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We use this protocol and it's working

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Cloning by Gibson Assembly

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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.

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