

Synfluenza (Synthetic Influenza) Clone Set, Recombinant in *Escherichia coli*, Plate 6 (Hemagglutinin)

Catalog No. NR-45095

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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-45095 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-45095 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 6 (Hemagglutinin), NR-45095.”

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 6, (NR-45095)¹

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
A01	A/Maryland/01/2007 (H1N1)	HUMAN_H1N1_HA_M000146:1 135630346029	EU199325.1	158188051	3759	3'-5'
A02	A/Thailand/CU-B97/2009 (H1N1)	HUMAN_H1N1_HA_M000473:1 135630361412	GU271950.1	270358811	3758	5'-3'
A03	A/New York/928/2006 (H3N2)	HUMAN_H3N2_HA_M000036:1 135630361426	CY019325.1	122855224	3745	3'-5'
A04	A/Colorado/14/2007 (H3N2)	HUMAN_H3N2_HA_M000057:1 135630362507	EU516054.1	168828112	3745	5'-3'
A05	A/Texas/72/2007 (H3N2)	HUMAN_H3N2_HA_M000110:1 135630347182	EU516076.1	168828156	3746	3'-5'
A06	A/Auckland/616/2005 (H3N2)	HUMAN_H3N2_HA_M000180:1 135630362075	CY022573.1	149780663	3745	3'-5'
A07	A/Denmark/63/2006 (H3N2)	HUMAN_H3N2_HA_M000295:1 135630346511	EU103652.1	156691513	3745	3'-5'
A08	A/Illinois/UR06-0600/2007 (H3N2)	HUMAN_H3N2_HA_M000406:1 135630346119	CY030197.1	168481166	3746	3'-5'
A09	A/Cheongju/H380/2007 (H3N2)	HUMAN_H3N2_HA_M000435:1 135630360853	FJ009466.1	196481107	3745	3'-5'
A10	A/Qingdao/2144/2009 (H3N2)	HUMAN_H3N2_HA_M000544:1 135630346385	CY050089.1	301637953	3745	3'-5'
A11	A/Texas/08/2007 (H3N2)	HUMAN_H3N2_HA_M000598:1 135630363577	EU199344.1	158188089	3744	5'-3'
A12	A/Arizona/WRAIR1142P/2009 (H3N2)	HUMAN_H3N2_HA_M000669:1 135630363786	CY069437.1	302424588	3745	3'-5'
B01	A/Solomon Islands/3/2006 (H1N1)	HUMAN_H1N1_HA_M000181:1 135630348694	EU124177.1	156992240	3758	3'-5'
B02	A/New York/20343/2010 (H3N2)	HUMAN_H3N2_HA_M000007:1 135630361975	CY072214.1	304373518	3745	3'-5'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
B03	A/Qingdao/2181/2009 (H3N2)	HUMAN_H3N2_HA_M000037:1 135630361445	CY050093.1	301637961	3745	5'-3'
B04	A/Massachusetts/12/2007 (H3N2)	HUMAN_H3N2_HA_M000058:1 135630350908	EU516050.1	168828104	3745	3'-5'
B05	A/Djibouti/N04787/2009 (H3N2)	HUMAN_H3N2_HA_M000112:1 135630360996	CY062333.1	295441920	3746	5'-3'
B06	A/Taiwan/1452/2007 (H3N2)	HUMAN_H3N2_HA_M000185:1 135630362751	CY040130.1	237688997	3744	3'-5'
B07	A/New York/394/2005 (H3N2)	HUMAN_H3N2_HA_M000349:1 135630350368	CY002736.1	75181124	3743	3'-5'
B08	A/Hawaii/12/2007 (H3N2)	HUMAN_H3N2_HA_M000407:1 135630360837	EU516034.1	168828084	3745	3'-5'
