Week 12: 24. August 2015 – 28. August 2015

24. August 2015

1) Expression of MEDH2 into *E. coli* BL21 for expression test and solubility assay
   - Measure OD_{600} of each preculture
   - Inoculate an 5 ml expression culture (LB+Kan [50 µg/ml]) with an OD_{600} of 0.3
   - Grow the expression culture for 1 hour at 37 °C shaking at 220 rpm.
   - Measure OD_{600} again (Optimal for induction of protein expression is an OD_{600} between 0.4- 1.0)
   - Take a 1 ml sample in an uninduced stage and harvest the cell by centrifugation at 8000 x g for 5 minutes
   - Induce protein expression by adding IPTG (Final Concentration: 1mM).
   - Incubate the culture for 3 hours at 37 °C shaking at 220 rpm.
   - Take 1 ml sample 3 hours after induction and harvest the cells by centrifugation at 8000 x g for 5 minutes
   - Harvest the rest 3 ml by centrifugation at 8000 x g for 5 minutes→ used for solublity assay
   - Keep the cell pellets at 4 °C
   - Treat the negative control the same way!

25. August 2015

1) SDS-PAGE and Coomassie staining to verify successful pilotexpression of MEDH2
   - Prepare Samples for SDS-Page:
     - Add 100 µl 1x SDS-Loading Buffer
     - Boil the sample at 95 °C for 20 minutes
   - Run SDS-Page
     - 12 % Seperating Gel
     - load 10 µl
   - Stain for 1 hour in Coomassie Staining Solution
   - Destain overnight

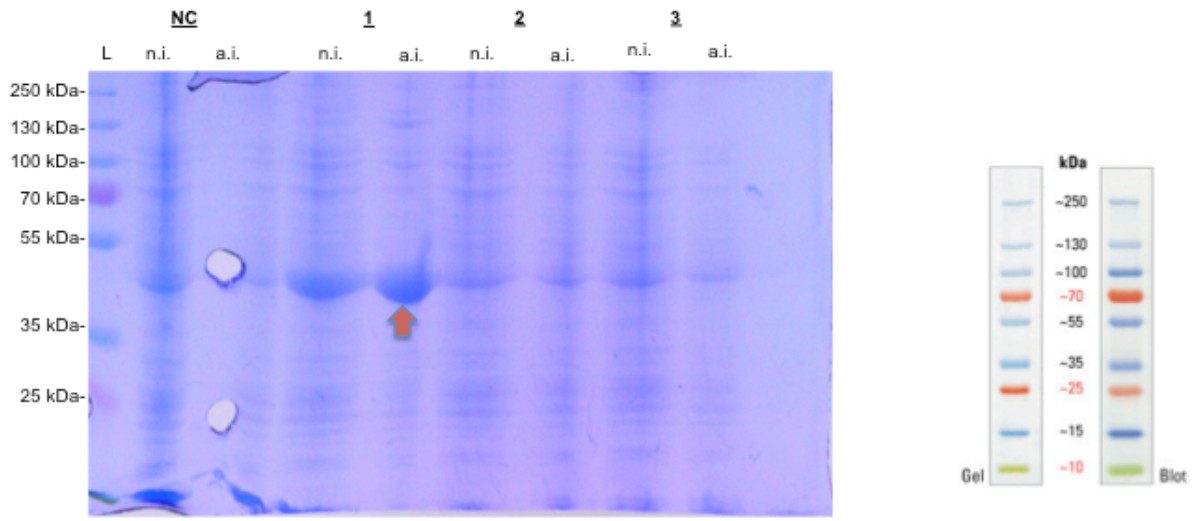

Figure 1: Pilotexpression of MEDH2 in *E. coli* BL21. Coomassie staining of heterologously expressed MEDH2 in pET-28. 1 ml samples were taken in an uninduced stage (n.i.) and 3 hours after induction (a.i.) with 1 mM IPTG. Theoretical weights: MEDH2- 40.15 kDA. Orange arrows determine the appropiate protein. As ladder PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (ThermoScientific) was used. As negative control (NC) selfligated pET-30 was used for expression in *E. coli* BL21. Proteins were separated by SDS-PAGE.

