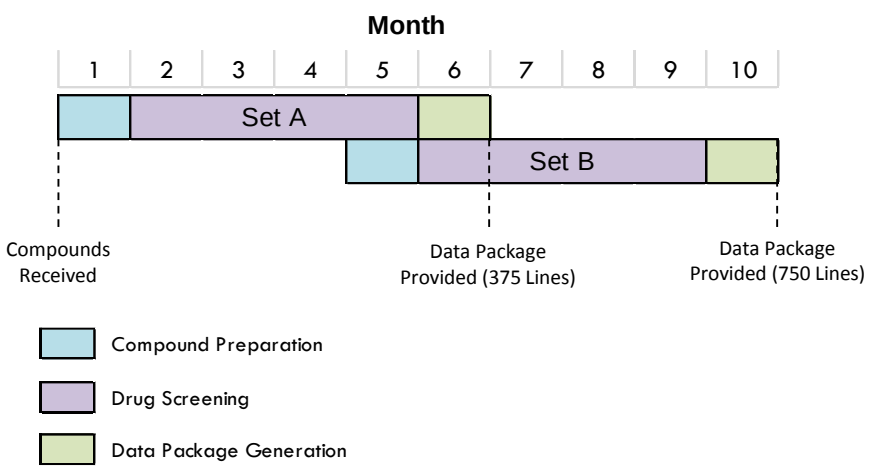
Screening Methods & Quality Controls
The GDSC Cell Line Screen

This document outlines the cell lines, screening methods and quality control procedures of the GDSC Cell Line Screens.

Rapid Screen Schedule



The GDSC cell line collection is divided into two (A & B) representative sets of 375 lines (750 total). Screening alternates between cell line sets, with each taking 4 months to complete (see Gantt chart).

A subset of cell lines (7) are included in both cell line sets and are screened in technical triplicate on six occasions, typically the start middle and end of each cell line set. This generates 18 replicates for every compound across each of the seven lines provided all plates meet quality controls and enables reproducibility to be investigated.

Cell Lines

Over 90% of the cell lines are commercially available from repositories and cell banks. They have been well characterised and cover a variety of tissues and cancer types. For all models within the screen detailed information can be found at: *cellmodelpassports.sanger.ac.uk*

To facilitate high throughput screening all cell lines are maintained and screened in one of two media types; DMEM/F12 or RPMI.

To prevent cross-contamination or mis-identification all cryovials banked for use in the screen are analysed using a panel of 95 SNPs (Fluidigm, 96.96 Dynamic Array IFC). The data obtained is compared against a set of reference SNP profiles which has been authenticated by short tandem repeat (STR) and referenced back to the supplying repositories.

Culture Media

RPMI:
RPMI (*Gibco: 52400-025*)
10% FBS
1% PenStrep
4.5mg/ml Glucose
1mM Sodium Pyruvate

DMEM/F12:
DMEM/F12 (*Gibco: 31330-038*)
10% FBS
1% PenStrep

Screening Assay

Cells are transferred into 1536-well plates in 7.5 µl of their respective growth medium using XRD384 (FluidX) dispensers. The seeding density of each cell line has been optimised to ensure they remain in exponential growth phase throughout the duration of the assay.

Following cell dispensing assay plates are incubated at 37°C in a humidified atmosphere at 5% CO_2 for 24 hours. The cells are then dosed with the test compounds using an Echo555 (Labcyte), final DMSO concentrations are typically 0.1% for single agents or 0.2% for combinations. The duration of drug treatment is 72 hours.

Cell viability is measured at the end of the assay by adding 2.5 µl of CellTiter-Glo 2.0 (Promega) to each well, plates are then incubated at room temperature for 10 minutes. Quantification of luminescence is performed using a Paradigm (Molecular Devices) plate reader.

Day1 Plates
In parallel with dosing, an additional plate for each cell line is read using the same reagents as above (CellTiter-Glo 2.0). These plates (Day1 Plates) have undergone no treatment and can be used to determine growth estimates over the duration of the assay.

Key Features:

- **1536 Well**
- **Optimised Cell Seeding**
- **72h Drug Treatment**
- **ATP Readout (CellTiter-Glo 2.0)**

GDSC Assay Timeline

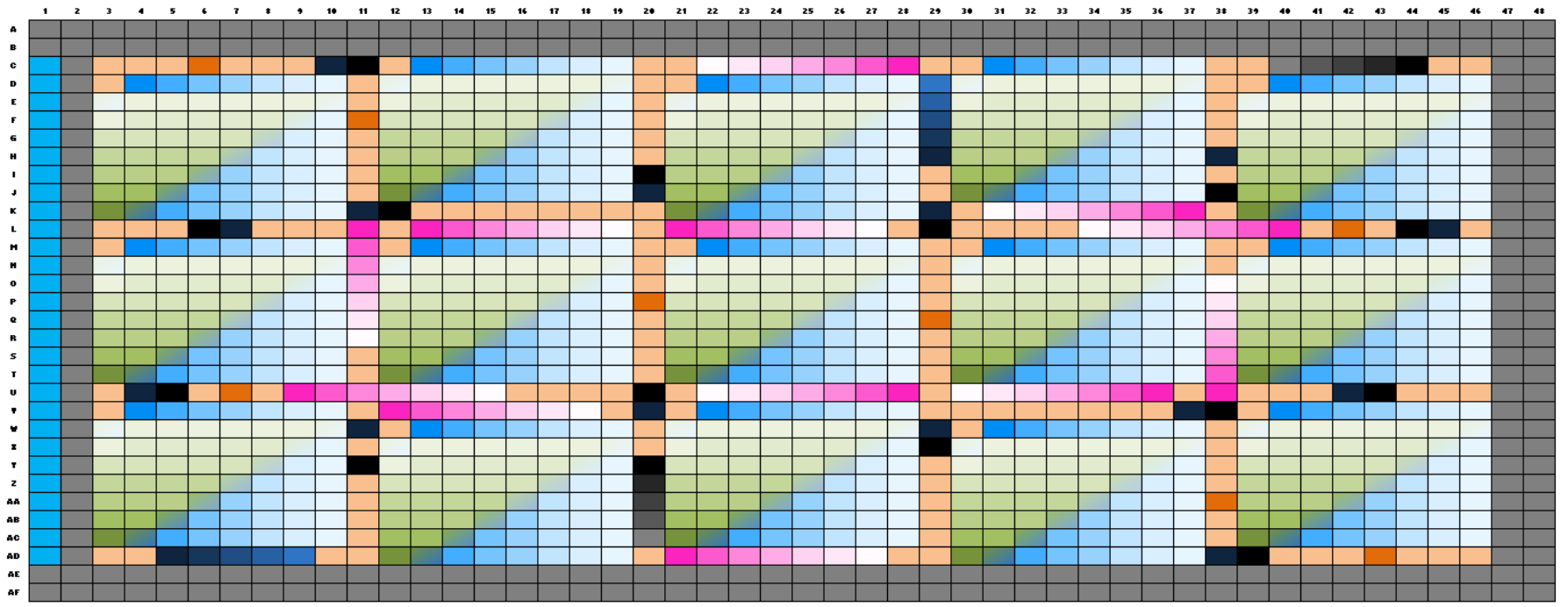
Compound Storage

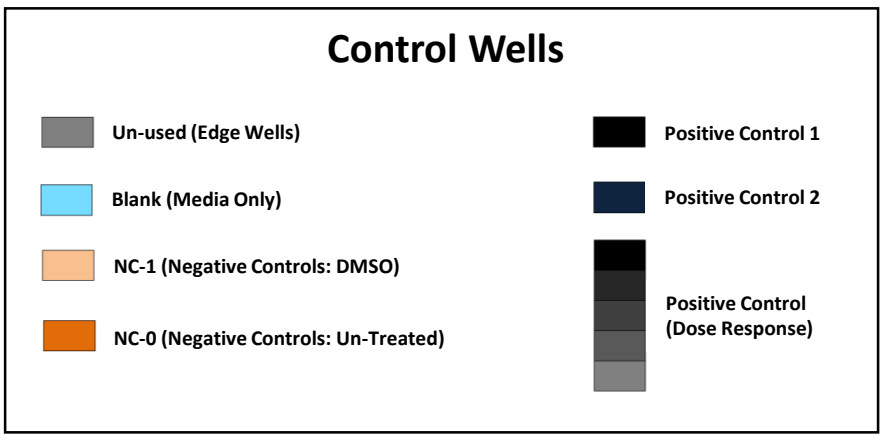
DMSO solubilised compounds are stored at room temperature in a low humidity (<12%), low oxygen (<2.5%) environment using Storage Pods (Roylan Developments). Water solubilised compounds are maintained at 4°C.

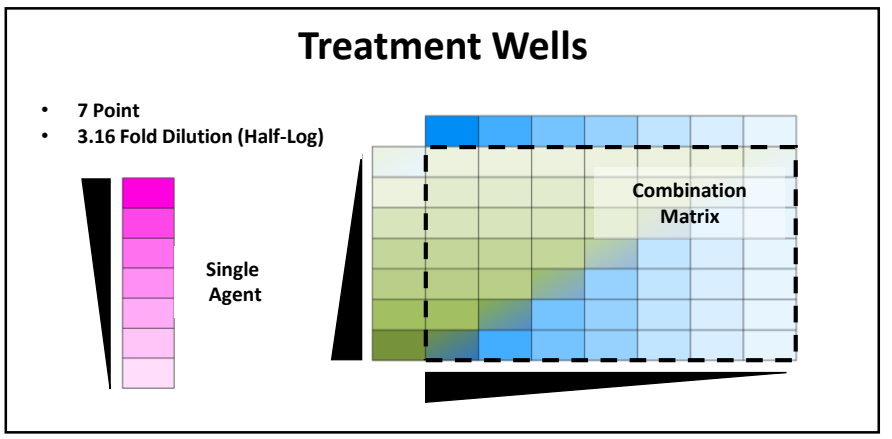
Compounds are arrayed into 1536-well source plates (1536-Well Low Dead Volume Microplates, Labcyte) for use in the screen. These plates are audited prior to any screening run in order to monitor DMSO hydration levels, plates are required to be >85% DMSO for dosing. In addition, source plates are routinely monitored for compound precipitation throughout each screening cycle.

Plate Design

Each plate used within the screen is designed to be self contained, including all of the controls and components of a combination required for the analysis. Below is an example of a plate layout used in the current screen.







Control wells are distributed across the plate to capture any variation in cell growth/number during the assay. Two positive control compounds are used; Staurosporine & MG-132. The edge wells are not used due to the effects of evaporation observed within these wells.
Each plate design once populated with test compounds is referred to as a “Drugset” and is assigned a unique identifier.

Assay Plate Quality Control

Defined quality control criteria are applied to each plate and rapid screen set.
An assay plate is required to have a negative control coefficient of variation (CV) below 0.18 which is calculated using the DMSO treated wells (NC-1).

$$CV = \sigma_N / \mu_N$$

With σ_N the standard deviation of the negative controls and μ_N the mean of the negative controls.

Plate QC – Well Types:

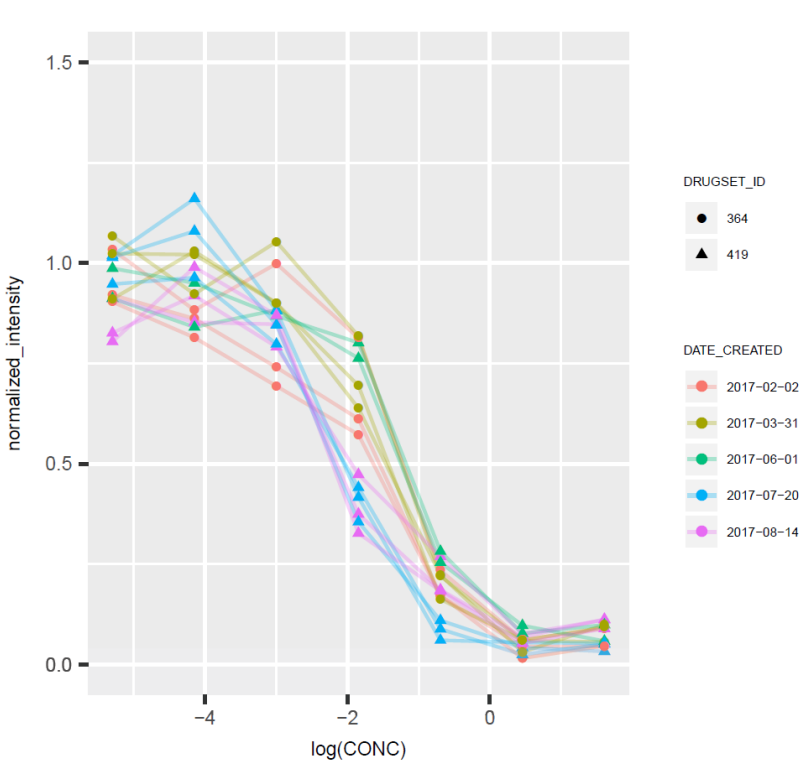
	Description	n	Tag
Negative Controls	Untreated	8	NC-0
	DMSO Treated	147	NC-1
Positive Controls	MG-132 Treated	16	PC1-D1
	Staurosporine Treated	16	PC2-D1
	Media Only	28	B

The effect of DMSO on cell viability is also assessed using the untreated and DMSO treated negative control wells. The DMSO concentration in the negative control wells is equivalent to that of the combination treatment wells (0.2%). Plates are required to have an NC-0/NC-1 ratio of between 0.8-1.2 calculated using the mean of each negative control.
Z-factors are calculated using the negative control (NC-1) and each positive control (PC1, PC2 & B). Where cell lines are sensitive to a positive control (NC-1/PC ratio ≥ 4) the Z-Factor is required to be above 0.3 (a small proportion of lines ~5% have a lower threshold of 0.2).

Z-factor = 1 - 3*(σ_P+ σ_N) / (μ_N-μ_P)
With σ_N and σ_P the standard deviation of the negative and positive controls, and μ_N and μ_P the mean of the negative and positive controls, respectively.

Across all plates in a screen the mean and median Z-Factors will be >0.4.

Compound Quality Control



Expected profile – consistent response.

We compare the response of each drug across all the technical and biological replicates for the seven replicate cell lines to identify any systematic error or inconsistency.
Drugs are flagged as problematic when they demonstrate the following:

- Significant inconsistency across two or more dose points.
- The behaviour is observed in two or more of the replicate lines.

Compounds meeting these criteria are failed and removed from the data package. We communicate details of any failed compounds as soon as possible.

Replicate Cell Lines

- **A375 (Skin)**
- **HT-29 (Large Intestine)**
- **PC-14 (Lung)**
- **U-2-OS (Bone)**
- **SW620 (Large Intestine)**
- **C32 (Skin)**
- **MHH-ES-1 (Bone)**