Author: Allyssa Hinkle
Entry 7/24: No entry title yet
In Project: Journal screening
No tags associated

created: 18.10.2020 13:51
updated: 26.10.2020 13:53

25.06.2020 – 08.07.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

25.06.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii (protocol iGEM 2019)"

01.07.:

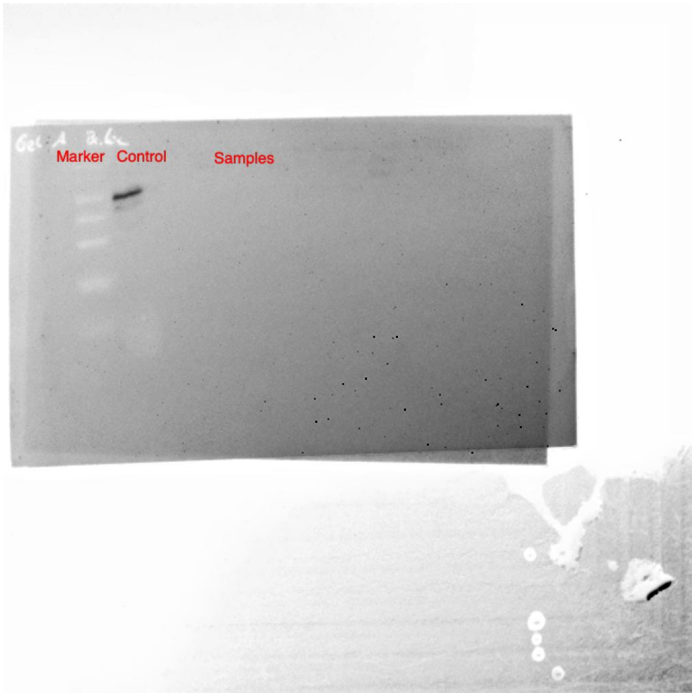
- Loaded samples onto SDS gel (proceeded after protocol "SDS-gel electrophoresis")
- Scheme:
  - Gel A (BaLac): Marker, control, BaLac 1 – 12, BaLac 26 (pos. from screening on 26.06.2020), marLac without SP20 (pos. from screening on 26.06.2020)
  - Gel B (marLac): Marker, control, marLac 1 – 12, marLac 17(pos. from screening on 26.06.2020)
- Western Blot according to protocol "Western Blot"

02.07.:

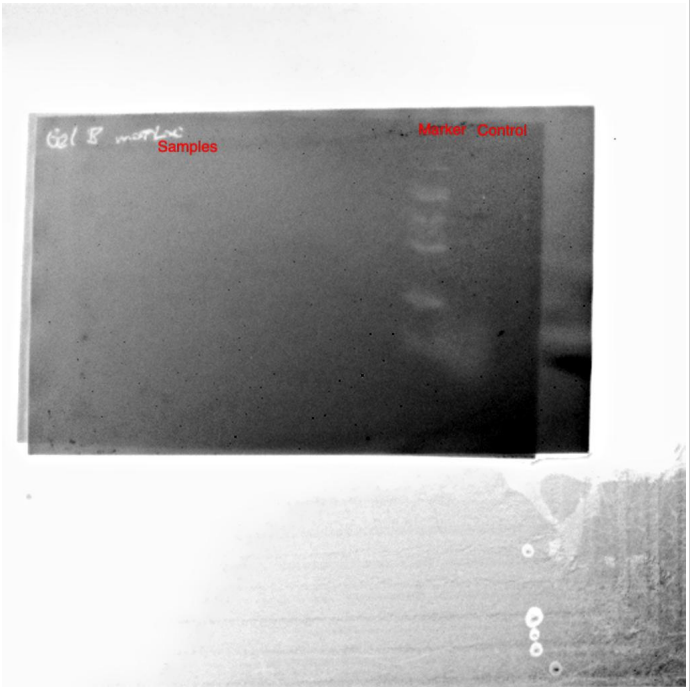
- Ponceau-S staining
- -> showed protein on the membrane

- ECL detection

Gel A (BaLac)



Gel B (marLac)



- No protein was detected in the supernatant
- Signal from the positive control was weak or undetectable: repeated SDS page and Western Blot:

07.07.

- Loading scheme was the same
- Performed SDS page according to protocol
- Performed Western Blot according to protocol

08.07.:

- Ponceau-S staining:
- -> showed protein on the membrane

- ECL detection:

20.07.09_Screening_cCA+Balac+SP20HAHis_Wiederholung.Tif

20.07.09_Screening_cCA+Marlac+SP20HAHis_Wiederholung.Tif

- Controls were still very faint

Author: Allyssa Hinkle
Entry 8/24: No entry title yet
In Project: Journal screening
No tags associated

created: 18.10.2020 17:15
updated: 26.10.2020 13:54

06.07.2020 – 07.07.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (pellets)

06.07.:

- Lysed pellets from screening of supernatant (25.06.2020 – 02.07.2020 pAR-cCA-BaLac-SP20-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His) according to protocol "Cell harvest and cell disruption for chlorophyll determination"
- Results from chlorophyll determination:

Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1	Abs (645	Abs. (663	Chlorop./	V for 2 ug	Chlorop					
2	b1	0.157	0.287	4.1						
3	b2	0.164	0.323	3.8						
4	b4	0.12	0.24	5.1						
5	b5	0.141	0.271	4.5						
6	b6	0.141	0.257	4.6						
7	b7	0.198	0.37	3.2						
8	b9	0.141	0.2	5						
9	b10	0.179	0.284	3.8						
10	b11	0.173	0.274	3.9						
11	b12	0.164	0.263	4.1						
12	m1	0.141	0.196	5.1						
13	m2	0.214	0.27	3.5						
14	m3	0.202	0.242	3.7						
15	m4	0.169	0.173	4.7						
16	m5	0.217	0.251	3.5						
17	m6	0.2	0.227	3.8						
18	m7	0.215	0.25	3.5						
19	m9	0.215	0.266	3.5						
20	m10	0.21	0.234	3.7						
21	m11	0.205	0.216	3.8						
22	m12	0.191	0.19	4.2						
23	k	0.2	0.228	3.8						
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Sheet1

labfolder_table_6519667_3.xlsx

- Loaded amount in column D onto SDS gel
- Scheme: Marker, pos (B5), pos (sALB), samples (BaLac/marLac) 1 – 12

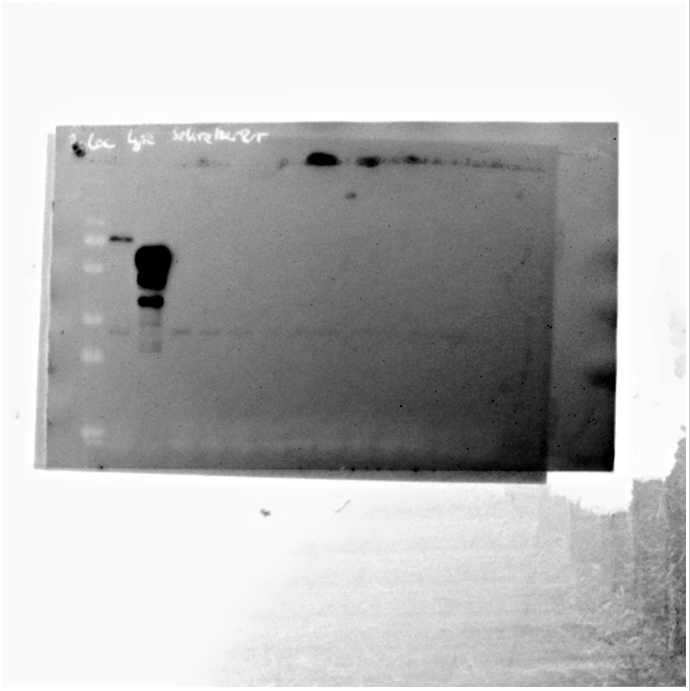
07.07.:

- Performed Western Blot according to protocol "Western Blot"
- showed protein on the membrane

- Results from Western Blot:

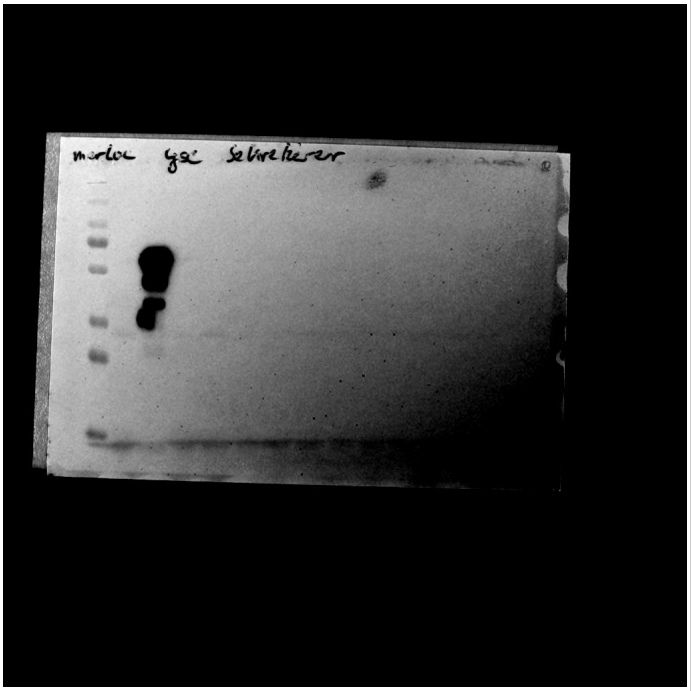
BaLac, 5 min, marker merged

BaLac, 5 min, increased contrast

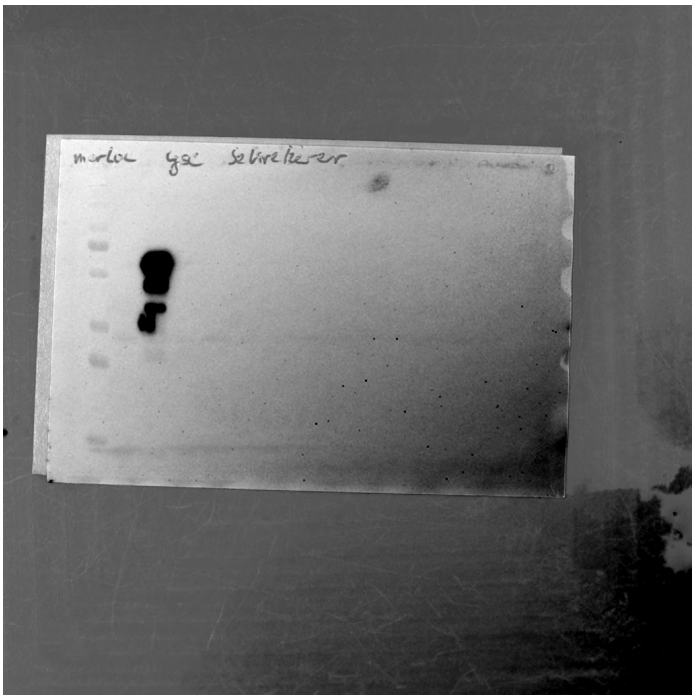


marLac, 5 min

marLac, 5 min, increased contrast, marker merged



marLac, 5 min, increased contrast, marker merged



marLac, 5 min

- Protein of interest was not detected

Author: Allyssa Hinkle
Entry 9/24: No entry title yet
In Project: Journal screening
No tags associated

created: 18.10.2020 17:53
updated: 19.10.2020 16:15

15.07.2020 – 21.07.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: BaLac 15 – 25 and marLac 15 – 24

15.07.:

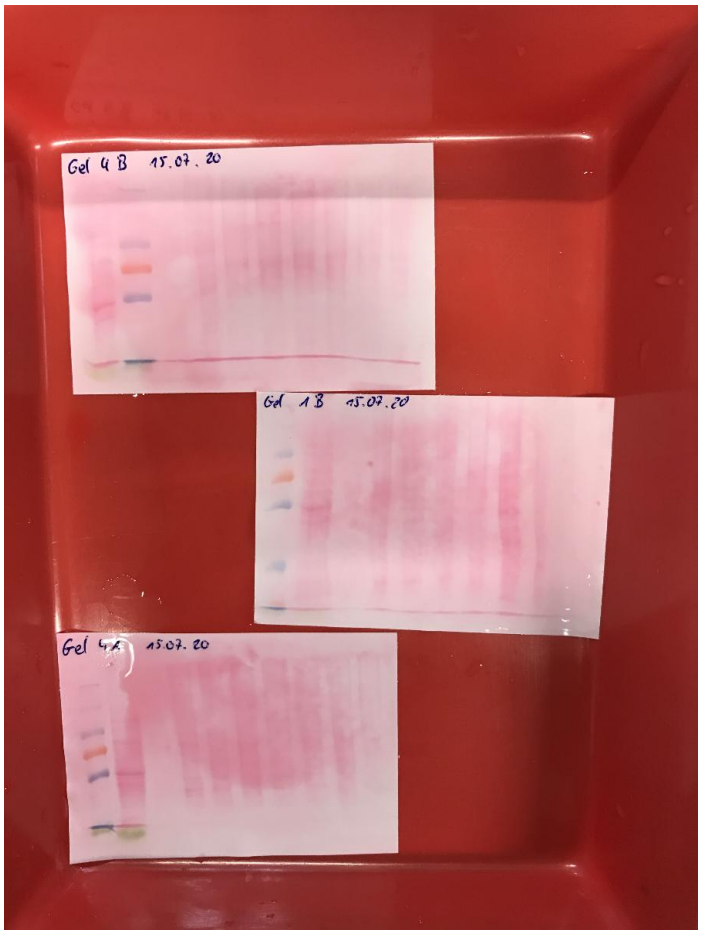
- Harvested in the exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii (protocol iGEM 2019)"

- Loaded samples onto SDS gel (proceeded after protocol "SDS-gel electrophoresis")
- Scheme:
  - Gel 4A: pos (BaLac), Marker, pos. control, marLac 15 – 21
  - Gel 4B: Marker, pos (BaLac), pos. control, BaLac 15 – 21
  - Gel 1B: Marker, pos (BaLac), pos. control, BaLac 22, BaLac 23, BaLac 24, marLac 22, marLac 23, marLac 24, BaLac 25
- Western Blot according to protocol "Western Blot"

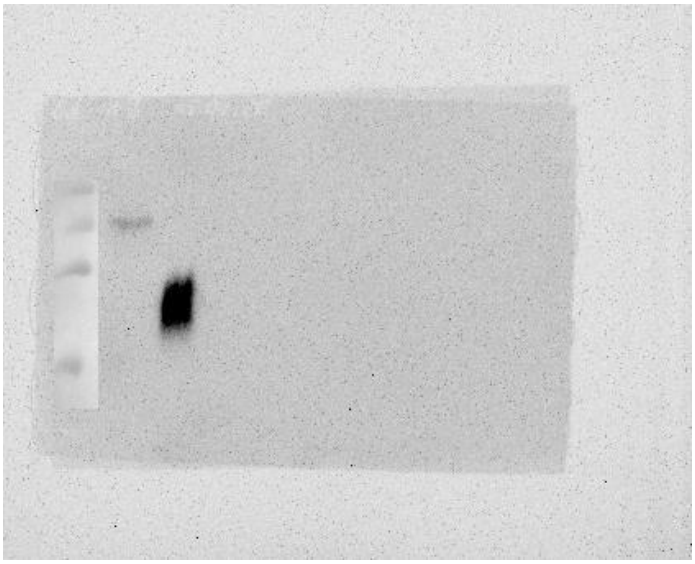
16.07.:

- Ponceau-S staining:

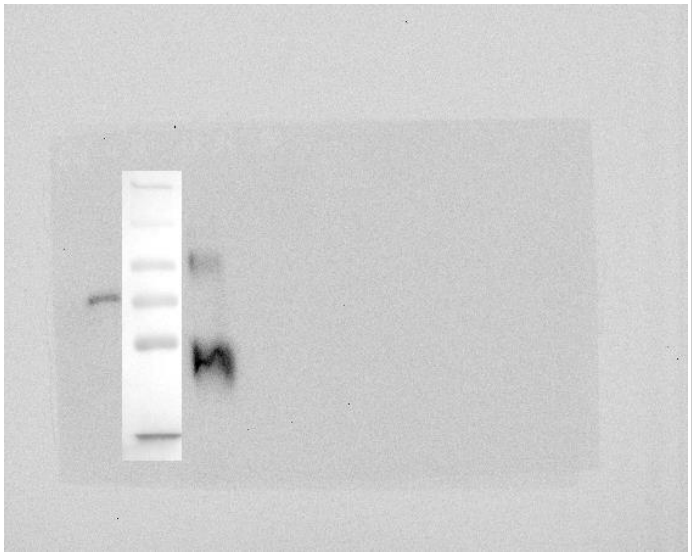
Ponceau



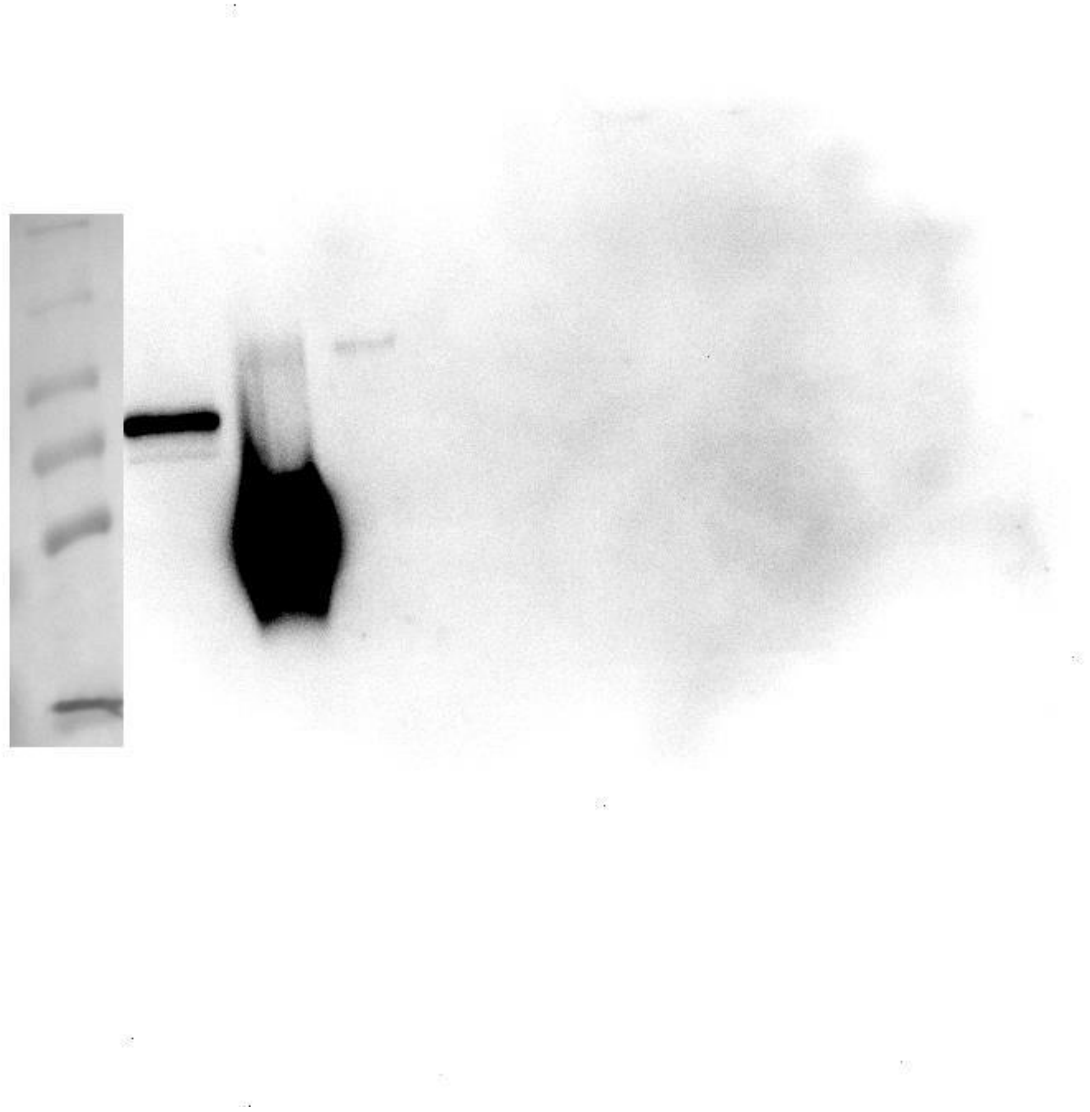
Gel 1B 4x4 binning 3 min



Gel 4B 3x3 binning 4 min



Gel 4A 2x2 binning 1.5 min



- Gel 4A showed signal

To clarify wether the protein is secreted of the signal on gel 4A comes from lysed cells we incubated the membranes with rpl1-antibody. Incubated gels 4B and 1A again with anti-HA antibody.

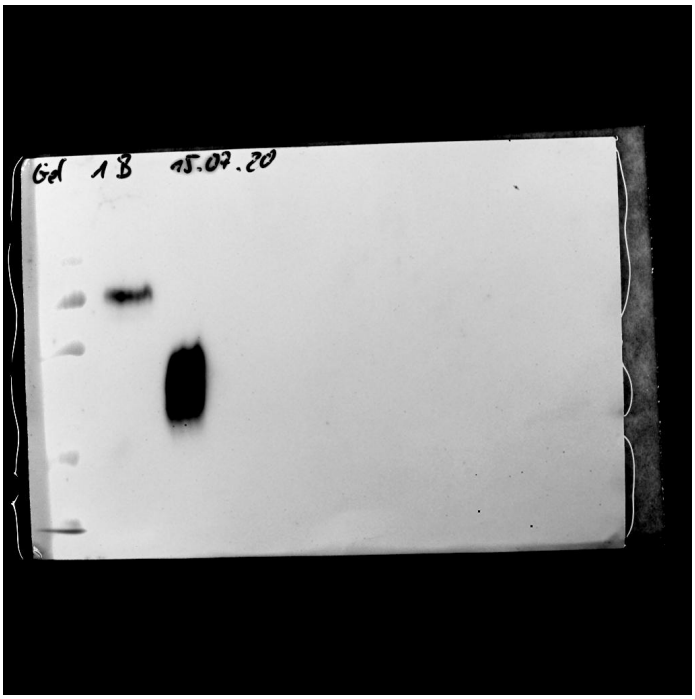
20.07.:

- Incubated gel 4A over night with rpl1-antibody
- Incubated other gels over night with anti-HA antibody

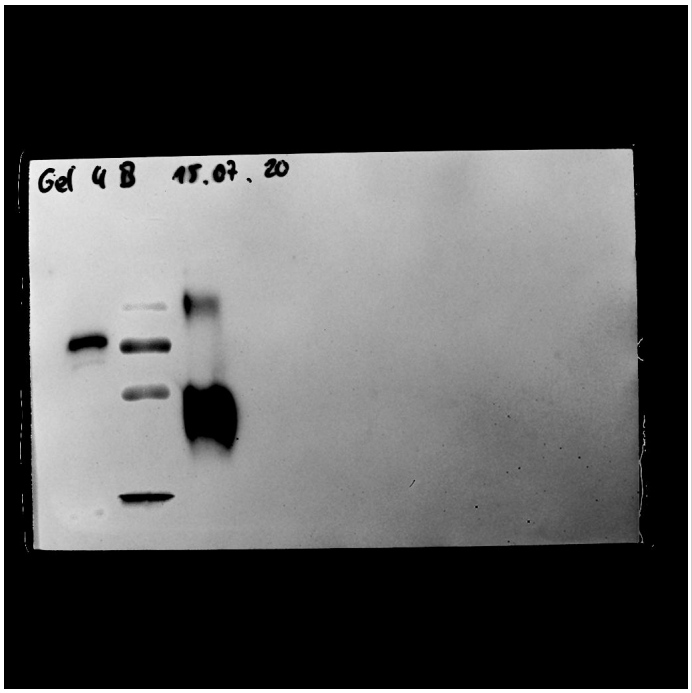
21.07.:

- Incubated gels with according secondary antibodies
- ECL detection results:

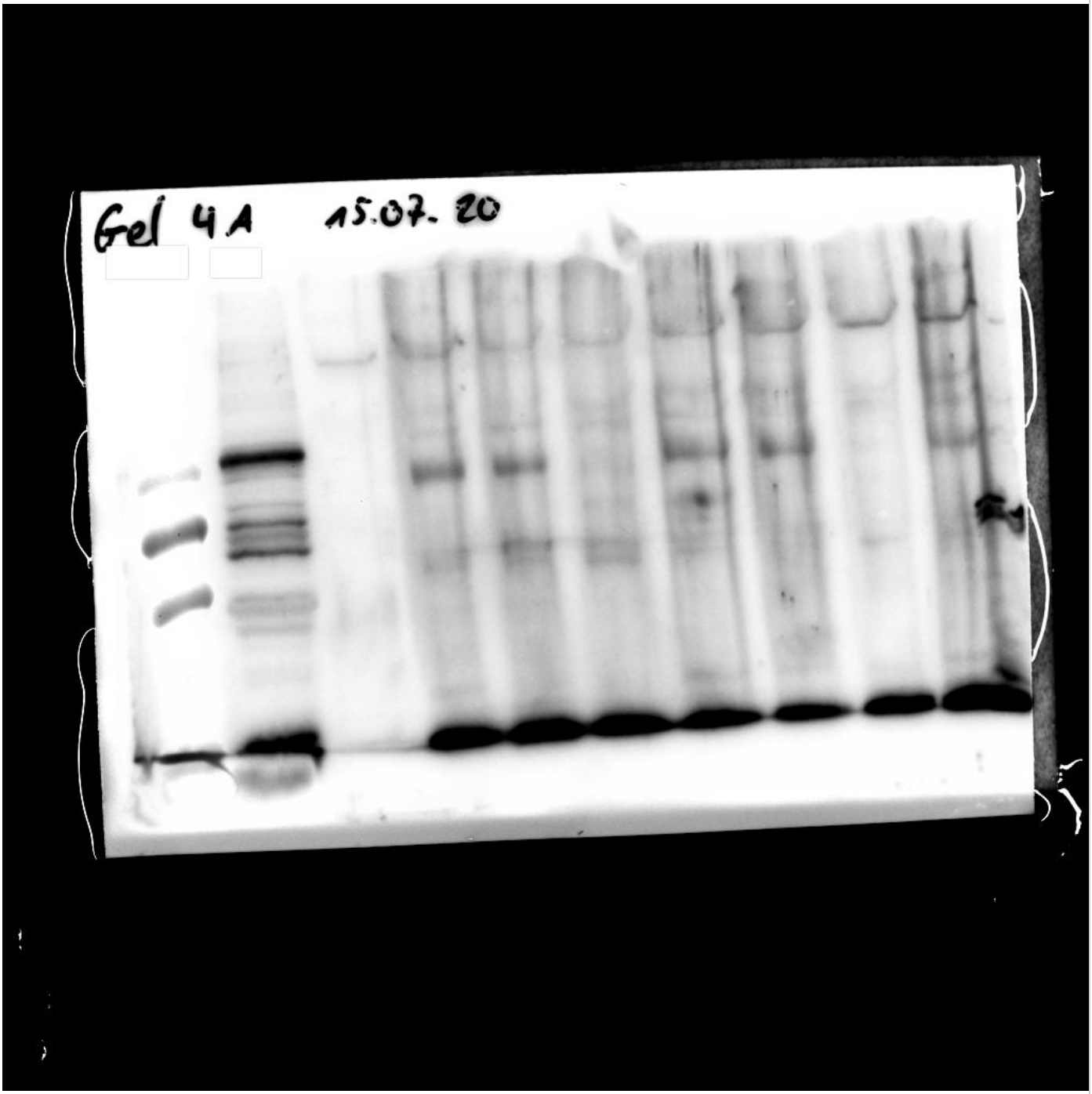
Gel 1B, 5 min



Gel 4B, 5 min



Gel 4A, 30 s



- Gel 4A showed not much protein from lysed cells.
- Other gels showed no signal.

Author: Allyssa Hinkle
Entry 10/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 09:30
updated: 19.10.2020 16:14

27.07.2020 – 29.07.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: BaLac 31 – 35 and marLac 15, 33 – 35

27.07.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

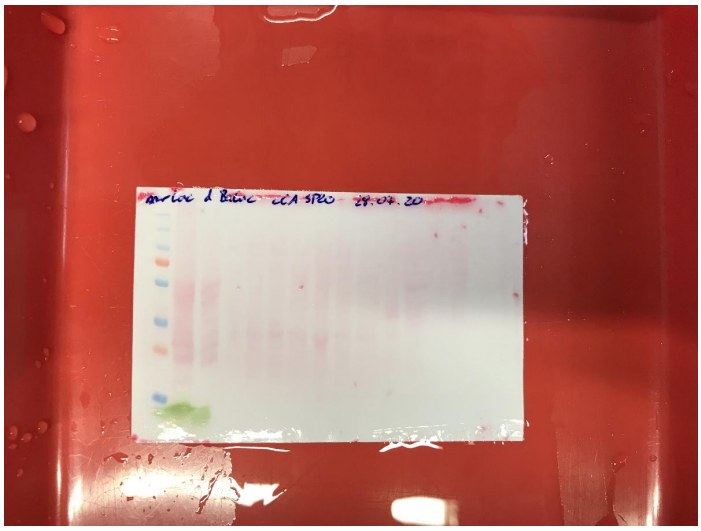
28.07.:

- Performed SDS-Gel according to protocol "SDS gel-electrophoresis"
- Loading scheme:
  - pos, marker, marLac 15, marLac 33 – 34, BaLac 31 – 35, marLac 35, B+, M+, marker

29.07.:

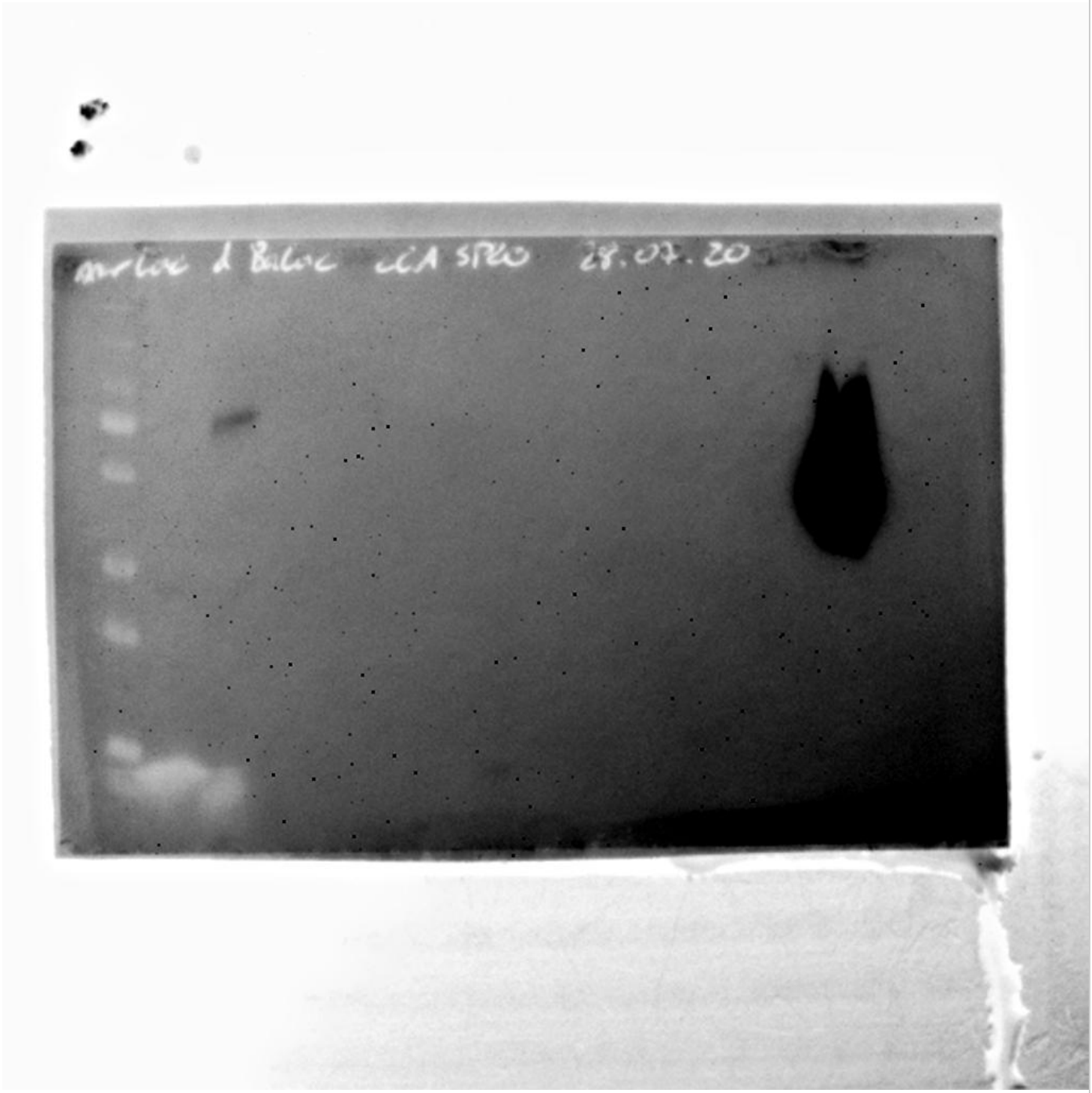
- Performed Western Blot according to protocol "Western Blot"
- Ponceau-S staining:

30-07-2020_B+M.png



- Results from ECL-detection:

BaLac and marLac, 10 min, merged with marker

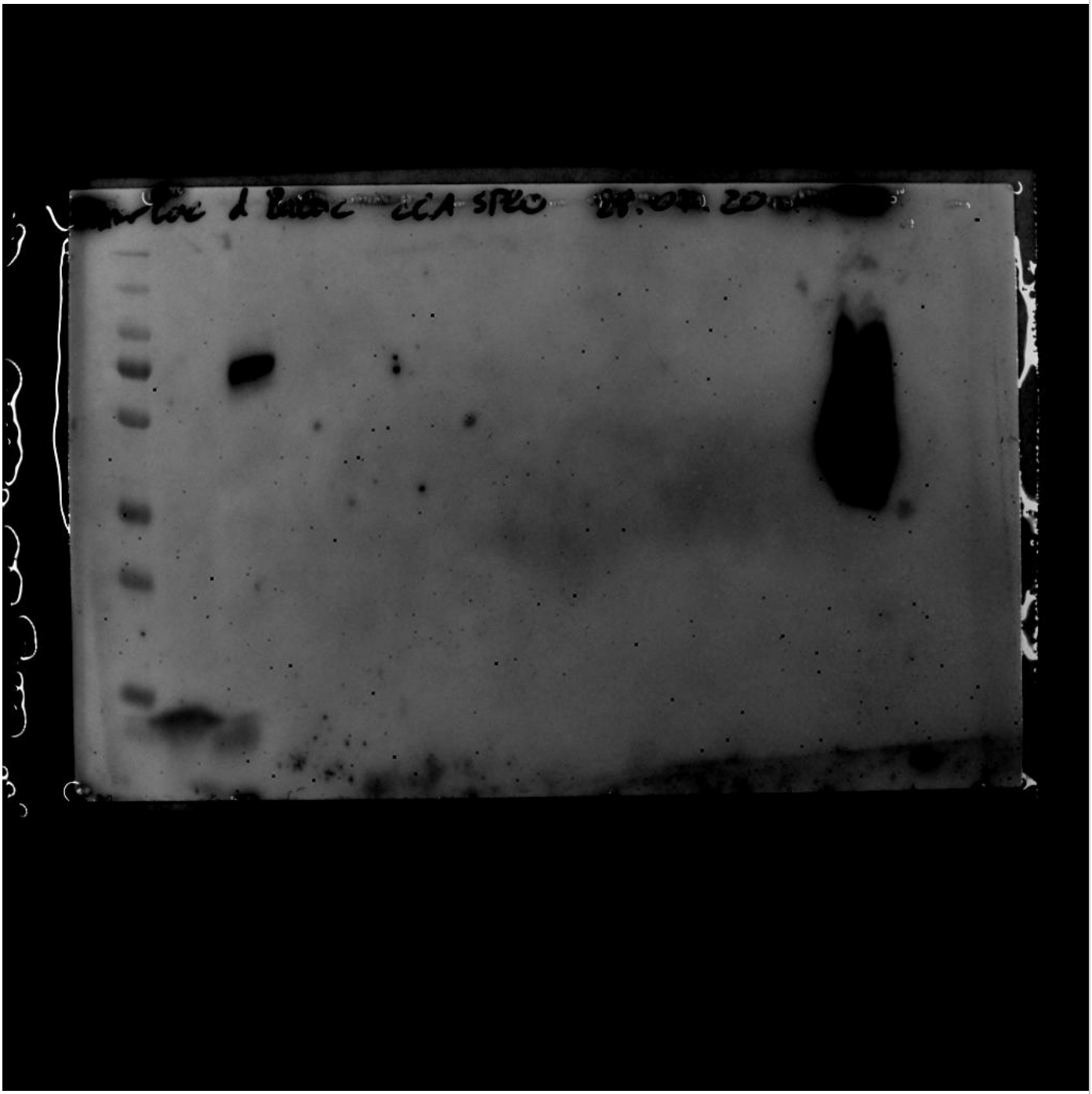


- Western Blot showed no signal from protein of interest.
- Incubated membrane again with primary antibody (anti-HA) over night.

