Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 1 (Neuraminidase)

Catalog No. NR-45090

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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-45090 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 pUC19_CmR (chloramphenicol) vector to establish gene segment clones in Transformax™ EPI300™ competent (Epicentre®) *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 12.5 µg/mL chloramphenicol 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-45090 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 12.5 µg/mL chloramphenicol
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 1 (Neuraminidase), NR-45090."

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.

Disclaimers:

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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.

Support Provided by NIAID

Table 1: Synfluenza Clone Set, Plate 1 (NR-45090)[1]

Well	Strain	Clone Name	Locus (CDS)	Gene ID[3]	Vector Total Size	Insert Orientation
A01	A/laughing gull/Delaware Bay/6/2006 (H7N3)	AVIAN_H7N3_NA_M000029	CY037081.1	221329115	3939	5'-3'
A02	A/turkey/Israel/747/2005 (H9N2)	AVIAN_H9N2_NA_M000057	EF492282.1	153799652	3952	5'-3'
A03	A/duck/Korea/CBU08107/2008 (H9N2)	AVIAN_H9N2_NA_M000106	HQ221658.1	312191533	3952	5'-3'
A04	B/Maryland/06/2009	HUMAN_FLUB_NA_M000484	GQ451481.1	255529614	4043	5'-3'
A05	A/Lisboa/2/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000138	CY067799.1	302026407	3944	5'-3'
A06	A/Qingdao/1268/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002008	CY050120.1	301638015	3944	5'-3'
A07	A/Mexico City/MCIG01/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002536	CY054297.1	283832012	3944	5'-3'
A08	A/Tennessee/UR06-0414/2007 (H1N1)	HUMAN_H1N1_NA_M000819	CY027453.1	158957889	3948	5'-3'
A09	A/Qingdao/1329/2009 (H3N2)	HUMAN_H3N2_NA_M000038	CY050126.1	301638027	3953	5'-3'
A10	A/Kisii/7568/2008 (H3N2)	HUMAN_H3N2_NA_M000156	HQ214386.1	306494509	3953	5'-3'
A11	A/Oregon/UR06-0289/2007 (H3N2)	HUMAN_H3N2_NA_M000208	CY026845.1	158453804	3953	5'-3'
A12	A/California/VRDL308/2009 (H3N2)	HUMAN_H3N2_NA_M000355	CY068467.1	302183196	3953	5'-3'
B01	A/Anas crecca/Germany/Wv177/2005 (H7N7)	AVIAN_H7N7_NA_M000019	AM933237.1	183579757	3950	5'-3'
B02	A/chicken/Israel/1638/2006 (H9N2)	AVIAN_H9N2_NA_M000072	FJ464613.1	215536374	3952	5'-3'
B03	A/duck/Tsukuba/4/2005 (H9N2)	AVIAN_H9N2_NA_M000111	AB472019.1	238809425	3952	5'-3'
B04	A/Texas/45071344/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000020	CY052809.1	272879813	3944	5'-3'
B05	A/Korea/CJ112/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000171	HM189500.1	295915348	3944	5'-3'
B06	A/Barcelona/INS378/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002038	CY073767.1	306412402	3944	5'-3'
B07	A/Kenya/0029/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002607	HQ214273.1	306494363	3944	5'-3'
B08	A/California/12/2008 (H3N2)	HUMAN_H3N2_NA_M000004	GQ895001.1	258456342	3953	5'-3'
B09	A/Nanjing/1/2009 (H3N2)	HUMAN_H3N2_NA_M000044	GU907119.1	289900248	3953	5'-3'
B10	A/Cheongju/H411/2008 (H3N2)	HUMAN_H3N2_NA_M000159	FJ009486.1	196481095	3953	5'-3'
B11	A/Ohio/07/2006 (H3N2)	HUMAN_H3N2_NA_M000228	EU516037.1	168825207	3953	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID[3]	Vector Total Size	Insert Orientation
B12	A/Kentucky/UR07-0068/2008 (H3N2)	HUMAN_H3N2_NA_M000442	CY037561.1	224022529	3953	5'-3'
C01	A/duck/Shiga/B149/2007 (H7N7)	AVIAN_H7N7_NA_M000024	AB472031.1	238809451	3950	5'-3'
