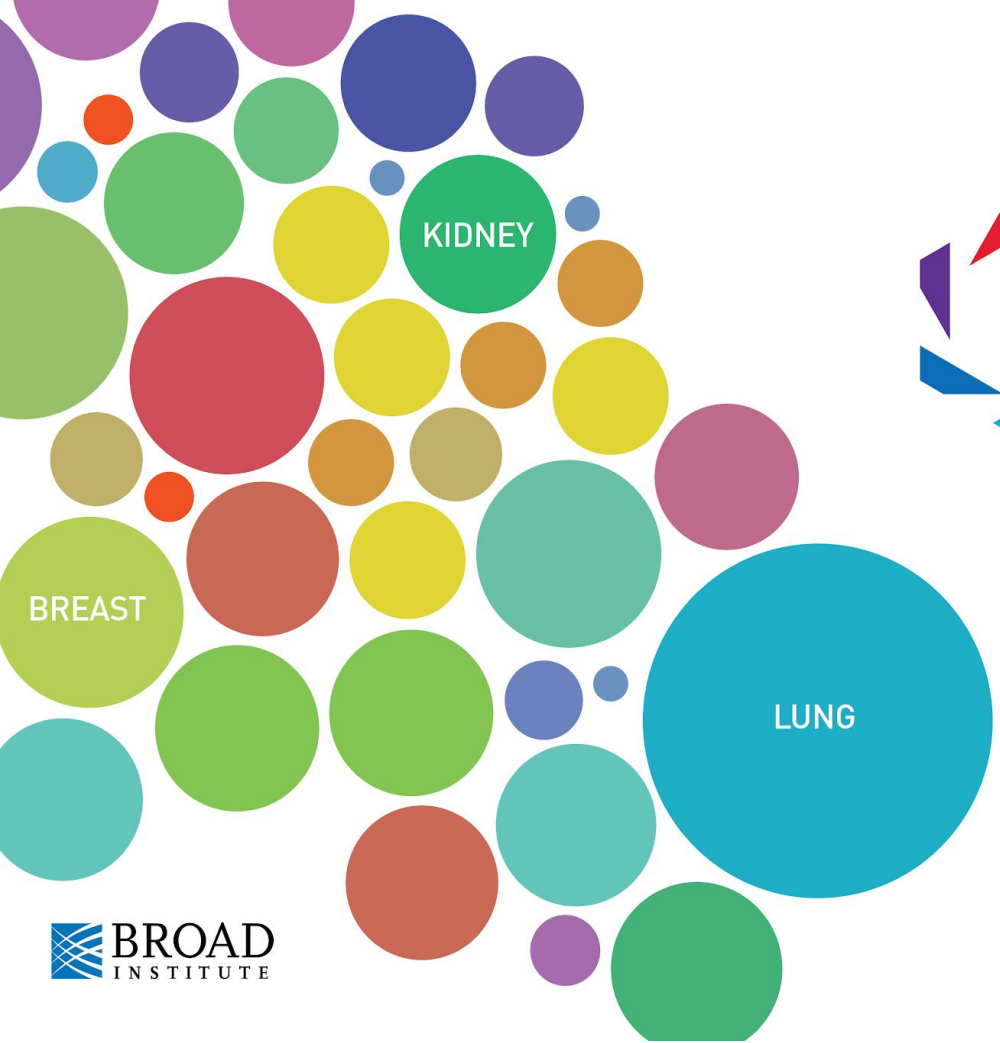


PRISM

MULTIPLEXED CELL LINE PROFILING

BROAD INSTITUTE

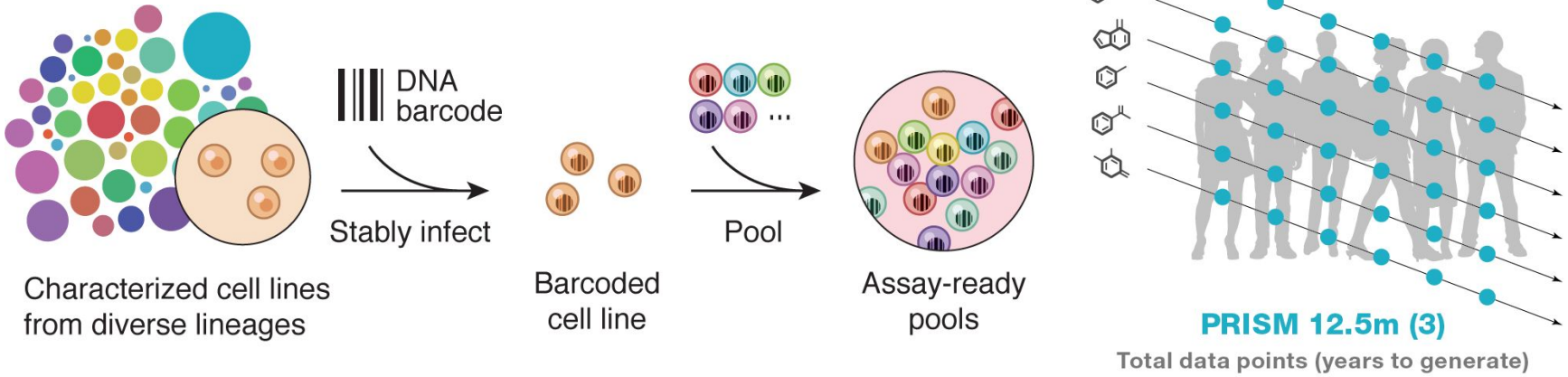
Data processing in MTS screens

PRISM overview

- A viability screen of 489 solid (or 489+278 solid, mixed, and suspension) cell lines in pools
- A primary screen to estimate potency and selectivity
- Does not replace individual cell line dose-response characterization
- Constantly improving (assay, pools, cell lines, etc.)
- Many options for downstream analysis
- Rich genomic and functional characterization of cell lines enables powerful biomarker analyses

