OriginALS notebook:

Week 24 – 22/7/18 – 31/7/18

25/7/18:

Who's at the lab:
Einan

Today's goals:

1. Colony PCR for BV2 cells transfected with px601-f4/80-g2 (10 days after transfection)
2. Colony PCR for C8-D30 cells transfected with AAV-Steap4-VP64 (10 days after transfection)

Description:
The following samples were produced of each transfection experiment:
1.

medium	pAc-GFP pos. BV2	BV2 electroporation with px601-f4/80-g2 (2.5µg)	BV2 electroporation with px601-f4/80-g2 (2.5µg)	BV2 electroporation with px601-f4/80-g2 (5µg)	BV2 electroporation with px601-f4/80-g2 (5µg)	BV2 electroporation with px601-f4/80-g2 (9µg)	BV2 electroporation with px601-f4/80-g2 (2.5µg)

2.

C8-D30 transfection with pAc-GFP	C8-D30 transfection with pAc-GFP	C8-D30 transfection with pAc-GFP	C8-D30 transfection with AAV-Steap4-VP64	C8-D30 transfection with AAV-Steap4-VP64	C8-D30 transfection with AAV-Steap4-VP64

Colony PCR was done on wells from each transfection treatment as follows:

a. 1 BV-2 cells were extracted using a tip in 200 µl of medium.
b. 2 C8-D30 cells were incubated with 200 µl of trypsin (after washing with PBS) for 5 minutes. Then, trypsin was countered by dilution with medium (1:4).
    i. ~50,000 cells were taken for each Eppendorf (after the amount of cells was determined using the cell counter).
    ii. Pellet cells (1min full speed in a table top should suffice) and remove supernatant.
    iii. Wash by re-suspending in 5-10mM Tris pH 8.5, pellet again (100 µl).
    iv. Re0suspend cells in 10ul of the above Tris buffer, heat 15' at 99 degrees Celcius.
    v. Cool on ice, centrifuge 1min at full speed.
    vi. Supernatant was transferred to a new PCR Eppendorf and stored in -20°C.

Tasks for next time:
PCR with appropriate primers and with appropriate controls on all samples and run on agarose gel.

29/7/18:

Who's at the lab:
MorS

The experiment:
Making reactive astrocytes' experiment at the four 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:
Thawing of BV2 cells to 24 wells plate 1#

Description:
In the 24 well (plate 1#), I seeded 150,000 cells of microglia for one well.
The procedure was performed for 1 flask:

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

Results:
falcon number 1 – 8950000 cells so I need to seed 17ηL for 1 well.

- 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 -10:02 AM the plate was putted.
- Take new flask and transfer the liquid from the falcon to the flask ,add proper medium of BV2 (9 ml net) and put in the incubator.

		BV2	BV2+LPS	BV2+LPS	BV2+LPS	
		medium **X**	medium **X** 48 hr.	medium **X** 24 hr.	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 24 hr. "Exr."	medium **X** 24 hr.	Medium C8D30
			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium C8D30

Tasks for next time:

1. Add LPS to the specific wells in the 24 wells plate of BV2 (1#). (48 hr. Exp.)
2. Thawing more BV2 cells to 24 wells plate 1# (24 hr. Exp.)
3. Thawing of astrocytes cells (C8D30) to 24 wells plate 2# (48 hr. Exp.)

30/7/18:

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

Today's goals:

1. Thawing more BV2 cells (new) to 24 wells plate 1# (24 hr. Exp.)
2. At 10:05 AM add LPS to the specific wells in the 24 wells plate of BV2. (48 hr. Exp.)
3. Thawing of astrocytes cells (C8D30) to 24 wells plate 2# (48 hr. Exp.)

Description:

1. In the 24 well (plate 1#), I seeded 150,000 cells of microglia for one well.
   The procedure was performed for 1 flask:
   a. Starting material: confluent flask of 25xcm^2. (the same flask from Sunday)
   b. Pre-warm to 37 degrees cell medium.
   c. Aspirate medium from flask.
   d. Add 1ml medium the flask, wash and remove the medium.
   e. Add 1ml new medium to the flask.
   f. Scrape gently with sterile scraper.
   g. Mix well without creating bubbles!!!
   h. Transfer the medium to falcon.
   i. Counting cells:
      ii. Mix 80 µl PBS + 10 µl medium with cells + 10 µl Tripan blue.
      iii. Put the glass cover on the cell counter leave a bit of space in the edges
      iv. Pipette gently and slowly 10 µl of mix to each side of cell counter (top & bottom) between the glass cover and the cell counter
      v. Transfer the cell counter under the microscope. focus on the center of the cell counter.
      vi. In each corner of the cell counter, there is a 4X4 square. Count only the living cells.
      vii. Average the 4 counts and use the following calculation to determine the number of cells in 1 ml.
   j. Living cells conc. = Average x 10^5

Results:
Falcon number 1 – 3875000 cells so I need to seed 39ηL for 1 well.

