# Gel Optics Lab Book

**01.08**

Transfer C. Voigt's Bacteria to several plates

+Goal:Transfer C. Voigt's Bacteria to several plates

+Methods: bacteria were plated on Cam plates and no-antibiotic plates

+Results: Bacterial growth was only observed on the "no-antibiotic" plates, This shows that the strain sent does not have pJFR1 plasmid and predectablly no plasmids

**02.08**

Trimetoprim liquid/ agar LB preparation:

+Goal: Prepare plates and Medium with Trimetoprim antibiotic

+Methods:

-weigh 5.0mg of Trimetoprim

-dilute (1/10 dilution) in 0.5mL of DMSO to 10mg/mL

- dilute 250microL in 250mL liquid LB to get 10microg/mL

-dilute 250microL in 250mL of agar LB to get 10microg/mL

    -poor in 11plates

    -place plates in fridge

-Results:250mL of trimetoprim liquid LB, 11plates of trimetoprim LB agar

Transformation of BBa_K1031803 into DH5alpha cells:

+Goal: transform BBa_K1031803 into DHalpha5 cells:

+Methods:

-Place 10uL of dH2O in plate 6 well 19E of 2017 distribution kit

- 2 tubes (1 real+ 1control) of 50µL DH5-alpha thawed on ice for 15min

- 1µL of resuspended DNA added into each tube

-Remaining DNA in the freezer (-20°C)

- Close and incubate on ice for 30 minutes

- Heat shocked at 42°C for 20 seconds

- Incubate on ice for 5 minutes

- Pipette 950µL of LB into each tube

- Incubate at 37°C for 1 hour, shaking at 300 rpm

- Pipette 100µL of each tube on petri plates and spread with glass beads (10%)
- Spin down cells at 6800g for 3 mins
- Discard 800µL of the supernatant
- Resuspend the cells in the remaining 100µL
- Pipette each transformation onto petri plates (90%)
- Incubate overnight at 37°C

+Results: The Negative Control Cam plate (without DNA) had 6 colonies looking like E. coli
Cam plates had colonies, the plate inseminated with the centrifuged cells had more colonies

**<u>03.08</u>**

<u>+Picking K1031803 DH5alpha colonies</u>

+Goal:grow transformed plates for miniprep
+Methods:
- Picked 3 single colonies
- Liquid culture in 10mL LB + Chloramphenicol (co) in 15mL Falcon tube
- Streak every toothpick on Cam plate
- Overnight growth at 37°C, shaking at 130rpm
-Picked colonies from the negative control (no DNA) and restreaked on Cam plate

+Results: 04.08-->LB liquid cultures showed normal growth

<u>Transformation of BBa_K1031803 into DH5alpha cells:</u>
+Goal: transform BBa_K1031803 into DHalpha5 cells:
+Methods:
- 2 tubes (1 real+ 1control) of 50µL DH5-alpha thawed on ice for 15min (kept for longer as cells were not thawing)
- 1µL of BBa_K1031803 DNA from the freezer (-20°C)
- incubated on ice for 30 minutes (35-40 min for dry water bath to turn on)
- Heat shocked at 42°C for 20 seconds

- Incubate on ice for 5 minutes
- Pipette 950µL of LB into each tube
- Incubate at 37°C for 1 hour, shaking at 300 rpm
- Pipette 100µL of each tube on petri plates and spread with glass beads (10%)
- Spin down cells at 6800g(=8000rpm) for 3 mins
- Discard 800µL of the supernatant
- Resuspend the cells in the remaining 100µL (! couldn't discard 800uL, less liquid remaining, left some for plating)
- Pipette each transformation onto petri plates (90%) (pipetted whatever was remaining in there (less than100uL))
- Incubate overnight at 37°C

+Results: Colonies on the 90% plate, 1colony on 10% plate,No colony on the negative control, Plates left in 37C incubator over the weekend

**04.08:**

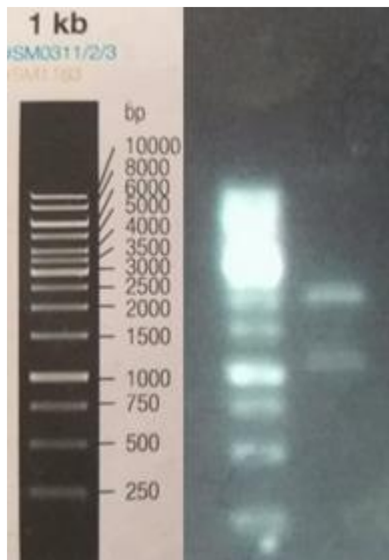
+Experiment: Miniprep and Nanodrop measure of K1031803:
+goal: obtain K1031803 plasmid from DH5alpha cells prepared on 03.08
+Methods:
-Harvested overnight culture with 2min 8000rpm centrifugation (3tubes)
-discarded the supernatant
-added 250uL of resuspension solution and vortexed until pellet was resuspended
-added 250yLof lysis solution and inverted 4-6times
-added 350uL of neutralization solution and inverted 4-6times
-centrifuged at 8000rpm for 5min
-poured supernatant of three falcon tubes in 2 genejet spin column
-centrifuged for 1min -discarded the flow-through
-(added 500uL of wash solution and centrifuged for 30s-discarded supernatant) done twice
-centrifuged for 1min
-transferer the column to clean Eppendorf tubes
-added 50uL of elution buffer and incubated 2mins
-centrifuged for 2min

-collected flow through
+Results:
-nanodrop measured: 123.0ng/uL of DNA
-DNA was frozen at -20C

+Experiment: Digest with NotI and run Gel electrophoresis of K1031803
+Goal: check for the right bands of K1031803 (the insert between the biobrick pre/suffix is 1.06KB long, the rest of the plasmid is 2.07KB long)
+Methods:
-The following were added to a clean eppendorf:
    -Molecular Water
    -10XFastDigest Green Buffer
    -1ug of K1031803 DNA (8.1uL of the 123.0ug/uL solution)
    -1ug of fast digest NotI
-tube was mixed and spinned down and incubated at 37C for 5min
-inactivated with 5min incubation at 65C

-Gel for electrophoresis was prepared by diluting 0.5g of agar into 5mL of TAEbuffer
-Microwaved for 30s - waited to cool down to the touch
-added 5uL of CyberSafe
-Poured in the mold with the Well comb
-placed 10uL of digested K1031803 and 2uL of 6XLD (loading dye) in new Eppendorf
-placed 10uL of resulting mix in gel well
-run the gel with a 1Kb scale for 30min

+Results:




-Two bands were visible (1kB and 2kB) corresponding to our biobrick size

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**<u>07.08:</u>**

<u>Transformation of pJFR1 into DH5alpha cells:</u>

<u>+Goal: transform pJFR1 into DHalpha5 cells:</u>

+Methods:

- 2 tubes (1 real+ 1control) of 50µL DH5-alpha thawed on ice for 15min
- 10µL of mH2O was transferred into the eppendorf provided by Voigt's lab labelle PJF1.

-1uL of the suspended DNA was Nanodropped and the concentration obtained was 49.9ng/uL

-1uL of the suspended DNA (49.9ng total DNA) was pipetted into a 50µL DH5-alpha eppendorf, 1uL of mH2O was pipetted into a 50µL DH5-alpha eppendorf (control)

- incubated on ice for 30 minutes (35-40 min for dry water bath to turn on)
- Heat shocked at 42°C for 20 seconds
- Incubate on ice for 5 minutes
- Pipette 950µL of LB into each tube
- Incubate at 37°C for 1 hour, shaking at 300 rpm
- Pipette 100µL of each tube on petri plates and spread with glass beads (10%)
- Spin down cells at 6800g(=8000rpm) for 3 mins
- Discard 800µL of the supernatant

- Resuspend the cells in the remaining 100µL (! couldn't discard 800uL, less liquid remaining, left some for plating)
- Pipette each transformation onto petri plates (90%) (pipetted whatever was remaining in there (less than100uL))
- Incubate overnight at 37°C

+Results: Colonies on the 90% plate, 1colony on 10% plate,No colony on the negative control, Plates left in 37C incubator over the weekend

**08.08-09.08:**

pJFR1 colonies were picked grown and minpreped by Aleks
pJFR2 was transformed in DH5alpha

**10.08**

+10.08A:Goal: PCR of pJFR1 and K1031803
+methods:
--Minipreped K1031803 (123ng/uL) and pJFR1 (180.9ng/uL) DNA was diluted (1/10) in mH2O.
-Primers o.17.005, o.17.006, o.17.009 ano.17.010 were resuspended in mH2O to 100uM as advised by IDT
--Primers were diluted to 25uM by placing 1uL of 100uM primer into 3uL of mH2O

-36uL of mH2O
-10uL of 5X phusion HF buffer
-1uL of dNTP
-1uL of the 25uM of forward and reverse primers (to reach conc of 0.5uM)
-1uL of associated DNA,
-0.5uL of Phusion
Were added to PCR tubes

