

An automated high-throughput lighting system for screening photosynthetic microorganisms in plate-based formats

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Supplementary Files

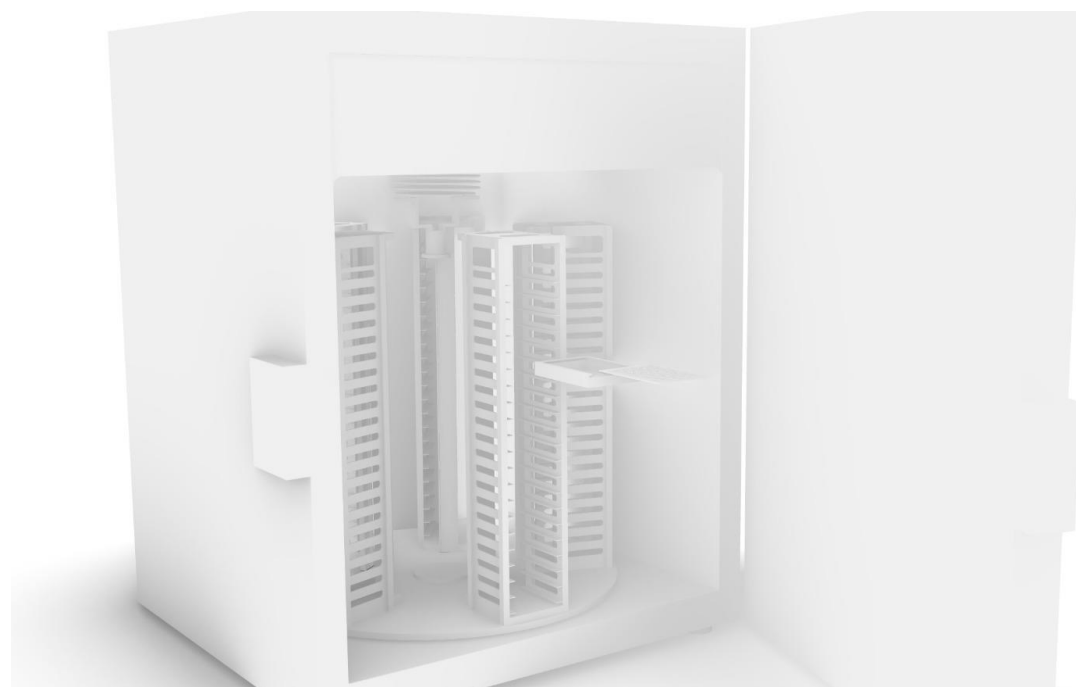
Supplementary data, scripts, and 3D models were placed in a GitHub repository at the following address: https://github.com/hallamlab/lighting_system_design_2024. The following table shows the contents of the repository and a brief description of each file.

File	Topic	Directory	Description
Noonan_HTP_lighting_system_figures.Rmd	RMarkdown	.	RMarkdown generating all data figures
Noonan_BG11-RSM_JMP_data_processing.Rmd	RMarkdown	.	RMarkdown for RSM data reformatting for input into JMP
PHERASTAR_2x2_data_processing.py	Python	.	OD data processing
PHERASTAR_standard_data_processing.py	Python	.	OD data processing
varioskan_data_parsing.py	Python	.	OD data processing
LED_driver.ino	Arduino	.	Arduino script for controlling LED intensity
parts-list.csv	System	system-design/	Parts list
2973_plate-type_growth_data.csv	Design / Validation	data/system-design/	Culture OD data
2973_seal_growth_data.csv	Design / Validation	data/system-design/	Culture OD data
2973_spectrum_growth_data.csv	Design / Validation	data/system-design/	Culture OD data
photodiode_array_data.csv	Design / Validation	data/system-design/	Photodiode data
sensor_array_data.csv	Design / Validation	data/system-design/	Photodiode data
layout_info.csv	Design / Validation	data/system-design/	Plate layout info
echo_evaporation_data.csv	Design / Validation	data/system-design/	Evaporation data
2973_BG11_growth_data.csv	Primary Use-Cases	data/use-cases/primary/	Culture OD data
2973_BG11_plate_layout.csv	Primary Use-Cases	data/use-cases/primary/	Plate layout info

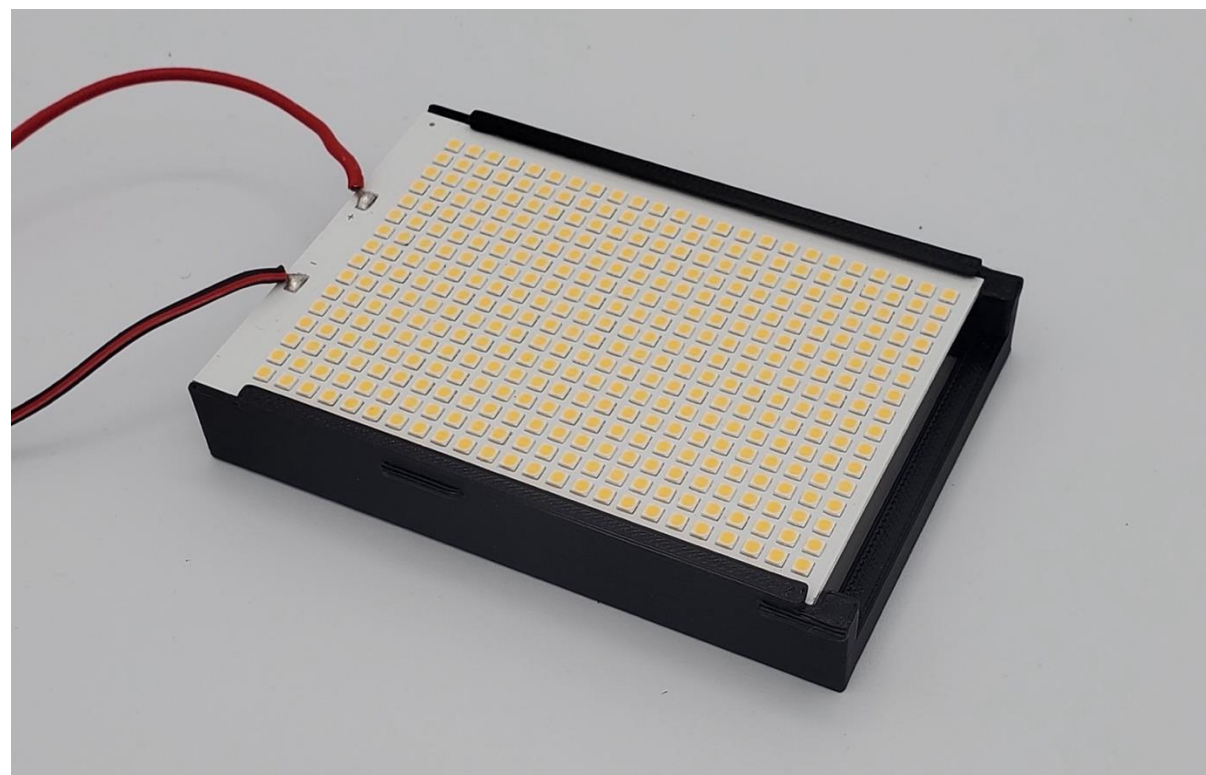
2973_BG11_plate_metadata.csv	Primary Use-Cases	data/use-cases/primary/	Culture metadata
antibiotics_growth_data.csv	Primary Use-Cases	data/use-cases/primary/	Culture OD data
antibiotics_plate_layout.csv	Primary Use-Cases	data/use-cases/primary/	Plate layout info
antibiotics_plate_metadata.csv	Primary Use-Cases	data/use-cases/primary/	Culture metadata
layout_info.csv	Primary Use-Cases	data/use-cases/primary/	Plate layout info
BG11_JMP_layout.csv	BG-11 RSM	data/use-cases/BG11-RSM/	Plate layout info
BG11_opt_OD_data.csv	BG-11 RSM	data/use-cases/BG11-RSM/	Culture OD data
flask_biomass_data_100uM.csv	BG-11 RSM	data/use-cases/BG11-RSM/	Culture biomass data
bioactive_library_info.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Screening Library Information
bioactive_screen1_OD_data.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Culture OD data
bioactive_round2_OD_data.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Culture OD data
bioactive_screen_round2_hit_compound_info.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Compound metadata
bioactive_screen_round3_layout.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Plate layout info
bioactive_screen_round3_OD_data.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Culture OD data
bioactive_gradient_data.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Culture OD data
gossypetin_scan_data.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Culture OD data
layout_info.csv	Bioactive Screen	data/use-cases/bioactive-screening/	Plate layout info
slipring_connector.stl	Design / Validation	3D model (.stl)	Model of slipring connector
LED_array_support.stl	Design / Validation	3D model (.stl)	Model of LED array support structure

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Supplementary Figures

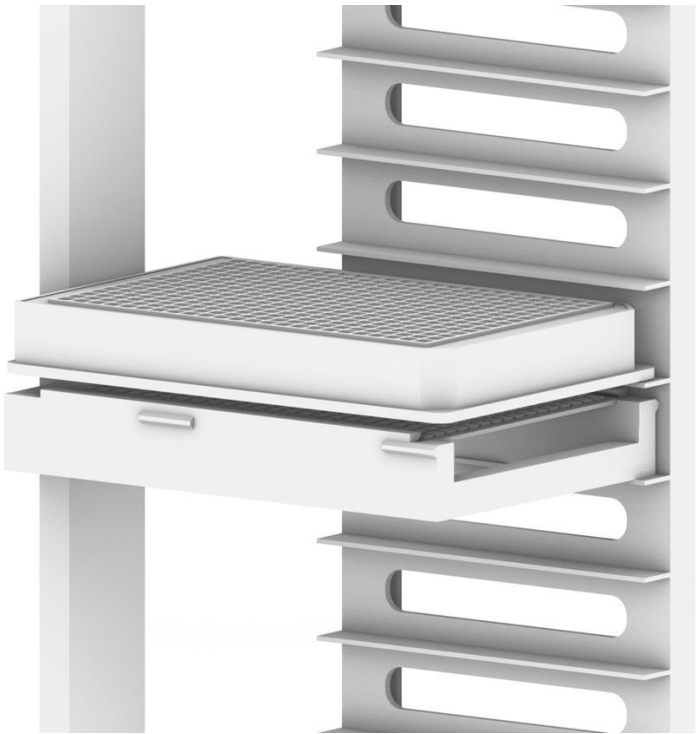


Supplementary Figure 1. Integration of lighting system into Cytomat 5 C450.

