Microscopy: Raji – Jurkat conjugates (protocol to image a fluorescent protein in Jurkat cells on a confocal microscope)

A) Live imaging

Transfection of Jurkat cells

→ make sure your cells are perfectly fine before you even start your experiment, dilute your cells the day before transfection

→ also Raji cells must be perfectly fit and healthy, otherwise you waste your time

- suspend 15 µg plasmid DNA with 200 µl pure RPMI in 4 mm electroporation cuvette
- 10 Mio Jurkat cells per sample
- spin, resuspend in 300 µl pure RPMI
- transfer to electroporation cuvettes with DNA, mix very well with DNA
- electroporate with 0.31 V / 960 µF
- with yellow tip carefully and slowly transfer 2x 200 µl cell suspension into 8 ml full medium in 6-well plate (make sure to leave the clump of dead cells on top of the electroporation solution behind, with the last 100 µl of the suspension)
- incubate overnight

Sorting of transfected cells

- filter transfected cell suspension through Filcon Microcap filters (keeps back clumps of dead cells and DNA)
- spin down, resuspend in 500 µl fresh full medium
- incubator / 2-3 hours
- carefully sort
- spin down sorted cells, resuspend in 200 µl full medium
- incubate in 96-well plate (can be imaged immediately or the next day)

Loading Raji cells with SEE superantigen

- 5 Mio Raji
- spin down, resuspend with 1 ml full medium (best in eppendorf tube)
- + 10 ng/ml SEE superantigen (final conc.; Toxin Technology #ET404, stock solution 100x in PBS)
- incubator / 1 h
- wash 3x with CM (conjugation medium = RPMI without phenol red + 0.5% FCS)

- resuspend in 1 ml CM
- seed at 1 Mio / 200 µl in MatTek dishes
- incubator / 20 min (in low FCS Raji cells bind to the normal positive charge of the glass)
- wash 1x with CM, incubate with 2 ml CM

Preparation of Jurkats

- 5 Mio Jurkat cells
- spin down, resuspend in 1 ml CM

Live imaging of conjugates

- take a few fluorescent Jurkat cells in a MatTek dish (whatever fluorophor you want to look at), put them on an inverted microscope
- use a 63x objective, focus on the cells
- adjust the settings to the cells with average brightness (avoid very bright cells, they won't behave normally!!!)
- also adjust the brightfield (transmitted light) image
- put MatTek dish with Raji cells on inverted microscope, well pre-heated to keep the focus stable (a few hours) *(the microscope should have CO_2 control, don't buffer with HEPES, Raji / Jurkats don't like it)*
- if necessary, readjust the brightfield image
- switch to the eyepiece
- carefully add 200 µl (= 1 Mio) Jurkat cells on top of Raji cells
- through the eyepiece observe the cells sinking down
- search for a fluorescent cell with average brightness
- start time-lapse imaging
- if possible, between images of your time course switch back to the eyepiece and readjust the focus

B) Fixed-cell imaging

Preparation of coverslips

- put coverslips in 24-well plate
- overlay with 0.02% Poly-L-lysine (SIGMA #P8920, diluted 1:5 in a. dest.)
- RT / 1 h
- wash 3x with PBS

Loading Raji cells with SEE superantigen

- 5 Mio Raji

- (spin down and resuspend in 1 ml full medium (best in eppendorf tube)
- + 10 ng/ml SEE superantigen (final conc.)
- incubator / 1 h
- wash 3x with CM (conjugation medium = RPMI without phenol red + 0.5% FCS)
- resuspend in 250 µl CM

Preparation of Jurkats

- 5 Mio Jurkat cells
- spin down, resuspend in 250 µl CM

Conjugation and fixation

- mix 100 µl (2 Mio) Raji cells with 100 µl (2 Mio) Jurkat cells in an Eppendorf tube
- incubate at 37C for the required time
- add 1.5 ml fixative
- RT / 5 min
- add to coverslips in 24-well plate
- centrifuge plates 5 min at 3,000 rpm (the fixative crosslinks cells to polylysine)
- wash 1x with PBS + 0.1 M ammonium chloride (from 1 M stock solution)
- wash 3x with PBS
- → *at this stage the cells can be stored for at leasta few days at 4C, or permeabilised / stained with antibodies*
- carefully rinse in H_2O (dip in beaker with distilled water)
- add a drop of 10 µl of ProLong Gold Mounting Medium (Molecular Probes # P36930) to glass slide
- mount coverslips upside down onto mounting medium
- RT / dark / overnight (at least; the mounting medium must dry completely!)
- fridge
- → *once the mounting medium has completely dried, the slides can be stored at -20C for several years*

Solutions

Fixative

→ wear safety goggles!

- 4% paraformaldehyde + 2% sucrose in 25 ml PBS
- heat in water bath to 65°C for 10 min

- + 400 µl 1 M NaOH
- 65°C / 10 min
- cool down to room temperature
- at pH-meter readjust the pH to 7-7.5 with 1 HCl