Technical Note

Power Up Your Assay Development: **DOE Made Easy on icon96™**

Introduction

Optimization of PCR assays for assay development (AD) workflows often involves multiple interdependent factors, including thermocycling conditions, enzyme levels, buffer chemistry, and oligonucleotide concentrations. Conventional thermocyclers restrict flexibility because all wells on a plate must share the same cycling conditions. This forces researchers to split experiments across multiple runs, leading to inflated workload, increased consumable use, and longer turnaround times.

Design of Experiments (DOE) is a powerful framework for navigating these complex parameter spaces in AD workflows. A classic study by Whitcomb & Kraber (Stat-Ease)[1] demonstrated optimization across nine factors, including three thermocycler settings and six reagent parameters. On legacy thermocyclers, such studies require split-plot designs in which thermocycler factors are fixed across entire plates.

Figure 1. icon96™, the only thermocycler with 96 independently controlled wells.

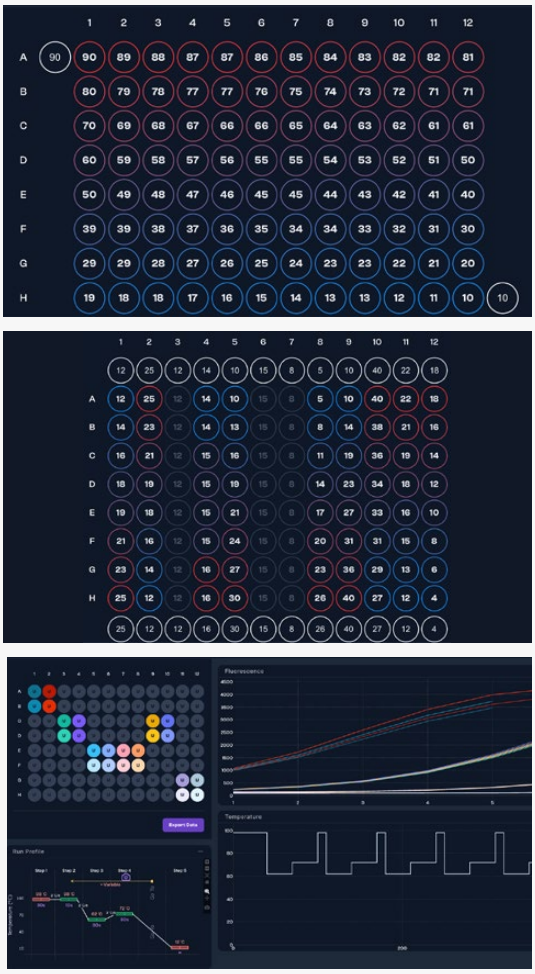

Figure 2. icon96 combines thermocycling and real-time analysis with:

- **96 independent wells** for fully randomized DOE designs.
- **Linear gradients** enabling broad temperature sweeps.
- **Intuitive visualization tools** for amplification curves, temperature profiles, and reagent conditions.

The icon96 platform, powered by iconPCR, removes this constraint. With 96 independently controlled wells, more than 80°C linear gradients, and built-in AutoNorm™ real-time normalization, icon96 allows multiple thermocycler conditions to be optimized within a single plate. This application note models how Whitcomb & Kraber's DOE could be executed on icon96.

DOE Workload Comparison

In the Whitcomb & Kraber DOE design, the six reagent parameters (forward primer concentration, reverse primer concentration, DNA probe concentration, $MgCl_2$ concentration, Tween concentration, and polymerase concentration) can all be tested within a single PCR plate.

- Legacy thermocyclers: While some models include temperature gradient options, they lack true per-well control. Because 32 wells per plate are consumed by reagent combinations, gradient features cannot be applied simultaneously. Each thermocycler condition (annealing temperature, denature temperature, denature time) must therefore be run as a separate plate (Figure 2A).
- icon96: Independent well control allows multiple annealing and denature temperatures to be run in the same plate (Figure 2B). This reduces the number of plates required by 50%. Unlike conventional systems, edge effects are eliminated with icon96 since each well is independently monitored and precisely maintained at its set temperature, allowing utilization of the entire plate.

