Autor: Robin Stei
Eintrag 1/6: cultivation of UDAR and UDAR 4787 and measuring in the Berthold Tristar2
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 27.08.2019 13:53
aktualisiert: 17.09.2019 10:49

2223.08.2019

- dilution series with UDAR 4787 (1:2, 1:4, 1:8, ..., 1:128)

- extracted 2 ml of the UDAR 4787 cultivation into a Falcon-Tube

    - diluted it with 18 ml of BG11

    - measured the OD, wich should be around 5

- submitted 6 ml BG11 in all 50 ml Falcon-Tubes

- starting by the solution with the OD of ~5, 6 ml of the solutions were transfered into the next Falcon-Tube with the next smaller dilution

        - repeated until every dilution was finished

- pipetted 200 µl of the dilutions into the 96-well-plates (flat wells) following the scheme

1:128	1:64	1:32	1:16	1:8	1:4	1:2	OD 5
1:64	1:64	1:32	1:16	1:8	1:4	1:2	OD 5
1:32	1:32	1:32	1:16	1:8	1:4	1:2	OD 5
1:16	1:16	1:16	1:16	1:8	1:4	1:2	OD 5
1:8	1:8	1:8	1:8	1:8	1:4	1:2	OD 5
1:4	1:4	1:4	1:4	1:4	1:4	1:2	OD 5
1:2	1:2	1:2	1:2	1:2	1:2	1:2	OD 5
OD 5	OD 5	OD 5	OD 5	OD 5	OD 5	OD 5	OD 5

- sealing 3 of the plates with the greiner bio-one foil and one of them with the divbio foil

- setting them up in the incubator at 42 °C, 5 % CO_2 and different light intensities

    - Plate 1 at a outer spot

- Plate 2 at a middle spot with ~375 µE

- Plate 3 at a middle spot with ~375 µE and the divbio foil

- Plate 4 at a inner spot with ~850 µE

Results:

- after 30 minutes the greiner foil already bloated and the single wells were'nt separated anymore

    - for the divbio foil it was just bloating over a few wells that were not filled

- after 72 hours2

    - Plate 1 still showed green cultures and in the higher concentrated wells the cells got lumpy

    - Plate 2 showed yellowish cultures for the 1:8 - 1 dilutions.

    - Plate 3 showed yellowish cultures in the well plates with the higher concentrations, while the low concentrated ones were dead.

    - Plate 4 showed mostly dead cells and a few of the high concentrated ones with yellow cultures.

26.08.2019

Testing higher dilutions in 96-well-plates

- extracted 1 ml of the UDAR 4787 cultivation into a Falcon-Tube

    - diluted it with 10 ml of BG11

    - measured the OD -> 5,06

- submitted 5 ml BG11 in four 50 ml Falcon-Tubes

- starting by the solution with the OD of 5,06, 5 ml of the solutions were transfered into the next Falcon-Tube with the next smaller dilution

        - repeated until the dilutions 1:2, 1:4, 1:8, 1:16 were finished

- pipetted 200 µl of the dilutions into the 96-well-plates (round bottom wells)

    - 1 in column 2, 1:2 in column 4, 1:4 in column 6, 1:8 in column 8, 1:16 in column 10+11

- sealing them with the standard cover of the 96-well-plates

- setting them up in the incubator at 42 °C, 5 % CO_2 and different light intensities

- Plate 1 at a outer spot
- Plate 2 at a middle spot with ~375 µE
- Plate 3 at a middle spot with ~850 µE with the divbio foil

Results:

- after 24 h
    - Plate 1 showed little green spots but the culture-solutions were slightly green
    - Plate 2 showed little green/brown spots and the culture-solutions were slightly green/brown
    - Plate 3 showed white spots and colorless medium

28.08.2019

Repeated the experiment from the 26.08.2019

- the cultures showed the same results
- after resuspending for two days the the plates at the corner spots in the incubator with ____ µE showed green cultures with a pellet on the ground
- the plates at the middle spot with ____ µE showed green/yellow cultures and pellets
- all wells lost a different amount of solution because of evaporation