C02	A/chicken/Korea/SH0802/2008 (H9N2)	AVIAN_H9N2_NA_M000091	HQ221659.1	312191535	3952	5'-3'
C03	B/Taiwan/500/2005	HUMAN_FLUB_NA_M000006	CY030849.1	170027557	4043	5'-3'
C04	A/Texas/45103737/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000025	CY052513.1	272840576	3944	5'-3'
C05	A/Seoul/1785/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000172	CY060458.1	295146958	3944	5'-3'
C06	A/Texas/JMS397/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002069	CY061085.1	294612245	3944	5'-3'
C07	A/Texas/45033774/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002692	CY052593.1	272849358	3944	5'-3'
C08	A/Qatar/AF02/2008 (H3N2)	HUMAN_H3N2_NA_M000007	CY037305.1	223995411	3953	5'-3'
C09	A/Colorado/UR06-0206/2007 (H3N2)	HUMAN_H3N2_NA_M000049	CY025351.1	157281586	3953	5'-3'
C10	A/Dakar/WRAIR0011T/2009 (H3N2)	HUMAN_H3N2_NA_M000169	CY069303.1	302424269	3953	5'-3'
C11	A/Mecklenburg-Vorpommern/4/2006 (H3N2)	HUMAN_H3N2_NA_M000230	FJ231859.1	208972497	3953	5'-3'
C12	A/Japan/WRAIR1037P/2009 (H3N2)	HUMAN_H3N2_NA_M000449	CY069319.1	302424307	3953	5'-3'
D01	A/chicken/Iran/B326/2005 (H9N2)	AVIAN_H9N2_NA_M000019	EU678375.1	188039107	3952	5'-3'
D03	B/Missouri/01/2009	HUMAN_FLUB_NA_M000158	GQ340642.1	251825418	4043	5'-3'
D04	A/Guangdong/1105/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000028	GU471695.1	284027634	3944	5'-3'
D05	A/Lisboa/85/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000173	CY067907.1	302026659	3944	5'-3'
D06	A/Wisconsin/629-D00357/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002127	CY057029.1	291097533	3944	5'-3'
D07	A/Shanghai/P1/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002695	AB539741.1	299758075	3944	5'-3'
D08	A/Memphis/5/2005 (H3N2)	HUMAN_H3N2_NA_M000009	CY068892.1	302372442	3953	5'-3'
D09	A/Boston/61/2008 (H3N2)	HUMAN_H3N2_NA_M000055	CY044742.1	256387332	3953	5'-3'
D10	A/California/VRDL291/2009 (H3N2)	HUMAN_H3N2_NA_M000170	CY067207.1	300885524	3953	5'-3'
D11	A/Morioka/35/2005 (H3N2)	HUMAN_H3N2_NA_M000241	AB433820.1	183396540	3953	5'-3'
D12	A/Novosibirsk/707/2009 (H3N2)	HUMAN_H3N2_NA_M000455	CY053673.1	281487528	3953	5'-3'
E01	A/chicken/Iran/68/2006 (H9N2)	AVIAN_H9N2_NA_M000020	HM165472.1	295916502	3952	5'-3'
E02	A/chicken/Yunnan/BaoShan1/2006 (H9N2)	AVIAN_H9N2_NA_M000097	EU216103.1	158552047	3949	5'-3'
E03	B/Mississippi/UR06-0348/2007	HUMAN_FLUB_NA_M000171	CY030643.1	169731703	4043	5'-3'
E04	A/Lisboa/35/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000035	CY067811.1	302026435	3944	5'-3'
E05	A/Guangdong/0872/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000238	HM780490.1	301137210	3944	5'-3'
E06	A/Canada-MB/RV1975/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002185	GQ402241.1	254575345	3944	5'-3'
E07	A/Canada-QC/RV1759/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002710	GQ402235.1	254575333	3944	5'-3'
E08	A/Ontario/1252/2007 (H3N2)	HUMAN_H3N2_NA_M000015	EU399753.1	165880682	3953	5'-3'
E09	A/Managua/31/2007 (H3N2)	HUMAN_H3N2_NA_M000080	CY032551.1	189231111	3953	5'-3'
E10	A/Kentucky/UR07-0037/2008 (H3N2)	HUMAN_H3N2_NA_M000173	CY037529.1	224022453	3953	5'-3'
E11	A/Delaware/WRAIR1240/2009 (H3N2)	HUMAN_H3N2_NA_M000244	CY069519.1	302424782	3953	5'-3'
E12	A/New York/33/2008 (H3N2)	HUMAN_H3N2_NA_M000462	FJ549029.1	218217944	3953	5'-3'
F01	A/chicken/Pakistan/UDL-02/2006 (H9N2)	AVIAN_H9N2_NA_M000028	CY038436.1	228015785	3952	5'-3'
F02	A/shorebird/Delaware/249/2006 (H9N2)	AVIAN_H9N2_NA_M000100	CY043914.1	255630947	3952	5'-3'
F03	B/Nepal/1087/2005	HUMAN_FLUB_NA_M000200	DQ343810.1	85541462	4043	5'-3'
