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NEBuilder – Version 4.0 – 10/21

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Featured Tools



For help with finding the right products and protocols for each step of your next traditional cloning experiment try **NEBcloner[®]** at NEBcloner.neb.com



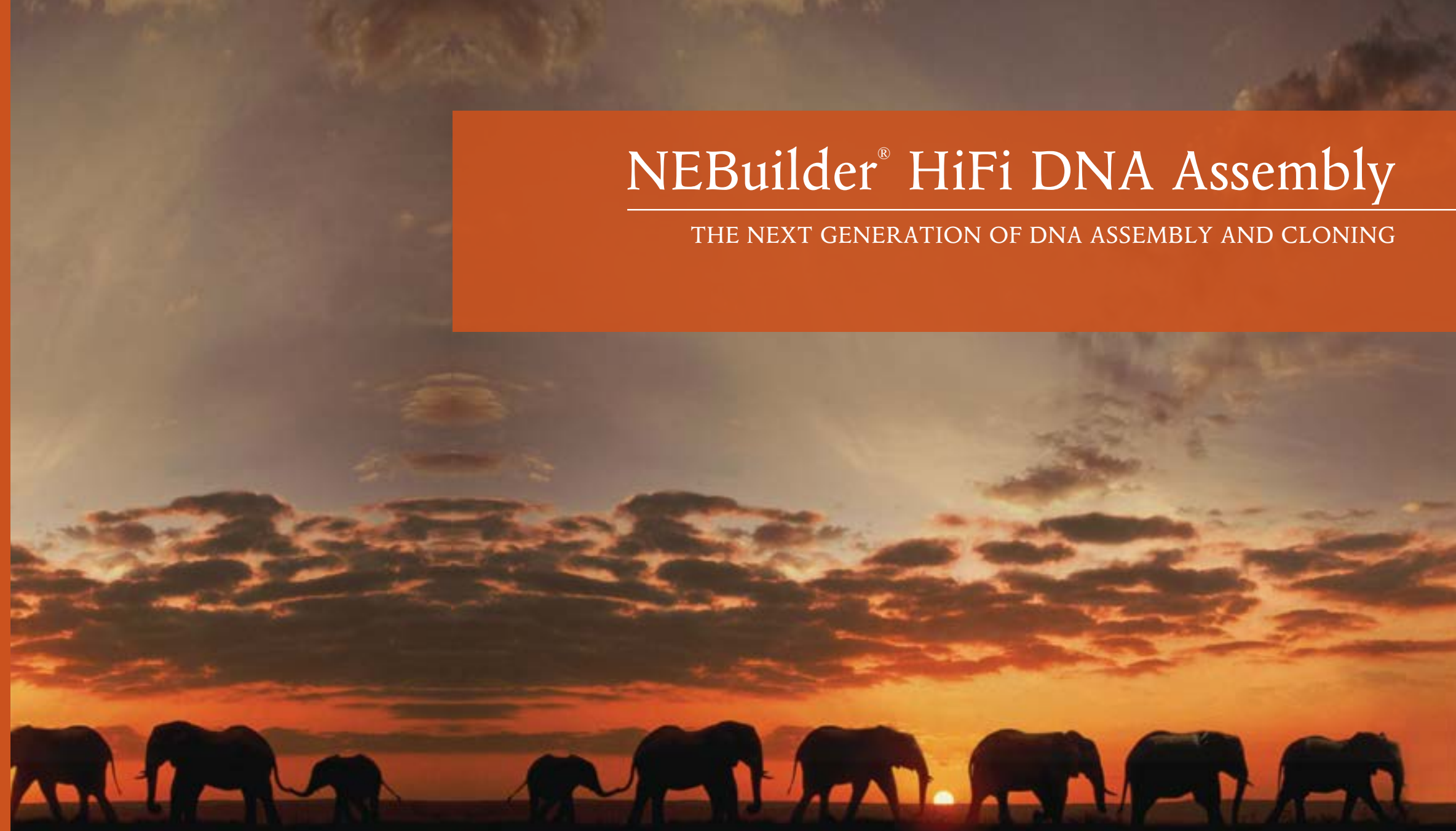
For help with designing primers for DNA assembly, try **NEBuilder[®] DNA Assembly Tool** at NEBuilder.neb.com



Download the **NEB AR App** for iPhone[®], iPad[®] or Android[™]. Scan the augmented reality butterfly icon located on the corner of the page to find videos, tutorials and immersive experiences.

