Jun 14, 2023

Cloning by Gibson Assembly

DOI
dx.doi.org/10.17504/protocols.io.rm7vzxmp8gx1/v1

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Protocol Citation: Eric ECS Cordeiro-Spinetti 2023. Cloning by Gibson Assembly . **protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzxmp8gx1/v1**

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

Created: June 14, 2023

Last Modified: June 14, 2023

Protocol Integer ID: 83426

Keywords: molecular cloning method, synthetic biology project, joining of multiple dna fragment, dna ligase, major workhorse of synthetic biology project, gibson assembly, multiple dna fragment, synthetic biology company, polymerase, dna, telesis bio, enzymatic activity, exonuclease, robust exonuclease, single strand region, isothermal reaction, annealed single strand region

Abstract

Gibson assembly is a **molecular cloning** method that allows for the joining of multiple **DNA** fragments in a single, isothermal reaction. It is named after its creator, Daniel G. Gibson, who is the chief technology officer and co-founder of the **synthetic biology** company, Telesis Bio. - Wikipedia

Daniel G. Gibson, of the J. Craig Venter Institute, described a robust exonuclease-based method to assemble DNA seamlessly and in the correct order, eponymously known as Gibson Assembly. The reaction is carried out under isothermal conditions using three enzymatic activities: a 5′ exonuclease generates long overhangs, a polymerase fills in the gaps of the annealed single strand regions, and a DNA ligase seals the nicks of the annealed and filled-in gaps. This method has been widely adopted and is a major workhorse of synthetic biology projects worldwide.

Troubleshooting

1 PCR (vector and insert)
Tm vector primers = oC
Tm insert primers = oC

2 Clean-up PCR products with 1 µL Dpn1 for 00:30:00 at 37 °C 30m

3 Purify PCR products and resuspend in lowest volume possible (5-10 uL)

4 Set up Gibson ligation
Vector = 50-100ng
Molar ratio Vector/Insert = 1:1-3

5 Add to Gibson Master Mix

6 Incubate for 01:00:00 at 50 °C 1h

7 Transfer 1-2 µL into 50µL suspension of E.coli

8 Incubate on ice for 00:30:00 30m

9 Heat shock at 40 °C for 30 seconds

10 Transfer to 300 µL of outgrowth media

11 Incubate in shaker for 01:00:00 at 37 °C 1h

12 Plate on antibiotic containing plate and grow Overnight 1h

13 Select colonies for sequencing

Protocol references

https://www.addgene.org/protocols/gibson-assembly/

https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/gibson-assembly

http://nebiocalculator.neb.com/#!/ligation