Spatially Aware Plate Layouts

Topic ASA BIOP DL Webinar

Beate Ehrhardt
Institute for Mathematical Innovation
University of Bath

1. Motivation & Background
2. Statistical methodology
3. Live demo

Motivation

Multi-well plate experiments are fundamental to modern biochemistry, pharmacology, and molecular biology

They enabling **high-throughput screening** and systematic investigation of multiple experimental interventions

Challenge: Spatial Bias
Measurements are often systematically affected by spatial bias across the plate

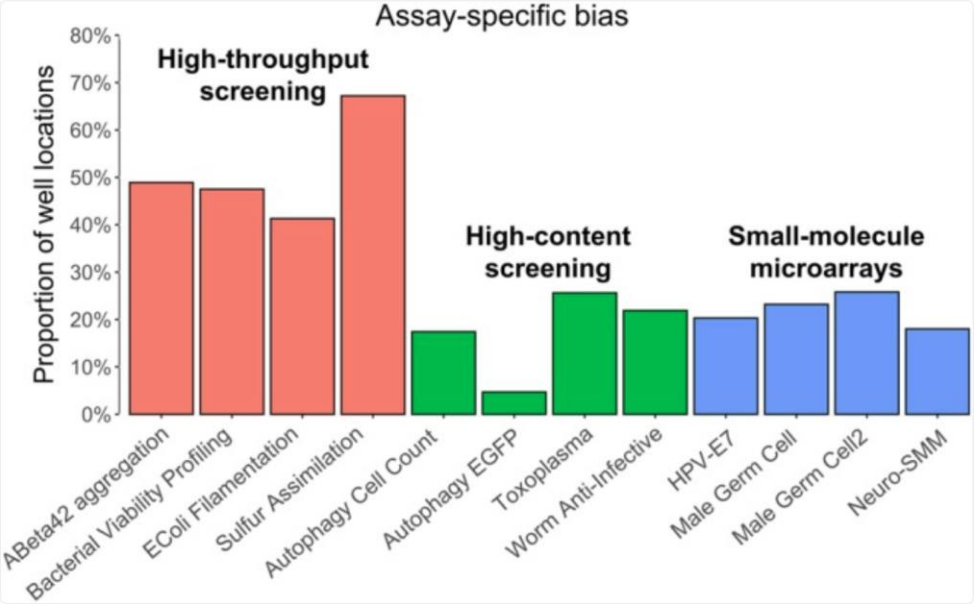
Potential sources
- Evaporation
- Cell or reagent degradation
- Pipette malfunctioning
- Variation in incubation time
- Temporal drift during measurement
- Thermal gradients (incubation or readout)
- Lighting or optical non-uniformity in plate readers

How Bias Manifests
- Over- or under-estimation of true signals
- Characteristic patterns:
  - Bowl-shaped distortions
  - Linear gradients

Consequence
- Increased false positive and false negative rates

Most screening technologies are prone to spatial bias

Mazoure at al. 2017



Setting

- 12 experimental assays from the ChemBank
- All wells same experiment
- Compare each well location with the remaining plate
- Report proportion of wells significantly different

Results

- All screening technologies are prone to spatial bias
- Particularly strong in HTS assays
- Use additive or multiplicative model to correct for the bias

Mazoure at al. 2017: Mazoure, Bogdan, Robert Nadon, and Vladimir Makarenkov. "Identification and correction of spatial bias are essential for obtaining quality data in high-throughput screening technologies." *Scientific reports* 7.1 (2017): 11921.

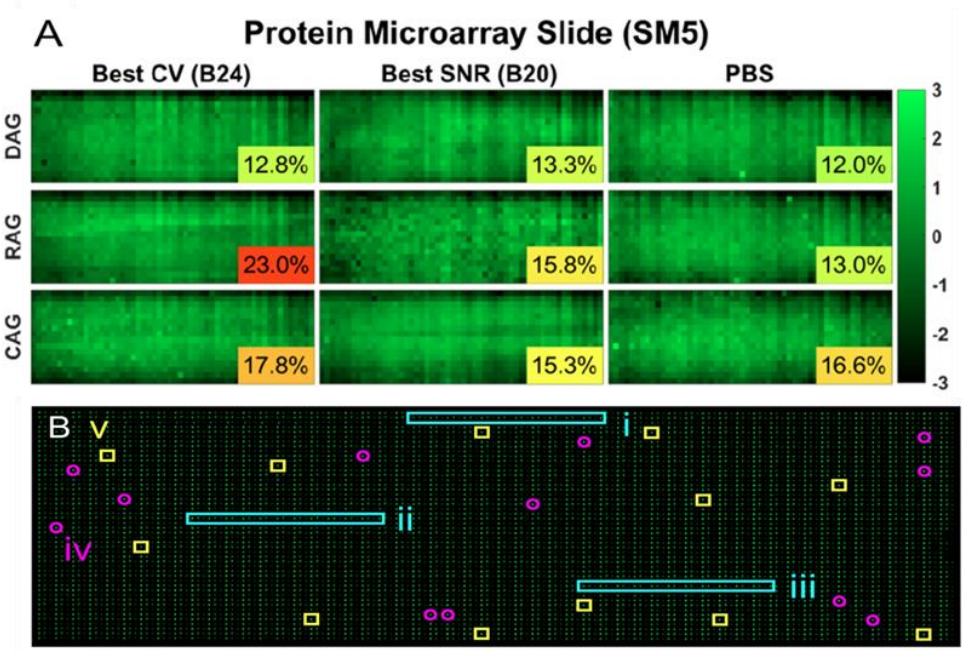
Structural random assignments capture most accurately the assay variation

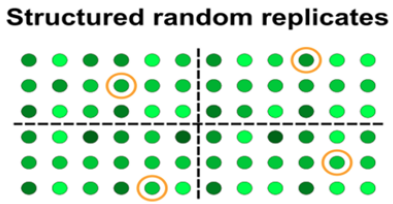
Normandeau at al. 2021

- Spatial bias in antibody microarrays
- Varying glass slides, buffer and antibodies

- Coefficient of variation:
  4.6% - 50%
- Spatial effects across plate

- Comparing assignment of positive control
  - Local (i – iii)
  - Random (iv)
  - Structured random (v)
- Structural random best estimates of assay variation





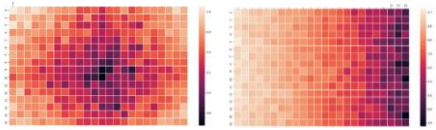
i)
ii) Local replicates
iii)
iv) Random replicates
v) Structured random replicates

Normandeau at al. 2021: Normandeau, Frédéric, et al. "Spatial bias in antibody microarrays may be an underappreciated source of variability." *ACS sensors* 6.5 (2021): 1796-1806.

