Protocol for Efficent Ligation and Cloning of DNA Fragments with 2-bp overhangs.

Ranjan and Rajagopal (2010). Efficent ligation and cloning of DNA fragments with 2bp overhangs. Analytical Biochemistry 402, 91-92.

Note: The authors used this method for NdeI-HindIII insert ligation. So one of the overhangs is a standard 4bp overhang.

2bp overhang enzymes from NEB:

AseI, AsiSI, BfaI, BspDI, BstBI, ClaI, CviAII, CviQI, HhaI, HinP1I, HpaII, MseI, MspI,

NarI, NdeI, PacI, PvuI,

Method:

1. Digest and purify vector and insert.

2. Place 150 ng of digested vector in an eppendorph tube

3. Add insert so vector:insert ratio is 1:3.

4. Heat DNA mixture to 55 C for 2 minutes and then flash freeze at -20 C for 10 minutes.

5. Add remaining ligation components.

   i.e. if ligation is 10 μL

   x μL vector +insert
   2 μL 10X T4 DNA Ligase Buffer
   3μL (24% w/v) PEG 6000
   Bring up to 9 μL with molecular grade water

   Add 1 μL of T4 DNA Ligase (2000 Units).

6. Let ligation reaction sit overnight at room temperature.

7. Transform bacteria (use 4μL of ligation for chemically competent Cells.

24% PEG 6000 solution:

2.4 g PEG 6000 add molecular grade water to 10 mL and filter sterilize