30.07.:

- Results from ECL detection:

BaLac and marLac, 10 min, merged with marker



- The positive result for marLac 15 could not be reproduced. Maybe mixed up samples. Positive result could be BaLac 15 or BaLac 19. Repeated Western Blot with these two cultures.

Author: Allyssa Hinkle
Entry 11/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 10:31
updated: 19.10.2020 10:58

29.07.2020 – 30.07.2020: TCA precipitation of colony 15 from construct pAR-cCA-marLac-SP20-HA-RGS-8His

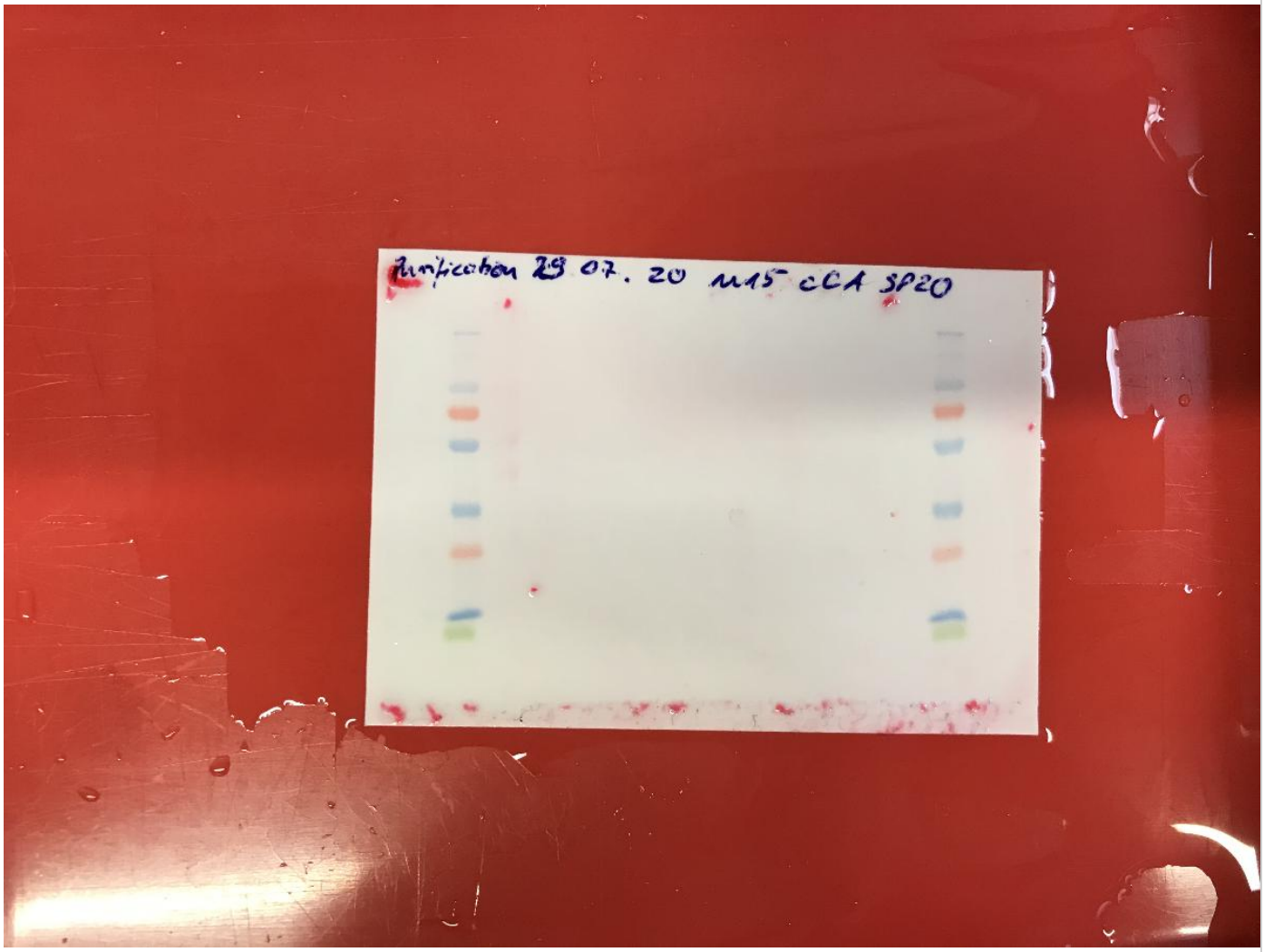
29.07.:

- Performed TCA precipitation according to protocol "TCA precipitation"
- Performed SDS page. Loading scheme:
  - Marker, Elution 1 – 5, Washing step, Flowthrough, Rinse 1 (+Triton 1:1), Rinse 2 (+Triton 1:2); loaded 15 µL for each sample and 3 µL marker.
- Performed Western Blot according to protocol "Western Blot"

30.07.:

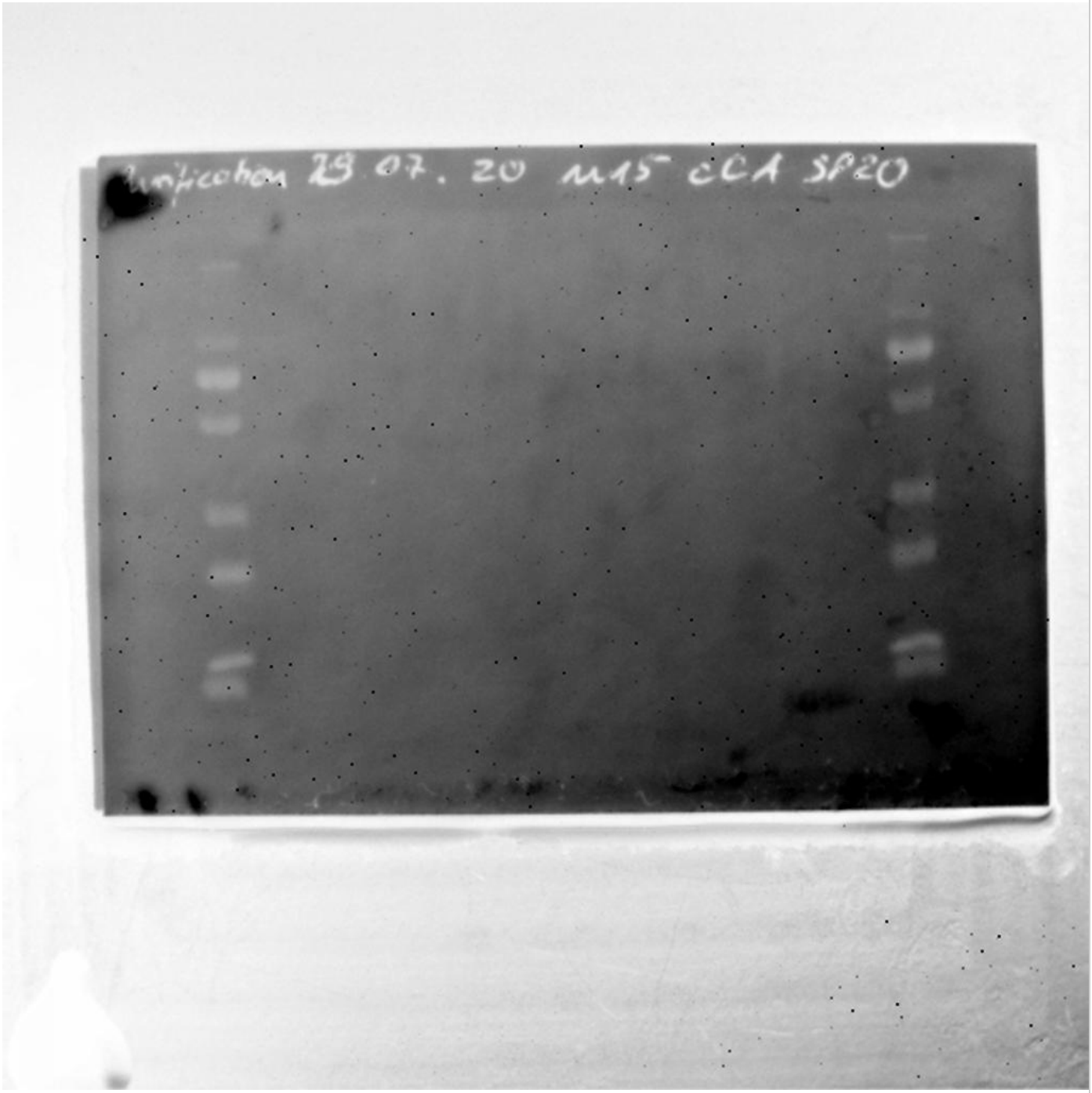
- Ponceau-S staining:

30-07-2020_Purification.png



- Ponceau showed only very faint bands on first elution step.
- Performed ECL detection:

2020.07.30_MarLac_15_column.Tif



- Protein of interest could not be detected.

Author: Allyssa Hinkle
Entry 12/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 10:58
updated: 20.10.2020 10:37

03.08.2020 – 05.08.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant + lysed pellets)

Screened colonies: BaLac 15, BaLac 19, BaLac 26 – 30, marLac 27 – 31

03.08.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

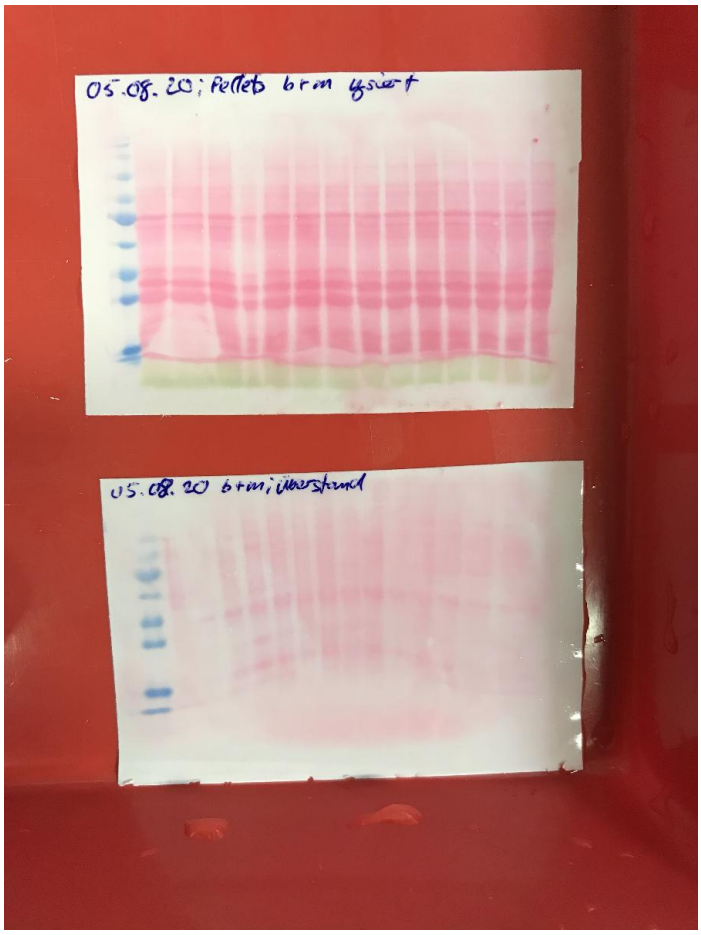
04.08.:

- Performed SDS page according to protocol
- Loading schemes:
  - Marker, pos., neg., BaLac 15, BaLac 19, BaLac 26 – 30, marLac 27 – 31
- Performed Western Blot according to protocol.

05.08.:

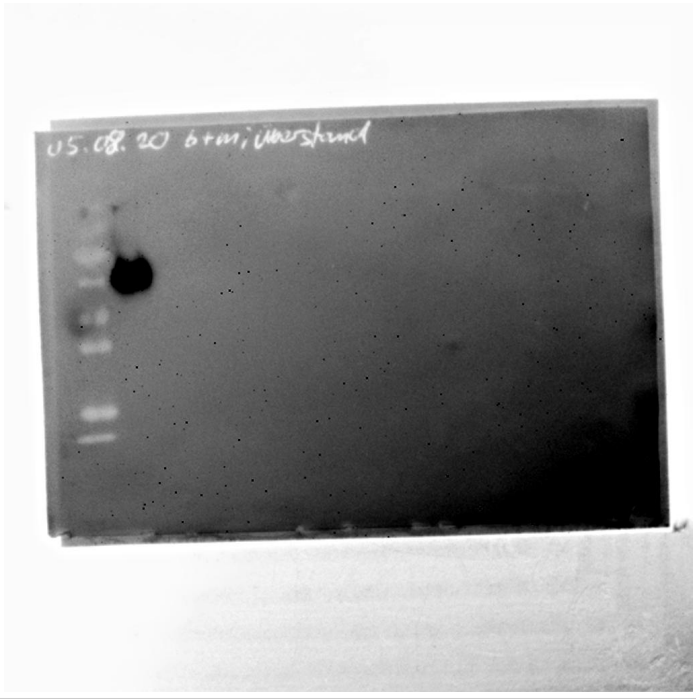
- Ponceau-S staining:

05-08-2020.png

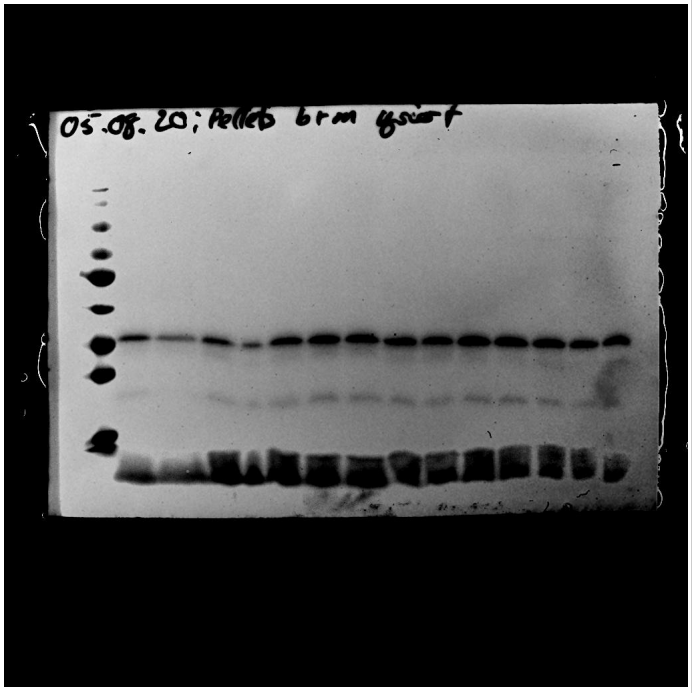


- ECL-detection:

Supernatant, 5 min, marker merged



Pellet, 5 min, marker merged



Author: Allyssa Hinkle
Entry 13/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 11:09
updated: 19.10.2020 16:14

03.08.2020 – 05.08.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: BaLac 37 – 43; marLac 32, 36 – 45

03.08.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

04.08.:

- Performed SDS page according to protocol
- Loading schemes:
  - Marker, pos., neg,, BaLac 37 – 43 (A)
  - Marker, pos., neg,, marLac 32, 36 – 45 (B)
- Performed Western Blot according to protocol.

