

Spatially Aware Plate Layouts

Topic ASA BIOP DL Webinar

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



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

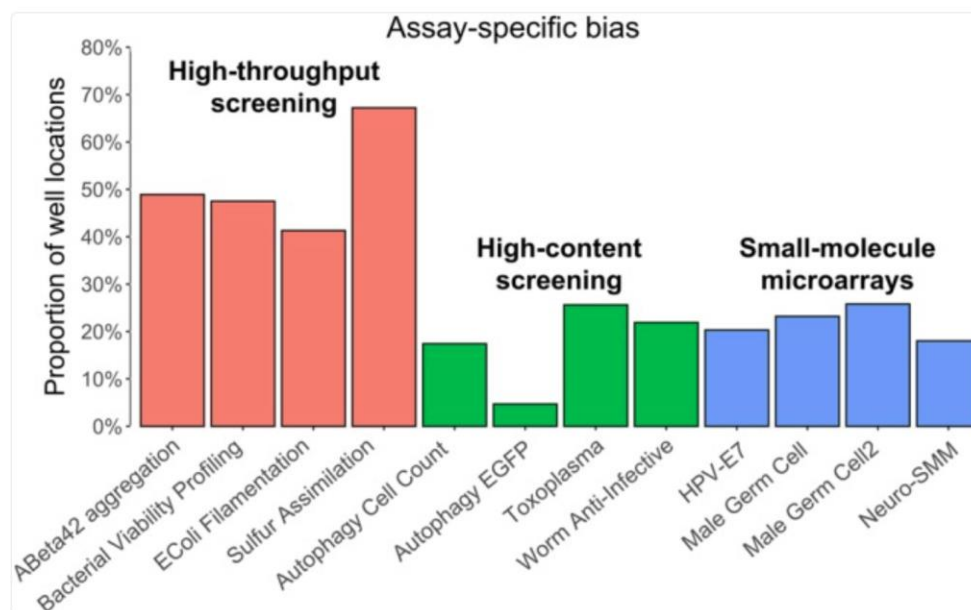
Consequence

- Increased false positive and false negative rates



Most screening technologies are prone to spatial bias

Mazoure et 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

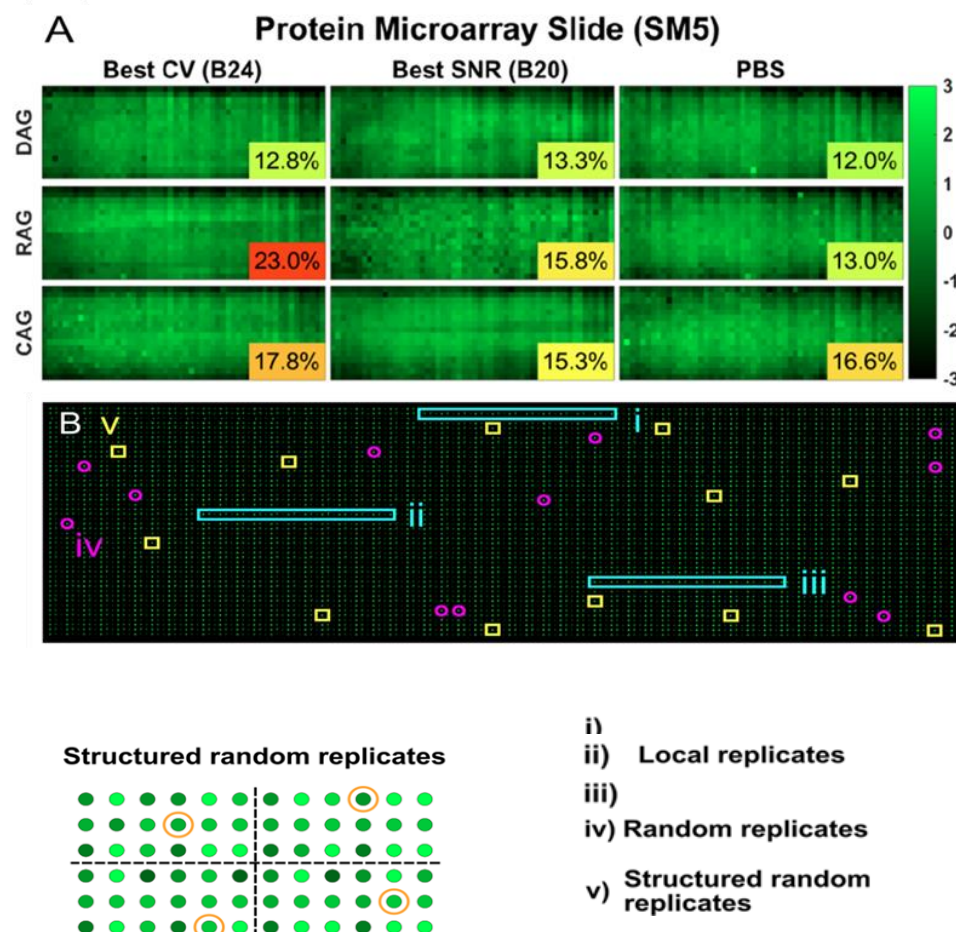
Results

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

Structural random assignments capture most accurately the assay variation

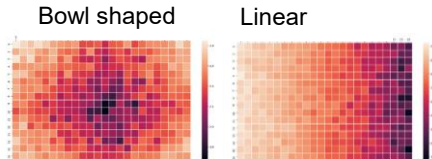
Normandeau et al. 2021

- 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

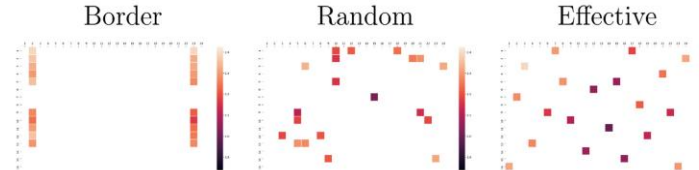


Spatial balance in randomisation improves accuracy & reduces errors

Plate effects



Placement of negative control



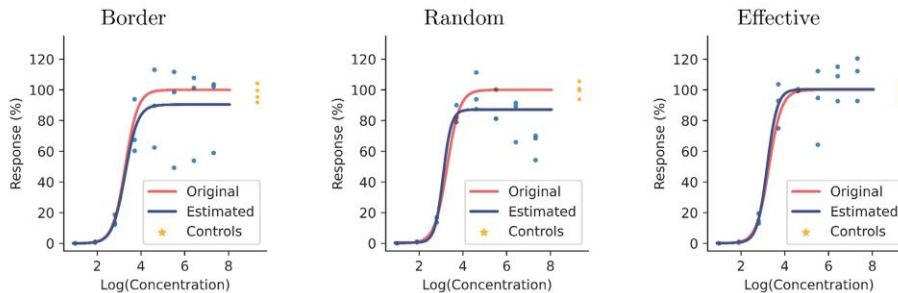
Rodríguez 2023

Simulation study:

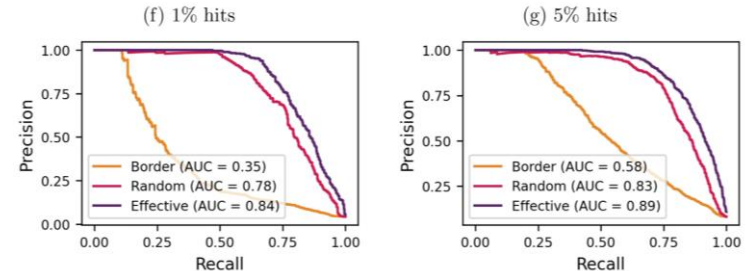
- 20 negative controls
- 384 well plate

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

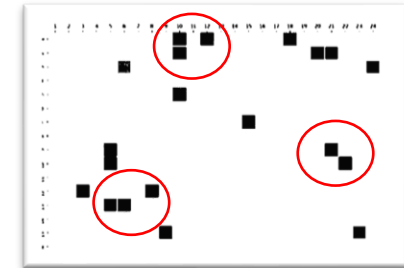
Experimental design (statistical best practice)

- Randomisation of controls and treatments [1–3]
- Evidence: spatial balance in randomisation reduces bias

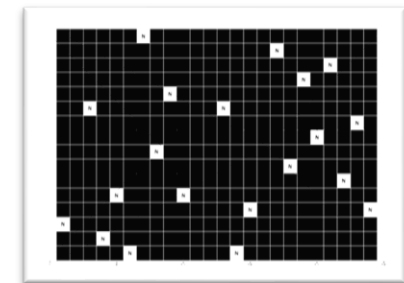
Practical limitations

