OriginALS notebook:

Week 16 – 27/5/18 – 31/5/18

27/5/18:

Who's at the lab:
MorP

Today's goals:

1. Check sequencing result of pX601-F4/80-EGFP-g2, pX601-F4/80-EGFP-g5 (2 colonies), pX601-F4/80-EGFP-g6 (2 colonies), pX601-F4/80-EGFP-g7 (2 colonies)
2. Freeze DH5a pX601-F4/80-EGFP-g1 (2 starters)

Description:

1. Sequencing results:
   a. pX601-F4/80-EGFP-g2: good assembly
   b. pX601-F4/80-EGFP-g5 (2 colonies):
      i. The HA tag seq was cut during the assembly, the promoter looks good, bad gRNA seq.
      ii. good assembly
   c. pX601-F4/80-EGFP-g6 (2 colonies):
      i. Good assembly, short seq length.
      ii. Good assembly, short seq length, there's a one nucleotide mutationafter U6 and before the gRNA
   d. pX601-F4/80-EGFP-g7 (2 colonies):
      i. good assembly, short seq length.
      ii. the HA tag seq was cut during the assembly, the promoter looks good, bad gRNA seq

I prepare starters for -80 glycerol stock freeze of the following bac:

- DH5a pX601-F4/80-EGFP-g2
- DH5a pX601-F4/80-EGFP-g5 #2
- DH5a pX601-F4/80-EGFP-g6 #1

- DH5a pX601-F4/80-EGFP-g6 #2 (with mut)
- DH5a pX601-F4/80-EGFP-g7 #1

for each sample I prepared 2 starters:
10ml LB + 10µl Amp + 100µl Bac from the mini-prep starters

2. Freeze DH5a pX601-F4/80-EGFP-g1 (2 starters) according to -80 glycerol freezing protocol. stock location:

- DH5a pX601-F4/80-EGFP-g1 #1 - 4.5.1.3 C4
- DH5a pX601-F4/80-EGFP-g1 #2 - 4.5.1.3 D4

Tasks for next time:
freeze starters.

28/5/18:

Who's at the lab:
Mors

The experiment:
Making reactive astrocytes experiment's in the second time, the activation includes:

A. Activation with medium of BV2 + LPS from microglia plate.
B. Activation with 3 cytokines (IL1a, C1q and TNFa) in the astrocyte medium.

Today's goals:

1. Splitting of C8-D30 cells to 24 wells plate and to 12 well plate for the activation with the medium + LPS and for the activation with the cytokines accordantly.
2. Splitting of BV2 cells to 24 wells plate (it is used for the activation with the medium + LPS).

Description:

1. In the 24 well plate (plate 1#), we need 80,000 cells for one well and in the 12 well plate (plate 2#), we need 150,000 cells for one well.
    a. The procedure was performed for 3 flasks:
        - Warm C8-D30 medium.
        - Remove and discard culture medium.

- Briefly rinse the cell layer with 1ml Trypsin and discard the 1 ml Trypsin.
- Add 2.0 mL of new Trypsin solution to flask and observe cells under an inverted microscope until cell layer is dispersed (5 minutes).
- Add 6.0 mL of complete growth medium and aspirate cells by gently pipetting.
- Move the 8 mL value to falcon (15 ml value).
- Put the falcon in the centrifuge for 5 min, 1500 RCF, 21°C.
- Remove the medium and add 1ml new medium and suspend the cells.
- Counting cells-

b. Mix 80 µl PBS + 10 µl medium with cells (from the falcon) + 10 µl Tripan blue and pipet the mix.
c. Put the glass cover on the cell counter leave a bit of space in the edges.
d. Pipette gently and slowly 10 µl of mix to each side of cell counter (top & bottom) between the glass cover and the cell counter
e. Transfer the cell counter under the microscope. Focus on the center of the cell counter.
f. In each corner of the cell counter, there is a 4X4 square. Count only the living cells (living cell will look in each 4x4 square.
g. Average the 4 counts and use the following calculation to determine the number of cells in 1 ml.
    - Living cells conc. = average x 10^5 in 1mL medium (divide in 1000 for 1ηL)

Results:

- Falcon number 1 – 587,500 (1 ml) cells so we need to seed 136ηL for 1 well (enough for 7 wells).
- Falcon number 2 - 362,000 (1 ml) cells so we need to seed 221ηL for 1 well (enough for 5 wells).
- Falcon number 3 - 975,000 (1 ml) cells so we need to seed 154ηL for 1 well (enough for 7 wells).
- Falcons 1,2 were used for the 24 well plate and the falcon 3 was used for the 12 wells plate.