Spatial balance in randomisation improves accuracy & reduces errors

Plate effects

Bowl shaped Linear

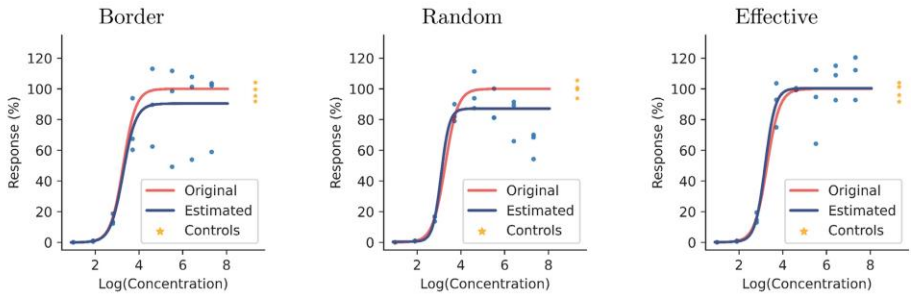


Placement of negative control

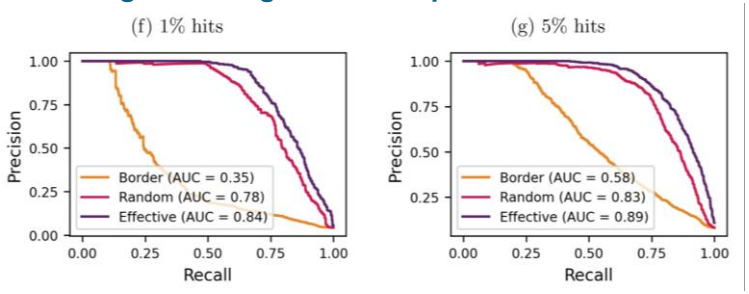
Rodríguez 2023
Simulation study:
- 20 negative controls
- 384 well plate

Results

More accurate dose-response curves



For drug screening: increased precision



➢ **Spatial balance in randomisation as a great potential**

Rodríguez 2023: Rodríguez, María Andreína Francisco, Jordi Carreras Puigvert, and Ola Spjuth. "Designing microplate layouts using artificial intelligence." *Artificial Intelligence in the Life Sciences* 3 (2023): 100073.

Solution for spatial bias

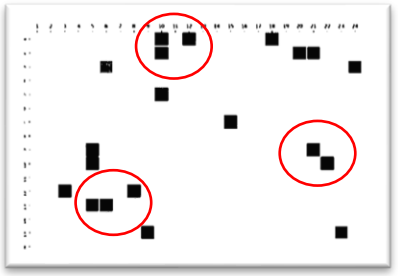
Downstream correction (standard practice)
- Normalisation methods(B-score, LOESS, robust Z-scores, additive or multiplicative models)
- Plate corrections(min–max scaling, fold change)

➢ Key assumption: approximately balanced plate layout

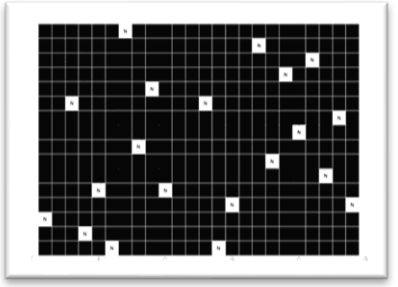
Experimental design (statistical best practice)
- Randomisation of controls and treatments [1–3]
- Evidence: spatial balance in randomisation reduces bias

Practical limitations
- Existing approaches often incompatible with lab constraints
  - Cannot exclude edge wells
  - Do not support column-wise treatments
- Naïve randomisation → local clustering → poor spatial coverage

Naïve Random



SAPL



1: Normandeau, Frédéric, et al. "Spatial bias in antibody microarrays may be an underappreciated source of variability." *ACS sensors* 6.5 (2021): 1796-1806.

2: Mazoure, Bogdan, Robert Nadon, and Vladimir Makarenkov. "Identification and correction of spatial bias are essential for obtaining quality data in high-throughput screening technologies." *Scientific reports* 7.1 (2017): 11921.

3: Malo, Nathalie, et al. "Statistical practice in high-throughput screening data analysis." *Nature biotechnology* 24.2 (2006): 167-175.

New methodology: Controlled randomisation

UNIVERSITY OF BATH

Method
- Understand the experimental needs
- Formalise as design constraints
- Integrate into randomisation

Generate layouts satisfying
- Randomisation
- Spatial balance
- Feasibility in practice

Relationship to existing methods
- Same optimality criterion as Rodríguez (2023)
- Reduces repeats in rows, columns and 3 x 3 areas
- Much broader range of experimental constraints

Spatially **A**ware **P**late **L**ayouts

Results
- Improves dose–response estimation
- Increases precision in drug screening

} compared to naïve randomisation

- Practically feasible designs across diverse experimental settings

1: Normandeau, Frédéric, et al. "Spatial bias in antibody microarrays may be an underappreciated source of variability." *ACS sensors* 6.5 (2021): 1796-1806.

2: Mazoure, Bogdan, Robert Nadon, and Vladimir Makarenkov. "Identification and correction of spatial bias are essential for obtaining quality data in high-throughput screening technologies." *Scientific reports* 7.1 (2017): 11921.

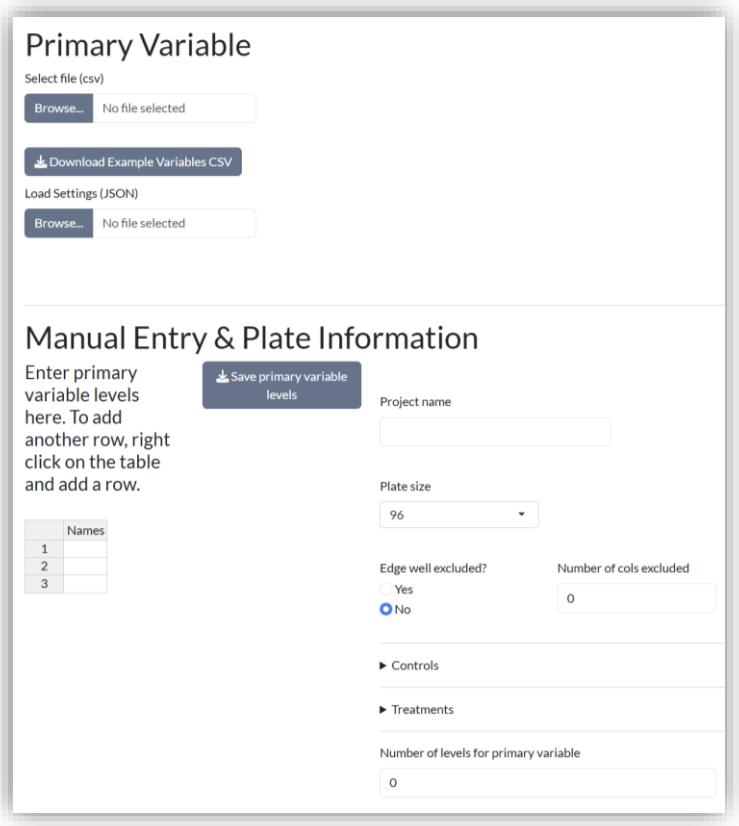
3: Malo, Nathalie, et al. "Statistical practice in high-throughput screening data analysis." *Nature biotechnology* 24.2 (2006): 167-175.

IMI

Spatially Aware Plate Layouts

R shiny app designed to support robust experimental design and reduce spatial bias in nonclinical high-throughput assays.

- SAPL explicitly manages **spatial balance** (e.g., control dispersion, edge buffering, row/column balance),
- while assigning wells at random leading an **unbiased** plate map.
- SAPL reduces time to generate a plate map from **1 day to 2 min** (for 384 well plate),
- Reduces **bias** and **improves reproducibility**
- Empowers scientists (**no coding expertise** required).