29.08.2019

Platereader measurement for the Berthold Tristar²

- submitted 25 ml BG11 in four 100 ml flasks
    - inoculated with: cultures from the day before
        - 427 µl for the UDAR culture to get a starting OD of 0,1
        - 853 µl for the UDAR culture to get a starting OD of 0,2
        - 348 µl for the UDAR 4787 culture to get a starting OD of 0,1
        - 696 µl for the UDAR 4787 culture to get a starting OD of 0,2

- started incubation at 5 PM with 42 °C, 5 % CO_2 and 130 rpm

- measured the OD on 30.08.2019 at 11 AM

  - UDAR 0,1 = 0,430 (1:2) -> OD = 0,86

  - UDAR 0,2 = 0,500 (1:2) -> OD = 1

  - UDAR 4787 0,1 = 0,235 (1:2) -> OD = 0,470

  - UDAR 4787 0,2 = 0,434 (1:2) -> OD = 0,868

- measured the OD on 30.08.2019 at 2:30 PM

  - UDAR 0,1 = 0,496 (1:2) -> OD = 0,992

  - UDAR 0,2 = 0,530 (1:2) -> OD = 1,060

  - UDAR 4787 0,1 = 0,481 (1:2) -> OD = 0,962

  - UDAR 4787 0,2 = 0,480 (1:2) -> OD = 0,960

- then measured the fluoreszenz of the UDAR 4787 compared to the UDAR in the Berthold Tristar2 and the Tecan (MPI)

  - shown in 300819_UDAR-Messung

  - the table with the header "UDAR 4787 0,1 ohne UDAR 0,1" / "UDAR 4787 0,2 ohne UDAR 0,2" shows the UDAR 4787 data without the background measured by the UDAR 0,1/UDAR 0,2

Results:

- the data shows a significant difference between the UDAR and UDAR 4787 samples

  - with a high STD of 21892

300819_UDAR-Messung

	A	B	C	D	E	F	G	H	I	J
1	Measurement									
2										
3	Measurement Data									
4		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
5	**A**	621523	707223	706038	714333	699397	714214	710500	717339	735097
6	**B**	726274	691874	686257	711218	695769	692091	701318	698699	711788
7	**C**	694346	686448	683871	680465	678696	713839	710647	703546	702407
8	**D**	690800	672539	693622	684206	687634	699785	707383	698249	721124

9	E	682684	685664	668780	679370	700257	698539	713623	698371	714528
10	F	683431	682066	683417	707477	684552	708008	697544	698261	722169
11	G	687199	661411	684363	683303	776206	704630	705945	721035	712188
12	H	692601	670037	679934	695335	706040	688542	697266	694067	705546
13										
14	Statistics									
15		Measurement:								
16		30/08/2019 12:50:25								
17										
18		Data File:								
19		UDAR_4787_0,2.wgd								
20										
21		Parameter File:								
22		iGEM_Fluoreszenz.wgp								
23										
24		Reader:								
25		BertholdTech TriStar2, Driver Version: 1.04 (1.0.4.0), S/N: 20-1111, Embedded Version: 1.05								
26		Plate Type: Greiner 96								
27		Name .... UDAR_Test								
28		Counting Time [s] 0.10								
29		Measurement Mode .... by Plate								
30		Halogen Lamp Lamp Energy [%] 100								
31		Excitation Filter .... F485 Emission Filter F535								
32		Second Measurement .... No								
33										
34		Program:								
35		ICE, Version 1.0.9.0								
36										
37		Operating System:								
38		Microsoft Windows 10 Education (64-bit)								
39										
40										
41										
42	UDAR_4787_0,2.wgd iGEM_Fluoreszenz.wgp									
43										
44							**UDAR 0,2**			
45		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
46	**A**	45430	49550	50318	49948	50738	49557	50147	49806	50752
47	**B**	51150	49970	49522	50830	46737	51762	50311	49892	49778
48	**C**	45913	49941	49799	49657	49856	50254	49963	49280	50076
49	**D**	51784	46659	49693	49821	49828	50233	49493	49685	49650
50	**E**	49209	49252	50155	49266	49572	50539	49586	50062	50126
51	**F**	49657	49551	49472	49536	50631	49287	49728	49849	49721
52	**G**	49124	49344	49280	49586	49849	49714	49287	49721	49309
53	**H**	49635	49564	50318	48179	49742	50254	46041	43295	49735
54										

