Ligation and transformation protocol AraC3 and C3 vectors

Materials

- T4 buffer
- T4 ligase
- Vector (C3 or AraC3)
- Insert DNA
- dH_2O
- KCM

Protocol

Ligation

1. Use NEBioCalculator to calculate molar ratios.
2. Thaw and resuspend the T4 DNA Ligase Buffer at room temperature.
3. Set up the reaction in a microcentrifuge tube on ice, according to the pipetting scheme below.
    - *(T4 DNA Ligase should be added last.*
    - *Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. Alternatively, a 1:5 ratio can be used.)*
4. Gently mix the reaction by pipetting up and down and microfuge briefly.
5. incubate at 16°C overnight
6. Heat inactivate at 65°C for 10 minutes.
7. Chill on ice and transform 5 µl of the reaction into 50 µl competent cells according to protocol found on the next page.

Reagent	Insert	Negative
T4 buffer	4.0 µl	4
Vector	0.5 µl	0.5
Insert	… µl	-
H2O	… µl	… µl
T4 Ligase	1.0 µl	1
Final volume	20.0 µl	20.0 µl

Transformation

1. Cool down sterilized water
2. Take competent cells out of -80°C and thaw on ice for 15-20 min (Do not let them get warm).
3. Prepare transformation mixtures according to scheme below
    a. Positive control: construct know to have gene of interest
    b. Negative control: no DNA
4. Cool the mix on ice for 1 min
5. Add 10 µl of Bacteria
6. Incubate for 30 min on ice
7. Heat shock for 1 min at 42°C
8. Incubate for 3 min on ice
9. Add 100 µl medium
10. Incubate for 1-2 hours at 37°C
11. Take 50µl transformed bacteria and plate them
12. Incubate the plate overnight at 37°C

Reagent	Sample	Positive ctrl	Negative ctrl
KCM	2.0 µl	2.0 µl	2.0 µl
DNA	5.0 µl	1.0 µl	x
dH_2O	3.0 µl	7.0 µl	8.0 µl
Final volume	10.0 µl	10.0 µl	10.0 µl