Week 4: 29. June 2015- 3. July 2015

29. June 2015

1) Gel-Extraction of *mmoD* and pET-30
   - Use the samples frozen at 26. June 2015
   - Use the Wizard® SV Gel and PCR Clean-Up System Kit (Promega) and follow the provided manual
2) Ligation of *mmoD* into pET-30
   - Pipetting scheme according to the Protocol Ligation with T4 DNA Ligase (NEB)
   - Control Reaction: pET-30 Selfligation
   - Incubate the reaction 30 minutes at room temperature
3) Transformation of *E. coli* TOP10 cells with ligation reaction pET-30+*mmoD* and pET-30 selfligated
   - 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
4) Isolation of genomic DNA from *Methylococcus capsulatus*
   - Use NucleoSpin® Tissue Kit (Machery-Nagle) and follow the provided manual

30. June 2015

- The Ligation does seems to work, the control plate, containg the transformed *E. coli* TOP10 with the selfligated pET-30 had an equal amount of colonys compared to the transformed *E. coli* TOP10 with the pET-30+*mmoD* Ligation

1) Digest of *mmoB, mmoX* and pET-30 with NdeI/EcoRI
   - Pipetting scheme according to the protocol restriction digest
   - First adding NdeI and incubate the Reaction at 37 °C for 1 hour, afterwards heat inactivation of NdeI at 65 °C for 2 minutes. Adding 1 ul of EcoRI and incubate the digest again for 1 hour at 37 °C
   - Verify 10 µl of the digestion on agarose gel
   - Control: undigested pET-30

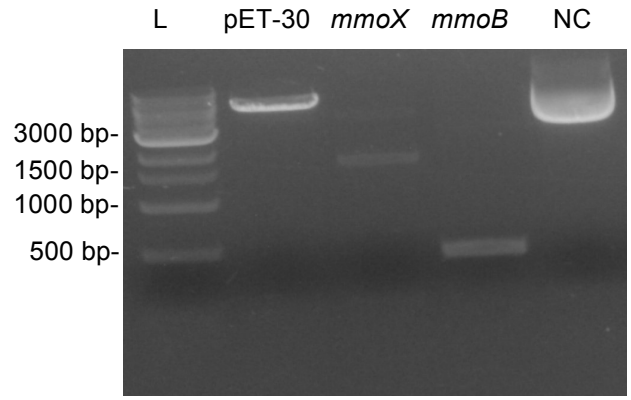

Figure 1: Digest of *mmoX, mmoB* and pET-30 with NdeI/ EcoRI. 10 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: pET-30-5269 bp, *mmoX*- 1605 bp, *mmoB*- 447 bp. Undigested pET-30 was included as negative control (NC). As ladder (L) 1kB Ladder (NEB) was used.

2) Purification of digested *mmoB, mmoX* and pET-30
   - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual
3) Ligation of *mmoX* and *mmoB* into pET-30
   - Pipetting scheme according to the Protocol Ligation with T4 DNA Ligase (NEB)
   - Control Reaction: pET-30 Selfligation
   - Incubate the reaction at 16 °C overnight
4) Amplification of *mmoG* adding Restriction sites (NdeI/EcoRI) for cloning into pET-30
   - Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase protocol
   - Primer: mmoG_E1/E2,
   - Template: PCR Product *mmoG* for TOPO cloning (26. June 2015)

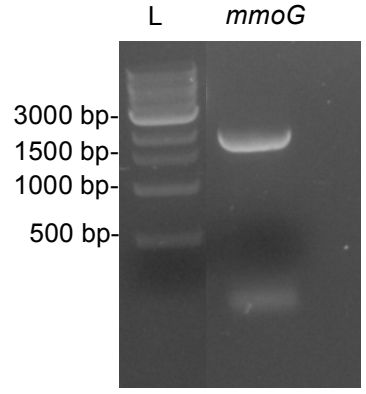

Figure 2: Amplification of *mmoG* adding restriction sites for cloning into pET-30. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected size: *mmoG*. As lader (L) 1kB Ladder (NEB) was used.

