05_03_Seamless-cloning-reaction

DIENSTAG, 1.6.2021

Goal-Setting

- Cloning of an insert and transformation into competent cells

Terms / abbreviations

- cPCR = Colony polymerase chain reaction
- RT = Room temperature

Risk areas

- None

Required materials and / or information

- Chemicals:
  - Chemical competent cells (E. coli C3040I)
  - Desired insert
  - pMA_217 vector
- Materials:
  - 1.5 mL tubes, Eppendorf
  - Agar plates (Ampicillin 100 µg/mL)
  - GeneArt™ Seamless Cloning and Assembly Enzyme Mix, Thermo Fisher Scientific
  - Ice
  - Pipettes, Eppendorf
  - S.O.C. Medium, Thermo Fisher Scientific or self-made
  - Timer

Templates, devices, software

- ThermoMixer C, Eppendorf

Preliminary work

- 05_01_Preparation-of-S.O.C.-medium
- 06_03_Casting-agar-plates

Operation

1. Pipette 5 µL enzyme mix into a cup (on ice)
2. The insert/vector ratio is 4:1. The amount of the insert is 200 ng. In addition, 50 ng of the vector is pipetted to the enzyme mix. The volume should not exceed 5 µL.
3. Incubate for 20 min at RT and incubate for 5 min on ice afterwards
4. Add 50 µL of chemical competent cells (E. coli C3040I)
5. Incubate on ice for 20 min
6. Heat shock at 42 °C for 45 s and incubate on ice for 3 min afterwards
7. Add 300 µL S.O.C. medium
8. Incubate for 30 min at 37 °C and 300 rpm
9. Plating out the medium on an agar plate and incubate them for 12-16 h at 37 °C

Troubleshooting

- Expected heights of the bands after cPCR for pMA_217 vector:
    - Uncut vector: 763 bp
    - Re-ligated vector: 190 bp
    - Vector with control insert: 732 bp
    - Vector with AT-rich insert: 190 + ~insert length bp

Follow-up work

- None