Week 10: 10. August 2015- 14. August 2015

10. August 2015

1) Digest of *hps* with NcoI and EcoRI for cloning into pET-28
   - Pipetting scheme according to the protocol Restriction Digest
   - Incubate the digest again for 1 hour at 37 °C
   - Verify 10 µl of the digestion on agarose gel

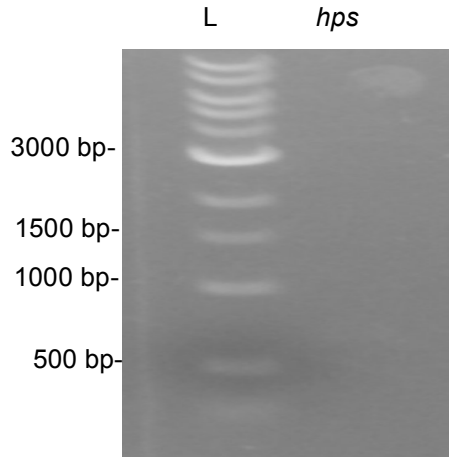

Figure 1: Digest of *hps* with NcoI/EcoRI. 10 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: *hps*-671 bp. As ladder (L) 1kB Ladder (NEB) was used.

2) Digest of *hps, phi* and *medh2* with EcoRI/ PstI for cloning into pSB1C3
   - Pipetting scheme according to the protocol Restriction Digest
   - Incubate the Reaction for 1 hour at 37 °C
   - Verify 10 µl of the digestion on agarose gel

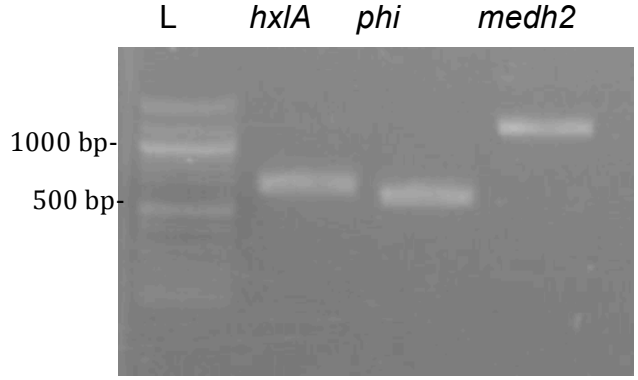

Figure 2: Digest of *hps, phi* and *medh2* with EcoRI/ PstI for cloning into pSB1C3. 10 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: *hps*-671 bp, phi-591 bp, *medh2*-1203 bp. As ladder (L) 100bp Ladder (NEB) was used.

3) Purification of digested *hps, phi* and *medh2*
   - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual
4) Ligation of *hps, phi* and *medh2* into pSB1C3 using T4 DNA Ligase
   - Pipetting scheme according to the Protocol Ligation with T4 DNA Ligase (NEB)
   - Use dephosphorylated pSB1C3
   - Control Reaction: pSB1C3 Selfligation
   - Incubate the reaction at 16 °C overnight

11. August 2015

1) Digest of *hps* with NcoI/ EcoRI for cloning into pET-28
   - Pipetting scheme according to the protocol Restriction Digest
   - Incubate the digest again for 1 hour at 37 °C
   - Verify 10 µl of the digestion on agarose gel

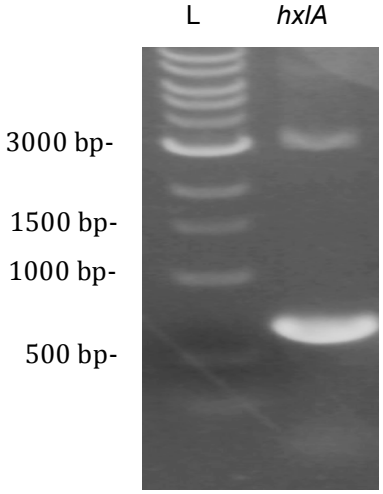

Figure 3: Digest of *hps* with NcoI/EcoRI. 10 µl of Digest were checked on 1% (w/v) agarose gel. Expected sizes: *hps*-671 bp. As ladder (L) 1kB Ladder (NEB) was used.

2) Purification of digested *hps*
   - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual
3) Ligation of *hps* and *medh2* into pET-28
   - Pipetting scheme according to the Protocol Ligation with T4 DNA Ligase (NEB)
   - Use dephosphorylated pET-28-EcoRI/XhoI (for *medh2*) and pET-28-NcoI/EcoRI (for *hps*)
   - Control Reaction: pET-28 Selfligations

- Incubate the reaction at 16 °C overnight

4) Transformation of *E. coli* TOP10 cells with pSB1C3+*hps*, pSB1C3+*phi* and pSB1C3+*medh2*
   - 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+Clm [25 µg/ml] and incubate overnight at 37 °C

