sfGFP process optimization, quad 96-well incubation: processopt_20261008

Titers, replicate CVs and comparison with the 384-well run jewett_20260824 (same 80 designs, same lysate lot)

2026-10-09

Summary

- **Median titer 2.71 g/L** over 318 design wells: 80 designs × 4 replicates, less two isolated dropouts. The target control reached 2.65 g/L (n = 8), and the no-template negatives read 0.04 g/L.
- **Best designs** are Ginkgo3-based:
  - glucose 19 mM: 4.23 g/L
  - K-phosphate 35 mM: 4.08 g/L
  - NTP 2 mM: 4.04 g/L
  - K-phosphate 30 mM: 3.95 g/L
- **Replicates are tight.** Median replicate CV is 7.2%, against 14.6% in the 384 run. Only 4 of 80 designs exceed 20% CV, against 32 in 384.
- **Against the 384-well run jewett_20260824 (39-L lysate)**, the quad 96-well format yields **7.3× more sfGFP** (median per-design fold; IQR 4.5–13.6). The two runs share identical recipes and the same lysate lot. The median design well is 2.71 g/L here against 0.36 g/L in 384, and the target control is 2.65 against 0.35 g/L.
- **The ranking does not transfer between formats.** Spearman ρ = −0.12 across the 80 designs, and none of the top 10 designs is shared. The 384 top 9 are all PANOx; in quad, PANOx ranks 58–72 and gains only 2.7×, against 7.3× for Ginkgo3 and 15× for RFopt.
- **Only the K-phosphate response is preserved** (block ρ = 0.84). In quad, the low-Mg recipes that fail in 384 make 1.8–2.7 g/L. Glucose, which lowers yield in 384, raises it in quad.
- **The format effect is reproducible.** The previous quad run (processopt_20260922) showed the same pattern against this 384 reference: 5.8× fold, ρ = 0.02 and no shared top-10 designs.

Runs compared

	processopt_20261008 (this run)	**jewett_20260824_20260722-39-L (reference)**
Design	`r1_sfgfp_2026-08-24_d77ff894`, 80 designs × 4	same plate design; 80/80 sample ids, identical recipes
Format	Echo hitpick into 384 → four 96-well DWPs (40 µL reactions) → compression to a 384 read plate	20 µL reactions in a standard 384 CFPS plate, read directly
Lysate	20260722-39-L	20260722-39-L
Base buffer	20260512-2-BB	**20260721-4-BB**
Stamp → read	≈20.1–20.3 h incubation, compression, then a re-read about 3.6 h later	≈20 h (bravo stamps end to Spark read)
Read	Spark re-read, samples diluted 2× by hand (standards undiluted)	single Spark read
Standards	6 levels, 3.72–118.9 µM, 8 wells/level; log-log fit, R^2 0.991	15 levels, 0.93–119 µM, 3 wells/level; R^2 0.993
Excluded dropouts	N21, O18	D21, K20, L20, N14, N21
Datastore	experiment 104985	experiment 103950

