

## DNA Fragment Ligation

1. Combine the following on ice:

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

2. Vector insert ratio should be about 1:3-- lower ratios may decrease insertion efficiency and higher ratios may lead to concatamerization 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