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T4 Ligation

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for DNA ligation using T4 ligase

Materials

Reagents:

- T4 ligase (Thermofisher, 15224041)
- 5x T4 ligation Buffer
- Competent E. coli cells (NEB, C3040H)
- LB plates with necessary antibiotic



T4 Ligation Protocol (Thermofisher, 15224041):

1h 39m

- 1 *T4 DNA Ligase catalyzes the joining of two cohesive- or blunt-ended strands of DNA between the 5'-phosphate and the 3'-hydroxyl groups of adjacent nucleotides.*

- 2 1. For initial reaction, Mix:

1h 39m

Component	Volume (uL)
5x reaction Buffer	4
Vector DNA	X
Insert DNA	Y
H2O	15-X-Y
T4 DNA Ligase	1

X and Y should be calculated for 3 Insert:1 vector molar ratio

Example of Molar Ratio calculation for 3000bp vector with 500bp PCR product insert:

	A	B	C	D	E
	Component	Length of DNA (bp)	Molar ratio	ng of DNA	Volume of 50ng/ul solution
	Vector	3000	1	50	1 ul
	PCR Fragment	500	3	25	0.5 ul
	H2O				8.5 ul

2. Incubate at room temperature for 00:15:00 .

3. Transform Product into E. coli

a) Add 2 μL of product to 50 μL of TOP10 cells and pipette up and down slowly to mix.

b) Incubate for 00:20:00 on ice.

c) Heat shock bacteria in 42 $^{\circ}\text{C}$ waterbath for 00:01:00 .

d) Incubate on Ice for 00:03:00 .

e) Add 100 μL of SOC media and shake in warm room for 01:00:00 .



f) Plate bacteria onto LB-Antibiotic Plate and spread cells with plate spreader to get individual colonies.

g) Incubate overnight in  37 °C warm room.

h) Pick colonies for miniprep growth and sequencing.