

# NEBuilder<sup>®</sup> HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING



*be* INSPIRED  
*drive* DISCOVERY  
*stay* GENUINE

# Introducing NEBuilder HiFi

## New to DNA Assembly?

NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5'- and 3'-end mismatches. Available with and without competent *E. coli*, this flexible kit enables simple and fast seamless cloning utilizing a new proprietary high-fidelity polymerase. Find out why NEBuilder HiFi is the next generation of DNA assembly and cloning.

## 4 Reasons to choose NEBuilder HiFi

### 1 Save time

Enjoy simple and fast seamless cloning in as little as 15 minutes.

### 2 Flexibility

Use one system for both "standard-size" cloning and larger gene assembly products, up to 11 fragments.

### 3 Compatible with downstream applications

DNA can be used immediately for transformation or as template for PCR or RCA.

### 4 Adaptable

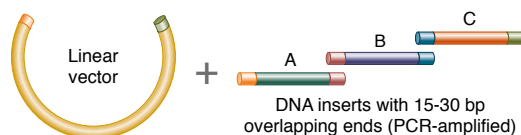
Adapts easily for multiple DNA manipulations, including site-directed mutagenesis.

### Overview of the NEBuilder HiFi DNA Assembly cloning method

#### DNA Preparation

##### From:

- PCR
- Restriction enzyme digestion
- Synthetic DNA (e.g., gBlocks)

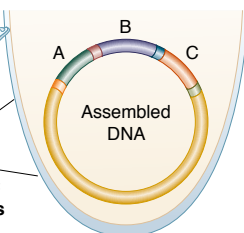


#### NEBuilder HiFi DNA Assembly Master Mix

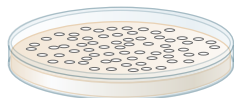
##### Single-tube reaction

- Exonuclease chews back 5' ends to create single-stranded 3' overhangs
- DNA polymerase fills in gaps within each annealed fragment
- DNA ligase seals nicks in the assembled DNA

Incubate at 50°C for 15-60 minutes



#### Transformation



#### DNA Analysis



RE Digest

OR



Colony PCR

OR



Sequencing

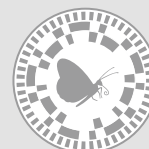
### Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L	10/50 reactions
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 reactions

### Explore and Discover

Download\* the NEB Augmented Reality (AR) app and enjoy videos, tutorials and immersive experiences by scanning the icons.

How does NEBuilder HiFi DNA Assembly work?



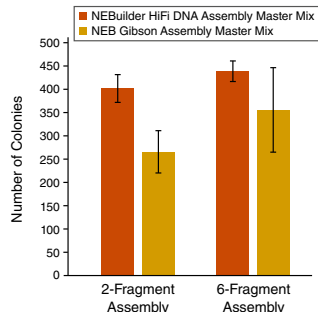
\*see back cover for details

# DNA Assembly

## Using NEB Gibson Assembly<sup>®</sup> or In-Fusion<sup>®</sup> HD?

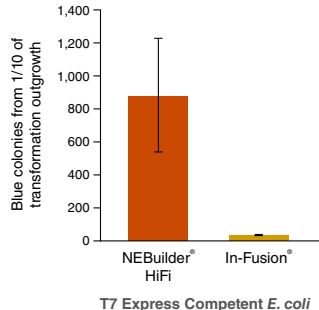
NEBuilder HiFi DNA Assembly offers several advantages over NEB Gibson Assembly and In-Fusion HD. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two double-stranded DNA fragments with a single-stranded DNA oligo (data not shown). NEBuilder HiFi DNA Assembly is the clear choice for efficient and accurate DNA assembly.

### NEBuilder HiFi DNA Assembly offers improved efficiency and accuracy over NEB Gibson Assembly



Reactions were set up in a 2- and 6- fragment assembly reaction according to recommended reaction conditions. NEBuilder HiFi DNA Assembly results in larger numbers of colonies over NEB Gibson Assembly, for both 2- and 6-fragment assemblies.

### NEBuilder HiFi DNA Assembly successfully joins fragments with 3'- and 5'-end mismatches, unlike In-Fusion HD



Reactions were set up using the NEBuilder Positive Control supplied with the NEBuilder HiFi DNA Assembly Master Mix, according to recommended protocols. Two  $\mu$ l of assembly were transformed into T7 Express Competent *E. coli* (NEB #C2566). 1/10 of outgrowth was spread on Ap<sup>R</sup>, IPTG and X-Gal plates. Blue colonies indicate correct assembly. Unlike In-Fusion HD, NEBuilder HiFi corrects mismatched ends and completes the assembly.