F04	A/Lisboa/76/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000037	CY067880.1	302026596	3944	5'-3'
F05	A/Australia/47/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000348	CY055766.1	290053143	3944	5'-3'
F06	A/Texas/45093214/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002211	CY052705.1	272864025	3944	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID[3]	Vector Total Size	Insert Orientation
F07	A/Rome/633/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002725	CY055347.1	290465810	3944	5'-3'
F08	A/Georgia/07/2009 (H3N2)	HUMAN_H3N2_NA_M000021	GQ895020.1	258456352	3953	5'-3'
F09	A/Massachusetts/12/2007 (H3N2)	HUMAN_H3N2_NA_M000112	EU516186.1	168827324	3953	5'-3'
F10	A/New York/22/2006 (H3N2)	HUMAN_H3N2_NA_M000178	EU100646.1	156123525	3953	5'-3'
F11	A/Berlin/30/2006 (H3N2)	HUMAN_H3N2_NA_M000267	FJ231853.1	208972485	3953	5'-3'
F12	A/New York/359/2005 (H3N2)	HUMAN_H3N2_NA_M000464	CY002002.1	71564675	3953	5'-3'
G01	A/duck/Yunnan/Yuxi2/2007 (H9N2)	AVIAN_H9N2_NA_M000031	EU216106.1	158552053	3940	5'-3'
G02	A/laughing gull/Delaware/12/2006 (H9N2)	AVIAN_H9N2_NA_M000101	CY041428.1	238823817	3952	5'-3'
G03	B/Taiwan/3001/2006	HUMAN_FLUB_NA_M000266	CY033950.1	194351907	4043	5'-3'
G04	A/Tallinn/INS431/2010 (H1N1)	HUMAN_H1N1PDM_NA_M000054	CY073735.1	306412329	3944	5'-3'
G05	A/Wisconsin/629-D01903/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000450	CY050993.1	268634097	3944	5'-3'
G06	A/Texas/JMS383/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002212	CY060981.1	294611838	3944	5'-3'
G07	A/Texas/JMS413/2010 (H1N1)	HUMAN_H1N1PDM_NA_M002768	CY061189.1	294612479	3944	5'-3'
G08	A/Western Australia/76/2005 (H3N2)	HUMAN_H3N2_NA_M000026	CY017101.1	117571095	3953	5'-3'
G09	A/Thailand/380/2007 (H3N2)	HUMAN_H3N2_NA_M000137	AB501503.1	261399555	3953	5'-3'
G10	A/New York/5/2006 (H3N2)	HUMAN_H3N2_NA_M000192	CY013234.1	110734349	3953	5'-3'
G11	A/Bethesda/956/2006 (H3N2)	HUMAN_H3N2_NA_M000284	GU968161.1	290745577	3953	5'-3'
G12	A/Washington/10/2006 (H3N2)	HUMAN_H3N2_NA_M000483	EU100651.1	156123535	3953	5'-3'
H01	A/chicken/Israel/282/2005 (H9N2)	AVIAN_H9N2_NA_M000050	EF492277.1	153799642	3952	5'-3'
H02	A/Bewick's swan/Netherlands/5/2007 (H9N2)	AVIAN_H9N2_NA_M000103	CY041276.1	238837342	3952	5'-3'
H03	B/Busan/976/2008	HUMAN_FLUB_NA_M000328	GU323425.1	283137868	4043	5'-3'
H04	A/Athens/INS329/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000101	CY072376.1	304419825	3944	5'-3'
H05	A/Korea/01/2009 (H1N1)	HUMAN_H1N1PDM_NA_M000513	GQ132185.1	229892681	3944	5'-3'
H06	A/District of Columbia/03/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002239	GQ894858.1	257786960	3944	5'-3'
H07	A/Wisconsin/629-D00636/2009 (H1N1)	HUMAN_H1N1PDM_NA_M002810	CY058032.1	291361363	3944	5'-3'
H08	A/California/VRDL352/2009 (H3N2)	HUMAN_H3N2_NA_M000036	CY068035.1	302136776	3953	5'-3'
H09	A/Denmark/10/2006 (H3N2)	HUMAN_H3N2_NA_M000152	EU103932.1	156692063	3953	5'-3'
H10	A/Bethesda/NIH12-D0/2008 (H3N2)	HUMAN_H3N2_NA_M000200	GU294117.1	281335529	3953	5'-3'
H11	A/Thailand/CU-B1672/2009 (H3N2)	HUMAN_H3N2_NA_M000347	GU271976.1	270358793	3953	5'-3'
H12	A/Thailand/545/2008 (H3N2)	HUMAN_H3N2_NA_M000543	AB501488.1	261399525	3953	5'-3'

[1]All information in this table was provided by J. Craig Venter Institute at the time of deposition.
[2]All clones contain full length inserts, HA inserts are 1716 to 1803 base pairs, NA inserts are 1453 to 1557 base pairs.
[3]Genbank gene ID

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898