Screen Description: **96 hours** incubation time after drug addition

Total Screen: 4(+3) Assay plates

Barcodes: D007T01 D030T01 D015T01

M003A M002K

Schedule (write Date below): initials

Preparation □

Cell Seeding □

Library Dilution + Preparation □

Live-Imaging □

Perturbation + Medium Change □

Prepare Fixing □

Dead-Cell Staining + Fixing □

Perming and Staining □

Cell Titer Glow Assay □

Imaging □

Preliminary Data Analysis □

**Critical Remarks:**

**Cassette:**

Tracer Sheet: Screen HC1092-6

**Overview:** Preparation of screen

**Preparation of screen:**

|  |  |  |
| --- | --- | --- |
| Multidrop in place? | □ | |
| Small cassette? | □ | |
| Standard cassette? | □ | |
| AD3+ and PBS for priming of multidrop | □ | |
| 2% SDS stock for cleaning | □ | |
| Check materials for seeding | □ |
| Check if there is >8TB of storage space on collab-bernd-fischer | □ |
| Prepare cold ENA+PG(human)/WENRAS-PG(mouse) for BME dilution | □ |
| Prepare EXCEL Screening SOP for needed BME amount and further reagents | □ |
| Prepare necessary BME and let it thaw over night | □ |
| Prepar barcodes for screening plates | □ |

**Remarks:**

*Plan with 60ml of BME if no further information is available*

Tracer Sheet: Screen HC1092-6

**Overview:** Cell seeding and preparation **on ice**

**Equipment/Materials:**

|  |  |
| --- | --- |
| Prepare EXCEL Screening SOP for needed BME amount and further reagents | □ |
| 1 Aliquot of prepared CTG reagent, use the same batch | □ |
| BME R1/Matrigel/BME V2 residues for seeding of remaining fragments | □ |
| Cell scrapers | □ |
| 96well microscopy plate with black sealing film | □ |
| DNAse (1:1000) 300.000 U/ml [ul] | □ |
| Fresh TrypLE (bottle) | □ |
| 40um filter | □ |
| Syringe Adapter for 40um filter | □ |
| 2% SDS solution | □ |