Tubes were PCRed with associated annealing temperature:
DNA// Primers//Annealing
pJFR1//o.17.005 and o.17.006// 63.2C
K1031803//o.17.009-o.17.010//63.2C

+Results: PCR products were run on gel (twice), no DNA bands were observed, PCR products were stored at -20C

**11.08:**

+11.08A:Goal run gel for PCR product of 10.08A (pJFR1 and K1031803)
+Methods:
-Gel for electrophoresis was prepared by diluting 0.5g of agar into 50mL of TAEbuffer
-Microwaved for 30s - waited to cool down to the touch
-added 5uL of CyberSafe
-Poured in the mold with the Well comb
-loaded gel with 5uL of PCR products pJFR1 and K1031803 from 10.08A
-Laoded gel with 1KB Ladder
-Run gel for 30min
+Results: The full ladder was observed but no mark was observed on the lanes of the PCR product

+11.08B:Goal: Transformation of pJFR3, and K516030 in DHalpha5 Cell
+Methods:10uL were added to the well 9I of plate 1 2017 and to the pJF3 tubes of Voigt's lab.
-Suspended pJFR3 DNA was nanodropped and a reading of 9.4ng/uL was observed
-DHalpha5 were thawed on ice for 1hour (! not 15 min!)
-1uL of the suspended DNA (9.4ng total DNA for pJFR3) was pipetted into a 50µL DH5-alpha eppendorf, 1uL of mH2O was pipetted into a 50µL DH5-alpha eppendorf (control)
- incubated on ice for 30 minutes (35-40 min for dry water bath to turn on)
- Heat shocked at 42°C for 40 seconds
- Incubate on ice for 5 minutes
- Pipette 950µL of LB into each tube
- Incubate at 37°C for 1 hour, shaking at 300 rpm
- Pipette 100µL of each tube on petri plates and spread with glass beads (10%)
- Spin down cells at 6800g(=8000rpm) for 3 mins
- Discard 800µL of the supernatant

- Resuspend the cells in the remaining 100µL
- Pipette each transformation onto petri plates (90%) (pipetted whatever was remaining in there ( a bit more than100uL))
- Incubate overnight at 37°C

+Results: No growth on pJFR3 plates and control, no growth on K516030 plates and control Transformation needs to be restarted!

+11.08C:Goal Prepare Cam plates and CAM and Trim LB medium

-5mg of Trimethoprime were placed in 50mL of LB medium (0.5mg/mL= 10X solution)

-17.5mg of Cloramphenicol were placed in 50mL of LB medium (0.35mg/mL = 10Xsolution)

-500uL of 10X solution was placed in 4.5mL of fresh LB medium ( 1X solution)

-5mL falcon tubes and 10X solution were stored in the fridge.

**<u>12.08:</u>**

<u>+12.08A:Goal: PCR of pJFR1 and K1031803</u>

+methods:

- K1031803 (123ng/uL) and pJFR1 (180.9ng/uL) DNA was diluted (1/10) in mH2O.

-Primers o.17.005, o.17.006, o.17.009 ano.17.010

    -59.6uL of mH2O

    -16uL of 5X phusion HF buffer

    -1.6uL of dNTP

    -0.5uL of the 100uMof forward and reverse primers ( Primers o.17.005, o.17.006, for pJF1; o.17.009 and.17.010 for K1031803, to reach conc of 0.625uM)

    -1uL of associated DNA (!**mistake**: puts way too much template DNA!)

    -0.8uL of Phusion

-Were added to eppendord tubes to a total of 80uL

-The 80uL were split in 4 PCR tubes (A, C E and H) which were run at different annealing -temperature (A:70C, C:67C, E:60.7C, H:55C)

-Tubes were PCRed with associated annealing temperature:

-PCR was run for 30cycles with 20uL volume

+Results:
See 12.08C

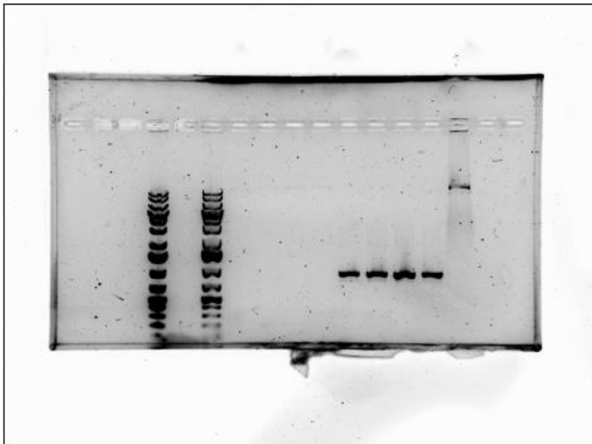
+12.08B: +Goal Digest pjFR1 with XbaI to observe 3Kb, 2.3Kb and 5.8kB bands

+Methods:
-fast digest protocol
-neutralised at 65C for 20min
+Results: check 12.08C gel

+12.08C::Goal run gel for PCR product of 10.08A (pJFR1 and K1031803)

+Methods:
-Gel for electrophoresis was prepared by diluting 0.5g of agar into 50mL of TAEbuffer
-Microwaved for 30s - waited to cool down to the touch
-added 5uL of CyberSafe
-4uL of PCR products pJFR1 (A,C,E,H)and K1031803 (A,C,E,H)from 12.08A were placed in eppendorf containing 1uL of 6X LD dye
-Laoded gel with 1KBplus Ladder in following order:
Ladder /pJFR1 (A/C/,E/H) / K1031803 (A/C/E/H)/ pJFR1 XbaI digest from 12.08B
-Run gel for 33min
+Results: K1031803 PCR product produced a band at 1Kb: PCR was successful
pJFR1 produced a slight band above 20Kb this was probably caused by the large amount of DNA (1uL) placed in the PCR reaction. This 20Kb band was also found (stronger) on the XbaI digest of pJF1. This was probably caused by the larger ammount (2uL) placed in digestion tube

PAUL 08-12 pJFR1 A,C,E,H K1031803 A,C,E,H pJF1 XbaI DIGEST



<u>+12.08D: Goal: Pick pJF2 colonies from Aleks (10.08) and grow in spectinomycin media</u>

+Methods:

-Colonies were labelled 1 to 3 and placed in Spec media and incubated for 48H at 37C

-Labelled colonies streaked on spec plate (plate used before but no growth + 500uL of fresh spectinomycin LB streaked on the plate before streaking colonies)

+Results: growth of the three colonies on the spec plates, growth of control colony

**+14.08:**

+14.08A: Miniprep pJF2

+Methods:

-miniprep pJF2 colonies from 12.08D

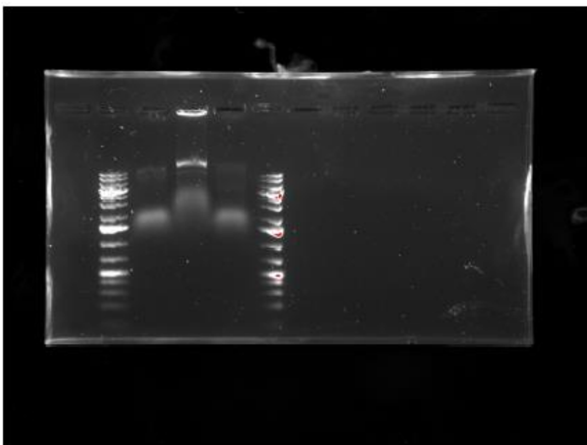
+Results:

-three tubes with concentration: 1: 63.3ng/uL, 2: 229.3ng/uL, 3:144.1ng/uL

+14.08B: +Goal:digest pJF2(2) from 14.08A and pJF1(2) from 12.08D with PstI FD. We should observe a 3.1Kb a 0.77kB and a 1.95Kb fragments for pJF2 and a 5.2Kb and 5.8Kb Fragment for pJF1

+Methods: Followed Thermo protocol
-ran a gel and added the pJFI XbaI digest from 12.08B
+Results
Left to Right: Ladder// pJF1 + PstI // pJF1XbaI // pJF2 PstI// ladder:

PAUL 14.08 pJF1 PJF2 DIGESTS



Conclusion: the bands are inconclusive we do not observe the pattern expected

**+15.08.17**

+15.08A Goal: as K156030 and pJF3 transformation didn't give any colonies and as pJF2 and pJF1 digestion did not give the right bands when digested. pJF1, pJF2, pJF3 and K156030 were transformed in Dh5alpha cells

+Methods:
- 5 tubes (4 real+ 1control) of 50µL DH5-alpha thawed on ice for 15min
-1uL of the suspended DNA of pJF1, pJF2, pJF3 and K516030 was pipetted into a 50µL DH5-alpha eppendorf, 1uL of mH2O was pipetted into a 50µL DH5-alpha eppendorf (control)

