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

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

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

1. DpnI digest for 1 hour at 37 0C.
2. Gel extract (optional).
3. Transform 10 µL into chemically competent cells.