**Cell preparation:**

|  |  |  |
| --- | --- | --- |
| Prepare all reagents from above | □ | |
| Prepare S2 cell culture hood | □ | |
| Turn on water bath to 37C and prewarm TrypLE | □ | |
| Turn on Fast-Cool of centrifuge | □ | |
| Check **every plate** for contaminations quikly in the S2 cell culture | □ | |
| |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | | **Name:** |  |  |  |  |  |  |  | | **Number of Wells:** |  |  |  |  |  |  |  | | **Score (1-5) per line:** |  |  |  |  |  |  |  | | **Passage of each line:** |  |  |  |  |  |  |  | | **Date of seeding:** |  |  |  |  |  |  |  | |  | |
| collect organoids with cell scraper, *scraping etc. might needs to be done batchwise* | □ | |
| collect organoids with old medium and add to 15 ml falcon | □ |
| *Operator 1 and 2 scrape a line together* |  |
| spin at 400g;3min;4C | □ |
| Prepare 1 50ml Falcons with filter (per line) | □ |
| Prepare 200ml of AD3+ DNAse medium by adding 150ul DNAse | □ |
| get rid of medium | □ |
| Add 1ml prewarmed TrypLE | □ |
| 20xup+down w/ 1000ul pipette | □ |
| Add 3ml TrypLE | □ |
| **Time:** |  |
| digest for 5 minutes | □ |
| *If not done before: Prepare 50ml falcon and 40um filter with connector ring (1/line)* | □ |
| *If not done before: Prepare CTG dilution row by pipetting medium (see below)* | □ |
| **Time:** |  |
| 20xup+down | □ |
| add 5ml AD3**+ DNAse** | □ |
| *collect all suspensions of the same line in one 15 ml tube, be careful and filter residues* | □ |
| prewet a 40um in a 50ml falcon with 3 ml of AD3+DNAse | □ |
| Processed lines operator 1:  Processed lines operator 2: |  |
| add concentrated cell suspension | □ |
| add 5ml AD3+DNAse | □ |
| Add 3ml prewarmed TrypLE on a blocked filter with 1000ul pipette | □ |
| **Time:** |  |
| digest for 5 minutes | □ |
| **Time:** |  |
| Add 3ml AD3+DNAse to the TrypLE | □ |
| Remove the red cap from the filter to unblock and let the suspension go through | □ |
| Add 3ml AD3+DNAse | □ |
| repeat this procedure until the majority of fragments are digested | □ |
| **Total rounds of digestion:** |  |
| Spin filtrate in multiple 15ml falcons at 800g, 5min, 4C | □ |
| Remove TrypLE and filters from the hood | □ |
| Prepare the 1:2 dilution row by adding 40ul of medium to wells 2-5 of the 96w plate | □ |
| Pool pellets from the same line and resuspend in 10ml of the supernatant | □ |
| take 2x80ul aliquot in a microscopy 96 well plate | □ |
| perform 2x5 serial 1:2 dilutions | □ |
| check that the volume in every of the 2x5 wells equals 40ul | □ |
| **Time:** |  |
| add 40ul of freshly thawn CTG reagent to every well and seal the bottom w/ black foil | □ |
| **Time:** |  |
| incubate plate for 15 minutes in the dark with an aluminium-lid | □ |
| Turn on Mithras reader, default settings for 96 well plate | □ |
| **Time:** |  |
| perform readout with Mithras flourescence reader | □ |
| Open EXCEL sheet from latest Organoid\_Screening SOP | □ |
| Enter Photon counts of Mithras readout | □ |
| Print Report and attach below | □ |
|  |  |
|  |  |

**Remarks:**

|  |  |
| --- | --- |
|  |  |

**Documentation and QC:**

|  |  |
| --- | --- |
| **Name of EXCEL file for density calculation:** |  |
| **Lot of the BME used: (add sticker)** |  |
| **Photons per well: (e.g. 5000 photons)** |  |

organoid fragments should be homogenous with 10-20 cells/ fragment

the number of single cells should be relatively small

**Remarks:**

**Plate preparation:**

|  |  |
| --- | --- |
| prepare waste funnel and EtOH for multidrop | □ |
| prepare a Multidrop standard casette ORGANOID and prime with EtOH | □ |
| **Name of cassette:** |  |
| relax the casette and let the EtOH flow back for ~5 minutes | □ |
| open a big centrigfuge, clean it with EtOH cloths and rub the plate-rotor | □ |
| *With more than 12 plates, a second centrifuge is needed. Run it prewarmed at ca. 27C* | □ |
| activate a big centrigfuge (no cooling), let it run empty with plate-rotor, 2880g, 20 min. | □ |
| turn on plate-loc | □ |
| test plate-loc | □ |
| *Group plates library-wise for centrifugation, bar-codes in the center* | □ |
|  |  |
| **Perform thes following steps after measuring the amount of available organoids:** |  |
| Prepare cold ENA+PG(human)/WENRAS-PG(mouse) for BME dilution | □ |
| Prepare BME by dilution with necessary medium to a concentration of 6.25mg/ml | □ |
| Prepare a container and ice box for plate transfer and cooling for upcoming steps | □ |
| prime the casette with ice-cold PBS | □ |
| prime the casette with ice-cold AD3+ | □ |
| prepare all necessary plates | □ |
| Set settings to 384 standard, PROMISE\_sed (**10ul**, fast, full plate) | □ |
| Mix the acute BME mix 3 times carefully with a 50ml pipette, Insert tubing | □ |
| prime the casette with ice-cold BME mixture | □ |
| **Time:** |  |
| run the dispension of the plates | □ |
| **Order of plates:** |  |
| **Visually check for pipetting mistakes** |  |
| Seal all plates with plate-loc (~175C; 2,5s), **do not stack plates** |  |
| **Order of plates:**   |  |  | | --- | --- | | **HTS** | **Storage Room**  **(if applicable)** | |  |  | |  |  | |  |  | |  |
| Add plates into big prewarmed centrifuge, run at 2880g, 20min, ramp up/down = 4 | □ |
| **Time:** |  |
| **Plate IDs and centrifuge:** |  |
| prime the casette with min. 25ml of 2%SDS solution **immediatly** | □ |
| Do not relax the casette and let the 2% SDS solution incubate the tips for 5 min. | □ |
| Prime with EtOH | □ |
| Relax the casette and let the EtOH flow back for ~5 minutes | □ |
| Remove plates from centrifuge and add to 37C incubator (do not incubate for >4h) | □ |
| Arrange plates by cell line | □ |
| Turn centrifuge back on for next round | □ |
| **Time:** |  |
|  |  |

**Remarks:**

**Cell seeding:**

|  |  |
| --- | --- |
| Define amount of needed cell suspension | □ |
| Transfer the neccessary amount to a 15ml falcon | □ |
| *Transfer the necessary amount of min. one plate to a second falcon for seeding on OTs* | □ |
| Spin 15ml falcon at 800g, 5min, 4C | □ |
| use a 50ml falcon for BME mixing, *or use a precooled 200ml cell culture flask* | □ |
| prepare BME by dilution with adequate growth medium (+/-PG) to 1mg/ml BME | □ |
| *The 15ml falcon with pellet for OT seeding is taken up in 850ul of BME V2, 10mg/ml* | □ |
| *Store the 15ml falcon for later seeding* | □ |
| Remove supernatant from spinned 15ml falcon | □ |
| Remove last residues with a 1000ul pipette | □ |
| resuspend a pellet of organoids in 5ml of the BME mix, rinse thoroughly | □ |
| Transfer the 1ml of BME-cell mix to the prepared BME solution in 50/200ml vessel | □ |
| activate a big centrigfuge (no cooling), let it run empty with plate-rotor, 4000rpm, 20 min | □ |
| prepare a Multidrop standard casette and prime with EtOH | □ |
| **Name of cassette:** |  |
| relax the casette and let the EtOH flow back for 5 minutes | □ |
| prime the casette with ice-cold PBS +P/S | □ |
| prime the casette with ice-cold AD3+ | □ |
| prepare all necessary plates, order by cell line | □ |
| set settings to 384 standard, PROMISE\_cell (**50**ul, slow, full plate) | □ |
| Mix the acute BME mix 10 times carefully with a coated 25/50 ml pipette | □ |
| Insert the tubing into the 1st cell-BME mix | □ |
| **prime the casette with ice-cold BME mixture (for every line)** | □  **Time:** |
| **Run the dispension while swirling the vessel, take care not so aspirate an air-gap** | □ |
| **Visually check for pipetting mistakes** |  |
| Seal all plates with plate-loc (~175C; 2,5s), **do not stack plates** | □ |
| Arrange plates according to library | □ |
| **Time:** |  |
| Repeat steps for every line, rinse the tubings with PBS/EtOH/PBS/AD3+ inbetween | □ |
| **Time(s) and Line(s):** |  |
| prime the casette with min. 50ml of 2%SDS solution | □ |
| Do not relax the casette and let the 2% SDS solution incubate the tips for 5 min. | □ |
| Add plates into big prewarmed centrifuge, run at 4000rpm, 20min. | □ |
| **Time:** |  |
| **Plate IDs and centrifuge:** |  |
| *If applicable: Restart the Evos microscope* | □ |
| Start seeding of all remaining and **necessary** residues | □ |
| Check water-basin of HTS incubator and refill | □ |
| Rinse the Multidrop with EtOH, relax the tubing |  |
| Shut down centrifuge and PlateLoc | □ |
| Clean up the Multidrop | □ |
| Dispense medium to spotted culture-plates and add to S2 incubator | □ |
| Take example images of every seeded line with the Axiovert | □ |
| Add all sealed plates into the HTS incubator *in a metal rack by library group* | □ |
| **Time:** |  |
| Shut down hood (if using not-S2 hoods) | □ |
| Set timer for medium dispensing of seeded plates | □ |
| Clean up the Mithras reader | □ |
| Check all used benches | □ |
| Shut down cell culture cabinet | □ |
| Arrange plates by library group | □ |