- 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 -9:55 AM the plate was putted.

		BV2	BV2+LPS	BV2+LPS	BV2+LPS	
		medium **X**	medium **X** 48 hr.	medium **X** 24 hr.	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 24 hr. "Exr."	medium **X** 24 hr.	Medium C8D30

			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium C8D30

2. Add, at 10:05 AM, 34 microliters of LPS to specific wells in the plate.
   The transferring was finished at 10:32 AM.3. In the 24 well (plate 2#), I seeded 45,000 cells of astrocytes for one well.

		BV2	BV2+LPS	BV2+LPS	BV2+LPS	
		medium **X**	medium **X** 48 hr.	medium **X** 24 hr.	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium BV2
			medium **X** 48 hr.	medium **X** 24 hr. "Exr."	medium **X** 24 hr.	Medium C8D30
			medium **X** 48 hr.	medium **X** 48 hr. "Exr."	medium **X** 24 hr.	Medium C8D30

The procedure was performed to 3 flasks:

a. Warm C8-D30 medium
b. Remove and discard culture medium.
c. Briefly rinse the cell layer with 1ml Trypsin and discard the 1 ml Trypsin.

d. Add 2.0 mL of new Trypsin solution to flask and observe cells under an inverted microscope until cell layer is dispersed (5 minutes).
e. Add 6.0 mL of complete growth medium and aspirate cells by washing.
f. Move the 8 mL value to falcon (15 ml value).
g. Put the falcon in the centrifuge for 5 min, 1500 RCF, 21°C.
h. Remove the medium and add 1ml new medium and suspend the cells.
i. Counting cells-
    i. Mix 80 µl PBS + 10 µl medium with cells (from the falcon) + 10 µl Tripan blue and pipet the mix.
    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.
    vi. Average the 4 counts and use the following calculation to determine the number of cells in 1 ml.
j. Living cells conc. = average x 10^5 in 1mL medium (divide in 1000 for 1ηL)

Results:

- Falcon number 1 – take 225ηL for 1 well (enough for 4 wells).
- Falcon number 2 - take 257ηL for 1 well (enough for 4 wells).
- Falcon number 2 - take 360ηL for 1 well (enough for 3 wells).

Later:

- Add 1.6 mL medium to the 24 wells.
- Put the specific value from all the falcons.
- Mix the plate gently.
- Put the plate in the autoclave overnight:
  12:10 AM – plate 2#

Medium from BV2	3 cytokines +C8D30	3 cytokines +C8D30	Medium from BV2+LPS	Medium from BV2+LPS	Medium from BV2+LPS	
Medium C8D30 **Y 5** 48 hr. control	Medium C8D30 **Y 4** 48 hr. without cytokines		Medium C8D30 **Y 3** 48 hr.	Medium C8D30 **Y 2** 24 hr.	Medium C8D30 **Y 1** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 10** 24 hr.	Medium C8D30 **Y 9** 48 hr.	Medium C8D30 **Y 8** 48 hr.	Medium C8D30 **Y 7** 48 hr.	Medium C8D30 **Y 6** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 15** 24 hr.	Medium C8D30 **Y 14** 48 hr.	Medium C8D30 **Y 13** 48 hr.	Medium C8D30 **Y 12** 24 hr.	Medium C8D30 **Y 11** 24 hr.	ACM from BV2 + Medium C8D30
	Medium C8D30 **Y 20** 24 hr.	Medium C8D30 **Y 19** 48 hr.	Medium C8D30 **Y 18** 48 hr.	Medium C8D30 **Y 17** 48 hr.	Medium C8D30 **Y 16** 24 hr.	ACM from BV2 + Medium C8D30

Tasks for next time:

1. Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines. (48 hr. Exp.)
2. At 10:38 AM add LPS to the specific wells in the 24 wells plate of BV2. (24 hr. Exp.)
3. Thawing of new astrocytes cells (C8D30) to 24 wells plate 2# (24 hr. Exp.)

31/7/18:

Continue the reactive astrocytes experiment's.
Today's goals:

1. Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines. (48 hr. Exp.)

2. At 10:38 AM add LPS to the specific wells in the 24 wells plate of BV2. (24 hr. Exp.)
3. Thawing of new astrocytes cells (C8D30) to 24 wells plate 2# (24 hr. Exp.)