Key features

Validation: Extensively beta-tested with functional genomics scientists at AstraZeneca across diverse experimental settings

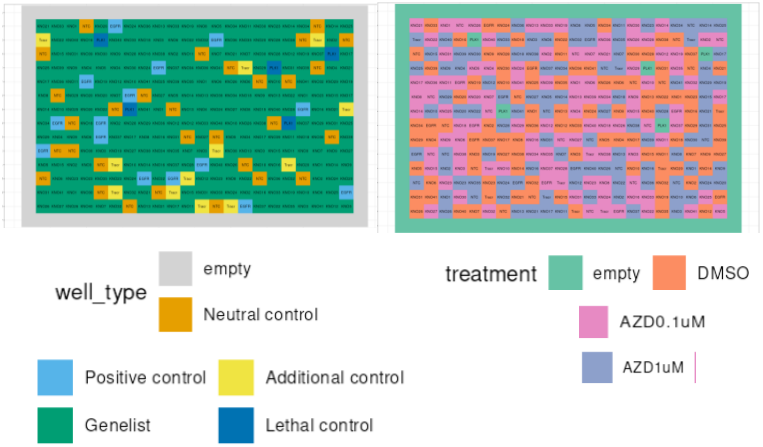
Input

- Plate formats: **24-, 96-**, and **384-well**
- **Flexible** inclusion of multiple positive, neutral, and lethal controls
- Accommodates **multiple treatments**, assigned freely or by column
- Generates **multiple plates** with shared or varying design features
- Customisation options:
  - Exclude outer rows/columns
  - Neutral controls in corners
  - Pair neutral and positive controls

Output

- **Quality control**: repetitions in rows, columns, and local 3x3 areas
- **Visual feedback**: Displays plate layout by treatment and well type
- **Multiple export options**: E.g. excepts echo pick list as an input and generates a plate map file compatible with echo dispensing system
- **Reproducibility**: Downloadable and reloadable input configurations for
  - transparency,
  - record-keeping,
  - ease for repeated use

Visualisation

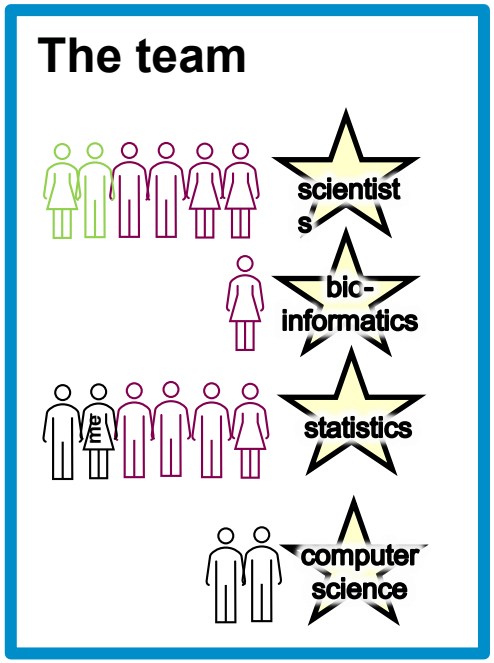


Multiple export formats, e.g., compatible with echo dispensing system

	Source Plate Name	Source Well	Transfer Volume	GeneID	Destination	Destination Well
2	Source Plate[2]	A1	480	KO1	Destination	D4
3	Source Plate[2]	A1	480	KO1	Destination	K10
4	Source Plate[2]	A1	480	KO1	Destination	K12
5	Source Plate[2]	A1	480	KO1	Destination	L16
6	Source Plate[2]	A1	480	KO1	Destination	O16
7	Source Plate[2]	A1	480	KO1	Destination	C18
8	Source Plate[2]	B1	480	KO2	Destination	K4
9	Source Plate[2]	B1	480	KO2	Destination	E7

Background

- Originally designed for functional genomics in R&D in AstraZeneca in **2023**
- Conceptional development with a broad team:
  - Scientists
  - Bioinformaticians
  - Statisticians
- Beta testing
- Integrated into standard workflow
- In **2025**: raise awareness & improve usability
  - Open access version
- Since May **2026**: Generalize tool
  - AstraZeneca
  - Functional Genomics Screening Lab, Cambridge, UK



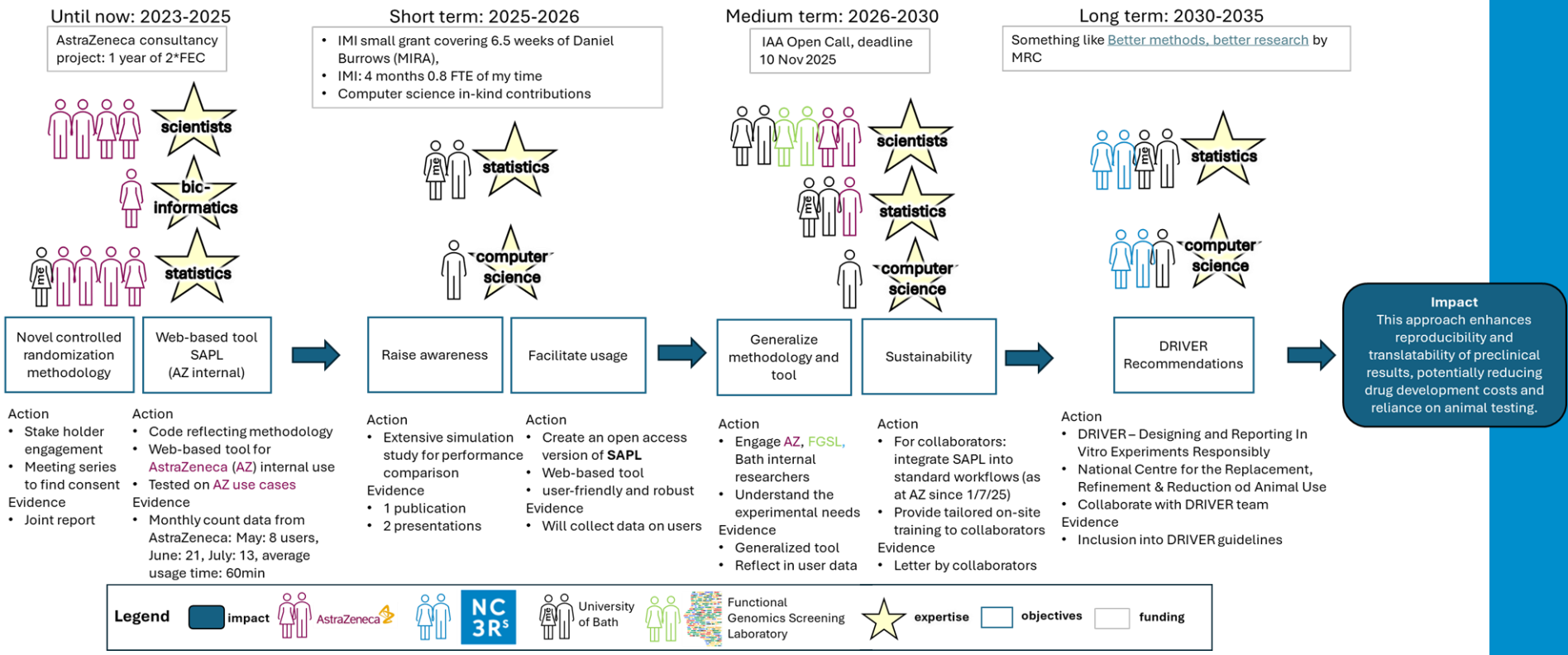
Legend

AstraZeneca

University of Bath

Functional Genomics Screening Laboratory

Long-term plan



Statistical methodology

Overview

1.Define plate

- Plate size: 24, 96, 384
- Discarded wells due to edge effects: #rows, #columns

2.Position controls

- Place neutral, positive, lethal controls, (and additional)
- by controlled randomisation