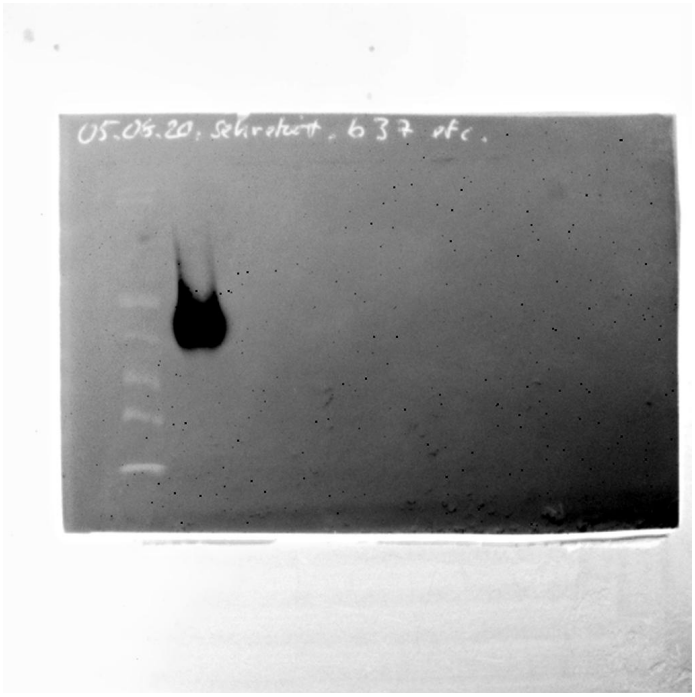
05.08.:

- Ponceau-S staining:

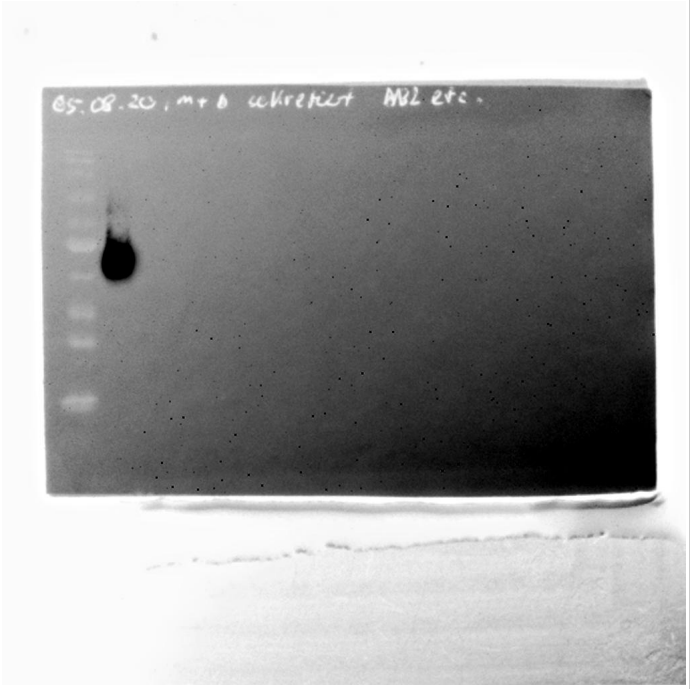
Ponceau

- Performed ECL detection:

A, 10 min, marker merged



B, 10 min, marker merged



- Protein of interest could not be detected.

Author: Allyssa Hinkle
Entry 14/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 11:26
updated: 19.10.2020 16:13

12.08.2020 – 13.08.2020: Screening colonies of constructs pAR-cCA-BaLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: BaLac 13, 14, 36, 43 – 50; marLac 13, 14, 25, 26 (two weeks old), 26 (10 days old), 26 (one week old), 45 – 49

12.08.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

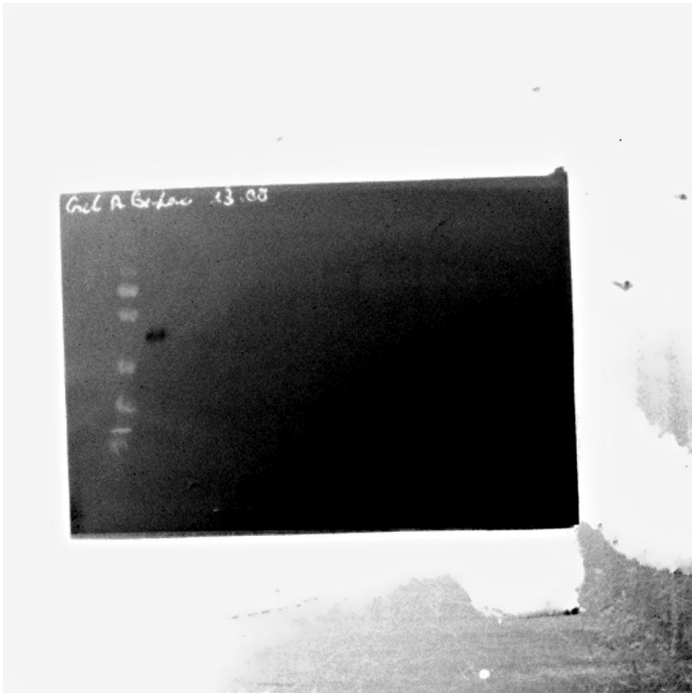
13.08.:

- Performed SDS page according to protocol
- Loading schemes:
  - Marker, pos., neg,, BaLac 37 – 43 (A)
  - Marker, pos., neg,, marLac 32, 36 – 45 (B)
- Performed Western Blot according to protocol.
- Ponceau-S staining:

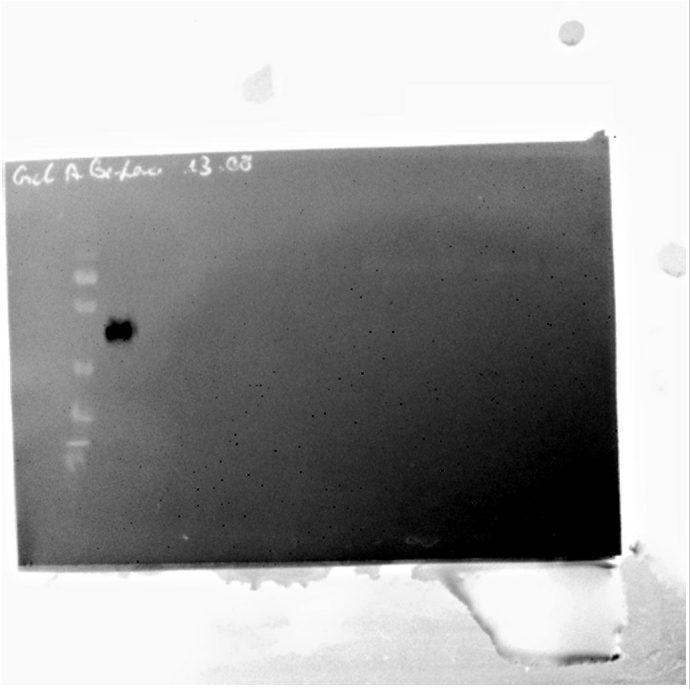
Ponceau

- Performed ECL detection:

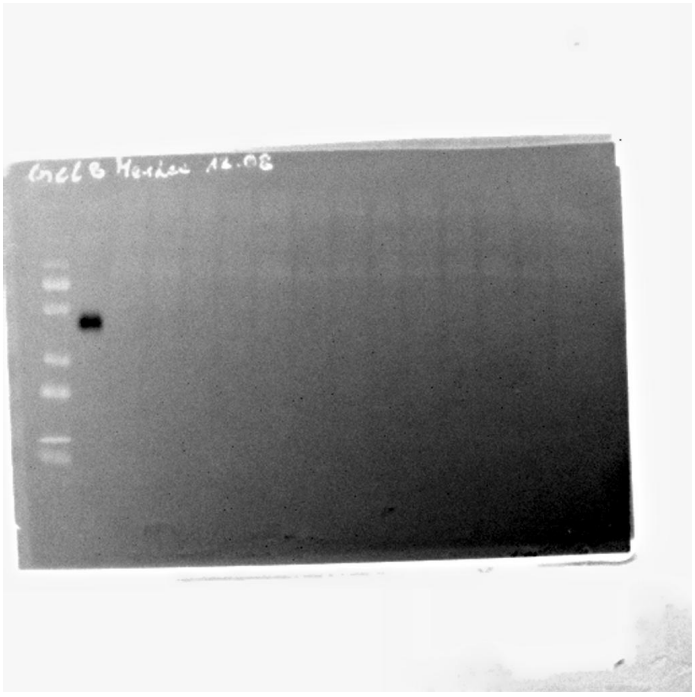
Gel A, 5 min, marker merged



Gel A, 10 min, marker merged



Gel B, 5 min, marker merged



Gel B, 10 min, marker merged

- Protein of interest could not be detected.

Author: Allyssa Hinkle
Entry 15/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 11:49
updated: 26.10.2020 13:54

19.08.2020 – 10.09.2020: Screening colonies of constructs pAR-cCA-BaLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: BaLac 1 – 50

19.08.:

- Inoculated fluid cultures of BaLac

25.08.:

- Havested cultures from BaLac in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

- Inoculated fluid cultures of marLac

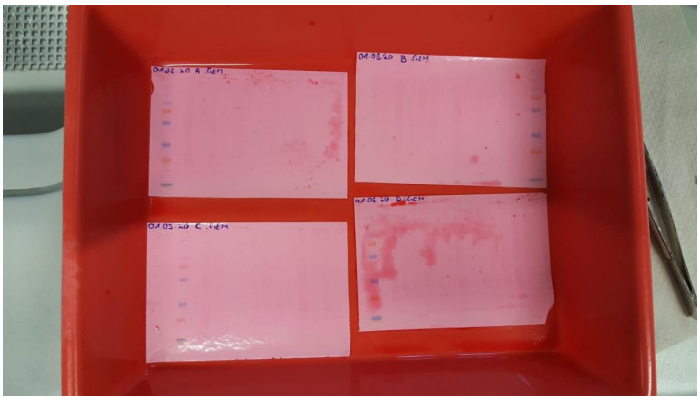
31.08.:

- Havested cultures from marLac in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

01.09.:

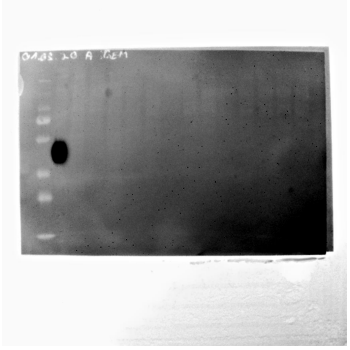
- Performed SDS page of fluid cultures from BaLac according to protocol
- Loading schemes:
  - A: Marker, pos., neg,, BaLac 1 – 12
  - B: Marker, pos., neg., BaLac 13 – 24
  - C: Marker, pos., neg,, BaLac 25 – 36
  - D: Marker, pos., neg., BaLac 37 – 48
- Performed Western Blot according to protocol.
- Ponceau-S staining:

Ponceau

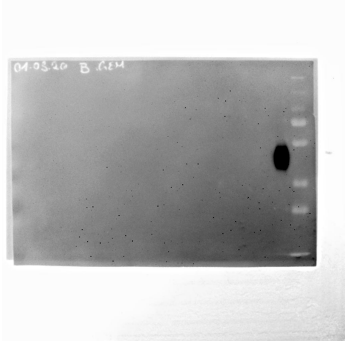


- Performed ECL detection of cultures from BaLac

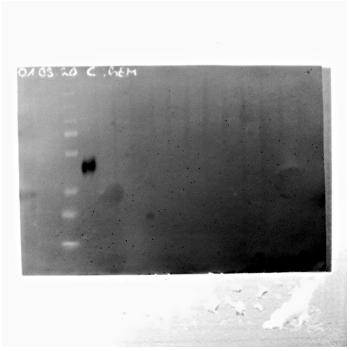
2020.09.02_A_10_min.Tif



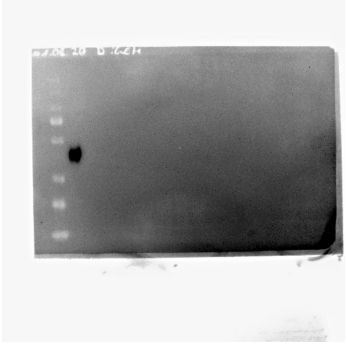
2020.09.02_B_10_min.Tif



2020.09.02_C_10_min.Tif



2020.09.02_D_5_min.Tif



- Protein of interest could not be detected.

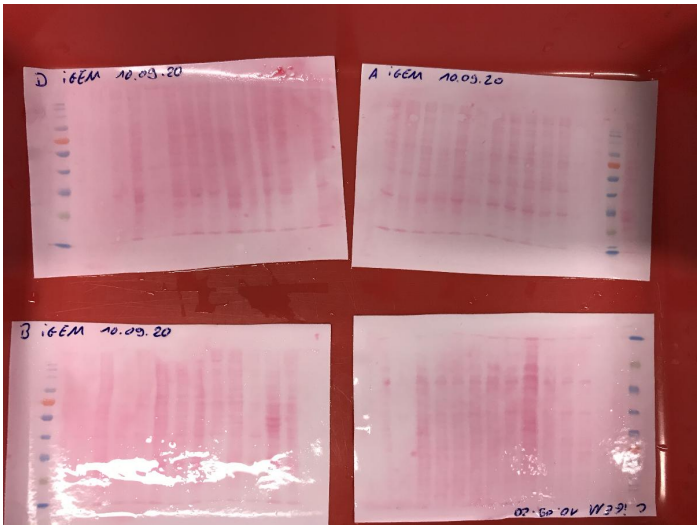
10.09.:

- Performed SDS page of fluid cultures from marLac according to protocol
- Loading schemes:
  - A: Marker, pos., neg,, marLac 1 – 12
  - B: Marker, pos., neg., marLac 14 – 24
  - C: Marker, pos., neg,, marLac 25 – 36
  - D: Marker, pos., neg., marLac 37 – 48
- Performed Western Blot according to protocol.

11.09.:

- Ponceau-S staining:

10-09-2020.png



- Performed ECL detection
- Protein of interest could not be found

Author: Allyssa Hinkle
Entry 16/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 11:58
updated: 26.10.2020 13:55

03.09.2020: Screening colonies of constructs pAR-cCA-BaLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (pellets)

Screened colonies: BaLac 1 – 50

03.09.:

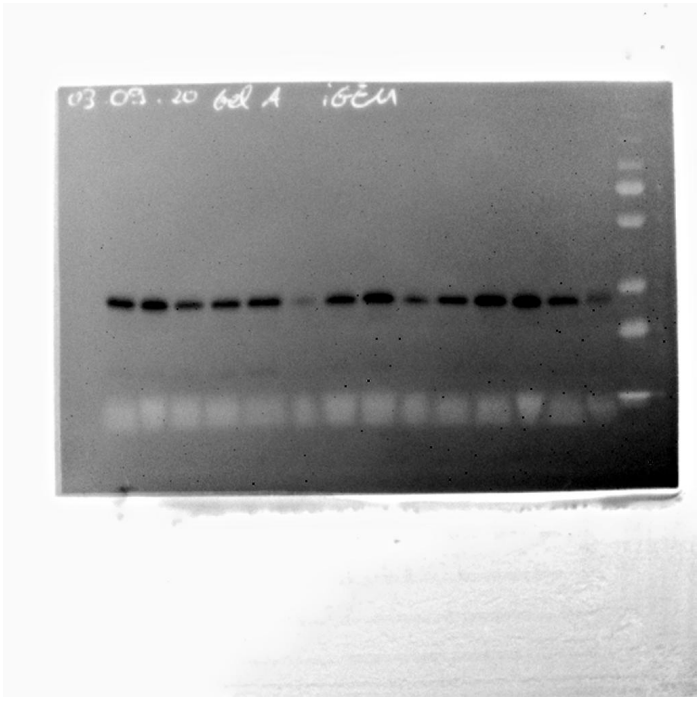
- Lysed pellets of BaLac according to protocol "Cell harvest and cell disruption for chlorophyll determination"

- Performed SDS page of fluid cultures from BaLac according to protocol
- Loading schemes:
  - A: Marker, pos., neg,, BaLac 1 – 12
  - B: Marker, pos., BaLac 13 – 24, neg.
  - C: Marker, pos., neg,, BaLac 25 – 36
  - D: Marker, pos., neg,, BaLac 37 – 45
- Performed Western Blot according to protocol.
- Ponceau-S staining:

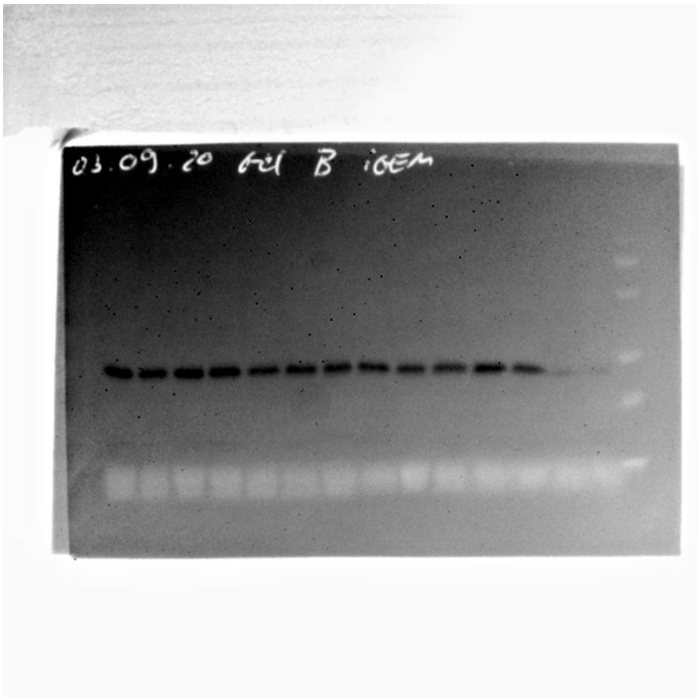
Ponceau

- Performed ECL-detection:

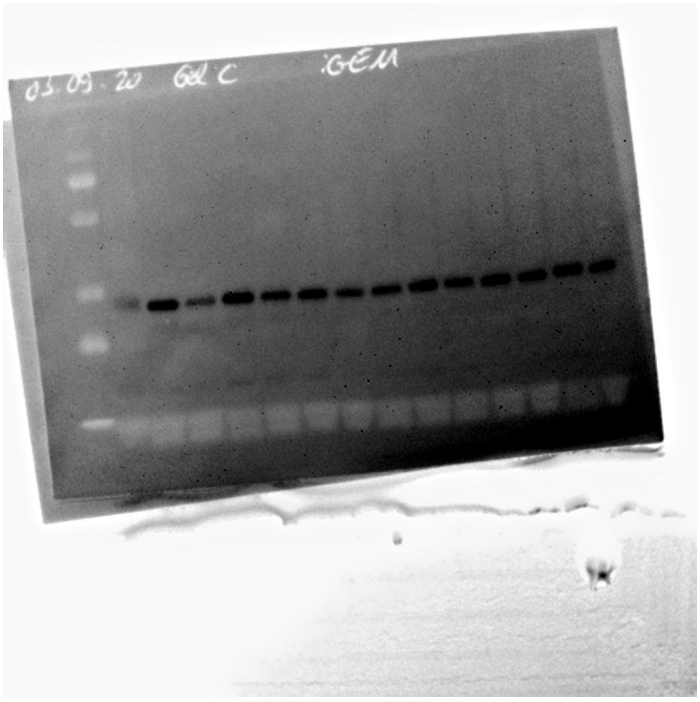
2020.09.03_A_10_min_Lyse.Tif



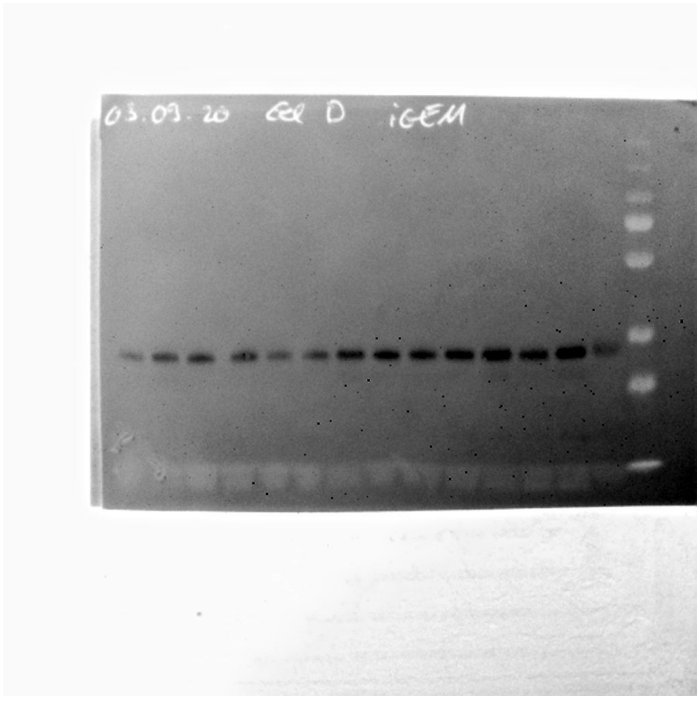
2020.09.03_Screening_B_10_min_Lyse.png



2020.09.03_Screening_C_10_min_Lyse.Tif



2020.09.03_Screening_D_10_min_Lyse.Tif



- Protein of interest could not be detected.

Author: Allyssa Hinkle
Entry 17/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 16:12
updated: 20.10.2020 10:39

16.09.2020 – 22.09.2020: Screening colonies of constructs pAR-cCA-BaLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Screened colonies: 1 – 24

16.09..:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

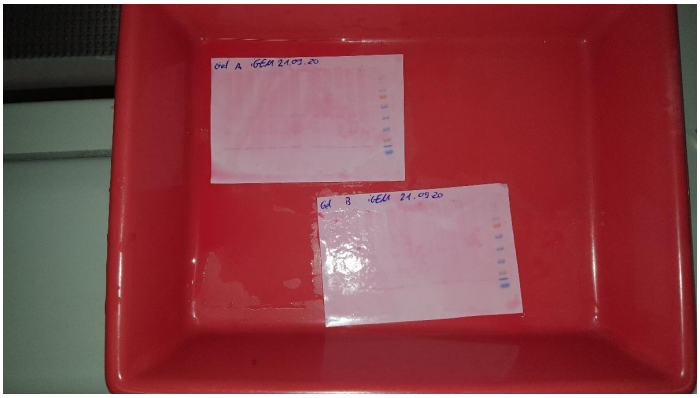
21.09.:

- Performed SDS page of fluid cultures from BaLac according to protocol
- Loading schemes:
  - B: Marker, pos., neg,, BaLac 1 – 12
  - A: Marker, pos., neg., BaLac 13 – 23
- Performed Western Blot according to protocol.

22.09.:

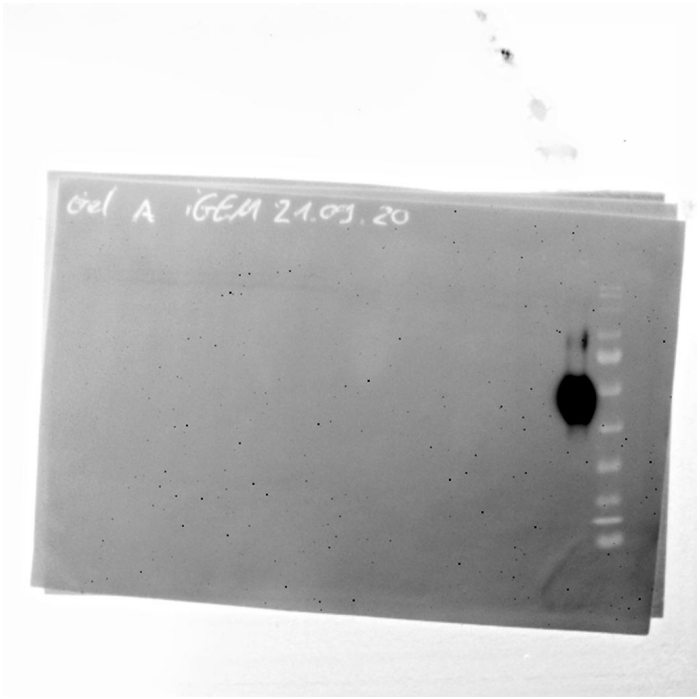
- Ponceau-S staining:

Ponceau

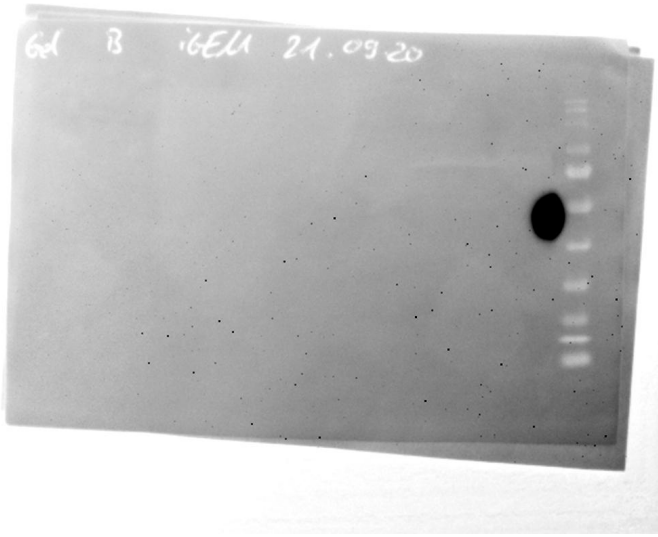


- Performed ECL detection:

Gel A, 10 min, merged



Gel B, 10 min, merged



- Protein of interest was not detected.

Author: Allyssa Hinkle
Entry 18/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 16:43
updated: 26.10.2020 14:17

28.09.2020: Freeze and thaw of constructs pAR-BaLac-3xHA-cyt and ABTS-activity assay

28.09.:

- Havested 50 mL of culture BaLac 5 (positive transformant) in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Freeze and thaw (cell lysis)"
- Used supernatant for ABTS assay:

Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1	Application: Tecan i-control				Tecan i-control , 1.1	1.1.0				
2	Device: infinite 200Pro				Serial number: 1510001295				Serial number of	
3	Firmware: V_4.25_05/15_Infinite (May 1				MAI, V_4.25_05/15_Infinite (May 11 201				5/13.53.33)	
4										
5	Date:	28.09.2020								
6	Time:	17:48:05								
7										
8										
9	System				BRONCO					
10	User				Bronco\Tecan					
11	Plate				Greiner 96 Flat Bottom Transparent Polystyrene Cat. No.:					
12	Plate-ID (Stacker)									
13										
14	Wait (Plat				On	Target Temperature				
15										
16	Wait (Plate Tempera				Valid Range: 20.9 -					
17										
18	List of actions in this measurer									
19	Kinetic									
20	Absorban									
21										
22										
23	Label: bis-pNPP									
24	Kinetic Measurement									
25	Kinetic duration				4:00:00					

26	Interval Time				0:05:00					
27	Mode				Absorbance					
28	Wavelength				415	nm				
29	Bandwidth				10	nm				
30	Number of Flashes				10					
31	Settle Time				0	ms				
32	Part of Plate				C5-F8					
33	Start Time	28.09.2020 17:48:06								
34										
35										
36	Cycle Nr.	Time [s]	Temp. [°C	C5	C6	C7	C8	D5	D6	D7
37	1	0	23,9	0,905799	0,0979	0,0604	0,0628	0,3064	1,541800	0,8281
38	2	299,8	30,5	0,812399	0,0974	0,0604	0,0627	0,682900	1,548499	0,8599
39	3	599,8	30,1	1,247200	0,0973	0,0603	0,0627	1,085600	1,559999	0,8944
40	4	899,9	30,4	1,595499	0,0974	0,0604	0,0627	1,3628	1,569200	0,9172
41	5	1199,9	30,1	1,475000	0,0972	0,0604	0,0627	1,529199	1,578799	0,9344
42	6	1499,9	30,7	1,415400	0,0972	0,0603	0,0627	1,634999	1,592399	0,9524
43	7	1800	30,4	1,378700	0,0972	0,0603	0,0627	1,748999	1,601699	0,9642
44	8	2100	30,1	1,360800	0,0973	0,0603	0,0627	1,825199	1,6128	0,9835
45	9	2400	30	1,345800	0,0972	0,0604	0,0627	1,751299	1,620900	0,9955
46	10	2700,1	30,3	1,330600	0,0971	0,0604	0,0627	1,584499	1,626099	1,0038
47	11	3000,1	30,6	1,315299	0,0972	0,0604	0,0628	1,027400	1,6322	1,0142
48	12	3300,1	30,6	1,299700	0,0972	0,0604	0,0627	1,314399	1,636600	1,0255
49	13	3600,2	30,5	1,284199	0,097	0,0604	0,0628	1,263499	1,639000	1,0335
50	14	3900,2	30,6	1,269099	0,0971	0,0604	0,0627	1,265499	1,642099	1,0440
51	15	4200,2	30,6	1,2543	0,0972	0,0603	0,0628	1,263499	1,644799	1,0542
52	16	4500,2	30,7	1,238800	0,0972	0,0603	0,0628	1,259899	1,647099	1,0634
53	17	4800,3	30,4	1,224599	0,0972	0,0604	0,0628	1,258000	1,648800	1,0672
54	18	5100,3	30,5	1,210000	0,0972	0,0604	0,0628	1,255399	1,651000	1,0716
55	19	5400,3	30,5	1,195299	0,0971	0,0604	0,0627	1,251999	1,652899	1,0743
56	20	5700,4	30,5	1,181399	0,0972	0,0604	0,0628	1,248299	1,656499	1,0776
57	21	6000,4	30,5	1,167000	0,097	0,0604	0,0627	1,246299	1,656399	1,0747
58	22	6300,4	30,4	1,153599	0,0971	0,0604	0,0627	1,242599	1,654899	1,0722
59	23	6600,5	30,4	1,139799	0,097	0,0604	0,0627	1,239300	1,656900	1,0735
60	24	6900,5	30,5	1,125900	0,097	0,0604	0,0628	1,235900	1,653399	1,0693
61	25	7200,5	30,4	1,112699	0,0971	0,0604	0,0627	1,233800	1,654700	1,0704
62	26	7500,6	30,5	1,099300	0,0971	0,0604	0,0627	1,229099	1,653699	1,0692
63	27	7800,6	30,4	1,085600	0,0971	0,0604	0,0628	1,226400	1,653499	1,0691
64	28	8100,6	30,5	1,072499	0,097	0,0604	0,0627	1,223500	1,653	1,0686
65	29	8400,7	30,5	1,060199	0,0971	0,0604	0,0627	1,220000	1,653100	1,0666
66	30	8700,7	30,6	1,047700	0,0972	0,0604	0,0627	1,217499	1,653499	1,0673
67	31	9000,7	30,7	1,034999	0,0972	0,0604	0,0628	1,213700	1,653599	1,0669
68	32	9300,7	30,6	1,022699	0,0971	0,0604	0,0628	1,210299	1,653699	1,0662
69	33	9600,8	30,6	1,010200	0,0971	0,0604	0,0627	1,207999	1,651900	1,0649
70	34	9900,8	30,6	0,9982	0,0971	0,0604	0,0628	1,205100	1,652699	1,0642
71	35	10200,8	30,6	0,985700	0,0971	0,0604	0,0627	1,200999	1,653	1,0622
72	36	10500,9	30,7	0,973900	0,0971	0,0604	0,0627	1,197700	1,652099	1,0615
73	37	10800,9	30,7	0,962000	0,0971	0,0604	0,0628	1,194200	1,652099	1,0619

74	38	11100,9	30,7	0,950299	0,097	0,0604	0,0627	1,194000	1,652199	1,06099
75	39	11401	30,7	0,938899	0,097	0,0604	0,0627	1,189700	1,651999	1,05939
76	40	11701	30,7	0,927500	0,0971	0,0604	0,0628	1,187100	1,651700	1,05999
77	41	12001	30,5	0,916000	0,0971	0,0604	0,0627	1,184700	1,651700	1,05949
78	42	12301,1	30,6	0,9048	0,0972	0,0604	0,0627	1,181599	1,651499	1,0596
79	43	12601,1	30,2	0,893499	0,0971	0,0604	0,0627	1,178599	1,652400	1,0589
80	44	12901,1	30,2	0,882399	0,0969	0,0604	0,0627	1,176900	1,6523	1,05980
81	45	13201,2	30,2	0,871699	0,0972	0,0604	0,0628	1,173099	1,651499	1,05799
82	46	13501,2	30,6	0,860899	0,0971	0,0604	0,0627	1,171900	1,651399	1,05700
83	47	13801,2	30,5	0,850300	0,0971	0,0604	0,0628	1,169299	1,652500	1,05910
84	48	14101,3	30,4	0,840099	0,0971	0,0604	0,0628	1,167799	1,651299	1,05729
85	49	14401,3	30,3	0,829699	0,0971	0,0604	0,0627	1,163900	1,651800	1,05669

Sheet1

labfolder_table_6519939_2.xlsx

image.png

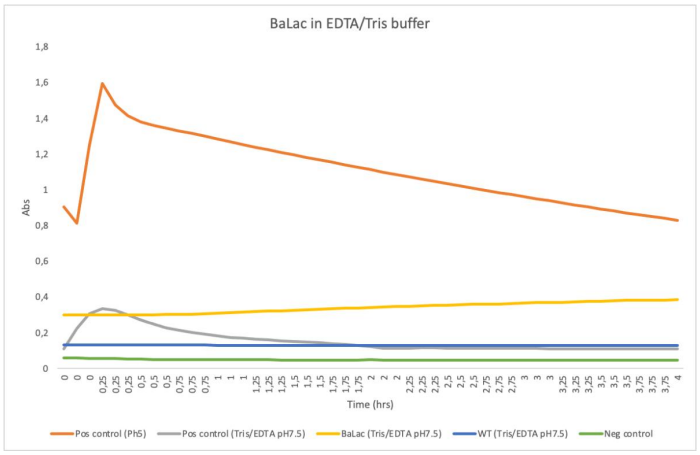
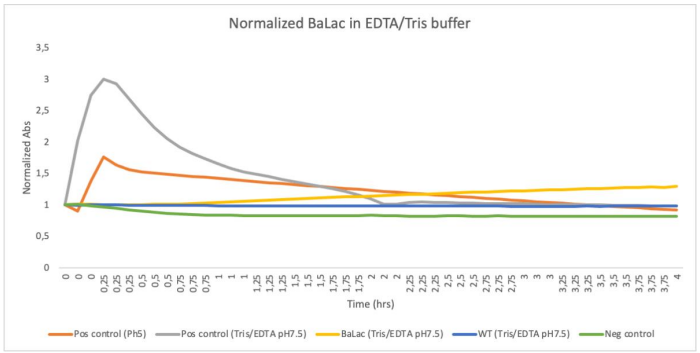


image.png



Author: Allyssa Hinkle
Entry 19/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 17:23
updated: 26.10.2020 13:55

05.10.2020 – 06.10.2020: Freeze and thaw of constructs pAR-BaLac-3xHA-cyt and ABTS-activity assay

05.10.:

- Havested 50 mL of culture BaLac 5 (positive transformant) in exponential phase (cell density < $1x10^7$ cells/mL) if not stated otherwise
- Proceeded after protocol "Freeze and thaw (cell lysis)", used citrate-phosphate buffer (pH 5 and pH 6.5 for lysis)
- Used supernatant for ABTS assay:

Data and graphs from activity assay

- Performed SDS page according to protocol
- Performed Western Blot according to protocol.