-  incubated on ice for 30 minutes
- Heat shocked at 42°C for 20 seconds
- Incubate on ice for 5 minutes
- Pipette 950µL of LB into each tube
- Incubate at 37°C for 1 hour, shaking at 300 rpm
- Pipetted 100µL of each tube on petri plates and spread with glass beads (10%)

-Mistake in the controls: placed 100uL the transformed cells on the 10% part of the control plate (**do not pay attention to the 10% controls)**

- Spin down cells at 6800g(=8000rpm) for 3 mins
- Discard 800µL of the supernatant
- Resuspended the cells in the remaining 100µL
- Pipetted each transformation onto petri plates (90%),

-Pipetted the right control cells on the 90% control plates

` Incubate overnight at 37°C

+Results:

K516030: 90%: 59 colonie; 10%: 2colo, Control:0

pJF1: 90%: tntc;10%:tntc; control:0

pJF2: Spectinomycin solution added didn't dry so bacteria made lawn, not a culture: control had also a lawn of bacteria!

pJF3:90%: TNTC; 10%164, Control:0

**<u>15.08B+Goal: PCR pJF1(1st transfo), pJF2(1st transfo), mRFP1 (haotian's) and 2 PCR controls (K592009 and I732100):</u>**

+methods:

- pJFR1(1) (180.9ng/uL) and pJFR2 (144ng/uL), mRFP DNA was diluted (1/100) in mH2O.

-Primerso.17.007, o.17.008, o.17.015, o.17.016, o.17.029, o.17.030 were resuspended in mH2O to 100uM as advised by IDT

    -36.5uL of mH2O
    -10uL of 5X phusion HF buffer
    -1uL of dNTP

-0.5uL of the 100uM of forward and reverse primers (to reach conc of 1uM) (pJF1: 17.005, o.17.006, pJF2: 17.007, o.17.008, mRFP1: .17.015, o.17.016 , Control1/2:.17.029, o.17.030.)

-1uL of associated DNA (final DNA mass:,pJFR1(1):1.80ng; pJFR2: 1.44ng, mRFP1:4-5ng, control)

-0.5uL of Phusion

Were added to PCR tubes

Tubes were PCRed with associated annealing temperature:

DNA// Primers//Annealing//Elongation time (s)//product length

pJFR1//o.17.005 and o.17.006// 63.2C// 3min//5.9kb

pJFR2//o.17.007-o.17.008//65.3C//1min10s//2.2kb

mRFP///o.17.015-o.17.015//65.2C//30//0.87kb

K592009//o.17.029-o.17.030//63.5C//30//0.67kb_________I732100//o.17.029-o.17.030//63.5C//30//1.1kb

+Results:

**+16.08.17:**

**16.08A: Run gel for PCR 15.08B:**

+methods

-Gel for electrophoresis was prepared by diluting 0.5g of agar into 50mL of TAEbuffer

-Microwaved for 30s - waited to cool down to the touch

-added 5uL of CyberSafe

-4uL of PCR products from 15.08A were placed in eppendorf containing 1uL of 6X LD dye

-Laoded gel with 1KBplus Ladder in following order:

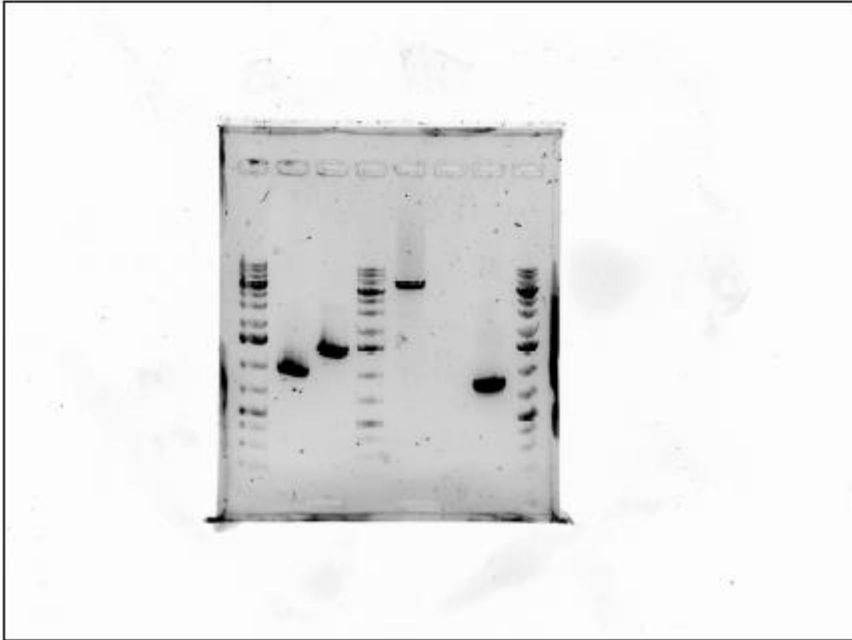
Ladder /Control1// /control2// Ladder/pJF1// pJF2//mRFP

Run gel for 25min

+Results:

Ladder /Control1// /control2// Ladder/pJF1// pJF2//mRFP

PC 16.08 C1 C2 PJF1 PJF2 mRFP



Both control worked, pJF1(2,T1) worked, mRFP1 worked.

**+16.08B: Picked Colonies from 15.08A;**

+Methods: picked colonies for pJF1, pJF2, pJF3 and K516030

For pJF2 bacteria were picked on the lawn in 3 different places

For all plates, control was sampled by swabbing the control plates of the transformation and streaked (even if control did not give visible colonies)

**+17.08.17:**

**17.08A: Miniprep pJF1, pJF2, pJF3, K516030 from 16.08B**

+methods: The protocol was run once with tubes 1 and 2 of each culture but gave very little DNA for all samples. A shitty pipette giving little accuracy was used. The minprep was repeated with cultures 3 and another miniprep kit and other pipetted which worked fine.

-0.5mL of cultures were placed in 0.5mL of 60% glycerol for culture stock and frozen at -20C

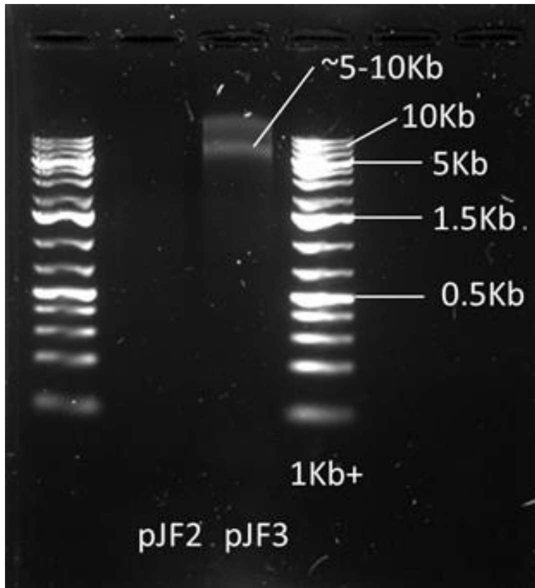
-Harvested overnight culture with 2min 8000rpm centrifugation (3tubes)

-discarded the supernatant
-added 250uL of resuspension solution and vortexed until pellet was resuspended
-added 250yLof lysis solution and inverted 4-6times
-added 350uL of neutralization solution and inverted 4-6times
-centrifuged at 8000rpm for 5min
-poured supernatant of three falcon tubes in 2 genejet spin column
-centrifuged for 1min -discarded the flow-through
-(added 500uL of wash solution and centrifuged for 30s-discarded supernatant) done twice
-centrifuged for 1min
-transferer the column to clean Eppendorf tubes
-added 50uL of elution buffer and incubated 2mins
-centrifuged for 2min
-collected flow through
+Results:
-nanodrop measured:
pJF1(T2-3): 192.6-196.9.0ng/uL; A260/A280:1.98-1.99
pJF2(T2-3):99.9-100.7ng/uL; A260/A280:2.06-2.07
pJF3(3):  102.2-102.2ng/uL; A260/A280: 1.75-1.69
K516030(3): 165.3 - 154.9 ng/uL; A260/A280:2.01-2.01

**<u>17.08B: Digest pJF2, pJF3 with XbaI and PstI:</u>**

+Methods:
-The following were added to a clean eppendorf:
    -Molecular Water 15uL
    -10XFastDigest Green Buffer: 2uL
    -7uL of pJF2 or pJF3 DNA (total mass:0.7ug)
    -1ug of fast digest PstI and 1ug of XbaI
-37C for 5min:

Results:
Left to right: 1Kb+//pJF2//pJF3//1Kb+




pJF3 is supposed to give two close bands: 3.7Kb and a 4.4Kb. However here the gel is inconclusive

**+18.08.17**

+18.08A:

Digest pJF2 and pJF3 with NotI: should observe a 5.8Kb for pJF2 and a 0.8Kb 2.4Kb and 6.2Kb for pJF3

-Methods, Digest and gel load made by Aya

Results

The bands are extremely faint but pJF3 definetely shows several bands as expected and pJF2 shows one also as expected.

