Lab notes - overview

TUESDAY, 6/18/2019

Welcome to the lab journal of Team QRoningen. Whenever you did something in the lab please add it here. It will be greatly appreciated, once we have to put stuff in the Wiki. :) (Maybe even more convenient when we all get our own account and can actually assign these notes to people automatically. For now we can get chatroom names like chocolatecookie_1760)

WEDNESDAY, 6/19/2019

for a mini tutorial go to the "Tutorial" entry

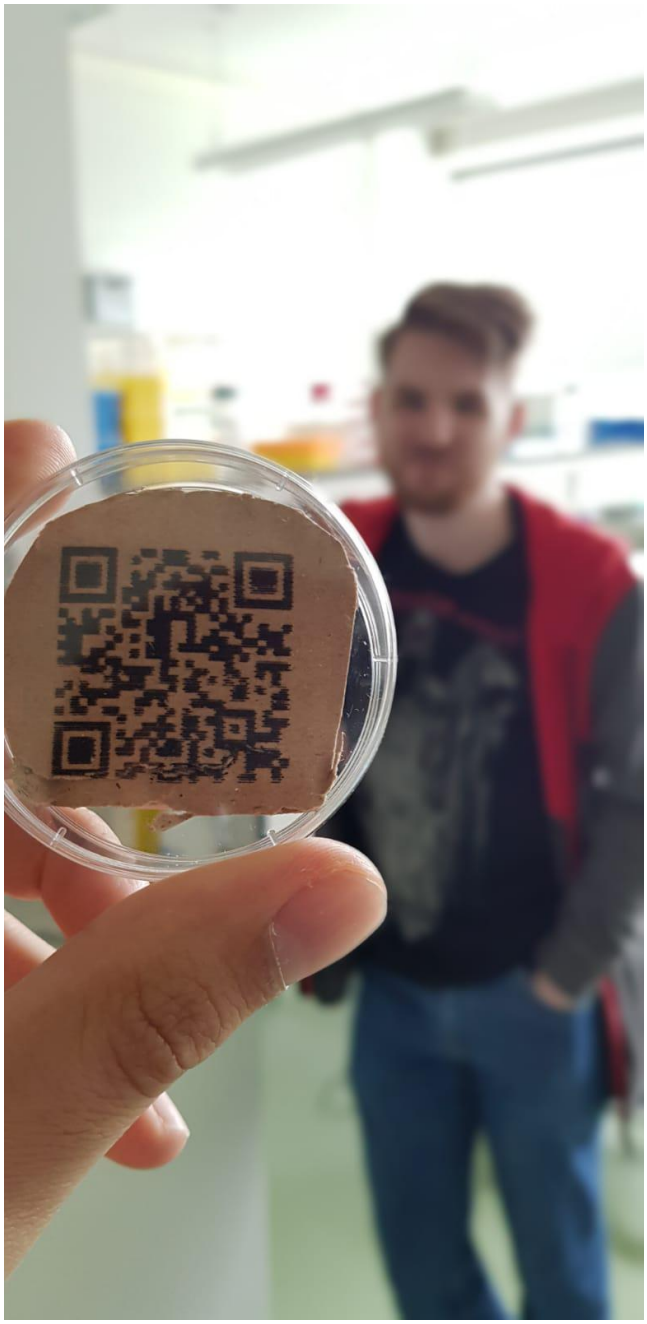
SATURDAY, 6/22/2019

22/06 (Sander) used competent BL21(DE3) cells from doulix and plated them on 2 agar plates. STREAK; with pipette tip streaked out one dip of fluid. SPREAD: plated residual fluid, which was most, out on another plate.

MONDAY, 7/8/2019

Michelle, Lieke : Picked 4 of the colonies spread by sander to start liquid culture (BL21DE3) (4 flask with 10 ml of LB) -> grow o/n in shaker (200 rpm, 37 °C)
Geet, Marc, Mariano: fixed issues with laser -> laser engraved QR code

QR (instagram) code engraved on cardboard with la ser



TUESDAY, 7/9/2019

Plan for today: pour some plates for dry team to experiment with agar & plate some bacteria

the OD of the 4 cultures: 2.63, 2.64, 2.75, 2.25

For yesterday: we checked different spotting/ drawing methods of the code (spotting with pipette S, drawing with loop D, drawing with toothpick DT, drawing with tooth pick on paper and stamping the paper StampT) with different dilutions of bacteria 370 µl of culture (OD 2.75) where diluted with 630 µl water to yield OD 1; this was used to make a dilution series (add 100 µl of the dilution to 900 µl of water)

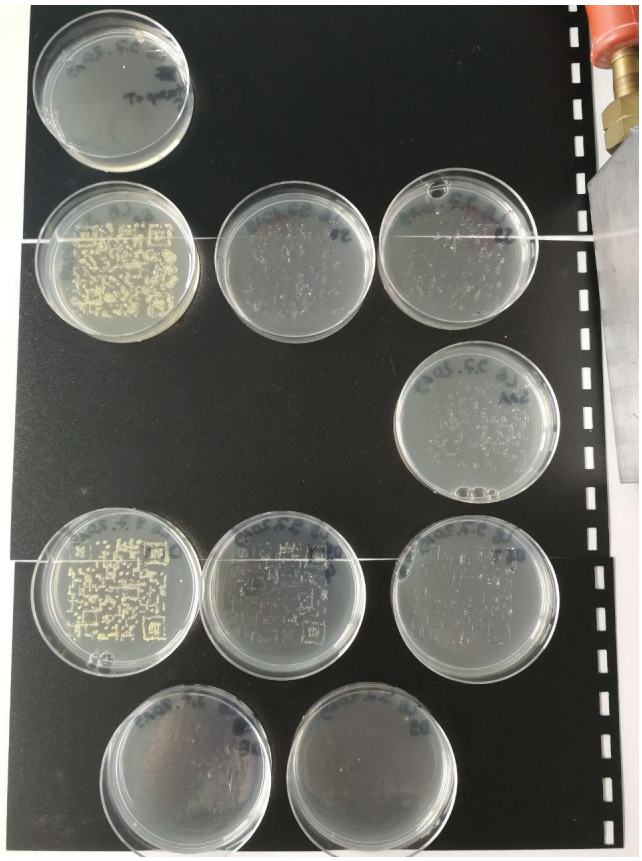
two plates were tested to see a full plane of bacteria the next day - 200 µl of 3 and 400 µl of 5

dilution series & used for & outcomes

	A	B	C	D	E	F	G
1	name	dilution, OD = 10 * 10 ^-x	used for	outcome		used for	outcome
2	1	1					
3	2	2	DT	Pattern visible in the morning		200 µl for plate	full plate but not layer
4	3	3	S	Pattern visible in the morning			
5	4	4	DT	not visible		400 µl for plate	not visible
6	5	5	StampT	not visible			
7	6	6	DT	not visible			
8	7	7	S	not visible			
9	8	8	D	not visible			
10	9	9	S, D	not visible	not visible		
11	10	10					
12	11	11	S	not visible			

comments on figure below from left to right and top to bottom: StampT5, S3, S7, S9, S11, DT2, DT4, DT6, D8, D9

platesfrom0709imagedonmorning0710



WEDNESDAY, 7/10/2019

PLAN : The toothpick method is the most efficient and precise to draw the pattern -> try again with higher bacteria concentrations and bigger QR code/ bigger plate

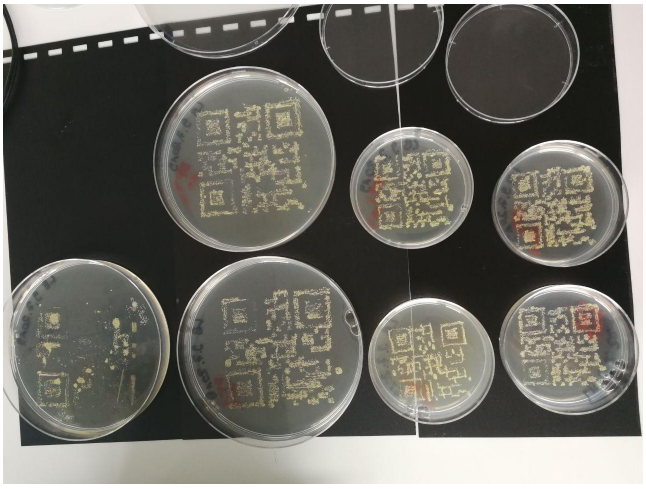
new dilution series, Culture grew to OD 0.43: Took 1 ml -> 500μl and water each for the next one
and the used QR code encodes QRoningnen instead of the link to our insta -> easier, less to draw

dilution, used for

	A	B	C	D
1	name	OD	used for	used for
2	1	0.43		
3	2	0.215		
4	3	0.1075	95 mm QR DT, 2x 50 mm QR DT	400 ul for 95 diameter plate for lawn 2x
5	4	0.05375	95 mm QR DT, 2x 50 mm QR DT, 95 mm QR D	
6				
7				
8				
9				
10				

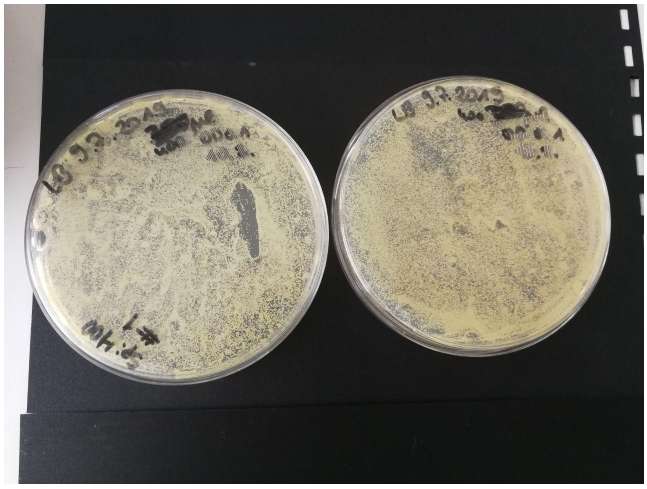
comments on figure below from left to right and top to bottom: DT4, DT3, DT4, D4, DT3, DT3, DT4

platesfrom0710imagedoon0711morning



the drawing of the QR by hand using a tothpick worked fine but the pattern is NOT scannable. Loops do not work!
The lawn of bacteria we get is not filling up the whole plate (400 uL, OD 0.1, diameter 95 mm)
comments on figure below from left to right: lawn by 400 ul of 3, lasering 400 (left), idk what right (right)

platesfrom0710imaged0711morning



from the filled plates that were engraved with the laser there is only a full lawn of bacteria, no sign of dying bacteria -> we found out from literature that it is actually the heat killing the bacteria not the light -> go for lower spead in glass dish or black agar -> char coal was ordered and Michelle will ask around for glass dishes (someone should have that -.-)