- 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: Mazouze, 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.

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 Aware Plate Layouts

Results

- Improves dose–response estimation
 - Increases precision in drug screening
 - Practically feasible designs across diverse experimental settings
- } compared to naïve randomisation

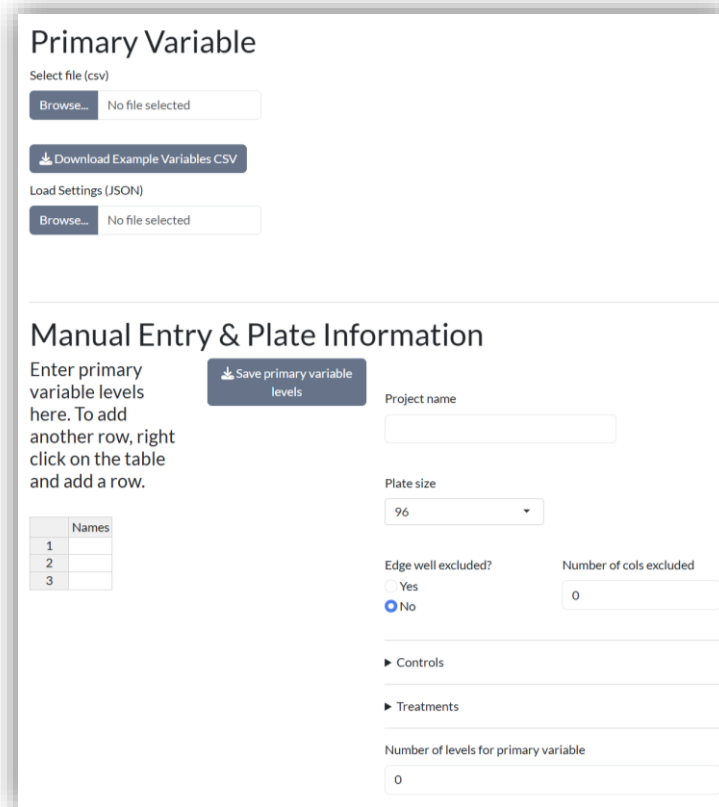
- 1: Normandeau, Frédéric, et al. "Spatial bias in antibody microarrays may be an underappreciated source of variability." *ACS sensors* 6.5 (2021): 1796-1806.
- 2: Mazouze, Bogdan, Robert Nadon, and Vladimir Makarenkov. "Identification and correction of spatial bias are essential for obtaining quality 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.



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).



The screenshot shows the SAPL R shiny app interface. It has a 'Primary Variable' section with file upload buttons for CSV and JSON. Below is the 'Manual Entry & Plate Information' section, which includes a 'Save primary variable levels' button, a 'Project name' field, a 'Plate size' dropdown (set to 96), and radio buttons for 'Edge well excluded?' (set to 'No'). There is also a 'Number of cols excluded' field (set to 0). At the bottom, there are expandable sections for 'Controls' and 'Treatments', and a 'Number of levels for primary variable' field (set to 0).

	Names
1	
2	
3	



Key features

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

Input

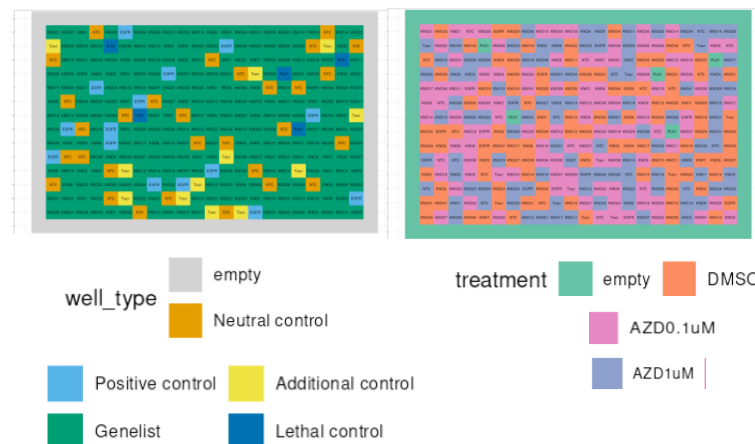
- Plate formats: **24-**, **96-**, and **384-well**
- **Flexible** inclusion of multiple positive, neutral, and lethal controls
- Accommodates **multiple treatments**, assigned freely or by column
- Generates **multiple plates** with shared or varying design features
- Customisation options:
 - Exclude 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. exports 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

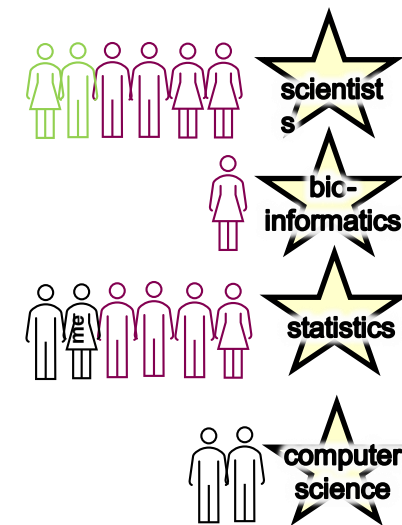
	Source Plate Name	Source Well	Transfer Volume	GeneID	Destination	Destination Well
1	Source Plate[2]	A1		480 KO1	Destination	D4
2	Source Plate[2]	A1		480 KO1	Destination	K10
3	Source Plate[2]	A1		480 KO1	Destination	K12
4	Source Plate[2]	A1		480 KO1	Destination	L16
5	Source Plate[2]	A1		480 KO1	Destination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



Background

- Originally designed for functional genomics in R&D in AstraZeneca in **2023**
- Conceptual 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

The team



Legend



Long-term plan

Until now: 2023-2025

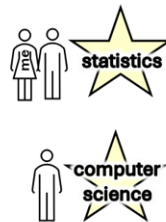
AstraZeneca consultancy project: 1 year of 2*FEC



- Action**
- Stake holder engagement
 - Meeting series to find consent
- Evidence**
- Joint report
- Action**
- Code reflecting methodology
 - Web-based tool for AstraZeneca (AZ) internal use
 - Tested on AZ use cases
- Evidence**
- Monthly count data from AstraZeneca: May: 8 users, June: 21, July: 13, average usage time: 60min

Short term: 2025-2026

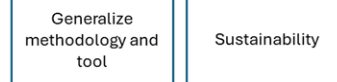
- IMI small grant covering 6.5 weeks of Daniel Burrows (MIRA),
- IMI: 4 months 0.8 FTE of my time
- Computer science in-kind contributions



- Action**
- Extensive simulation study for performance comparison
- Evidence**
- 1 publication
 - 2 presentations
- Action**
- Create an open access version of SAPL
 - Web-based tool
 - user-friendly and robust
- Evidence**
- Will collect data on users

Medium term: 2026-2030

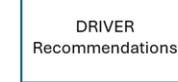
IAA Open Call, deadline 10 Nov 2025



- Action**
- Engage AZ, FGSL, Bath internal researchers
 - Understand the experimental needs
- Evidence**
- Generalized tool
 - Reflect in user data
- Action**
- For collaborators: integrate SAPL into standard workflows (as at AZ since 1/7/25)
 - Provide tailored on-site training to collaborators
- Evidence**
- Letter by collaborators

Long term: 2030-2035

Something like [Better methods, better research](#) by MRC



- Action**
- DRIVER – Designing and Reporting In Vitro Experiments Responsibly
 - National Centre for the Replacement, Refinement & Reduction of Animal Use
 - Collaborate with DRIVER team
- Evidence**
- Inclusion into DRIVER guidelines

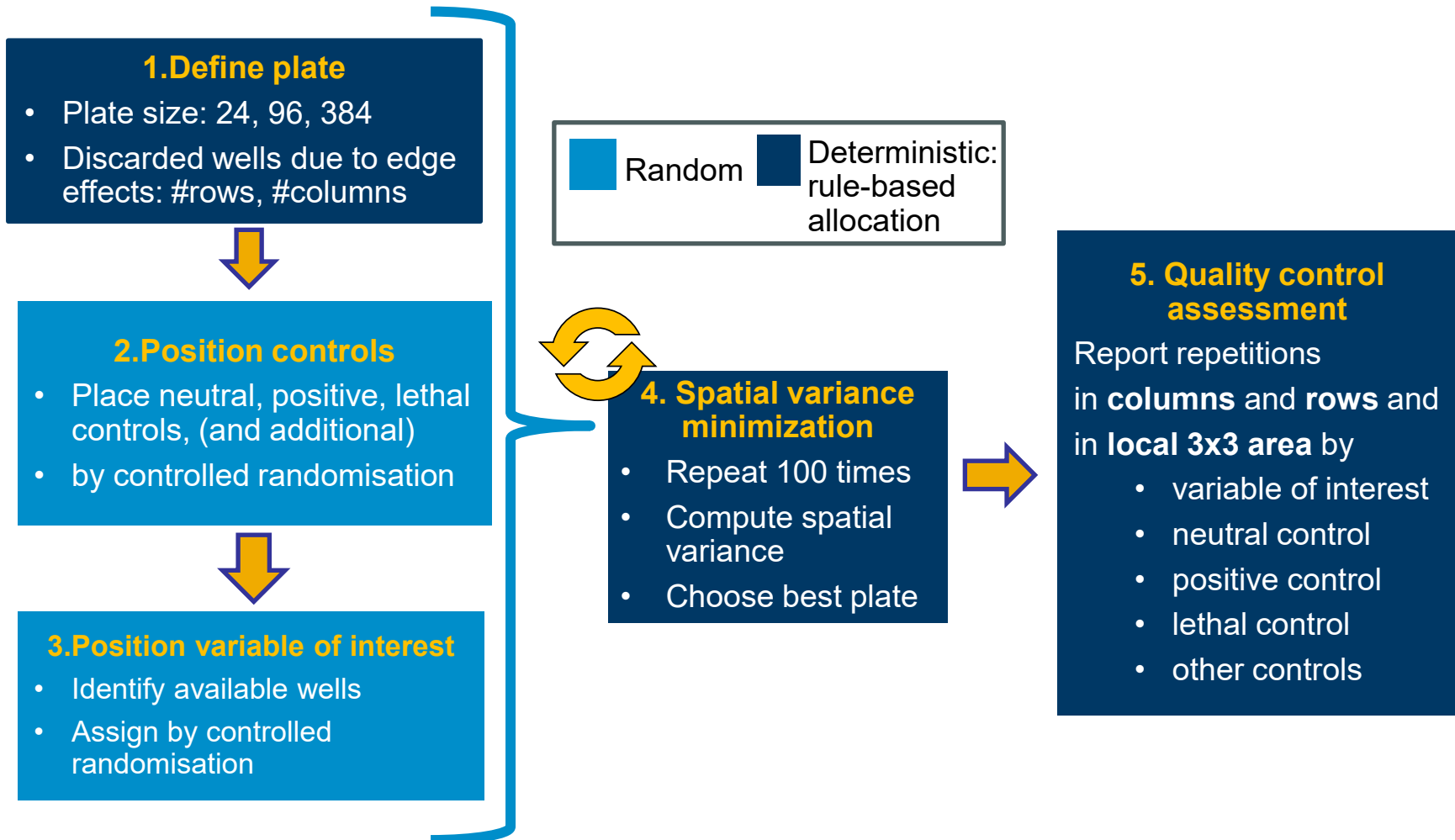
Impact
This approach enhances reproducibility and translatability of preclinical results, potentially reducing drug development costs and reliance on animal testing.

Legend



Statistical methodology



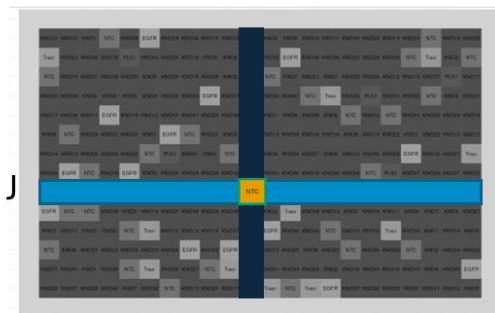


2. Position controls: controlled randomisation



Draw at random
without
replacement

J 12

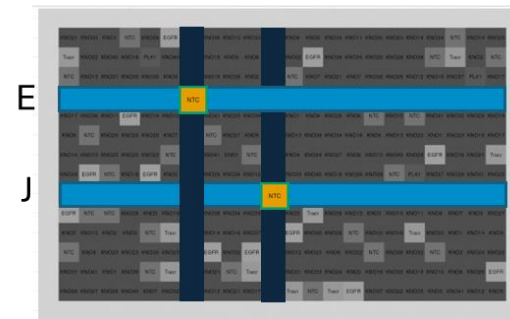


12



Draw at random
without
replacement

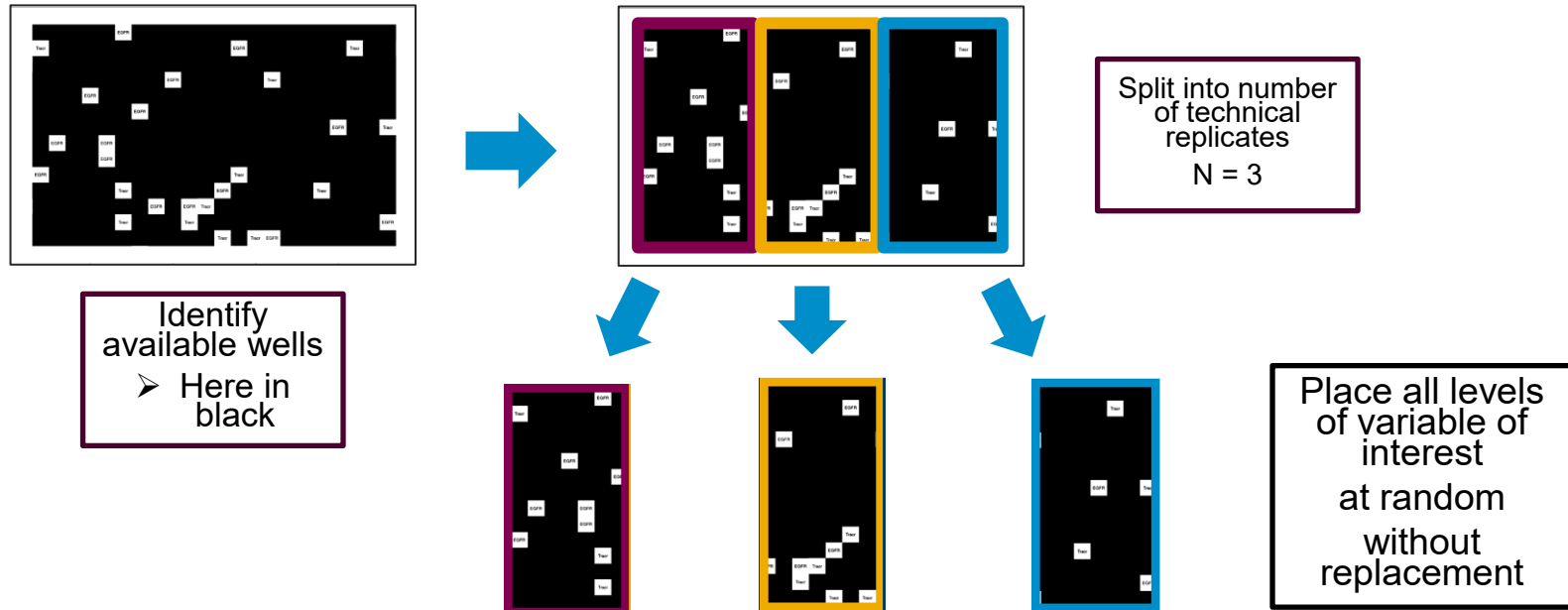
E 8



8 12

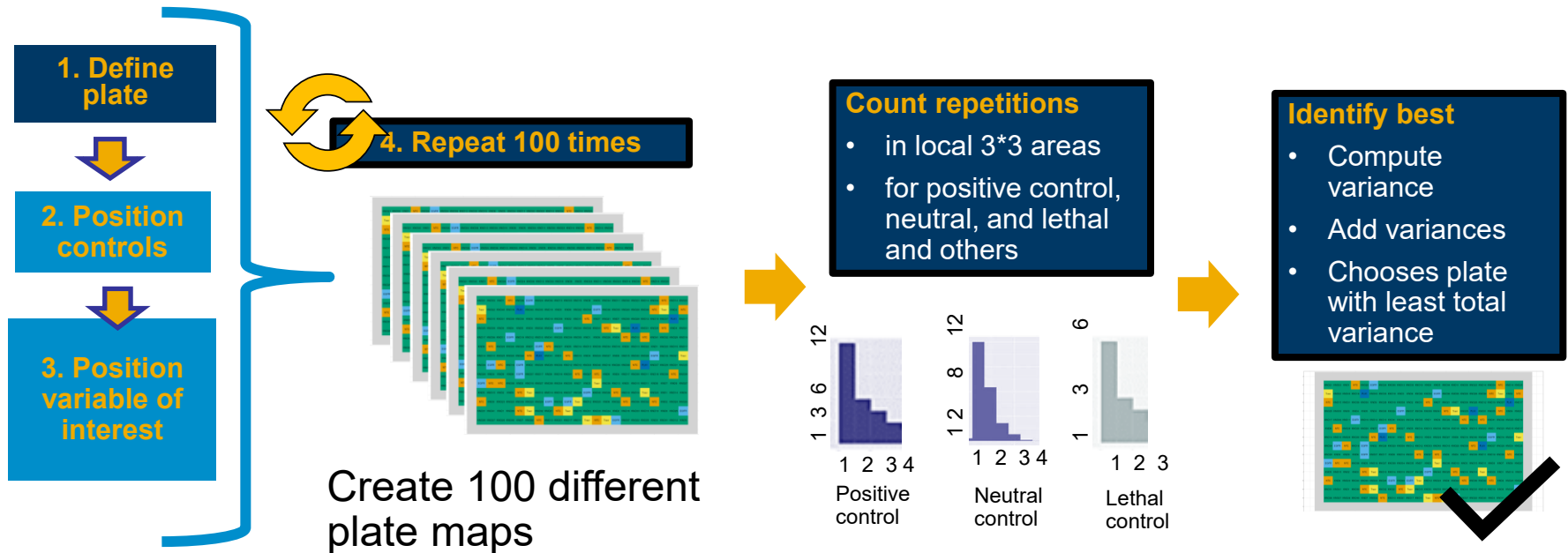
➤ Reduces the repetition of the controls in rows and columns

3. Position variable of interest: controlled randomisation



➤ Reduces the repetition of the levels of the variable of interest in columns

4. Spatial variance minimization I



➤ 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:

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

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



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, #columns

 Random  Deterministic: rule-based

2. Position controls

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

Scenario 1: no treatment
Scenario 2 & 3: treatment

- dispensable at random
- in columns

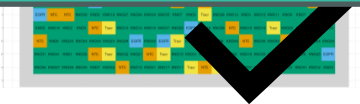
Quality control assessment

tions
and rows and
area by
le of interest
l control
re control
control

- other controls

3. Position variable of interest

- Identify available wells
- Assign by controlled randomisation



Live demo



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



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



Features SAPL



Enter data I: primary variable

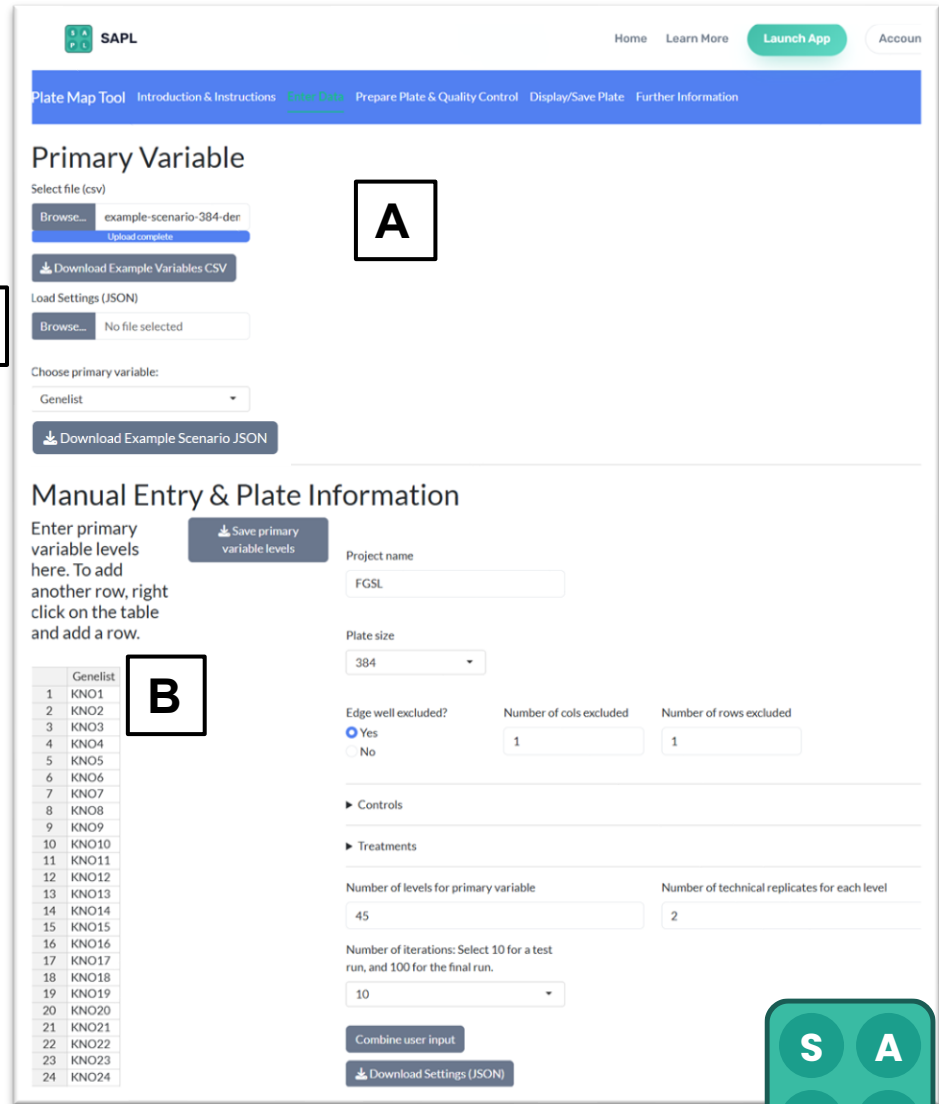
Enter **primary variable** = **gene knockout (gKO)**

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

Project name
FeedbackEvent

Plate size
384

Edge well excluded?
☒ Yes
☐ No

Number of cols excluded
1

Number of rows excluded
1

▼ Controls

Number of different positive controls
2

	name	no_reps
1	P1	6
2	P2	4

Number of different neutral controls
2

	name	no_reps
1	N1	7
2	N2	3

Place neutral control in the corners?
☐ Yes
☒ No

Pair positive and neutral control?
☐ Yes
☒ No

Number of different lethal controls
1

	name	no_reps
1	Leth	2

Number of different additional controls
1

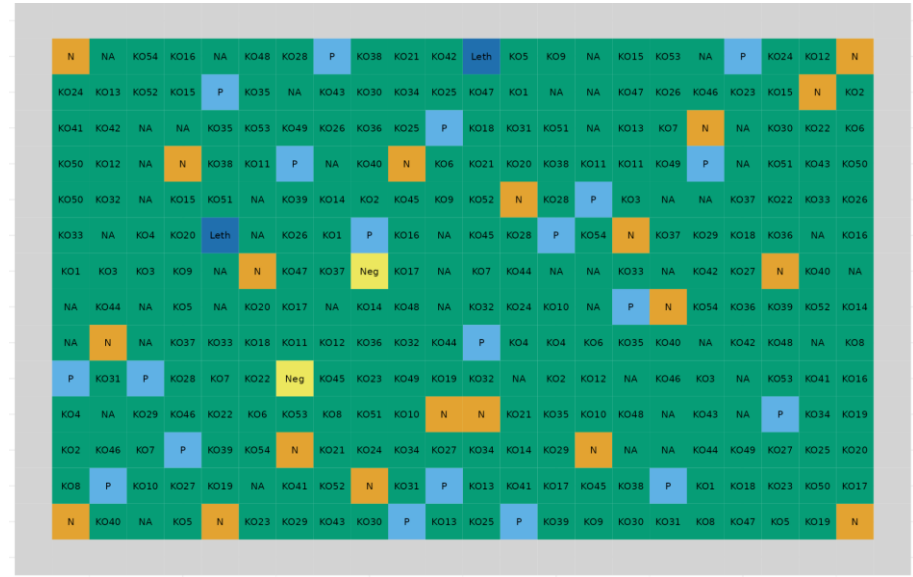
	name	no_reps
1	Neg	2



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

▼ Treatments

Number of different treatments

3

J

	name
1	AZD 0.1uM
2	AZD 1uM
3	GSK 1 uM

Combine control(s) with treatment?

☒ neutral

☒ positive

☐ additional

K

Vehicle present?

☒ Yes

☐ No

L

Vehicle Name

V

Treatment dispensed in columns?

☒ Yes

☐ No

M

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

▼ Treatments

Number of different treatments

3

	name
1	AZD 0.1uM
2	AZD 1uM
3	GSK 1 uM

Combine control(s) with treatment?

☒ neutral

☒ positive

☐ additional

Vehicle present?

☒ Yes

☐ No

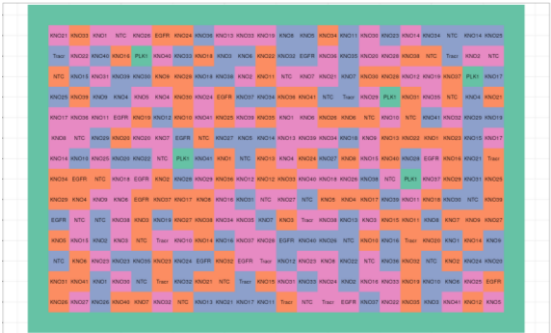
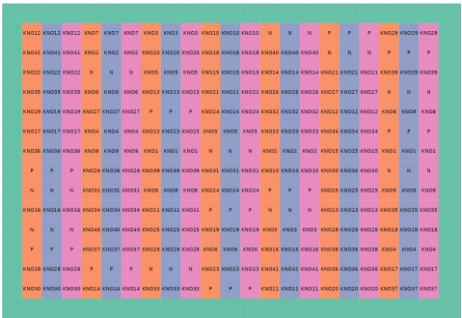
Vehicle Name

V

Treatment dispensed in columns?

