

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 3 (Hemagglutinin)

Catalog No. NR-45092

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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-45092 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-45092 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 3 (Hemagglutinin), NR-45092.”

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 3 (NR-45092)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/ruddy turnstone/Delaware/509531/2007 (H5N1)	AVIAN_H5N1_HA_M000008:1 135630348803	GU186517.1	269315808	3751	3'-5'
A02	A/mallard/Maryland/802/2007 (H5N1)	AVIAN_H5N1_HA_M000031:1 135630347764	CY053877.1	282269023	3750	3'-5'
A03	A/duck/France/05066b/2005 (H5N1)	AVIAN_H5N1_HA_M000105:1 135630350874	AJ971297.1	145207498	3750	3'-5'
A04	A/duck/Vietnam/8/2005 (H5N1)	AVIAN_H5N1_HA_M000132:1 135630350133	DQ366322.1	86753759	3761	3'-5'
A05	A/chicken/Czech Republic/11242-38/2007 (H5N1)	AVIAN_H5N1_HA_M000177:1 135630347904	EU443553.1	168805377	3762	3'-5'
A06	A/duck/France/080036/2008 (H5N1)	AVIAN_H5N1_HA_M000214:1 135630352694	CY046182.1	281427635	3744	5'-3'
A07	A/chicken/Henan/A-7/2006 (H5N1)	AVIAN_H5N1_HA_M000260:1 135630345425	HM172080.1	295915572	3762	3'-5'
A08	A/duck/Lang Son/201/2005 (H5N1)	AVIAN_H5N1_HA_M000297:1 135630348798	GU186724.1	269315766	3758	3'-5'
A09	A/chicken/Laos/1/2008 (H5N1)	AVIAN_H5N1_HA_M000317:1 135630351504	AB435522.1	189046947	3758	5'-3'
A10	A/chicken/Laos/P0130/2007 (H5N1)	AVIAN_H5N1_HA_M000355:1 135630345979	CY040974.1	238628174	3758	3'-5'
A11	A/duck/Egypt/106d/2010 (H5N1)	AVIAN_H5N1_HA_M000420:1 135630348867	HQ198287.2	307557858	3759	5'-3'
A12	A/quail/Egypt/3050NAMRU3-CLEVB77/2007 (H5N1)	AVIAN_H5N1_HA_M000502:1 135630350506	EU371904.1	166034486	3761	5'-3'
B01	A/shorebird/Delaware/472/2007 (H5N1)	AVIAN_H5N1_HA_M000010:1 135630348962	CY043928.1	255631202	3751	3'-5'
B02	A/mallard/Ontario/499/2005 (H5N1)	AVIAN_H5N1_HA_M000032:1 135630347846	EF405825.1	146746659	3749	5'-3'
B03	A/mute swan/Aktau/1460/2006 (H5N1)	AVIAN_H5N1_HA_M000106:1 135630350895	FJ434373.1	213688725	3750	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B04	A/duck/Vietnam/1/2005 (H5N1)	AVIAN_H5N1_HA_M000133:1 135630350116	DQ366306.1	86753755	3762	5'-3'
B05	A/duck/Thai Ninh/07-86/2007 (H5N1)	AVIAN_H5N1_HA_M000189:1 135630347203	GU050499.1	267846960	3759	5'-3'
B06	A/chicken/Xinjiang/67/2005 (H5N1)	AVIAN_H5N1_HA_M000217:1 135630351906	HM172099.1	295915610	3762	5'-3'
B07	A/chicken/Huadong/4/2008 (H5N1)	AVIAN_H5N1_HA_M000261:1 135630345361	HQ677023.1	313757897	3763	5'-3'
B08	A/teal/Germany/Wv632/2005 (H5N1)	AVIAN_H5N1_HA_M000299:1 135630348768	CY061885.1	295054818	3751	3'-5'
B09	A/chicken/Anhui/1089/2007 (H5N1)	AVIAN_H5N1_HA_M000318:1 135630351467	FJ784842.1	224181157	3760	5'-3'