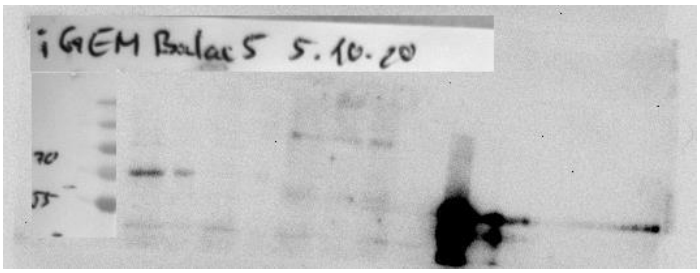
06.10.:

- Ponceau-S staining:

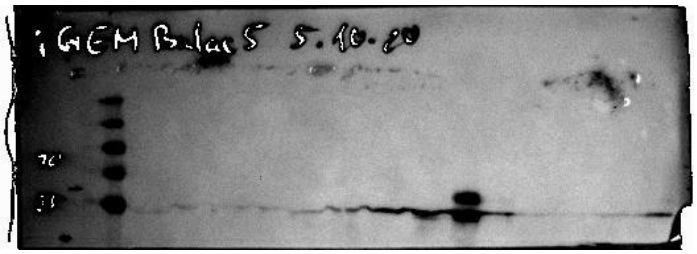
20201006_094800.jpg

- Performed ECL detection:

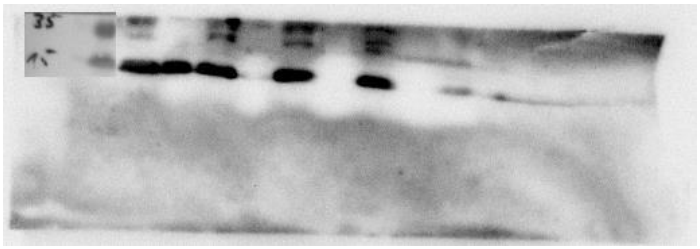
anti HA, 2x2 binning, 4 min



CF1b, 2x2 binning, 4 min



S11 antibody 2x2 binning 11 s



- Lysis didn't work properly with pH (see S11)

Author: Allyssa Hinkle
Entry 20/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 18:15
updated: 26.10.2020 14:20

09.10.2020 – 13.10.2020: Test if copper concentration has an effect on expression level of laccase/laccase activity. Screen for laccase activity

09.10.:

- Measured cell density

Colony	Cu concentration in medium [µM]	cell density [10^6 cells/mL]; measurement 1	cell density [10^6 cells/mL]; measurement 2	cell density [10^6 cells/mL]; mean	harvest [mL]	pH of added lysis buffer
B5	20	4.872	5.410	5.141	176	5
B5	20	4.275	5.000	4.638	195	6.4
B5	2	3.256	3.069	3.163	285	5
B5	2	2.829	2.330	2.580	350	6.4
UVM 4	2	7.141	7.405	7.273	124	1: 5; 2 & 3: 6.4
sALB	2	5.699	5.880	5.790	156	1: 5; 2 & 3: 6.4
UVM 4	20	5.316	4.985	5.151	175	1: 5; 2 & 3: 6.4
sALB	20	5.274	4.811	5.058	179	1: 5; 2 & 3: 6.4

- Calculated harvest volume: 2 x 580x10^6 x 350 = 9x10^8 cells
- Filled up to 350 mL
- Harvested 100 mL per replicate

12.10.:

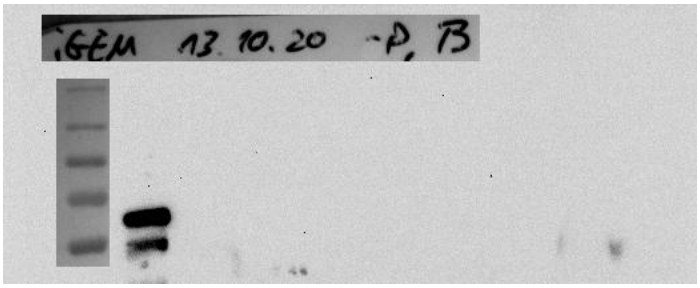
- Proceeded after modified freeze and thaw protocol
- Performed SDS page according to protocol, Loading scheme:
  - A, +P (insoluble fraction): Marker, pos., samples (order of table; 20 µL)
  - B, +P (membrane fraction): Marker, pos., samples (order of table; 20 µL)
  - A, -P (whole lysate): Marker, pos., samples (order of table; 20 µL)
  - B, -P (soluble fraction): Marker, pos., samples (order of table; 20 µL)
- Performed Western Blot according to protocol.

13.10.:

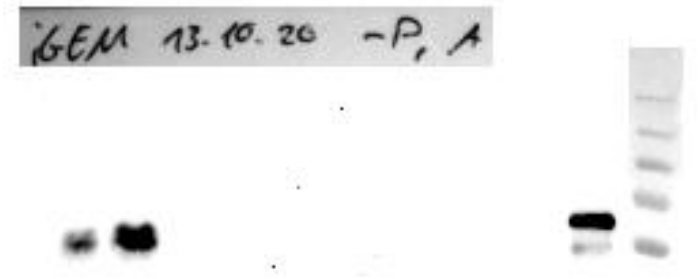
- Ponceau-S staining:
- -> protein on membrane

- ECL detection:

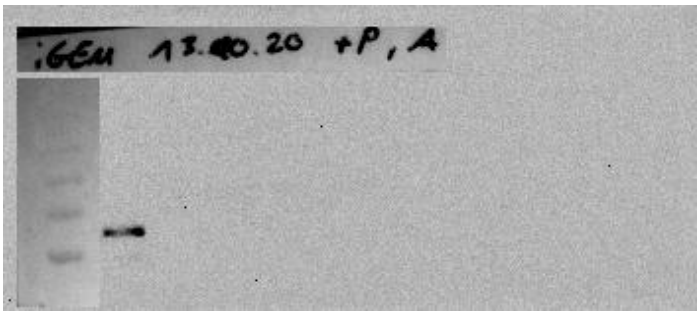
2020-10-14_B5_HA_freeze_and_thaw_2x2_binning_5_min_-P_B_lysate,1-1._Scan+Marker_image.tif



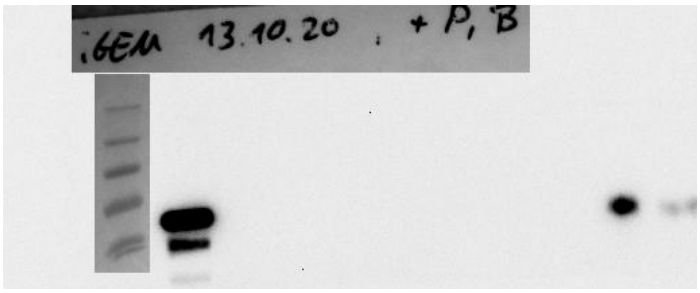
2020-10-14_B5_intra_freeze_and_thaw_-P_A_5_min_4x4_binning_supernatant,1-1._Scan+Marker_image.tif



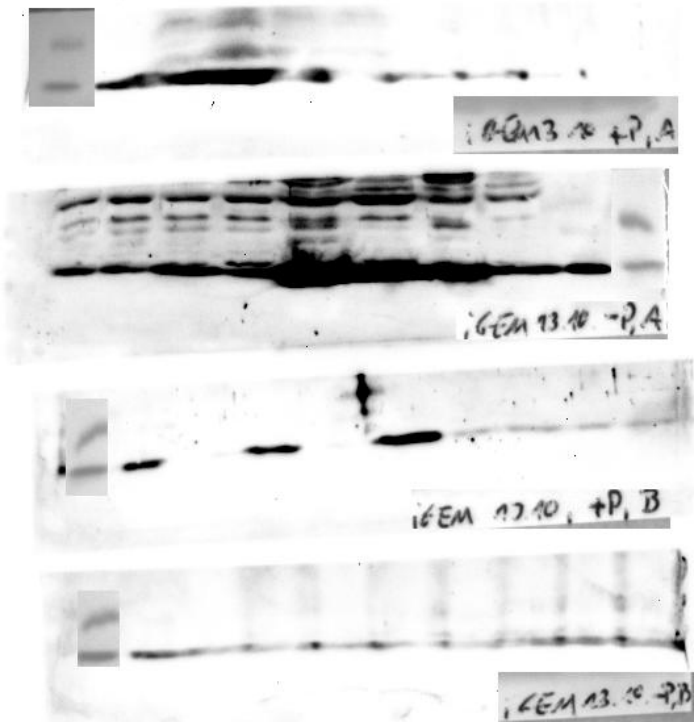
2020-10-14_B5_intra_HA_freeze_and_thaw_2min_37sek_3x3_binning,1-1._Scan+Marker_image.tif



2020-10-14_B5_intra_HA_freeze_and_thaw_2x2_binning_5_min_+P_B_triton_supernatant,1-1._Scan+Marker_image.tif



2020-10-14_B5_intra_S11_freeze_and_thaw_2x2_binning_5_min_all,1-1._Scan+Marker_image.tif



- Signal from protein of interest could not be detected

- Performed Bradford assay according to protocol ("Bradford assay")

- Performed ABTS activity assay with soluble fraction:

Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1	Application: Tecan i-control				Tecan i-control , 1.1	1.1.0				
2	Device: infinite 200Pro				Serial number: 1510001295				Serial number of	
3	Firmware: V_4.25_05/15_Infinite (May 1				MAI, V_4.25_05/15_Infinite (May 11 201	5/13.53.33)				
4										
5	Date:	13.10.2020								
6	Time:	16:40:42								
7										
8										
9	System				BRONCO					
10	User				Bronco\Tecan					
11	Plate				Greiner 96 Flat Bottom Transparent Polystyrene Cat. No.:					
12	Plate-ID (Stacker)									
13										
14	Wait (Plat				On	Target Temperature				

15										
16	Wait (Plate Tempera				Valid Range: 20.9 -					
17										
18	List of actions in this measurer									
19	Kinetic									
20	Absorban									
21										
22										
23	Label: bis-pNPP									
24	Kinetic Measurement									
25	Kinetic duration				4:00:00					
26	Interval Time				0:05:00					
27	Mode				Absorbance					
28	Wavelength				415	nm				
29	Bandwidth				10	nm				
30	Number of Flashes				10					
31	Settle Time				0	ms				
32	Part of Plate				A5-H8					
33	Start Time	13.10.2020 16:40:42								
34										
35										
36	Cycle Nr.	Time [s]	Temp. [°	A5	A6	A7	A8	B5	B6	B7
37	1	0	26,6	0,1656	0,4756	0,389	0,098	0,1058	0,3549	0,3684
38	2	299,8	29,9	0,2529	0,4786	0,3928	0,0984	0,1076	0,3705	0,3776
39	3	599,9	30,4	0,3538	0,480500	0,395200	0,0982	0,1107	0,384299	0,3888
40	4	899,9	30,2	0,464199	0,481999	0,398600	0,0983	0,1147	0,399100	0,4016
41	5	1199,9	30	0,580900	0,4853	0,402700	0,0984	0,1192	0,4163	0,4172
42	6	1499,9	30,3	0,701300	0,4896	0,408100	0,0981	0,1248	0,437999	0,4366
43	7	1800	30,6	0,822399	0,495299	0,413399	0,0979	0,13	0,4619	0,4526
44	8	2100	30,2	0,9443	0,501699	0,419999	0,0979	0,1357	0,4901	0,4726
45	9	2400	30,4	1,068600	0,508199	0,4269	0,0979	0,141299	0,514500	0,4923
46	10	2700,1	30,4	1,192000	0,513100	0,4332	0,0979	0,1471	0,5341	0,5120
47	11	3000,1	30,4	1,315099	0,518599	0,439799	0,0978	0,152700	0,563700	0,5303
48	12	3300,1	30	1,437399	0,525200	0,4454	0,0979	0,1582	0,586300	0,5508
49	13	3600,2	30,5	1,5596	0,531499	0,4506	0,0981	0,1639	0,610599	0,5791
50	14	3900,2	30,4	1,673699	0,536099	0,454299	0,0978	0,169400	0,619899	0,6112
51	15	4200,2	30	1,780799	0,540499	0,4614	0,0978	0,174799	0,637899	0,6298
52	16	4500,3	30,7	1,881399	0,545199	0,4677	0,0978	0,1804	0,656499	0,6316
53	17	4800,3	30,6	1,973099	0,550599	0,475100	0,0979	0,186	0,662999	0,6209
54	18	5100,3	30,4	2,057300	0,5557	0,4813	0,0978	0,1919	0,664200	0,6065
55	19	5400,4	30,3	2,132800	0,561900	0,4885	0,0981	0,1981	0,670899	0,6104
56	20	5700,4	30,1	2,201299	0,566200	0,493499	0,0979	0,203799	0,695800	0,6243
57	21	6000,4	30	2,259700	0,571399	0,4991	0,0979	0,2095	0,713599	0,6374
58	22	6300,5	30,7	2,310600	0,578000	0,505500	0,0979	0,2154	0,723599	0,6539
59	23	6600,5	30,7	2,352799	0,583	0,511200	0,0979	0,221300	0,735499	0,6664
60	24	6900,5	30,7	2,387700	0,587499	0,515699	0,0979	0,2272	0,746299	0,6794
61	25	7200,5	30,5	2,4152	0,592100	0,520699	0,0979	0,2332	0,759	0,6969
62	26	7500,6	30,4	2,438899	0,597800	0,526300	0,0979	0,2392	0,773899	0,7070

63	27	7800,6	30,3	2,459700	0,6031	0,531899	0,0979	0,245100	0,784600	0,722
64	28	8100,6	30,1	2,468600	0,607800	0,537500	0,0979	0,251399	0,794099	0,7326
65	29	8400,7	30,1	2,479799	0,612699	0,5424	0,0979	0,257	0,803300	0,7411
66	30	8700,7	30	2,487400	0,617299	0,547200	0,0979	0,262899	0,814700	0,7494
67	31	9000,7	30	2,4921	0,622399	0,552200	0,0979	0,268799	0,823899	0,7555
68	32	9300,8	30,1	2,489200	0,626600	0,557299	0,0979	0,274899	0,832300	0,7662
69	33	9600,8	30,4	2,488199	0,630800	0,5625	0,098	0,2809	0,840799	0,7735
70	34	9900,8	30,4	2,486399	0,635500	0,567200	0,098	0,287	0,849399	0,7827
71	35	10200,9	30,6	2,484100	0,6401	0,571200	0,0979	0,292800	0,857800	0,7907
72	36	10500,9	30,7	2,479899	0,6444	0,576200	0,0979	0,2985	0,865700	0,7996
73	37	10800,9	30,7	2,476799	0,648599	0,581499	0,0979	0,3044	0,874199	0,8076
74	38	11101	30,6	2,469500	0,652800	0,585900	0,0979	0,310200	0,880599	0,8127
75	39	11401	30,5	2,463999	0,657000	0,591400	0,0979	0,3161	0,888599	0,8202
76	40	11701	30,4	2,457900	0,661199	0,595099	0,0979	0,321900	0,894599	0,8266
77	41	12001,1	30,4	2,452899	0,665400	0,599099	0,0979	0,327499	0,901300	0,8317
78	42	12301,1	30,4	2,445100	0,670099	0,605099	0,0979	0,3335	0,9084	0,8378
79	43	12601,1	30,4	2,439300	0,674700	0,609799	0,0979	0,3391	0,9163	0,8449
80	44	12901,1	30,4	2,432199	0,6789	0,611899	0,0979	0,345	0,922900	0,8493
81	45	13201,2	30,4	2,424900	0,682699	0,616900	0,0979	0,3502	0,928200	0,8546
82	46	13501,2	30,4	2,416699	0,686999	0,6196	0,098	0,356099	0,933700	0,8601
83	47	13801,2	30,5	2,410700	0,691200	0,624100	0,0979	0,3619	0,941999	0,8642
84	48	14101,3	30,4	2,403100	0,694199	0,627300	0,098	0,367700	0,949400	0,8690
85	49	14401,3	30,3	2,397099	0,697799	0,630500	0,0979	0,373299	0,958199	0,8745
86										
87										

Sheet1

labfolder_table_6519964_10.xlsx

Author: Allyssa Hinkle
Entry 21/24: No entry title yet
In Project: Journal screening
No tags associated

created: 19.10.2020 18:43
updated: 26.10.2020 14:21

09.10.2020 – 16.10.2020: Test if copper concentration has an effect on expression level of laccase/laccase activity. Screen for laccase activity. Higher cell number.