B09	A/California/VRDL362/2009 (H3N2)	HUMAN_H3N2_HA_M000444:1 135630363476	CY068081.1	302138133	3745	3'-5'
B10	A/California/VRDL285/2009 (H3N2)	HUMAN_H3N2_HA_M000545:1 135630360664	CY068433.1	302183115	3744	5'-3'
B11	A/California/VRDL291/2009 (H3N2)	HUMAN_H3N2_HA_M000601:1 135630362176	CY067205.1	300885519	3744	5'-3'
B12	A/Managua/4348.01/2007 (H3N2)	HUMAN_H3N2_HA_M000685:1 135630360698	CY039439.1	229433745	3745	3'-5'
C01	A/Florida/19/2008 (H1N1)	HUMAN_H1N1_HA_M000250:1 135630361878	GQ475642.1	255959933	3757	5'-3'
C02	A/Managua/2/2007 (H3N2)	HUMAN_H3N2_HA_M000016:1 135630361866	CY032509.1	189231011	3744	5'-3'
C03	A/Wyoming/02/2006 (H3N2)	HUMAN_H3N2_HA_M000043:1 135630351236	EU199252.1	158187905	3744	3'-5'
C04	A/Canterbury/20/2005 (H3N2)	HUMAN_H3N2_HA_M000061:1 135630362236	CY008340.1	85833267	3745	5'-3'
C05	A/Denmark/50/2006 (H3N2)	HUMAN_H3N2_HA_M000119:1 135630347129	EU103759.1	156691727	3745	3'-5'
C06	A/Hong Kong/CUHK40022/2005 (H3N2)	HUMAN_H3N2_HA_M000191:1 135630351369	EU856996.1	194268874	3746	3'-5'
C07	A/Wisconsin/67/2005 (H3N2)	HUMAN_H3N2_HA_M000357:1 135630362186	CY034116.1	194352332	3746	5'-3'
C08	A/Thailand/CU272/2007 (H3N2)	HUMAN_H3N2_HA_M000408:1 135630346562	EU021270.1	154224781	3746	3'-5'
C09	A/Japan/WRAIR1037P/2009 (H3N2)	HUMAN_H3N2_HA_M000454:1 135630363689	CY069317.1	302424303	3745	3'-5'
C10	A/California/VRDL361/2009 (H3N2)	HUMAN_H3N2_HA_M000548:1 135630346232	CY068073.1	302138114	3745	3'-5'
C11	A/Australia/19/2009 (H3N2)	HUMAN_H3N2_HA_M000644:1 135630352000	CY061898.1	295191777	3745	3'-5'
C12	A/California/VRDL233/2009 (H3N2)	HUMAN_H3N2_HA_M000691:1 135630360314	CY068273.1	302182721	3744	3'-5'
D01	A/Novosibirsk/3/2009 (H1N1)	HUMAN_H1N1_HA_M000259:1 135630349468	CY053658.1	281487498	3759	5'-3'
D02	A/California/VRDL136/2009 (H3N2)	HUMAN_H3N2_HA_M000020:1 135630361795	CY064815.1	298347673	3744	5'-3'
D03	A/Denmark/67/2006 (H3N2)	HUMAN_H3N2_HA_M000046:1 135630362714	EU103802.1	156691813	3746	5'-3'
D04	A/Hiroshima/52/2005 (H3N2)	HUMAN_H3N2_HA_M000064:1 135630350636	EU283414.1	161778957	3745	5'-3'
D05	A/Morioka/22/2006 (H3N2)	HUMAN_H3N2_HA_M000121:1 135630348975	AB434113.1	183396612	3746	5'-3'
D06	A/Western Australia/68/2005 (H3N2)	HUMAN_H3N2_HA_M000202:1 135630350443	CY016004.1	115290969	3744	3'-5'
D07	A/Taiwan/70002/2007 (H3N2)	HUMAN_H3N2_HA_M000360:1 135630363721	CY039399.1	229433648	3744	3'-5'
D08	A/Colorado/15/2007 (H3N2)	HUMAN_H3N2_HA_M000409:1 135630346553	EU199343.1	158188087	3745	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
D09	A/Thailand/CU-B1672/2009 (H3N2)	HUMAN_H3N2_HA_M000462:1 135630363817	GU271974.1	270358817	3745	5'-3'
D10	A/Qingdao/2199/2009 (H3N2)	HUMAN_H3N2_HA_M000552:1 135630360272	CY050095.1	301637965	3745	3'-5'