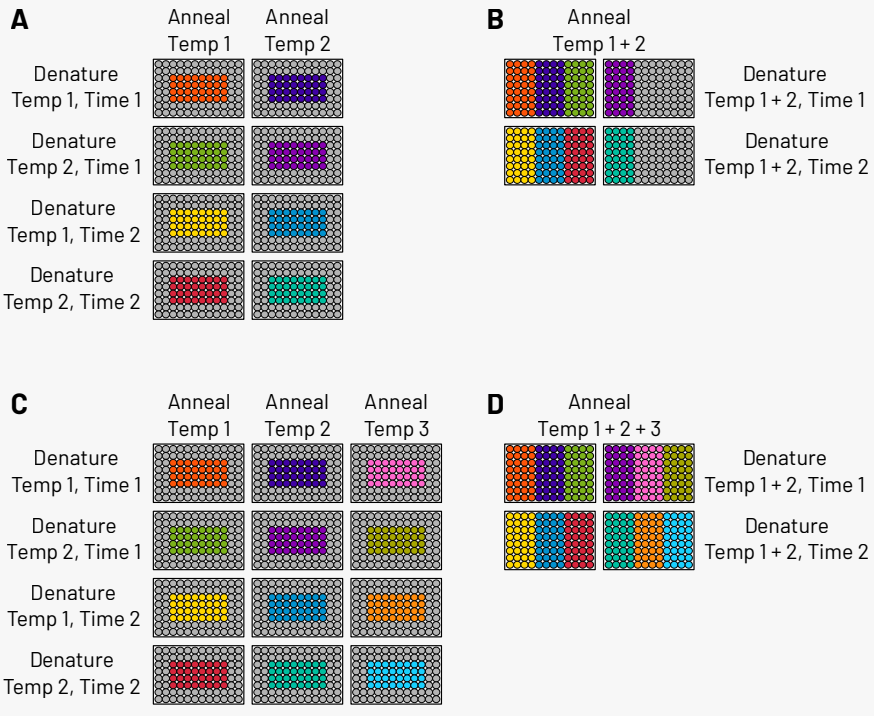

Figure 3. Example of DOE plate setup A) Legacy thermocycler conditions are locked plate-wide, requiring 8 separate PCR runs. B) icon96 allows multiple annealing and denature conditions in a single plate, reducing runs by 50%. C) Adding an extra annealing temperature to legacy systems increases plates by 50%, totaling 12 runs. D) Addition of another annealing temperature can be incorporated into existing plates with no increase in runs on icon96.

Scalability Example

Adding a single annealing temperature to the DOE increases the number of plates on legacy thermocyclers by 50% (Figure 2C). On icon96, the same additional temperature can be incorporated within unused wells without adding plates (Figure 2D).

Scaling the number of factors further illustrates the impact of icon96. For example, a design testing six PCR factors with five annealing and three denature conditions would require 15 plates on a legacy system but only 5 plates on icon96, saving 20 hours (h). At the high end, designs with 10 annealing temperatures and multiple denature settings can demand 60 plates on legacy thermocyclers, but just 10–20 plates on icon96. In these cases, the time savings can exceed 80–100 h, in addition to the dramatic reduction in consumable use (Table 1).

# PCR Factors	# Anneal Temps	# Denature Temps	# Denature Times	# Legacy Plates Needed	#icon96 Plates Needed	Time Savings (h)
4	2	2	1	4	**1**	**6**
4	5	3	1	15	**3**	**24**
4	2	2	2	8	**2**	**12**
4	3	2	2	12	**2**	**20**
4	10	3	2	60	**10**	**100**
6	2	2	1	4	**2**	**4**
6	5	3	1	15	**5**	**20**
6	2	2	2	8	**4**	**8**
6	3	2	2	12	**4**	**16**
6	10	3	2	60	**20**	**80**

Table 1. Plates required for DOE on legacy thermocyclers vs. icon96. Time savings calculated at 2 h per plate. Across these representative designs, icon96 reduces plate usage by 50–80%, corresponding up to 100 h of time saved depending on study size.

Conclusion

icon96 is revolutionizing PCR assay development. DOE studies that once demanded dozens of plates and untold hours at the thermocycler can now be completed with fewer plates, reduced hands-on time, and richer data—all while trimming consumable usage. By eliminating split-plot headaches and building in per-well normalization, icon96 makes full-factorial optimization truly practical and scalable. For assay developers, that means faster iteration, smarter reagent use, and higher-quality data every step of the way.

Every assay development journey is a bit different—let's make yours easier. Reach out to discover how icon96 can turbocharge your optimization workflows.

References

1. Whitcomb, P. J., & Kraber, S. (2007). PCR Process Optimized via Split-Plot DOE. Stat-Ease, Inc. White Paper. Minneapolis, MN.

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