Defining the Seeding Cell Concentration

Goal

Seeding different cell concentrations on a 96-well plate and monitor the growth of the cells. The information acquired will give us an optimal concentration of cells to be seeded, in order to avoid overgrowth in further experiments.

Samples

- Cell lines tested: HEK-293 and HeLa CCL-2 lc (This cell line was developed in Prof.Dr. Yaakov Benenson's laboratory using HeLa CCL-2 cells. This cell line was generated from AAVS1 LOCUS insertion via talen. In that way, these cells are constitutively producing citrine and luciferase.).

Materials

- Dulbecco's Modified Eagle Medium (Gibco-DMEM)
- Penicillin and streptomycin
- 100% Fetal Bovine Serum (FBS)

Equipment

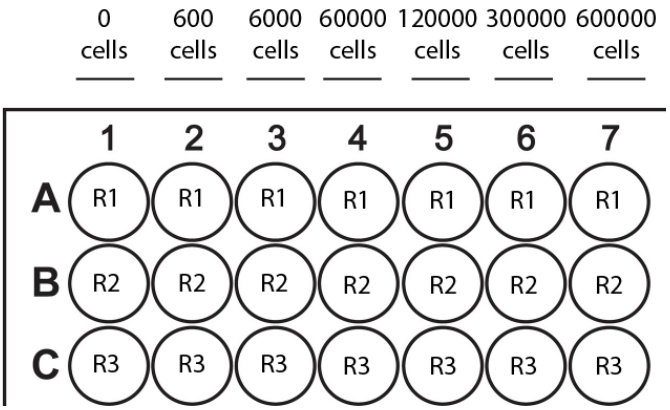
- CO_2 incubator for mammalian culture incubation
- 96-well Greiner microplate, black, clear bottom, chimney well, medium binding
- Nikon Eclipse-*TI* microscope equipped with a Hammamatsu C10600 ORCA – R^2 Digital camera and a Nikon Intesilight C-HGFE illuminator for fluorescence microscopy.

Procedure

Day 1

SEEDING CELLS

1. Cells were seeded on a 96-well plate up to a volume of 100 μL as depicted on the picture below:



2. Incubation of cells for 24 hours at 37oC, 5% CO_2 in DMEM medium supplemented with 1% (w/v) streptomycin and 1% (w/v) penicillin and 2% (w/v) of sterile filtered fetal bovine serum (FBS). The percentage of FBS was reduced from the standard 10% (w/v) FBS that is used for mammalian-cell cultivation in order to avoid cell overgrowth.

Day 2

IMAGING

3. After 24 hours, the cell growth was assessed via microscopy.