D11	A/Egypt/N13658/2009 (H3N2)	HUMAN_H3N2_HA_M000646:1 135630352166	CY062352.1	295441958	3745	5'-3'
D12	A/New Hampshire/01/2009 (H3N2)	HUMAN_H3N2_HA_M000780:1 135630363177	GQ385889.1	254564351	3745	5'-3'
E01	A/New York/63/2009 (H1N1)	HUMAN_H1N1_HA_M000336:1 135630362259	GQ475968.1	255960936	3757	5'-3'
E02	A/South Australia/23/2005 (H3N2)	HUMAN_H3N2_HA_M000025:1 135630361695	CY016659.1	115607726	3743	5'-3'
E03	A/Ohio/UR07-0105/2008 (H3N2)	HUMAN_H3N2_HA_M000051:1 135630362470	CY037575.1	224022562	3745	3'-5'
E04	A/California/VRDL207/2009 (H3N2)	HUMAN_H3N2_HA_M000076:1 135630362002	CY068782.1	302371737	3746	3'-5'
E05	A/Denmark/62/2006 (H3N2)	HUMAN_H3N2_HA_M000125:1 135630361759	EU103653.1	156691515	3745	3'-5'
E06	A/Canterbury/270/2005 (H3N2)	HUMAN_H3N2_HA_M000206:1 135630350421	CY008652.1	87043096	3746	3'-5'
E07	A/Denmark/164/2005 (H3N2)	HUMAN_H3N2_HA_M000373:1 135630353741	EU103790.1	156691789	3745	3'-5'
E08	A/Alabama/UR06-0545/2007 (H3N2)	HUMAN_H3N2_HA_M000419:1 135630345865	CY025859.1	157282778	3746	3'-5'
E09	A/California/VRDL211/2009 (H3N2)	HUMAN_H3N2_HA_M000463:1 135630363947	CY068233.1	302182626	3745	5'-3'
E10	A/New York/1670/2009 (H3N2)	HUMAN_H3N2_HA_M000555:1 135630360409	CY050452.1	268513441	3745	5'-3'
E11	A/Montana/04/2009 (H3N2)	HUMAN_H3N2_HA_M000651:1 135630352992	GQ385872.1	254564339	3744	3'-5'
E12	A/New York/UR07-0160/2008 (H3N2)	HUMAN_H3N2_HA_M000831:1 135630361309	CY044381.1	256385587	3745	5'-3'
F01	A/California/VRDL134/2009 (H1N1)	HUMAN_H1N1_HA_M000362:1 135630362512	CY064799.1	298347654	3758	5'-3'
F02	A/California/VRDL138/2009 (H3N2)	HUMAN_H3N2_HA_M000027:1 135630361663	CY064823.1	298347692	3743	5'-3'
F03	A/Canterbury/104/2005 (H3N2)	HUMAN_H3N2_HA_M000052:1 135630362456	CY008356.1	85833229	3746	3'-5'
F04	A/New York/3135/2009 (H3N2)	HUMAN_H3N2_HA_M000084:1 135630364043	CY050580.1	268516173	3746	5'-3'
F05	A/Tokyo/Ut-Sk-1/2007 (H3N2)	HUMAN_H3N2_HA_M000126:1 135630361733	CY049748.1	285138736	3745	5'-3'
F06	A/Denmark/07/2005 (H3N2)	HUMAN_H3N2_HA_M000209:1 135630362089	EU103781.1	156691771	3745	5'-3'
F07	A/Ohio/06/2006 (H3N2)	HUMAN_H3N2_HA_M000374:1 135630353680	EU516029.1	168828074	3744	3'-5'
F08	A/New Jersey/05/2007 (H3N2)	HUMAN_H3N2_HA_M000420:1 135630347500	EU199279.1	158187959	3744	5'-3'
F09	A/Pennsylvania/PIT20/2008 (H3N2)	HUMAN_H3N2_HA_M000480:1 135630362135	CY035166.1	208344178	3745	3'-5'
F10	A/New York/3687/2009 (H3N2)	HUMAN_H3N2_HA_M000582:1 135630363460	CY055083.1	284466048	3746	3'-5'
F11	A/California/30/2007 (H3N2)	HUMAN_H3N2_HA_M000661:1 135630353427	EU516067.1	168828138	3745	3'-5'