09.10.:

- Measured cell densities and diluted cultures

Colony	Cu concentration in medium [µM]	cell density [10^6 cells/mL]; measurement 1	cell density [10^6 cells/mL]; m
B5	20	4.872	5.410
B5	20	4.275	5.000
B5	2	3.256	3.069
B5	2	2.829	2.330
UVM 4	2	7.141	7.405
sALB	2	5.699	5.880
UVM 4	20	5.316	4.985
sALB	20	5.274	4.811

14.10.:

- Measured cell densities and harvested cultures

Name	Colony	Cu concentration in medium [µM]	cell density [10^6 cells/mL]; measurement 1	cell density [10^6 cells/mL]; measurement 1	cell density [10^6 cells/mL]; mean
I	B5	20	2.837	3.325	3.081
III	B5	2	3.265	~~4.413~~	~~3.839;~~ 3.265
V	UVM 4	2	3.186	3.338	3.262
VI	sALB	2	3.191	2.619	2.905
VII	UVM 4	20	3.202	3.125	3.164
VIII	sALB	20	2.999	2.852	2.926

- All cultures were harvested at a density around $3x10^6$ cells/mL
- Harvested 150 mL per replicate
- Proceeded after modified freeze and thaw protocol

15.10.:

- Performed Bradford assay with soluble fraction:

Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1										
2										
3	User: Vinnie									
4	Path: E:\FLUOstar Omega\Vinnie\Data\									
5	Test ID: 187									
6	Test Name: bradford vincent									
7	Date: 15.10.2020									
8	Time: 19:58:43									
9	Absorbance			Absorbance values are displayed as OD						
10										
11										
12										
13		1. Raw Data (595)								
14		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
15	**A**	0,808	0,841	0,832	0,928	0,941	0,982	0,855	0,901	0,93
16	**B**	1,24	1,064	1,131	1,027	0,912	0,975	0,904	0,827	0,83
17	**C**	1,715	1,584	1,682	1,123	1,104	1,252	1,017	0,966	1,15
18	**D**	2,021	2,072	2,232	1,071	1,128	1,055	1,232	1,079	1,(
19	**E**	2,267	2,292	2,249	0,867	0,903	0,857	0,929	0,947	0,88
20	**F**	2,389	2,492	2,451	0,879	0,845	0,83	1,002	0,974	0,87
21	**G**	1,335	1,402	1,428	0,893	0,878	0,874	1,189	1,254	1,22
22	**H**	0,782	0,809	0,785	0,888	0,895	0,89	1,032	1,035	0,93
23										
24		2. Blank corrected raw data (595)								
25		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
26	**A**	0,016	0,049	0,04	0,136	0,149	0,19	0,063	0,109	0,14
27	**B**	0,448	0,272	0,339	0,235	0,12	0,183	0,112	0,035	0,04
28	**C**	0,923	0,792	0,89	0,331	0,312	0,46	0,225	0,174	0,30
29	**D**	1,229	1,28	1,44	0,279	0,336	0,263	0,44	0,287	0,27
30	**E**	1,475	1,5	1,457	0,075	0,111	0,065	0,137	0,155	0,09
31	**F**	1,597	1,7	1,659	0,087	0,053	0,038	0,21	0,181	0,08
32	**G**	0,543	0,61	0,636	0,101	0,086	0,082	0,397	0,462	0,43
33	**H**				0,096	0,103	0,098	0,24	0,243	0,14
34										
35		3. Average based on Blank corrected (595)								
36		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
37	**A**	0,035	0,039	0,041	0,161	0,111	0,133	0,084	0,13	0,14
38	**B**	0,415	0,245	0,368	0,153	0,139	0,179	0,134	0,146	0,1

39	**C**	0,982	0,84	0,95	0,263	0,199	0,296	0,234	0,242	0,2
40	**D**	1,323	1,378	1,352	0,151	0,225	0,288	0,322	0,193	0,2
41	**E**	1,571	1,582	1,545	0,034	0,054	0,076	0,133	0,14	0,1
42	**F**	1,663	1,782	1,759	0,068	0,048	0,012	0,209	0,193	0,1
43	**G**	0,502	0,569	0,627	0,13	0,059	0,058	0,37	0,295	0,2
44	**H**				0,087	0,039	0,036	0,174	0,102	0,2
45										
46										
47		1	3	4	6	8	10	12		
48		0,038333	0,342666	0,566	0,924	1,351	1,566	1,7705		
49										
50										
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Sheet1

labfolder_table_6519988_3.xlsx

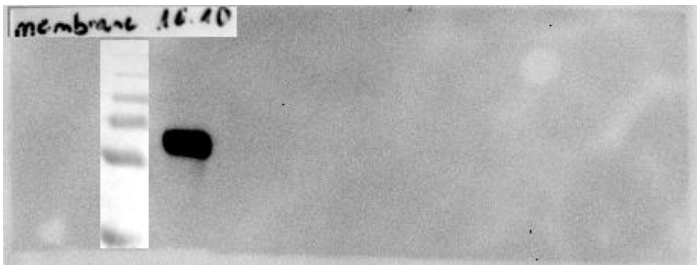
16.10.:

- Performed SDS page according to protocol, Loading scheme:
  - Gel 4 (insoluble fraction): Marker, pos., samples (order of table)
  - Gel 3 (membrane fraction): Marker, pos., samples (order of table)
  - Gel 1 (soluble fraction): Marker, pos., samples (order of table)
- Loaded 15 µg whole protein calculated from mean (in soluble fraction and the same volume for the other fractions as well)
- (I1, I2, I3, I4 and II1, II2, II3, II4... are replicates from culture I or culture II respectively. Definition see table above.)
- Performed Western Blot according to protocol.
- Ponceau-S staining:

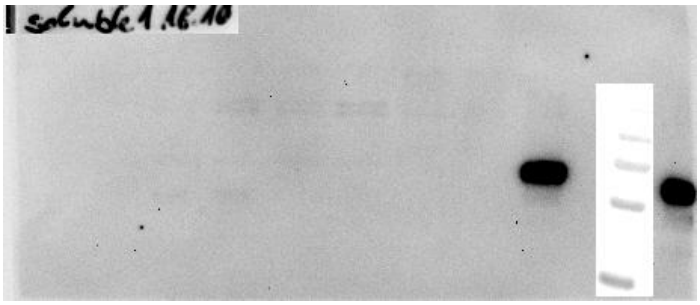
- Performed ABTS activity assay (plate layout see table above; volumes pipetted see table above):

- Performed ECL detection:

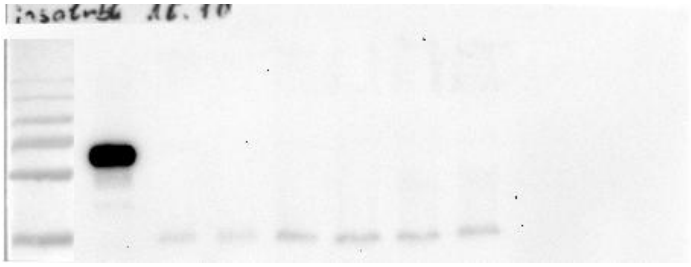
B5_intra_HA_freeze_and_thaw_membrane_3x3_binning_5_min,1-1._Scan+Marker_image.tif



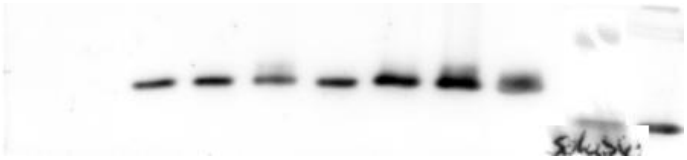
HA_freeze_and_thaw_3x3_binning_5_min_soluble,1-1._Scan+Marker_image.tif



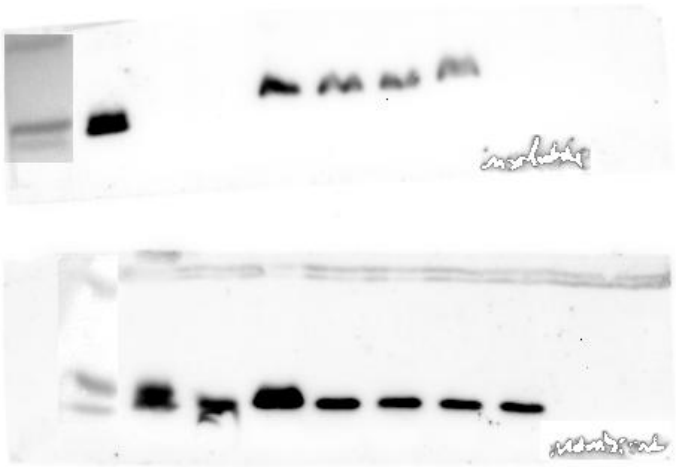
B5_intra_freeze_and_thaw_3x3_binning_5_min_insoluble,1-1._Scan+Marker_image.tif



B5_intra_S11_freeze_and_thaw_soluble_3x3_binning_50_sek,1-1._Scan+Marker_image.tif



B5_intra_freeze_and_thaw_membrane_and_insoluble_3x3_binning_3,5_min,1-1._Scan+Marker_image.tif



Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1	User: XXXX									
2	Path: E:\FLUOstar Omega\Vinnie\Data\									
3	Test ID: 189									
4	Test Name: iGEM2020									
5	Date: 15.10.2020									
6	Time: 23:01:42									
7	ID1: 18.06.2020_Lysat-intra-BaLac,MarLac-secreted									
8	Absorbance			Absorbance values are displayed as OD						
9										
10										
11	Used range(s): Range 1;									
12	**WellRow**	**WellCol**	**Content**	**ıta (415)1**	**ıta (415)2**	**ta (415)3**	**·ta (415)4**	**·ta (415)5**	**·ta (415)6**	**·ta (415)**
13				0	5	10	15	20	25	
14	A	1	ample X1	0,0435	0,0422	0,0421	0,0418	0,0423	0,0423	0,04
15	A	2	ample X2	0,0429	0,0426	0,0423	0,0426	0,0428	0,0425	0,042
16	A	3	ample X3	0,1381	0,1948	0,259	0,3295	0,3995	0,4694	0,539
17	A	4	ample X4	2,017	2,1158	2,1791	2,2082	2,2485	2,2627	2,339
18	A	5	ample X5	2,1725	2,1761	2,2077	2,2066	2,2144	2,2643	2,252
19	A	6	ample X6	0,1041	0,1014	0,0989	0,0968	0,0956	0,0937	0,093
20	A	7	ample X7	2,2358	2,1169	1,9599	1,7688	1,5991	1,3862	1,217
21	A	8	ample X8	0,1885	0,1884	0,1869	0,1878	0,1897	0,1915	0,192
22	A	9	ample X9	0,2453	0,2501	0,2544	0,2591	0,2617	0,2634	0,267
23	A	10	mple X10	0,0434	0,0432	0,0426	0,0432	0,0422	0,0425	0,043
24	A	11	mple X11	0,0612	0,0596	0,0596	0,0602	0,0591	0,0597	0,060
25	A	12	mple X12	0,063	0,0606	0,0593	0,0598	0,0604	0,0599	0,059
26	B	1	mple X13	0,053	0,0511	0,0506	0,0504	0,0501	0,0508	0,050
27	B	2	mple X14	0,0414	0,0398	0,0391	0,0395	0,0397	0,0397	0,039
28	B	3	mple X15	1,0246	1,7127	2,3966	2,6795	2,8758	2,983	3,040
29	B	4	mple X16	1,7883	1,822	1,8815	1,9522	2,0314	2,1195	2,115
30	B	5	mple X17	1,9051	1,9657	2,077	2,1242	2,1676	2,2139	2,230
31	B	6	mple X18	0,1905	0,1792	0,1766	0,1678	0,1563	0,1474	0,130

32	B	7	mple X19	0,2121	0,1996	0,1865	0,1786	0,167	0,1526	0,13
33	B	8	mple X20	0,213	0,2256	0,2418	0,2568	0,2718	0,2864	0,30
34	B	9	mple X21	0,1932	0,2101	0,2235	0,2372	0,251	0,2659	0,27
35	B	10	mple X22	0,0464	0,045	0,0447	0,0452	0,045	0,0455	0,04
36	B	11	mple X23	0,0441	0,042	0,0415	0,0426	0,0421	0,0428	0,04
37	B	12	mple X24	0,0723	0,071	0,0707	0,0713	0,0706	0,071	0,07
38	C	1	mple X25	0,0462	0,044	0,0432	0,0437	0,0431	0,0438	0,04
39	C	2	mple X26	0,043	0,041	0,0407	0,0409	0,0413	0,0409	0,0
40	C	3	mple X27	0,0474	0,0486	0,0511	0,0541	0,057	0,0574	0,05
41	C	4	mple X28	3,5	3,5	3,5	3,3259	3,5	3,5	3
42	C	5	mple X29	3,1495	3,2505	3,2395	3,1326	3,0062	3,1127	3,06
43	C	6	mple X30	0,0444	0,0428	0,042	0,0419	0,0419	0,0429	0,04
44	C	7	mple X31	0,0447	0,0434	0,0425	0,0432	0,0432	0,0436	0,04
45	C	8	mple X32	0,2557	0,2628	0,2664	0,2719	0,2772	0,2789	0,28
46	C	9	mple X33	0,4039	0,409	0,4134	0,4187	0,4236	0,4281	0,4
47	C	10	mple X34	0,0439	0,0417	0,041	0,0421	0,0419	0,0414	0,04
48	C	11	mple X35	0,0581	0,0558	0,0554	0,0555	0,0558	0,0556	0,05
49	C	12	mple X36	0,0512	0,0495	0,0485	0,0489	0,0486	0,0485	0,04
50	D	1	mple X37	0,0624	0,0605	0,0596	0,0603	0,0598	0,0606	0,05
51	D	2	mple X38	0,0492	0,0463	0,0454	0,046	0,0459	0,0465	0,04
52	D	3	mple X39	0,0988	0,1464	0,1999	0,2612	0,3238	0,3853	0,45
53	D	4	mple X40	1,3506	1,4232	1,4921	1,429	1,4871	1,4809	1,50
54	D	5	mple X41	1,6698	1,6898	1,6546	1,6172	1,6455	1,7053	1,75
55	D	6	mple X42	0,0489	0,0468	0,0464	0,047	0,0468	0,0472	0,04
56	D	7	mple X43	2,1679	1,7801	1,4232	1,1479	0,9538	0,7468	0,63
57	D	8	mple X44	0,2267	0,2348	0,2425	0,2536	0,2656	0,2781	0,28
58	D	9	mple X45	0,2284	0,2314	0,2431	0,2524	0,2598	0,2679	0,28
59	D	10	mple X46	0,0427	0,0406	0,0404	0,0403	0,0404	0,041	0,04
60	D	11	mple X47	0,0424	0,0406	0,0398	0,04	0,0399	0,0403	0,04
61	D	12	mple X48	0,0592	0,0579	0,0575	0,0574	0,0573	0,0564	0,05
62	E	1	mple X49	0,0736	0,0713	0,0691	0,0707	0,072	0,0717	0,07
63	E	2	mple X50	0,0597	0,057	0,0558	0,0561	0,0553	0,0556	0,05
64	E	3	mple X51	0,0385	0,0559	0,0696	0,0506	0,0355	0,0353	0,03
65	E	4	mple X52	2,0101	2,0585	2,0784	2,0769	2,1025	2,1382	2,16
66	E	5	mple X53	1,8071	1,8367	1,8417	1,8588	1,8723	1,9003	1,
67	E	6	mple X54	0,9534	0,9476	1,0137	0,9811	0,8876	0,9477	0,93
68	E	7	mple X55	2,5587	2,525	2,5622	2,4797	2,4494	2,3909	2,33
69	E	8	mple X56	0,1644	0,1643	0,1695	0,172	0,1728	0,1759	0,17
70	E	9	mple X57	0,2572	0,2554	0,2581	0,2614	0,2649	0,2676	0,27
71	E	10	mple X58	0,0491	0,0477	0,0462	0,0467	0,0457	0,0462	0,04
72	E	11	mple X59	0,0415	0,0389	0,0386	0,0385	0,0376	0,0387	0,0
73	E	12	mple X60	0,0431	0,0412	0,0398	0,0405	0,0408	0,041	0,04
74	F	1	mple X61	0,0463	0,0439	0,0438	0,0439	0,0442	0,0439	0,04
75	F	2	mple X62	0,048	0,0458	0,0455	0,0455	0,045	0,0459	0,04
76	F	3	mple X63	0,0376	0,035	0,035	0,0348	0,0347	0,0345	0,0
77	F	4	mple X64	1,4424	1,3776	1,3623	1,3899	1,3934	1,4308	1,45
78	F	5	mple X65	1,4383	1,3782	1,4042	1,4413	1,4554	1,4431	1,65
79	F	6	mple X66	0,045	0,0425	0,0416	0,0419	0,0414	0,0413	0,04