PRISM pooled viability screening

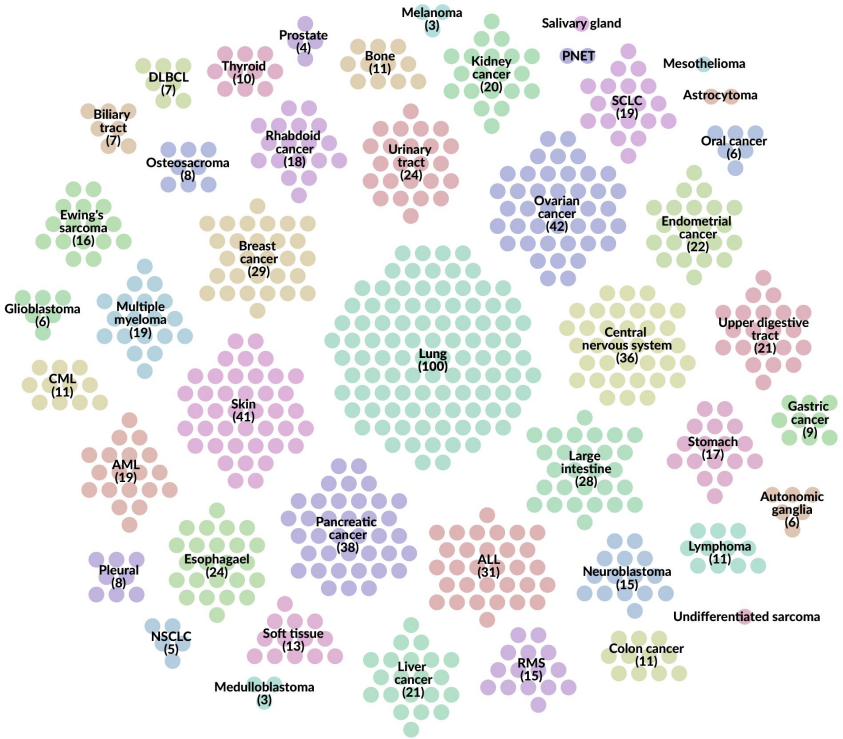
Requires fewer resources and allows for increase in scale



- Cell lines are adapted to RPMI-1640, infected and selected with blasticidin
- Stocks of individual cell lines are made and QC'ed
- Cell lines are pooled based on estimated doubling time in pools of 25 cell lines

PRISM utilizes ~750 cancer cell lines

Solid and Suspension/Hematopoietic cell lines represent the diversity of CCLE

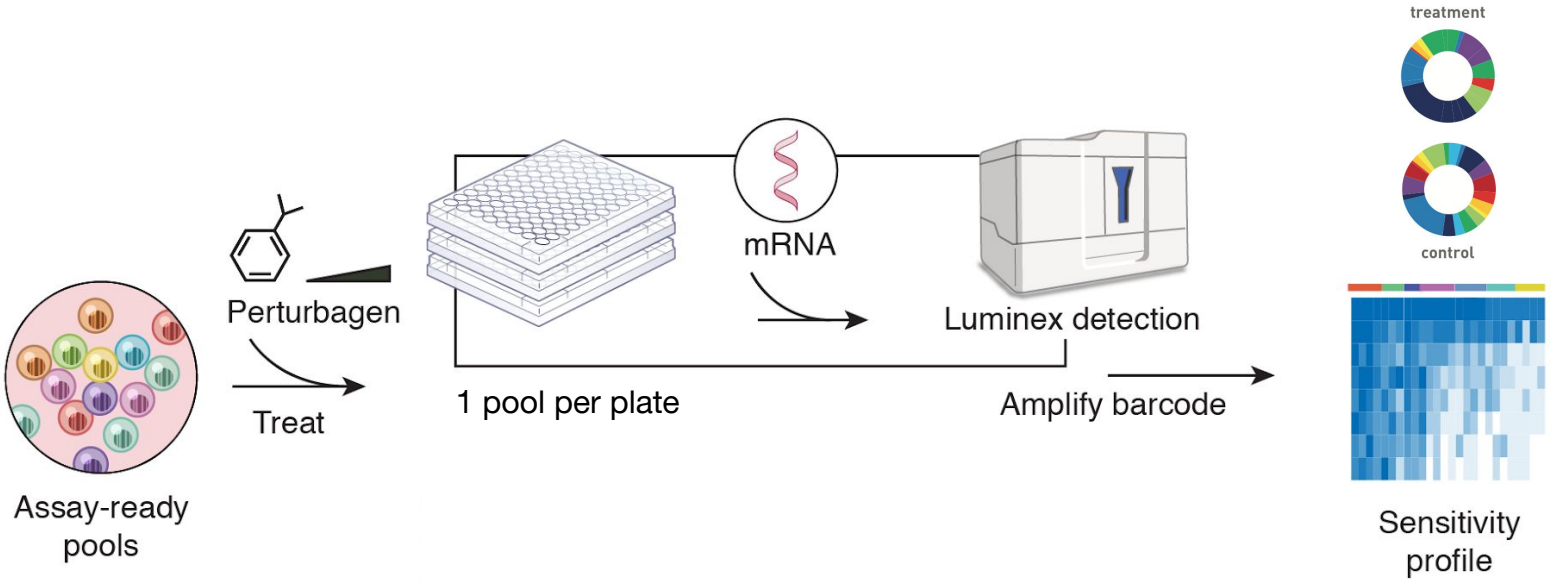


Differences between two cell sets

	PR500	PR300
Kinds of cell lines	Solid/ Adherent	115 hematopoietic, 135 pediatric and 50 solid/ loosely adherent
# of cell lines in cell set	489	278
# of cell lines per well	22-25	20-25
# of cells per cell line	~50	~80
# of cells per well	1250	2000
FBS	10%	20%
Cell thaw procedure	Thawed and treated on same day	Thawed and recovered, then plated 3 days after thaw

