Scratch Assays_Version1.2

Protocol:

Arrange at least 24 hours before starting the assay:

- Reserve CellIQ device in the online booking system. Get access to the system by asking the system administrator M. Ofterdinger.
- Turn the CellIQ device on (green switch of back side of the cabinet) and set desired temperature on the display on the front door, next to the sample slot (e.g. 37.0°C)

Following wound closure with CellIQ- Machine:

- Thaw cells and grow them to a confluent state in DMEM + Supplements (e.g. 10%FCS, PenStrep, L-Glutamine) in 10 cm dishes, change media after 24 hours to remove dead cells.
- Seed 300.000 cells in a 12 well plate and grow them to a confluent state (approx 48 hours) in DMEM
- Add a wound to the confluent cell layer with the tip of a 200 µL pipette tip. E.g. diagonally from the left upper corner to the right lower corner. Try to scratch as fast as possible to avoid curves in the scratch, but not too harsh to avoid separation of the celllayer from the plate.
- Remove media and wash twice with PBS
- Add 1.5 mL L15 media (Lobkovitz-15 500 mL with 10% FCS and Pen/Strep)
- Close Plate with a strip of parafilm to avoid evaporation
- Place the 12 well plate in the CellIQ device, well A1 being in the upper right corner.
- Open the Cell-IQ Imagen program
    - Settings:
        - Incubator – Set – plate type – e.g. Nunc 12 well plate – OK
        - Edit – Reset cycle 1
        - Select positions, (3x3 grid if necessary)
        - Imaging – Set grabbing interval: e.g. 30 minutes
        - Save cycle 1

    - Imaging – Set saving directory
- Start imaging, start without gas – yes
- Stop imaging approximately 24 hours later. Save pictures immediately to the network drive

Datamining:

1. Very slow version using Cell-IQ Analyzer
    - Use Cell-IQ Analyzer program (possible at the Imaris station. Ask M. Offterdinger for help)
    - Load photos to the workstation, open an example-photo
    - Make a library: Pick samples – Area Counter – Make Protocol – Mark Background – Choose a minimum of 30 Background samples – Mark Cell phase 1 - Choose a minimum of 30 cell samples
    - Build protocol – test with one image (red area = recognized cells, gray area = background)
    - Save sample library
    - Save protocol
    - Start of the protocol: Run Protocol – choose your protocol – choose your photo folder – create a destination folder – If you collected 3x3 grids tell the program which photos it should analyze for each well (e.g. 1,5,9) – next – next – set time borders if wanted – START
    - After finishing analysis run the program opens automatically the curve analysis program. Export results in excel file (Data-Export all curves to .xml).
    - Open excel file and calculate % closure over time by using the formula as published in Goetsch et al. 2011.
2. Faster version using CellProfiler (recommended in most cases)
    - Download and install CellProfiler (www.cellprofiler.org)
    - Download CellProfiler pipeline
    - Open CellProfiler

- File> Load pipeline: ScratchAssay
- Enter default input folder
- Enter default output folder
- Select “Analyze Images”
- Wait a while but significantly less than for version 1
- Processed Images and and Excel compatible csv file will be saved in the output folder
- Drawback: Won’t work properly if the cells are not quite confluent (outside the actual scratch)