NEBuilder® HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING

NEW ENGLAND Biolabs®

Why choose NEBuilder HiFi

New to DNA Assembly?

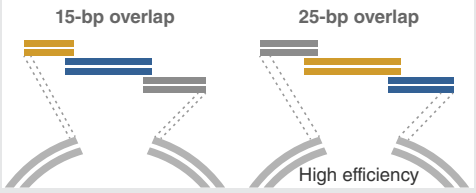
NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5´- and 3´-end mismatches. Available with and without competent *E. coli*, this flexible kit enables simple and fast seamless cloning utilizing a new proprietary high-fidelity polymerase. Find out why NEBuilder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

Not your average DNA assembly reagent

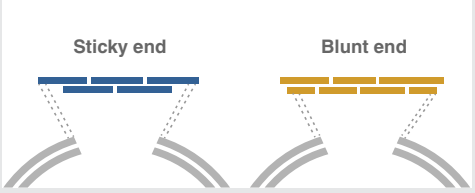
2-fragment assembly

ssOligo & dsDNA assembly

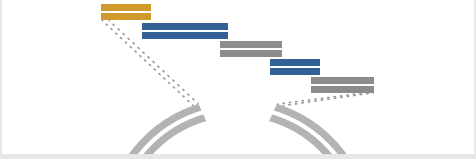
4-fragment assembly



Annealed-oligo assembly

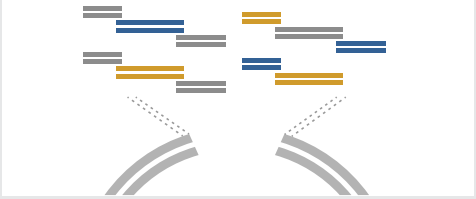


6-fragment assembly

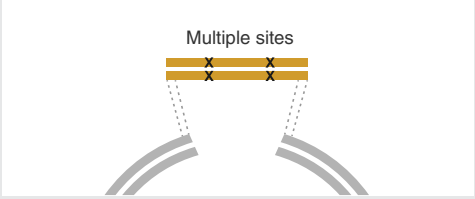


3´- and 5´-end mismatch assembly

12-fragment assembly



Site-directed mutagenesis



6 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 mismatch and ssOligo assembly.

5 Site-directed mutagenesis

Use to perform multi-site mutagenesis.

6 Increased stability

Store at -20°C, with improved stability over competition.

Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix*	E2621S/L/X	10/50/250 reactions
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 reactions

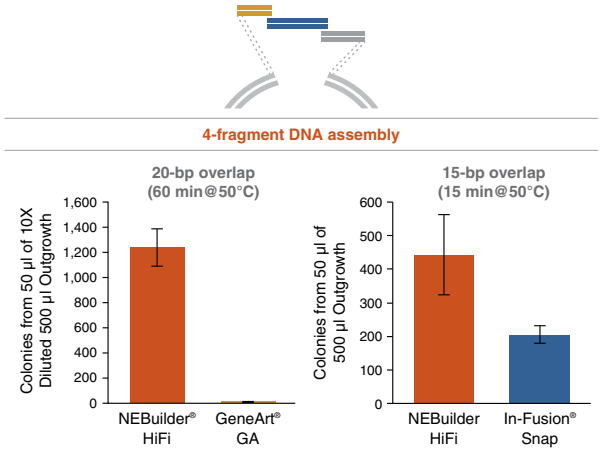
** Available in larger volumes. Please contact custom@neb.com for details.*

i DNA Assembly?

Using GeneArt® Gibson Assembly® or In-Fusion® Snap Assembly?

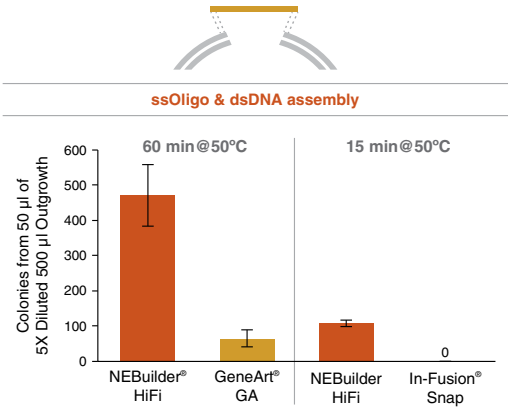
NEBuilder HiFi DNA Assembly offers several advantages over GeneArt Gibson Assembly and In-Fusion Snap Assembly. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5´- and 3´-end mismatches, lower DNA input requirements and the ability to bridge two 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.

NEBuilder HiFi DNA Assembly offers improved efficiency in 4-fragment assembly reactions



Four fragments (~20 fmol) with 20 bp overlap were assembled using NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621), GeneArt Gibson Assembly Mix (Thermo Fisher® #A46627) and In-Fusion Snap Assembly Master Mix (Takara Bio USA #638947) to create a pUC19 vector. Assembly reactions were performed at 50°C for 60 min or 15 min. 2 µl of each assembled mix was transformed into NEB 5-alpha Competent E. coli (NEB #C2987) and spread on LB/Amp plates with IPTG and X-Gal. Blue colonies that indicated correct assembly were counted. NEBuilder HiFi DNA Assembly Mix yields more colonies than both competitors.

NEBuilder HiFi DNA Assembly offers improved efficiency in assembly of a ssDNA oligo with a linearized vector



One pmol of ssDNA oligos were assembled with a linearized vector (30 ng of CRISPR Nuclease Reporter DNA) using NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621), GeneArt Gibson Assembly Mix (Thermo Fisher #A46627) and In-Fusion Snap Assembly Mix (Takara Bio USA #638947). Assembly reactions were performed at 50°C for 60 min or 15 min. 2 µl of the assembled mix was transformed into NEB 5-alpha Competent E. coli NEB #C2987). 20 colonies were further screened by PCR to confirm the presence of inserts. Greater than 95% of colonies tested from NEBuilder HiFi and GeneArt Gibson Assembly reactions contained proper inserts, although GeneArt Gibson Assembly yielded fewer colonies. In-Fusion Snap did not yield any successful colonies. NEBuilder HiFi DNA Assembly Master Mix outperformed both GeneArt Gibson Assembly and In-Fusion Snap Assembly.

Comparison of DNA Assembly Reaction Types

	NEBuilder HiFi DNA Assembly		GeneArt Gibson Assembly		In-Fusion Snap Assembly	
	Assembly efficiency	Covalently sealed?*	Assembly efficiency	Covalently sealed?*	Assembly efficiency	Covalently sealed?*
2-fragment assembly						
No mismatch	+++	Yes	++	Yes	++	No
3´- and 5´-end mismatch	+++	Yes	++	Yes	X	No
4-fragment assembly						
15-bp overlap & no mismatch	+++	Yes	++	Yes	++	No
25-bp overlap & no mismatch	+++	Yes	++	Yes	++	No
Oligo assembly						
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

Visit **NEBuilderHiFi.com** to view additional performance data, tutorials and much more

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

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For help with designing primers for DNA assembly, try **NEBuilder® DNA Assembly Tool** at **NEBuilder.neb.com**

Did you know that many of these products can be purchased in large volumes and custom formats? Learn more at **www.neb.com/customizedsolutions**