Gibson Assembly

Introduction

This protocol is the "NEBuilder HiFi DNA Assembly Reaction Protocol" from New England BioLabs.

Protocol

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.

HiFi DNA Assembly Protocol

1. Set up the following reaction on ice:

	Recommended Amount of Fragments Used for Assembly		
	2–3 Fragment Assembly*	4–6 Fragment Assembly**	NEBuilder 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 H_2O	10-X µl	10-X µl	0
Total Volume	20 µl††	20 µl††	20 µl

* 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. 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). 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 (for further details see FAQ section).

3. Transform NEB 5-alpha or 10-beta Competent *E. coli* cells (provided in the cloning kit, bundle or purchased separately from NEB) with 2 µl of the chilled assembled product, following the transformation protocol.