01.09.2020

Transformation BarLac:

Thaw Eppi BL21, prepare batch with self-prepared plasmid:

• Eppi 4: 1 µl BaLac pGEX-6P-1

Rest see above

Towbin buffer: 500 ml

Tris-HCl pH 8.3: 1.514 g

Glycine: 7.206 g

TBS: 1L

Tris-HCl pH 7.4: 6.057 g

NaCl: 8.18 g

Take 500 ml of this and add 500 µl Tween to get TBS-T

02.09.2020

Place the plates of the transformer from September 1st, 2020 in the 37 ° C incubator (approx. 7:00 a.m.)

- Before that, plates were at room temperature

Inoculation of overnight cultures see protocol (approx. 4:00 p.m.)

- Volume: 50 ml in 500 ml flask with 1 baffle
- 3 replicas, as the colonies were still very small

03.09.2020

IPTG stock solution: 1M, 5ml → M = 238.3 g / mol

- c * v * M = m →1.1915g with distilled water. Top up, stored at -20 ° C until use

Production see protocol

OD:

approach	dilution	measured OD	calculated OD
Overnight cukture	1:10	0,427	4,27
flask 1	-	0,068	0,068
flask 2	-	0,068	0,068
flask 3	-	0,068	0,068

flask 4	-	0,068	0,068

- Flask in 37 ° C shaker at 09:24, first carried flask 1 and 2 over, then flask 3 and 4; shaker had only heated up to 31 ° C
- After 90 min the first OD measurement, as it was not yet at 0.5, incubation for another 15 min
- After 105 min OD measurement:

approach	dilution	measured OD
Kolben 1	-	0,525
Kolben 2	-	0,531
Kolben 3	-	0,523
Kolben 4	-	0,539

- After induction with IPTG, incubate at 30 ° C for 5 hours (from 11:50 am)
- Sample taken from flask 2 (Ba VI 3.9.20)

OD sample taken from the post induction after 5h

approach	dilution	measured OD	calculated OD
flask 2	1:10	0,405	4,05

<u>Harvest cells see protocol:</u>
→ pellet 10.42 g

07.09.2020

<u>Cell lysis see protocol</u>
- Without adding the protease inhibitor

<u>Purification of the pellet from 03.09.2020 overnight</u>
- Columns at room temperature

<u>Dialysis with PrescissionProtease in the appropriate buffer see protocol</u>
- Overnight at 4°C

Preparation of samples for gel:

	VI	NI	lysate	DF	wash	E1	E2	E4	E6
water/sample [µl]	29,86	227,8	40	40	40	40	40	40	40
SDS [µl]	9,95	75,9	20	20	20	20	20	20	20

08.09.2020

Finish dialysis see protocol

For purification of Ba via the second column, see protocol

- Dialysis in pH 7 over night

Pour 10% gels see protocol

09.09.2020

Dialysis see protocol

- Pick up from pH7 and pour into a falcon
- Pipette into a new tube and dialyze for 1.5 hours at pH 5.5
- then for 1.5 hours in pH 4 dialysis series

o since the dialysis stopped turning when we got into the room, we dialyzed it half an hour longer

