

**Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 12 (Neuraminidase)**

**Catalog No. NR-45830**

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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-45830 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-45830 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 12 (Neuraminidase), NR-45830.”

**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).

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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 12 (NR-45830)<sup>1</sup>**

Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
A01	A/chicken/Thailand/ICRC-195/2007 (H5N1)	AVIAN_H5N1_NA_M0008 51:1135630355890	EU669188.1	190195351	3380	5'-3'
A02	A/chicken/Shandong/A-5/2006 (H5N1)	AVIAN_H5N1_NA_M0008 66:1135630355255	HM172194.1	295915848	3380	3'-5'
A03	A/chicken/Fujian/1/2007 (H5N1)	AVIAN_H5N1_NA_M0008 74:1135630354744	HM172169.1	295915798	3380	5'-3'
A04	A/chicken/Pakistan/UDL-02/2008 (H9N2)	AVIAN_H9N2_NA_M0000 14:1135630357882	CY038468.1	228015861	3449	5'-3'
A05	A/chicken/Israel/375/2007 (H9N2)	AVIAN_H9N2_NA_M0000 34:1135661843456	GQ120540.1	238627230	3449	3'-5'
A06	A/avian/Saudi Arabia/910135/2006 (H9N2)	AVIAN_H9N2_NA_M0000 59:1135630356904	GU050289.1	267845163	3448	3'-5'
A07	A/chicken/Yunnan/Diqing/2006 (H9N2)	AVIAN_H9N2_NA_M0000 95:1135630355169	EU216098.1	158552037	3447	3'-5'
A08	A/chicken/Jiande/01/2009 (H9N2)	AVIAN_H9N2_NA_M0001 24:1135630358245	HM036347.1	293338854	3439	3'-5'
A09	A/chicken/Henan/L1/2008 (H9N2)	AVIAN_H9N2_NA_M0001 57:1135630355407	EU935063.1	195934145	3449	3'-5'
A10	B/Alaska/01/2009	HUMAN_FLUB_NA_M000 165:1135661847102	GQ895061.1	257815148	3540	5'-3'
A11	B/Hong Kong/36/2005	HUMAN_FLUB_NA_M000 220:1135661842746	EU879085.1	194140659	3540	5'-3'
A12	B/New Jersey/11/2008	HUMAN_FLUB_NA_M000 269:1135661845353	GQ340597.1	251825352	3540	3'-5'
B01	A/chicken/Thailand/ICRC-618/2008 (H5N1)	AVIAN_H5N1_NA_M0008 52:1135630355862	EU875388.1	194269701	3381	3'-5'
B02	A/chicken/Xinjiang/27/2006 (H5N1)	AVIAN_H5N1_NA_M0008 67:1135630355251	HM172207.1	295915874	3381	5'-3'
B03	A/northern pintail/Aomori/1001/2008 (H7N7)	AVIAN_H7N7_NA_M0000 04:1135661842728	AB517633.1	269964882	3448	3'-5'

