Gibson Assembly Cloning

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

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ABSTRACT

Gibson assembly requires a vector backbone and one or more inserts that have been PCR amplified. The inserts should have 15-20 base pairs of overlap at ligation sites, so primers used to amplify the inserts should contain a tail overhang with this homology. The vector backbone should be linear.

A Gibson reaction uses a master mix that can be homemade or commercially purchased. Add the appropriate amount of master mix, then your vector and insert at 2-3 fold molar excess for a 20 µl reaction. Exact ratios will need to be optimized based on the size of your vector and inserts and how many inserts you have. Incubate this reaction at 50°C for 60 minutes. Then transform this reaction into DH5 alpha (or competent cell of choice) and plate on LB agar with appropriate antibiotic.

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87471

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MATERIALS

PEG-8000
Tris-HCl pH 7.5
$MgCl_2$
DTT
dNTPs [NEB, N0447S]
NAD
10U/µl T5 exonuclease [NEB, M0663S]
2U/µl Phusion polymerase [Thermo, F530S]
40U/µl Taq Ligase [NEB, M0208S]
Sterile water

Competent cells (DH5α) [Thermo, 18258012]
PCR machine
LB, LB agar
antibiotic of choice

Prepare Gibson master mix

1 Make 5 ml of 5x reaction buffer

1.1 25% PEG-8000 1.25 g
500 mM Tris-HCl pH 7.5 2.5 ml
50 mM $MgCl_2$ 0.25 ml of 1M
50 mM DTT 0.25 ml of 1M
1 mM each dNTPs 0.05 ml each of 100mM
5 mM NAD 0.5 ml of 50 mM

2 Prepare master mix

2.1 Master Mix
320 µl 5X Reaction Buffer (above)
0.64 µl 10U/µl T5 exonuclease
20 µl 2U/µl Phusion
160 µl 40U/µl Taq Ligase
700 µl Water

3 Use master mix as 1.5x. Snapfreeze in 15 µL aliquots and store at -80 °C.

Gibson assembly

4 Prepare linearized vector backbone by restriction enzyme digestion or PCR amplification.

5 Prepare inserts by PCR amplification. Ensure that each insert has 15-20 base pairs of overlap with other inserts/vector at ligation sites, added by primer design.

6 Take a 15 ul aliquot of 1.5x Gibson master mix on ice. Add 50-100 ng of vector with 2-3 fold molar excess of PCR inserts, and sterile water if necessary, to make total reaction volume up to 20 µL.

7 Mix well and let incubate in PCR machine for 01:00:00 at 50 °C 1h

Plasmid preparation 1h

8 After incubation is complete, transform into competent cells (DH5α) and plate onto LB agar with appropriate antibiotic. Let this grow at 37 °C Overnight.

9 Pick a single colony to grow in 5 ml LB culture at 37 °C Overnight and purify plasmid to check sequencing.