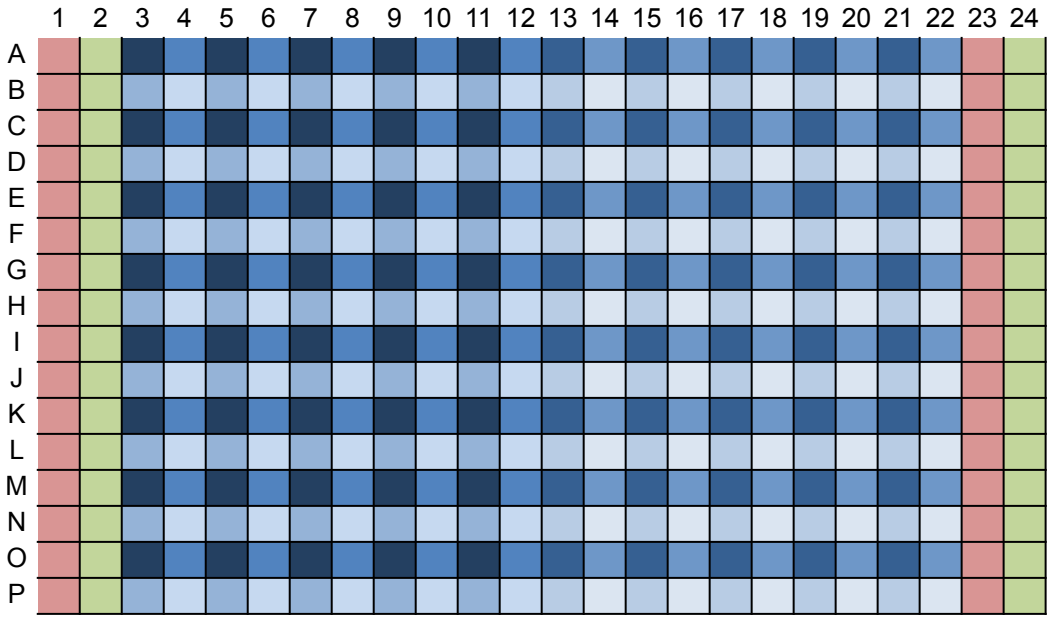
PRISM viability 5-day assay

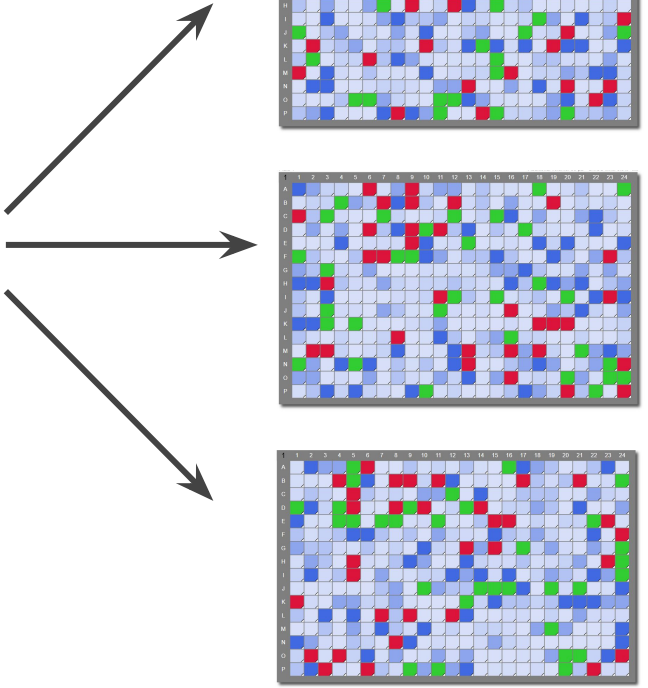
Pooled cytotoxicity assay on up to ~750 barcoded cell lines