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Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
B04	A/chicken/Pakistan/UDL-01/2006 (H9N2)	AVIAN_H9N2_NA_M0000 16:1135661845169	CY038428.1	228015766	3449	5'-3'
B05	A/chicken/Uchal/8293/2006 (H9N2)	AVIAN_H9N2_NA_M0000 38:1135661843591	HM370058.1	298364227	3440	3'-5'
B06	A/chicken/Israel/182/2008 (H9N2)	AVIAN_H9N2_NA_M0000 79:1135630354042	GQ120536.1	238627222	3450	3'-5'
B07	A/chicken/Yunnan/Dehong2/2007 (H9N2)	AVIAN_H9N2_NA_M0000 96:1135661842765	EU216097.1	158552035	3447	5'-3'
B08	A/chicken/Yunnan/Chuxiong1/2007 (H9N2)	AVIAN_H9N2_NA_M0001 25:1135630358267	EU216093.1	158552027	3437	3'-5'
B09	A/watercoot/Haryana/5844/2005 (H9N2)	AVIAN_H9N2_NA_M0001 59:1135630355393	FJ605502.1	220172388	3449	5'-3'
B10	B/New York/01/2007	HUMAN_FLUB_NA_M000 177:1135661846692	EU515981.1	168824927	3540	3'-5'
B11	B/Taiwan/417/2005	HUMAN_FLUB_NA_M000 222:1135661842825	CY038265.1	225676764	3540	5'-3'
B12	B/Houston/B850/2005	HUMAN_FLUB_NA_M000 271:1135661845459	CY018343.1	119515716	3541	5'-3'
C01	A/chicken/Sukhothai/NIAH114843/2008 (H5N1)	AVIAN_H5N1_NA_M0008 54:1135630355817	FJ750821.1	224037366	3381	3'-5'
C02	A/chicken/Jiangsu/18/2008 (H5N1)	AVIAN_H5N1_NA_M0008 68:1135630355156	HM172171.1	295915802	3381	3'-5'
C03	A/magpie/Korea/YJD174/2007 (H7N7)	AVIAN_H7N7_NA_M0000 17:1135661844004	FJ750856.1	224181022	3448	3'-5'
C04	A/chicken/Pakistan/UDL-03/2007 (H9N2)	AVIAN_H9N2_NA_M0000 17:1135630357908	CY038452.1	228015823	3448	3'-5'
C05	A/chicken/Iran/RZ70/2008 (H9N2)	AVIAN_H9N2_NA_M0000 43:1135661844461	HM855267.1	302140426	3450	5'-3'
C06	A/chicken/Pakistan/UDL-01/2005 (H9N2)	AVIAN_H9N2_NA_M0000 86:1135661843151	CY038412.1	228015728	3450	3'-5'
C07	A/mallard/Portugal/27972-B139/2007 (H9N2)	AVIAN_H9N2_NA_M0001 07:1135630357542	HM849013.1	301322557	3448	3'-5'
C08	A/chicken/Shandong/A1/2009 (H9N2)	AVIAN_H9N2_NA_M0001 27:1135630358209	HQ225838.1	307087711	3441	3'-5'
C09	A/chicken/Henan/1.2/2008 (H9N2)	AVIAN_H9N2_NA_M0001 60:1135630354834	FJ534539.1	217385755	3433	5'-3'
C10	B/Mississippi/UR06-0340/2007	HUMAN_FLUB_NA_M000 186:1135661846366	CY030635.1	169731684	3535	3'-5'
C11	B/California/5/2005	HUMAN_FLUB_NA_M000 225:1135661843752	EU879086.1	194140860	3541	5'-3'
C12	B/Taiwan/383/2005	HUMAN_FLUB_NA_M000 275:1135661845432	CY033814.1	194351584	3541	3'-5'
D01	A/chicken/Thailand/Kanchanaburi/CK-160/2005 (H5N1)	AVIAN_H5N1_NA_M0008 55:1135630355784	DQ334762.1	85062770	3380	5'-3'
D02	A/chicken/Liaoning/A-1/2007 (H5N1)	AVIAN_H5N1_NA_M0008 69:1135630355130	HM172206.1	295915872	3380	5'-3'
D03	A/whooper swan/Norway/10_438/2006 (H7N7)	AVIAN_H7N7_NA_M0000 22:1135661844504	FM179762.1	194305238	3447	3'-5'
D04	A/chicken/Pakistan/UDL-02/2005 (H9N2)	AVIAN_H9N2_NA_M0000 22:1135630356445	CY038420.1	228015747	3437	5'-3'
D05	A/avian/Saudi Arabia/910134/2006 (H9N2)	AVIAN_H9N2_NA_M0000 48:1135661844520	GU050281.1	267845161	3449	5'-3'
D06	A/chicken/Israel/953/2007 (H9N2)	AVIAN_H9N2_NA_M0000 87:1135630355789	GQ120547.1	238627244	3450	5'-3'
D07	A/duck/Vietnam/OIE-2327/2009 (H9N2)	AVIAN_H9N2_NA_M0001 08:1135661844882	AB545592.1	288556950	3449	5'-3'
D08	A/bird/Guangxi/A1/2006 (H9N2)	AVIAN_H9N2_NA_M0001 33:1135630358716	EU086288.1	157286394	3450	3'-5'

