

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 5 (Hemagglutinin)

Catalog No. NR-45094

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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-45094 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-45094 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 5 (Hemagglutinin), NR-45094.”

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 5, (NR-45094)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/Thailand/CU-H9/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0204:1135630350355	GQ866959.1	257786794	3761	3'-5'
A02	A/Addis Ababa/WR2848N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0409:1135630346437	CY071818.1	303385747	3750	3'-5'
A03	A/Athens/INS339/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0502:1135630352480	CY072446.1	304419982	3760	3'-5'
A04	A/California/07/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0613:1135630362585	FJ966974.1	227831807	3760	3'-5'
A05	A/Guangdong/2282/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0773:1135630361498	CY058834.1	293597628	3759	3'-5'
A06	A/California/VRDL129/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1030:1135630362676	CY066407.1	300218678	3760	3'-5'
A07	A/Mexico City/WR1310N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1769:1135630352219	CY050043.1	265693533	3758	5'-3'
A08	A/Delaware/07/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2335:1135630360823	GQ338355.1	243031578	3761	3'-5'
A09	A/San Salvador/WR0167N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2520:1135630353912	CY049883.1	265691959	3755	3'-5'
A10	A/Tessengerlo/INS191/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2719:1135630362271	CY063051.1	295843212	3760	3'-5'
A11	A/Taipei/WR1485T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2842:1135630353116	CY071634.1	303385353	3754	3'-5'
A12	A/Dhule/NIV9433/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2967:1135630363192	HM204572.1	295883969	3760	5'-3'
B01	A/Ashgabat/WR0865N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0205:1135630350410	CY071426.1	303384905	3753	3'-5'
B02	A/Santo Domingo/WR1068N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0431:1135630348051	CY049979.1	265692908	3753	3'-5'
B03	A/New York/6941/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0581:1135630348656	CY061938.1	295207868	3761	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B04	A/Salekhard/01/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0625:1135630362864	GU367327.1	282555880	3759	5'-3'
B05	A/Dongcheng/SWL45/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0818:1135630360511	CY064393.1	298286936	3760	5'-3'
B06	A/Ancona/86/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1064:1135630361894	GU576522.1	284999388	3760	3'-5'
B07	A/Madrid/INS134/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1854:1135630362970	CY062763.1	295842557	3761	5'-3'
B08	A/Pune/NIV21115/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2383:1135630363118	HM204578.1	295883981	3760	5'-3'
B09	A/Nagasaki/HA-10-4/2010 (H1N1)	HUMAN_H1N1PDM_HA_M00 2541:1135630360332	AB551874.1	292657216	3761	3'-5'
B10	A/Athens/INS154/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2735:1135630353784	CY063646.1	296240669	3760	3'-5'
B11	A/Mexico City/WR1301N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2893:1135630348133	CY050011.1	265693220	3753	5'-3'
B12	A/Ontario/25913/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2986:1135630362672	CY060550.1	294544904	3760	5'-3'
C01	A/Bangkok/INS426/2010 (H1N1)	HUMAN_H1N1PDM_HA_M00 0207:1135630350326	CY071343.1	303304638	3760	3'-5'
C02	A/Santo Domingo/WR1057N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0434:1135630348345	CY049947.1	265692588	3757	3'-5'
C03	A/Qingdao/383/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0582:1135630361608	CY050271.1	302026286	3760	3'-5'
C04	A/Pensacola/INS38/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0662:1135630361950	CY056156.1	290144549	3760	3'-5'
C05	A/Mexico City/WR1696T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0819:1135630346015	CY071722.1	303385542	3754	3'-5'