Cells are plated into assay-ready plates

Cells are seeded onto plates containing (randomized) compounds

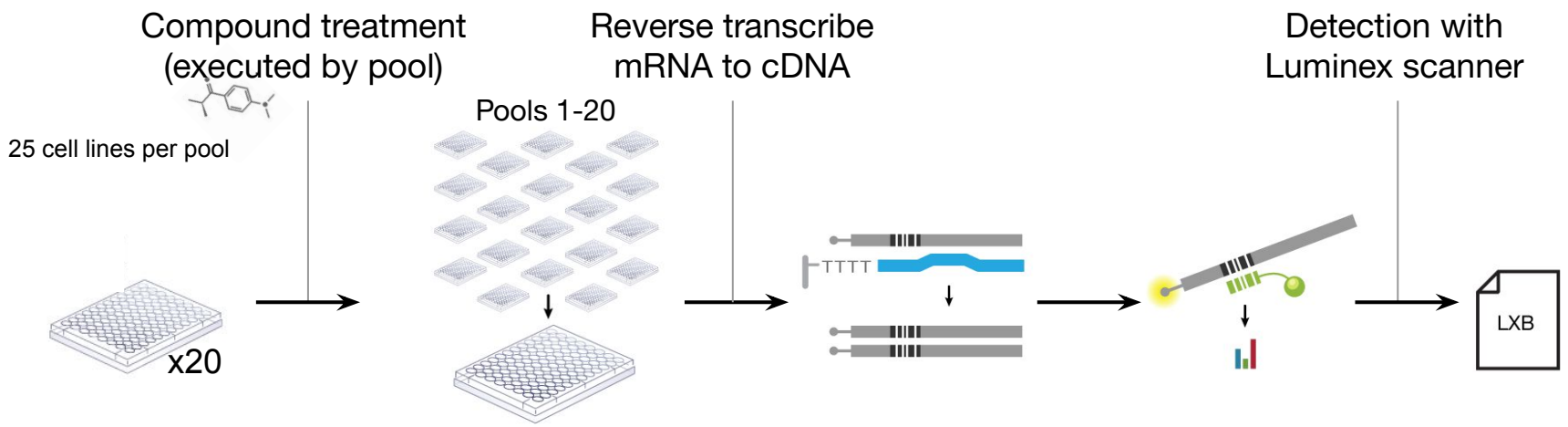




Empty/Positive Control
DMSO
Compound

mRNA detection: Luminex assay

Lysate generation, amplification and detection



Data pre-processing

- Raw files from the luminex scanner: CSV and one LXB per well.
  - Each LXB contains fluorescence values (FI) for each detected analyte. FI values indicate how much amplicon was bound to a given analyte (bead), which in turn corresponds to the unique 24nt barcode that the cell line was labelled with.
  - The CSV files contain summary statistics such as the bead counts and Median Fluorescence Intensity (MFI) for each analyte in each well.

Data pre-processing

- Initial quality control filters:
  - *Bead count*. The number of beads detected is used to filter out poor-signal wells. Wells with a bead count median < 20 are removed.
  - *Control signal intensity*. Spiked-in pooled lysate controls are used to assess technical quality of the assay process on a per-well basis from lysate pooling through detection. Wells with median signal < 600 units are removed.
- If the total number of wells that fail exceeds 10% (i.e >39 wells) the entire plate is re-processed

Data processing

- Our immediate goal is to make all the data processing and downstream analysis reproducible and transparent
  - https://github.com/broadinstitute/prism_data_processing
- *Caveat*: the pipeline is evolving each run (e.g., based on feedback we receive from collaborators)
  - Any form of feedback is very welcome!

Data Processing - Overview

Luminex machine reports **median fluorescence intensity** (MFI) for each **analyte** (barcoded **bead**)

log2

Compound x dose x replicate

Cell lines / analytes / beads

log2(MFI)

Normalize with respect to inert barcodes in same well on the same plate

Compound x dose x replicate

Cell lines / analytes / beads

Barcode normalized log2(MFI)

Filter cell lines that fail QC

Compound x dose x replicate

Cell lines / analytes / beads

Barcode-normalized log2(MFI)

Normalize with respect to median of DMSO wells on the same detection plate

Compound x dose x replicate

Cell lines / analytes / beads

log2(viability)

Correct pool-based batch effects. ComBat is applied to each compound-dose combination independently

Compound x dose x replicate

Cell lines / analytes / beads

ComBat-corrected log2(viability)

Take median of replicates

Cell lines / analytes / beads

Compound x dose

Median log2(viability)

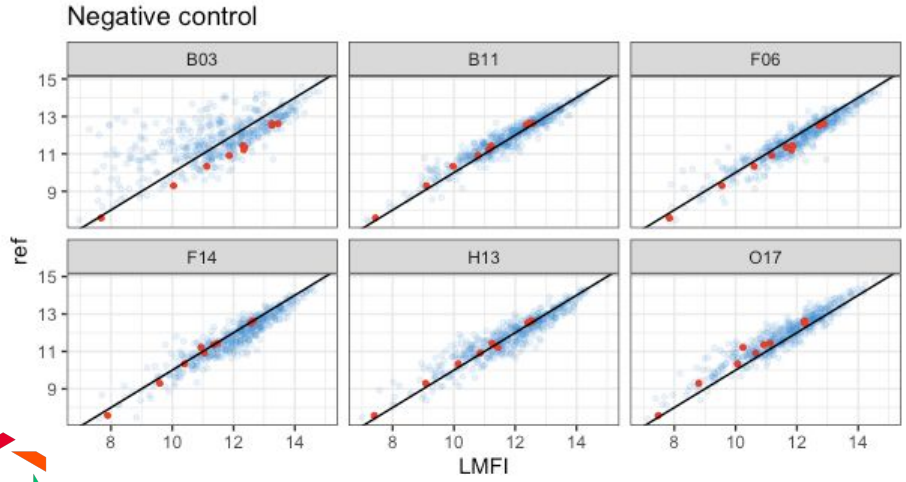
Fit dose response curves

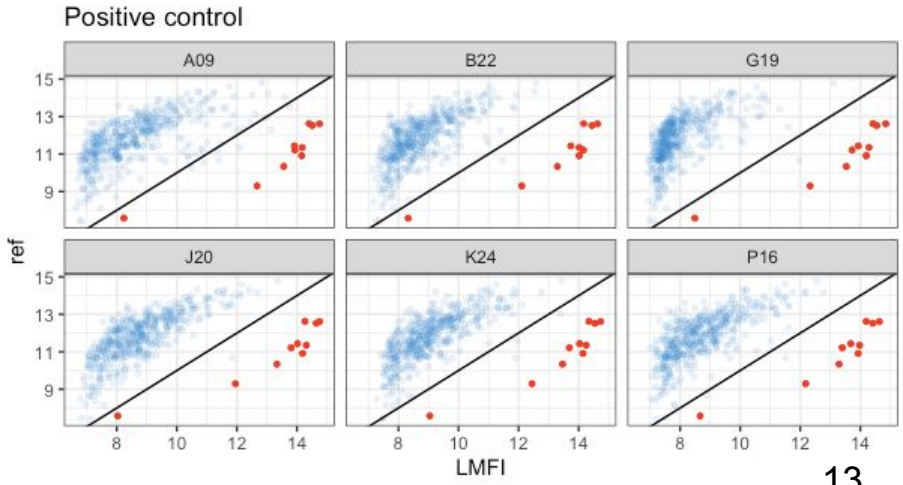
Cell lines x Compound

DRC parameters (AUC, IC50)

Invariant normalization

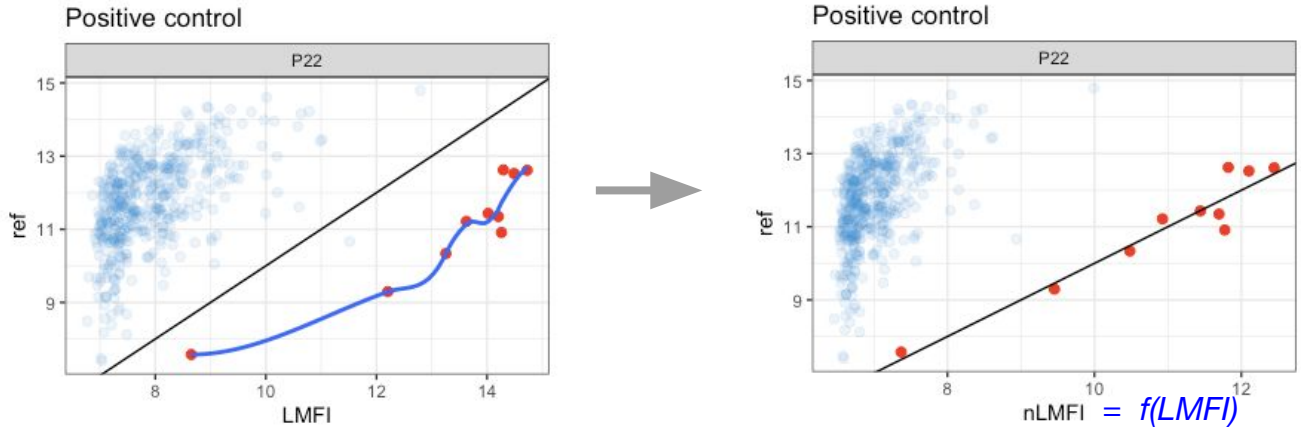
- 10 control barcodes are spiked in after lysate generation.
- Goal: correcting for amplification and detection artifacts.
- In the raw data files, control barcodes are denoted in the pool_id column (= “CTLBC”).





Invariant normalization

- For each plate, a reference profile is generated (median DMSO log2(MFI)).
- For each profile, we fit a monotonic-increasing smooth transformation (p-spline) using only the spike-ins.
- All cell-line log2(MFI) values pass through this transformation.



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Compound x dose

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Median log2(viability)

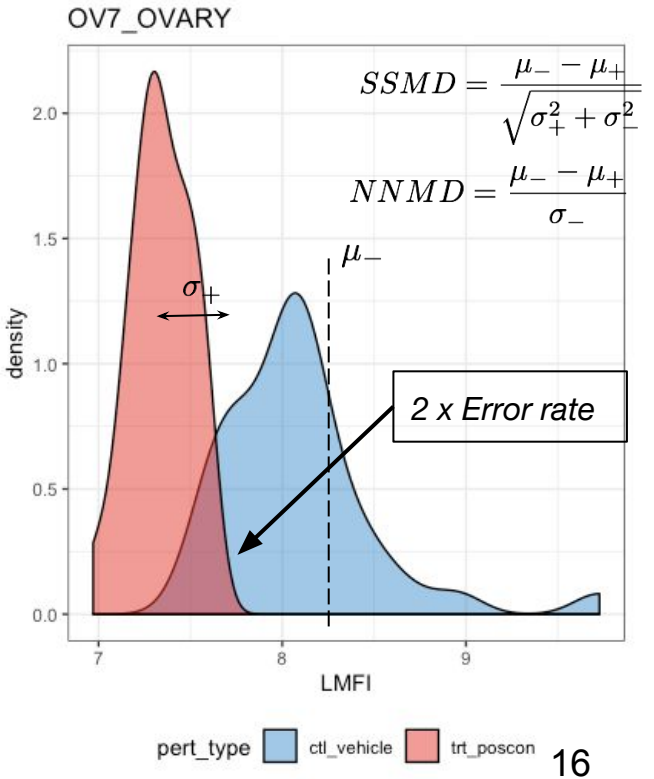
Fit dose response curves

Cell lines x Compound

DRC parameters (AUC, IC50)

QC: Control Separation

- Post-detection, we assess separation between positive and negative controls.
  - Historically, we used SSMD > 2
  - Recent screens have used error rate < 0.05
- Filters applied per detection plate × cell line.
- Compound × cell line plates filtered if not at least 2/3 replicates with good separation.



QC: Control Separation Notes

- NNMD is fairly similar to SSMD except it does not punish high deviation in positive controls.
- Error rate is more interpretable and robust to outliers but oblivious to the dispersion in the negative controls.

