Gibson protocol from RJ communications

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Gibson protocol

PROTOCOL CITATION

Elizabeth Fozo 2021. Gibson protocol from RJ communications. **protocols.io** https://protocols.io/view/gibson-protocol-from-rj-communications-bq48myzw

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ABSTRACT

Gibson protocol

Protocol from RJ and SH

1 Perform vector and insert amplification using **Q5** PCR
- For template, use 10 and 1 ng.

2 Determine which reactions had successful amplification via agarose gel electrophoresis, use the reaction with a strong/bright band and the lowest amount of starting template for the following steps.

3 Common: Treat vector with DpnI if final selection method would also select for vector template.

3.1 **Do not PCR purify before treating with DpnI unless you need to do gel extraction. DpnI has activity in the Q5 buffer.

3.2 Rare: treat inserts with DpnI if, for some reason, the template would be selectable with the final selection method.

4 PCR purify template and inserts using Qiagen kit or gel extract
- Gel extract only if looks like there are multiple bands on agarose gel from step 2. Otherwise, PCR purify. Gel extraction causes a large loss of DNA

5 Determine the DNA concentration of each piece using a fluorimeter (nanodrop can be less reliable according to Dr. Fozo).
- You can also do a concentration check on the gel using the ladder for confirmation if you're not sure about the concentration given by nanodrop or fluorimeter.

6 Calculate concentrations and volumes you will need to performGibsonassembly reaction before setting up the reaction(s).This is especially important if you are doing multiple reactions.
If you do not have enough of each piece, repeat the previous steps until you do

7 Perform Gibson assembly reaction

7.1 Assemble in Eppendorf tube
- 10µL 2X Gibson assembly Master Mix
- DNA as needed

1. 50 to 100 ng vector, 5:1 or 3:1 molar ratio insert:vector for each insert. Use Nebiocalculator.neb.com
2. The total volume of vectors and insert should not exceed 10µL

- ddH_2O to **20µL**
- Include a no-insert control only if required

7.2 Incubate 1hr at 48^{o}C in heat block (can go longer than 1hr)

8 **Highly suggested:** Perform PCR check of Gibson assembly prior to transformation to see if Gibson was successful.
- Dilute Gibson 1:4 in ddH_2O prior to addition to PCR reaction.

9 Transform 2µL Gibson assembly into TOP10 cells (or another appropriate strain)using electroporation procedure. Rescue with 500µL media (SOC, etc.), plate 100-125µL rescue on 4-5selective plates.
- Also, transform a positive control of whole empty vector with an equivalent amount of DNA to vector in reaction divided by 10 (transforming 2µL of 20µL reaction).

10 Streak isolated colonies, 4-8 for each Gibson, for isolation on appropriate selective media.

11 Screen colonies after isolation streaking using colony PCR for successful Gibson (same PCR as step 8). Use empty vector as control for amplification.

12 Quickly determine presumptively positive isolated colonies from colony PCR, perform steps to prepare -80^{O}C temporarily frozen stocks.

- Do not wait long to take a colony from the plate and move to -80^{O}C, especially for *E. faecalis*. This prevents the accumulation of mutations.

13 At the same time as step 12, isolate plasmid and send out for sequencing if required to confirm no unwanted mutations have occurred and that your colony is fully isolated.

14 If sequencing looks good, move from temporary -80^{O}C to long-term -80^{O}C storage by communicating with Dr. Fozo.