SEP 23, 2019

HiFi Gibson Assembly (Protocol for the NEBuilder® HiFi DNA Assembly Master Mix)

Alba Balletbó¹

¹Wageningen University

iGEM Wageningen 2019

Alba Balletbó

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.7kchksw

Protocol Citation: Alba Balletbó 2019. HiFi Gibson Assembly (Protocol for the NEBuilder® HiFi DNA Assembly Master Mix). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7kchksw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Sep 23, 2019

Last Modified: Sep 23, 2019

PROTOCOL integer ID:
 28004

ABSTRACT

This is the protocol for DNA Assembly using the NEBuilder® HiFi DNA Assembly Master Mix.

GUIDELINES

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, [NEBcalculator](#).

$\text{pmols} = (\text{weight in ng}) \times 1,000 / (\text{base pairs} \times 650 \text{ daltons})$

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.

MATERIALS

MATERIALS

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

1 Set up the following reaction on ice (to 20µl total volume):

	2-3 Frag ment Asse mbly	4-6 Frag ment Asse mbly
DNA Ratio	Vecto r:Inser t = 1:2	Vecto r:Inser t = 1:1
Total amou nt of Frag ment s	0.03- 0.2 pmols	0.2- 0.5 pmols
NEBui lder HiFi DNA Asse mbly Mast er Mix	10 µL	10 µL
Deioni zed H2O	10 - X µL	10 - X µL
Total Volu me	20 µL	20 µL

- 2 Incubate samples in a thermocycler at 50°C for 15 minutes (when 2 or 3 fragments are being assembled) or 60 minutes (when 4–6 fragments are being assembled).
- 3 Following incubation, store samples on ice or at –20°C for subsequent transformation.
- 4 Transform into chemically competent cells (1-5µL) or in electrocompetent cells (1 µL, diluted 10x or 5 µL, after purification to remove salts).