C06	A/Athens/INS262/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1065:1135630361916	CY067095.1	300725731	3760	5'-3'
C07	A/Guangzhou/GIRD07/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1906:1135630361888	HM014332.1	291297432	3759	5'-3'
C08	A/Port Au Prince/WR2294T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2386:1135630363052	CY071802.2	304365671	3761	3'-5'
C09	A/Qingdao/1006/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2550:1135630361134	CY050100.1	301637975	3761	3'-5'
C10	A/Mexico City/WR1306N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2745:1135630353212	CY050019.1	265693297	3754	3'-5'
C11	A/Mexico City/WR1307N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2894:1135630348283	CY050027.1	265693377	3756	5'-3'
C12	A/Singapore/TLL52/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 3100:1135630363866	GQ527166.1	256600041	3760	3'-5'
D01	A/Mexico City/WR1090N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0217:1135630351212	CY071458.1	303384974	3751	5'-3'
D02	A/Santo Domingo/WR1058N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0435:1135630348476	CY049955.1	265692669	3756	3'-5'
D03	A/Wisconsin/629-D01190/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0584:1135630361624	CY057926.1	291361124	3760	5'-3'
D04	A/Wisconsin/629-D02018/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0664:1135630361980	CY058300.1	291620857	3761	3'-5'
D05	A/Mexico City/WR1692T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0820:1135630345688	CY071706.1	303385508	3754	5'-3'
D06	A/Ancona/97/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1113:1135630362012	GU576524.1	284999390	3759	5'-3'
D07	A/Mexico City/WR1747N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1926:1135630345882	CY071770.1	303385645	3755	3'-5'
D08	A/Madrid/INS113/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2410:1135630361214	CY062675.1	295842311	3755	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D09	A/Australia/36/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002555:1135630361144	CY055700.1	290040576	3760	5'-3'
D10	A/Ankara/WRAIR1426N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002782:1135630347471	CY073072.1	304433499	3760	3'-5'
D11	A/Mexico City/WR1708T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002929:1135630345467	CY071762.1	303385628	3753	5'-3'
D12	A/Tehran/310/2009 (H1N1)	HUMAN_H1N1_HA_M000011:1135630347029	HM144134.1	295647386	3757	5'-3'
E01	A/Texas/JMS414/2010 (H1N1)	HUMAN_H1N1PDM_HA_M000250:1135630349158	CY061195.1	294612492	3760	3'-5'
E02	A/Santo Domingo/WR1059N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000436:1135630348242	CY049963.1	265692749	3757	3'-5'
E03	A/Thailand/THB0441/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000596:1135630362982	CY054621.1	295446030	3760	3'-5'
E04	A/Ankara/20/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000691:1135630361003	GU369664.1	282933985	3760	5'-3'
E05	A/Shanghai/60T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000862:1135630361563	GQ411908.1	254728923	3761	5'-3'
E06	A/Ancona/69/2009 (H1N1)	HUMAN_H1N1PDM_HA_M001132:1135630363754	GU576517.1	284999382	3760	3'-5'
E07	A/Wisconsin/629-S0464/2009 (H1N1)	HUMAN_H1N1PDM_HA_M001954:1135630361329	CY051487.1	268635261	3761	5'-3'
E08	A/California/VRDL119/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002428:1135630360954	CY066327.1	300218475	3759	5'-3'
E09	A/Hunan Changsha/SWL4346/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002603:1135630362563	GU371271.1	282768189	3759	5'-3'
E10	A/Ankara/WR1429T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002783:1135630347581	CY071594.1	303385266	3754	3'-5'
E11	A/Ankara/WRAIR1440T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002930:1135630353761	CY073096.1	304433550	3760	3'-5'
E12	A/Azarbayejan-Sharghi/147/2005 (H1N1)	HUMAN_H1N1_HA_M000028:1135630347619	HM144111.1	295647340	3757	5'-3'
F01	A/Silver Spring/WRAIRSP510P2/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000281:1135630346084	CY073286.1	304433968	3760	5'-3'
F02	A/Athens/INS162/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000483:1135630362056	CY062883.1	295842834	3761	5'-3'
F03	A/California/04/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000602:1135630362355	GQ280797.1	240130134	3760	3'-5'
