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NEBuilder HiFi DNA Assembly Reaction (E2621) V.2

New England Biolabs[1]

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NEBuilder HiFi DNA Assembly Master Mix was developed to improve the efficiency and accuracy of DNA assembly. This method allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. This method has been used to assemble either single-stranded oligonucleotides or different sizes of DNA fragments with varied overlaps (15–80 bp). It has utility for the synthetic biology community, as well as those interested in one-step cloning of multiple fragments due to its ease of use, flexibility and simple master-mix format. The reaction includes different enzymes that work together in the same buffer (see Figure 1 in Guidelines):

- The exonuclease creates single-stranded 3´ overhangs that facilitate the annealing of fragments that share complementarity at one end (the overlap region)
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

The end result is a double-stranded fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation of *E coli*.

NEBuilder HiFi DNA Assembly kits are available in various formats: with NEB 5-alpha chemically competent cells (Cloning Kit, NEB #E5520), as a bundle with NEB 10-beta chemically competent cells (Bundle for Large Fragments, NEB #E2623) and without competent cells (Master Mix, NEB #E2621) . NEB 5-alpha chemically competent cells are excellent for routine assemblies of 15 kb or less. NEB recommends NEB 10-beta Competent *E. coli* (High Efficiency, NEB #C3019) or NEB 10-beta Electrocompetent *E. coli* (NEB #C3020) for assemblies larger than 15 kb. If the assembled genes contain repetitive sequences, NEB Stable Competent *E. coli* (NEB #C3040) should be used.

DOI

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https://www.neb.com/protocols/2014/11/26/nebuilder-hifi-dna-assembly-reaction-protocol

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NEBuilder HiFi DNA Assembly Master Mix, DNA assembly, InFusion, cloning, E2621

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Figure 1: Overview of the NEBuilder HiFi DNA Assembly Method