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Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
D09	B/North Dakota/01/2009	HUMAN_FLUB_NA_M000 015:1135661843286	GQ340574.1	251825316	3540	5'-3'
D10	B/Massachusetts/01/2007	HUMAN_FLUB_NA_M000 189:1135661846400	EU515933.1	168824867	3540	3'-5'
D11	B/Nepal/1106/2005	HUMAN_FLUB_NA_M000 231:1135661843660	DQ343803.1	85541441	3541	5'-3'
D12	B/Gyeonggi/592/2005	HUMAN_FLUB_NA_M000 289:1135661844898	DQ231543.1	77744408	3541	3'-5'
E01	A/chicken/Kalasin/NIAH3776/2005 (H5N1)	AVIAN_H5N1_NA_M0008 56:1135630355715	AB450613.1	210144876	3381	5'-3'
E02	A/chicken/Guizhou/7/2008 (H5N1)	AVIAN_H5N1_NA_M0008 70:1135630354641	HM172188.1	295915836	3381	3'-5'
E03	A/mallard/Netherlands/9/2005 (H7N7)	AVIAN_H7N7_NA_M0000 25:1135661844640	CY077010.1	311978458	3448	5'-3'
E04	A/chicken/Pakistan/UDL-04/2006 (H9N2)	AVIAN_H9N2_NA_M0000 24:1135630356513	CY038404.1	228015709	3449	5'-3'
E05	A/chicken/Israel/29/2005 (H9N2)	AVIAN_H9N2_NA_M0000 51:1135630356727	EF492274.1	153799636	3449	3'-5'
E06	A/chicken/Israel/1548/2006 (H9N2)	AVIAN_H9N2_NA_M0000 89:1135630355826	FJ464628.1	215536657	3449	5'-3'
E07	A/duck/Hokkaido/238/2008 (H9N2)	AVIAN_H9N2_NA_M0001 10:1135661845411	AB485601.1	224028211	3449	5'-3'
E08	A/chicken/Guangxi/17/2007 (H9N2)	AVIAN_H9N2_NA_M0001 40:1135630356036	GU722370.1	289471548	3441	5'-3'
E09	B/North Carolina/WRAIR1582P/2009	HUMAN_FLUB_NA_M000 021:1135661843070	CY069567.1	302424895	3540	3'-5'
E10	B/Texas/UR06-0541/2007	HUMAN_FLUB_NA_M000 191:1135661846078	CY037425.1	224021856	3541	5'-3'
E11	B/Hawaii/03/2009	HUMAN_FLUB_NA_M000 242:1135661843514	GQ451544.1	255529701	3541	5'-3'
E12	B/Canada/88/2007	HUMAN_FLUB_NA_M000 290:1135661846283	GQ423426.1	254927584	3540	3'-5'
F01	A/duck/Hunan/1930/2007 (H5N1)	AVIAN_H5N1_NA_M0008 60:1135630355318	FJ784768.1	224181344	3381	3'-5'
F02	A/chicken/Tibet/6/2008 (H5N1)	AVIAN_H5N1_NA_M0008 71:1135630354826	HM172202.1	295915864	3381	5'-3'
F03	A/northern shoveler/Interior Alaska/8BM3470/2008 (H9N2)	AVIAN_H9N2_NA_M0000 01:1135661844960	CY079648.1	315620327	3449	3'-5'
F04	A/thick-billed murre/Alaska/44085-155/2006 (H9N2)	AVIAN_H9N2_NA_M0000 26:1135630356586	HM060026.1	294712873	3448	3'-5'
F05	A/chicken/Israel/178/2006 (H9N2)	AVIAN_H9N2_NA_M0000 52:1135661843975	EF492276.1	153799640	3449	5'-3'
F06	A/chicken/Korea/HC0410/2009 (H9N2)	AVIAN_H9N2_NA_M0000 90:1135630355430	HQ221675.1	312191567	3448	5'-3'
F07	A/chicken/Korea/HC09/2009 (H9N2)	AVIAN_H9N2_NA_M0001 15:1135661845257	HQ221660.1	312191537	3450	3'-5'
F08	A/chicken/Yunnan/Dali/2007 (H9N2)	AVIAN_H9N2_NA_M0001 52:1135630355582	EU216095.1	158552031	3446	3'-5'
F09	B/Oklahoma/WRAIR1587P/2009	HUMAN_FLUB_NA_M000 022:1135661843050	CY069574.1	302424914	3541	3'-5'
F10	B/Arizona/146/2005	HUMAN_FLUB_NA_M000 192:1135661846033	DQ343821.1	85541495	3541	3'-5'
F11	B/Busan/2422/2008	HUMAN_FLUB_NA_M000 247:1135661844533	GU323436.1	283137901	3541	5'-3'
F12	B/Florida/01/2007	HUMAN_FLUB_NA_M000 295:1135661846234	EU515980.1	168824924	3540	3'-5'
G01	A/chicken/Hunan/1793/2007 (H5N1)	AVIAN_H5N1_NA_M0008 61:1135630355291	FJ784767.1	224181342	3382	5'-3'