55					UDAR 4787 0,2					
56	0	1	2	3	4	5	6	7	8	9
57	A	621523	707223	706038	714333	699397	714214	710500	717339	735097
58	B	726274	691874	686257	711218	695769	692091	701318	698699	711788
59	C	694346	686448	683871	680465	678696	713839	710647	703546	702407
60	D	690800	672539	693622	684206	687634	699785	707383	698249	721124
61	E	682684	685664	668780	679370	700257	698539	713623	698371	714528
62	F	683431	682066	683417	707477	684552	708008	697544	698261	722169
63	G	687199	661411	684363	683303	776206	704630	705945	721035	712188
64	H	692601	670037	679934	695335	706040	688542	697266	694067	705546
65										
66					UDAR 4787 0,2 ohne UDAR 0,2					
67		1	2	3	4	5	6	7	8	9
68	A	576093	657673	655720	664385	648659	664657	660353	667533	684345
69	B	675124	641904	636735	660388	649032	640329	651007	648807	662010
70	C	648433	636507	634072	630808	628840	663585	660684	654266	652331
71	D	639016	625880	643929	634385	637806	649552	657890	648564	671474
72	E	633475	636412	618625	630104	650685	648000	664037	648309	664402
73	F	633774	632515	633945	657941	633921	658721	647816	648412	672448
74	G	638075	612067	635083	633717	726357	654916	656658	671314	662879
75	H	642966	620473	629616	647156	656298	638288	651225	650772	655811
76										
77	**Mittelwert**	**635869.5**	**632928.9**	**635965.6**	**644860.5**	**653949.75**	**652256**	**656208.75**	**654747.1**	**665712.5**
78	Standa392.863									
79	Mittelwert der Mittelwerte		**653121**							
80										
81										
82										
83										
84										
85										

UDAR_0,1 | UDAR_0,2 | UDAR_4787_0,1 | UDAR_4787_0,2

03.09.2019 - 04.09.2019

2nd Platereader measurement for the Berthold Tristar²

- starting cultures:

    - UDAR with OD = 4,33

    - UDAR 4787 with OD = 3,97

- inoculated 4 cultures with different starting ODs at 5 PM

    - 577 µl of the UDAR were used for the 0,1 OD culture

    - 1155 µl of the UDAR were used for the 0,2 OD culture

    - 629 µl of the UDAR 4787 were used for the 0,1 OD culture

    - 1259 µl of the UDAR 4787 were used for the 0,2 OD culture

- measured the OD on 04.09.2019 at 9:30 AM

Time of OD Measuring	9:30 AM	11 AM	12 AM	1:30 PM	15:50 PM
UDAR 0,1	0,212	0,253		0,332	0,509
UDAR 0,2	0,487	0,563		0,664	0,911
UDAR 4787 0,1	0,225		0,252	0,316	0,492
UDAR 4787 0,2	0,378		0,427	0,516	0,741

- the OD's were totally different and there werent done any further measurings with the Berthold Tristar2

Autor: Chun-Ho Ip
Eintrag 2/6: Inoculation of UDAR and UDAR 4787
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 31.08.2019 17:41
aktualisiert: 12.09.2019 16:23

29.8.2019:

Inoculate UDAR

- desinfection steril bench + 1h UV

- 50 mL BG11 was added into two flasks (250 mL)

- take flask with UDAR culture out from incubator

- shake well and took 1-2 mL of the culture with a measuring pipette

- took 25 µL out from the measuring pipette and gave into one flask

- put rest into another flask

- put back into incubator (5% CO_2, 42 ˚C, 110 rpm)

Inoculate UDAR 4787

- desinfection steril bench + 1h UV

- 50 mL BG11 was added into two flasks (250 mL)

- 25 µL spec solution was added

- take flask with UDAR 4787 culture out from incubator

- shake well and took 1-2 mL of the culture with a measuring pipette

- took 25 µL out from the measuring pipette and gave into one flask

- put rest into another flask

- put back into incubator (5% CO_2, 42 ˚C, 110 rpm)

02.9.2019: Inoculate UDAR and UDAR 4787 (cf. 29.8.2019)

