Protocol for Ligation

Prepare the reaction system as the following:

Component	volume
The product from gel extraction	35ng
Double-stranded	
oligonucleotides(product from	2μL/each
phosphorylation modification)	
T4 DNA ligase	1μL
10×T4 DNA ligase buffer	$2\mu L$
ddH ₂ O	Up to 20μL

Incubate the reaction system at 16 $^{\circ}$ C for 2 h.

Heat it at 65 $^{\circ}$ C for 10 min to end the reaction.