Ligation

Material

T4 Ligase from New English biolabs

10x T4 ligase buffer

DNA fragments

ddH_2O

Procedure

1. Calculate the amounts of insert DNA and vecter.
2. Make up a master mix of everything int EP tubes and microcentrifuge it for 30-60s (10.0μL ligation system)

   T4 Ligase 0.5μl

   10x T4 ligase buffer 1.0μl

   ddH_2O (8.5 - vector and insert volume) μl

   6:1 molar ratio of insert to vector (~10ng vector)

   -------- 10.0μL Total
3. Incubate at 60℃ for 1 hour or even longer.