03.09.2019: OD Meausurement of the cultures from 02.9.2019

After 24h: OD(UDAR) = 0.547

OD(UDAR478) = 0.195

After 48h: OD(UDAR) = 1.776 Dilution (1:5): OD(UDAR) = 0.768

OD(UDAR4787) = 1.639 Dilution (1:5): OD(UDAR4787) = 0.642

04.9.2019: Inoculate UDAR and UDAR 4787 (cf. 29.8.2019)

OD Meausurement of the cultures from 02.9.2019

After 48h: OD(UDAR) = 1.776 Dilution (1:5): OD(UDAR) = 0.768

OD(UDAR4787) = 1.639 Dilution (1:5): OD(UDAR4787) = 0.642

06.9.2019: Inoculate UDAR and UDAR 4787 (cf. 29.8.2019)

08.9.2019: Inoculate UDAR and UDAR 4787 (cf. 29.8.2019)

09.9.2019: Inoculate UDAR and UDAR 4787 with 1 mL (cf. 29.8.2019)

	$OD_{star}t$ (14:00 h)	OD (10.09, 11:00 h)	doubling time
UDAR	0.038	2.3	4-5 h ? lack pahse ?
UDAR 4787	0.022	1.7	4-5 h ? lack phase ?

10.9.2019: Inoculate UDAR and UDAR 4787 (cf. 29.8.2019) and Lack phase experiment

	$OD_{star}t$ (11:00 h)	OD (13:00 h)	OD (15:00 h)	OD (17:00 h)	doubling time
UDAR	0.098	0.12	0.18	0.22	
UDAR 4787	0.083	0.1	0.12	0.13	

experiment end bc CO2 from incubator empty...

Autor: Chun-Ho Ip
Eintrag 3/6: cultivation on agar plates
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 04.09.2019 22:32
aktualisiert: 21.09.2019 18:30

04.09.2019 cultivation on agar plates

- steri bench, 1h UV

- took 1.5 mL from culture of 4.9.2019 into epi

- centrifuged (2 min, 2000 rpm)

- discarded supernatent

- resuspended the green cell pellet

- put 50 µL BG11-Media on BG11-agar plate

- put 25 µL and 50 µL of cell pellet on each agar plate and spread untill dry

- seal the plate with parafilm

- put into incubator (15:00 h, 42˚C, 130 rpm, 5% CO_2, at the border of the incubator where light is not too strong)

10.09.19 colonies are visible

13.09.19 inculate colonie into liquid media (BG11)

- pick colony and drop pipette tip into 25 mL BG11, (add 12,5 µL Spec tp 4787).

- inuculate at low light, 42ºC, 130 rpm, 5% CO_2

- after 24 h, looks really green,

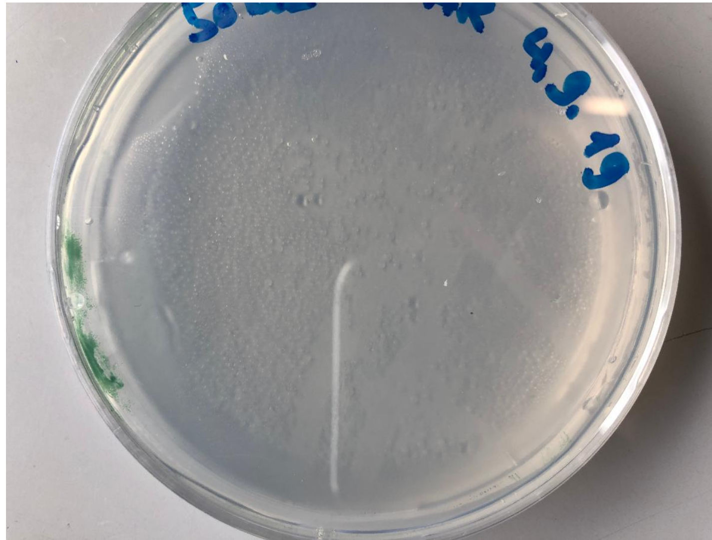
-inoculate to new cultur with OD=0.1; after 20 h OD = 2.57

