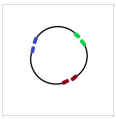

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Nested Gibson Assembly

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1 Works for me dx.doi.org/10.17504/protocols.io.bbikikcw

Protist Research to Optimize Tools in Genetics (PROT-G) JCVI West Protocols

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ABSTRACT

This method can be used to increase the efficiency of Gibson Assemblies containing many pieces and/or difficult to assemble DNA fragments.

GUIDELINES

Use this protocol if your Gibson Assembly repeatedly fails. This method will decrease the amount of DNA pieces going into your Gibson, thereby increasing efficiency of assemblies originally containing a lot of pieces. This protocol has also been used to successfully get colonies from Gibson Assemblies that contained difficult to work with DNA (i.e. AT-rich fragments or significant amounts of secondary structure in certain DNA pieces). Even if a Gibson Assembly failed overall, some of the DNA pieces in the reaction may still be ligated together. By PCR amplifying these already assembled fragments you can then repeat the Gibson Assembly with less pieces.

MATERIALS

NAME	CATALOG #	VENDOR
Gibson Assembly Master Mix - 50 rxns	E2611L	New England Biolabs
Zymo DNA Clean & Concentrator - 5	D4014	Zymo Research
PrimeSTAR Max DNA Polymerase	R045A	Takarabio
NEB 5-alpha Competent E. coli cells	C2987H	New England Biolabs

MATERIALS TEXT

Thermocycler
Agarose
Gel box
UV imager
Waterbath
Incubator
LB broth and plates with antibiotics

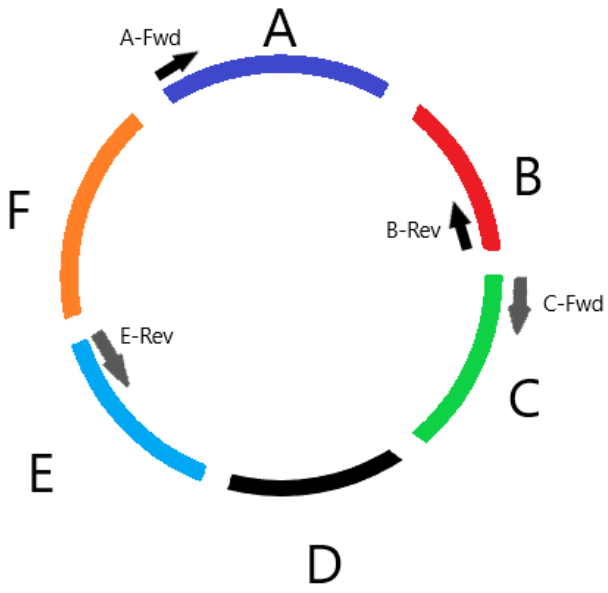
BEFORE STARTING

If your transformation of a Gibson assembly failed to yield any colonies or testing revealed that your assembled plasmids are incomplete, then save the remainder of your Gibson Assembly. The Gibson Assembly will function as template for PCR.

Select primers

1 Using the same primers that you used to amplify your DNA pieces for the Gibson Assembly, you can pick new primer pairs to amplify multiple fragments that have already been ligated together from the failed Gibson reaction.

Example: By using the A-Fwd and B-Rev primers in a PCR reaction, fragments A and B can be amplified as one piece. The C-Fwd and E-Rev primers can also be used to amplify fragments C, D, and E as a single piece. The Gibson Assembly can then be repeated with a total of 3 fragments instead of 6.
Fragment- 1. A-B
2. C-E
3. F



Example of a 6-piece Gibson Assembly being amplified to reduce the total number of pieces to 3

PCR

2 Run the PCR reactions using the chosen primer pairs from step 1 and the failed Gibson Assembly as the template.

Reaction Mixture
25 µl PrimeSTAR Max DNA mastermix
22 µl DI water
1 µl Forward primer [10 µM]
1 µl Reverse primer [10 µM]
1 µl Failed Gibson Reaction
---
50 µl Total

Cycling Conditions
1. 98°C for 10 sec.
2. Tm-5°C for 15 sec.
3. 72°C for 10 sec./kb
* Run steps 1-3 for 30 cycles

Run a gel

3 Run the PCR fragments amplified from step 2 on a .8% - 1% agarose gel to ensure that each of the reactions was successful.

Note: If a PCR reaction of some of the pieces failed, then repeat step 1 with different primer pairs to amplify a different set of fragments together. This also acts as a diagnostic tool to determine which DNA pieces are not assembling efficiently.

Purify DNA

4 Purify the remainder of each of the PCR reactions. We use a Zymo Research DNA Clean and Concentrator kit.

Gibson Assembly

5 Set up Gibson Assembly in a PCR tube
1 µl each DNA fragment (we dilute each of our fragments to 30 fmol)
5 µl Gibson Assembly Master mix (NEB)
x µl DI water
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10 µl total

*Incubate the Gibson Assembly in a thermocycler at 50°C for 1 h.

Transform

6 Mix 5 µl Gibson Assembly with 25 µl NEB 5-alpha chemically competent cells and hold on ice 5-10 min

7 Heat shock cells at 45°C for 30 sec and immediately place back on ice for 5 min

8 Add 270 µl SOC to the cells and incubate at 37°C for 1 h

9 Plate 50 µl cells on an LB plate containing the appropriate antibiotics and grow overnight at 37°C

Verify Transformants

10 Grow resulting colonies in LB broth + antibiotics and perform colony PCR, restriction digestions, and/or sequencing to verify that the assembled plasmids are correct.