07_01 Biotin Streptavidin Immobilization

SAMSTAG, 9.10.2021

Goal-Setting

- Immobilization of a biotin-labelled primer on a streptavidin-tagged magnet bead
- A magnet stick is covered with a plastic envelope to perform TdT reactions in solution

Terms / abbreviations

- None

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
  - 50 mM Tris, 150 mM NaCl (pH 7.5)
  - Endotoxin free water, Invitrogen
- Material:
  - 2 mL tubes (autoclaved)
  - 1000 µL pipette tip
  - Lighter
  - Neodym Stabmagnet 4x60 mm, Maqna
  - PureCube HiCap StrepTactin MagBeads, Cube Biotech
  - Scissors
  - Trash bags, Th. Geyer GmbH & Co. KG

Templates, devices, software

- Magnet rack

Preliminary work

- None

Operation

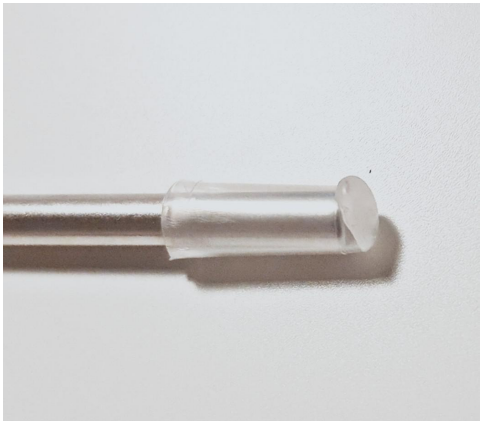
Preparation of magnet beads

1. Put the reaction tube of streptavidin-tagged magnet beads into a magnet rack
2. Pipette the solution and discard
3. Dissolve the beads again in 50 mM Tris, 150 mM NaCl (pH 7.5)

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

Magnet stick with envelope



Immobilization

1. Dilute 10 µL of streptavidin-tagged beads in 100 µL biotin-labelled primer
    a. Shake the tube with beads before usage!
2. Incubate for 10 min and flick the tube from time to time
3. Add a magnet stick with envelope
4. Incubate for 5 min and stir from time to time
5. Transfer the magnet stick with envelope together with the bound beads and primers into a new tube with endotoxin free water
6. Incubate for 5 min
7. The immobilized primers are now ready to use

Elution

1. Carefully pipette 30 µL of elution buffer around the envelope of the stick and stir

Disposal

- Autoclave trash bags, discard in S1 waste

Troubleshooting

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

Follow-up work

- 03_01 Thermofisher Protocol for TdT Tailing Reaction