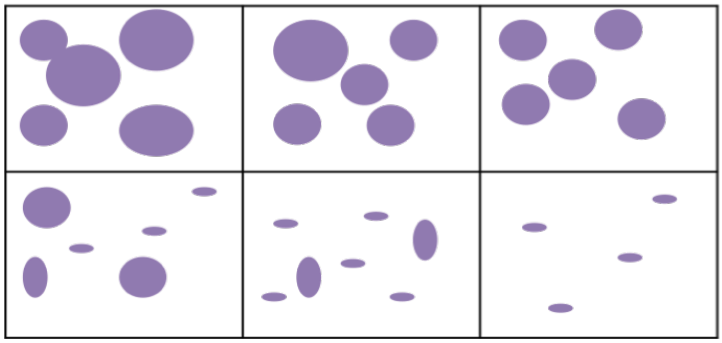
Clonogenic Growth Assay

6 well plate



Day 1: Plate 100-200 cells per well with 2mL media for 6 well plate

- Lift and split cells like normal and put leftover cells in 15mL conical
- Vortex and count using 20µL per chamber slide
- Calculate for 100 or 200 cells depending on the cell line (divide amount you want ie: 200 cells by amount you have cells/mL and multiply by 1000 to get amount of µL/well)
    - Calculate for a 1:10 dilution by moving decimal to the right one place
    - Multiply that number by the amount of wells you are going to plate (ie: 14 wells for 2-6well plates)
- Make your 1:10 dilution by putting 9mL fresh media into a new 15mL conical followed by 1mL of your cell suspension and mix.
    - Put the amount of your 1:10 dilution that you calculated into a 50mL conical with fresh media at 2mL per well (ie: 28mL for 14 wells)
        - Mix by inverting tubes a couple of times
- Plate 2mL of cell mixture and rock a few times
- Return to incubator overnight

Day 2: Switch to 2mL drug treatment

- Aspirate media off of cells
- Replace with 2mL of your drug media
- Return to incubator

Day 5-7: Check on colonies and feed 1x per week with fresh drug treatment

Day 7+: Check on colonies and develop when colonies are big enough but not overgrown

-Usually 10-14 days

Develop:

- Aspirate media from each well
- Rinse with 1mL 1x PBS; aspirate
- Add 200µl - 250µl Crystal Violet per well and incubate 30min
- Aspirate Crystal Violet
- Rinse in DI water
- Let air dry upside down
- Take Photos, Photoshop (crop, grayscale, auto levels, save as tiffs), MetaMorph

Crystal Violet (0.5% Crystal Violet in 6% Gluteraldehyde):

- 6mL 50% Gluteraldehyde
- 44mL MQ H2O
- 0.25g Crystal Violet