03/07/17 Agar Plates Preparation(Unfinished)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

- Antibiotics (Amp 1000X, Kan 50 mg/ml, Cam 25 mg/ml)

Procedure

Morning Preparation

1. prepare 500ml LB agar and autoclave.
2. Prepare 500ml LB and autoclave
3. autoclave extra 500ml bottles
4. Perpare kan stock and anyother missing antibiotics.
5.

03/07/17 Transformation of irrE to 10-β cells

Introduction

Before doing the irrE experiments, certain types of cells are prepared as negative control.

Materials

›

- › 10-β cells (Competent already), two tubes, one will be transformed and one will not.
- › irrE mini-prep from former experiments

Procedure

Nanodrop

1. 25 µg/µl mesurements taken with zeroed nanodrop by elution buffer used in the mini-prep kit.

Transformation

2. Take 100µg of plasmids and add it to cells
3. Mix and incubate for 30 seconds in 42 drgree incubator.
4. Put the tube in ice for 5 mins.
5. Add LB 1ml.
6. Incubate at 37 degrees in the shaking incubator for 30 mins
7. Plate (100 µl, rest, no antibiotic cells 25µl)
8. Incubate overnight.

P.S. Supplmentery materials for section 7

9. plasmid 100µl
10. centrifuged the cell for 2:20 at top speed
11. discard the supernatant and resuspend the cells with small amount of supernant left in the tube
12. Plate the cells.
13. Trasnsformant cells tube is not marked by 'X'

14.


iGEM
R.F
04/07/17
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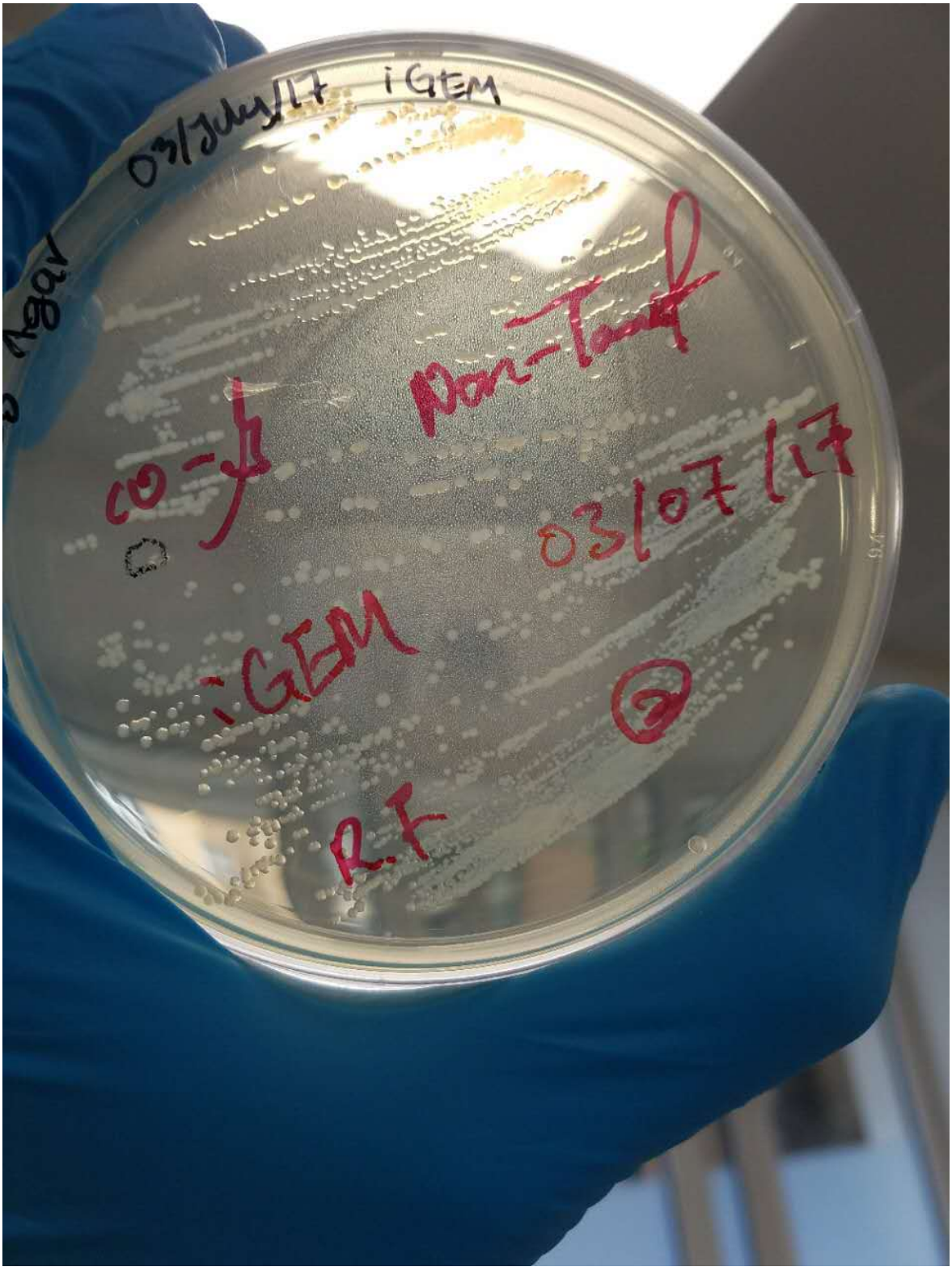

03/July/17 iGEM
co-f
Par-Tet
03/07/17
iGEM
R.F

04/07/17 Make overnight cultures & make plates

Introduction

Make overnight cultures & make plates

Materials

›

- NEB 10-β cells
- stellar pUC 57 irrE cells
- DH5 ALPHA
- LB media
- Ampicillin

Procedure

Make overnight cultures

Make 5 LB plain plates

05/07/17 Formal irrE Experiment

Introduction

Take the overnight culture and measure the optical density of each. Make glycerol stocks of each of them (500µl glycerol + 500µl culture)

Materials

›

› Four cells prepared as controls

Procedure

UV experiments

1. 5µl each culture and 1995µl PBS resuspension. Max. Dose as X1 X3 X5
2. The exact distribution is shown as below.
3. After resusoension, plate 20 µl in to corresponding plates
4. Culture them at 37 degree c overnight

D) Dilute (Darren's) = ✓ — resuspend w/ 2ml PBS

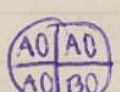
3. Make glycerol stocks of each of them (500µl glycerol + 500µl culture) ✓

4. UV experiments (triplicates) 5µl each culture + 1995µl PBS

		UV dose		
		1	3	5
A	OUV	x1	x3	x5
A	OUV	x1	x3	x5
A	OUV	x1	x3	x5
B	OUV	x1	x3	x5
B	OUV	x1	x3	x5
B	OUV	x1	x3	x5
C	OUV	x1	x3	x5
C	OUV	x1	x3	x5
C	OUV	x1	x3	x5
D	OUV	x1	x3	x5
D	OUV	x1	x3	x5
D	OUV	x1	x3	x5

← 8x 6well plates

20µl on each quadrant



For A & B plates should have Amp!

For C & D plates should NOT have antibiotic

5. Plate 20µl into coresponding plate's quadrant!

05/07/17 Optical Densities of Four Cells

Introduction

1/8 dilution is done and optical densities of 4 cells used in the irrE experiments are measured.

Materials

›

- NEB 10-ß (No antibiotics resistances)
- NEB 10-ß (irrE transformed)
- Stellar pUC 57 irrE
- DH5α cells

Procedure

OD

1. DH5α cells 0.442 X 8 = 3.536
2. Stellar pUC 57 irrE 0.468 X 8 = 3.744
3. NEB 10-ß (irrE transformed) 0.329 X 8 = 2.632
4. EB 10-ß (No antibiotics resistances) 0.405 X 8 = 3.240

Incubation and Labelling

5. stored at -20 degree
6. 50% glycerol and stock amount 25% glycerol pure.
7. Label the plates

Dosage time measurement

8. For single maximum dosage exposure, the time taken si measured
9. 2'53"41/2'49"70

    2'59"37/ 2'58"60 (C3/D3/C5/D5)
    2'57"79/ 2'57'70 (C5/D5)

11/07/17 irrE experiments and plates making

Introduction

Repeat IrrE experiments

Materials

- 2x 10ml cultures LB
- +ve NEB 10B IrrE
- 10µl Amp (glycerol stock)
- -ve NEB 10B (glycerol stock)

Procedure

Make 10 Amp and plain plates

12/07/17 irrE Exposure

Introduction

IrrE Exposure

Materials

- NEB IrrE Amp
- NEB IrrE
- PBS
  -

Procedure

IrrE Exposure

1. Centrifuge 2ml of overnight cultures (+NEB IrrE Amp & NEB IrrE)
2. Resuspend with 2ml PBS
3. 5µl + 1995µl PBS
4. Plates from Rosaline
5. 8 Plates
6. + 30/60/90/120/150/180/uncovered (90mins)
7. - 30/60/90/120/150/180/uncovered (90mins)

12/07/17 UV IrrE experiments

Introduction

UV IrrE experiments

Materials

-
  - PBS
  - IrrE
  - Ampicillin
  - LB media

Procedure

1. Spin down 2ml of each culture
2. Resuspend cells with 2ml PBS
3. Obtain a bigger plate (instead of 6 well plate)
4. Plate

13/07/17 Interlab - Cell Competent Test Prep

Introduction

Making Cam (chloroamphenical) plates (20)

Materials

-
  - CAM
  - LB-Broth

Procedure

1. CAM con. in plates: 25mg/ml
2. 1:1000 dilution, final c=25µg/ml
3.

14/07/17 Making Agar CAM plates

Introduction

Making 7 Amp plates and 9 plain plates

Materials

-
  - IrrE
  - PBS
  - Ampicillin

Procedure

1. Spin 2ml of IrrE and resuspend 2ml of PBS for 5mins
2. 5µl + 1995µl PBS and plate 20µl with Amp and plain
3.

17/07/17 Colonies Counting

Introduction

Colonies Counting

Materials

- N/A
  - N/A

Procedure

Positive Group

1. A.Uncovered: 0/ Covered: 3 (incl. lawn)
2. B.Uncovered: 0 / Covered: 33 (excl. yellower colonies)
3. C.Uncovered: 0 / Covered: 27 (excl. lawn)
4. D.Uncovered: 0 / Covered: 0
5. E.Uncovered: 0 / Covered: 0
6. F.Uncovered: 0 / Covered: 0

Negative Group

7. G. Uncovered: 3 / Covered: 0
8. H. Uncovered: 0 / Covered: 0
9. I. Uncovered: 0 / Covered: 68
10. J. Uncovered: 9 / Covered: 80 (excl. yellower colonies)
11. K. Uncovered: 8 (excl. yellower colonies) / Covered: 4
12. L. Uncovered: 15 / Covered: 43

17/07/17 Colony Count Pictures

Introduction

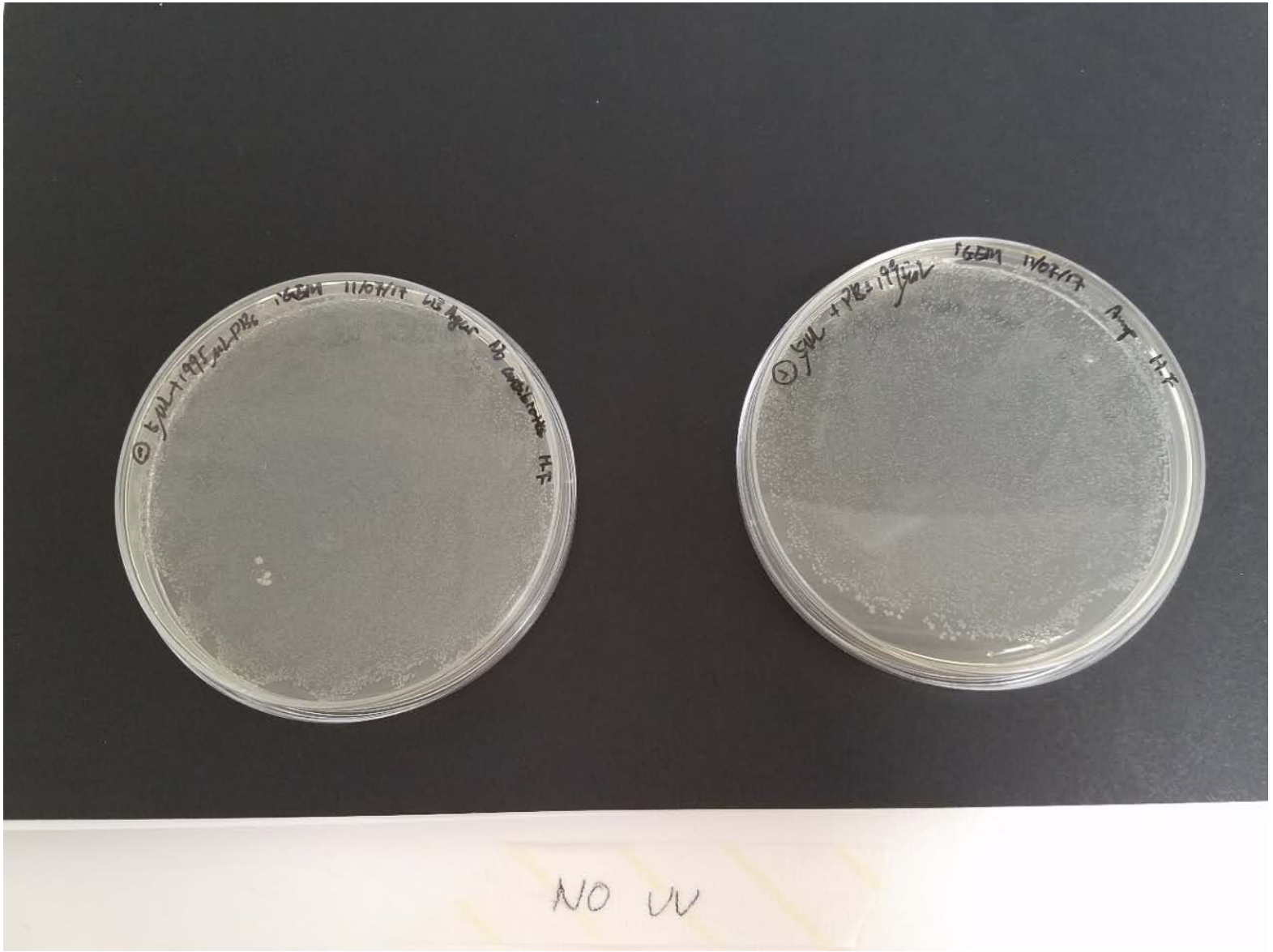
Get started by giving your protocol a name and editing this introduction.Colony Count Pictures

Materials

›

›

Procedure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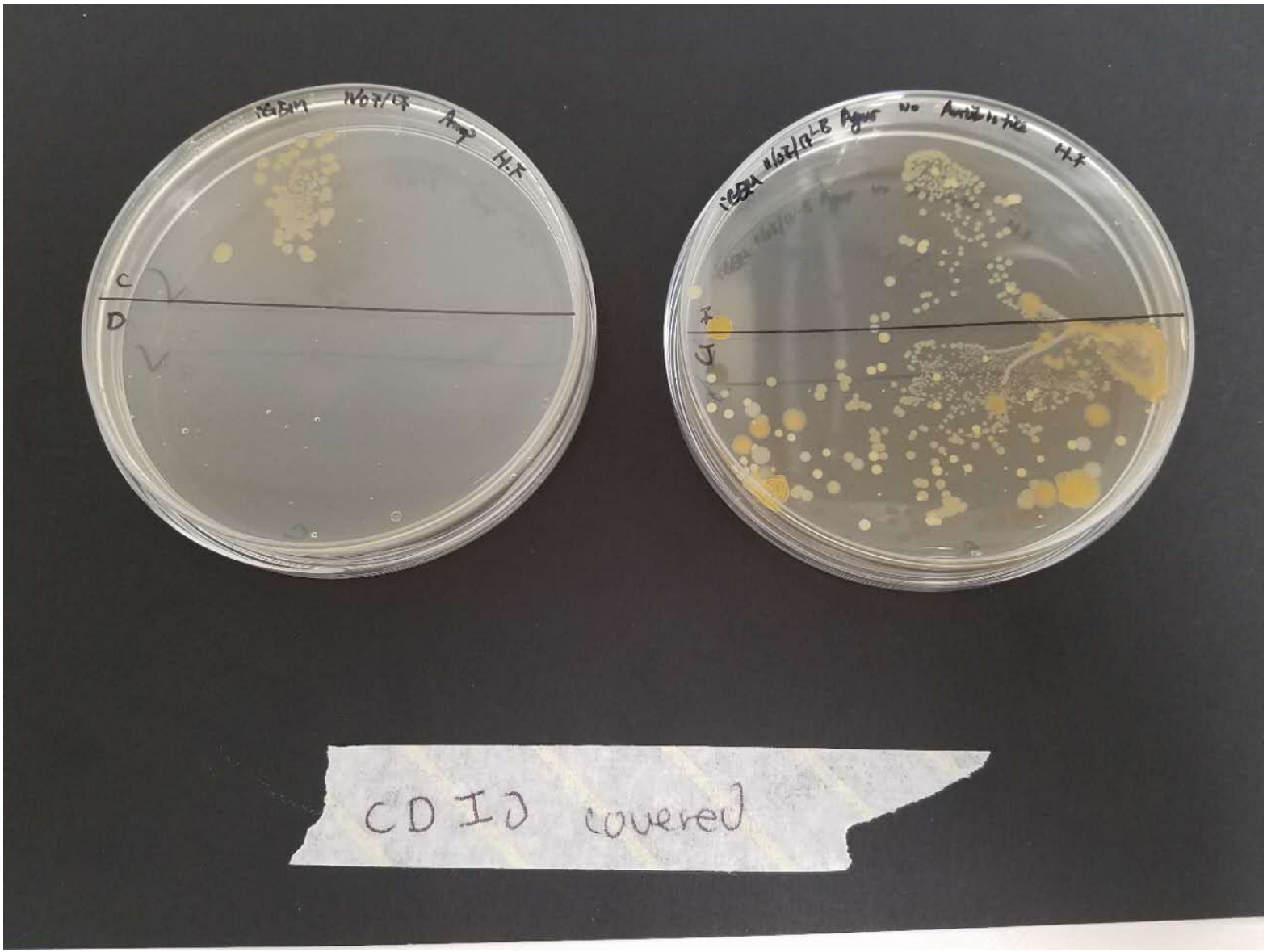
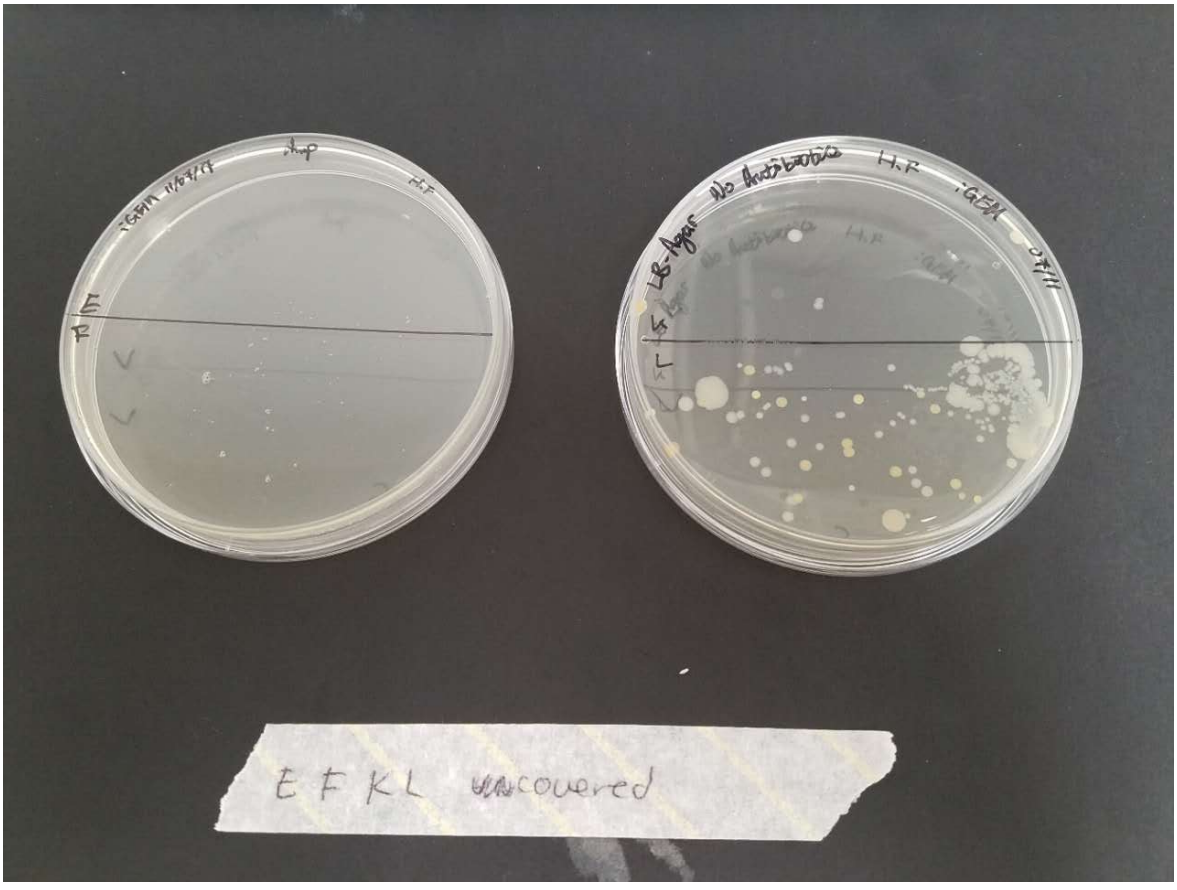
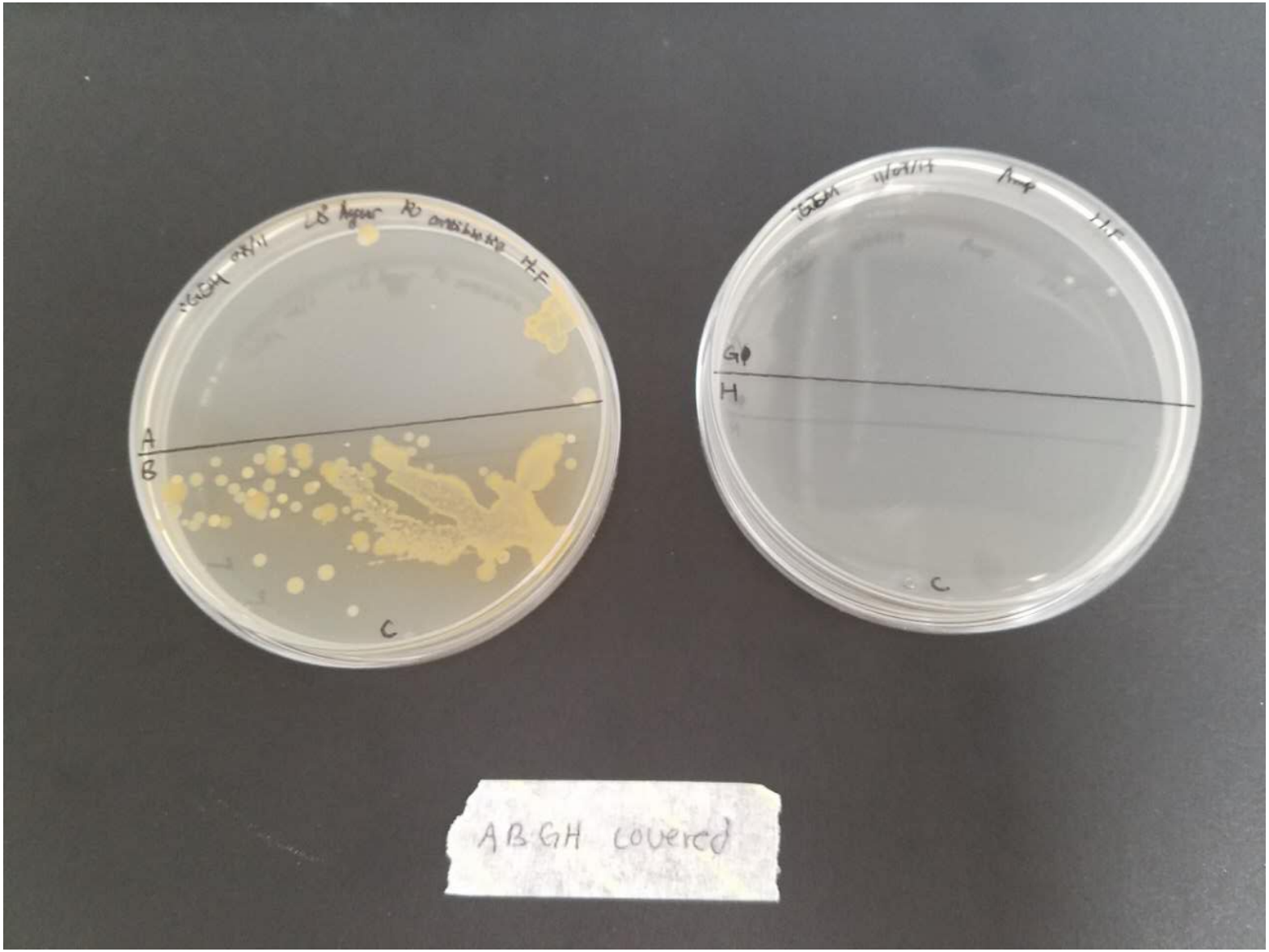
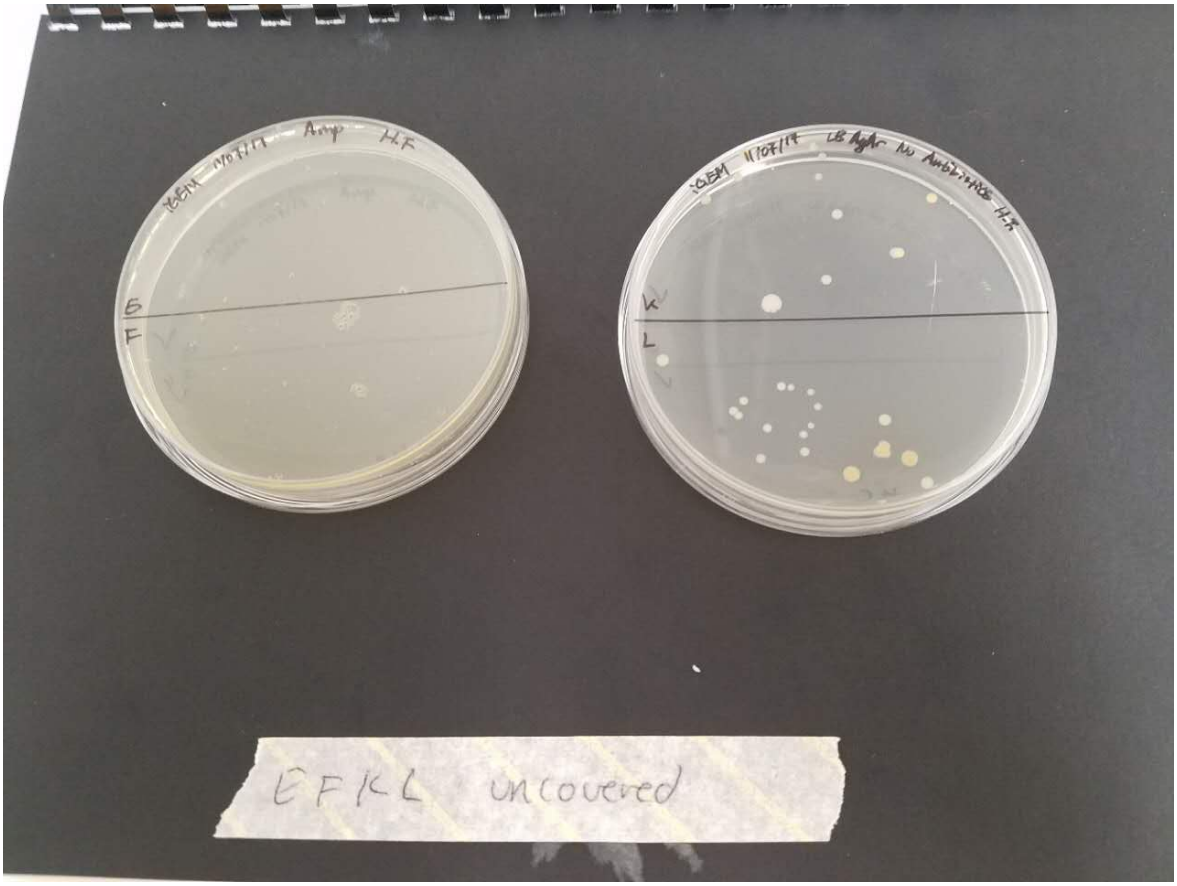

