Lab book

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July 11, 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 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
- CFU/mL in the D5 cultures

Sample	CFU/mL
SBW25-1 LB	$*10$
SBW25-2 LB	$*10$
SBW25-3 LB	$*10$
SBW25-1 M9	$*10$
SBW25-2 M9	$*10$
SBW25-3 M9	$*10$
MG1655-1 LB	$7,60 * 10^8$
MG1655-2 LB	$7,80 * 10^8$
MG1655-3 LB	$6,10 * 10^8$
MG1655-1 M9	$1,04 * 10^9$
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REL606-4 LB	$3,70 * 10^8$
REL606-5 LB	$3,50 * 10^8$
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	$1,14 * 10^9$

- Inoculated the glycerol stocks with 1 mL of the respective O/N cultures, and put at -80°C

12/07/2019

- Count *P. fluorescens* D5 plates
- Move cultures to 28°C room (upstairs, first door on the right)