Data Processing - Overview

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DRC parameters (AUC, IC50)

Computing Viability: Log Fold-Change

- Log2(fold-change) viabilities computed per plate × cell line combination: $LFC = LMFI - median\,(LMFI_{DMSO})$
  - Does not allow differentiation between cytotoxic and cytostatic responses.
  - High variance (noise) in DMSO complicates interpretation.
  - As a heuristic, we use log2(0.3) (30% viability) as the threshold to consider a cell line as sensitive.

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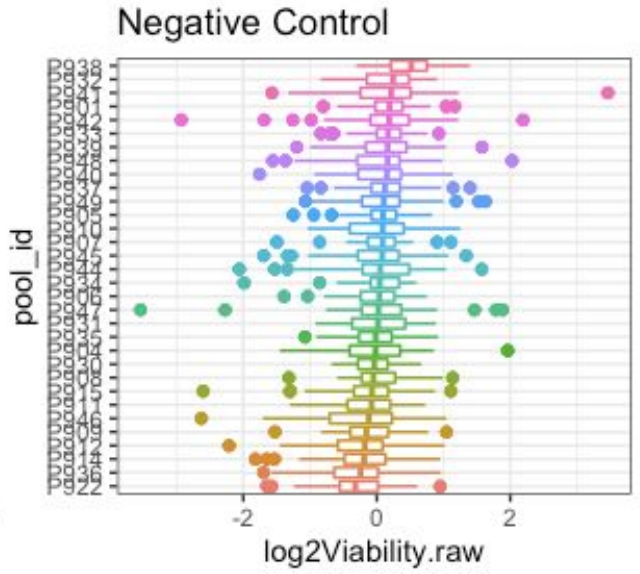
Fit dose response curves

Cell lines x Compound

DRC parameters (AUC, IC50)

Pool Effects: ComBat

- Pool effects in viability profiles are often often as strong as the biological biomarkers.
- Use ComBat[1] to correct for pool effects, so that each pool has similar mean and variance.



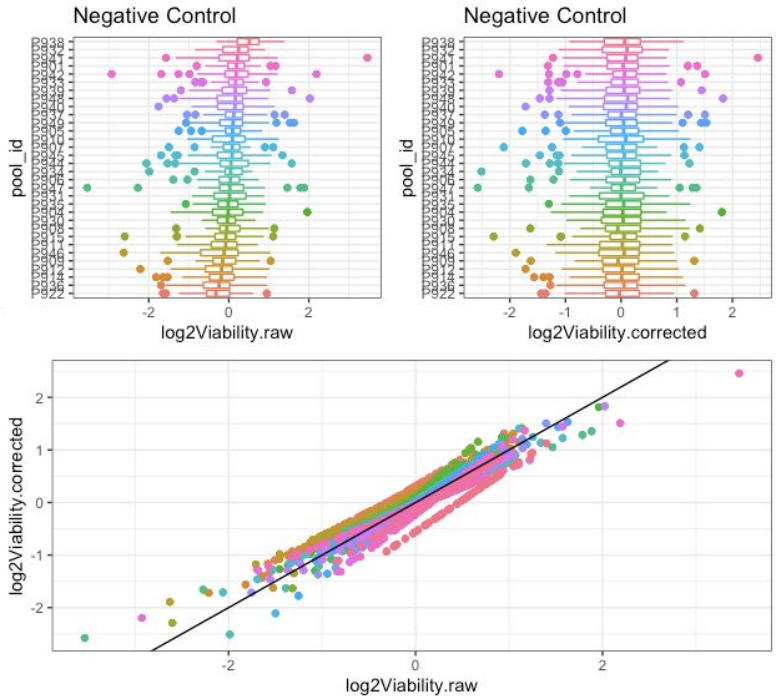
$$Y_{ijg} = \alpha_g + X\beta_g + \gamma_{ig} + \delta_{ig}\varepsilon_{ijg},$$

i : pool id, g : probe (dummy),
j : cell line

[1] Johnson, WE, Rabinovic, A, and Li, C (2007). Adjusting batch effects in microarray expression data using Empirical Bayes methods. *Biostatistics* **8**(1):118-127.

Pool Effects: ComBat

- ComBat is a pretty light-handed correction.
  - Most points are around the main diagonal in the pre- vs post-correction scatter.
- Note: the pool effects are driven by two main artifacts:
  - Assay plates.
  - Growth rate differences.



ComBat: Notes

- The risk of ComBat is if a compound truly selectively kills a particular pool.
  - Deconvolving the artifacts and the true signal is not trivial.
  - When designing pools, we are careful to ensure particular lineages or genomic features are not overrepresented.

- Note: that this is the first part PR300 and PR500 data touches each other.

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Take median of replicates

Compound x dose

Cell lines / analytes / beads

Median log2(viability)

Fit dose response curves

Cell lines x Compound

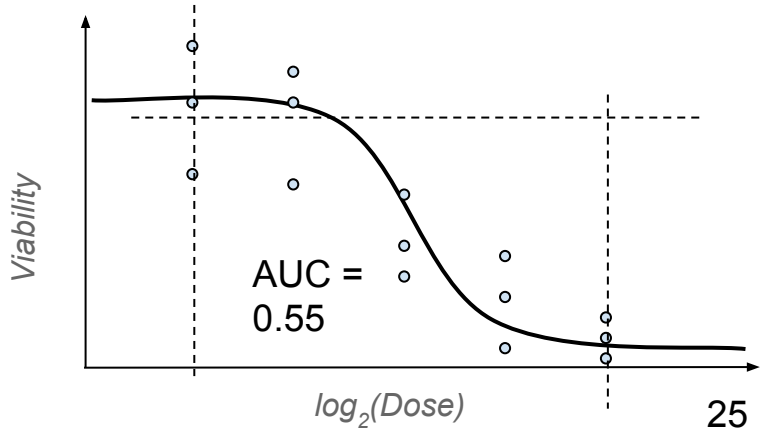
DRC parameters (AUC, IC50)

Curve fitting

- useS the 4-parameter log-logistic model (R packages dr4pl or drc):

$$Viability(Dose) = LowerLimit + \frac{UpperLimit - LowerLimit}{1 + (Dose/EC50)^{-Slope}}$$

- Compute AUC and IC50
  - AUCs are more stable.
  - IC50s are universal: comparable across compounds, not assays.



