

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 4 (Hemagglutinin)

Catalog No. NR-45093

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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-45093 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-45093 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 4 (Hemagglutinin), NR-45093.”

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.

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Table 1: Synfluenza Clone Set, Plate 4, (NR-45093)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/chicken/Egypt/06959-NLQP/2006 (H5N1)	AVIAN_H5N1_HA_M000622:1 135630351181	EU372947.1	165973143	3762	3'-5'
A02	St Jude H5N1 influenza seed virus 163243	AVIAN_H5N1_HA_M000652:1 135630345972	DQ659326.1	108671042	3751	5'-3'
A03	A/chicken/Central Java/UT3091/2005 (H5N1)	AVIAN_H5N1_HA_M000683:1 135630349971	GQ122395.1	238627713	3760	5'-3'
A04	A/chicken/Indonesia/Wates83/2005 (H5N1)	AVIAN_H5N1_HA_M000712:1 135630353571	EU124054.1	156989863	3759	3'-5'
A05	A/chicken/Vietnam/NCVD-016/2008 (H5N1)	AVIAN_H5N1_HA_M000733:1 135630352366	FJ842476.1	225729095	3765	3'-5'
A06	A/crested myna/Hong Kong/1178/2009 (H5N1)	AVIAN_H5N1_HA_M000777:1 135630347110	AB521163.1	258612358	3761	5'-3'
A07	A/duck/Vietnam/1/2005 (H5N1)	AVIAN_H5N1_HA_M000858:1 135630353328	CY016827.1	115609697	3759	3'-5'
A08	A/pigeon/Thailand/VSMU-11-KRI/2005 (H5N1)	AVIAN_H5N1_HA_M000930:1 135630349563	AB450573.1	210144796	3762	3'-5'
A09	A/chicken/Hebei/126/2005 (H5N1)	AVIAN_H5N1_HA_M000988:1 135630353887	EU243146.1	159895920	3761	3'-5'
A10	A/northern shoveler/Washington/44249-749/2006 (H7N3)	AVIAN_H7N3_HA_M000080:1 135630351093	CY076293.1	310699739	3714	5'-3'
A11	A/chicken/Israel/282/2005 (H9N2)	AVIAN_H9N2_HA_M000057:1 135630351396	EF492225.1	153800240	3725	5'-3'
A12	A/Athens/INS336/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000031:1135630361454	CY072422.1	304419928	3760	3'-5'
B01	A/turkey/Egypt/7/2007 (H5N1)	AVIAN_H5N1_HA_M000625:1 135630346714	CY055191.1	289629458	3763	5'-3'
B02	A/avian/Russia/5354-vac/2005 (H5N1)	AVIAN_H5N1_HA_M000653:1 135630345835	EU147293.1	161897910	3762	5'-3'
B03	A/chicken/West Java/HAMD/2006 (H5N1)	AVIAN_H5N1_HA_M000684:1 135630349935	EU124163.1	156991630	3761	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B04	A/chicken/Egypt/R6/2007 (H5N1)	AVIAN_H5N1_HA_M000713:1 135630353609	EU183332.1	158389166	3758	3'-5'
B05	A/mallard/Italy/3401/2005 (H5N1)	AVIAN_H5N1_HA_M000738:1 135630352272	CY021397.1	134048372	3750	5'-3'
B06	A/chicken/Primorje/1/2008 (H5N1)	AVIAN_H5N1_HA_M000785:1 135630347680	EU676174.1	188572102	3759	5'-3'
B07	A/chicken/Vietnam/TY9/2005 (H5N1)	AVIAN_H5N1_HA_M000862:1 135630353167	EU118134.1	156523781	3759	3'-5'
B08	A/chicken/Vietnam/NCVD11/2005 (H5N1)	AVIAN_H5N1_HA_M000936:1 135630349691	CY036719.1	218663924	3762	3'-5'
B09	A/chicken/Hebei/102/2005 (H5N1)	AVIAN_H5N1_HA_M000989:1 135630353843	EU243133.1	159895922	3765	5'-3'