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Well	Strain	Clone Name	Locus (CDS)	Gene ID <sup>3</sup>	Vector Total Size	Insert Orientation
G02	A/chicken/Hunan/1/2009 (H5N1)	AVIAN_H5N1_NA_M0008 72:1135630354806	HM172195.1	295915850	3380	3'-5'
G03	A/avian/Egypt/920431/2006 (H9N2)	AVIAN_H9N2_NA_M0000 02:1135661844952	GU050304.1	267845167	3449	3'-5'
G04	A/chicken/Israel/114/2007 (H9N2)	AVIAN_H9N2_NA_M0000 29:1135661843685	FJ464614.1	215536391	3449	3'-5'
G05	A/turkey/Israel/89/2005 (H9N2)	AVIAN_H9N2_NA_M0000 53:1135661844029	EF492275.1	153799638	3448	3'-5'
G06	A/chicken/Yunnan/Honghe/2007 (H9N2)	AVIAN_H9N2_NA_M0000 92:1135661842980	EU216099.1	158552039	3446	3'-5'
G07	A/chicken/Kunming/123/2007 (H9N2)	AVIAN_H9N2_NA_M0001 19:1135661845328	HQ398347.1	311350310	3431	5'-3'
G08	A/chicken/Yunnan/Yuxi1/2007 (H9N2)	AVIAN_H9N2_NA_M0001 53:1135630355494	EU216105.1	158552051	3446	3'-5'
G09	B/Taiwan/1734/2006	HUMAN_FLUB_NA_M000 031:1135661846596	CY030857.1	169822845	3541	3'-5'
G10	B/Texas/UR06-0343/2007	HUMAN_FLUB_NA_M000 195:1135661845915	CY030809.1	169822750	3541	5'-3'
G11	B/Georgia/09/2005	HUMAN_FLUB_NA_M000 248:1135661844574	CY019485.1	125662991	3540	5'-3'
G12	B/Taiwan/71523/2007	HUMAN_FLUB_NA_M000 320:1135661842873	CY033966.1	194351945	3540	3'-5'
H01	A/chicken/Suphanburi/NIAH108192/2005 (H5N1)	AVIAN_H5N1_NA_M0008 65:1135630355206	AB450611.1	210144872	3381	5'-3'
H02	A/chicken/Shandong/A-1/2009 (H5N1)	AVIAN_H5N1_NA_M0008 73:1135630354785	HM172191.1	295915842	3381	5'-3'
H03	A/chicken/Iran/RZ53/2008 (H9N2)	AVIAN_H9N2_NA_M0000 13:1135630358148	GU071973.1	261863875	3449	5'-3'
H04	A/goose/Yunnan/chuxiong07/2007 (H9N2)	AVIAN_H9N2_NA_M0000 30:1135661843499	EU644483.1	188504870	3447	3'-5'
H05	A/chicken/Israel/554/2005 (H9N2)	AVIAN_H9N2_NA_M0000 54:1135630356793	EF492279.1	153799646	3449	3'-5'
H06	A/chicken/Yunnan/Simao1/2007 (H9N2)	AVIAN_H9N2_NA_M0000 94:1135630355180	EU216100.1	158552041	3445	3'-5'
H07	A/goose/Yunnan/wenshan09/2007 (H9N2)	AVIAN_H9N2_NA_M0001 20:1135630358340	EU644488.1	188504880	3437	5'-3'
H08	A/chicken/Zibo/B1/2008 (H9N2)	AVIAN_H9N2_NA_M0001 55:1135661843017	EU935062.1	195934143	3443	5'-3'
H09	B/Alaska/03/2009	HUMAN_FLUB_NA_M000 152:1135661847408	GQ451525.1	255529677	3541	5'-3'
H10	B/Myanmar/M170/2007	HUMAN_FLUB_NA_M000 196:1135661845892	FJ229870.1	206812574	3540	3'-5'
H11	B/Missouri/01/2008	HUMAN_FLUB_NA_M000 249:1135661844592	EU566957.1	170181666	3541	3'-5'
H12	B/California/17/2007	HUMAN_FLUB_NA_M000 321:1135661842916	EU515976.1	168824912	3541	5'-3'

<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