**<u>18.08B: Goal PCR pJF2, PJF3 and K516030 as a positive control:</u>**

<u>+methods:</u>

- pJFR2(T2-3) (100ng/uL) and pJFR3 (T2-3) (102ng/uL), K516030(160ng/uL)

-Primers o.17.007, o.17.008, o.17.015, o.17.016, o.17.029, o.17.030 were resuspended in mH2O to 100uM as advised by IDT

-37uL of mH2O

-10uL of 5X phusion HF buffer

-1uL of dNTP

-0.5uL of the 100uM of forward and reverse primers (to reach conc of 1uM) ( pJF2: 17.007, o.17.008,pJF3: 17.013, o.17.014 ;K516030: .17.003, o.17.004 )

-0.5uL of associated DNA (final DNA mass:,pJFR2(T2-3):1.ng; pJFR3(T2-3): 1.0ng, K516030:1.6ng, l)

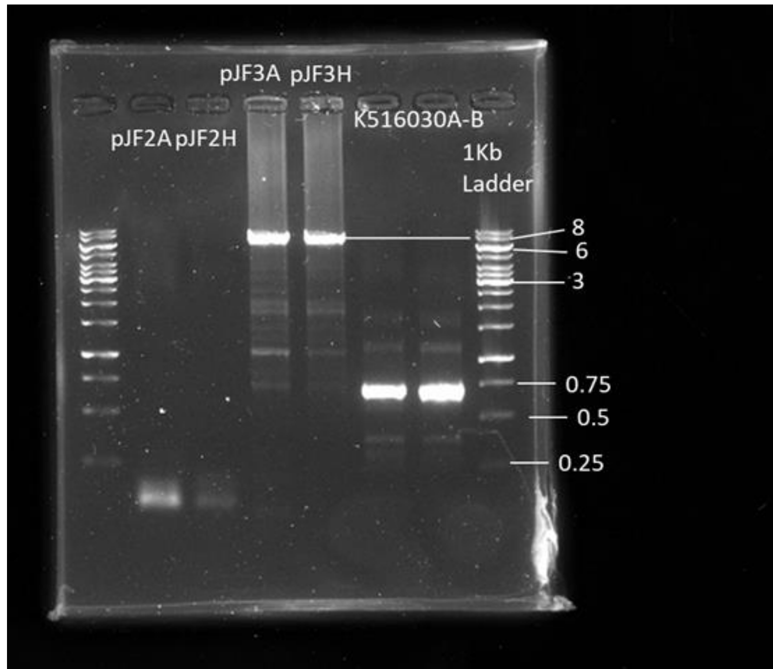
-0.5uL of Phusion

Were added to PCR tubes

Tubes were PCRed with associated annealing temperature:

DNA// Primers//Annealing//Elongation time (s)//product length
pJFR2//o.17.007 and o.17.008// A:62.5C, H:59C// 1min15s//2.3kb
pJFR3//o.17.013-o.17.014//A:63.5C, H:60.0C//3min50//7.3kb
K516030///o.17.003-o.17.004//63.4C//30s//0.87kb

Results:




pJF3A and H worked, K5160390 Worked.
pJF2 didnt work. But pJF2A produced a stronger >0.25Kb band

**<u>19.08.17</u>**

<u>+Goal Prepare pJF1(T1-2), pJF2, and mRFP PCR product for Golden Gate clonning</u>

<u>+Methods: DpnI Digestion +QIAQuick PCR purification</u>

<u>+DpnI digestion:</u>

-DpnI FD protocol (with inactivation)

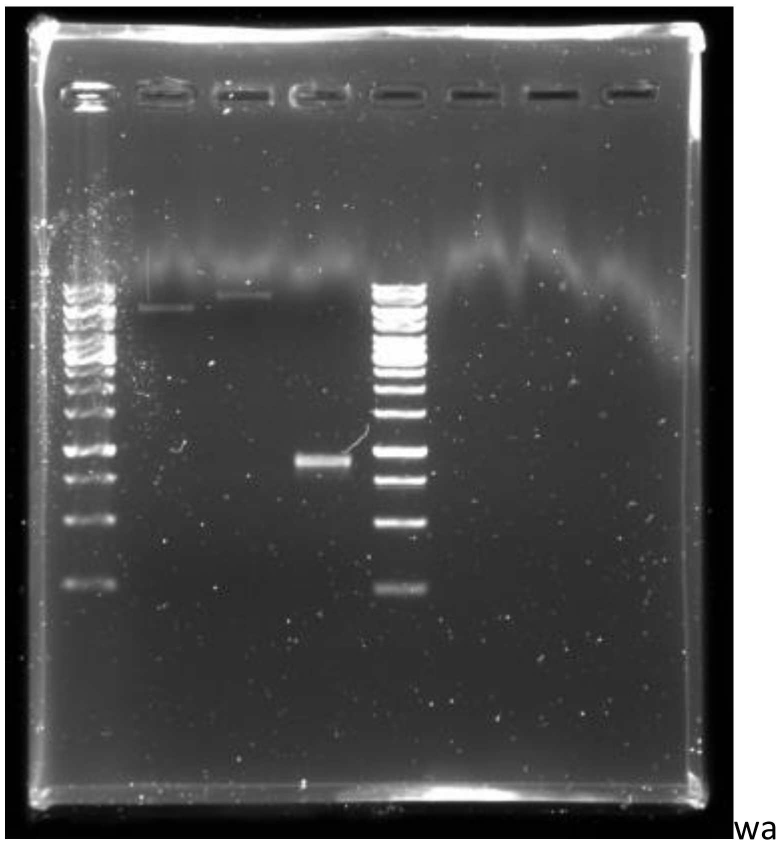
<u>+QIAquick:</u>

-100uL added to the 20uL volume obtained from DpnI digestion (5x volume added)

-120uL volume of buffer + DNA placed in QIAQuick and spinned at 13Krpm for 60s

-Washed with 750uL of PE buffer and centrifuged 60s

-flowthroug discarded and recentrifuged for 60s
-column placed in fresh Eppendorf (1.5mL)
-30uL of EB buffer placed in column, left for 1min and spinned for 60s
-flowthrough placed in column again and reflushed by spinning for 60s
-5uL of resulting product loaded with 1mL of Loading buffer and electrophesised.
+results:
Left to right: Ladder 1Kb// pJF1(T1-2)// pJF3 // mRFP// 1Kb ladder

