Lab book

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September 2, 2019

02/07/2019

- 12 am: I prepared 1L of M9 medium whith Anuradha, who prepared another 1L
- 3 pm: streaked *P. fluorescens* SBW25 with Anuradha from the stock at -80°C in position IIIA3-29
  - I streked 1 LB plate and 1 M9 plate
  - Anuradha streaked 1 LB plate and 1 M9 plate
  - Plates put at 28 °C

03/07/2019

- 9.30 am: streaked *E. coli* K12 MG1655 from the stock at -80°C in position IIIA1-22 and * E. coli* B REL606 form the stock in position IIIA1-14 with Gunda
  - I streked 2 LB plates and 2 M9 plates (1 for each strain)
  - Gunda streaked 2 LB plates and 2 M9 plates (1 for each strain)
  - Plates put at 28 °C
- I tried to write the primers for discrimitating the strains by myself

04/07/2019

- 9.00 am: Put all the cultures in 4 mL of LB/M9 at the appropriate temperature (28/37°C, 200rpm)
  - Labeled all the dilution plates for tomorrow, put glass beads inside
  - Put glycerol in vials for tomorrow, vials labeled
  - Put plates with streaks of the strains at 4°C
- Checked primers with Gocke: need to change something because I did not check for partial matches
- Afternoon: I have rewritten the primers and sent them to Gocke

05/07/2019

- Medium controls for the O/N cultures look all clean, cultures are all well grown except for SBW25-1 M9 (nothing visible) and SBW25-2 M9 (really faint)
- 9.00 am: inoculated all the glycerol stocks except for SBW25-1 M9, which did not show any growth
  - Stocks put at -80°C in the bottom shelf
  - Overnight cultures put at 4°C
  - SBW25-1 M9 put back at 28°C
- 10.15 am: meeting with Arne
  - We decided to talk to other theory people to discuss the modelling part
- 11.30 am: inoculation of the long term cultures (4 mL of LB or M9) with 4 μL of the overnight cultures
  - 05/07/19 is my day 0 (D0) of the long term experiment)
  - Long term cultures are labeled as strain(SBW25/MG1655/REL606)-sample(1-3, 1-6 for REL606) medium(LB/M9)

        * LB is labeled in black, M9 in blue
    - Long term cultures put at 28/37°C as appropriate
    - Overnight cultures put back at 4°C
    - SBW25-1 M9 seems to show an incredibly faint cloudiness, I decided to use it to inoculate the respective long term culture
        * I will wait this afternoon for making the glycerol stock
    - Long term cultures put at the 28/37°C with MCs, SBW25-1 M9 put at 28°C
- 5 pm: SBW25-1 M9 shows visible growth
    - Glycerol stock inoculated and put at -80°C
    - Stocks are labeled with the date (05/07/19), medium, strain, sample number, day of the experiment (D0), my initials (SP)
    - LB vials labeled in black and M9 vials in blue
- Inoculated all the dilution plates (52 plates) with 100 μL taken from the respective dilution of the O/N cultures in Ringer's (D0 dilution plates)
    - There are 2 plates for each sample, at different dilutions (10^-7 and 10^-6 for LB, 10^-6 and 10^-5 for M9)
    - 3 SBW25 LB samples (1-3), 3 MG1655 LB samples (1-3), 6 REL606 LB samples (1-6)
    - 3 SBW25 M9 samples (1-3), 3 MG1655 M9 samples (1-3), 6 REL606 M9 samples (1-6)
    - Medium controls for LB and M9, at 28°C and 37°C (4 in total)

06/07/2019

- Counted the dilution plates for *E. coli*, and put at 4°C

07/07/2019

- Counted the dilution plates for *P. fluorescens*, and put at 4°C
- Plate SBW25-2 LB D0 10^{-7} contaminated with green mould
    - Put parafilm around it to avoid spreading of spores
- Created spreadsheets for glycerol stock and dilution plates
- SBW25 did not produce many colonies in LB (10^-7 and 10^-6 dilutions)
    - Next time I will try with 10^-6 and 10^-5
- CFU/mL in the D0 O/N cultures used for inoculating the long term cultures