☒ Yes

☐ No



treatment None AZD 0.1uM AZD 1uM V



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

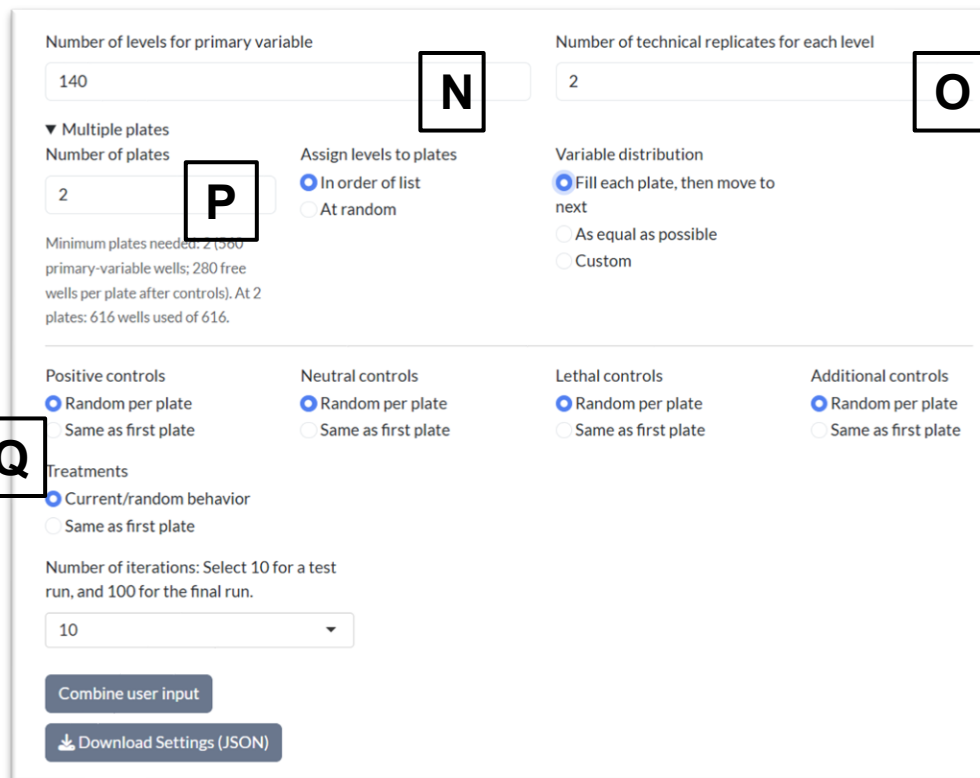
- How many wells with the exact same filling would you like per gene?

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



The screenshot shows a web form for entering data for multiple plates. The form includes the following fields and options:

- Number of levels for primary variable:** A text input field containing the value 140, labeled with a black box containing the letter **N**.
- Number of technical replicates for each level:** A text input field containing the value 2, labeled with a black box containing the letter **O**.
- Multiple plates:** A section with a dropdown menu set to "Multiple plates" and a text input field containing the value 2, labeled with a black box containing the letter **P**.
- Assign levels to plates:** Two radio button options: "In order of list" (selected) and "At random".
- Variable distribution:** Three radio button options: "Fill each plate, then move to next" (selected), "As equal as possible", and "Custom".
- Minimum plates needed:** A text input field containing the value 2, labeled with a black box containing the letter **Q**.
- Positive controls:** Two radio button options: "Random per plate" (selected) and "Same as first plate".
- Neutral controls:** Two radio button options: "Random per plate" (selected) and "Same as first plate".
- Lethal controls:** Two radio button options: "Random per plate" (selected) and "Same as first plate".
- Additional controls:** Two radio button options: "Random per plate" (selected) and "Same as first plate".
- Treatments:** Two radio button options: "Current/random behavior" (selected) and "Same as first plate".
- Number of iterations:** A text input field containing the value 10.
- Buttons:** "Combine user input" and "Download Settings (JSON)".



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

Number of iterations: Select 10 for a test run, and 100 for the final run.

10

▼

Combine user input

Download Settings (JSON)

R

S

T



Enter data V: final section

R: Number of iterations

S: Combine user input

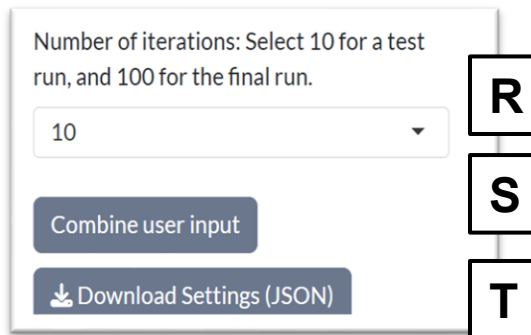
When you press this button all data above will be joint to one input file.

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Transparency

Speed up the process for next experiment if similar



Number of iterations: Select 10 for a test run, and 100 for the final run.

10

Combine user input

Download Settings (JSON)