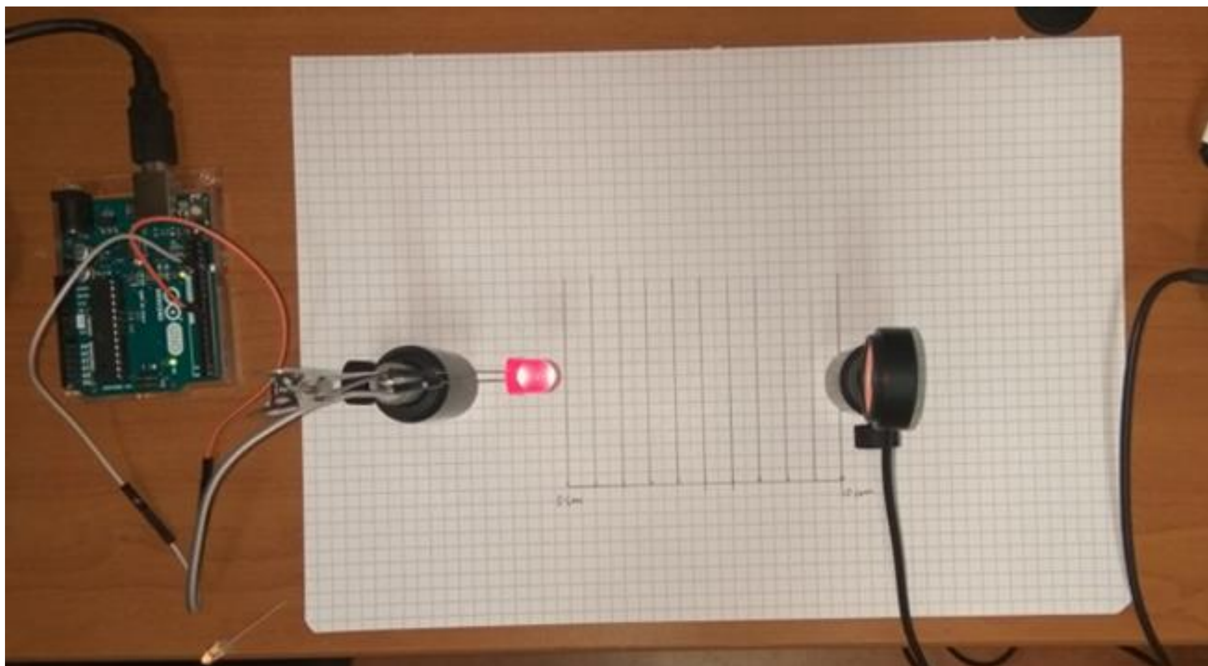

wa

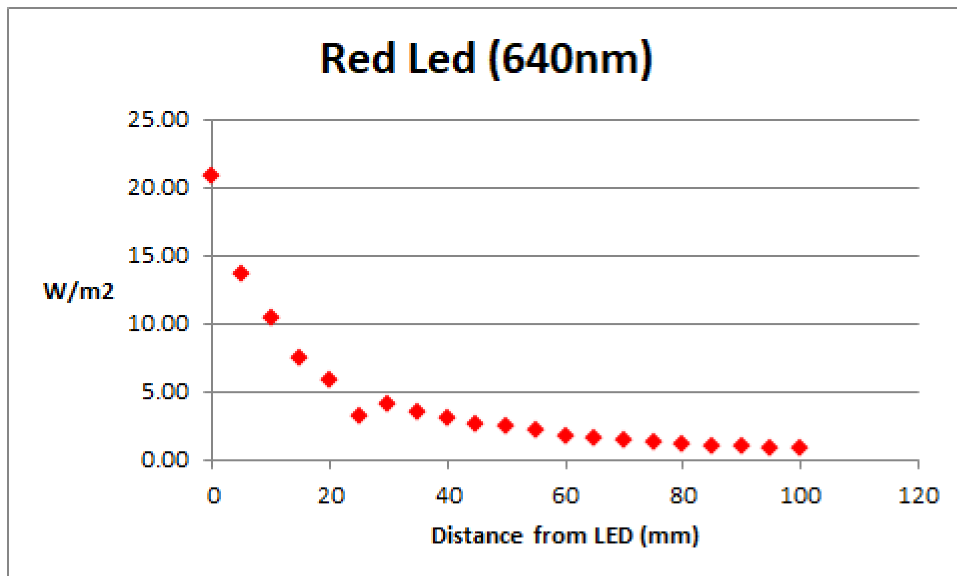
**31.08.17**

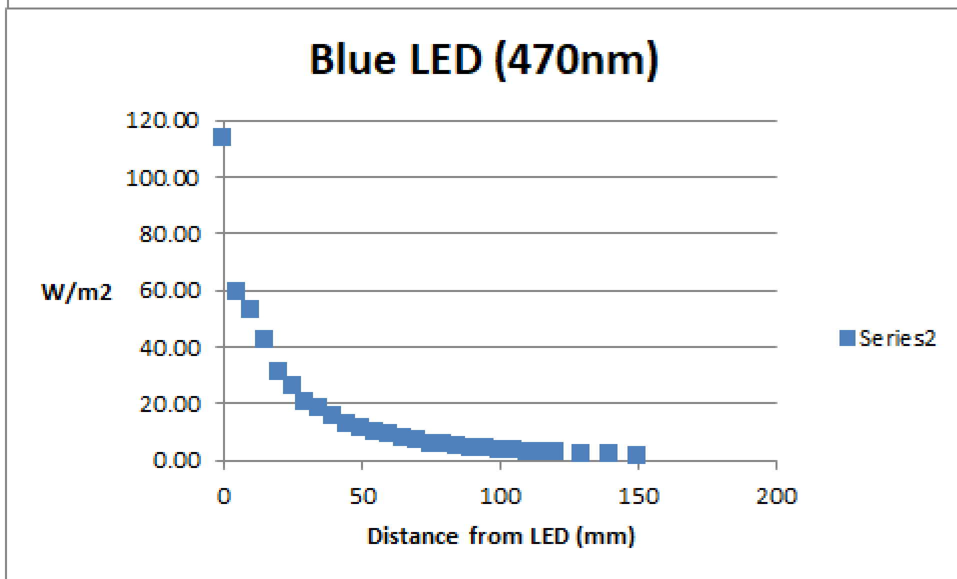
M9 media making:
+added 780mL of distillied water
+200mL of 5x M9 media
+200uL of 1M $MgSO_4$
+20mL of 20% glucose
+100uL of 1M $CaCl_2$
+autoclaved

+M9 media with 0.5% Gelrite:
+Autoclaved M9 media (0.33L)
+1.66g of Gelrite
+autoclaved

+31.08A: measure light itensity of Blue and Red led.
+red and blue leds were powered with arduino uno(+5V)
+Light intensity was measured with photosensor S120VC (Thorlabs) and Power Meter Kit PM100D (Thorlab)






The blue and red light intensity needed are blue=2.8nm , red=11.1nm , therefore the distance from the LED must be blue=11-12cm, red=1-2cm

**05.09:**

+goal: Find the concentration of gel which sets for Agar, agarose. Gelrite

+methods: Gels were prepared (autoclave) and plated at:

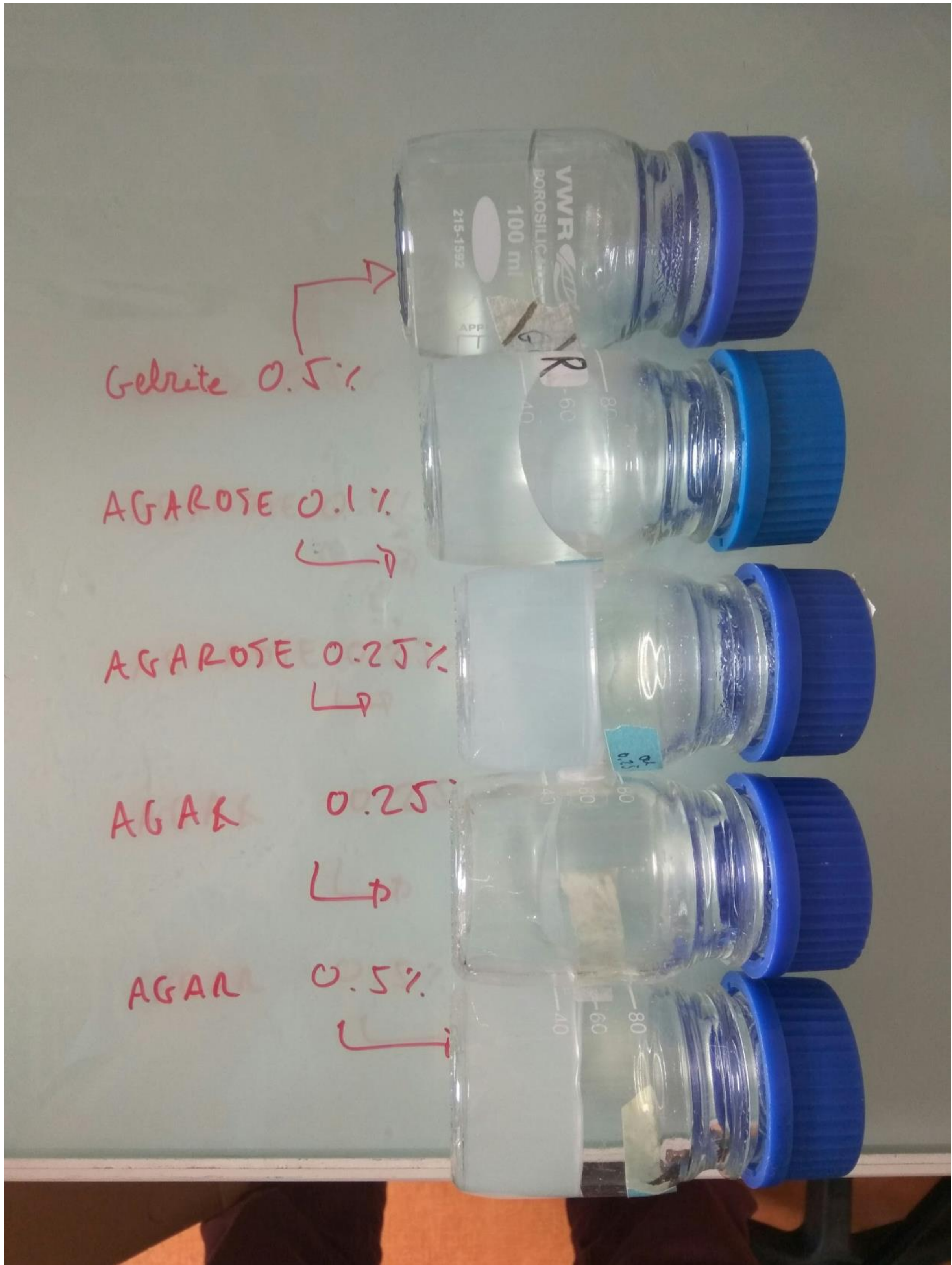
| Gelrite | 0.5% (munich experiments) | 0.25% | 0.15% | 0.05% |
|---|---|---|---|---|
| Agarose | 1%(manufact uers | 0.5% | 0.25% | 0.1% |

| | | | | |
|---|---|---|---|---|
| | recommand.) | | | |
| Agar | 1% (manufacturers recommand.) | 0.5% | 0.25% | 0.1% |

