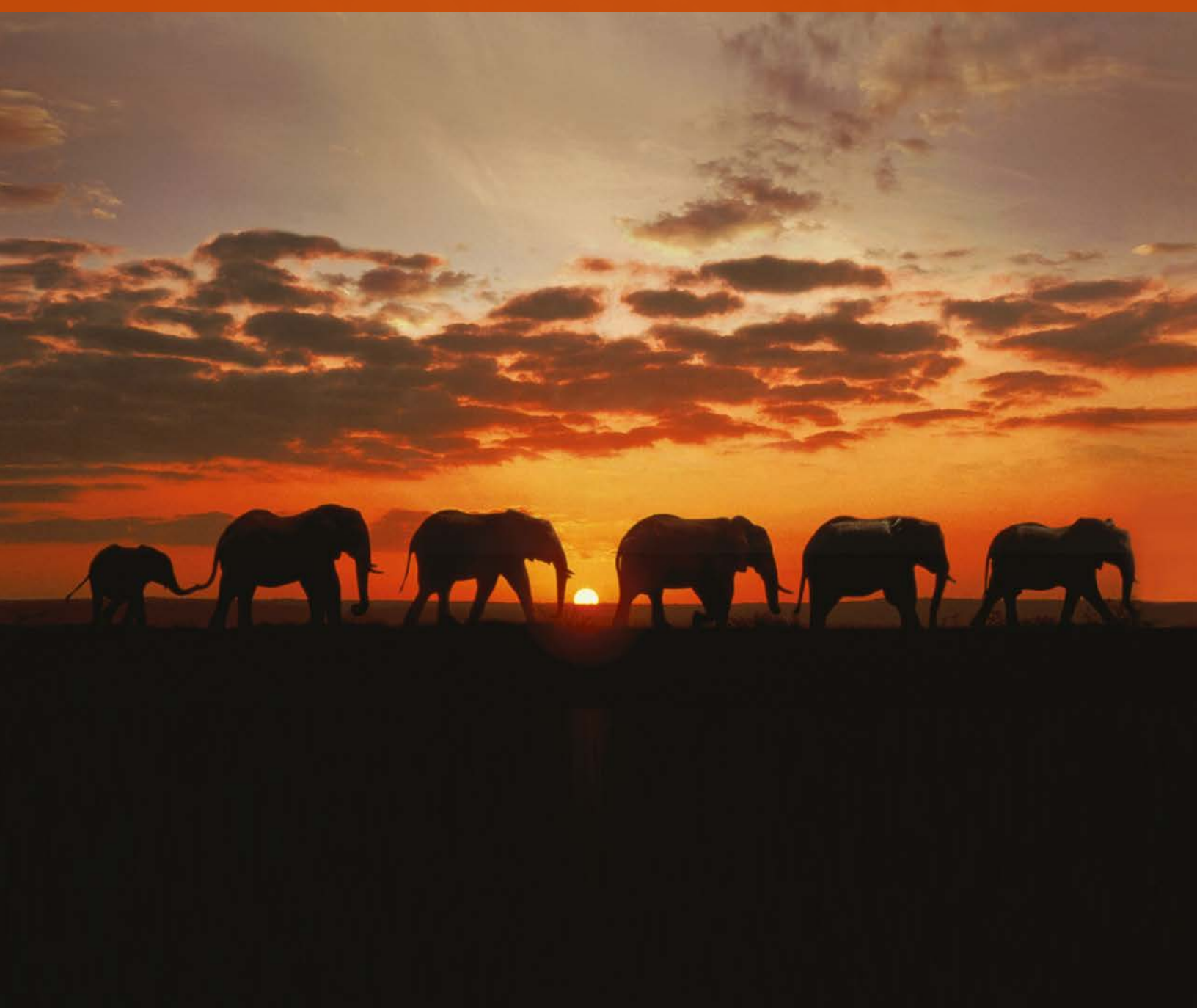


# NEBuilder<sup>®</sup> HiFi DNA Assembly

THE NEXT GENERATION OF DNA ASSEMBLY AND CLONING



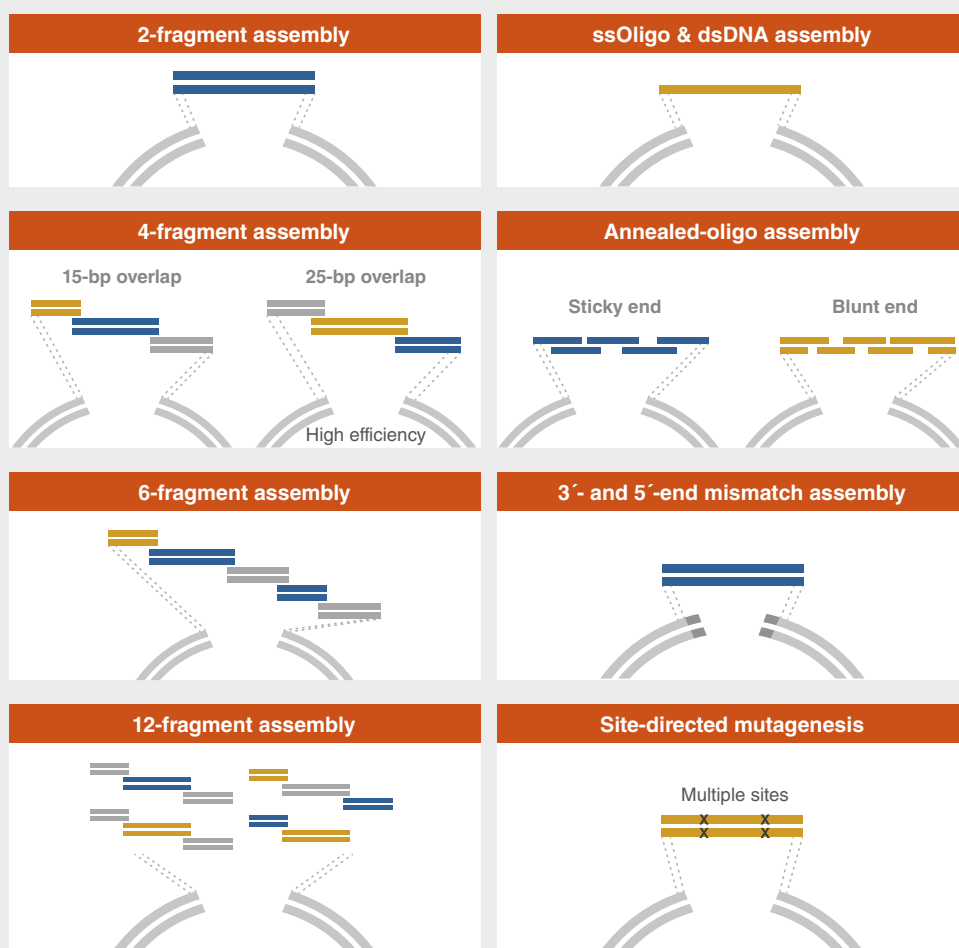
*be* INSPIRED  
*drive* DISCOVERY  
*stay* GENUINE

# 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 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

## Ordering Information

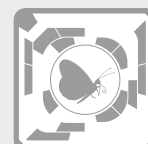
PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix*	E2621S/L/X	10/50/250 reactions
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 reactions

\* Available in larger volumes. Please contact [custom@neb.com](mailto:custom@neb.com) for details.



DOWNLOAD THE NEB AR APP\*

How does  
NEBuilder HiFi DNA  
Assembly work?



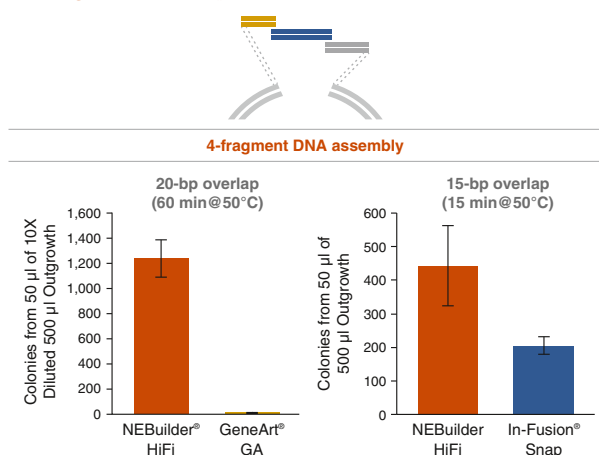
\*see back cover for details

# i DNA Assembly?

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

