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CPEC Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.6t7hern

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

J. Quan and J. Tian, "Circular polymerase extension cloning of complex gene libraries and pathways," PloS one, vol. 4, no. 7, p. 6441, 2009.

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

2 Add 100 ng of the linearized vector backbone and equimolar amounts of the other assembly pieces to a 25 ml total volume assembly reaction mixture as follows:

linearized vector backbone (100 ng)
+ each additional assembly piece (to equimolar with backbone)
+ 5 ml 5X HF Phusion Reaction Buffer
+ 1 ml 10 mM dNTPs
+ 0.75 ml DMSO
+ 0.5 ml 2U/ml Phusion Polymerase
+ _____dH_2O to
25 ml

3 Perform the assembly reaction in a thermocycler as follows:

30 sec @ 98 C 1 cycle
10 sec @ 98 C }
30 sec @ 55 C } 1 to 15 cycle(s)**
length* (kb) x 15 sec @ 72 C }
10 min @ 72 C 1 cycle

*The total length of the assembled product (in kb)
**The number of repeated cycles should exceed the number of assembly pieces

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.