C
D
CDIJ covered


E F K L uncovered


ABGH uncoated


A
B
C
GH
H
C
ABGH covered


S
F
K
L
EFKL uncovered

1.

17/07/17 Setting up irrE cultures

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

- › M9 Salts
- › Sodium phosphate pentahydrate
- › Potassium phosphate
- › Sodium chloride
- › Ammonium chloride
- › Magnesium sulfate
- › Calcium chloride
- › Glucose

Procedure

Setting up 2x 10ml cultures IrrE+

Prepare M9 minimal media

1. M9 salts: 5x concentrate
2. 800ml distilled/deionized water
3. 64g sodium phosphate pentahydrate
4. 15g potassium phosphate (dibasic)
5. 2.5g NaCl
6. 0.5g ammonium chloride
7. Stir unti dissolved
8. adjust volume to 1000ml
9. Sterilize by autoclauing or filtering
10. Add: 2ml 1M $MgSO_4$, 0.1 1M $CaCl_2$, 20ml of 20% glucose
11. Add 15g agar

20/07/17 irrE Media Test

Introduction

Test the viability of irrE transformed *E.coli* cells in Lubricant, LB and mixture of Lubricant and LB by measuring the optical density of cells.

Materials

- NEB 10-β Cells
- Lubricant
- LB Media
- Spectrophotometer
- Cuvette

Procedure

1.

20/07/17 IrrE minimal Media & lubricant

Introduction

IrrE minimal media & lubricant

Materials

-
  - M9
  - Ampicillin
  - IrrE

Procedure

50ml of each media + corresponding cell:

1. 40% lubricant & 60% M9 (IrrE-)
2. 40% lubricant & 60% M9 + Amp (IrrE+)
3. M9 (IrrE-)
4. M9 + Amp (IrrE+)
5. LB (IrrE-)
6. LB + Amp (IrrE+)
7. 40% lubricant & 60% LB (IrrE-)
8. 40% lubricant & 60% LB (Irre+)

23/06/17 Media Preparation

Introduction

To prepare 500 mL LB media and 500mL LB-Agar media from prepared powder media.
To make CamR stocks.
Pouring plates.

Materials

-
  - LB Agar
  - Water
  - CamR

Procedure

Premade/prepared-powder media

1. For LB media (500mL), 12.5g of LB was added and water was added to make upto 500ml.
2. For LB-Agar media (500mL), 18.5g of LB-Agar was added and water was added to make upto 500mL.
3. Stir at 300 rpm.

CamR stocks 25mg/mL

4. 20mg Cam in 10 mL 99% ethanol
5. Mixed and vortexed to dissolve.
6. Divided into 10 aliquots of 1mL each. Final conc. is 25mg/mL

Pouring Plates

7. 40mL molten LB agar + 40µl of 25mg/mL Cam, mixed in falcon tube.
8. Pour 2 plates at a time X 10 times.
9. Stored at **4°C Frige**

26/06/17 Glycerol and PBS Buffer Preparation

Introduction

Prepare 50% Glycerol

Materials

›

- milliQ water
- Glycerol Stock
- Syringe and Syringe Filter
- PBS
- Deionised water
- Cellulose Acetate Synringe Filter

Procedure

Sterilisation

1. Glycerol is filtered to keep the sterility
2. milliQ water is filter in the same way using 0.2 μm cellulose acetate filter.

Mixing

3. Mix two solutions and Vortex

PBS Preparation

4. Measure 200 mL of deionised water in measuring cylinder.
5. add PBS tablets to bottle. Fill up with 200 mL deionised water.
6. Vortex mixture。
7. Filter 50 mL of PBS. Repeat step to make another tube
8. 70% Ethanol solution for spray is also made for sterilisation.

26/06/17 Overnight Culture

Introduction

Grow the bacteria cunture (pUC 37 and pET 29) overnight to see if the stock ampicillin is working properly.
Negative control with different antibiotic resistance was used to compare.

Expectation: KanR *E. coli* pET 29 dies and pUC grows if Amp works as the former one does not have resistance of Ampicillin.
OD of pET 29 is expected to be 1-2 or more if grow properly.
AmpR= Ampicillin Resistance
KanR= Kanamycin Resistance

Materials

›

- Amp Stellar pUC 37 X 2
- KanR-*E.coli* + pET 29 X 2 (Negative control)
- 15mL of LB media in falcon tubes
- Glycerol stock (Given by Mike Sibley) Stellar, pUC 27 irrE Amp(10/10/16) kept at **-20°C** (From iGEM 2016)

Procedure

Culture Preparation

1. After labelling the tubes and added the strain into tubes, add 15µl of 1000X Amp to each tube.
2. Innoculate two tubes with strains from glyserol stock into falcon tubes by use of tips.
3. Grow at 37°C shaking incubator at 250 rpm.

Prepare Agar Plates From Liquid LB

4. Label LB Agar, 100µg/mL Amp.
5. Add 40 mL of LB Agar Media into falcon tubes
6. Add 40 µl of Amp and leave on bench for solidifying.

27/06/17 Overnight Culture Collection and Mesurements

Introduction

The culture inculated overnight is collected and Optical density is measured if things works out as expected.
Dilution is carried out in order to measure an OD between 0-1.

Materials

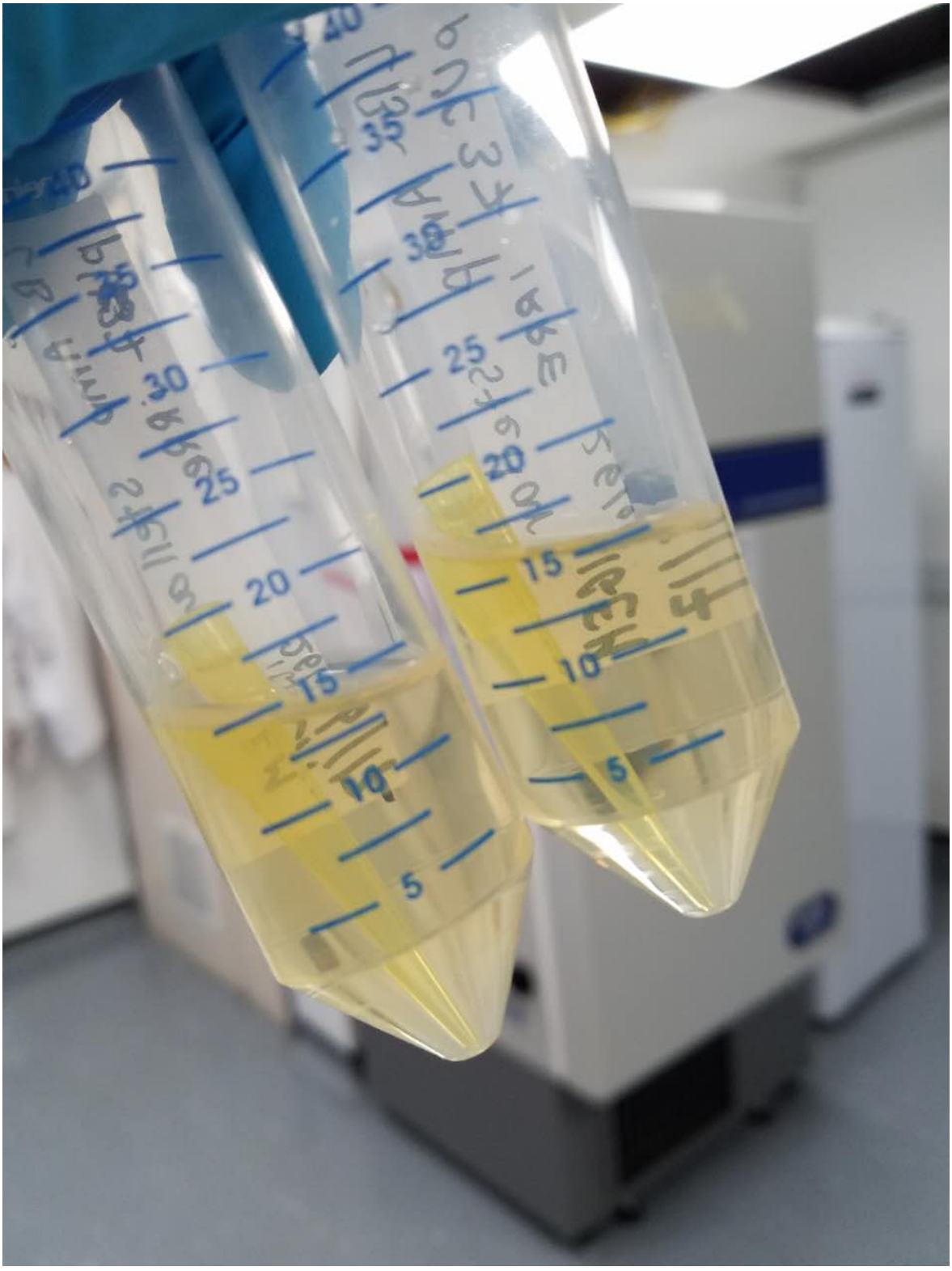
›

- Spectrophotometer (Borrowed from 2.21)
- Cuvette (Borrowed from 2.21)
- miliQ Water

Procedure

1 in 8 Dilution and take the measurements (The procedure applies to the second culture)

1. Ppipette 0.5 µl of culture into cuvette and add same volume of milliQ water.Mix by pipetting up and down 6 times, 1/2 dulution is made.
2. pipette out 0.5µl of 1/2 dilution out into new cuvette and add same amount of milliQ water to make 1/4 dilution.
3. Repeat step 2 to make 1/8 dilution.
4. Zero the spectrophotometer and put the cuvette with culture in. Take measurements of OD, The exact OD is the measured OD times 8.
5. Data listed below: OD of pUC 37 irrE = 0.35 X 8 = 2.80
6. Another group: OD = 0.349 X 8 = 2.792


40
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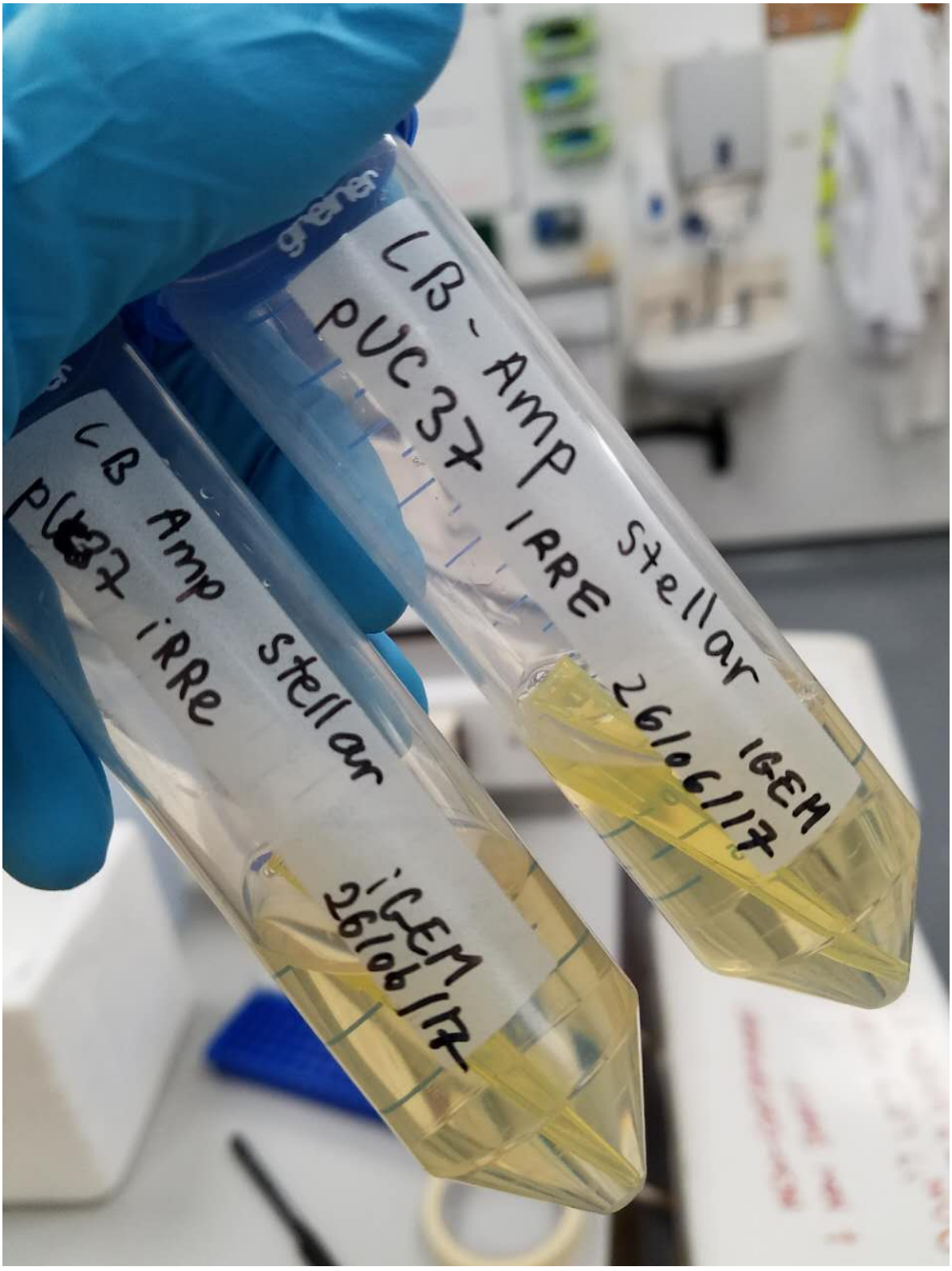

LB - AMP
PUC37
iRRE
Stellar
iGEM
26/06/17
LB Amp
PUC37
iRRE
Stellar
iGEM
26/06/17

27/06/17 Mini-prep of irrE

Introduction

Conduct a Mini-prep for Diagnostic electrophoresis and keep a copy of the plasmids.

Materials

›

› Mini-Prep Kit #K0502

Procedure

Refer to protocol

1. Total of 2 preps are made. Stored at **4°C frige.**
2. The resuspension and RNA is kept at **4°C frige.** DO NOT TAKE OUT UNTIL MINI-PREP.
3. Mini-prep labelled as iGEM 27/06/17 irrE pUC 37 R.F

27/06/17 UV irrE Experiments

Introduction

To resuspend cells and plate cells onto plates. Once finish the plating, the cells are exposed to maximum UV dose for set time (depends on the mashine. A fixed amount of UV is emitted to cells at certain rate.)

