

Blunt end ligation

To ligate the linear gBlock synthesized by Integrated DNA Technologies a blunt end ligation was performed using the T4 DNA Ligase from NEB. The gBlock was ordered with 5' phosphorylation.

1. Set up the following reaction in a microcentrifuge tube on ice.

Table 1: Components of the blunt end ligation reaction.

Component	20 μL reaction
T4 DNA Ligase Buffer (10x)	2 μ L
Vector DNA	100 ng
T4 DNA Ligase	1.0 μ L
Nuclease-free water	To 20 μ L

2. Gently mix the reaction by pipetting up and down.
3. Incubate at 16°C overnight.
4. Heat inactivate at 65°C for 10 minutes.
5. Chill on ice and transform 1-5 μ L of the reaction into 50 μ L competent cells.