Plates were left to cool and oriented vertically to check if gel remain on the plate

Results:

Gelrite stands well at 0.5% - 0.25% was very liquid




Agarose: 0.25% was very solid - 0.1% was liquid

Agar:0.5% very solid - 0.25% liquid

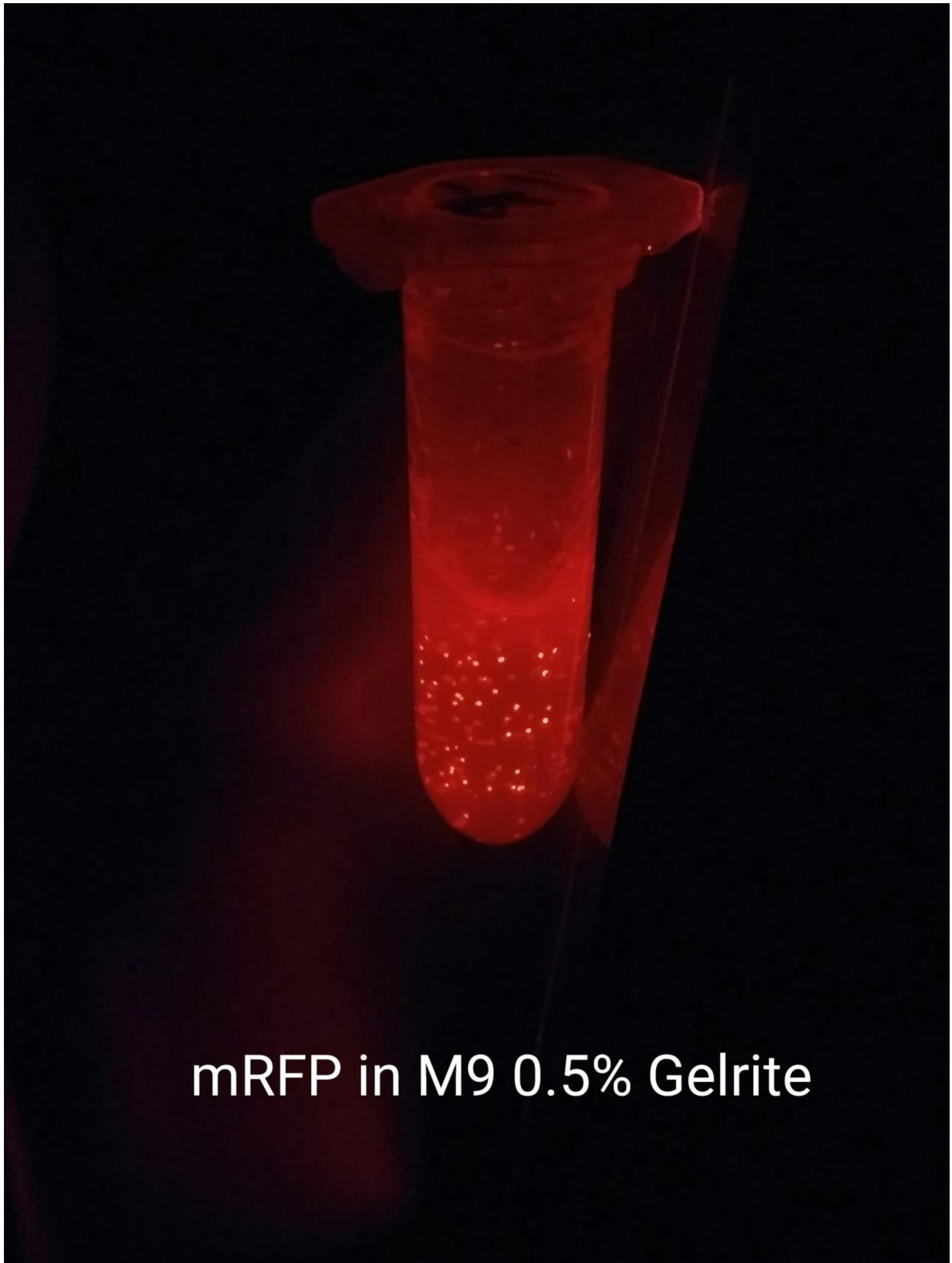
Goal: record transmittance of lowest concentration of solid gel:
Result:

**12.09:**

**Goal: dilute mRFP E. coli in gelrite M9**

Methods: mRFP E. coli was grown overnight in cloremphenicol
The OD600 of the culture diluted to 50% was measured to 26.6
The culture was diluted to an OD600=2.43 to obtain a working culture
The working culture was serially diluted (1/10) 8times
The 6th, 7th, 8th dilution were plated (0.5mL on agar plate)
The 5th, 6th, 7th dilutions were diluted (1/10: 100uL in 900uL falcontube) in M9Gelrite with cloremphenicol at 50C which was immediately cooled in at 25C water batch

Results: the plated count was 45, 6 and 0 for the 6th, 7th, 8th dilution indicating a working cell density of 7.95CFU/mL.
The falcon tubes gave 24.5, and 2 cfu/mL for the 7th and 8th dilution suggesting that diluting in 50C M9 doesn't induce bacteria killing




**18.09:**

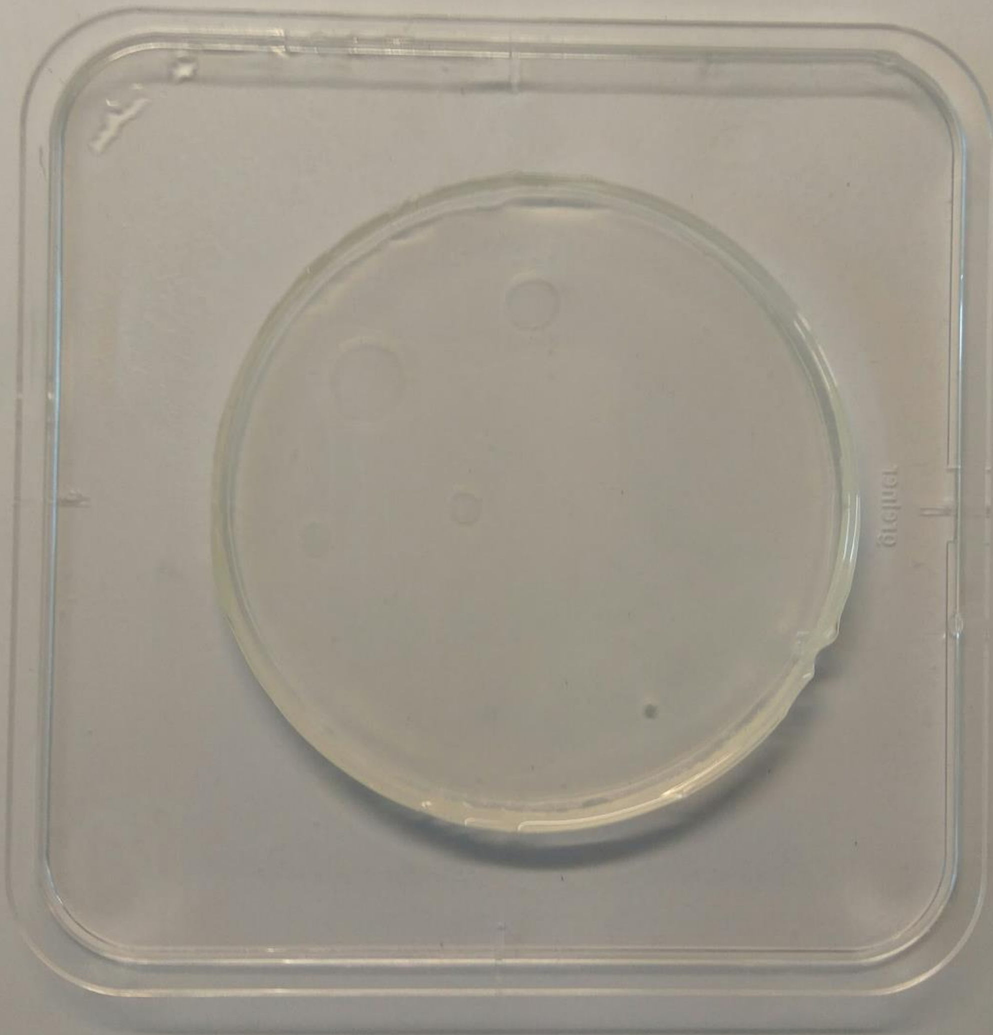
**<u>Goal: test lowest Alginate at which solid gel is still obtained</u>**

Methods: Alginate (0.5, 1, 1.5 , 2% W/V) and CaCl2 100mM was autoclaved

**19.09:**

10mL of 2.5% LB agar containing 100mM of CaCl2 was set in plates
30mL of 0.5; 1; 1.5; 2% Alginate was pooted on top of solid agar.
Plates were left at room temp for 24h
Results:
At All aglginate concentrations, alginate layers solidified.
At 0.5 and 1% the Alginate layer did not stick to the agar layer allowing to detach the disc of alginate from underlying agar

At 1.5 and 2% the Alginate had fused with agar preventing the detachment



**20.09**

Setting low concentration Alginate on top CaCl2 agar:

Goal: as previous experiments showed that 0.5%. Alginate produced strong gel when set overnight over agar, I tried with 0.25% and 0.1%:

Methods: 10mL of 1.5% agar containing 100mM of $CaCl_2$ was set in plates 30mL of 0.25, 0.1%; Alginate was poored on top of solid agar.