Materials

- irrE cells
- Centrifuge
- PBS resuspension solution
- UV crosslinker
- LB-Agar Plates

Procedure

Resuspension

1. Centrifuge 2 mL of irrE cell culture at top speed for 5 mins
2. Resuspend using PBS buffer, vortex. Notation="RE"

Plating

3. Two plates are plated as 8 quaters. One for UV and one for non-UV.
4. Labelling and contents 2mL each innoculation

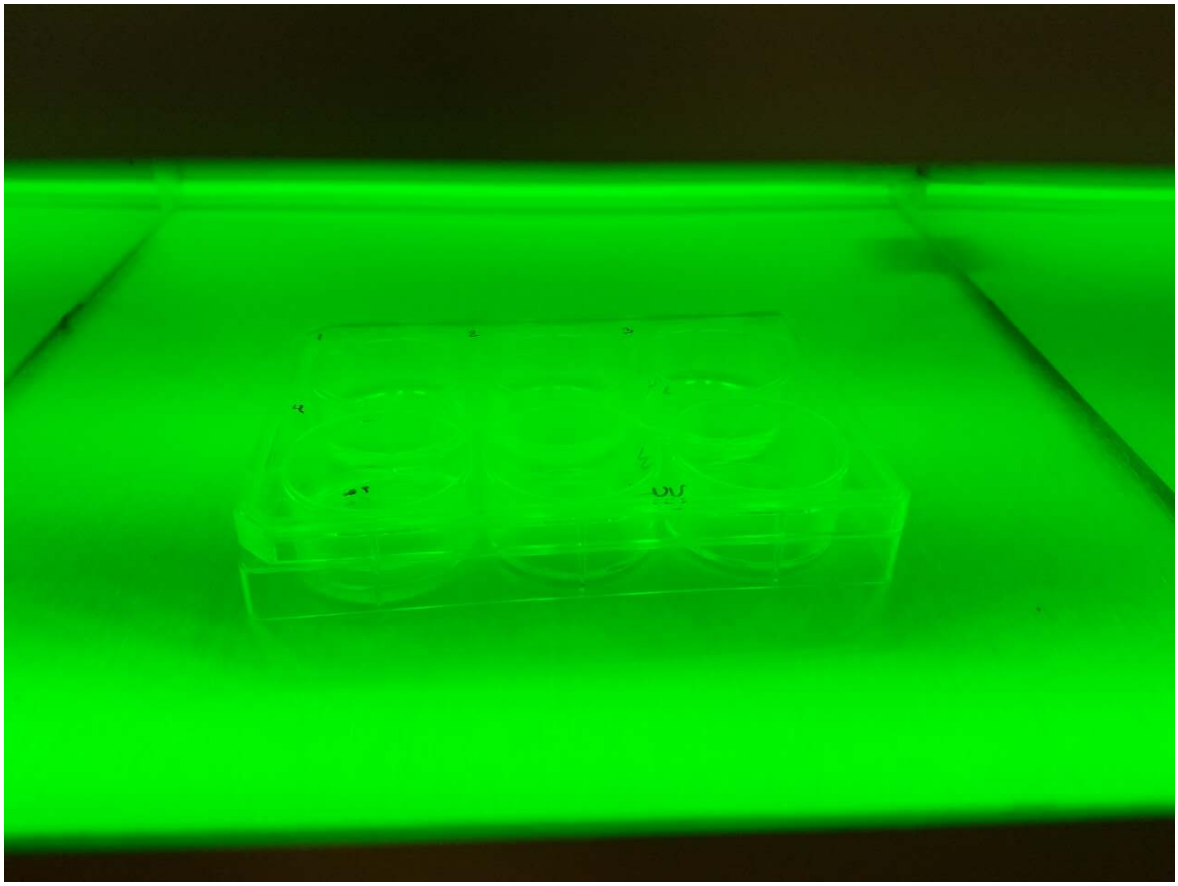
    UV

    I. 400 µl RE + 1600 µl PBS
    II. 50 µl RE + 1950 µl PBS
    III. 5 µl RE + 1995 µl PBS
    IV. 200 µl RE+ 1800 µl PBS

    Non-UV
    V. 50 µl RE + 1950 µl PBS
    VI. 400 µl RE + 1600 µl PBS
    VII. 5 µl RE + 1995 µl PBS
    VIII. 200 µl RE = 1800 µl PBS

UV Radiation

5. Maximum dose is given. Total of 2 mins 35 secs taken to finish the required dosage.
6.




ENERGY
TIME
PRESET
START
STOP
RESET
ENTER
CL-1000 Ultraviolet Crosslinker
UVP

Incubation

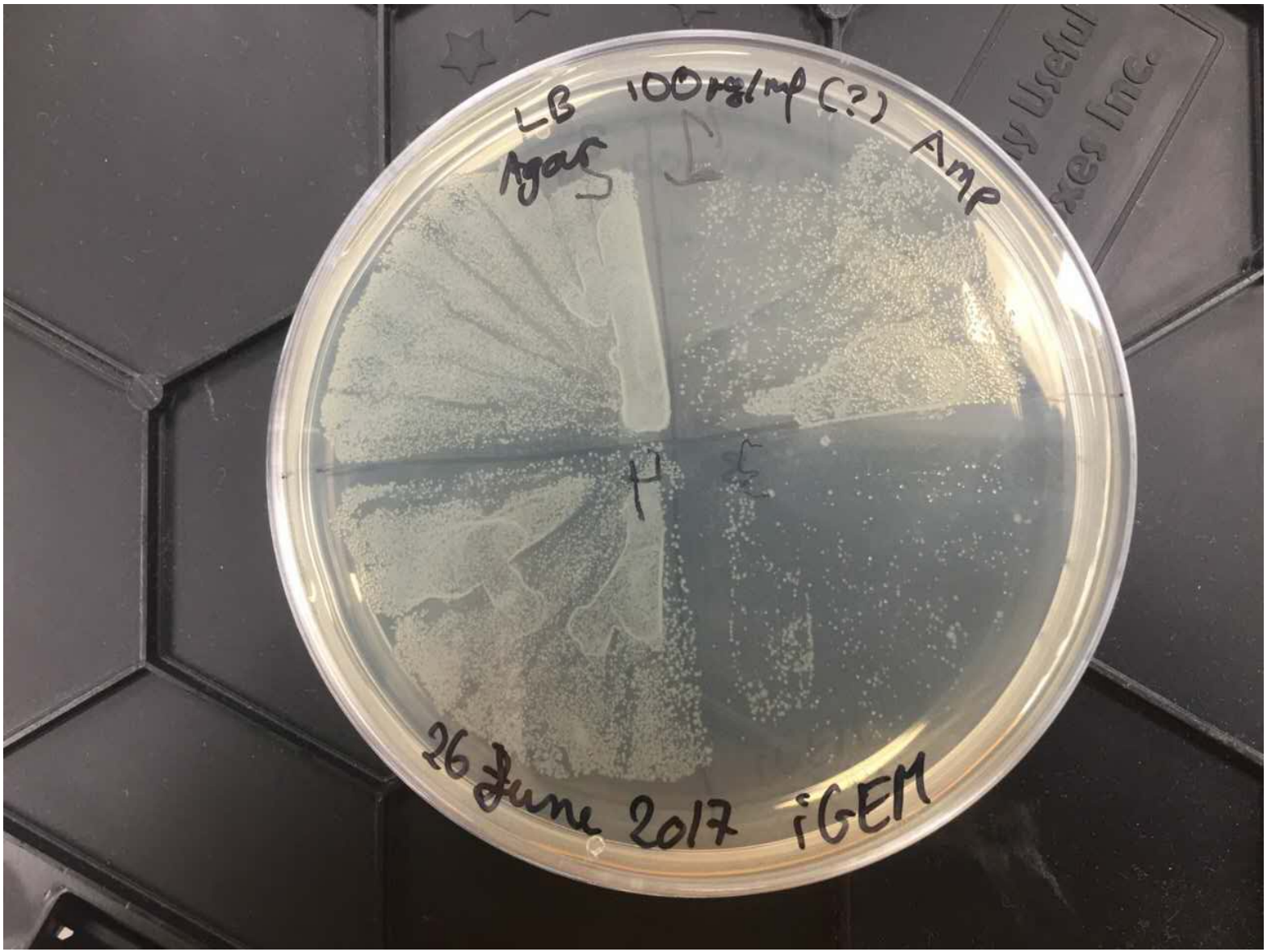
7. UV and Non-UV plates are incubated **37°C Incubator** overnight**.**

Results 28/06/17

Colonies Count (By Eyes)
III. 484 colonies
VII. 549 colonies

notes:
there is a lawn of colonies that is denser than the rest of the colonies but it was counted as it looks like it cannot be from another region.
VII. (without lawn) 216 colonies.


LB
Agar
100 µg/ml (?) Amp
26/June/17 iGEM


LB
Agar
100 µg/ml (?)
Amp
26 June 2017
iGEM

28/06/17 Perfection to Previous irrE, Comparison to wild type cells. (Negative control)

Introduction

Continuation of previous experiments. Both irrE habouring cells and wild type cells are cultured overnight and be prepared for plating.

Materials

- Amp
- pUC 37 irrE cells
- pET 29 Wild type cells
- Kan

Procedure

Cells

1. The irrE cells pUC 37 are with Ampicillin. 5mL
2. Wild type cells pET29 cells are with kanamycin. 5mL

Innoculation

3. Prepare 5 mL of LB media in a falcon tube
4. tip the irrE cell stock and leave the tip in the falcon with LB media.
5. Add 3 µl of Kan at concentration of 50 µg/mL.
6. Repeat step 3
7. tip the wild type cell stock and leave the tip in the falcon with LB media.
8. Add 5 µl of X1000 amp.
9. Incubate both in shaking incubator overnight.

Failure Experiment (IMG only)

Introduction

Get started by giving your protocol a name and editing this introduction.

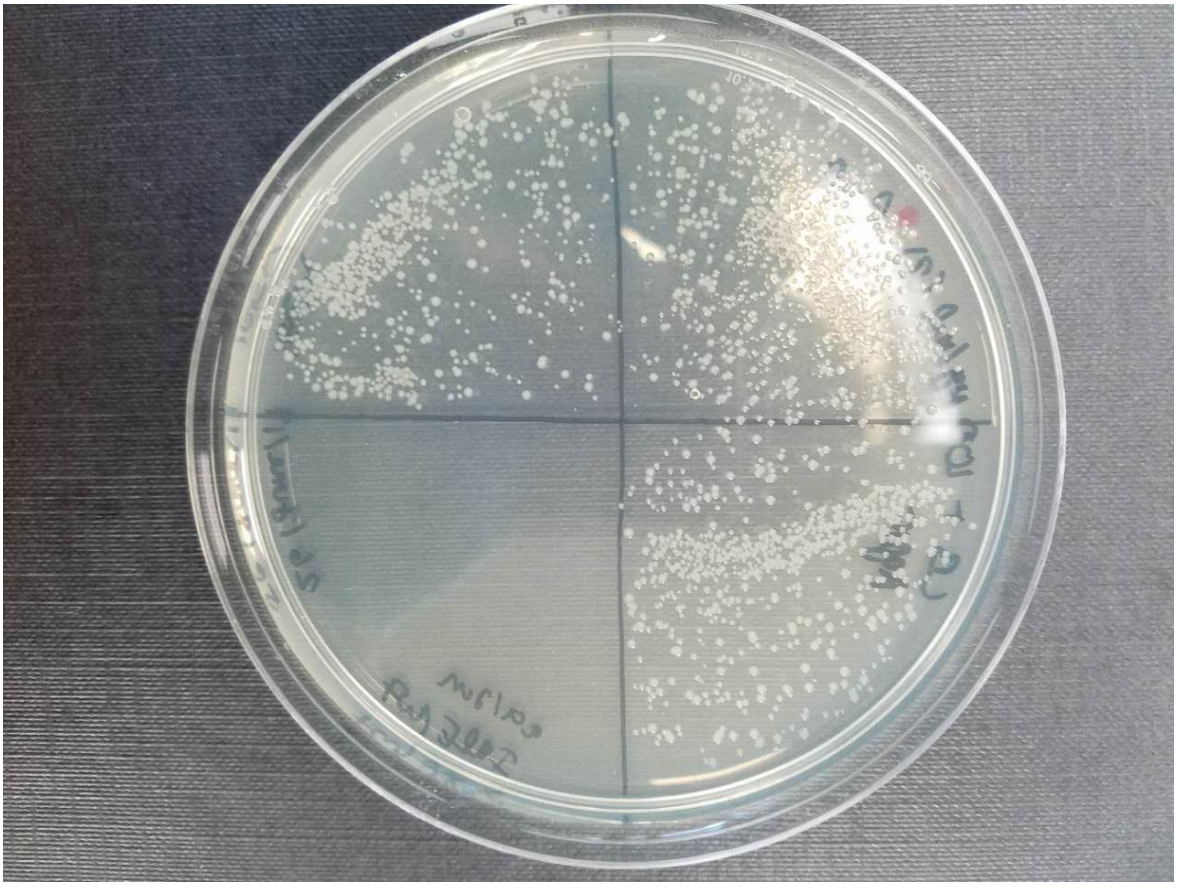
Materials

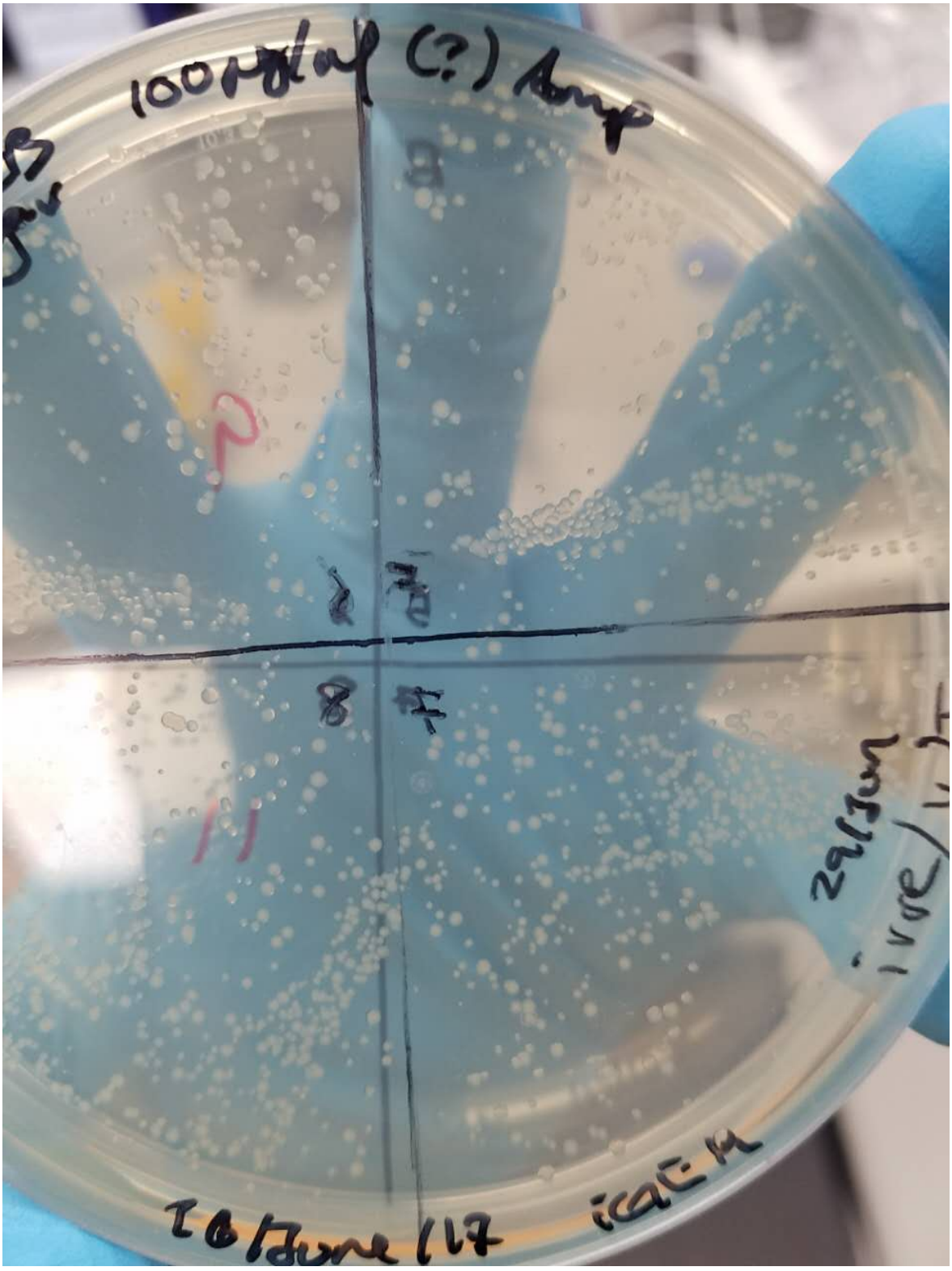
›

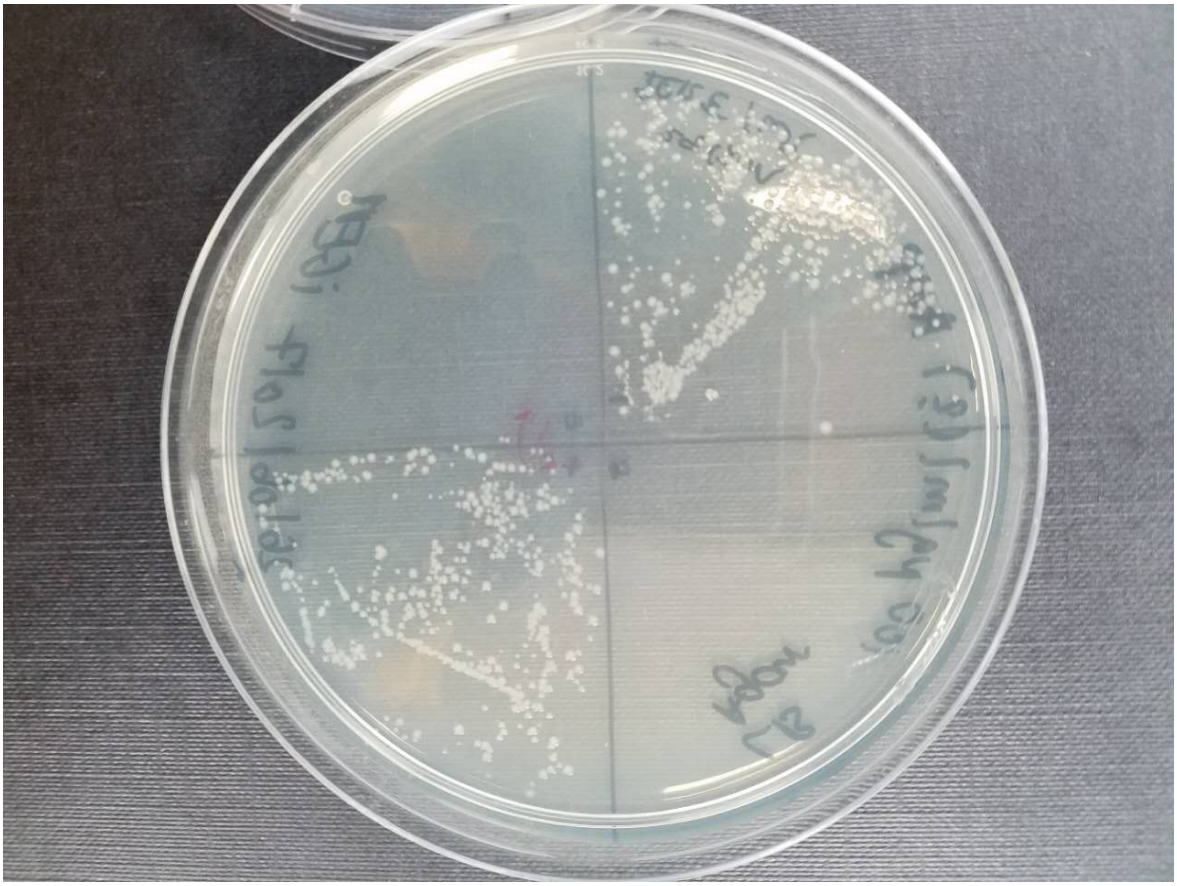
›

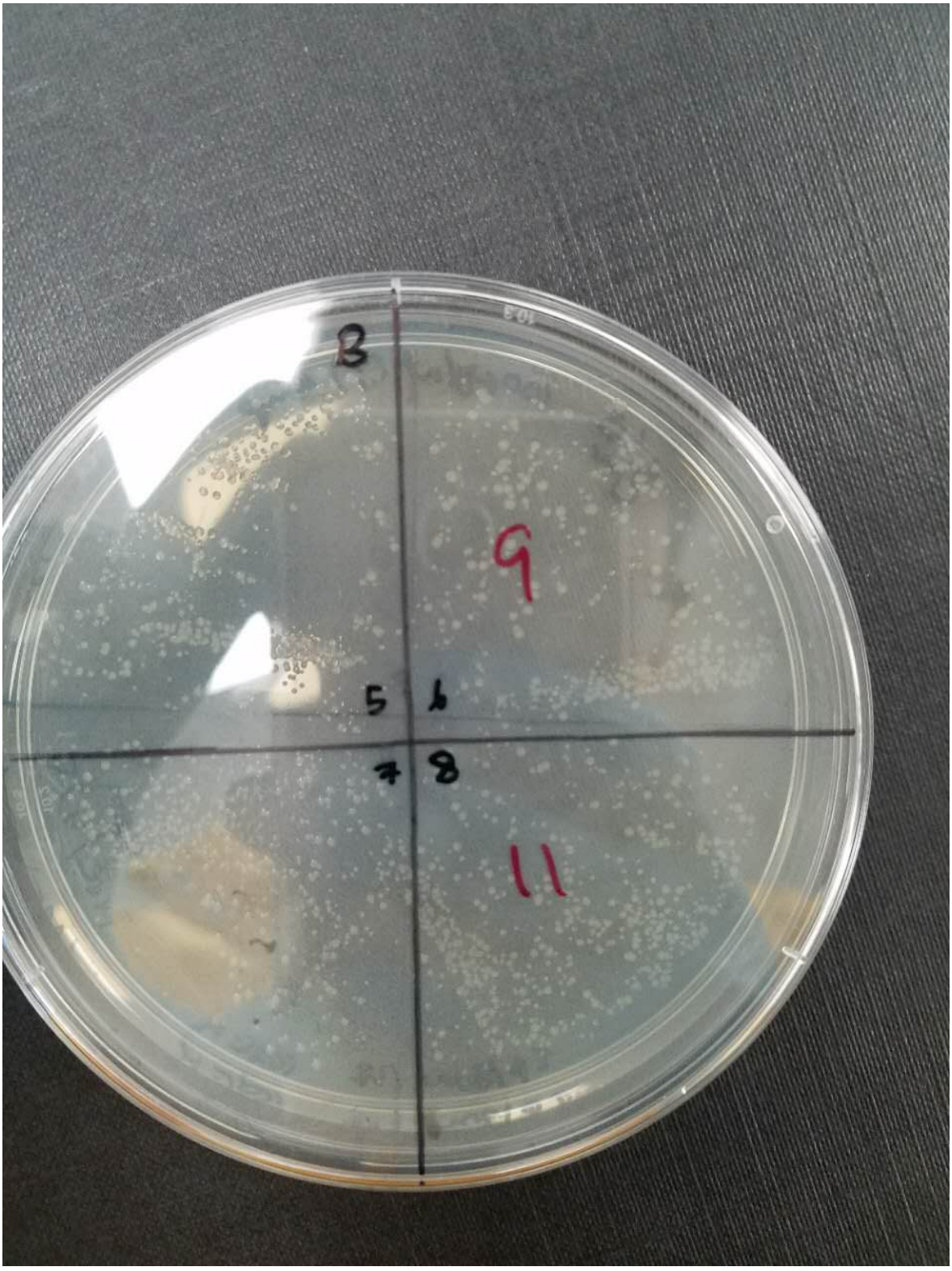
Procedure

1.




