NEBuilder HiFi DNA Assembly Reaction (E2621)

NEB

Abstract

This is the protocol for DNA Assembly using the NEBuilder® HiFi DNA Assembly Master Mix (E2621).

Citation: NEB NEBuilder HiFi DNA Assembly Reaction (E2621). **protocols.io**
dx.doi.org/10.17504/protocols.io.cwaxad
Published: 10 Feb 2015

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

NEBuilder HiFi DNA Assembly Master Mix - 10 rxns E2621S by New England Biolabs

Protocol

Step 1.

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

	Recommended Amount of Fragments Used for Assembly		
	2–3 Fragment Assembly*	4–6 Fragment Assembly**	Positive Control†
Recommended DNA 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

PROTOCOL

. E2621 DNA Assembly Reaction

CONTACT: New England Biolabs

ANNOTATIONS

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

Step 1.1.
Vector DNA

ANNOTATIONS

Shine Sun 19 Apr 2016
pLenti Guide puro
Weijun Liu 15 Aug 2016

pLentiCRISPRV2

Step 1.2.
Insert fragments DNA

Step 1.3.
NEBuilder HiFi DNA Assembly Master Mix

REAGENTS

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Step 1.4.
Deionized H2O

Step 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).

Step 3.
Following incubation, store samples on ice or at –20°C for subsequent transformation.

ANNOTATIONS

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Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).

Step 4.

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