

Ligation

Introduction

Ligation with T4 ligase.

Materials

- Insert
- Plasmid
- Ligase buffer
- Ligase T4
- Nuclease-free water

Procedure

1. Calculate the amount of the insert
2. Pipette the needed amounts; don't forget the control (ligation control)!

Example:

	Real sample
Vector	7 μL
Insert p17	3 μL
Ligase buffer 10x	2 μL
Ligase T4	1 μL
ddH ₂ O	7 μL
Complete volume	20 μL

3. Incubate for 1 hour at around 22°C (RT)
4. Stop the ligation by heating on 65°C for 10 min.