B10	A/northern shoveler/California/44287-164/2007 (H7N7)	AVIAN_H7N7_HA_M000019:1 135630350530	CY076381.1	310703507	3715	3'-5'
B11	A/chicken/Guangdong/02/2007 (H9N2)	AVIAN_H9N2_HA_M000115:1 135630353340	FJ231863.1	206812649	3725	5'-3'
B12	A/Canada-AB/RV1644/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0039:1135630347942	GQ465679.1	255734907	3760	3'-5'
C01	A/chicken/Liaoning/23/2005 (H5N1)	AVIAN_H5N1_HA_M000635:1 135630347301	HM172453.1	295916414	3762	3'-5'
C02	A/chicken/Taput/BBPV1-576/2005 (H5N1)	AVIAN_H5N1_HA_M000668:1 135630349199	EU124083.1	156989918	3787	3'-5'
C03	A/muscovy Duck/Jakarta/HABWIN/2006 (H5N1)	AVIAN_H5N1_HA_M000687:1 135630350083	EU124149.1	156991602	3762	5'-3'
C04	A/chicken/Guizhou/7/2008 (H5N1)	AVIAN_H5N1_HA_M000715:1 135630353667	HM172079.1	295915570	3759	5'-3'
C05	A/chicken/Indonesia/Belitung Timor1631-18/2006 (H5N1)	AVIAN_H5N1_HA_M000739:1 135630352310	EU124201.1	156992290	3786	5'-3'
C06	A/duck/Hunan/8/2008 (H5N1)	AVIAN_H5N1_HA_M000787:1 135630347648	GU182166.1	268527178	3759	5'-3'
C07	A/duck/Vietnam/TG24-O1/2005 (H5N1)	AVIAN_H5N1_HA_M000866:1 135630352710	AM183677.1	109941945	3759	3'-5'
C08	A/duck/Cambodia/D1KC167/2006 (H5N1)	AVIAN_H5N1_HA_M000954:1 135630346417	HQ200483.1	306494067	3762	3'-5'
C09	A/environment/Indiana/08OS2857/2008 (H7N3)	AVIAN_H7N3_HA_M000007:1 135630353275	CY079484.1	315440820	3714	3'-5'
C10	A/mallard/California/HKWF1971/2007 (H7N7)	AVIAN_H7N7_HA_M000022:1 135630350695	CY033380.1	194310507	3714	3'-5'
C11	A/gadwall/Netherlands/1/2006 (H9N2)	AVIAN_H9N2_HA_M000157:1 135630346781	CY043864.1	255630164	3726	3'-5'
C12	A/Cherry Point/WR1355/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0047:1135630349047	CY050067.1	265693755	3758	5'-3'
D01	A/wild duck/Liaoning/8/2006 (H5N1)	AVIAN_H5N1_HA_M000638:1 135630347215	HM172084.1	295915580	3763	5'-3'
D02	A/chicken/Indonesia/Wates80/2005 (H5N1)	AVIAN_H5N1_HA_M000670:1 135630349527	EU124052.1	156989859	3762	5'-3'
D03	A/chicken/West Java/SMI-ENDRI1/2006 (H5N1)	AVIAN_H5N1_HA_M000689:1 135630350011	EU124162.1	156991628	3763	3'-5'
D04	A/chicken/Nigeria/08RS848-75/2007 (H5N1)	AVIAN_H5N1_HA_M000716:1 135630353494	CY048507.1	283552599	3761	3'-5'
D05	A/chicken/Langkat/BBPV1-576/2005 (H5N1)	AVIAN_H5N1_HA_M000742:1 135630351556	EU124082.1	156989916	3763	5'-3'
D06	A/chicken/Egypt/1/2008 (H5N1)	AVIAN_H5N1_HA_M000796:1 135630348594	CY061552.1	294716488	3761	5'-3'
D07	A/chicken/Uthaihani/NIAH115067/2008 (H5N1)	AVIAN_H5N1_HA_M000869:1 135630352642	FJ743696.1	224037344	3762	5'-3'
D08	A/duck/Vietnam/Hau Giang680F/2005 (H5N1)	AVIAN_H5N1_HA_M000965:1 135630346971	EU124172.1	156992230	3763	3'-5'
D09	A/northern shoveler/California/28327/2007 (H7N3)	AVIAN_H7N3_HA_M000012:1 135630352422	CY076421.1	310703683	3715	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D10	A/ruddy turnstone/Delaware/892/2006 (H7N7)	AVIAN_H7N7_HA_M000024:1 135630350675	EU684262.1	187384849	3714	5'-3'