**Remarks:**

Tracer Sheet: Screen HC1092-6

**Library dilution + Preparation:**

**(has to be done 24h in advance, not earlier)**

|  |  |
| --- | --- |
| Prepare DW blocks (both are in the upper shelve in the storage room) | □ |
| Prepare Storage plates | □ |
| CAVE: the following guideline applies to 4 lines per run.  The dimensions have to be revised if the screen size exceeds this limit |  |
| Prepare ENA or comparable medium without Y: | □ |
| Prepare 45ml\*number of libraries (4 in HC1092) of AD3+ | □ |
| Let library plates thaw at RT | □ |
| Check if sufficient one-time robot (white) 384w reservoirs are in stock | □ |
| prepare a Multidrop standard casette ORGANOID and prime with EtOH | □ |
| **Name of cassette:** |  |
| relax the casette and let the EtOH flow back for ~5 minutes | □ |
| Check index plates visually: | □ |
| prime the casette with ice-cold PBS +P/S | □ |
| prime the casette with ice-cold AD3+ | □ |
| prepare all necessary deepwell blocks (max. Vol 200ul, depending on number of plates) | □ |
| **Number:** *4* |  |
| **Vol:** *200ul* |  |
| **Medium:** *ENA-PGY* |  |
| set settings to 384 DW22, **(applicable Vol) ul**, fast, full plate | □ |
| Insert the tubing into the medium | □ |
| prime the casette with medium | □ |
| run the dispension of the plates | □ |
| Prime the casette with 20ml of ice-cold PBS | □ |
| Seal all plates with alu seals (no plate-loc) | □ |
| Spin thawed library aliquots at 180g, 1 minutes, RT | □ |
| Turn on the robot, PC and start the Biomek software | □ |
| Login, no password | □ |
| Prepare (no. Of libs\*2) 384 30ul **brown** well tips (30ul): brown+barrier | □ |
| Prepare ((no. Of libs+1)\*2) 384 30ul **green** well tips (50ul): 30ul XL | □ |
| Open the Fill\_plates routine | □ |
| Home all axis | □ |
| Fill a clean 384w reservoir with 90ml of AD3+ | □ |
| Prepare 384well storage plates and label with date, screen-name and 100x/10x | □ |
| Run the Fill\_plates routine (results in 45ul filled plate) | □ |
| Open the Library\_dilution routine | □ |
| Prepare all objects, use the new tipboxes on the loader position | □ |
| Run the Library\_dilution routine | □ |
| Seal all plates with alu-seal | □ |
| *Repeat above according to number of libraries* | □ |
| ***CAVE: In HC1092 L08 needs to pipetted into two 10x dilution plates***  ***b/c a CTG is done as well.*** |  |
| *Let medium plates sit at RT during pipetting, store at 4C for long break o/n* |  |
| Store drug-medium mixture at 4C | □ |
| Store library at -80C | □ |
| *Use the clean\_tips routine to rinse tips* ***CAVE: use the right tip size*** | □ |
| Clean robot and shut it down | □ |

**Remarks:**

*Robot settings*

|  |  |
| --- | --- |
| CCP | KiStem |
| 1 | 1 |
| 5 | 5 |
| 20 | 20 |
| 18 | 18 |
| 5 | 5 |
| 45 | 45 |
| 45 | 45 |
| **55** | **65** |

