Protein Expression of Cas13 Lbu, Lsh, Lwa and the TEV protease

Aim of the Experiment

This protocol is used for the expression of Cas13a proteins and the TEV protease. Therefore, *E. coli* strains suitable for protein expression should be transformed with the respective plasmids. For use in this protocol, these expression plasmids were all under the control of an IPTG-inducible T7-promoter. Prior to protein expression it is highly recommended to exclude potential point mutations in the transformed plasmids via sequencing and to prepare cryostocks of the transformed bacteria for long-term use.

Materials

- Cryostocks of *E. coli* Rosetta p2CT-His-MBP-Cas13a-Lbu-WT, *E. coli* Rosetta p2CT-His-MBP-Cas13a-Lsh-WT, *E. coli* BL21 star pSB1C3-His-SUMO-Cas13a-Lwa-WT, *E. coli* BL21 star pSB1C3-His-BBa_K1639008 (TEV)
- 2x YT medium (Carl Roth, Germany)
- Isopropyl-β-D-1-thiogalactopyranoside (IPTG, Carl Roth, Germany)
- Chloramphenicol (Cm, Sigma Aldrich, Germany)
- Carbenicillin (Carb, Sigma Aldrich, Germany)
- Nanophotometer (Implen, Germany)
- Incubator at 37 °C and 16 °C
- Centrifuge (Rotana 460 R, Hettich, Germany)

  General: Autoclave media and sterile filter IPTG and antibiotics before use.

Protein expression

1. Prepare a pre-culture in LB medium with appropriate antibiotics from a cryostock. (Reminder: Rosetta cells carry a second plasmid with a Cm resistance, so in this case do not forget to add this antibiotic).

2. Incubate overnight at 37 °C while shaking.
3. Next morning, dilute the pre-culture 1:100 in sterile 2x YT medium in a Erlenmeyer flask.
4. Incubate at 37 °C while shaking and measure the OD_{600} regularly.
5. Induce with 1 mM IPTG when cells reach a density of OD_{600} = 0.6-0.8.
6. After induction, incubate overnight at 16 °C.
7. Centrifuge the culture for 30 min at 5000 rcf and 4 °C
8. Discard most of the supernatant and resuspend in 50 ml remaining medium and transfer to 50 ml tube.
9. Centrifuge for 10 min at 4500 rcf and 4 °C and discard supernatant.
10. Store or incubate the pellet at least for 1 h at -80 °C to facilitate cell lysis.