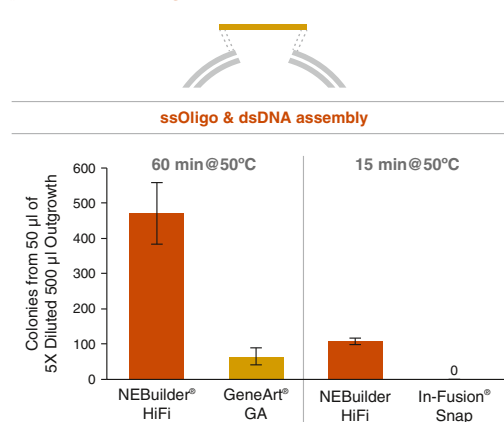
NEBuilder HiFi DNA Assembly offers several advantages over GeneArt Gibson Assembly and In-Fusion Snap Assembly. These include: higher accuracy due to the use of a high-fidelity polymerase, the ability to assemble both 5'- and 3'-end mismatches, lower DNA input requirements and the ability to bridge two 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?*
<b>2-fragment assembly</b>						
No mismatch	+++	Yes	++	Yes	++	No
3'- and 5'-end mismatch	+++	Yes	++	Yes	X	No
<b>4-fragment assembly</b>						
15-bp overlap & no mismatch	+++	Yes	++	Yes	++	No
25-bp overlap & no mismatch	+++	Yes	++	Yes	++	No
<b>Oligo assembly</b>						
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](http://NEBuilderHiFi.com) to view additional performance data, tutorials and much more



