21.07.2020

Transformation see protocol

- Thawed 2 Eppis each with 200 µl overnight bacterial culture from E.coli BL21 strain
- Prepare 2 approaches on the flame:
  - Eppi 1: 1 µl sterile H_2O
  - Eppi 2: 1 µl self-prep marLac

22.07.2020

Prepare 2YT medium: on three liter

- 48 g Trypton (16 g per L)
- 30 g Yeast (10 g per L)
- 15 g NaCl (5 g per L)

overnight culture:

- in 5 ml LB medium for glycerine
- in 45 ml LB medium for production
- overnight at 37°C 170rpm

23.07.2020

glycerine stock:

- OD of overnight culture: 0,417
- 2 ml of preculture for 2 min and 13.000 rpm in the centrifuge
- resuspend cell pellet in 400 µl LB and 2µl ampicillin
- add 600 µl sterilem glycerine, invert, incubate 15 min on ice
- storage cells at -80°C

production of marLac, 30°C, 3h:

- OD of overnight culture 1:10 dilution: 0,708
- 4 5 L sterile Erlenmeyer flesks fill with 1 L of sterile 2YT medium
  - 1 ml Amp (c = 1 µl/m of 100 µM Stock)
  - 0,1 ml $CuSO_4$ (c = 0,1 mM of 1 M Stock)
  - add 10 ml of overnight culture
- Start OD:

1	2	3	4
0,028	0,03	0,026	0,028

- put it in the shaker at 95 rpm and 37°C, until the culture reaches a OD of 0,5
  - let it grow for 2 hours

1	2	3	4
0,561	0,814	0,787	0,872

- remove pre-induction sample (VI)
- add 500 µl IPTG (12:00 Uhr)
- After 3h (15:00 Uhr) with 30°C remove after induction sample (induction)

dilution	1	2	3	4
1:10	0,255	0,282	0,309	0,315
-	2,55	2,82	3,09	3,15

- fill it in 1 L centrifuge beaker and balance it
- in the centrifuge for 15 min, 9000 rpm and 4°C
- discard the supernatant and put the pellet in a Falkon (all 4 pellets in one Falkon)
    - do everything on ice
- weigh the pellet and store it on -20°C

➔ m_{Pellet}: 11,53 g

27.07.2020

Cell disruption and first affinity chromatography.

- See protocol
- dilution of not induced sample and induced sample:

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sample	**Marker**	**not ind.**	**ind.**	**Lysate**	**flow through**	**Wash eluate**	**E1**	**E2**	**E4**	**E6**
Loading volume	5 µl	10 µl	10 µl	10 µl	10 µl	10 µl	15 µl	15 µl	15 µl	15 µl

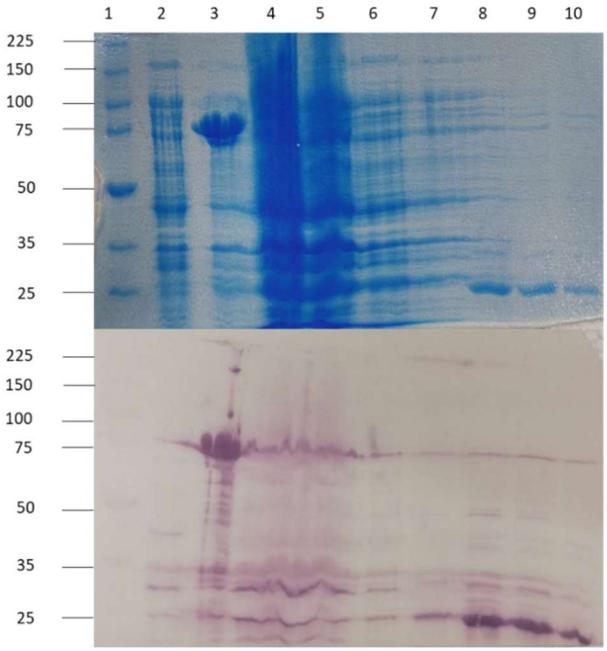
	OD	water	SDS
Not induced	0,561	10,51	2,6
induced	2,55	143,4	35,8

- then run the SDS Gel for 50min and 200V

Westernblot

- see protocol

E.*coli* BL21 (DE3) mar pGEX-6P-1 with $CuSO_4$, medium: 2YT; 3h; 30°C



1: marker

2: before induction sample

3: after induction sample (3h, 30°C)