1. July 2015

1) Transformation of *E. coli* TOP10 cells with pET-30+*mmoX*, pET-30+*mmoB* and pET-30 selflligated

- 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

2. July 2015

- The ligation was successful, control reaction with selfligated pET-30 showed less clones compared to the ligation reactions

1) Colony-PCR to screen for clones containing the pET-30+*mmoX* or pET-30+*mmoB*

- Constructs: pET-30+*mmoX*, pET-30+*mmoB*
- Pipetting scheme and PCR program according to PCR with Taq-DNA Polymerase Protocol
- Primer: mmoB_E1/E2, mmoD_E1/E2
- Check 6 Clones per Construct
- Positive Control: add 1 µl of pSC1B3+*mmoX* or pSC1B3+*mmoB*
- Negative Control: add 1 µl MilliQ Water

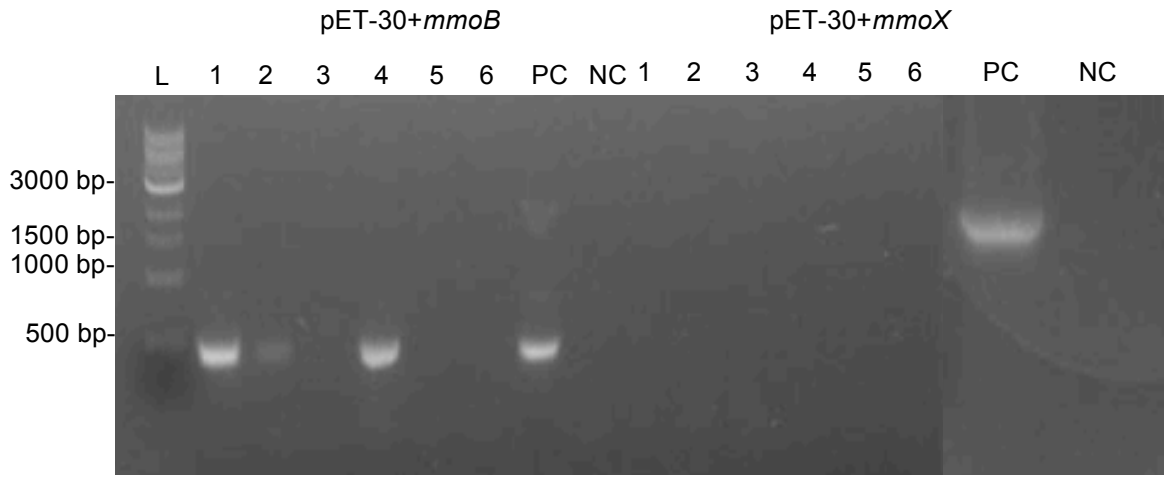

Figure 3: Colony-PCR to identify clones containing pET-30+*mmoX* or pET-30+*mmoB* expression constructs. Numbers 1-6 determine the checked clone. As positive control (PC) 1 µl pSC1B3+*mmoX* or pSC1B3+*mmoB* was added. As negative control (NC) 1 µl MilliQ Water was added. 10 µl of PCR were analzyed on 1 % (w/v) agarose gel. Expected sizes: mmoB-453 bp ,mmoX-1611 bp. As ladder (L) 1 kB Ladder (NEB) was used.

- The pET-30+*mmoB* expression plasmid seems to be successful cloned

1) Inoculation of liquid culture for plasmid isolation of pET-30+*mmoB* and pET-30 selfligated

- Inoculate clone 2 and 4 with 5 ml LB+Kan [50 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.
- Inoculate one of the clones of the pET-30 selfligation with 5 ml LB+Kan [50 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.

3. July 2015

1) Plasmid Isolation of pET-30+*mmoB*

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