OriginALS notebook:

Week 18 – 10/6/18 – 16/6/18

10/6/18:

Who's at the lab:
Mors

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

a. Activation with MCM/ACM + 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 2#
2. Splitting of BV2 cells to 24 wells plate 1#

Description:

1. In the 24 well (plate 2#), we need 80,000 cells of astrocytes for one well.
   The procedure was performed to 2 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-

   - Mix 80 µl PBS + 10 µl medium with cells (from the falcon) + 10 µl Tripan blue and pipet the mix.
   - Put the glass cover on the cell counter leave a bit of space in the edges.
   - Pipette gently and slowly 10 µl of mix to each side of cell counter (top & bottom) between the glass cover and the cell counter
   - Transfer the cell counter under the microscope. focus on the center of the cell counter.
   - 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.

- 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 – 512,500 (1 ml) cells so we need to seed 156ηL for 1 well (enough for 7 wells).
- Falcon number 2 - 375,000 cells so we need to seed 213ηL for 1 well (enough for 5 wells).

After:

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

7:48 AM – plate 2#

		3 cytokines +C8D30	Medium from LPS	Medium from BV2+LPS	Medium from BV2+LPS	
			Medium C8D30 **Y 3**	Medium C8D30 **Y 2**	Medium C8D30 **Y 1**	MCM from BV2 + Medium C8D30
		Medium C8D30 **Y 5**			Medium C8D30 **Y 4**	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 10**	Medium C8D30 **Y 9**	Medium C8D30 **Y 8**	Medium C8D30 **Y 7**	Medium C8D30 **Y 6**	ACM from BV2 + Medium C8D30
		Medium C8D30 **Y 12**			Medium C8D30 **Y 11**	ACM from BV2 + Medium C8D30

2. in the 24 well (plate 1#), we need 150,000 cells of microglia for one well (accidently I put 157500 cells in each 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 – 7875000 cells so we need to seed 20ηL for 1 well (enough for 8 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:

   6:25 AM the plate was putted.

			BV2	BV2+LPS	BV2+LPS	
			medium **X**	medium **X**	medium **X**	Medium BV2
					medium **X**	Medium BV2
			medium **X**	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.

11/6/18:

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

Today's goals:
At 6:25 AM add LPS to the specific wells in the 24 wells plate of BV2.

Description:
Add, at 6:25 AM, 3.4 microliter of LPS to specific wells in the plate.
The transferring was finished at 6:34 AM.

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

Tasks for next time:
Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines.

12/6/18:

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

Today's goals:
Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines.

Description:

1. At 6:34 AM remove the old medium from the plate 2# .
2. Transfer 1.5 ml of the medium from the BV2 plate to plate 2# (1,2,3,4,6,7,8,11).
3. Put new 1.5 mL medium of astrocytes in wells number – 5,9,10,12.

		3 cytokines +C8D30	Medium from LPS	Medium from BV2+LPS	Medium from BV2+LPS	
			Medium C8D30 **Y 3**	Medium C8D30 **Y 2**	Medium C8D30 **Y 1**	MCM from BV2
		Medium C8D30 **Y 5**			Medium C8D30 **Y 4**	MCM from BV2
	Medium C8D30 **Y 10**	Medium C8D30 **Y 9**	Medium C8D30 **Y 8**	Medium C8D30 **Y 7**	Medium C8D30 **Y 6**	ACM from BV2
		Medium C8D30 **Y 12**			Medium C8D30 **Y 11**	ACM from BV2

4. Add 3 cytokines to the specific wells (5,9,12):
    - 0.45 microliter TNFa
    - 0.45 microliter IL1a
    - 0.6 microliter C1q

5. Put the plate 2# in the incubator overnight. (7:09 AM)

Tasks for next time:
After 48 hr. – collect the medium from each well and make a Western Blot analyzation. In addition, calculate the number of the cells in each well, make a comparison to the number in the beginning.

14/6/18:

Who's at the lab:
Mors

Today's goals:
After 48 hr. – collect the medium from each well and make a Western Blot analyzation. In addition, calculate the number of the cells in each well, make a comparison to the number in the beginning.

Description:
** I was not able to calculate the number of the cells in each well in the plate of C8D30 again, because the cells didn't detached from the plate. So, we need to practice this procedure before we will do the experiment again **

1. I collected the medium from each well (1.4 ml) to 12 Eppendorf's and putted in the freezer at 4 degrees.
2. I performed the Western Blot analysis according to the protocol Western Blot

Results:

* The bends were very weak, therefore we need to repeat the exp. again and make a new stock of antibodies with skin milk.