Sample	CFU/mL
SBW25-1 LB	$1,00 * 10^8$
SBW25-2 LB	$7,00 * 10^7$
SBW25-3 LB	$5,00 * 10^7$
SBW25-1 M9	$7,60 * 10^7$
SBW25-2 M9	$8,70 * 10^7$
SBW25-3 M9	$1,41 * 10^8$
MG1655-1 LB	$7,60 * 10^8$
MG1655-2 LB	$7,80 * 10^8$
MG1655-3 LB	$6,10 * 10^8$
MG1655-1 M9	$1,04 * 10^9$
MG1655-2 M9	$9,00 * 10^8$
MG1655-3 M9	$7,00 * 10^8$
REL606-1 LB	$3,40 * 10^8$
REL606-2 LB	$2,80 * 10^8$
REL606-3 LB	$3,40 * 10^8$
REL606-4 LB	$3,70 * 10^8$
REL606-5 LB	$3,50 * 10^8$

Sample	CFU/mL
REL606-6 LB	$1,12 * 10^9$
REL606-1 M9	$1,45 * 10^8$
REL606-2 M9	$4,70 * 10^8$
REL606-3 M9	$7,40 * 10^8$
REL606-4 M9	$5,80 * 10^8$
REL606-5 M9	$5,90 * 10^8$
REL606-6 M9	$1,14 * 10^9$

08/07/2019

- Dilution plates of D0 checked with Jenna and then trashed
- I have labeled the plates, cryovials and tubes for D5, which will be on Wednesday 10/07
- Primers for telling apart SBW25, MG1655 and REL606 ordered with Anuradha
  - SBW25_unique1_fw: 5'-ATACTACGACTCCAGAGCGATGG-3'
  - SBW25_unique1_rv: 5'-GTTCAGCGTCTGCGTGGCTTG-3'
  - SBW25 expected product size: 1024 bp
  - REL606_unique1_fw: 5'-CAGTGGATTGTGGTTTGTTGCC-3'
  - REL606_unique1_rv: 5'-GGCTGGTACTTTTCAGGTCGG-3'
  - REL606 expected product size: 1138 bp
  - MG1655_unique1_fw: 5'-CTGAATCGGTCATGATGATGGGGACTG-3'
  - MG1655_unique1_rv: 5'-TTCAGGCGGACTTACTATCCCG-3'
  - MG1655 expected product size: 1241 bp

09/07/2019

- Put glycerol on the cryovials for tomorrow (10/07/19), beads in the labeled plates and medium (2 mL) in the tubes for the O/N cultures

10/07/2019

- This is my D5
- Dilutions of the LTSP tubes in 2 96-wells [1 for LB and 1 for M9] plates in the following way
  - 10^{-1} -> 5 μL culture in 45 μL Ringer's
  - Other steps -> 20 μL previous step in 180 μL Ringer's
  - MC -> 5 μL MC in 45 μL Ringer's
- Dilution plates
  - Check the dilutions!
  - Plated 100 μL from the respective dilutions for the cultures
  - Plated 50 μL from the 10^{-1} dilution for MC [the total volume!]
  - SBW25 LB -> plated 10^{-6} and 10^{-5}
  - SBW25 M9 -> plated 10^{-6} and 10^{-5}
  - MG1655 LB -> plated 10^{-7}, 10^{-6} and 10^{-5}
  - MG1655 M9 -> plated 10^{-6} and 10^{-5}
  - REL606 LB -> plated 10^{-7} and 10^{-5}
  - REL606 M9 -> plated 10^{-6} and 10^{-5}
- O/N cultures for the D5 glycerol stocks
  - 2 μL of the LTSP culture in 2 mL medium
- LTSP cultures brought back to 4 mL (added 0-500 μL mqH_2O upon necessity)
- Since some spare tubes with medium were available, inoculated them with mqH_2O to test for contamination

  - 1 LB tube put at 28°C, 1 M9 tube put at 37°C

11/07/2019

- The plate MC LB 37°C D5 is contaminated
  - The tube itself looks clean
  - I have plated its 10^{-1} dilution, and it has 112 colonies that look like MG1655
  - The dilution plates of MG1655 and REL606 at 10^{-7} look fine, with 3-5 colonies each, far fewer than the MC
  - I think the contamination could come from the dilution step, or a wrong plating
  - I have plated again 50 μL form the LTSP MC LB 37°C on an LB plate
  - I have washed the well in the 96 plate that I have used for inoculating the MC plate with 100 μL Ringer's, and used them for inoculating another LB plate
  - Tomorrow I will see if these too gets contaminated
- MG1655-1 LB is contaminated with a patina (bead tracks) in both dilutions (10^{-6} and 10^{-5}), and the same is true for REL606-4 LB 10^{-6}
  - Colonies are still visible, and I have counted MG1655-1 LB 10^{-5} since it was the only countable one for that replicate
  - The plate MC M9 37°C is clean
- Counted *E. coli* D5 plates
- REL606 in LB shows small colonies alongside the normal ones in all the replicates, which are absent from MG1655
  - The small colonies have more defined margins
  - I took 2 pictures with the microscope and showed them to Jenna, we will have a look at them tomorrow
  - Jenna said that they look like contamination, but it is unlikely that I have contaminated all the REL606 replicates
  - At the microscope with India Ink they look like bacteria, but are smaller than the ones from the normal colonies
    * Not motile, as expected for REL606
    * We will test with the primers when they arrive
- Inoculated the glycerol stocks with 1 mL of the respective O/N cultures, and put at -80°C

12/07/2019

- Counted *P. fluorescens* D5 plates
- The replica plating of 50 μL of 10^{-1} MC LB 37°C is clean, while the plate obtained by washing the well used for the MC LB 37°C D5 plate (which was contaminated) with 100 μL Ringer's shows the same contamination
  - I conclude that the contamination came from the dilution step, so I can ignore it
- SBW25-3 LB 10^{-6} shows what I think is a wrinkly spreader
- SBW25-2 LB 10^{-6} has a bacterial patina (bead tracks) and is uncountable
- Wrinkly spreaders appeared in LB
  - SBW25-2 LB 10^{-5} has 5 big WS
  - SBW25-3 LB 10^{-6} has 1 small WS, 10^{-5} has 2 small WS
- CFU/mL in the D5 cultures

Sample	CFU/mL
SBW25-1 LB	$6.8 * 10^7$
SBW25-2 LB	$6.2 * 10^7$
SBW25-3 LB	$1.21 * 10^8$
SBW25-1 M9	$2.96 * 10^8$

Sample	CFU/mL
SBW25-2 M9	$2.98 * 10^8$
SBW25-3 M9	$2.39 * 10^8$
MG1655-1 LB	$4.2 * 10^8$
MG1655-2 LB	$3.8 * 10^8$
MG1655-3 LB	$2.8 * 10^8$
MG1655-1 M9	$5.28 * 10^8$
MG1655-2 M9	$7.4 * 10^8$
MG1655-3 M9	$6.3 * 10^8$
REL606-1 LB	$2.04 * 10^8$
REL606-2 LB	$2.37 * 10^8$
REL606-3 LB	$3.11 * 10^8$
REL606-4 LB	$2.15 * 10^8$
REL606-5 LB	$1.61 * 10^8$
REL606-6 LB	$1.90 * 10^8$
REL606-1 M9	$7.0 * 10^8$
REL606-2 M9	$4.3 * 10^8$
REL606-3 M9	$7.8 * 10^8$
REL606-4 M9	$6.31 * 10^8$
REL606-5 M9	$5.2 * 10^8$
REL606-6 M9	$6.4 * 10^8$

- Initial considerations looking at the CFU/mL at D0 and D5
  - M9 has consistently higher readings than LB at D5 for all strains
    - Probably because in M9 cells are smaller, so more of them can grow in the same volume
  - Readings at D5 are much more consistent than at D0
  - MG1655 is consistently decreasing from D0 to D5
  - REL606 is slowly decreasing in LB, and stationary in M9
  - SBW25 is stationary in LB, and increasing (!) consistently in M9
    - It started lower, so it is maybe just normalizing
- The LTSP cultures were moved to the 28°C room in a static rack at 3 pm of Friday
  - This would be D8 at 3pm
- 3 pm: LTSP cultures moved to 28°C room in a static rack because of the power outage

13/07/2019

- 11 pm: LTSP cultures moved back to the shakers because the power outage is over

15/07/2019

- We will make dilutions and stocks on D10 (wednesday), D12 (friday) D15 (monday) and D17 (wednesday) because we are near the supposed death of REL606 (D16)
- Labeled all the dilution plates, tubes and criovials for D10 and D12
- Put medium in D10 tubes and glycerol in criovials
- Made O/N cultures in 2 mL LB from stocks of SBW25 (IIIA1-5), MG1655 (IIIA1-22) and REL606 (IIIA1-14) for extracting genomic DNA to be used in the test PCR
- I made a mistake on the labels: what I considered D10 is actually D12, and D12 is D14
  - Tomorrow I will correct it in RED ink in the plates, and with the same ink used for the stocks

16/07/2019

- The primers (SBW25_unique1_fw, SBW25_unique1_rv, MG1655_unique1_fw, MG1655_unique1_rv, REL606_unique1_fw, REL606_unique1_rv) have arrived
  - Made stock solution 100 μM
  - Made working stock 5 μM (10μL of stock in 200 μL total)
- Extracted genomic DNA from O/N cultures of SBW25, MG1655 and REL606
  - 1.5 mL eppis put at -20°C in my rack
- I made a test PCR for the primers
  - 94°C 10'+30*(94°C 30'‘+60°C 30'‘+72°C 1')+72°C 5'
  - Samples labeled A1, A2, A3, A0, B1, B2, B3, B0, C1, C2, C3, C0
    - A -> SBW25 specific primers
    - B -> MG1655 specific primers
    - C -> REL606 specific primers
    - 1 -> SBW25 genomic DNA as a template
    - 2 -> MG16555 genomic DNA as a template
    - 3 -> REL606 genomic DNA as a template
    - 0 -> no template (H_2O)
  - Samples run in the following order in a 50 mL 1% gel, for 45 minutes at 80V
    - Ladder A1 A2 A3 A0 B1 B2 B3 B0 C1 C2 C3 C0 Ladder Empty
  - The bands look as expected, the primers work
    - Gel image saved as 16-07-19_img1.pdf
- Label of the D12 (ex D10) plates corrected with red marker
- Label of the D14 (ex D12) plates corrected with various markers because it did not write well
- D10 (actually D12) and D12 (actually D14) tubes not corrected because they will never be mixed up
- Glycerol stocks of D12 (ex D10) and D14 (ex D12) corrected with the same ink used

17/07/2019

- Inoculated O/N tubes for D12
- Inoculated dilution plates D12
- Put medium in the D14 O/N tubes
- Put glycerol in the D14 vials
- I made a PCR for testing the small REL606 colonies observed in D5
  - I used the primer pair REL606_unique1_fw/REL606_unique1_rv
  - 12 samples
    - 1 small colony from each REL606 replicate (6 in total, taken from the D5 plates)
    - 2 big (normal) colonies from 2 of the REL606 replicates (taken from the D5 plates)
    - 1 positive control with REL606 genomic DNA
    - 1 negative control with water
  - 94°C 10'+30*(94°C 30'‘+60°C 30'‘+72°C 1')+72°C 5'
  - Samples run in the following order in a 50 mL 1% gel, for 45 minutes at 80V
    - Ladder 1S 2S 3S 4S 5S 6S 1B 2B Ctr+ Ctr- Ladder Empty Empty Empty
    - Both small colonies and big colonies are positive, the positive and negative control are as expected
    - Image of the gel saved as 17-07-19_img1.pdf
- Counted the number of small colonies and big colonies in the D5 plates (REL606 10^{-5})
  - REL606-1 big->68 small->119
  - REL606-2 big->73 small->149
  - REL606-3 big->82 small->198
  - REL606-4 big->50 small->147
  - REL606-5 big->58 small->118
  - REL606-6 big->62 small->96

