30.07.2020

production of BaLac, 17°C, overnight:

- see protocol
- OD overnight culture: (dilution 1:10) 0,499

➔ OD: 4,99

Start OD:

	1	2	3	4
OD	0,027	0,031	0,029	0,036

let it grow in 37°C room 95 rpm until the OD of 0,5

- after 120 min:

	1	2	3	4
OD	0,577	0,564	0,596	0,536

- Take a sample from 1 and 3 for the pre-induction sample (VI)
- cool down 20min to room
- in 17°C room for 95 rpm over

glycerine stocks:

- see protocol
- OD overnight culture: (dilution 1:10) 0,911

➔ OD: 9,11

isolated smear:

- Smear of BL21 mar with the transformants of the 21.07.2020
- Smear of the glycerine stocks DH5α mar
  - dip lightly with a flamed inoculattion loop into the thawed stock
  - three stroke smear

31.07.2020

cell harvest see protocol

OD of overnight culture:

dilution	1	2	3	4
1:10	0,431	0,408	0,382	0,410
-	4,31	4,08	3,82	4,10

- take samples from 1 and 3 for after induction sample (induced)
- weigh cell pellet, freeze it at -20°C

➔ Pellet with protein inhibitor: 6,89 g

➔ Pellet without protein inhibitor: 6,76 g

03.08.2020

buffer production see protocol

- Protease buffer (2 L)
- Elution buffer (25 ml)
- Towbin buffer (500 ml)

Protein purification of BaLac see protocol

- two approach with each from 2 L of the production
- Add to the pellet PBS until 18 ml is reached
    - After ultra-sonification add to one approach protease inhibitor cocktail
    - Stock: 1 tablet per 10 ml, so we take 2 tablets for 20 ml
- all steps with the column need to be done on ice, the dialysis is in the 4°C room Dialyse with protease see protocol

Test of new protease to see which concentration cuts:

- Approach 1: 1 ml of eluate (without inhibitor) and 1 ml of the PreScission Protease
- Approach 2: 1 ml of eluate (without inhibitor) and 500 µl of the PreScission Protease
- The remainder of the eluate without inhibitor is mixed with 16 µl of the known PreScission Protease
- Each in its own dialysis tube, the next day after taking a sample for the gel is reunited
- Dialysis overnight at 4 ° C
- Gel for protein purification (1st column)
- See log for charging scheme
- WB blotting for protein purification (1st column) see protocol

LB agar preparation see protocol

04.08.2020

Phosphate buffer:

- Na2HPO4: 0.2 M
  M = 177.99 g / mol
  Total volume: 4 L
  • Weigh 142.39 g and fill up to 4 L with Mili-Q-Water

- Citric acid: 0.1 M
  M = 210.14 g / mol
  Total volume: 3 L
  • Weigh 63.04 g and make up to 3 L with Mili-Q-Water

- pH 7: 1647 ml Na2HPO4
  353 ml Citric acid

- pH 5.5: 1137.5 ml Na2HPO4
  862.5 ml Citric acid

- pH 4: 771 ml Na2HPO4
  1229 ml Citric acid

2. Affinity chromatography see protocol

- Everything in the 4 ° C room
- Three approaches of the eluate without inhibitor to test the protease concentration and the known PreScission protease are combined in Falkon, after which 60 µl aliquots for the gel were taken from each
- Eluate without inhibitor and with inhibitor are purified again on 2 different columns
- Dialysis in pH7 for 1.5 h
- Dialysis in pH 5.5 for 1.5 h
- Dialysis in pH 4 for 1.5 h

See protocol for concentration

→ With protease inhibitor (was given directly to the assay team): c = 6.355 µM; V = 500 µl

→ Without protease inhibitor (was frozen, but then stored at -20 ° C instead of -80 ° C): c = unknown, V = 500 µl