100
(?) Amp
29/3/2017




B
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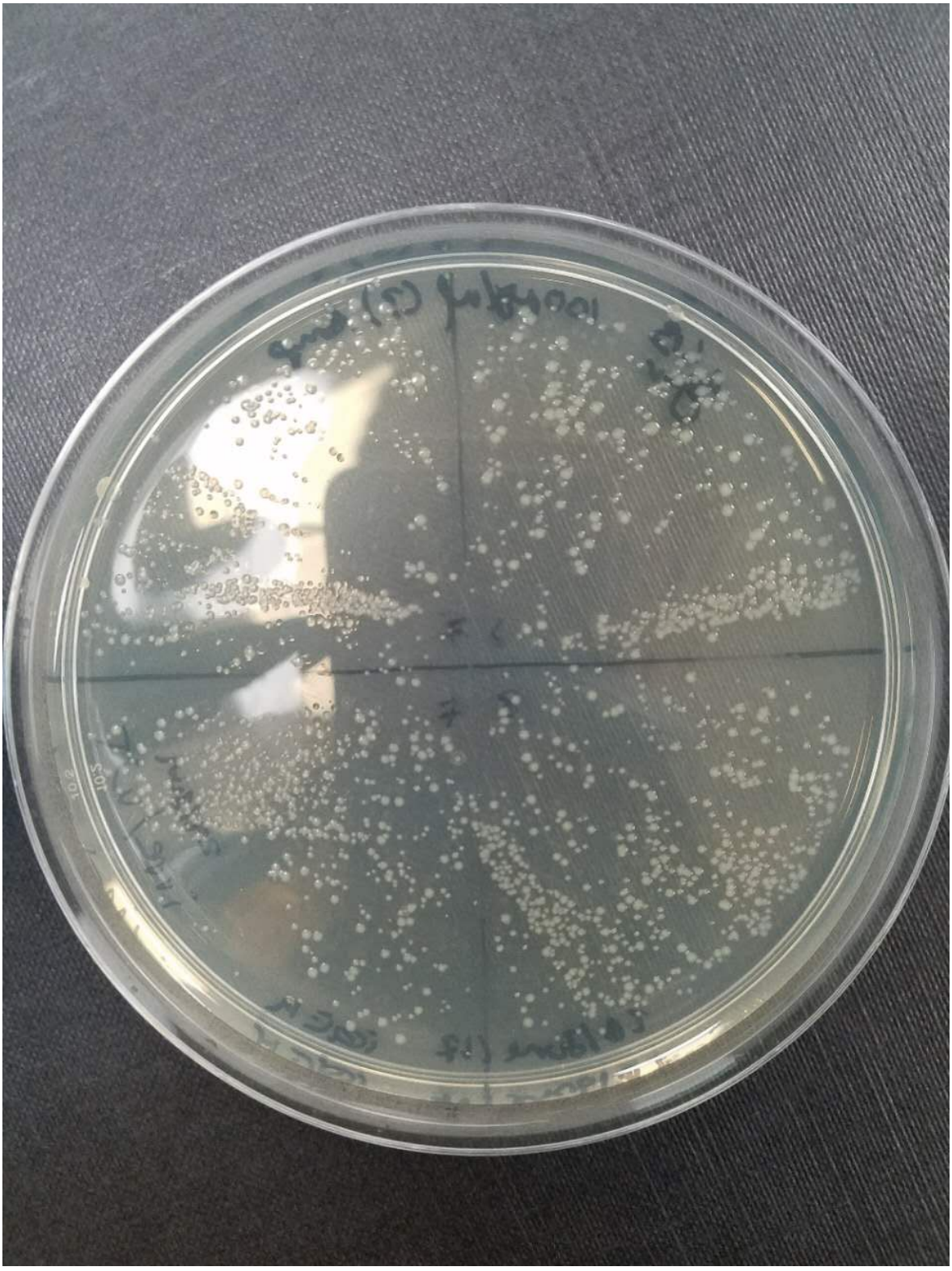
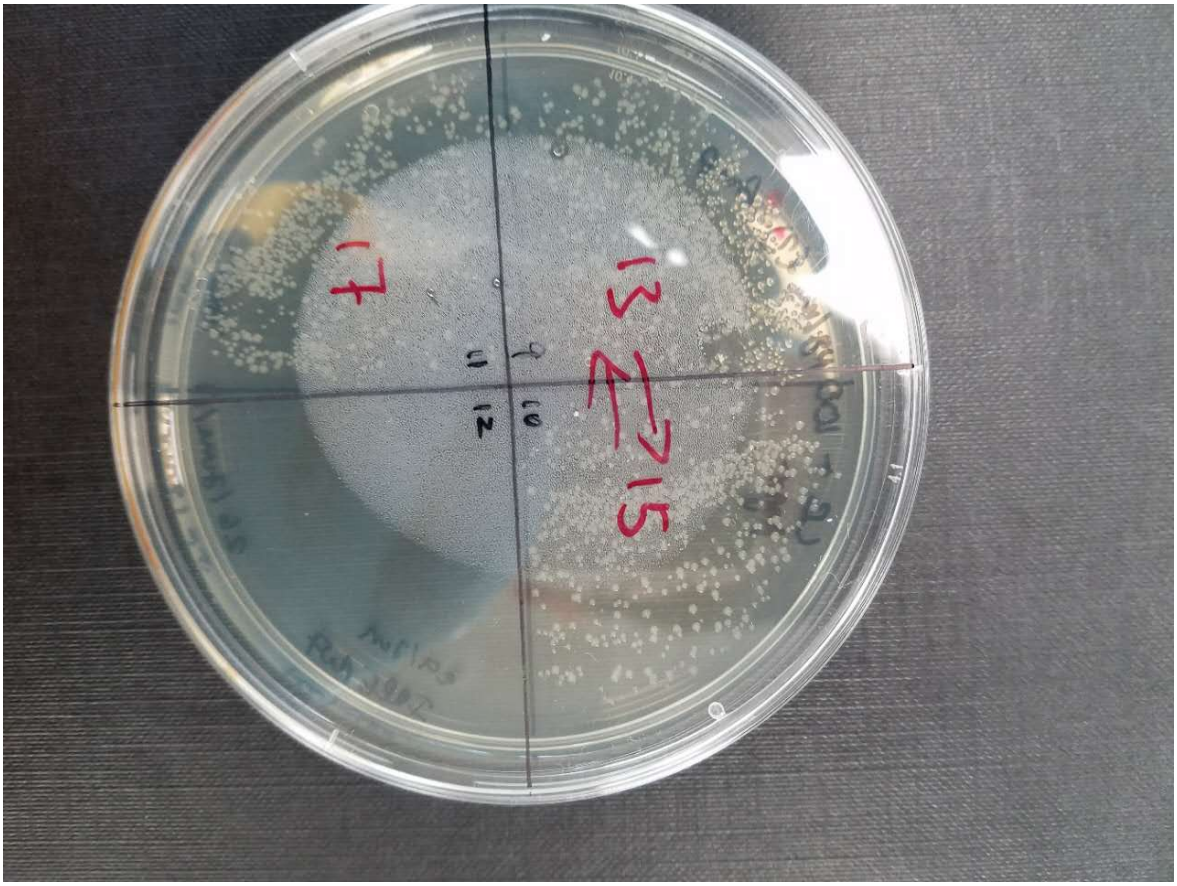
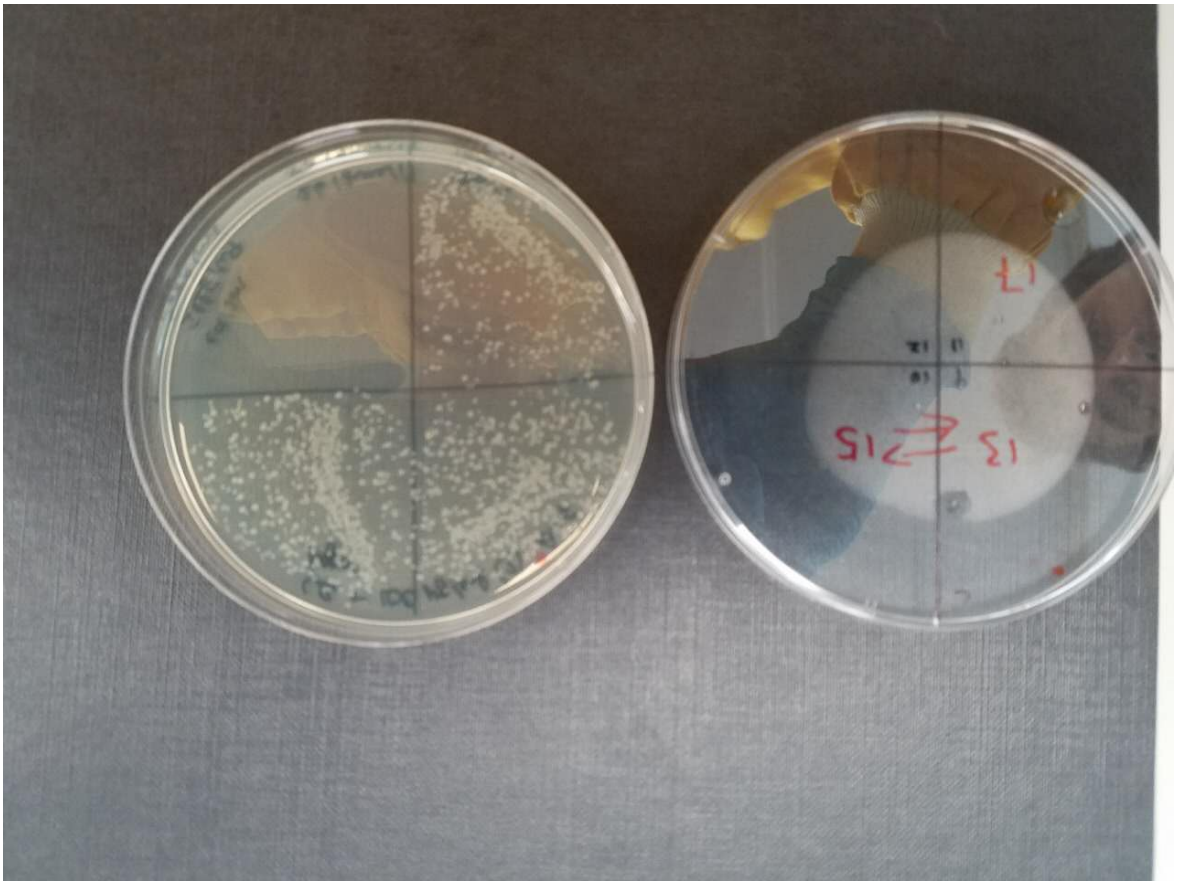



L7
L3
L5


L7
L3
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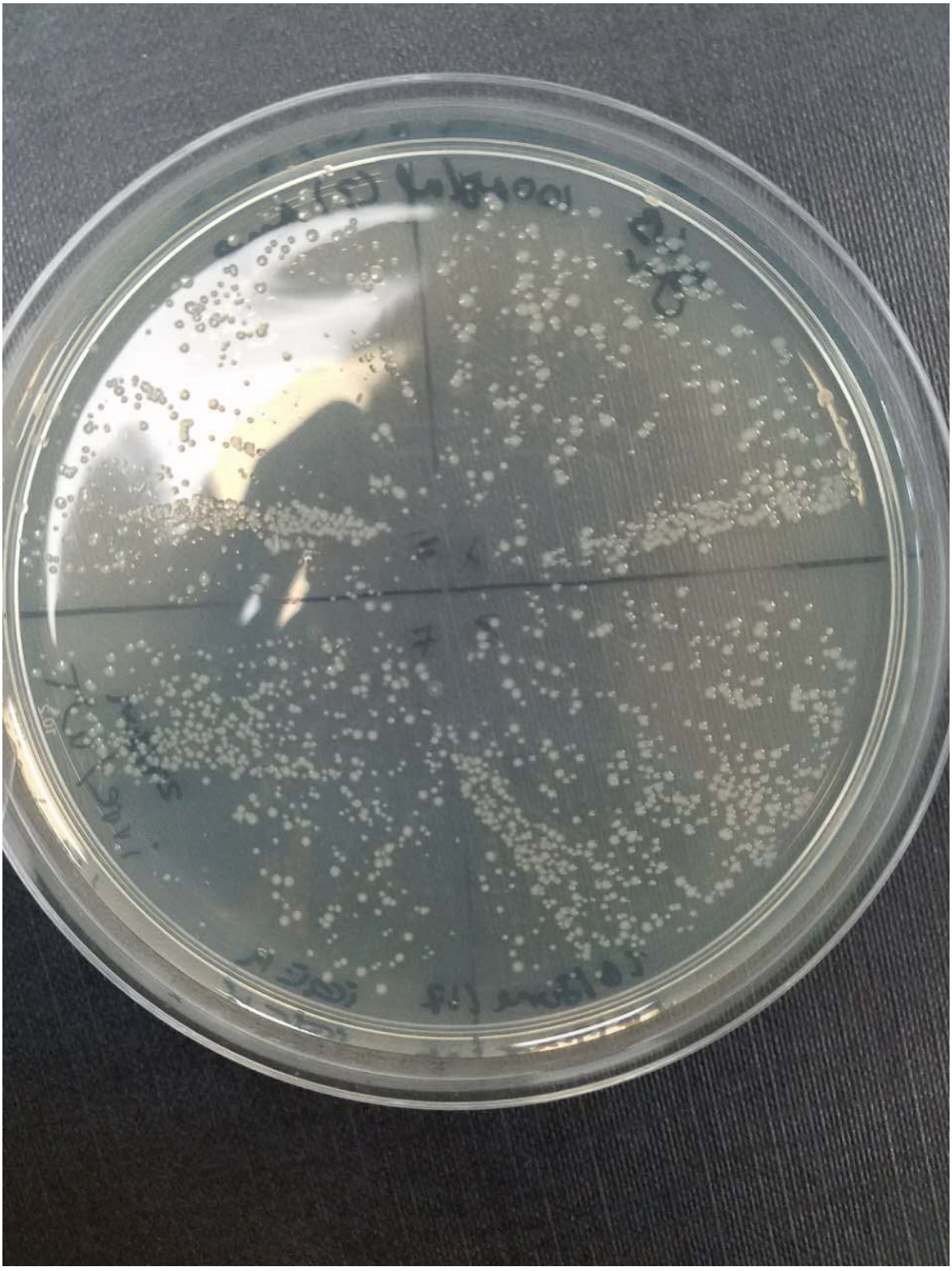
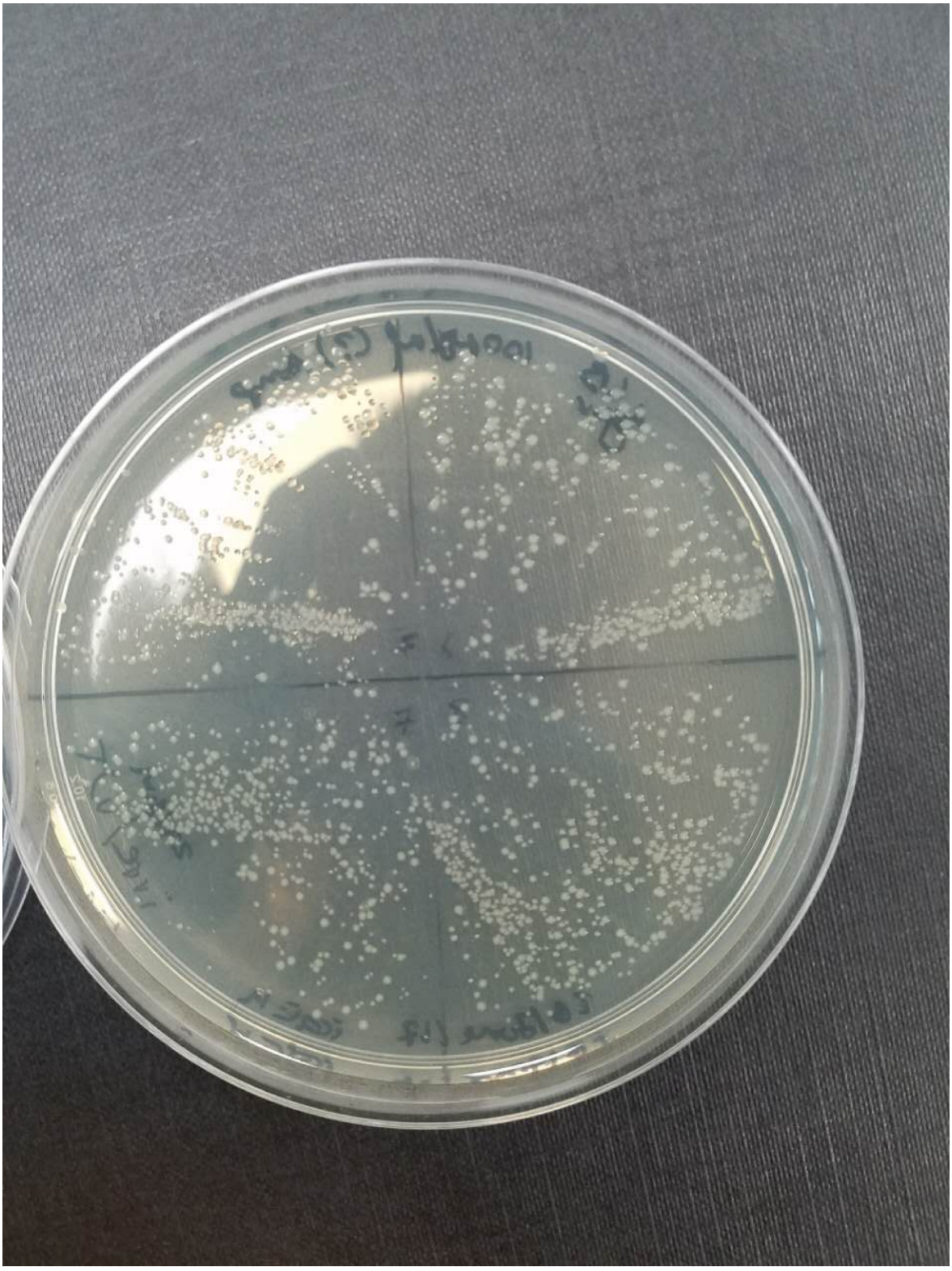
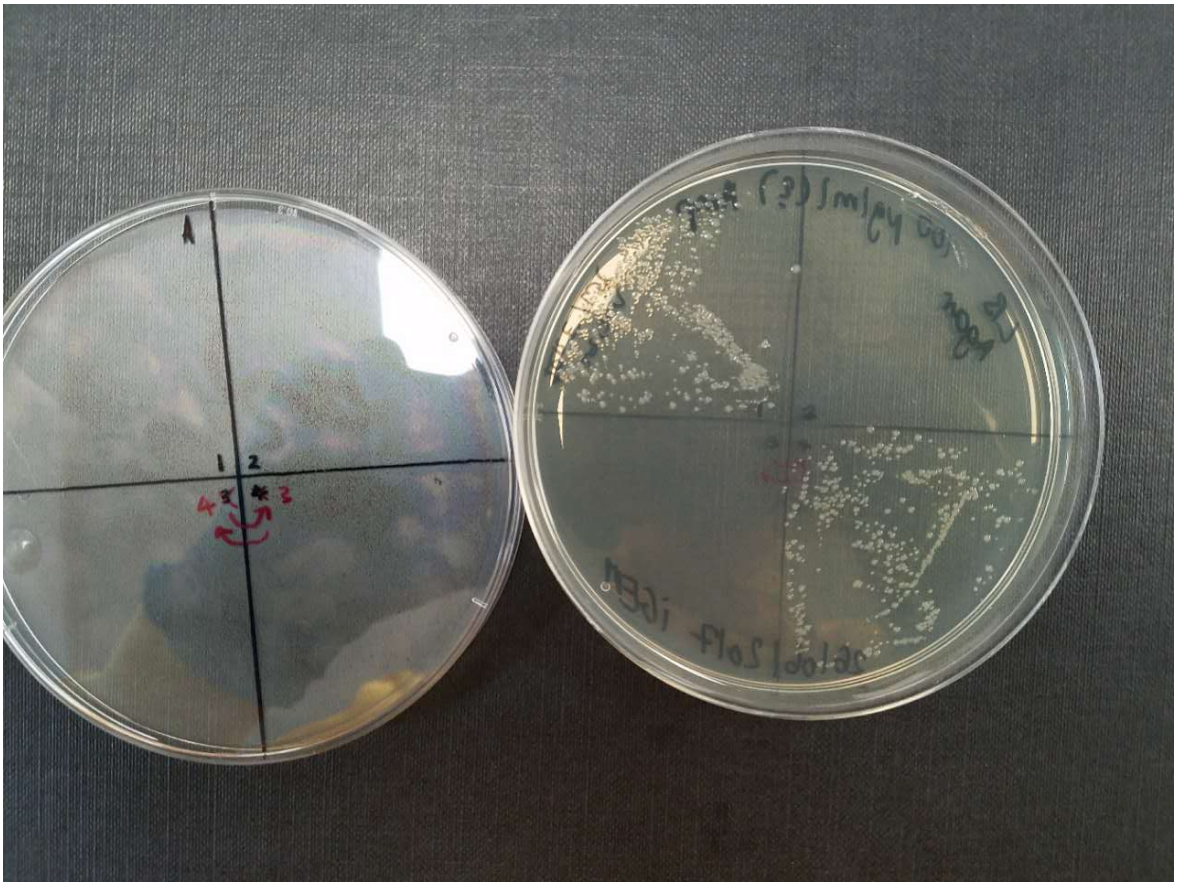





1
2
4
3

Part A. Testing expression levels of fusion proteins:

1. Intimin'-SpyTag
2. Intimin'-SpyCatcher

Experiments in brief:

1. Two cell lines expressing either fusion protein will be combined and will measure sedimentation levels or using a particle sizer
    a. Wild type + wild type - measure sedimentation
    b. Wild type + SpyTag/Catcher - measure sedimentation (after DNAcome)
2. Visualize protein localization using GFP tagged SpyTag or SpyCatcher added externally onto cells expressing surface binding partner (cell-protein interaction)
3. Visualize expression levels of each fusion protein using SDS PAGE gel
    a. Run wild-types through the SDS PAGE.
4. His tag purification of complex formed

Protein Expression protocol

Day n-1, Late afternoon
Set up starter culture:
10ml medium/antibiotic
1 colony from plate/glycerol stock
37 oC o/n

Day 1
Subculture o/n culture into fresh medium/antibiotic.
Volume: more than enough to split into later cultures.
Dilution: Add o/n culture to 1-2% final volume.
37 oC, 2h Measure OD600
Blank w clean medium.