20.09.19 cultivation on agar plate with "new" UDAR

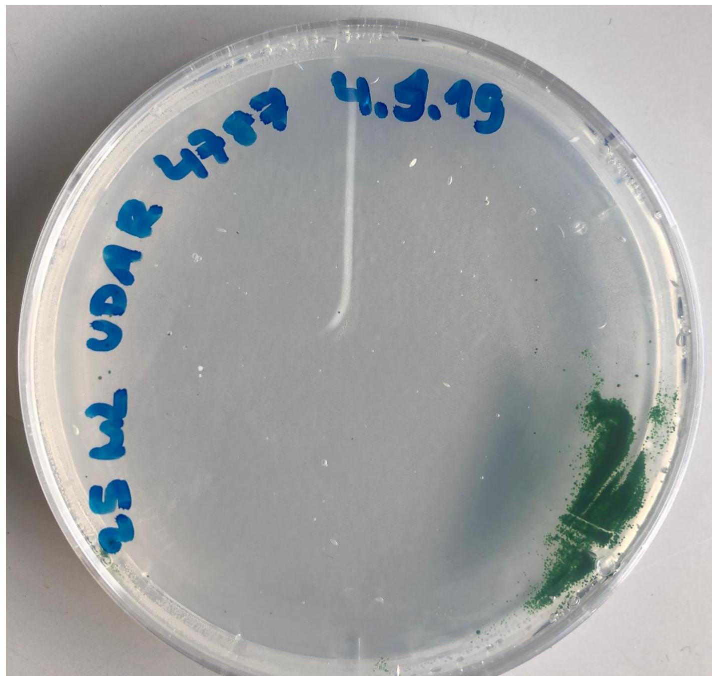
- did the same like on 04.09.19 (siehe oben), but with culture with different ODs (= 1,94 and 1,5)

-start incubator 12:00 h ....

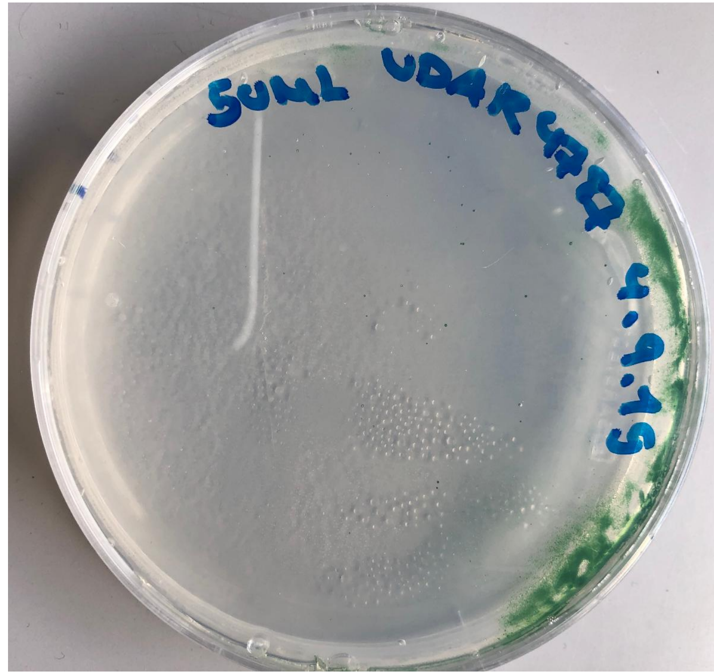
10.09.19_Plate_UDAR_50_μL.jpeg



10.09.19_Plate_UDAR4787_25_μL.jpeg



10.09.19_Plate_UDAR4787_50_μL.jpeg



Autor: Chun-Ho Ip
Eintrag 4/6: BG11
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 09.09.2019 16:37
aktualisiert: 09.09.2019 16:42

BG11:

preparation at steri bench or bunsen burner

- 700 mL dest. H_2O
- 20 ml HEPES buffer (1M, PH 8)
- 10 mL Stock 1
- 10 mL Stock 2
- 10 mL Stock 3
- 1 mL Stock 4
- 1.5 g $NaNO_3$
- fill up to 1 L
- autoclave

Autor: Chun-Ho Ip
Eintrag 5/6: New cultivation of UDAR
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 12.09.2019 16:23
aktualisiert: 20.10.2019 19:02

11.09.19 New cultivation of UDAR

- inoculate UDAR culture to OD=0.1 from MPI culture (17:00h)

12.09.19 Inoculation of UDAR (see: #Inoculation of UDAR and UDAR 4787 - entry #2 in project 'cultivation of UTEX' (Chun-Ho Ip, 12.09.2019))