Description:

1. Continue activation the astrocytes:
   a. At 10:00 AM remove the old medium from the plate 2# .
   b. Add 1.5 ml new medium of C8D30.
   c. Add to the specific wells – 0.45 microliter TNFa, 0.45 microliter IL1a and 0.6 microliter c1q. (9,14,19)
   d. Transfer 1.5 ml of the medium from the BV2 plate to plate 2# (3,5,7,8,13,17,18).
   e. Put the plate 2# in the incubator overnight. (10:38 AM)

Medium from BV2	3 cytokines +C8D30	3 cytokines +C8D30	Medium from BV2+LPS	Medium from BV2+LPS	Medium from BV2+LPS	
Medium C8D30 **Y 5** 48 hr. control	Medium C8D30 **Y 4** 48 hr. without cytokines		MCM from BV2 **Y 3** 48 hr.	Medium C8D30 **Y 2** 24 hr.	Medium C8D30 **Y 1** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 10** 24 hr.	Medium C8D30 +cytokines **Y 9** 48 hr.	MCM from BV2 **Y 8** 48 hr.	MCM from BV2 **Y 7** 48 hr.	Medium C8D30 **Y 6** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 15** 24 hr.	Medium C8D30 +cytokines **Y 14** 48 hr.	ACM from BV2 **Y 13** 48 hr.	Medium C8D30 **Y 12** 24 hr.	Medium C8D30 **Y 11** 24 hr.	ACM from BV2 + Medium C8D30
	Medium C8D30 **Y 20** 24 hr.	Medium C8D30 +cytokines **Y 19** 48 hr.	ACM from BV2 **Y 18** 48 hr.	ACM from BV2 **Y 17** 48 hr.	Medium C8D30 **Y 16** 24 hr.	ACM from BV2 + Medium C8D30

2. Add, at 10:38 AM, 34 microliters of LPS to specific wells in the plate.
   The transferring was finished at 10:53 AM.

		BV2	BV2+LPS	BV2+LPS	BV2+LPS	
		medium X	medium X 48 hr.	medium X 24 hr.	medium X 24 hr.	Medium BV2
			medium X 48 hr.	medium X 48 hr. "Exr."	medium X 24 hr.	Medium BV2
			medium X 48 hr.	medium X 24 hr. "Exr."	medium X 24 hr.	Medium C8D30
			medium X 48 hr.	medium X 48 hr. "Exr."	medium X 24 hr.	Medium C8D30

3. In the 24 well (plate 2#), I seeded new 45,000 cells of astrocytes for one well. The procedure was performed from 6 flasks:

    a. Warm C8-D30 medium
    b. Remove and discard culture medium.
    c. Briefly rinse the cell layer with 1ml Trypsin and discard the 1 ml Trypsin.
    d. Add 2.0 mL of new Trypsin solution to flask and observe cells under an inverted microscope until cell layer is dispersed (5 minutes).
    e. Add 6.0 mL of complete growth medium and aspirate cells by washing.
    f. Move the 8 mL value to falcon (15 ml value).
    g. Put the falcon in the centrifuge for 5 min, 1500 RCF, 21°C.
    h. Remove the medium and add 1ml new medium and suspend the cells.
    i. Counting cells-
        I. Mix 80 µl PBS + 10 µl medium with cells (from the falcon) + 10 µl Tripan blue and pipet the mix.
        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.
        VI. Average the 4 counts and use the following calculation to determine the number of cells in 1 ml.
    j. Living cells conc. = average x 10^5 in 1mL medium (divide in 1000 for 1ηL)

Results:
Falcon number 1 (50 ml) – take 300ηL for 1 well (enough for 9 wells).

- Add 1.6 mL medium to the 24 wells.
- Put the specific value from all the falcons.
- Mix the plate gently.
- Put the plate in the autoclave overnight:

13:40 AM – plate 2#

Medium from BV2	3 cytokines +C8D30	3 cytokines +C8D30	Medium from BV2+LPS	Medium from BV2+LPS	Medium from BV2+LPS	
Medium C8D30 **Y 5** 48 hr. control	Medium C8D30 **Y 4** 48 hr. without cytokines		Medium C8D30 **Y 3** 48 hr.	Medium C8D30 **Y 2** 24 hr.	Medium C8D30 **Y 1** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 10** 24 hr.	Medium C8D30 **Y 9** 48 hr.	Medium C8D30 **Y 8** 48 hr.	Medium C8D30 **Y 7** 48 hr.	Medium C8D30 **Y 6** 24 hr.	MCM from BV2 + Medium C8D30
	Medium C8D30 **Y 15** 24 hr.	Medium C8D30 **Y 14** 48 hr.	Medium C8D30 **Y 13** 48 hr.	Medium C8D30 **Y 12** 24 hr.	Medium C8D30 **Y 11** 24 hr.	ACM from BV2 + Medium C8D30
	Medium C8D30 **Y 20** 24 hr.	Medium C8D30 **Y 19** 48 hr.	Medium C8D30 **Y 18** 48 hr.	Medium C8D30 **Y 17** 48 hr.	Medium C8D30 **Y 16** 24 hr.	ACM from BV2 + Medium C8D30

<u>Tasks for next time:</u>
Activation the astrocytes directly with LPS + medium from the BV2 plate and with the cytokines. (24 hr. Exp.)