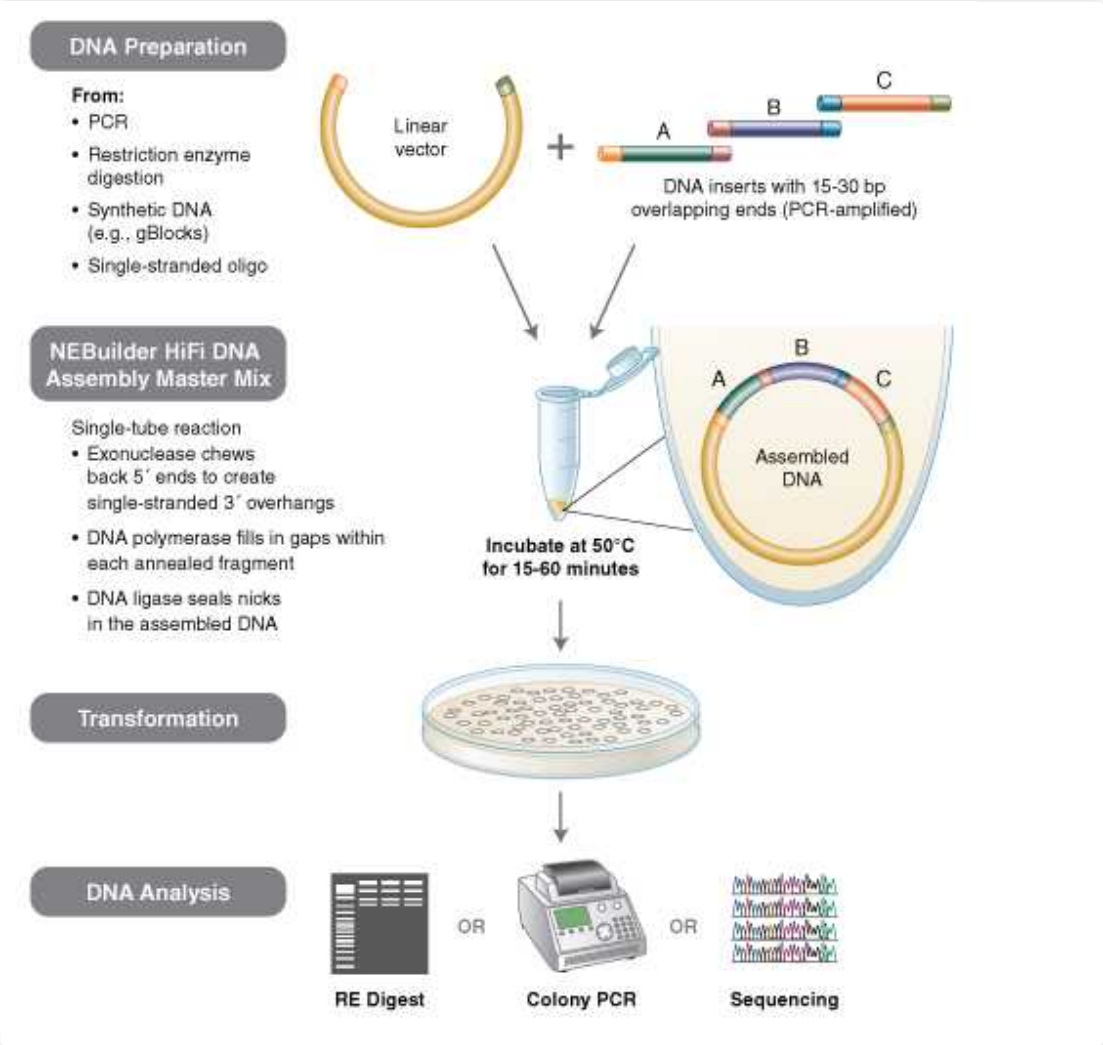
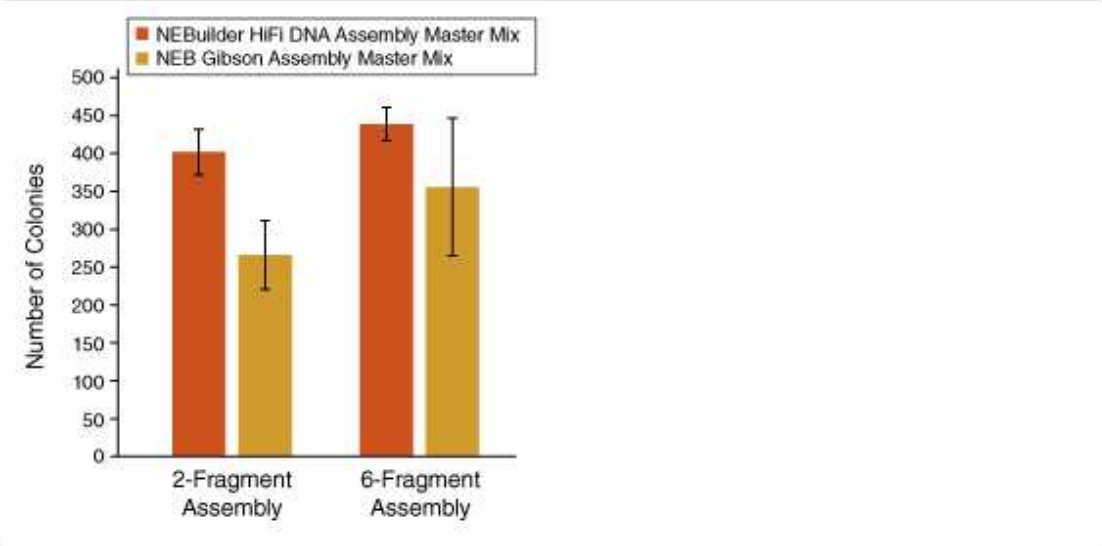