PRISM sensitivity profiles are correlated with DepMap feature sets to identify biomarkers

depmap

Genomics

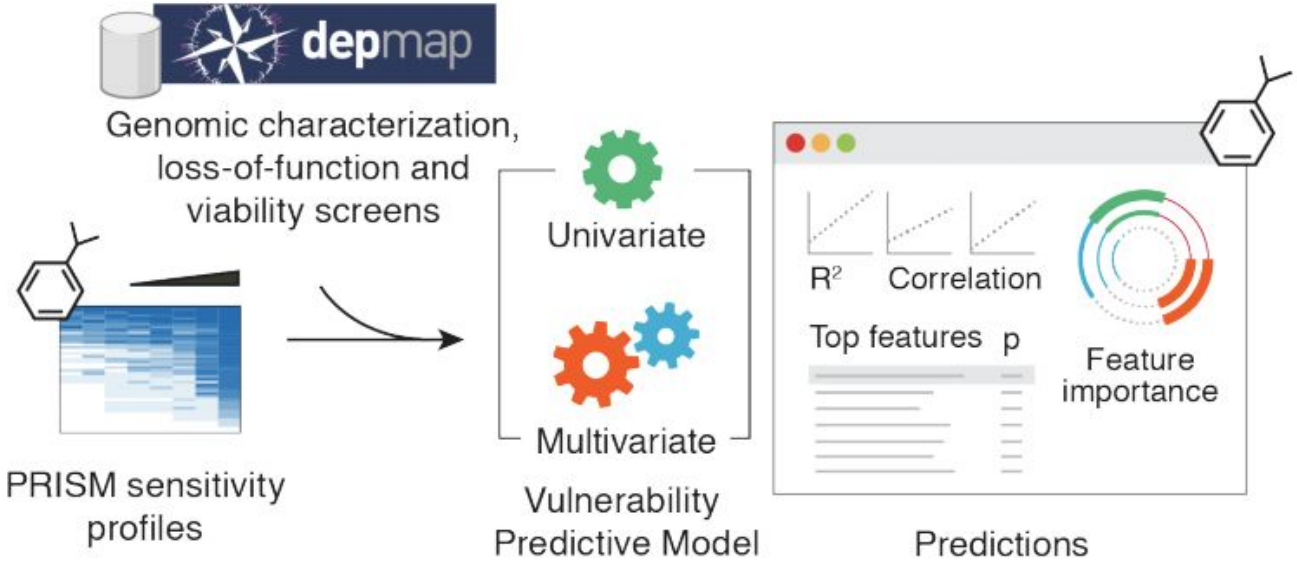
Mutations
Copy number
Methylation

Expression

Single gene-expression
Pathway level-expression
mRNA

Other

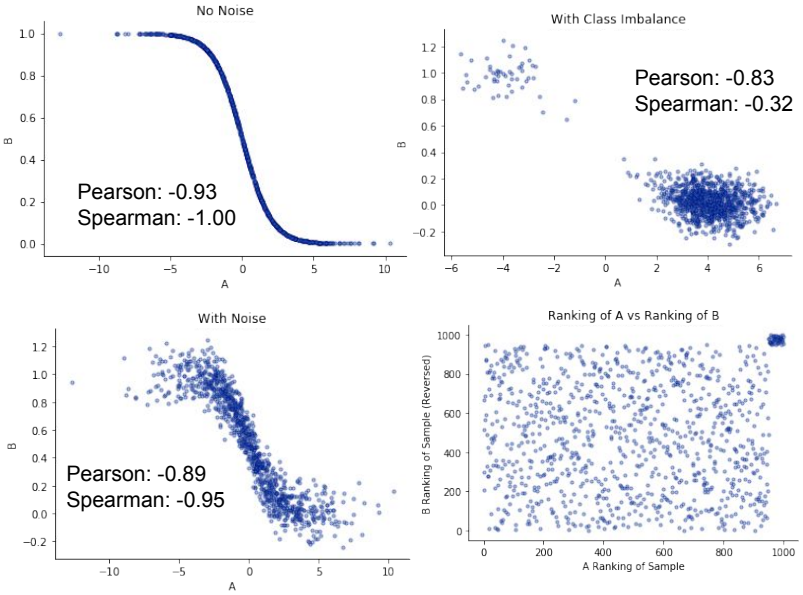
Protein (RPPA)
Metabolomics
Achilles (CRISPR/RNA)
PRISM Repurposing



Univariate Analysis

- For each depmap.org dataset* and PRISM profile**:
  - Compute Pearson correlations between each feature and the profile
- Compute p-values:
  - Fisher's z-transform, limma
  - Bootstrap: not feasible for bulk computations!
- Correct for multiple hypotheses:
  - Benjamini-Hochberg

Josh Dempster



* mutation, gene expression, CRISPR, lineage, metabolomics, miRNA, copy number, protein (RPPA)
** log2(Viability), AUC, log2(IC50)

Multivariate Analysis

- We train random forest and elastic net (α=0.5) models using:
  - CCLE features (copy-number alterations, RNA expression, mutation status, and lineage annotation)
  - CCLE features + RPPA (protein) + CRISPR + metabolomics + miRNA.
- As with univariate analyses, we analyze data for both individual concentration points and overall fit curves

Multivariate Analysis

- For each model, we report the cross-validated R-squared values and Pearson scores (the correlation between the model predictions and PRISM profiles). These describe how accurate the model is.
  - For each feature of each model, the feature importances are computed (after normalizing to the sum of the importances to 1 in each model) and tabulated along with the accuracy measures.