Tracer Sheet: Screen HC1092-6

**Drug treatment:**

|  |  |
| --- | --- |
| Remove a plate of every line, check for contaminations | □ |
| Take 2 10x images per plate in defined positions (DMSO treated later), auto-exposure | □ |
| **Positions (see attachments):** *B02/ O23* |  |
| **Location saved:** |  |
| Remove medium deep wells and let them warm up to 37C in the S1 cell culture inc. | □ |
| Warm up 10x medium-drug plates at RT | □ |
| Turn on the robot, PC and start the Biomek software | □ |
| Login, no password | □ |
| Open the Drug\_Dispensing routine | □ |
| Home all axis | □ |
| **Check if sufficient PBS system liquid is available** | □ |
| Prepare Isopropanol one-time Reservoir with ~ 50ml of 70% Isopropanol | □ |
| Move PBS, 10x Drug medium and organoid medium deepwell to their position | □ |
| Check all variables and objects in the routine (Be aware of both 30 and 30xl tips!) | □ |
| Remove lid from tipboxes | □ |
| Remove seals of ENA+PG-Y, PBS and Drug Medium blocks | □ |
| Turn on PlateLoc | □ |
| Prepare robot-compatible plate lids from seeding step | □ |
| If applicable: Remove one module from incubator (one plate of each line, start with L02) | □ |
| **Time:** |  |
| Remove sealing foils from plates and add lids under the hood in the HTS room | □ |
| Transfer plates to a hermetic container | □ |
| Arrange plates on the robot deck | □ |
| Run routine | □ |
| Change the PBS reservoir every two runs | □ |
| Transfer plates to a hermetic container | □ |
| Add sealing foils to screening plates in the HTS room and save lids once again in bag | □ |
| Wipe with an EtOH cloth | □ |
| Return plates back to incubator | □ |
| **Time:** |  |
| **Duration:** |  |
| *If applicable: Remove further modules and repeat* | □ |
| ***Time:*** |
| ***Re-perform workflow*** |
| ***Time:*** |
| ***Duration:*** |
| *Check both Durations* | □ |
| *Return plates back to incubator* | □ |
| ***Time:*** |
|  |
|  |

**Remarks:**

*KiStem1/2 = L02, L03*

Tracer Sheet: Screen HC1092-6

**Live-Imaging:**

|  |  |
| --- | --- |
| Turn on Incell 6000 | □ |
| Prepare Overlord routine with „\_wlid“ protocol | □ |
| Remove plates from the incubator | □ |
| Add lids under the hood and transfer to transport container | □ |
| **Time:** |  |
| Arrange in Incell 6000 bay 1 | □ |
| Start imaging | □ |
| Return plates, seal and repeat with new set | □ |

Tracer Sheet: Screen HC1092-6

**Overview:** Preparation of screen

**Preparation of Fixation + Staining:**

|  |  |
| --- | --- |
| Prepare PFA/PBS + BSA buffer and store at 4C | □ |
| Prepare PERM + BSA buffer and store at 4C | □ |
| Check the system liquids of the robot | □ |