Induce if mid-log phase. (Log phase OD600 = 0.4-0.8. Mid-log phase is around 0.6.) (If not yet log phase/mid-log phase, return culture to incubator, check again in 20-30mins.)

Split culture into -+ induction (or whatever conditions you're trying). (Uninduced control is pretty important.)
Add inducer to relevant concentration (eg. IPTG to 1 mM).
Incubate: 37 oC, 2-3 h or 30 oC, 3-6 h or 16-25 oC o/n

Harvest cells:
Take final OD.
Pellet 2.5 OD cells in cooled microfuge (usually around 1-2ml culture). (4min, 8,000 xg, 4oC)
- wash in 500 µl cold buffer*, re-pellet
- resuspend in lysis buffer*
*Buffers to use here will depend on application. You don't want the wash buffer to be a lysis buffer, so Tris-Cl of ~ pH 7.5 w NaCl between 50-150 mM is ideal.

Lysis buffer: can be like wash buffer if sonicating. Can also add reagents for lysis, eg. detergents like Tween or Triton. Most commonly I add lysozyme to 1mg/ml final concentration and incubate at for 30 oC, 20min.

50 mM Tris-HCl pH 7.5
100 mM NaCl
1 mM DTT (for intracellular proteins)
5% glycerol (possibly)

I keep the final buffer to 250 µl usually. This means you'll have 2.5OD cells/250 µl = 0.01 OD/µl, which is a good concentration, as 10 µl of this (0.1OD) is a decent # cells and a decent amount of proteins to load onto SDS-PAGE.

Lysis either:
(1) sonication: cup horn sonicator, settings 100% amplitude, 2min, 30s on/30s off.
(2) lysozyme (see above) - typically followed by sonication as well.
(3) can freeze pellet after washing (see above), and on thawing, either do sonication or lysozyme method.
Separation of fractions: spin in cooled microfuge: 30' at 16,000 xg, 4oC pellet will contain **insoluble proteins**, membrane fractions. **Soluble** material can be transferred to new eppie. pellet can be resuspended in 250 µl 1X LB.

Prep samples for gel: Soluble: 15 µl sample + 15 µl 2xLB [= 30 µl, of which to load 20 µl on gel] boil 95oC, 5min
Insoluble: boil whole 250 µl as above.

Day 2

Run SDS-PAGE gels to determine whether your protein was expressed.
Typically, MW ladder (NEB colour prestained/broad range, 5 µl), soluble fractions, insoluble fractions. Soluble: load 20 (of 1:1 sample:2xLB), Insoluble: load 5 µl (of sample in 1xLB).
*Insoluble fractions run better if they've been passed through a syringe to break up DNA.

Typically: 80-100 V until samples get through stacking gel, then 120V+. This takes ~2h total. (Technically possible to run it much faster, resolution is very poor though.)
Stain: instant blue, 30min RT on rocker. Destain in dH2O, 30min or o/n.
Capture on Licor

Part B. Testing covalent interaction between fusion proteins in vitro

SDS-PAGE to detect amide bond formation between SpyTag-fusion protein and SpyCatcher

Proteins mixed at 10uM in PBS pH7.4 at 24*C for 3hr (quantifications performed in triplicate)
Stop reaction by heating samples in SDS loading buffer on a Bio-Rad C1000 thermal cycler at 95 °C for 7 min.
SDS-PAGE on 14% polyacrylamide gels, at 200 V for approximately 1 h.
Gels stained with Instant Blue Coomassie stain (Triple Red Ltd.) and band intensities were quantified using a Gel Doc XR imager and Image Lab 3.0 software (Bio-Rad).

Reactions for pH-dependence: Mixing 10 μM of each protein in 40 mM Na2HPO4 with 20 mM citric acid pH 7.0 (phosphate-citrate) for the indicated time at 25 °C, or at the indicated temperature and pH. (PBS alone would not enable proper buffering over the pH range explored.)
For determining temperature dependence: all reactions were incubated in a Bio-Rad C1000 thermal cycler at 4, 25, and 37 °C with a heated lid to prevent evaporation.
To calculate the rate constant, SpyTag-MBP and SpyCatcher at 10 μM were mixed in triplicate in phosphate-citrate and incubated at 25 °C for 1, 3, or 5 min, in the linear part of the reaction. Samples were then heated to 95 °C for 7 min in SDS loading buffer and analyzed on 14% SDS-PAGE with Coomassie staining. Unreacted SpyCatcher concentration was quantified from band intensity as above. 1⁄[unreacted Spy Catcher] was plotted against time and a straight line, whose gradient corresponds to the second order rate constant, was fitted using the "LINEST" linear least squares curve-fitting routine in Excel. The units were converted from μM−1 min−1 to M−1 s−1.

→ could include GFP to see in vivo localization of fusion proteins

Part C. Testing covalent interaction between fusion proteins

- Combine cultures, one expressing SpyTag fusion protein (that had the highest expression levels) with another culture expressing the SpyCatcher fusion protein (which had the highest expression levels)
- Repeat protein expression protocol
- Isolate protein extracts using His column
  → ensure His tag is on SpyCatcher

Cell aggregation:
http://www.pnas.org/content/100/23/13259.full

Microscopy and Data Acquisition. To record the motion of individual cells in the clusters, the slides were left undisturbed for ≈20 min on the microscope stage, which was found to be the optimal time required to form tight clusters that did not drift. Images were acquired within the window of 20–40 min because after 40 min many cells lost motility and the clusters had a tendency to disperse. This result is probably caused by exhaustion of oxygen by bacterial respiration. Cells grown in LB medium typically loose motility under a coverslip after ≈20 min. The cluster size in LB medium is comparable to that observed in minimal media (M9 glycerol). Both dark field and fluorescence microscopy were used to characterize the macroscopic cluster properties and the motility of single cells. Images were acquired at 15 frames/s by using a Nikon E800 microscope equipped with a cooled CCD camera (CoolSNAP$_{HQ}$, Roper Scientific, Duluth, GA).
Data Analysis. Cells were tracked by using Metamorph (Universal Imaging Systems), which yielded the coordinates of each fluorescent cell for every frame of the video. Cells that left the field of view were not tracked. These data were then analyzed by using a tumble detection algorithm similar to Alon *et al.* (19) implemented in MATLAB (Mathworks, Natick, MA). Cells that swum at <75% of the mean speed were discarded from the data set. Success of the program was verified by visual examination of recordings and was found to be 90% accurate. The errors were largely due to cells that did not slow down while changing direction. It is possible that these may not in fact be tumble events but instead collisions with other cells or with the slide or coverslip. The center of a cluster was determined by averaging dark field images acquired over 6.5 s. In total, from the trajectories of 32 individual cells expressing GFP found in 28 clusters, 97 tumble events were registered. To determine the cell density profile, 19 clusters consisting only of cells expressing GFP were analyzed. The mean swimming speed of cells was determined to be 27 μm/s, which is the projected 2D speed. The actual speed will be a slightly greater because the system is only quasi-2D; i.e., the height of the system was ≈40 μm, which is approximately one-fifth of the typical cluster diameter.

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0107445
Fluorescence microscopy
For the selection lines derived from *E. coli* MGAY, when the cells reached an absorbance 0.2 (600 nm), they were placed on a slide that was layered with a 1.5% agarose pad and covered with a cover slip to immobilize the cells. Observations were made using differential interference contrast (DIC) and fluorescence imaging on Zeiss Axioimager M-1 upright microscope (100X/1.40 Oil DIC M27) with a color digital camera controlled by Axio software 4.8. Images both DIC/fluorescence were taken using exposure time of 30 millisecond illumination. The resulting images were processed and analyzed using Image J 1.44c software [25]. The fluorescence image alone was used for quantification of fluorescence intensity reflecting aggregate size whereas composite image formed by merging DIC and fluorescence was used to determine the location of protein aggregates in a cell.

Part D: Light induction

Introduce construct of best fusion protein partners in plasmid downstream of the light induction control promoter

1. Seed cultures from freshly transformed plates and grow overnight in 5 ml of LB medium supplemented with appropriate antibiotics at 37°C with shaking (225 rpm). Where specified, *N*-(3-oxo-hexanoyl)-l-HSL was added to the cultures at the indicated concentrations.
2. Overnight cultures were diluted into 5 ml of fresh pre-warmed M9 medium [(12.8 g $Na_2HPO_4 \cdot 7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl), 1 M $MgSO_4$, 1 M $CaCl_2$, 0.2% (w/v) casamino acids and 20 mM glucose as a sole carbon source. As appropriate, antibiotics] for 2–3 h exponential outgrowth at 37°C with shaking (225 rpm).

3. Cultures aliquoted (0.5 ml) into 0.5 ml of pre-warmed M9 medium in triplicates of total 1 ml volume in a flat-bottom 12-well microplate (Nunc™). The microplate was incubated at 37°C with shaking (120 rpm) in a mini shaker incubator with illumination or kept in dark (wrapped in black cloth covering all edges) between each cycle of measurements.
4. Cells are illuminated using a custom built 3 × 4 LED blue light panel (465 nm) with adjustable ON/OFF pulsing and intensity for individual wells.

IF WE SOMEHOW HAVE GFP expression as well, we could observe directly junction between cells that have adhered, fluoresce

Time series OD (600 nm) and fluorescence (GFP: excitation 485 nm, emission 528 nm and RFP: excitation 540 nm, emission 600 nm) were read using Synergy™ HTX Multi-Mode Microplate Reader (BioTek). In all experiments, auto-fluorescence was measured using a negative control strain lacking the fluorescence reporter. The fluorescence/OD_{600} (Fluo/OD_{600}) at a specific time for a sample culture was determined after subtracting from each of the technical triplicate readings of the negative control cultures (fluorescence free) at the same time. The fluorescence synthesis rate (Fluo.$OD_{600}{}^{-1}$/min) of any sample at time t, was calculated by taking the difference of Fluo/OD_{600} values from two time points and dividing the result by the time interval δt. Normalization of Fluo/OD_{600} values was calculated into a new arbitrary range (min = 0) to (max = 1) with the observed original dataset min and max values.

Otherwise we would just perform protein expression assays post light induction vs no light induction and His tag column

24/07/17
Experiment:
Culture Preparation

Introduction:
Prepare the overnight culture for experiments on bacterial viabilities in lubricants and M9 media. The experiments are based on the irrE transformed and non-transformed cells. Another test is also done as reference on the original strain from which the plasmid was obtained.

Materials:
ON Culture 1 = NEB 10-ß irrE transformed cells (Positive) + LB + 20 µl Ampicillin
ON Culture 2 = NEB 10-ß irrE non-transformed cells (Negative, wild type) + LB
ON Culture 3 = irrE Stellar cells (plasmid original transformed strain) + LB +20 µl Ampicillin

20 ml each for 3 cultures.

Method:
Normal inoculation method.
Keep everything sterilized and culture is supposed to be autoclaved.
Take optical densities tomorrow.

Appendix:


Cell adhesion control experiments

Experiments in brief:

1. Two cell lines expressing either fusion protein will be combined and will measure sedimentation levels or using a particle sizer
    a. Wild type + wild type - measure sedimentation
    b. Wild type + SpyTag/Catcher - measure sedimentation (after cells come)
2. Visualize expression levels of each fusion protein using SDS PAGE gel
    a. Run wild-types through the SDS PAGE
3. Visualize protein localization using GFP tagged SpyTag or SpyCatcher added externally onto cells expressing surface binding partner (cell-protein interaction).
4. His tag purification of complex formed

31/07/17
Experiment:
Cell Growth against oxidative stress, high salinity environment and lack of nutrients.

Introduction:
Test the growth conditions of IrrE plasmid harboring cells and negative control in media with various stress. The oxidative stress is brought by hydrogen peroxide. The high salinity environment is created by high molarity NaCl solution. The M9 media is a good environment to test the cell growth in lack of nutrients.

The experiment can be referred to previous paper. Certain modification on the concentration of H_2O_2 and NaCl so that more data can be collected and be characterized. Both positive and negative control should have plasmid but positive has IrrE plasmid and negative has plain plasmid.

Materials:
Plate reader
ON culture of +/-
96 x well plates
Ampicillin (Depends on the vector)
LB broth

Methods: (+/-=control groups, 3= triplicates as biological repeats)
Take 1 ml of ON culture, measure OD_{600}

- **H_2O_2 + M9/LB**
    1. Pellet 1 ml cell culture and re-suspend with 1 ml LB with 15 µl of H_2O_2 for 10 mins
    2. Pellet and re-suspend with LB/M9
    3. Measure the optical density and calculate equivalent volume of OD_{600}=0.1
    4. Inoculate to 12 tubes (3+&3- in LB)/ (3+&3- in M9)

- **NaCl + M9/LB**
    1. Measure the optical density and calculate equivalent volume of OD_{600}=0.1
    2. Add NaCl to LB/M9 culture to 1 M.
    3. Inoculate to 12 tubes (3+&3- in LB)/ (3+&3- in M9)

- **M9/LB Media**
    1. Measure the optical density and calculate equivalent volume of OD_{600}=0.1
    2. Inoculate to 12 tubes (3+&3- in LB)/ (3+&3- in M9)

The results should provide sets of comparing data.

1. Growth in 3 stresses conditions. (Stress only variable and has the negative control all)
2. Cell growth in Pure M9 and LB as reference. (Both Negative and Positive)

Appendix:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2635966/

For M9 + Salt.

+

M9	M9 + H_3O_2	M9 + NaCl
① M9		
② M9	M9 H_3O_2	M9 + NaCl
③ M9	M9 + H_3O_2	M9 + NaCl

LB

① LB	LB H_3O_2	LB NaCl
② LB	LB H_3O_2	LB NaCl
③ LB	LB H_3O_2	LB NaCl

−

M9	M9 + H_3O_2	M9 NaCl
① M9		
② M9	M9 H_3O_2	M9 NaCl
③ M9	M9 H_3O_2	M9 NaCl

LB

① LB	LB H_3O_2	LB NaCl
② LB	LB H_3O_2	LB NaCl
③ LB	LB H_3O_2	LB NaCl

20 mL LB × 18

01/08/17
Experiment:
IrrE Positive and Negative Hydrogen Peroxide Resistance Preliminary Screening

Introduction:
To pre-test the viability of IrrE harbouring strains and negative control strain in the presence of 1.5% and 1.0% of H_2O_2. This provides the information for the culturing experiments in next session.

Materials:
LB
H_2O_2
Agar plates and agar plates with 100 µg/ml Ampicillin
Spreaders

Methods:

1. Take the ON culture of both strains

Centrifuge 3 tubes of 1 ml of each, labelled as LB, LB+1.0% H_2O_2
, LB+1.5% H_2O_2. Do the same labelling on negative ones.

2. Re-suspend 2 tubes labelled as LB with fresh LB

Re-suspend 2 tubes labelled as LB+1.0% H_2O_2 with 990 µl of LB and 10 µl of H_2O_2

3. Re-suspend 2 tubes labelled as LB with fresh LB

Re-suspend 2 tubes labelled as LB+1.0% H_2O_2 with 985 µl of LB and 15 µl of H_2O_2

4. Incubate all tubes in 37˚C water bath for 10 mins
5. Take 4 tubes with H_2O_2 and centrifuge. Then re-suspend with fresh LB

4 Plates are used, labelled as: (with quarters) Q = Quarter
+ Group Amp 1.0%: 1stQ = LB 2ndQ= LB+1.0% H_2O_2 3rdQ=1/10 LB+1.0% H_2O_2 4thQ = N/A
+ Group Amp 1.5%: 1stQ = LB 2ndQ= LB+1.5% H_2O_2 3rdQ= 1/10 LB+1.5% H_2O_2 4thQ = N/A
- Group Agar 1.0%: 1stQ = LB 2ndQ= LB+1.0% H_2O_2 3rdQ= 1/10 LB+1.0% H_2O_2 4thQ = N/A
- Group Agar 1.5%: 1stQ = LB 2ndQ= LB+1.5% H_2O_2 3rdQ= 1/10 LB+1.5% H_2O_2 4thQ = N/A

Plate as labelled accordingly.
Incubate the plates ON to see the colonies.

25/07/17
Experiment:
Cell Growth in M9 media and Lubricant

Introduction:
Monitor the growth of irrE cells in different media by measuring the optical densities. The cells are cultures for 8hrs. Measurements are taken every 20 mins when the cells duplicate.

Materials:
ON culture 1 2 3
12 Shaking flasks
Pipette gun
Lubricant
M9 solution
LB media

Method:
Take optical densities of each ON culture and dilute them into same cell density.

Prepare 50ml of culture 1/2/3/4
1= M9 solution
2= LB media
3= Lubricant + LB media (Lubricant concentration 40%v/v)
4= Lubricant + M9 solution (Lubricant concentration 40%v/v)

Label 12 flasks as below
- (+) Culture 1/2/3/4
- (-) Culture 1/2/3/4
- (s+) Culture 1/2/3/4 12 flasks in total.

Inoculate accordingly into each flask.
The number of cells inoculated must be same for three cultures.
The method is described below:
The Equation was $C_1V_1=C_2V_2$, the concentration (C) and volume (V) before and after dilution.
OD_{600} of ON culture 1 2 3 (1/8 dilution)
C_1=0.154*8=1.232 C_2V_2=0.5
C_2=0.160*8=1.280 C_2V_2=0.5
C_3=0.214*8=1.712 C_2V_2=0.5
After inoculating
Take optical density of 12 cultures every 20 mins for 8 hours.

Appendix:
Data was saved on the Excel in the same folder.


New Brunswick Scientific

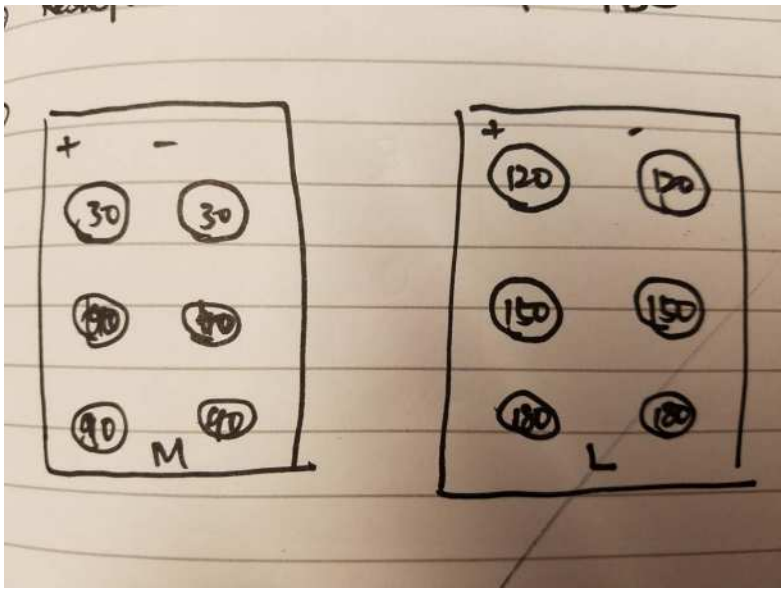
12/07/17
Experiment:
irrE Exposure Experiments III

Introduction:
Test the viability of cells transformed with irrE plasmids (+) as positive group with the comparison to non-transformed ones (-). The viabilities of two groups are compared against period of exposure. The overnight culture was prepared in advance. Additionally, the plated is covered with aluminum to prevent *E.coli* photo-reactivation and another reference group is un-covered.

Materials:
Sterile 6-well plates
ON culture of both Positive and negative groups.
Autoclaved Spreaders
LB Plates
LB Plates with Ampicillin
Sterile PBS solution

Methods:
Sterilize everything used involving cells.
Centrifuge 2 ml of ON culture of each and re-suspend with PBS.
Take out 5 µl of both resuspension cultures.
Pipette cell culture onto the according wells. (as shown in the appendix)
Re-suspend with 1995 µl of PBS.
Expose the plates in the UV cross-linker for the period required.
Both culture re-suspensions are exposed for 30/60/90/120/150/180 mins and get covered with aluminum foil.
Prepare one plate for UV exposed but not uncovered as reference. The Exposure time is 90 mins.
Take 20 µl of the culture after exposure and plate it onto plates with according antibiotics.
Repeat the steps when exposure period increases.