Future work

- Pipeline optimization:
  - Better documentation and reproducible code.
  - Collect more data on whether to combine PR500 and PR300 for biomarker analyses or not.
- Dose-response curves:
  - Constraints:
    - Fixed or free asymptotes?
    - Capping AUCs?
    - Corrected or raw viability values?
- Batch/pool correction?

Future work

- Reliable estimation of growth rate inhibition metrics.
  - Traditional viability metrics are susceptible to variation in the doubling time of cell lines.
  - GR metrics are a nice alternative:

$$GR(c) = 2^{\frac{\log_2(x(c)/x_0))}{\log_2(x_{ctrl}/x_0)}} - 1$$

    - Challenges:
      - large variation in day 0 abundances
      - assumes constant growth rate throughout assay duration
      - logistical challenges

Future work

- Better characterization of assay noise.
  - MFI values are overdispersed; Naive Bayes model may capture the noise structure better.
- Better account of uncertainty.
  - Confidence intervals and standard errors via bootstrap.
- Better biomarker models.

Acknowledgements

PRISM Team


Jen Roth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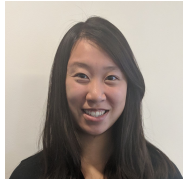
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Tenzin Sangpo


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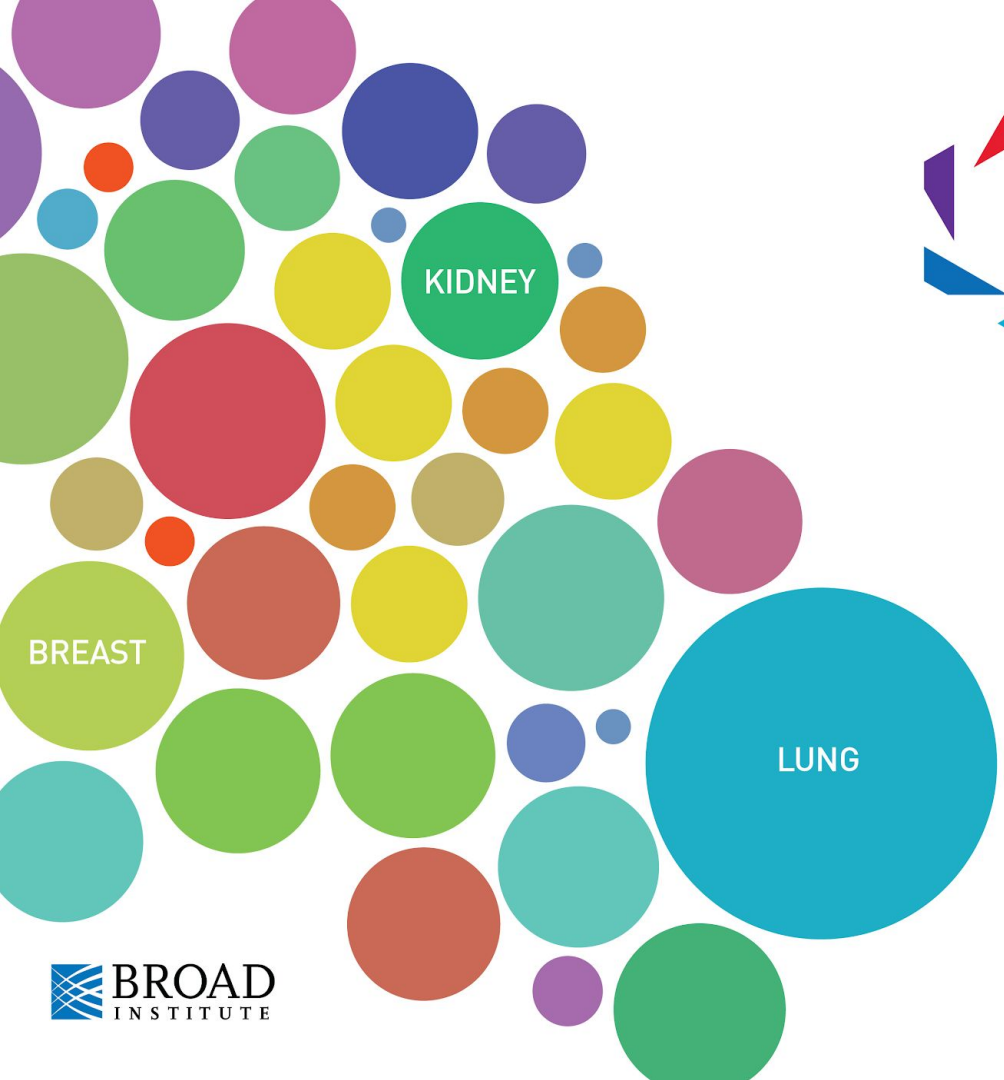
Joshua Sacher
Ryan Philbin



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