**Remarks:**

Tracer Sheet: Screen HC1092-6

**Dead Cell Dye addition:**

|  |  |
| --- | --- |
| Open EXCEL sheet to calculate needed amount of dead cell dye | □ |
| Spin Dead Cell Dye | □ |
| Prepare necessary amount of Dead Cell Dye in a black 15ml (50ml) falcon | □ |
| **Lot of Dead Cell Dye (add sticker):** |  |
| Vortex falcon vigorously | □ |
| prepare a Multidrop small casette and prime with EtOH | □ |
| **Name of cassette:** *organoid II (small)* |  |
| relax the casette and let the EtOH flow back for 5 minutes | □ |
| Remove a plate of every line, check for contaminations | □ |
| Prepare robot-compatible plate lids under the hood/ use PlateLoc | □ |
| prime the casette with ice-cold PBS +P/S | □ |
| prime the casette with ice-cold AD3+ | □ |
| Remove all plates from the incubator | □ |
| Transfer plates in container | □ |
| set settings to 384 standard, PROMISE\_DCD | □ |
| Remove seals of plates during dispension and add lids under hood afterwards | □ |
| Insert the tubing into the dye mix | □ |
| Run dispension after removing the lids temporarley | □ |
| Add lids back on plates | □  **Time:** |
| After all plates are treated with 5ul of the dye, move plates to HTS incubator for 4h | □ |
| **Time:** |  |
| Clean up Multidrop and shut down hood | □ |
| Move Dead Cell Dye to -20C | □ |
| Check that all reagents are prepared for upcoming fixation | □ |
|  |  |
|  |  |

**Remarks:**

Tracer Sheet: Screen HC1092-6

**Fixation, Perm, Staining:**

|  |  |
| --- | --- |
| Remove PFA 4% and Perm buffer from the coldroom and let it warm up at RT | □ |
| Remove CTG reagent from the -20 and let it warm up at 4C | □ |
| **Lot of CTG (no sticker):** |  |
| Turn on the robot, PC and start the Biomek software | □ |
| Login, no password | □ |
| Prepare 384 30ul **max** well tips | □ |
| **Name of tipbox:** |  |
| Open the **Fix\_cells** routine | □ |
| Home all axis | □ |
| Toggle number of plates | □ |
| Check if sufficient PBS system liquid is available | □ |
| Remove all plates from the incubator | □ |
| **Time:** |  |
| Place plates on deck with correct lids | □ |
| Remove tape from tipbox and store it | □ |
| run the fixation of the plates | □ |
| Seal plates with Plateloc | □ |
| **Time:** |  |
| ***For HC1092: store the plates at 4C in a dark place, process in less than 7 days for imaging*** | □ |

**Remarks:**

*Set time to 2400s for fixation of 24 plates*

|  |  |
| --- | --- |
| Tracer Sheet: Screen HC1092-6  **Perm, Staining:**  **Time:** |  |
| Refresh deck; Prepare **Perm routine, use intransparent lids** | □ |
| prepare new tipbox for every perm/stain step | □ |
| run the perm of the plates | □ |
| shut off light whenever possible | □ |
| Prepare the staining buffer using the BSA stock | □ |
| Calculate amount of staining solution with EXCEL sheet | □ |
| Vortex flask | □ |
| Refresh deck; Prepare **Stain** routine | □ |
| run the stain of the plates (2h incubation time) | □ |
| Set timer for 3:20h | □ |
| **Time:** |  |
| Use PlateLoc to seal plates | □ |
| Clean up robotics | □ |
| Discard Tipbox | □ |
| Shut down the robot and PC | □ |
| Start Microscopy of screening plates immediatly with the first library-module | □ |

**Remarks:**

*Set time to 2400s for fixation of 24 plates*

*Day1:*

*Day2:*

*Day3:*

Tracer Sheet: Screen HC1092-6

**Cell Titer Glow (perform on the day of fixation!)**

|  |  |
| --- | --- |
| **Time:** |  |
| Refresh deck; Prepare **CTG routine** | □ |
| prepare new tipbox | □ |
| Fill ca. 50ml of CTG reagent into standard reservoir |  |
| run the routine of the 4-6 plates | □ |
| shut off light after 4pm | □ |
| **Time:** |  |
| **Sticker with Lot ID:** |  |

**Appendix:**

PROMISE\_SEED

* 384, standard
* 10ul
* standard cassette
* fast
* full plate

PROMISE\_DCD

* 384, standard
* 5ul
* small cassette
* fast
* full plate

PlateLoc Settings

* settings: 2.5 sec, 182 °C
* let plates cool-down before stacking

Wells to watch in this experiment