

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 cultures 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*, *hxlB* 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, *hxlB*_E1/E2, *medh2*_E1/E2,
- Template: pSC1B3+*mmoX*, pSC1B3+*mmoY*, pSC1B3+*mmoZ*, pSC1B3+*mmoB*, pSC1B3+*mmoD*, pCR4+*hxlB*, pCR4+*medh2*

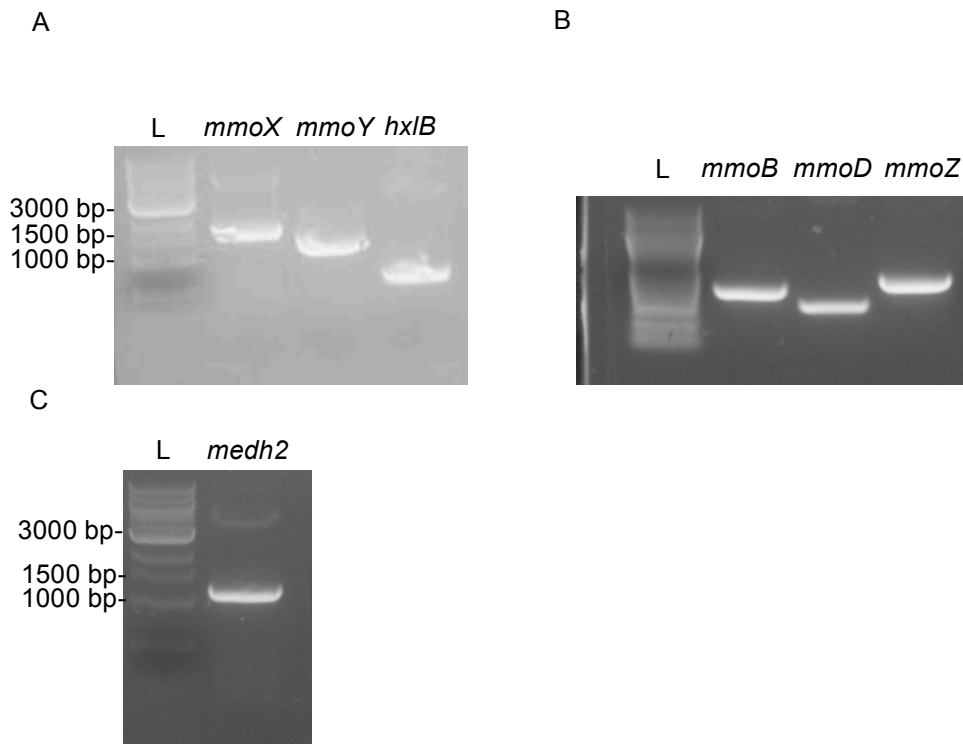


Figure 4: Amplification of *mmoX*, *mmoY*, *mmoZ*, *mmoB*, *mmoD*, *hxlB* and *medh2* adding restriction sites for cloning into pET-30. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected sizes: *mmoX*-1611 bp,

mmoY-1197 bp, *hxlB*-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*, *hxlB* 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.