Michelle will also ask if there is a clean bench we can use so that the plates will not be contaminated and we don't have to waste all that gas
The first laser trials were performed on this date, for more information check the "Laser Experiments" tab

THURSDAY, 7/11/2019

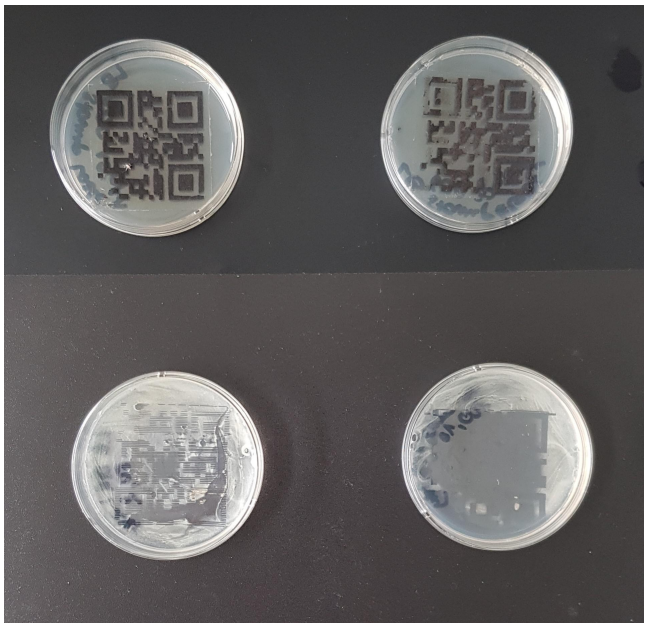
Checking the results from the cultures arranged on Wednesday (Yesterday) it seems that the laser is not affecting the bacteria as visible in the picture
Two experiments on small dishes were performed at slower speeds to ensure the heat of the laser kills the bacteria. This time with a white surface under the laser to avoid melting the plastic

FRIDAY, 7/12/2019

PLAN: in absence of wet lab team members today the plates from yesterday will be checked, and two more laser experiments will be perfdrmed on cultures previously perpared by Michelle

Comments on figure below from left to right and top (Stamps) to bottom (laser etched):

20190712_111442.jpg



1:

Both plates on top using the "stamp" method have no characteristic growth that may hint at the proper deposition of the bacteria on the agar

Both plates on the bottom can be seen to have the form of a QR code, although they are not readable. The right plate seems to be empty in the center, maybe due to heat distribution of the laser, or the bacteria needs more time to grow

These experiments show a better layer distribution of bacteria, making it easier to identify the shape of the QR code, and making it readable for the camera

The plates are left in the incubator one more night to ensure if the stamps need more time, and maybe so do the laser ethched cultures

Two more cultures were laser etched to validate the laser process (for more info on the details of these experiments refrer to the "Laser Experiments" tab

SATURDAY, 7/13/2019

PLAN: to check the cultures stored in the incubator on Friday (Yesterday) for any changes and store them in the fridge

qrqrqr.jpg

In this picture we can see the plates from yesterday left to grow one more night. Indeed it appears that the cultures needed more time to manifest themselves and now the QR codes at the bottom are clearly visible, although the agar seems to have displaced (tore), probably due to the handling the previous day. The cardboard stamps seem to yield no conclusive results, so we will try making a rubber stamp next time.

20190713_145252.jpg

The last laser experiments performed yesterday seem to yield great results, with the right plate in the picture being scannable (Experiment number 9 in the laser table)

MONDAY, 7/15/2019

stamps and laser experiments (summary please)

1) extrapolating laser printing parameters(speed and density) from agar plate engraving

2) testing various materials with engraver to conclude wood as a good stamping material

3) engraving at least 2 samps to proceed with further testing
4)Conclusion: currently we have 2 methods to print out a bio QR code.
inoculation of culture e.coli o/n for experiments tomorrow

TUESDAY, 7/16/2019

PLAN: black agar, Vibrio agar, tryout stamp and black agar with E. coli, recover Vibrio, inventory of fridge (figure out what reagents we need to order), inoculate for tomorrow, glycerol stocks with biobricks from molgen

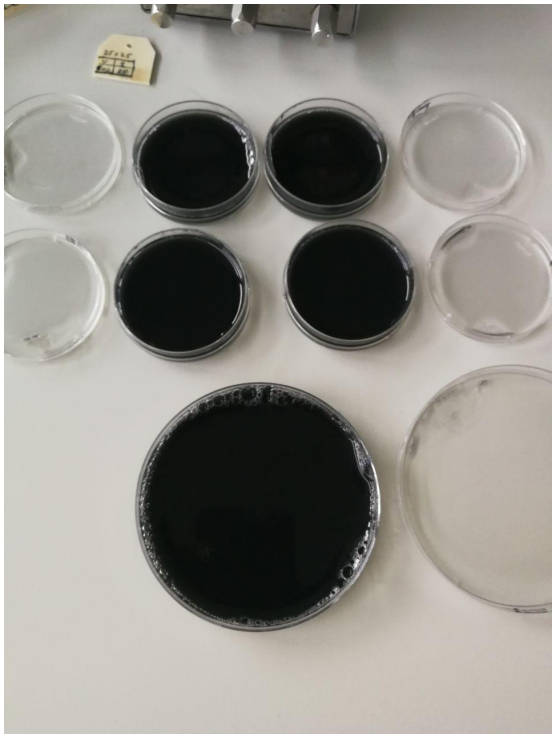
made black agar plates: 49 ml normal LB agar + 1 ml of 150 g/l charcoal (autoclaved) -> plates are called LB coal
made vibrio agar plates: 49 ml normal LB agar + 1 ml of 100 g/l NaCl (autoclaved) -> plates are called LB V
made standard agar plates
Sander made inventory of the fridge and freezer find it in projects -> inventory -> find the entry you like
Yashika made the glycerol stocks from bacteria with the biobricks from MolGen

Biobricks from MolGen glycerolstocks

	A	B	C	D	E
1	Name	Location	Brick	resistance	fragment
2	1	-80	R0051	Amp	lambda cI regulated promoter
3	2	-80	I13500	Cm	RBS-GFP
4	3	-80	J23110	Amp	constitutive promoter
5	4	-80	J06602	Cm	RBS-mCherry
6	5	-80	R0040	Cm	Tetr repressible promoter
7	6	-80	B0015	Cm	double terminator
8	7	-80	I13502	Cm	RBS-RFP
9	8	-80	R0010	Cm	lacI regulated promoter
10	9	-80	K808000	Cm	AraC and pbad
11	10	-80	K117000	Cm	lysis gene
12	11	-80	J04500	Cm	LacI+rbs
13	12	-80	J04500	Amp	RFP (1A3)
14	13	-80	J04500	Kan	RFP (1K3)
15	14	-80	J04500	Cm	RFP (1C3)
16	15	-80	J04500	Tet	RFP (3T5)
17	16	-80	J04500	Cm	RFP (3C5)
18	17	-80	J04500	Amp	RFP (4A5)
19	18	-80	J04500	Cm	RFP (4C5)
20	19	-80	K741002	Amp	GFP (1A3)
21	20	-80	K741002	Cm	GFP (1C3)
22	21	-80	K741002	Cm	GFP (3C5)
23	22	-80	K741002	Cm	GFP (4C5)
24	23	-80	J23102	amp	Constitutive promoter family member J23102
25	24	-80	J23106	amp	Constitutive promoter family member J23106
26	25	-80	J23116	amp	Constitutive promoter family member J23116
27	26	-80	S0107	cm	tetR
28	27	-80	R0010+K2592025	Km	LacI regulated promoter+Purple blue chromoprotein amilCP

experiments with laser: filled two small plates with 2.25 ml (small plate) or 4 ml (big plate) culture of OD 0.2 and let the laser kill in the pattern of QR code
experiment with stamp: dip the stamp in the culture of OD 0.2 and touch it to 2 normal agar plates consecutively; dip the stamp again and leave it o/n to try and touch it the next day

photo_2019-07-17_16-25-25.jpg

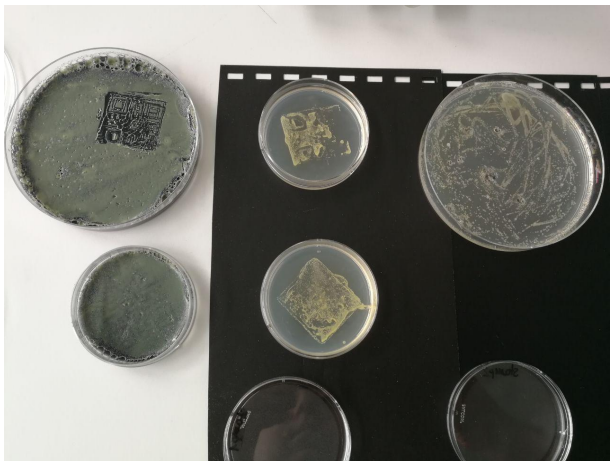


plates with LB agar, supplemented with 3 g/L charcoal

WEDNESDAY, 7/17/2019

PLAN: test if stamp with culture from yesterday works, isolate plasmids with Biobricks from MolGen group

photo_2019-07-17_16-25-18.jpg



plates from left to right and top to bottom: **1)** filled with even layer of E. coli, (used as ink with wooden stamp) failed edging (1000 mm/min, 12 lines/mm, Laser Experiments 12), **2)** wooden stamp that was dipped into liquid culture of OD 0.2 (E. coli) was touched immediately to 5) for 5 min and afterwards to 2) for 5 minutes, **3)** recovery of Vibrio from glycerol stock grown on LB plate overnight at 37 °C, **4)** even layer of E.coli failed edging 800 mm/min 12 lines/mm (Laser Experiments 11), **5)** see 2)

photo_2019-07-17_16-25-09.jpg

LB coal filled with E. coli, (2.25 ml, OD 0.2, removed and dried), edged at 250 mm/min, 12 lines /mm; used for stamping

Sander isolated the Plasmids from Molgen group

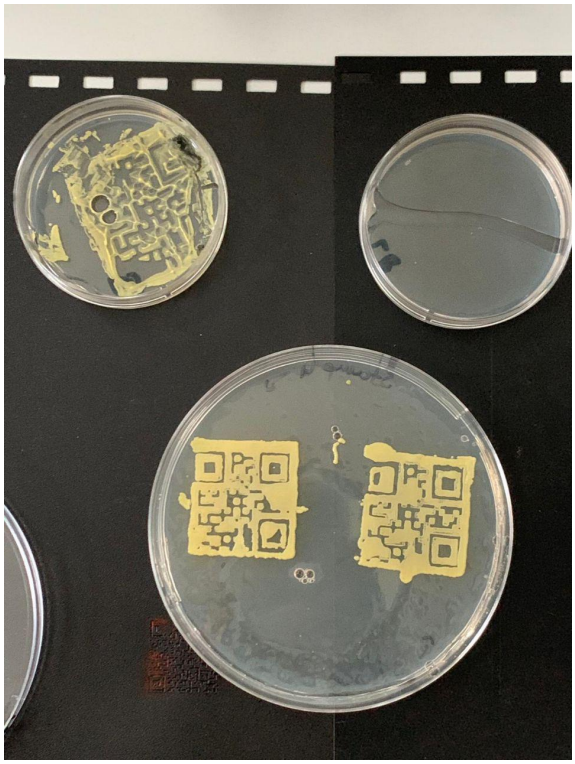
Plasmids with Biobricks received from MolGen, Sally Box19A

	A	B	C	D	E
1	Name	Location	Brick	resistance	fragment
2	1	A1	R0051	Amp	lambda cI regulated promoter
3	2	A2	I13500	Cm	RBS-GFP
4	3	A3	J23110	Amp	constitutive promoter
5	4	A4	J06602	Cm	RBS-mCherry
6	5	A5	R0040	Cm	Tetr repressible promoter
7	6	A6	B0015	Cm	double terminator
8	7	A7	I13502	Cm	RBS-RFP
9	8	A8	R0010	Cm	lacI regulated promoter
10	9	B1	K808000	Cm	AraC and pbad
11	10	B2	K117000	Cm	lysis gene
12	11		J04500	Cm	LacI+rbs
13	12		J04450	Amp	RFP (1A3)
14	13		J04450	Km	RFP (1K3)
15	14		J04450	Cm	RFP (1C3)
16	15		J04450	Tet	RFP (3T5)
17	16		J04450	Cm	RFP (3C5)
18	17		J04450	Amp	RFP (4A5)
19	18		J04450	Cm	RFP (4C5)
20	19		K741002	Amp	GFP (1A3)
21	20		K741002	Cm	GFP (1C3)
22	21		K741002	Cm	GFP (3C5)
23	22		K741002	Cm	GFP (4C5)
24	23		J23102	Amp	constitutive promoter family member J23102
25	24		J23106	Amp	constitutive promoter family member J23106
26	25		J23116	Amp	constitutive promoter family member J23116
27	26		S0107	Cm	tetR
28	27		R0010+K592025	Km	LacI regulated promoter+ Purple-blue chromoprotein amilCP

FRIDAY, 7/19/2019

Plan: make media for Vibrio, make plates for CFU experiment

photo_2019-07-21_17-09-33.jpg



From left to right and top to bottom: **1)** grown edged QR code cut out and used as stamp, **2)** stamp dipped into OD 0.2 E.coli, used twice (see day before) and left o/n touched to this plate for 5 min and grown overnight, **3)** wooden stamp touched to even layer of E. coli and touched to this plate (first left then immediately right)

recipe for LBV (1 L): 30 g NaCl, 10 g triptone, 5 g yeast extract (if needed 20 g agar)
50 plates (small) were poured for each LB and LBV medium as preparation for the CFU experiment

SATURDAY, 7/20/2019

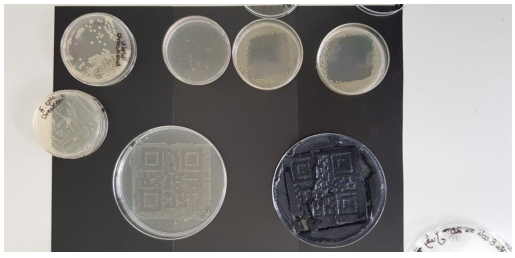
Plan: plan cloning, agar stamp on big coal plate, test vibrio edging, test closed plate with E. coli, test bigger amount of information (paragraph on big plate)

for details on laser experiments go to lab journal -> QR - drylab -> Laser Experiments (experiments 13-16)
planned strains are listed in lab journal -> strain design -> planned strains, this entry focusses on the growing conditions.

SUNDAY, 7/21/2019

Plan: agarose stamp, big wooden stamp, find sth on viscous culture, make clear and coal LBV plates

photo_2019-07-21_17-09-17.jpg



left to right, top to bottom: **1) & 2)** black agar (2) edged to from a stamp, covered with even layer of E. coli, that stamp touched to plate (1) and incubated o/n at 37 °C, **3) & 4)** streak out of E. coli and V. natriegens to pick single colonies, **5)** E. coli filled plate edged with lid on, **6) & 7)** plates filled with even layer of Vibrio (Laser Exp #14 top, Laser Exp #13 bottom)

conclusions: try higher speed with Vibrio plating, try E. coli again without the lid
made 100 ml of each LBV clear and coal agar medium, autoclaved, poored small plates
edge big plate with e.coli (paragraph), edge two small plates with vibrio (different conditions since before the whole square died), edge small agar stamp, edge small stamp

MONDAY, 7/22/2019

Plan: cfu/mL Determination, E. coli reproducibility edge, try with filled plate as ink,

(Sander) As the relation between OD and cell concentration is dependent on the spec you use as well as on the cell you are working with, you have to run the experiments yourself (read OD for actively growing cells and plate dilutions for efficient counting). As a comparison, a rough estimate for E. coli is 10^9 cfu /mL /OD_{600nm} unit.

E. coli CFU determination:

Methods

a liquid broth was made of *E. Coli* and *V. Natriegens.* From time to time, 750 uL was taken to determine $OD_{600.}$, 100 uL was taken and a dilution series was made in MilliQ. 0.2 mL of the dilution series was then used to inoculate small agar plates. The fluid was spread out using a Drigalski Spatula.

Results

Cfu determination E.co.xlsx

Relationship: 1 x 10^9 x $OD/_{600nm}$ + 6 x 10^7 = CFU usually u use -> (1 x 10^9 x $OD/_{600nm}$

V. Natriegens viability in dilution series

V. Natriegens showed no growth, except for the 10^{-1} dilution plate.

Hypothesis: Since *V. Natriegens* is a halophile, they might die due to the lack of NaCl in the MilliQ solution.

Procedures:

A liquid broth was made of *V. Natriegens.* 750 uL was taken to determine OD_{600}, 100 uL was taken and a dilution series was made in MilliQ, H20 and NaCl (30g/L) and LBV media. the 10-1, 10-3, 10-5, 10-7, 10-9 dilution series were plated. 200 uL of the dilution series was used to inoculate small agar plates., The fluid was dispersed using a Drigalski Spatula.

V. Natriegens viability in dilution series

	A	B	C	D	E	F
1	Dilution Solution	10-1	10-3	10-5	10-7	10-9
2	H2O	y	N	N	N	N
3	H2O (30g/L NaCl)	Y	Y	Y	63	1
4	LBVibrio	Y	Y	Y	110	24

V. Natriegens CFU determination:

Methods

a liquid broth was made of *V. Natriegens.* From time to time, 750 uL was taken to determine $OD_{600.}$, 100 uL was taken and a dilution series was made in MilliQ. 0.2 mL of the dilution series was then used to inoculate small agar plates. The fluid was spread out using a Drigalski Spatula.

Results

Copy of CFU vibrio.xlsx

Relationship: 2 x 10^8 x OD_{600nm} + 2 x 10^7 -> use 2 x 10^8 x OD_{600nm}

what can be observed is that for the same amount of OD, e. coli has about 5 times as much CFU

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build agar stamp: poured full black agar plate, lasered at 10 mm/min, density 7, sticked with super glue into the plate, dipped into OD 0.5 Vibrio culture, and touched to LBV plates

photo_2019-07-23_09-38-03.jpg

from left to right, top to bottom: **1) & 2)** Vibrio fully plated and lasered grown overnight twice (LS # 13, 14), **3)** closed lid lasered on fully plated E. coli (LS #15), **4)** lasered fully plated E. coli (LS # 16), **5)** big stamp, covered with OD 0.2 E. coli touched to the plate, **6) & 7)** Vibrio fully plated and lasered grown overnight (LS # 17,18) **8), 9) & 10)** Vibrio filled, grown over night, 10 was used coat a wooden stamp

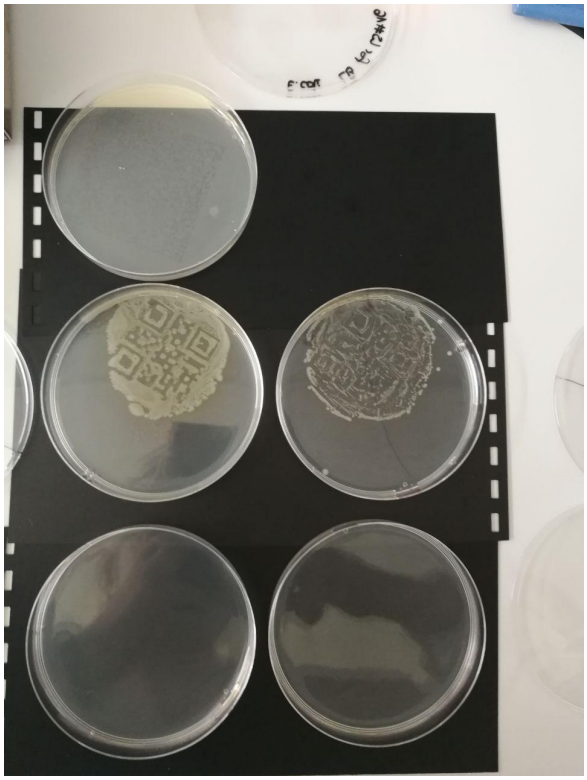
photo_2019-07-23_09-38-06.jpg

from left to right, top to bottom: **1), 2) & 3)** Trial 1: wooden stamp soaked in OD 0.5 with Vibrio touched to the agar first (1), third (2) or forth time (3) **4), 5), 6) & 7)** Trial 2: wooden stamp soaked in OD 0.5 with Vibrio touched to the agar first (4), second (5), third (6) or forth time (7), **8) & 9)** Trial 3: wooden stamp soaked in OD 0.5 with Vibrio touched to the agar first (8), second (9) time , **10)**

