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NEBuilder – Version 2.0 – 9/16

NEBuilder® HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING



Need help designing primers for DNA assembly?



Try NEBuilder Assembly Tool at NEBuilder.neb.com

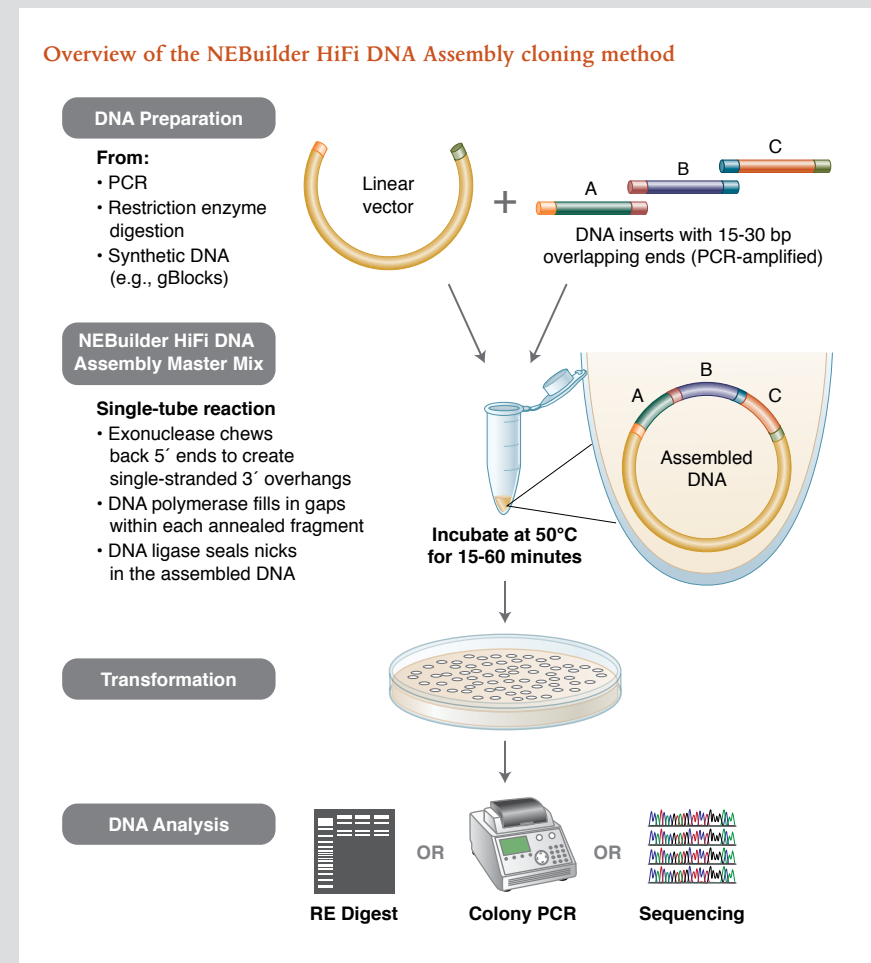


be INSPIRED
drive DISCOVERY
stay GENUINE

Why choose NEBuilder HiFi DNA Assembly?

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.



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
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 rxns

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IN-FUSION® is a registered trademark of ClonTech Laboratories, Inc.

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.

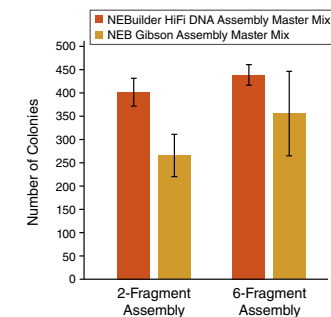


▶ Visit NEBuilderHiFi.com to view our video tutorials

Using NEB Gibson Assembly® or In-Fusion® HD?

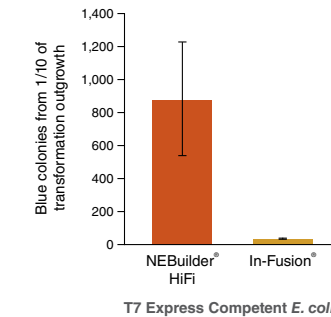
NEBuilder HiFi DNA Assembly offers several advantages over NEB Gibson Assembly and In-Fusion HD. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two double-stranded 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 µl of assembly were transformed into T7 Express Competent *E. coli* (NEB #C2566). 1/10 of outgrowth was spread on Ap^r, IPTG and X-Gal plates. Blue colonies indicate correct assembly. Unlike In-Fusion HD, NEBuilder HiFi corrects mismatched ends and completes the assembly.

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?*
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

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



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