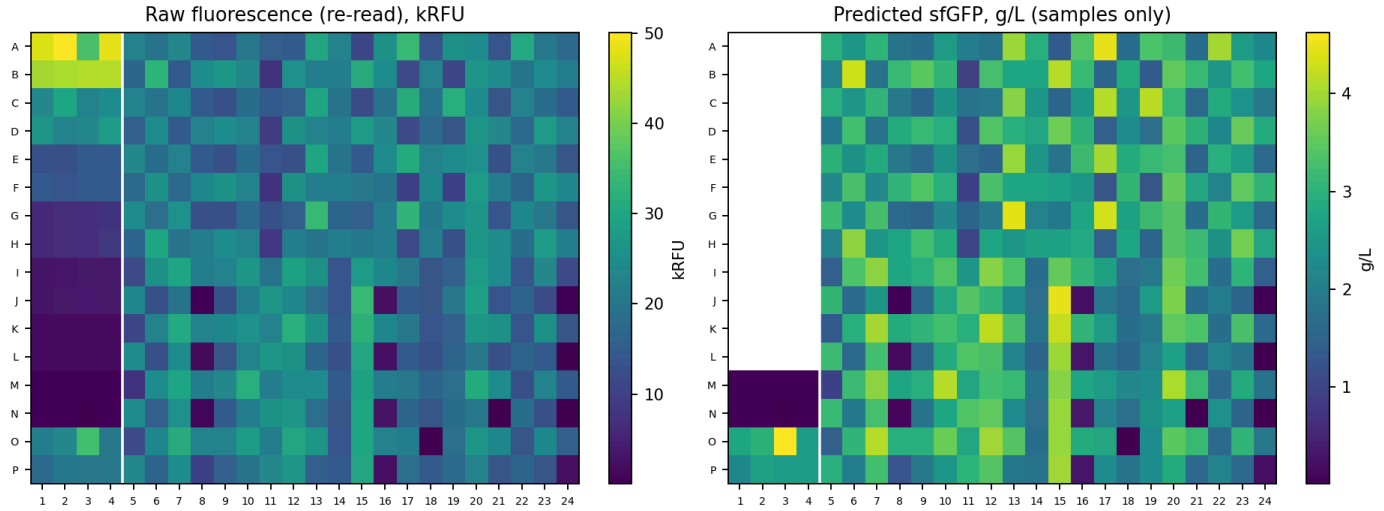
Design overlap. All 80 design sample ids are shared. Every Echo-dispensed reagent concentration matches per design, and the lysate, base buffer and template volumes are identical. The lysate lot is the same, verified on both lysate stamps (1878185, 1878180) and the 384 CFPS plate. The base buffer lot differs, which is a confound alongside the format and run date.

Read used. In the first read the samples exceeded the top standard. The samples, but not the standards, were then diluted 2× by hand and re-read (protocol run `d3db49c5`), and only the re-read is used. The data confirm the dilution: between reads the standards rose by the gain change (slope 1.53) and the samples fell (slope 0.75), which implies a **2.03×** sample dilution. All sample predictions are corrected by 2×.

Plate identity. Each quadrant's designs were placed on the 384 read plate using the Echo transfer log of the hitpick plate. All 312 recipe wells match exactly under one quadrant arrangement and no other.

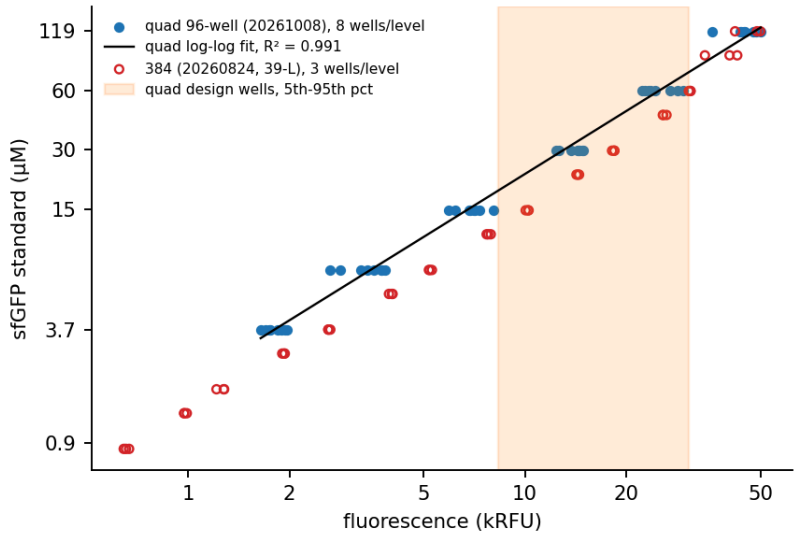
Dropouts. In both runs, a well is excluded when it reads below 20% of the median of its replicates and those replicates are healthy (≥ 3 kRFU). Designs that are low in every replicate are kept.

