OCTOBER

MONDAY 04/10/2021

I. Protein purification GGA 1.2

- resuspension of cell pellet with 5 ml binding buffer (pH 8.1)
- retsch mill cylinders were precooled in liquid nitrogen
- cell suspensions were put in liquid nitrogen in cylinders until frozen
- shaken in retsch mill for 1:30 min with 30/s frequency
- cylinder frozen in liquid nitrogen
- repeated three times
- cell powder resuspended to 40 ml with binding buffer in new falcon tubes
- centrifugation at 4°C, 30 min, 11.000 g
- used gravity column was prepared
- 2 x wash with 15 ml ddH2O
- 15 ml binding buffer
- close column, load centrifugation supernatant
- close with parafilm, incubate for 1 h at 4°C, shaking slowly (90 rpm)
- unbound protein was flown through, sampled for SDS Page
- 5 x 25 ml binding buffer
- 2 x 25 ml washing buffer, sampled for SDS Page
- 5 ml 100 mM Imidazol elution buffer, flow through was collected completely, sampled for SDS Page
- 25 ml 400 mM Imidazol elution buffer, flow through was collected completely, sampled for SDS Page
- flow through was transferred to concentrator tubes, concentrated to 0.75 ml

concentration: 0.350 mg/ml

- centrifugation for 60 min at 4°C, 4.000 g
- SDS Page samples were mixed with loading buffer, incubated 10 min at 90°C
- all samples were frozen and stored at -20°C for further analysis, concentrated protein was stored at -80°C
- Resin was recovered by adding 15 ml elution buffer, incubating for 30 min
- 0.5 M NaOH was incubated for 30 min
- H2O wash
- storage in 30 % at 4°C, column closed with parafilm

TUESDAY 05/10/2021

I. SDS-gel electrophoresis GGA 1.2 and 3.1

Two 8 % polyacrylamide gels were loaded with 20 µl of prepared samples of the protein purification of GGA 1.2 and 3.1 . The gels was run for 20 minutes at 100 V and 50 min at 120 V.

GGA 1.2: L, unbound protein, 1. Wash, 2. Wash, E 100mM Imidazole, E 400mM Imidazole, E 400mM Imidazole concentrated

GGA 3.2: L, unbound protein, 1. Binding Buffer, 1. Wash, 2. Wash, E 100mM Imidazole, E 400mM Imidazole, E 100mM Imidazole concentrated, E 400mM Imidazole concentrated

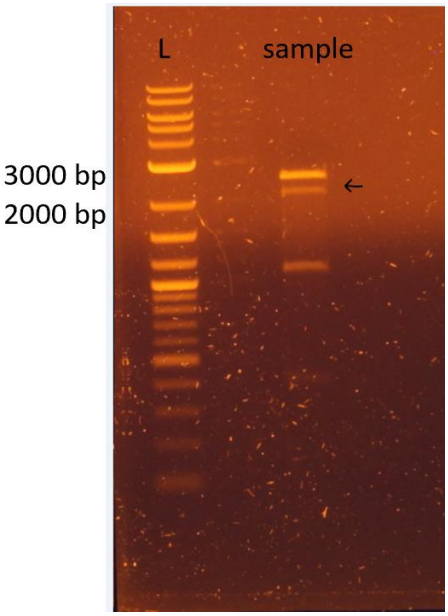
II. Digestion psB1K3

psB1K3 was digested with pSTI and EcoRI for a ligation with the Amorphadiensyntase

Table 05102018-1: Digest recipe.

500 ng psB1k3 (from the 22.06.2021, 51.279 ng/µL)	
Fast digest Green Buffer	2.5 µL
Fast digest pSTI	1 µL
Fast digest EcoRI	1 µL
H2O	10.7 µL

Reaction ran for 20 min at 37°C. The Enzymes were inactivated for 5 min at 80°C.



III. Gel Extraction

- Gel extraction of pSB1K3 with Monarch DNA Gel Extraction Kit by NEB.
- Weight: 0.1694g
- 678 µL Dissolving buffer was added
- Worked by the Kit protocol
- 2x Elution with 7.5 µL H20 (50°C) and 1 min incubation time
- total volume: 15µL

Table 05102018-2: Measured concentration of the extracted plasmid.

Product	concentration [ng/µL]	230/260	280/260
digested pSB1KA	5.052	0.02	3.62

IV. Preparing Plates

New camp and kan plates were made. 1 µL Antibiotic per mL Agar

V. Ligation

Ligation of pSB1K3 and Amorphadiensynthase

Mix:

- 14 µL / 70 ng of pSB1K3
- 14.31 µL / 57.27 ng of Amporphadiensynthase
- 3 µL of 10x T4 Ligase Buffer
- 1 µL T4 Ligase

Incubation overnight at 16°C

VI. Plates contol

Kan and camp plates were tested with a negative control. tested with DH5alpha

WEDNESDAY 06/10/2021

I. Dot Blot

Dot Blot of purified Proteins of GGA 1.2 and 3.1 with Antibodies from the HPI. Didn´t work, since we don´t have X-ray film

II. Transformation of the Ligation

The ligation of the pSB1K3 and Amorphadiensynthase got transformed in DH5alpha.

THURSDAY 07/10/2021

I. Competent cells

We decided to make new competent cells, since our Kan-negativcontrols always had Colonies on them. Even after making new Plates.

We used DH5alpha from the AK Ignatova.

- DH5alpha and 400 mL LB-Medium
- Grow the cells at 37°C till OD = 0.2-0.4
- Culture was chilled on ice for 5 min
- Centrifugation 5000 rpm, 10 min at 4 °C
- Resuspension of the cells in 32 mL cold Ca/Glyceril buffer on ice
- Incubation for 15 min
- Reapeat of Centrifugation and resuspension
- resuspended cells were incubationg for 30 minutes in Ca/glycerol Buffer for 30 min
- Cells were collected by centrifugation at 500 rpm, 10 min at 4°C
- Resuspension in 4.8 mL Ca/glycerol buffer
- 75 µL Aliquotes, frozen in liquid-N2 and stored at 80°C
- labeled: DH5alpha 10/21

II. Transformation of the Ligation

rerun of the Transformation of the Ligation product (psB1K3 + Amophadiensynthase), because the negative control had colonies. We used for the Transformation the DH5alpha cells from the AG Ignatova.

III. SDS-gel electrophoresis GGA 1.2 and 3.1

Two 10 % polyacrylamide gels were loaded with 20 µl of prepared samples of the protein purification of GGA 1.2 and 3.1 . The gels was run for 10 minutes at 100 V and 60 min at 120 V. Overnight stained in Comassie blue