iGEM 2018 InterLab Lab Notebook Edinburgh_UG

19th Tuesday
E. coli K-12 strain DH5-alpha strain was obtained by collaborating with Edinburgh iGEM Overgraduate team. (Appendix 1)

E. coli DH5-alpha was transformed with plasmids (all in pSB1C3) from the Kit Plate 7 as stated in the iGEM interlab 2018 protocol.The transformation was done using following heat-shock transformation protocol (Appendix 2)
Star time of the transformation: 14:00
All the transformed cells were left in the incubator from 17:00 until 9:00 next morning (16 hours).

20th Wednesday
All transformation worked well as cells grew up in all the plates. (see *Figure 1*)

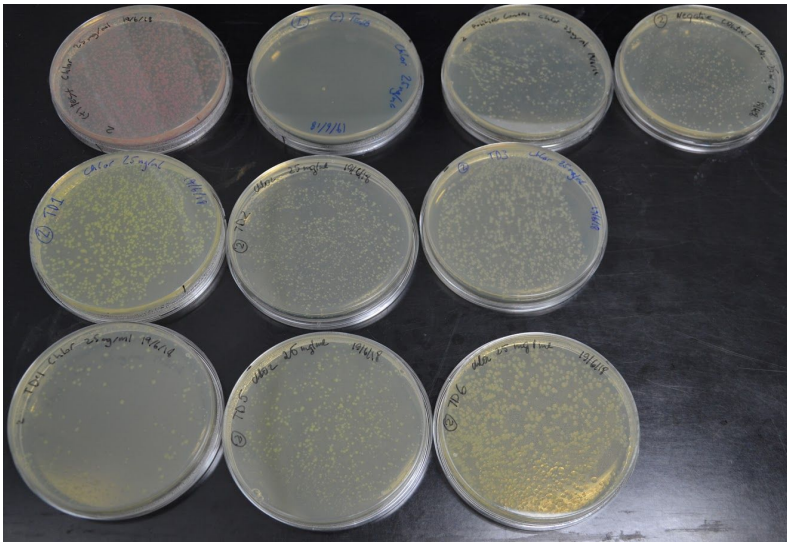

Figure 1. Transformation of compatent cells with the plasmids required for iGEM 2018 InterLab study

Time 13:30 2 colonies were picked from transformed cells and transferred to LB+Chlor media, following iGEM 2018 interlab protocol.

Calibration was carried out according to the protocol, except instead of pipetting to mix before reading on the plate-reader, the plate was shaken with the shaking function on the plate reader. *Also, due to unavailability of ddH2O, single distilled water was used instead.*

21st Thursday
Due to restricted accessibility to plate reader 50µL of cells were transferred to 5 ml LB+chlor media and was grown overnight (18h) at 37℃ and 220 rpm.

25th Monday
Re-inoculated from overnight cultures stored in fridge over weekend (100 µl culture + 4.9 ml LB-cam).
Absorbance at 600 nm obtained after first dilution of the overnight culture is shown in Table 1. As absorption is linearly proportional to concentration (A=ecl, where A- absorption, e - absorption coefficient, c- concentration and l- path length, usually 1 cm) using proportion rule, volumes required to get an absorption of 0.02 in 12 ml sample were calculated. Mean volume required was calculated as:

	Dilution 1		Dilution no.2	
	Colony no.1	Colony no.2	Colony no.1	Colony no.2
Positive Control	0.475	0.469	0.044	0.048
Negative Control	0.503	0.496	0.064	0.061
Test Device 1	0.440	0.429	0.077	0.045
Test Device 2	0.517	0.498	0.076	0.052
Test Device 3	0.507	0.478	0.063	0.032
Test Device 4	0.476	0.453	0.068	0.040
Test Device 5	0.484	0.477	0.080	0.033
Test Device 6	0.489	0.476	0.098	0.027

Absorption measurement results after the second dilution did not achieve the absorption required. However, the measurement itself is not in the spectrophotometer accurate reading range and the %error here is very high. Therefore, InterLab protocol was being followed foward despite the absorption of 0.02 was not obtained.

26th Tuesday
Followed protocol for day 3. TD6 from UG was used for OG TD6 (no growth seen for OG). Plates stored in fridge overnight for flow cytometry on Wednesday.

27th Wednesday
Performed flow cytometry. Re-inoculated positive and negative controls for CFU experiment

28th Thursday & 29th Friday
Followed protocol for CFU

Appendix 1

Competent cells protocol

Introduction

OG iGEM 2018 protocol for competent cells based on the iGEM webpage protocol. As you are working without antibiotics using this protocol, you need to be as sterile as possible to avoid contaminating the competent cell culture.

Materials

- SOB medium
    - 0.5% (w/v) yeast extract
    - 2% (w/v) tryptone
    - 10 mM NaCl
    - 2.5 mM KCl
    - 20 mM MgSO4
- CCMB80 buffer
    - 10 mM KOAc pH 7.0 (10 ml of a 1M stock/L)
    - 80 mM CaCl2.2H2O (11.8 g/L)
    - 20 mM MnCl2.4H2O (4.0 g/L)
    - 10 mM MgCl2.6H2O (2.0 g/L)
    - 10% glycerol (100 ml/L)
- LB medium 5 ml
- Cells culture

Procedure

Day 1

1. Pick a single colony from a plate into 5 ml of LB medium and shake overnight and during the following day at ~ 25 °C (big water bath in G.129).

