CellPyAbility protocols
The results of these experiments can be rapidly analyzed via the CellPyAbility software.

The below protocols offer a brief description of our dose-response experiments comparing two cell lines across 10 concentrations of drug (referred to as growth delay assay, or GDA) and our synergy dose-response experiments of one cell line and 59 unique concentration combinations.

We aim to publish a detailed manuscript describing these protocols along with some use cases in cancer biology. For now, we hope this overview suffices. If there are questions, please feel free to contact me at james.elia@yale.edu.

GDA protocol:
In a cell-culture-treated 96-well plate (cell plate), the outermost wells are filled with 150 uL of DPBS to buffer evaporation. Two cell lines are passaged at 70-90% confluency, counted, and plated in 100 uL of medium at 1000 cells/well (10,000 cells / mL) for rapidly dividing lines and 2000 cells/well for slow dividing lines (10 wells in technical triplicate for two cell lines). Cells will therefore be in B-G and 2-11.

24 hours later, in a separate 96-well plate (drug plate), make a 100x concentration of the top concentration of drug desired in A11, then make serial dilutions into A10 … A3 with only vehicle in A2. For example, if the top concentration of drug desired on cells is 1 µM, A11 will contain 100 µM of drug in solvent.

In B2-B11 of the drug plate, add 310.4 uL of media to each well. Then, transfer 9.6 uL of drug from A2-A11 into B2-B11, which dilutes the drug from 100x to 3x. 320 uL allows for 50 uL x 6 rows with 20 uL excess volume.

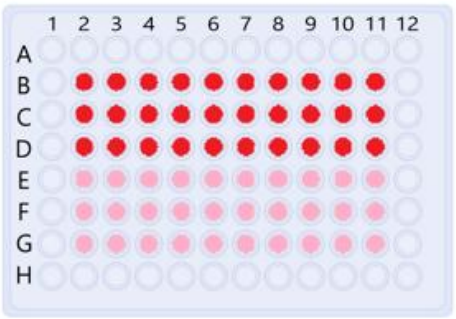
Thoroughly mix the 3x drug-media solution and transfer 50 uL to each well of each row in the cell plate for a final concentration of 1x (1% solvent). This can be repeated for however many drugs desired (one row of 100x drug, one row of media, 4 drugs per 96-well drug plate).

120 hours later, medium is removed from the cell plates, plates are washed with 50 uL of DPBS per well, cells are fixed with 4% formaldehyde, formaldehyde is removed, then cells are stained with 10 µg/mL Hoechst 33342 (or another nuclear stain) for 30 minutes with gentle agitation.

The stain is removed and 50 uL of DPBS is added to each well; plates can be stored and imaged later at this step. Plates are imaged at 4x magnification with a DAPI filter. We use a Cytation 3 microplate imager with a 377,447 filter. Twelve images are taken to capture the entire well, then stitched together, so each plate generates 60 TIF files total. A directory containing all 60 TIF images can be directly analyzed using the CellPyAbility GDA module.

Growth Delay Assay

PBS in outer wells
to buffer evaporation



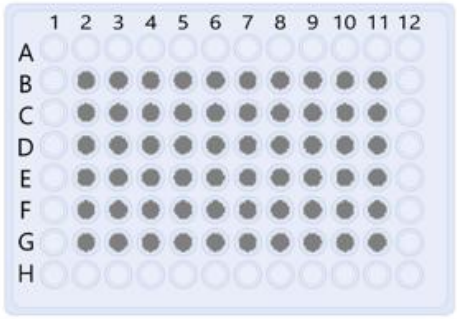
24 hours

log_x serial dilutions

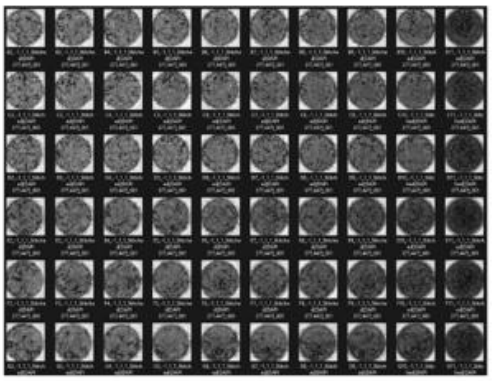
Day 0
Plate two cell lines in one plate
(technical triplicate)

Day 1
Treat cells with drug gradient
(column 2: vehicle control)

120 hours



Plates can be
stored at this step



When convenient
Image each well
with a plate reader
(60 stitched images)

Day 6
Remove media, wash with PBS,
fix with 4% formaldehyde,
stain with Hoechst (DNA dye)

Notes:

Synergy protocol:

The synergy protocol follows a nearly identical workflow to the GDA protocol with two main exceptions:

Two drugs are used in combination (one increasing across the rows like the GDA, one increasing down the columns, unlike the GDA), so the concentration must be doubled (200x) to keep the cells at 1% DMSO/solvent. Since each well is now a unique combination of drugs or vehicle, three cell plates are used for technical triplicate.

Only one cell line/cell condition can be assessed (given three plates/one biological replicate).

In three cell-culture-treated 96-well plates (cell plates), the outermost wells are filled with 150 uL of DPBS to buffer evaporation. One cell line is passaged at 70-90% confluency, counted, and plated in 100 uL of medium at 1000 cells/well (10,000 cells / mL) for rapidly dividing lines and 2000 cells/well for slow dividing lines (10 wells in technical triplicate for two cell lines). Cells will therefore be in B-G and 2-11.