## Benefits over NEB Gibson Assembly

- Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly.
- Join DNA fragments together more efficiently, even with larger fragments or low DNA inputs.
- Use NEBuilder HiFi in successive rounds of assembly, because it removes 5'- and 3'-end mismatches. (Save time by avoiding time-consuming PCR amplification steps.)\*\*
- Bridge two ds-fragments with a synthetic ssDNA oligo for simple and fast construction (e.g. linker insertion or gRNA library)\*\*
- Switch from other systems easily, as NEBuilder HiFi is compatible with Gibson Assembly- designed (and other) fragments
- No licensing fee requirements from NEB for NEBuilder products

\*\* View our tutorials at [NEBuilderHiFi.com](http://NEBuilderHiFi.com)

## Comparison of DNA Assembly Reaction Types

	NEBuilder HiFi DNA Assembly		NEB Gibson Assembly		In-Fusion HD	
	Assembly efficiency	Covalently sealed?*	Assembly efficiency	Covalently sealed?*	Assembly efficiency	Covalently sealed?*
<b>2-fragment assembly</b>						
No mismatch	+++	Yes	++	Yes	++	No
3'- and 5'-end mismatch	+++	Yes	++	Yes	X	No
<b>4-fragment assembly</b>						
15 bp overlap & no mismatch	+++	Yes	++	Yes	++	No
25 bp overlap & no mismatch	+++	Yes	++	Yes	++	No
<b>Oligo assembly</b>						
3'- and 5'-overhang	+++	Yes	++	Yes	X	No
Blunt end & no mismatch	+++	Yes	++	Yes	X	No
ssOligo & vector	+++	Yes	NP	Yes	X	No

\* Assembled products are treated with T5 exonuclease followed by PCR. Only covalently sealed products resistant to T5 exonuclease digestion can serve as templates for PCR and yield PCR product.

+++ Performs best; recommended  
 ++ Performs well; but other product(s) perform better  
 + Performs, but not recommended  
 X Does not perform  
 NP Experiment not performed



For help designing primers, try NEBuilder Assembly Tool at [NEBuilder.neb.com](http://NEBuilder.neb.com)

GERMANY & AUSTRIA  
New England Biolabs GmbH  
Brüningstr. 50, Geb B852  
65926 Frankfurt/Main, Germany  
Tel: +49/(0)69/305-23140  
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)  
Free Call: 00800/246 52277 (Austria)  
info.de@neb.com

[www.neb-online.de](http://www.neb-online.de)

#### FRANCE

New England Biolabs France SAS  
Genopole Campus 1, Bâtiment 6  
5 rue Henri Desbrères  
91030 Evry cedex, France  
Tel.: 0800 100 632 (Customer Service)

Tel.: 0800 100 633 (Technical Service)  
FAX.: 0800 100 610  
info.fr@neb.com

[www.neb-online.fr](http://www.neb-online.fr)

#### HEADQUARTERS:

##### USA

New England Biolabs, Inc.  
Telephone: (978) 927-5054

Toll Free (USA Orders): 1-800-632-5227

Toll Free (USA Tech): 1-800-632-7799

Fax: (978) 921-1350

info@neb.com

[www.neb.com](http://www.neb.com)

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#### AUSTRALIA

New England Biolabs, Inc.  
Toll Free: +61 1800 934 218  
info.au@neb.com

#### CANADA

New England Biolabs, Ltd.  
Toll Free: 1-800-387-1095  
info.ca@neb.com

#### CHINA, PEOPLE'S REPUBLIC

New England Biolabs (Beijing), Ltd.  
Telephone: 010-82378265/82378266  
info@neb-china.com

#### JAPAN

New England Biolabs Japan, Inc.  
Telephone: +81 (0)3 5669 6191  
info.jp@neb.com

#### SINGAPORE

New England Biolabs, PTE. Ltd.  
Telephone: +65 63859623  
sales.sg@neb.com

#### UNITED KINGDOM

New England Biolabs (UK), Ltd.  
Call Free: 0800 318486  
info.uk@neb.com

## Your local NEB distributor:

BeNeLux:  
BIOKÉ  
Tel: (+31) 71 720 0220  
(BE: 0800 - 71640)  
Fax: (+31) 71 891 0019  
info@bioke.com  
www.bioke.com