Day 2

1. Autoclave a 1 l glass flask
2. Prepare 260 ml of SOB medium and autoclave
3. Prepare CCMB80 buffer; 100 ml per 250 ml SOB culture.
4. Inoculate 250 ml of SOB in the 1 l flask with 1 ml of the preculture and shake overnight at ~ 20 °C (big water bath in G.129)

Day 3

1. Check OD_{600} first thing in the morning and keep monitoring. Desired OD_{600} nm = 0.3. **Aim for lower, not higher OD, if you cannot hit this mark.**
2. When the cells are getting close to the desired OD, precool the big centrifuge to 4 °C (will take ~ 15 min).
3. Fill an ice bucket halfway with ice. Use the ice to pre-chill as many 50 ml Falcon tubes as needed.
4. Once the cells are ready, put them on ice for 5 min. **After this keep the cells as much as possible on ice**.
5. Transfer the culture to the 50 ml Falcon tubes.
6. Weigh and balance the tubes in the centrifuge adaptors by using the light brown scale next to the PCR machines.
7. Centrifuge at 3000 g at 4°C for 10 minutes.
8. Decant supernatant into the 1 l culture flask used for previously for growing.

9. Short spin in the big centrifuge.
10. Remove supernatant (SN) with a 1 ml pipette.
11. Gently (**cells are fragile at this point**) resuspend the pellet in each of the 5 Falcon tubes in 16 ml ofice cold CCMB80 buffer using a 25 ml plastic pipette and pipette boy. Resuspend until all white 'particles' are gone from the suspension
12. CRITICAL
13. Pipette buffer against the wall of the centrifuge bottle to resuspend cells. Do not pipette directly into cell pellet.
14. Combine the resuspended cells into 2 50 ml Falcon tubes
15. Incubate on ice for 20 minutes
16. Centrifuge again at 3000 g at 4 °C for 10 min.
17. Decant supernatant into the 1 l culture flask used for previously for growing.
18. Short spin in the big centrifuge
19. Remove SN with a 1 ml pipette
20. Resuspend each of the two cell pellets in 5 ml of ice cold CCMB80 buffer.
21. Incubate on ice for 20 minutes.
22. Pour dry-ice into a flat blue ice bucket
23. Add 1.5 ml eppendorf tubes with open lids to the dry ice. Wear safety glasses.
24. Into the chilled tubes pipette 200 µl cells, close the lid
25. Long-term storage is at -80 °C; flash freezing does not appear to be necessary.

SOB medium

Mix all material for medium in autaclaved lab glassware.
Adjust to pH = 7.5 prior to use. This requires approximately 25 ml of 1M NaOH per liter.
260 ml is the minimum and yielded for us about 40 x 200 µl competent cell aliquots; it's better to make double SOB medium e.g. 600 ml in case something goes wrong or the culture overgrows.

CCMB80 buffer

100 ml per 250 ml SOB culture.
Mix all material for buffer in lab glassware.
Adjust pH **down** to 6.4 with 0.1N HCl, if necessary. Adjusting pH up will precipitate manganese dioxide from Mn containing solutions.
Sterile filter and store at 4°C.
Slight dark precipitate appears not to affect its function.

Appendix 2

Chemical transformation by heat shock

Introduction

Protocol for chemical transformation of *E.coli* by heat shock.

Materials

- SOC 10ml
  Pre-heated at 37^{o}C
- Competent cells 200 µl
  Keep on ice
- Plasmids solution 20µl
  Or 0.1 µl per 1 µl of competent cells
- Centrifuge tubes 1.5 ml x 2 (eppi)
- Agar plates x 2
  Pre-heated at 37^{o}C

Procedure

1. Place a small glass bottle with sterile SOC (10 ml) in the 37 °C incubator to prewarm. Also prewarm at this point the plates for spreading out the transformation.
2. Thaw on ice one eppi (200 µl) of self-made competent Top10 cells per ligation to be transformed. **Keep the competent cells as much as possible on ice**.
3. Add the entire ligation (20 µl) to the cells (or 0.1 to 1 µl for plasmids to be re-transformed) and mix by tapping gently. **Do not mix cells by pipetting, their membrane is fragile and shear force can kill cells at this point**.
4. Incubate on ice for 30 minutes (if possible, mix once or twice by gently tapping again).
5. Switch on the water bath immediately after adding the DNA to the cells and set to 42 °C (check temperature with a thermometer, sometimes the setting needs to be adjusted to achieve measured 42 °C. (**In our lab, set to 39.5 °C to achieve 42 °C**)
6. Heat shock the cells by incubating the eppis for 60 seconds in the 42 °C water bath. **Do not mix or shake**.
7. Remove the eppis from the 42 °C bath and quickly place on ice, incubate for 2 min.
8. Add 500 µL of pre-warmed SOC medium (SOC is a rich medium; use proper sterile technique to avoid contamination.)
9. Secure the eppis in a small microcentrifuge rack with tape and place in the 37°C shaking incubator for 60- 90 min (the rack should stick down with its short side so that the eppis lie parallel to the platform for maximum aeration). Warning: if you label only the lids, the tape will remove the labelling when you strip it off after incubation. Label the side of the Eppendorf and secure the label with masking tape.
10. Plate 50- 100 µl of the cells on one selective pre-warmed plate. Spin down the remainder of the cells at 3800 rpm for 3 min at RT and pour off the SN until about 50 µl of liquid remain in the tube. Resuspend the pellet in this remaining liquid by pipetting up and down with a 200 µl pipette and plate the entire resuspension onto a second plate.
11. Wrap the plates loosely in cling film and incubate upside down at 37°C o.n.