F12	A/Wisconsin/03/2007 (H3N2)	HUMAN_H3N2_HA_M000856:1 135630352596	EU516105.1	168828206	3744	3'-5'
G01	A/Indiana/07/2009 (H1N1)	HUMAN_H1N1_HA_M000368:1 135630362565	GQ475933.1	255960912	3757	5'-3'
G02	A/California/VRDL156/2009 (H3N2)	HUMAN_H3N2_HA_M000031:1 135630361534	CY066519.1	300218940	3745	5'-3'

Well	Strain	Clone Name	Locus (CDS)	Gene ID ³	Vector Total Size	Insert Orientation
G03	A/Qingdao/1115/2009 (H3N2)	HUMAN_H3N2_HA_M000054:1 135630362544	CY050102.1	301637979	3746	5'-3'
G04	A/Qingdao/1118/2009 (H3N2)	HUMAN_H3N2_HA_M000107:1 135630361272	CY050106.1	301637987	3745	5'-3'
G05	A/Hong Kong/CUHK7221/2005 (H3N2)	HUMAN_H3N2_HA_M000129:1 135630361828	EU857085.1	194269052	3745	5'-3'
G06	A/Waikato/9/2005 (H3N2)	HUMAN_H3N2_HA_M000245:1 135630351585	CY014111.1	113171442	3744	5'-3'
G07	A/Denmark/60/2006 (H3N2)	HUMAN_H3N2_HA_M000384:1 135630363076	EU103669.1	156691547	3745	3'-5'
G08	A/Texas/UR06-0480/2007 (H3N2)	HUMAN_H3N2_HA_M000421:1 135630347422	CY025747.1	157282512	3745	3'-5'
G09	A/California/VRDL352/2009 (H3N2)	HUMAN_H3N2_HA_M000489:1 135630362107	CY068033.1	302136771	3744	5'-3'
G10	A/Thailand/CU-B110/2009 (H3N2)	HUMAN_H3N2_HA_M000586:1 135630363128	GQ902809.1	258578589	3745	3'-5'
G11	A/Texas/UR06-0418/2007 (H3N2)	HUMAN_H3N2_HA_M000663:1 135630363967	CY025851.1	157282759	3745	5'-3'
G12	A/New York/67/2009 (H3N2)	HUMAN_H3N2_HA_M000864:1 135630353159	GQ385915.1	254564371	3744	3'-5'
H01	A/Novosibirsk/4/2009 (H1N1)	HUMAN_H1N1_HA_M000425:1 135630361372	CY053664.1	281487510	3759	5'-3'
H02	A/New York/928/2006 (H3N2)	HUMAN_H3N2_HA_M000032:1 135630348517	CY020093.1	131055994	3744	3'-5'
H03	A/Oregon/UR06-0221/2007 (H3N2)	HUMAN_H3N2_HA_M000056:1 135630350870	CY028371.1	163964507	3745	3'-5'
H04	A/Qingdao/1295/2009 (H3N2)	HUMAN_H3N2_HA_M000108:1 135630361257	CY050123.1	301638021	3745	3'-5'
H05	A/California/UR06-0547/2007 (H3N2)	HUMAN_H3N2_HA_M000152:1 135630349400	CY026243.1	157368183	3744	5'-3'
H06	A/Waikato/12/2005 (H3N2)	HUMAN_H3N2_HA_M000268:1 135630363704	CY014135.1	113171499	3744	5'-3'
H07	A/Denmark/178/2005 (H3N2)	HUMAN_H3N2_HA_M000387:1 135630363095	EU103794.1	156691797	3744	5'-3'
H08	A/California/VRDL204/2009 (H3N2)	HUMAN_H3N2_HA_M000428:1 135630361100	CY068766.1	302371566	3745	5'-3'
H09	A/South Carolina/14/2009 (H3N2)	HUMAN_H3N2_HA_M000528:1 135630361279	GQ895050.1	258456390	3745	3'-5'
H10	A/California/VRDL263/2009 (H3N2)	HUMAN_H3N2_HA_M000587:1 135630363152	CY068353.1	302182911	3746	5'-3'
H11	A/Texas/63/2007 (H3N2)	HUMAN_H3N2_HA_M000664:1 135630364000	EU516064.1	168828132	3745	5'-3'
H12	A/Minnesota/14/2008 (H3N2)	HUMAN_H3N2_HA_M000869:1 135630363913	FJ179354.1	198241603	3745	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