**Pseudo-time experiment**

Aim: to capture features that describe specific steps of SARS-CoV2 viral cycle

**Material:**

* 384 well plate Perking Elmer
* Tissue culture medium:

DMEM High glucose + 2% Heat Inactivated FBS + 1% Pen/Strep Gibco 5,000 U/mL Penicillin and 5,000 ug/mL Streptomycin) + 1% HEPES (1M Buffer Solution) + 1% NEAA (100X MEM- non-essential amino acids, Gibco + 1% L-Glutamine (5 mL) (200 mM 100X)

* Phosphate buffer Phosphate buffered saline (Sterile – Gibco), either +/+ for infection and -/- for staining
* Paraformaldehyde solution 20%, dilution 1/5 prepared daily
* Triton X-100
* BSA, Roche 03116956001 and Goat serum (Gibco 16220-064)

**Procedure:**

1. Seed 3000 Huh7 in 14 384-well plates, black clear bottom (6 plates per cell line) and allow cells to adhere overnight
2. drug treatment with reference compounds for 4 hours

-anti IFNalpha and beta Receptor antibody (10ug/ml) -Remdesevir (15nM)

-Entecavir (500nM) -Z-FA-FMK (50nM)

-Hydroxycloroquine (2 uM) -Eliglustat (4 nM) ON pre-treatment

-Lactoferrin (100 ug/mL)

Plate layout

Note that “Row B is uninfected and the periphery is uninfected too”



1. Infection in BSL3 with SARS-CoV2 WA1 strain (MOI 1) at 37’C for one hour in PBS+/+
2. Replace viral inoculum with DMEM 2% FBS and incubate for
   1. 1h
   2. 3h
   3. 6h
   4. 12h
   5. 18h
   6. 24h
   7. 48h
3. At each time point: fix plate with 4% PFA for 30minutes
4. Permeabilize with 0.3% Triton X-100 (please advise for FISH application)
5. Block in antibody buffer (1.5% BSA, 1X TBS Tween20 (0.0025%), 1% Goat serum in PBS -/-)
6. Stain with antibody dye-sets in antibody buffer (viral primary overnight, secondary for 2-4h)