

07_02_Cyclic-synthesis

MITTWOCH, 13.10.2021

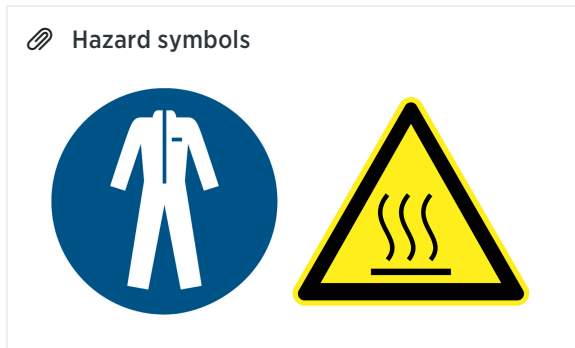
Goal-Setting

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

Terms / abbreviations

- dNTP = Deoxynucleoside triphosphate

Risk areas



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](#)

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 contains TdT buffer, the TdT, the desired nucleotide, and endotoxin-free water for a 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. Washing step for 5 min (like in step 3)
8. Dissolve the biotin-streptavidin bond by carefully pipetting 30 μ L 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 contaminate other experiments

Follow-up work

- [📄 03_04_PCR-for-ssDNA-samples](#)