Week 3: 22. June 2015- 26. June 2015

22. June 2015

1) Invitro mutagenesis of *hps* to remove XbaI Restriction Site by PCR

a) PCR Reaction

- Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase protocol
- Primer: hps_mut1/mut2
- Template: pCR4+*hps*

b) DpnI Treatment

- Add 1 µl of DpnI to the PCR Reaction and incubate at 37 °C for 1 hour

c) Transformation of *E. coli* TOP10 cells with DpnI treated PCR reaction

- Add 1 µl of DpnI treated PCR 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

23. June 2015

1) Inoculation of liquid culture for plasmid isolation of pCR4+*hps*-mut XbaI
   - Inoculate one clone with 5 ml LB+Amp [100 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.
2) Amplification of *mmoC* 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: mmoC_E1/E2,
   - Template: pCR4+*mmoC*

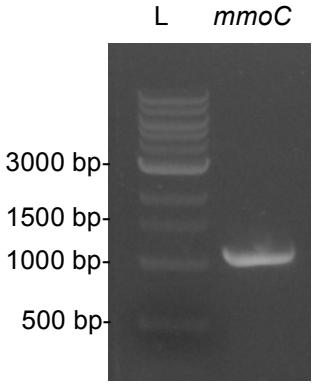

Figure 1: Amplification of *mmoC* adding restriction sites for cloning into pET-30. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected size: *mmoC*-1088 bp. As lader (L) 1kB Ladder (NEB) was used.

3) Purification of generated *mmoC* PCR product

- Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual

24. June 2015

1) Plasmid Isolation of pCR4+*hps*-mut XbaI

- Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.
- Send for sequencing
  - Sequencing showed that the mutagenesis was not successful.

26. June 2015

1) Double digest of *mmoX, mmoY, mmoZ, mmoB, mmoC, mmoD, phi* and pET-30 with NdeI/EcoRI

- Pipetting scheme according to the protocol Restriction Digest
  - Exception: NdeI and EcoRI were added at the same time
- Incubate the Reaction for 1 hour at 37 °C
- Verify complete digestion on agarose gel
- Cut out the digested fragment and keep at -20 °C prior to Gel Extraction

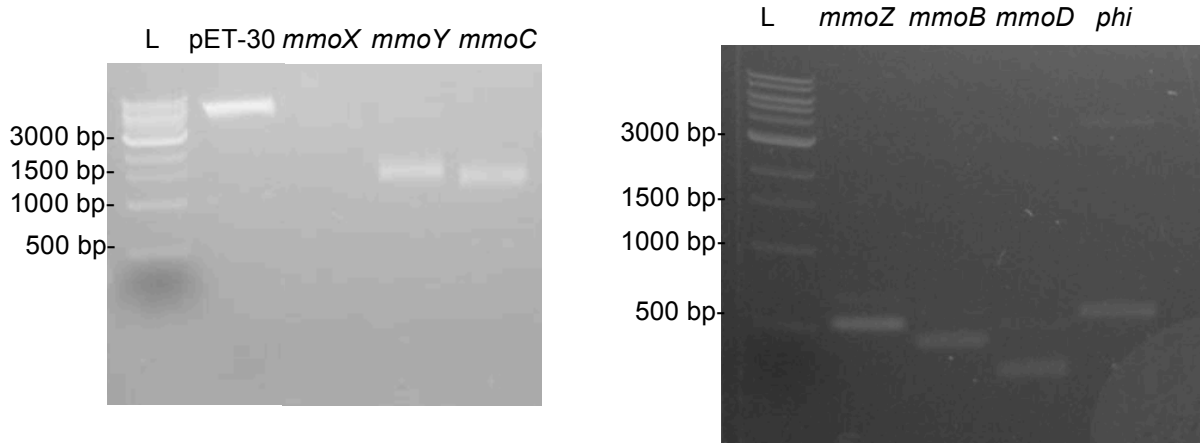

Figure 2: Digest of *mmoX, mmoY, mmoZ, mmoB, mmoC, mmoD, phi* and pET-30 with NdeI/EcoRI. 50 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: pET-30-5269 bp , *mmoX*-1605 bp, *mmoY*-1191 bp, *mmoC*-1082 bp, *mmoZ*-546 bp, *mmoB*-447 bp, *mmoD*-333 bp, *phi*-586 bp. As ladder (L) 1kB Ladder (NEB) was used.

2) Double digest of pET-28 with NcoI/EcoRI

- Pipetting scheme according to the protocol Restriction Digest
- Incubate the Reaction for 1 hour at 37 °C

- Verify complete digestion on agarose gel
- Cut out the digested fragment and keep at -20 °C prioir to Gel Extraction

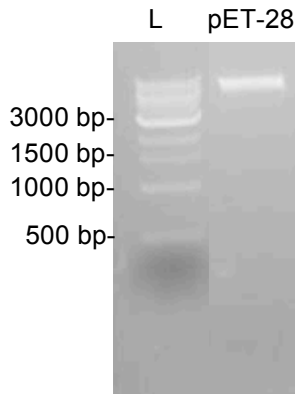

Figure 3:Digest of pET-28 with NcoI/EcoRI. 50 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: pET-28-5265 bp. As ladder (L) 1kB Ladder (NEB) was used.

4) Amplification of *mmoG* for cloning

- Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase Protocol
- Primer: mmoG_P1/P2
- Template: genomic DNA M.capsulatus

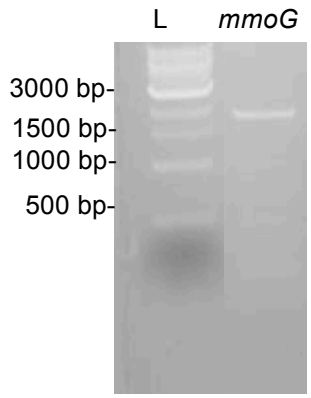

Figure 4: Amplification of *mmoG* for TOPO cloning. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected sizes: *mmoG*-2249 bp. As ladder (L) 1kB Ladder (NEB) was used.

5) Purification of generated *mmoG* PCR product for TOPO cloning

- Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual