Lab book

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July 16, 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

Future