Appendix:


+
-
30
30
90
90
M
+
-
120
120
150
150
180
180
L

23/July/17 IrrE UV & Media experiments

UV

- 2ml of Stellar+ / 10β+ / 10β- → spin
- resuspend in 2ml PBS

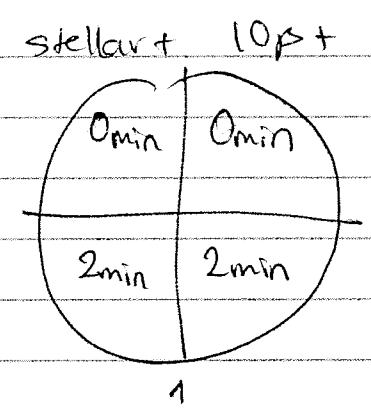
	Stellar+	10β+	10β-
0min	✓	✓	✓
2min	✓	✓	✓ *
5min	✓	✓	✓ +3min
10min	✓	✓	✓ +5min
15min	✓	✓	✓ +5min
20min	✓	✓	✓ +5
30min	✓	✓	✓ +10 min

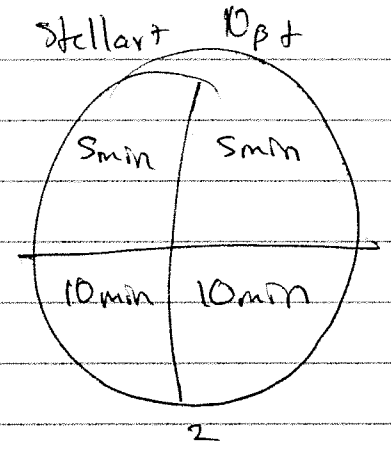
LB Plates

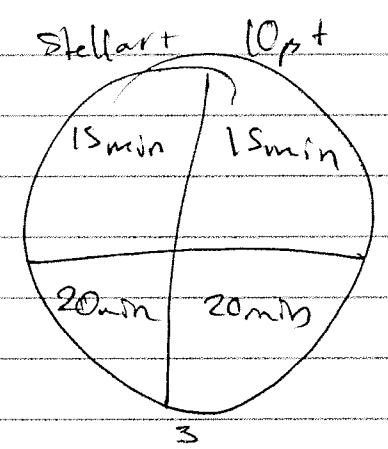
Stellar+	10β+
0 ✓	0 ✓
✓ +2	✓
✓ +3	✓
~~+5~~	
~~+10~~	✓
~~+10~~	
+15min ✓	

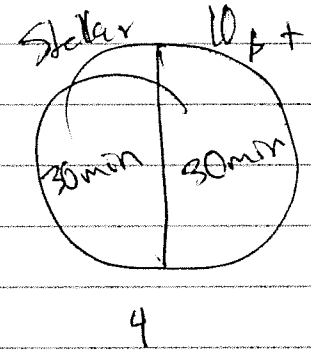
* did this one at a time

- add 5µl culture onto 6 wells x3 (3x6well plates) and 5µl on 1 eppy x3 (3 eppies)
- add 1995µl PBS onto wells / eppies
- expose 6well plates to UV according to table
- Plate [redacted]µl onto









4x Amp plates

7x LB plates for 10β-

7x LB plates for 10β+

7x LB plates for Stellar+

28/July/17 IrrE UV & Media experiment

UV

- 2ml of Stellar+ / 10β+ / 10β- → spin
- resuspend in 2ml PBS

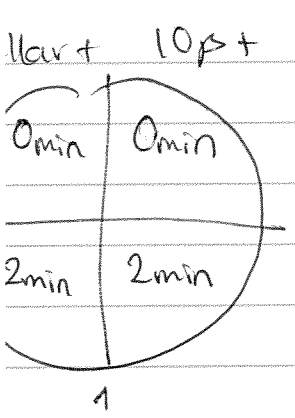
	Stellar+	10β+	10β-
0min	✓	✓	✓
2min	✓	✓	✓ *
5min	✓	✓	✓ +3min
10min	✓	✓	✓ +5min
15min	✓	✓	✓ +10min
20min	✓	✓	✓ +5
30min	✓	✓	✓ +10 min

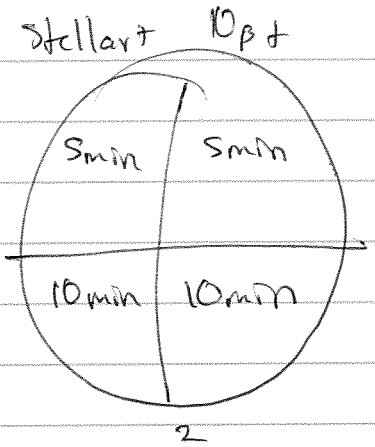
*did this one at a time

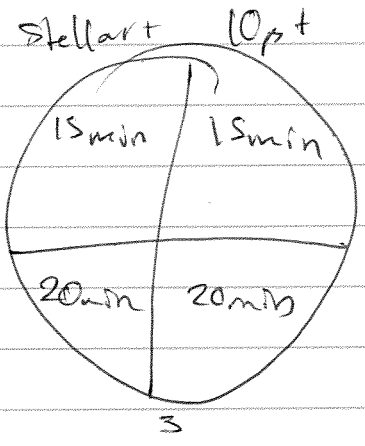
LB Plates

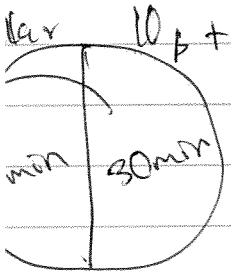
Stellar+	10β+
0 ✓	0 ✓
✓ +2	✓
✓ +3	✓
~~+5~~	
+10	✓
~~+10~~	
+15min	✓

- add 5µl culture onto 6 wells x3 (3x6well plates) and 5µl on 1 eppy x3 (3 eppies)
- add 1995µl PBS onto wells / eppies
- expose 6well plates to UV according to table
- Plate [redacted]µl onto









4x Amp plates

LB plates for 10β -

LB plates for 10β +

LB plates for Stellar +

M1E1_DH5α E.coli E-cadherin transformation_19.07.2017

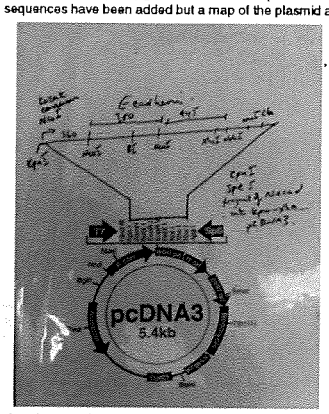
Aim

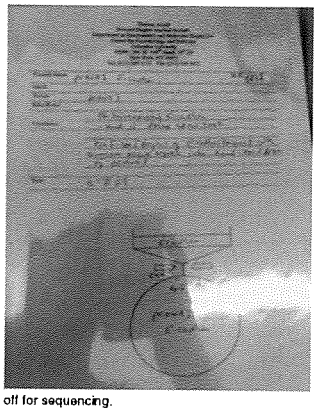
amplification of E-cadherin plasmid: transformation of DH5alpha E.coli with E-cadherin (mouse)

iGEM Members: Anima Sutradhar & Camillo Moschner
Supervisor: Alex Cotton

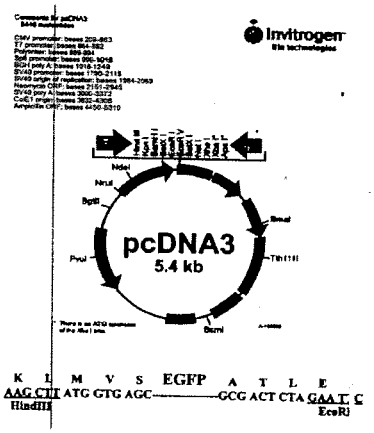
Background

Prof. Stephen Price (CDB) gave the iGEM team (Camillo Moschner) 2 µL (5µg/µL) of pcDNA3 plasmid with E-cadherin (mouse); no sequences have been added but a map of the plasmid and a log entry:





We therefore need to amplify the existing plasmid to be able to send it off for sequencing.



pcDNA3 map from the internet (comparison)

Results

The transformation was succesful and yielded 1,000+ colonies.

Reagents

DH5alpha E.coli (NEB)
pcDNA3 (containing E-cadherin [mouse])
dH2O
SOC medium

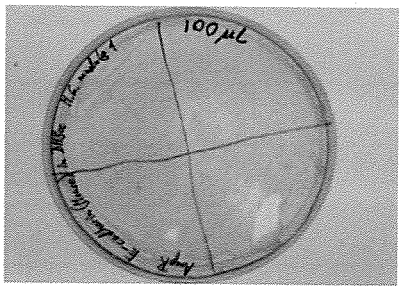
Transformation Protocol (NEB - High Efficiency Transformation Protocol (C2987H/C2987I))

1) Dilute 2 µL (5µg/µL)* of pcDNA3 with 18 µL dH2O → new concentration 0.5µg/µL
2) thaw one tube of DH5alpha cells
3) Pippete 50 µL DH5alpha cells into a 1.5 mL Eppendorf tube
4) Add 5 µL (2.5 µg/5µL) to the 1.5 mL Eppendorf tube
5) Put 15 µL of pcDNA3 (rest) into the freezer

6) Put on ice for 10min (normally 30min but since we are not interested in efficiency we speeded up the standard protocol)
7) Put 1.5 mL Eppendorf in the waterbath at 42°C (Heat-shock treatment) for exactly 30s
8) Add 800 µL of SOC medium to the 1.5 mL Eppendorf tube
10) Incubate with shaking (220 RPM) at 37°C for 1h
11) Add 100 µL / 50 µL / 20 µL to AmpR LB agar plates & streak them evenly
12) Inoculate overnight at 37°C (approximately 16 hours)

Discussion

DH5alpha show strong growth on the AmpR LB agar medium; ideal for further culturing of the cells



M1E2_Creating liquid culture of DH5α E.coli (E-cadherin +)_20.07.2017

Aim

amplification of E-cadherin plasmid: creating a liquid culture of the freshly transformed DH5alpha E.coli with E-cadherin (mouse

iGEM Members: Anima Dakota & Camillo Moschner

Supervisor: Tom Hickman

Background

see M1E1

Results

The transformation was succesful and yielded 1,000+ colonies.

Reagents

transformed DH5alpha E.coli (containing pcDNA3 with E-cadherin)

LB media

Ampicillin

Protocol

1) Transfer 10 mL LB medium in a Falkentube (2x)
2) Add 10 µL of Ampicllin from the 1,000X to create 1X (2x)
3) Use inoculation loop to inoculate 1 colony from M1E1 into the Falkentube (2x)
4) Inoculate overnight at 37°C at 220 RPM overnight

Discussion

-

M1E3_Plasmid DNA Purification of DH5α E.coli (E-cadherin +)_21/24.07.2017

Aim

Purification of of E-cadherin plasmid: performing a mini-prep on transformed *E. coli* DH5alpha with E-cadherin (mouse)

iGEM Members: Anima Sutradhar & Camillo Moschner
Supervisor: Tom Hickman

Background

See M1E1 and M1E2

Results

We successfully extracted and purified the pcDNA3 plasmid containing E-cadherin. The elution is pure and no contaminants could be detected by NanodropOne analysis.

The final concencatration of plasmid in elution buffer:

Sample 1: 788.907 (ng/uL) - 48 µL left in the freezer

Sample 2: 855.815 (ng/uL) - 48 µL left in the freezer

Reagents

Resuspension Solution
Lysis Solution
Neutralization Solution
Wash Solution (Concentrated)
Elution Buffer

Protocol (ThermoScientific GeneJET Plasmid Miniprep Kit - Purification Protocol A)

1) Resuspend the pelleted cells in **250 µL of the Resuspension Solution**. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2) Add **250 µL of the Lysis Solution** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.

3) Add **350 µL of the Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times. **Note:** it is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudy.

4) Centrifuge for 5 min to pellet cell debris and chromosomal DNA.

5) Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. **Note:** close the bag with GeneJET Spin Columns tightly after each use!

6) Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.

7) Add **500 µL of the Wash Solution** (diluted with ethanol prior to first use to the GeneJETspin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.

8) Repeat the wash procedure (step 7) using **500 µL of the Wash Solution**.

9) Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.

10) Transfer the GeneJETspin column into a fresh 1.5 mL microcentrifuge tube (not included). Add **50 µL of the Elution Buffer** to the center of GeneJETspin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min.

11) Discard the column and store the purified plasmid DNA at -20°C.

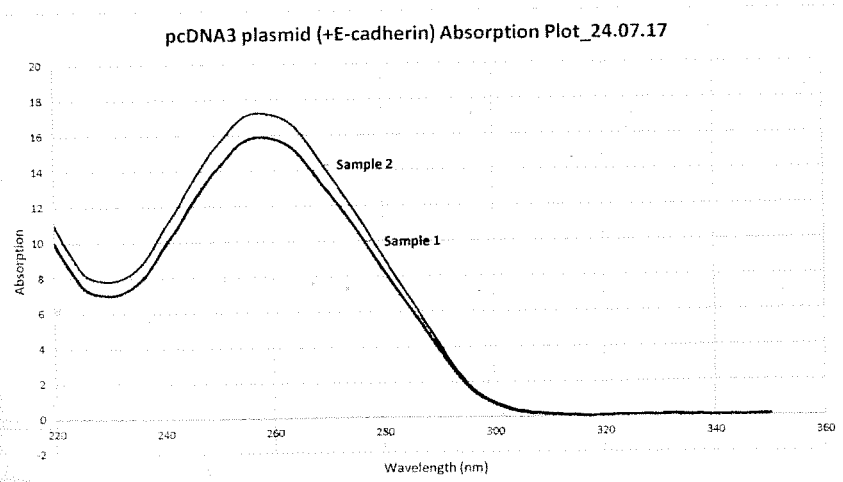
12) Measure the concentration and analyse the purity of the purified plasmid with the Nanodrop: use 2 µL for analysis (24.07.)

→ 12500 RPM for microcentrifuge.

Discussion

This was the first time we used 'ThermoScientific Nanodrop One'. It was successful and easy to use. The DNA elution is remarkably pure and the detailed data is attached in the digital version of this protocol (see google drive: UCL iGEM 2017>Lab-protocols>Mammalian Group)

Insignificant difference between the two samples might be due to picking different colonies, different growth rates or intrinsic impresicion in pipetting.



Sample Name	Nucleic Acid(ng/uL)
Sample 1	788.907
Sample 2	855.815

Gibson Assembly

mm -- pg -- ng

#2 - Intimin SpyTag (0.2 pmol/4)

	x 0.04		
ITA	21 ng/µl	2.1 µl	+ 10 µl MM ✓
ITB	21 ng	2.1 µl	+ 1.45 µl dH_2O ✓
ITC1	10.6 ng	1.06 µl	
ITC2	11.1 ng	1.11 µl	tot = 20 µl
Plasmid 2070 (25 ng/µl)	54.6 ng	2.18 = 8.55	

#3 - Intimin SpyCatcher (0.2 pmol/4)

	x 0.05		
ICA	32.2 ng	3.22 µl	+ 20 µl MM ✓
ICB	32.9 ng	3.29 µl	+ 8.91 dH_2O

Transformation

- Thaw on ice
- 2 µl assembly + 50 µl CK
- Ice 10'
- 42°C 35 sec
- Ice 2 min
- 950 µl SOC
- 37°C 60' shaking 250 rpm
- 100 µl on plate
- Spin + Plated rest

- 50°C x 60 min Incubation (4-6 fragments)
- Transformation (5 min 2-3 fragm

$c = \frac{n}{V}$

Gibson Assemblies

ng/ul

2	[DNA]	Purity
1	146.4	2.04
2	~~146.4~~ 148.2 ~~2.04~~	2.06
3	155.3	1.98
4	141.1	1.98

148.2 2.06

3	[DNA]	Purity
1	125.1	2.02
2	138 ng/ul	- 2.05
3	115.6	- 1.99
4	126.1	- 1.94

5	[DNA]	Purity
1	128.9	2.05
2	128.3	1.93
3	163.2	2.09
4	168.7	2.07

~~µg — µl~~

$\frac{1000\,ng}{50} = 20\,ng$

Test digest Gibson Assembly

On ice

EcoRI - 1µl
DNA - 1µg
10x Buf - 5µl
Tot = 50µl
43

- 1hr 37°C
- 20min 65°C

DNA	#2	#3	#5	
	6.85µl	8µl	7.8µl	1
	+36.15	+35	+35.2	
	6.8µl	7.25µl	7.8µl	2
	+36.2	+35.75	+35.2	
	6.45µl	8.7µl	6.1µl	3
	+36.55	+34.3	+36.9	
	7.1µl	7.94µl	5.93µl	4
	+35.9	+35.06	+37.07	

2x loeej tot

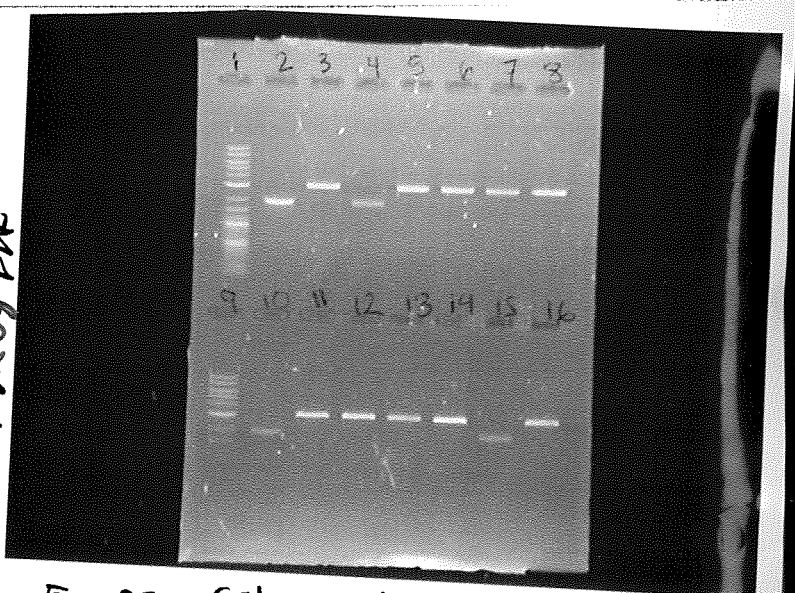
Run gel agarose 14 wells MW, Plasmid, 1-12

~~#2~~ ~~#3~~ 10µl DNA + 2µl ~~buffer~~ dye = 12µl total

~~#5~~ → 20µl DNA + 4µl dye = 24µl total

100 volts 45min

- MW ladder (2-log DNA ladder) (lanes 1, 9)
- Linearized pSB1C3 (lanes 2,10)
- pSB1C3-SpyTag EcoRI digested (lanes 3-6)
- pSB1C3-SpyCatcher EcoRI digested (lanes 7 & 8,11 (2))
- pSB1C3-GFP·Catcher EcoRI digested (lanes 13-16)



EcoRI Gibson Assembly MW, plasmid #2 (1-4), #3 (1-4), #5 (1-4)

- spin 3-5 sec
- dilute
- vortex

- 80°C is - 20 min
- vortex
- centrifuge

1000 ng / 100 µl

500 ng / 50 µl

Received gBlocks → 10 ng/µl

G-STAG

PRSA

PRSB

Intimin CatcherA

ICB

GCA

GC Bamb

222 PRG

ICCamb

RP222

onday 7 Aug

Repeating Transformation w minipreps 2 3 5

- 1µl (~100ng) + 50µl competent cells, flick gently
- ice 20min
- 42°C 30 sec
- ice 2min
- + 950 µl SOC/LB
- 37°C 45 min

#
- Plate

Tuesday 8 Aug

EcoRI 1hr 37°C / 20min 65°C
PstI 1hr 37°C / 20min 85°C
(DpnI 30min 37°C / 20min 80°C)
→ Cutsmart buffer

Enzyme 1µl	5-8µl PCR
DNA 1µg	1µl Cutsmart
10x buff 5µl	1µl DpnI
dH2O 50-xµl	dH2O 10-xµl
tot = 50µl	tot = 10µl

Gel 1
1. MW
2. pSB1C3 (5µl)
3. #2.1
4. #2.1 EcoRI (3.1 kb)
5. #2.1 NotI
6. #2.1 EcoRI, PstI (and D
7. MW

Gel 2
1. MW
2. pSB1A3
x
3. pSB1A3 EcoRI, PstI (+D
x
4. pSB1A3 NotI
5. MW

10 Aug 17

→ 2-3 fragments 0.02 - 0.5 pmoles
→ 4-5 fragments 0.2 - 1.0 pmoles
Optimized: 50ng-100 ng vector ⅓ 2-3x more concentrated fragments

		Pmol	pg	ng	µl	
#1 Intimin GFP SpyTag (1:1)						10 ng/µl
783	IGTA	0.05	0.026 pg		2.6 µl	13.14 DNA µl
783	IGTB	0.05	0.026 pg		2.6 µl	15 µl GMix = 30 µl rxn
373	IGTC1	0.05	0.012 pg		1.2 µl	1.86 µl dH_2O
409	IGTC2	0.05	0.013 pg		1.3 µl	
777	IGTD	0.05	0.026 pg		2.6 µl	
2155	plasmid PSB1A3	0.05	0.071 pg	71 ng	2.84 µl	
#2 Intimin SpyTag (1:1)						25 ng/µl
783	ITA	0.05	0.026 pg		2.6 µl	
785	ITB	0.05	0.026 pg		2.6 µl	10.74 DNA µl
400	ITC1	0.05	0.013 pg		1.3 µl	15 µl GMix = 30 µl rxn
421	ITC2	0.05	0.014 pg		1.4 µl	4.26 µl dH_2O
2155	plasmid PSB1A3	0.05	0.071 pg	71 ng	2.84 µl	
#3 Intimin SpyCatcher (1:1)						
975	ICA	0.05	0.032 pg		3.2 µl	11.64 DNA µl
997	ICB	0.05	0.033 pg		3.3 µl	15 µl GMix = 30 µl rxn
711	ICC	0.05	0.023 pg		2.3 µl	3.36 µl dH_2O
2155	plasmid PSB1A3	0.05	0.071 pg	71 ng	2.84 µl	
#4 GFP spytag (3:1)						
979	G-Stag	0.10	0.065 pg	65 ng	6.5 µl	18.5 µl DNA
2155	plasmid PSB1A3	0.035	0.07 pg	50 ng	2 µl	10 µl GMix = 20 µl rxn
						1.5 µl dH_2O
#5 GFP SpyCatcher (2:1)						
642	GCA	0.07	0.30 pg	30 ng	3 µl	8 µl DNA
720	GCB	0.07	0.33 pg	30 ng	3 µl	10 µl Gmix = 20 µl rxn
2155	plasmid PSB1A3	0.035	0.05 pg	50 ng	2 µl	2 µl dH_2O

- Incubate 50°C for 60 min
- Drop dialysis

→ then Transform! (Pellet)

Thu Aug 10 – Plasmid digest (remove RFP)