B10	A/quail/Mingalardone/866/2007 (H5N1)	AVIAN_H5N1_HA_M000357:1 135630346156	AB474084.1	218775022	3759	3'-5'
B11	A/chicken/Indonesia/Wates77/2005 (H5N1)	AVIAN_H5N1_HA_M000430:1 135630346759	EU124051.1	156989857	3759	3'-5'
B12	A/chicken/Egypt/0891/2008 (H5N1)	AVIAN_H5N1_HA_M000528:1 135630349498	CY041298.1	238821992	3763	3'-5'
C01	A/oriental magpie robin/Hong Kong/9298/2009 (H5N1)	AVIAN_H5N1_HA_M000012:1 135630349095	AB557633.1	294831538	3761	3'-5'
C02	A/mallard/Ontario/499/2005 (H5N1)	AVIAN_H5N1_HA_M000034:1 135630347703	EF491876.1	146746663	3751	3'-5'
C03	A/chicken/Tambov/570-2/2005 (H5N1)	AVIAN_H5N1_HA_M000118:1 135630348020	DQ864716.1	111380019	3763	5'-3'
C04	A/goose/Vietnam/3/2005 (H5N1)	AVIAN_H5N1_HA_M000134:1 135630350055	DQ366314.1	86753757	3762	5'-3'
C05	A/muscovy duck/Son La/07-85/2007 (H5N1)	AVIAN_H5N1_HA_M000190:1 135630351807	GU050491.1	267846958	3758	5'-3'
C06	A/chicken/Xinjiang/53/2005 (H5N1)	AVIAN_H5N1_HA_M000218:1 135630351875	HM172094.1	295915600	3762	3'-5'
C07	A/chicken/Vietnam/NCVD-swab19/2008 (H5N1)	AVIAN_H5N1_HA_M000266:1 135630345746	FJ842486.1	225697347	3762	5'-3'
C08	A/duck/Egypt/D2Li234/2007 (H5N1)	AVIAN_H5N1_HA_M000301:1 135630351074	AB497015.1	296178222	3762	5'-3'
C09	A/chicken/Hubei/2856/2007 (H5N1)	AVIAN_H5N1_HA_M000331:1 135630353946	FJ784851.1	224181175	3759	5'-3'
C10	A/duck/Mong Cai/07-58/2007 (H5N1)	AVIAN_H5N1_HA_M000376:1 135630347357	GU050443.1	267846946	3759	5'-3'
C11	A/chicken/Egypt/09279S-NLQP/2009 (H5N1)	AVIAN_H5N1_HA_M000433:1 135630346849	GU002690.1	260104168	3761	5'-3'
C12	A/chicken/Egypt/1012sf/2010 (H5N1)	AVIAN_H5N1_HA_M000529:1 135630349419	HQ198275.2	307557849	3760	5'-3'
D01	A/chicken/Nigeria/08RS848-110/2007 (H5N1)	AVIAN_H5N1_HA_M000014:1 135630349037	CY048131.1	283551704	3762	5'-3'
D02	A/turkey/Egypt/0959-NLQP/2009 (H5N1)	AVIAN_H5N1_HA_M000039:1 135630347762	GU002679.1	260104146	3762	3'-5'
D03	A/chicken/Assam/142007/2008 (H5N1)	AVIAN_H5N1_HA_M000125:1 135630348316	GU066389.1	261265437	3762	3'-5'
D04	A/chicken/India/WB-NIV16924/2009 (H5N1)	AVIAN_H5N1_HA_M000142:1 135630349451	GQ917227.1	259023897	3763	5'-3'
D05	A/chicken/Vietnam/NCVD-03/2008 (H5N1)	AVIAN_H5N1_HA_M000191:1 135630351797	FJ842481.1	225697337	3768	5'-3'
D06	A/chicken/Xinjiang/27/2006 (H5N1)	AVIAN_H5N1_HA_M000219:1 135630351826	HM172091.1	295915594	3762	3'-5'
D07	A/chicken/Shandong/A-5/2006 (H5N1)	AVIAN_H5N1_HA_M000273:1 135630349826	HM172082.1	295915576	3762	5'-3'
D08	A/duck/Egypt/1063s/2010 (H5N1)	AVIAN_H5N1_HA_M000304:1 135630350986	HQ198293.2	307557861	3758	5'-3'
D09	A/goose/Hubei/65/2005 (H5N1)	AVIAN_H5N1_HA_M000336:1 135630353885	HM172070.1	295915552	3758	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D10	A/goose/Yunnan/3798/2006 (H5N1)	AVIAN_H5N1_HA_M000381:1 135630346963	CY030905.1	198285744	3759	5'-3'