Gel for 2nd column see protocol

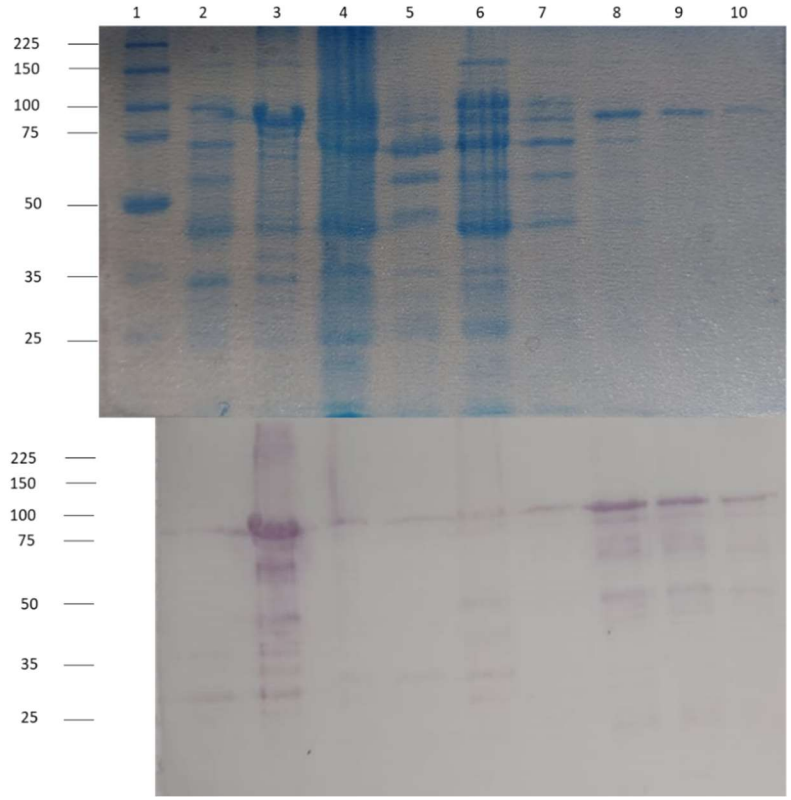
- Loading scheme for protein without protease inhibitor, with test of the protease concentration:

	1	**2**	**3**	**4**	**5**	**4**	**5**	**6**	**7**	**8**
Sample	**Marker**	**elution fraction (before digestion)**	**Elution fraction (after digestion)**	**Elution fraction 1 ml, Protease 1 ml**	**Elution fraction 1 ml, Protease 500 µl**	**D1**	**D2**	**D3**	**D4**	**Elution fraction (eluted GST)**
Volume	5 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl
Sample buffer	-	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20µl	20 µl
Boil time	-	5 min	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	5 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl

Western blotting to gel from May 4th, 2020 (2nd column) see protocol

Western blot detection of the WB from May 3rd, 2020 (1st column) see protocol

E.*coli* BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ without protease inhibitor medium: 2YT; overnight; 17°C (1.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

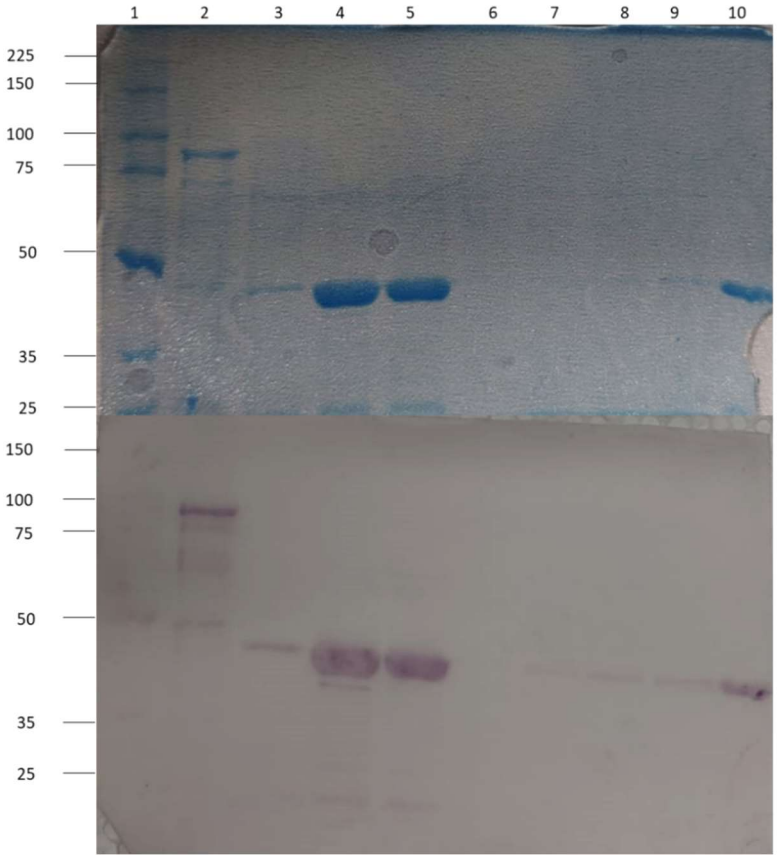
9: eluate 4

10: eluate 6

MW with GST: 89,34 kDa

MW without GST: 61,59 kDa

E.*coli* BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ without protease inhibitor medium: 2YT; overnight; 17°C (2.column)



