

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 10 (Neuraminidase)

Catalog No. NR-45828

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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-45828 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-45828 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 10 (Neuraminidase), NR-45828.”

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 10 (NR-45828)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/duck/Vietnam/8/2007 (H5N1)	AVIAN_H5N1_NA_M0003 07:1135630358662	CY029609.1	172052942	3381	3'-5'
A02	A/open-billed stork/Nakhonsawan/BBD3516M/2005 (H5N1)	AVIAN_H5N1_NA_M0003 34:1135630359714	EF112342.1	132653874	3380	5'-3'
A03	A/chicken/Egypt/12378N3-CLEVB/2006 (H5N1)	AVIAN_H5N1_NA_M0003 62:1135661846662	EF486241.1	134037017	3381	3'-5'
A04	A/Cygnus olor/Italy/742/2006 (H5N1)	AVIAN_H5N1_NA_M0003 88:1135630354506	DQ533581.1	95020619	3382	5'-3'
A05	A/chicken/Reshoty/02/2006 (H5N1)	AVIAN_H5N1_NA_M0004 61:1135630359226	CY047485.1	261278962	3382	3'-5'
A06	A/wild duck/Bavaria/13/2007 (H5N1)	AVIAN_H5N1_NA_M0004 76:1135661846964	GU046777.1	260677781	3381	5'-3'
A07	A/chicken/Egypt/1889N3-SM26/2007 (H5N1)	AVIAN_H5N1_NA_M0005 26:1135630354614	EF486243.1	134037021	3382	5'-3'
A08	A/chicken/Kuwait/KISR2/2007 (H5N1)	AVIAN_H5N1_NA_M0005 54:1135661846410	CY029950.1	168203440	3381	5'-3'
A09	A/chicken/Hebei/126/2005 (H5N1)	AVIAN_H5N1_NA_M0005 71:1135630358399	EU243145.1	159895918	3381	5'-3'
A10	A/peacock/Chakshahzad/NARC9238/2007 (H5N1)	AVIAN_H5N1_NA_M0005 90:1135661844699	CY034732.1	199580419	3381	3'-5'
A11	A/chicken/Vietnam/6/2005 (H5N1)	AVIAN_H5N1_NA_M0006 01:1135630357195	CY016845.1	115953003	3381	3'-5'
A12	A/chicken/Simalanggang/BPPVI/2005 (H5N1)	AVIAN_H5N1_NA_M0006 21:1135630354603	DQ493013.1	93008452	3381	5'-3'
B01	A/chicken/Cambodia/047LC4/2005 (H5N1)	AVIAN_H5N1_NA_M0003 12:1135630357478	HQ200563.1	306493929	3381	5'-3'
B02	A/duck/Schaerding/1398/2006 (H5N1)	AVIAN_H5N1_NA_M0003 39:1135661846951	GQ355842.1	254546496	3382	3'-5'
B03	A/chicken/Egypt/08124S-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0003 63:1135630359335	GQ184285.1	238800855	3381	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B04	A/buzzard/Denmark/6370/2006 (H5N1)	AVIAN_H5N1_NA_M0004 27:1135661845077	EF523697.1	149999416	3381	5'-3'
B05	A/bar-headed goose/Mongolia/1/2005 (H5N1)	AVIAN_H5N1_NA_M0004 62:1135661846544	AB239304.1	81687146	3382	3'-5'
B06	A/chicken/Rostov-on-Don/35/2007 (H5N1)	AVIAN_H5N1_NA_M0004 81:1135630360166	EU401753.1	166798263	3382	3'-5'
B07	A/chicken/Egypt/1129N3-HK9/2007 (H5N1)	AVIAN_H5N1_NA_M0005 27:1135630354580	EF486240.1	134037015	3381	3'-5'
B08	A/great crested grebe/Germany/R1406/2007 (H5N1)	AVIAN_H5N1_NA_M0005 57:1135630359212	AM914013.1	219969225	3381	5'-3'
