Cell preparation and spotting (SCI)

Note: Keep samples and reagents on ice. When spotting cells, spot them promptly after uptake into the nozzle. Do not let samples sit in the nozzle for an extended period of time.

In advance

- **To resuspend CFSE: Add 18 uL DMSO.**
- **To resuspend Live/Dead Red Stain: Add 50 uL DMSO.**
- **Stains should be kept in small single-use aliquots (~2 uL) at -20°C to reduce freeze/thawing. Throw out aliquots after thawing/using once.**
- **Prepare or thaw a solution of 0.22uM filtered 1X PBS with 0.04% BSA (400 µg/ml). For difficult samples, substitute this solution for all PBS steps in both cell handling and staining. This helps reduce clumping.**

Robot set up

1. Degas system liquid as outlined in general robot SOP and prime with PDC90 type 4 or cell qualified nozzle.
2. Start chiller.
3. Set dewpoint control to -1.00.
4. Fill fresh water bottle and empty waste water bottle.
5. Fill both back 2mL SciClean basin (20uL SciClean + 2 mL PCR water).

Cell C/H digestion from tissues

Prepare cells from cryopreserved stomached cells/organoids from xenograft:

- Blunted tips may help the pipetting steps for large tissue samples. Take care to recover tissue that may stick to the inside of the tip.
- Save all supernatants at all wash steps just in case the cells yield is low and we need to recover all cells.
- Supernatant from the collagenase/hyaluronidase incubation may be saved for gDNA extraction. This superntatant is often cloudy.

1. Quick thaw cryopreserved stomached cells/organoids at 37°C.
2. Transfer sample to an eppie tube and top up to 1.5ml with DMEM or MEGM.
3. Spin down (1050-1100rpm, 5min), set aside supernatant. This is to remove DMSO from freeze media.
4. Add 0.5mL collagenase/hyaluronidase (StemCell) aliquot and top up to 1.5 mL with MEGM. Pipette up and down to dislodge tissue pellet.
5. Incubate at 37C for two hours, pipetting up and down the sample every ~30min for the first hour, and every 15-20 min for the second hour (when most of the digestion occurs).
6. Pipette up and down with a P1000 for 1 minute to break up tissue. Tissue will not typically fully digest at this step.
7. Spin down (1050-1100rpm, 5min). Keep supernatant for gDNA prep.
8. Resuspend in 500uL trypsin, pipette up and down 1 minute, top up with FBS to 1.5mL and spin down (1050-1100rpm 5min). Set aside supernatant. Tissue is often not fully digested at this step.
9. Add 1mL dispase (StemCell) and pipette up and down 1 minute. Take a 5uL aliquot for hemocytometer and spin down (1050-1100rpm 5min). Set aside supernatant. Tissue is often not fully digested at this step.
10. If cells are fresh (not cryopreserved), you need to lyse the red blood cells:
    - Add 0.5-1 mL of ammonium chloride (0.8% NH4Cl, StemCell).
    - Incubate at room temp. for 1-2min.
    - Top up with media and centrifuge for 5 min at 1050-1100rpm
11. Resuspend these digested cells in PBS + 0.04% BSA in appropriate volume to achieve a good concentration (~ 1 million/ml).
12. Pass cells twice through a 70uM filter to remove remaining undigested tissue. If a large piece of tissue remains in the first filter, set aside in an eppie tube of PBS.

Cell staining

1. Spin down cells and discard the supernatant **(all spins: 200g/1200rpm, 10min).**
2. For ~50,000 cells or less: mix 100 uL PBS with 0.4 uL CFSE and 0.4 uL Live/Dead Red stain.
    - Scale volumes up proportionally for more cells (ie. for ~100,000 cells, mix 200 uL PBS with 0.8 uL CFSE and 0.8 uL Live/Dead Red stain).

1. Resuspend cells in dye mix and incubate at 37°C (clean TC incubator) for 20 minutes.
2. Spin down cells and resuspend in PBS for a final concentration of 1 cell/nL (1 million cells/mL)
    - At this concentration, spot 1 nL of cells per well for a ~0.37% occupancy (Poisson distribution).

Nuclei staining

1. Double the volume of cells with the Sigma Nuclei EZ lysis buffer (ie. for 100 uL of cells, add 100 uL of EZ lysis buffer).
2. Spin down nuclei and discard the supernatant **(all spins: 500g/2500rpm, 10min).**
3. For ~50,000 nuclei or less: mix 50uL PBS + 50uL EZ lysis buffer with 0.4uL CFSE and 0.4uL Live/Dead Red stain.
    - Scale volumes up proportionally for more nuclei (ie. for ~100,000 nuclei, mix 200 uL PBS with 0.8 uL CFSE and 0.8 uL Live/Dead Red stain).

1. Resuspend nuclei in dye mix and incubate at 37°C (clean TC incubator) for 20 minutes.
2. Spin down nuclei and resuspend in a 1:1 mix of PBS/EZ lysis buffer for a final concentration of 1 nucleus/nL (1million nuclei/mL)
    - At this concentration, spot 1 nL of nuclei per well for a ~0.37% occupancy (Poisson distribution).
    - Note that nuclei should not be cryopreserved, this makes for poor results with DLP.

> ### *Cell staining with Syto9*
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> Note that this nucleic acid stain can only be used on cells spotted into chips with **no prespotted primers**, or it will light up the entire well and imaging the cell is impossible. Also note that SYTO9 photobleaches rapidly and cannot be used for CellenOne spotting.
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> 1. Add 0.15uL Syto9 to 49.85uL cells in PBS (50,000 cells).

Cell quantification with hemocytometer

1. Assess the quantity and viability of cells with 5uL of sample and 5uL of trypan on the hemocytometer.
2. Save an image of the cells with the date.

Cell spotting (by poisson, without CellenONE, see CellenONE protocol for details on running the automated software)

1. Check cell concentration by spotting into the top row of a chip. Select the volume you spot at assuming the concentration determined in the staining steps is correct (e.g. 1 nL per well for 1 million cells/mL). Use the microscope to count the cells in this row, and the cell count calculator to determine the correct volume to spot the cells into the chip with to achieve 37% occupancy.
2. Spot cells into fields of interest using spot volume determined in previous step.
3. Create a JIRA ticket and a database entry for each chip cells are spotted in (see "Data entry"). If multiple regions of a chip are spotted for different libraries, designate them as the chip id plus a letter (ex. A90564A, A90564B). If a chip is only being used in a small region, and the plan is to use it again, mark the library as the chip id plus 'A'.
4. Spin down chip for 1 minute.
5. Place chip face down in microscope stage with the cut corner to the far right. Ensure the clips to the very edge of the chip (clips in the center of the chip will distort it, which causes issues for imaging).
6. Move to Eppie1Nozzle1 and pump out any cells, or if using modified wash station, pump into the camera eppie.
7. Wash flush strong, SciClean wash tray1, wash flush strong (wash cycle to remove any cells from nozzle).

> ### *Filter cells*
>
> Filtering cells can help clear clumps which may help if you are having difficulty spotting. To filter cells:
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> 1. Pre-wet the 50um filter from a FACS tube with 100uL of PBS (can cut off the lid of an eppie and place the filter on).
> 2. Discard PBS.
> 3. Add cells to filter and spin very briefly in tube spinner (4-5 rotations, Mendal Mini).

Data entry

In the Colossus under 'create libraries', add the IDs of the chips you are spotting into and record relevant sample details such as viability and passage number.

Next...

- **Chip imaging and calling (SCI)**