18/07/2019

- Inoculated glycerol vials with D12 O/N
- Counted *E. coli* D12 dilution plates
- In REL606 LB the small colonies are now (D12) the vast majority
  - There are also some very tiny colonies, much smaller than the small ones (see REL606 LB D12 pictures)
- Small colonies begun to appear in MG1655 LB
  - The distinction between small and big is less clear than in REL606
  - MG1655-1 big->45 small->0
    - The 10^{-5} dilution had 387 colonies, of which around 70 were small (difficult to count the exact number)
  - Mg1655-2 big->22 small->12
  - Mg1655-3 big->21 small->21
- Jenna proposed to do a NGS project with hers 6-months old *P. fluorescens* samples
  - The samples are called Pf longterm 1-4
    - Pf longterm 1 should be large colony, smooth, fluorescent
    - Pf longterm 2 should be small colony, smooth
    - Pf longterm 3 should be very small colony, smooth
    - Pf longterm 4 should be medium-sized colony, possible colanic acid switcher
  - They should be stored in IIIA3 29-32 but they are actually in IIIA3 24-28 (?, check)
  - I have streaked them on LB plates, but I will probably streak them again on saturday so to be able to put the overnights on monday and do a genomic extraction on tuesday
- Labeled D17 plates and O/N tubes

19/07/2019

- Inoculated plates and tubes D14
- Prepared vials D17
- Read D12 SBW25 plates
- Updated the number of colonies in *E. coli* M9 D12, since many colonies were small and not visible yestarday
- Still no diversity in M9 for all of the strains, in LB SBW25 shown many WS, big and small

20/07/2019

- Inoculated D14 glycerol stocks
- Prepared D17 tubes
- Put *E. coli* D14 plates in the fridge

21/07/2019

- Counted D14 plates
- Streaked again Pf longterm plates for NGS (see 18/07/2019)

22/07/2019

- Inoculated D17 plates and O/N tubes

Template	$MgCl_2$ (5x)	Buffer green (10x)	CES 5x	dNTPs	SBW25 FW	SBW25 RV	GoTaq	H_2O	TOT
0.5 μL	3 μL	5 μL	5 μL	1 μL	4 μL	4 μL	0.5 μL	2 μL	25 μL

- I have checked by PCR a single colony from each Pf longterm sample (1-4) with SBW25-specific primers
  - The sample was obtained by touching a pipette tip on the colony and rinsing it in 20 μL of sterile Ringer's in a 96 well
    - 0.5 μL of this suspension were used as a template in the PCR
  - 94°C 10'+30*(94°C 30'‘+60°C 30'‘+72°C 1')+72°C 5'
  - Used SBW25 genomic DNA as a positive control and Ringer's as a negative control
  - Run as Ladder(100bp)-1-2-3-4-Ctr+-Ctr-
  - All samples are positive, controls as expected
  - Gel in 22-07-2019_img1
- I have set up the O/N cultures for Pf longterm 1-4, for genomic extraction
  - The cultures are done in 4 mL LB in 13 mL tubes
  - The tubes were inoculated with 2 μL of the suspension used for the PCR

23/07/2019

- I have counted the D17 *E. coli* plates
  - MG1655 in M9 is going down sharply, next time I will plate 10^{-4}, 10^{-2}, 10^{0}
  - MG1655-2 M9 is extremely high compared to the other replicates (100 fold)
    - This is consistent in both the dilution plates
    - Also the OD of the O/N tube is visibly different
      - MG1655-1 and -3 M9 do not show any visible growth in the O/N tube, but MG1655-2 M9 does
- Michael Lachmann talk on the origin of life
  - Selection can happen in absence of reproduction

24/07/2019

- Inoculated the tubes and plates for D19
- I have counted the D17 *P. fluorescens* plates
- Michael Lachmann talk on plasticity
  - Genomes do not encode the whole phenotype
  - Phenotype is partially encoded by the environment
  - The genome has an optimal nuber of parameters for modelling a phenomenon, so to give smart responses and avoid overfitting

25/07/2019

- MG1655 in M9 shows a marked decreas in CFU/mL, and replicate 2 is much more viable than the other replicates
  - I plated all the MG1655 M9 in LB plates to check if they develop different colony morphologies, especially MG1655-2
    - I plated 100 μL of 10^{-4} for replicate 2, and 10^{-1} for the others
- I counted the *E. coli* plates for D19
  - Some colonies in M9 plates (MG1655) are still too small to be counted, will do that tomorrow
- I will not be here on the weekend, so I will do next plating on monday (D24)

26/07/2019

- Counted all of the D19 plates
- Next plating will be done in D24
- Some colonies in SBW25 look weird
  - Color is less intense, they are rounder, smaller
- I made a PCR for testing some colonies of MG1655 M9 plated on LB and for the strange SBW25 LB colonies
  - The 3 MG1655 M9 colonies tested are positive, they are MG1655
  - The SBW25 LB colonies are all positive, except for 1 (colony B1 on the plate)
  - I have also tested the Pf longterm genomic DNA with the respective primers (all positive)
  - I have used one MG1655 colony as a control for the SBW25-specific primers and vice-versa
  - Template vas obtained from a 20 μ suspension of the colony in mqH_2O
  - See 26-07-2019_img1
  - I have used 0.5 μL of template in every case
  - One of the small SBW25 colonies (marked as B1 on the plate) is negative
    - Could be an error in picking the colony?

29/07/2019

- Inoculated D24 tubes and plates
  - I have plated/made stocks of only the REL606 (LB + M9) and MG1655 M9
- Prepared ggplot graphs for the meeting with Jenna

30/07/2019

- Meeting with Jenna
  - The results are interesting, although different from what we expected
  - The WGS part of the project will probably begin in 2 weeks
    - In the meantime I should install breseq and learn how it works
  - We will plate every 2-3 days from now on
- Counted D24 plates
- Prepared D26 tubes and plates
- Genomic DNA of Pf longterm (1-4) run in an 1% agarose gel 90V 1h by Anuradha (5 μL)
  - It shows a sharp band above 10 kb, I will ask Jenna if it can be genomic
  - Saved as 30-07-2019_img1.bmp

31/07/2019

- Inoculated tubes and plates D26
- I have made 3 additional stocks with MG1655(1-3) M9 because they are almost dead
  - I have spun down 4 mL of O/N and resuspended it in a bit more than 1 mL
  - I have used 1 mL of this with 800 μL glycerol saline for the stock

01/08/2019

- Prepared tubes and plates D28
- Counted MG1655 LB plates D26
- REL606 LB $10^{[}-3]$ has too many colonies to be counted, but they are really small and less than on D24
- The other plates need to grow more, will count them tomorrow
- In M9 there is essentially nothing in all the plates

  - Since it is a new batch, to check wether there is a problem with the medium I poured 1 mL of O/N REL606 with visible growth in 2 clean M9 plates (1 for each batch)
- Initiated box 3 for the glycerol stocks
- I started updating the glycerol stock spreadsheet
- I have installed breseq

02/08/2019

- Counted D26 plates
- M9 plates are fine, there are colonies
- I have inoculated D28 tubes and plates
- I make more M9 plates

03/08/2019

- Inoculated D28 tubes and plates
- Prepared D31 tubes and plates
- Counted D26 plates

05/08/2019

- I counted M9 D28 plates
- Inoculated tubes and plates D31

06/08/2019

- Finished counting D28 plates
- Inoculate dglycerol stock D28
- Counted some D31 plates (MG1655 LB)
- Labeled tubes and plates for D39 (Tuesday after return from Italy)
- 1 LB plates of SBW25-2 presents a colony that is a bit different from the others
  - It is similar to smooth morph but is bigger and less defined
  - Gokce said that it could be fuzzy spreader
  - Jenna said it could just be a bigger colony
  - I prepared an O/N in 2 mL LB from the colony and took pictures
  - I will make a glycerol stock from the O/N

07/08/2019

- Went to lab early morning because from today to 12/08 I will be in Italy with Yaren
- Counted the remaining D31 plates
- Inoculated glycerol stock for possible fuzzy spreader (SBW25-2 LB D31)
  - I labeled it with red marker as “SBW25-2 fuzzy spreader LB D31”

13/08/2019

- Inoculated tubes and plates D39
  - Non-inoculated LB tubes labeled for the O/N of D39 prepared on 06/08/2019 were all contaminated by mold
  - I prepared new tubes with a fresh LB bottle
- Measured Pf longterm samples for NGS with NanoDrop for sequencing (will probably be done tomorrow)

