Labbook Cell Culture

Tuesday, 08.14.2018
Preparation of 50 ml Basic Culture Media and media exchange in 4 bottles
EMEM+Glutamine 42 ml
FBS from Heidi 3,75 ml
FBS 3,75 ml
Pen/Strep 0,5 ml

Wednesday, 08.15.2018
Preparation of 2x 50 ml Basic Culture Media
EMEM+Glutamine 42 ml
FBS 7,5 ml
Pen/Strep 0,5 ml

Christoph made 6 aliquots of 5ml Trypsin in 15 ml tubes, stored in -20°C

Splitting of P13 Cells from 4 Bottles

- aspirate media,
- wash with 7 ml PBS
- aspriate PBS
- apply 1 ml Trypsin and incubate 3 min at 37°C, tap bottle
- inhibit and rinse Trypsin with 4 ml Basic Culture Media
- unite all cells in the last bottle and transfer to 50 ml tube
- counting of cells: 396, 485 and 405 cells per big square in an improved Neubauer chamber -> ~4,300.000 cells per ml.
- 12 Aliquots of 1 ml cells in 15 ml tubes
- Centrifuge 3 min at 300*g and 4°C, discard supernatant
- Resuspend in 1,5 ml cooled Freezing Media (17,1 ml Basic Culture Media + 0,9 ml DMSO) and transfer in cooled cryotubes
- cooled 12,5 min at 4°C and 12,5 min at -20°C, transferred to -80°C at Jansen Lab (Labeled SHSY5Y P14, 15.8.18)
- Innocculation of 180µl united cells in 8,5 ml Basic Culture Media in 2 new bottles

Thursday, 08.16.2018
Looked at Cells under microscope: adherent, few floating cells. Low confluence

Friday, 08.17.2018
Changed medium, cells were unequally distributed in the flask. At the bottom of the flask were nearly no cells and the bottleneck were most of the cells

Monday, 08.20.2018
Cells splitted: p14->p15
Started 2 flasks, à 0.35 x 10^6 Zellen
Thawed 1 vial p14
Started 6-well plate for differentiation
per well 0.1 x 10^6 cells in 2 ml basic culture medium

Tuesday, 08.21.2018
Making of 50 mM stock solution retinoic acid in DMSO
Aliquotisiation of 10 µl (50 mM) retinoic acid, ca. 30 reaction tubes
Change of media in differentiation
New media: Differentiation media 1 (2 ml)

Wednesday, 08.22.2018
Change of BCM in 2 Bottles P15 SHSY5Y

Friday, 08.24.2018
Change of Media in 2 Bottles P15 SHSY5Y
Sterile filtration of 1 ml Retinoic acid in 36 10µl Aliquots - Stored at -20°C
Change of Differentiation Media 1 in 6 Wells P15 SHSY5Y.
Aspirated the thawed P14 SHSY5Y
Asked AG Feil for access to Microscope with camera. -> Monday ist Michael back, he is using it right now

Monday, 08.27.2018
Splitting of P15 Cells
10:00 Cell count: 76, 46, 67 and 35 -> 560.000 cells/ml. 115µl of cell suspension in each 35 mm dish, adding 2ml of Basic culture Medium. Equals to 64.400 cells per dish
Seeding of 1,5 ml cell suspension in 2 new bottles. Adding 9 ml of BCM, incubating at 37°C. Equals to 840.000 cells per bottle.

Splitting of 1. Differentiation onto uncoated dishes. One had a fungus, and we put parafilm on it to protect the others.
200µl of Trypsin per dish, incubate at 37° for 3 Minutes and inhibit with 2ml Differentiation Media 1. Rinse well and conjugate into a 15ml Tube. Reeseeding of 2ml cell suspension onto new 6-well plates.
Discard the fungus and aspirate with lots of ethanol after 5 min.