	OD (17:00)	13.09 (9:30)	13.09 (11:30)	13.09 (13:30)	13.09 (16:00)	13.09 (18:00)	doubling time	comment
UDAR	0.1	0.3	0.45	0.72	1.15	1.26		

13.09.19 Inoculation of UDAR

inoculate new culture from exponential culture (13.09, 13:30)

	OD (14:00)	13.09 (16:00)	13.09 (18:00)	13.09 (20:00)	
UDAR	0.11	0.17	0.226		

15.09.19 Inoculation of UDAR

inoculate new culture from exponential culture

	OD (10:00)	15.09 (17:00)	
UDAR	0.1	0.35	

Test cultivation in 24-Wellplate: inoculate Wellplate with UDAR OD=0.1 (17:00 h), 42˚C, 130 rpm, ca. 500 µE.

16.09.2019 14:00 h Photometer

	1	2	3
A	0,909	0,899	0,92
B	0,99	0,905	0,85
C	0,903	0,982	0,94
D	0,843	0,923	1,03

-> cultivation works really good and no precipitation :)

16.09.2019 14:00 Platereader Tecan Maya

	1	2	3
A	0,93983816	0,90301246	0,8945607
B	0,88188297	0,86316831	0,86739419
C	1,02616728	0,98964349	1,01922475
D	1,10977975	1,19912733	1,22025683

-> no comparision, made an calibration curve -> drive Chuni files

16.09.19 - 22.10.19 Daily Inoculation of UDAR and UDAR 4787 at 9:00 and 21:00 to OD=0.1 to keep the culture in exponential phase

- cultures looks really nice and healthy

Autor: Robin Stei
Eintrag 6/6: cultivation of UDAR and UDAR 4787 in 24 and 48-well-plates
In Projekt: cultivation of UTEX
Keine Tags verwendet

erstellt: 17.09.2019 10:50
aktualisiert: 20.10.2019 19:31

09.09.2019

First try to cultivate UDAR in 24-well-plates

starting UDAR culture with OD = 1,79

- inoculated two times 12 ml BG11 with OD 0,1 and 0,2

  - starting OD = 0,13 and 0,23

  - started at 5:30 PM with 41 °C, 5 % CO_2 and 250 rpm

    - the temperature changed from 41 °C to 37 °C because of some problems with the incubator and later it had to be 38 °C because of some agar-plates

- measuring the OD on 10.09.2019

starting OD\measuring time	11 AM	1 PM	3:30 PM	5 PM
0,1	0,883	1,090	1,276	1,780
0,2	1,145	1,554	1,652	2,180

- inoculated another 24-well-plate with cultures from this 24-well-plate

  - started cultivating at 6:15 PM on 10.09.2019 with 38 °C, 5 % CO_2 and 250 rpm

- measured the OD on 11.09.2019 at 4:30 PM

  - all wells growed and showed a good green color

  - one of the 0,1 starting OD-wells had a even brighter green (located in the corner of the well-plate)

  - so the OD of this well, another 0,1 well, and one 0,2 well

  - 0,1 with the brighter green had a OD of 6,04

  - 0,1 OD = 3,05

  - 0,2 OD = 3,32

    - the total cultivation time was 22:15 h

    - estimated 5 doubling times to reach around OD = 3.2 starting by 0,1 and 4 doubling times starting by 0,2

- without lag-phase the cultures doubled every 4.45 h for 0.1 and every 5.56 h for 0,2

- measured the OD on 13.09.2019 at 11:15 AM

  - all wells growed and showed a good green color

  - so the OD of a 0,1 well and one 0,2 well was measured

  - 0,1 OD = 9,94

  - 0,2 OD = 9,9

    - after a total of 65 h cultivation time

13.09.2019

started another well-plate with the starting culture of the UDAR from the MPI OD = 3,2

  - used 24 and 48 well-plates to compare

    - for the 48-well-plate the wells were inocculated with OD 0,1, 0,2 and some cultures from agar-plates (300 µl)

    - the 24 well was only incoculated with 0,1 and 0,2

  - the 0,1 and 0,2 wells for 24 and 48 were also differentiated by the volume of BG11 (300µl and 500 µl)

