

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 13 (Neuraminidase)

Catalog No. NR-45831

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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-45831 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-45831 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 13 (Neuraminidase), NR-45831.”

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 13 (NR-45831)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	B/California/03/2008	HUMAN_FLUB_NA_M000329:1135661842705	FJ480174.1	214003721	3540	3'-5'
A02	B/Florida/02/2008	HUMAN_FLUB_NA_M000353:1135661844311	FJ152013.1	197239625	3541	3'-5'
A03	A/Iran/16677/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000004:1135661842566	HM581912.1	299152147	3437	3'-5'
A04	A/Athens/INS396/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000081:1135661845492	CY071169.1	303304247	3443	3'-5'
A05	A/Nagasaki/HA-58/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000194:1135661846169	AB537490.1	271280810	3441	5'-3'
A06	A/Managua/2604.02/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000297:1135661844144	CY072616.1	304420378	3442	3'-5'
A07	A/Amman/WR1335T/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000420:1135661847487	CY071588.2	304365656	3442	5'-3'
A08	A/Myanmar/60/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002026:1135661844393	GU014797.1	260104052	3440	3'-5'
A09	A/California/VRDL112/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002551:1135661845195	CY066273.1	300218354	3442	5'-3'
A10	A/Kenya/0007/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002682:1135661847207	HQ214306.1	306494429	3441	3'-5'
A11	A/Rome/665/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002727:1135661843562	CY055403.1	290465922	3440	3'-5'
A12	A/Texas/JMS364/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002769:1135661842340	CY060893.1	294611640	3442	3'-5'
B01	B/Incheon/2501/2008	HUMAN_FLUB_NA_M000331:1135661842647	GU323437.1	283137904	3541	5'-3'
B02	B/Pennsylvania/01/2007	HUMAN_FLUB_NA_M000354:1135661844291	EU515889.1	168824801	3541	5'-3'
B03	A/New York/6668/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000016:1135661847216	CY056765.1	291094286	3442	3'-5'

