Protocol for ligation

1.Test the concentration of the DNA sample(s);
2.Pipet the following into a 0.2ml microfuge tube:
Linearized vector DNA: around 100ng
Insert DNA (at 3:1 molar excess over vector): variable
10x ligation buffer: 1μL
T4 DNA Ligase (NEB): 1μL
ddH_2O: up to 10 μl
3.Vortex thoroughly and spin briefly to collect drops;
4.Incubate the mixture at 16 degree for 16h;
5.Use the ligation mixture for transformation