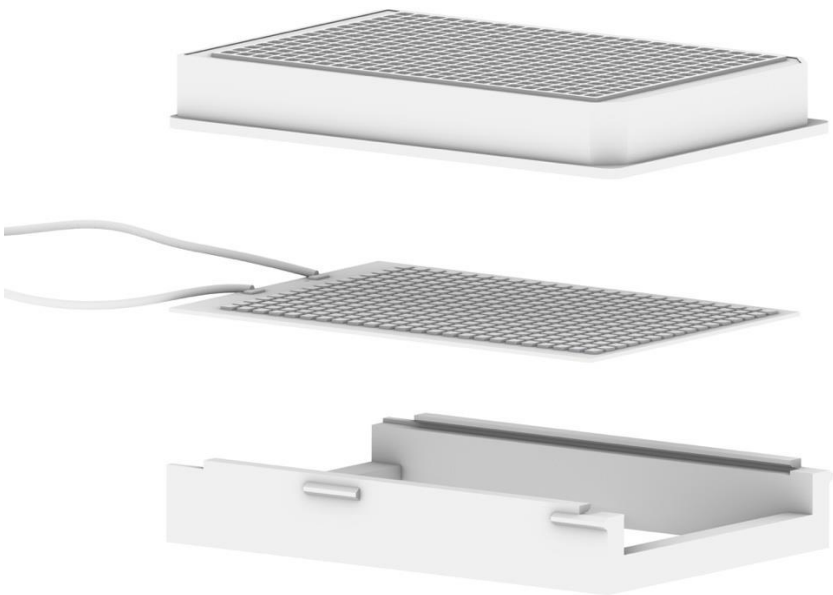


Supplementary Figure 2. LED array in support structure

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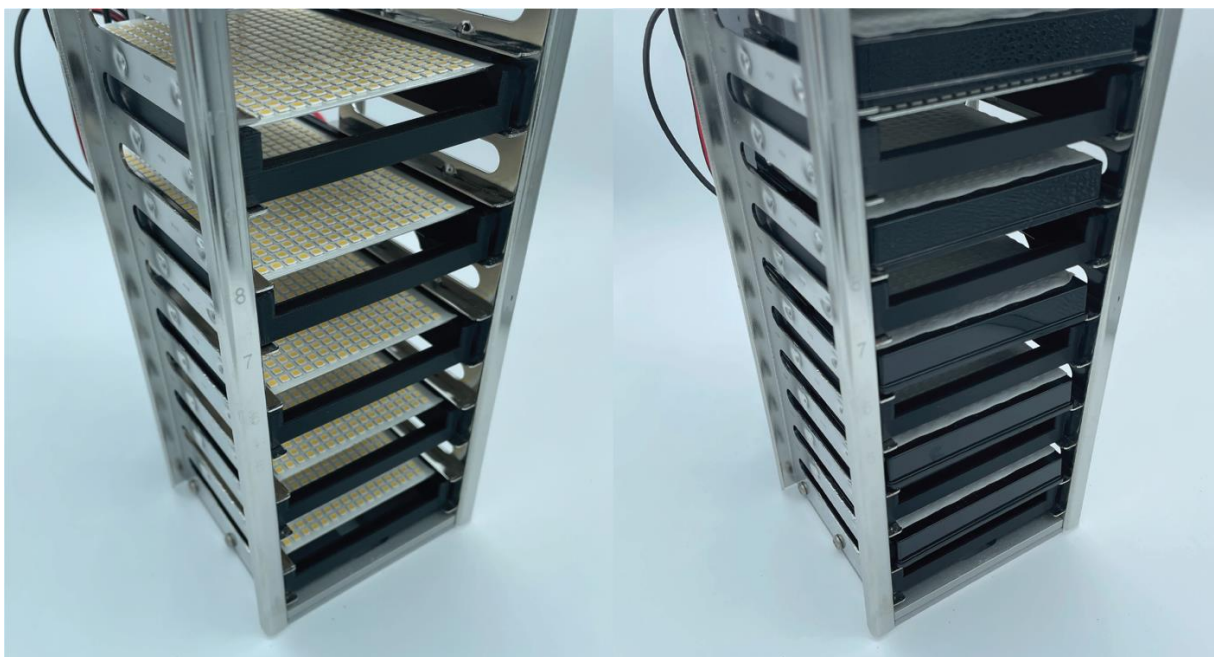
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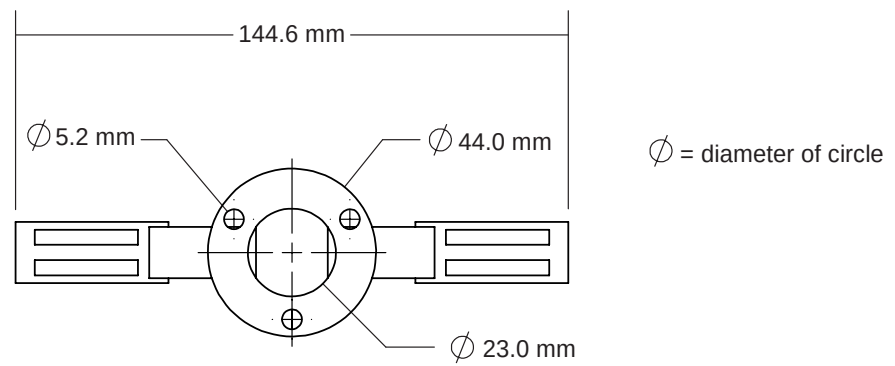
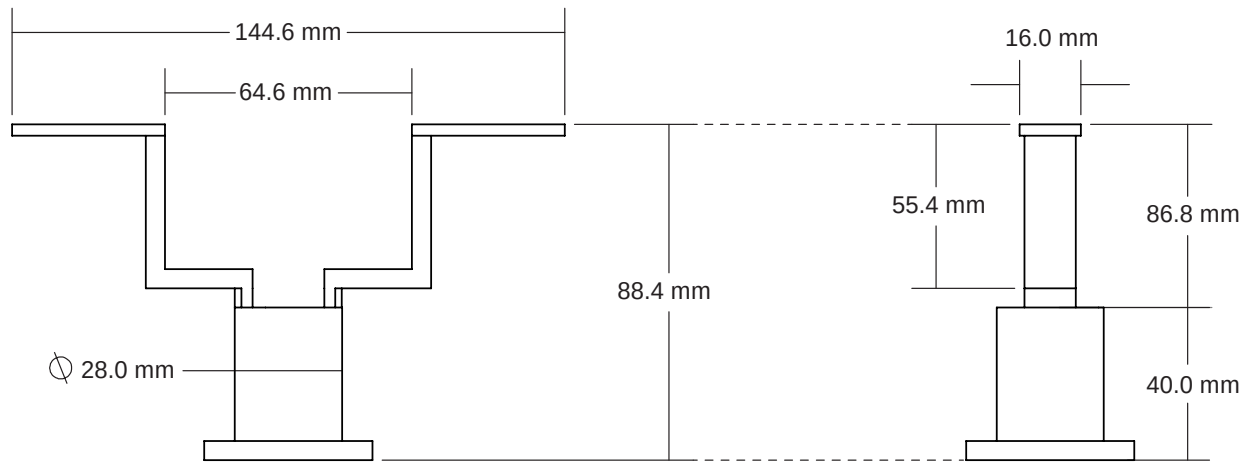
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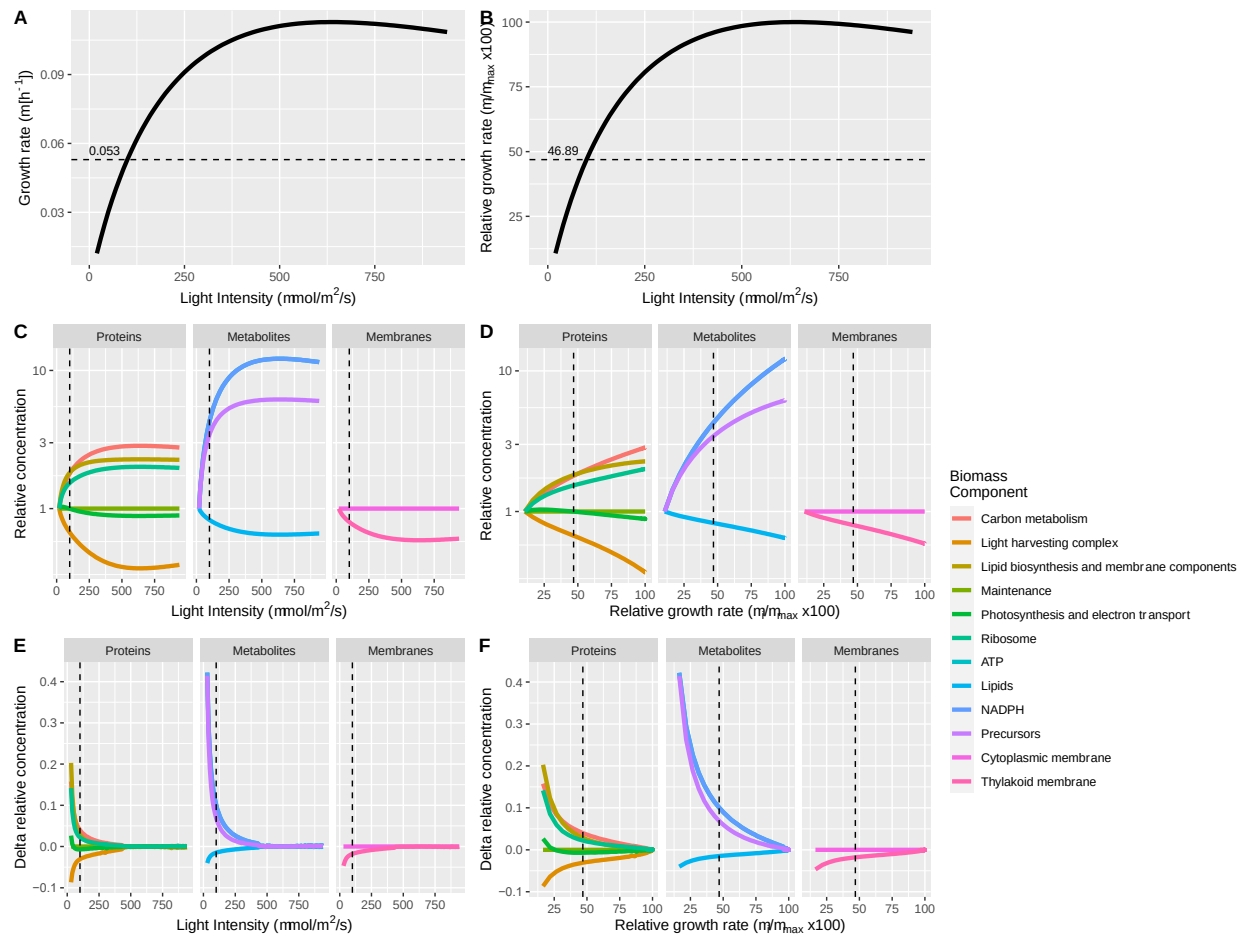
Supplementary Figure 3. Assembled and disassembled lighting system in Cytomat 5 C450 rack.



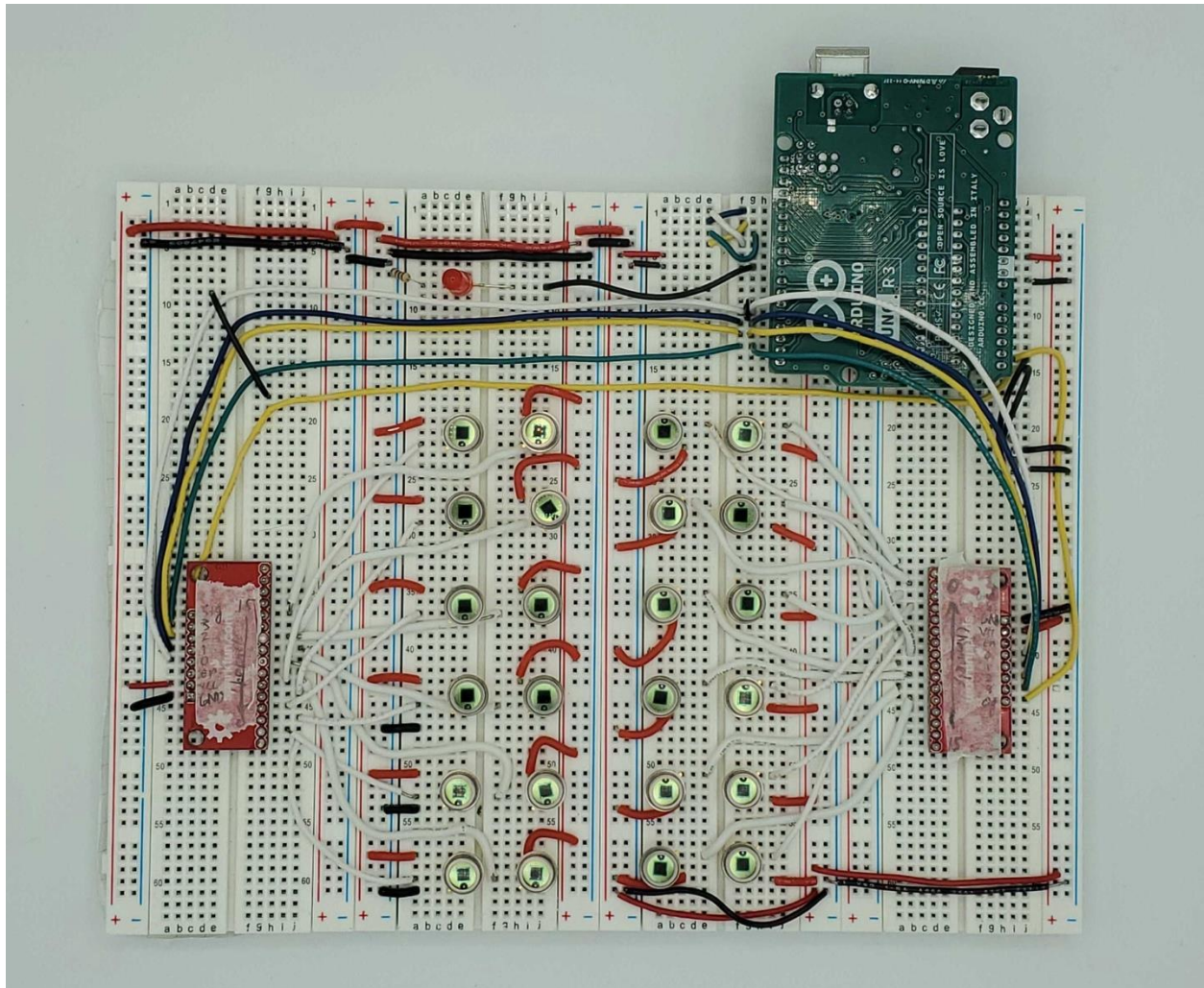
Supplementary Figure 4. LED arrays in Cytomat 5 C450 rack, with and without plates.



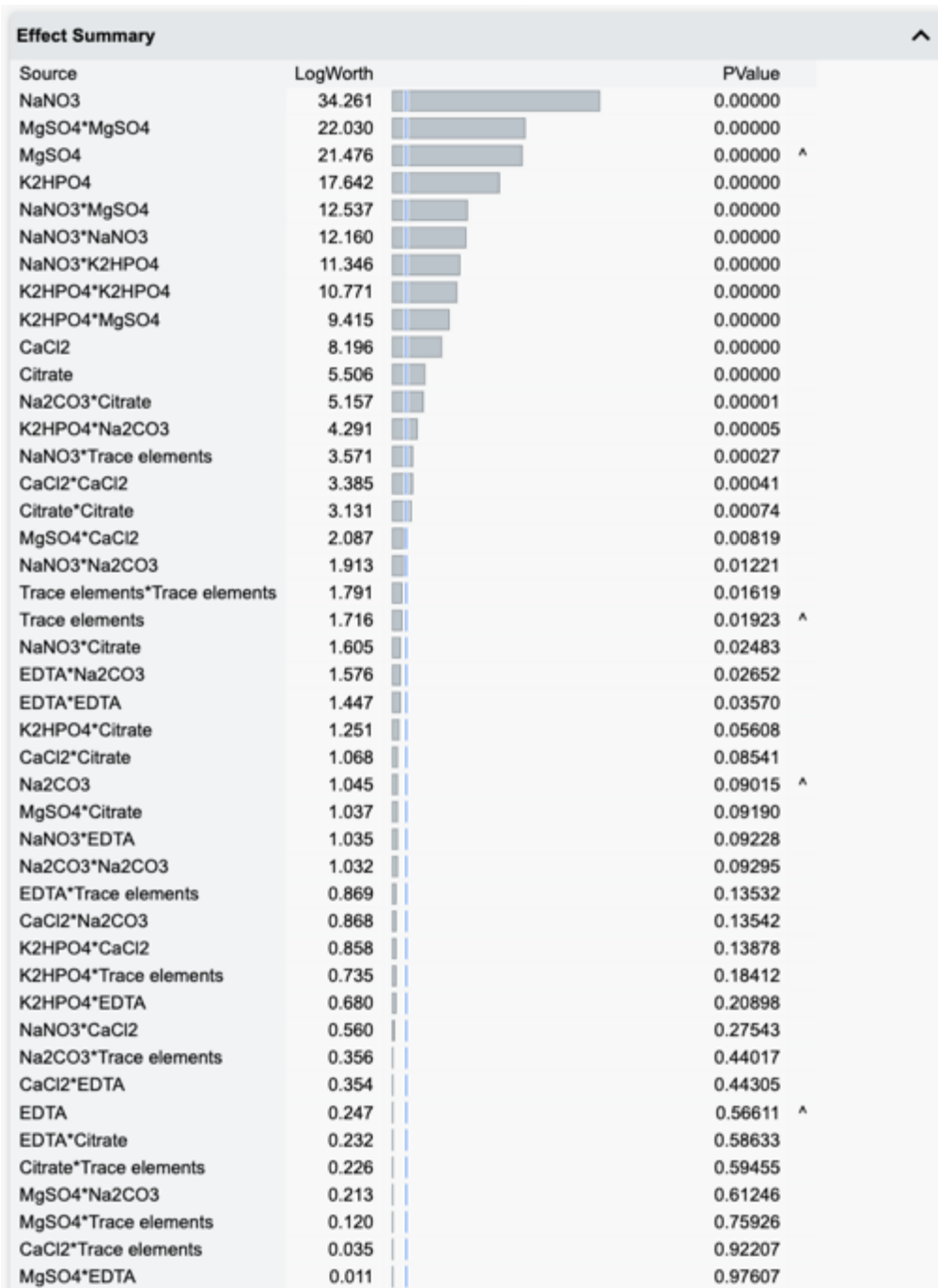
42
43 **Supplementary Figure 5.** Slipping connector.



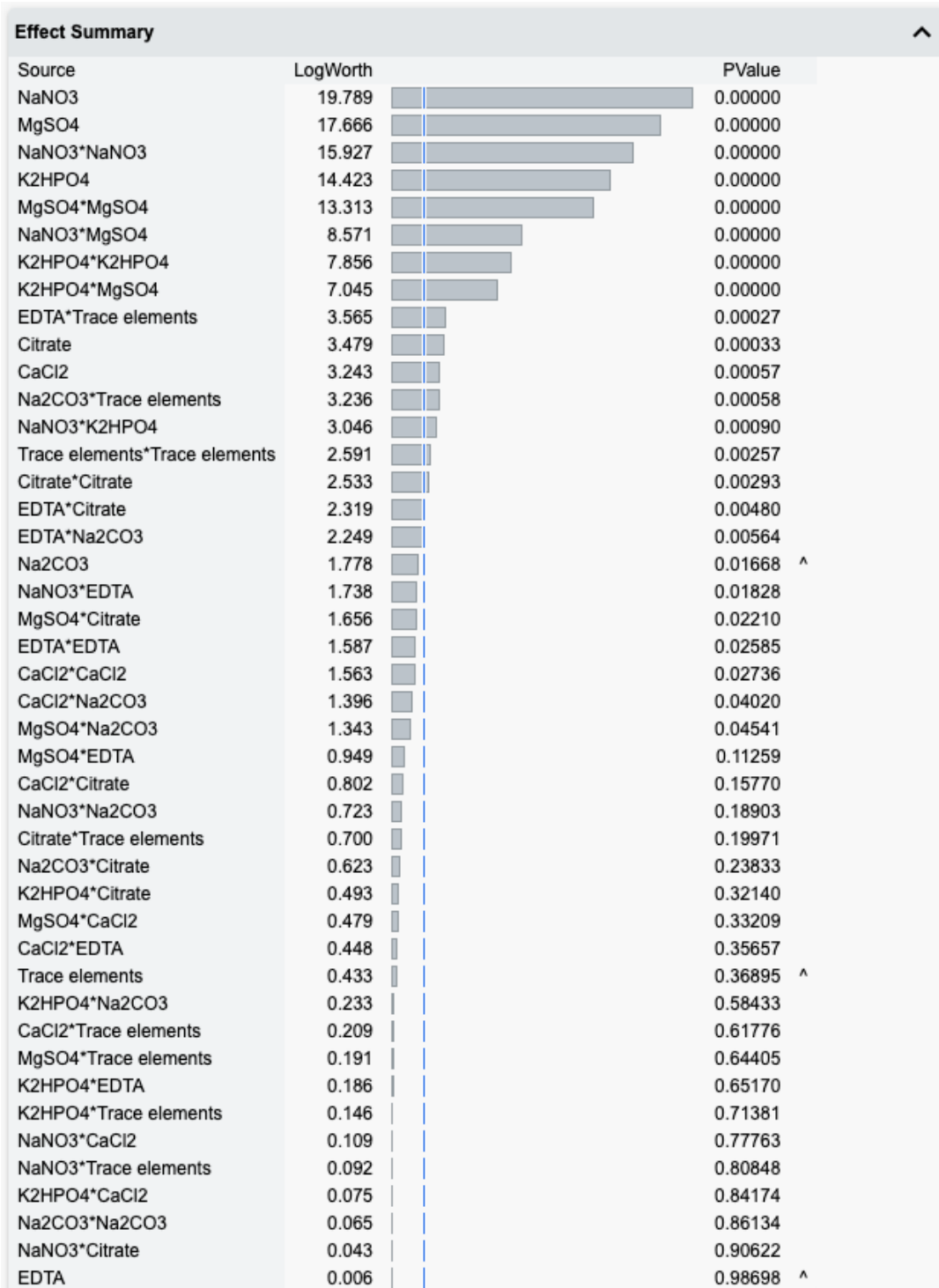
Supplementary Figure 6. Protein economy modeling of *Synechocystis* sp. PCC 6803.



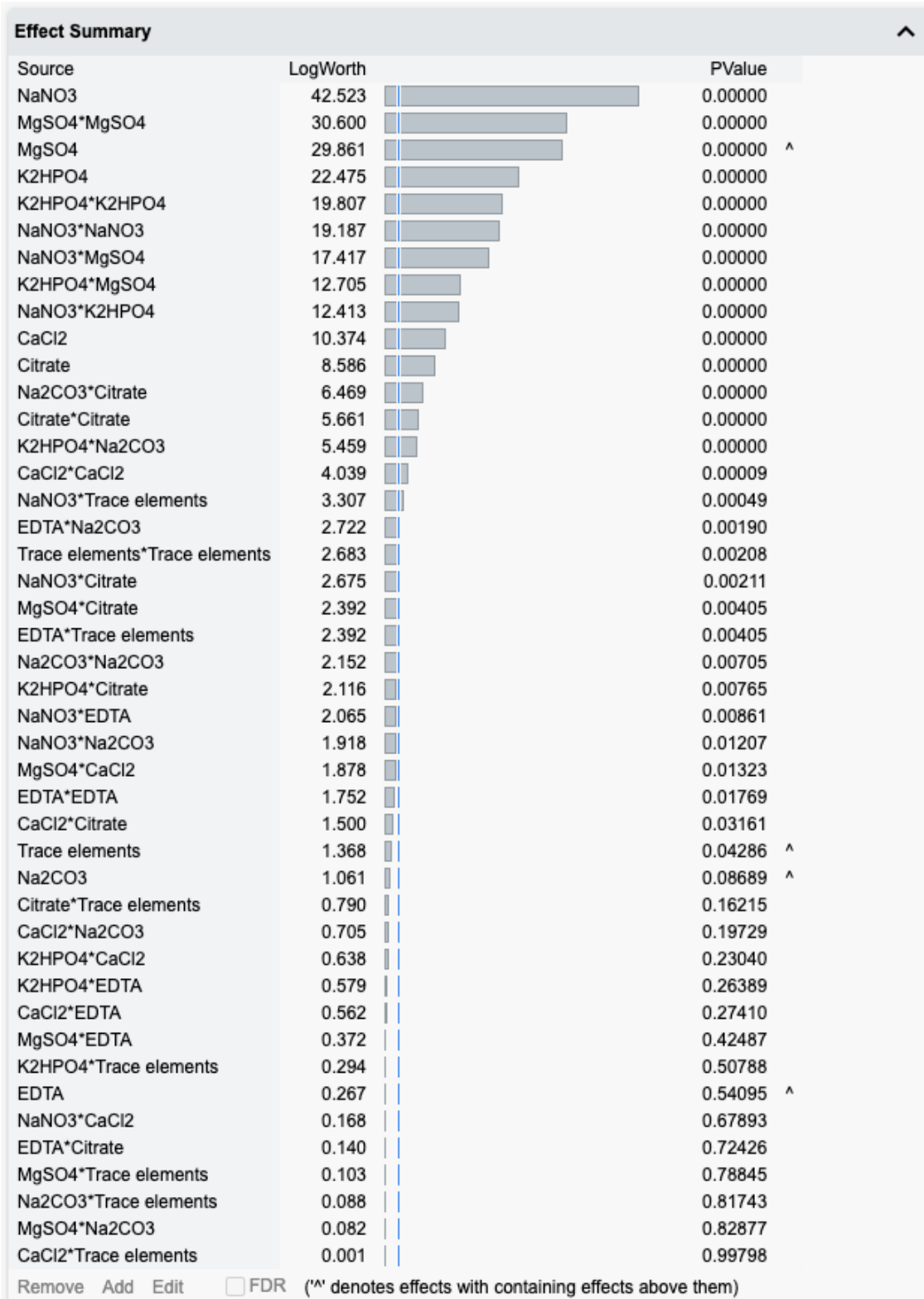
Supplementary Figure 7. 24 photodiode array to investigate the relationship between LED array to microplate distance and evenness of light distribution.



Supplementary Figure 8. *S. elongatus* UTEX2973 with NaHCO₃ JMP effect summary.



Supplementary Figure 9. *S. elongatus* UTEX2973 without NaHCO₃ JMP effect summary.



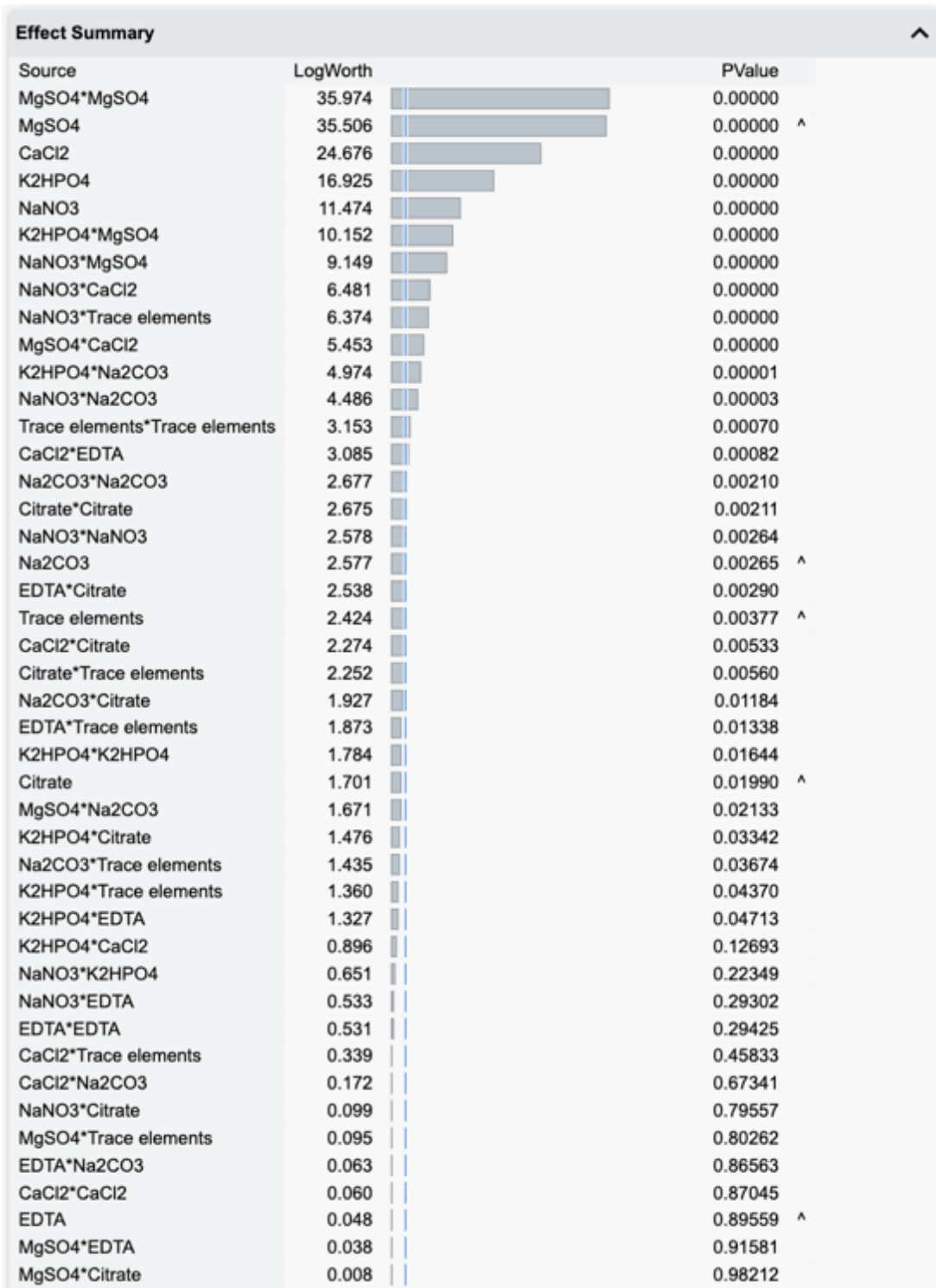
Supplementary Figure 10. *C. reinhardtii* UTEX90 with NaHCO₃ JMP effect summary.

Source	LogWorth	PValue
NaNO3	32.955	0.00000
CaCl2	32.954	0.00000
MgSO4*MgSO4	31.124	0.00000
Na2CO3*Citrate	21.772	0.00000
NaNO3*NaNO3	20.026	0.00000
MgSO4	18.818	0.00000 ^
K2HPO4*K2HPO4	17.945	0.00000
Citrate	13.173	0.00000 ^
NaNO3*MgSO4	12.516	0.00000
MgSO4*CaCl2	10.824	0.00000
K2HPO4	10.637	0.00000 ^
Citrate*Citrate	9.898	0.00000
EDTA*EDTA	8.529	0.00000
NaNO3*CaCl2	8.266	0.00000
NaNO3*Trace elements	8.047	0.00000
K2HPO4*CaCl2	7.799	0.00000
K2HPO4*Citrate	7.596	0.00000
K2HPO4*Na2CO3	6.805	0.00000
NaNO3*K2HPO4	6.792	0.00000
NaNO3*Na2CO3	5.385	0.00000
K2HPO4*MgSO4	4.125	0.00008
Na2CO3	3.416	0.00038 ^
MgSO4*Citrate	3.385	0.00041
CaCl2*CaCl2	2.793	0.00161
CaCl2*Citrate	2.318	0.00481
EDTA*Citrate	2.132	0.00738
Na2CO3*Trace elements	2.000	0.00999
K2HPO4*EDTA	1.954	0.01112
NaNO3*Citrate	1.894	0.01276
Trace elements	1.735	0.01843 ^
CaCl2*EDTA	1.631	0.02340
EDTA	1.529	0.02959 ^
EDTA*Trace elements	1.512	0.03078
K2HPO4*Trace elements	1.390	0.04078
CaCl2*Na2CO3	1.303	0.04977
Na2CO3*Na2CO3	1.241	0.05738
CaCl2*Trace elements	0.745	0.18002
Citrate*Trace elements	0.713	0.19360
MgSO4*EDTA	0.517	0.30438
Trace elements*Trace elements	0.384	0.41323
EDTA*Na2CO3	0.310	0.49022
NaNO3*EDTA	0.252	0.55933
MgSO4*Trace elements	0.132	0.73770
MgSO4*Na2CO3	0.118	0.76146

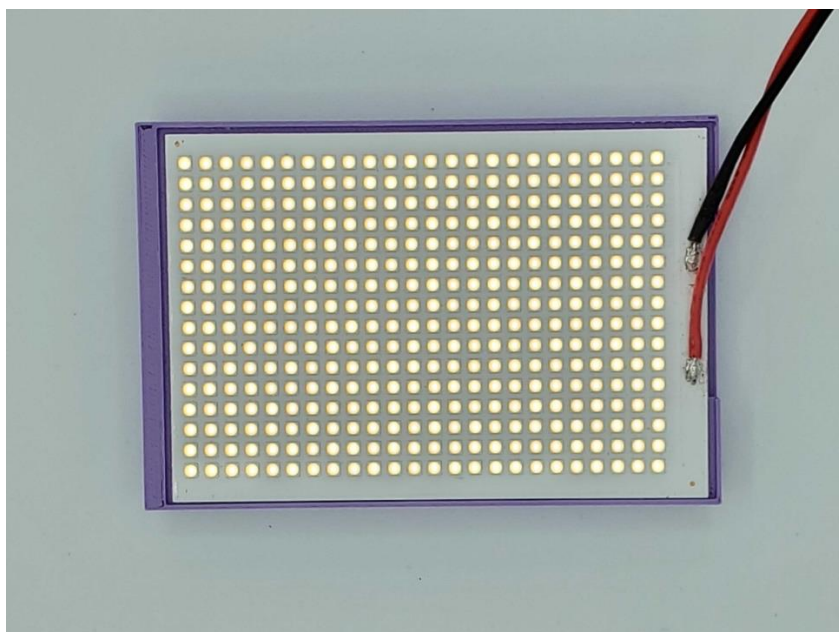
Supplementary Figure 11. *C. reinhardtii* UTEX90 without NaHCO₃ JMP effect summary.

Effect Summary			
Source	LogWorth	PValue	
MgSO4	27.202	0.00000	
CaCl2	12.609	0.00000	
MgSO4*MgSO4	9.995	0.00000	
NaNO3*Trace elements	5.899	0.00000	
K2HPO4	3.938	0.00012	
NaNO3*K2HPO4	3.854	0.00014	
Trace elements	3.034	0.00092	^
K2HPO4*CaCl2	2.991	0.00102	
K2HPO4*MgSO4	2.880	0.00132	
Na2CO3*Na2CO3	2.644	0.00227	
EDTA*EDTA	2.334	0.00463	
NaNO3*MgSO4	2.042	0.00908	
CaCl2*Citrate	1.995	0.01012	
NaNO3*NaNO3	1.991	0.01021	
K2HPO4*K2HPO4	1.894	0.01277	
NaNO3*Na2CO3	1.885	0.01303	
NaNO3*CaCl2	1.859	0.01383	
EDTA*Na2CO3	1.559	0.02759	
NaNO3	1.556	0.02781	^
EDTA*Citrate	1.489	0.03245	
K2HPO4*Trace elements	1.373	0.04234	
MgSO4*Citrate	1.371	0.04258	
EDTA	0.442	0.36123	^
NaNO3*EDTA	0.433	0.36863	
Citrate*Trace elements	0.377	0.41996	
CaCl2*EDTA	0.328	0.47029	
Na2CO3*Citrate	0.293	0.50962	
Citrate*Citrate	0.283	0.52093	
MgSO4*Na2CO3	0.282	0.52263	
Na2CO3	0.251	0.56121	^
CaCl2*Trace elements	0.243	0.57195	
K2HPO4*Na2CO3	0.226	0.59361	
K2HPO4*EDTA	0.226	0.59482	
Na2CO3*Trace elements	0.204	0.62457	
CaCl2*CaCl2	0.190	0.64617	
MgSO4*EDTA	0.163	0.68734	
Trace elements*Trace elements	0.135	0.73218	
K2HPO4*Citrate	0.123	0.75315	
MgSO4*CaCl2	0.107	0.78099	
MgSO4*Trace elements	0.100	0.79411	
NaNO3*Citrate	0.097	0.80067	
CaCl2*Na2CO3	0.021	0.95374	
Citrate	0.007	0.98351	^
EDTA*Trace elements	0.000	0.99957	

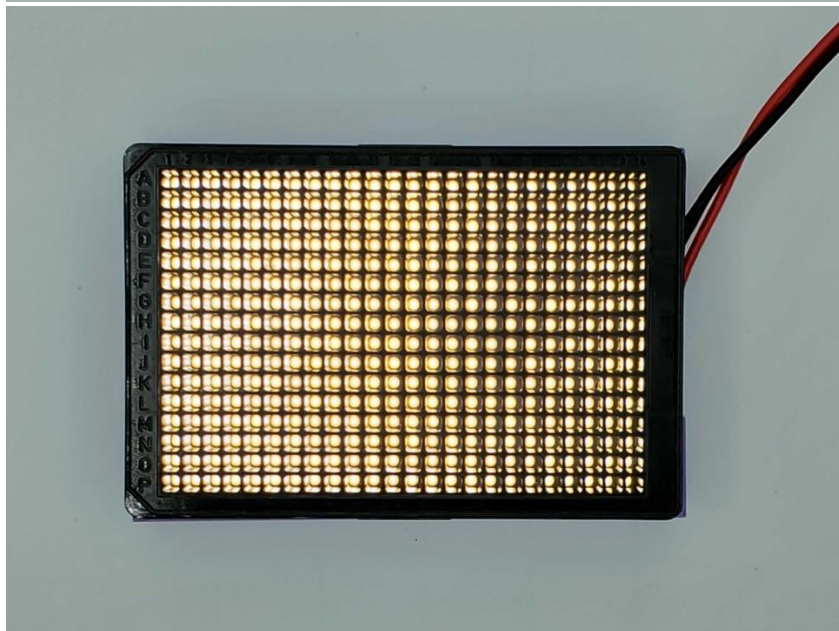
Supplementary Figure 12. *Nostoc hatei* CUBC1040 with NaHCO₃ JMP effect summary.



