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Gibson Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.6t5heq6

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

D. G. Gibson, "Enzymatic assembly of DNA molecules up to several hundred kilobases," Nature Methods, vol. 6, no. 5, pp. 343-345, 2009.

1 Prepare 6 ml of 5X ISO Buffer in a 15 ml falcon tube as follows:

3 ml 1 M Tris-HCl pH 7.5
+ 150 ul 2 M MgCl2
+ 240 ul 100 mM dNTP mix (25 mM each: dGTP, dCTP, dATP, dTTP)
+ 300 ul 1 M DTT
+ 1.5 g PEG-8000
+ 300 ul 100 mM NAD
+ _____dH_2O to
6 ml
Store at -20 C in 320 ml aliquots.

2 Prepare 1.2 ml of Gibson assembly master mix as follows:

320 ul 5X ISO Buffer
+ 0.64 ul 10 u/ml T5 exonuclease*
+ 20 ul 2 u/ml Phusion polymerase
+ 160 ul 40 u/ml Taq ligase
+ _____dH_2O to
1.2 ml
Store at -20 C in 15 ml aliquots.

*This is optimized for 20-150 bp sequence homology overlaps

3 Thaw a 15 ml aliquot of the Gibson assembly master mix and keep on ice until use.

4 Measure the DNA concentration (ng/ml) of each assembly piece.

5 Add 100 ng of the linearized vector backbone and equimolar amounts of the other assembly pieces to the thawed 15 ml master mix in a 20 ml total volume assembly reaction mixture as follows:

linearized vector backbone (100 ng)
+ each additional assembly piece (to equimolar with backbone)
+ 15 ml Gibson assembly master mix
+ _____dH_2O to
20 ml

6 Incubate the assembly reaction at 50 C for 60 minutes, and then place on ice.

7 Transform 5 ml of the assembly reaction into 100 ml of competent E. coli and/or run a diagnostic agarose gel to check for successful assembly.