B09	A/grebe/Novosibirsk/29/2005 (H5N1)	AVIAN_H5N1_NA_M0005 72:1135661845714	DQ230523.1	78450389	3381	3'-5'
B10	A/chicken/India/WB-NIV16915/2008 (H5N1)	AVIAN_H5N1_NA_M0005 91:1135630357503	GQ917226.1	259023895	3381	5'-3'
B11	A/chicken/West Bengal/155505/2009 (H5N1)	AVIAN_H5N1_NA_M0006 03:1135630357182	GU272000.1	270358870	3381	5'-3'
B12	A/chicken/Purworejo/BBVW/2005 (H5N1)	AVIAN_H5N1_NA_M0006 22:1135630354726	DQ492997.1	93008420	3381	5'-3'
C01	A/duck/Vietnam/2B-TV/2008 (H5N1)	AVIAN_H5N1_NA_M0003 19:1135630357514	FJ812003.1	225219885	3382	3'-5'
C02	A/turkey/Islamabad-Pakistan/NARC-7871/02/2007 (H5N1)	AVIAN_H5N1_NA_M0003 41:1135661847332	EU401797.1	166239967	3381	3'-5'
C03	A/chicken/Krasnodar/01/2006 (H5N1)	AVIAN_H5N1_NA_M0003 64:1135630359307	DQ676836.2	115520953	3381	5'-3'
C04	A/whooper swan/Mongolia/3/2005 (H5N1)	AVIAN_H5N1_NA_M0004 28:1135661845105	AB239311.1	81687174	3381	5'-3'
C05	A/grey lag goose/Denmark/6692/2006 (H5N1)	AVIAN_H5N1_NA_M0004 66:1135661846571	EF523698.1	149999418	3382	5'-3'
C06	A/goose/Yunnan/3644/2005 (H5N1)	AVIAN_H5N1_NA_M0004 85:1135630359971	EF124329.1	118584243	3381	5'-3'
C07	A/chicken/Egypt/0891/2008 (H5N1)	AVIAN_H5N1_NA_M0005 30:1135630359786	CY041300.1	238821997	3381	3'-5'
C08	A/common pochard/Switzerland/WV4080110/2008 (H5N1)	AVIAN_H5N1_NA_M0005 62:1135661846771	GU563331.1	284795144	3382	5'-3'
C09	A/chicken/Egypt/1709-5/2008 (H5N1)	AVIAN_H5N1_NA_M0005 75:1135630358455	EU717856.1	188572137	3382	3'-5'
C10	A/bar-headed goose/Qinghai/1-HVRI/2006 (H5N1)	AVIAN_H5N1_NA_M0005 93:1135630357840	HM172183.1	295915826	3382	5'-3'
C11	A/chicken/West Bengal/100879/2008 (H5N1)	AVIAN_H5N1_NA_M0006 08:1135630354307	GU083639.1	261890026	3380	5'-3'
C12	A/chicken/Central Java/UT3091/2005 (H5N1)	AVIAN_H5N1_NA_M0006 23:1135630354691	GQ122493.1	238627450	3380	5'-3'
D01	A/duck/Vietnam/5/2007 (H5N1)	AVIAN_H5N1_NA_M0003 26:1135630357992	CY029585.1	172052885	3380	5'-3'
D02	A/duck/Fujian/10160/2005 (H5N1)	AVIAN_H5N1_NA_M0003 45:1135630360071	EF124315.1	118584215	3382	3'-5'
D03	A/chicken/Xinjiang/28/2006 (H5N1)	AVIAN_H5N1_NA_M0003 71:1135630354555	HM172193.1	295915846	3381	3'-5'
D04	A/wild duck/Liaoning/8/2006 (H5N1)	AVIAN_H5N1_NA_M0004 46:1135630358472	HM172190.1	295915840	3382	5'-3'
D05	A/Cygnus olor/BIH/1/2006 (H5N1)	AVIAN_H5N1_NA_M0004 67:1135630359253	FN186008.1	283483552	3381	3'-5'
D06	A/chicken/Xinjiang/54/2005 (H5N1)	AVIAN_H5N1_NA_M0004 86:1135630359954	HM172204.1	295915868	3381	3'-5'
D07	A/chicken/Hebei/326/2005 (H5N1)	AVIAN_H5N1_NA_M0005 31:1135630359820	DQ349118.1	85681814	3380	5'-3'
D08	A/houbara bustard/Saudi Arabia/6732-1/2007 (H5N1)	AVIAN_H5N1_NA_M0005 63:1135661846710	EU445682.1	168805331	3380	3'-5'
D09	A/chicken/Egypt/0831-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0005 76:1135630358438	GQ184267.1	238800819	3381	3'-5'