  - I sent the nanodrop reads and the sample labels to Jenna

14/08/2019

- MG1655-1 and -3 M9 do not show any colony on 10^0, 100 μL
  - Jenna plated them when I was in Italy with Yaren, and I repeated the plating on D39
  - I will stop plating them
  - I have inoculated for each of them 2 mL LB and 2 mL M9 with 200 μL of the long term culture, to see if something grows
    - Immediately after inoculation, the tubes have the following OD
      - MG1655-1 LB -> 0.14
      - MG1655-3 LB -> 0.09
      - MG1655-1 M9 -> 0.16
      - MG1655-3 M9 -> 0.10
- Medium control LB D39 37°C is contaminated by what seems like small wrinkly spreaders
  - I will let it grow at 28 °C to see what happens
- Counted some D39 plates
- Inoculated D39 criovials
  - I started box 4 in the -80 °C
- I am helping Gokce with her plating
- MG1655-2 M9 has way too many colonies in both dilutions (-4, -3)
  - I suppose a dilution error
  - I plated again -4 and -3 (D40)

15/08/2019

- Counted D39 plates
- MG1655-1 and -3 M9 are definetely dead
  - The O/N coltures prepared yesterday with 200 μL of inoculum in 2 mL of M9 and LB do not show any growth (OD measurement) * MG1655-1 LB -> 0.07 * MG1655-3 LB -> 0.05 * MG1655-1 M9 -> 0.09 * MG1655-3 M9 -> 0.07
- PCR of some D39 colonies with the respective primer pair
  - 2 SBW25 LB colonies that looked like fuzzy spreaders
    - I am not sure if they are FS, but I am calling them like this for convenience in my dataset
  - 2 MG1655-2 M9 colonies, randomly
    - This is the only replicate still alive
  - 2 REL606 LB small colonies
  - For each primer pair water was used as a control
  - Everithing as expected, except for MG1655-2, where only one colony was positive
    - Maybe was a PCR problem, will test more colonies tomorrow
- MC D39 LB 37°C, which was contaminated and put at 28°C to see if the contamination was *P. fluorescens*, shows now yellow colonies
  - They are really hard, and it seems they did not grow
  - At the microscope they look like non-motile bacteria
- I looked some of my coltures at the microscope
  - SBW25 is, as expected, motile
  - REL606 is, as expected, non-motile
  - Strangely, MG1655 is also not motile

19/08/2019

- I performed control PCRs of MG1655-2 M9, which is consistently more viable than the other replicates

  - Liquid MG1655-2 M9 culture with the 3 different primer pairs
    - I used 0.5μL of the Ringer's suspension from the 10^{-1} dilution used for plating
      - It is made of 45 μL Ringer's and 5 μL of culture
  - I did also a PCR of 5 different colonies from the D40 dilution plate with MG1655 primers
  - I did also 3 negative controls for the 3 primer pairs
  - Everithing went as expected, there are no contaminations
  - The gel was run the day after (20/08) and the sample were put at -5 for the night
- Plated and made O/N for D45

20/08/2019

- Counted part of the D45 plates

21/08/2019

- Finished counting D45 plates
- From now on I will write the report
  - The tubes are still in the incubator

30/08/2019

- I analysed NGS data for Pf longterm
- Pf longterm 1
  - Pf longterm 1 should be large colony, smooth, fluorescent
  - Mutation in Dna binding response regulator (PFLU_RS27420)
- Pf longterm 2
  - Pf longterm 2 should be small colony, smooth
  - Big deletion in position 3326589 (Δ 213799 bp)
  - Many small deletions
  - Mutation in Dna binding response regulator (PFLU_RS27420)
    - Same mutation in Pf longterm 1
- Pf longterm 3
  - Pf longterm 3 should be very small colony, smooth
  - Big deletion in position 3326589 (Δ 213799 bp)
  - Many small deletions
  - Mutation in Dna binding response regulator (PFLU_RS27420)
    - Same mutation in Pf longterm 1
  - It has the same identical mutations of Pf longterm 2 (?) maybe there is some confusion
- Pf longterm 4
  - Pf longterm 4 should be medium-sized colony, possible colanic acid switcher