Week 11: 17. August 2015 – 23. August 2015

19. August 2015:

1) Digest of *pET-28a* with EcoRI/ XhoI
    - Pipetting scheme according to the protocol Restriction Digest
    - Incubate the Reaction at 37 °C for 1 hour
    - Verify 10 µl of the digestion on agarose gel

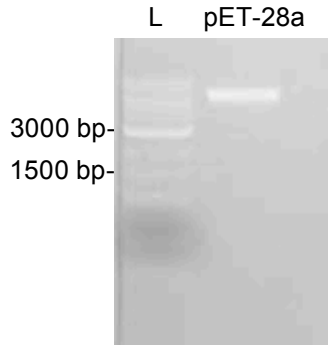

Figure 1: Digest of *pET-28a* with EcoRI/ XhoI. 10 µl of digest were checked on 1% (w/v) agarose gel. Expected sizes: pET-28a- 5265 bp. As ladder (L) 1kB Ladder (NEB) was used.

2) Purification of digested pET-28a
    - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual
3) Dephosphorylation of opened pET-28a using shrimp alkaline phosphatase
    - Pipetting scheme according to the protocol Shrimp Alkaline Phosphatase Treatment (Fermentas).
4) Ligation of *medh2* into pET-28a
    - Pipetting scheme according to the Protocol Ligation with T4 DNA Ligase (NEB)
    - Use dephosphorylated pET-28a
    - Control Reaction: pET-28a Selfligation
    - Incubate the reaction at 16 °C overnight

20. August 2015

1) Transformation of *E. coli* TOP10 cells with pET-28a+*medh2* and pET-28a Selfligation
    - Add 5 µl of ligation reaction to chemically competent *E. coli* TOP10 cells.
    - Following the protocol for transformation of chemical competent *E. coli* cells
    - Plate on LB+Kan [50 µg/ml] and incubate overnight at 37 °C

21. August 2015

1) Colony-PCR to screen for clones containing the pET-28+*medh2*

- Constructs: pET-28+*medh2*
- Pipetting scheme and PCR program according to PCR with Taq-DNA Polymerase Protocol
- Primer: medh2_E1/E2
- Check 5 Clones per Construct
- Positive Control: add 1 µl of pCR4+medh2
- Negative Control: add 1 µl MilliQ Water

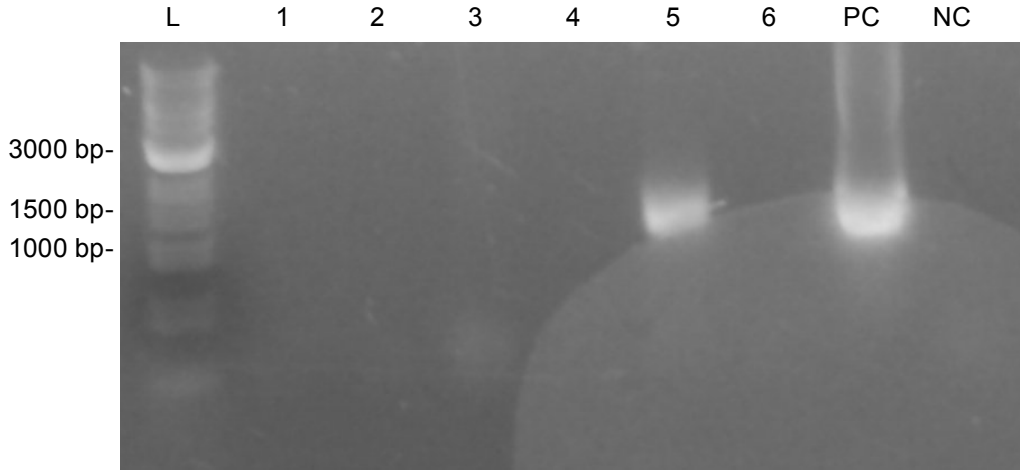

Figure 2: Colony-PCR to identify clones containing pET-28+*medh2* expression construct. Numbers 1-5 determine the checked clone. C determines the selfligated pet-28. As positive control (PC) 1 µl pCR4+*medh2* was used. As negative control (NC) 1 µl MilliQ Water was added. 10 µl of PCR were analzyed on 1 % (w/v) agarose gel. Expected sizes: *medh2*-1203 bp. As ladder (L) 1 kB Ladder (NEB) was used.

2) Inoculation of liquid culture for plasmid isolation of pET-28+*medh2* and pET-28 selfligated

- Inoculate one clone with 5 ml LB+Kan [50 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.

22. August 2015

1) Plasmid Isolation of pET-28+*medh2* and pET-28 selfligated

- Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.

2) Transformation of *E. coli* BL21 cells with pET-28+*medh2* and pET-28 selfligated

- Add 1 µl of pET-28+*medh2* or pET-28 selfligated to chemically competent *E. coli* BL21 cells.
- Following the protocol for transformation of chemical competent *E. coli* cells
- Plate on LB+Kan [50 µg/ml] and incubate overnight at 37 °C

23. August 2015

Inoculation of preculture for protein expression of MEDH2

- Inoculate three clones with 5 ml LB+Kan [50 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.
- Inoculate 5 ml LB+Kan [50 µg/ml] with *E. coli* containing the selfligated pET-28 and incubate overnight at 37 °C shaking at 220 rpm.