R

S

T



Enter data V: final section

R: Number of iterations

S: Combine user input

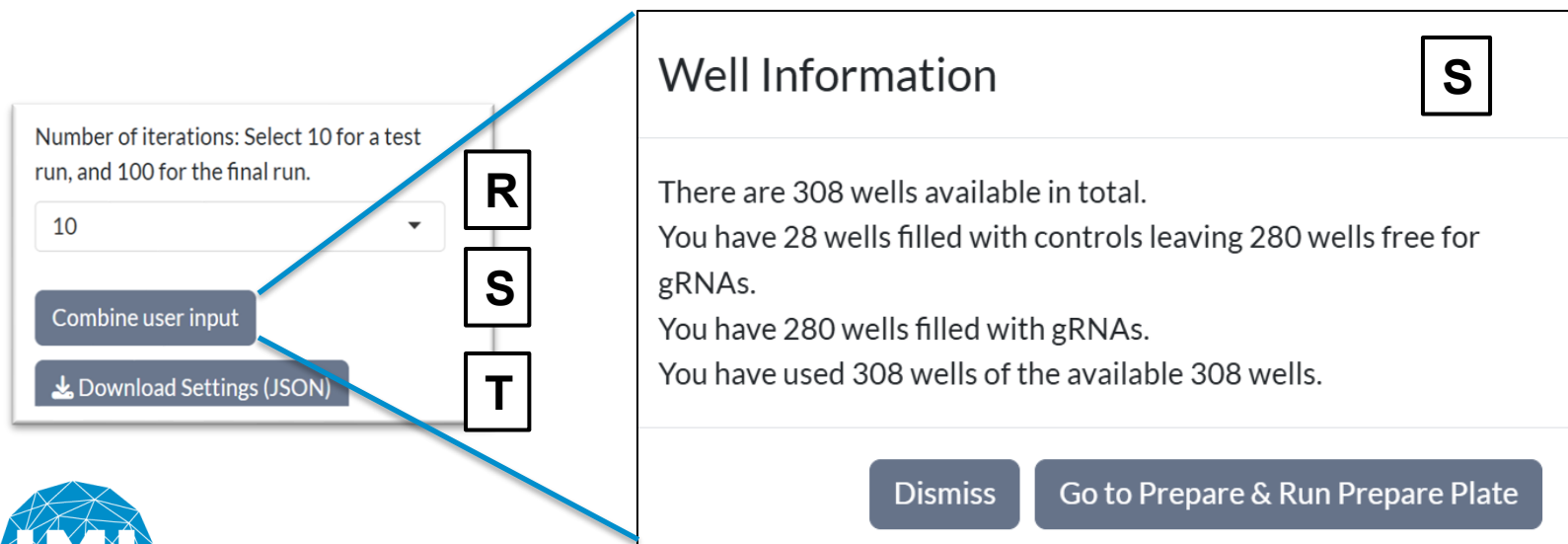
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Transparency

Speed up the process for next experiment if similar



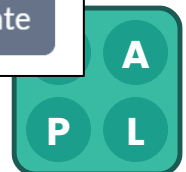
Well Information **S**

There are 308 wells available in total.
You have 28 wells filled with controls leaving 280 wells free for gRNAs.
You have 280 wells filled with gRNAs.
You have used 308 wells of the available 308 wells.

Dismiss Go to Prepare & Run Prepare Plate

Callouts:

- R:** Points to the 'Number of iterations' dropdown menu.
- S:** Points to the 'Combine user input' button.
- T:** Points to the 'Download Settings (JSON)' button.



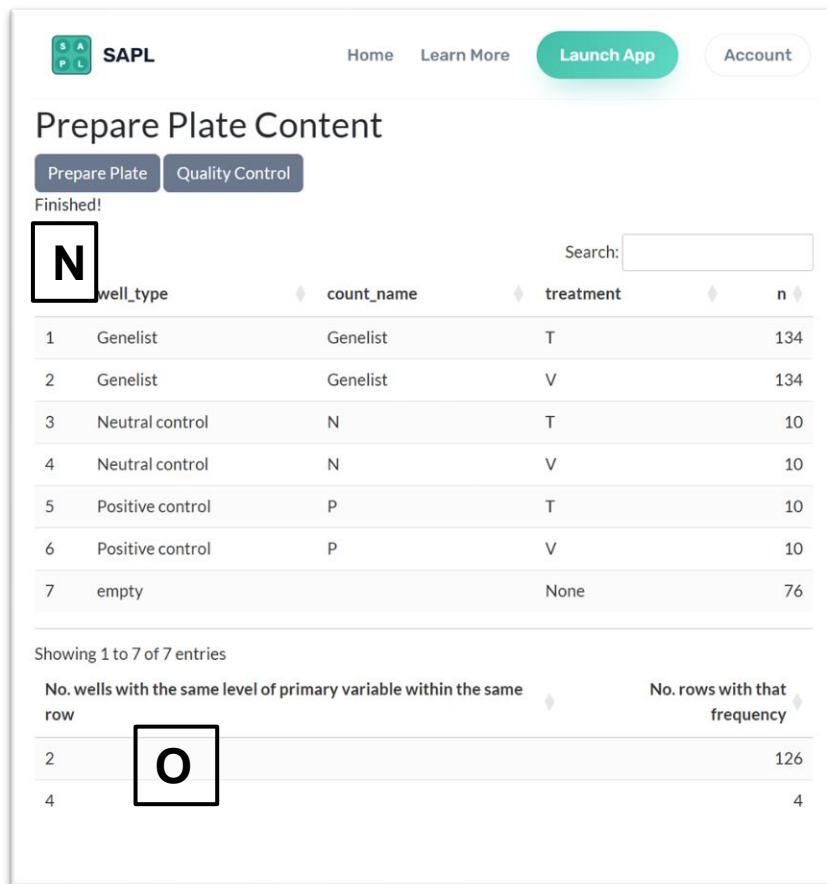
Prepare Plate & Quality Control

N: Table of counts of wells by

- well type
- name
- Treatment

O: quality control

- Tables of counts for repetitions by
 - Rows
 - Columns
 - 3x3 areas



SAPL Home Learn More Launch App Account

Prepare Plate Content

Prepare Plate Quality Control

Finished!

N

Search:

	well_type	count_name	treatment	n
1	Genelist	Genelist	T	134
2	Genelist	Genelist	V	134
3	Neutral control	N	T	10
4	Neutral control	N	V	10
5	Positive control	P	T	10
6	Positive control	P	V	10
7	empty		None	76

Showing 1 to 7 of 7 entries

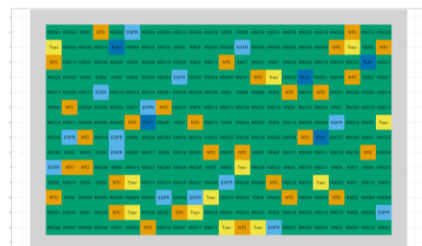
No. wells with the same level of primary variable within the same row	No. rows with that frequency
2	126
4	4

O

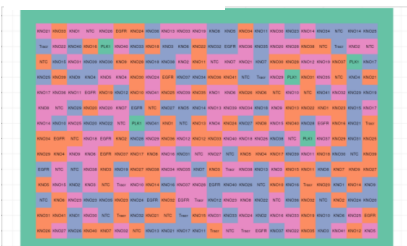
Display & Save Plate

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

