

# A Constraint Programming Approach to Microplate Layout Design

María Andreína Francisco Rodríguez<sup>[0000–0001–8745–9858]</sup> and  
Ola Spjuth<sup>[0000–0002–8083–2864]</sup>

Department of Pharmaceutical Biosciences, Uppsala University, Sweden  
`{maria.andreina.francisco,ola.spjuth}@farmbio.uu.se`

## Introduction

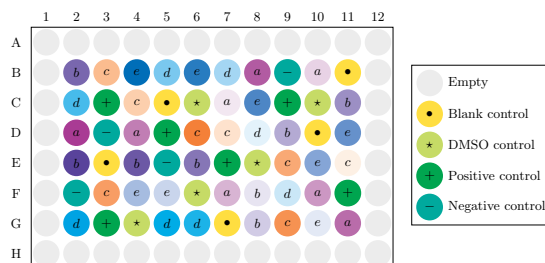
Microplates were introduced in the early 1960s [3], and since then they have become an integral part of daily laboratory work in many disciplines, including drug discovery and clinical routine testing [2]. A *microplate* is a flat surface that typically consist of a 2:3 matrix of wells, such as 96 or 384 wells. A 96-well microplate can be seen in Figure 1. Microplates can be handled both manually and by robots, and they are the main media used in high-throughput assay systems. An *assay* [1] is procedure for assessing or measuring the presence, amount, or function of a particular target (like a cell or a drug). Most assays give one result per well, but it is also possible to perform time-resolved assays with one measurement per time-point per well. In high-throughput settings, thousands of measurements are generated by automatic plate handling robots.

Assays commonly exhibit a systematic variation across the geometry of the plate. Factors such as well location, temperature, and humidity are unequally distributed and can affect the results to the point of rendering the assay unusable. In consequence, we need controls to be distributed in a statistically secured way over the plates. A *control* is a compound with the same characteristics as those in the experimental group, but which is being subjected to a different kind of treatment [8]. Controls are designed with the goal of accounting for the effects of variables other than what is being tested, thus increasing the reliability of the results. Proper experimental design, including blocking and randomization of experimental samples and conditions, can help reduce unwanted bias and control for potential plate or batch effects.

Traditionally in biomedical research, microplate layouts have been designed manually, following patterns that intuitively distribute controls and compounds over several plates. More recently, some tools have been developed [2,9], most of which still require a human in the loop, and none of which is easily customizable.

Our goal is to design an automated laboratory system capable of iteratively designing experiments, execute them, evaluate them, and based on the results, repeat the process again. Towards this goal, we designed a flexible model that 1) helps researchers plan well-designed experiments reducing the rate of (partial) microplate rejection, and 2) will be a key component of our automated robotic laboratory system. We also believe this is the *first* attempt to use constraint programming to design microplate layouts.

**Fig. 1.** Example of a microplate with 96 wells. Letters represent compounds, while the 8 color intensities represent the concentrations.



## Problem Description

Given the details of an experiment, the aim of the Microplate Layout Design Problem is to decide the content of each well for a set of microplates in such a way that we can account for variations due to the well location and reduce the rate of microplate rejection. Typically, the user specifies the number of compounds and concentrations to be tested, as well as the number of *replicas*, that is, how many times each compound should be tested in all the concentrations. The user also indicates the amounts and types of controls needed. Based on discussions with experienced researchers, we have formulated the following hard constraints:

- The outermost rows and columns should be left empty in order to reduce errors due to the *edge effect* [6]. The *edge effect* is a discrepancy between the center and outer wells primarily caused by evaporation [4].
- For each compound, all concentration levels of a given replica must appear on the same plate.
- If possible, the replicated compounds should appear on a different plate.
- All controls are distributed evenly across all plates.
- Controls of the same kind are separated by at least 2 wells in any direction.
- The contents of the wells should be randomly distributed.

We also implemented some variants and extensions to the microplate layout design problem, which can be summarized as follows: 1) the amount of each type of control is given per plate, 2) plate dimensions given in rows and columns, 3) the location of (some of) the controls is given, 4) replicas must be located on different wells, 5) allowing the use of the outermost wells, 6) specifying the distance between controls of the same type.

We implemented our model and its variants in MiniZinc [7], with Gecode [5] as solver. Initial results are quite promising, solving all instances in a few seconds.

## Conclusion

We developed first fast and flexible model, and although it is very much tailored for our work at the Pharmaceutical Bioinformatics research group at Uppsala University, it is general enough that it can be adopted by other groups. We are currently working on generalizing and improving our model even further, which we will then integrate into our laboratory information system, and which will later be a part of the automated robotic laboratory system we are building.

## References

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