



Aug 27,
2019

CPEC Protocol

N.J. Hillson^{1,2}

¹JBEI, ²LBNL

1

Works for me

dx.doi.org/10.17504/protocols.io.6t7hern



Mike Fero



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
+ _____dH₂O 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.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited