07_02_Cyclic-synthesis

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Goal-Setting

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

Terms / abbreviations

- dNTP = Deoxy nucleoside 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

Operation

Preparation of magnet stick

1. Desinfect the magnet stick or clean it by wiping with dry tissue
2. Cut off the upper and lower part of the pipette tip
3. Put the magnet stick into the pipette tip and melt the lower end using a lighter

Immobilized TdT reaction

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 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

Follow-up work

- 07_03_PCR-for-cyclic-synthesis-samples