D11	A/duck/Egypt/08425S-NLQP/2008 (H5N1)	AVIAN_H5N1_HA_M000435:1 135630346871	GQ184250.1	238800937	3761	5'-3'
D12	A/duck/Egypt/9399NAMRU3-CLEVB202/2007 (H5N1)	AVIAN_H5N1_HA_M000539:1 135630350252	EU371921.1	166034520	3757	5'-3'
E01	A/chicken/Nigeria/08RS848-24/2006 (H5N1)	AVIAN_H5N1_HA_M000017:1 135630348999	CY048251.1	283551989	3761	3'-5'
E02	A/chicken/Egypt/0847-NLQP/2008 (H5N1)	AVIAN_H5N1_HA_M000091:1 135630351437	GQ184228.1	238800893	3759	3'-5'
E03	A/crow/Assam/142119/2008 (H5N1)	AVIAN_H5N1_HA_M000126:1 135630348192	GQ478333.1	255985344	3756	3'-5'
E04	A/chicken/West Bengal/170564/2009 (H5N1)	AVIAN_H5N1_HA_M000146:1 135630349361	GU083661.1	261890048	3762	5'-3'
E05	A/avian/Hong Kong/1993/2007 (H5N1)	AVIAN_H5N1_HA_M000195:1 135630351707	GU050341.1	267846920	3759	3'-5'
E06	A/chicken/Xinjiang/28/2006 (H5N1)	AVIAN_H5N1_HA_M000220:1 135630352125	HM172090.1	295915592	3761	5'-3'
E07	A/chicken/Liaoning/A-1/2007 (H5N1)	AVIAN_H5N1_HA_M000275:1 135630349629	HM172078.1	295915568	3762	3'-5'
E08	A/chicken/Vietnam/10/2005 (H5N1)	AVIAN_H5N1_HA_M000305:1 135630352355	CY016867.1	115953503	3759	5'-3'
E09	A/duck/Hunan/3315/2006 (H5N1)	AVIAN_H5N1_HA_M000343:1 135630353581	FJ784855.1	224181183	3760	5'-3'
E10	A/duck/Yunnan/47/2006 (H5N1)	AVIAN_H5N1_HA_M000382:1 135630346895	HM172109.1	295915630	3763	3'-5'
E11	A/chicken/Egypt/07202-NLQP/2007 (H5N1)	AVIAN_H5N1_HA_M000459:1 135630346682	EU496389.1	168810710	3762	5'-3'
E12	A/chicken/Egypt/9388NAMRU3-CLEVB149/2007 (H5N1)	AVIAN_H5N1_HA_M000540:1 135630349761	EU371913.1	166034504	3763	5'-3'
F01	A/black duck/North Carolina/674-694/2006 (H5N1)	AVIAN_H5N1_HA_M000024:1 135630348629	EF607853.1	157169325	3750	5'-3'
F02	A/peregrine falcon/Hong Kong/810/2009 (H5N1)	AVIAN_H5N1_HA_M000094:1 135630351510	AB521159.1	258612350	3759	3'-5'
F03	A/chicken/Sulawesi Selatan/UT2093/2005 (H5N1)	AVIAN_H5N1_HA_M000129:1 135630349867	GQ122393.1	238627709	3762	5'-3'
F04	A/chicken/West Bengal/155505/2009 (H5N1)	AVIAN_H5N1_HA_M000151:1 135630346523	GU271998.1	270358866	3762	3'-5'
F05	A/chicken/Ha Nam/07-83/2007 (H5N1)	AVIAN_H5N1_HA_M000209:1 135630352577	GU050475.1	267846954	3760	3'-5'
F06	A/chicken/Pyigyitagon/204/2006 (H5N1)	AVIAN_H5N1_HA_M000224:1 135630352040	AB474081.1	218775016	3761	5'-3'
F07	A/chicken/West Java/GARUT-MAY/2006 (H5N1)	AVIAN_H5N1_HA_M000281:1 135630349242	EU124153.1	156991610	3763	3'-5'
F08	A/chicken/Vietnam/17/2005 (H5N1)	AVIAN_H5N1_HA_M000310:1 135630352869	CY017059.1	116070364	3760	5'-3'
F09	A/chicken/Hunan/1793/2007 (H5N1)	AVIAN_H5N1_HA_M000344:1 135630353622	FJ784847.1	224181167	3759	5'-3'
F10	A/wild duck/Hunan/021/2005 (H5N1)	AVIAN_H5N1_HA_M000400:1 135630349902	EU329177.1	162296058	3759	5'-3'
F11	A/chicken/Egypt/0815-NLQP/2008 (H5N1)	AVIAN_H5N1_HA_M000462:1 135630346177	GQ184221.1	238800879	3763	5'-3'
F12	A/chicken/Nigeria/08RS848-70/2007 (H5N1)	AVIAN_H5N1_HA_M000545:1 135630349576	CY048475.1	283552521	3763	3'-5'
