This protocol based on a protocol on openwetware somewhere.
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∅∅∅∅∅ T4 Ligase Protocol

T4 allows for the fusion of free DNA ends provided they are complementary or blunt and phosphorylated. Ligase relies on ATP, which is provided in the buffer at a concentration of 10mM.

A series of ligations will be set up, as ligation effectiveness is unreliable and heavily dependent on complex factors including DNA length, concentration, etc. DNA stocks should ideally be purified after a digestion, but ligations can still work even if fragments contain NEBuffer or residual (inactivated) restriction enzyme.

The total volume in the PCR tube should come to 20μL. Any volume left below 20μL should be filled with ddH2O.

**Do not attempt to ligate fragments amplified by PCR unless you are confident they are clean and possess minimal background!*

Compounds
10x T4 ligase buffer
T4 ligase (in glycerol)
ddH2O or autoclaved diH2O
Linearized vector (in EB or ddH2O)
Linearized insert(s) (in EB or ddH2O)

Materials
5μL pipette, tips
PCR tubes

External protocols:
Heat shock transformation

For single insert construction, set up three ligation mixtures:

Ligation 1 (1-1):	Ligation 2 (3-1):	Ligation 3 (5-1):
1μL **Plasmid**	1μL **Plasmid**	1μL **Plasmid**
1μL **Insert**	3μL **Insert**	5μL **Insert**
1μL **Ligase buffer**	1μL **Ligase buffer**	1μL **Ligase buffer**
1μL **T4 Ligase**	1μL **T4 Ligase**	1μL **T4 Ligase**
6μL **ddH2O**	4μL **ddH2O**	2μL **ddH2O**

For double insert/3A construction, set up two ligation mixtures:

Ligation 1 (1-1-1):	Ligation 2 (3-3-1):
1μL **Plasmid**	1μL **Plasmid**
1μL **Insert 1**	3μL **Insert 1**
1μL **Insert 2**	3μL **Insert 2**
1μL **Ligase buffer**	2μL **Ligase buffer**
1μL **T4 Ligase**	1μL **T4 Ligase**
5μL **ddH2O**	10μL **ddH2O**

1. Incubate the mixtures at **22°C (room temperature)** for **1-3 hours**.
   ⇒ *Optionally, you can heat shock after this period at 65 ℃ for 15 minutes to deactivate the ligase.*

2. Plate solutions on appropriate antibiotic plates using the **heat shock transformation** protocol.