F04	A/New York/6775/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000707:1135630363655	CY056787.1	291094335	3759	3'-5'
F05	A/Mexico City/WRAIR1773T/2010 (H1N1)	HUMAN_H1N1PDM_HA_M000898:1135630362414	CY073126.1	304433617	3760	3'-5'
F06	A/Texas/44313703/2009 (H1N1)	HUMAN_H1N1PDM_HA_M001174:1135630360970	CY052671.1	272859474	3759	5'-3'
F07	A/La Paz/WR0096T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002226:1135630347066	CY049851.1	265691643	3755	3'-5'
F08	A/Mexico City/WR1668T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002449:1135630345819	CY071666.1	303385422	3753	5'-3'
F09	A/Hangzhou/06/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002628:1135630363021	HM214474.1	296034999	3760	3'-5'
F10	A/Mexico City/WR1695N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002811:1135630352924	CY071714.1	303385525	3754	5'-3'
F11	A/Quito/WR1589N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M002952:1135630352551	CY071650.1	303385388	3755	5'-3'
F12	A/Azarbayejan-Sharghi/179/2006 (H1N1)	HUMAN_H1N1_HA_M000032:1135630345386	HM144112.1	295647342	3754	3'-5'
G01	A/Santo Domingo/0574/2009 (H1N1)	HUMAN_H1N1PDM_HA_M000319:1135630351253	CY049907.1	265692192	3757	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G02	A/Mexico City/WR1673N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0488:1135630350465	CY071674.1	303385439	3755	3'-5'
G03	A/California/04/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0607:1135630362445	FJ966082.1	227809829	3759	3'-5'
G04	A/Mexico City/WR1762T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0744:1135630350834	CY071778.1	303385662	3754	3'-5'
G05	A/Taipei/WR0194N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0920:1135630345523	CY071410.1	303384870	3754	3'-5'
G06	A/California/WR1315P/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1192:1135630360484	CY071490.2	304365627	3760	3'-5'
G07	A/Guangdong/01/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2239:1135630346344	HQ011408.1	302315382	3770	5'-3'
G08	A/Nagasaki/HA-12/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2475:1135630361588	AB530464.1	262318503	3756	3'-5'
G09	A/Madrid/INS129/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2650:1135630360240	CY062747.1	295842515	3760	5'-3'
G10	A/Mexico City/WR1706T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2827:1135630352257	CY071754.1	303385611	3755	5'-3'
G11	A/Singapore/TLL53/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2962:1135630351854	GQ527163.1	256600035	3759	5'-3'
G12	A/Kyoto/08K056/2009 (H1N1)	HUMAN_H1N1_HA_M000044: 1135630360668	CY043537.1	288541899	3758	5'-3'
H01	A/Santo Domingo/0574T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0327:1135630350953	CY049915.1	265692270	3766	3'-5'
H02	A/Bahrain/N11890/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0499:1135630362778	CY062411.1	295442369	3761	3'-5'
H03	A/Mexico City/WR1675T/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0612:1135630351056	CY071682.1	303385457	3754	3'-5'
H04	A/Australia/13/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 0749:1135630362425	CY055573.1	290021458	3760	5'-3'
H05	A/New York/5755/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1004:1135630362340	CY056571.1	291093849	3761	5'-3'
H06	A/Mexico City/WR1297N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 1767:1135630352305	CY050003.1	265693138	3756	5'-3'
H07	A/Bishkek/WR0882N/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2291:1135630351301	CY071442.1	303384940	3754	5'-3'
H08	A/New York/3351/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2489:1135630361397	CY041814.1	242032053	3761	5'-3'
H09	A/New York/4866/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2683:1135630347316	CY051799.1	268635963	3759	3'-5'
H10	A/Nagasaki/HA-59/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2836:1135630364239	AB535746.1	270124448	3761	5'-3'
H11	A/Wisconsin/629-D01154/2009 (H1N1)	HUMAN_H1N1PDM_HA_M00 2964:1135630351963	CY050372.1	268507261	3761	5'-3'
H12	A/Guangzhou/1684/2006 (H1N1)	HUMAN_H1N1_HA_M000132: 1135630345505	EU382987.1	170762605	3759	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