Analysis of Compound Combination Experiments

With recent improvements in automation and liquid handling, compound combination experiments can be executed at higher throughput than ever before. However, software tools have not kept up with this increase and often still require either a manual, per-combination workflow or cover just the synergy calculation without the necessary pre-processing steps. In this application note we describe how compound combination experiments should ideally be analysed.

The earliest work formulating a systematic methodology for assessing combination effects was done by Loewe and Fraser (1928, 1872). The principal assumption is that two compounds combined in one organism may have a different effect than the two compounds individually. The whole purpose of compound combination studies is to identify and quantify effects such as synergism and antagonism in the organisms under study.

### Plate layout

In compound combination experiments, each well carries either one compound (mono-therapeutic curves) or two (combination curves). The pipetting scheme can be classic with concrete concentration matrices on each plate, or optimised for higher throughput experiments where the layout is not obvious by looking at the plates. In either case, it is important to have a software system that stores the layout of the well content and provides this information to a suitable data analysis system.

### Data normalisation

The raw data coming from the instrument has to be normalised with respect to control wells, for instance by applying a 2-point normalisation for inhibition experiments. Depending on the application, a more complex normalisation might be required, where for example cell growth is taken into account by incorporating the initial cell count measured shortly after cell seeding.

### Curve fitting

With two compounds in every well, two sets of dose response curves have to be processed: one compound is fitted while the concentration of the other compound is kept constant. In cell growth assays, the response can exceed the stimulation control, and the fitting process has to account for this. It also has to take into account that if one of the compounds is considered an outlier and masked, the same well cannot be valid in the other fit. The fitted mono-therapeutic or combination curves can be overlaid for a first visual inspection of the results.

### Model generation

Literature describes different models that can be used for synergy calculations; here we will just mention Loewe additivity, which differentiates and quantifies synergistic or antagonistic effects of compounds acting on the same target with the same mechanism.

To calculate the additive effect for each combination well, the two mono-therapeutic curves are combined by applying the additivity model. Similarly, synergistic or antagonistic effects are predicted by applying the respective models. A plot showing the resulting activity matrix for the model can act as an important visual reference.

### Effect assessment and quality control

The last step to determine synergism is to subtract the measured activities for each combination well from the result of the model calculation. The differences are summed up, and if the sum is zero, there is no synergism. For a large combination experiment with hundreds or thousands of combinations, the numerical results (often expressed as Synergy Scores) are sorted to identify promising combinations. The visualisation of measured and modelled data, as well as their differences, is a crucial QC step to make sure the individual combinations were measured successfully. An additional, common way to express synergy while assessing its proper measurement is through isobolograms.

For more information:

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Figure 1: Overlay in Genedata Screener for combination screening: Different dose response curves of compound A at increasing concentrations of compound B, showing the shift of IC50 as dependent of compound.

Figure 2: Results of a single combination from a large combination screen as visualised in Genedata Screener.