Protein expression and purification of Lsh/Lwa/Lbu with Ni-NTA Agarose

Always work on ice. The volume of the starting culture is assumed to be 1 L. Depending on the actual volume, change the volumes values accordingly.

Aim of the experiment

This experiment can be used to purifiy Cas13a in IPTG-induced bacterial strains.

Materials

- Lysis buffer
  - 50 mM Tris-HCl pH 7.0
  - 500 mM NaCl
  - 5% (v/v) Glycerol
  - 1 mM TCEP
- Washing buffer (add Imidazol to 25 mM final concentration to lysis buffer)
  - 50 mM Tris-HCl pH 7.0
  - 500 mM NaCl
  - 5% (v/v) Glycerol
  - 1 mM TCEP
  - 25 mM Imidazol
- Elution buffer (add Imidazol to 250 mM final concentration to lysis buffer)
  - 50 mM Tris-HCl pH 7.0
  - 500 mM NaCl
  - 5% (v/v) Glycerol

  - 1 mM TCEP
  - 250 mM Imidazol
- 5 mL colum
- 1 M DTT
- PSMF

Procedure

Protein expression

1. Prepare a pre-culture (e.g 100 ml) by scratching cell material with a pipette tip and then dipping in into medium.
2. Incubate O/N.
3. Next morning, dilute 1:100 the O/N culture to a new flask.
4. Measure OD_{600} regularly.
5. At 0.6-0.8 OD_{600} induce with 1 mM IPTG.
6. Incubate O/N.

Cell lysis

1. Centrifuge culture, 4500 g, 30 min, 4 $^\circ C$
2. Discard supernatant. Resuspend in 50 ml medium.
3. Transfer to 50 ml Falcon tubes.
4. Centrifuge, 4500 g, 10 min, 4 $^\circ C$.
5. Discard supernatant.
6. Incubate the pellets at least for 1h, -80 $^\circ C$.
7. Thraw on ice.
8. Add 50 μl DTT to 50 ml lysis buffer.
9. Add PMSF to a final concentration of 1 mM to the lysis buffer.

10. Resuspend pellet in the 50 mL of the lysis buffer.
11. Sonicate (20 s pulse, 50% amplitude, 10 s pause, 8 min). If necessary split the resuspension beforehand.
12. Centrifuge, 6000 g, 30 min, 4 $^\circ C$.

Prepare Ni-NTA agarose

1. For every 4 ml lysate, pipette 1 ml Ni-NTA agarose into a 15 ml tube. Cut the tip off to avoid shredding the matrix.
2. Briefly centrifuge the agarose. (Spin, 15 s).
3. Remove supernatant. Add 2 mL lysis buffer. Gently mix by inverting.
4. Briefly centrigue the agarose. (Spin, 15 s).

Protein purification

1. After step 12 from Cell lysis is done, add the lysate to the matrix.
2. Mix by shaking, 4 $^\circ C$, 60 min.
3. Load 2.5 ml of the lysate-Ni-NTA mixture into a 5 ml column and into a 15 ml Falcon tube.
4. Remove the column's bottom cap.
5. Wait until the column stops dripping.
6. Repeat steps 3-5 until all lysate has been loaded.
7. Collect flowthrough for SDS-PAGE analysis.
8. Put the column in a new Falcon tube.
9. Wash with 2.5 ml washing buffer.
10. Collect flowthrough for SDS-PAGE analysis.
11. Repeat steps 9-10 once.
12. Put the column in a new Falcon tube.
13. Elute the protein with 0.5 mL elution buffer.

14. Collect flowthrough for SDS-PAGE analysis.
15. Repeat steps 13-14 three times.
16. Analyze fraction on SDS-PAGE.
17. Store fractions at 4 $^{\circ}C$.