TUESDAY, 7/23/2019

Plan: prepare media for VMT

photo_2019-07-23_11-51-21.jpg



from left to right, top to bottom: **1)** E.coli grown 2 nights with high res QR, **2), 3), 4) & 5)** agar stamp touched to plate first (2), second (3), third (3), forth (4) and fifth (5) time after dipping into OD 0.5 and dried

conclusions: E. coli should get a color to give more contrast

photo_2019-07-23_11-51-31.jpg

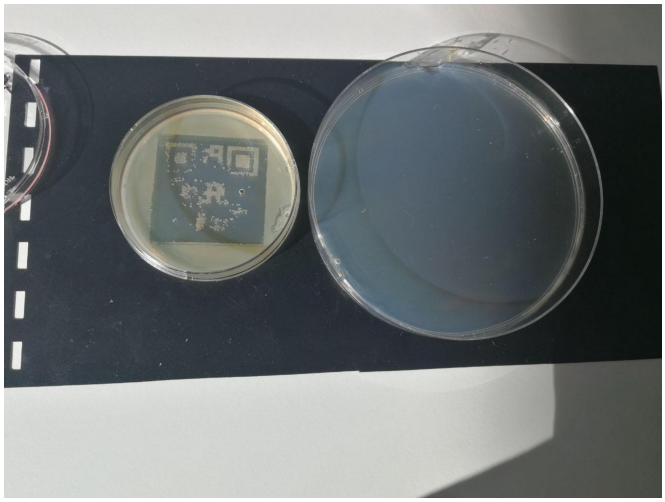
Vibrio fully plated and lasered LS # 19

not scannable because of cut off corners

WEDNESDAY, 7/24/2019

Plan: VMT day 1, make plates

photo_2019-07-24_09-37-30.jpg



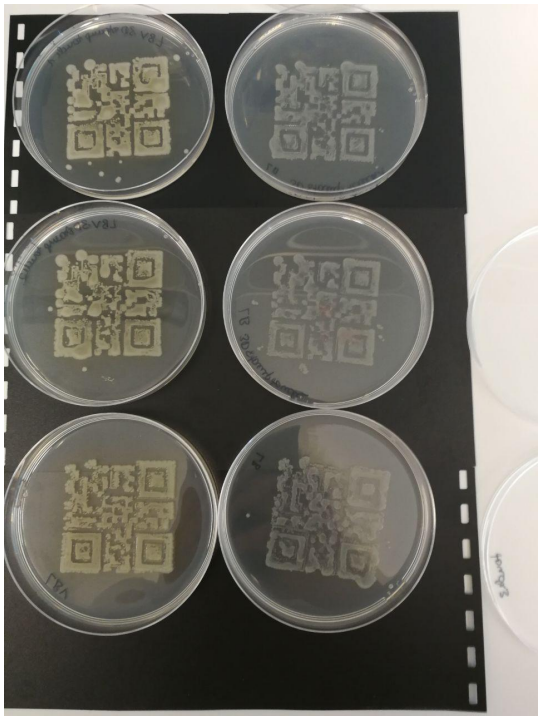
from left to right: **1)** LS # 20, **2)** LS # 21

conclusions: line density of 15 with this speed to high -> lower density or higher speed, maybe wrong plate lead to no growth

THURSDAY, 7/25/2019

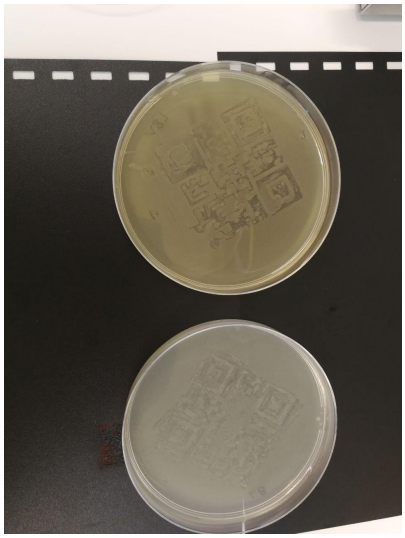
Plan: VMT day 2, replica plating,

photo_2019-07-25_09-54-19.jpg



stamping with the 3D printed stamp: left Vibrio, right E. coli, from top to bottom: first, second, third touch

photo_2019-07-25_09-54-23.jpg



used for inking the stamp (see picture above for stamping)

conclusions: optimize stamping -> pointy shape of stamp, less OD ink

FRIDAY, 7/26/2019

Plan: VMT day 3, replica plating,

Growth Curves V. Natriegens.

growth vnat.png

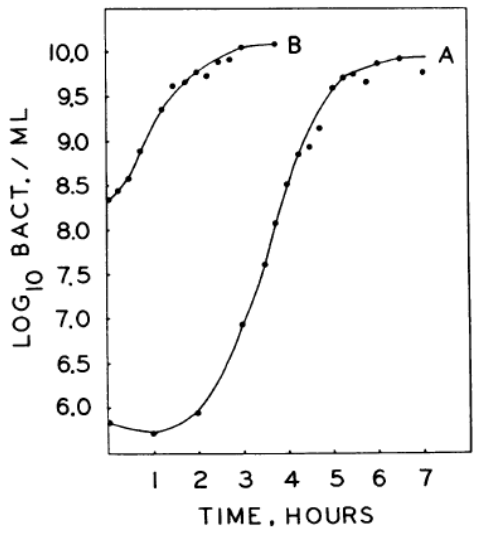
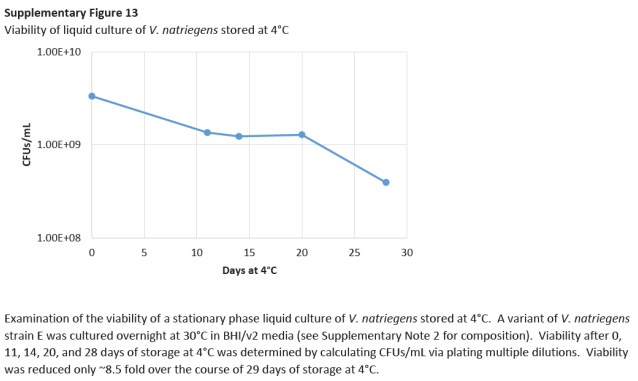


FIG. 1. *Growth curve of Pseudomonas natriegens. Numbers of microorganisms were estimated by the plate count method. Curve A: two loopfuls of a stationary-phase culture were used as inoculum for 30 ml of brain heart infusion medium containing 1.5% sea salt in 500-ml Erlenmeyer flasks on a rotary shaker at 37 C. Curve B: 5 ml of a logarithmic-phase culture were used as inoculum for 50 ml of medium.*

viability in 4 graden.png



Key points from literature:

V. Natriegens have a lag-phase when inoculating using low density inoculums. (they like having friends)

Growth increases steadily until 37 degrees, when going higher growth slows down and stops at 42 degrees.

Vibrio natriegens_growthcurves on different media.docx

V. Natriegens, a marine bacterium with a generation time of less than 10 minutes..pdf

SO: rich media, like BHI media and media supplemented with ocean salts seem to give the best growth. However, to the best of my knowledge, nobody has figured out wether the amount of sodium ions in the concentration change the growth of this bacterium. HENCE the following experiment was designed:

Growth of V. Natriegens in culture supplemented with MgCL2 and KCl with different concentrations of NaCl.

MEDIUM PREPARATION:
Perform in triplicate/duplicate , 8 different conditions, so 16 or 24 vials.
Recipy for media: L-Broth (0.5g NaCl/L), MgCl, KCl, NaCl variable, 25 mL. Concentrations and milligrams per 25mL are calculated and given in the excel file.

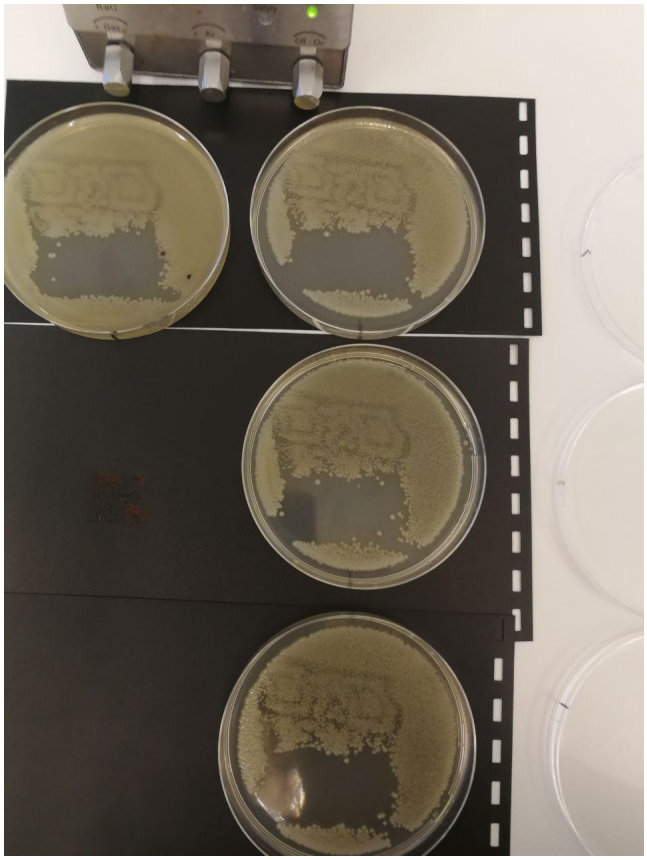
Salts in medium.xlsx

PROCEDURE:

- Single colony was picked and resuspended in 300 μl LBV (30g/l salt)
- 10 μl of cell suspension was added to 25 ml growth media in an 100 ml shaking-flask
- Incubation at ???? rpm; 37°C
- OD600 and Time were measured.