NEBuilder[®] HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING



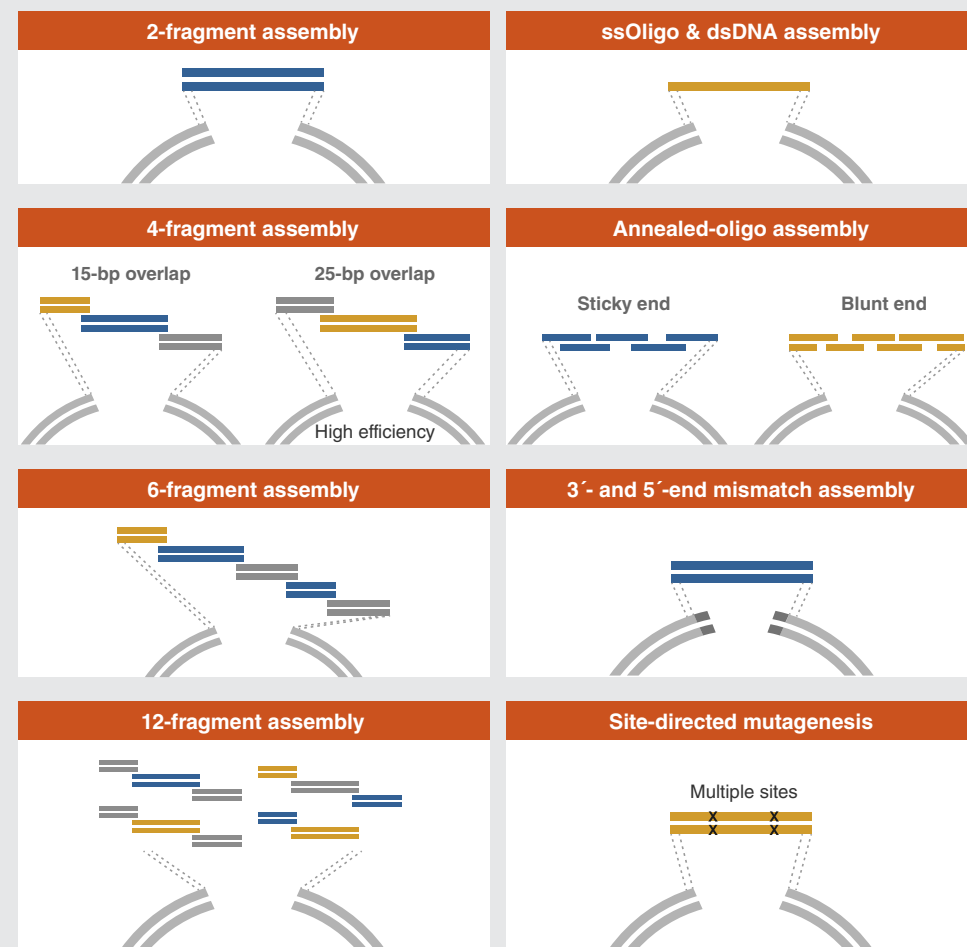
be INSPIRED
drive DISCOVERY
stay GENUINE

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.

Not your average DNA assembly reagent



Ordering Information

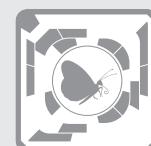
PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix*	E2621S/L/X	10/50/250 reactions
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 reactions

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

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 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.
- 5 Save money**
No licensing fees from NEB for NEBuilder products
- 6 Increased stability**
Store at -20°C, with improved stability over competition

DOWNLOAD THE NEB AR APP*



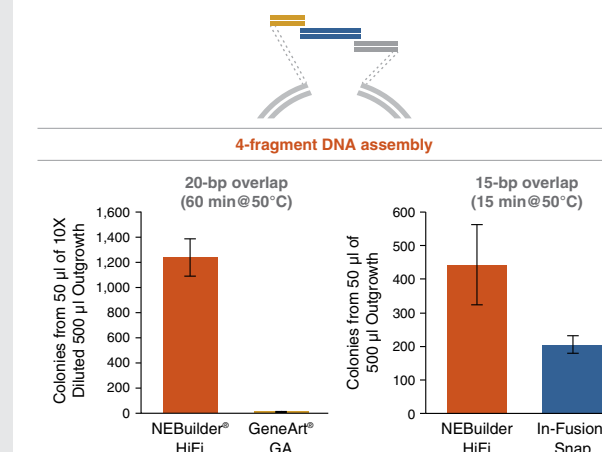
How does NEBuilder HiFi DNA Assembly work?

*see back cover for details

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

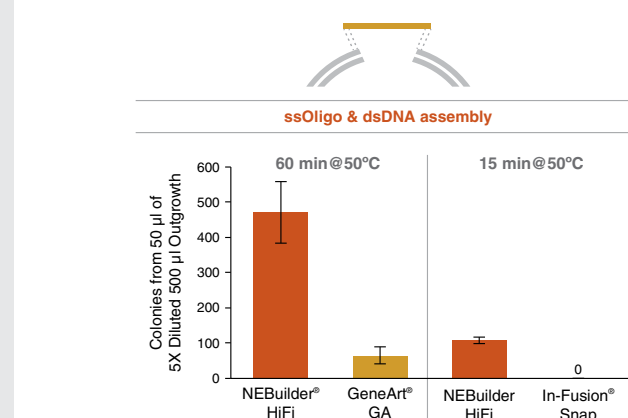
NEBuilder HiFi DNA Assembly offers several advantages over GeneArt Gibson Assembly and In-Fusion Snap Assembly. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two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