D11	A/chicken/Shandong/lx331/2007 (H9N2)	AVIAN_H9N2_HA_M000189:1 135630349787	FJ190140.1	202071381	3721	5'-3'
D12	A/Cherry Point/WR0080/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0048:1135630349089	CY049828.1	265691398	3758	5'-3'
E01	A/bar-headed goose/Qinghai/3/2005 (H5N1)	AVIAN_H5N1_HA_M000639:1 135630347388	HM172454.1	295916416	3762	5'-3'
E02	A/chicken/Indonesia/R60/2005 (H5N1)	AVIAN_H5N1_HA_M000674:1 135630349329	AM183670.1	109941931	3761	3'-5'
E03	A/chicken/Indonesia/Siak1631-2/2006 (H5N1)	AVIAN_H5N1_HA_M000692:1 135630348527	EU124197.1	156992285	3763	3'-5'
E04	A/chicken/Benin/6693-16/2007 (H5N1)	AVIAN_H5N1_HA_M000720:1 135630353010	EU436612.1	167613756	3762	5'-3'
E05	A/chicken/Deli Derdang/BBPVI/2005 (H5N1)	AVIAN_H5N1_HA_M000745:1 135630345641	EU124091.1	156989932	3762	3'-5'
E06	A/chicken/Thailand/Sukhothai/NIAH6-3- 0013/2005 (H5N1)	AVIAN_H5N1_HA_M000804:1 135630351408	FJ265577.1	209164567	3762	3'-5'
E07	A/open-billed stork/Nakhonsawan/BBD3516M/2005 (H5N1)	AVIAN_H5N1_HA_M000888:1 135630353836	EF112290.1	132450096	3763	3'-5'
E08	A/duck/Laos/A0301/2007 (H5N1)	AVIAN_H5N1_HA_M000970:1 135630347074	CY040934.1	238628079	3758	5'-3'
E09	A/ruddy turnstone/Delaware Bay/121/2007 (H7N3)	AVIAN_H7N3_HA_M000016:1 135630352524	CY036783.1	218664076	3714	3'-5'
E10	A/mallard/New Zealand/1365-355/2005 (H7N7)	AVIAN_H7N7_HA_M000026:1 135630350757	CY061618.1	294861158	3714	3'-5'
E11	A/chicken/Shandong/lx929/2007 (H9N2)	AVIAN_H9N2_HA_M000196:1 135630349260	FJ190120.1	202071341	3705	3'-5'
E12	A/Cherry Point/WR0101/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0049:1135630348708	CY049867.1	265691801	3759	3'-5'
F01	A/grebe/Novosibirsk/29/2005 (H5N1)	AVIAN_H5N1_HA_M000642:1 135630347882	DQ230521.1	78450309	3762	3'-5'
F02	A/chicken/Indonesia/Bangka Seletan1631- 21/2006 (H5N1)	AVIAN_H5N1_HA_M000679:1 135630349797	EU124203.1	156992292	3786	5'-3'
F03	A/turkey/Egypt/F2/2006 (H5N1)	AVIAN_H5N1_HA_M000696:1 135630348388	EU183322.1	158389146	3761	3'-5'
F04	A/chicken/Nigeria/08RS848-76/2007 (H5N1)	AVIAN_H5N1_HA_M000721:1 135630353063	CY048515.1	283552618	3762	3'-5'
F05	A/chicken/Pidie/BPPV1/2005 (H5N1)	AVIAN_H5N1_HA_M000748:1 135630345718	EU124093.1	156989936	3763	3'-5'
F06	A/chicken/Nigeria/08RS848-92/2007 (H5N1)	AVIAN_H5N1_HA_M000810:1 135630351248	CY048603.1	283552827	3762	5'-3'
F07	A/chicken/Thailand/ICRC-V143/2007 (H5N1)	AVIAN_H5N1_HA_M000906:1 135630347993	EU233416.1	159139249	3779	3'-5'
F08	A/duck/Anhui/56/2005 (H5N1)	AVIAN_H5N1_HA_M000972:1 135630347143	HM172107.1	295915626	3760	5'-3'
F09	A/northern shoveler/California/HKWF1026/2007 (H7N3)	AVIAN_H7N3_HA_M000025:1 135630351659	CY039580.1	229598473	3714	3'-5'
F10	A/mute swan/Hungary/5973/2007 (H7N7)	AVIAN_H7N7_HA_M000027:1 135630350774	GQ240813.1	241829453	3714	5'-3'
F11	A/chicken/Zhejiang/HF3/2009 (H9N2)	AVIAN_H9N2_HA_M000230:1 135630352073	GU471865.1	284930074	3725	5'-3'