SATURDAY, 7/27/2019

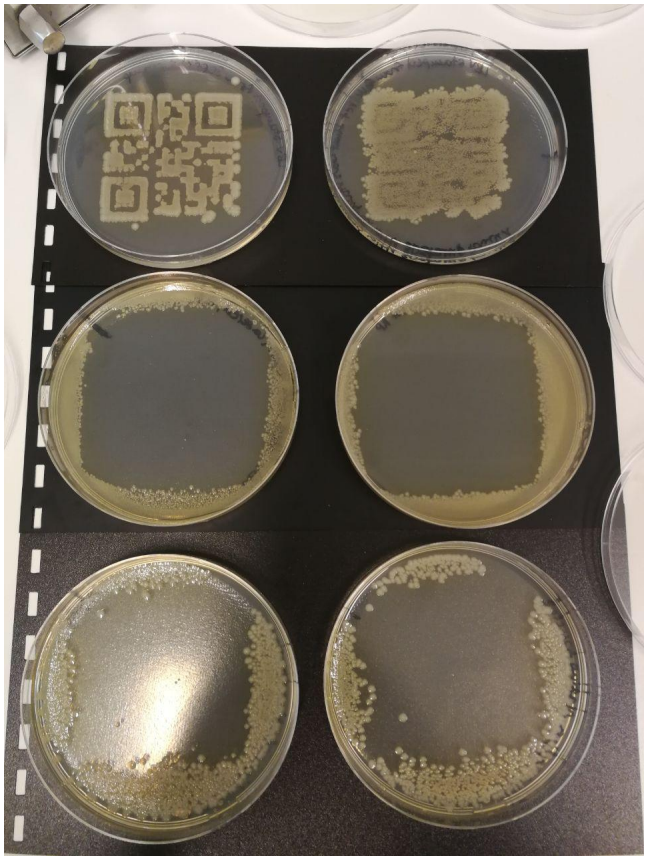
photo_2019-07-27_14-56-28.jpg



left to right, top to bottom: **1)** fully filled plate with an even layer of vibrio, lasered (22) to yield QR pattern and then used for replica plating, **2), 3) & 4)** touched to the velvet of replica plating for the first (2), second (3) or third (4)

SUNDAY, 7/28/2019

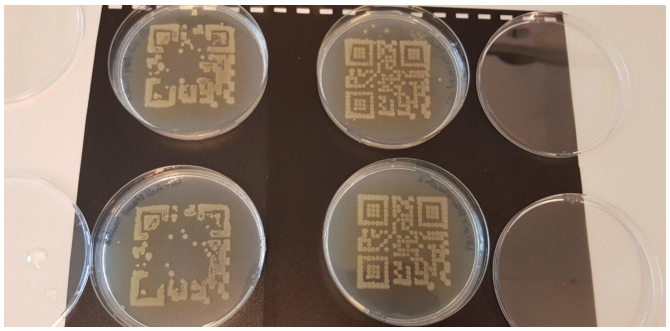
WhatsApp Image 2019-07-30 at 09.21.55.jpeg



From left to right, top to bottom: **1)** stamped with 3D printed stamp **2)** touched to velvet and velvet touched to agar **3) & 4)** Laser experiments 24 & 25 **5) & 6)** replica plated from plates above

MONDAY, 7/29/2019

WhatsApp Image 2019-07-30 at 09.22.33.jpeg



Top to bottom, left to right: 3D printed stamp solid (**1&2**) and pixelated (**3&4**), first touch upper row and second touch lower row

experiment stamping with different agar concentrations. OD viscosity stamp vibrio
method: - a single colony from a solid culture was resuspended in liquid culture (10 mL) LBV medium and incubated for 1.5 hours

- 10 LBV tubes with different agar concentrations were made during the incubation period.
-

TUESDAY, 7/30/2019

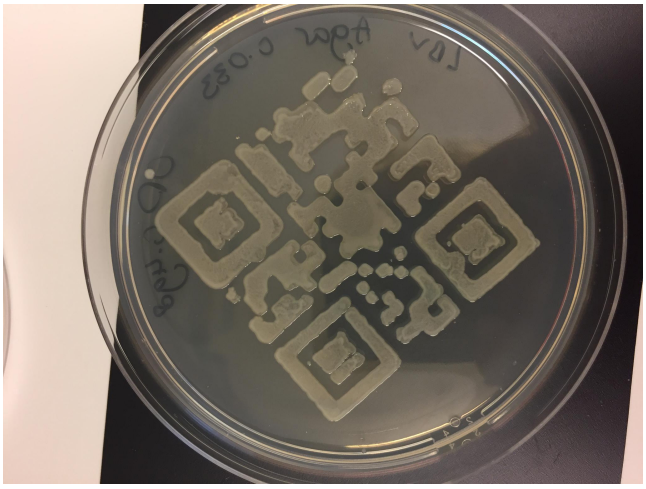
stamping with different vicosity cultures.
procedure: - 0.750 ml bacteria was added to LBV media with different agar concentrations

- OD600 was measured against blank after which the cultures were incubated in shaker for 12 min.

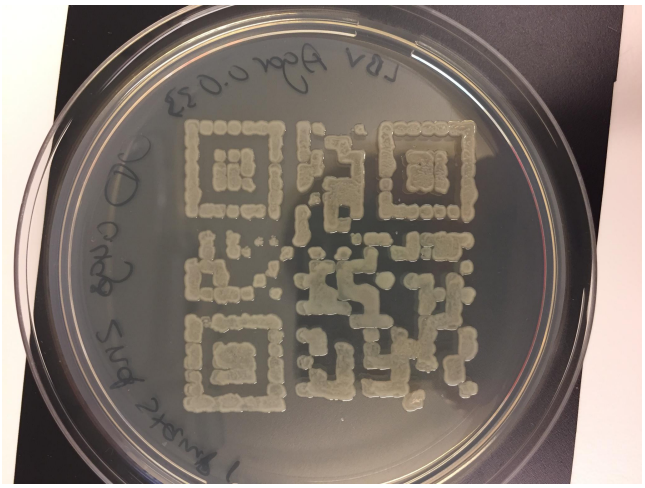
- OD600 was measured again and stamps were made.
- Cultures were incubated in shaker for 12 min again.
- OD600 was measured and spamps were made
- Cultures were incubated for an hour after which the OD600 was measured again and stamps were made.

After moistorizing the stamp and before the actual stamping with bacteria, the stamps were dried for 5 minutes.

