19.5.2015

TUESDAY, 5/19

Present: Arto, Petra, Tuukka, Anna
9:15 Enter lab, started making chloramphenicol plates (see protocol) and planning the work
11.30 Finishing with chloramphenicol plates, started making solution for comp cells
1. 0.1 M $CaCl_2$
2. 0.1 M $CaCl_2$ solution containing 15% glycerol
11.55 Finishing with the solutions

Solutions:
50ml 0,1M $CaCl_2$ solution:

45ml sterile H_2O
+ 5ml 1,0M $CaCl_2$
-> prepare in the sterile hood
-> put in +7 fridge to use tomorrow for competent

10ml 0,1M $CaCl_2$ in 15% glycerol:

(85% glycerol stock
85% x X = 15% x 10ml
X = (15 x 10ml)/85 = 1,7647 ml -> 1,8ml)

7,2ml H_2O
+1,8ml 85% glycerol
+1ml 1M $CaCl_2$

Made an o/n culture of E. coli TOP10 in sterile LB media. Culture prepared in the sterile hood (no antibiotic resistance used) and put in +37°C shaking.

20.5.2015

WEDNESDAY, 5/20

1. Refreshed TOP10 o/n culture at 9.00am
   250ml LB broth + 500µl o/n culture in sterile conditions
   -> +37°C
2. Followed the chem competent cells protocol in half volume
   -Measured OD600, 0.3573 @ 10:45
   -Put bacteria to ice bth @ 10:58
   -Moved the chilled culture into one 50 mL falcon tube and centrifuged at 4C at 4000RPM for 10 minutes @11:13
   -Dircarded supernatant, added 20 ml of ice-cold 0.1M $CaCl_2$ solution@ 11.50. Put cells on ice @ 11.50.
3. In step 13, we made 110µl aliquots

-> stored in -80°C freezer

afternoon: present Anna and Petra

14.00 Start with transformation efficiency kit

- spinned the DNA tubes from the kit 30 sec , 9000 rpm

-Followed the protocol (protocol done on the bench)

    -We included #37 from 2014 teams samples as a control.

        -C[#37]:145ng/µl

        -Diluted 1:50

        -> c[#37]: 0,290ng/µl

            -> we used 1µl of this

    -Incubation on ice 35mins

-Plating:

    Ctrl plated on three chloramphenicol plates: 20µl, 20µl and 20µl per plate.

    50pg plated on two chloramphenicol plates, 20µl on each

    Rest of the samples plated on one plate each, 20µl per plate per sample

    -> this was done because Martina is very sceptical that the amount of DNA in iGEM's transformation efficiency kit will produce any colonies. There's no point in wasting 3 plates for each of these.

->plates in +37°C o/n

Amounts of DNA on each plate:

    Ctrl 0,290ng into 50µl of competent cells -> 0,0058ng/ul

        Plate with 20µl -> 20µl x 0,0058ng/µl = <u>0,116ng</u>

    50pg into 50µl of competent cells -> 1pg/µl

        Plate with 20µl -> 20µl x 1pg = 20pg = <u>0,02ng</u>

    20pg into 50 µl of competent cells -> 0,4pg/µl

        Plate with 20µl -> 20µl x 0,4pg/µl = 8pg = <u>0,008ng</u>

    10pg into 50µl of competent cells -> 0,2pg/µl

        Plate with 20µl -> 20µl x 0,2pg/µl = 4pg = <u>0,004ng</u>

    5pg into 50µl of competent cells -> 0,1pg/µl

        Plate with 20µl -> 20µl x 0,1pg/µl = 2pg = <u>0,002ng</u>

    0,5pg into 50µl of competent cells -> 0,01pg/µl

        Plate with 20µl -> 20µl x 0,01pg/µl = 0,2pg = <u>0,0002ng</u>

15.00 Making of o/n culture of BL21 to make competent cells tomorrow. Bit of glycerol stock transferred into 2,5ml LB with a transferring loop.

    -> +37C shaker next to laminar room

21.5.2015

THURSDAY, 5/21

Refreshed the o/n culture at 9am

- 50µl of o/n culture into 50ml fresh LB media (in the sterile hood)

Morning: present Petra and Tuukka

10.36 Measured the OD: 0.3066
10.39 Put the BL21 bottle to ice for 15 min

Followed the protocol in half volume

11.18 Resuspended cells put to ice for 30 min
12.06 Incubation on ice stopped later because centrifuge wasn't free and we had to wait another centrifuge to cool down.
Centricuged at 3900 rpm since in was the maximum of the other fuge.

Calculating transformation plates
0,5 pg: 0 colonies
5 pg: 0 colonies
10 pg: 0
20 pg: 15
50 pg 1: 27
50 pg 2: 27
50 pg average: 27
#37 1: 494
#37 2: 692
#37 3: 530
#37 average: 548

Transformation efficiencies (cells/ug)
0,5 ng: 0
5 ng: 0
10 ng: 0
20 ng: 1875000
50 ng: 1350000
#37: 4724138

Transformations for propagations:

Table1

Plasmid name	Plate	Well
AH001	Plate 1	19E
AH002	Plate 3	13A
AH003	Plate 4	4G
AH004	Plate 2	2J
AH005	Plate 4	1H
AH006	Plate 3	3F
AH007	Plate 4	2H
AH008	Plate 4	6B

Followed the kit plasmid propagation protocol and removed the plasmids from the plate according to table 1.
-> stored in -20°C

Proceeded with the transformation protocol (TOP10 cells).
- included a H2O control (1µl water instead of DNA

-> followed the protocol
-> there is some variance in the recovery time on ice (according to protocol 5mins) due to making so many samples at the same time. (1-2min variance)
-> made double plates of each sample, one plate with 10µl, one with 20µl
-> +37°C o/n

Testing BL21 competent cells' transfomation efficiency

- Done as yesterday with TOP10 cells
    - 1 tube for each concentration from the kit
    - 1 tube for last year's #37 (1:50 dilution)
    - Added a H2O control (1µl of water instead of DNA)

-> followed the protocol (transformation efficiency kit)
-> there is some variance in the recovery time on ice (according to protocol 5mins) due to making so many samples at the same time. (1-2min variance)
-> Double plates for #37 and 50pg
-> 20µl on each plate
-> +37°C o/n

22.5.2015

FRIDAY, 5/22

Present: Petra, Tuukka, Tamannae
13.30 Enter lab, started making 3 stocks of chloramphenicol plates. Two of them were made based on 500ml agar with 368 µl chloramphenicoll so that the concentration was 25 µg/ml. However, when melting the third agar with 50W, the agar explosed in microwave causing the loss of 100 ml agar. Thus, third stock was made of 400ml agar and 294 µl chloramphenicoll and separated from two first batch. The explosed stock plates was left to incubate in the room temperature over the weekend, so the possible contaminations could be detected.

All propagations were fine except for weak RBS AH005 and the backbones AH007, AH008.

AH007 was the ampicillin backbone and AH008 the kanamycin backbone, which explains why they did not grow on chloramphenicol plates.

BL21 competent cells produced colonies but they were not red. We are not sure if our comps are OK.