Plate heatmap



Raw fluorescence of the re-read (left) and dilution-corrected sfGFP for reaction wells (right). Columns 1–4 hold the reserved wells: standards in rows A–L, negatives in M–N, target controls in O–P. The standards are blanked on the right because their g/L is not a titer. Dark design wells are mostly the PANOx low-Mg and zero-K-phosphate designs, plus the two dropouts N21 and O18. The bright reserved well O03 is a target-control replicate reading 4.6 g/L against 2.2–3.0 g/L for the others.

Standard curves

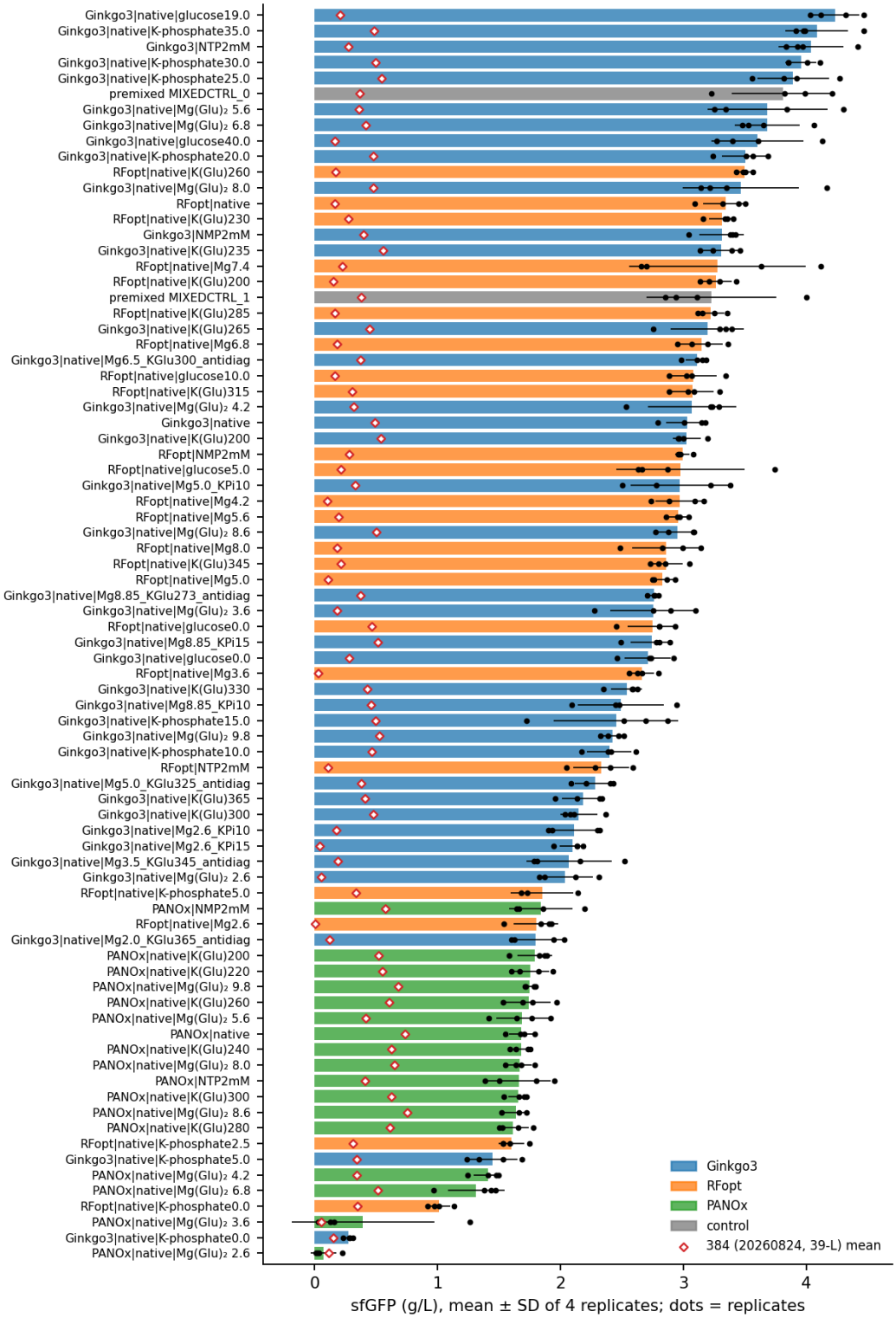


sfGFP standards of both runs against fluorescence, with this run's log-log fit. The shaded band is the central 90% of this run's design wells, all within its calibrated range. Back-calculated quad levels are 3.71, 7.47, 15.5, 33.3, 57.1 and 110 µM, with replicate CV 7–13% per level.

Each run is calibrated on its own standards. The concentration scale is the same: the top standard is 118.9–119 µM in both runs, and the quad standards pass through the same compression as the samples.

The curves differ in shape. Over the sample range, the same fluorescence maps to about 1.3× more sfGFP on the quad curve than on the 384 curve. The reads were taken at different gains, so this is not a direct matrix comparison. Even if all of it were a calibration bias, it would account for at most 1.3× of the 7.3× fold.

Titers by design



Mean ± SD sfGFP per design in this run (dots are the four replicates), coloured by base formulation. Red diamonds are the 384-run mean for the same design.

