28.09.2020

New smear of the plates from 24.09.

29.09.2020

Overnight cultures of

- BL21 mar
- Rosetta Ba
- AD494 Ba
  → Taken from the plates of 28.09.
  → 25 ml each in 250 ml flasks

30.09.2020

Expression
Overnight culture OD:

strain	dilution	OD (measured)	OD (calculated)	Volume per flask [ml]
AD494 Ba	1:10	0,321	3,21	4,67
Rosetta Ba	1:10	0,152	1,52	9,86
BL21 mar	1:10	0,486	4,86	3,08

- 2 L each are produced
- 2 times 1 L in 5 L flasks
- 100 µl copper sulfate

Antibiotics:

strain	Antibiotics
AD494 Ba	Kan 300 µl Amp 500 µl
Rosetta Ba	Cam 2000 µl Kan 300 µl Tet 200 µl Amp 500 µl
BL21 mar	Amp 1000 µl

The volume of the overnight culture calculated above was pipetted into different flasks to set an OD of 0.015

Stamm	Start OD	1:30 h	2:10 h	2:25 h	3:10 h	3:40 h	4:40 h	5:20 h	5:30 h
AD494 Ba 1	0,019	0,042			0,281	0,485			
AD494 Ba 2	0,02	0,046	0,103		0,268	0,475			

Rosetta Ba 1	0,009	0,023			0,092		0,233	0,429	0,46
Rosetta Ba 2	0,01	0,021	0,048		0,091		0,220	0,452	0,458
BL21 mar 1	0,022	0,105	0,336	0,6					
BL21 mar 2	0,01	0,074	0,318	0,484					

Green = induced with 500 µl IPTG

Samples taken:

- AD494 Ba: sample 1
- Rosetta Ba: sample 1
- BL21 mar: sample 2

→ Production takes place at 17°C overnight

01.10.2020

OD of colonies produced overnight:

strain	dilution	OD measured	OD calculated
AD494 Ba 1	1:10	0,158	1,58
AD494 Ba 2	1:10	0,147	1,47
Rosetta Ba 1	1:10	0,094	0,94
Rosetta Ba 2	1:10	0,090	0,9
BL21 mar 1	1:10	0,390	3,90
BL21 mar 2	1:10	0,302	3,02

- A 1 ml sample is taken from each colony

Harvest cells see protocol

- Pellet that has formed in one liter after centrifugation and each belongs to a strain is combined -> you get a pellet of 2 liters

Pellet:

AD494 Ba	2,69 g
Rosetta Ba	1,63 g
BL21 mar	6,97 g

05.10.2020

Buffer preparation:

- Protease buffer: 2 L

  Tris-HCl 12.11 g

  NaCl 17.53 g

EDTA 0.585 g

DTT 0.31 g

- Elution buffer:

  Tris-HCl pH8: 25 ml

  Glutathione: 0.076 g

For digestion and purification of the BL21 mar and AD494 Ba pellets (harvested on October 1st and stored at -20°C) see protocol

- Adding PBS to resuspend:
- BL21 mar: fill up to the 20 ml mark
- AD494 Ba: fill up to the 10 ml mark

1st column affinity purification

- Dialysis see protocol
- Add 10 µl PreScission Protease each time

06.10.2020

2nd column affinity purification

- Phosphate buffer pH7 overnight

07.10.2020

Phosphate buffer pH 5.5

- 1,5h in 4°C room

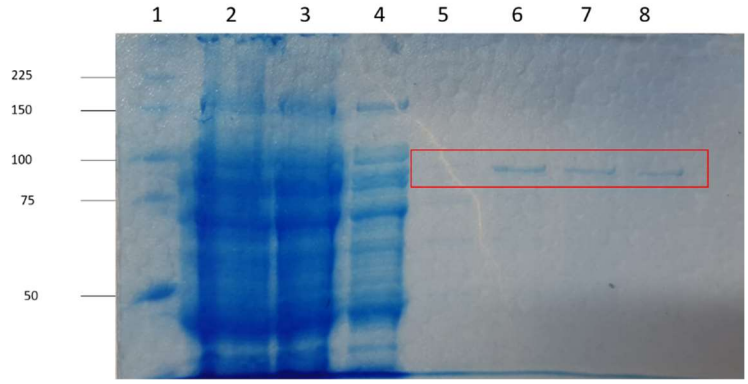
Phosphate buffer pH 4

- 1,5h in 4°C room

concentrate

- see protocol

E. coli AD494 Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 17°C overnight, 1st column



1: marker

2: before induction sample

3: after induction sample (3h, 30°C)

4: lysate

5: supernatant

6: wash

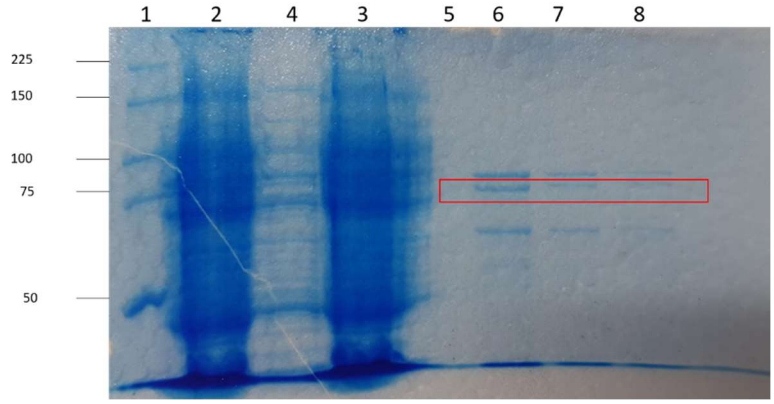
7: eluate 1

8: eluate 2

9: eluate 4

10: eluate 6

E. coli BL21 (DE3) mar pGEX-6P-1 with $CuSO_4$ medium: 2YT; 17°C overnight, 1st column



1: marker

2: before induction sample

3: after induction sample (3h, 30°C)

4: lysate

5: supernatant

6: wash

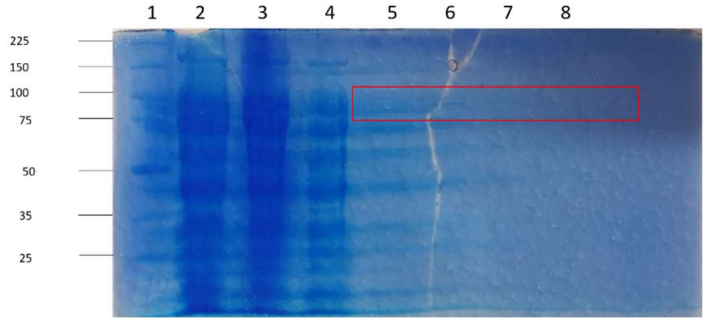
7: eluate 1

8: eluate 2

9: eluate 4

10: eluate 6

E. coli Rosetta gami Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 17°C overnight, 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