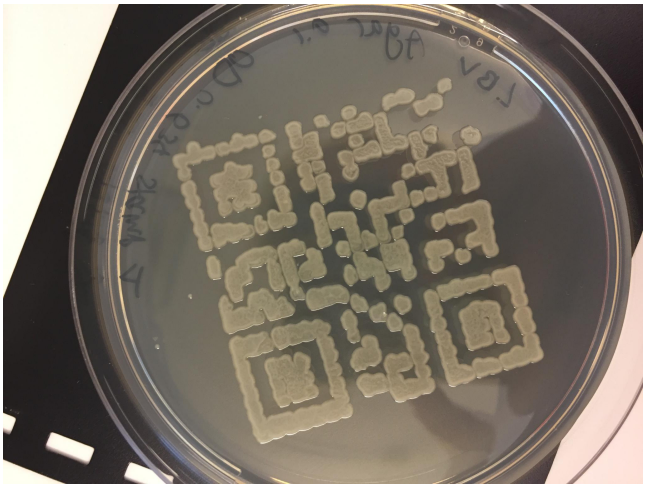
LBV agar 0.033 OD 0.498 Stamp 1



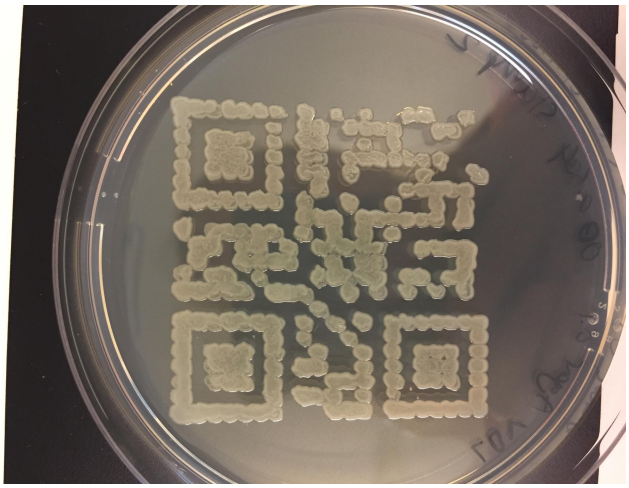
LBV agar 0.033 OD = 0.498 stamp 2



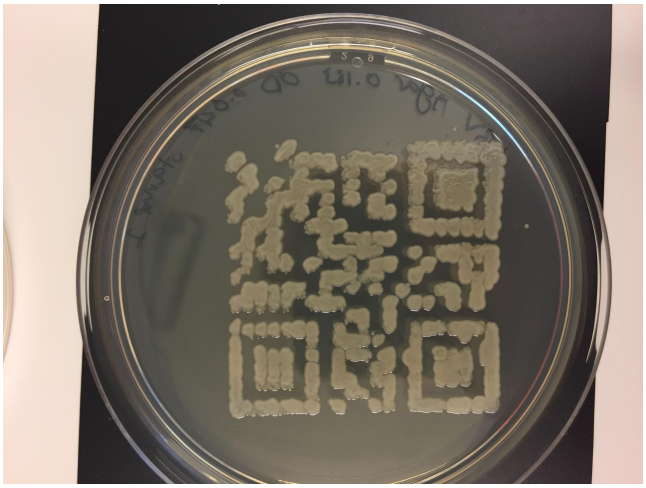
LBV agar 0.1 OD 0.634 stamp 1



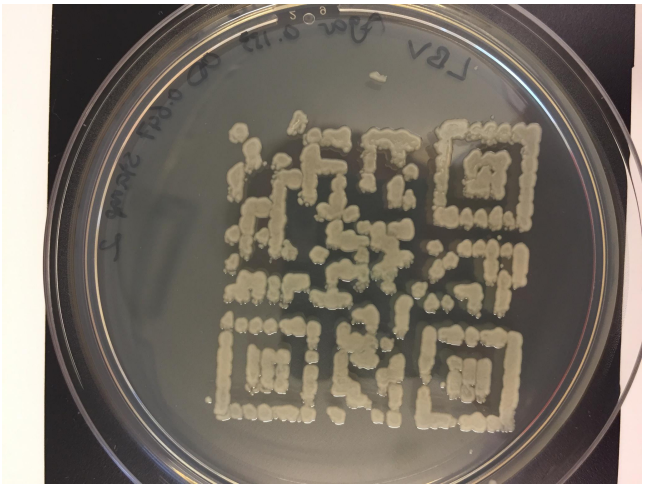
LBV agar 0.1 OD 0.634 stamp 2



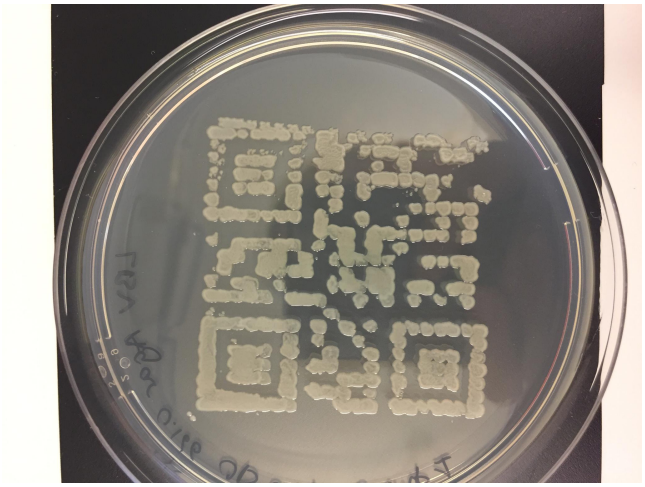
LBV agar 0.133 OD 0.647 stamp 1



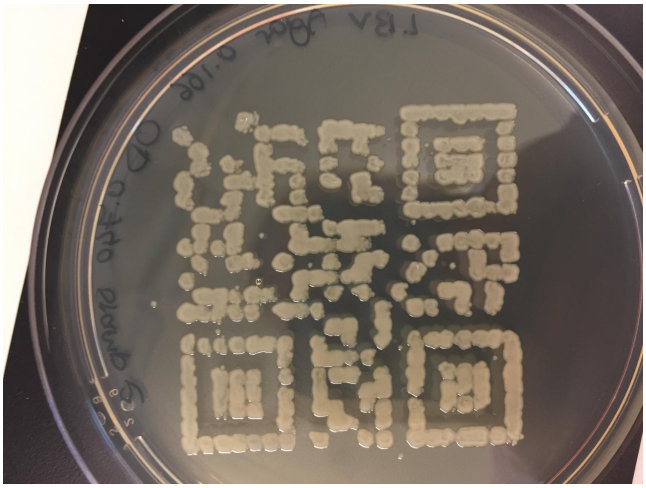
LBV agar 0.133 OD 0.647 stamp 2



LBV agar 0.166 OD 0.740 stamp 1

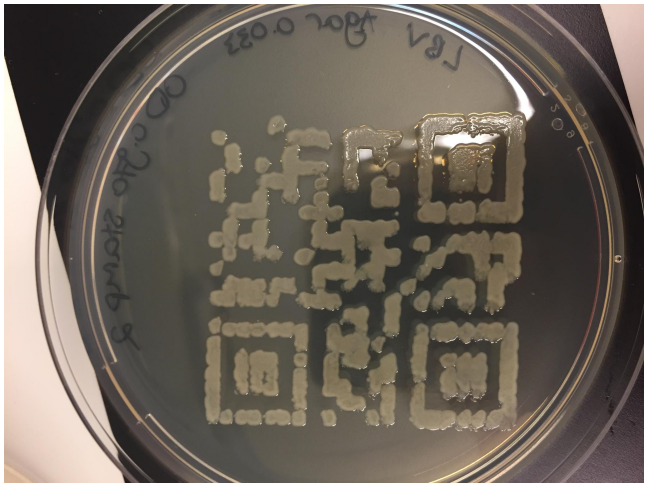


LBV agar 0.166 OD 0.740 stamp 2



LBV agar 0.033 OD 0.970 stamp 1

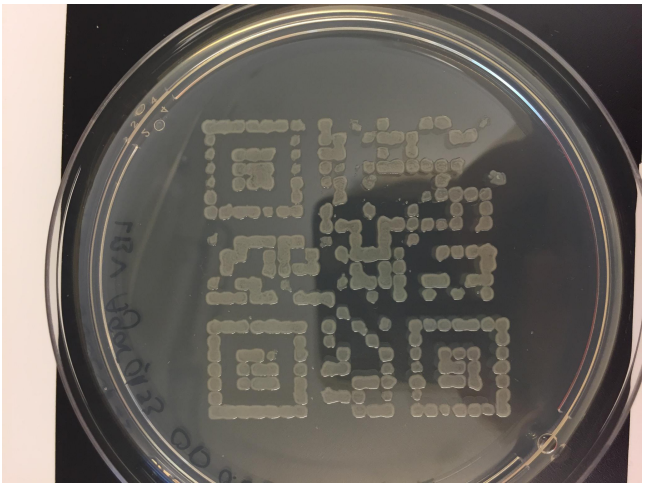
LBV agar 0.033 OD 0.970 stamp 2



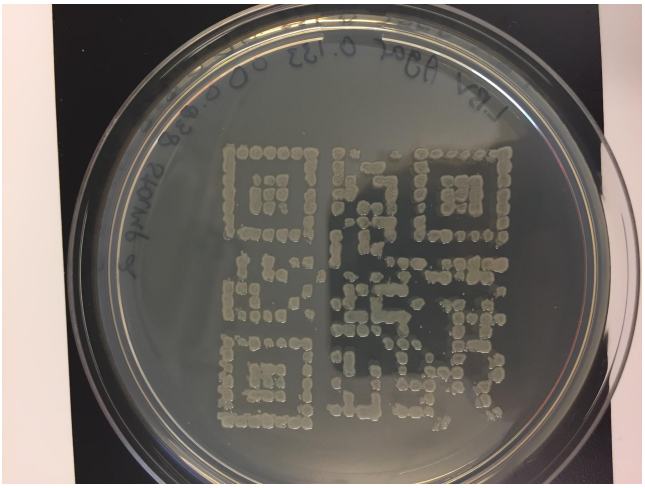
LBV agar 0.1 OD 1.072 stamp 1

LBV agar .01 OD 1.072 stamp 2

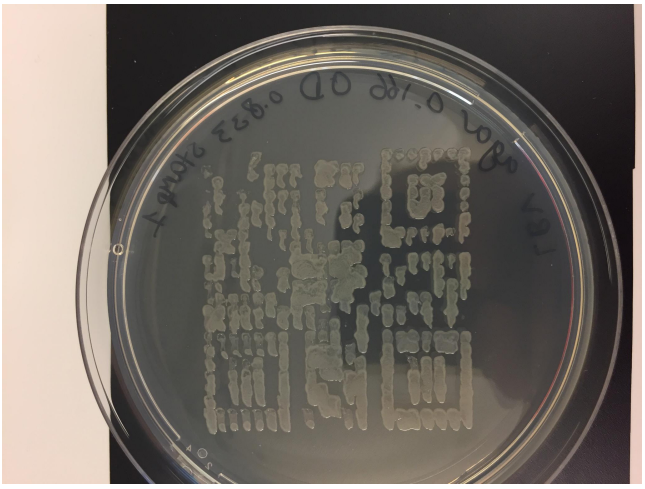
LBV agar 0.133 OD 0.838 stamp 1



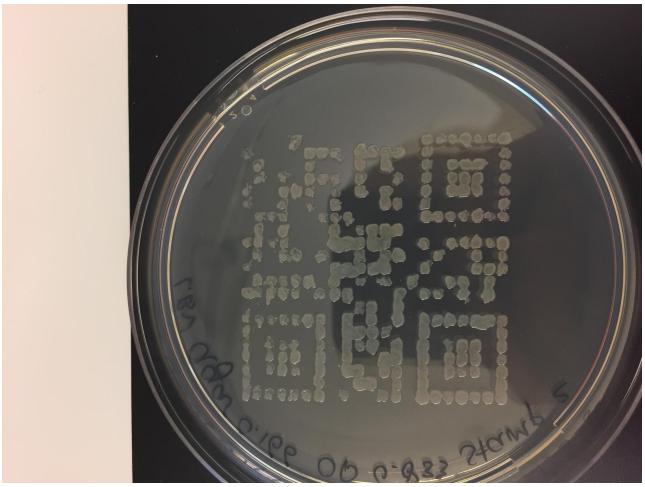
LBV agar 0.133 OD 0.838 stamp 2



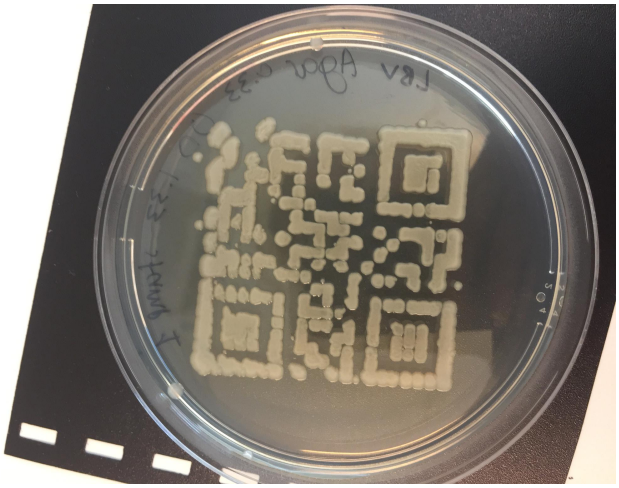
LBV agar 0.166 OD 0.833 stamp 1



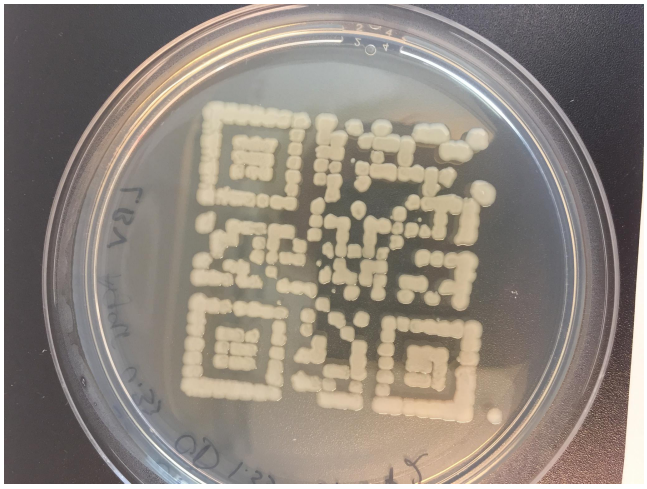
LBV agar 0.166 OD 0.833 stamp 2



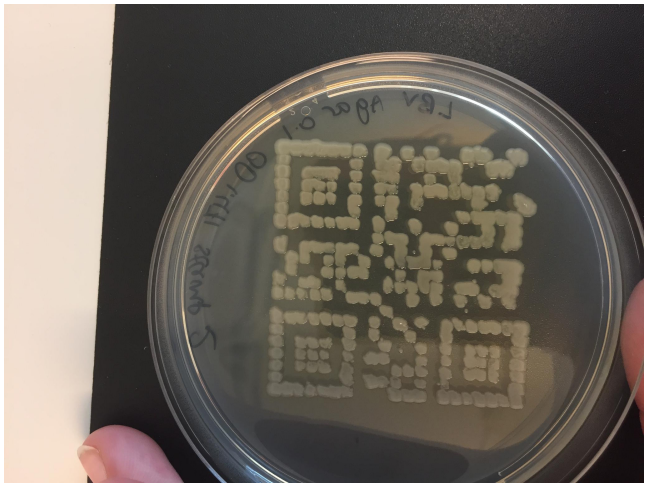
LBV agar 0.033 OD 1.332 stamp 1



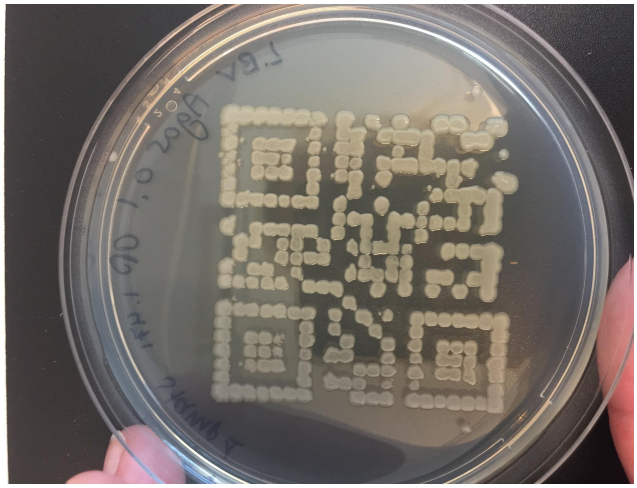
LBV agar 0.033 OD 1.332 stamp 2



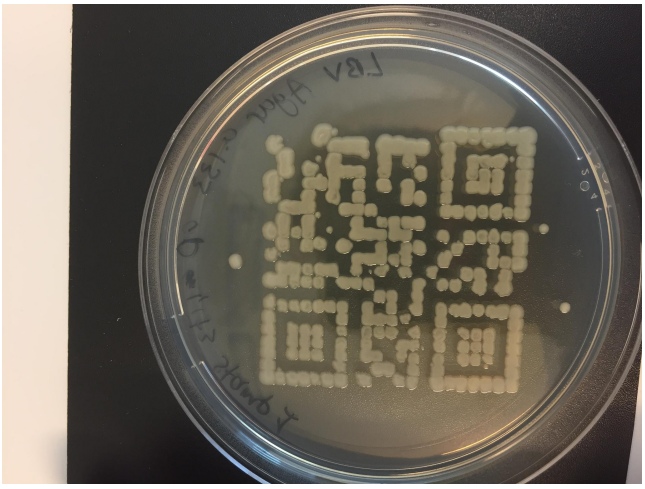
LBV agar 0.1 OD 1.471 stamp 2



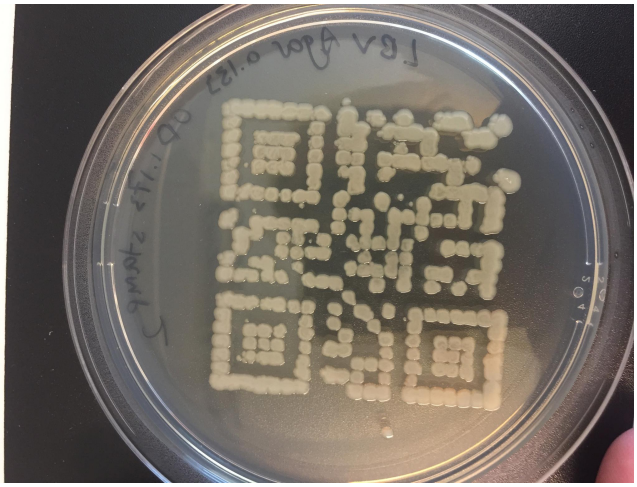
LBV agar 0.1 OD 1.471 stamp 1



LBV agar 0.133 OD 1.173 stamp 1

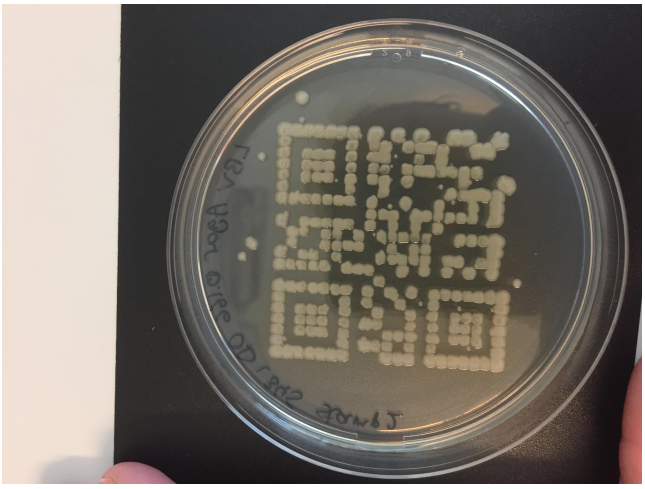


LBV agar 0.133 OD 0.173 stamp 2

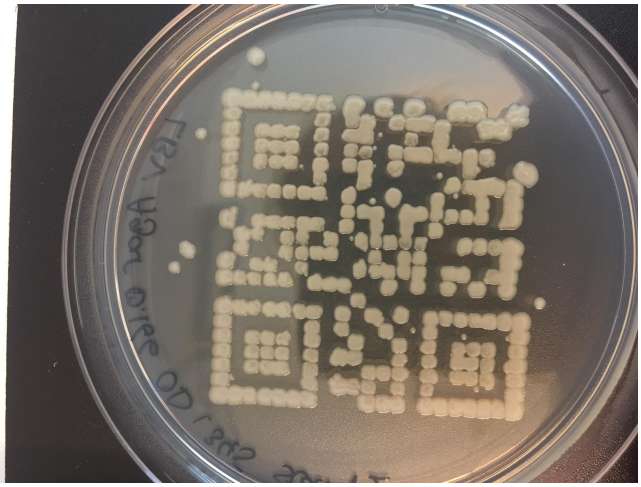


