Sep 23, 2024

T4 Ligation

DOI
dx.doi.org/10.17504/protocols.io.q26g71po8gwz/v1

Carolina Lopez[1]

[1]Washington University

Cecilia Escudero
wustl

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q26g71po8gwz/v1

Protocol Citation: Carolina Lopez 2024. T4 Ligation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71po8gwz/v1

Protocol status: Working
We use this protocol and it's working

Created: May 28, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 100755

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
H20	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 50 ng/ul solution
	Vector	3000	1	50	1 ul
	PCR Fragment	500	3	25	0.5 ul
	H20				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 °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.