3.Position variable of interest

- Identify available wells
- Assign by controlled randomisation

Random

Deterministic: rule-based allocation

4. Spatial variance minimization

- Repeat 100 times
- Compute spatial variance
- Choose best plate

5. Quality control assessment

Report repetitions in **columns** and **rows** and in **local 3x3 area** by

- variable of interest
- neutral control
- positive control
- lethal control
- other controls

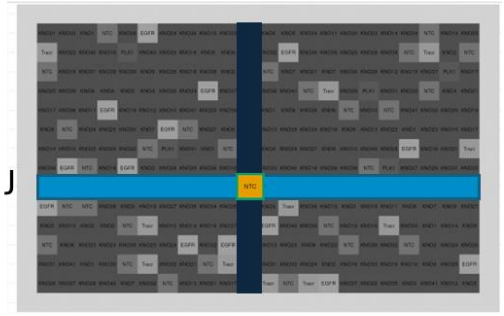
2. Position controls: controlled randomisation

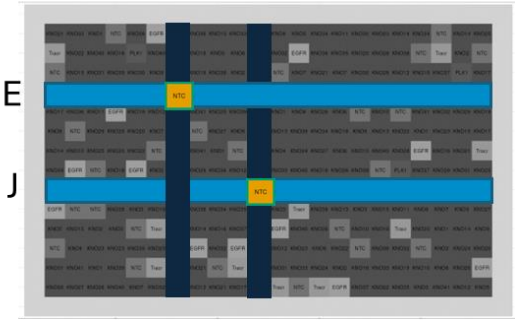
Draw at random without replacement

Draw at random without replacement

J 12

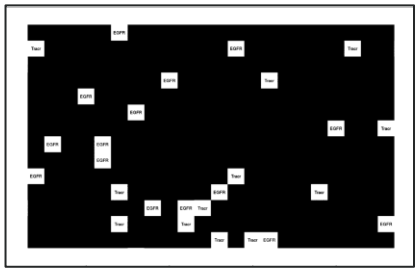
E 8





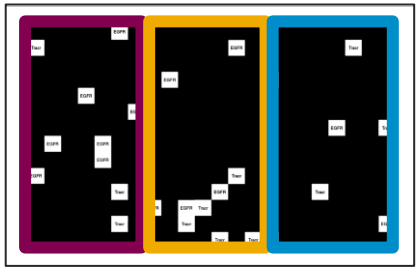
- **Reduces the repetition of the controls in rows and columns**

3. Position variable of interest: controlled randomisation



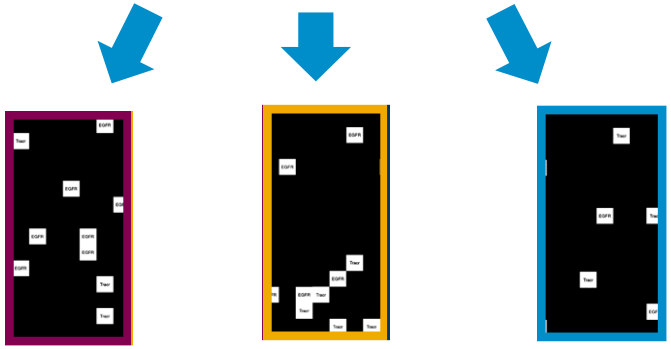
Identify available wells

- Here in black



Split into number of technical replicates

N = 3



Place all levels of variable of interest

at random

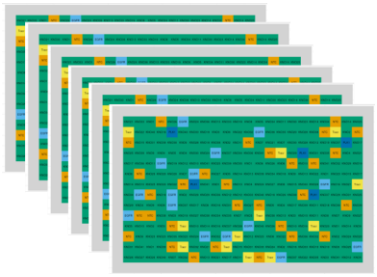
without replacement

- **Reduces the repetition of the levels of the variable of interest in columns**

4. Spatial variance minimization I

1. Define plate
2. Position controls
3. Position variable of interest

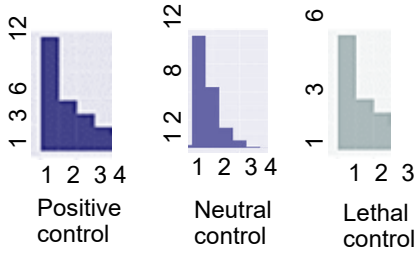
4. Repeat 100 times



Create 100 different plate maps

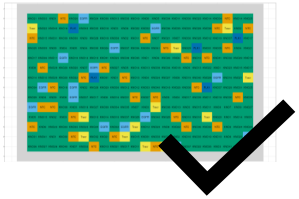
Count repetitions

- in local 3*3 areas
- for positive control, neutral, and lethal and others



Identify best

- Compute variance
- Add variances
- Chooses plate with least total variance



➢ **Reduces the repetition of controls in 3x3 area**

4. Spatial variance minimization II

For every local 3x3 area, how many wells are filled with control type C_i :

$$\phi_i(p,q) = \sum_{u=p}^{p+2} \sum_{v=q}^{q+2} \mathbf{1}_{f(u,v)=C_i}$$

Compute the variance of these counts for each control type:

$$\sigma_i^2 = \mathrm{Var}(\{\phi_i(p,q) : (p,q) \in \mathcal{P}\})$$

Define the spatial variance as the weighted sum of variances, including a penalty term for the highest count. For a plate map f :

$$J(f) = \sum_{i=1}^{k} w_i \sigma_i^2 + \lambda \max_{i,p,q} \phi_i(p,q)$$

Choose the plate map f with the lowest J

Final step: quality control assessment

1.Define plate

- Plate size: 24, 96, 384
- Discarded wells due to edge effects: #rows, #columns

2.Position controls

- Place neutral, positive, lethal controls, (and additional)
- by controlled randomisation

3.Position variable of interest

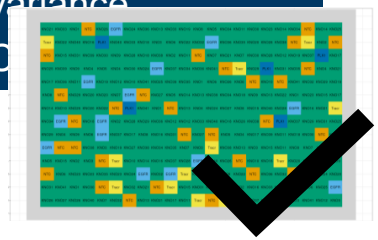
- Identify available wells
- Assign by controlled randomisation

Random

Deterministic: rule-based allocation

4. Spatial variance minimization

- Repeat 100 times
- Compute spatial variance
- C



5. Quality control assessment

Report repetitions in **columns** and **rows** and in **local 3x3 area** by

- variable of interest
- neutral control
- positive control
- lethal control
- other controls