Product Information Sheet for NR-45828

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D10	A/chicken/Xinjiang/16/2005 (H5N1)	AVIAN_H5N1_NA_M0005 94:1135630357815	HM172208.1	295915876	3381	5'-3'
D11	A/house crow/Hong Kong/5288/2007 (H5N1)	AVIAN_H5N1_NA_M0006 14:1135630354130	CY036191.1	212507496	3382	5'-3'
D12	A/teal/Vietnam/MBP5/2006 (H5N1)	AVIAN_H5N1_NA_M0006 24:1135630354684	FJ811998.1	225219875	3442	5'-3'
E01	A/chicken/Cambodia/LC1AL/2007 (H5N1)	AVIAN_H5N1_NA_M0003 28:1135630358007	HQ200574.1	306493927	3380	5'-3'
E02	A/chicken/India/WB-NIV16924/2009 (H5N1)	AVIAN_H5N1_NA_M0003 50:1135630359012	GQ917228.1	259023899	3381	3'-5'
E03	A/chicken/Huadong/4/2008 (H5N1)	AVIAN_H5N1_NA_M0003 72:1135630355061	HQ677024.1	313757899	3382	3'-5'
E04	A/black-neck crane/Qinghai/7/2006 (H5N1)	AVIAN_H5N1_NA_M0004 47:1135630358538	FJ602835.1	219564202	3382	5'-3'
E05	A/chicken/Nigeria/1071-9/2007 (H5N1)	AVIAN_H5N1_NA_M0004 69:1135630359294	EU148398.1	157399786	3381	3'-5'
E06	A/chicken/Nigeria/228-5/2006 (H5N1)	AVIAN_H5N1_NA_M0004 87:1135630359942	EU697218.1	187764050	3382	5'-3'
E07	A/chicken/Egypt/06553-NLQP/2006 (H5N1)	AVIAN_H5N1_NA_M0005 32:1135661847173	GQ184254.1	238800793	3382	5'-3'
E08	A/chicken/Egypt/0847-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0005 64:1135630359367	GQ184271.1	238800827	3380	5'-3'
E09	A/chicken/Egypt/08194S-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0005 78:1135630358335	GQ184287.1	238800859	3382	3'-5'
E10	A/chicken/Xinjiang/17/2005 (H5N1)	AVIAN_H5N1_NA_M0005 95:1135630357802	HM172176.1	295915812	3380	5'-3'
E11	A/black-crowned night heron/Hong Kong/659/2008 (H5N1)	AVIAN_H5N1_NA_M0006 16:1135630354174	CY036231.1	212508533	3381	3'-5'
E12	A/chicken/Bali/UT2091/2005 (H5N1)	AVIAN_H5N1_NA_M0006 25:1135630354680	GQ122469.1	238627442	3382	5'-3'
F01	A/duck/Vietnam/NCVD-1/2007 (H5N1)	AVIAN_H5N1_NA_M0003 29:1135630357940	CY030241.1	211997776	3381	5'-3'
F02	A/goose/Yunnan/5539/2005 (H5N1)	AVIAN_H5N1_NA_M0003 54:1135630358972	EF124218.1	118584021	3382	5'-3'
F03	A/duck/Eastern China/58/2005 (H5N1)	AVIAN_H5N1_NA_M0003 74:1135630355102	EU429764.1	167859493	3381	3'-5'
F04	A/turkey/Saudi Arabia/6732-6/2007 (H5N1)	AVIAN_H5N1_NA_M0004 50:1135630358827	EU596411.1	183238977	3381	3'-5'
F05	A/chicken/Xinjiang/68/2005 (H5N1)	AVIAN_H5N1_NA_M0004 70:1135630359866	HM172192.1	295915844	3380	5'-3'
F06	A/turkey/Turkey/1/2005 (H5N1)	AVIAN_H5N1_NA_M0004 98:1135661844366	EF619973.1	148340538	3381	3'-5'
F07	A/Canada goose/Bavaria/4/2007 (H5N1)	AVIAN_H5N1_NA_M0005 33:1135630359758	GU046761.1	260677765	3380	3'-5'
F08	A/chicken/Vietnam/NCVD-016/2008 (H5N1)	AVIAN_H5N1_NA_M0005 68:1135630359550	HM444790.1	297660476	3380	3'-5'
F09	A/turkey/Egypt/07203-NLQP/2007 (H5N1)	AVIAN_H5N1_NA_M0005 87:1135630358791	GQ184260.1	238800805	3380	3'-5'
F10	A/chicken/Hebei/A-8/2009 (H5N1)	AVIAN_H5N1_NA_M0005 96:1135630357793	HM172197.1	295915854	3381	3'-5'