(1)

	2x
– DpnI digest	
5–8 µl PCR product	16 µl PCR product
1 µl cutsmart	2 µl cutsmart
1 µl DpnI	2 µl DpnI
10 – x dH2O	~~dH2O~~

Tot volume = 10 µl

– 37 °C for 30 min

– 80 °C for 20 min

Remember! to recalc w/ diluted [plasmid]

(2) Gibson Assembly (see prev page)

(3) Dialysis ½ check on gel

→ Gel 1 ~~2~~ 2-log MW ladder

1	2	3	4	5	6	7	8
MW	Plasmid	fragments #2		fragments #3		MW	~~RFP~~

→ Gel 2

1	2	3	4	5	6	7	8
MW	~~Plasmid~~ C-ve	fragment #4		fragments #5		MW	

(4) Transformation

Repeated Gibson #2 #3 #4 #5

inoculated 5 colonies from each plate

1hr 37°C 60°C 20min

→ PSB1A3 : 2155
PSB1A3-RFP : ~2000

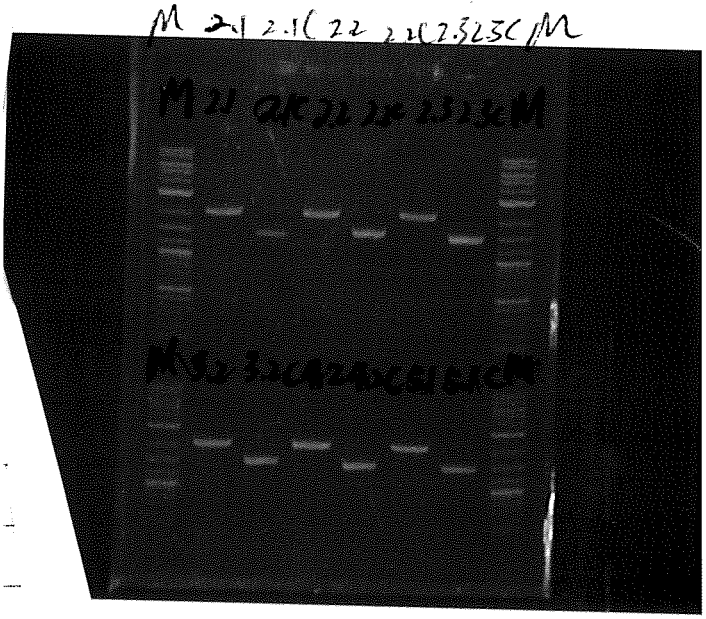
Test digest

	EcoRI / PstI	EcoRI / SphI
#2 Intimin-Tag:	2266bp, 2114	1295, 3085
#3 Intimin-Catcher:	2599, 2,114	1313, 3400
#4 GFP - Tag:	EcoRI / PstI: 977, 2114	BsaAI / pstI ✗: 572, 2519
#5 GFP - Catcher:	EcoRI / PstI: 1318, 2114	BsaAI / PstI ✗: 913, 2519
#6 Plasmid - RFP:	EcoRI / PstI: 680, 2544	/ Plasmid EcoRI / PstI: 41, 2134

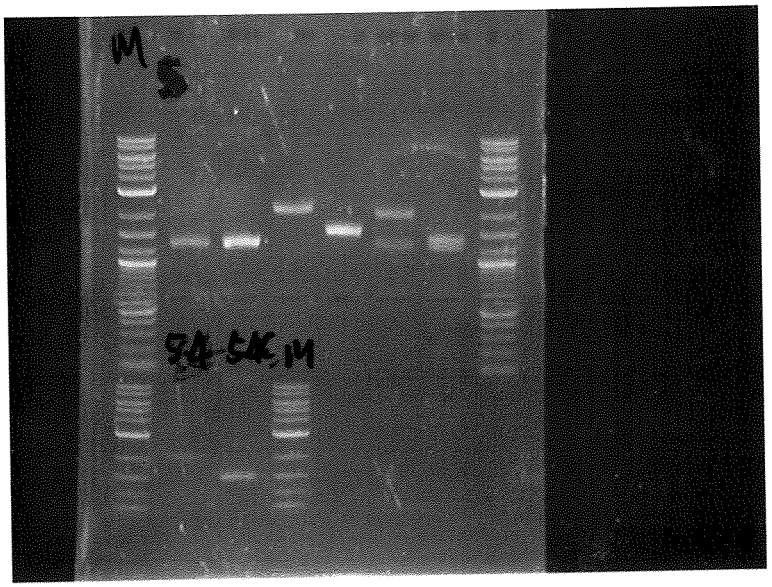
On ice	EcoRI/PstI	EcoRI SphI	BsaAI/PstI ✗
- enzyme 1	1µl	- EcoRI 1µl	
- enzyme 2	1µl	- SphI 1µl	
- DNA	1µg	- DNA 1µg	
- 10x Buffer	5µl	- 10x Buf 5µl	
- dH2O	to 50µl	- dH2O to 50µl	
	Accumet		
	37°C, 5-15min	37°C 5-15min	
	65°C 20min	65°C 20min	

MW | uncut Plasmid | cut Plasmid | #2 uncut | #2 cut | #3 uncut | #3 cut | #4 uncut

#5 uncut | #5 cut



GEL 1



GEL 2

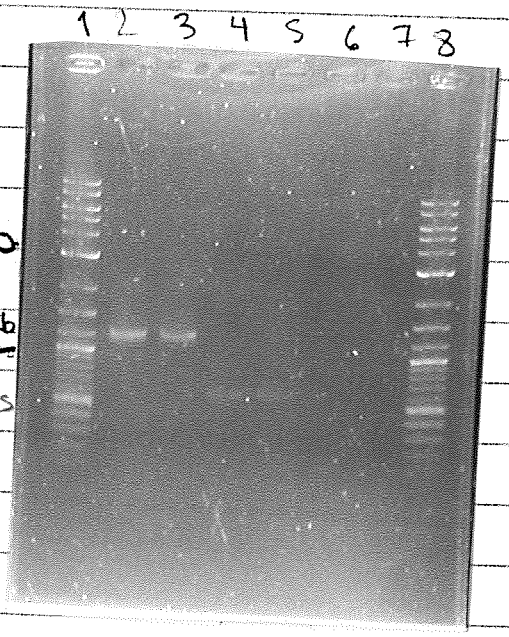
5.4 5.4C

M= Marker

2.1 ~2.3	Intimin Tag	C= uncut Control
2.1C~2.3C	undigested Control	
3.2	Intimin Catcher	
4.2	GFP Tag	
5.1~5.4	GFP Catcher	

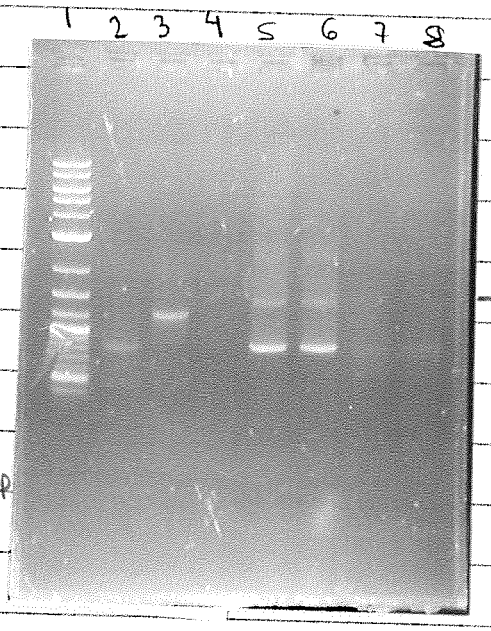
PCR 1

Gel 2 (20μl each well)



1 - MW (5μl)
2 - GFP-Catcher Amb (20μl) (#11) 1362 bp ✓
3 - GFP-Catcher Amb (20μl) (#11) " bp ✓
4 - Lux (20μl) (#12) 5984 bp
5 - Lux (20μl) (#12 " bp
6 - Lux Rep (20μl) (#13) 5931 bp
7 - Lux Rep (20μl) (#13) " bp
8 - MW (5μl)

Gel 1 (20 - 40μl on wells)



1 - MW (5μl)
2 - Intimin SpyTag (#2) 40μl 2389bp
✓3 - GFP-SpyCatcher (#5) 40μl 1362bp
4 - Intimin SpyCatcher (#3) 40μl 2683
✓5 - tRNA synthetase (#8) 20μl 1493 bp
6 - tRNA synthetase (#8) 20μl " bp
7 - Intimin Catcher Amb (#10) 20μl 2683 bp
8 - Intimin Catcher Amb (#10) 20μl " bp

1ng 10ng/μl
~ 0.1μl

Aug 24 2017

① QS PCR ×10 (new primers) 50μl Rxn

QS 2×MM	25μl	250μl		DNA 0.1μl
10μM F.	2.5μl	25μl	#11	+ 49.9 from MM
10μM R.	2.5μl	25μl		
DNA	x	x		x 8
dH_2O	to 50μl	to 500μl		
	(19.9μl)	- 199μl dH_2O		

② QS PCR ~~MM~~ XS (same primers) DNA ~~0~~ 1μl

QS 2×M	12.5μl	62.5		+ 24μl XS
10μM F	1.25μl	6.25	#2 #3 #10	
10μM R	1.25μl	6.25		
DNA	x		#12 #13	
dH_2O	to 25μl	45μl		
	9			

98°C 30sec

×35
- 98°C 10sec 57°C
- 57°C 30sec
- 72°C ~~3min~~ ~~3min~~ 4min

72°C 2min

4°C ∞

30 sec per kb

Friday 25 2017

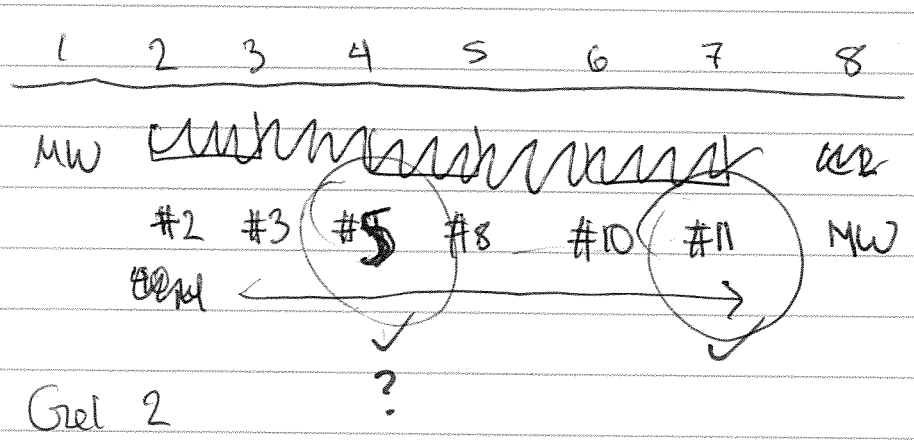
PCR purify

25 + 5 dy
= 30 µl

Run on gels

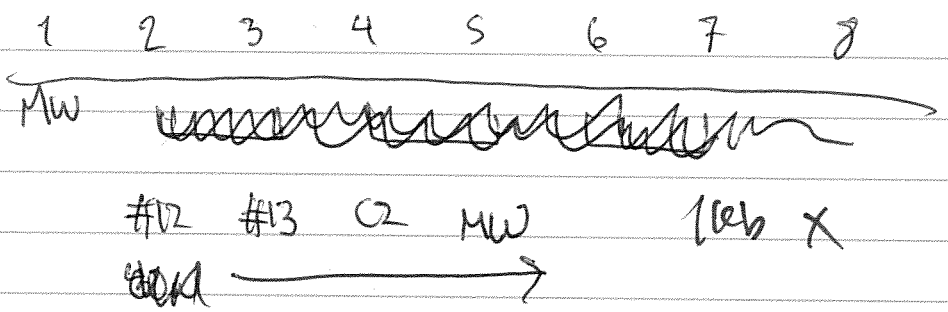
5 + 1
30 + 6

Gel 1



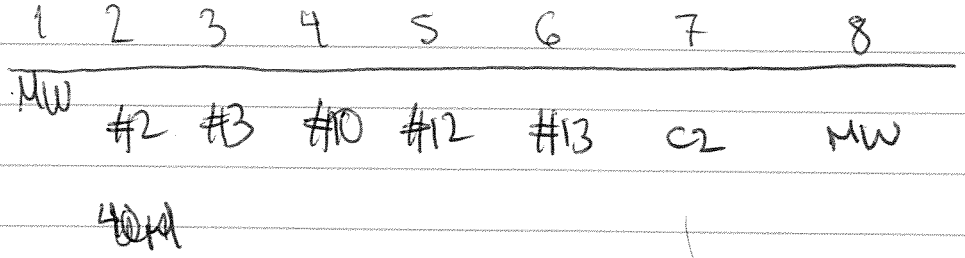
25 µl DNA + S
load 30 µ

Gel 2

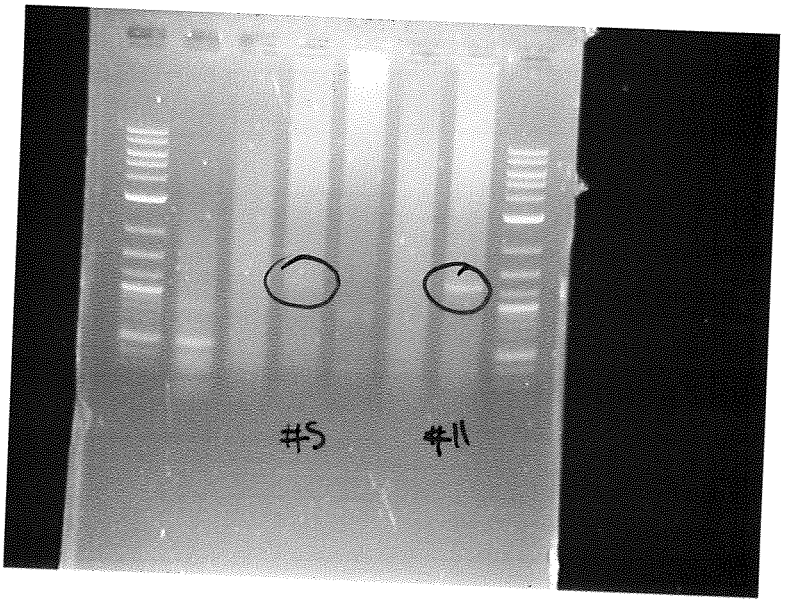


1 Gel X

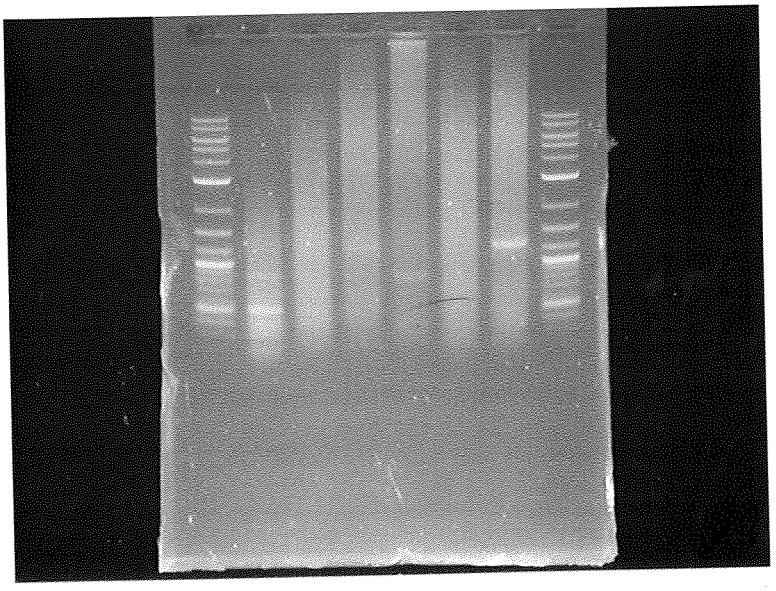
Gel 3



Nothing worked

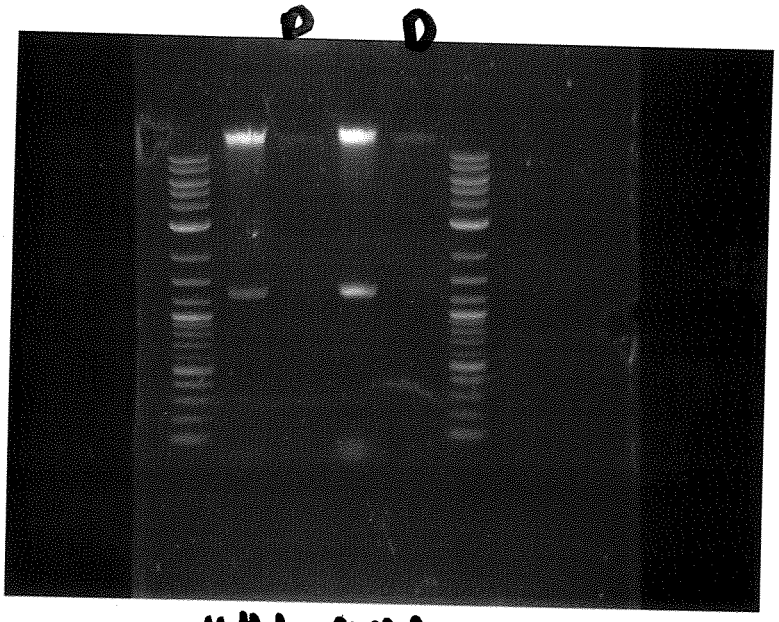


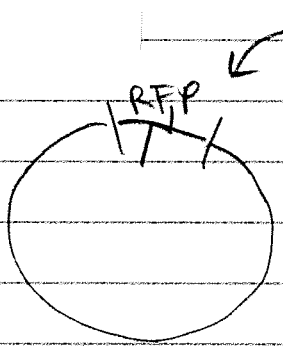
GEL 1.1



GEL 1.2

M 4.4.1 4.4.1 D 4.4.2 4.4.2 D M





Notes from Paola

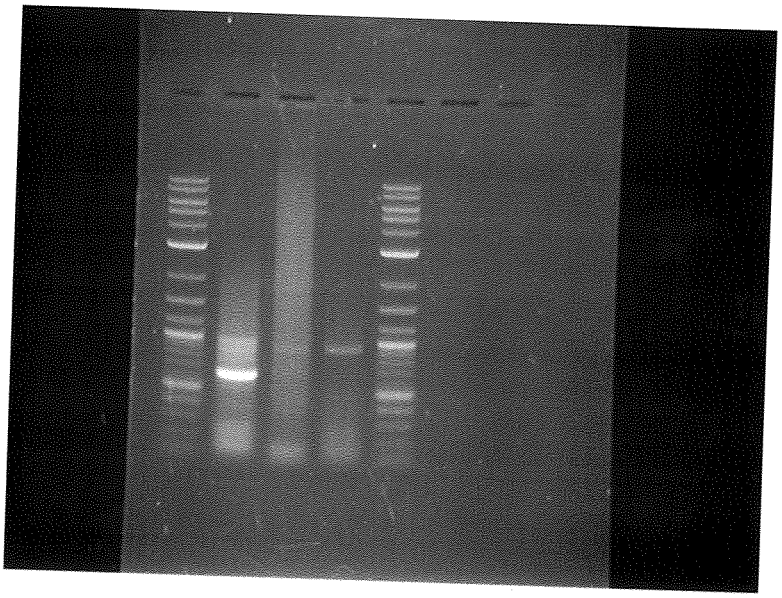
~~[illegible]~~ fixed

- Restriction digest / Clone #5 #11 ~~#4~~ #

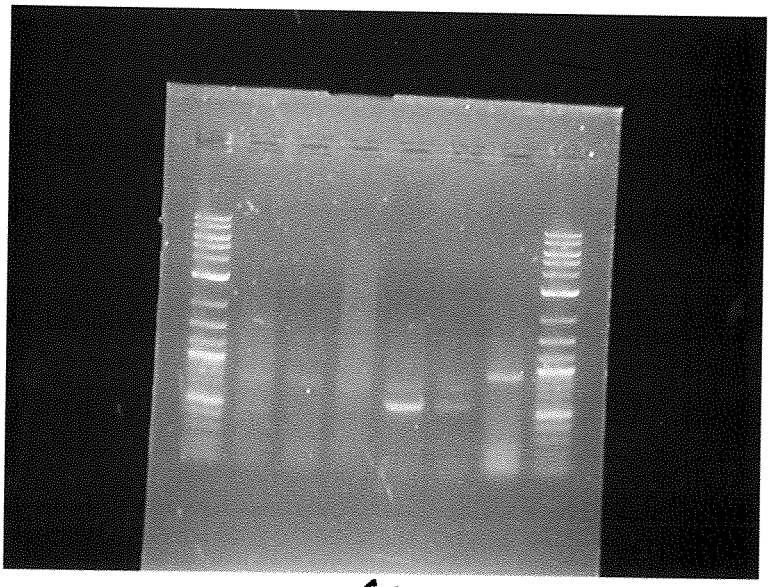
~~non PCR amplified~~
PCR amplified twice
EcoRI / PstI

- In fusion #2, #3, #5, #8, #10, #11, #12, #13 (all)

- PCR amplify #8 w/ first & ~~third~~ second primers & gel extract 1-5kb band



GEL 2



GEL 3

First Primers longer &

In fusion w/ Plasmid
1:2

4:45

#2

	µl
ITA ✓	0.88
TTB ✓	0.88
TTC1 ✓	0.45
ITC2 ✓	0.47
PSB1C3 X.S ✓	0.87

+ 1µl NEB 2.1 buffer ✓
+ 5.25µl dH$_2$O ✓
+ 0.2µl NEB Polymerase ✓

#

#3

	µl
ICA ✓	1.1
ICB ✓	1.1
ICC ✓	0.8
PSB1C3 ✓	0.87

+1µl NEB 2.1 buff ✓
+4.93 dH$_2$O ✓
+ 0.2µl NEB Poly ✓

~2 min 30 ✓

#10

	µl
ICA ✓	1.1
ICB ✓	1.1
ICCamb ✓	0.8
PSB1C3 ✓	0.87

+1 µl NEB 2.1 Buf ✓
+ ~~0.99~~ 4.93 dH$_2$O ✓
+0.2 µl NEB poly

~2 min 30 ✓

#12 / **#13**

X2

#12	#13	
Wx1 ✓	PLRep Wx1 ✓	
Wx2 ✓		
Wx3 ✓		
Wx4 ✓		0.98µl ✓
Wx5 ✓		
Wx6 ✓		
Wx7 ✓		0.50µl ✓
Wx8 ✓		0.61µl
PSB1C3 ✓		0.87µl ✓