4: lysate

5: supernatant

6: wash

7: eluate 1

8: eluate 2

9: eluate 4

10: eluate 6

MW with GST: 75,79 kDa

MW without GST: 48,18 kDa

Dialysis oft the eluate:

- cut off a section of a dialysis tube, approx 10 cm
- attach with a clamp the lower end of the tube
- pipette the eluate in the dialysis tube
- add 20 µl Pressicion protease
- attach the top end with a clamp too and fix it on a swimmer
- in 1 L (100-times amount of buffer) overnight for 4°C let the dialysis happen while stirring gently

28.07.2020

buffer: see protocol

- TBS-T (pH=7,4) 1 L
- TBS (pH=7,4) 500 ml
- Tris-HCl 50 mM

2YT medium: for 4 L

- 64 g Trypton
- 40 g Yeast
- 20 g NaCl

2. affinity chromatography:

- see protocol
- put the eluate again in a dialysis tube and dialysis it in pH7 for 1,5h:
    - buffer: phosphatbuffer pH 7, see protocol

concentration:

- see protocol

➔ Mar: c = 28,45 µM; V = 800 µl

Sample preparation and loading scheme.

	1	2	3	4	5	6	7	8	9
Sample	**elution fraction (before digestion)**	**Elution fraction (after digestion)**	**D1**	**D2**	**D3**	**D4**	**Elution fraction (eluted GST)**	**Marker**	**Protease**
Volume	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	5 µl	10 µl
Sample buffer	20 µl	20 µl	20 µl	20 µl	20 µl	20µl	20 µl	-	2,5 µl
Boil time	5 min	5 min	5 min	5 min	5 min	5 min	5 min	-	5 min
			Centrifuge all samples 5 min at 11.000g before loading						
Loading volume	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	5 µl	5 µl

- run the SDS gel for 50min 200 V

Westernblot:

- see protocol

Transformation:

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- Prepare 2 approaches on the flame:
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29.07.2020

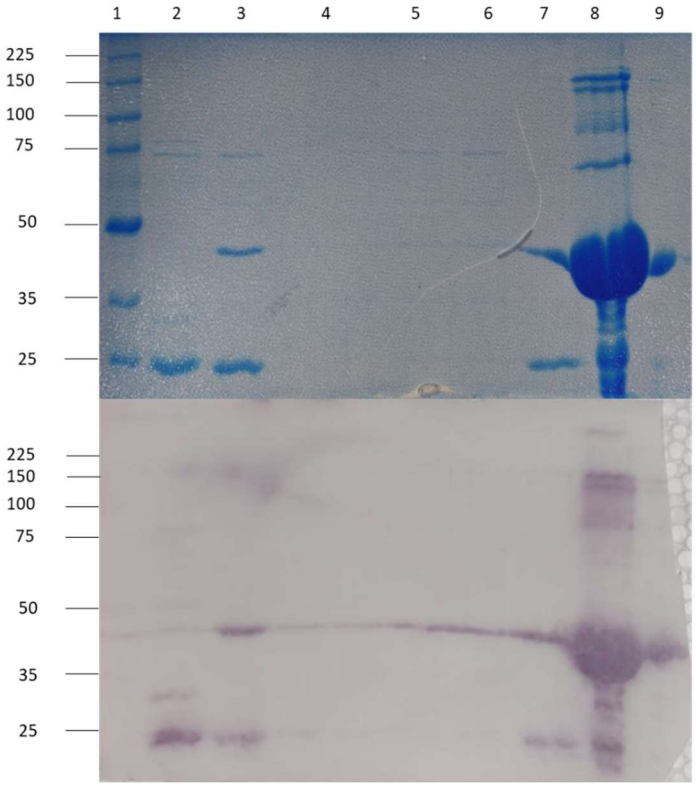
pour LB-Agar-plates

- see protocol

detect the Westernblot

- see protocol

E.*coli* BL21 (DE3) mar pGEX-6P-1 with $CuSO_4$, medium: 2YT; 3h; 30°C



1: marker

2: protein before dialysis

3: protein after dialysis

4: D1

5: D2

6: D3

7: D4

8: eluate

9: protease

MW with GST: 75,79 kDa

MW without GST: 48,18 kDa

overnight culture:

- see protocol
- for the BaLac-production on the 30.07.2020
    - 50 ml in 500 ml flask without chicane
    - 50 µl ampicillin
    - inoculate with the tranformants of the 28.07.2020
- for BL 21 BaLac glycerine stocks:
    - 5 ml LB in a test tube
    - 5 µl ampicillin
    - inoculate with the tranformants of the 28.07.2020