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: 89,34 kDa

MW without GST: 61,59 kDa

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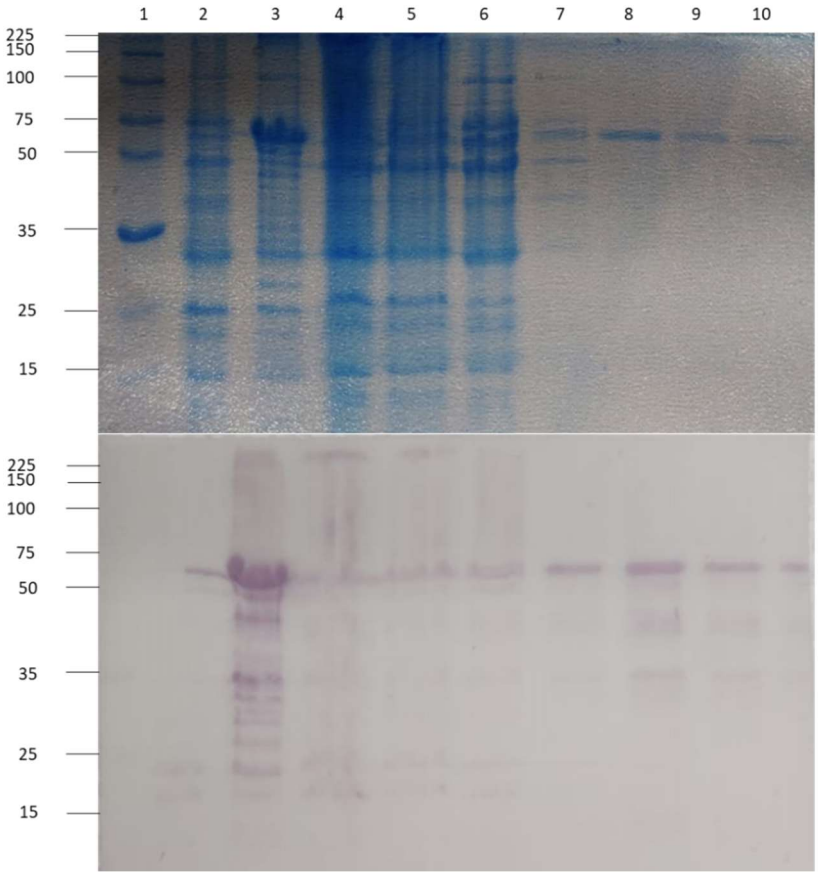

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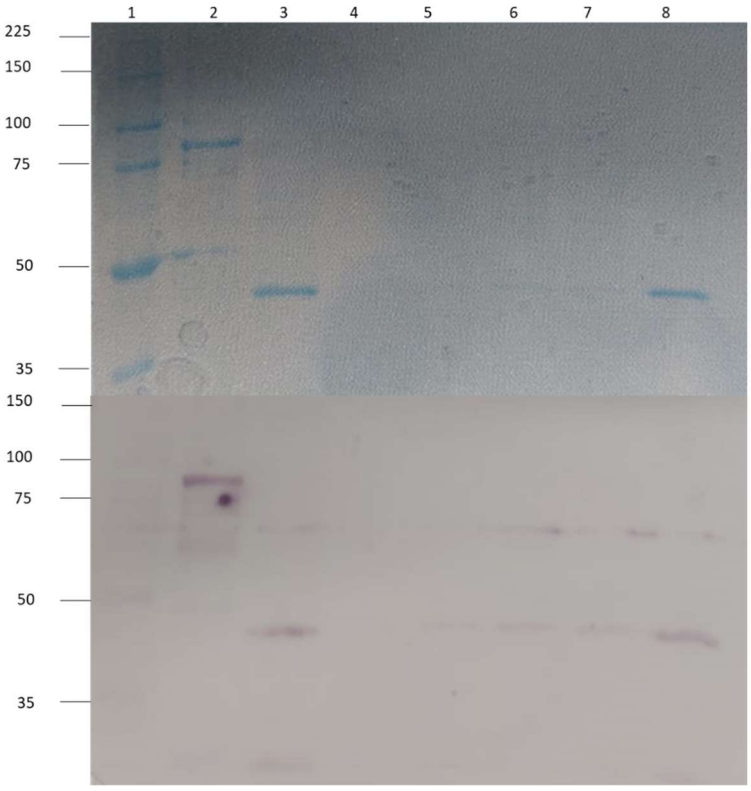

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