  - couldn't measure them on the following days but the cultures all showed a good green color

    - except the ones inoculated with cultures from agar-plates

    - also there were little aggregates in every culture swimming around

- after nearly 72 hours the cultures still showed green colors but the small volumes evaporated a lot more than the bigger volumes and were nearly dried out

  - also the wells inoculated with the cultures from agar-plates showed green cultures

16.09.2019

Started the first try of the parts-measuring protocoll

- inoculating well-plates with the UDAR 4787 with OD 0,1, let them grow till exponential growth 0,6 - 0,8, then inoculating a new well-plate with the cultures from the first well-plate by OD 0,1, let them grow to 0,6 - 0,8 and measure the fluorescence of the YFP by a platereader

- inoculated a 24 and a 48 well-plate with UDAR 4787 OD = 0,1
    - used a culture that was inoculated at 0,4 and grew to 0,436 in 6:30 h
        - the preculture was incoculated from a culture with OD = 4,63
- the well-plates did not grow over night

17.09.19

Inoculated 2 UDAR 4787 cultures by picking some colonies from a agar plate

18.09.19

The colonie cultures showed good growth with a slight green color and OD = 0,356

- inoculated cultures with OD 0.05, 0.1 and 0.2 at 9 AM
- OD showed below

	2:30 PM	4:40 PM	5:30 PM	7 PM
UDAR 4787 (starting OD=0,05)	0,142	0,242	0,282	0,410
UDAR 4787 (starting OD=0,1)	0,282	0,512	0,548	0,754
UDAR 4787 (starting OD=0,2)	0,542	0,908		

When the cultures reached OD 0.6 - 0.8 they were used to inoculate 24-well-plates

- the well-plates were incubated at 42 °C, 5 % CO_2, ~ 500 µE and 130 rpm
    - incubated the well-plate from the 0.2 culture at 5 PM and the well-plate from the 0.1 culture at 7 PM
- OD couldnt be measured over night because the platereader showed clearly wrong data
    - was measured at 19.09.2019 1:30 PM
        - 24-well-plate inoculated (to OD 0.1) from the 0.1 culture - OD = 1.025

- 24-well-plate inoculated (to OD 0.1) from the 0.2 culture - OD = 2.010

20.09.2019

Inoculated a 24-well-plate with UDAR 4787 to OD 0.1 at 9 PM

- 500 µl incubated at 42 °C, 5% CO_2, ~500 µE, 130 rpm

21.09.2019

OD measuring of the 24-well-plate from the day before

- 11:15 AM -> OD = ~ 0.374
- 9 PM -> OD = 2.08

Inoculated a new 24-well-plate to OD 0.1

- 10:30 AM -> OD = 1.8

23.09.2019

Inoculated 8 wells of a 24-well-plate to OD 0.1 (simulating the last steps of the part-measuring-protocol)

- 5:30 PM -> OD = 0.132

01.10.2019

Updated the parts measurement protocol

- to test the protocol the row A of a 24 well plate will be inoculated with UDAR 4787 OD=0.1 and the well B6 will be inoculated with UDAR OD=0.1
- after they reach 0.6-0.8 the rowsC and D will be inoculated with OD 0.1 and B4 and B5 will be inoculated with OD=0.1 of well B6
    - transfering from A1 to C1 and D1 (analog for every other well)
- after they reached OD=0.6-0.8 their OD and fluorescence was measured via plate reader
    - and later on via photometer to compare the different measurements (for OD)

02.10.2019 - 17.10.2019

We prepared for the parts measurement by testing and updating the workflow

- we used UDAR and UDAR 4787 (with fluorescence proteine)

- in the beginning we just measured the OD of the cultures to see how they grow in the well plates
  - with this we also wanted to find out whether the OD of the plate reader is also comparable to the OD of the photo meter
- after this we started to measure the fluorescence
  - analyzed which measurement method (how many measuring points are needed and how big the gap to the border of the well should be)

Some of the results are listed in the table below (well-plate-measurement_overview)

- the fluorescence and the OD were measured after "Parts Measurement Protocol"
- the OD values of the plate reader and the photometer could not be correctly compared with a calibration curve. although a large database was used, the calibration curve did not show any suitable values in further tests

well-plate-measurement_overview.xlsx