2) <u>Solubility Assay, SDS-PAGE and Coomassie staining to verify soluble expression of MEDH2</u>

- Solubility Assay
    - Resuspend the cell pellet in 600 µl PBS buffer
    - Add glas beads
    - Use machine to disrupt the cells
    - Centrifuge at 16,000 x g for 5 minutes
    - Transfer 600 µl supernatant to a new tube—> soluble fraction
    - Resuspend the pellet in 300 µl PBS buffer → Insoluble fraction
- Prepare Samples for SDS-Page:
    - Add 200 µl 4x SDS-Loading Buffer
    - Boil the sample at 95 °C for 20 minutes
- Run SDS-Page
    - 12 % Seperating Gel
    - load 10 µl
- Stain for 1 hour in Coomassie Staining Solution
- Destain overnight

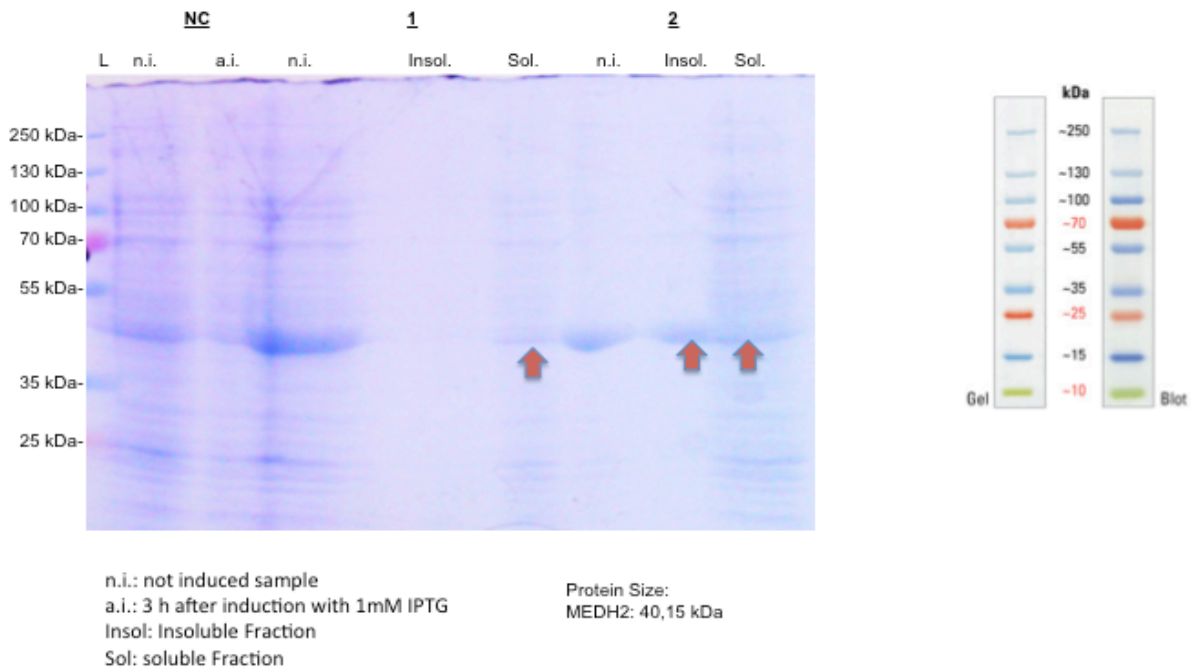

Figure 2: Solubility assay of MEDH2. Coomassie staining of solubility assay of MEDH2. Proteinexpression was performed for 3 hours. Cell pellet was lysed and separated into soluble (sol) and insoluble (insol) fraction. As control 1 ml sample was taken in an uninduced stage. Theoretical molecular weight: MEDH2-40.15 kDA. Orange arrows determine the appropiate protein. As ladder PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (ThermoScientific) was used. As negative control (NC) selfligated pET-28 was used for expression in *E. coli* BL21.