Sep 23, 2019

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

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

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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**

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

Created: September 23, 2019

Last Modified: September 23, 2019

Protocol Integer ID: 28004

Keywords: hifi gibson assembly, protocol for dna assembly, gibson assembly, dna assembly, assembly, dna

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, **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.

Materials

MATERIALS

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

Troubleshooting

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

		2-3 Fragment Assembly	4-6 Fragment Assembly
	DNA Ratio	Vector:Insert = 1:2	Vector:Insert = 1:1
	Total amount of Fragments	0.03-0.2 pmols	0.2-0.5 pmols
	NEBuilder HiFi DNA Assembly Master Mix	10 µL	10 µL
	Deionized H2O	10 - X µL	10 - X µL
	Total Volume	20 µL	20 µL

2 Incubate samples in a thermocycler at 50ºC 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 –20ºC 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).