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Ligation

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ABSTRACT

Method of plasmid construction connection (T4 DNA ligase)

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MATERIALS TEXT

MATERIALS

T4 DNA Ligase, 500u **Promega Catalog #M1804**

T4 DNA Ligase Buffer **Thermo Fisher Catalog #46300018**

system

1 **1.5 µL VECTOR DNA**

4.5 µL Insert DNA

0.5 µL T4 DNA ligase

2 µL 5X T4 DNA Ligase Buffer

12.5 µL ddH2O

step1

2 Set up a system as required, all operations on ice Mix ligation system with 100µL of competent and ice bath for at least 30min (thorough contact of DNA and cell, enabling DNA to get inside cell walls)

step2

3 42˚C heat shock for 90s (competent cell swell under high temperature, cell membrane and cell wall fully contact, enabling DNA to flow inside the cell)
Ice bath for 5min (Cell shrinks and DNA is dragged through the cell membrane)

step3

4 Pipette 200µL of full nutrient culture (in clean bench), place in a shaker at 37˚C for 1h

5 Spread the entire sample on the plate, observe after 16h~20h

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