Plates were left at room temp for 24h

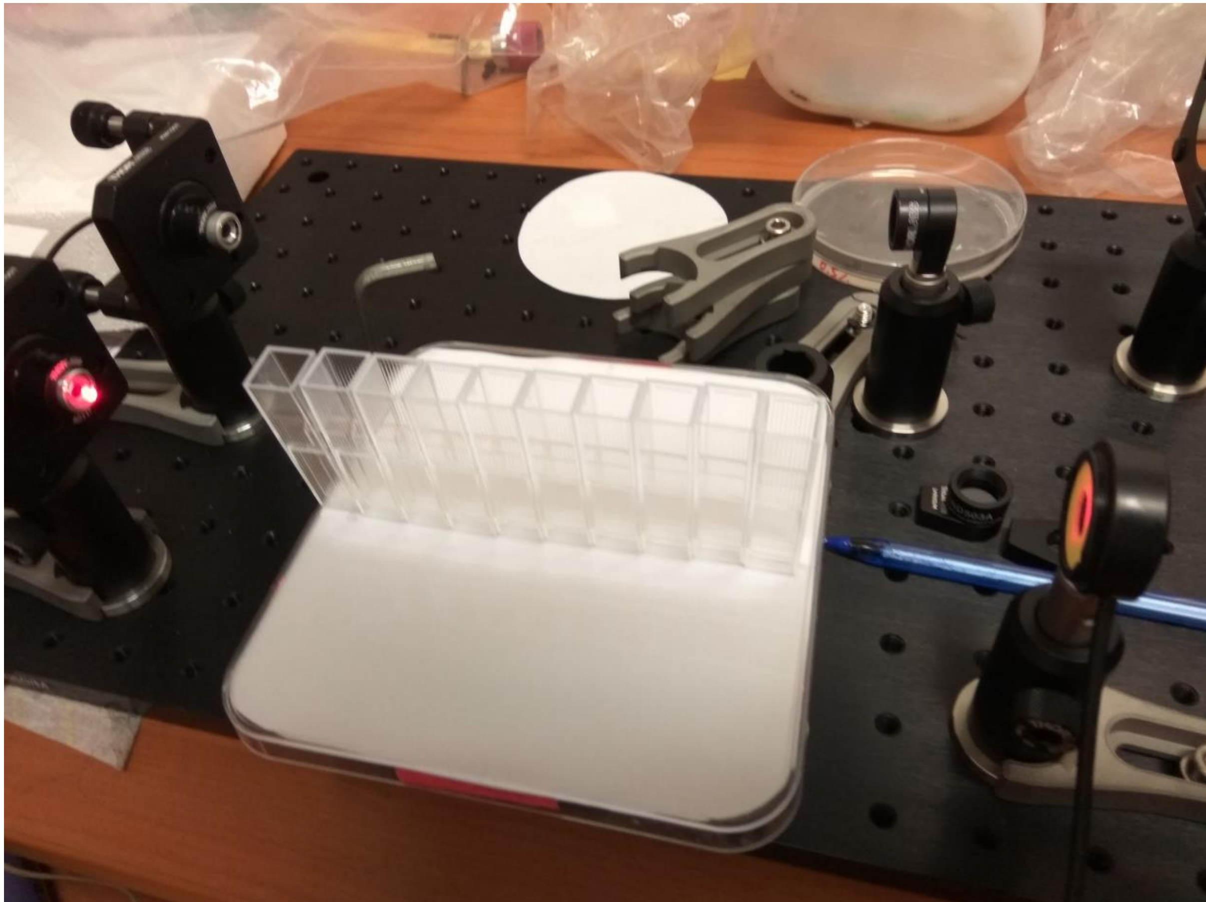
Results: both 0.25% and 0.1% Alginate concentration gave gels but the 0.1% was extremely light and soft **. 0.25% Alginate should be used.**

0.25% ALG(30mL) overnight on
1.5%Agar (10mL 100mM $CaCl_2$)

**23.09:**

Goal:measure light diffusion through Gelrite0.5% and Alginate 0.5%

Methods: pictures of gels with laser blue and laser red were taken with a webcam and processed on Matlab.
To relate the relative intensity lost through the gel due to absolute light intensity measures I attempted to cut Gelrite with a scalpel. However the gel was too liquid to form blocks and flowed out of shape. Therefore I chose to stack cuvettes of gels in the pass of the laser to measure light loss with distance. A baseline measure of empty cuvette was taken for blue and red lasers.



**26.09**

Goal: observe if bacteria dilute in gels

Methods:pSB3C5 containing e coli (with E1010 mRFP) were diluted in saline to OD600 1.204.

Dilution: 0.5mL of saline in first 5mL of gel, 250uL in next 5mL of gel and 50uL in next 5mL of gel

Glr 0.5% 70C 1% 85C

Agar 0.5% 70C 1% 70C

Alginate 0.25% 0.5% 1%: 1.6mL of Agar (100mM CaCl2) was left to solidify in falcon tube, 3.4mL of Alginate was added on top (dilutions: 0.34ml, 0.17mL, 34uL)

Results: Fluorescent growth was observed on the surface of the gel but no fluorescent colonies were observed in the gel

**01.10**

Goal: observe bacteria in gel

An overnight of mRFP ecoli grown in LB (OD600= 12) was diluted to OD 2.06. the solution was diluted 1/100 6 times in 0.8% saline.

Gelrite and Agar: 0.5% and 1% gels were melted in the microwave and 1.8mL was placed in 2 mL tubes and left in an electric bheater at 80C. 2uL of CAM was added and 200uL of mRFP bacteria was quickly added and plunged in a cold water bath.

Alginate: 0.5ml of agar containing 100mM of CaCl2 was placed in 2mL tubes.

When solid, 1.35mL of Alginate ( 0.25, 0.5 and 1%) was added to the tube. 150uL of mRFP bacteria were added to the tube

Tube1: 6th dilution in gel

Tube2: 5th dilution

Tube3: 4th dilution

Results: Fluorescent growth was observed on the surface of the gel but no fluorescent colonies were observed in the gel

**05.10**

Goal observe bacteria diffusion in gels

Gelrite and Agar: 0.5% and 1% gels were melted in the microwave and 10mL was placed in tubes containing 100uL CAM(100X).

Alginate: 5ml of agar containing 100mM of CaCl2 was placed in tubes containing 50uL CAM (100X)

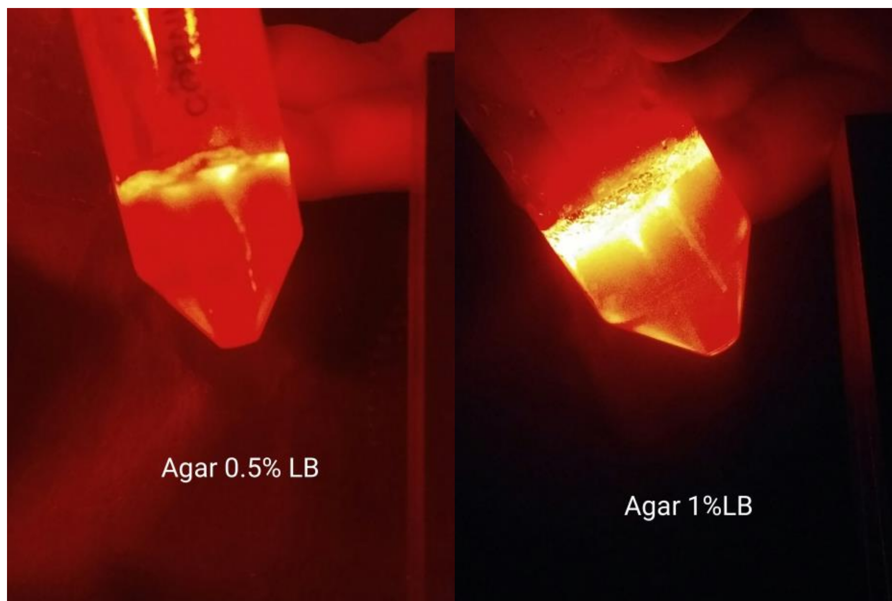
When solid, 100uL 100X CAM and 10mL of Alginate ( 0.25, 0.5 and 1%) was added to the tube.

