630356441	AB517664.1	256403014	3381	5'-3'
H12	A/chicken/Thailand/ICRC-V586/2008 (H5N1)	AVIAN_H5N1_NA_M0008 48:1135630356316	EU669199.1	190195373	3387	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