17:45: Change to DM1 in the second differentiation

Tuesday, 08.28.2018
Got plasmids pcDNA3 and pcDNA3.1- from Markus AG Feil, made a 100µg/µl stock, stored at -20°C in 2017 box
Resuspended Cre from 2013 plate 5 well D5.
Thawed chemically competent DH5a on ice, added 1 µl DNA (Cre, pcDNA3 and pcDNA3.1-). Incubated on ice 30 min.

Wednesday, 08.29.2018
Change of DM1 in differentiation 2
Change of BCM in the 2 bottles of P16 cells
Resuspend 100µg human fibronectin in 1 ml sterile PBS. 3 Aliquots of 250µl and 2 125µl. Freezing at -20°C
Coating of 1 well 25 µg FN with 1250µl and 1 well with 12,5µg in 500µl.
Wraped the Plate in Parafilm and incubate at 4°C overnight
Got 1 g of BSA from AG Lerche.

Picked 3 clones from pcDNA3.1- and from the Cre plate, innoculated in 10 ml LB overnight.

Thursday, 08.30.2018
Resuspended 1 g of BSA in 10 ml sterile PBS and aliquoted 500µl, freeze at -20°

Used 1 aliquot to block the fibronectin coated wells. Added 4,5ml PBS to 500µl 100mg/ml to a concentration on 10mg/ml. Blocked with 2,5 ml per well. Spilled some while applying parafilm. Problem?

Miniprep with Sol I, II and III without column from 8ml overnight cultures. Protocoll from gust
Spinned down 8 min, 10.000rpm at 4°.

DNA content Minipreps

pcDNA3.1-/1	666,3 ng/µl
pcDNA3.1-/2	406,1 ng/µl
pcDNA3.1-/3	526,8 ng/µl
Cre 1	433,4 ng/µl
Cre 2	541,7 ng/µl
Cre 3	500,5 ng/µl

Splittet differentiation 1 in a new 6 well plate.

Friday, 08.31.2018
Changed to DM3 in differentiation 1, added 2µl retinoic acid.
Changed DM1 in differentiation 2.
Changed BCM in 1 bottle. Discarded 1.

Monday, 09.03.2018
Thawed a new veil of P14 in 8,5ml EMEM at 37°C. Centrifuged at 1000g for 2 Minutes. Discard supernatant and resuspendet the pellet in 10ml BCM. Poured in bottle.
Changed DM3 in first differentiation, added 2µl retinoic acid (seemed flaky)
Changed DM2 in second differentiation

Tuesday, 09.04.2018
Changed BCM in the thawed P14 flask.

Wednesday, 09.05.2018
Changed DM3 in first differentiation
Changed DM2 in second differentiation

Thursday, 09.06.2018
Splittet bottle of P14 cells, resuspended in 10ml BCM: 357.500 cells/ml. 37, 27, 28, 51 cells per big square.
platet each 270µl of cell suspension in 6 wells with each 1ml BCM.
Seeded 1,75ml of cell suspension into 8ml BCM into a new bottle.

Friday, 09.07.2018
Aliquoted 50ml FBS into 7,5ml, freeze at -20°C
Prepared 50ml BCM
Prepared 50 ml DM1, 4 Aliquots of 12ml
Changed BCM in Bottle
Changed to DM1 in differentiation 3
Changed DM3 in differentiation 1

Monday, 09.10.2018
Changed BCM in P15 Bottle
Changed DM1 in differentiation 3
Changed DM3 in differentiation 1

Wednesday, 09.12.2018
Change of BCM in cutlure flask P15
Change of DM1 in differentiation 3
aspriation of differentiation 1

Thursday, 09.13.2018
Preparation of new Basic culture media
splitting of differentiation 3 into a new 6-well plate with DM1
Splitting of P15 culture flask.
dilutet 1:10 and countet 81,86,61,54,72,111 -> 775.000 cells/ml
seeding of 1,1 ml into a new flask (P16), adding 12 ml media.
and seeding of 130µl per well into 6-well plates. Adding 2 ml BCM.