Concentration of the protein see protocol

→ 31.106 µM

→ 350 µM

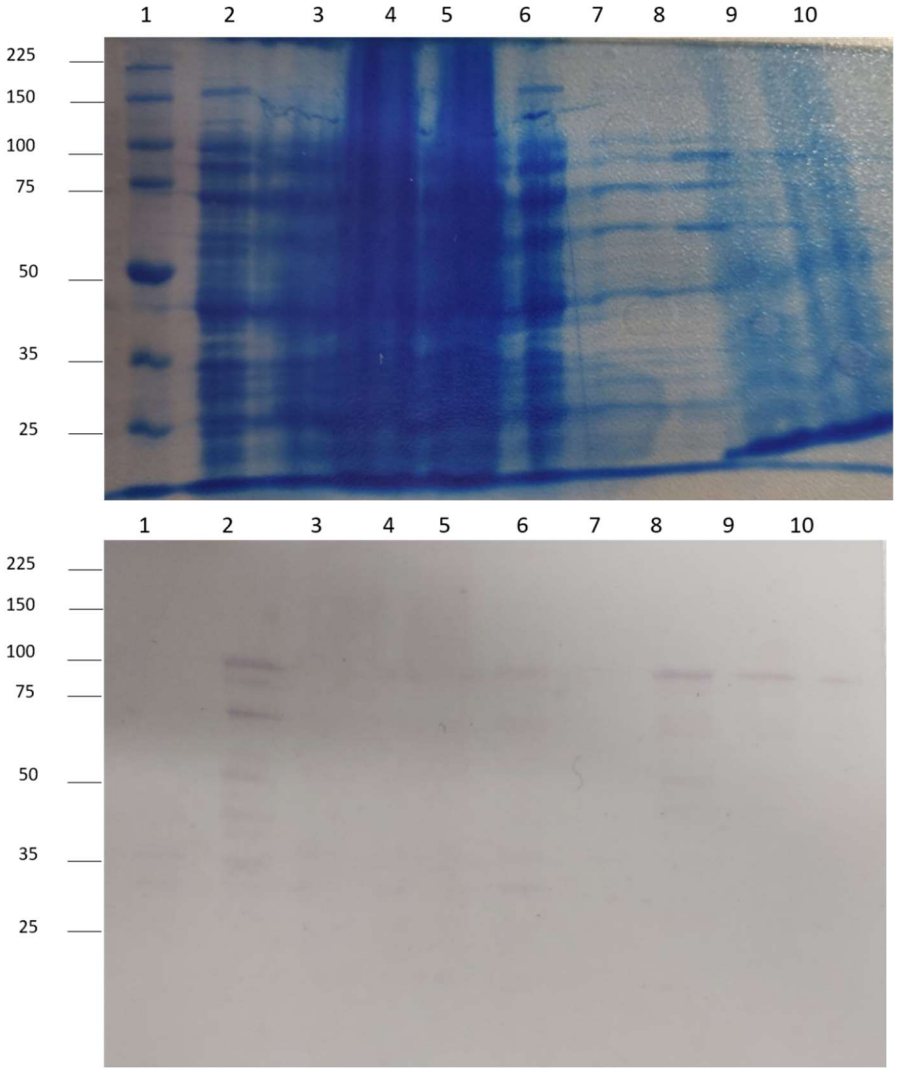
Gel for the 07.09. and 08.09. load and run log

- For order scheme of the 1st pillar, see protocol
- See the protocol for the order scheme for the 2nd pillar

Western blot for the 07.09. and 08.09. blotting overnight

- Incubate the blocking solution at 4°C overnight

E. coli BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 5h; 30 ° C 1st column



1: marker

2: before induction sample

3: after induction sample (3h, 30°C)

4: lysate

5: supernatant

6: wash

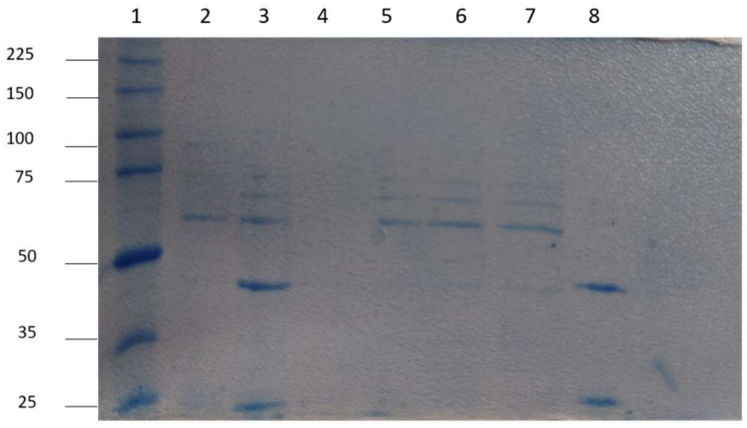
7: eluate 1

8: eluate 2

9: eluate 4

10: eluate 6

E. coli BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 5h; 30 ° C 2nd column



1: marker

2: protein before dialysis

3: protein after dialysis

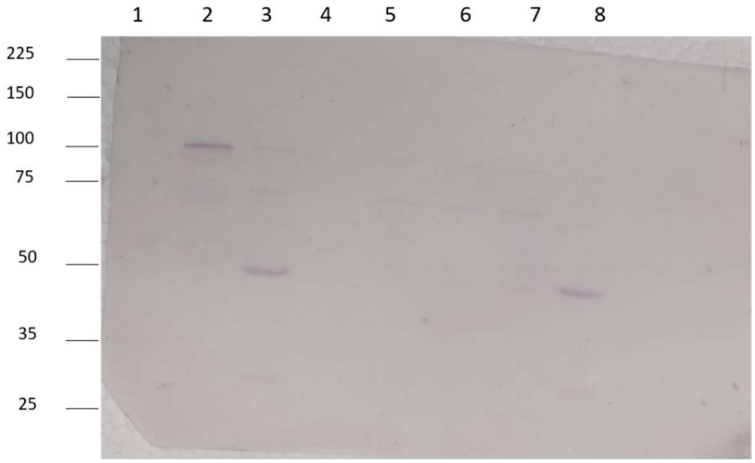
4: D1

5: D2

6: D3

7: D4

8: eluate



10.09.2020

Detect Western blots from 09.09.2020 see protocol