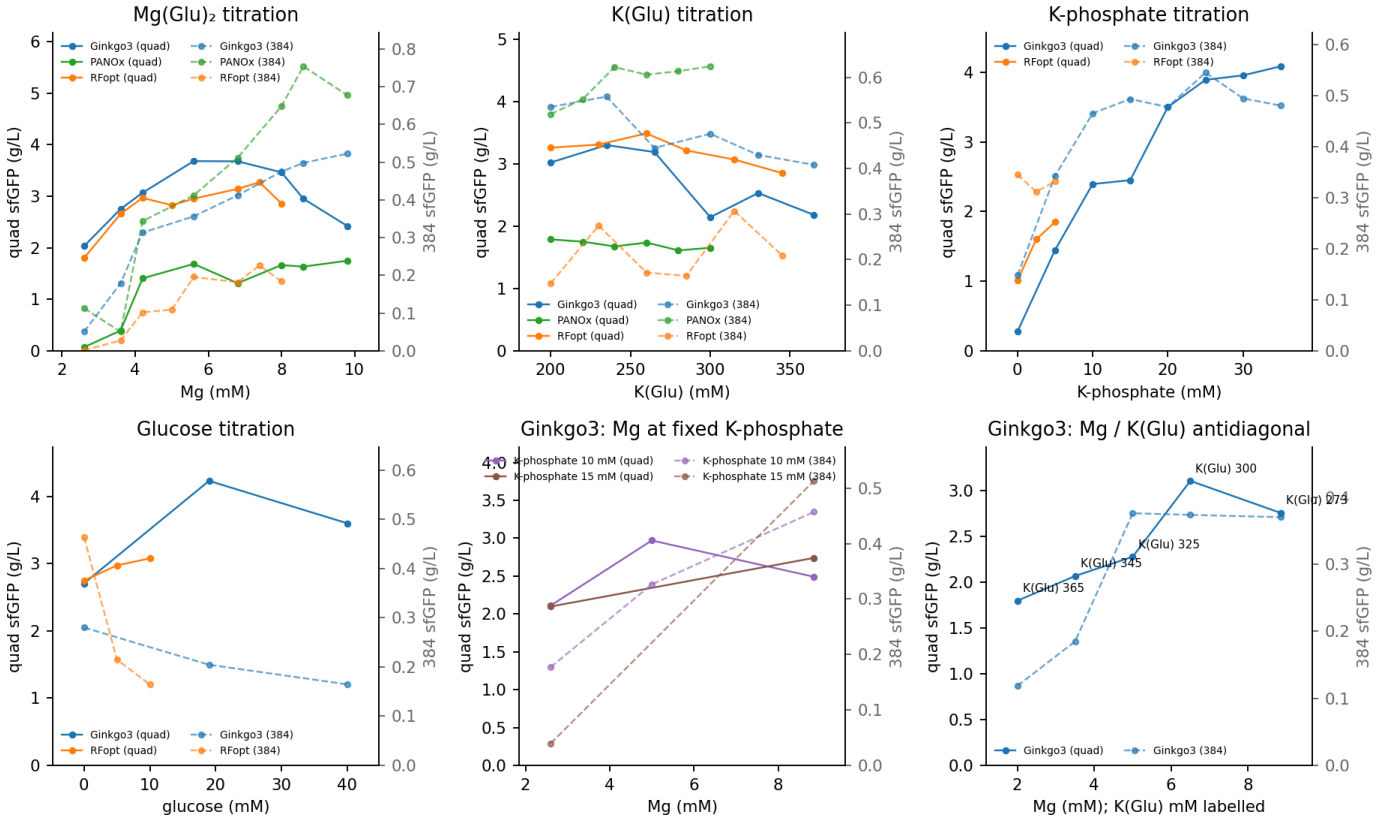
Design	Quad g/L (mean ± SD)	CV %	Quad rank	384 g/L	384 CV %	384 rank	Fold
Ginkgo3 | glucose 19	4.23 ± 0.20	5	1	0.20	42	57	20.7
Ginkgo3 | K-phosphate 35	4.08 ± 0.26	6	2	0.48	12	22	8.5
Ginkgo3 | NTP 2 mM	4.04 ± 0.26	6	3	0.27	53	53	14.8
Ginkgo3 | K-phosphate 30	3.95 ± 0.12	3	4	0.49	6	19	8.0
Ginkgo3 | K-phosphate 25	3.89 ± 0.29	8	5	0.55	22	12	7.1
Premixed control 0	3.81 ± 0.42	11	6	0.37	5	40	10.3
Ginkgo3 | $Mg(Glu)_2$ 5.6	3.68 ± 0.49	13	7	0.36	9	41	10.3
Ginkgo3 | $Mg(Glu)_2$ 6.8	3.68 ± 0.26	7	8	0.41	15	31	8.9
Ginkgo3 | glucose 40	3.60 ± 0.38	10	9	0.17	20	66	21.9
Ginkgo3 | K-phosphate 20	3.50 ± 0.19	5	10	0.48	19	23	7.3
RFopt | K(Glu) 260	3.49 ± 0.06	2	11	0.17	15	64	20.3
Ginkgo3 | $Mg(Glu)_2$ 8	3.46 ± 0.47	14	12	0.47	7	25	7.3
…							
PANOx | $Mg(Glu)_2$ 4.2	1.41 ± 0.11	8	75	0.34	58	43	4.1
PANOx | $Mg(Glu)_2$ 6.8	1.31 ± 0.23	18	76	0.51	29	17	2.6
RFopt | K-phosphate 0	1.01 ± 0.09	9	77	0.35	3	42	2.9
PANOx | $Mg(Glu)_2$ 3.6	0.39 ± 0.58	148	78	0.05	60	76	7.7
Ginkgo3 | K-phosphate 0	0.28 ± 0.03	12	79	0.15	28	70	1.9
PANOx | $Mg(Glu)_2$ 2.6	0.07 ± 0.10	144	80	0.11	197	72	0.6

Top 10 in the 384 run, for reference:

384 rank	Design	384 g/L	Quad g/L (rank)
1	PANOx | $Mg(Glu)_2$ 8.6	0.75	1.63 (71)
2	PANOx | native	0.73	1.68 (66)
3	PANOx | $Mg(Glu)_2$ 9.8	0.68	1.75 (63)
4	PANOx | $Mg(Glu)_2$ 8	0.65	1.66 (68)
5	PANOx | K(Glu) 300	0.62	1.66 (70)
6	PANOx | K(Glu) 240	0.62	1.68 (67)
7	PANOx | K(Glu) 280	0.61	1.61 (72)
8	PANOx | K(Glu) 260	0.61	1.74 (64)
9	PANOx | NMP 2 mM	0.57	1.84 (58)
10	Ginkgo3 | K(Glu) 235	0.56	3.31 (16)

$Mg(Glu)_2$, K(Glu), K-phosphate and glucose are in mM. In the recipe's `condition_label`, "Mg(Glu)22.6" means $Mg(Glu)_2$ at 2.6 mM. All 80 designs are in `table_designs.csv`.

Titration responses



solid = quad 96-well (20261008) (left axis); dashed = 384 (20260824, 39-L) (right axis, scaled by the median fold 7.3x); design means

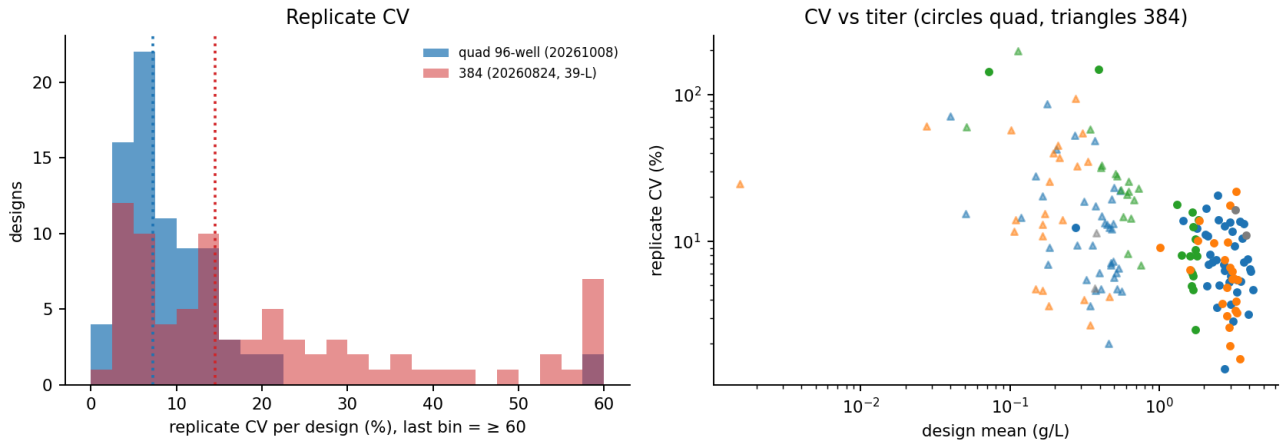
One panel per design block. This run is solid on the left axis; the 384 run is dashed on the right axis, scaled by the median fold so the two shapes overlay.

Titration	Family	Quad optimum	384 optimum
Mg(Glu)₂ (mM)	Ginkgo3	5.6	9.8
	PANOx	9.8	8.6
	RFopt	7.4	7.4
K(Glu) (mM)	Ginkgo3	235	235
	PANOx	200	300
	RFopt	260	315
K-phosphate (mM)	Ginkgo3	35	25
	RFopt	5	0
Glucose (mM)	Ginkgo3	19	0
	RFopt	10	0

- **K-phosphate is the shared lever.** Removing K-phosphate costs Ginkgo3 15× in quad and 3.7× in 384. In both formats, yield rises to a plateau at 20–35 mM. This block agrees at ρ = 0.84.
- **Low Mg is rescued in quad.** In 384, RFopt falls about 65× between Mg 4.2 and 2.6 mM (0.10 → 0.0015 g/L), and Ginkgo3 falls from 0.31 to 0.05 g/L. In quad, the same recipes make 1.8–2.0 g/L.
    - The Ginkgo3 optimum shifts down from 9.8 to 5.6 mM, and yield declines above it.
    - The exception is PANOx, which fails at Mg ≤ 3.6 mM in both formats.

