

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 (50 μ l of 1M stock)
 - 2mM ATP (100 μ l 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 10 μ l with dH₂O.
2. Add 10 μ l 2X buffer and mix.
3. Add 1 μ l 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 1-10 μ g/ml for efficient ligation. Insert:vector ratios between 2 and 6 are optimal for single insertions.

Reference:

NEB catalog, p.100