LBV agar 0.166 OD 1.345 stamp 1

LBV agar 0.166 OD 1.345 stamp 2



LBV agar 0.166 OD 1.345 stamp 2



Not much difference between the stamps. the main difference is whether I had shaky hands or not. Conclusion: viscosity of the 'ink' does not really matter.

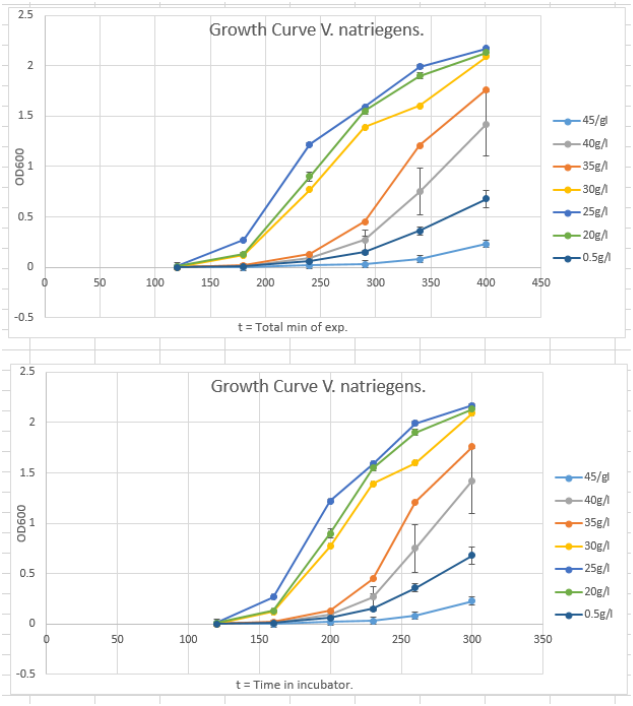
WEDNESDAY, 7/31/2019

WhatsApp Image 2019-07-30 at 09.26.02.jpeg

Results Growth Curve experiment:

Results Growth Curve V Nat dif NaCl.xlsx

Growth Curves V.Nat in NaCL dep medium.png



RECIPY FOR OPTIMIZED MEDIA:

500 ml H20

12.25 g NaCl

1.56 g KCl

23.5 g MgCl.(6H2O)<-- this is for HEXAHYDRATE MgCl, for regular MgCl, recalculate. (concentration should be: 231.4)

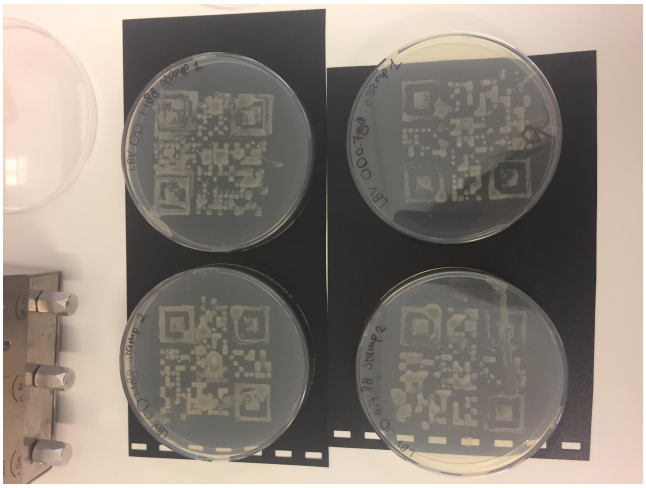
7.75 g/L L broth (contains 0.5 g/l NaCl)

THURSDAY, 8/1/2019

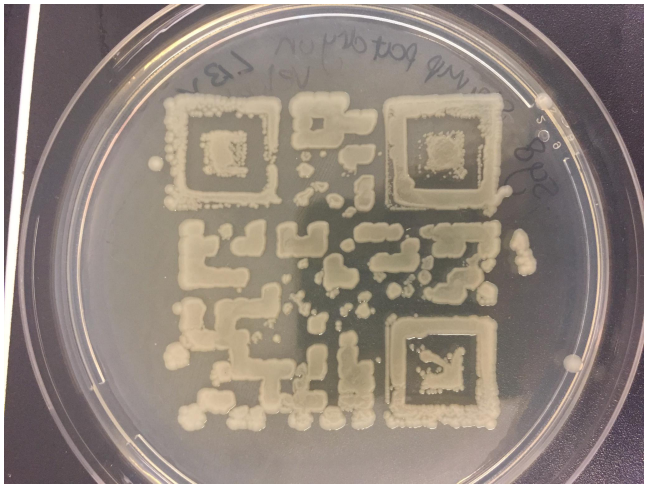
stamp is patted dry on velvet and then left to dry on there for 1 minute before it is stamped on LBV agar plate

2 stamps with OD of 1.188 and 2 stamps with OD of 0.788 and 1 stamp with OD 0.598

left OD 1.188 right OD 0.788



LBV OD 0.598 velvet dried stamp



I feel like the new stamp is a bit bent in the middle because i had to press really hard to get any bacteria in the middle. Also maybe the fridge is too cold or something because there is condens on the agar even though they are laying upside down, this makes the bacteria also smudge through the plate. Still, patting the stamp dry with the velvet could work, I dont feel like these bad results are because of the velvet.