G01	A/turkey/Virginia/505477-17/2007 (H5N1)	AVIAN_H5N1_HA_M000026:1 135630348519	GU051957.1	269826078	3749	3'-5'
G02	A/mallard/Hokkaido/24/2009 (H5N1)	AVIAN_H5N1_HA_M000095:1 135630351626	AB530992.1	264160589	3750	3'-5'
G03	A/chicken/Egypt/0960-NLQP/2009 (H5N1)	AVIAN_H5N1_HA_M000130:1 135630350209	GU002680.1	260104148	3762	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G04	A/swan/Bavaria/5/2007 (H5N1)	AVIAN_H5N1_HA_M000158:1 135630346686	GU046762.1	260677835	3762	3'-5'
G05	A/muscovy duck/Ha Nam/07-84/2007 (H5N1)	AVIAN_H5N1_HA_M000210:1 135630352738	GU050483.1	267846956	3759	3'-5'
G06	A/domestic green-winged teal/Hunan/67/2005 (H5N1)	AVIAN_H5N1_HA_M000226:1 135630351930	EU430496.1	167716900	3761	3'-5'
G07	A/chicken/Ghana/2534/2007 (H5N1)	AVIAN_H5N1_HA_M000288:1 135630349134	EU373734.1	166065489	3762	5'-3'
G08	A/scaly-breasted munia/Hong Kong/2433/2007 (H5N1)	AVIAN_H5N1_HA_M000313:1 135630352890	CY036133.1	212506194	3760	3'-5'
G09	A/chicken/Vietnam/200/2005 (H5N1)	AVIAN_H5N1_HA_M000345:1 135630353525	EU930876.1	195934512	3760	5'-3'
G10	A/duck/Hubei/2911/2007 (H5N1)	AVIAN_H5N1_HA_M000401:1 135630349926	FJ784852.1	224181177	3759	5'-3'
G11	A/chicken/Egypt/34-2/2008 (H5N1)	AVIAN_H5N1_HA_M000475:1 135630353386	CY062604.1	295789199	3762	3'-5'
G12	A/chicken/Nigeria/08RS848-74/2007 (H5N1)	AVIAN_H5N1_HA_M000552:1 135630345723	CY048499.1	283552578	3762	5'-3'
H01	A/mute swan/Michigan/451072-2/2006 (H5N1)	AVIAN_H5N1_HA_M000028:1 135630348576	EF607855.1	157169329	3749	5'-3'
H02	A/chicken/Guangdong/1/2005 (H5N1)	AVIAN_H5N1_HA_M000101:1 135630350920	EU874899.2	225165033	3760	5'-3'
H03	A/duck/Egypt/0964-NLQP/2009 (H5N1)	AVIAN_H5N1_HA_M000131:1 135630350151	GU002681.1	260104150	3763	5'-3'
H04	A/Peking duck/Germany/R1959/2007 (H5N1)	AVIAN_H5N1_HA_M000166:1 135630345873	FM165530.1	219916867	3761	5'-3'
H05	A/duck/Ha Tinh/07-53/2007 (H5N1)	AVIAN_H5N1_HA_M000211:1 135630352780	GU050428.1	267846942	3759	3'-5'
H06	A/goose/Islamabad/NARC7757/2007 (H5N1)	AVIAN_H5N1_HA_M000245:1 135630346995	CY034725.1	199580405	3761	3'-5'
H07	A/chicken/Yunnan/chuxiong01/2005 (H5N1)	AVIAN_H5N1_HA_M000292:1 135630348920	EU635874.2	190576654	3758	5'-3'
H08	A/duck/Hai Duong/07-40/2007 (H5N1)	AVIAN_H5N1_HA_M000315:1 135630352972	GU050389.1	267846932	3760	3'-5'
H09	A/duck/Hubei/Hangmei01/2006 (H5N1)	AVIAN_H5N1_HA_M000348:1 135630353509	EU594349.1	193227286	3759	3'-5'
H10	A/goose/Egypt/1057/2010 (H5N1)	AVIAN_H5N1_HA_M000406:1 135630348450	HQ198283.2	307557856	3759	3'-5'
H11	A/chicken/Egypt/5NLQP-CLEVB307/2008 (H5N1)	AVIAN_H5N1_HA_M000491:1 135630352014	FJ686835.1	222875635	3761	5'-3'
H12	A/chicken/Mahachkala/05/2006 (H5N1)	AVIAN_H5N1_HA_M000614:1 135630350856	DQ676830.1	108782511	3697	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