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One Part CPEC and quick change

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1 Works for me dx.doi.org/10.17504/protocols.io.6vdhe26

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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.

GUIDELINES

This protocol works well for CPEC-style DNA assemblies for which there is only one assembly piece per assembly reaction (e.g., when deleting a portion of an existing plasmid vector, or inserting/replacing short sequences within an existing vector). In such instances, j5 will have designed two full-length primers (containing the requisite flanking homology sequences and possibly embedded short insert sequences) to amplify a DNA template to yield an assembly piece for subsequent CPEC assembly. This protocol uses these same two j5-designed full-length primers but is a preferred alternative to regular CPEC assembly.

1 Setup 2 PCR-like reactions, each with only one of the two full-length primers:

Sterile water 13.5 µL
GC buffer 5 µL
DMSO (30% stock) 2.5 µL
dNTPs (10 mM stock) 0.5 µL
Primer (10 µM stock) 2.5 µL
Mini-prepped template DNA 0.5 µL
Polymerase (Pfu) 0.5 µL
Total 25 µL

98 °C	30s	
98 °C	10s	repeat 4x
Tm+3 °C	20s	
72 °C	15s/kb of DNA	
4 °C	hold	

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2 Combine both reactions and add 1 µL more of polymerase.

98 °C	30 sec	

98 °C	10 sec	repeat 18x
Tm+3 °C	20 sec	
72 °C	15 sec/kb of DNA	
72 °C	5 min	
4 °C	hold	

3 DpnI digest for 1 hour at 37 C.

4 Gel extract (optional).

5 Transform 10 µL into chemically competent cells.

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