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

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Alba Balletbó<sup>1</sup>

<sup>1</sup>Wageningen University

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Alba Balletbó

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** September 23, 2019

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## 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:Inse rt = 1:2	Vecto r:Inse rt = 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
Deion ized H <sub>2</sub> O	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).