Supplementary Figure 13. *Nostoc hatei* CUBC1040 without NaHCO₃ JMP effect summary.



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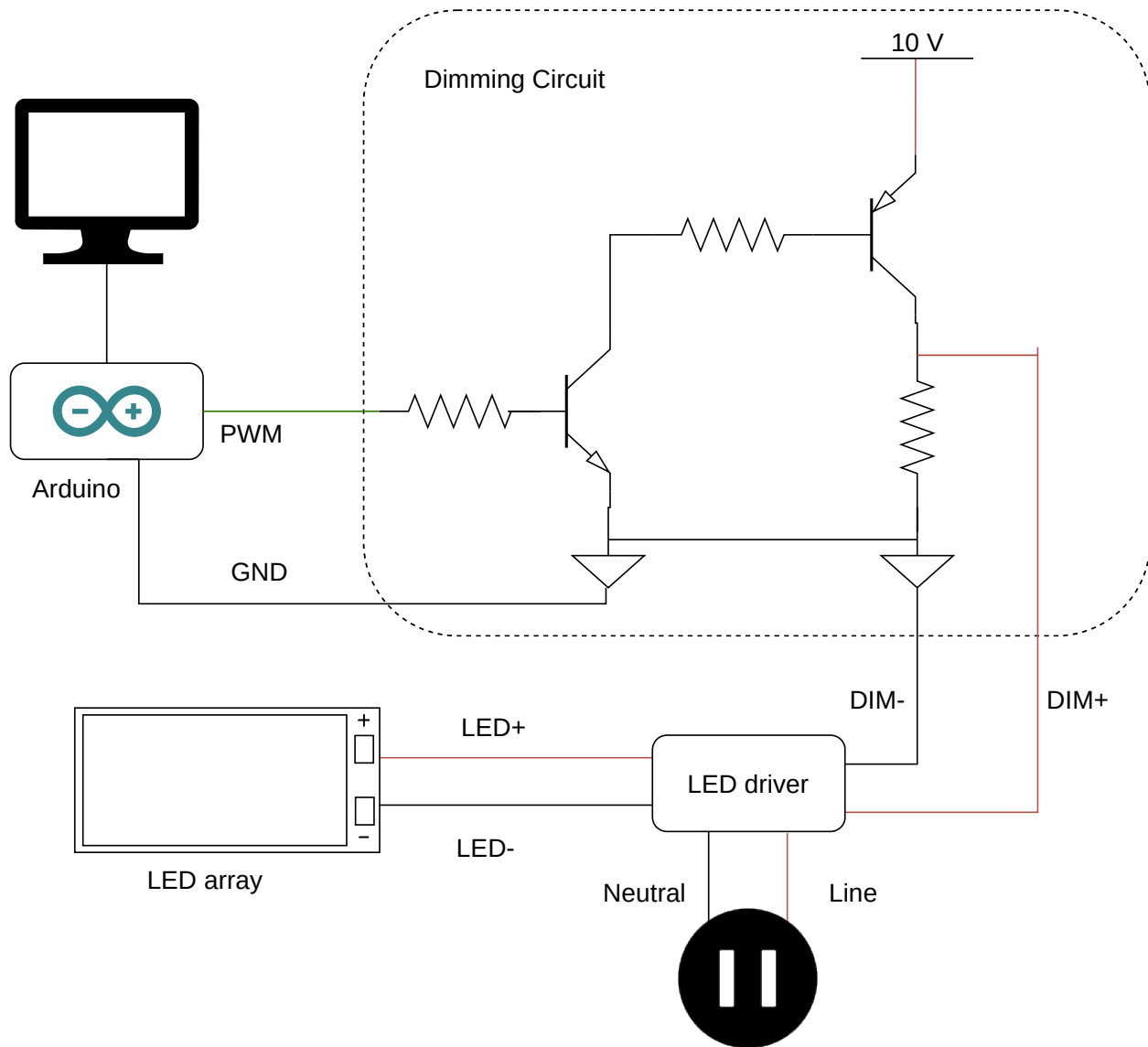
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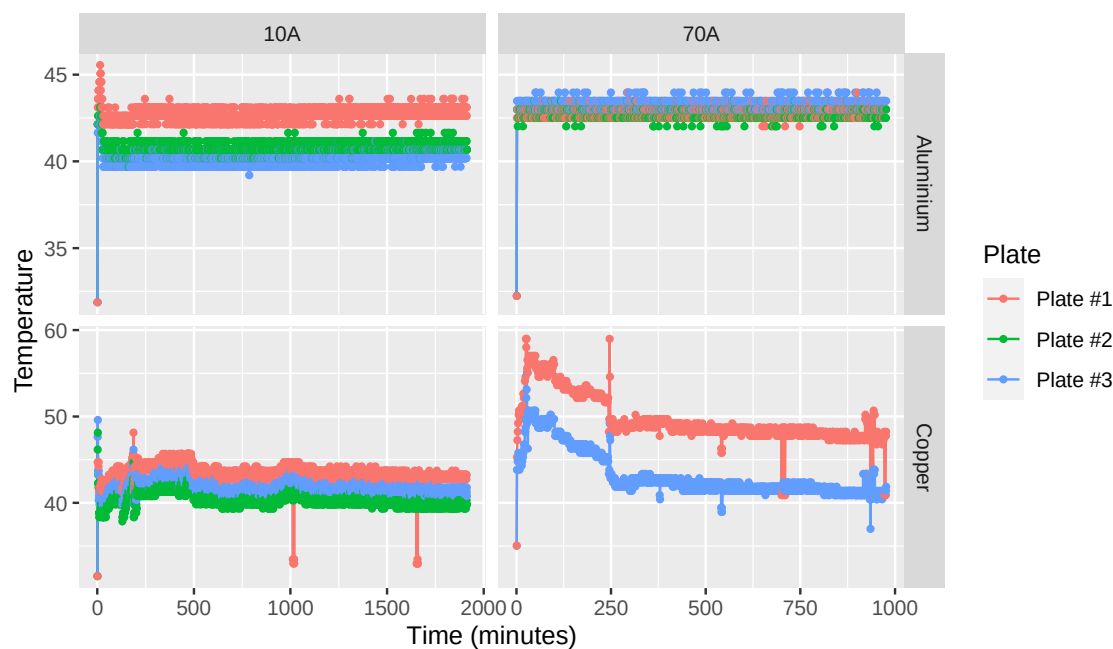
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Supplementary Figure 14. Microplate and LED array

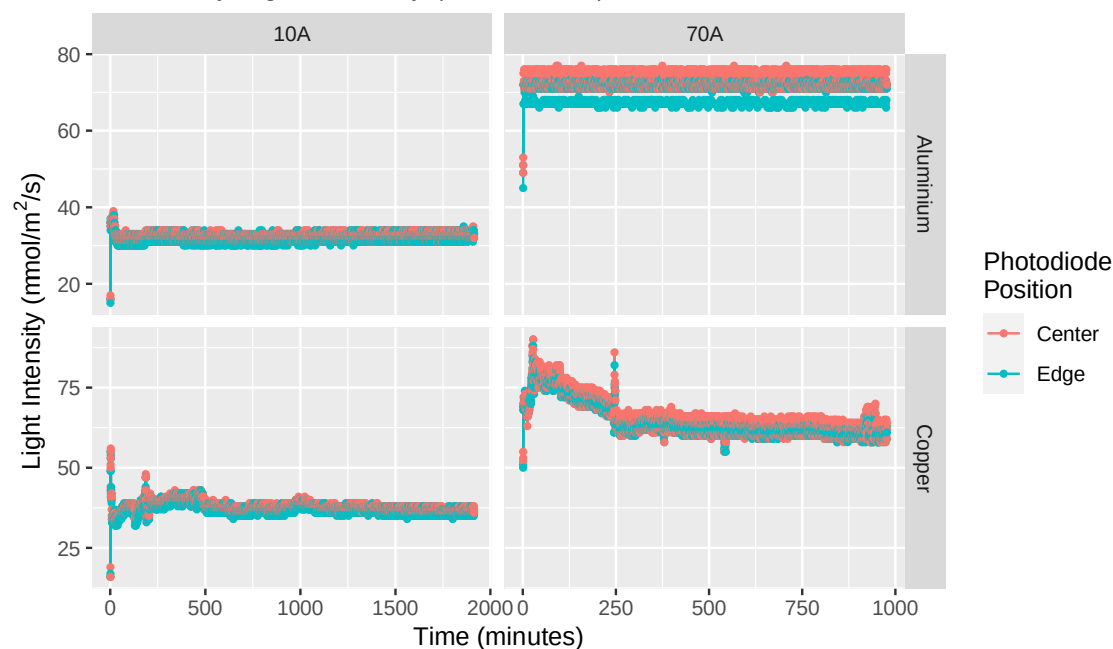


Supplementary Figure 15. Electrical circuit design. The positive and negative connections on the LED array connect to the V+ and V- wires on the LED driver, which provides a defined amount of power to the LED arrays. The LED driver is also connected to a wall outlet via the neutral and line wires. The DIM+ and DIM- wires connect to the dimming circuit. A 12V power supply is also connected to the dimming circuit. The Arduino PWM pin 10 also connects to the dimming circuit. The dimming circuit consists of transistors and resistors.

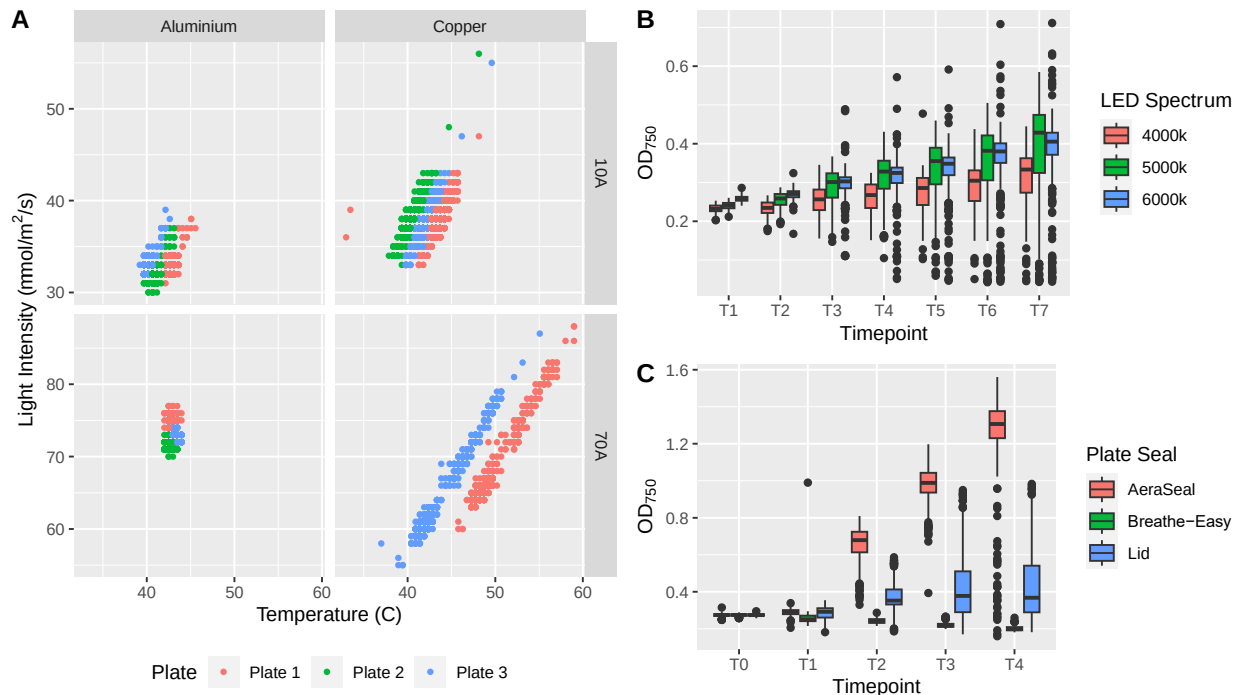
A LED Array Temperature (0–16 hours)



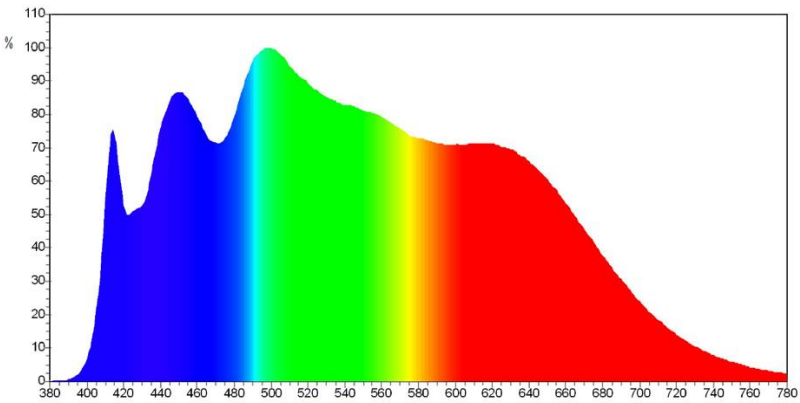
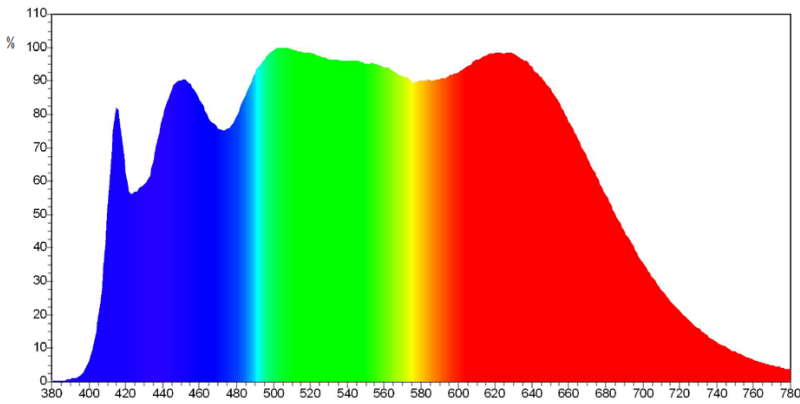
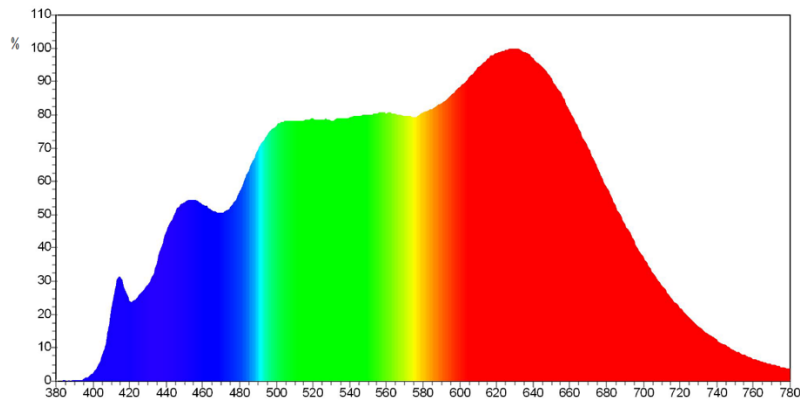
B LED Array Light Intensity (0–16 hours)



Supplementary Figure 16. 16-hour intensity and temperature data. LED arrays mounted on super-conducting aluminium plates resulted in less variability in plate temperature and light intensity, in comparison to copper plates. Points in a represent single values measured each minute over 16 hours, with colours representing replicate LED arrays. Plate 2 data (green) were removed from the bottom-right panel of (A) because of a faulty connection in the sensor circuit.

