Cell Screen-SA (CeSSA)

CeSSA is a fee-for-service laboratory that provides services in high throughput screening of compounds or RNAi on cell-culture or enzymatic based models of disease. We can also use our robotic equipment to automate other methods used in laboratory research (e.g. ELISA). Our facility operates to standards that are compatible with the National Drug Discovery Centre (NDDC), see below for further information.

Workflow through CeSSA

Note this is an idealised workflow and can vary depending on the project. There may be back and forth between steps.

1. Initial project discussion:

CeSSA and collaborator discuss the project objectives, models to use, assays and expected outcomes. This should include a basic assessment of controls, see section below, ***Screening Assay***.

2. Defining screening general settings:

a. Size of the screen (replicates)
b. Plate format
c. Library selection
d. Treatment time and concentration
e. Readouts

3. Defining data analysis:

If CeSSA is to analyse the screen data, then analysis requirements, general measures/outcomes and reporting expectations should be finalised.

4. Protocol adjustment for HTS:

a. CeSSA reviews the proposed protocol, screening concentrations, replicates and data analysis requirements and makes changes where necessary to make it suitable for screening/our equipment. ***Additional assay development could be required in case the assay to be use is not adjustable or HTS.***
b. All changes to the protocol must be tested to confirm consistency of results: This should be preferable to be done by Collaborators in their laboratory. CeSSA can take over of this part given the Collaborator supply reagents, cells, materials (if need it). Also, a comprehensive summary of the data from previous experiments should be provided as well as any additional data as required. These tests should include re-assessment of the positive and negative control and calculation of Z-prime and signal -to-background. See ***Assay Development*** and ***Screening Preparations*** below.
c. Protocol is finalised.

5. Protocol setting at CeSSA:

When assay development requirements are met, the development required at CeSSA is scheduled. This includes:

a. Writing and testing instrument protocols including liquid handling and plate-scheduling. All liquid handling is tested for %CV and %SE.
b. Individual testing of assay components if necessary (e.g. testing cell wash steps on our instruments).
c. Complete testing of the screening protocol on small scale (1-2 plates), using controls. Screening statistics determined and confirmed as acceptable (e.g. Z-prime, signal-to-background, %CV of controls).
d. Other tests as outlined in **CeSSA QC Checklist**.

6. Collaborator approves **Final Screening Protocol**. This also include approving and providing all reagent/cell/material as required.

7. Final protocol testing (Trial):

A reasonable small number of **trial plates** are to be run through the protocol including automation to check system/protocol readiness for Screening.

8. Screen is scheduled:

On successful testing of complete screening protocol, the final screen is scheduled at CeSSA.

Screening Assay

Screening essentially involves repeating an assay several times, ideally only testing one variable (the test compound/siRNA). Assay reproducibility is therefore crucial to achieving quality screening data. You should be very familiar with your assay protocol and have positive and negative controls. The assay should be in 96-well or 384-well format.

In the first instance, assay reproducibility can be demonstrated by calculation of:

- %CV for each control
- Z-prime.
- Signal:Background (e.g. positive:negative control)
- At the very minimum there should be values from three independent, identically prepared experiments, with triplicates within each experiment.
- These tests should use the same brand/type of cells, plates and reagents as you will use for the screen.

Typically, Z-prime should be >0.5 and S:B>3, as a minimum.

Assay Development

Usually, assay protocols need to be adapted to fit our equipment. We will advise the specific changes needed but they can include such adaptations as:

- Increasing or decreasing incubation times so we do not reach a "plate bottleneck" in which two plates need to be at the same piece of equipment at the same time.
- Decreasing washing steps. Generally, automation is used for washing and this type of washing is more harsh than manual washing. We reduce the number of wash steps to reduce the possibility that cells will be removed from the plate during automated washing. If automated washing is required for your screen protocol we will typically test this with your plates and cells early in the process.
- Combining reagent additions e.g. staining with two dyes at once. This can reduce wash steps (see above) and also total screening time.
- Increasing/reducing well volumes, to prevent plate artefacts (e.g. evaporation of outer wells during prolonged incubation periods) or reduce reagent quantities.

More information about best practises for assay development can be found in the NDDC requirements and in the NIH assay guidance manual.

Development or data that is required for most assays includes:

- Conversion of assay to 384-well. We can do this conversion for an additional fee. Or can supply plates for you to adapt your assay in your own laboratory. A good place to start is to reduce cell numbers by ~1/3 and volumes by ~1/4.
- Testing reagent stability over the time it will take to use the reagent during screening. For example, if plate 1 is dispensed at time = 0 and plate 11 is dispensed at time =40 minutes, is the reagent being dispensed still stable after 40 minutes and under what conditions (room temperature, ice?). This applies to both commercial reagents and "in house" reagents such as purified proteins.
- Protocol for thawing of cells, if needed. E.g. if we need 2 confluent flasks on screen day, when should we thaw and split the cells to reach this cell quantity?

Screening Preparations and Reagents

- Cell and reagent stocks should be the same throughout the duration of CeSSA's work-up (see **CeSSA QC Checklist**) and all screen replicates.
- We will advise on specific amounts required. We always request more reagent than needed, in case of (human or instrument) error or other unforeseen circumstances.

General Considerations for Screening

- When screening 1000s of compounds/RNAi the method to be used should be as simple as possible. Generally, more complicated and lengthy methods (e.g. live-cell imaging, imaging 3D cell culture) are best left for use on "hit" compounds/RNAi identified in the primary screen.

- Consider the variables you will measure, how they contribute to your hypothesis or question and how you will analyse the data from these variables. Positive and negative controls are needed for all variables you are measuring. Ideally the controls will produce z-prime value > 0.5 and signal-to-background >3, though controls/assays with lower values can sometimes be used. The calculation for Z-prime is available in an online tool.

- For RNAi libraries we recommend Dharmacon. For compound libraries we recommend Compounds Australia. Compounds Australia can also store and dispense libraries which are currently held within your laboratory (CeSSA does not provide this service). We have a small, in-house, libraries that could be suitable for some projects.

- We typically recommend Perkin Elmer plates for imaging work. E.g. Viewplate, Cell carrier.

- Promega has some exceptional assays and reporters for high-throughput screening. Although costs may seem high the suitability of these reagents for screening generally makes them more cost-effective in the long-run.