12. August 2015

1) Colony-PCR screen for *medh2, hps* and *phi* in pSB1C3
   - Constructs: pSB1C3+hps, pSB1C3+phi and pSB1C3+medh2
   - Pipetting scheme and PCR program according to PCR with Taq-DNA Polymerase Protocol
   - Primer: medh2-BioBrick Fwd/Rev, phi-BioBrick Fwd/Rev, hps-BioBrick Fwd/Rev
   - Check 6 Clones
   - Positive Control: add 1 µl of pSB1C3+*mmoB*
   - Negative Control: add 1 µl MilliQ Water

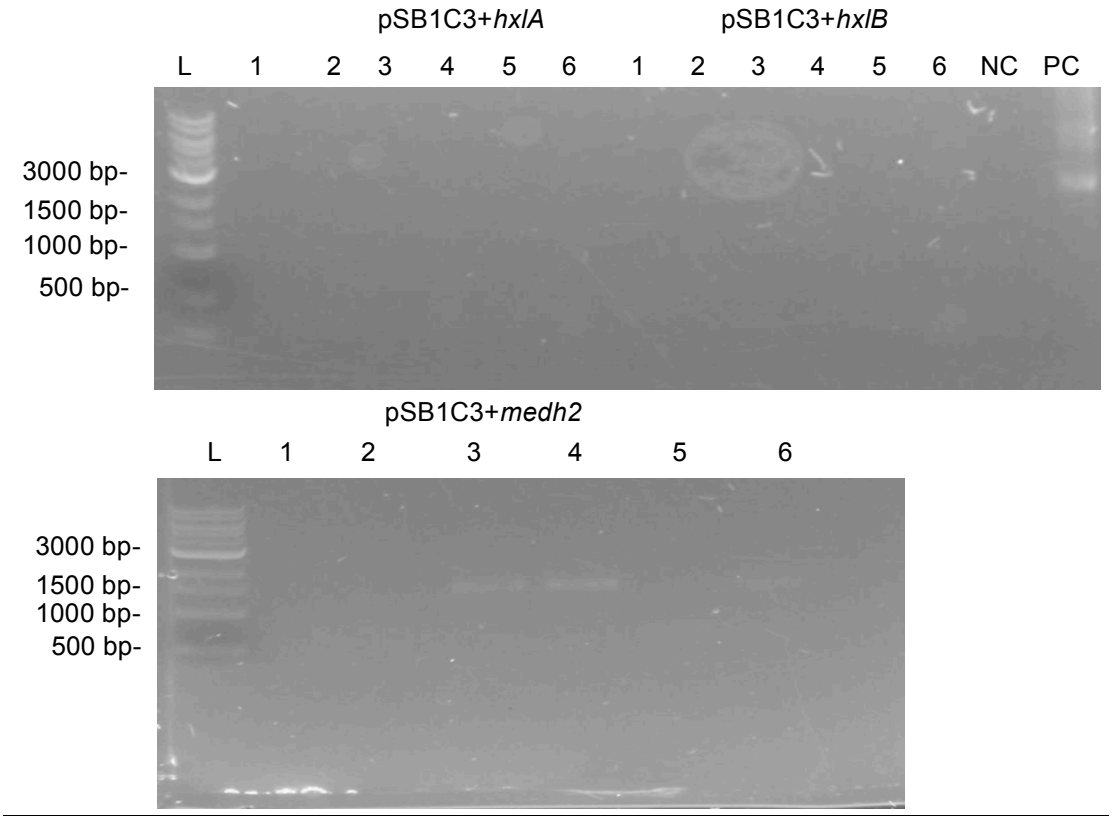

Figure 4: Colony-PCR to identify clones containing pSB1C3+*hps,* pSB1C3+*phi* and pSB1C3+*medh2* Biobrick constructs.

PCR with Taq-DNA Polymerase to identify pSB1C3+*hps,* pSB1C3+*phi* and pSB1C3+*medh2*. Numbers 1-6 determine the checked clone. As positive control (PC) 1 µl pCR4+*medh2* (A) and pSB1C3+*mmoB (B) 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: *hps*-671 bp, phi-591 bp, *medh2*-1203 bp. As ladder (L) 1 kB Ladder (NEB) was used.

- **The colony-PCR failed, nevertheless we inoculated some cultures for plasmid isolation.**

2) Inoculation of liquid culture for plasmid isolation of pSB1C3+*hps*, pSB1C3+*phi* and pSB1C3+*medh2*
   - Inoculate clone with 5 ml LB+Clm [25 µg/ml] and incubate overnight at 37 °C shaking at 220 rpm.

3) Transformation of *E. coli* TOP10 cells with pET-28+*hps*, pET-28+*medh2* and pET-28 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+Clm [25 µg/ml] and incubate overnight at 37 °C

13. August 2015

- **Ligation of *hps* and *medh2* into pET-28 failed**

1) Plasmid Isolation of pSB1C3+*hps*, pSB1C3+*phi* and pSB1C3+*medh2*
   - Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.
   - Send the plasmids for sequencing with BioBrick Sequencing Primer FWD/Rev
     - Sequencing revealed 100 % integrity of our BioBrick Constructs