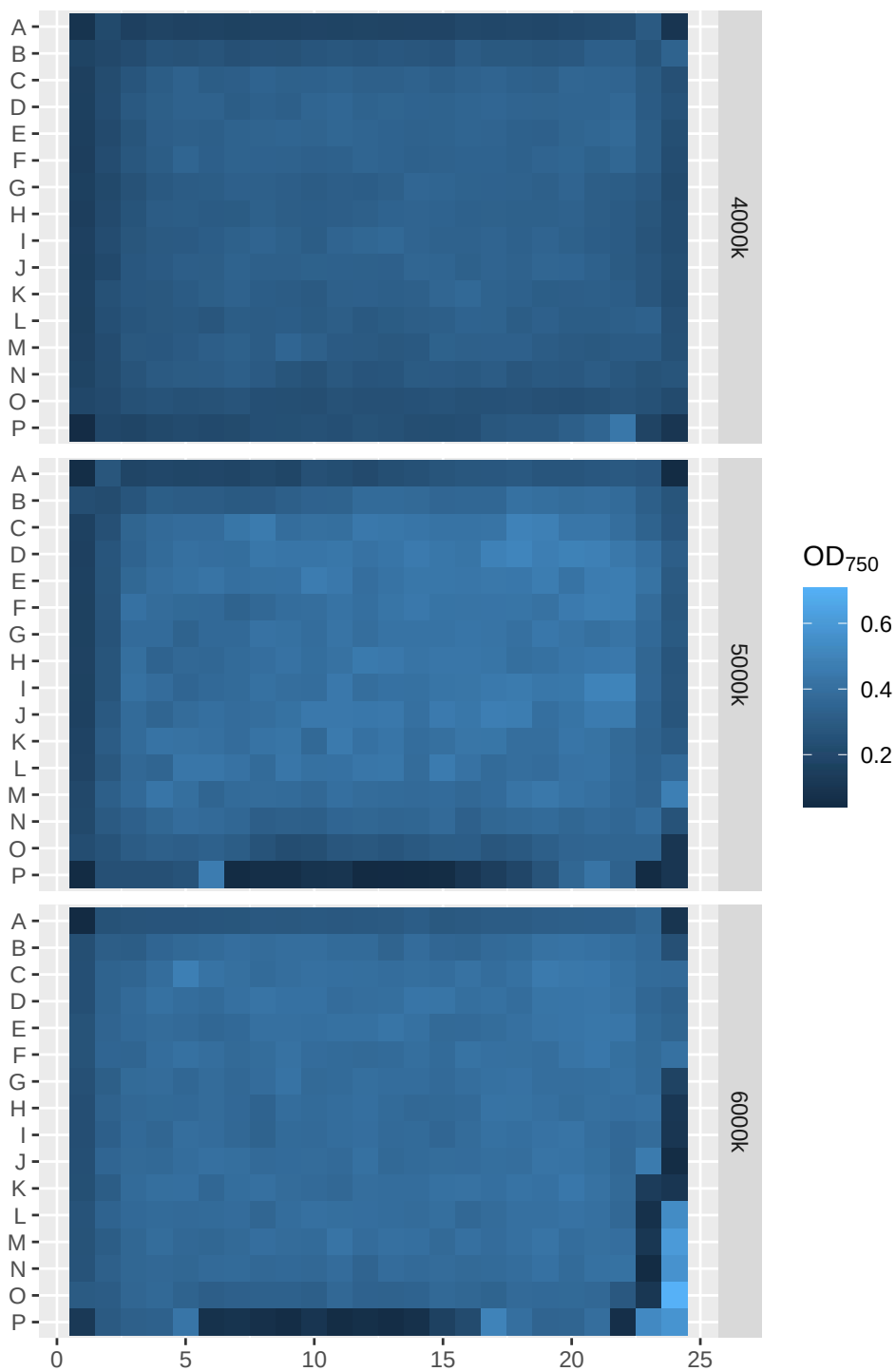


Supplementary Figure 17. System component validation. Sensor data and *Synechococcus elongatus* UTEX2973 growth rate-based experiments were used to validate and select specific components of the lighting system hardware and design. A) LED arrays mounted on superconducting aluminium plates resulted in less variability in plate temperature and light intensity, in comparison to copper plates, at 10 amps (10A) and 70 amps (70A) of current. Points in a represent single values measured each minute over 16 hours, with colours representing replicate LED arrays. Plate 2 data (green) were removed from the bottom-right panel because of a faulty connection in the sensor circuit. B) 5091 K broad-spectrum LEDs resulted in the fastest median growth rate across 384-well microplates. Black points represent outliers resulting from strong edge effects. C) Woven breathable seals resulted in significantly faster median growth rate across 384-well microplates, in comparison the microplate lids and non-woven breathable seals.



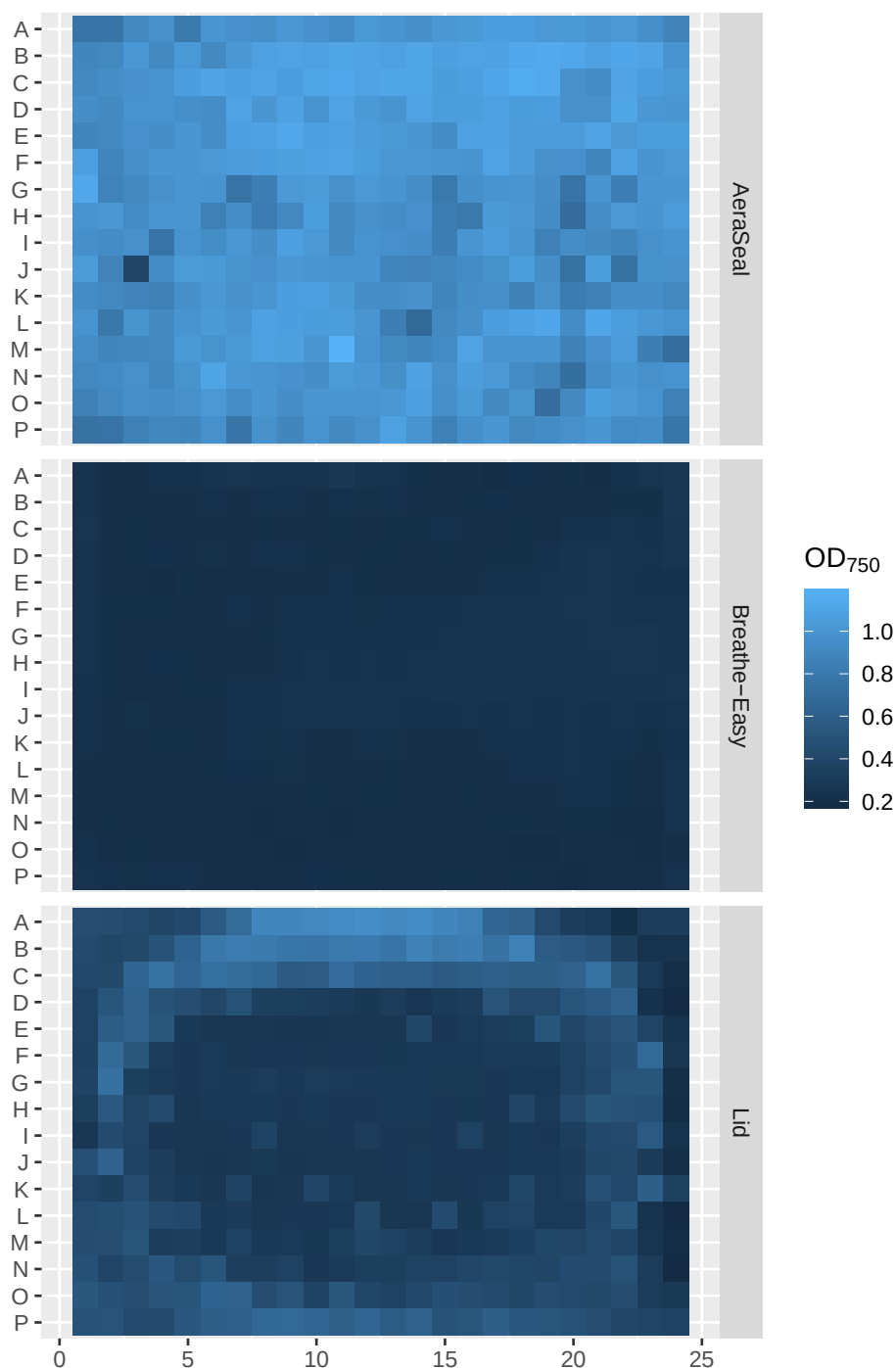
Supplementary Figure 18. LED emission spectra (4044 K, 5091 K and 6083 K).

UTEX2973 by LED Array Spectrum (T6)

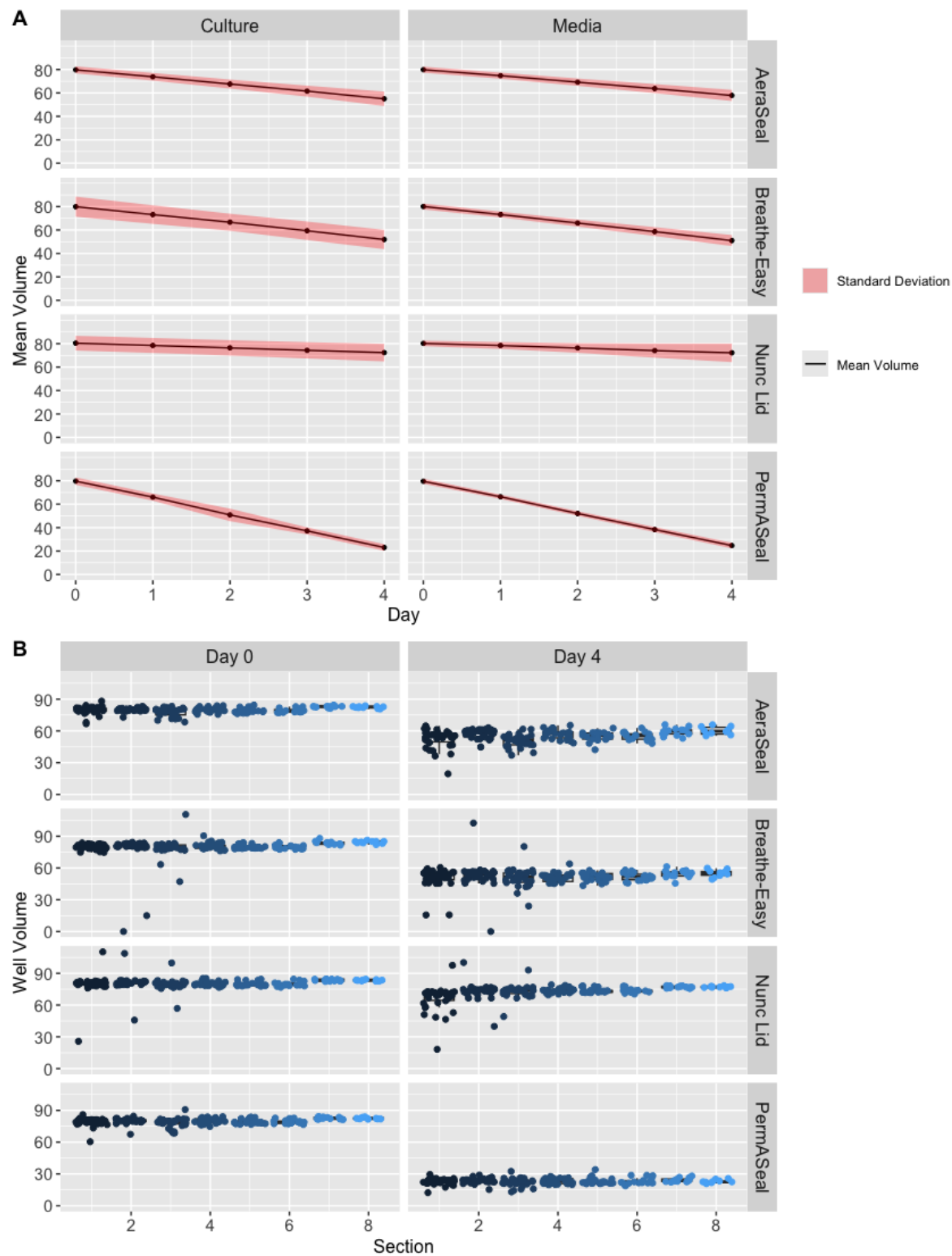


Supplementary Figure 19. Spectrum test heatmaps.

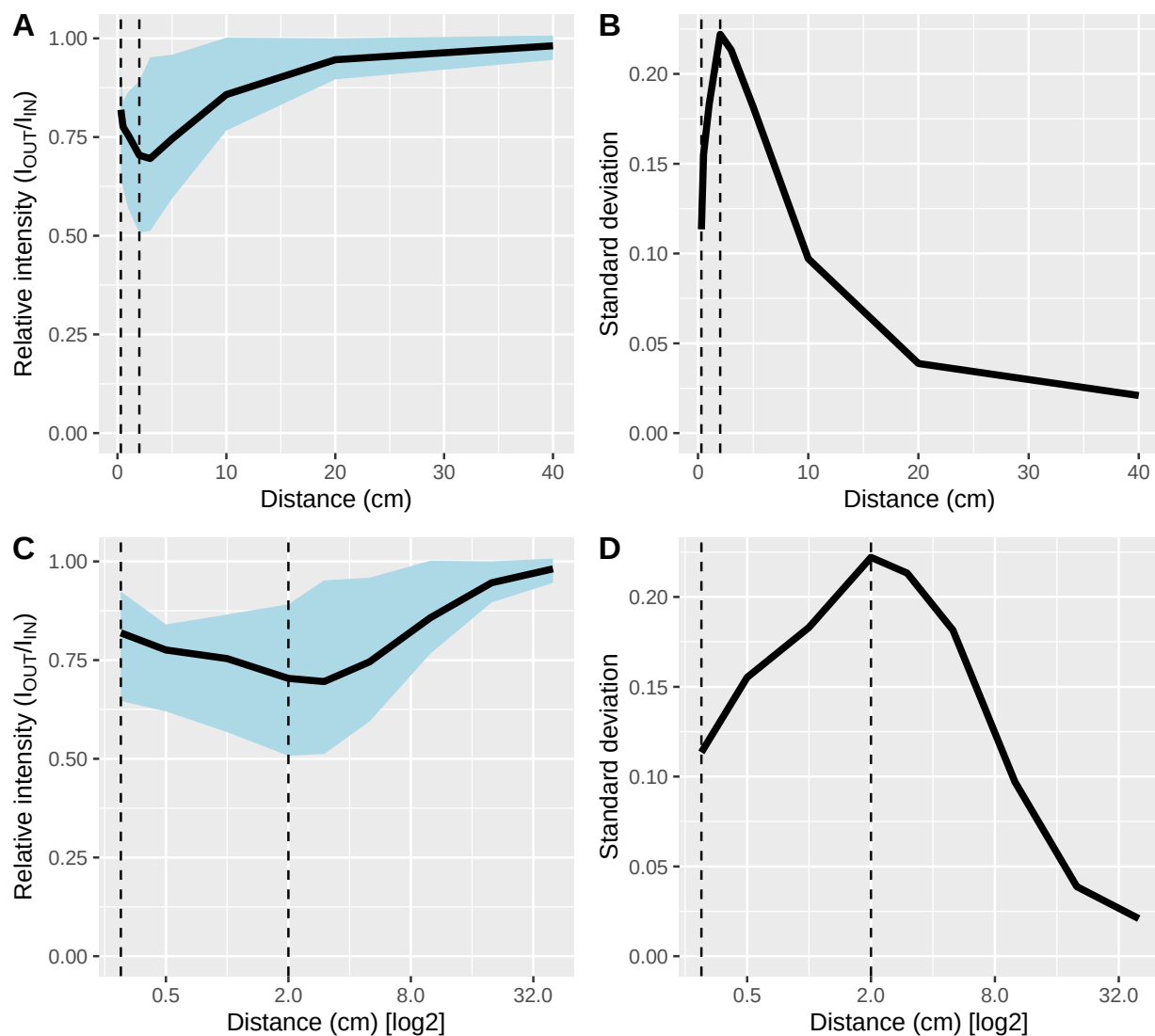
UTEX2973 by Plate Seal (T3)