Final step: quality control assessment

1.Define plate

- Plate size: 24, 96, 384
- Discarded wells due to edge effects: #rows, #co

2.Position cor

- Place neutral, pos controls, (and add
- by controlled rand

3.Position variable of interest

- Identify available wells
- Assign by controlled randomisation

Random | Deterministic: rule-based

Scenario 1: no treatment
Scenario 2 & 3: treatment

- dispensable at random
- in columns

y control sment

tions

nd **rows** and

area by

le of interest

l control

e control

control

- other controls

Live demo

Accessing the tool & support

Tool (public): https://sapl.cs.bath.ac.uk

For questions, advice, or feedback: be323@bath.ac.uk

Training videos: https://sapl.cs.bath.ac.uk/video-guides

Company internal version

- With license agreement/ collaboration agreement
- For free
- Source code
- Cannot share the tool
- Agree to track the usage (how many users, which countries)
- Agree to give feedback/ write letter of reference

UNIVERSITY OF BATH

Live demo:
https://sapl.cs.bath.ac.uk

Features SAPL

Enter data I: primary variable

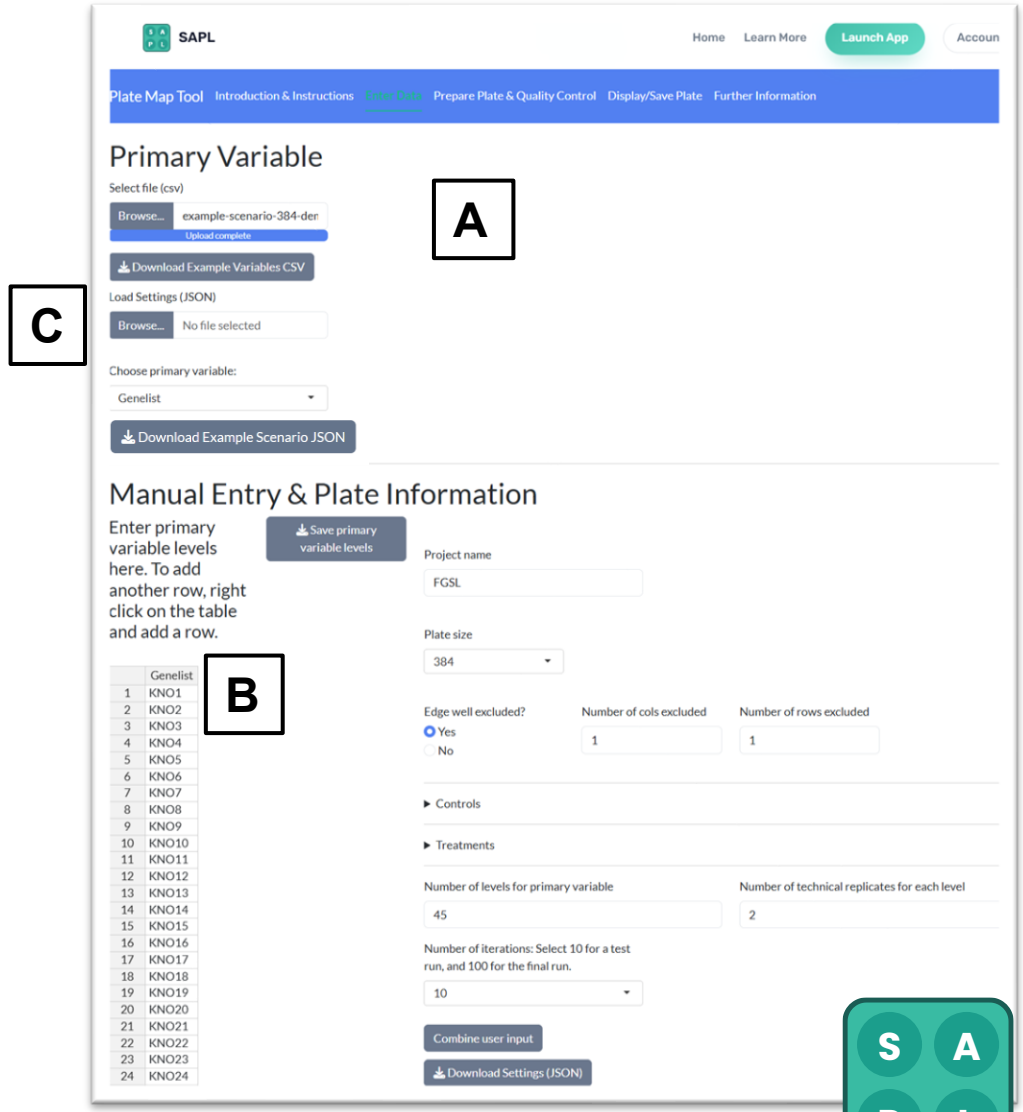
Enter **primary variable = gene knockout (gKO)**

A: Load in an existing list as csv file

- choose the column with gKOs
- other columns will be ignored
- avoid columns with no name
- For primary variable of interest: avoid spaces in the column name

B: Enter manually

C: Load settings (JSON)



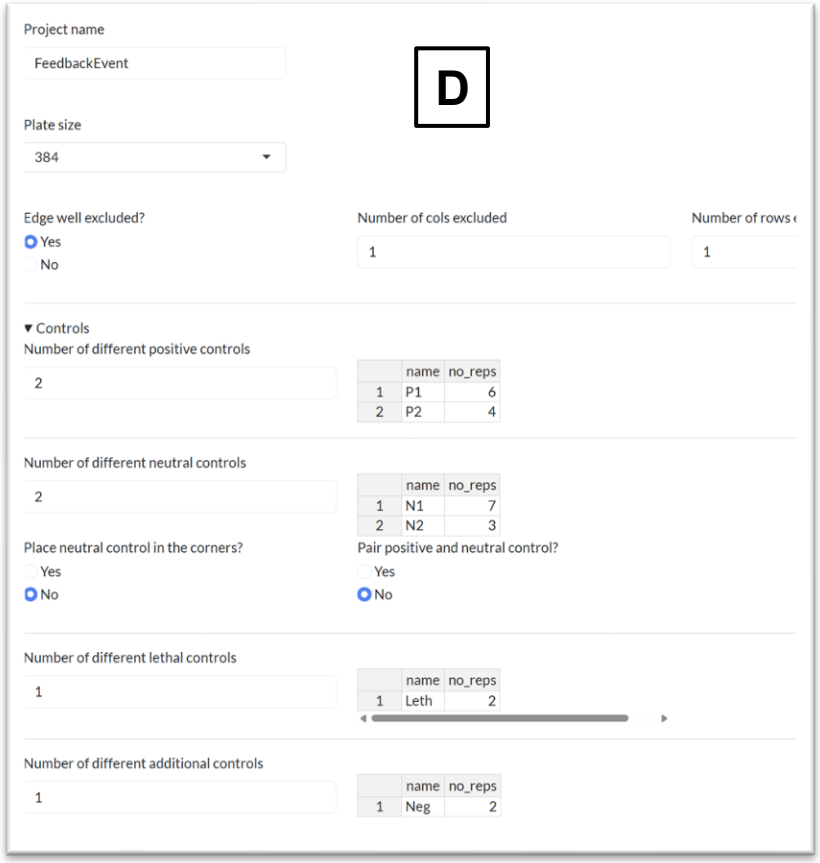
Enter data II: Plate Information & Controls

D: Plate Information

- You need to specify a project name
- Plate size: 24-, 96-, and 384-well plates
- Edge well excluded? (optional)
  - Specify how many rows and
  - How many columns
- New option: “exclude only the corner wells”

