QUICK LIGATION

Reagents:

- 2X Quick Ligation Buffer
  - 132mM Tris-HCl (3.3 ml of 1M stock)
  - 20mM MgCl2 (0.5 ml of 1M stock)
  - 2mM DTT (50μl of 1M stock)
  - 2mM ATP (100μl of 500mM stock)
  - 15% PEG 6000 3.75 ml
    - 25 ml total

Add ATP last and keep buffer on ice. The pH should be 7.6. Aliquot and store at -20°C.

- T4 DNA Ligase

Procedure:

1. Combine vector and insert in 1.5 ml tube and bring total volume to 10μl with dH2O.
2. Add 10μl 2X buffer and mix.
3. Add 1μl ligase, mix, and incubate at room temperature for 5 minutes.
4. Chill on ice and transform.

Comments:

1. Ligation efficiency is optimal between 5-15 minutes. Longer incubation times will result in lower transformation efficiency.
2. The concentration of vector + insert should be between 1-10μg/ml for efficient ligation. Insert:vector ratios between 2 and 6 are optimal for single insertions.

Reference:

NEB catalog, p.100