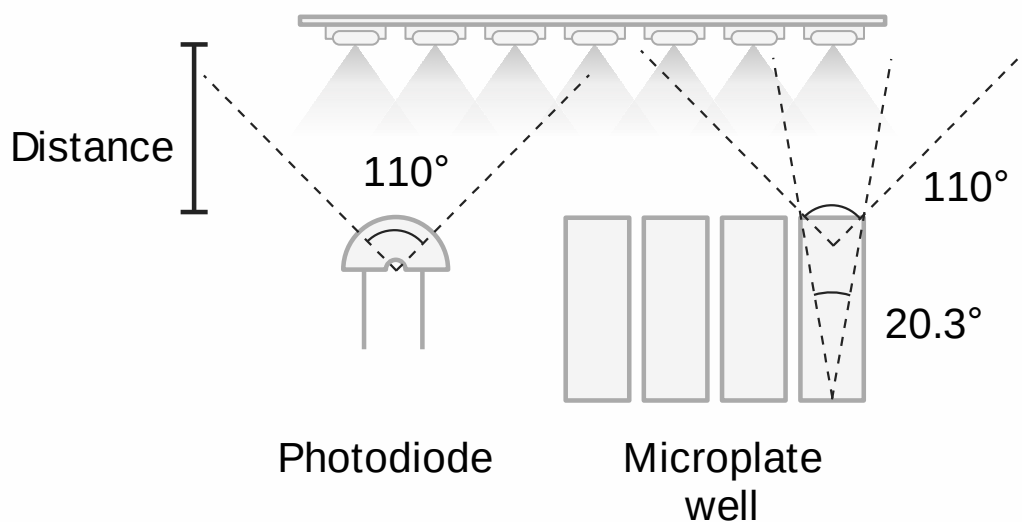
Supplementary Figure 20. Seal test heatmaps. Plate sealed with an AeraSeal seal (top) showed the highest median growth rate and most even distribution of growth across the microplate. Breathe-Easy plate (middle) showed very little growth. The plate with a microplate lid showed an uneven distribution of growth, likely resulting from minimal airflow from the edges of the microplate.



Supplementary Figure 21. Evaporation rates are regular and vary by seal type (A). Woven AeraSeal and PermaSeal seals show no significant variability in well volumes after 96 hours. Rayon Breathe-Easy seals and Nunc lids both show significant variability in volumes after 96 hours (B).

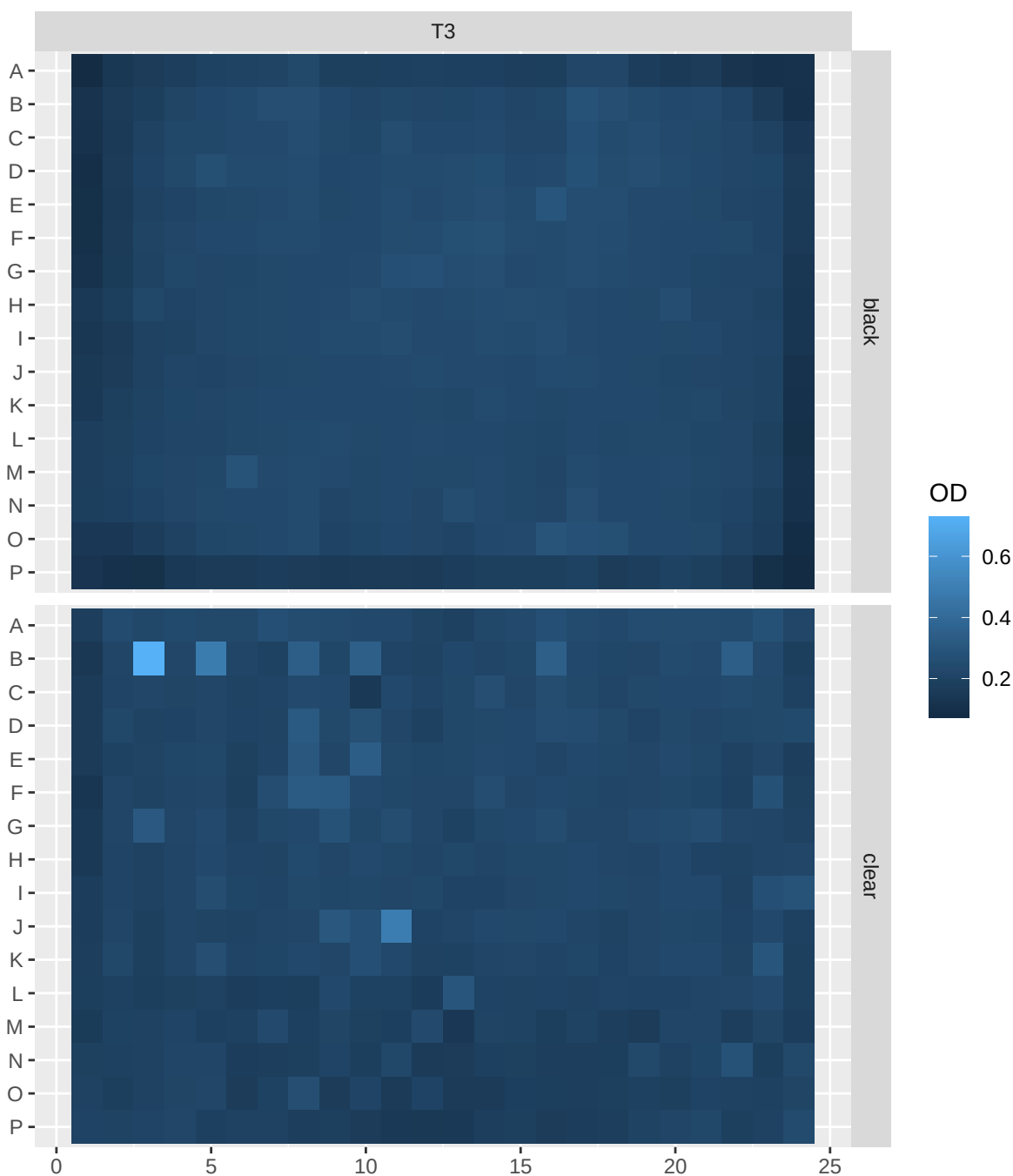


Supplementary Figure 22. Photodiodes likely over-estimate the variability in light intensity between inside and outside of the microplate because of the wide angle that is measured. The angle relevant to culture illumination is narrower, because of well dimensions.



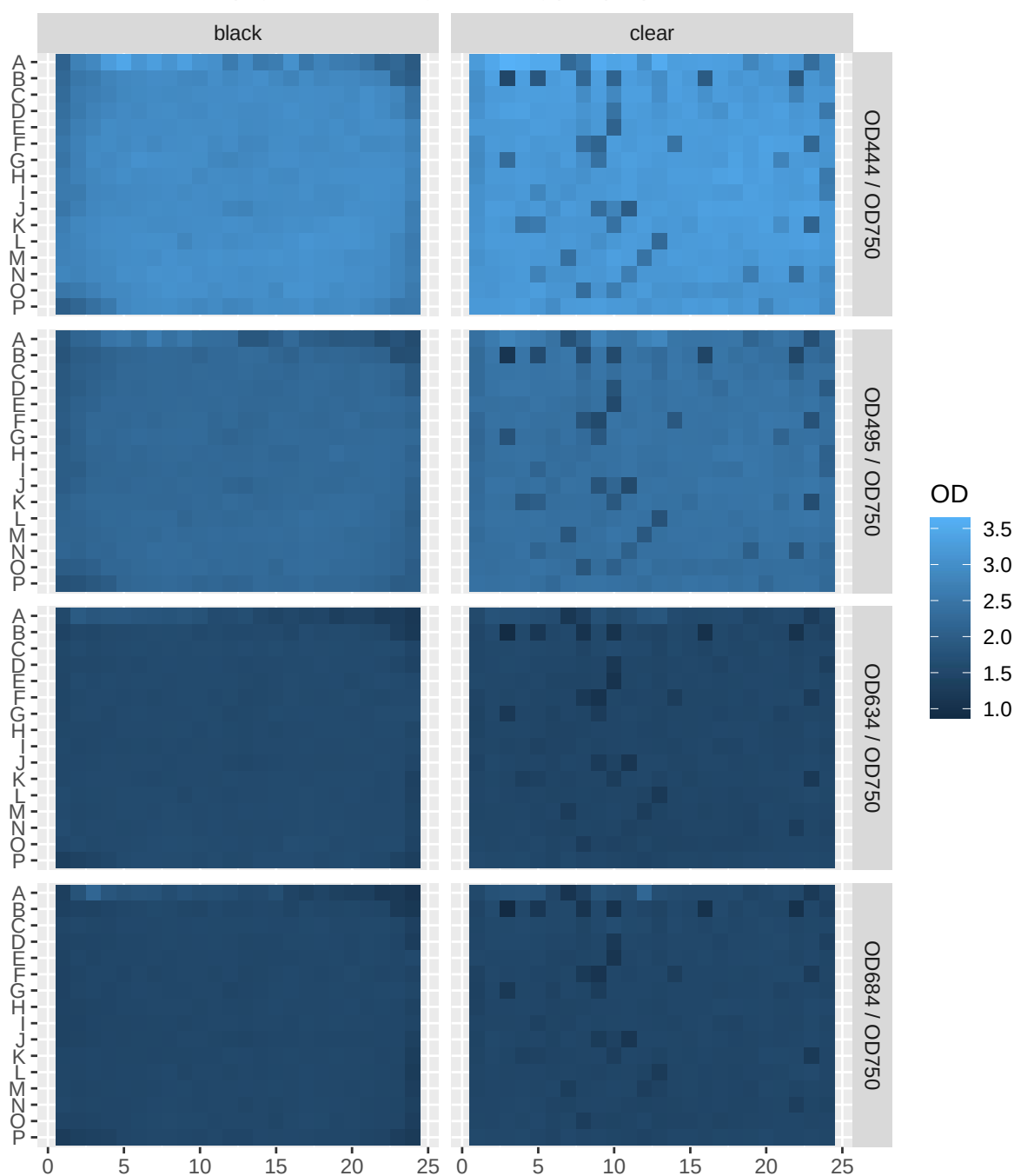
Supplementary Figure 23. Photodiodes likely over-estimate the variability in light intensity between inside and outside of the microplate because of the wide angle that is measured. The angle relevant to culture illumination is narrower, because of well dimensions.

UTEX2973 by Plate Type (T3 / replicate #1)



Supplementary Figure 24. OD₇₅₀ values across 384-well microplate in black or clear microplates (T3). The black plate (top) showed a more even distribution of growth across inside wells. The clear plate showed a more variable distribution of growth across the plate, but with less pronounced edge effects.

Relative Photopigment Abs by Plate Type (T3)



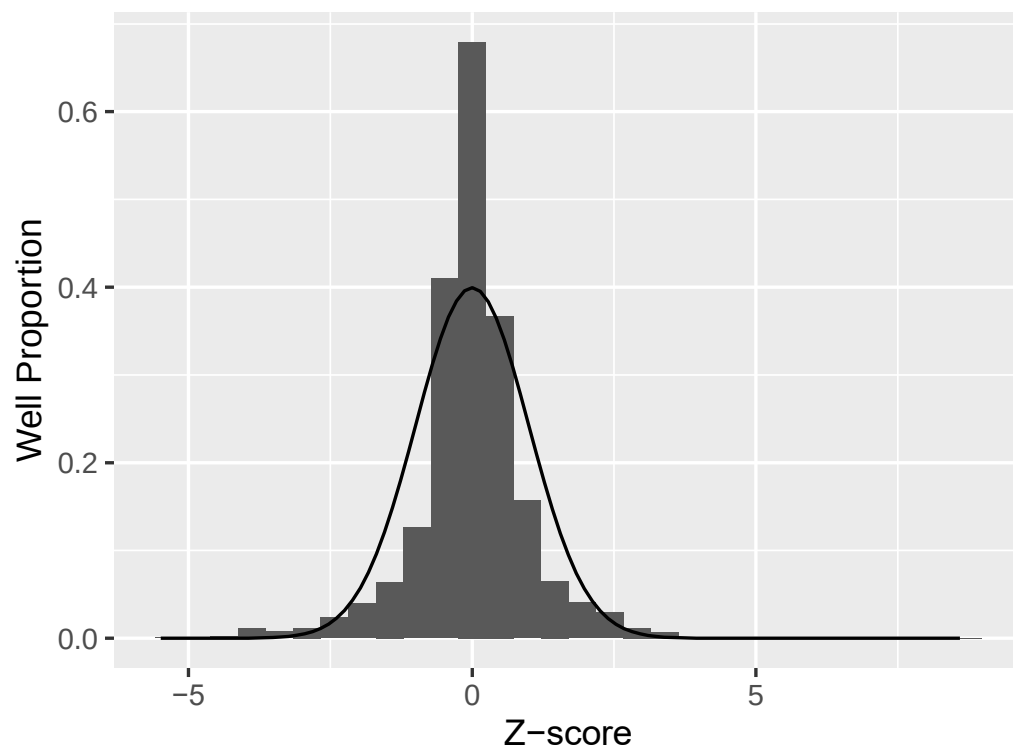
Supplementary Figure 25. Relative photopigment absorbance of cultures across 384-well microplate in black or clear microplates (T3). Rows show relative photopigment absorbance at 4 absorbance peaks (OD₄₄₄, OD₄₉₅, OD₆₃₄, and OD₆₈₄). Columns show black and clear microplates, with black microplates showing less variability across inside wells, but more pronounced edge effects.



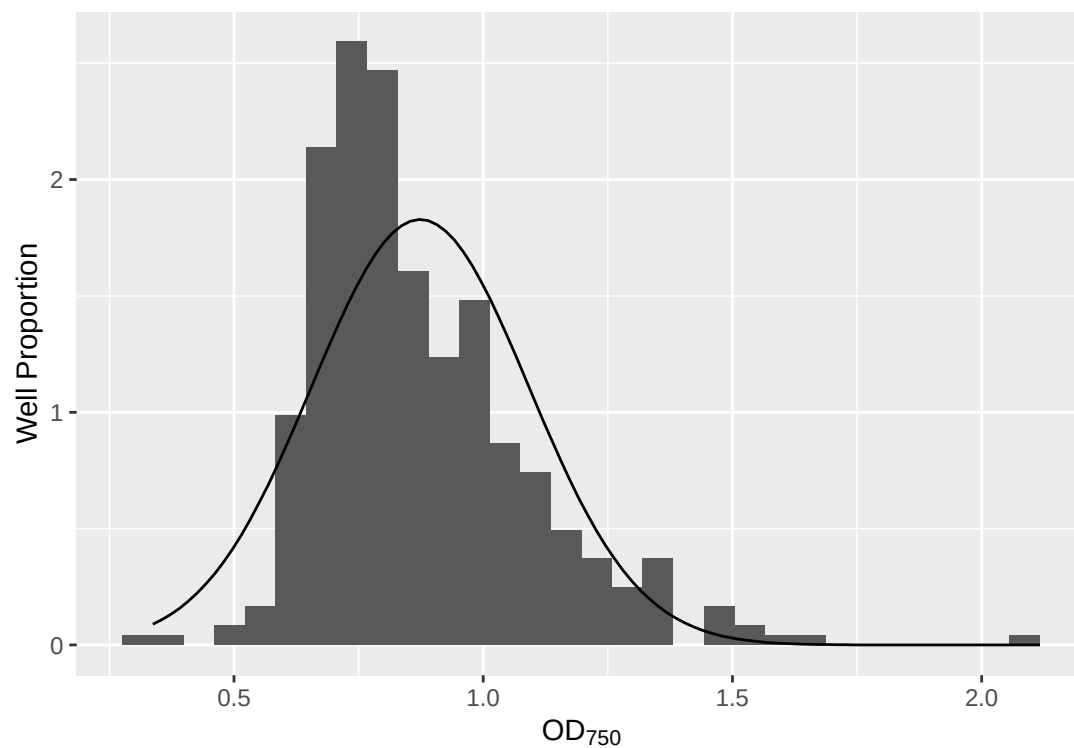
Supplementary Figure 26. *Synechococcus elongatus* UTEX2973 with antibiotic treatments in a black-well 384-well plate.