E: Enter Controls

- Multiple positive controls: Each with name and number of replicates
- Multiple neutral controls:
  - Each with name and number of replicates

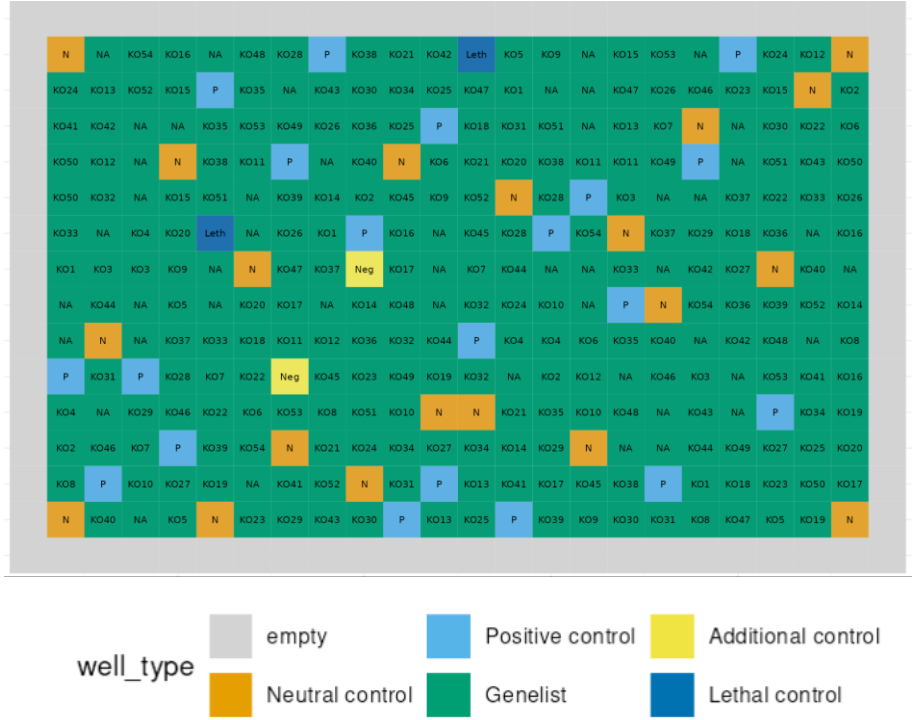


E

Enter data II

D: Plate Information

- You need to specify a project name
- Plate size: 24-, 96-, and 384-well plates
- Edge well excluded? (optional)
  - Specify how many rows and
  - How many columns
- New option: “exclude only the corner wells”



E: Enter Controls

- Multiple positive controls: Each with name and number of replicates
- Multiple neutral controls:
  - Each with name and number of replicates
  - Place neutral controls in the corners
  - Pair positive and neutral control

Enter data III: Treatment

J: Number of treatments

Multiple treatments

Same treatment different concentration

“Intervention”, like different serum concentrations

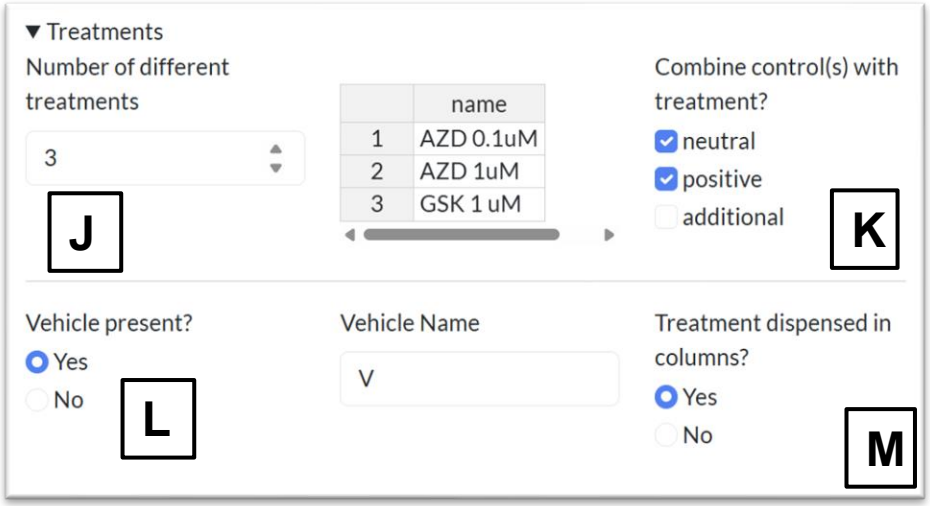
K: Combine with controls

Do you want to treat the neutral controls?

Do you want to treat the positive controls?

Do you want to treat the additional controls?

L: Is there a vehicle present



Enter data III: Treatment

J: Number of treatments

Multiple treatments

Same treatment different concentration

“Intervention”, e.g. varying serum concentrations

K: Combine with controls

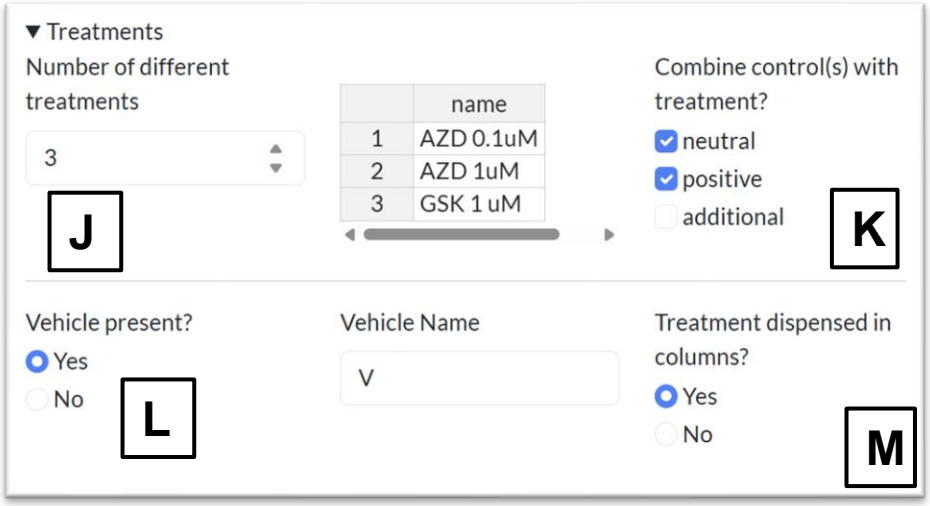
Do you want to treat the neutral controls?

Do you want to treat the positive controls?