Later:

- Add 1.5 mL medium to all the 24 wells and to all the 12 wells.
- Take specific value from all the falcons.
- Mix the plate gently.
- Put the plate in the autoclave overnight:

10:20 AM – plate 1#
10:30 AM – plate 2#

PLATE 1#:

		Medium from BV2	Medium from LPS	Medium from BV2+LPS	Medium from BV2+LPS	
		Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30
					Medium C8D30 **Y**	Medium C8D30
	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30
					Medium C8D30 **Y**	Medium C8D30

PLATE 2#:

	Just cytokines	C8D30	3 cytokines + C8D30	

	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30
		Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30
		Medium C8D30 **Y**	Medium C8D30 **Y**	Medium C8D30

2. In the 24 well plate, we need 150,000 cells for one well.

The procedure was performed for 1 flask:

- Starting material: confluent flask of 25xcm^2
- Pre-warm to 37 degrees cell medium.
- Aspirate medium from flask.
- Add 1ml medium the flask, wash and remove the medium.
- Add 1ml new medium to the flask.
- Scrape gently with sterile scraper.
- Mix well without creating bubbles.
- Transfer the medium to falcon.
- counting cells-
    i. Mix 80 µl PBS + 10 µl medium with cells + 10 µl Tripan blue.
    ii. Put the glass cover on the cell counter leave a bit of space in the edges
    iii. Pipette gently and slowly 10 µl of mix to each side of cell counter (top & bottom) between the glass cover and the cell counter
    iv. Transfer the cell counter under the microscope. focus on the center of the cell counter.
    v. In each corner of the cell counter, there is a 4X4 square. Count only the living cells (living cell will look in each 4x4 square.
    vi. Average the 4 counts and use the following calculation to determine the number of cells in 1 ml.
- Living cells conc. = Average x 10^5

Results:

- Falcon number 1 – 2,212,500 cells so we need to seed 68ηL for 1 well (enough for 15 wells).

Later:

- Add 1.7 mL medium of C8-D30/BV2 to the 24 wells.
- Put the specific value from all the falcons.
- Mix gently the plate.
- Put the plate in the autoclave overnight:

8:50 AM the plate was putted.

		BV2	LPS	BV2+LPS	BV2+LPS	
		medium **X**	medium	medium **X**	medium **X**	Medium BV2
					medium **X**	Medium BV2
		medium **X**	medium	medium **X**	medium **X**	Medium C8D30
					medium **X**	Medium C8D30

Tasks for next time:

1. Add LPS to the specific wells in the 24 wells plate of BV2.

29/5/18:

Continue the reactive astrocytes experiment.
Who's at the lab:
Mors

Today's goals:
1. At 8:50 AM add LPS to the specific wells in the 24 wells plate of BV2.

Description:

1. Add, at 8:50 AM, 3.4 microliter of LPS to specific wells in the

		BV2	LPS	BV2+LPS	BV2+LPS	
		medium X	medium	medium X	medium X	Medium BV2
					medium X	Medium BV2
		medium X	medium	medium X	medium X	Medium C8D30
					medium X	Medium C8D30

plate. The transferring was finished at 8:56 AM.

Tasks for next time:

1. Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines.

30/5/18:

Continue the reactive astrocytes experiment.

Who's at the lab:
Mors
Today's goals:

1. Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines.

Description:

1. At 8:56 AM remove the old medium from the plate 1#.
   Transfer the medium from the BV2 plate to late 1#.

		Medium from BV2	Medium from LPS	Medium from BV2+LPS	Medium from BV2+LPS	
		Medium was transferred Y **4**	Medium was transferred Y **3**	Medium was transferred Y **2**	Medium was transferred Y **1**	Medium BV2
					Medium was transferred Y **5**	Medium BV2
	Medium C8D30 Y **10**	Medium was transferred Y **9**	Medium was transferred Y **8**	Medium was transferred Y **7**	Medium was transferred Y **6**	Medium C8D30
					Medium was transferred Y **11**	Medium C8D30

Put new 1.5 mL medium in well number 11.

At 9:30 AM remove the old medium from the plate 2#, put 1 mL new medium in the wells and add 3 cytokines to the specific wells (4 wells):
0.3 microliter TNFa
0.3 microliter IL1a
0.4 microliter C1q

	Just cytokines	C8D30	3 cytokines + C8D30	

	Medium C8D30 **C** Y	Medium C8D30 **B** Y	Medium C8D30 **A** Y	Medium C8D30
		Medium C8D30 **E** Y	Medium C8D30 **D** Y	Medium C8D30
		Medium C8D30 **G** Y	Medium C8D30 **F** Y	Medium C8D30

Tasks for next time:

1. After 24 hr. - freezing the medium from the 2 plates (1# and 2#).

31/5/18:

Who's at the lab:
Mors

Today's goals:
After 24 hr. - freezing the medium from the 2 plates (1# and 2#)

Description:

1. At 9:00 AM transfer 200 microliter from the wells in the plate 1# to falcons (15 mL value), pipit gently.
2. Return the plate to the incubator.
3. Put falcons in the centrifuge (1500 RCF, 10 min, 4 degrees).
4. Transfer the liquid from the falcons to Eppendorf's (150 microliter).
5. Put the Eppendorf's in the freezer in -80 degrees. (9:15 AM)
6. At 9:25 AM transfer 200 microliter from the wells in the plate 1# to falcons (15 mL value)., pipit gently.
7. Return the plate to the incubator.
8. Put falcons in the centrifuge (1500 RCF, 10 min, 4 degrees).
9. Transfer the liquid from the falcons to Eppendorf's (150 microliter).
10. Put the Eppendorf's in the freezer in -80 degrees. (9:34 AM) (There is a " ' " sign).

Tasks for next time:

1. After 48 hr. - freezing the medium from the 2 plates (1# and 2#)