

Lab book

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July 23, 2019

02/07/2019

- 12 am: I prepared 1L of M9 medium with Anuradha, who prepared another 1L
- 3 pm: streaked *P. fluorescens* SBW25 with Anuradha from the stock at -80°C in position IIIA3-29
 - I streaked 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 from the stock in position IIIA1-14 with Gunda
 - I streaked 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 discriminating 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 μ L 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 μ L 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'-GTTTCAGCGTCTGCGTGGCTTG-3'
 - SBW25 expected product size: 1024 bp
 - REL606_unique1_fw: 5'-CAGTGGATTGTGGTTTGTGGCC-3'
 - REL606_unique1_rv: 5'-GGCTGGTACTTTTCAGGTCGG-3'
 - REL606 expected product size: 1138 bp
 - MG1655_unique1_fw: 5'-CTGAATCGGTCATGATGATGGGGACTG-3'
 - MG1655_unique1_rv: 5'-TTCAGGCGGACTTACTATCCCCG-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 μ L culture in 45 μ L Ringer's
 - Other steps -> 20 μ L previous step in 180 μ L Ringer's
 - MC -> 5 μ L MC in 45 μ L Ringer's
- Dilution plates
 - Check the dilutions!
 - Plated 100 μ L from the respective dilutions for the cultures
 - Plated 50 μ L 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 μ L of the LTSP culture in 2 mL medium
- LTSP cultures brought back to 4 mL (added 0-500 μ L mqH₂O upon necessity)
- Since some spare tubes with medium were available, inoculated them with mqH₂O 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 μ L from 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 μ L 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 μ L 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 μ L 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 μ M
 - Made working stock 5 μ M (10 μ L of stock in 200 μ L 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 -> MG1655 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

	<i>MgCl</i> ₂	Buffer green	CES		SBW25	SBW25			
Template	(5x)	(10x)	5x	dNTPs	FW	RV	GoTaq	<i>H</i> ₂ <i>O</i>	TOT
0.5 μ L	3 μ L	5 μ L	5 μ L	1 μ L	4 μ L	4 μ L	0.5 μ L	2 μ L	25 μ L

- 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 μ L of sterile Ringer's in a 96 well
 - * 0.5 μ L 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 μ L 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
- Michael Lachmann talk on the origin of life
 - Selection can happen in absence of reproduction

Future