- **Higher K(Glu) costs more in quad.** Ginkgo3 loses about 35% between 235 and 300 mM in quad and 15% in 384. Across the K(Glu) block the ranking anti-correlates (ρ = −0.75), driven by the family order.
- **Glucose reverses sign.** It raises Ginkgo3 from 2.7 to 4.2 g/L at 19 mM in quad, but lowers yield in 384 (0.28 → 0.20 g/L).

Replicate CVs



Left: distribution of per-design replicate CVs in both runs, with dotted lines at the medians. Right: CV against design mean (log axes); circles are quad and triangles are 384.

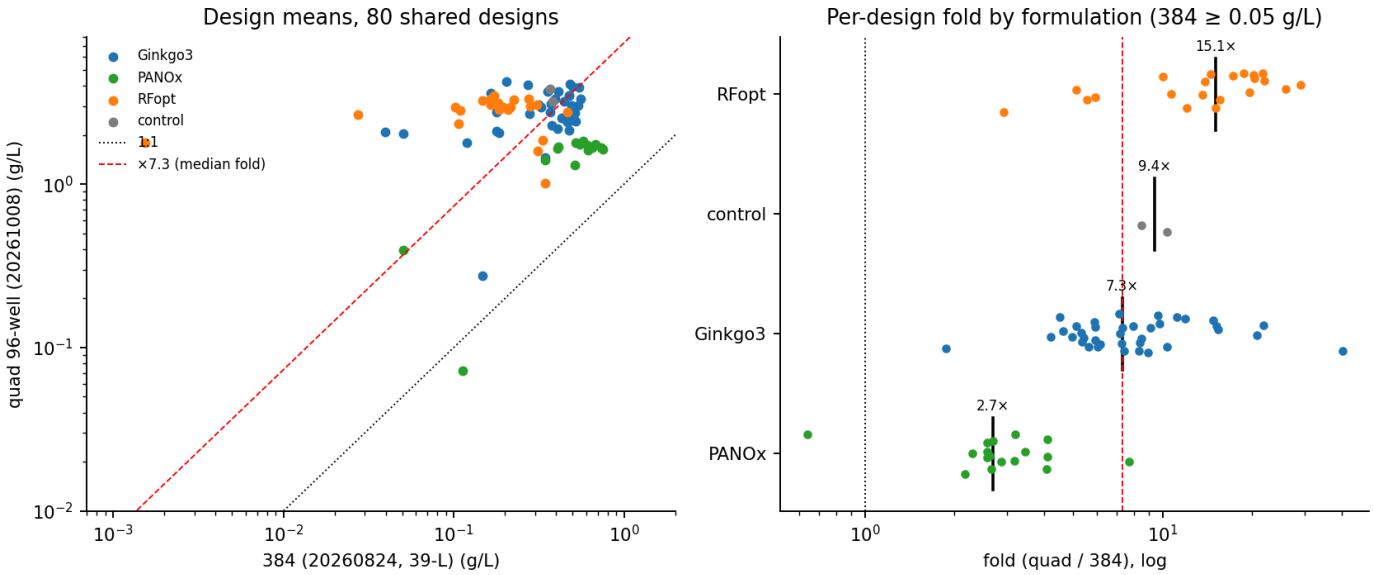
	processopt_20261008 (quad)	jewett_20260824 (384)
Median replicate CV	7.2%	14.6%
Designs with CV > 20%	4	32
Design 10th–90th percentile (g/L)	1.61–3.61	0.11–0.58

The quad format halves the replicate CV. In 384, CV rises steeply below ~0.3 g/L, where many recipes sit. In quad, nearly every design is above 1 g/L, so few designs are in the noisy low-signal range.