80	F	7	mple X67	0,0522	0,0502	0,0486	0,0483	0,0479	0,0478	0,048
81	F	8	mple X68	0,1757	0,174	0,1766	0,181	0,1861	0,1906	0,195
82	F	9	mple X69	0,1721	0,1724	0,1751	0,1777	0,1811	0,1864	0,189
83	F	10	mple X70	0,0373	0,0351	0,0345	0,0343	0,0343	0,0342	0,034
84	F	11	mple X71	0,0455	0,0431	0,0421	0,0426	0,0427	0,0431	0,042
85	F	12	mple X72	0,0457	0,0431	0,0423	0,0424	0,0426	0,0427	0,043
86	G	1	mple X73	0,053	0,0502	0,05	0,0498	0,0495	0,0496	0,050
87	G	2	mple X74	0,0498	0,0467	0,0461	0,0467	0,0469	0,0467	0,046
88	G	3	mple X75	0,0415	0,0389	0,038	0,0383	0,0374	0,0383	0,037
89	G	4	mple X76	2,9395	2,9853	3,0171	2,8202	2,9446	2,7395	2,874
90	G	5	mple X77	2,0901	2,0728	2,0773	2,0366	2,0433	2,0341	2,037
91	G	6	mple X78	0,0535	0,0507	0,0502	0,0497	0,0493	0,0493	0,048
92	G	7	mple X79	0,0446	0,0418	0,0408	0,0415	0,0412	0,0413	0,041
93	G	8	mple X80	0,2801	0,2803	0,2797	0,284	0,2845	0,2852	0,284
94	G	9	mple X81	0,3408	0,3451	0,3474	0,3515	0,3564	0,3604	0,362
95	G	10	mple X82	0,0548	0,0522	0,0516	0,0526	0,0517	0,0513	0,051
96	G	11	mple X83	0,0469	0,0446	0,0437	0,0439	0,0437	0,0438	0,043
97	G	12	mple X84	0,0471	0,0444	0,0445	0,0444	0,0438	0,0444	0,044
98	H	1	mple X85	0,0456	0,0437	0,0425	0,0427	0,0423	0,0431	0,042
99	H	2	mple X86	0,0641	0,0611	0,0614	0,0614	0,0607	0,0605	0,061
100	H	3	mple X87	0,0487	0,046	0,0453	0,046	0,0457	0,0456	0,045
101	H	4	mple X88	1,2256	1,2493	1,2694	1,2839	1,3072	1,323	1,339
102	H	5	mple X89	1,1483	1,1745	1,1922	1,212	1,2253	1,2416	1,259
103	H	6	mple X90	0,0472	0,0437	0,0432	0,043	0,0427	0,0433	0,042
104	H	7	mple X91	0,044	0,0414	0,0406	0,0412	0,0408	0,0414	0,041
105	H	8	mple X92	0,2171	0,2125	0,2146	0,2179	0,2216	0,2251	0,227
106	H	9	mple X93	0,2147	0,2128	0,2182	0,2245	0,2272	0,2306	0,232
107	H	10	mple X94	0,0601	0,0581	0,0567	0,057	0,0575	0,057	0,057
108	H	11	mple X95	0,0538	0,0515	0,0507	0,0512	0,0504	0,0509	0,050
109	H	12	mple X96	0,0588	0,0553	0,0545	0,0546	0,0535	0,0535	0,053

Sheet1

labfolder_table_6519988_14.xlsx

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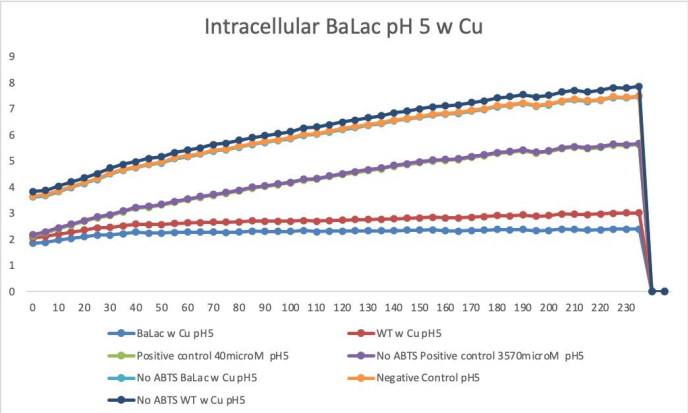

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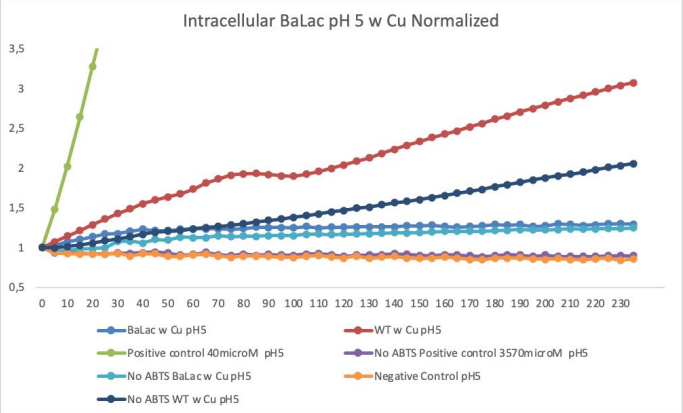

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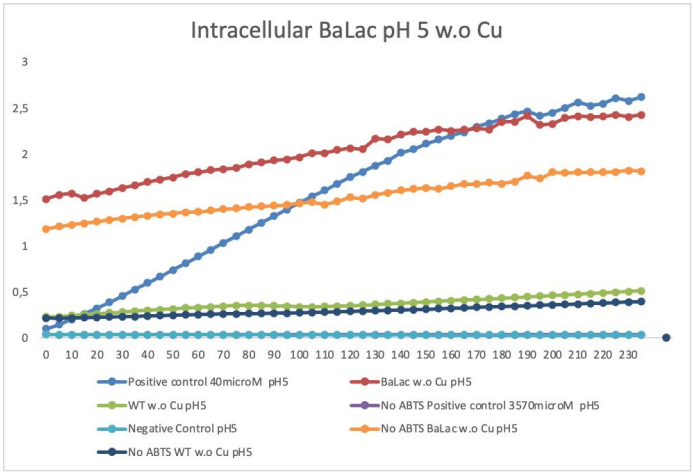

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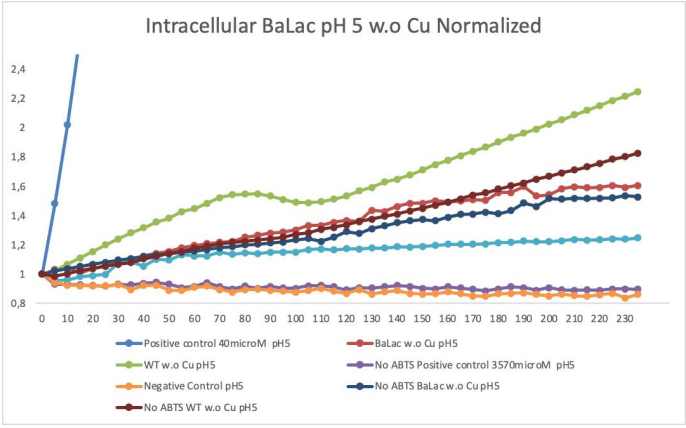


Author: Allyssa Hinkle
Entry 22/24: No entry title yet
In Project: Journal screening
No tags associated

created: 20.10.2020 10:01
updated: 20.10.2020 12:05

13.10.2020 – 16.10.2020: Screened colonies from constructs pAR-cCA-BaLac-SP20-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His again with increased copper concentration in medium.

Let colonies grow in TAP-NH4 medium with increased copper concentration (20 µM copper instead of 2 µM)

Screened colonies: 1 – 24

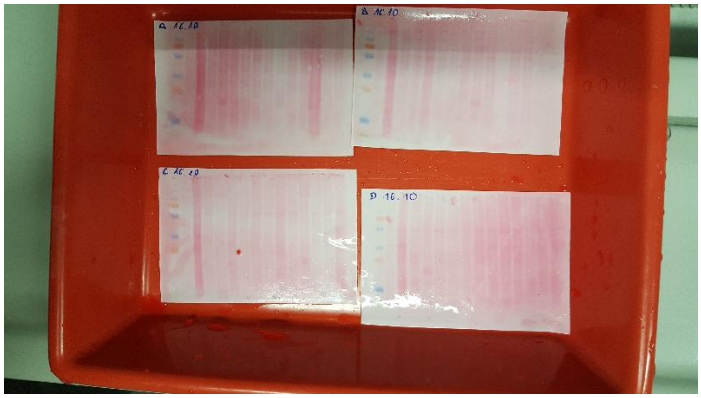
13.10.:

- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

16.10.:

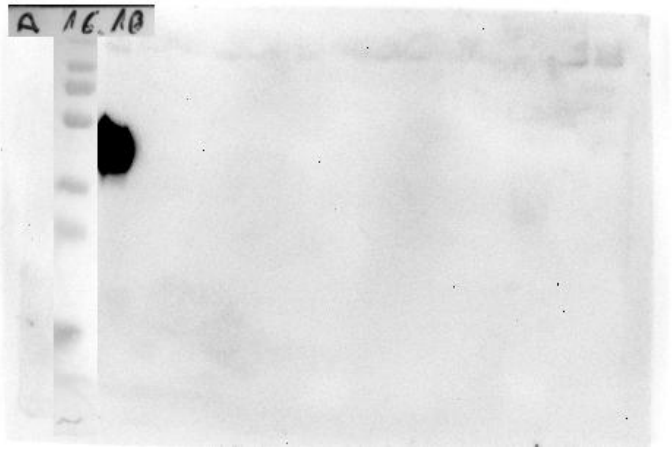
- Performed SDS page of fluid cultures from BaLac according to protocol
- Loading schemes:
  - A: Marker, pos., neg,, BaLac 1 – 12
  - B: Marker, pos., neg., BaLac 13 – 24
  - C: Marker, pos., neg., marLac 1 – 12
  - D: Marker, pos., neg., marLac 13 – 24
- Performed Western Blot according to protocol.
- Ponceau-S staining:

Ponceau



- Performed ECL detection:

cCA_balac_1-12_SP20_HA_RGS_8His_20uM_Cu_5_min_3x3,1-1._Scan+Marker_image.tif



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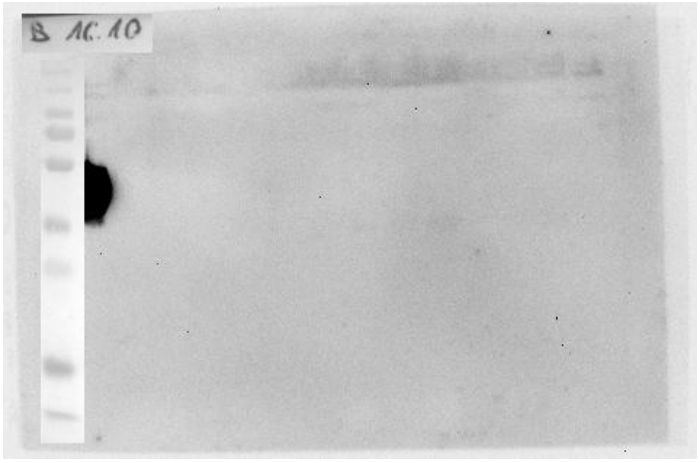

cCA_marlac_1-12_SP20_HA_RGS_8His_20uM_Cu,2-1._Scan+Marker-merged_raw.tif

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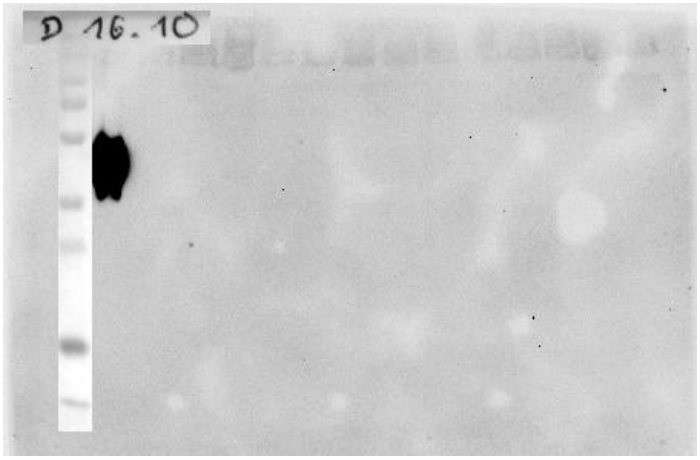


Author: Allyssa Hinkle
Entry 23/24: No entry title yet
In Project: Journal screening
No tags associated

created: 20.10.2020 10:34
updated: 21.10.2020 16:22

Author: Allyssa Hinkle
Entry 24/24: No entry title yet
In Project: Journal screening
No tags associated

created: 21.10.2020 16:22
updated: 26.10.2020 13:56

31.08.2020 – 08.09.2020: Screening construct pAR-cCA-marLac-SP20-HA-RGS-8His for positive transformants using SDS-Page and Western Blot (supernatant)

Colonies 1 – 48

31.08.:

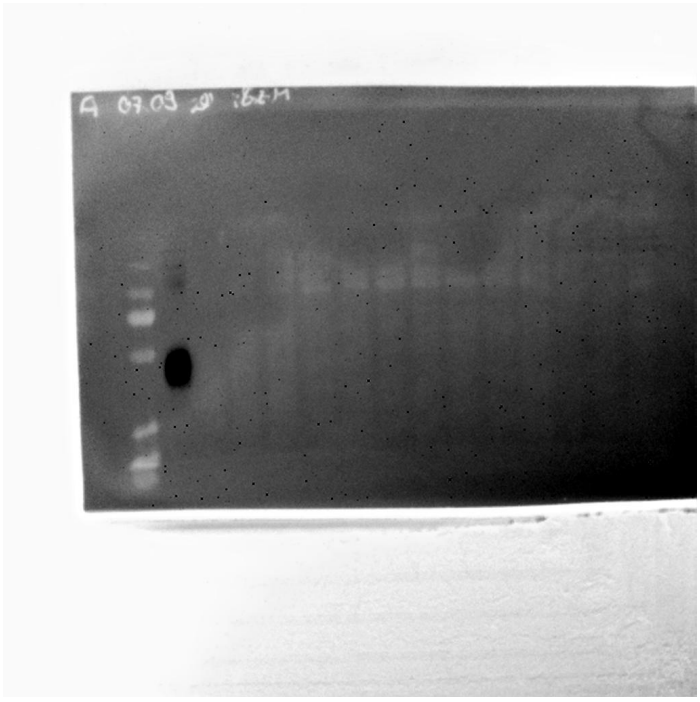
- Havested cultures in exponential phase (cell density < $1x10^7$ cells/mL)
- Proceeded after protocol "Screening of Secreting Chlamydomonas reinhardtii"
- Volume harvested: 6 mL instead of 2 mL

07.09.:

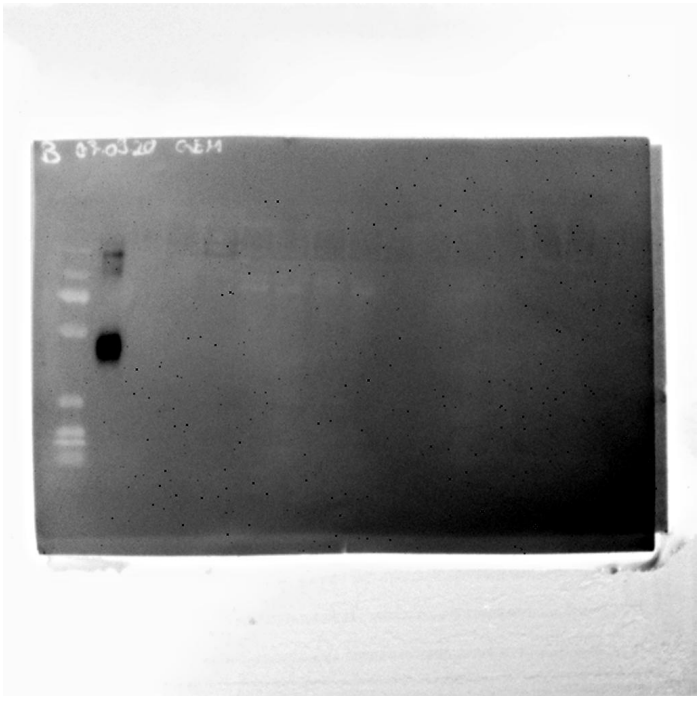
- Performed SDS page of fluid cultures from BaLac according to protocol
- Loading schemes:
  - A: Marker, pos., neg,, marLac 1 – 12
  - B: Marker, pos., neg., marLac 13 – 24
  - C: Marker, pos., neg., marLac 25 – 36
  - D: Marker, pos., neg., marLac 37 – 48
- Performed Western Blot according to protocol.
- Ponceau-S staining:
- protein on membrane

- Performed ECL detection

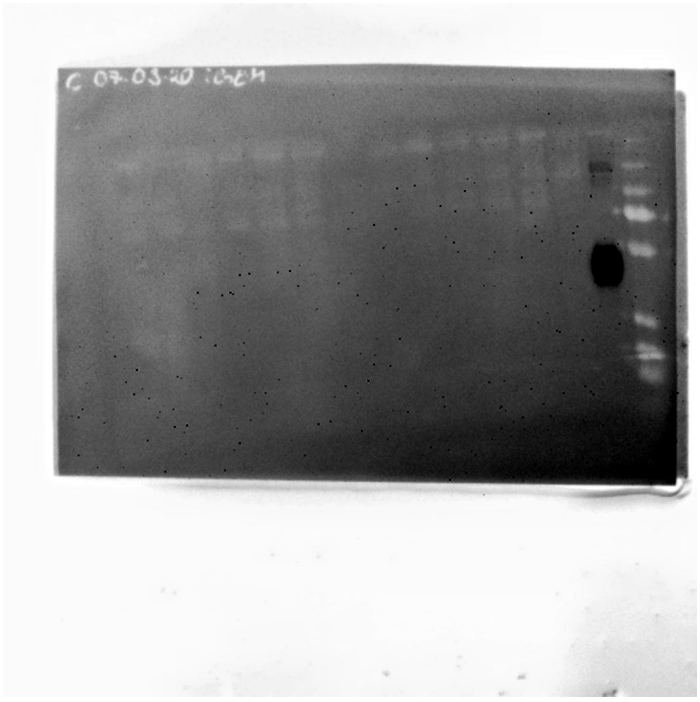
2020.09.07_Screening_A_10_min.Tif



2020.09.07_Screening_B_10_min.Tif



2020.09.07_Screening_C_10_min.Tif



2020.09.07_Screening_D_10_min.Tif