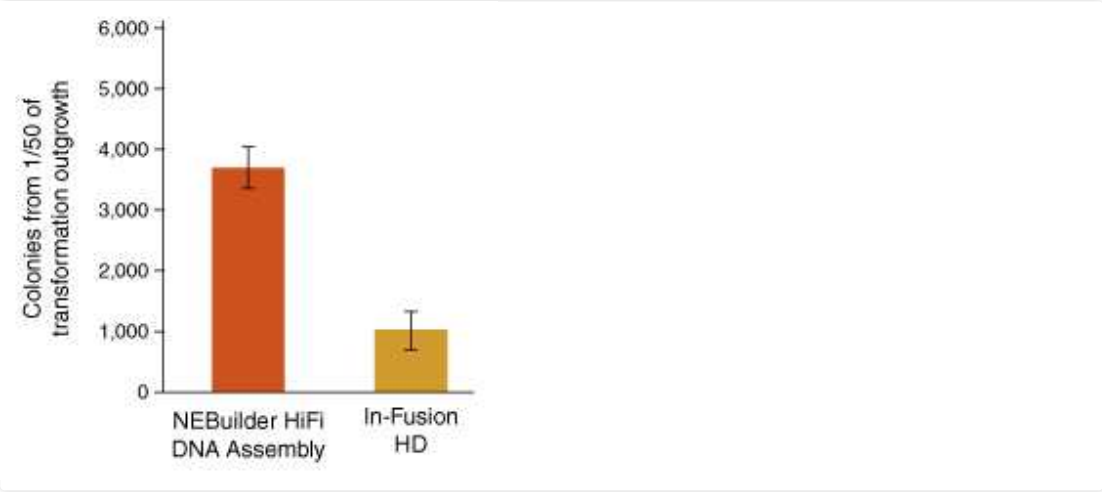
Figure 2: NEBuilder HiFi DNA Assembly offers improved efficiency and accuracy over NEB Gibson Assembly



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.

View additional performance data compared to NEB Gibson Assembly

Figure 3: NEBuilder HiFi delivers higher colony yield than In-Fusion HD



Two-fragment reactions were set up using the positive control from the In-Fusion HD Cloning Kit (Clontech Takara Bio USA, Inc), according to recommended protocols. 2 µl of assembly reaction was transformed into supplied competent cells. 1/50 of outgrowth was spread on an Ap^R plate.

View additional performance data compared to In-Fusion HD

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MATERIALS

NEBuilder HiFi DNA Assembly Master Mix - 50 rxns **New England Biolabs Catalog #E2621L**

NEBuilder HiFi DNA Assembly Master Mix - 10 rxns **New England Biolabs Catalog #E2621S**

NEBuilder HiFi DNA Assembly Master Mix - 250 rxns **New England Biolabs Catalog #E2621X**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Optimal Quantities
NEB recommends a total of **0.03–0.2 pmols** of DNA fragments when 1 or 2 fragments are being assembled into a vector, and **0.2–0.5 pmols** of DNA fragments when 4–6 fragments are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend the following formula, or using the tool, NEBiocalculator.

pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)
50 ng of 5000 bp dsDNA is about 0.015 pmols
50 ng of 500 bp dsDNA is about 0.15 pmols

The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm or estimated from agarose gel electrophoresis followed by ethidium bromide staining.

1 

Set up the following reaction **On ice** (to **20 µL** total volume):

A	B	C	D
	Recommended Amount of Fragments Used for Assembly		
	2–3 Fragment Assembly*	4–6 Fragment Assembly**	Positive Control †
Recommended DNA Molar Ratio	vector:insert = 1:2	vector:insert = 1:1	
Total Amount of Fragments	0.03–0.2 pmols* X µl	0.2–0.5 pmols** X µl	10 µl
NEBuilder HiFi DNA Assembly Master Mix	10 µl	10 µl	10 µl
Deionized H2O	10-X µl	10-X µl	0
Total Volume	20 µl ††	20 µl ††	20 µl

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* Optimized cloning efficiency is 50–100 ng of vector with 2-fold excess of each insert. Use 5-fold molar excess of any insert(s) less than 200 bp. Total volume of unpurified PCR fragments in the assembly reaction should not exceed 20%. To achieve optimal assembly efficiency, design 15-20 bp overlap regions between each fragment.
** To achieve optimal assembly efficiency, design 20-30 bp overlap regions between each fragment with equimolarity of all fragments (suggested: 0.05 pmol each).
† Control reagents are provided for 5 experiments.
†† If greater numbers of fragments are assembled, increase the volume of the reaction, and use additional NEBuilder HiFi DNA Assembly Master Mix.

2 Determine whether your reactions will involve assembly of 2-3 fragments or 4-6 fragments and move forward with the following steps:
Step 2 includes a Step case.
2-3 fragments
4-6 fragments

step case

2-3 fragments

3 

Incubate samples in a thermocycler at **50 °C** for **00:15:00** .

4 

Following incubation, store samples **On ice** or at **-20 °C** for subsequent transformation.

Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases.

5 

Transform NEB 5-alpha Competent *E. coli* cells (provided in the cloning kit, bundle or purchased separately from NEB) with **2 µL assembled product** , following the transformation protocol.

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