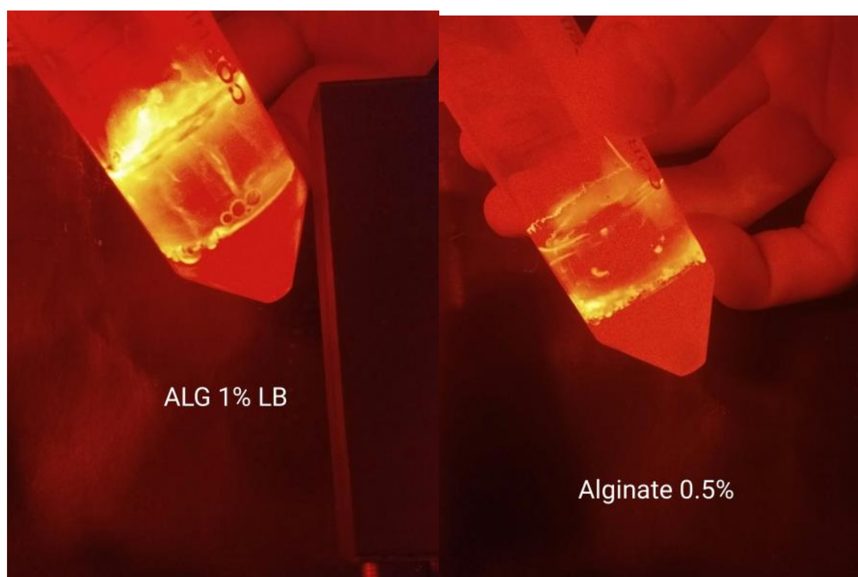
Gels were left at room temperature for 24h.

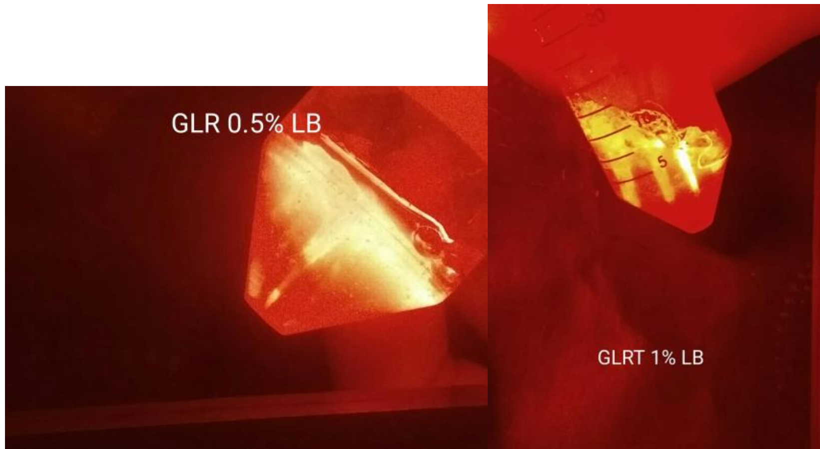
PSB3C5 containing EColi were picked with sterile pipette from red colonies and the tip was pricked three times at different places in the gel

Gels were incubate at 37C

Results:






**14.10**

**Goal: record temperature of gelation**

Gels were melted and temperature was measured as they cooled down with an infrared thermometre ( Volcraft IR250-85)

Temperature of gelation:

| | Gelrite LB | Agar LB |
|---|---|---|
| 0.5% | 51C | 25.6C bubbles start being trapped at 29-30C |
| 1% | 28C (no LB) | 33.5C |

Gels were left to solidify and placed in 37C incubator to check if gels were solid at this temperature

| | Gelrite LB | Agar LB |
|---|---|---|
| 0.5% | Solid | |
| 1% | | |

**Laser measurement:**

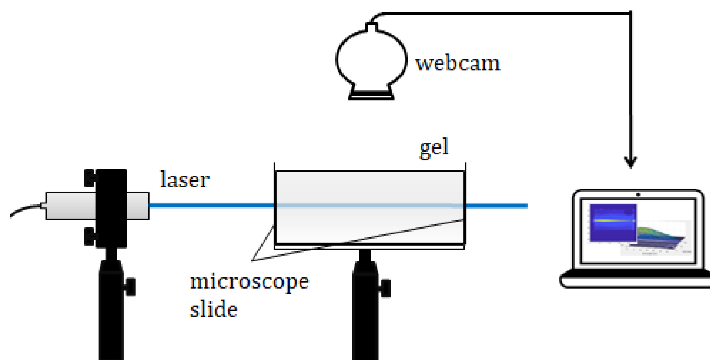
**Goal:** Measure and predict light intensity in Alginate, Gelrite and Agar exposed to Blue and Red laser.

Gel at 1% concentration with normal 2.5% LB was casted in a 5.7x9.0x2.8cm mold

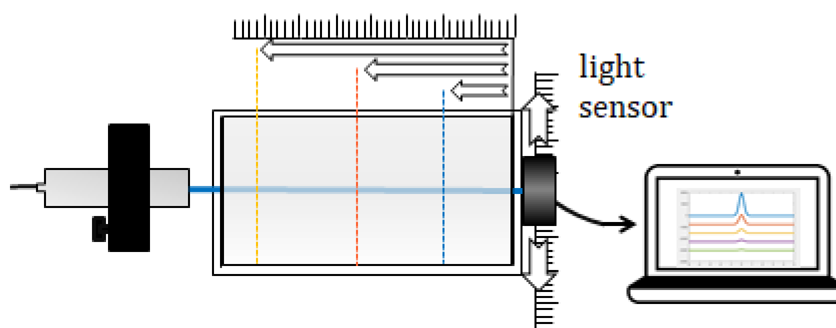
(for Alginate: 1 volume of Agar containing 100mM CaCl2 was casted at the bottom of the mold and cooled, 2 volumes of Alginate 1% was poored on top and left to solidify overnight)

The gel was removed out of the mold and two clean microscope slides were stuck to its long extremities taking care to avoid trapping bubbles between the gel and the slide (distilled water was sometimes squeezed between the gel and the slide to remove bubbles).

The Gel was placed on a flat transparent support and a laser beam was passed through the gel with a perpendicular incidence (axis of laser beam: xaxis, axis perpendicular to the xaxis on the flat plane: y axis, depth axis: z-axis) . A picture of the gel was acquired with a webcam placed perpendicularly above the gel.




Subsequently, a light sensor (S120VC connected to PM100D, Thorlabs) was placed behind the gel in the path of the laser beam. The sensing aperture of the sensor (9.5mm diameter) was covered with a wood cover with a 1mm2 (9.5mmx0.105mm) rectangular opening slit. The light intensity was read in the path of the laser and the position of the sensor was shifted gradually away from the laser path (thanks to a caliper fixed to the optic table) recording the decreasing light intensity at each position (-20mm to +20mm) on each side of




the laser.

To reduce the path length of the laser within the gel, the measurements were repeated with different lengths of gels.

Results:
The image acquired with the webcam

<u>**Resin solidification experiments**</u>:
**Goal**: Compare the size and mass of resin particles cured with a light intensity tuned with a filter of given absorbance or with gelrite 0.5% with a path length giving equivalent absorbance.

**Methods**: The blue Laser was passed through different optical filters with given transmission. An absorbance cuvette filled with 0.5mL of light curing resin S-Pro (kindly provided by our sponsor Spot-A Materials) was placed behind the filter in the path of the blue laser for 1 min. A small particle of resin was observed to solidify against the inside wall of the cuvette. The overload of liquid resin was then washed away with 70% ethanol and the solidified particle was detached from the wall and weighted. The size of the particle was also measured with a picture and ImageJ.

The procedure was repeated by replacing the filter with a volume of Gelrite 0.5% (LB 25g/L). The length of Gelrite was set to match the absorbency of the optical filter based on our model by solving:

$I(x)= I0*10^{(-ax)}$
$x= Log(\% \text{ transmission})/(-a)$

With ‘a’ the parameter directing laser intensity decay, estimated to -0.03919 mm-1 for this Gelrite.
I0 being the laser intensity before entering the gel and I(x) the intensity after passing through x length of gel

<u>**Inducing bacterial response in gel:**</u>

**Goal**: Show that light can activate bacteria in a given gel proving that the prediction of light intensity are globaly light sensitive bacteria can be activated within a gel

**Goal get light intensity at any place in gel:**

**1)get Gauss through gel at 1% find Spread and see that main effect is due to Absorption. Calculate A =log(i0/i)**

**2)Check that absorption is similar in cuvette**

**3)Get absorption in cuvette at 0.5% Use spread data from previous gel and use it to predict the intensity in gel**

**4) measure gauss again in gel to show accuracy**