+1µl NEB 2.1 Buf ✓ — #13 - (85)
+0.94 dH$_2$O
+0.2µl NEB Poly ✓

~~only 86~~ ~ .7 (#13)

5,47
5,88
6,89
7,76

.856

Infusion 1.7

	µl	10 µl Rxn
	1.7	
-IB	1.7	+ 1 µl Buf ✓
ITC1	0.87	+ 2.79 µl dH2O ✓
ITC2	0.87	+ 0.2 µl NEB poly
pSB1C3	0.87	

#3	µl	10 µl Rxn
ICA	2.1	+ 1.0 Buf ✓
ICB	2.1	+ 2.23 dH2O ✓
ICC	1.5	+ 0.2 NEB Poly
pSB1C3	0.87	

#10	µl	10 µl Rxn
ICA	2.1	+ 1.0 µl Buf ✓
ICD	2.1	+ 2.23 µl dH2O ✓
ICCamb	1.5	+ 0.2 NEB Poly
pSB1C3	0.87	

#12 #13	µl	15 µl Rxn
Lux1 / PLRepLux1	1.9	
2	1.9	
3	1.9	+ 1 µl Buf ✓
4	1.9	+ 4.35 dH2O
5	1.9	+ 0.2 µl NEB Poly
6	1.9	
7	0.98	
8	1.2	
pSB1C3	0.87	

2 min 30 sec bench
↓
on ice
↓
transform

Control

pSB1C3 1µl

+ 1 µl Buf

+ 0.2 µl NEB Poly

+ 7.8 µl dH2O

Thu Aug 31

A.1 [illegible] Restriction cloning - Not1 digestion

#4 G-8tag
#6 222PRG
#7 RB222
~~#9 Pgl (?)~~ we don't have it yet

#5 (b4 & after PCR 1-2)
#8 (b4 PCR 1/2)
#11 (b4 & after PCR 1-2)
~~#10, 12, [illegible]~~

~~Not1 - 1µl~~
~~DNA - 1µg (100) [illegible]~~
~~10xBuf - 5µl~~
~~dH2O - to 50µl~~

~~Not1 - 1µl~~
~~DNA - 0.1µg~~
~~10xBuf - 1µl~~
~~dH2O - to 10µl~~

~~Transform [illegible]'s constructs~~

~~25µl rxn~~

~~Not1 - 5µl~~
~~DNA - 0.5µg~~
~~10x Buf - 2.5µl~~
~~dH2O - to 25µl~~

~~37°C o/n~~

	DNA	dH2O
~~9~~	~~50µl~~	
~~6~~	~~50µl~~	
~~7~~		

10 µl rxn

~~Not1 - 1µl~~
~~DNA - 0.1µg~~
~~10x Buf - 1µl~~
~~dH2O - to 10µl~~

Construct	DNA	dH2O
#4	10µl	
#6	10µl	
#7	10µl	

~~Transform Esters constructs~~

15 µl Rxn		Construct	DNA	dH_2O
Not 1	1.0 µl	#4, 6, 7	10 µl	2.4 µl
DNA	0.1 µg	#5 B4		
10X Buf	1.5 µl	#8 B4		
dH_2O	to 15 µl	#11 B4		
		#5.2 (after)		
		#11.2 (after)		

Eldin - Transformation x4

1. Thawed 4 NEB 10-β competent cells for 10 minutes
2. To each cell tube, added 2 µL of the respective DNA:
   1. PHB - 34 minutes
   2. iNP - 30 mins
   3. iNP-RBS - 32 mins
   4. ② - 30 mins
   5. ②+ - 33 mins
   6. dCAS9 - 32 mins
   7. pUC19 - control - 30 mins
3. All added for at least 30 minutes to incubate ahead for all tubes
4. Each was put under heat shock 42°C for 30 s.
5. All put for 5 mins in ice
6. 950 µL SOC put into each tube

Control ligation 1 197 ng/µl • 4µl
2 140 ng/µl (4)? • 4µl
2 210 ng/µl (4)? •

	DNA		dH2O
#10.1	140 ng/µl	4µl	17.5 •
#10.2	170 ng/µl	4µl	17.5 •
#10.3	180 ng/µl	4µl	17.5
#10.4	140 ng/µl	4µl	17.5
#10.5	120 ng/µl	4µl	17.5
[illegible]	[illegible]		
#13.1	100 ng/µl	5µl	16.5 •
#12.1	190 ng/µl	4µl	17.5 •
#2.1	170 ng/µl	4µl	17.5 •
#2.2	180 ng/µl	4µl	17.5 •
#3.1	157 ng/µl	4µl	17.5 •
#4.1	110 ng/µl (?)	5µl	16.5 m
#4.2	190 ng/µl	4µl	17.5 •
#4.3	120 ng/µl	4µl	17.5 •
#4.4	280 ng/µl (2)	2µl	18.5
#4.5	210 ng/µl	2.5µl	19.0

25µl Rxn

Not 1 - 0.5µl
DNA - 0.5µg (500ng)
10X Buf - 2.5µl
dH_2O - to 25µl

0.5 x 17	8.5µl	3µl into each
2.5 x 17	42.5	

25 - 3.5 = 21.5

17 reactions

Thursday

Gel 1

1	2	3	4	5	6	7	8
MW	ctrl 1	ctrl 2	9.2	4.3	6.1	6.2	MW

Gel 2

1	2	3	4	5	6	7	8
MW	ctrl 1	ctrl 2	7.1	7.2	9.1	9.2	MW

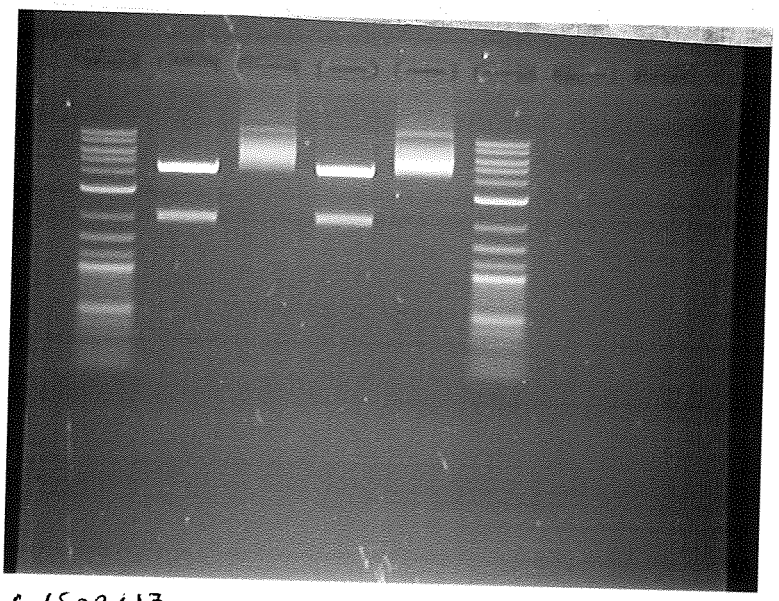
Gel 3

1	2	3	4	5	6	7	8
MW	ctrl 1	ctrl 2	10.1	10.2	12.1	13.1	MW

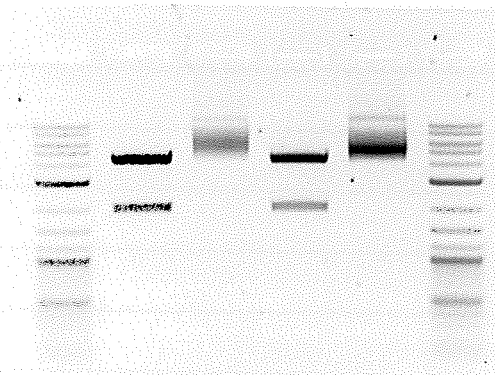
Gel 4

1	2	3	4	5	6	7	8
MW	ctrl 1	ctrl 2	2.1	2.2	3.1	MW	PSD1C3 vnew

dist.
PHB (plate 4)



6/Sep/17
PSB1C3 – PHB
MW, NotI cut col1, uncut, NotI cut col2, uncut



6/Sep/17
PHB PSB1C3
MW, NotI cut col1, uncut, NotI cut col2, uncut

Thursday

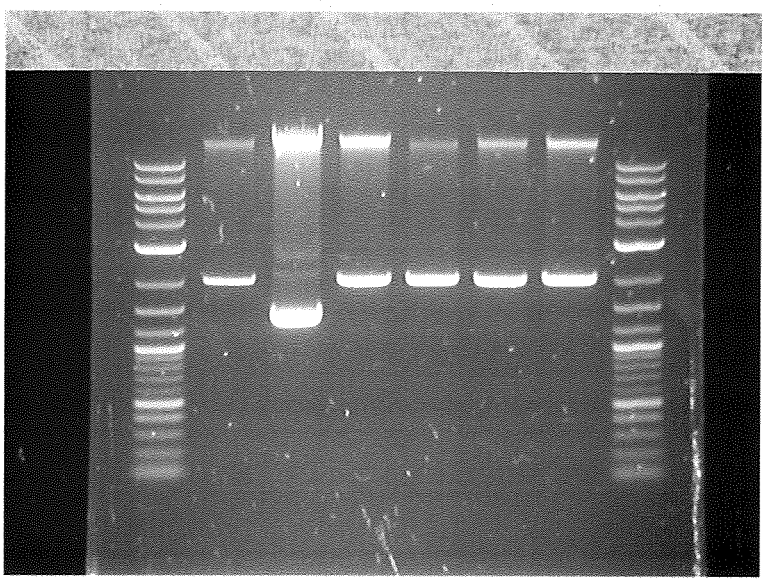
* pSB1C3 · NotI → ligation control xs
no insert, colony 1 cut w/ NotI

• pSB1C3 → ligation control xs
mini prep, uncut, colony 1

Gel 1

lanes	1	2	3	4	5	6	7	8
		*	•					
	MW	pSB1C3 NotI	pSB1C3	4.2	4.3	6.1	6.2	MW

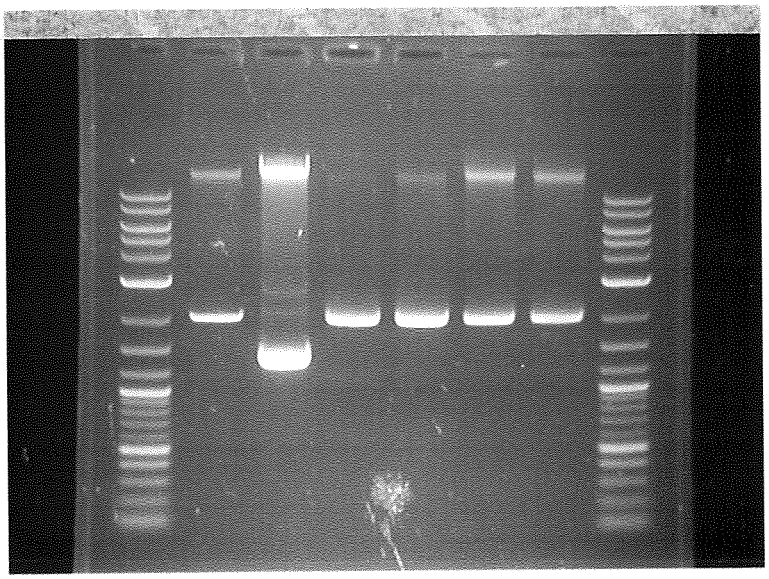
24 µl into wells
6 µl controls / MW



Gel 1
7/Sep/13

Gel 2

lanes	1	2	3	4	5	6	7	8
		*	•					
	MW	pSB1C3 NotI	pSB1C3	7.1	7.2	9.1	9.2	MW

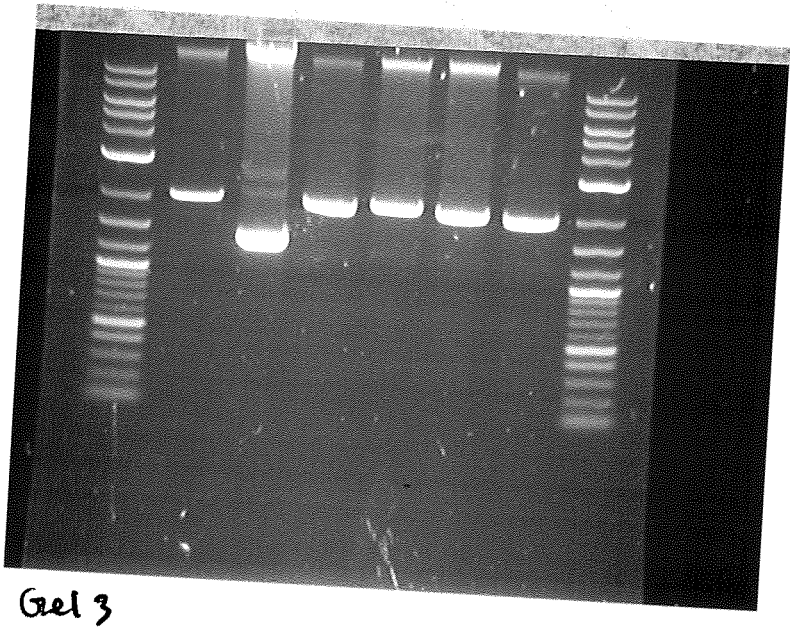


Gel 2

* PSB1C3 NOT1 – C

Gel 3

lanes	1	2	3	4	5	6	7	8
	MW	PSB1C3 NOT1	PSB1C3	10.1	10.2	12.1	13.1	MW

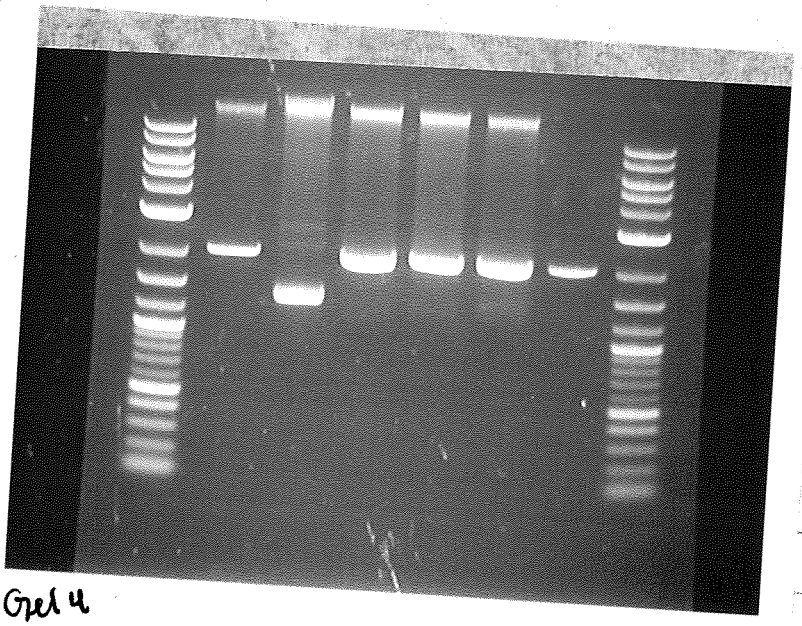


Gel 3

7/Sep/17

Gel 4

lanes	1	2	3	4	5	6	7	8
	MW	PSB1C3	PSB1C3	2.1	2.2	3.1	PSB1C3	MW



Gel 4

7/Sep/17

8/5/19

- Make pSB1C3-RFP cultures x8 → Ricardo
- miniprep 8 cultures
- Enzyme digest
- Treat w/ Alkaline phosphatase
- Run on gel & gel extract
- Do ligation w/out insert

1) [50µl Rxn]

XbaI & SpeI Digestion

DNA - (1µg)	
XbaI - 1µl	
SpeI - 1µl	
10x Buffer - 5µl	
dH_2O to 50µl	

[50µl Rxn]

EcoRI / PstI Digestion

DNA - (1µg)	
EcoRI - 1µl	
PstI - 1µl	
10x Buffer - 5µl	
dH_2O to 50µl	

1hr at 37°C

DNA - 43

Cultures	[DNA]	1µg to add	dH_2O
1	228.8	5µl	38
2	312.2	4µl	39
3	317.8	4µl	39
4	264.8	5µl	38
5	279.0	4µl	37
6	261.1	4µl	~
7	296.8	4µl	~
8	302.3	4µl	~

7 EISE

② Alkaline phosphatase digestion

NOT this o

Protocol from NEB [rSAP]

rSAP Reaction Buffer (10x)	2μl	37°C f
DNA	1μl (~1pmol for 3Kb plasmid)	65°C fo
rSAP (1 unit/μl)	1μl	
dH2O	to 20μl	

Protocol from Promega [CIAP]

This one!!

DNA (10pmol)	40μl	- 37°C for 30 min
CIAP 10x buffer	5μl	- Add diluted CIAP (gent
Diluted CIAP (0.01 U/μl)	up to 5μl	- 37°C for another 30 min
	50μl	(65°C for 5min)

need to dilute

$(20\ U/\mu l)(V) = (0.01\ U/\mu l)(40\mu l)$

$V = 0.02\ \mu l$

$+ 39.98\ dH_2O$

AP

40

⇒ 40 μl of 0.01 U/μl Alkaline phosphatase (CIAP)

8 Reaction

$(2 + 198)\ \mu l$ ← $\frac{1}{100} \times 20$

AP H2O

0.2

$\frac{0.4}{1000} = 0.0$

Alkaline Phosphatase Dilution

take

RP = Reaction + Phosphatase

1) $(20\ u/\mu l)(V) = (0.1\ u/\mu l)(40)$

$20V = 4$

5

8 µl AP + 32 µl dH_2O

⇓

2) $(0.1\ u/\mu l)(V) = (0.02)(40\ \mu l)$

$0.1V = 0.8$

8 µl + 32 µl dH_2O

= 40 µl of 0.02 u/µl

228ng - 1µl
1000ng = 4.39µl (~4.40) + 39.61 dH2O (~39.6)

original [pSB1C3] = 228 ng/µl
col 1 8sep17

~~[struck out]~~

~4.40
4.39 DNA
+ 38.61 dH2O
~38.6

NotI digestion (nuclease free water)

①
- NotI - 1µl
- ✓ DNA - 1µg
- ✓ 10X Buf - 5µl
- ✓ dH2O - to 50µl

1hr @ 37°C
→ 85°C 20min

② +1 unit CIP (1µl)
37°C for 30min

~~[struck out]~~
↓ PCR purify

XbaI / SpeI digestion

③
- XbaI - 1µl
- SpeI - 1µl
- ✓ DNA - 1µg
- ✓ 10X Buf - 5µl
- ✓ dH2O - to 50µl

→ 1hr @ 37°C
85°C 20min

④ + 1unit CIP
37°C for 30min

↓ PCR purify

[DNA] ① pSB1C3 - NotI = 21.8ng/µl
② pSB1C3 - NotI + CIP = 22ng/µl
③ pSB1C3 - XbaI/SpeI = 28.3ng/µl
④ pSB1C3 - XbaI/SpeI + CIP = 20ng/µl

↓ run on gel

Band / Conc.

pSB1C3 uncut
cut
cut + CIP

↓ gel extract

[cut + CIP] = 7.1 ng/µl
mass spec
[cut] = 3.2ng/µl
↓ run on gel

[cut + CIP] =

↓ run on gel

Band / Conc.

pSB1C3 uncut
cut
cut + CIP

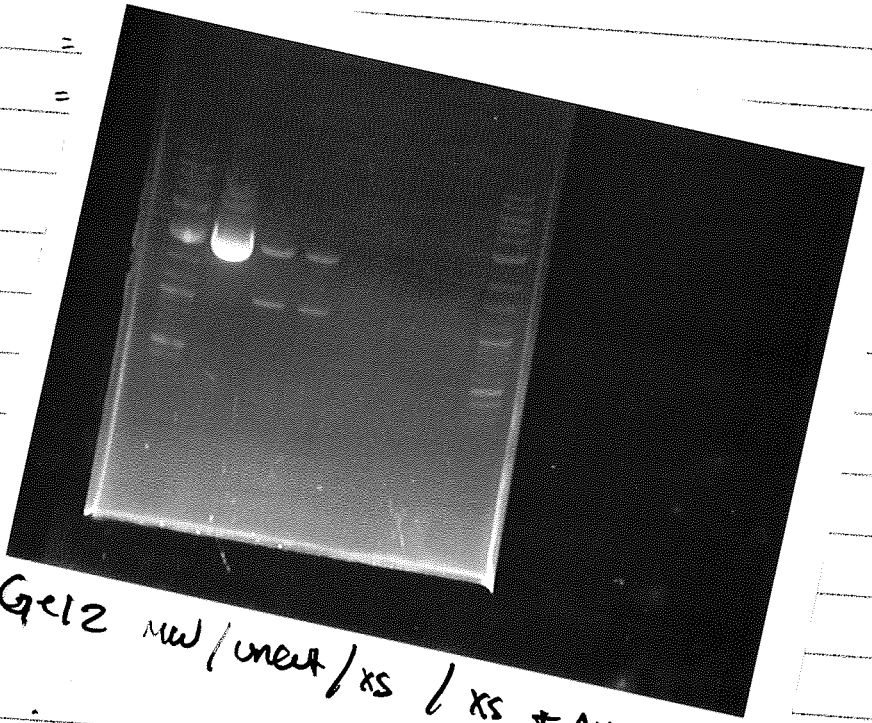
↓ gel extract

[cut + CIP] = 4.9 ng/µl
mass spec
[cut] = 5.7 ng/µl
↓ run on gel

[cut + CIP] =

Morning
Anima
20/09/2017

- Miniprep of 11.2 (bacterial)
  1, 2, 3, 4 (mammalian).
- Nanodrop measurements:
  11.2 = 100.6 ng/μL
  1 = 596.4 ng/μL
  2 =
  3 =
  4 =



Gel 2 MW / uncut / XS / XS + AMR phosph /