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



Required materials and / or information

- Chemicals:
 - o 1x Buffer BXT Strep-Tactin®XT elution buffer, IBA Life Science
 - o 20 nM biotin-labelled primer, Ella Biotech
 - o 50 mM Tris, 150 mM NaCl (pH 7.5)
 - o Endotoxin free water, Invitrogen
- Material:
 - o 2 mL tubes (autoclaved)
 - \circ 1000 μL pipette tip
 - o Lighter
 - o Neodym Stabmagnet 4x60 mm, Magna
 - o PureCube HiCap StrepTactin® MagBeads, Cube Biotech
 - Scissors

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

Immobilization

- 1. Dilute 10 μ L of streptavidin-tagged beads in 100 μ L 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
 - a. Following TdT reactions should be perfomed in 100 µL volume

Elution

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

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_02_Cyclic-synthesis