ARIA

DNA ligation Protocol

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DNA ligation

Introduction

Thanks to DNA ligation, we are able to join both ends of the linear DNA molecule. In this way, we can obtain a circular plasmid backbone.

Materials

- *T4 DNA Ligase*
- *T4 DNA Ligase Buffer*
- DNA sample (from PCR cleanup)

Procedure

Recommendations

1. The reaction consisting of the elements and reagents in the table need to be set up into a microcentrifuge tube on ice.

   - *T4 DNA Ligase should be added last.*
   - *T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*
   - *The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.*
   --> Molar ratios can be computed by using the NEBioCalculator.

DNA ligation: T4 DNA ligase

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

2. Gently **mix** the reaction by pipetting up and down and microfuge briefly.

3. For cohesive (sticky) ends, **incubate** at 16°C overnight or room temperature for 10 minutes.

4. For blunt ends or single base overhangs, **incubate** at 16°C overnight or room temperature for 2 hours.

   --> Alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation.

5. **Heat** inactivate at 65°C for 10 minutes.

6. **Chill** on ice and transform 1-5 µl of the reaction into 50 µl competent cells (NZY5α Competent cells, *E. Coli*) [1].

Bibliography

1. New England Biolabs. (n.d.-a). Ligation Protocol with T4 DNA Ligase (M0202). Retrieved October 17, 2021, from Neb.com website: https://international.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202