F11	A/chicken/East Java/UT6044/2007 (H5N1)	AVIAN_H5N1_NA_M0006 17:1135630354289	GQ122535.1	238627464	3380	5'-3'
F12	A/chicken/Bali/UT2092/2005 (H5N1)	AVIAN_H5N1_NA_M0006 30:1135630355142	GQ122475.1	238627444	3382	5'-3'
G01	A/duck/Vietnam/NCVD-7/2007 (H5N1)	AVIAN_H5N1_NA_M0003 30:1135630359638	CY030289.1	211997890	3382	3'-5'
G02	A/duck/Egypt/07322S-NLQP/2007 (H5N1)	AVIAN_H5N1_NA_M0003 57:1135630358912	GQ184261.1	238800807	3381	3'-5'
G03	A/chicken/Vietnam/G62/2005 (H5N1)	AVIAN_H5N1_NA_M0003 75:1135630355118	EU118142.1	156523799	3382	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G04	A/chicken/West Bengal/81760/2008 (H5N1)	AVIAN_H5N1_NA_M0004 51:1135630358816	GU083619.1	261890030	3381	3'-5'
G05	A/chicken/Xinjiang/78/2005 (H5N1)	AVIAN_H5N1_NA_M0004 71:1135630359601	HM172198.1	295915856	3381	5'-3'
G06	A/chicken/Egypt/0850-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0005 14:1135630354091	GQ184272.1	238800829	3382	5'-3'
G07	A/greylag goose /Bavaria/11/2007 (H5N1)	AVIAN_H5N1_NA_M0005 45:1135630360129	GU046773.1	260677777	3380	3'-5'
G08	A/chicken/Hebei/102/2005 (H5N1)	AVIAN_H5N1_NA_M0005 69:1135630359571	EU243134.1	159895924	3380	5'-3'
G09	A/chicken/Shandong/A-10/2006 (H5N1)	AVIAN_H5N1_NA_M0005 88:1135630358775	HM172212.1	295915884	3381	5'-3'
G10	A/chicken/Egypt/0813-NLQP/2008 (H5N1)	AVIAN_H5N1_NA_M0005 97:1135630357725	GQ184264.1	238800813	3381	3'-5'
G11	A/chicken/Navapur/Nandurbar/India/7966/2006 (H5N1)	AVIAN_H5N1_NA_M0006 18:1135630354257	DQ862474.1	110816474	3380	3'-5'
G12	A/chicken/Indonesia/R60/2005 (H5N1)	AVIAN_H5N1_NA_M0006 34:1135630355379	AM183682.1	109941955	3381	3'-5'
H01	A/duck/Vietnam/NCVD06/2005 (H5N1)	AVIAN_H5N1_NA_M0003 32:1135630359729	CY034776.1	206731521	3382	3'-5'
H02	A/Mergus albellus/Slovakia/Vh212/2006 (H5N1)	AVIAN_H5N1_NA_M0003 59:1135661846340	AM911074.1	219969061	3382	5'-3'
H03	A/Cygnus olor/Croatia/1/2005 (H5N1)	AVIAN_H5N1_NA_M0003 76:1135630355010	CY016821.1	115608073	3380	5'-3'
H04	A/chicken/Ghana/3158-NAMRU3/2007 (H5N1)	AVIAN_H5N1_NA_M0004 58:1135630358924	EF624250.1	148628582	3381	5'-3'
H05	A/duck/Tuva/01/2006 (H5N1)	AVIAN_H5N1_NA_M0004 75:1135630359583	DQ861293.1	112820199	3381	5'-3'
H06	A/chicken/Egypt/3/2006 (H5N1)	AVIAN_H5N1_NA_M0005 25:1135661842625	EU146881.1	157104076	3382	5'-3'
H07	A/mallard/Bavaria/10/2007 (H5N1)	AVIAN_H5N1_NA_M0005 50:1135661846443	FJ183473.1	198385699	3382	3'-5'
H08	A/chicken/Hebei/706/2005 (H5N1)	AVIAN_H5N1_NA_M0005 70:1135630358411	EU243127.1	159895938	3380	5'-3'
H09	A/great cormorant/Tibet/12/2006 (H5N1)	AVIAN_H5N1_NA_M0005 89:1135630358710	HM172179.1	295915818	3381	5'-3'
H10	A/duck/Hunan/1204/2006 (H5N1)	AVIAN_H5N1_NA_M0005 98:1135630357714	EF124322.1	118584229	3380	3'-5'
H11	A/chicken/Vietnam/NCVD12/2005 (H5N1)	AVIAN_H5N1_NA_M0006 20:1135630354630	CY035900.1	211593636	3381	3'-5'
H12	A/duck/Eastern China/59/2005 (H5N1)	AVIAN_H5N1_NA_M0006 35:1135630355446	EU429759.1	167859483	3380	3'-5'

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