Medium Change iPSC

The medium of the iPSC cells will be changed other everyday.

Need:

- Stemflex medium (Life technologies; A3349401)

Protocol:

1. Take out the Stemflex medium in the morning
    - (It needs to be at room temperature)
2. Take off the old medium in the dish's
    - **(Change tips every other cell line!)**
3. At new Stemflex medium to every dish
    - 1,5ml for the 3,5cm dish
    - 3ml for the 6cm dish
4. Place the cells back in the incubator

Geltex coating

Need:

- Geltrex; dilute 5mL of Geltrex (Life technologies; A1413202) in 495mL of DMEM F-12 (Life technologies; 11320074)
- Culture dishes

Protocol:

1. Add geltrex to the new dishes
    - 1,5ml for the 3,5cm dish
    - 3ml for the 6cm dish
2. Leave the geltrex-dishes o/n in the incubator (or at least 1 hour)
3. The next day, take the geltrex from the dishes and fill the dishes with medium.

Splitting iPSC cell lines

Need:

1. Stemflex medium (Life technologies; A3349401)
2. 0,5mM EDTA
3. 1x PBS (Life technologies; 14190169)
4. Rock-inhibitor (Dilute 1:1000 in medium) (Axon Biochemicals; AXON 1683)
5. Geltrex coated dishes

Protocol:

1. Check which dish needs to be split, the other is the back-up
    - Check for amount of cells, but also differentiation
2. Dilute the rock-inhibitor 1:1000 in Stemflex
    - Amount of medium needed: 1,5ml per new dish + 1ml per cell-line + 1ml extra
3. Take off the geltrex from the dishes and add 1,5ml Stemflex with y27
4. Take off the medium in every dish, and wash cells with PBS
5. Take off the PBS, add EDTA to the cells and transfer the cells to the incubator for 2 minutes and 10 seconds
    - 1,5ml EDTA to the 3,5cm dish
    - 3ml EDTA to the 6cm dish
6. Take off the EDTA after 2 minutes and 10 seconds
7. Shoot 1ml of Stemflex with y27 on the dish (once!), the cells should come off
8. Take 35µl of cells and add them to the new dish (add double the amount to the other dish)
9. Check if there are enough small colonies on the new dish
    - If not, add more cells
10. Store in the incubator, and check the cells the next day
11. If the cells look good, change medium
    - Add 2ml to the dishes instead of 1,5ml
12. If there are not enough colonies on the new dish, do the splitting again with the back-up dish

Freezing iPSCs

Need:

- Cold Freezing medium: FBS (Sigma-Aldrich; F7524)/ 10%DMSO
    - Amount of Freezing medium: 1ml per vial + 1ml extra, filter before use
- PBS (Life technologies; 14190169)
- 0,5mM EDTA
- Stemflex medium (Life technologies; A3349401)

Protocol:

1. Take off the medium
2. Wash dishes with 2ml of PBS, and remove it
3. Add EDTA and leave in the incubator for 2 minutes
4. Prepare a 15mL tube with 6mL of Stemflex medium
5. Remove the EDTA
6. Add 3-4ml of PBS, shoot 1ml on the cells each time, on a different spot
7. Collect PBS in the 15ml tube
8. Shoot 1ml of Stemflex medium on the cells to detach them more, and add to the 15ml tube
9. Centrifuge tube for 4 minutes at 200rpm
10. Remove supernatant
11. Resuspend pellets in cold FBS/10% DMSO
    - Resuspending volume depends on amount of vials
    - 1x 6cm dish is enough for 2 vials.
12. Put the vials in the purple box with the ring inside and bring to the -80°C
    - **The ceramic ring must be inside the purple box and at room temperature**
13. Next day, place vials in Liquid Nitrogen

Thawing iPSC's

Need:

- 1x 6cm dish, coated with geltrex per vial (Life technologies; A1413202)
- Rock-inhibitor (Axon Biochemicals; AXON 1683)
- HuES medium
  - DMEM-F12 (Life technologies; 11320074)
  - 100 ml KOSR (Life technologies; 10828028)
  - 5 ml L-glutamine (Life technologies; 25030024)
  - 5 ml NEAA (Life technologies; 11140035)
  - 2,5 ml P/S (Life technologies; 15140122)
  - 3,6ul 2-mercaptoethanol (add to NEAA in fumehood)
- Stemflex (Life technologies; A3349401) with rock-inhibitor (y27, 1:1000)

Protocol:

1. Take out the tube from the Liquid Nitrogen and keep it on dry ice
2. Thaw the cells in the warm water-bath
   - **Be carefull, tube can explode**
3. Pipet the cells in the HuES medium and spin down at 200rcf for 4 minutes.
   - Use at least 7 times more HuES medium volume then cell volume.
4. Take of the geltrex from the dishes
   - Prepare at least 1 hour in advance.
5. Remove the supernatant from the cell-pellet
6. Dissolve the pellet in 1ml Stemflex
7. Pipet cells in the new dishes
8. Place the cells in the incubator