One Part CPEC

Loosely based on http://dx.doi.org/10.1371/journal.pone.0006441 and quick change.

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 ^{0}C	30s	
98 ^{0}C	10s	**repeat 4x**
Tm+3 ^{0}C	20s	
72 ^{0}C	15s/kb of DNA	
4 ^{0}C		

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

98 ^{0}C	30 sec	
98 ^{0}C	10 sec	**repeat 18x**
Tm+3 ^{0}C	20 sec	
72 ^{0}C	15 sec/kb of DNA	
72 ^{0}C	5 min	
4 ^{0}C		

3. DpnI digest for 1 hour at 37 ^{0}C.
4. Gel extract (optional).
5. Transform 10 μL into chemically competent cells.