Product Information Sheet for NR-45831

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B04	A/New York/5271/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000100:1135661842548	CY057288.1	291358746	3441	5'-3'
B05	A/San Diego/INS101/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000195:1135661846124	CY061229.1	294612569	3440	5'-3'
B06	A/Athens/INS259/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000304:1135661845303	CY067073.1	300725682	3442	5'-3'
B07	A/Italy/127/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000536:1135661846481	GQ392031.1	254355273	3442	5'-3'
B08	A/Athens/INS412/2010 (H1N1)	HUMAN_H1N1PDM_NA_M002051:1135661844747	CY071273.1	303304481	3435	3'-5'
B09	A/Russia/74/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002552:1135661845209	CY053730.1	282160618	3441	3'-5'
B10	A/Kenya/0018/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002689:1135661847162	HQ214303.1	306494423	3442	5'-3'
B11	A/Mexico/47N/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002734:1135661844481	CY040890.1	238623293	3442	3'-5'
B12	A/Texas/44312415/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002774:1135661843173	CY052340.1	280978580	3441	5'-3'
C01	B/Chungnam/1081/2008	HUMAN_FLUB_NA_M000333:1135661842671	GU323427.1	283137874	3540	5'-3'
C02	B/Pennsylvania/02/2007	HUMAN_FLUB_NA_M000358:1135661844275	EU515942.1	168824885	3541	5'-3'
C03	A/Pensacola/INS40/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000018:1135661847278	CY056166.1	290146187	3441	3'-5'
C04	A/New York/6293/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000125:1135661843048	CY056701.1	291094142	3442	5'-3'
C05	A/New York/4981/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000211:1135661847026	CY056429.1	291092969	3441	3'-5'
C06	A/Mexico City/WR1310N/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000309:1135661845376	CY050045.1	265693552	3442	3'-5'
C07	A/Wisconsin/629-D01014/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000628:1135661842565	CY057784.1	291360389	3442	3'-5'
C08	A/Bangkok/INS424/2010 (H1N1)	HUMAN_H1N1PDM_NA_M002093:1135661846358	CY071329.1	303304607	3442	5'-3'
C09	A/Managua/5665.01/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002597:1135661846675	CY058142.1	291620502	3442	3'-5'
C10	A/Texas/JMS356/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002691:1135661846898	CY060845.1	294611532	3441	5'-3'
C11	A/Athens/INS163/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002739:1135661844620	CY062893.1	295842857	3442	5'-3'
C12	A/Ontario/313762/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002783:1135661842856	CY060624.1	294545654	3441	3'-5'
D01	B/Alaska/03/2008	HUMAN_FLUB_NA_M000342:1135661842445	GQ340621.1	251825388	3541	5'-3'
D02	B/Texas/1/2006	HUMAN_FLUB_NA_M000359:1135661844240	CY015383.1	114942523	3540	3'-5'
D03	A/Managua/462.01/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000026:1135661846911	CY058542.1	292495142	3441	5'-3'
D04	A/Frankfurt/INS402/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000131:1135661846587	CY071209.1	303304337	3442	3'-5'
D05	A/Wisconsin/629-S1309/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000240:1135661846550	CY057616.1	291359842	3442	3'-5'
D06	A/Guangzhou/GIRD07/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000346:1135661846528	HM014326.1	291297419	3442	5'-3'
D07	A/Athens/INS339/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000751:1135661845729	CY072448.1	304419987	3440	3'-5'
D08	A/California/07/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002397:1135661847257	GQ377078.1	253828632	3442	3'-5'
D09	A/Lisboa/78/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002600:1135661843905	CY067886.1	302026610	3442	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D10	A/Texas/46233104/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002693:1135661846809	CY060821.1	294611478	3442	5'-3'
D11	A/Texas/46172731/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002742:1135661844093	CY058040.1	291361381	3441	3'-5'
D12	A/Odense/INS177/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002807:1135661845341	CY062973.1	295843037	3440	5'-3'
E01	B/Arkansas/01/2007	HUMAN_FLUB_NA_M000344:1135661842378	EU566960.1	170181673	3539	3'-5'
E02	B/Orchon/2505/2007	HUMAN_FLUB_NA_M000372:1135661843634	EU791323.1	189484140	3541	3'-5'
E03	A/Singapore/SS004/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000034:1135661846625	CY067194.1	300872377	3442	3'-5'
E04	A/New York/6903/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000135:1135661846660	CY061908.1	295195801	3442	3'-5'
E05	A/Texas/47/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000255:1135661843106	GQ894921.1	257787006	3441	5'-3'
E06	A/Kenya/0021/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000360:1135661847342	HQ214297.1	306494411	3442	3'-5'
E07	A/Shizuoka/793/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000818:1135661847004	GU014803.1	260104058	3441	3'-5'
E08	A/Managua/4209.04/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002435:1135661845854	CY073667.1	305390313	3442	3'-5'
E09	A/Athens/INS161/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002606:1135661843958	CY062877.1	295842821	3442	5'-3'
E10	A/District of Columbia/INS44/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002698:1135661846756	CY056869.1	291094524	3442	5'-3'
E11	A/Iran/14089/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002755:1135661842604	HM581920.1	299152163	3442	5'-3'
E12	A/New York/0461/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002818:1135661845563	CY062060.1	295237063	3442	5'-3'
F01	B/Arkansas/01/2007	HUMAN_FLUB_NA_M000345:1135661842430	EU779540.1	189303176	3540	3'-5'
F02	B/Delaware/04/2007	HUMAN_FLUB_NA_M000383:1135661843369	EU516016.1	168824993	3540	5'-3'
F03	A/Mexico City/005/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000045:1135661846380	CY050881.1	268633845	3442	5'-3'
F04	A/Boston/141/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000141:1135661846320	CY064678.1	297616147	3441	5'-3'
F05	A/Managua/3490.03/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000260:1135661843239	CY073699.1	305390385	3442	3'-5'
F06	A/Guangdong/0752/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000378:1135661842526	HM780478.1	301137182	3442	5'-3'
F07	A/Niigata/749/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000823:1135661847473	GU014767.1	260104022	3441	5'-3'
F08	A/Netherlands/602/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002467:1135661844776	CY039528.2	258589192	3442	5'-3'
F09	A/California/VRDL18/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002643:1135661842941	CY055473.1	290002165	3440	5'-3'
F10	A/New York/3074/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002717:1135661843726	CY047312.1	260181146	3441	5'-3'
F11	A/Mexico city/CIA1/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002758:1135661842605	CY062492.1	295691607	3440	5'-3'
F12	A/San Salvador/WRAIR1109N/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002829:1135661846054	CY073035.1	304433417	3442	5'-3'
G01	B/Daejeon/3808/2008	HUMAN_FLUB_NA_M000347:1135661844685	GU323443.1	283137922	3540	3'-5'
G02	B/Vienna/23/2007	HUMAN_FLUB_NA_M000385:1135661843321	FJ183465.1	198400359	3541	3'-5'