F12	A/California/20/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0050:1135630349288	GQ377085.1	253828576	3760	3'-5'
G01	A/chicken/Domododovo/MK/2007 (H5N1)	AVIAN_H5N1_HA_M000648:1 135630345593	FJ667188.1	224180930	3763	3'-5'
G02	A/chicken/Papua/TA5/2006 (H5N1)	AVIAN_H5N1_HA_M000680:1 135630350303	EU124158.1	156991620	3762	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G03	A/guinea fowl/Burkina Faso/1347-20/2006 (H5N1)	AVIAN_H5N1_HA_M000698:1 135630348115	EU277841.1	160369970	3761	3'-5'
G04	A/duck/chicken/Nigeria/08RS848-120/2007 (H5N1)	AVIAN_H5N1_HA_M000726:1 135630352837	CY048179.1	283551818	3763	5'-3'
G05	A/great crested grebe/Tyva/22/2010 (H5N1)	AVIAN_H5N1_HA_M000750:1 135630345807	HQ131674.1	304419376	3758	3'-5'
G06	A/chicken/Togo/4106-1/2007 (H5N1)	AVIAN_H5N1_HA_M000813:1 135630351132	EU373738.1	166065497	3763	5'-3'
G07	A/brown-headed gull/Thailand/VSMU-28-SPK/2005 (H5N1)	AVIAN_H5N1_HA_M000914:1 135630348674	EF178528.1	145337054	3762	5'-3'
G08	A/chicken/West Bengal/239020/2010 (H5N1)	AVIAN_H5N1_HA_M000976:1 135630347609	CY061294.1	309354814	3762	3'-5'
G09	A/chicken/SK/HR-00011/2007 (H7N3)	AVIAN_H7N3_HA_M000027:1 135630351745	EU500860.1	168487797	3732	5'-3'
G10	A/chicken/Iran/TH186/2007 (H9N2)	AVIAN_H9N2_HA_M000008:1 135630353295	EU477245.1	169212587	3725	3'-5'
G11	A/chicken/Zhejiang/HE1/2009 (H9N2)	AVIAN_H9N2_HA_M000247:1 135630347272	GU471870.1	284930084	3726	5'-3'
G12	A/Texas/45034157/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0102:1135630363100	CY052607.1	272851484	3761	5'-3'
H01	A/Cygnus cygnus/Iran/754/2006 (H5N1)	AVIAN_H5N1_HA_M000650:1 135630345929	CY016779.1	115608011	3762	5'-3'
H02	A/chicken/Madiun/BBVW1420/2005 (H5N1)	AVIAN_H5N1_HA_M000681:1 135630350266	EU124147.1	156991598	3761	3'-5'
H03	A/duck/Cote d'Ivoire/1787-18/2006 (H5N1)	AVIAN_H5N1_HA_M000699:1 135630348170	CY016803.1	115609536	3761	5'-3'
H04	A/chicken/Nigeria/08RS848-118/2007 (H5N1)	AVIAN_H5N1_HA_M000732:1 135630352378	CY048163.1	283551780	3762	5'-3'
H05	A/black-headed gull/Tyva/115/2009 (H5N1)	AVIAN_H5N1_HA_M000757:1 135630346311	GQ338084.1	251825470	3759	5'-3'
H06	A/chicken/Nigeria/1071-7/2007 (H5N1)	AVIAN_H5N1_HA_M000857:1 135630353312	EU148388.1	157399763	3761	5'-3'
H07	A/chicken/Thailand/Kamphaengphet/NIAH6-3-0006/2005 (H5N1)	AVIAN_H5N1_HA_M000927:1 135630349188	FJ265573.1	209164559	3762	5'-3'
H08	A/chicken/Hebei/326/2005 (H5N1)	AVIAN_H5N1_HA_M000979:1 135630347444	DQ343150.1	85062564	3761	5'-3'
H09	A/cinnamon teal/California/JN1310/2007 (H7N3)	AVIAN_H7N3_HA_M000059:1 135630350601	CY076856.1	311540617	3714	5'-3'
H10	A/chicken/Yunnan/BaoShan3/2007 (H9N2)	AVIAN_H9N2_HA_M000044:1 135630351015	EU216080.1	158552001	3711	3'-5'
H11	A/Nagasaki/HA-10-20/2010 (H1N1)	HUMAN_H1N1PDM_HA_M00 0003:1135630346487	AB558542.1	295841743	3759	3'-5'
H12	A/Nagasaki/HA-58/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0169:1135630350073	AB536769.1	270288624	3760	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