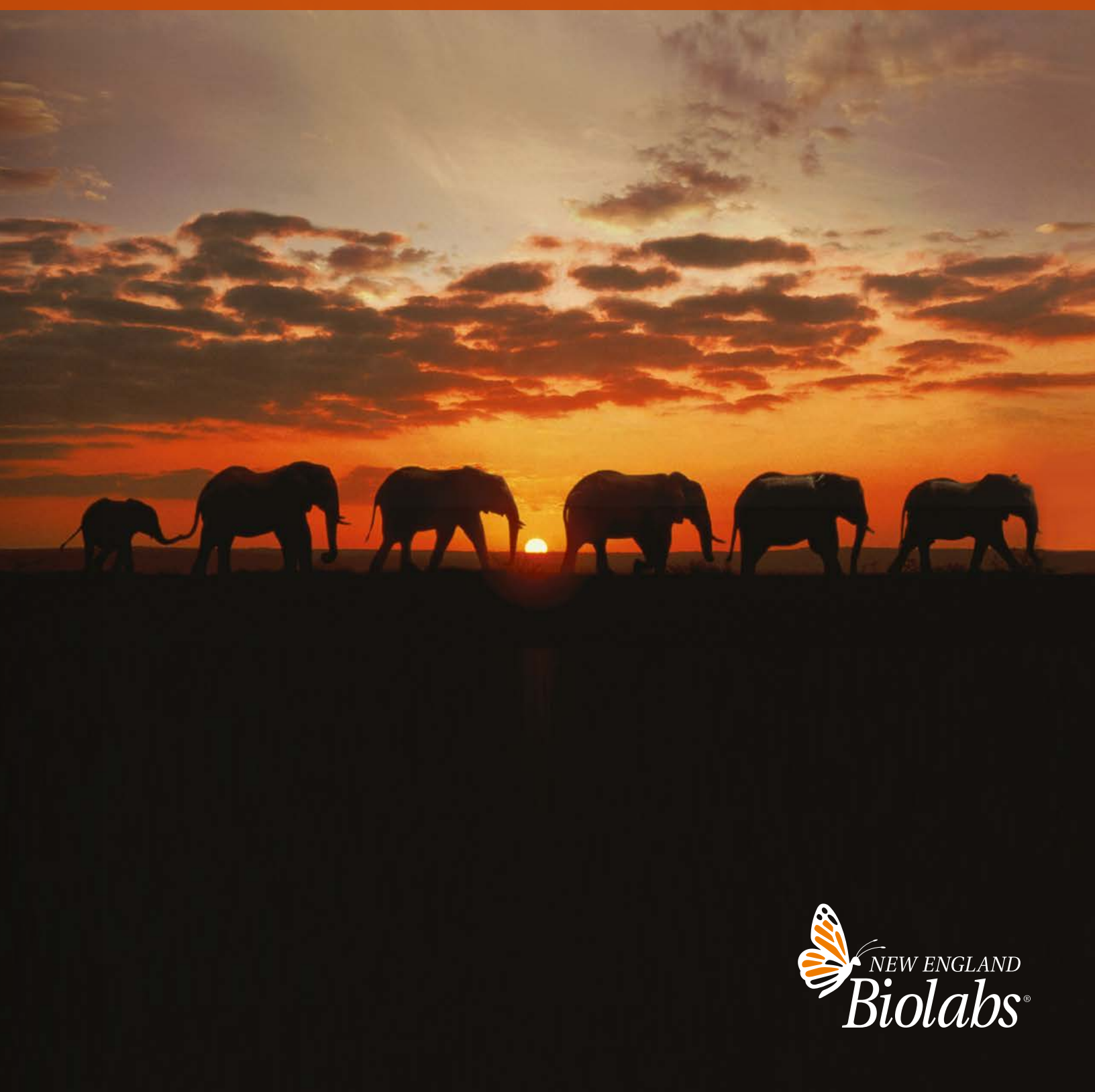


NEBuilder[®] HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING

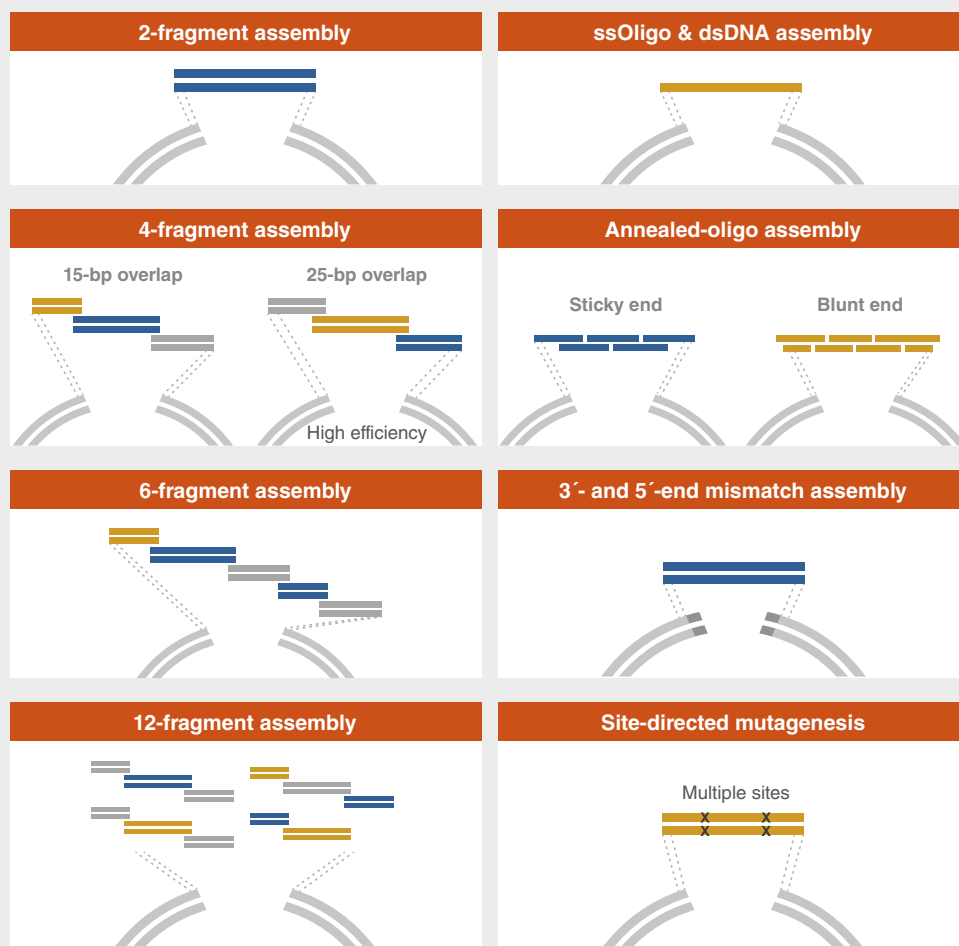


Why choose NEBuilder HiFi

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



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

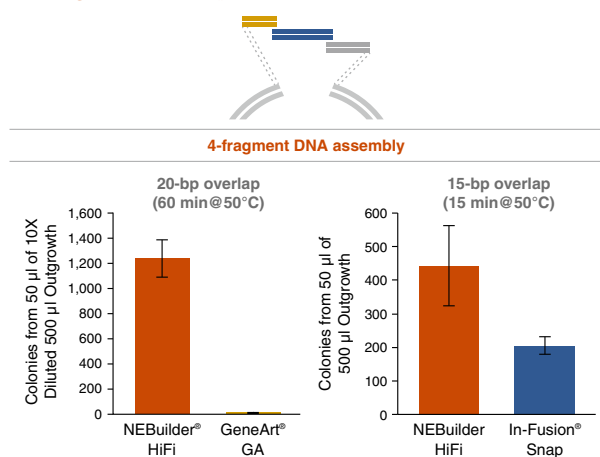
* Available in larger volumes. Please contact custom@neb.com for details.

i DNA Assembly?

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

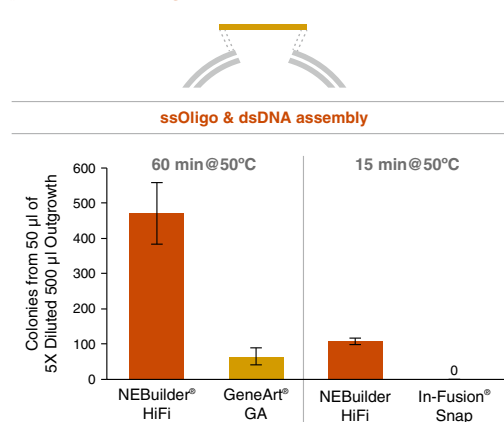
NEBuilder HiFi DNA Assembly offers several advantages over GeneArt Gibson Assembly and In-Fusion Snap Assembly. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two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