For help designing primers, try NEBuilder Assembly Tool at [NEBuilder.neb.com](http://NEBuilder.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](http://www.neb-online.de)

#### HEADQUARTERS:

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  
info@neb.com  
www.neb.com

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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](http://NEBcloner.neb.com)



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

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(BE: 0800 - 71640)  
info@bioke.com  
www.bioke.com

BULGARIA  
ELTA 90M Ltd.  
Tel: (+359) 2 983 96 49  
Fax: (+359) 2983 2211, 9274 232  
office@elta90.eu  
www.elta90.com

CROATIA:  
Diagnostica Skalpeli d.o.o.  
Tel: (+385) 1 377 8484  
Fax: (+385) 1 377 8585  
skalpeli@skalpeli.hr  
www.skalpeli.hr

CZECH REPUBLIC:  
BioTech a.s.  
Tel: (+420) 2727 017 39  
info@ibiotech.cz  
www.ibiotech.cz

DENMARK:  
BioNordika Denmark A/S  
Tel: (+45) 39 56 20 00  
Fax: (+45) 39 56 19 42  
info@bionordika.dk  
www.bionordika.dk

FINLAND:  
BioNordika Oy  
Tel: (+358) 207 410 270  
info@bionordika.fi  
www.bionordika.fi

GREECE:  
BIOLINE SCIENTIFIC  
Tel: (+30) 210 522 6547  
Fax: (+30) 210 524 4744  
info@bioline.gr  
www.bioline.gr

HUNGARY:  
Kvalitex Kft.  
Tel: (+361) 340 4700  
Fax: (+361) 339 8274  
info@kvalitex.hu  
www.kvalitex.hu

ITALY:  
EUROCLONE S.P.A.  
Tel: (+39) 02 38 1951  
info@euroclone.it  
www.euroclonegroup.it

ISRAEL:  
Ornat BioChemicals &  
Lab Equipment  
Tel: (+972) 8 947 7077  
Fax: (+972) 8 936 3034  
ornatbio@ornat.co.il  
www.ornat.co.il

LITHUANIA:  
Nanodiagnostika, Ltd.  
Tel: (+370) 525 052 44  
lina@nanodiagnostika.lt  
www.nanodiagnostika.lt

NORWAY:  
BioNordika AS  
Tel: (+47) 2303 5800  
info@bionordika.no  
www.bionordika.no

POLAND:  
Lab-JOT Ltd. Sp.z o.o. Sp.k.  
Tel: (+48) 2233 598 84  
Fax: (+48) 2233 598 19  
biuro@labjot.com  
www.labjot.com

PORTUGAL:  
Werfen  
Tel: (+351) 214 247 300  
dac-pt@werfen.com  
www.pt.werfen.com

ROMANIA:  
BioZyme  
Tel: (+40) 264 523 281  
contact@biozyme.ro  
www.biozyme.ro

RUSSIA and CIS:  
SkyGen LLC  
Tel: (+88) 0033 312 26  
info@skygen.com  
www.skygen.com

SERBIA:  
Alfa Genetics d.o.o.  
Tel: (+38) 1011 2084 252  
office@alfagenetics.rs  
www.alfagenetics.rs

SPAIN:  
Werfen  
Tel: (+34) 900 822 290  
customerservice-es@werfen.com  
www.es.werfen.com

SLOVAK REPUBLIC  
BioTech s.r.o.  
Tel: (+421) 254 7744 88  
Fax: (+421) 232 2010 48  
info@ibiotech.sk  
www.ibiotech.sk

SLOVENIA:  
Mikro+Polo d.o.o.  
Tel: (+386) 2614 33 00  
Fax: (+386) 2614 33 20  
podpora@mikro-polo.si  
www.mikro-polo.si

SOUTH AFRICA:  
Inqaba Biotechnical Industries  
(Pty) Ltd  
Tel: (+27) 1234 358 29  
info@inqababiotech.co.za  
www.inqababiotech.co.za

SWEDEN, ESTONIA, LATVIA,  
LITHUANIA:  
BIONORDIKA SWEDEN AB  
Tel: (+46) 830 60 10  
info@bionordika.se  
www.bionordika.se

SWITZERLAND:  
BioConcept Ltd.  
Tel: (+41) 6148 680 80  
Fax: (+41) 6148 680 00  
info@bioconcept.ch  
www.bioconcept.ch

TURKEY:  
EKA BIOLAB TEKNOLOJİ  
Tel: (+90) 312 248 13 24  
Email: [info@ekabiolab.com](mailto:info@ekabiolab.com)  
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