Product Information Sheet for NR-45831

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G03	A/Perm/CRIE-ZTS/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000050:1135661845841	HM189325.1	295916576	3441	3'-5'
G04	A/Guangdong/1202/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000151:1135661847272	GU562469.1	284472228	3440	5'-3'
G05	A/California/VRDL8/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000286:1135661842712	CY066497.1	300218890	3441	3'-5'
G06	A/Taipei/WR0193T/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000410:1135661847130	CY071404.1	303384856	3441	5'-3'
G07	A/Canada-MB/RV2023/2009 (H1N1)	HUMAN_H1N1PDM_NA_M001744:1135661844058	GQ402227.1	254575317	3441	3'-5'
G08	A/Frankfurt/INS301/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002468:1135661844799	CY069148.1	302378734	3442	3'-5'
G09	A/Houston/1H/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002675:1135661847509	CY052961.1	272901405	3441	3'-5'
G10	A/Canada-SK/RV2486/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002723:1135661843481	GQ465696.1	255734953	3441	3'-5'
G11	A/California/VRDL2/2010 (H1N1)	HUMAN_H1N1PDM_NA_M002762:1135661842399	CY066457.1	300218800	3442	3'-5'
G12	A/Athens/INS341/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002834:1135661845700	CY072464.1	304420023	3441	3'-5'
H01	B/Guangzhou/01/2007	HUMAN_FLUB_NA_M000352:1135661844443	EU305616.1	161377396	3541	3'-5'
H02	B/Pennsylvania/03/2009	HUMAN_FLUB_NA_M000486:1135661845528	GQ451477.1	255529608	3541	5'-3'
H03	A/India/Nsk_NIV10348/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000051:1135661845877	HM241719.1	296491114	3441	5'-3'
H04	A/San Diego/INS15/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000160:1135661847021	CY056861.1	291094506	3442	3'-5'
H05	A/New York/4294/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000294:1135661844122	CY065045.1	298850417	3441	3'-5'
H06	A/New Hampshire/11/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000411:1135661847141	GQ894852.1	257786956	3442	5'-3'
H07	A/Texas/45072128/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002012:1135661843798	CY052825.1	272882697	3441	5'-3'
H08	A/Texas/30/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002544:1135661844188	GQ323553.1	241959928	3442	5'-3'
H09	A/San Diego/WR1634P/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002676:1135661847436	CY073388.1	304434196	3442	5'-3'
H10	A/Rome/651/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002726:1135661843537	CY055375.1	290465866	3442	5'-3'
H11	A/Texas/46172734/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002767:1135661842313	CY058048.1	291361399	3441	5'-3'
H12	A/Athens/INS340/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002835:1135661845627	CY072456.1	304420005	3442	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