07_02 Cyclic Synthesis

MITTWOCH, 13.10.2021

Goal-Setting

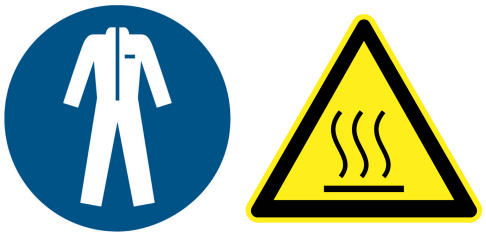
- Immobilized TdT-reaction with biotin-labelled primer and streptavidin-tagged magnet beads to create transitions

Terms / abbreviations

- dNTP = Deoxynucleoside triphosphate

Risk areas

Hazard symbols



Required materials and / or information

- Chemicals:
  - 1x Buffer BXT Strep-Tactin XT elution buffer, IBA Life Science
  - 20 nM biotin-labelled primer, Ella Biotech
  - Endotoxin free water, Invitrogen
  - dNTPs, ThermoFisher
- Material:
  - 2 mL tubes (autoclaved), Eppendorf
  - 1000 µL pipette tip
  - Lighter
  - Neodym Stabmagnet 4x60 mm, Maqna
  - PureCube HiCap StrepTactin MagBeads, Cube Biotech
  - Scissors

Templates, devices, software

- None

Preliminary work

- 07_01 Biotin Streptavidin Immobilization
- Create a mastermix according to 03_01 Thermofisher Protocol for TdT Tailing Reaction

Operation

- The following workflow is an example of a cyclic synthesis, it is not yet optimized

1. Add 100 µL of the biotin-labelled primer and add 10 µL of the magnet beads. Incubate for 10 min
2. Add the magnet with the envelope and incubate for 5 min

3. Put the magnet with the envelope in a tube with endotoxin free water as a washing step for 5 min
4. Put the magnet with the envelope in a tube, which containing a mastermix for TdT reaction for 15 min
5. Washing step for 5 min (like in step 3)
6. Repeat steps 4 and 5 until the desired transitions are created
7. Dissolve the biotin-streptavidin bond by carefully pipetting 30 µL of elution buffer around the envelope, stir, and incubate 10 min

Troubleshooting

- Try to perform every experiment isolated from other experiments involving magnets, as magnet sticks can move and contamine other experiments

Disposal

- Autoclave trash bags, discard in S1 waste

Follow-up work

- 03_04 PCR for ssDNA Samples