BULGARIA  
ELTA 90M Ltd.  
Tel: (+359) 2 983 96 49  
Fax: (+359) 2 9832211  
office@elta90.eu  
www.elta90.com

CROATIA:  
Diagnostica Skalpeli d.o.o.  
Tel: (+385) 1 377 8484  
Fax: (+385) 1 377 8585  
skalpeli@skalpeli.hr  
www.skalpeli.hr

CZECH REPUBLIC:  
BioTech a.s.  
Tel: (Toll Free) 0800 124683  
Fax: 02 72701742  
info@ibitech.cz  
www.ibitech.cz

DENMARK:  
BioNordika Denmark A/S  
Tel: (39) 56 20 00  
Fax: (39) 56 19 42  
info@bionordika.dk  
www.bionordika.dk

FINLAND:  
BioNordika Oy  
Tel: (+358) 207 410 270  
info@bionordika.fi  
www.bionordika.fi

GREECE:  
BIOLINE SCIENTIFIC  
Tel: (0030) 210 522 6547  
Fax: (0030) 210 524 4744  
info@bioline.gr  
www.bioline.gr

HUNGARY:  
Kvalitex Kft.  
Phone: (1) 340 4700  
Fax: (1) 339 8274  
info@kvalitex.hu  
www.kvalitex.hu

ITALY:  
EUROCLONE S.p.A.  
Free call: 800-315911  
Tel: +39 02 381 951  
Fax: +39 02 339 137 13  
info@euroclone.it  
www.euroclonegroup.it

ISRAEL:  
Ornat BioChemicals &  
Lab Equipment  
Tel: (+972) 8 947 7077  
Fax: (+972) 8 936 3034  
ornatbio@ornat.co.il  
www.ornat.co.il

NORWAY:  
BioNordika AS  
Tel +47 23 03 58 00  
Fax +47 23 03 58 01  
info@bionordika.no  
www.bionordika.no

POLAND:  
Lab-JOT Ltd. Sp.z o.o. Sp.k.  
Tel: +48 22 335 988 4  
Fax: +48 22 335 981 9  
biuro@labjot.com  
www.labjot.com

PORTUGAL:  
Werfen  
Tel: +351 214 247 312  
Fax +351 21 417 26 74  
dac-pt@werfen.com  
www.pt.werfen.com

ROMANIA:  
BioZyme  
Tel: +40 264 523281  
contact@biozyme.ro  
www.biozyme.ro

RUSSIA:  
SkyGen LLC  
Tel: +88 00 333 1226  
info@skygen.com  
www.skygen.com

SERBIA:  
Alfa Genetics d.o.o.  
Tel: +38 10 11 2084252  
office@alfagenetics.rs  
www.alfagenetics.rs

SPAIN:  
Werfen  
Tel: +34 902 20 30 90  
Fax: +34 902 22 33 66  
customerservice-es@werfen.com  
www.es.werfen.com

SLOVAK REPUBLIC  
BioTech s.r.o.  
Tel: +421 2 54 77 44 88  
Fax: +421 2 32 20 10 48  
info@ibitech.sk  
www.ibitech.sk

SLOVENIA:  
Mikro+Polo d.o.o.  
Tel: +386 2 614 33 00  
Fax: +386 2 614 33 20  
info@mikro-polo.si  
www.mikro-polo.com

SOUTH AFRICA:  
Inqaba Biotechnical Industries  
(Pty) Ltd  
Tel: +27 123 43 58 29  
Fax: +27 86 677 8409  
info@inqababiotec.co.za  
www.inqababiotec.co.za

SWEDEN, ESTONIA, LATVIA,  
LITHUANIA:  
BIONORDIKA SWEDEN AB  
Tel: +46 8 30 60 10  
Fax: +46 8 30 60 15  
info@bionordika.se  
www.bionordika.se

SWITZERLAND:  
BioConcept Ltd.  
Tel: +41 (0)61 486 80 80  
Fax: +41 (0)61 486 80 00  
info@bioconcept.ch  
www.bioconcept.ch

TURKEY:  
EKA BIOLAB TEKNOLOJİ  
Tel: +90/312 248 13 24  
Email: info@ekabiolab.com  
www.ekabiolab.com

## Featured Tools



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For help with designing primers for DNA assembly, try **NEBuilder® DNA Assembly Tool** at [NEBuilder.neb.com](http://NEBuilder.neb.com)



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