DRUG SCREEN USING MAMMALIAN CELLS

This procedure has been developed for large-scale drug screens (up to 6000 compounds) using mammalian cells. We used Hela HSE-Luc cells to detect for the induction of the heat shock response. We pool the drug compounds into a "master mix" before adding to the cells (6 drugs combined at 5μM each). Thus, 6000 drugs can be initially screened using eleven 96-well plates of cells. We then analyze for induction of luciferase expression using Promega's Bright-Glo Luciferase Assay detection system.

Materials:

expression cells (containing reporter construct)
hemacytometer
8-well multi-channel pipettor
sterile multi-channel reservoir trays
96-well tissue culture plate (Corning #3596 flat-bottom cell culture)
Round bottom 96-well plate for drug "master plate" (Falcon #3076 U-bottom)
96-well plate for luminometer (Corning #3912 white polystyrene flat bottom)
Luminometer (we have a Molecular Devices L*max* luminometer)

Reagents:

cell culture media, 1xPBS, 1xTrypsin, G418 (200μg/μl)
DMSO (Sigma D-5879)
Bright-Glo Luciferase Assay reagent (Promega E2620)
Drugs (we obtained the drugs as 100μg lyophilized on 96-well plates)
Celesterol (100μM) for positive control
$CdCl_2$ (1mM) for positive control

To split cells to 96-well plate:

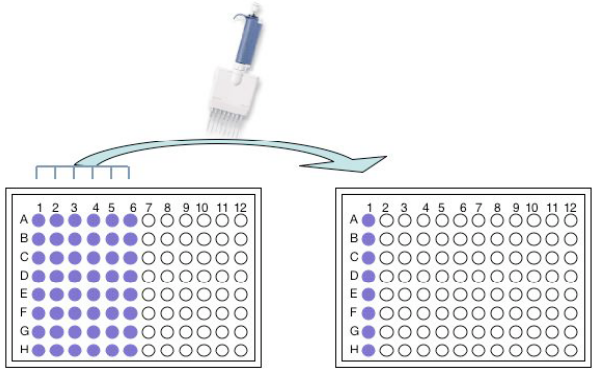
1. Grow cells in tissue culture flask or plate (Hela HSE-Luc cells grow in DMEM/10%FBS with G418 geneticin at 200μg/ml). Allow to grow until ~80% confluent.
2. Split cells to tissue culture 96-well plate as indicated below when ~80% confluent (and ~24 hours before drug treatment):
    1) remove media
    2) rinse plate with 1xPBS
    3) add 1xTrypsin to cover the plate
    4) incubate at 37°C ~5min
    5) add 8ml media to plate; collect cells and transfer to a 15ml conical tube
    6) centrifuge to pellet cells 2000rpm for 2min
    7) resuspend pellet in volumes suggested below and mix
    8) remove 10μl to count cells using the hemacytometer.

Hela Hsp70-Luc cells if ~80% confluent		
plate size	resuspension volume	approx. number of cells
100mm		
150mm	1.5-2.0 ml	4,500,000 cells

9) For one 96-well plate you will need 700,000 cells in 10ml (to have a little extra).
   for example, if 200,000 cells/ml:
   200,000 cells/ml / 700,000 cells = 0.286 ml cell suspension + 9.71ml media = 700,000 cells/10ml
10) In a tissue culture hood, combine cells and media in a sterile multi-channel pipet reservoir tray and mix well.
11) plate 100µl per well to a 96-well plate (7000cells). *Note: you will need extra wells of cells for controls. We did controls in duplicate on the same plate.*
12) cells should be ~80% confluent after 18-24 hours.

Drug Treatment

1. Resuspend lyophilized (100µg) drugs in 100µl DMSO (to bring the concentration to 1µM/µl). Dissolve the drugs by pipetting up-and-down several times. Once resuspended the plates may be stored at –20°C (the DMSO will take ~1 hour to thaw at room temp once frozen).
2. Prepare a master mix plate using a sterile round-bottom 96-well plate. Take 5µl of each drug in column 1 of the original plate and transfer to column 1 in the master (round-bottom) plate. Next, take 5µl of each drug in column 2 of the original plate and transfer to column 1 of the master plate (same column as before). Repeat until 5µl from columns 1-6 of the original plate have been combined into column 1 in the master plate. Each master plate well will contain 30µl total.



Original plate Master plate (30µl per well)

3. Transfer the entire 30µl drug mix from each well on master plate to prepared 96-well plate of cells (containing 7000 cells in 100µl per well (for 5µM final concentration of each drug). Controls we used:

negative	positive	stock	dilute to:	
no treatment	5µM Celesterol	10mM	100µM	1µl in 99µl DME/10%FBS
DMSO (30µl)	35µM $CdCl_2$	10mM	1mM	10 µl in 90µl DME/10%FBS

4. Incubate cells with drugs for 18hr.

Luciferase Assay

1. Prepare Bright-Glo according to instructions. Aliquot into 15ml conical tubes (make ~10.5ml aliquots) and wrap in foil. Store aliquots at –70°C.
2. Remove cells from incubator and allow to sit at room-temp for 5min (to allow to cool to ~room temp as Bright-Glo reagent is temperature sensitive).
3. Pour Bright-Glo reagent into multi-channel reservoir tray and add 100µl Bright-Glo reagent directly to each well containing 100 µl of cells (Bright-Glo reagent is used at a volume of 1:1). *Note: be aware that the Bright-Glo luciferase reagent is time-sensitive so try to work as fast as possible and avoid having the reagent at room temp longer than necessary.*
4. Incubate 5min at room temp to allow the cells to lyse (cover plate with foil as luciferase assay is light sensitive.
5. Transfer 200µl of each well to white luminometer plate.
6. Read plate immediately in the Lmax luminometer.
    1) Turn on L*max* luminometer (switch is in the back of the instrument).
    2) Insert the 96-well plate into the plate-slot.
    3) Open the "Bright-Glo" plate reading program on the desktop.
    4) Once the program is opened, click "READ". *Note: it takes about 15min for the luminometer to read a 96-well plate. Therefore, if you have multiple plates you should stagger the luciferase procedure so the reaction to be read by the plate reader immediately after the 5min incubation.*

Comments:
It is useful to keep in mind that pooling the compounds may hide the detection of a potentially positive response. If one drug out of the six in the combined pool is toxic enough to kill the cells (or prohibit a heat shock response) a very weak induction—if at all—will be detected.

We screen the drugs at 5µM, however, some drugs have been found to have wide-ranging optimization conditions.

References:

Matsumoto, Shuji et.al. Toxicology

Johnson, P.H. et. al, *Multiplex gene expression analysis for high-throughput drug discovery: screening and analysis of compounds affecting genes overexpressed in cancer cells*. Mol Cancer Ther. 2002 Dec;1(14):1293-304.