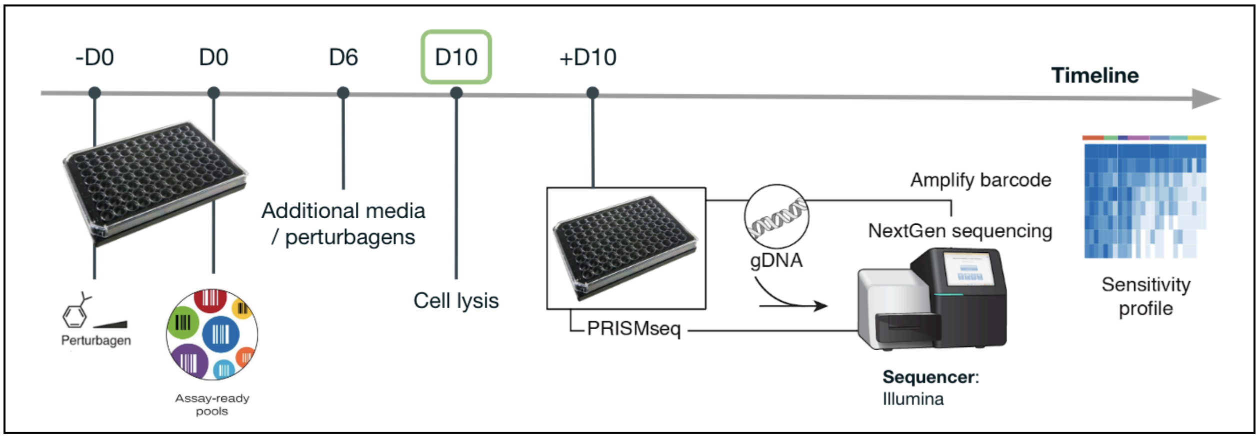
PRISM Extended Assay Workflow

The PRISM 10-day viability assay is an alternative to the standard 5-day PRISM MTS screens, and utilizes the ‘PR500’ adherent cell set for longer drug incubation. Test agents are added to assay-ready plates (ARPs) at 5 doses with serial dilutions (dilution factor selected by collaborator at time of submission) and screened in triplicate against pooled cell lines for 10 days. Between 22-50 cell lines are seeded per well. At day 6 pools are re-dosed, and at day 10 cells are lysed and genomic DNA is isolated. Barcode sequences are amplified by PCR and quantified using NextGen Sequencing (*Illumina*). Relative barcode abundance (compared to DMSO control) is then used to generate cell line sensitivity profiles for each test agent and concentration tested.



Overview of the PR500 10-day Extended PRISM assay Screens (EPS):

EPS	
Assay Endpoint	10 days
Readout	NGS
Cell set	PR500
Dosing Format	5 pt, custom dilution series, in triplicate
Culture Conditions	Pools of ~22-50 cell lines each 96-well plates 467- 1,833 cells/well
Validation Compound	KSQ-4279
Volume Required	● 3-fold dilution (33%): 600 uL of 1000X top dose ● 2-fold dilution (50%): 720 uL of 1000X top dose

EPS Data Analysis and Deliverables:

Data reports for test agents in the extended assay will look similar to standard MTS data packages, including:

- Report files
    - Summary of analyzed data for each test agent
        - Log-fold change
        - Viability of each cell line at each drug dose (displayed as a heatmap)
        - Individual dose data

- Dose-response curves
    - Computed for each cell line that displays sensitivity to drug treatment
    - Used to calculate parameters like AUC

- Raw data
    - Normalized read-count values
    - Viability data (and replicate-collapsed viability data)
    - Detailed cell line quality control data

- Feature set correlations
    - Interactive plots and tables for univariate (continuous and discrete associations) and multivariate (Random Forest model) analyses using lineage, mutation, gene expression, CRISPR knockout, Repurposing and other DepMap data

- Matrix versions
    - Data correctly formatted for upload into the DepMap data explorer tool