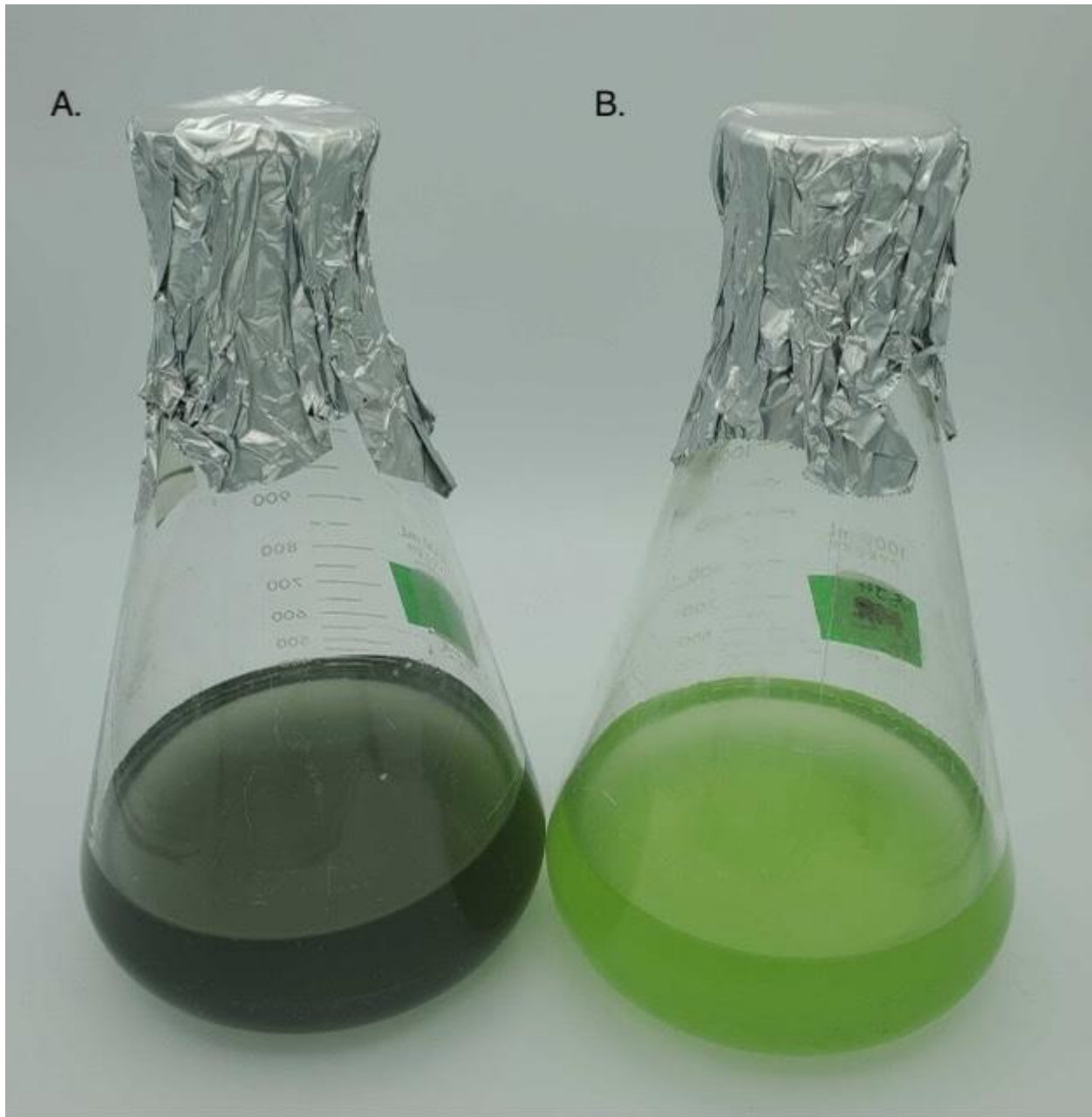
Supplementary Figure 27. OD_{750} values of individual cultures in medium optimization experiment. Only data with NaHCO₃ is shown here (+ Ci). Colour indicates when compound in row label was included in test condition (orange) or not (green). Dashed lines indicate median values of central compositions (standard BG-11 composition).



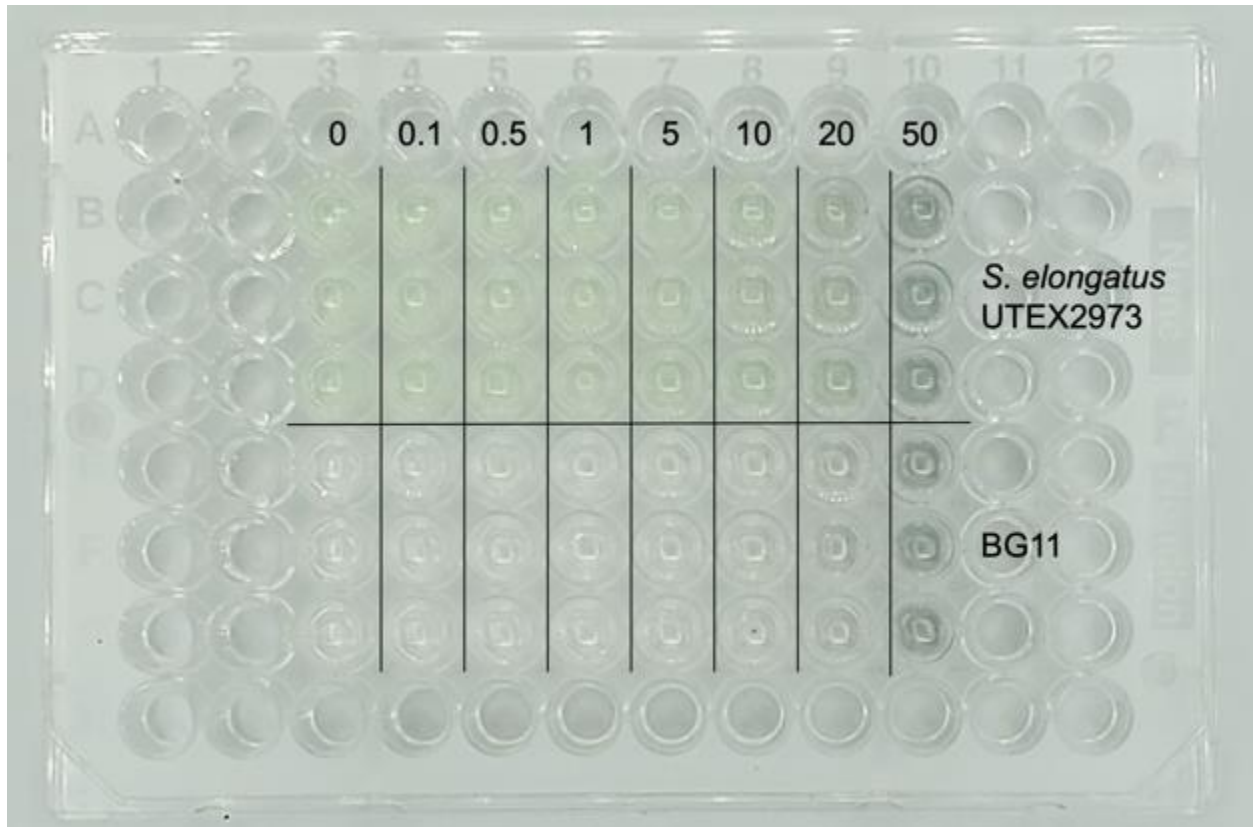
Supplementary Figure 28. Distribution of Z-scores from round 1 of bioactive screen, compared to normal distribution with same mean and standard deviation.



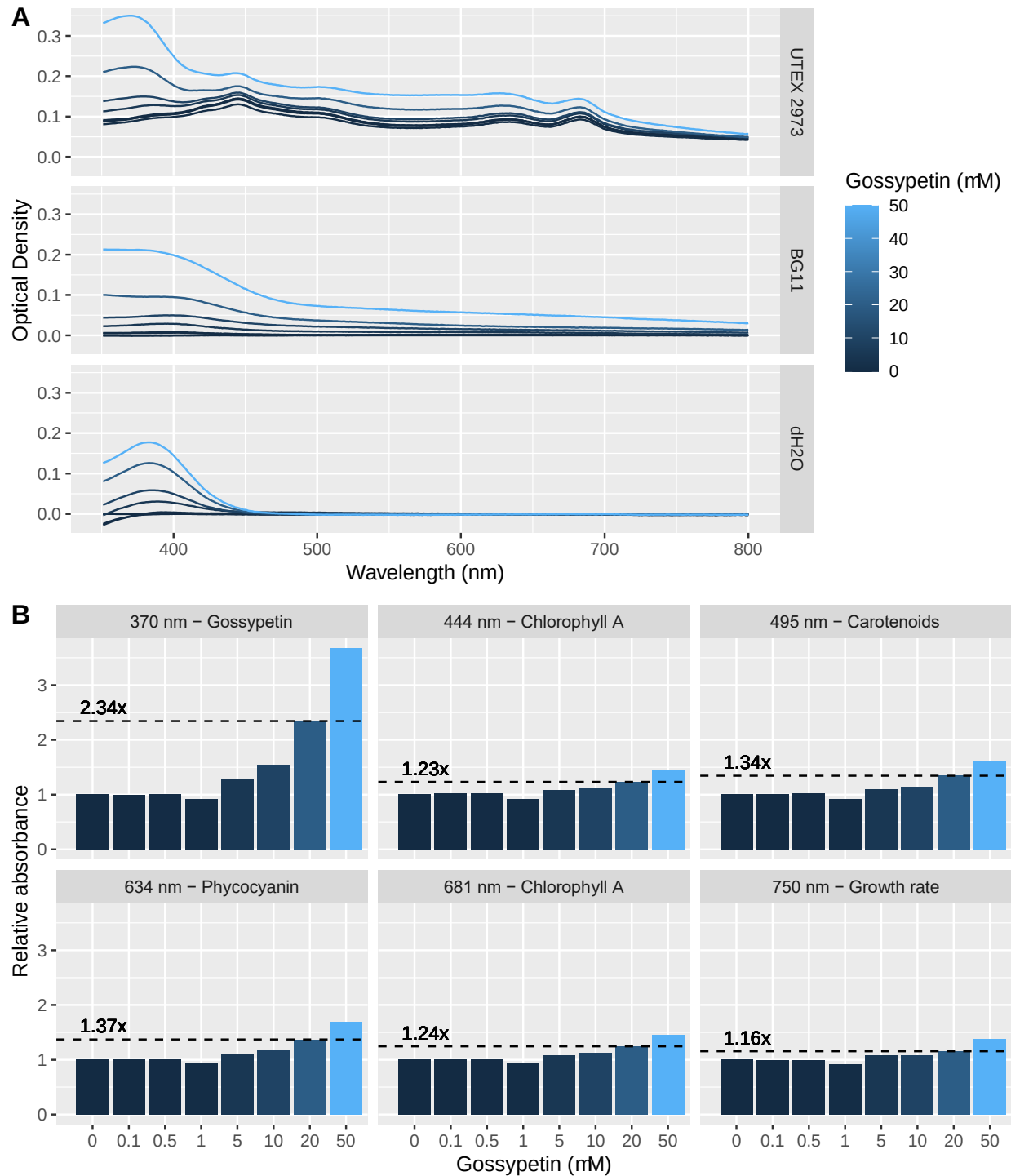
Supplementary Figure 29. Distribution of OD₇₅₀ values from round 2 controls of bioactive screen, compared to normal distribution with same mean and standard deviation.



Supplementary Figure 30. Impact of 20 μM gossypetin treatment on *S. elongatus* UTEX2973 culture (A), compared to untreated culture (B).



Supplementary Figure 31. Gossypetin concentration gradient in *S. elongatus* UTEX2973 culture and BG-11 medium.



Supplementary Figure 32. A) The impact of gossypetin treatment on absorbance of a culture at OD₇₅₀ of 0.2 between 350 nm and 800 nm. B) The impact of gossypetin treatment on absorbance of a culture at OD₇₅₀ of 0.2 at important wavelengths. Y-axis indicates fold-increase in absorbance over untreated culture. Dashed line and labelled value indicate the fold-increase with 20 μ M gossypetin treatment.

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Supplementary Tables

NaNO3	K2HPO4	MgSO4	CaCl2	EDTA	Na2CO3	Citrate	Trace elements	Replicates
0	0	0	0	0	0	0	0	24
-1	-1	-1	-1	1	1	0	1	6
-1	-1	-1	0	-1	0	-1	-1	6
-1	-1	0	0	0	-1	0	1	6
-1	-1	1	-1	-1	-1	1	0	6
-1	-1	1	1	-1	1	-1	1	6
-1	-1	1	1	-1	1	1	-1	6
-1	-1	1	1	1	0	1	0	6
-1	0	-1	-1	-1	1	1	1	6
-1	0	-1	-1	0	-1	-1	0	6
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0	-1	-1	-1	0	1	1	-1	6
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0	-1	1	0	1	1	-1	0	6
0	0	-1	0	-1	1	0	0	6
0	0	-1	1	-1	0	-1	-1	6
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0	1	1	0	-1	0	0	-1	6

0	1	1	1	1	1	1	1	6
1	-1	-1	-1	-1	1	-1	1	6
1	-1	-1	-1	1	-1	1	1	6
1	-1	-1	1	-1	-1	1	-1	6
1	-1	-1	1	1	1	0	-1	6
1	-1	0	-1	1	-1	-1	-1	6
1	-1	1	-1	-1	1	-1	-1	6
1	-1	1	-1	0	0	-1	1	6
1	-1	1	0	-1	1	1	1	6
1	-1	1	0	1	-1	1	-1	6
1	-1	1	1	-1	-1	-1	0	6
1	0	-1	-1	-1	-1	0	-1	6
1	0	-1	0	1	1	-1	1	6
1	0	0	-1	1	1	1	0	6
1	0	0	1	0	0	0	0	6
1	0	1	1	1	-1	0	1	6
1	1	-1	0	1	0	1	0	6
1	1	-1	1	-1	1	1	-1	6
1	1	-1	1	0	-1	-1	-1	6
1	1	0	0	-1	-1	1	1	6
1	1	0	1	-1	1	-1	1	6
1	1	1	-1	0	0	1	-1	6
1	1	1	-1	0	1	0	1	6
1	1	1	-1	1	-1	-1	0	6
1	1	1	1	1	1	-1	-1	6

Supplementary Table 1. BG-11 optimization 8-factor design for response surface modelling using central a central composite design.

