Week 2: 15. June 2015 – 19. June 2015

15. June 2015:

1) Transformation of *E. coli* TOP10 cells with plasmids containing the sMMO subunits
   - Add 1 µl of pSC1B3+sMMO subunit to chemically competent *E. coli* TOP10 cells.
   - Following the protocol for transformation of chemical competent *E. coli* cells
   - Plate on LB+Clm [25 µg/ml] and incubate overnight at 37 °C
2) Purification of generated *mmoC* PCR product (11. June 2015)
   - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual
3) Streaking out of *E. coli* stocks containing the pET-30 or pET-28 Expression vector
   - Streak out on LB+Kan [50 µg/ml] and incubate overnight at 37 °C

16. June 2015

- Transformation of *E. coli* Top10 cells with pSC1B3+sMMO subunits was successful.
- Streaking out of *E. coli* stocks containing pET-30 or pET-28 was successful.

1) Inoculation of liquid cultures for plasmid isolation of pSC1B3+ sMMO subunits
   - Inoculate one clone per construct with 5 ml LB+Clm [25 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.
2) Inoculation liquid culutures for plasmid isolation of pET-30 and pET-28
   - Inoculate one clone per Vector with 5 ml LB+Kan [50 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.

17. June 2015

1) TOPO cloning of *mmoC* into pCR4 Vector
   - Using the Zero Blunt Topo cloning Kit (Invitrogen)
   - See Topo cloning Protocol for pipetting scheme
2) Transformation *E. coli* TOP10 cells with TOPO cloning reaction
   - Add 2 µl of TOPO reaction to chemically competent *E. coli* TOP10 cells.
   - Following the protocol for transformation of chemical competent *E. coli* cells
   - Plate on LB+Amp [100 µg/ml] and incubate overnight at 37 °C
3) Plasmid Isolation of pSC1B3+ sMMO Subunit gene

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

4) Plasmid Isolation of pET-30 and pET-28 vector
   - Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.

18. June 2015

1) Inoculation of liquid culture for plasmid isolation of pCR4+*mmoC*
   - Inoculate one clone with 5 ml LB+Amp [100 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.
2) Amplification of *mmoX, mmoY, mmoZ, mmoB, mmoD, phi* adding Restriction sites (NdeI/ EcoRI) and amplification of *medh2* adding restriction sites (EcoR/ XhoI) for cloning into pET-30
   - Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase protocol
   - Primer: mmoX_E1/E2, mmoY_E1/E2, mmoZ_E1/E2.1, mmoB_E1/E2, mmoD_E1/E2, phi_E1/E2, medh2_E1/E2,
   - Template: pSC1B3+*mmoX*, pSC1B3+*mmoY*, pSC1B3+*mmoZ*, pSC1B3+*mmoB*, pSC1B3+*mmoD*, pCR4+*phi*, pCR4+*medh2*

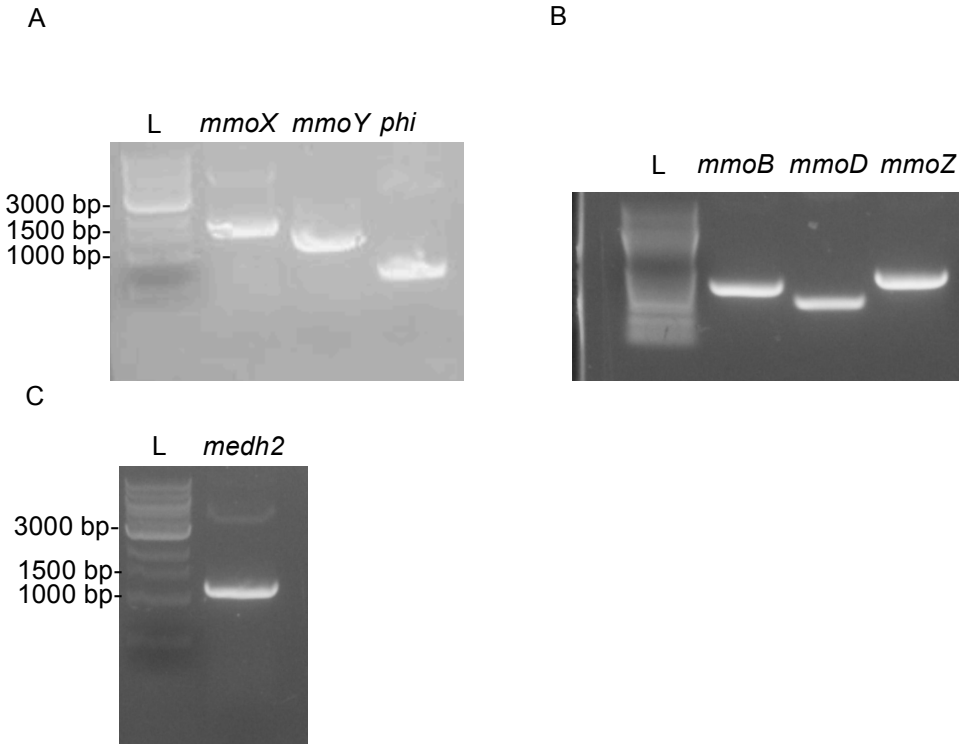

Figure 4: Amplification of *mmoX, mmoY, mmoZ, mmoB, mmoD, phi* and *medh2* adding restriction sites for cloning into pET-30. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected sizes: *mmoX*-1611 bp,

mmoY-1197 bp, *phi*-591 bp, *mmoB*-453 bp, *mmoD*-339 bp, *mmoZ*- 552 bp, *medh2*-1203 bp. As ladder (L) 1kB Ladder (NEB) was used,except for B). There 100 bp ladder (NEB) was used.

19. June 2015

1) Plasmid Isolation of pCR4+*mmoC*
   - Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.
   - Send for sequencing
     - Correct Sequence confirmed
2) Purification of generated *mmoX, mmoY, mmoZ, mmoB, mmoD, phi and medh2* PCR product with added restriction sites for cloning into pET-30
   - Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.