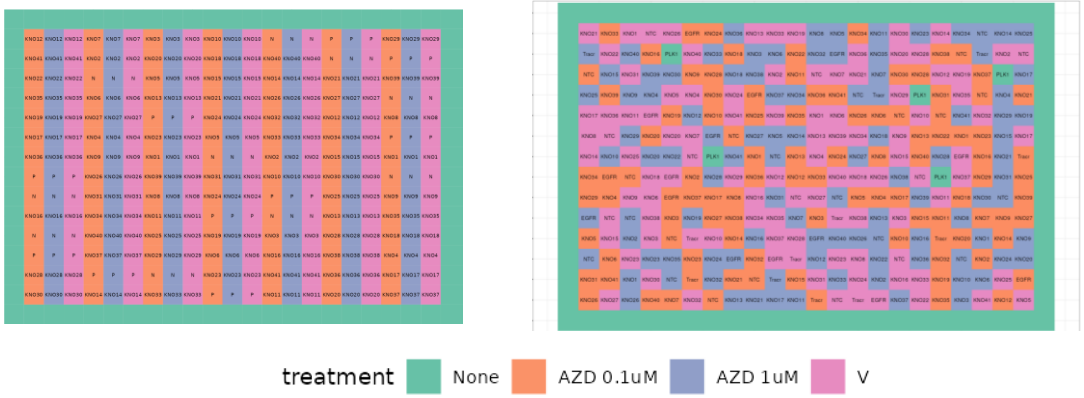
Do you want to treat the additional controls?

L: Is there a vehicle present

M: Treatment



dispensed in columns OR freely



Enter data IV: Multiple plates

N: Number of levels of primary variable

- How many genes from the list shall be used?

O: Number of technical replicates for each level

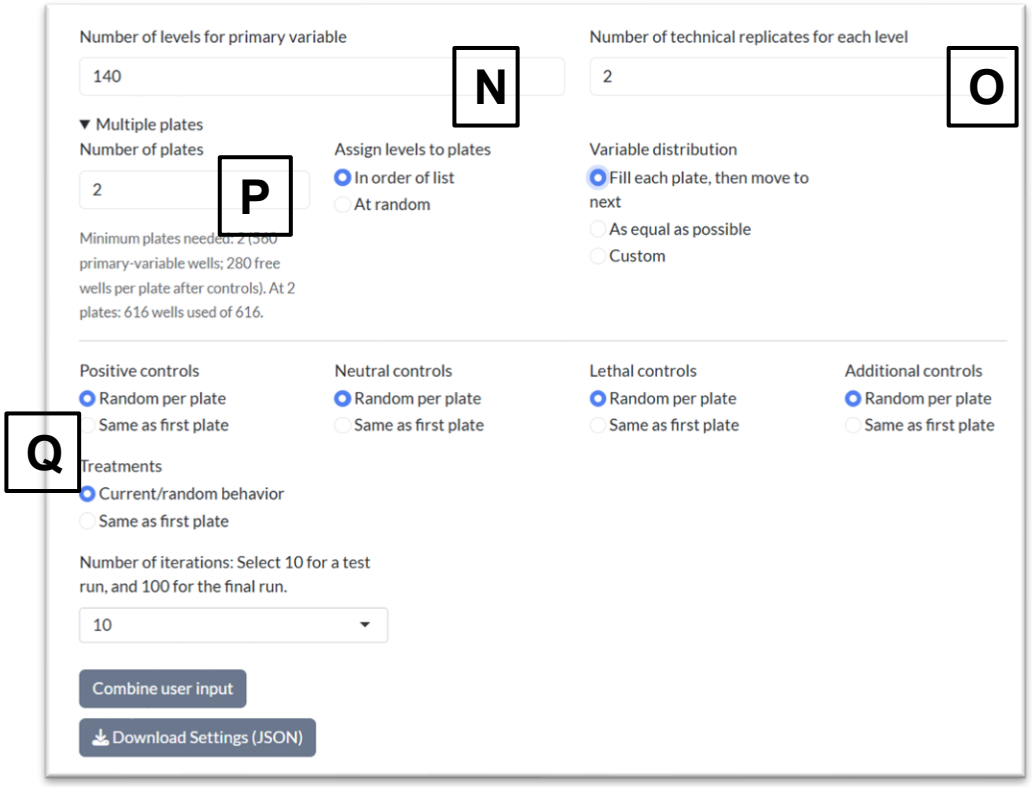
- How many wells with the exact same filling would you like per gene?

P: Choose the number of plates

- Take gene names in the order of the list OR at random
- Fill each plate then move to next OR as equal as possible

Q: How would you like the controls and the treatment to be added?

- Same as on the first plate OR randomly



Enter data V: final section

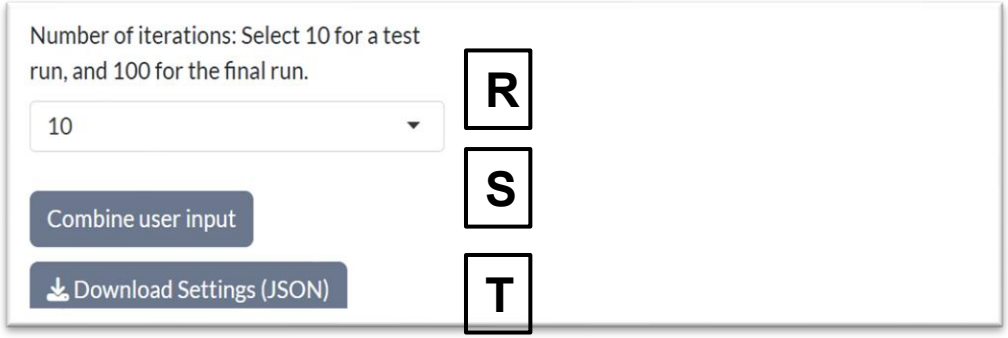
R: Number of iterations

S: Combine user input

When you press this button all data above will be joint to one input file.
If you make changes above you need to press the button again.

T: For the final figures, press “Download Settings” and save file with your protocol.

Transparency
Speed up the process for next experiment if similar



Enter data V: final section

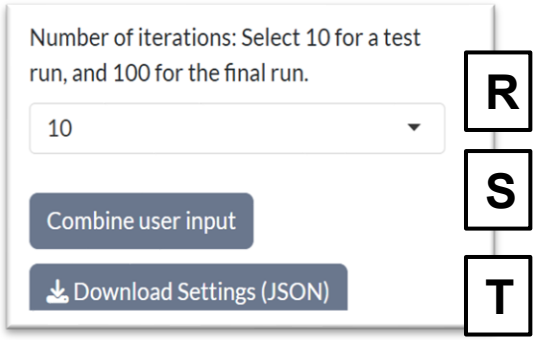
R: Number of iterations

S: Combine user input

When you press this button all data above will be joint to one input file.
If you make changes above you need to press the button again.

T: For the final figures, press “Download Settings” and save file with your protocol.

Transparency
Speed up the process for next experiment if similar



Enter data V: final section

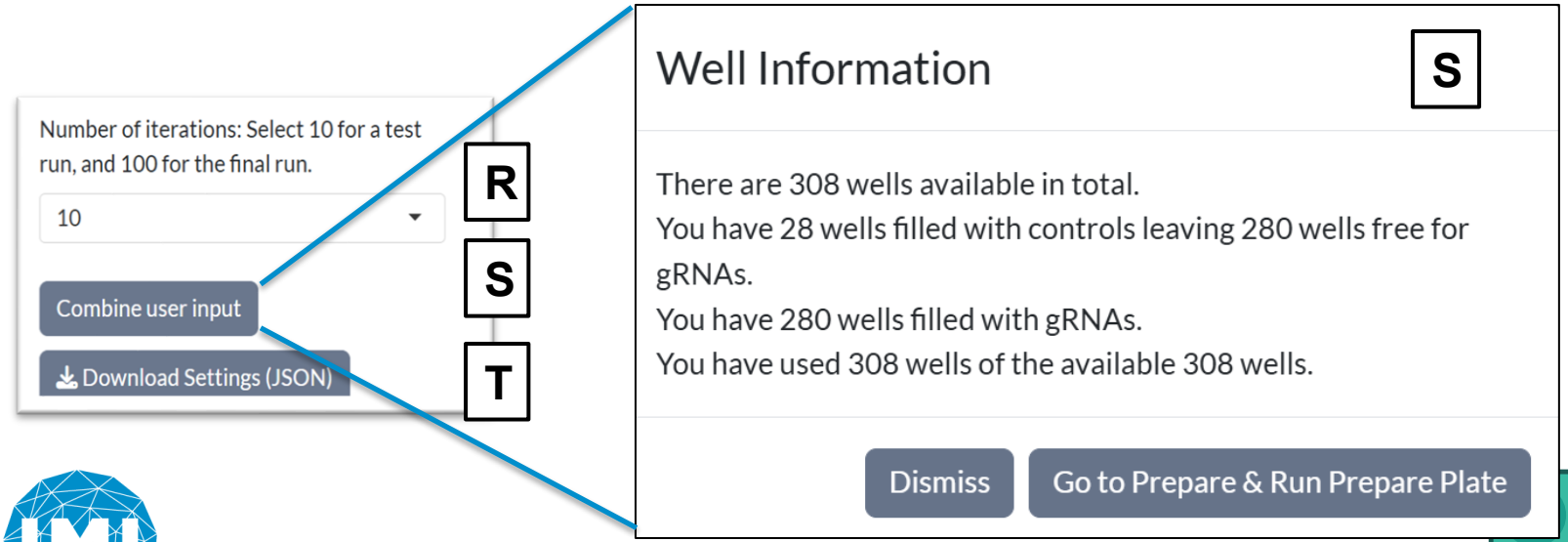
R: Number of iterations

S: Combine user input

When you press this button all data above will be joint to one input file.
If you make changes above you need to press the button again.

T: For the final figures, press “Download Settings” and save file with your protocol.

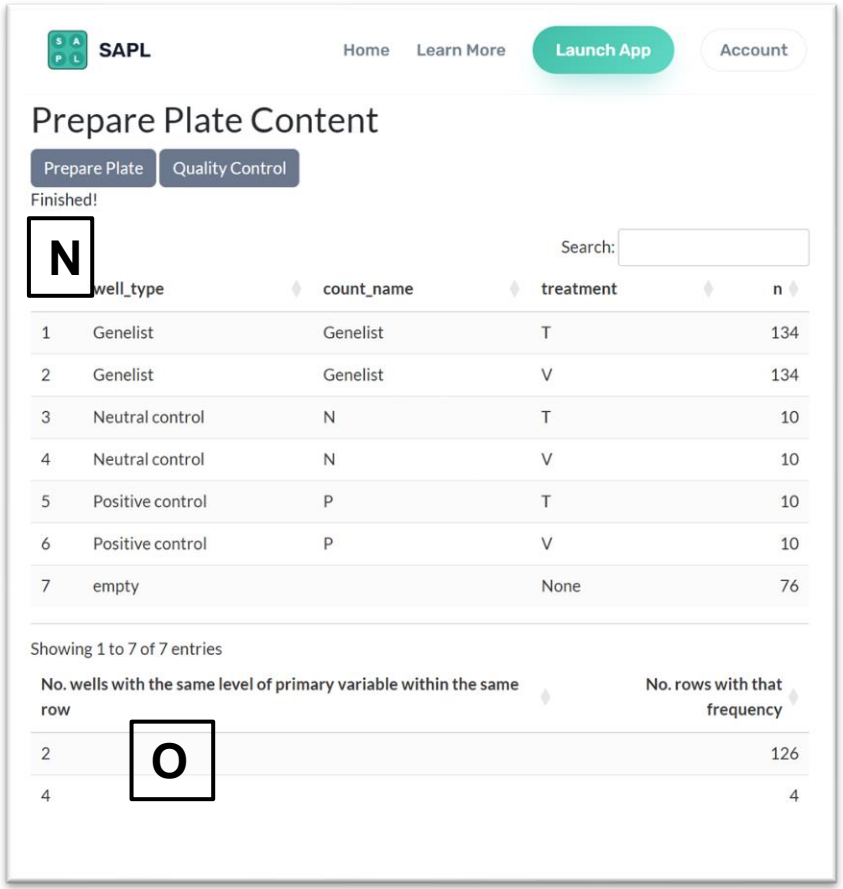
Transparency
Speed up the process for next experiment if similar



Prepare Plate & Quality Control

N: Table of counts of wells by
- well type
- name
- Treatment

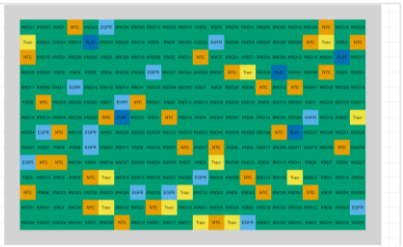
O: quality control
- Tables of counts for repetitions by
  - Rows
  - Columns
  - 3x3 areas



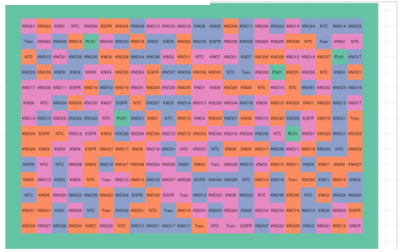
Display & Save Plate

Visualisation

Primary Variable Map



Treatment Map



Save plate

Save Plate as .xlsx

	A	B	C	D	E	F	G
1	row	column	well	Genelist	treatment	gene_treatm	well_type
20	C	2	C2	KNO62	T	KNO62_T	Genelist
21	D	2	D2	KNO58	T	KNO58_T	Genelist
22	E	2	E2	KNO61	T	KNO61_T	Genelist
23	F	2	F2	N	T	N_T	Neutral control
24	G	2	G2	KNO65	T	KNO65_T	Genelist

Attach plate to original data and save as .xlsx

Compatible with echo dispensing system

	1	2	3	4	5	6	7
1	Source Plate Name	Source Well	Transfer Volume	GeneID	Destination	Destination Well	Treatment
2	Source Plate[2]	A1	480	KO1	Destination	D4	DMSO
3	Source Plate[2]	A1	480	KO1	Destination	K10	AZD1uM
4	Source Plate[2]	A1	480	KO1	Destination	K12	AZD0.1uM
5	Source Plate[2]	A1	480	KO1	Destination	L16	AZD0.1uM
6	Source Plate[2]	A1	480	KO1	Destination	O16	AZD1uM
7	Source Plate[2]	A1	480	KO1	Destination	C18	DMSO
8	Source Plate[2]	B1	480	KO2	Destination	K4	AZD0.1uM
9	Source Plate[2]	B1	480	KO2	Destination	E7	AZD1uM

Accessing the tool & support

Tool (public): https://sapl.cs.bath.ac.uk

For questions, advice, or feedback: be323@bath.ac.uk

Training videos: https://sapl.cs.bath.ac.uk/video-guides

Company internal version

- With license agreement/ collaboration agreement
- For free
- Source code
- Cannot share the tool
- Agree to track the usage (how many users, which countries)
- Agree to give feedback/ write letter of reference

Coming in summer on our website: Q&A