

NEBuilder[®] HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING



Introducing NEBuilder HiFi

New to DNA Assembly?

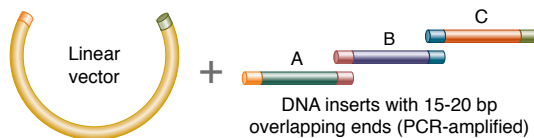
NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5'- and 3'-end mismatches. Available with and without competent *E. coli*, this flexible kit enables simple and fast seamless cloning utilizing a new proprietary high-fidelity polymerase. Find out why NEBuilder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

Overview of the NEBuilder HiFi DNA Assembly cloning method

DNA Preparation

From:

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

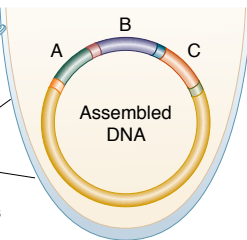


NEBuilder HiFi DNA Assembly Master Mix

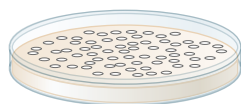
Single-tube reaction

- 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

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 12 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.



▶ Visit NEBuilderHiFi.com to view our video tutorials

Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L/X	10/50/250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 rxns

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DNA Assembly

Already cloning with Gibson Assembly®?

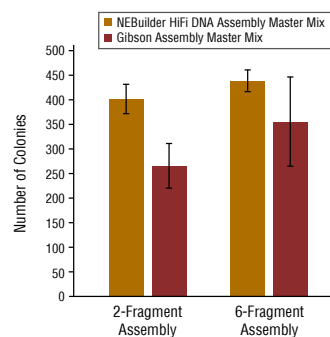
NEBuilder HiFi DNA Assembly offers several advantages over Gibson Assembly. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two double-stranded DNA fragments with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

Benefits over Gibson Assembly

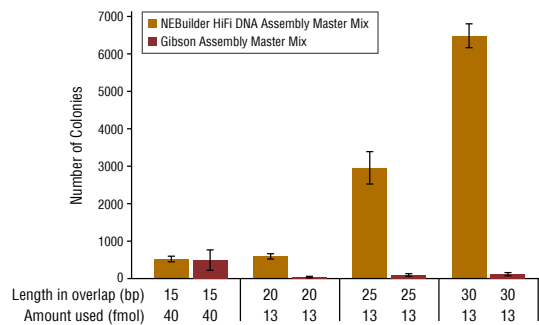
- Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly.
- Join DNA fragments together more efficiently, even with larger fragments or low DNA inputs.
- Use NEBuilder HiFi in successive rounds of assembly, because it removes 5'- and 3'-end mismatches. (Save time by avoiding time-consuming PCR amplification steps.)
- Bridge two ds-fragments with a synthetic ss-DNA 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

NEBuilder HiFi DNA Assembly offers improved efficiency and accuracy

A. With different numbers of fragments

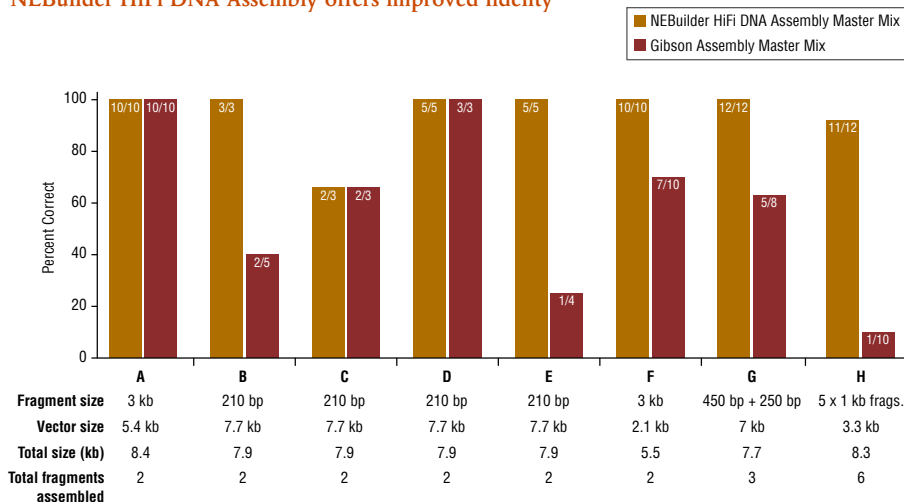


B. With lower amounts of DNA by increasing overlap length



A. Reactions were set up in a 2- and 6- fragment assembly reaction according to recommended reaction conditions.
 B. Reactions were set up in a 4- fragment assembly reaction according to recommended reaction conditions.
 Amount of DNA and size of overlap is shown.

NEBuilder HiFi DNA Assembly offers improved fidelity



Fidelity of assembled products was compared between NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621) and Gibson Assembly Master Mix (NEB #E2611). Experiments were performed using various fragment and vector sizes following suggested protocols. Experiments B through E vary because sequences of fragments are different. Experiments F and H were performed with fragments containing 3'-end mismatches.



For help designing primers, try NEBuilder Assembly Tool at NEBuilder.neb.com

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
e-mail: info@neb.com
www.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

France

New England Biolabs France
Telephone : 0800 100 632
info.fr@neb.com

Japan

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

Singapore

New England Biolabs, PTE. Ltd.
Telephone +65 6776 0903
sales.sg@neb.com

United Kingdom

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

www.neb-online.de



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