Time

Allow take 3-3.5 hours, add one more hour when seeding on ibidi dishes in the drop

Materials

- Fibronectin (Sigma; Cat num. F1141-5mg; lot …)

- Pluronic Acid (Cat bum. 59005, lot P40614-101164)
- ddH20
- RPMI (+)
- KOSR
- Rock Inhibitor
- Enzyme T
- Enzyme T diluent
- FBS
- PBS
- Counting Slide
- DIshes/slides
  - Ibidi dish with the beads coated surface (dry); 3.14cm2
  - 6well with 2cm stencil; 9.5cm2

Preparation steps

Solutions

Fibronectin

- Dilute 1mg/mL fibronectin to 50ug/mL in PBS
- Amount:
  - 0.75mL per dish
  - 2mL per 1w6

Pluroinc Acid

- Dilute to 1% from 10% stock in ddH20
- Amount:
  - 3mL per dish
  - 3mL per 1e6 NOTE: filter through 22um filter

Stop solution

- dilute FBS to 20% in PBS
- Amount:
  - 2mL per 12well
  - 1mL per 24 well

Replating medium

- 10% KOSR in RPMI(+), add 1to2000 Rock Inhibitor
- Amount:
  - 3mL per ibidi dish
  - 3mL for 6well dish
  - 1mL for counting of cells

Enzyme T

- dissolve 33uL/mL of buffer X
- Amount:
  - 400uL per 1w12 well
  - 200uL per 1w24 well

Process

Coat and sterilze the plates

- Ibidi:
  - Add 750uL drop on the ibidi dish, spread to edges using pipette (is very hydrophobic)
- 1w6:
  - Add 2mL swirl to make sure all surface is coated
- Incubate 1hr at RT
- Aspirate FN, Wash with 3mL PBS 3x
- Add pluronic 3mL (both ibidi or 1w6)
- Incubate 30min at RT (Ideally start harvesting cells at this point)
- wash with PBS 3mL 3x

Collect cells

- wash with PBS
- add diluted enzyme T (200uL/24 well, 400uL/12 well)
- incubate 8-10min
- Add Stop solution (300ul/24well, 600ul/12 well)
- Collect by pipetting, disrupt to single cells
- Add (500ul; 1mL) to wash down
- Spin at 300g for 3-5min
- Resuspend in 0.5mL replating media (for 24 well, for the rest, 1mL is enough)
- count

Seed cells

- create a solution
  - Ibidi: approx 100k cells/mL in replating solution
  - 1w6: approx 30k cells/mL
- Deposit:
  - Ibidi: as a drop approx 600uL, spread to the edges to fill the whole surface of insert
  - 1w6: 3mL in the dish
- Add media (Ibidi only):
  - Let the cells to sit down for 1 hour (check after 30 min if its okey), but 1hr is better
  - Fill the rest of the vessel with the replating medium (2mL)

Change media

- change media day after
- afterwards cheange media to RPMI (+)
- change every second day
- Measure from the day 3 onwards