Friday, 09.14.2018
Change to DM2 in 3.differentiation
Coat with fibronectin in 24 well plates, 250µl of 20ng/µl.
placed 4 glass plates before coating. Wraped the Plate in Parafilm and incubate at 4°C overnight
Changed to DM1 in differentiation 4

Monday, 09.17.2018
Block 24well plate with 20mg/ml with 1ml. Wraped the Plate in Parafilm and incubate at 37°C for 90 minutes
Pipetting scheme

	A	B	C	D	E	F
1	x					
2	x	x	x	x	x	x
3	x	x	x	x	x	x
4	x	x	x	x		

Prepare Differentiation MEdia 1 and Aliquot
Change of DM1in differentiation 4.
1:1 Split of differentiation 3 into blocked 24well plate.
Discard P16 Flask.

Tuesday, 09.18.2018
5x 250µl STocks of BDNF in Neurobasal Media with 1x B-27 ->1,2 ml
5x 100µl Stocks of bdcAMP in H2O 510µl
Preparation of DM3:
Change to DM3 in differantiation 3

Thursday, 09.20.2018
Splitting of differentation 4 into uncoated 6well plates with DM1
Friday, 09.14.2018
Changed DM3 in diff 3
Changed to Dm2 in Diff 4
Coated 24 well falcon Plate with 11,8 µg of fibronectin: 200µl PBS vorgelegt, dilutet 300µl of 1mg/ml stock into 1350µl PBS. Added 65µl of dilution to each well. Incubate at 4°C over weekend covered with parafilm.

Monday, 09.24.2018
Blocked with BSA for 90 min at 37°C.
Splitting of Diff 4 on to coated plates
Change of Media in Diff 3
Thawed 1 veil SHSY5Y in a small flask
Prepared BCM

Tuesday, 09.25.2018
Changed to DM3 in Diff 4
Changed BCM in SHSY5Y

Wednesday, 09.26.2018
Prepared DM3
Changed DM3 in Diff 3

Thursday, 09.27.2018
Splitted SHSY5Y into 2 wells in 24well plate with

Friday, 09.28.2018
Picked coverslips of 2 Differentiated cells 3 and of the 2 undifferentiated SHSY5Y
wash with 1 ml of PBs and discard PBS
Added 1 ml of Paraformaldehyde and incubatet at room temperature for 20 min.
washed 5 times with PBS, handed over to AG Lerche for immuno staining.
Changed DM3 in Diff 3 and 4

Monday, 10.01.2018
Change of Media in Diff 3 and 4

Thursday, 10.04.2018
Preparation in DM3
Change of Media in Diff 3 and 4

Friday, 10.05.2018
Changed Media in Diff 3 and Diff 4

Monday, 10.08.2018
Preparation of BCM and DM3
Changed Media in Diff 3 and Diff 4
Thawed 2 veils of SHSY5Y P14

Tuesday, 10.09.2018
Changed Media in 2 flasks SHSY5Y

Wednesday, 10.10.2018
Changed Media in Diff 3 and Diff 4

Thursday, 10.11.2018
Thawed 1ml mT/mG MEF cells from liquid Nitrogen after thawing protocol from SHSY5Y in DMEM with 10%FBS and 500µl Pens/Strep distributed by AG Feil.

Friday, 10.12.2018
Changed Media in MEF
Distributed coverslips in 2 24-well plates.
Splitted 2 Flasks undifferentiatet SHSY5Y, 6.410.000 cells/ml in 10ml BCM.
Innoculatet a new Flask P15 with 140µl splitted cells and 10 ml BCM
Innoculated 20 ml BCM with 310µl cells and distributed 500µl per well on a 24-well plates.
Added 5 ml BCM to the remaining 8 and distributed it into 24-well plate.

Saturday, 10.13.2018
Prepared BCM
Prepared Differentiation Media 3
Changed Media in Diff 3 and Diff 4

Monday, 10.15.2018
Change of Media in MEF
Change of Media in undifferentiated cells.

Wednesday, 10.7.2018
Splitting of MEF: 180.000 cells per ml. Seeding of 500µl per well 4x in 24well plate. Rest of 8 ml reseedet into big flask, added 4 ml of media.