The two quad designs above 100% CV are PANOx $Mg(Glu)_2$ 2.6 and 3.6 mM. Both are near zero in every replicate except one partial well, and both are also failures in 384. The other high-CV quad designs are RFopt Mg 7.4 (22%) and Ginkgo3 K-phosphate 15 (21%).

The fold is not a position effect. Each design's four replicates sit in one quadrant, and the median fold per quadrant is 5.6–9.0×, the spread expected from each quadrant holding a different design mix.

Comparison with jewett_20260824 (384)



Left: design means of the 80 shared designs (log axes), with the 1:1 line and the median 7.3× fold. Right: per-design fold by formulation family for designs with 384 ≥ 0.05 g/L (77 of 80); bars mark the family medians.

Metric	Value
Shared designs	80 / 80, identical recipes
Median per-design fold, quad / 384 (IQR)	7.3× (4.5–13.6), n = 77
Fold by family	Ginkgo3 7.3×, RFopt 15.1×, PANOx 2.7×, premixed controls 9.4×
Spearman ρ, design means	−0.12
Pearson r, log design means	0.15
Top-10 overlap	0 / 10
Median family rank (quad / 384)	Ginkgo3 36 / 34, RFopt 32 / 60, PANOx 68 / 9

Agreement by design block (Spearman ρ of design means, quad vs 384):

Block	n	ρ
K-phosphate titration	11	**0.84**
Mg / K(Glu) antidiagonal	5	0.70
Mg at fixed K-phosphate	5	0.70
$Mg(Glu)_2$ titration	24	−0.03
Nucleotide swap	6	−0.54
Glucose titration	6	−0.71
K(Glu) titration	18	−0.75

The family order inverts:

- **384:** PANOx > Ginkgo3 > RFopt.

- **Quad:** Ginkgo3 ≈ RFopt > PANOx.

PANOx gains least from the format (2.7×) and RFopt gains most (15×), most of all at Mg ≤ 5 mM (fold 26–1,200×). The previous quad run against the same reference showed the same family pattern (PANOx 3.2×, RFopt 12.8×).

Possible explanations, not yet tested:

- **Evaporative concentration in the DWP.** Losing part of the volume during the 30 °C incubation in a 2 mL deep well would raise every salt concentration. That would lower the apparent Mg and K(Glu) optima and lift the low-Mg recipes into the working range. A post-incubation volume or weight check on the DWPs would test it.
- **Better aeration.** A thin reaction film under a large headspace would support oxidative energy regeneration. That fits the higher overall yield and the relative gain of RFopt and Ginkgo3 over PANOx.
- **Base buffer lot.** 20260512-2-BB here against 20260721-4-BB in 384 is a confound. It cannot be separated from the format without a same-buffer side-by-side.

Caveats

- **Standard scale.** Absolute g/L follows the analysis convention: the standard stamp concentration is treated as reaction-equivalent, which puts both runs on the same 3.7–119 µM scale.
    - The quad standards share the compression dilution with the samples, but they are not incubated in the DWP.
    - If the reactions lose volume during incubation, quad g/L overstates product per nominal reaction volume.
- **Calibration shape.** Over the sample range, the curves differ by about 1.3× (see *Standard curves*). This is small against the 7.3× fold.
- **Confounds beyond format.** The two runs were made on different dates with different reagent preparations and base buffer lots, in different reaction volumes (40 µL quad, 20 µL in 384). Read handling also differed: this run used a hand-diluted re-read; the 384 run used a single direct read.
- **Diluted target controls.** The target controls (rows O–P) were diluted with the samples and are corrected. The negatives sit at background, so whether they were diluted cannot be resolved.
- **Local analysis only.** The results have not been uploaded to the datastore experiment.