

## ligation

1. Assemble the reaction mixture- Total volume of 10uL:
  - vector DNA 25-50ng
  - insert DNA 25-50ng
  - 5 × Ligase Reaction Buffer 4uL
  - dH<sub>2</sub>O to complete to 10 uL
2. Add 1µl of DNA Ligase to the reaction mixture on ice.
3. Incubate the reaction for 5 min at room temperature.
4. Use transformation protocol to transform ligated products in to competent cells.