DNA Fragment Ligation

1. Combine the following on ice:

Reagent	1x(volume in ul)	[final]
10x Ligase Buffer	2	1x
H_2O	Vol req for 20 ul	-
Vector DNA	3 <= fmoles <= 30	-
Insert DNA	9 <= fmoles <=90	-
T4 DNA Ligase	1	1 U
Total	**20**	-

2. Vector insert ratio should be about 1:3-- lower ratios may decrease insertion efficiency and higher ratios may lead to cancatamerization of inserts. Additionally, 100ng <= total DNA <= 500ng
3. Incubate at 16C overnight or at RT for 30 minutes(overnight ligation is preferred)
4. Heat inactivate enzyme at 65C for 15 minutes