Gibson Assembly® Protocol (E5510)

Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found here.

Materials

- Gibson Assembly Cloning Kit
  - Gibson Assembly® Master Mix
  - NEBuilder® Positive Control
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - SOC Outgrowth Medium
  - pUC19 Transformation Control Plasmid
- DNA Polymerases (for generating PCR products)
  - Recommended: Q5® High-Fidelity DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase, or Q5 Hot Start High-Fidelity 2X Master Mix
- LB (Luria-Bertani) plates with appropriate antibiotic

Procedure

Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to* ***input your total DNA fragment volume in cells B3 and C3*** *for assemblies with 2-3 fragments and 4-6 fragments, respectively.*

   - NEB recommends a total of ***0.02–0.5 pmols of DNA fragments when 1 or 2 fragments*** *are being assembled into a vector and* ***0.2–1.0 pmoles 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 using NEB's online tool, NEBioCalculator.*
   - 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.

Table1

	A	B	C	D
1		**2-3 Fragments Assembly**	**4-6 Fragments Assembly**	**Positive Control ****
2	**Concentration Range of DNA fragments**	02 - .5 pmols*	.2 - 1.0 pmols*	0 pmols
3	**Total Volume of Fragments (µl)**			10
4	**Gibson Assembly Master Mix (2x) (µl)**	10	10	10
5	**Deionized Water (µl)**	#VALUE!	#VALUE!	0
6	**Total Volume (µl) *****	20	20	20

**Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.*
*** Control reagants are provided for 5 experiments with the Gibson Asembly Kit.*
**** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.*

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.

   Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).
   **Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

   00:15:00

3. Store samples on ice or at –20°C for subsequent transformation.

4. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the chemcial transformation protocol or electro competent cells transformation protocol