- Design of Constructs (BioBricks)

1. Construct I:
   (LacI regulated promoter + rbs) + Lysis gene + RFP coding device psB1K3.

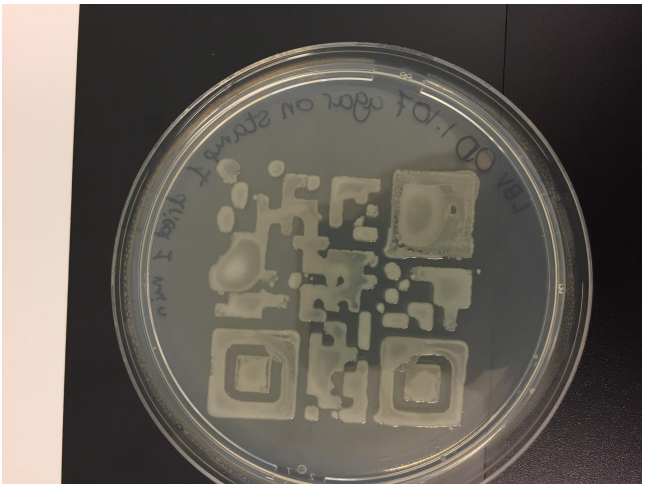
   Procedure:

   3A assembly of the BioBricks cloned in psB1K3 RFP coding device.
   Colonies bearing RFP and w/o RFP appeared on the plates with LB+Km
   Colony PCR using primers VF2 and VR was carried out.

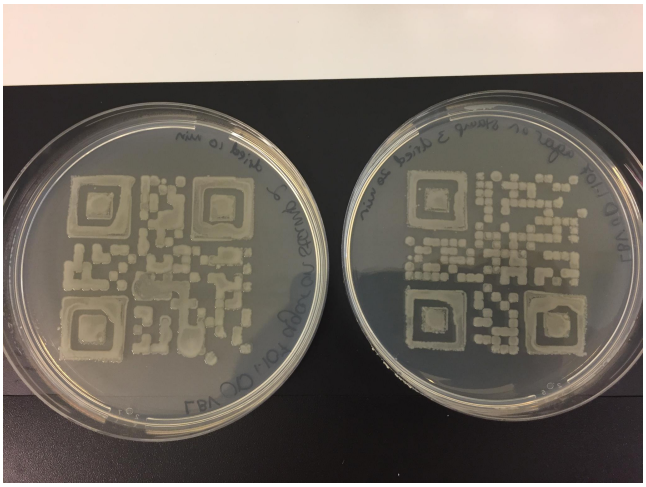
TUESDAY, 8/6/2019

Stamp agar plate on stamp.
stamp was fixed on wooden base with dubble sided stickey tape, it does not move so this works.
the stamp has to dry a long time, 20 min.

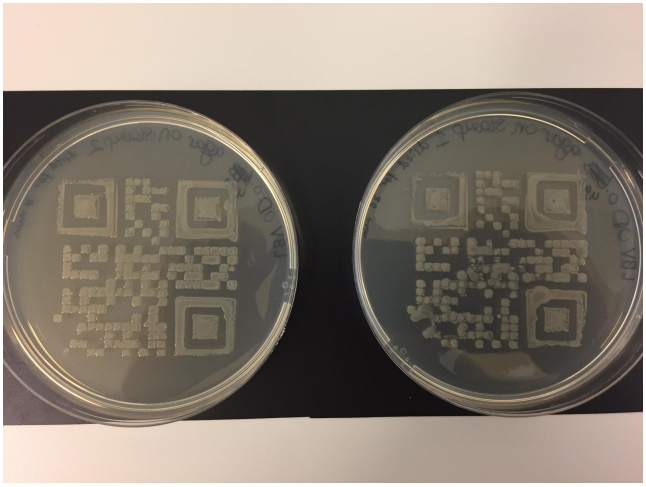
agar on stamp OD 1.107, stamp dried 2 min



agar on stamp OD 1.107 left: stamp dried 10 min. right: stamp dried 20 min



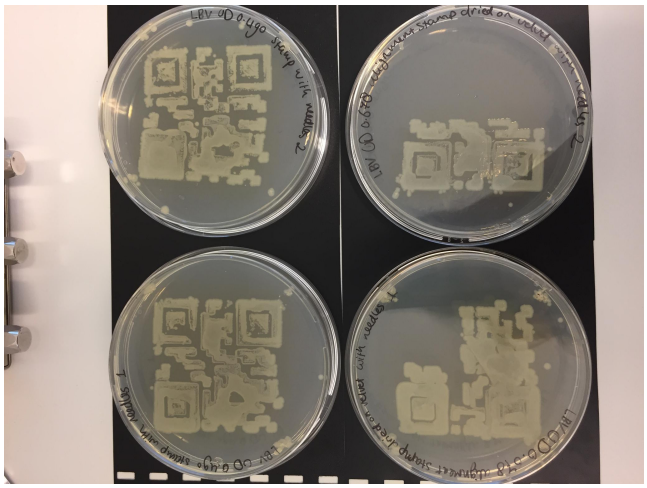
Agar on stamp OD 0.490. The stamp dried for 20 min.



Next time: could try drying the stamp on velvet to make it faster.
Experience: It is easier to remove the agar from the stamp after stamping. Removing the stamp from the agar is harder because there is no handle and thus I shake more and sometimes even touch the agar with my fingers. The stamp was quite hard to remove from the wooden base though, it may have bent a bit during this process.
Observation: when observing these plates it seems that the agar plates may also have moved while removing it from the stamp, because the squares are unequal in width/thickness of the lines.

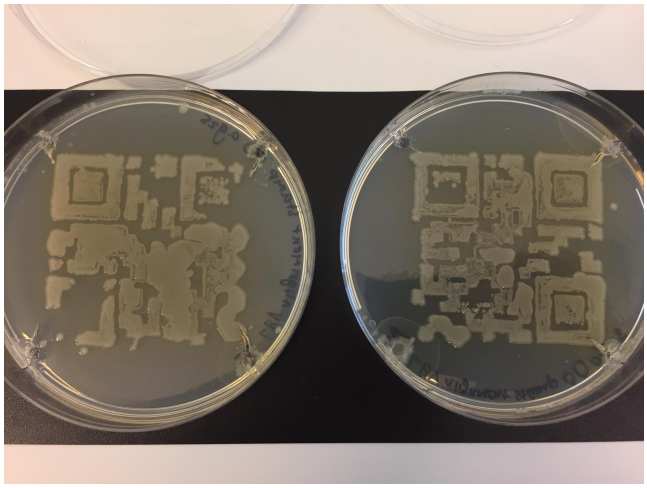
Alignment stamping with needles.
Holes were poked with hot needles on the same places of the two alignment stamps (in the corners).
Experience: the stamping was horrible, the needles did not stay straight so pressing the stamp down did not go well. Also when stamping the second alignment plate, you do not really see the holes the needles make and it is also hard again to get the needles in the same position because they do not stay put but move around.
Next time: not using needles but designing pegs on the stamp already for the same purpose. The pegs do not move, will be in the same position for both stamps and will be visible in the agar, hopefully.

alignment stamp with needles left: OD 0.490 right: OD 0.678



Stamping with 3D printed pegs on the stamp.
Observation: the alignment is fine, the pegs leave clear marks in the agar so you can see where we need to put the second stamp. downside: agar breaks, so maybe do it more carefully next time. Observing the blotchiness the stamp was probably not dry enough yet to start stamping with it.
experience: the stamps are easy to remove from the agar, I do not touch my fingers to the agar. Still, try to do it more carefully to combat the blotchiness and ripping of agar.

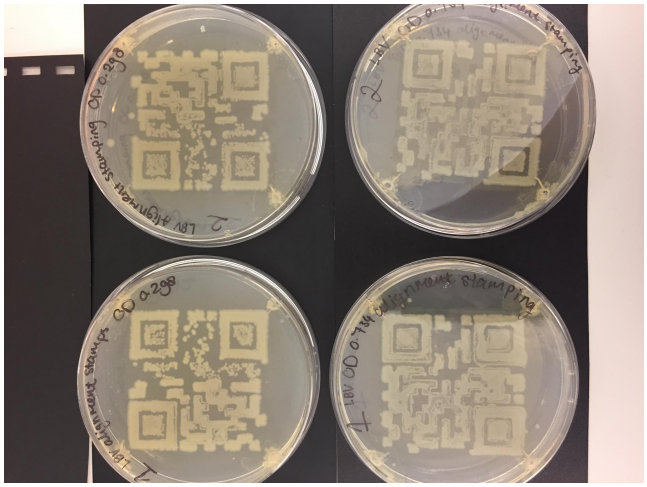
Alignment stamping with pegs. OD 0.925



THURSDAY, 8/8/2019

The stamps need to dry more evenly. I try to dry them on the velvet by tapping them so the excess is removed. Then I pat them on the velvet, then I let them air dry upside down on the velvet, then I start stamping.

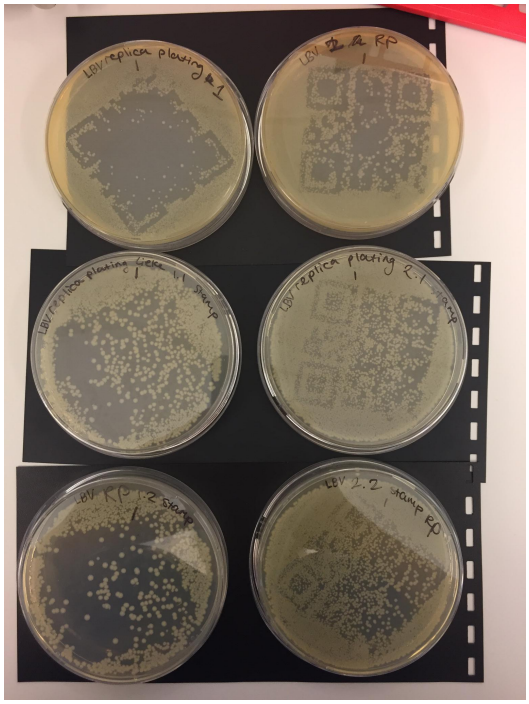
Left: OD 0.298. Right: OD 0.734



Result: they dry quicker and more evenly on the velvet. I pressed them down on the agar plate way to hard which is why the agar is really ripped and the lines are quite thick. Need to try to do it more softly. The aligning is not hard to do at all so this is nice.

Replica plating:
The lasering was a bit tricky, this can also be seen in RP trail 1 where the laser did not successfully laser the QR code. Trial 1 I pressed hard on the velvet, trial 2 I pressed softly. Seeing as these results suck, I should try again.

IMG_0521[1].JPG



FRIDAY, 8/9/2019

Soft stamping.

PROPOSAL BIO-LASER PRINTER:

Idea + Sup info.docx