24 hours later, in a separate 96-well plate (drug plate), make a 200x concentration of the top concentration of drug desired in A11, then make serial dilutions into A10 … A3 with only vehicle in A2. For example, if the top concentration of drug desired on cells is 1 μM, A11 will contain 200 μM of drug in solvent. Repeat this process with G1 as the 200x of the top concentration, making serial dilutions into F1 … C1 with only vehicle in B1.

In B-G and 2-11 of the drug plate, add 310.4 uL of media to each well. Then, transfer 4.8 uL of drug from A2-A11 into every well with cells (drugging each row), which dilutes the drug from 200x to 3x. Then, transfer 4.8 uL of drug from B1-G1 into every well with cells (drugging each column), which dilutes the drug from 200x to 3x. 320 uL allows for 50 uL x 6 wells with 20 uL excess volume, so this process can drug 6 cell plates as easily as 3 cell plates. This is useful for testing synergy in two cell lines simultaneously.

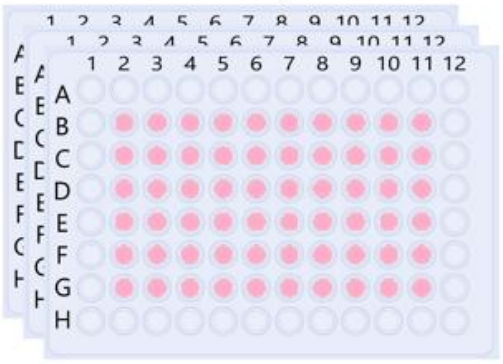
Thoroughly mix the 3x drug-media solution and transfer 50 uL to each well of each cell plate for a final concentration of 1x (1% solvent). As stated above, this can be done for six cell plates, and a biological replicate only takes three cell plates, so two cell lines/cell conditions can be tested if desired.

120 hours later, medium is removed from the cell plates, plates are washed with 50 uL of DPBS per well, cells are fixed with 4% formaldehyde, formaldehyde is removed, then cells are stained with 10 μg/mL Hoechst 33342 (or another nuclear stain) for 30 minutes with gentle agitation.

The stain is removed and 50 uL of DPBS is added to each well; plates can be stored and imaged later at this step. Plates are imaged at 4x magnification with a DAPI filter. We use a Cytation 3 microplate imager with a 377,447 filter. Twelve images are taken to capture the entire well, then stitched together, so each plate generates 60 TIF files total. A directory containing all 180 TIF files can be directly analyzed using the CellPyAbility synergy module.

Synergy Assay

PBS in outer wells
to buffer evaporation

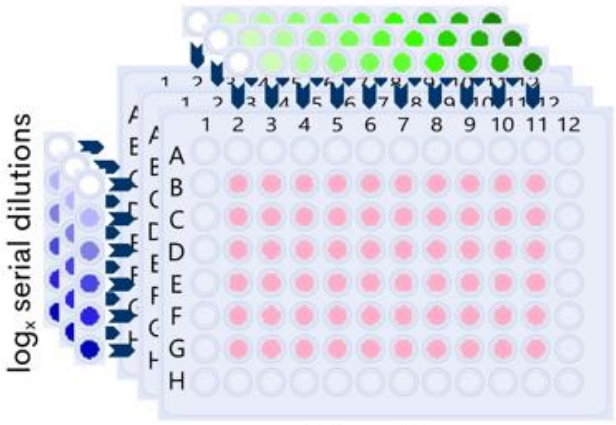


Day 0

Plate one cell line in three plates
(technical triplicate)

24 hours

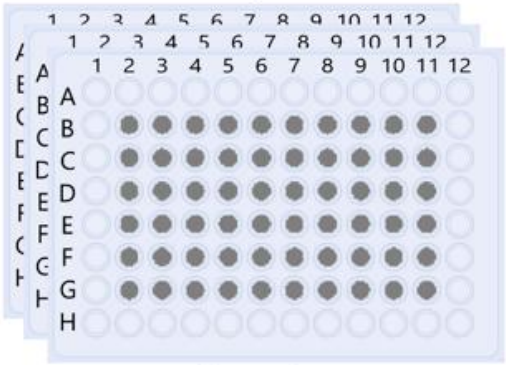
$\log_x$ serial dilutions



Day 1

Treat cells with horizontal and
vertical drug gradient

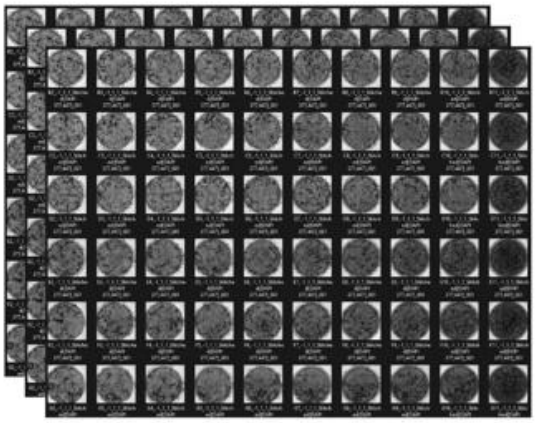
120 hours



Day 6

Remove media, wash with PBS,
fix with 4% formaldehyde,
stain with Hoechst (DNA dye)

Plates can be
stored at this step



When convenient

Image each well
with a plate reader
(180 stitched images)

Notes: