Large Row-Constrained Supersaturated Designs for High-throughput Screening

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This work is a team effort

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Outline

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High Throughput Screening

Used in drug discovery and chemical biology.

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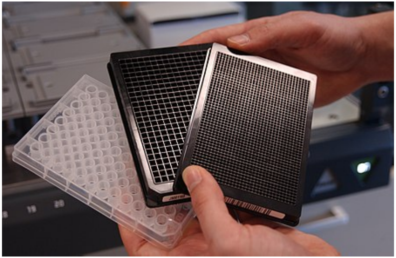

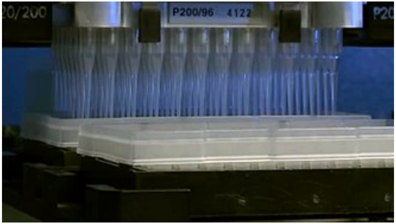

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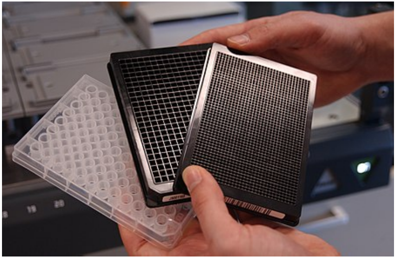

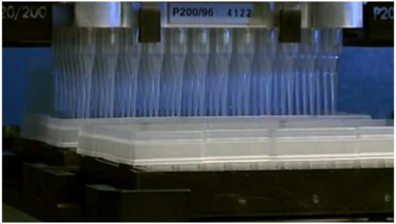

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Later, we'll discuss two examples related to the problem of antibiotic-resistant bacteria.

Take-Home Message

In many high-throughput screening campaigns, **pooling** multiple compounds per well will improve hit rates and reduce false positives.

HTS Design and Analysis Approaches

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- Each compound is treated as a factor with two levels (absent, present)
- Each well is treated as an independent experimental unit
- Each well receives *multiple* compounds, *but no more than c compounds per well*
- The number of compounds k can be more than the number of wells n, so use machinery of *supersaturated designs*

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SSDs generate data to estimate $\mathbf{y} = \beta_0 \mathbf{1} + X\boldsymbol{\beta} + \boldsymbol{\epsilon}$, where X is $n \times k$.

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SSDs generate data to estimate $\mathbf{y} = \beta_0 \mathbf{1} + X\boldsymbol{\beta} + \boldsymbol{\epsilon}$, where X is $n \times k$. We use the Lasso to estimate $\boldsymbol{\beta}$.

Standard Supersaturated Design Criterion, UE(s^2)

Let $L = [\mathbf{1}, X]$ and $S = L^{'}L$ with elements s_{ij}.

Unconditional $E(s^2)$-criterion:

$$\text{UE}(s^2) = \binom{k+1}{2}^{-1} Q(X)$$

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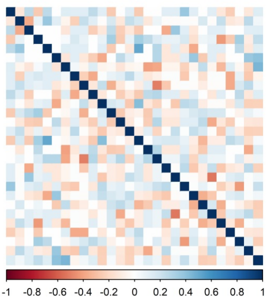

S matrix for a 16×28 $UE(s^2)$-optimal design

Pooling in the Literature

Pooling multiple compounds in a single well has been previously proposed and studied (e.g., see reviews by Kainkaryam and Woolf 2009).

Elkin et al. (2015) calls pooling "controversial ... with a long history of limited success" but shows a successful example of a two compounds/well campaign.

Some recent works have proposed new pooling procedures: Ji et al. (2023) and Liu et al. (2024). Both use statistical methods to screen; neither study their approaches as supersaturated designs, nor do they compare with competing methods.

Outline

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CRowS Designs

We say an $n \times k$ design X is a Constrained Row Screening design if $n \leq k$, $[X]_{ij} = x_{ij}$, and the design solves the following optimization problem:

$$\min \quad UE(s^2) \tag{1}$$

$$\text{s.t.} \quad x_{ij} \in \{-1, 1\} \ \forall i, j \tag{2}$$

$$\sum_{j=1}^{k} x_{ij} \leq 2c - k \ \forall i \tag{3}$$

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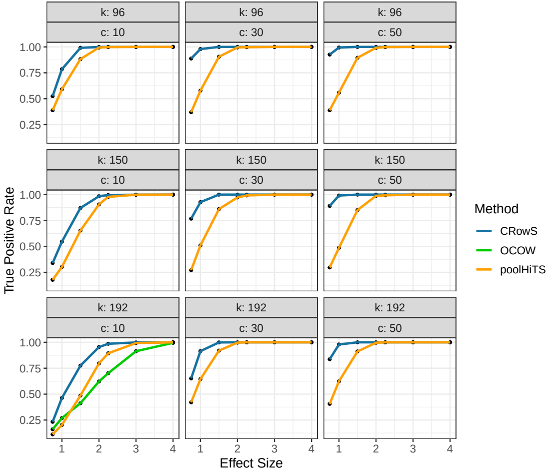
Comparisons: True Positive Rate



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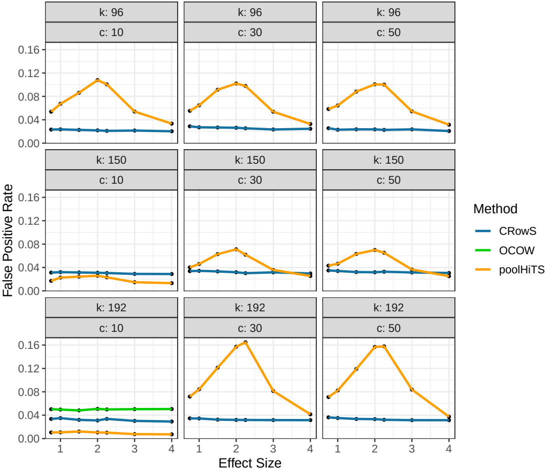

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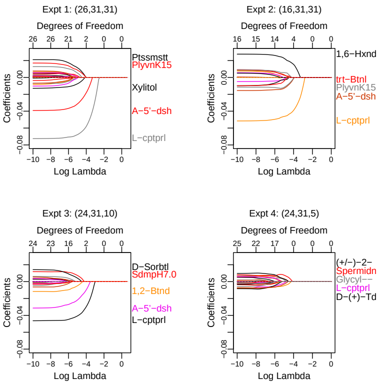
Detecting Inhibitors of Metallo-β-lactamases



- Working with Rick Page's lab at Miami University
- Goal to find inhibitors of enzyme that causes bacterial resistance to antibiotics
- Four mini-experiments, spiked with a known inhibitor, `L-captopril`

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Searching for New Antimicrobials

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Goal: identify compounds that hit on Wildtype but not on Knockout

Pilot Studies with 320 Wells

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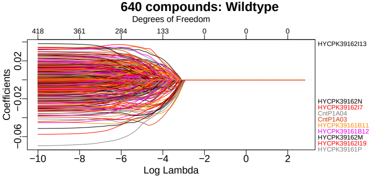
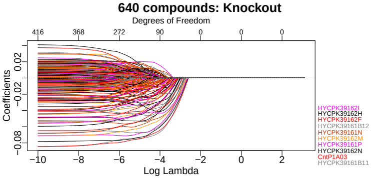
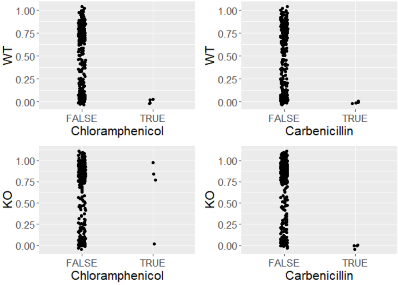






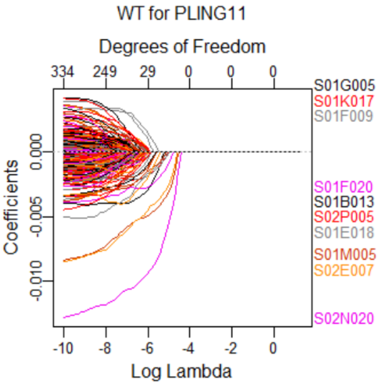
CntP1A04==Chloramphenicol and CntP1A03==Carbenicillen

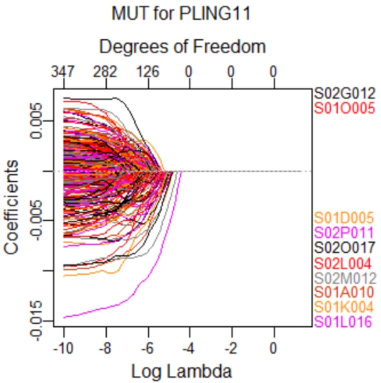
Initial 10,000+ Compound Screen

METHOD

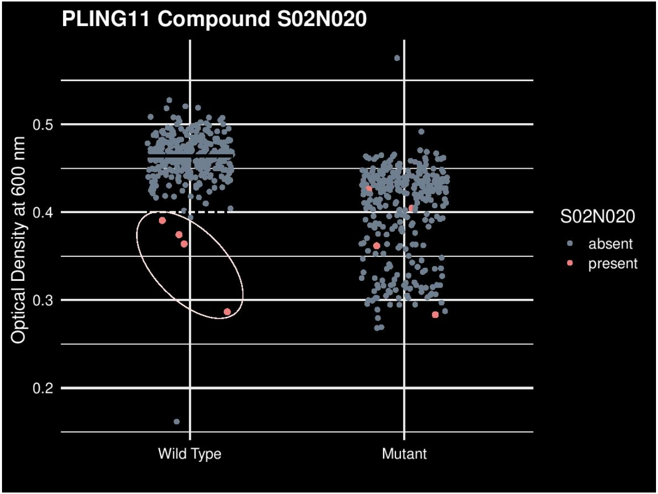
1. **Plan** the pools using a CRowS design
2. **Construct** the pools using a liquid handling system
3. **Obtain and clean** the assay data
4. **Generate** an initial hit list using the Lasso
5. **Trim** hit list as desired by isolating compounds that show consistent inhibition across replications

Out of 16 “PLINGs”, PLING 11 is notable





S02N020 from PLING 11 is promising



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Summary of Work

Our contributions:

1. CRowS, a statistically principled and high-quality solution to the pooling problem in HTS
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2. Interactions?

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Ongoing and future work: more applications; comparisons with other methods; handling interactions; theoretical development to better understand and construct the designs

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Thank you!

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Find me on Twitter/X (@ByranSmucker) and LinkedIn

arXiv preprint: “Large Row-Constrained Supersaturated Designs for High-throughput Screening” (Smucker et al. 2024)

Outline

Features of Row-Constrained SSDs: $(n = 96, k = 144)$