Ligation

Aim

Ligation of one or more DNA fragments previously digested with restriction enzymes for further experiments.

Procedure

Initial notes:
The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.
The T4 DNA Ligase should be added last.

Mix the following components on ice.

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)	2 µl
Vector DNA*	variable
Insert DNA*	variable
T4 DNA ligase	1 µl
RNase-free water	To 50 µl

*Molar ratios can be calculated with the University of Dusseldorf Ligation Calculator.

Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

Incubate at 16°C overnight or at room temperature for 10 minutes.
Heat-inactivate at 65°C for 10 minutes.

Sources

This protocol is a modified version of the original Ligation Protocol with T4 DNA Ligase provided by NEB®.