Plate ID	OD750	Z-score	Chemical name	Formula
1209	high	2.64	Metronidazole	C6H9N3O3
1209	high	2.69	Naphazoline hydrochloride	C14H15ClN2
1209	high	2.87	Sulindac	C20H17FO3S
1209	high	4.26	Ifenprodil tartrate	C25H33NO8
1212	high	3.23	Methionine sulfoximine (L)	C5H12N2O3S
1215	high	2.66	Clioquinol	C9H5ClINO
1215	high	2.55	2-Aminobenzenesulfonamide	C6H8N2O2S
1215	high	3.25	Nimodipine	C21H26N2O7
1215	high	2.63	Paroxetine Hydrochloride	C19H21ClFNO3
1221	high	2.56	ANDIROBIN	C27H32O7
1221	high	8.59	ISOROTENONE	C23H22O6
1221	high	3.88	DEOXYKHIVORIN	C32H42O9
1221	high	2.63	VULPINIC ACID	C19H14O5
1221	high	2.55	12a-HYDROXY-5-DEOXYDEHYDROMUNDUSERONE	C19H18O6
1221	high	4.81	ERGOSTEROL	C28H44O
1221	high	2.52	IRIDIN	C24H26O13
1224	high	3.14	PERILLIC ACID (-)	C10H14O2
1224	high	3.4	STRYCHNINE METHIODIDE	C22H25IN2O2
1224	high	6.33	BENZYL ISOTHIOCYANATE	C8H7NS
1224	high	3.55	GOSSYPETIN	C15H10O8
1227	high	2.53	PIPERACILLIN SODIUM	C23H26N5NaO7S
1230	high	3.6	PSEUDOEPHEDRINE HYDROCHLORIDE	C10H16ClNO
1230	high	7.04	PYRVINIUM PAMOATE	C49H43N3O6
1230	high	2.69	CISPLATIN	H6Cl2N2Pt
1236	high	3.18	METHYL 7-DESHYDROXYPYROGALLIN-4-CARBOXYLATE	C13H10O6
1236	high	2.62	ACETAMINOSALOL	C15H13NO4
1236	high	2.59	TADALAFIL	C22H19N3O4
1236	high	2.66	TRIFLURALIN	C13H16F3N3O4
1236	high	2.78	RETINYL ACETATE	C22H32O2
1239	high	3.55	DICHLORISONE ACETATE	C23H28Cl2O5
1239	high	3.19	BEPHENIUM HYDROXYNAPTHOATE	C28H29NO4
1243	high	2.61	DL-alpha-Methyl-p-tyrosine	-
1243	high	4.38	N-Acetyl-L-Cysteine	-
1243	high	4.33	4-Chloromercuribenzoic acid	-
1243	high	3.62	ML-9	-
1243	high	2.76	R(+)-6-Bromo-APB hydrobromide	-
1243	high	7.11	Clotrimazole	-

1247	high	3.29	Cyclothiazide	-
1247	high	2.5	($\pm$)-CPP	-
1247	high	2.87	(S)-3,5-Dihydroxyphenylglycine	-
1247	high	2.88	Carvedilol	-
1247	high	3.59	L-3,4-Dihydroxyphenylalanine methyl ester hydrochloride	-
1251	high	2.63	ODQ	-
1251	high	2.86	Nordihydroguaiaretic acid from Larrea divaricata (creosote bush)	-
1255	high	3	Tyrphostin AG 1478	-
1255	high	2.84	Trifluoperazine dihydrochloride	-
1255	high	2.58	Quipazine, 6-nitro-, maleate	-
1255	high	3.41	Tyrphostin AG 490	-
1255	high	2.93	K114	-
1255	high	2.51	($\pm$)-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride	-
1255	high	3.39	U-101958 maleate	-
1255	high	3.78	($\pm$)-Taxifolin	-
1258	high	2.69	Muscarine Cl, (+)-	-
1258	high	2.82	Mycophenolic acid	-
1258	high	2.72	Pinocembrin	-
1258	high	2.64	Bilobalide	-
1258	high	3.35	Ochratoxin A	-
1258	high	3.31	Methysticin	-
1258	high	3.15	Homoeriodictyol (-)	-
1209	low	-2.84	Disulfiram	C10H20N2S4
1209	low	-2.57	Metampicillin sodium salt	C17H18N3NaO4S
1212	low	-2.85	Terconazole	C26H31Cl2N5O3
1212	low	-3.87	Dequalinium dichloride	C30H40Cl2N4
1212	low	-2.66	Ketoconazole	C26H28Cl2N4O4
1212	low	-2.63	Niridazole	C6H6N4O3S
1212	low	-2.98	Ceforanide	C20H21N7O6S2
1212	low	-2.63	Tobramycin	C18H37N5O9
1212	low	-3.85	Riboflavine	C17H20N4O6
1215	low	-2.69	Moxalactam disodium salt	C20H18N6Na2O9S
1215	low	-2.81	Sulfameter	C11H12N4O3S
1215	low	-2.79	Nafcillin sodium salt monohydrate	C21H23N2NaO6S
1215	low	-2.82	Alexidine dihydrochloride	C26H58Cl2N10
1215	low	-3	Cefmetazole sodium salt	C15H16N7NaO5S3
1215	low	-2.54	Cephalothin sodium salt	C16H15N2NaO6S2
1215	low	-2.55	Cefuroxime sodium salt	C16H15N4NaO8S

1221	low	-3.44	DIHYDROROTENONE	C23H24O6
1224	low	-3.05	ARTEMISININ	C15H22O5
1224	low	-5.21	PLUMBAGIN	C11H8O3
1224	low	-2.59	DEGUELIN(-)	C23H22O6
1227	low	-3.57	SANGUINARINE SULFATE	C20H15NO8S
1227	low	-3.84	PYRITHIONE ZINC	C10H10N2O2S2Zn
1227	low	-3.26	DISULFIRAM	C10H20N2S4
1227	low	-4.11	RIBOFLAVIN	C17H20N4O6
1227	low	-3.95	GENTIAN VIOLET	C25H30CIN3
1227	low	-3.66	TOBRAMYCIN	C18H37N5O9
1227	low	-3.57	BLEOMYCIN (bleomycin B2 shown)	C58H94N20O26S4
1230	low	-3.93	PHENYLMERCURIC ACETATE	C8H8HgO2
1230	low	-3.93	THIMEROSAL	C9H9HgNaO2S
1230	low	-2.61	AZITHROMYCIN	C38H72N2O12
1230	low	-2.52	BACAMPICILLIN HYDROCHLORIDE	C21H28CIN3O7S
1236	low	-3.93	DEMECLOCYCLINE HYDROCHLORIDE	C21H22Cl2N2O8
1236	low	-3.69	2-THIOURACIL	C4H4N2OS
1236	low	-3.4	METAMPICILLIN SODIUM	C17H18N3NaO4S
1236	low	-3.57	DICHLOROPHENE	C13H10Cl2O2
1236	low	-3.82	PENTACHLOROPHENOL	C6HCl5O
1236	low	-3.58	CARMOFUR	C11H16FN3O3
1236	low	-3.79	CEFACLOX	C15H14CIN3O4S
1236	low	-2.75	PROPAZINE	C9H16CIN5
1236	low	-3.42	FLOXURIDINE	C9H11FN2O5
1236	low	-2.53	COUMOPHOS	C14H16ClO5PS
1236	low	-3.76	PROMETRYN	C10H19N5S
1236	low	-3.68	DIURON	C9H10Cl2N2O
1236	low	-3.35	HYGROMYCIN B	C20H37N3O13
1236	low	-3.18	GLIPIZIDE	C21H27N5O4S
1239	low	-4.66	CEFTAZIDIME	C22H22N6O7S2
1239	low	-5.49	AURANOFIN	C20H34AuO9PS
1239	low	-3.06	ENDRIN	C12H8Cl6O
1243	low	-3.35	Bay 11-7085	-
1243	low	-2.77	Apomorphine hydrochloride hemihydrate	-
1247	low	-3.15	Dequalinium chloride hydrate	-
1247	low	-3.57	L-3,4-Dihydroxyphenylalanine	-
1247	low	-3.26	5-fluoro-5'-deoxyuridine	-
1251	low	-4.02	Minocycline hydrochloride	-
1251	low	-3.77	BBMP	-

1251	low	-3.24	1-Phenyl-3-(2-thiazolyl)-2-thiourea	-
1251	low	-2.96	Ofloxacin	-
1251	low	-3.71	Lomefloxacin hydrochloride	-
1251	low	-3.05	7-Nitroindazole	-
1251	low	-2.93	Indatraline hydrochloride	-
1251	low	-3.35	SB 242084 dihydrochloride hydrate	-
1251	low	-3.8	Pentamidine isethionate	-
1251	low	-3.94	TBB	-
1251	low	-3.46	Methocramine tetrahydrochloride	-
1251	low	-2.78	(-)-Naproxen sodium	-
1251	low	-3	JWH-015	-
1251	low	-2.97	L-Methionine sulfoximine	-
1251	low	-3.07	L-687,384 hydrochloride	-
1255	low	-2.74	SR 59230A oxalate	-
1255	low	-3.93	NU6027	-
1255	low	-4.15	1,10-Phenanthroline monohydrate	-
1255	low	-3.7	Tetraethylthiuram disulfide	-
1255	low	-3.12	Thio-L-citrulline	-
1255	low	-4.76	Bay 11-7082	-
1255	low	-4.54	Sanguinarine chloride	-
1255	low	-4.34	Vancomycin hydrochloride from <i>Streptomyces orientalis</i>	-
1255	low	-2.74	Pinacidil	-
1258	low	-4.26	Remerine HCl	-
1258	low	-3.8	Capreomycin sulfate	-
1258	low	-3.92	Rosamicin	-
1258	low	-3.13	Tubercidin	-
1258	low	-3.93	Tunicamycin B	-
1258	low	-3.64	Mitomycin C	-
1258	low	-3.97	Cerulenin	-
1258	low	-3.96	Gliotoxin	-
1258	low	-2.7	Ryanodine	-
1258	low	-2.57	Cotininecarboxylic acid, trans-4-	-

Supplementary Table 2. Bioactive screening round 1 hits and associated Z-scores. Compounds without formulas indicate formula was not included in library database.

Chemical name	Significant Measurements	Hit Type	Rep 1 (x-fold)	Rep 2 (x-fold)	Rep 3 (x-fold)
BEPHENIUM HYDROXYNAPTHOATE	2	higher	1.39	2.07	1.27
COUMOPHOS	2	higher	1.3	1.18	1.51
DL-alpha-Methyl-p-tyrosine	2	higher	1.27	1.4	1.16
METHYL 7-DESHYDROXYPYROGALLIN-4-CARBOXYLATE	2	higher	1.14	1.36	1.98
GOSSYPETIN	2	higher	1.08	1.63	2.05
Rosamicin	2	lower	0.98	0.49	0.56
IRIDIN	2	higher	0.92	1.67	1.67
ISOROTENONE	2	lower	0.69	0.63	0.71
Minocycline hydrochloride	3	lower	0.65	0.51	0.49
Sulfameter	3	lower	0.62	0.57	0.59
Lomefloxacin hydrochloride	3	lower	0.51	0.54	0.52
METAMPICILLIN SODIUM	3	lower	0.5	0.36	0.36
Cephalothin sodium salt	3	lower	0.47	0.46	0.49
SB 242084 dihydrochloride hydrate	3	lower	0.46	0.47	0.4
AZITHROMYCIN	3	lower	0.45	0.64	0.5
Tobramycin	3	lower	0.42	0.36	0.4
Indatraline hydrochloride	2	lower	0.41	0.74	0.57
Mitomycin C	3	lower	0.41	0.39	0.47
Alexidine dihydrochloride	2	lower	0.41	0.61	0.67
PENTACHLOROPHENOL	3	lower	0.41	0.35	0.32
Ofloxacin	3	lower	0.41	0.49	0.44
ARTEMISININ	3	lower	0.4	0.48	0.58
Pentamidine isethionate	3	lower	0.39	0.4	0.37
DICHLOROPHENE	3	lower	0.38	0.47	0.45
Vancomycin hydrochloride from <i>Streptomyces orientalis</i>	3	lower	0.36	0.31	0.36
DEMECLOCYCLINE HYDROCHLORIDE	3	lower	0.36	0.39	0.38
Dequalinium chloride hydrate	3	lower	0.36	0.32	0.29
BACAMPICILLIN HYDROCHLORIDE	3	lower	0.35	0.32	0.42
Cefmetazole sodium salt	3	lower	0.35	0.36	0.37
Moxalactam disodium salt	3	lower	0.35	0.33	0.33
TBB	3	lower	0.34	0.41	0.41
CEFTAZIDIME	3	lower	0.33	0.38	0.4
Niridazole	3	lower	0.33	0.31	0.36
Metampicillin sodium salt	3	lower	0.32	0.3	0.26
BBMP	3	lower	0.32	0.35	0.33

1-Phenyl-3-(2-thiazolyl)-2-thiourea	3	lower	0.31	0.44	0.37
Ceforanide	3	lower	0.31	0.31	0.28
Tunicamycin B	3	lower	0.31	0.23	0.24
PROMETRYN	3	lower	0.31	0.42	0.43
Methoctramine tetrahydrochloride	3	lower	0.31	0.51	0.51
Nafcillin sodium salt monohydrate	3	lower	0.3	0.36	0.34
Sanguinarine chloride	3	lower	0.3	0.3	0.32
AURANOFIN	3	lower	0.3	0.33	0.25
Apomorphine hydrochloride hemihydrate	3	lower	0.3	0.38	0.39
SANGUINARINE SULFATE	3	lower	0.3	0.29	0.29
Cerulein	3	lower	0.29	0.25	0.29
1,10-Phenanthroline monohydrate	3	lower	0.29	0.37	0.38
Capreomycin sulfate	3	lower	0.29	0.32	0.34
CARMOFUR	3	lower	0.29	0.39	0.45
DIURON	3	lower	0.29	0.33	0.36
FLOXURIDINE	2	lower	0.28	0.55	0.64
Bay 11-7082	3	lower	0.28	0.27	0.31
Bay 11-7085	3	lower	0.27	0.34	0.32
Cefuroxime sodium salt	3	lower	0.26	0.31	0.32
Tubercidin	3	lower	0.25	0.29	0.37
PLUMBAGIN	3	lower	0.25	0.25	0.26
HYGROMYCIN B	3	lower	0.25	0.35	0.44
Disulfiram	3	lower	0.23	0.18	0.2
DISULFIRAM	3	lower	0.23	0.29	0.25
Tetraethylthiuram disulfide	3	lower	0.23	0.29	0.23
GENTIAN VIOLET	3	lower	0.23	0.14	0.13
PYRITHIONE ZINC	3	lower	0.21	0.24	0.27
BLEOMYCIN (bleomycin B2 shown)	3	lower	0.2	0.24	0.26
PHENYLMERCURIC ACETATE	3	lower	0.2	0.2	0.13
Glutathione	3	lower	0.19	0.23	0.25
Riboflavin	3	lower	0.16	0.14	0.24
Dequalinium dichloride	3	lower	0.13	0.25	0.32
RIBOFLAVIN	3	lower	0.12	0.08	0.11
THIMEROSAL	3	lower	0.12	0.12	0.13
Remerine HCl	3	lower	0.09	0.11	0.08

Supplementary Table 3. Round 2 compounds matching selection criterion.

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Compound name	Supplier	Formula	MolWt	CAS number
Gossypetin	BOC Sciences	C ₁₅ H ₁₀ O ₈	318.2	489-35-0
Bephenium Hydroxynapthoate	Sigma	C ₂₈ H ₂₉ NO ₄	443.5	3818-50-6
Iridin	AChemBlock	C ₂₄ H ₂₆ O ₁₃	522.5	491-74-7
Coumaphos	Sigma	C ₁₄ H ₁₆ ClO ₅ PS	362.8	NA
DI-Alpha-Methyl-P-Tyrosine	Sigma	NA	195.2	NA
Gentian Violet	Sigma	C ₂₅ H ₃₀ CIN ₃	408	548-62-9
Phenylmercuric Acetate	Sigma	C ₈ H ₈ HgO ₂	336.7	62-38-4
Disulfiram	Sigma	C ₁₀ H ₂₀ N ₂ S ₄	296.5	97-77-8

252 **Supplementary Table 4.** Bioactive compound supplier.